	Formular
	Portofoliu lucrări științifice

UNIVERSITATEA DE ȘTIINTE AGRONOMICE ȘI MEDICINĂ VETERINARĂ DIN BUCUREȘTI
Domeniul de studii universitare de doctorat: Horticultură

PORTOFOLIU LUCRĂRI STIINȚIFICE

Candidat: TUDOR P. Valerica, Dr./ din 18.10.2011, Conferențiar Universitar/din 01.10.2020

1.	Iulian Andrei SZEIKELY, **Valerica TUDOR***, Răzvan Ionuț TEODORESCU, 2024. *Biodiversity in vegetable culture and its role in the sustainability of agroecosystems. A review*, Scientific Papers Series "Management, Economic Engineering in Agriculture and Rural Development", Vol. 24, Issue 4, PRINT ISSN 2284-7995, E-ISSN 2285-3952, 805-815, WOS:001437484400038, IF=1.00, https://www.webofscience.com/wos/woscc/full-record/WOS:001437484400038
2.	Oana Hera, Monica Sturzeanu, Loredana Elena Vîjan*, **Valerica Tudor**, Răzvan Teodorescu, 2023. *Biochemical Evaluation of Some Fruit Characteristics of Blueberry Progenies Obtained from 'Simultan×Duke'*, ACS Omega 2023, 8, 21, DOI: 10.1021/acsomega.3c00466, 18603-18616, WOS:001011060900001, IF=3.70 https://www.webofscience.com/wos/woscc/full-record/WOS:001011060900001
3.	Andrei Daniel TUDOR, Ciprian BOLOHAN, **Valerica TUDOR***, Răzvan Ionuț TEODORESCU, 2022. *Main active components of goji berry and their nutritional importance – a review*, Journal of Applied Life Sciences and Environment https://jurnalalse.com Review Article, doi.org/10.46909/alse-52053, Vol. 55, Issue 2 (190)/2022, pp 111-132, https://jurnalalse.com/wp-content/uploads/ALSE2-22-01.pdf
4.	Mihalascu Costel, Bolohan Ciprian, **Tudor Valerica***, Mihalache Mircea, Teodorescu Razvan, 2020. *Research on the growth and development of some varieties of Lavandula angustifolia (Mill.) in the South-East of Romania*, Rom Biotechnol Lett. 2020; 25(6): 2180-2187, Received for publication, July, 28, 2020 Accepted, August, 28, 2020, Vol. 25, Nr.6 /2020, Rom Biotechnol Lett. 2020, doi: 10.25083/rbl/25.6/2180.2187, ISSN print: 1224-5984 ISSN online: 2248-3942, 2180-2187, WOS:000581736800022, IF=0.765, https://www.webofscience.com/wos/woscc/full-record/WOS:000581736800022
5.	Dan POPESCU, Adrian ASĂNICĂ, **Valerica TUDOR**, 2021. *Start of the blueberry breeding program at the University of Agronomic Sciences and Veterinary Medicine of Bucharest*, Scientific Papers. Series B, Horticulture. Vol. LXV, No. 2, 2021 Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653, 50-55,WOS:000756054100007, https://www.webofscience.com/wos/woscc/full-record/WOS:000756054100007
6.	Asănică A., **Tudor Valerica**, Badea Adriana, Micu M.M., 2014. *Sweet cherry breeding programme at Istrita farm*, Analele Universității din Craiova, Vol. XIX (LV) – 2014, ISSN 1453-1275, pp 5-14, https://www.cabidigitallibrary.org/doi/full/10.5555/20153293456
7.	Jeni Veronica Țiu, Răzvan Ionuț Teodorescu, **Valerica Tudor***, Mircea Mihalache, Adrian Asănică, Sorin Mihai Cîmpeanu, 2016. *The Effect of Drip Irrigation on Several Physical and Chemical Features of Soil*, Romanian Biotechnological Letters, Vol. 21, No. 4, received for publication July 4, 2015, accepted April 18 2016, pp 11669-11676, WOS:000385259500015, IF=0.381, https://www.webofscience.com/wos/woscc/full-record/WOS:000385259500015
8.	Adrian Asănică, **Valerica Tudor**, Catița Plopa, Mihaela Sumedrea, Adrian Peticilă, Răzvan Teodorescu, Valentina Tudor, 2016. *In vitro embryo culture of some sweet cherry genotypes*, Agriculture and Agricultural Science Procedia (5th International Conference "Agriculture for Life, Life for Agriculture", 2016), Volume 10, ISSN: 2210-78432016, DOI: 10.1016/j.aaspro.2016.09.049, 172–177, WOS:000387504900025, https://www.webofscience.com/wos/woscc/full-record/WOS:000387504900025
9.	**Valerica Tudor**, Carmen Manole, Răzvan Teodorescu, Adrian Asănică, Iuliana Diana Bărbulescu, 2015. *Analysis of some phenolic compounds and free radical scavenging activity of strawberry fruits during storage period*, Agriculture and Agricultural Science Procedia (Conference Agriculture for Life, Life for Agriculture), Vol. 6, doi: 10.1016/j.aaspro-2015.08.053, pp 157-164, WOS:000380846200024, https://www.webofscience.com/wos/woscc/full-record/WOS:000380846200024
10.	V. Bălan, **Valerica Tudor**, E. Topor, M. Corneanu, 2007. *Apricot Genetics and biotechnology in Romania*, Invited review, The European Journal of Plant Science and Biotechnology, ©2007 Global Science Books, ISSN 1752-3842, pag 153-169, http://www.globalsciencebooks.info/Online/GSBOnline/images/0712/EJPSB_1(2)/EJPSB_1(2)152-169o.pdf

Data 09.04.2025

Candidat: Conf. univ. dr. Tudor Valerica

Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 24, Issue 4, 2024
PRINT ISSN 2284-7995, E-ISSN 2285-3952

BIODIVERSITY IN VEGETABLE CULTURE AND ITS ROLE IN THE SUSTAINABILITY OF AGROECOSYSTEMS. A REVIEW

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Abstract

Biodiversity plays an essential role in vegetable agroecosystems, providing multiple benefits for soil health, natural pest control, and climate change adaptation. This article examines the role of biodiversity in vegetable cultivation and the impact of diversified agricultural practices on the sustainability and health of agroecosystems. The research methods were represented by: studying specialized and scientific literature, structuring the references on the topics of interest, critical evaluation of the ideas belonging to different authors, exposing our own ideas and drawing the main conclusions on the role played by biodiversity in vegetable culture in the sustainability of agroecosystems. The study shows that diversifying vegetable crops through species rotation, intercropping, the use of cover crops, and applying organic mulch is essential for maintaining biodiversity in agroecosystems. These methods contribute to soil health, reduce the need for chemical inputs, and enhance ecological stability. Thus, they represent effective solutions for promoting sustainable agriculture and a healthier, more balanced agricultural environment, offering a solid foundation for resilient and adaptable agriculture.

Key words*: biodiversity, agroecosystems, legumes, cover crops, sustainable agriculture*

INTRODUCTION

The intensification of modern agriculture has led to the adoption of monoculture practices, which has increased the risk of biodiversity loss and affected the stability of agroecosystems [4]. Biodiversity, especially in vegetable cultivation, plays a fundamental role in supporting soil health, attracting natural pest predators, and maintaining a stable ecological balance. Various vegetable species and the use of ecological practices, such as crop rotation and intercropping, contribute to biodiversity conservation and provide a foundation for the development of sustainable agriculture [3], [13].

Plant diversity in vegetable agroecosystems contributes to adaptation and resilience to climate variations. Agricultural systems with a high diversity of species can respond better to climate stress, such as drought or extreme temperatures, through ecological mechanisms that help maintain soil moisture and productivity [18], [85]. The study by [61] showed that crop diversification can enhance agroecosystem resilience, allowing them to adapt more easily to climate variability. For example, cover crops and intercropped crops help maintain lower soil surface temperatures, reduce moisture loss, and protect crops from extreme temperatures. In the long term, implementing diversification practices in vegetable cultivation could reduce dependence on synthetic pesticides and fertilizers, thereby contributing to increased ecological and food security [61].

Vegetable species play an essential role in maintaining and enhancing the biodiversity of agroecosystems. They contribute to soil health, natural pest control, and support balanced ecosystems through complex nutrient cycles and beneficial interactions with microorganisms and local fauna. The diversity of vegetable species includes not only traditional varieties but also legumes, cover crops, and crops with increased genetic resistance, all of which contribute to a sustainable agricultural system.

Biodiversity in agroecosystems helps conserve water and prevent soil erosion by providing permanent ground cover and improving soil structure.

Cover crops and intercropped plants help safeguard soil from wind and water erosion, enhancing water retention and minimizing nutrient loss through runoff.

[20] study demonstrated that cover crops like clover and mustard effectively mitigate soil erosion and enhance water retention, promoting long-term soil fertility and stability.

Another study, conducted by [58], highlights the benefits of biodiversity in reducing soil erosion and conserving water, showing that better-structured soil has a greater capacity for water infiltration, thus preventing soil degradation and preserving water resources.

Aromatic and medicinal plants, such as rosemary (*Rosmarinus officinalis*) and lavender (*Lavandula* spp.), are particularly valuable for attracting natural predators and pollinators, thereby contributing to pest control and improving vegetable productivity. These plants have a high content of essential oils that deter many harmful species while attracting beneficial insects, such as bees and parasitic wasps. According to the research by [92], adding aromatic plants to vegetable crops increases faunal biodiversity and may help reduce the need for pesticides.

A study by [67] showed that the use of organic mulch in vegetable cultivation supports a greater diversity of beneficial soil organisms, such as bacteria and fungi. Organic mulch also contributes to stabilizing nutrient levels and improving soil health, thus facilitating long-term productivity.

The purpose of this paper is to examines the role of biodiversity in vegetable cultivation and the impact of diversified agricultural practices on the sustainability and health of agroecosystems.

MATERIALS AND METHODS

Information sources were represented by specialized and scientific publications on innovative technologies, reports, projects and studies at both national and international levels were consulted.

The studied literature was structured based on the topics of interest and the authors' ideas have been critically evaluated and commented.

The content of this research work includes the results of the bibliographic analysis focused on specific vegetable farming practices, such as crop rotation, the use of cover crops and the application of organic mulch, with the aim of synthesizing best practices and their impact on sustainability.

RESULTS AND DISCUSSIONS

Legumes and their contribution to biodiversity conservation

The production of legumes provides numerous ecosystem services, facilitating land use diversification and supporting biodiversity in agroecosystems due to their ability to fix atmospheric nitrogen in the soil.

In the European Union, protein crops occupy only 1.5% of arable land, compared to 14.5% globally [101]. A relatively small percentage of peas (*Pisum sativum* L.) (11–15%) and fava beans (*Vicia faba* L.) (9–14%) cultivated in Europe are designated for human consumption, with the majority being allocated for animal feed, a less efficient method of protein production for human diets [17]. In Northern Europe, peas (*Pisum sativum* L.) and fava beans (*Vicia faba* L.) have long been integral crops in agricultural practices [94]. Protein-rich crops such as fava beans (*Vicia faba* L.), peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), lupins (*Lupinus albus* L.), and soybeans (*Glycine max* L.) possess the unique ability to fix atmospheric nitrogen, making them essential for low-input farming systems that aim to lower greenhouse gas emissions [60].

These crops can provide significant amounts of nitrogen to subsequent crops in the rotation, reducing the need for mineral fertilizers [101]. In addition, they improve soil health and support crop protection [51]. Other studies have shown that adding legumes to cereal rotations has positive effects on cereal yields and gross margins, compared to monocultures [102].

[71] demonstrate that intercropping maize (*Zea mays* L.) with legumes increases N uptake. Additionally, nitrogen fixation

provides significant residual benefits, enhancing the productivity of subsequent maize crops [1].

Recently, legume crops have gained importance due to rising prices for animal feed and food proteins, fertilizers and fuel, in addition to sustainability concerns. The impact of introducing legume (*Vicia faba* L.) into dominant cereal crop production systems, typical of southwestern Finland, was investigated by [98].

Traditional varieties and indigenous species of vegetables

The wide array of traditional crops once cultivated on a sustainable scale in various regions of the world has been replaced by a limited selection of major crops grown in large-scale monocultures [49]. This shift has significantly reduced the diversity of species upon which global food security depends [96]. Over the past 50 years, reliance on commercial hybrids and advanced cultivars, coupled with the marginalization of traditional local species, has drastically diminished horticultural and agricultural biodiversity. Additionally, factors such as habitat loss, climatic changes, and evolving cultural practices have further narrowed the spectrum of non-commercial crops commonly utilized by humans. Globally, vegetable genetic resources are being lost at an estimated rate of 1%–2% annually [25], primarily due to changes in how the human population exploits the world's edible plant resources [86].

There is a need of investment in research breeding and cultivar development in traditionally open-pollinated cultivars and in the minor and so-called “forgotten” vegetables. More investments in this area will mean cheaper cultivars for growers to choose from and more preservation of vegetable biodiversity.

Indigenous and traditional vegetables exhibit remarkable biodiversity, thriving in specific marginal soil and climatic conditions with minimal reliance on external inputs [28], [48]. Incorporating these traditional vegetables into current production systems enhances their heterogeneity, which in turn improves resilience to both abiotic and biotic stresses [74].

[61] highlights examples where diverse agroecosystems successfully suppressed pests and diseases while buffering against climate variability.

Several solanaceous crops, including species of Solanum, Capsicum, and Physalis, are cultivated beyond their original centers of domestication. Recently, there has been growing interest in novel Solanaceae crops for European cultivation [79], [87], [89], [88], [68], [69]. This emerging focus underscores the potential of lesser-known species, warranting further exploration and research. As climate change continues to shift environmental parameters, many of these crops could potentially expand beyond their traditional climatic zones [86].

The initial step in launching new breeding programs for indigenous vegetable crops is their thorough characterization, a process that should begin promptly across various countries. The assumption that these crops are inherently and permanently "resistant to pests and diseases" compared to conventional or globally cultivated vegetable crops is likely a misconception. When indigenous crops transition to mainstream production, they will inevitably face a broad spectrum of pests and diseases, potentially undermining farm productivity. To ensure sustainable and profitable cultivation, defensive strategies such as selective breeding, grafting, integrated pest management, and robust agronomic practices will be essential to achieve high-quality and sufficient crop yields [55].

Cover crops

Cover crops, such as red clover (*Trifolium pratense* L.) and rapeseed (*Brassica napus* L.), are essential for soil protection against erosion and improving microbial biodiversity. These crops provide constant soil cover, preventing nutrient runoff and offering a favorable habitat for beneficial microorganisms and insects. In a study conducted by [19], it was shown that the use of cover crops reduces the need for chemical inputs and supports soil structure by increasing microbial activity.

Cover crops also contribute to pest control by stimulating populations of natural predators and pollinators, thus maintaining an

ecological balance within the agroecosystem [59].

Cover crops are plants grown between production cycles of main crops to protect the soil from erosion, increase organic matter content, and improve soil fertility or as feed for animals. They also provide habitat for a wide range of beneficial organisms, including predatory insects, bacteria, and fungi.

The decomposing residues of brassica cover crops, through the release of glucosinolates, aid in the control of parasitic nematodes [81]. In addition, during the same phase, they can cause chemical and physical changes in the soil and facilitate root penetration of the next crop and act as a buffer for the soil [50]. Several studies confirm that the use of legume cover crops in crop rotations, such as clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.), and graminaceous cover crops, such as ryegrass (*Trifolium pratense* L), oat (*Medicago sativa* L.), and barley (*Hordeum vulgare* L.*)*, enhance the yields of the following cash crop [52], [64], [24].

Studies conducted by [19], demonstrate that cover crops such as red clover (*Trifolium pratense* L.) and mustard (*Brassica napus* L.) improve soil health by enriching it with nutrients, reducing the need for chemical fertilizers, and helping regulate pest populations. Additionally, [59] emphasized that the use of cover crops increases biodiversity by attracting pollinators and through biological pest control.

There are various crop alternatives to be used as vegetative cover, such as grains, legumes, root crops and oil crops. All of them are of great benefit to the soil, however some cover crops have certain attributes, which need to be kept when planning a rotation scheme [2], [23]. It is important to start the first years of conservation agriculture with cover crops that leave a lot of residues on the soil surface, which decompose slowly (because of the high carbon/Nitrogen ratio, an indicator for nitrogen limitation of plants and other organisms). The adoption of cover crops in agro-ecosystems provides multiple benefits to the agro-ecosystems.

To maximize the agro-ecological functions, complementing and synergizing the effects, cover crops are usually cultivated in a mixture. Very important, in the constitution of these mixes is to use the functional complementarity of the species [21]. The potential application of crop mixtures (involving cereal, legume, and even crucifer cover crops) is an issue of strategic interest when designing low-C cropping systems such as in Mediterranean areas.

In addition, Brassicaceous over crops are chosen to improve soil penetration resistance due to taproot growth but are also used as a highly effective catch crop [11]. For example, mixing radish with rye can mitigate both soil compaction and soil erosion risks due to the bio-drilling potential of radish and abundant aboveground biomass cover produced by rye [22], [82].

Radish (*Raphanus raphanistrum* subs. *sativus*), a widely used and highly beneficial cover crop, catch soil nutrients, especially nitrogen [93]. In that sense, the use of cruciferous species as cover crops could allow the natural control of potential diseases.

[99] showed that radish (*Raphanus raphanistrum* subsp. *sativus*) or brown mustard (*Brassica juncea* L.) as a biofumigant crop could be effective against plant-parasitic nematodes without compromising on soil health or changing the structure of the nematode community. Furthermore, [6] revealed that using radish (*R. sativus*) and arugula (*Eruca sativa*) as winter cycle plants before plants that are susceptible to the root-knot nematode *Meloidogyne arenaria* would help to reduce gall index, egg masses and consequently damage and also increase crop yields. Last, leguminous vover crops are recognized as the most effective when maximizing nitrogen (N) input becomes the priority [35]. Indeed, leguminous cover crops can deposit significant amounts of N in the soil during growth and have the ability to acidify the rhizosphere by facilitating the uptake of insoluble phosphorous into the soil [72], [100]. According to [91], the presence of *Trifolium subterraneum,* for three consecutive years, determined a considerable increase in ammoniacal nitrogen, nitric nitrogen, and the N cycle bacteria.

Furthermore, [39] revealed that the mitigation effect of the legume (vetch) cover crops mainly due to the reduction of synthetic N inputs in the subsequent cash crop as well as a decrease in indirect N_2O emissions from NO_3^- leaching and an increase in C sequestration due to an intensive photosynthetic activity.

[29] have observed that the specific use of *Trifolium repens* and *Vicia villosa* as cover crops can improve soil quality and yield in apple orchards. In long-term cropping systems such as orchards, cover crops have economic benefits because, in addition to protecting soil against water and wind erosion, they can contribute, through residue deposition, to nutrient recycling, increased soil health and reduced mineral fertilization needs [27]. This could be useful on vineyard and olive tree systems that are generally affected by erosion due to the high loss of organic matter and excessive tillage operations [7]. Other tree systems that have the same issues are those consisting of almond [65], apricot [56] and persimmon orchards [83]. Several studies showed that the cultivation of cover crops is an effective solution to minimize soil erosion in these orchards caused by intensive tillage, excessive mineral fertilizer applications and herbicide use and, therefore, preserve soil from the risk of desertification [54], [8].

[36] observed that the use of cover crops could reduce soil losses by 3.8 to 0.7 Mg ha^{-1} in a vineyard.

[76] showed that using cover crops reduces by 27% annual water runoff and can be used as an agronomic strategy for improving water use efficiency.

Aromatic and medicinal plants

Intercropping medicinal and aromatic plants with various horticultural crops plays a significant role in reducing post-harvest yield losses, preserving fruit quality, and extending shelf life during storage. Additionally, the essential oil content, yield, and composition of medicinal and aromatic plants are influenced by the interspecific competition present within the intercropping system. [32], [77].

According to the experimental result of [57] intercropping of some aromatic plants with tomato (*Solanum lycopersicum* L.) protect the infestation of *Tuta absoluta* on tomato. The inclusion of rosemary (*Rosmarinus officinalis* L.) with onion elevated yield advantage and competitiveness over sole planted crop per unit area as indicated by higher LER and relative crowding coefficient. This enables to prevent the insect pest attack on onion [5]. Planting marigolds (*Calendula officinalis L.)* between tomatoes protects the tomato plants from harmful root-knot nematodes in the soil and increase the marketable fruit yield of tomato by trapping different insects and pest attack and the like [31]. Marigold repels nematodes, tomato worm, slugs and general garden pests [38] found that intercropping of tomato with African marigold (*Tagetes erecta* L.) reduced early blight (*Alternaria solani*) of tomato (*Solanum lycopersicum* L.). Intercropping marigold (*Tagetes erecta* L.) for nematode management also appeared to reduce numbers of aphids and whiteflies, and resulted in lower levels of virus in tomato [103].

Tomato (*Solanum lycopersicum* L.) and basil (*Ocimum basilicum* L.) are common pairs that are intercropped [70]. Several studies reported the performance of intercropping of aromatic and medicinal plant species with selected major horticultural crops in different countries as cited by [70], the experimental results of [73], [75]. [37] reported intercropping of onion (*Allium cepa* L.) with basil (*Ocimum basilicum* L.) at a 1:1-row arrangement could provide farmers with the best yield advantage and income over sole planting of component (onion) crops. Basil and tomato are companion plants that have similar lighting and watering needs, some even say tomatoes (*Solanum lycopersicum* L.) taste better when they neighbour basil (*Ocimum basilicum* L.) [12].

Application of organic mulch

The application of organic mulch is an effective technique for conserving soil moisture, reducing weed growth, and improving soil structure [80]. Organic mulch helps increase soil biodiversity by providing a habitat and a constant source of organic matter for soil organisms. It can also

contribute to maintaining soil temperature and improving water retention capacity.

Numerous studies have demonstrated that intercropping and living mulches can positively influence pest and disease management as well as weed control [40], [41], [45], [46], [47], [53], [90] . However, this outcome cannot be universally applied and must be evaluated on a case-by-case basis [66]. The effectiveness of these practices often depends on various factors, including the cropping systems and the arthropod species involved in the experiments.

[30] highlight the overall positive impact of the living mulch technique on plant-soil systems, evidenced by increased soil biodiversity and the absence of significant negative effects on pest abundance.

In the study by [30], the effects of a 'cover crop–vegetable cash crop' intercropping system on arthropod dynamics and biodiversity were analyzed across four European countries: Italy, Denmark, Germany, and Slovenia. Soil arthropod fauna served as an indicator for comparing the ecosystem services provided by living mulch systems versus sole crop systems. The findings revealed that the living mulch technique had no adverse effect on the infestation of cabbage caterpillar (*Pieris* spp.), a key pest of cabbage [14], [15], [16].

In Denmark, aphid populations were notably higher in the sole crop system compared to the living mulch system. In Italy, a high rate of larval parasitization was observed, with parasitization levels reaching 88% in living mulch systems versus 63% in sole crop systems during one year of the study. Additionally, the living mulch positively influenced the activity density of Carabid beetles, enhancing species diversity and evenness in Italy and Slovenia and increasing the activity density of specific taxa in Slovenia and Denmark.

Overall, the results demonstrate that living mulch techniques contribute positively to arthropod biodiversity in plant-soil systems, enhancing soil biodiversity and showing no detrimental effects on the density of canopy pests. These findings suggest that living mulch can provide valuable ecosystem services while maintaining effective pest control [26], [33], [63].

In conclusion, a notable finding from our study is that the use of living mulch in cauliflower cultivation did not lead to an increase in pest infestation, demonstrating the absence of any detrimental effects associated with this technique.

When vegetables are undersown in living mulches or row intercropped with cover crops or other vegetable crops, they are found to reduce herbivorousinsects and damage caused by them [42], [43], [44], [97], [104].

These systems (living mulches, intercropping) create diverse habitats that are generally less favorable for herbivores and/or more conductive for natural enemies [84].

However, herbivore response to diverse habitats could not be explained by a single ecological theory and may depend on the behavior of herbivores (host finding, host acceptance, etc.) to the specific habitat type [44].

The primary mechanisms behind the pest-suppressive effects of living mulches and intercrops are attributed to the disruption of host-plant detection, chemically-based repellency, impacts on insect pests and their natural enemies, and potential competition between the cash crop and adjacent non-crop vegetation [34], [62]. Although these techniques can function independently as pest management strategies, their effectiveness is enhanced when integrated with other approaches, including chemical controls (used selectively, such as in trap cropping), cultural practices, and biological control methods [9], [10] [78]. Below, we discuss the main mechanisms underlying the effects of living mulches and intercrops on pest suppression [95].

Further development of such methods, as shown in Figure 1, promote biodiversity and provide favorable conditions for agriculture based on ecological principles are expected to reduce chemical inputs (e.g., insecticides) (a), thus impacting positively the society and the environment to move towards sustainability in vegetable production systems (b).

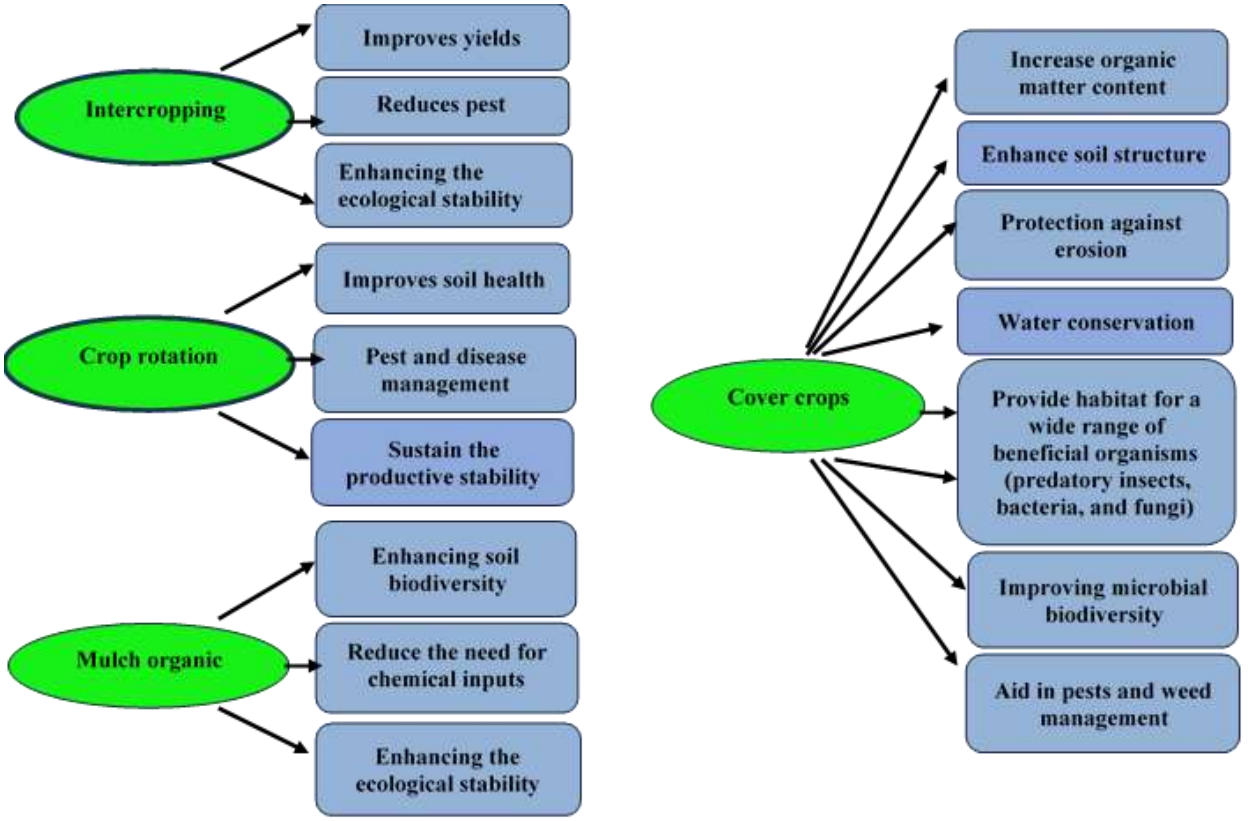

Fig. 1. Methods of diversifying vegetable crops for the sustainability of biodiversity in agroecosystems (a) and The ecological and economic impact of using these methods (b)
Source: Authors' own conception.

The economic impact of the use of various methods for diversifying vegetable crops

The use of a large variety of crops gives a new chance to farmers to develop their agri-business in terms of a higher production, diversification of the products obtained, improving the acquisition price at the farm gate, raising their incomes and profit.

Consumers will have at their disposal more alternatives from which to choose the most suitable one to cover their needs.

Industry will benefit of a large range of raw materials to transform them into new products which will enlarge the domestic market and also could become an object for commercialization on the international market.

In the rural communities, where agriculture plays the main role, new opportunities of jobs could be created by extending and diversifying the cultivated crops.

The new crops could increase value-added, create market niches and generate income streams along the value chain.

The implementation of a large range of vegetal crops and enlarging the use of various the methods to cultivate them in a friendly manner with the environment will contribute to production diversification, to the increase of revenues, to the reduction of the market dependence on commodity crops, and to the enhancement of the competitiveness of farmers in global markets [13].

CONCLUSIONS

There is a lot of scientific evidence that supports the adoption of cover crops as a valid solution for allowing the ecological transition of modern and intensive systems toward sustainable farming systems. Several beneficial effects could be accounted for following the cultivation of cover crops, such as the improvement of soil health, enhancement of nutrient cycling, carbon sequestration and reduction of greenhouse gas emissions, reduction of synthetic fertilizers, and economic returns [82]. Therefore, the

introduction of cover crops into agricultural systems can sustain the productive stability of cash crops and increase soil fertility through organic matter accumulation. Furthermore, cover crops may enhance soil structure, water conservation, and aid in pests and weed management.

Agricultural practices such as crop rotation, intercropping, the use of cover crops, and the application of organic mulch are essential for maintaining biodiversity in agroecosystems. These methods contribute to soil health, reduce the need for chemical inputs, and enhance ecological stability. Thus, they represent effective solutions for promoting sustainable agriculture and a healthier, more balanced agricultural environment, providing a solid foundation for resilient and adaptable farming.

REFERENCES

[1]Abdelsalam, A., Fouda, T., 2023, Determination of color properties of some seeds, Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development Vol. 23(1), 15-19.

[2]Ahmadnia, F., Ebadi, A., Hashemi, M., Ghavidel, A., 2020, Investigating the short time effect of cover crops on physical and biological properties of soil. J. Water Soil Conserv. 26, 277–290.

[3]Altieri, M. A., 1999, The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems & Environment, 74(1-3), 19-31.

[4]Armengot, L., Barbieri, P., Andres, C., Milz, J., Schneider, M., 2016, Cacao agroforestry systems have higher return on labor compared to full-sun monocultures. Agronomy for sustainable development, 36(4), 1-10.

[5]Ashenafi, N., Muleta G., Nibret T., 2020, Competitiveness and Yield Advantage of Carrot-Rosemary Intercropping over Solitary at Wondo Genet, Southern Ethiopia, International Journal of Research Studies in Agricultural Sciences, 6(8): 1-9.

[6]Aydınlı, G., Mennan, S., 2018, Brazilian Archives of Biology and Technology Biofumigation Studies by Using Raphanus sativus and Eruca sativa as a Winter Cycle Crops to Control Root-knot Nematodes. Agribus. Biotechnol. 61, 18180249.

[7]Barrena-González, J., Rodrigo-Comino, J., Gyasi-Agyei, Y., Fernández, M.P., Cerdà, A., 2020, Applying the RUSLE and ISUM in the Tierra de Barros Vineyards (Extremadura, Spain) to estimate soil mobilisation rates. Land 9, 93.

[8]Beniaich, A., Silva, M.L.N., Guimarães, D.V., Bispo, D.F.A., Avanzi, J.C., Curi, N., Pio, R., Dondeyne, S., 2020, Assessment of soil erosion in olive orchards (*Olea Europaea* L.) Under cover crops management systems in the tropical region of Brazil. Rev. Bras. Ciênc. Solo, 44.

[9]Bjorkman, M., Hamback, P. A., Ramert, B., 2007, Entomol. Exp. Appl., 124, 319-326.

[10]Bjorkman, M., Hamback, P. A., Hopkins, R. J., Ramert, B., 2010, Agric. Forest Entomol., 12, 123-132.

[11]Blanco-Canqui, H., Ruis, S.J., 2020, Cover crop impacts on soil physical properties: A review. Soil Sci. Soc. Am. J., 84, 1527–1576.

[12]Bomford, M.K., 2004, Yield, pest density and tomato flavor effects of companion planting in garden-scale studies incorporating tomato, basil and brussels sprout (Doctoral dissertation, West Virginia University).

[13]Branzova, P., 2024. New crops: potential and opportunities for the future of agriculture. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development Vol. 24(3), 107-118.

[14]Broad, S. T., Schellhorn, N. A., Lisson, S. N., Mendham, N. J., Corkrey, R., 2008, Host location and parasitism of Brevicoryne brassicae in diversified broccoli cropping systems. Entomol. Exp. Appl., 129, 166-171.

[15]Bryant, A., Brainard, D. C., Haramoto, E. R., Szendrei, Z., 2013, Cover crops mulch and weed management influence arthropod communities in strip-tilled cabbage, Environ. Entomol., 42, 293-306.

[16]Bryant, A., Coudron, T., Brainard, D., Szendrei, Z., 2014, Cover crop mulches influence biological control of the imported cabbageworm (Pierisrapae L., Lepidoptera: Pieridae) in cabbage, Biol. Control, 73, 75- 83.

[17]Bues, A., Preissel, S., Reckling, M., Zander, P., Kuhlman, T., Topp, K., Watson, C., Lindström, K., Stoddard, F. L., Murphy-Bokern, D., 2013, The environmental role of protein crops in the new common agricultural policy. European Parliament. https://www.europarl.europa.eu/RegData/etudes/etudes/join/2013/495856/IPOL-AGRI_ET(2013)495856_EN.pdf, Accessed on 04.11.2024

[18]Burghilă, C., Bordun, C., Cîmpeanu, S.M., Burghilă, D., Badea, A., 2016, Why mapping ecosystems services is a must in eu biodiversity strategy for 2020?. AgroLife Scientific Journal, 5(2), 28-37.

[19]Cavigelli, M. A., Mirsky, S. B., Teasdale, J. R., Spargo, J. T., Doran, J., 2013, Organic grain cropping systems to enhance ecosystem services. Ren. Agric. Food Syst 28, 145–159.

[20]Cavigelli, M. A., Teasdale, J. R., Conklin, A. E., 2008, Long-term agronomic performance of organic and conventional field crops in the mid-Atlantic region. Agron. J 100, 785–794.

[21]Chapagain, T., Lee, E.A., Raizada, M.N., 2020, The Potential of Multi-Species Mixtures to Diversify Cover Crop Benefits. Sustainability, 12, 2058.

[22]Chen, G., Weil, R.R., 2010, Penetration of cover crop roots through compacted soils. Plant Soil, 331, 31–43.

[23]Chetroiu, R., Ilie, D.M., 2024, Theoretical approach on the nitrogen potential contribution from sustainable natural fertilization with cattle manure. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development Vol. 24(3), 167-180.
[24]Chu, M., Jagadamma, S., Walker, F.R., Eash, N.S., Buschermohle, M.J., Duncan, L.A., 2017, Effect of Multispecies Cover Crop Mixture on Soil Properties and Crop Yield. Agric. Environ. Lett.2, 170030.
[25]Da Silva Dias J.C., 2010, Impact of improved vegetable cultivars in overcoming food insecurity. Euphytica 176:125–136, doi 10.1007/s10681-010-0237-5.
[26]Damicone, J. P., Edelson, J. P., Sherwood, J. V., Myers, L. D., Motes, J. E., 2007, Plant Dis., 91, 509-516.
[27]Daryanto, S., Fu, B., Wang, L., Jacinthe, P.A., Zhao, W., 2018, Quantitative synthesis on the ecosystem services of cover crops. Earth-Sci. Rev.185, 357–373.
[28]De la Peña, R.C., Ebert, A.W., Gniffke, P., Hanson, P., Symonds, R.C., 2011, Genetic adjustment to changing climates: vegetables. In Crop Adaptation to Climate Change, 1st Ed.
[29]Demir, Z., Işik, D., 2020, The impact of different cover crops, mechanical cultivation and herbicide treatment on the soil quality variables and yield in apple (*Malus domestica* borkh.) orchard with a coarse-textured soil. Tarim Bilim. Derg.26, 452–470.
[30]Depalo, L., Burgio, G., Von Fragstein, P., Kristensen, H.L., Bavec, M., Robačer, M., Campanelli, G., Canali, S., 2016, Impact of living mulch on arthropod fauna: analysis of pest and beneficial dynamics on organic cauliflower (*Brassica oleracea* L. var. botrytis) in different European scenarios, Renewable Agriculture and Food Systems: 32(3), 240–247 doi:10.1017/S1742170516000156.
[31]Dikr, W., Belete, K., 2021, Intercropping of African marigold (*Tagetes erecta* Linnaeus (Asteraceae) Varieties at Different Plant Density with Tomato (*Solanum lycopersicum* Linnaeus) on Yield Related Traits and Yield of tomato at Wondo Genet, Southern Ethiopia, Journal of Biology, Agriculture and Vol.11, No.6.
[32]Dikr, W., 2022, Role of Intercropping some Aromatic and Medicinal Plants with Fruit Vegetables Crops, a Review, Glob. Acad. J. Agri. Biosci, Vol.4(2), pp- 22-30. DOI: 10.36348/gajab.2022.v04i02.002,
[33]Fereres, A., 2000, Barrier crops as a cultural control measure of non-persistently transmitted aphid-borne viruses, Virus Res., 71, 221-231.
[34]Frank, D. L., Liburd, O. E., 2005, Effects of Living and Synthetic Mulch on the Population Dynamics of Whiteflies and Aphids, Their Associated Natural Enemies, and Insect-Transmitted Plant Diseases in Zucchini, Environ. Entomol., 34, 857-865.
[35]Gabriel, J.L., Quemada, M., 2011, Replacing bare fallow with cover crops in a maize cropping system: Yield, N uptake and fertiliser fate. Eur. J. Agron.,34, 133–143.
[36]García-Díaz, A., Marqués, M.J., Sastre, B., Bienes, R., 2018, Labile and stable soil organic carbon and physical improvements using groundcovers in vineyards from central Spain. Sci. Total Environ. 621, 387–397.
[37]Girma, A., 2015, Yield Advantage and Economic Benefit of Maize Basil Intercropping under Different Spatial Arrangements and Nitrogen Rates. Scholarly Journal of Agricultural Science, 5(8): 296-302.
[38]Gomez-Rodriguez, O., Zavaleta-Mejía E., González-Hernández, V.A, Livera-Munoz, M.E., Cárdenas-Soriano, E., 2003, Allelopathy and microclimatic modification of intercropping with marigold on tomato early blight disease development. Field Crop Research, 83: 27-34.
[39]Guardia, G., Aguilera, E., Vallejo, A., Sanz-Cobena, A., Alonso-Ayuso, M., Quemada, M., 2019, Effective climate change mitigation through cover cropping and integrated fertilization: A global warming potential assessment from a 10-year field experiment. J. Clean. Prod., 241, 118307.
[40]Hilje, L., Stansly, P. A., 2008, Living ground covers for management of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and *Tomato yellow mottle virus* (ToYMoV) in Costa Rica, Crop Prot., 27, 10-16.
[41]Hinds, J., Hooks, C. R. R., 2013, Population dynamics of arthropods in a sunn-hemp zucchini interplanting system, Crop Prot. 53, 6-12.
[42]Hooks, C. R. R., Johnson, M. W., 2002, Lepidopteran pest populations and crop yields in row 267 intercropped broccoli, Agric. Forest Entomol., 4, 117-125.
[43]Hooks, C. R. R., Johnson, M. W., 2004, Using undersown clovers as living mulches: effects on yields, lepidopterous pest infestations, and spider densities in a Hawaiian broccoli agroecosystem, Int. J. Pest Mgmt., 50, 115-120.
[44]Hooks, C. R. R., Johnson, M. W., 2006, Population densities of herbivorous lepidopterans in diverse cruciferous cropping habitats: Effects of mixed cropping and using a living mulch, BioCon., 51, 485-506.
[45]Hooks, C. R. R., Johnson, M. W., 2003, Impact of agricultural diversification on the insect 269 community of cruciferous crops, Crop Prot., 22, 223-238.
[46]Hooks, C. R. R., Hinds, J., Zobel, E., Patton, T., 2013a, Impact of crimson clover dying mulch on two eggplant insect herbivores, J. Appl. Entomol., 137, 170-180.
[47]Hooks, C. R. R., Hinds, J., Zobel, E., Patton, T., 2013b, The effects of crimson clover companion planting on eggplant crop growth, yield and insect feeding injury, Int. J. Pest Manage., 59, 287-293.
[48]Hughes, J. d'A., Ebert, A.W., 2013, Research and development of underutilized plant species: the role of vegetables in assuring food and nutritional security. Acta Hortic. 979, 79–91 http://dx.doi.org/10.17660.
[49]Ivanov, B., Krustev, V., Bojinov, B., 2024, Effectiveness and significance of crop yield forecast monitoring constituted on vegetation indices, Scientific

Papers Series Management, Economic Engineering in Agriculture and Rural Development Vol. 24(3), 457-464.
[50]Jabro, J.D., Allen, B.L., Rand Dangi, S.R., Campbell, J.W., 2021, Effect of Previous Crop Roots on Soil Compaction in 2 Yr Rotations under a No-Tillage System, Land 2021, 10, 202. https://doi.org/10.3390/land10020202.
[51]Jalli, M., Huusela, E., Jalli, H., Kauppi, K., Niemi, M., Hiemanen, S., Jauhiainen, L., 2021, Effects of Crop Rotation on Spring Wheat Yield and Pest Occurrence in Different Tillage Systems: A Multi-Year Experiment in Finnish Growing Conditions, Front. Sustain. Food Syst., Sec. Climate-Smart Food Systems,Vol.5, https://doi.org/10.3389/fsufs.2021.647335.
[52]Jensen, J.L., Thomsen, I.K., Eriksen, J., Christensen, B.T., 2021, Spring barley grown for decades with straw incorporation and cover crops: Effects on crop yields and N uptake. Field Crops Res., 270, 108228.
[53]Jones, G.A., Sieving, K.E., 2006, Intercropping sunflower in organic vegetables to augment bird predators of arthropods. Agriculture, Ecosystems & Environment 117:171–177.
[54]Kaye, J.P., Quemada, M., 2017, Using cover crops to mitigate and adapt to climate change. A review. Agron. Sustain. Dev. 37, 4.
[55]Keatinge, J.D.H., Wang, J.F., Dinssa, F.F., Ebert, A.W., Hughes, J. d'A., Stoilova, T., Ravishankar, M., 2015, Indigenous vegetables worldwide: their importance and future development, Acta Hort. 1102:1-20, DOI:10.17660/ActaHortic.2015.1102.1.
[56]Keesstra, S., Pereira, P., Novara, A., Brevik, E.C., Azorin-Molina, C., Parras-Alcántara, L., Jordán, A., Cerdà, A., 2016, Effects of soil management techniques on soil water erosion in apricot orchards. Sci. Total Environ 551–552, 357–366.
[57]Khafagy, I. F., 2015, The role of some aromatic plants intercropping on Tuta absoluta infestation and the associated predators on tomato. Egy. J. Plant Pro. Res, 3(2), 37-53.
[58]Lal, R., 2004, Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science, 304 (5677), 1623-1627.
[59]Landis, D.A., Wratten, S.D., Gurr, G.M., 2000, Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45:175–201.
[60]Lemke, R. L., Zhong, Z., Campbell, C. A., Zentner, R., 2007, Can pulse crops play a role in mitigating greenhouse gases from North American agriculture? Agronomy Journal, 99(6), 1719–1725.
[61]Lin, B. B., 2011, Resilience in Agriculture through Crop Diversification: Adaptive Management for Environmental Change. BioScience, 61(3), 183-193, http://dx.doi.org/10.1525/bio.2011.61.3.4.
[62]Manandhar, R., Hooks, C. R. R., Wright, M. W., 2009, Influence of cover crops and intercrop systems on *Bemisia argentifollii* (Hemiptera: Aleyrodidae) infestation and associated squash silverleaf disorder in zucchini, Environ. Entomol., 38, 442-449.
[63]Manandhar, R., Hooks, C. R. R., 2011, Using protector plants to reduce the incidence of papaya ringspot virus-watermelon strain in zucchini, Environ. Entomol., 40, 391-398.
[64]Marcillo, G.S., Miguez, F.E., 2017, Corn yield response to winter cover crops: An updated meta-analysis. J. Soil Water Conserv. 72, 226–239.
[65]Martínez Raya, A., Durán Zuazo, V.H., Francia Martínez, J.R., 2006, Soil erosion and runoff response to plant-cover strips on semiarid slopes (SE Spain). Land Degrad. Dev.17, 1–11.
[66]Masiunas, J.B., 1998, Production of vegetables using cover crop and living mulches—a review. Journal of Vegetable Crop Production 4:11–31.
[67]Mazzoncini, M., Sapkota, T.B., Barberi, P., Antichi, D., Risaliti R., 2011, Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content, Soil and Tillage Research,114(2), https://doi.org/10.1016/j.still.2011.05.001,165-174.
[68]Messinger, J., Lauerer, M., 2015, *Solanum quitoense*, A new greenhouse crop for central Europe: Flowering and fruiting respond to photoperiod. Sci. Hortic., 183, 23–30.
[69]Messinger, J., Martini, M.M.F., Rossi, G., Samuels, J., Lauerer, M., 2015, Successful pollination of the neotropical crop *Solanum quitoense* by Bombus terrestris: Behaviour, efficiency and yield. J. Appl. Entomol., 140(1-2), 124-134.
[70]Midekesa, C., Chala, C., 2022, Productivity of Intercropping with Tomato and Basil under Different Planting Densities at Wondo Genet, Ethiopia, Advances in Life Science and Technology, 9: 19-23.
[71]Mugi-Ngenga, E., Bastiaans, L., Zingore, S., Anten, N.P.R., Giller, K.E., 2022, The role of nitrogen fixation and crop N dynamics on performance and legacy effects of maize-grain legumes intercrops on smallholder farms in Tanzania, European Journal of Agronomy, Volume 141, https://doi.org/10.1016/j.eja.2022.126617.
[72]Mukherjee, R., Sen, S., 2021, Agricultural sustainability through nitrogen fixation: Approaches and techniques. Harvest , 6(1), 48–55.
[73]Neelam, R., Lokho, P., 2009, Intercropping of medicinal and aromatic plants with vegetable cropsareview.MFP News, 19(2): 14-18.
[74]Newton, A.C., Johnson, S.N., Gregory, P.J., 2011, Implications of climate change for diseases, crop yields and food security. Euphytica 179, 3–18 http://dx.doi.org/10.1007/s10681-011-0359-4.
[75]Nigussie, A., Lulie, B., Chala, M., 2017, Intercropping of Onion with Rosemary as Supplementary Income Generation at Wondo Genet Sidama zone, Southern Ethiopia. Acad. Res. J. Agri. Sci. Res., 5(2): 107-115.
[76]Novara, A., Cerda, A., Barone, E., Gristina, L., 2021, Cover crop management and water conservation in vineyard and olive orchards. Soil Tillage Res. 208, 104896.
[77]Paulus, D., Becker, D., Nava, G. A., Luckmann, D., Moura, C. D. A., 2019, Cultivation of mint (*Mentha*

x gracilis) in intercropping with fruit trees in an agroforestry system: Production and quality of essential oil. European J Med Plants, Vol.30 (1).
[78]Piñero, J.C., Manandhar, R., 2015, Effects of increased crop diversity using trap crops, flowering plants, and living mulches on vegetable insect pests. Review, Trends in Entomology Vol. 11, 91-109.
[79]Prohens, J., Rodriguez-Burruezo, A., Nuez, F., 2003, New crops: An alternative for the development of horticulture. Food Agric. Env., 1, 75–79.
[80]Pullaro, T. C., Marino, P. C., Jackson, D. M., Harrison, H. F., Keinath, A. P., 2006, Agric. Ecosyst. Environ., 115, 97- 104.
[81]Quintarelli, V., Radicetti, E., Allevato, E., Stazi, S.R., Haider, G., Abideen, Z., Bibi, S., Jamal, A., Mancinelli, R., 2022, Cover Crops for Sustainable Cropping Systems: A Review, Agriculture, 12(12), 2076; https://doi.org/10.3390/agriculture12122076.
[82]Rodino, S., 2024, Potential of agricultural biomass valorization for circular bioeconomy, Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development Vol. 24(3), 749 - 756.
[83]Rodrigo-Comino, J., Ponsoda-Carreres, M., Salesa, D., Terol, E., Gyasi-Agyei, Y., Cerdà, A., 2020, Soil erosion processes in subtropical plantations (Diospyros kaki) managed under flood irrigation in Eastern Spain. Singap. J. Trop. Geogr. 41, 120–135.
[84]Root, R. B., 1973, Organization of a Plant-Arthropod Association in Simple and Diverse Habitats: The Fauna of Collards (*Brassica Oleracea*), Ecol. Monogr., 43(1), 95- 124.
[85]Ruiz-González, M. X., Vicente, O., 2023, Agrobiodiversity: conservation, threats, challenges, and strategies for the 21st century, AgroLife Scientific Journal – Vol.12(1), 174-185.
[86]Samuels, J., 2015, Biodiversity of Food Species of the Solanaceae Family: A Preliminary Taxonomic Inventory of Subfamily Solanoideae, Resources 4, 277-322; doi:10.3390/resources4020277.
[87]Samuels, J., 2009, The Solanaceae-novel crops with high potential. Org. Grow., 9, 32–34.
[88]Samuels, J., 2014, *Capsicum pubescens*- The Rocoto Pepper: A novel crop Capsicum. Org. Grow., 28, 10–12.
[89]Samuels, J., 2013, Super Fruits. Permaculture 75, 58–61.
https://www.academia.edu/8142181Super_Fruits_Fruiting_Solanaceae_Species_of_Latin_America, Accessed on 04.11.2024
[90]Sans, F.X., Altieri, M.A., 2005, Effects of Intercropping and Fertilization on Weed Abundance, Diversity and Resistance to Invasion. 1st ISOFAR International Conference, Organic ePrints, https://orgprints.org/id/eprint/4468/ Accessed on 28.10.2024.
[91]Scavo, A., Restuccia, A., Lombardo, S., Fontanazza, S., Abbate, C., Pandino, G., Anastasi, U., Onofri, A., Mauromicale, G., 2020, Improving soil health, weed management and nitrogen dynamics by Trifolium subterraneum cover cropping. Agron. Sustain. Dev., 40, 18.
[92]Schipanski, M.E., Barbercheck, M., Douglas, M.R., Finney, D.M., Haider, K., 2014, A framework for evaluating ecosystem services provided by cover crops in agroecosystems. Agric. Syst. 125:12–22.
[93]Stocking Gruver, L., Weil, R.R., Zasada, I.A., Sardanelli, S., Momen, B., 2010, Brassicaceous and rye cover crops altered free-living soil nematode community composition. Appl. Soil Ecol. 45, 1–12.
[94]Stoddard, F. L., Hovinen, S., Kontturi, M., Lindström, K., Nykänen, A., 2009, Legumes in Finnish agriculture: History, present status and future prospects. Agricultural and Food Science, 18(3-4), 191–205
[95]Summers, C. G., Newton, A. S., Mitchell, J. P., Stapleton, J. J., 2010, Population dynamics of arthropods associated with earlyseason tomato plants as influenced by soil surface microenvironment, Crop Prot., 29, 249-254.
[96]Thies, E., 2000, Promising and Underutilized Species, Crops and Breeds. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ): Eschborn, Germany.
[97]Togni, P. H. B., Laumann, R. A., Medeiros, M. A. Sujii, E. R., 2010, Odour masking of tomato volatiles by coriander volatiles in host plant selection of *Bemisia tabaci* biotype B , Entomol. Exp. Appl., 136, 164-173.
[98]Tzemi, D., Rämö, J., Palosuo, T., Peltonen-Sainio, P., Wejberg, H., Lehtonen, H., 2024, The introduction of legume-based crop rotations: an impact assessment on cereal cropping farms in Finland, International Journal of Agricultural Sustainability, 22(1), https://doi.org/10.1080/14735903.2024.2335085.
[99]Waisen, P., Cheng, Z., Sipes, B.S., Wang, K.H., 2022, Biofumigation effects of brassicaceous cover crops on soil health in cucurbit agroecosystems in Hawaii, USA. Pedosphere 32, 521–531.
[100]Wang, Y., Lambers, H., 2020, Root-released organic anions in response to low phosphorus availability: Recent progress, challenges and future perspectives. Plant Soil 447, 135–156.
[101]Watson, C. A., Reckling, M., Preissel, S., Bachinger, J., Bergkvist, G., Kuhlman, T., Lindström, K., Nemecek, T., Topp, C. F., Vanhatalo, A., 2017, Grain legume production and use in European agricultural systems. Advances in Agronomy, 144, 235–303.
[102]Yigezu, Y. A., El-Shater, T., Boughlala, M., Bishaw, Z., Niane, A. A., Maalouf, F., Degu, W. T., Wery, J., Boutfiras, M., Aw-Hassan, A., 2019, Legume-based rotations have clear economic advantages over cereal monocropping in dry areas. Agronomy for Sustainable Development, 39(6), 58.
[103]Zavaleta-Mejía, E., Gómez, R.O., 1995, Effect of Tagetes erecta L. and Tomato (*Lycopersicon esculentum* Mill). Intercropping on some tomato pests. Fitopatología, 30: 33-45.
[104]Zhao, Q., Zhu, J. J., Qin, Y., Pan, P., Tu, H., Du, W., Zhou, W., Baxendale, F. P., 2014, Reducing whiteflies on cucumber using intercropping with less preferred vegetables, Entomol. Exp. Appl., 150, 19-27.

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Article

Biochemical Evaluation of Some Fruit Characteristics of Blueberry Progenies Obtained from 'Simultan × Duke'

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Cite This: *ACS Omega* 2023, 8, 18603−18616

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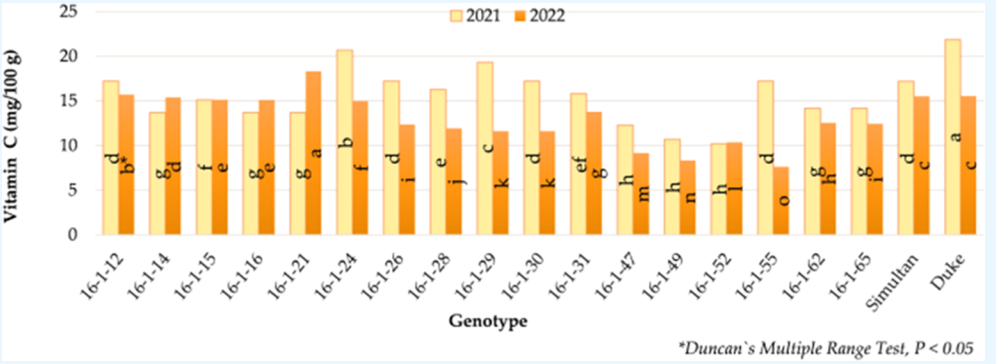


ABSTRACT: The popularity of *Vaccinium corymbosum* blueberry cv. has increased over time because its fruits are highly valued for their taste, aroma, and multiple ways of use. A field trial with two genotypes and their hybrids was organized during 2021−2022 at the Research Institute for Fruit Growing Pitesti-Maracineni, Romania. This paper proposes a comparative analysis of the quality of berries in 17 hybrids of the 'Simultan' and 'Duke' cultivars, selected by the size and the soluble solid content, in agreement with the objectives of the blueberry breeding program. The genotype influence on berry weight, total soluble solids, pH, vitamin C, total polyphenols, total flavonoids, total anthocyanins, lycopene, β-carotene, and antioxidant activity was determined considering the climatic factors. The results showed that fruit weight varied between 1.22 and 2.47 g, total soluble solids reached a maximum of 19.22 °Brix, and the pH oscillated between 3.14 and 3.89. Vitamin C content varied from 9.52 to 18.69 mg in 100 g fresh weight, with an average of 14.35 mg/100 g. Total polyphenol, flavonoid, and anthocyanin contents averaged 709.92 mg gallic acid equivalent in 100 g fresh weight, 165.48 mg catechin equivalent in 100 g fresh weight, and 81.88 mg cyanidin-3-*O*-glucoside equivalent in 100 g fresh weight, respectively. Results show that the strategy of growers to produce blueberries with a large diameter, visually attractive for traders and consumers, is not sufficient for repeat sales. Our study proves that large fruits do not have the highest content of bioactive compounds. Smaller berries had higher polyphenol, lycopene, and β-carotene contents. It is recommended that the selection of the hybrid in the breeding program also takes into account the content of bioactive compounds.

INTRODUCTION

The cultivated blueberry (*Vaccinium corymbosum*) is a tetraploid species native to North America.[1] The highbush blueberry is primarily characterized by its fruits, which are 2−4 times larger than those of the blueberry wild, and their content in nutrients exceeds that of black blueberry (*Vaccinium fuscatum*) or wild blueberry (*Vaccinium myrtillus*) from the spontaneous flora.[2]

Fresh blueberries contain ∼84% water, ∼9.7% carbohydrates, ∼0.6% proteins, and ∼0.4% fat. The dietary fibers represent ∼3.5% of fruit weight, and a portion of 100 g fresh blueberries provides ∼192 kJ and, also, ∼10 mg of vitamin C.[3] Blueberries are an excellent source of bioactive compounds, such as polyphenols, mainly flavonoids, procyanidins, flavonols, phenolic acids, and derivatives of stilbenes.[4−6] The main anthocyanins from the blueberry fruits are malvidin, delphinidin, petunidin, cyanidin, and peonidin, with the sugar moieties of glucose, galactose, and arabinose.[7] The anti-inflammatory and anticarcinogenic properties as well as the cardiovascular protective effects of blueberries[8,9] have been proven in multiple studies.

The antioxidant compounds present in blueberries seem to diminish the risk of coronary diseases and prevent the oxidation of cholesterol, thus lowering the risk of atherosclerosis with the possibility of averting neurodegenerative disorders.[10] The hypoglycemic and hypolipidemic effects of

Received: January 23, 2023
Accepted: April 24, 2023
Published: May 15, 2023

blueberry[11−13] have also been detected, which highlighted their potential to prevent (pre)diabetes.[14,15]

The highbush blueberry requires special conditions to grow and produce fruit, such as low soil pH (4.8−5.5) and good water drainage. Therefore, frequently, the substrate on which blueberries are grown is represented by a mixture of soil with peat, coniferous litter, manure, and coniferous sawdust, while sulfur applications help to correct the pH.[16]

The fruit of highbush blueberry is a spherical-turned or spherical berry, colored light blue to dark blue with an intense cerum layer, and can be consumed fresh, frozen, or dried. They are also used as raw materials for the food industry, in different processed forms, such as juices, syrups, jams, jellies, wine analogues, liqueurs, or food supplements, which are highly appreciated by consumers.[17−19] Taking into account the fact that during the processing of the above-mentioned products, the levels of polyphenolic compounds are diminished, the consumption of fresh fruits is much more beneficial, but the processed products represent important sources of phenolic compounds throughout the whole year.[4,6]

The highbush blueberry breeding program started in 1983 and there were obtained varieties with increased adaptability to the edapho-climatic conditions in the Southern Subcarpathian area, where soils with lower acidity prevail compared to the optimal requirements of the species.[20] Over the years, the main indicators taken into account for the evaluation of the degree of adaptation of the blueberry in different ecological conditions have been the growth speed of the bush, fruiting potential,[21] fruit quality, ripening period,[22−25] and the content of compounds with antioxidant action.[26] Thus, in the beginning, the emphasis was laid on the commercial aspect quantified by the size, color, taste, aroma, and the content of the main biochemical components.[23,24] Second, the emphasis was laid on the prolonged shelf-life of blueberries, quantified by the berry firmness and their high resistance to the action of mechanical factors during the technological flow (picking, handling, transport, packaging, and storage).[25,26] Third, the selection criterion was represented by the content of bioactive compounds with antioxidant action, such as vitamin C, polyphenols, and anthocyanins.[26]

Until now, through the highbush blueberry breeding activity, at the Research Institute for Fruit Growing Pitesti-Maracineni, some of these objectives have been achieved and valuable cultivars have been homologated: 'Azur' (1998), 'Safir' (1998), 'Augusta' (1999), 'Delicia' (2001), 'Simultan' (2001), 'Lax' (2002), and 'Pastel' (2019). As the next step, these cultivars were tested in different pedoclimatic conditions in the country and abroad and were also included in new breeding programs.

'Duke' and 'Simultan' cultivars are considered the best early-season cultivars available. The size and quality of the berries are very good, but late harvesting can negatively influence their taste and aroma.

To create genotypes/cultivars with a high level of bioactive compounds, visually attractive for traders and consumers, and productive in the edapho-climatic conditions from Romania, one has to select the appropriate parental forms. If we are aware of the correlations between the biochemical characteristics and the physical ones, this will allow us to select the parent pairs within the enhancement process aiming to obtain new varieties with a high content of bioactive compounds, which will be more beneficial for consumers.

The first trigger on which consumers' attention is focused is the fruit's appearance (size, shape, color). However, the repurchase decision of the fruits is frequently based on the taste experience, acquired previously. For this reason, the content of blueberries in organic acids, sugars, and tannins becomes of similar or even greater importance to the fruit size. Last but not least, for the fruits used to obtain food supplements, the purchase criterion is the content of bioactive compounds. In light of these considerations, the aim of breeding programs is not only to obtain large-sized fruits but also tasty fruits with the highest possible level of bioactive compounds. This paper proposes a comparative analysis of the berries quality in 17 hybrids of the 'Simultan' and 'Duke' cultivars, selected by the size and the soluble solid content, in agreement with the objectives of the blueberry breeding program. The genotype influence on berry weight, total soluble solids (TSS), pH, vitamin C, total polyphenols, total flavonoids, total anthocyanin, lycopene, β-carotene, and antioxidant activity (AA) was determined considering the plant age and climatic factors.

MATERIALS AND METHODS

Chemicals and Reagents. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,6-dichloroindophenol (DCPIP), sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium nitrite, disodium phosphate, aluminum chloride, methanol, acetone, *n*-hexane, ethanol, citrate/acetate buffer, gallic acid, catechin, vitamin C, cyanidin-3-*O*-glucoside, metaphosphoric acid, acetic acid, hydrochloric acid, citric acid, and Folin−Ciocalteu reagent were obtained from Merck, Darmstadt, Germany.

Plant Material. The fruits of two commercial cultivars of highbush blueberry, namely, 'Simultan' obtained from open pollination of 'Spartan' cv. and 'Duke' obtained from breeding following cross combination ('Ivanhoe × Earliblue') × 192-8 (E-30 × E × 11) and 17 selected progeny hybrids (16-1-12, 16-1-14, 16-1-15, 16-1-16, 16-1-21, 16-1-24, 16-1-26, 16-1-28, 16-1-29, 16-1-30, 16-1-31, 16-1-47, 16-1-49, 16-1-52, 16-1-55, 16-1-62, and 16-1-65) deriving from the two above-mentioned cultivars represent the plant material debated upon in this study. The selection of these hybrids was performed according to the main breeding objectives: fruit weight and soluble solid content.

The experiment was set up at the Research Institute for Fruit Growing Pitesti-Maracineni, Arges, Romania, within the Genetic and Breeding Department in 2019, in an experimental seedling plot. The experimental plot was organized in an open field according to a randomized design with three repetition plots. Selected hybrids and genitors were planted at a distance of 3 m × 1 m on a mixture of soil and peat (30 t/ha). The soil contains clay (17.6%), organic matter (1.84%), and pH (5.8). The experimental lot was irrigated by sprinklers, and Cropmax (0.5 L/ha) was used for foliar fertilization.

The blueberries were harvested at the full maturity stage (visually appreciated by the specific integral coloring of berries) between 15 June and 7 July (2021) and 10 and 30 June (2022) and analyzed immediately after the last harvest.

A WatchDog 900 ET weather station, located in the vicinity of the experimental lot, was used to record the evolution of climatic factors. The Pitesti-Arges area has a continental humid climate, Cfbx category.[27] The average multiannual temperature of the area (in the last 53 years) is 10 °C, and the annual amount of precipitation averages 678.1 mm. Taking into account the fact that blueberry bud-breaking occurred starting from the second half of March and the last harvest was made in the first week of July, Table 1 presents the meteorological

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

Table 1. Meteorological Parameters (Mean Temperature, Maxim and Minim Temperature Averages, Daily Thermal Amplitude, Sunshine Hours, Air Relative Humidity, and Rainfall) during March−July in 2021 and 2022, and Their Multiannual Values (1969−2021)

meteorological parameters		interval	March	April	May	June	July	average (temperatures, air relative humidity)/ sum (sunshine, rainfall)
air temperature (°C)	monthly average	2021	4.12	8.59	15.60	19.32	23.48	14.22
		2022	3.58	10.12	16.45	21.11	22.85	14.82
		1969−1921	4.86	10.39	15.35	18.94	20.71	14.05
	maximum temperature average	2021	10.50	15.04	22.25	26.55	31.05	21.08
		2022	10.26	17.42	24.37	29.18	31.52	22.55
		1969−1921	11.03	16.95	22.17	25.79	27.97	20.78
	minimum temperature average	2021	−1.35	2.57	9.03	13.40	16.38	8.01
		2022	−2.55	3.23	8.72	13.64	15.42	7.69
		1969−1921	−0.10	4.55	9.30	12.80	14.32	8.17
	daily thermal amplitude	2021	11.85	12.48	13.22	13.15	14.67	13.07
		2022	12.82	14.19	15.65	15.54	16.10	14.86
		1969−1921	11.12	12.40	12.87	12.99	13.65	12.61
sunshine hours (monthly sum, hours)		2021	160.30	176.80	266.23	259.91	288.16	1151.40
		2022	185.44	215.30	286.00	286.30	289.70	1262.74
		1969−1921	160.16	193.89	246.15	275.99	304.17	1180.36
air relative humidity (%)		2021	64.58	64.77	65.13	73.30	61.42	65.84
		2022	65.90	74.69	72.91	75.22	70.34	71.81
		1969−1921	71.39	68.75	71.89	72.64	70.82	71.10
rainfall (monthly sum, mm)		2021	66.80	38.40	65.40	104.00	33.50	308.10
		2022	19.40	88.00	72.60	25.60	25.30	230.90
		1969−1921	37.74	55.02	81.77	100.63	80.50	355.66

parameters from March to July in the years 2021 and 2022, along with the average values of the 1969−2021 period.

In March (both 2021 and 2022), average minimum temperatures dropped under the multiannual value, and the last frost occurred in March−April (2021 and 2022, data not presented). During the growing season, until harvest, the average temperature, the average maximum, and the thermal amplitude exceeded the multiannual averages, while the level of precipitation was lower. In particular, July 2021, June, and July 2022 were warmer and drier than normal.

Determination of Average Weight. By weighing a sample of 50 fruits for each genotype (15 plants/genotype), the average weight of fruits was determined and the results were expressed in g/fruit.

Determination of Total Soluble Solid Content. Soluble solids were determined using a Kruss DR201-95 refractometer and the results were reported as °Brix at 20 °C.

Determination of pH. pH values were measured in freshly extracted blueberry juice at 20 °C, using a Consort C-561 multimeter.

Determination of Total Polyphenol Content (TPC). The total polyphenol content (TPC) was determined according to the methodology suggested by Matić et al.[28] By the reaction of polyphenols with phosphotungstic acid, in an alkaline medium, a blue-colored compound is formed. This newly formed compound has maximum absorption at 760 nm. The results were expressed as mg gallic acid equivalent (GAE)/100 g fresh weight (FW).

Determination of Total Flavonoid Content (TFC). The total flavonoid content (TFC) was determined according to the methodology suggested by Tudor-Radu et al.[29] By the reaction of flavonoids with aluminum chloride, a yellow-orange-colored compound is formed. This newly formed compound has maximum absorption at 510 nm. The results were expressed as mg catechin equivalent (CE)/100 g fresh weight (FW).

Determination of Vitamin C Content. According to the colorimetric method and the methodology suggested by Omaye et al.,[30] the vitamin C content was determined by using 2,6-dichloroindophenol (DCPIP) at pH 3−4.5. Vitamin C reduces the DCPIP indicator to a colorless solution, causing a decrease in the absorption of an indicator at 520 nm. The results were expressed in mg vitamin C/100 g fresh weight (FW).

Determination of Total Anthocyanin Content (TAC). Total anthocyanin content (TAC) was determined by the pH differential method suggested by Di Stefano and Cravero.[31] The method determines the total monomeric anthocyanin content because the anthocyanin chromophore undergoes a reversible structural transformation as a function of the pH. The absorbance at 520 nm was measured after 30 min from the preparation of the blueberry extract samples in pH 0.6 (2% hydrochloric acid) and pH 3.5 (a phosphate buffer, containing 0.1 M citric acid and 0.2 M disodium phosphate) buffer. The results were expressed as cyanidin-3-*O*-glucoside equivalent (C3-GE)/100 g fresh weight (FW).

Determination of Lycopene and β-Carotene Levels. The lycopene and β-carotene content was determined according to the methodology proposed by Tudor-Radu et al.,[29] by the carotenoid extraction in a mixture of hexane/ethanol/acetone. The results were expressed in mg lycopene or β-carotene in 100 g fresh weight (FW), using molar extinction coefficients of both compounds at 470 and 503 nm.[32]

Determination of Antioxidant Activity. Total antioxidant activity was evaluated according to the radical scavenging capacity of DPPH free radicals based on the methodology suggested by Moon and Shibamoto[33] with some modifications. A solution of DPPH in methanol (0.116 mM) was prepared and 2.97 mL of DPPH solution was mixed with 0.03 mL of

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

Table 2. Variations in the Average Berry Weight, Total Soluble Solids (TSS), and pH in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

year	genotype	berry weight (g)	TSS (°Brix)	pH
2021	average	2.26 ± 0.58[a]	12.35 ± 4.81[b]	3.47 ± 0.40[a]
2022	average	1.45 ± 0.48[b]	14.85 ± 2.72[a]	3.32 ± 0.37[b]
2021 + 2022	16-1-12	2.16 ± 0.46[abc a,b]	13.47 ± 2.12[b]	3.46 ± 0.45[bcd]
	16-1-14	2.46 ± 0.13[ab]	12.77 ± 1.48[b]	3.86 ± 0.26[a]
	16-1-15	2.38 ± 0.32[ab]	12.25 ± 2.97[b]	3.16 ± 0.21[d]
	16-1-16	1.93 ± 0.49[abcd]	13.17 ± 2.03[b]	3.89 ± 0.29[a]
	16-1-21	1.76 ± 0.74[abcd]	13.82 ± 3.22[b]	3.22 ± 0.31[d]
	16-1-24	1.35 ± 0.78[cd]	12.45 ± 1.97[b]	3.35 ± 0.49[cd]
	16-1-26	1.44 ± 0.69[cd]	13.72 ± 3.19[b]	3.14 ± 0.38[d]
	16-1-28	1.60 ± 0.37[bcd]	11.30 ± 1.86[b]	3.37 ± 0.45[cd]
	16-1-29	1.73 ± 0.74[abcd]	15.02 ± 3.45[ab]	3.18 ± 0.31[d]
	16-1-30	1.33 ± 0.54[cd]	19.22 ± 8.34[a]	3.30 ± 0.50[cd]
	16-1-31	1.41 ± 0.51[cd]	19.13 ± 8.66[a]	3.30 ± 0.44[cd]
	16-1-47	2.47 ± 0.83[a]	13.43 ± 1.88[b]	3.17 ± 0.27[d]
	16-1-49	2.07 ± 0.80[abcd]	11.07 ± 0.15[b]	3.45 ± 0.14[bcd]
	16-1-52	1.86 ± 1.13[abcd]	12.45 ± 6.00[b]	3.37 ± 0.27[cd]
	16-1-55	2.33 ± 0.75[ab]	11.58 ± 2.22[b]	3.23 ± 0.10[d]
	16-1-62	1.22 ± 0.33[d]	14.42 ± 4.92[ab]	3.62 ± 0.31[abc]
	16-1-65	1.89 ± 0.75[abcd]	11.93 ± 4.58[b]	3.36 ± 0.24[cd]
	'Simultan'	1.93 ± 0.70[abcd]	11.92 ± 3.41[b]	3.33 ± 0.38[cd]
	'Duke'	1.96 ± 0.37[abcd]	15.32 ± 1.05[b]	3.74 ± 0.53[ab]
	mean	1.85	13.67	3.39
	std. deviation	0.68	4.12	0.39
	range	3.05	27.40	2.04
	minimum	0.60	7.80	2.76
	maximum	3.65	35.20	4.80
genotype influence sig. (*P*)		0.002	0.010	0.000
year influence sig. (*P*)		0.000	0.000	0.002
genotype × year influence sig. (*P*)		0.000	0.569	0.000

[a]Means of data collected in 2 years and standard deviation (2021−2022) are presented. [b]Means with the same letter are not significantly different at the 5% level.

methanolic extract of blueberry. The mixture was gently homogenized and kept to stand at room temperature for 30 min. Then, the absorbance of the mixture was spectrophotometrically measured at 517 nm.

Statistical Analysis. All analyses were performed in triplicate and data were reported as mean ± standard deviation (SD). Excel 2021 (XLSTAT) was used for data statistical analysis. One-way analysis of variance (ANOVA) and two-way ANOVA and Duncan's multiple range tests were performed.

RESULTS AND DISCUSSION

Tables 2−4 show the values for the average berry weight, total soluble solids, pH, polyphenols, flavonoids, anthocyanins, vitamin C, lycopene, β-carotene, and antioxidant activity with the indication of the values of minimum, maximum, mean, and standard deviation for the berries of the 'Simultan' and 'Duke' genitors and the hybrids of the 'Simultan' and 'Duke' cultivars.

A set of criteria was suggested as a minimum quality standard for fruits, such as pH between 2.25 and 4.25, acidity, expressed by citric acid, from 0.3 to 1.3%, over 10% soluble solids, and sugar-to-acid ratios from 10 to 33.[34] The fruits of any cultivar with a lower sugar-to-acid ratio (i.e., more acidic fruits) tend to maintain their integrity for a longer time.

Berry weight is one of the representative quality parameters for the commercial blueberry market, since the cultivars with good production and larger-sized fruits positively influence buyers' decisions.

As presented in Table 2, blueberry hybrid progeny quality indicators were significantly genotype-dependent. A significant variation between 2020 and 2022 was also noted, while, except for total soluble solids (TSS), the genotype × year effect showed that the genetic influence is still variable, depending on the environmental factors (and the age of the plants).

Berry weight for both years (2021 and 2022) has oscillated between 1.22 g for the 16-1-62 hybrid and 2.47 g for the 16-1-47 hybrid with a mean value of 1.85. Similar results were reported by Ancu et al.[35] who found values between 1.24 and 2.15 g for the fruits of seven Romanian blueberry varieties ('Simultan', 'Delicia', 'Lax', 'Compact', 'Augusta', 'Azur', and 'Blueray'). The average berry weight grown in Bosnia ('Earliblue','Bluegold','Bluecrop', and 'Goldtraube') ranged from 1.12 to 2.11 g,[36] in Korea (45 highbush blueberries cultivars) from 1.6 to 2 g,[37] and in Serbia ('Bluecrop', 'Jersey', and 'Earliblue', from two different locations) from 1.47 to 1.83 g.[38]

Soluble solid content is a measure of sweetness.[39] The higher the soluble solid content, the more convenient and desirable it is to process the blueberry fruits.[40] The total soluble solid content oscillated from 11.07 °Brix (16-1-49 hybrid) to 19.22 °Brix (16-1-30 hybrid) with a mean value of 13.67 °Brix. Similar results were reported by Ancu et al.[35] who obtained values between 12.51 and 16.09 °Brix for the fruits of seven Romanian blueberry varieties. The total soluble solid content in blueberry fruits from 45 commercial cultivars (39

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

Table 3. Variations in the Total Content of Polyphenols (TPC), Flavonoids (TFC), Anthocyanins (TAC), and Vitamin C in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

year	genotype	TPC (mg GAE/100 g FW)	TFC (mg CE/100 g FW)	TAC (mg C3-GE/100 g FW)	vitamin C (mg/100 g FW)
2021	average	378.13 ± 168.08^{b}	141.31 ± 52.94^{b}	76.61 ± 60.75^{b}	15.67 ± 3.04^{a}
2022	average	1041.72 ± 569.08^{a}	189.66 ± 68.75^{a}	87.13 ± 42.22^{a}	13.03 ± 2.79^{b}
2021 + 2022	16-1-12	1113.76 ± 0.87$^{ab\ a,b}$	195.15 ± 20.78^{c}	136.00 ± 39.81^{def}	16.45 ± 0.87^{c}
	16-1-14	866.15 ± 0.94^{abcd}	131.32 ± 5.63^{i}	66.94 ± 33.87^{c}	14.55 ± 0.94^{g}
	16-1-15	1186.09 ± 0.25^{a}	217.94 ± 42.78^{a}	106.00 ± 25.28^{b}	15.11 ± 0.25^{ef}
	16-1-16	663.14 ± 0.80^{abcd}	199.07 ± 66.29^{bc}	112.68 ± 10.34^{ab}	14.41 ± 0.80^{gh}
	16-1-21	1032.49 ± 2.53^{abc}	144.03 ± 23.03^{fg}	82.55 ± 24.68^{c}	16.01 ± 2.53^{d}
	16-1-24	1133.95 ± 3.13^{ab}	144.57 ± 29.64^{fg}	65.48 ± 14.90^{c}	17.84 ± 3.13^{b}
	16-1-26	1153.99 ± 2.66^{ab}	206.19 ± 102.94^{b}	135.43 ± 77.75^{a}	14.79 ± 2.66^{fg}
	16-1-28	366.37 ± 2.38^{cd}	221.86 ± 145.24^{a}	68.9 ± 40.02^{c}	14.11 ± 2.38^{h}
	16-1-29	721.70 ± 4.20^{abcd}	116.45 ± 8.15^{j}	63.97 ± 19.03^{c}	15.47 ± 4.20^{e}
	16-1-30	942.14 ± 3.06^{abcd}	142.23 ± 22.21^{gh}	66.68 ± 28.88^{c}	14.42 ± 3.06^{gh}
	16-1-31	402.35 ± 1.19^{cd}	199.44 ± 19.38^{bc}	132.98 ± 64.68^{a}	14.80 ± 1.13^{fg}
	16-1-47	387.96 ± 1.75^{cd}	106.23 ± 19.25^{k}	116.67 ± 117.83^{ab}	10.74 ± 1.75^{k}
	16-1-49	722.33 ± 0.57^{abcd}	150.95 ± 93.23^{f}	62.67 ± 4.03^{c}	9.52 ± 1.30^{m}
	16-1-52	427.70 ± 0.27^{cd}	178.38 ± 94.42^{d}	104.17 ± 35.68^{b}	10.30 ± 0.27^{l}
	16-1-55	487.03 ± 5.25^{bcd}	140.90 ± 85.22^{gh}	75.83 ± 40.39^{c}	12.42 ± 5.25^{j}
	16-1-62	290.54 ± 0.92^{d}	176.45 ± 44.01^{d}	29.14 ± 24.04^{d}	13.38 ± 0.91^{i}
	16-1-65	592.24 ± 0.98^{abcd}	159.13 ± 45.30^{e}	73.43 ± 27.43^{c}	13.32 ± 0.98^{i}
	'Simultan'	396.51 ± 2.21^{cd}	178.69 ± 27.24^{d}	29.00 ± 1.27^{d}	16.35 ± 1.05^{cd}
	'Duke'	689.31 ± 0.65^{abcd}	135.17 ± 33.93^{hi}	27.11 ± 12.26^{d}	18.69 ± 3.50^{a}
	mean	709.92	165.48	81.88	14.35
	std. deviation	534.38	65.73	52.34	3.20
	range	2073.34	296.54	281.04	14.69
	minimum	99.66	58.45	14.96	7.63
	maximum	2173.00	354.99	296.00	22.32
genotype influence sig. (P)		0.000	0.000	0.000	0.000
year influence sig. (P)		0.000	0.000	0.004	0.000
genotype × year influence sig. (P)		0.000	0.000	0.000	0.000

^{a}Means of data collected in 2 years and standard deviation (2021−2022) are presented. ^{b}Means with the same letter are not significantly different at the 5% level.

northern highbush and 6 half highbush blueberries) grown in Suwon, Korea ranged from 8.3 to 14.3 °Brix.[37] On average, the soluble solid content of the harvested fruits in Guasca, Colombia, from the cultivars 'Biloxi' and 'Sharpblue', was in a range of 12.4−14.5 °Brix.[39]

The pH of blueberries had a mean value of 3.39 and increased from 3.14 (16-1-26 hybrid) to 3.89 (16-1-16 hybrid). Similar data were reported by Aliman et al.[36] who found values for pH of 3.2−3.6 for the highbush blueberry and wild bilberry fruit grown in central Bosnia and Zorenc et al.[38] who found values for pH of 2.76−3.89 for the highbush blueberry fruits of three traditionally cultivated cultivars in Slovenia, 'Bluecrop', 'Earliblue', and 'Jersey'.

A significant influence of genotype was registered for berry weight, total soluble solids, and pH (P = 0.000−0.010).

As presented in Tables 3 and4, total phenolic, flavonoid, anthocyanin, vitamin C, lycopene, and β-carotene content and the antioxidant activity of the discussed genitors and hybrids varied significantly under the genotype influence (P = 0.000).

The total phenolic content represents a marker of antioxidant capacity and it is generally used as an antioxidant activity test. Phenolic compounds are known to inhibit free radicals and prevent the deformation of DNA.[40,41] Total phenolic content in blueberry fruits depends on the cultivar,[5] the growing conditions,[42,43] and the degree of maturity at the harvest of berries.[44]

The total phenolic content of the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids recorded an average value of 709.92 mg GAE/100 g FW and oscillated between 290.54 mg GAE/100 g FW (hybrid 16-1-62) and 1186.09 mg GAE/100 g FW (16-1-15 hybrid). Most of the analyzed samples (including genitors—'Duke', with 689.31 mg/100 g FW, and 'Simultan', with 396.51 mg GAE/100 g FW) presented a phenolic content under average, but 5 out of the 19 genotypes had total phenolic content higher than 1000 mg GAE/100 g FW.

The total phenolic content in blueberry fruits from 45 commercial cultivars grown in Suwon, Korea ranged from 170.9 to 385.7 mg GAE/100 g FW.[36] Lee et al.[45] obtained values for the total polyphenol content between 367 and 1286 mg GAE/100 g FW for *Vaccinium membranaceum* species and 677−1054 mg GAE/100 g FW for *Vaccinium ovalifolium* species, native to Pacific Northwest of North America. Prior et al.[3] reported values for the total phenolic content between 181 and 390 mg/100 g FW for *V. corymbosum* L. species ('Bluecrop', 'Jersey', 'Croatan', 'Duke', 'Rancocas', 'Rubel', 'O'Neal', 'Reveille', 'Blue Ridge', 'Cape Fear', 'Pender', and 'Bladen' cvs.). Dragovič-Uzelac et al.[46] reported for the 'Bluecrop' variety a higher amount of polyphenols than 'Duke' (blueberry cultivars grown in Northwest Croatia), while Prior et al.[3] obtained a higher total phenolic content value for the 'Duke' cultivar, grown in Chatsworth, New Jersey, United States. Gündeşli et al.[8] reported values between 158.4

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

Table 4. Variations in the Total Content of Lycopene and β-Carotene, and Antioxidant Activity (AA) in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

year	genotype	lycopene (mg/100 g FW)	β-carotene (mg/100 g FW)	AA (mmol Trolox/100 g FW)
2021	average	0.025 ± 0.025[b]	0.088 ± 0.091[b]	
2022	average	0.118 ± 0.054[a]	0.129 ± 0.094[a]	0.2022 ± 0.0075
2021 + 2022	16-1-12	0.070 ± 0.040[bcd a,b]	0.070 ± 0.040[def]	0.2039 ± 0.0004[fg]
	16-1-14	0.070 ± 0.050[bcd]	0.080 ± 0.050[cdef]	0.2046 ± 0.0003[ef]
	16-1-15	0.050 ± 0.010[cd]	0.020 ± 0.010[f]	0.1980 ± 0.0005[klm]
	16-1-16	0.060 ± 0.030[bcd]	0.060 ± 0.030[def]	0.2124 ± 0.0003[b]
	16-1-21	0.090 ± 0.140[abcd]	0.150 ± 0.140[bc]	0.2080 ± 0.0004[d]
	16-1-24	0.060 ± 0.040[bcd]	0.200 ± 0.040[b]	0.1973 ± 0.0004[lm]
	16-1-26	0.040 ± 0.030[d]	0.120 ± 0.030[cd]	0.2015 ± 0.0006[hi]
	16-1-28	0.020 ± 0.030[d]	0.110 ± 0.030[cde]	0.2099 ± 0.0004[c]
	16-1-29	0.030 ± 0.010[d]	0.030 ± 0.030[ef]	0.2210 ± 0.0003[a]
	16-1-30	0.080 ± 0.040[bcd]	0.100 ± 0.040[cde]	0.1964 ± 0.0005[m]
	16-1-31	0.080 ± 0.060[bcd]	0.120 ± 0.010[cd]	0.1988 ± 0.0004[jkl]
	16-1-47	0.070 ± 0.600[bcd]	0.070 ± 0.060[def]	0.1997 ± 0.0005[ijk]
	16-1-49	0.130 ± 0.010[ab]	0.120 ± 0.010[cd]	0.1846 ± 0.0005[n]
	16-1-52	0.130 ± 0.070[abc]	0.100 ± 0.070[cdef]	0.2033 ± 0.0006[fgh]
	16-1-55	0.050 ± 0.010[cd]	0.030 ± 0.010[ef]	0.2004 ± 0.0004[ij]
	16-1-62	0.050 ± 0.040[cd]	0.060 ± 0.040[def]	0.2024 ± 0.0009[gh]
	16-1-65	0.100 ± 0.080[abcd]	0.080 ± 0.080[cdef]	0.1973 ± 0.0045[lm]
	'Simultan'	0.100 ± 0.020[abcd]	0.210 ± 0.070[b]	0.2062 ± 0.0003[de]
	'Duke'	0.160 ± 0.060[a]	0.380 ± 0.050[a]	0.1968 ± 0.0002[lm]
	mean	0.070	0.110	0.2022
	std. deviation	0.060	0.100	0.0075
	range	0.260	0.450	0.0372
	minimum	0.000	0.000	0.1841
	maximum	0.260	0.450	0.2213
genotype influence sig. (P)		0.006	0.000	0.0000
year influence sig. (P)		0.000	0.000	
genotype × year influence sig. (P)		0.000	0.000	

[a]Means of data collected in 2 years and standard deviation (2021−2022) are presented. [b]Means with the same letter are not significantly different at the 5% level.

and 2784.45 mg GAE/100 g FW for the total polyphenol content in blueberry fruits, from different countries (Italy, Turkey, United States). Colak et al.[47] reported the total phenolic content ranging from 555 to 638 mg GAE/100 g FW in the wild bilberry population grown in Ardahan province located in eastern Anatolia, Turkey, and 327 mg GAE/100 g FW in the blueberry cultivar 'Bluecrop', that indicate lower values than all wild bilberry accessions. The polyphenol classification proposed by Vasco et al.[48] using low (<1 mg GAE/g), medium (1−5 mg GAE/g), and high (>5 mg GAE/g) values indicates that our blueberry samples are a good source of these compounds.

Flavonoids constitute the largest subgroup of polyphenols. Pietta et al.[49] stated that flavonoids were responsible for antioxidant activity. The total flavonoid content in blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids varied between 106.23 and 221.86 mg CE/100 g FW, having a mean value of 165.48 mg CE/100 g FW. It could be observed that most of the 'Simultan × Duke' hybrids had flavonoid contents higher than their genitors (135.17 mg CE/100 g FW for 'Duke' and 178.69 mg CE/100 g FW for 'Simultan') and the 16-1-28 hybrid was remarked (221.86 mg CE/100 g FW), followed by 16-1-15 (217.94 mg CE/100 g FW) and 16-1-26 (206.19 mg CE/100 g FW). Therefore, the 16-1-15 hybrid stood out as having the first higher total polyphenol content and the second higher total flavonoid content. Similar results were reported by Dróżdż et al.,[50] Häkkinen and Törrönen,[51] Koca and Karadeniz.[52] Studies[50−52] illustrate that total flavonoid amounts of the same blueberry cultivar can be different, with the climatic conditions and the cultivation techniques having a great influence.

Anthocyanin content presented an average of 81.88 mg C3-GE/100 g FW and varied significantly under the genotype effect. The highest level was found for the 16-1-12 hybrid, 136.00 mg C3-GE/100 g FW. All progenies had higher anthocyanin content than the genitors (29.00 mg C3-GE/100 g FW—'Simultan' and 27.11 mg C3-GE/100 g FW—'Duke'). The blueberry cultivar 'Bluecrop' grown in Turkey had a total anthocyanin content of 142 mg C3-GE /100 g FW.[53] Similar results were found by Okan et al.,[41] who reported TAC values between 43.03 and 295.06 mg C3-GE/100 g FW for 28 samples of blueberries from the Black Sea region situated in north-eastern Turkey, in 2012−2014 years. In blueberries of three cultivars in Slovenia, 'Bluecrop', 'Earliblue', and 'Jersey', the total anthocyanin content value was in the range of 103.0−241.9 mg/100 g FW and the anthocyanins constituted 35−55% of the total analyzed phenolics.[38]

Vitamin C acts in the human body as an antioxidant by preventing free-radical-induced damage to DNA, quenching oxidants that can lead to the development of cataracts, improving endothelial cell dysfunctions, and decreasing low-density lipoprotein-induced leukocyte adhesion.[54] Blueberries are known among the richest fruits in vitamin C, usually with

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

Table 5. Intensity of the Correlations between Analyzed Parameters in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

	TSS	pH	TPC	TFC	TAC	vitamin C	lycopene	β-carotene	antioxidant activity
berry weight (g)	−0.493[c]	0.143	−0.390[c]	−0.362[c]	0.000	0.153	−0.418[c]	−0.298[b]	−0.158
	0.000	0.129	0.000	0.000	0.998	0.104	0.000	0.001	0.241
TSS (°Brix)	1	−0.112	0.215[a]	0.105	0.118	−0.027	0.217[a]	0.191[a]	0.154
		0.235	0.022	0.266	0.210	0.777	0.020	0.042	0.252
pH		1	−0.121	−0.009	−0.109	0.021	−0.082	−0.008	−0.117
			0.199	0.924	0.250	0.827	0.387	0.935	0.387
TPC (mg GAE/100 g)			1	0.288[b]	0.303[b]	−0.044	0.432[c]	0.116	0.022
				0.002	0.001	0.642	0.000	0.218	0.870
TFC (mg CE/100 g)				1	0.450[c]	−0.163	0.133	−0.013	0.073
					0.000	0.083	0.159	0.889	0.587
TAC (mg C3-GE/100 g)					1	−0.193[a]	−0.129	−0.257[b]	0.169
						0.040	0.171	0.006	0.208
vitamin C (mg/100 g)						1	−0.187[a]	0.371[c]	0.288[a]
							0.046	0.000	0.030
lycopene (mg/100 g)							1	0.541[c]	−0.373[b]
								0.000	0.004
β-carotene (mg/100 g)								1	−0.120
									0.375

[a]Correlation is significant at the 0.05 level (two-tailed). [b]Correlation is significant at the 0.01 level (two-tailed). [c]Correlation is significant at the 0.001 level (two-tailed).

values in quite wide intervals, from 4.54 to 100 mg/100 g FW.[47]

Regarding the total vitamin C content in blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids, a mean value of 14.35 mg/100 g FW was determined. The vitamin C content oscillated around 9.52 mg/100 g FW (16-1-49 hybrid), while the highest content was obtained for 'Duke' cv. (18.69 mg/100 g FW). In this case, genitors contained higher vitamin C than most of their hybrids.

The vitamin C content in 10 highbush blueberry cultivars grown in Latvia[55] ('Northland', 'Spartan', 'Barkeley', 'Duke', 'Chippewa', 'Bluecrop', 'Jersey', 'Blueray', 'Chandler', and 'Bluejay') ranged between 6.9 and 11.8 mg/100 g FW, but the fruits of these cultivars were analyzed after freezing. The amount of vitamin C in fruits of three cultivars ('Reca', 'Elizabeth' and 'Bluegold') of the highbush blueberries grown in the Western forest-steppe of Ukraine in 2017−2019 years varied from 15.70 to 20.00 mg/100 g FW, with an average value of 17.30 mg/100 g FW for the 3 years ('Reca'), from 16.70 to 27.00 mg/100 g FW, with an average value of 20.17 mg/100 g FW ('Elizabeth'), and from 19.60 to 22.50 mg/100 g FW, with an average value of 20.90 mg/100 g FW ('Bluegold').[56] The experiment of Ukrainian researchers included cultivars with different ripening times and different countries of origin: 'Reca'—early season, New Zealand; 'Elizabeth' and 'Bluegold'—mid-season, USA. Correia et al.[57] claimed that the different contents of vitamin C in blueberries were a varietal trait that can be adjusted to the conditions of the year, ranging from 6 to 162 mg/100 g.

Carotenoids include diverse compounds such as lycopene, α- and β-carotene, lutein, and xanthophylls, and they are found in almost all colored vegetables. Scientific evidence is referring to the fact that lycopene and β-carotene are the primary bioactive components in fruits and vegetables that reduce cancer risk,[58−63] and the results from animal and cell-culture studies indicate even more beneficial cellular effects. These include antioxidant activity, inhibition of the cell cycle, and signaling pathways.[64]

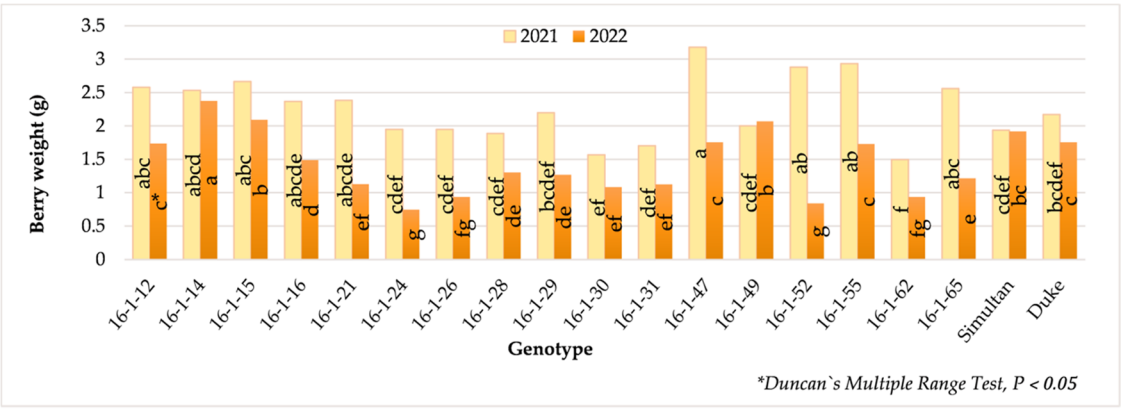
The mean values for lycopene and β-carotene content in the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table 4) were 0.07 mg lycopene/100 g FW and 0.11 mg β-carotene/100 g FW, respectively. The lycopene level in the fruits of 'Simultan' and 'Duke' cvs. and their progeny hybrids oscillated between 0.02 mg/100 g FW (16-1-28 hybrid) and 0.16 mg/100 g FW ('Duke'). Also, the β-carotene level varied from 0.02 mg/100 g FW (16-1-15 hybrid) to 0.38 mg/100 g FW ('Duke'). Similar to vitamin C, both analyzed carotenoids registered lower levels in hybrids than those in genitors, with some exceptions.

In conformity with multiple studies, the antioxidants from fruits and vegetables protect lipids, proteins, and nucleic acids against the oxidative damage produced by free radicals, which represents an important step in the fight against cancer, heart disease, and vascular and neurodegenerative diseases.[65,66] Antioxidants are present in large quantities in blueberries (genus *Vaccinium*).[67] A few epidemiological studies showed that some types of cancer are caused by specific dietary habits, i.e., people consuming fruits and vegetables regularly have a lower risk of cancer.[67,68] It has been proven that berries inhibit many stages of carcinogenesis and stimulate the apoptosis of cancer cells.[67]

Reducing power is generally linked with reducing substances, which have been shown to exert antioxidant action by breaking the free radical chain and donating a hydrogen atom.[52] Anthocyanins, phenolic acids, and flavonoids are the main bioactive compounds of blueberries,[69] with the antioxidant activity of the fruit being an indication of the functional value of the fruit. Scibisz and Mitek[70] demonstrated that the antioxidant capacity was strongly correlated with the content of total anthocyanins and total phenolics in fruits of 14 cultivars of highbush blueberry (*V. corymbosum* L.) grown in Poland in the years 2002−2004.

To measure the antioxidant capacities of blueberries, in this study, we used the DPPH radical scavenging activity test. It is known that low DPPH values indicate a high antioxidant capacity. Regarding the antioxidant activity in blueberry fruits

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616



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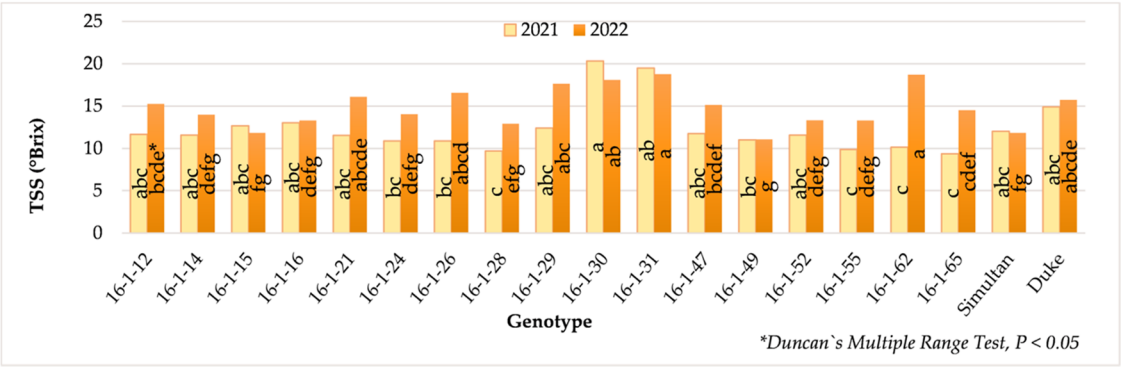

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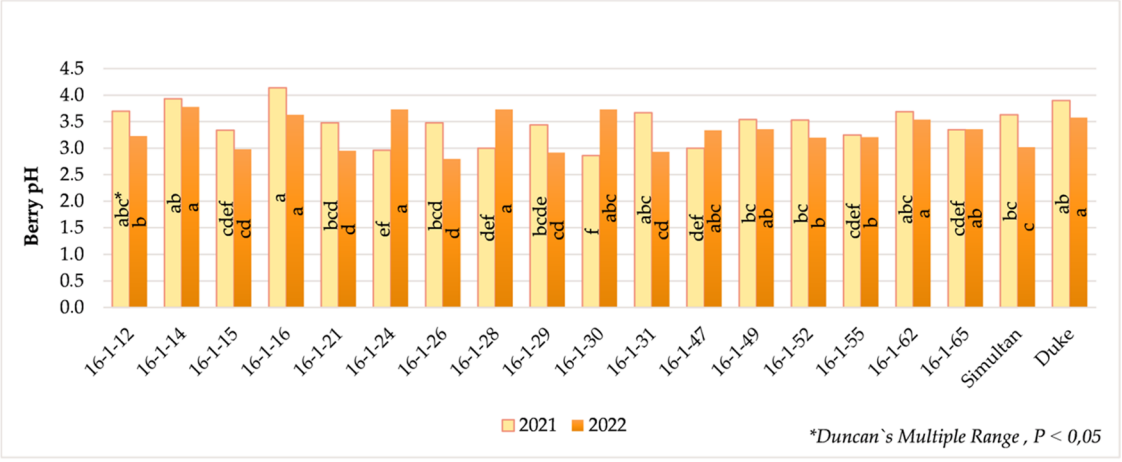

(c)

Figure 1. Genotype × year interaction effect on berry weight (a), blueberry TSS (b), and pH (c).

of 'Simultan' and 'Duke' cultivars and their progeny hybrids, the mean value was 0.2022 mmol Trolox/100 g FW and reached its lowest limit, 0.1846 mmol Trolox/100 g FW, for the 16-1-49 hybrid, while the highest one was 0.2210 mmol Trolox/100 g FW, for the 16-1-29 hybrid. Finally, regarding the previously discussed 16-1-29 hybrid, a slightly above-average vitamin C content (15.47 mg/100 g) and low levels of lycopene and β-carotene (0.03 mg/100 g) were also determined.

As shown in Table 5, a tendency was observed for small berries to accumulate more soluble solids than larger ones. It has also been shown that as berry weight decreases, the total level of phenolic compounds, flavonoids, and carotenoids increases. This dynamics of the components with antioxidant activity could reflect the dilution of the berry juice (caused by the water accumulation) in larger berries. This observation is sustained by the significant positive correlations of total soluble solids (TSS) to polyphenols, lycopene, and β-carotene (to which is added positive but insignificant correlations of TSS to flavonoids and anthocyanins) and the negative (although insignificant) correlation of TSS and pH, which would rather characterize dilution but not insufficient ripening of the berries.

Anthocyanins correlated negatively with vitamin C and β-carotene (even insignificantly with lycopene).

Vitamin C showed opposite sign correlations with lycopene (negative) and β-carotene (positive).

Among the components with antioxidant activity, vitamin C was significantly positively correlated to antioxidant activity,

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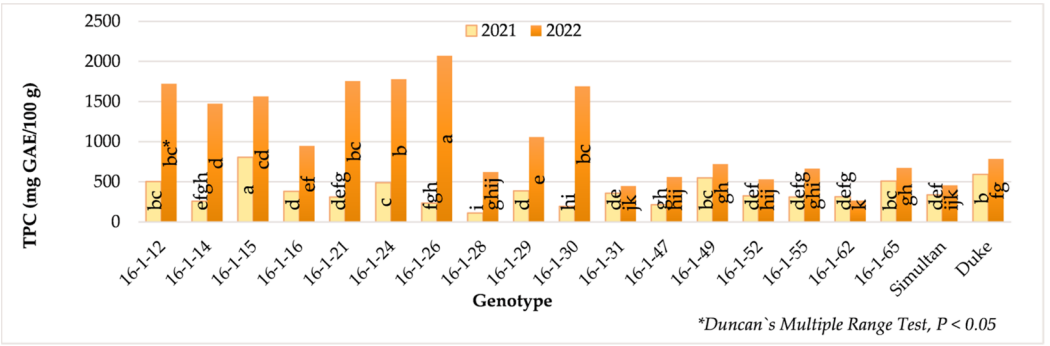
ACS Omega 2023, 8, 18603−18616



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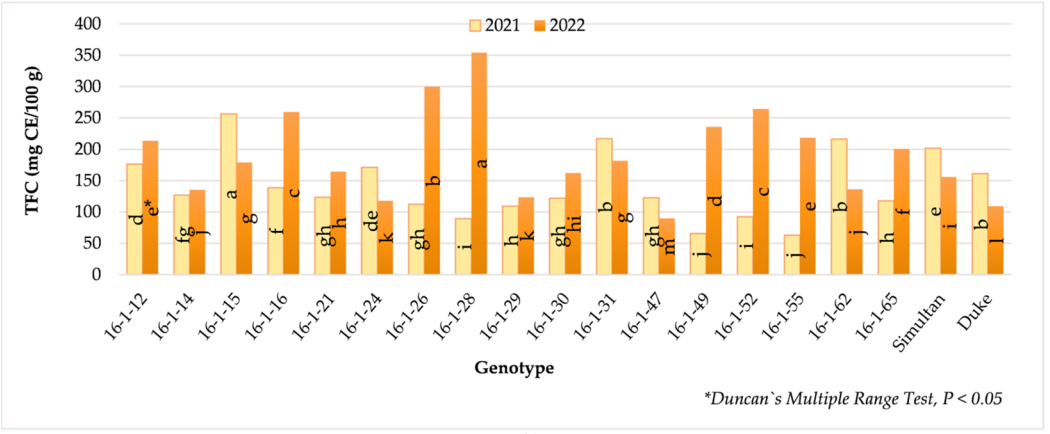

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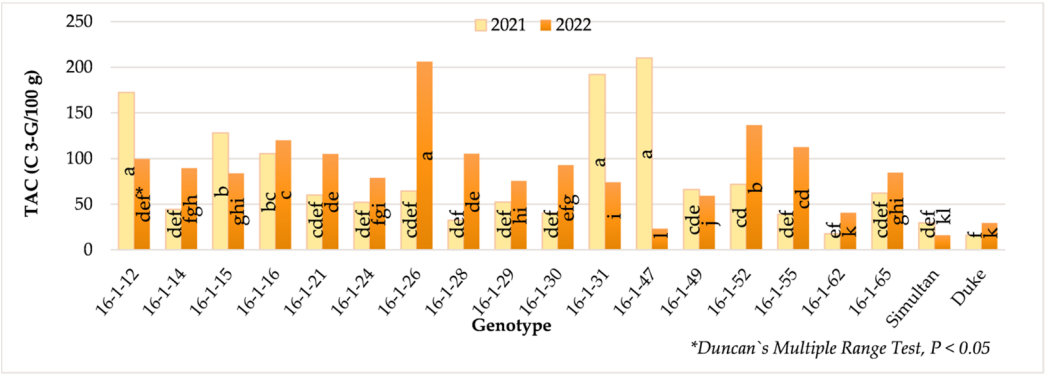

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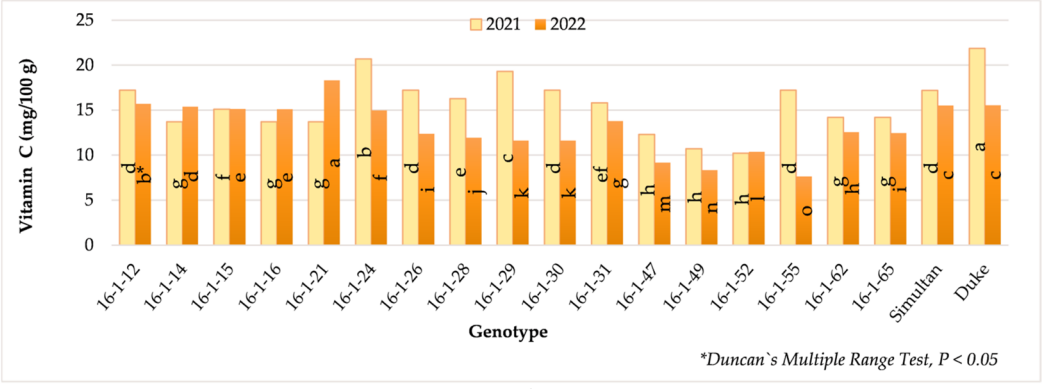

(d)

Figure 2. Genotype × year interaction effect on blueberry TPC (a), TFS (b), TAC (c), and vitamin C content (d).

although positive but insignificant correlations were established between phenolics (TPC, TFC, and TAC) and antioxidant activity. Carotenoids were negatively correlated to antioxidant activity (significant, lycopene and insignificant, β-carotene).

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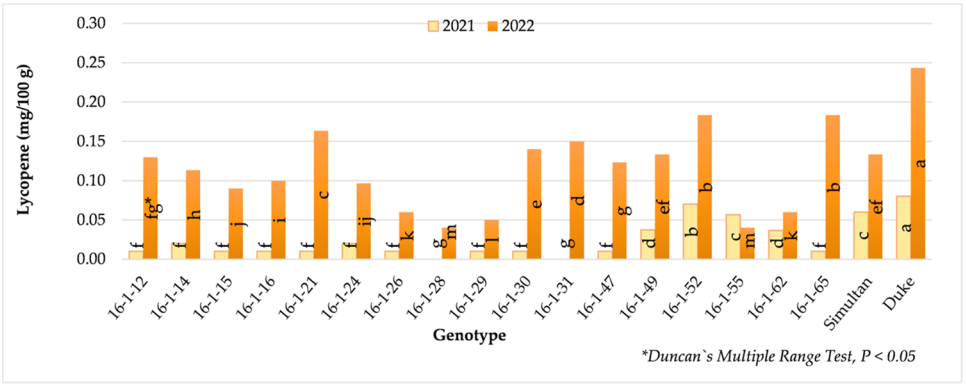

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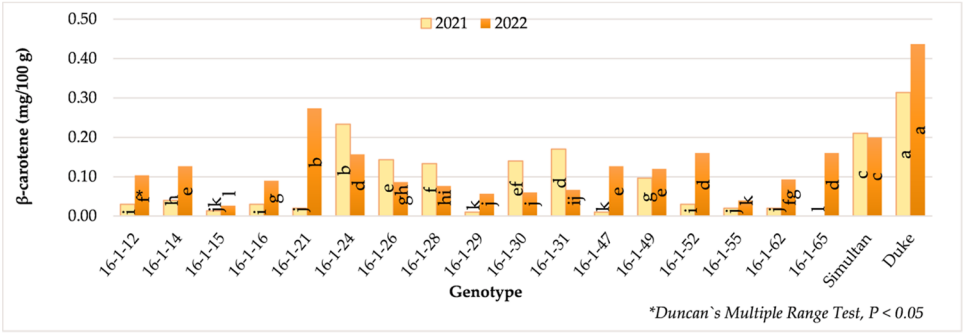

(b)

Figure 3. Genotype × year interaction effect on blueberry lycopene (a) and β-carotene content (b).

Table 6. One-Way ANOVA Test Results Regarding the Genotype Influence on Blueberry Quality Indicators, Depending on the Experimental Year

dependent variable	year		sum of squares	df	mean square	*F*	sig.
berry weight (g)	2021	between groups	12.125	18	0.674	3.645	0.000
	2022	between groups	12.205	18	0.678	38.096	0.000
total soluble solids (°Brix)	2021	between groups	474.062	18	26.337	1.219	0.295
	2022	between groups	297.021	18	16.501	5.329	0.000
pH	2021	between groups	517.933	18	28.774	188.734	0.000
	2022	between groups	24 719.145	18	1373.286	9595.745	0.000
polyphenols (mg GAE/100 g)	2021	between groups	1 469 407.286	18	81 633.738	27.527	0.000
	2022	between groups	17 635 256.189	18	979 736.455	74.360	0.000
flavonoids (mg CE/100 g)	2021	between groups	154 382.170	18	8576.787	127.464	0.000
	2022	between groups	263 806.003	18	14 655.889	627.102	0.000
TAC (mg C3-G/100 g)	2021	between groups	182 347.588	18	10 130.422	15.844	0.000
	2022	between groups	104 937.045	18	5829.836	93.866	0.000
vitamin C (mg/100 g)	2021	between groups	511.046	18	28.391	137.120	0.000
	2022	between groups	435.343	18	24.186	4893.811	0.000
lycopene (mg/100 g)	2021	between groups	0.033	18	0.002	73.217	0.000
	2022	between groups	0.161	18	0.009	339.163	0.000
β-carotene (mg/100 g)	2021	between groups	0.459	18	0.026	1307.228	0.000
	2022	between groups	0.491	18	0.027	470.859	0.000

As represented in Figures 1−3, significant differences were recorded between the 2 years of the study in terms of the determined parameters, while also highlighting a significant genotype × year interaction.

In general, berry weight and vitamin C content were higher in the first year of the study, while an increasing trend in the second year was observed for total soluble solids, polyphenols, and lycopene. Except for β-carotene, a greater influence of the genotypes was observed in the second year of the study on the fruit quality indicators (Table 6).

Therefore, as represented in Figures 1−3 and Tables S1−S3 in the Supporting Information, the highest berry weights were recorded in 2021 for hybrids 16-1-47, 16-1-55, 16-1-52 and in 2022 for 16-1-14, 16-1-15, and 16-1-49. Hybrids 16-1-30 and 16-1-31 had in both years of study high TSS values. Also, in 2022, among hybrids 16-1-26, 16-1-24, 16-1-21, 16-1-12, and 16-1-30, with a high level of polyphenols, only 16-1-26 stood out for its high flavonoid content. Last but not least, in 2021, hybrids 16-1-24 and 16-1-29 presented only slightly lower

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

vitamin C levels than Duke but significantly higher than the other descendants.

In the first year of the study (2021), the shrubs (in the third year from planting) produced few but large berries. In 2022, the number of fruit buds and implicitly of berries was higher, but their weight decreased. This can be explained by the fact that the plants did not have the proper photosynthetic capacity to invest in the berries produced in the second year of the study, especially in the conditions of more pronounced thermal stress in 2022 than that in 2021. In 2022, the absolute maxima in May, June, and July (31.5, 36.8, and 38.3 °C) were higher than those in 2021 (28.4, 34.0, and 36.8 °C). Since the plantation was irrigated and the atmospheric humidity was not lower in 2022, it cannot be a question of berry dehydration (and therefore a reduction of the berry weight and a concentration of the soluble solids caused by the dehydration). Cell division is sensitive to temperature, and therefore, it can influence the growth of the fruits. In this case, it is necessary to mention that the absolute minima in 2022 (−9.3 °C in March and −3.8 °C in April) were lower than those in 2021 (−6.2 °C in March and −3.3 °C in April). Higher temperatures in 2022 could also explain the higher TPC of the blueberries according to López et al.[71] and the reduction in vitamin C content.

In 2022, starting from May and until harvest, the number of hours with temperatures over 30 °C was higher than that in 2021. However, the minimum temperatures of this period were frequently below 15 °C (being generally lower than 2021), which determined higher amplitudes of temperature variation in the second year of the study. Practically, the plants benefited not only from warm and sunny days but also from cooler nights. Under these conditions, TPC recorded higher levels in 2022 than those in 2021. In addition, according to the authors cited by Nicholas et al.,[72] the increase in sunshine hours correlated with an increase in TPC, especially TAC (which belongs to the class of flavonoids). Dinis et al.[73] cited authors who referred to the fact that there was a well-documented relationship between total sugar content (TSC) and total anthocyanin content (TAC). Thus, higher levels of TSC in 2022 than those in 2021 proved that in 2022, the climatic conditions were more favorable for the physiological process development than in 2021. Additionally, there are some observations regarding the high temperatures to which the blueberry species are adapted. According to Zheng et al.,[74] the increase in environmental temperature from 25 to 30 °C (more frequent in the present study in 2022) contributed to the regular distribution of stomata, but not the subsequent increase in temperature from 30 to 40 °C. The authors also found that the optimal temperature for transpiration in the northern highbush blueberry is 38 °C. This means that the plants suffer less from the heat due to the superior cooling capacity compared to the southern highbush blueberry, at which the optimum temperature for transpiration was established at 34 °C. In general, the data in the literature refer to situations where the growth temperature correlates with the reduction of the TPC level not only in blueberries but also in other species, for example, grapevine.[73,75−78] These discrepancies may be due to temperature oscillations above the optimum of the respective species, taking into account the fact that an increase in temperatures below the optimum stimulates plant physiological processes, and an increase in temperatures above the optimum can cause their reduction.

Some authors state that the vitamin C level of blueberries is a characteristic of the variety, but it is also influenced by environmental conditions.[56,57] Regarding the oscillations of vitamin C, Correia et al.[57] reported, similar to our study, a reduction in the level of this compound under conditions of increasing temperature and duration of sunshine. Last but not least, Shevchuk et al.[56] found the highest levels of vitamin C in 'Reca', 'Elisabeth', and 'Bluegold' blueberry cultivars in conditions of lower temperatures and poor rainfall regimes.

CONCLUSIONS AND PERSPECTIVES

The strategy adopted for the improvement of additive polygenic parameters is the crossing of "similar" × "similar" parents, which already have a good level of parameters. The success of the selection was revealed by the individualization of superior genotypes for the character of the selection objective. The highest average weight was determined for hybrids 16-1-47 (2.47 g), 16-1-14 (2.46 g), 16-1-15 (2.38 g), and 16-1-55 (2.33 g), exceeding their genitors (1.93 g of Simultan and 1.96 g of Duke). Overall, among genotypes with high berry weight, eight had above-average other quality indicators: 16-1-12 (TPC, TFC, TAC, vitamin C, and antioxidant activity), 16-1-14 (TPC, vitamin C, and antioxidant activity), 16-1-15 (TPC, TFC, TAC, and vitamin C), 16-1-16 (TFC, TAC, and antioxidant activity), 16-1-21 (TSS, lycopene, β-carotene, TPC, TAC, vitamin C, and antioxidant activity), 16-1-29 (TSS, TPC, vitamin C, and antioxidant activity), 16-1-49 (lycopene, β-carotene, and TPC), and 16-1-52 (lycopene, TFC, TAC, and antioxidant activity). In most cases (except for carotenoids and vitamin C), the mentioned hybrids exceeded their genitors. These will be evaluated in the next breeding stages for registration as new cultivars.

Smaller blueberry hybrids had higher values of total soluble solids, total polyphenol and flavonoid content, lycopene, and β-carotene. Although berry size can influence the purchase decision and therefore the success of the blueberry market, it is important to remember that smaller fruits are richer in compounds with biological activity. As an overview, it can be concluded that berry quality was significantly influenced by both genotype and study year.

Even though the appearance (quantified by size, color, taste, and aroma) is still a decisive element for consumers regarding the fruit quality estimation, the results of our study highlighted that small blueberries accumulated more soluble solids and presented higher content of polyphenols, lycopene, and β-carotene than big ones.

Our results will strengthen the breeding process in the Research Institute for Fruit Growing Pitesti-Arges, which aims to create new valuable varieties of *V. corymbosum* L. with a significant complex of biologically active compounds, which makes the fruits of this crop a trendy food product. Similar to their genitors, hybrids have the advantage of showing early fruiting. Their berry quality is a genetically determined character. However, the significant variations between the two years of the study indicate the need to continue this research over a longer period, in order to observe the behavior of the plants in the environmental conditions of the Pitesti-Arges area, especially during summers with statistically assured warming trends.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00466.

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

Variations in average berry weight, total soluble solids (TSS), and pH of the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table S1); variations in the total content of polyphenols (TPC), flavonoids (TFC), anthocyanins (TAC), and vitamin C in blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table S2); and variations in the total content of lycopene and β-carotene, and antioxidant activity (AA) of the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table S3) (PDF)

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Author Contributions

L.E.V.: conceptualization, methodology, validation, investigation, writing—review and editing, and supervision; O.H.: conceptualization, software, investigation, resources, and writing—original draft preparation; M.S.: validation, resources, and writing—review and editing; V.T.: software; and R.T.: conceptualization and supervision. All authors have read and agreed to the published version of the manuscript

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the project "PROINVENT", Contract no. 62487/03.06.2022 POCU/993/6/13—cod SMIS: 153299 financed by The Human Capital Operational Programme 2014−2020 (POCU), Romania.

REFERENCES

(1) Hera, O.; Teodorescu, R.; Sturzeanu, M. Blueberry (Vaccinium corymbosum) breeding programme in the main cultivating countries. *Sci. Papers Ser. B. Hortic.* **2021**, *LXV* (1), 82−89.

(2) Botez, M.; Bădescu, G.; Botar, A. *Cultura arbuştilor fructiferi*; Editura Ceres: Bucureşti, 1984; p 186.

(3) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2686−2693.

(4) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunningham, D.; Vannozzi, S.; Prior, R. L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC−MS fluorescent detection method. *J. Agric. Food Chem.* **2002**, *50*, 4852−4860.

(5) Taruscio, T. G.; Barney, D. L.; Exon, J. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *J. Agric. Food Chem.* **2004**, *52*, 3169−3176.

(6) Wu, X.; Kang, J. Blueberries: Major Phytochemicals and Potential Health Effects in Cardiovascular Diseases. In *Berries: Properties, Consumption and Nutrition*; Tuberoso, C., Ed.; Nova Biomedical Books: New York, 2012; pp 83−104.

(7) Gao, L.; Mazza, G. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J. Food Sci.* **1994**, *59*, 1057−1059.

(8) Gündeşli, M. A.; Korkmaz, N.; Okatan, V. Polyphenol content and antioxidant capacity of berries: A review. *Int. J. Agric. For. Life Sci.* **2019**, *3*, 350−361.

(9) Routray, W.; Orsat, V. Blueberries and their anthocyanins: Factors affecting biosynthesis and properties. *Compr. Rev. Food Sci. Food Saf.* **2011**, *10*, 303−320.

(10) Ramassamy, C. Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: A review of their intracellular targets. *Eur. J. Pharmacol.* **2006**, *545*, 51−64.

(11) Huang, W.; Yao, L.; He, X.; Wang, L.; Li, M.; Yang, Y. X.; Wan, C. Hypoglycemic activity and constituents analysis of blueberry (*Vaccinium corymbosum*) fruit extracts. *Diabetes, Metab. Syndr. Obes.: Targets Ther.* **2018**, *11*, 357−366.

(12) Herrera-Balandrano, D. D.; Chai, Z.; Hutabarat, R. P.; Beta, T.; Feng, J.; Ma, K.; Li, D.; Huang, W. Hypoglycemic and hypolipidemic effects of blueberry anthocyanins by AMPK activation: *In vitro* and *in vivo* studies. *Redox Biol.* **2021**, *46*, No. 102100.

(13) Li, Z.; Tian, J.; Cheng, Z.; Teng, W.; Zhang, W.; Bao, Y.; Wang, Y.; Song, B.; Chen, Y.; Li, B. Hypoglycemic bioactivity of anthocyanins: A review on proposed targets and potential signaling pathways. *Crit. Rev. Food Sci. Nutr.* **2022**, 1−18.

(14) Nunes, S.; Vieira, P.; Gomes, P.; Viana, S. D.; Reis, F. Blueberry as an attractive functional fruit to prevent (pre)diabetes progression. *Antioxidants* **2021**, *10*, 1162−1185.

(15) Delpino, F. M.; Figueiredo, L. M.; Bielemann, R. M.; Cordeiro da Silva, B. G.; Dos Santos, F. S.; Mintem, G. C.; Flores, T. R.; Arcencio, R. A.; Nunes, B. P. Ultra-processed food and risk of type 2 diabetes: a systematic review and meta-analysis of longitudinal studies. *Int. J. Epidemiol.* **2022**, *51*, 1120−1141.

(16) Iancu, M.; Ancu, I.; Mladin, P.; Ancu, S.; Mladin, G.; Chitu, V. Influence of the planting substrate on blueberry growth and yield. *Bull. Univ. Agric. Sci. Vet. Med., Hortic.* **2008**, *65*, 308−313.

(17) Scibisz, I.; Mitek, M. Effect of processing and storage conditions on phenolic compounds and antioxidant capacity of highbush blueberry jams. *Pol. J. Food Nutr. Sci.* **2009**, *59*, 45−52.

(18) Prior, R. L.; Wilkes, S. E.; Rogers, T. R.; Khanal, R. C.; Wu, X.; Howard, L. R. Purified blueberry anthocyanins and blueberry juice alter development of obesity in mice fed an obesogenic high-fat diet. *J. Agric. Food Chem.* **2010**, *58*, 3970−3976.

(19) Barba, F. J.; Esteve, M. J.; Frigola, A. Physicochemical and nutritional characteristics of blueberry juice after high pressure processing. *Food Res. Int.* **2013**, *50*, 545−549.

(20) Hancock, J. Northern highbush blueberry breeding. *Acta Hortic.* **2006**, *715*, 37−40.

(21) Lyrene, P. M. Breeding Southern highbush and rabbiteye blueberries. *Acta Hortic.* **2006**, *715*, 29−36.

(22) Hancock, J. F.; Erb, W. A.; Goulart, B. L.; Scheerens, J. C. Blueberry hybrids with complex genetic backgrounds evaluated on mineral soils: cold hardiness as influenced by parental species and location. *Acta Hortic.* **1997**, *446*, 389−396.

(23) Scheerens, J. C.; Erb, W. A.; Goulart, B. L.; Hancock, J. F. Blueberry hybrids with complex genetic backgrounds evaluated on mineral soils: stature, growth rate, yield potential and adaptability to mineral soil conditions as influenced by parental species. *Fruit Var. J.* **1999**, *53*, 73−90.
(24) Scheerens, J. C.; Erb, W. A.; Goulart, B. L.; Hancock, J. F. Blueberry hybrids with complex genetic backgrounds evaluated on mineral soils: flowering, fruit development, yield and yield components as influenced by parental species. *Fruit Var. J.* **1999**, *53*, 91−105.
(25) Mladin, P.; Mladin, G.; Rădulescu, M. Rezultate preliminare privind ameliorarea genetică a afinului de cultură (*Vaccinium corymbosum* L.). *Lucr. Stiint. ICDP Pitesti—Maracineni* **1998**, *XIX*, 153−161.
(26) Mladin, P.; Mladin, G.; Ancu, I. Ameliorarea sortimentului de afin cu tufa înaltă la I.C.D.P. Pitesti - Maracineni. *Lucr. Stiint. ICDP Pitesti—Maracineni* **2007**, *XXIII*, 26−31.
(27) Paltineanu, C.; Chitu, E. Climate change impact on phenological stages of sweet and sour cherry trees in a continental climate environment. *Sci. Hortic.* **2020**, *261*, No. 109011.
(28) Matić, P.; Sabljić, M.; Jakobek, L. Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. *J. AOAC Int.* **2017**, *100*, 1795−1803.
(29) Tudor-Radu, M.; Vijan, L. E.; Tudor-Radu, C. M.; Tiţa, I.; Sima, R.; Mitrea, R. Assessment of ascorbic acid, polyphenols, flavonoids, anthocyanins and carotenoids content in tomato fruits. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2016**, *44*, 477−483.
(30) Omaye, S. T.; Turnbull, J. D.; Sauberlich, H. E.Selected Methods for the Determination of Ascorbic Acid in Animal Cells, Tissues, and Fluids. *Methods in Enzymology*; Elsevier B.V., 1979; Vol. *62*, pp 3−11.
(31) Di Stefano, R.; Cravero, M. C. I composti fenolici e la natura del colore dei vini rossi. *Enotecnico* **1989**, *25*, 81−87.
(32) Rubio-Diaz, D. E.; Francis, D. M.; Rodriguez-Saona, L. E. External calibration models for the measurement of tomato carotenoids by infrared spectroscopy. *J. Food Compos. Anal.* **2011**, *24*, 121−126.
(33) Moon, J. K.; Shibamoto, T. Antioxidant assays for plant and food components. *J. Agric. Food Chem.* **2009**, *57*, 1655−1666.
(34) Beaudry, R.*Blueberry Quality Characteristics and How They Can Be Optimized*. Annual Report; Michigan State Horticultural Society, 1992; pp 140−145.
(35) Ancu, I.; Mladin, P.; Iancu, M.; Mladin, G.; Ancu, S.; Nuta, A.; Nicola, C. Planting substrates effects on crop and quality fruit characteristics of some blueberry Romanian cvs. *Sci. Pap. Res. Inst. Fruit Grow. Pitesti, Rom.* **2009**, *XXV*, 121−124.
(36) Aliman, J.; Michalak, I.; Bušatlić, E.; Aliman, L.; Kulina, M.; Radović, M.; Hasanbegović, J. Study of the physicochemical properties of highbush blueberry and wild bilberry fruit in central Bosnia. *Turk. J. Agric. For.* **2020**, *44*, 156−168.
(37) Kim, J. G.; Kim, H. L.; Kim, S. J.; Park, K. S. Fruit quality, anthocyanin and total phenolic contents, and antioxidant activities of 45 blueberry cultivars grown in Suwon, Korea. *J. Zhejiang Univ., Sci., B* **2013**, *14*, 793−799.
(38) Zorenc, Z.; Veberic, R.; Stampar, F.; Koron, D.; Mikulic-Petrovsek, M. Changes in berry quality of northern highbush blueberry (*Vaccinium corymbosum* L.) during the harvest season. *Turk. J. Agric. For.* **2016**, *40*, 855−864.
(39) Cortés-Rojas, M. H.; Mesa-Torres, P. A.; Grijalba-Rativa, C. M.; Pérez-Trujillo, M. M. Yield and fruit quality of the blueberry cultivars Biloxi and Sharpblue in Guasca, Colombia. *Agron. Colomb.* **2016**, *34*, 33−41.
(40) Stajčić, S. M.; Tepić, A. N.; Djilas, S. M.; Šumić, Z. M.; Čanadanović-Brunet, J. M.; Ćetković, G. S.; Vulić, J. J.; Tumbas, V. T. Chemical composition and antioxidant activity of berry fruit. *Acta Period. Technol.* **2012**, *43*, 93−105.
(41) Okan, O. T.; Deniz, I.; Yayli, N.; Şat, I. G.; Öz, M.; Hatipoğlu-Serdar, G. Antioxidant activity, sugar content and phenolic profiling of blueberry cultivars: a comprehensive comparison. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2018**, *46*, 639−652.
(42) Mikulic-Petkovsek, M.; Schmitzer, V.; Slatnar, A.; Stampar, F.; Veberic, R. A comparison of fruit quality parameters of wild bilberry (*Vaccinium myrtillus* L.) growing at different locations. *J. Sci. Food Agric.* **2015**, *95*, 776−785.
(43) Giordani, E.; Biricolti, S.; Ancillotti, C.; Petrucci, W. A.; Gori, M.; Calistri, E.; Orlandini, S.; Furlanetto, S.; Del Bubba, M. Genetic diversity and changes in phenolic contents and antiradical activity of *Vaccinium myrtillus* berries from its southernmost growing area in Italy. *Genet. Resour. Crop Evol.* **2018**, *65*, 1173−1186.
(44) Castrejón, A. D. R.; Eichholz, I.; Rohn, S.; Kroh, L. W.; Huyskens-Keil, S. Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. *Food Chem.* **2008**, *109*, 564−572.
(45) Lee, J.; Finn, C. E.; Wrolstad, R. E. Comparison of anthocyanin pigment and other phenolic compunds of *Vaccinium membranaceum* and *Vaccinium ovatum* native to the Pacific Nortwest of North America. *J. Agric. Food Chem.* **2004**, *52*, 7039−7044.
(46) Dragović-Uzelac, V.; Savić, Z.; Brala, A.; Levaj, B.; Bursač Kovačević, D.; Biško, A. Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in the Nortwest Croatia. *Food Technol. Biotechnol.* **2010**, *48*, 214−221.
(47) Colak, A. M.; Kupe, M.; Bozhuyuk, M. R.; Ercisli, S.; Gundogdu, M. identification of some fruit characteristics in wild bilberry (*Vaccinium myrtillus* L.) accessions from Eastern Anatolia. *Gesunde Pflanz.* **2018**, *70*, 31−38.
(48) Vasco, C.; Ruales, J.; Kamal-Eldin, A. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chem.* **2008**, *111*, 816−823.
(49) Pietta, P.; Gardana, C.; Pietta, A. Flavonoids in Herb. In *Flavonoids in Health and Disease*; Rice-Evans, C. A.; Packer, L., Eds.; Marcel Dekker Inc.: New York, 2003; pp 62−89.
(50) Dróżdż, P.; Šėžienė, V.; Pyrzynska, K. Phytochemical properties and antioxidant activities of extracts from wild blueberries and lingonberries. *Plant Foods Hum. Nutr.* **2017**, *72*, 360−364.
(51) Häkkinen, S. H.; Törrönen, A. R. Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. *Food Res. Int.* **2000**, *33*, 517−524.
(52) Koca, I.; Karadeniz, B. Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey. *Sci. Hortic.* **2009**, *121*, 447−450.
(53) Celik, F.; Bozhuyuk, M. R.; Ercisli, S.; Gundogdu, M. Physicochemical and bioactive characteristics of wild grown bilberry (*Vaccinium myrtillus* L.) genotypes from Northeastern Turkey. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2017**, *46*, 128−133.
(54) Padayatty, S. J.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J. H.; Chen, S.; Corpe, C.; Dutta, A.; Dutta, S. K.; Levine, M. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J. Am. Coll. Nutr.* **2003**, *22*, 18−35.
(55) Kampuse, S.; Šnē, E.; Šterne, D.; Krasnova, I. Chemical composition of highbush blueberry cultivars. *Latv. J. Agron.* **2009**, *12*, 53−59.
(56) Shevchuk, L. M.; Grynyk, I. V.; Levchuk, L. M.; Yareshcenko, O. M.; Tereshcenko, Y. Y.; Babenko, S. M. Biochemical contents of highbush blueberry fruits grown in the Western forest-steppe of Ukraine. *Agron. Res.* **2021**, *19*, 232−249.
(57) Correia, S.; Gonçalves, B.; Aires, A.; Silva, A.; Ferreira, L.; Carvalho, R.; Fernandes, H.; Freitas, C.; Carnide, V.; Silva, A. P. Effect of harvest year and altitude on nutritional and biometric characteristics of blueberry cultivars. *J. Chem.* **2016**, *2016*, No. 8648609.
(58) Holzapfel, N. P.; Holzapfel, B. M.; Champ, S.; Feldthusen, J.; Clements, J.; Hutmacher, D. W. The potential role of lycopene for the prevention and therapy of prostate cancer: from molecular mechanisms to clinical evidence. *Int. J. Mol. Sci.* **2013**, *14*, 14620−14646.

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

(59) Willett, W. C. Vitamin A and lung cancer. *Nutr. Rev.* **2009**, *48*, 201−211.
(60) Lippman, S. M.; Benner, S. E.; Hong, W. K. Chemoprevention strategies in lung carcinogenesis. *Chest* **1993**, *103*, 15S−19S.
(61) Borek, C. Molecular mechanisms in cancer induction and prevention. *Environ. Health Perspect.* **1993**, *101 Suppl. 3*, 237−245.
(62) Hennekens, C. H. Antioxidant vitamins and cancer. *Am. J. Med.* **1994**, *97*, 2S−4S.
(63) Giovannucci, E.; Ascherio, A.; Rimm, E. B.; Stampfer, M. J.; Colditz, G. A.; Willett, W. C. Intake of carotenoids and retinol in relation to risk of prostate cancer. *JNCI, J. Natl. Cancer Inst.* **1995**, *87*, 1767−1776.
(64) Finley, J. W. Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and selenocompounds. *Ann. Bot.* **2005**, *95*, 1075−1096.
(65) Kalt, W.; Forney, C. F.; Martin, A.; Prior, R. L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem.* **1999**, *47*, 4638−4644.
(66) Howard, L. R.; Clark, J. R.; Brownmiller, C. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J. Sci. Food Agric.* **2003**, *83*, 1238−1247.
(67) Tsuda, H.; Kunitake, H.; Kawasaki-Takaki, R.; Nishiyama, K.; Yamasaki, M.; Komatsu, H.; Yukizaki, C. Antioxidant activities and anti-cancer cell proliferation properties of natsuhaze (*Vaccinium oldhamii* Miq.), shashanbo (V. bracteatum Thunb.) and blueberry cultivars. *Plants* **2013**, *2*, 57−71.
(68) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer. II. Mechanisms. *Cancer Causes Control* **1991**, *2*, 427−442.
(69) Guofang, X.; Xiaoyan, X.; Xiaoli, Z.; Yongling, L.; Zhibing, Z. Changes in phenolic profiles and antioxidant activity in rabbiteye blueberries during ripening. *Int. J. Food Prop.* **2019**, *22*, 320−329.
(70) Scibisz, I.; Mitek, M. Antioxidant properties of highbush blueberry fruit cultivars. *Electron. J. Pol. Agric. Univ.* **2007**, *10*, 34.
(71) López, J.; Uribe, E.; Vega-Gálvez, A.; Miranda, M.; Vergara, J.; Gonzalez, E.; Di Scala, K. Effect of air temperature on drying kinetics, vitamin C, antioxidant activity, total phenolic content, non-enzymatic browning and firmness of blueberries variety O'Neil. *Food Bioprocess Technol.* **2010**, *3*, 772−777.
(72) Nicholas, K. A.; Matthews, M. A.; Lobell, D. B.; Willits, N. H.; Field, C. B. Effect of vineyard-scale climate variability on Pinot noir phenolic composition. *Agric. For. Meteorol.* **2011**, *151*, 1556−1567.
(73) Dinis, L.-T.; Bernardo, S.; Conde, A.; Pimentel, D.; Ferreira, H.; Félix, L.; Gerós, H.; Correia, C. M.; Moutinho-Pereira, J. Kaolin exogenous application boosts antioxidant capacity and phenolic content in berries and leaves of grapevine under summer stress. *J. Plant Physiol.* **2016**, *191*, 45−53.
(74) Zheng, Y.; Li, R.; Sun, Y.; Xu, M.; Zhang, H.; Huang, L.; Zhu, Y.; Wang, H.; Li, G.; Liu, L.; Li, F.; Guo, L.; Zhang, X. The optimal temperature for the growth of blueberry (*Vaccinium corymbosum* L.). *Pak. J. Bot.* **2017**, *49*, 965−979.
(75) Hykkerud, A. L.; Uleberg, E.; Hansen, E.; Vervoort, M.; Mølmann, J.; Martinussen, I. Seasonal and yearly variation of total polyphenols, total anthocyanins and ellagic acid in different clones of cloudberries (*Rubus chamaemorus* L.). *J. Appl. Bot. Food Qual.* **2018**, *91*, 96−102.
(76) Bernal-Gallardo, J. O.; Molina-Torres, J.; Angoa-Pérez, M. V.; Cárdenas-Valdovinos, J. G.; García-Ruíz, I.; Ceja-Díaz, J. A.; Mena-Violante, H. G. Phenolic compound content and the antioxidant and antimicrobial activity of wild blueberries (*Vaccinium stenophyllum* steud.) fruits extracts during ripening. *Horticulturae* **2022**, *8*, No. 15.
(77) Arellanes-Juárez, N.; Benito-Bautista, P.; Zárate-Nicolás, B. H. Phenolic compound content in Biloxi blueberry grown in the Sierra Norte of Oaxaca, Mexico, harvested in warm season. *Acta Hortic.* **2023**, *1357*, 207−212.
(78) Bernardo, S.; Dinis, L. T.; Machado, N.; Barros, A.; Pitarch-Bielsa, M.; Malheiro, A. C.; Gómez-Cadenas, A.; Moutinho-Pereira, J. Uncovering the effects of kaolin on balancing berry phytohormones and quality attributes of Vitis vinifera grown in warm-temperate climate regions. *J. Sci. Food Agric.* **2022**, *102*, 782−793.

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

Journal of Applied Life Sciences and Environment
https://jurnalalse.com

Review Article
https://doi.org/10.46909/alse-552053
Vol. 55, Issue 2 (190) / 2022: 111-132

MAIN ACTIVE COMPONENTS OF GOJI BERRY AND THEIR NUTRITIONAL IMPORTANCE – A REVIEW

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Received: Oct. 27, 2022. Revised: Nov. 23, 2022. Accepted: Dec. 12, 2022. Published online: Jan. 26, 2023

ABSTRACT. The consumption of fruits, leaves, and roots of *Lycium barbarum* L. and *Lycium chinense* (Mill.) species has a long tradition, especially on the Asian continent, due to their health benefits. In recent decades, social and economic factors, along with scientific progress, have stimulated the expansion of the consumption and cultivation of goji plants on a global scale, but mostly in Western countries. The traditional therapeutic properties attributed to goji plants, scientifically demonstrated through clinical and pharmacological studies *in vitro* and *in vivo*, are due to a diversified content in antioxidants (polysaccharides, flavonoids, carotenoids, and antioxidant capacity). With the development of technological capabilities for the detection and extraction of biocompounds from plant resources (including from secondary metabolisms), the completeness of research on the beneficial and secondary effects of the use of these species in human nutrition has increased. In most of the published studies, the chemical profile of *L. barbarum* or *L. chinense* species was analysed in terms of the therapeutic benefits of the variety, the different plant components subjected to extraction, the prior processing of these components, the method of extraction of active biocompounds, and to some extent, the correlation of this profile with geographical origin. The objective of this study is to provide a comprehensive and updated summary on some chemical compounds with therapeutic effects from *Lycium spp*. plants, addressing the correlation of the phytochemical composition in relation to their cultivation area, in the perspective of identifying and creating new goji varieties with high adaptability to local pedoclimatic conditions.

Keywords: *L. barbarum*; *L. chinense*; chemical profile; cultivation areas.

Cite: Tudor, A.D.; Bolohan, C.; Tudor, V.; Teodorescu, R.I. Main active components of goji berry and their nutritional importance – a review. *Journal of Applied Life Sciences and Environment* **2022,** 55 (2), 111-132. https://doi.org/10.46909/alse-552053

INTRODUCTION

Due to the nutritional properties and the high content of different phytochemical compounds with multiple effects on health, goji fruits have received the generic name of 'superfruit' or 'superfood' (Van Straten and Griggs, 2006; Llorent-Martínez *et al.*, 2013; Kulczyński and Gramza-Michałowska, 2016; Chang *et al.*, 2018; Ma *et al.*, 2019; Jiao and Liu, 2020; Chang *et al.*, 2020; Wang *et al.*, 2022). Lately, the special attention generally enjoyed by the species of the *Lycium* genus emerges from the research carried out on them by numerous authors, including through reviews that cover an increasing amount of data. Qian *et al.* (2017a) showed that in a period of 41 years (1975-2016), more than 350 chemical compounds have been isolated from different parts of plants of the genus *Lycium* and described. Lu *et al.* (2017) mentioned the consumption of goji fruits due to the special taste as a 'unique aroma' and talked about the detection of 193 volatile substances in the fruits of *Lycium barbarium* L. grown in the Ningxia Region of China, and Kim *et al.* (2009) detected 130 volatile substances in the fruits of *Lycium chinensis* Miller (Chungchungnam-do, South Korea).

Geographical distribution and taxonomy

The generic name 'goji' is attributed to the fruit and, by extension, to the perennial shrubs of the family *Solanaceae*, genus *Lycium*. The genus includes between 75 (Miller, 2002) and 80 species (Levin and Miller, 2005; Zhang et al., 2018; Liu and Sun, 2020), with a fragmented distribution in temperate, arid, or semi-arid climate areas and temperatures between 15 °C and 40 °C (Jatoi *et al.*, 2017; Yao *et al.*, 2018a) (*Figure 1*). Carl Linneaus made the first description of the genus, with three of its species (*Lycium europaeum*, *Lycium barbarum*, and *Lycium afrum*), in his 1753 work, 'Species Plantarum' (Yao *et al.*, 2018a), where he gives the species name *L. barbarum*. Miller named the species *Lycium chinense* in 1768 (Kulczyński and Gramza-Michałowska, 2016). The first taxonomy, including 43 species of the genus *Lycium* from the Northern Hemisphere, was made by Hitchcock in 1932 (Yao *et al.*, 2018a).

The generic name 'goji' is a derivative of the term 'gouqi' (Potterat and Hamburger, 2008) or 'gou qi' (Chen *et al.*, 2018; Yao *et al.*, 2018a), resulting from the extrapolation of several native Chinese words and stated in this form for the first time in 1973 by Tanaduk Botanical Research Institute researchers (Amagase and Farnsworth, 2011 cited by Shahrajabian *et al.*, 2018). The first mention of the term 'gou qi' is found in "Shen Nong Ben Cao Jing", a book of Chinese origin written between 200 and 250 AD that describes agricultural practices and presents information about medicinal plants (Chen *et al.*, 2018).

The endemic species of the genus *Lycium* are mainly found in North America, South America, South of Africa, Asia, Europe, and Australia (*Table 1*). Three taxonomic varieties and seven species of goji are found in China (Zhang *et al.*, 2018). Analyses carried out by genetic sequencing (Miller *et al.*, 2011; Cao *et al.*, 2021) support the hypothesis that species of the genus *Lycium* migrated from South America to

North America and Africa, then to Eurasia, East Asia, and Australia.

L. barbarum and *L. chinense* were the species intensively promoted under the name of goji, and they spread worldwide from the point of view of cultivation for commercial purposes, on the background of the superior quality of the fruits, exploited as food with therapeutic effects.

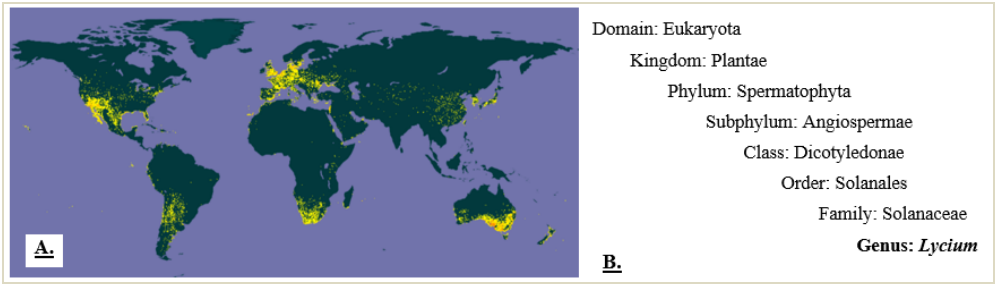

Figure 1 - Genus *Lycium* distribution and taxonomy. A) Global distribution of genus *Lycium* based on the records associated with the number of occurrences (yellow dots) of its species from 1600 to 2022 (cumulated values) in the Global Biodiversity Information Facility (GBIF, 2022). B) Genus *Lycium* taxonomic tree (CABI, 2022)

Table 1 - Species of the genus *Lycium*

Continent	*Lycium* spp.	Authors
Asia	*L. chinense, Lycium ruthenicum, L. barbarum, Lycium truncatum, Lycium dasystemum, Lycium cylindricum, L. chinense, Lycium yunnanense, Lycium changjicum, Lycium depressum*	Levin and Miller, 2005; Cao *et al.*, 2021
Europe	*Lycium ruthenicum*	Levin and Miller, 2005
	Lycium europeaum	Yao *et al.*, 2018
South of Africa	*Lycium arenicol, Lycium bosciifolium, Lycium cinereum, Lycium ferocissimum, Lycium hirsutum, Lycium. horridum, Lycium oxycarpum, Lycium pilifolium C, Lycium schizocalyx, Lycium shawii, Lycium sp. N-309, Lycium tenue, Lycium villosum, Lycium villosum,*	Levin and Miller, 2005
Australia	*Lycium australe*	Levin and Miller, 2005
North America	*L. barbarum, Lycium berlandieri, Lycium californicum, Lycium carolinianum, Lycium cooperi, Lycium exsertum, Lycium exsertum, Lycium fremontii, Lycium macrodon, Lycium pallidum, Lycium parishi, Lycium puberulum, Lycium Shockley, Lycium sp. 202, Lycium texanum, Lycium torreyi,*	Levin and Miller, 2005; Cao *et al.*, 2021; Shahrajabian *et al.*, 2018
South America	*Lycium ameghinoi, Lycium. americanum, Lycium andersonii, Lycium brevipes, Lycium cestroides, Lycium chilense, Lycium ciliatum, Lycium cuneatum, Lycium elongatum, Lycium fremontii, Lycium gilliesianum, Lycium infaustum, Lycium moronga, Lycium nodosum, Lycium vimineum*	Levin and Miller, 2005; Shahrajabian et al., 2018

Although the quality and productivity of other species or subspecies of the *Lycium* genus may be lower, they are valued locally under the same common name (goji) (Wetters *et al.*, 2018) or similar names (e.g., wolfberry or boxthorn). Based on the distribution of the two species, Yao *et al.* (2021) postulated the impossibility of differentiating the two based on their geographical origin, given the overlap of their distribution areas *(Figure 2)*.

According to some authors (Wenli *et al.*, 2021), goji is native from China, a perception justified by the fact that this country is the main grower of goji for commercial purposes (Sun *et al.*, 2017; Chen *et al.*, 2018). The most recent data (Yao *et al.*, 2018b) indicated an area of about 150 thousand hectares (at the level of 2015) that was cultivated mainly in the regions of north and northwest China in the provinces of Inner Mongolia (Inner Mongolia), Xinjiang, Gansu, Qinghai, Ningxia, and Hebei (Potterat and Hamburger, 2008; Sun *et al.*, 2017; Yao *et al.*, 2018b; Zhang *et al.*, 2018). Other authors (Kulczyński and Gramza-Michałowska, 2016) advance values of about 82,000 hectares in terms of cultivated area, with an annual production of about 95,000 tons. Moreover, the beginning of the domestication and cultivation of the species *L. barbarum* and *L. chinese* about 600 years ago in northwest China, the endemic character of the species *L. ruthenicum* for this area (Shahrajabian *et al.*, 2018; Zhang *et al.*, 2018), and the use of different component parts of goji plants (fruits, roots, leaves, calyx, bark, or even the whole plant) in traditional Chinese medicine or as food for about 4,000 years (Wang *et al.*, 2015; Yao *et al.*, 2018a) have facilitated the perception of goji as a native plant in China. This perception is to some extent justified for the commercial goji varieties that were obtained over 600 years of natural and artificial selection (Zhang *et al.*, 2018). In addition, in the last decades, significant government resources have been allocated to support the cultivation and promotion of the consumption of goji berries, especially *L. barbarum* and *L. chinense*, a fact that led to the expansion and international recognition of the two species, assimilated most frequently under the name of goji (Yao, 2019).

Goji was first introduced to Europe in the 18th century (Sopher, 2013 cited by Kulczyński and Gramza-Michałowska, 2016); however, since the 2000s, due to effective marketing strategies promoting goji berries as a quasi-miracle remedy for health and anti-aging (Potterat and Hamburger, 2008; Potterat, 2010; Jiao and Liu, 2020), the two species (*L. barbarum* and *L. chinense*) were introduced for cultivation in North America, southeastern Europe (Romania, Bulgaria), and in Mediterranean agro-climatic conditions (Italy, Portugal) (Amagase and Farnsworth, 2011) where new varieties were developed (Dzhugalov *et al.*, 2015; Protti *et al.*, 2017; Mocan *et al.*, 2018, 2019). In the southern region of Italy, *L. barbarum* is cultivated on about 38 ha, the largest plantation in Europe (Juan-Garcia *et al.*, 2019), and the fruits are sold as a fresh, dried, or processed products. The geographical origin of the fruit is essential from the point of view of quality because the chemical composition changes depending on the

climate, water, soil, and cultivation conditions (Li *et al.*, 2017). In Romania, Stavrescu-Bedivan *et al.* (2022) showed that the species *L. Barbarum* was reported in 37 counties out of a total of 41, including the Bucharest area.

Phytochemical constituents

The interest in researching goji species derives from the complexity of the plant's chemical composition. This is especially attributed to the content of different classes of biocompounds with antioxidant, anti-inflammatory, and antineoplastic effects (Chen *et al.*, 2018), with the predominant ones being polysaccharides, flavonoids, carotenoids, lipids, steroids, alkaloids, terpenoids, and phenolic compounds (Protti *et al.*, 2017; Chen *et al.*, 2018; Jiang *et al.*, 2021). In the different parts of the plant (reproductive: fruits, flowers, and seeds, but also vegetative: roots, leaves, and bark), *Lycium* spp. contain 355 active compounds that can be grouped into glycerogalactolipids (6%), phenylpropanoids (9%), coumarins (3%), lignans (4%), flavonoids (9%), amides (10%), alkaloids (20%), anthraquinones (1%), organic acids (9%), terpenoids (11%), peptides (1%), and sterols, steroids, and other constituents (16%) (Qian et al., 2017a; Jiao and Liu, 2020). Total phenolic content differed according to cultivation conditions and variety (*Table 2*). *Lycium* spp. fruit contain a total of 186 phenolic compounds, with flavonoids and phenolic acids being the predominant classes (Jiang *et al.*, 2021) in red goji berries (*L. barbarum*, *L. chinense*) and anthocyanins being the main polyphenols in black goji berries (varieties of *L. ruthenicum*) (Sun *et al.*, 2017).

Goji berries are a source of potentially bioactive substances, but the chemical profile could differ depending on their origin, cultivation, and/or processing method (Mocan *et al.*, 2019). Environmental conditions influence both the fruit appearance and the metabolic profile (Zhang *et al.*, 2012; Shen *et al.*, 2016) of goji plants, in terms of the amount of polysaccharides (*Table 3*), carotenoids (*Table 4*), flavonoids (*Table 5*) and betaine.

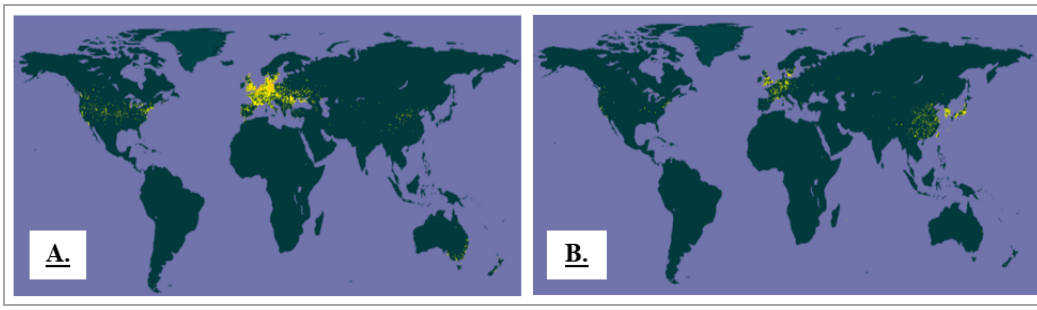

Figure 2 - Distribution maps of *L. barbarum* (A) and *L. chinense* (B); data reflect the number of occurences of the species (yellow areas) based on the number of occurrences from 1600 to 2022 (cumulated values) and are based on the Global Biodiversity Information Facility (GBIF, 2022)

Yang *et al.* (2021) identified strong correlations between chemical profile constituents and geographic distribution of *L. chinense* with higher concentrations of flavonoids and polysaccharides in cultivars from central and southern China. Zheng *et al.* (2010) observed such variations in a study on the fructose, glucose, sucrose, polysaccharide, and sugar content of *L. barbarum* and *L. chinense* fruits from different cultivars and regions. The research revealed that the pedological specificity of the cultivation area, from the point of view of pH, organic matter, and nitrogen content but also the salt content ($HCO3^-$, Na^+, Ca^{2+}, Mg^{2+}, and Cl^-), influences the accumulation of sugar in the fruits of goji. Bondia-Pons et al. (2014) highlighted the importance of the geographical origin in analysing the chemical profile of the plants, as it is influenced by climate and soil conditions and cultivation methods, which can change the chemical composition and impact the quality of the fruits or other parts of the plant.

Bertoldi *et al.* (2019) identified a higher total carotenoid content (355 mg/ 100 g DWcompared to 198 mg/100 g DW), a higher magnesium content (from 72 to 267 mg/100 g compared to values between 78 and 161 mg/100 g), and higher values of the micronutrient content (K, B, Cu, Mo, Se, Zn) in Italian goji berries compared to those of Asian origin.

The sterol content of goji berries differs according to the geographic area of cultivation (Cossignani *et al.*, 2018). The results of the research carried out by Cossignani *et al.* (2018) showed that goji berry samples of Italian origin had a higher content of β-sitosterol, whereas the content of Δ5-avenasterol and Δ-5,23-stigmastadienol was representative of fruits of Asian origin. Moreover, the content of essential fatty acids was higher for samples from China and Mongolia (61.0% and 61.6%) and lower (47.8%) for Italian fruits, and the content of phytosterols varied between 42.8 mg/100 g for Italian fruits and 130.1 mg/100 g for Mongolian ones. Wojdyło *et al.* (2018) analysed 21 new cultivars of *L. barbarum* obtained in a breeding program developed in Poland and identified differences in the biochemical profile and functional properties of the new varieties, postulating the opportunity to use only some of them as a source of bioactive compounds with high biological activity.

Yossa Nzeuwa *et al.* (2019) identified slight differences in the chemical profile of dried fruits from different varieties of *L. barbarum* and *L. chinense* cultivated in China (in the regions devoted to these species, like Ningxia, Xinjiang, Qinghai, and Gansu) and Nepal, without obtaining specific results to support the prevalence of a geographical area in terms of chemical composition (*Table 2*).

Research on the chemical composition and antioxidant activity of the dried fruits of *L. barbarum* (cultivated in Greece, China, and Mongolia) and *L. chinense* (cultivated in Greece) highlights differences both in the total carbohydrate content and the total phenolic content (Skenderidis *et al.*, 2019). The highest total carbohydrate content was recorded in *L. barbarum* fruits grown in Greece and harvested in August (490 mg/g dw), and the highest total phenolic content (10.9 mg/g dw) is identified in the Mongolian variety.

Moreover, *L. chinense* shows a lower concentration of carbohydrates and phenols compared to *L. barbarum* varieties (Skenderidis *et al.*, 2019). The antioxidant activity of *Lycium* spp. fruits has been validated by numerous authors (*Table 6*), and the values of this parameter vary depending on the variety/cultivar and the cultivation area.

Nutritional uses

Between 31 (Yao *et al.*, 2018) and 36 species (Yao, 2019) of the genus *Lycium* are exploited for food or medicinal purposes globally. *L. barbarum*, *L. chinense*, and *L. ruthenicum* are the species most often reported in Asia, especially in China (Yao *et al.*, 2018) where the fruits have been used in traditional medicine for thousands of years (Cossignani *et al.*, 2018). Goji berries are also used in traditional Korean, Vietnamese, Japanese, and Tibetan medicine (Potterat and Hamburger, 2008; Shahrajabian *et al.*, 2018). As food, the fruits are usually consumed fresh or dried, or they are processed as juices, tinctures, or jellies (Kulczyński and Gramza-Michałowska, 2016; Jiao and Liu, 2020). Along with the fruits, the bark and leaves of *Lycium* spp. are boiled for infusions, individually or in combination with *Chrysanthemum morifolium*, *Chrysanthemum indicum*, *Zizisiphus jujube*, or *Camellia sinensis* (Sun *et al.*, 2017). In Chinese cuisine, goji berries are often cooked before consumption and used in vegetable soups in combination with rice porridge, chicken, or pork (Kulczyński and Gramza-Michałowska, 2016; Sun *et al.*, 2017). Goji wines are another form of processing the fruits of these species (Xia *et al.*, 2016; Kulczyński and Gramza-Michałowska, 2016; Jiao and Liu, 2020). Traditionally, goji wine is obtained by macerating the fruit in an alcohol (Sun *et al.*, 2017), and fermented goji wines have gained special attention in recent years due to their distinctive aroma and taste (Xia *et al.*, 2016). In the last decades, *L. barbarum* and *L. chinense* have been promoted on a global scale as 'superfood' (Yao *et al.*, 2018) or 'superfruit' (Jiao and Liu, 2020), terms used to define a food rich in elements, nutrients, and antioxidants (Chang *et al.*, 2018) with properties to prevent or treat certain conditions (Jiao and Liu, 2020). Although *L. europeaum*, *L. intricatum*, and *L. shawii* are endemic species reported in the Mediterranean area and the Middle East, *L. pallidum* in North America, and *L. afrum* in Africa (Yao *et al.*, 2018), *L. barbarum* and *L. chinense* have been promoted for cultivation on a global scale and are culturally accepted under the name goji. The two species were initially promoted for consumption in Europe, America, and Australia in juice form (Potterat and Hamburger, 2008). Later, the fruits were integrated into cakes, protein bars, chocolate, muesli, sausages, and even soups (Potterat and Hamburger, 2008), but most frequently they are consumed as a food supplement or used in the form of tea (Sun *et al.*, 2017).

The popularization of goji consumption in Western countries is in close interdependence with the expansion of research on deepening the nutritional profile of the species but also investigating the potential for new uses of them for food purposes.

Table 2 - Total phenolic content (TPC) of *Lycium* spp. fruits in different areas of the world

Country (region)	*Lycium barbarum* L.		*Lycium chinense* Mill.		Authors, year
	Cultivar/variety	TPC Value	Cultivar/variety	TPC Value	
Greece, Thessaly	8 varieties	272.3 to 394.3 mg GAE/L	2 varieties	297.3 mg GAE/L 371.3 mg GAE/L	Skenderidis *et al.*, 2018
	nr	6.9 to 10.1 mg/g DW	nr	7.4 to 8.9 mg/g DW	Skenderidis *et al.*, 2019
Romania, Northwest	Erma	11.6 mg GAE/g DW	-	-	Mocan *et al.*, 2018
	Big Lifeberry	15.7 mg GAE/g DW			
Italy, Northern		nr *Licium* spp. 268.35 (mg_{GAE}/100 g_{FW})			Donno *et al.*, 2015
Switzerland Conthey Wallis, Southwestern	Big Lifeberry	1.20 mg GAE/g DW	-	-	Kosinska-Cagnazzo *et al.*, 2017
	No. 1	2.94 mg GAE/g DW			
	Red Life	1.11 mg GAE/g DW			
	Saxon	2.07 mg GAE/g DW			
	Sweet Lifeberry	1.51 mg GAE/g DW			
	Tibet	0.71 mg GAE/g DW			
Slovenia	nr	1319 mg GAE/kg	-	-	Mikulic-Petkovsek *et al.*, 2012
Turkey, Konya	nr	8.16^{WE} mg GAE/100 g	-	-	Ozkan et al., 2018
Central Anatolia		9.14^{ME} mg GAE/100 g	-	-	
Turkey, Manisa province	nr	208.6 mg GAE/100 g	-	-	Ağagündüz *et al.*, 2021
Serbia, Southern	No.1 (red goji)	162.4 mg GAE/100	-	-	Ilić *et al.*, 2020
	Amber Sweet Goji (yellow goji)	176.3 mg GAE/100			
China, NingXia Hui	R1	3.12 mg GAE/g DW	-	-	Islam *et al.*, 2017
	R2	2.87 mg GAE/g DW			
	R3	2.17 mg GAE/g DW			
	R4	4.48 mg GAE/g DW			
China, Ningxia	nr	7.854 mg GAE/g DW	-	-	Lu *et al.*, 2019
China, Gansu		7.690 mg GAE/g DW			
China, Qinghai		7.335 mg GAE/g DW			
China, Inner Mongolia		7.098 mg GAE/g DW			
China, Xinjiang		7.239 mg GAE/g DW			
China, Hebei Monsoon province	-	-	nr	8.86 mg GAE/g DW	Yossa Nzeuwa *et al.*, 2019
China, Delhi, Qinghai province	nr	9.82 mg GAE/g DW	-	-	
Nepal	nr	14.13 mg GAE/g DW	-	-	

FW = fresh weight; DW = dry weight; GAE = gallic acid equivalents; WE = water extract; ME = methanol extract; nr = not reported; R1 to R4 = varieties

Table 3 - Total polysaccharide content (TPsC) of *Lycium* spp. fruits in different areas of the world

Country (region)	*Lycium barbarum* L.		*Lycium chinense* Mill.		Authors, year
	Cultivar/variety	TPsC Value	Cultivar/variety	TPsC Value	
China, Ningxia Hui (20 ^{DAF})	Damaye	76.86 mg/g FW	-	-	Zhao *et al.*, 2015b
	Baihua	42.53 mg/g FW			
	Ningqi No.1	27.60 mg/g FW			
HaiKou, Chin	nr	23.13%	-	-	Yin and Dang, 2008
China, Ningxia, Qinghai, Xinjiang	nr	16–48 mg/g	-	-	Wang *et al.*, 2019 cited by Vidović *et al.*, 2022
China, Ningxia	Damaye	55.9 mg/g FW	Zhongguo	25.0 mg/g FW	Zhang *et al.*, 2016
	Ningji No.1	56.9 mg/g FW	Potaninii (Pojark.) A. M. Lu	49.6 mg/g FW	
	Baihua	62.7 mg/g FW	-	-	
	Auranticarpum K. F. Ching	54.5 mg/g FW			
Taiwan, Taipei^{LS}	nr	57.2^{CP} mg/g	-	-	Wang *et al.*, 2010
China, Ningxia	-	33.96 mg/g DW	-	-	Lu *et al.*, 2019
China, Gansu		30.57 mg/g DW			
China, Qinghai		25.58 mg/g DW			
China, Inner Mongolia		26.88 mg/g DW			
China, Xinjiang		26.78 mg/g DW			

DAF = days after blossom; CP = crude polysaccharide; LS = local store; DW = dry weight; FW = fresh weight; nr = not reported.

Table 4 - Total carotenoid content (TCC) of *Lycium* spp. fruits in different areas of the world

Country (region)	*Lycium barbarum* L.		*Lycium chinense* Mill.		Authors, year
	Cultivar/variety	TCC Value	Cultivar/variety	TCC Value	
Serbia, Southern	No.1	41.71 mg/100 g	-	-	Ilić et al., 2020
	Amber Sweet Goji	3.60 mg/100 g			
Italy, Southern	-	184.2[D] mg/100 g	-	-	Niro et al., 2018
		56.4[F] mg/100 g			
China, Ningxia Hui Autonomous Region	R1	233.08 µg/g	-	-	Islam et al., 2017
	R2	212.24 µg/g			
	R3	224.21 µg/g			
	R4	222.63 µg/g			
China, Zhongning county	nr/S4	508.90 µg/g FW	-	-	Liu et al., 2014
Poland, West - Wrocław	21 cultivars[M]	2129.44 mg/kg dm	-	-	Wojdyłoa et al., 2018
Ningxia Hui, China	*Barbarum* sp.	0.386%	*Chinense* sp.	0.444%	Peng et al., 2005
	Auranticarpum K.F. Ching	0.035%	Potaninii A.M. Lu	0.306%	
Italy, South Lazio	Big Lifeberry	1.1980[PU] mg/g[F]	-	-	Spano et al., 2021
		0.3274[PE] mg/g[F]			
	Sweet Lifeberry	1.2810[PU] mg/g[F]			
		0.4652[PE]			
China, Ningxia	nr	19.94 βCE/g DW	-	-	Lu et al., 2019
China, Gansu		19.89 βCE/g DW			
China, Qinghai		18.91 βCE/g DW			
China, Inner Mongolia		20.41 βCE/g DW			
China, Xinjiang		12.93 βCE/g DW			
China, Ningxia	Damaye	7.97 mg βCE/g FW	Zhongguo	5.94 mg βCE/g FW	Zhang et al., 2016
	Ningji No.1	11.33 mg βCE/g FW	Potaninii (Pojark.) A. M. Lu	9.85 mg βCE/g FW	
	Baihua	7.83 mg βCE/g FW	-		
	Auranticarpum K. F. Ching	3.64 mg βCE/g FW			

F = fresh; D = dried; S4 = stage four; M = mean; PU = pulp; PE = peel; FW = fresh weight; DW = dry weight; nr = not reported; dm = dry matter; R1 to R4 = varieties.

Table 5 - Total flavonoid content (TFC) of *Lycium* spp. fruits in different areas of the world

Country (region)	*Lycium barbarum* L.		*Lycium chinense* Mill.		Authors, year
	Cultivar/variety	TFC Value	Cultivar/variety	TFC Value	
Serbia, Southern	No.1	214.2 mg HE/100 g	-	-	Ilić *et al*., 2020
	Amber Sweet Goji	335.5 mg HE/100 g			
China, Ningxia Hui Autonomous Prefecture	R1	2.67 mg_{CAE}/g	-	-	Islam *et al*., 2017
	R2	2.78 mg_{CAE}/g			
	R3	2.69 mg_{CAE}/g			
	R4	3.16 mg_{CAE}/g			
Ningxia Hui Region, China	nr	314.8168 mg GAE/L	nr	270.1829 mg GAE/L	Qian *et al*., 2017b
Turkey, Konya, Central Anatolia	nr	1.78^{WE} mg CE/g extract	-	-	Ozkan *et al*., 2018
		2.63^{ME} mg CE/g extract			
Turkey, Southeastern Anatolia	NQ1	51.09 mg QE/g	-	-	Oguz and Erdogan, 2016
	Damaye	48.89 mg QE/g			
China, Ningxia	nr	5.278 mg RE/g DW	-	-	Lu *et al*., 2019
China, Gansu		5.567 mg RE/g DW			
China, Qinghai		3.702 mg RE/g DW			
China, Inner Mongolia		4.964 mg RE/g DW			
China, Xinjiang		3.177 mg RE/g DW			
Bulgaria, Plovdiv	JB1	1.9 mg/100 g	-	-	Dzhugalov and Denev, 2020
	JB2	5.7 mg/100 g			
	JB4	2.6 mg/100 g			
	JB10	3.5 mg/100 g			
China, Ningxia	Damaye	42.6 mg RE/g FW	Zhongguo	45.3 mg RE/g FW	Zhang *et al*., 2016
	Ningji No.1	54.7 mg RE/g FW	Potaninii (Pojark.) A. M. Lu	37.2 mg RE/g FW	
	Baihua	48.2 mg RE/g FW	-	-	
	Auranticarpum K. F. Ching	38.5 mg RE/g FW			

WE = water extract; ME = methanol extract; RE = rutin equivalent; DW = dry weight; FW = fresh weight; GAE = gallic acid equivalents; QE = quercetin equivalents; nr = not reported; JB= variety tested.

Table 6 - Antioxidant activity by (DPPH) of *Lycium* spp. fruits in different areas of the world

Country (region)	*Lycium barbarum* L.		*Lycium chinense* Mill.		Authors, year
	Cultivar/variety	DPPH Value	Cultivar/variety	DPPH Value	
Serbia, Southern	No.1	452.6 µmol TE/100 g	-	-	Ilić *et al.*, 2020
	Amber Sweet Goji	443.6 µmol TE/100 g			
Romania, Northwest	Erma	8.79 mg TE/g extract	-	-	Mocan *et al.*, 2018
	Big Lifeberry	9.35 mg TE/g extract			
China, Ningxia Hui Autonomous Prefecture	R1	16.07 µmol TE/g	-	-	Islam *et al.*, 2017
	R2	16.61 µmol TE/g			
	R3	16.46 µmol TE/g			
	R4	17.47 µmol TE/g			
Italy, Northern	nr *Lycium* sp. 19.36 mmol Fe^{2+}/kg				Donno *et al.*, 2015
Turkey, Manisa province	nr	33.4 µmol TE/g	-	-	Ağagündüz *et al.*, 2021
Turkey, Central Anatolia	nr	20.78%	-	-	Endes *et al.*, 2015
Turkey, Çivril Denizli province	G1	32.28 µM TE/g FW	-	-	Çolak *et al.*, 2016
	G2	56.82 µM TE/g FW			
	G3	87.48 µM TE/g FW			
	G4	65.14 µM TE/g FW			
China, Ningxia	Damaye	76.6 µM TE/g FW	Zhongguo	35.88 µM TE/g FW	Zhang *et al.*, 2016
	Ningji No.1	85.46 µM TE/g FW	Potaninii (Pojark.) A. M. Lu	53.49 µM TE/g FW	
	Baihua	77.47 µM TE/g FW	-	-	
	Auranticarpum K. F. Ching	62.28 µM TE/g FW			

TE = Trolox equivalent; FW = fresh weight; DPPH = 2,2-diphenyl-1-picrylhydrazyl; nr = not reported.

Dried and fresh goji fruits are a rich source of proteins and minerals (Niro *et al.*, 2017; Pires *et al.*, 2018). One hundred dry grams of goji berries can contain: 1) 10.2% (Niro *et al.*, 2017) –14.26% (Potterat, 2010) protein; 2) 101.3–190 mg of calcium; 3) 861.9–2,233 mg of potassium; 4) 3.4–9 mg of iron; 5) 0.17–0.5 mg of selenium; 6) 0.33–1.3 mg of vitamin B2 (riboflavin); 7) 29–148 mg of vitamin C; 8) 45.9–140 mg of magnesium; 9) 209.8–448 mg of natrium; and 10) 140–174.3 mg of phosphorus (Potterat, 2010; Niro *et al.*, 2017; Pedro *et al.*, 2018; Shahrajabian *et al.*, 2018).

Rybicka *et al.* (2021) compared dried goji berries with a selection of other dried fruits (raisins, prunes, sea buckthorn, rose hips, etc.; all commercially available in Poland), and the results obtained show the importance of dried goji berries as an alternative to snack foods, due to their high protein content (13.3%).

Other authors (Pop *et al.*, 2013; Bora *et al.*, 2019) successfully test the use of goji fruit and powder to improve the nutritional and sensory properties of muffins and cookies. Ducruet *et al.* (2017) successfully developed an antioxidant-rich beer based on goji berries.

Through a study on the development of yogurt microflora, Rotar *et al.* (2015) validates the fact that goji berries can be successfully used as a potentiator of the level of probiotics in yogurt. The high antioxidant content makes *Lycium* spp. plants a candidate for obtaining extracts with potential use in the food industry. Using non-toxic solvents, Pedro *et al.* (2018) obtained a goji fruit extract with applications as a natural antioxidant in the food industry, especially for edible oils. Other research (Juan-Garcia *et al.*, 2019) suggests the consumption of goji berries have a preventive or curative role in the case of phytotoxic effects generated by the mycotoxin beauvericin, attributed to *Fusarium* infestation of cereals and cereal-based products. Fadiloglu and Çoban (2019) proved the effectiveness of goji extracts as antimicrobial and antioxidant agents to extend the shelf life of smoked fish sausages by up to seven days.

Potential health and therapeutic uses

Through their chemical composition, goji fruits have a high potential in reducing oxidative stress, including preventing the effect of free radicals on the damage of proteins, lipids, and DNA (Ma *et al.*, 2019). They are also used to treat diabetes and a variety of problems related to blood circulation (Chen *et al.*, 2018). The Asian pharmacopeia postulates the therapeutic use of the fruits and peel of *L. barbarum* / *L. chinense* (Chinese pharmacopoeia) and sometimes of the aerial parts of *L. barbarum* and *L. europeaum* (Indian pharmacopoeia), while the European one includes only the dried fruits of *L. barbarum* (Yao *et al.*, 2018).

Various modern *in vitro* or *in vivo* clinical research validated the therapeutic effects of the species (Jiang *et al.*, 2021). Research by He *et al.* (2012) demonstrated that goji plants, through their content in polysaccharides, have the potential to be used in protection against cancer. This action is attributed

to the ability of *Lycium Barbarum* Polysaccharide to stop the cell cycle and inhibit some signalling flows, eliminating excess abnormal cells (Jin *et al.*, 2013). Li *et al.* (2007) proved the positive effects of *L. barbarum* polysaccharides in reducing the risk of lipid peroxidation and the decline of the total antioxidant capacity in the body of mice. Hsu *et al.* (2017) identified the ability of a carotenoid nanoemulsion from *L. barbarum* to inhibit the growth of HT-29 cancer cells, associated with colon cancer.

In vitro research (Huang *et al.*, 2019) reveals the positive effect of the pectic polysaccharide XLBP-I-I, extracted from the fruits of *L. barbarum*, in reducing endoplasmic reticulum stress and protecting cells against apoptosis induced by this type of stress. Gan *et al.* (2004) analysed the regulatory capacity of a polysaccharide-protein complex extracted from *L. barbarum* on the immune system and the size of S180 sarcoma tumours in mice, their results indicated a very significant effect on tumour weight in the case of treatment with 10 mg/kg polysaccharide-protein extract from *L. barbarum*, associated with immunostimulatory activity.

The phenolic compounds extracted from *L. barbarum* are correlated with the inhibition of lipid peroxidation and the increase of the antioxidant and hepatoprotective effects, applicable in the case of high-fat diets, according to the *in vivo* research (on laboratory mice) carried out by Cui *et al.* (2011). Ming *et al.* (2009) also observed the positive effect obtained as a result of the administration of polysaccharides extracted from *L. barbarum* in the sense of decreasing, on the background of antioxidant activity, both total cholesterol and LDL, HLD fractions, triglycerides, and glucose content in the blood and glycogen from the liver. Kulczyński and Gramza-Michałowska (2016) identified the reduction of cholesterol concentration (LDL and HDL) in the case of laboratory mice subjected to a high-fat diet, which were administered polysaccharide fractions extracted from *L. barbarum*.

Cheng and Kong (2011) demonstrate that the polysaccharides specific to the *L. barbarum* species have a significant impact in improving liver damage, preventing the progression of fatty liver associated with alcohol consumption, and improving the antioxidant activity of the body of laboratory mice. Other *in vivo* research postulates the hypoglycaemic and antidiabetic effect of the polysaccharide extract from *L. barbarum* in particular (Zhou *et al.*, 2009; Jing and Yin, 2010; Zhao *et al.*, 2015a), and the results are supported by *in vitro* studies (Wojdyło *et al.*, 2018).

CONCLUSIONS

The rediscovery of the food value and the commercial importance of goji fruit is reflected in the effort of the scientific literature of the last decades to validate and deepen the state of knowledge of the species of the genus *Lycium*.

Numerous studies have followed the chemical composition of *Lycium* spp. plants, mainly analysing the chemical profile of *L. barbarum* fruits, since this is generally accepted as the superior quality species from the point of view of human consumption. Therefore, future

research can also focus on *L. chinense*, also accepted as goji, or other species of the genus *Lycium*.

The scientific literature reports differences in the chemical composition and antioxidant action of goji plants; these changes are associated with the cultivar/variety, geographical origin, and the cultivation technology or the processing method.

Any change in the chemical profile of goji plants influences the nutritional or therapeutic effect on the body, in addition to affective consumption. Therefore, either their commercial destinations (food, medicinal, industrial) are adapted accordingly, or the possibility of acclimatization or creation can be deepened by new cultivars/varieties of goji in different areas to ensure the supply of these 'superfruits' locally.

Author Contributions: Conceptualization V.T.; methodology V.T., R.I.T; resources A.D.B.; writing T.V., C.B.; review T.V.; supervision R.I.T. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: There are no conflicts of interest related to this work.

REFERENCES

Ağagündüz, D.; Köseler-Beyaz, E.; Duman, S. Assessment of the Physicochemical and Antioxidant Profile of Dried Goji Berries (*Lycium barbarum*). *Progress in Nutrition*. **2021**, 23, 1-8. https://doi.org/10.23751/pn.v23i4.11321

Amagase, H.; Farnsworth, N.R. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). *Food research international*. **2011**, 44, 1702-1717. https://doi.org/10.1016/j.foodres.2011.03.027.

Bertoldi, D.; Cossignani, L.; Blasi, F.; Perini, M.; Barbero, A.; Pianezze, S.; Montesano, D. Characterisation and geographical traceability of Italian goji berries. *Food chemistry*. **2019**, 275, 585-593. https://doi.org/10.1016/j.foodchem.2018.09.098.

Bondia-Pons, I.; Savolainen, O.; Torronen, R.; Martinez, J.A.; Poutanen, K.; Hnhineva, K. Metabolic profiling of goji berry extracts for discrimination of geographical origin by non-targeted liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Food Research International.* **2014,** 63, 132-138. https://doi.org/10.1016/j.foodres.2014.01.067.

Bora, P.; Ragaee, S.; Abdel-Aal, S.M. Effect of incorporation of Goji berry by-product on biochemical, physical and sensory properties of selected bakery products. *LWT - Food Science and Technology*. **2019**, 112, 108225. https://doi.org/10.1016/J.LWT.2019.05.123.

CABI (Digital Library). *Lycium barbarum* (Matrimonyvine). Available online: https://www.cabi.org/isc/datasheet/31905 (accessed on 13 August 2022).

Cao, Y.L.; Li, Y.I.; Fan, Y.F. Wolfberry genomes and the evolution of *Lycium* (Solanaceae). *Communications Biology*. **2021**, 4, 671. https://doi.org/10.1038/s42003-021-02152-8.

Chang, S.K.; Alasalvar, C.; Shahidi, F. Superfruits: Phytochemicals, antioxidant efficacies, and health effects - A comprehensive review. *Critical Reviews in Food Science and Nutrition*. **2018**, 59, 1580-1604. https://doi.org/10.1080/10408398.2017.1422111.

Chen, J.; Chao, C.T.; Wei, X. Gojiberry breeding: current status and future prospects, In *Breeding and health benefits of fruit and nut crops*.

IntechOpen., London, United Kingdom, 2018, pp. 2-15.

Cheng, D.; Kong, H. The effect of *Lycium barbarum* polysaccharide on alcohol-induced oxidative stress in rats. *Molecules*. **2011**, 16, 2542-2550. https://doi.org/10.3390/molecules16032542.

Çolak, A.M.; Okatan, V.; Güçlü, S.F.; Korkmaz, N.; Polat, M. Chemical characteristics and antioxidant activities of four native goji (*Lycium barbarum* L.) genotypes. *"Scientific Papers" Iași University of Life Sciences (IULS), Series Horticulture*. **2016**, 59, 29-34. https://repository.uaiasi.ro/xmlui/handle/20.500.12811/2027.

Cossignani, L.; Blasi, F.; Simonetti, M.S.; Montesano, D. Fatty acids and phytosterols to discriminate geographic origin of *Lycium barbarum* berry. *Food analytical methods*. **2018**, 11, 1180-1188. https://doi.org/10.1007/s12161-017-1098-5.

Cui, B.; Liu, S.; Lin, X.; Wang, J.; Li, S.; Wang, Q.; Li, S. Effects of Lycium barbarum aqueous and ethanol extracts on high-fat-diet induced oxidative stress in rat liver tissue. *Molecules*. **2011**, 16, 9116-9128. https://doi.org/10.3390/molecules16119116.

Donno, D.; Beccaro, G.L.; Mellano, M.G.; Cerutti, A.K.; Bounous, G. Goji berry fruit (Lycium spp.): Antioxidant compound fingerprint and bioactivity evaluation. *Journal of functional foods*. **2015**, 18, 1070-1085.

Ducruet, J.; Rébénaque, P.; Diserens, S.; Kosińska-Cagnazzo, A.; Héritier, I.; Andlauer, W. Amber ale beer enriched with Goji berries – The effect on bioactive compound content and sensorial properties. *Food Chemistry*. **2017**, 226,109-118. https://doi.org/10.1016/j.foodchem.2017.01.047.

Dzhugalov, H.; Lichev, V.; Yordanov, A.; Kaymakanov, P.; Dimitrova, V.; Kutoranov, G. First results of testing Goji berry (*Lycium barbarum* L.) in Plovdiv region, Bulgaria. *Scientific Papers. Series B, Horticulture*. **2015**, 59, 47-50.

Dzhugalov, H.; Denev, P. Growth, reproductive characteristics and antioxidant activity of four goji berry (*Lycium barbarum* L.) Cultivars. *AGRICULTURAL SCIENCES - Agricultural University - Plovdiv*. **2020**, 12, 86-91. https://doi.org/10.22620/agrisci.2020.27.013.

Endes, Z.; Uslu, N.; Özcan, M.M.; Er, F. Physico-chemical properties, fatty acid composition and mineral contents of goji berry (*Lycium barbarum* L.) fruit. *Journal of agroalimentary processes and technologies*. **2015**, 21, 36-40.

Fadiloglu, E.E.; Çoban, M.Z. The Effects of Goji Berry (*Lycium barbarum* L.) Extract on Some Chemical, Microbiological and Sensory Characteristics of Liquid Smoked Common Carp (*Cyprinus carpio* L., 1758) Sausages. *Yuzuncu Yıl University Journal of Agricultural Sciences*. **2019**, 29, 702-710. https://doi.org/10.29133/yyutbd.632966

GBIF. Available online: https://www.gbif.org/species/2928808 (accessed on 17 August 2022)

He, N.; Yang, X.; Jiao, Y.; Tian, L.; Zhao, Y. Characterisation of antioxidant and antiproliferative acidic polysaccharides from Chinese wolfberry fruits. *Food Chemistry*. **2012**, 133, 978-989. https://doi.org/10.1016/j.foodchem.2012.02.018.

Hsu, H.J.; Huang, R.F.; Kao, T.H.; Inbaraj, B.S.; Chen, B.H. Preparation of carotenoid extracts and nanoemulsions from *Lycium barbarum* L. and their effects on growth of HT-29 colon cancer cells. *Nanotechnology*. **2017**, 28, 135103. https://doi.org/10.1088/1361-6528/aa5e86.

Huang, C.; Yao, R.; Zhu, Z.; Pang, D.; Cao, X.; Feng, B.; Paulsen, B.S.; Li, L.; Yin, Z.; Chen, X.; Jia, R.; Song, X.; Ye, G.; Luo, Q.; Chen, Z.; Zou, Y. A pectic polysaccharide from water decoction of Xinjiang *Lycium barbarum* fruit protects against intestinal endoplasmic reticulum stress.

International journal of biological macromolecules. **2019**, 130, 508-514. https://doi.org/10.1016/j.ijbiomac.2019.02.157.

Ilić, T.; Dodevska, M.; Marčetić, M.; Božić, D.; Kodranov, I.; Vidović, B. Chemical characterization, antioxidant and antimicrobial properties of goji berries cultivated in Serbia. *Foods*. **2020**, 9, 1614. https://doi.org/10.3390/foods9111614.

Islam, T.; Yu, X.; Badwal, T.S.; Xu, B. Comparative studies on phenolic profiles, antioxidant capacities and carotenoid contents of red goji berry (*Lycium barbarum*) and black goji berry (*Lycium ruthenicum*). *Chemistry Central Journal*. **2017**, 11, 1-8. https://doi.org/10.1186/s13065-017-0287-z.

Jatoi, M.A.; Jurić, S.; Vidrih, R.; Vinceković, M.; Vuković, M.; Jemrić, T. The effects of postharvest application of lecithin to improve storage potential and quality of fresh goji (*Lycium barbarum* L.) berries. *Food chemistry*. **2017**, 230, 241-249. https://doi.org/10.1016/j.foodchem.2017.03.039.

Jiang, Y.; Fang, Z.; Leonard, W.; Zhang, P. Phenolic compounds in Lycium berry: Composition, health benefits and industrial applications. *Journal of Functional Foods*. **2021**, 77, 104340. https://doi.org/10.1016/j.jff.2020.104340

Jiao, Y.; Liu, G. Goji Berry: a Novel Nutraceutical "Superfruit" for Florida Master Gardeners: HS1391, 10/2020. *EDIS*. 2020, 5, 1-5. http://dx.doi.org/10.32473/edis-hs1391-2020.

Jing, L.; Yin, L. Antihyperglycemic activity of polysaccharide from Lycium barbarum. *Journal of Medicinal Plants Research*. **2010**, 4, 023-026. https://doi.org/10.5897/JMPR09.301.

Jin, M.; Huang, Q.; Zhao, K.; Shang, P. Biological activities and potential health benefit effects of polysaccharides isolated from *Lycium barbarum* L. *International journal of biological macromolecules*. **2013**, 54, 16-23. https://doi.org/10.1016/j.ijbiomac.2012.11.023.

Juan-García, A.; Montesano, D.; Mañes, J.; Juan, C. Cytoprotective effects of carotenoids-rich extract from Lycium barbarum L. on the beauvericin-induced cytotoxicity on Caco-2 cells. *Food and Chemical Toxicology*. **2019**, 133, 110798. https://doi.org/10.1016/j.fct.2019.110798.

Kim, J.S.; Chung, H.Y. GC-MS Analysis of the Volatile Components in Dried Boxthorn (*Lycium Chinensis*) Fruit. *Journal of the Korean Society for Applied Biological Chemistry*. **2009**, 52, 516-524. https://doi.org/10.3839/jksabc.2009.088

Kosińska-Cagnazzo, A.; Weber, B.; Chablais, R.; Vouillamoz, J.F.; Molnar, B.; Crovadore, J.; François, F.; Andlauer, W. Bioactive compound profile and antioxidant activity of fruits from six goji cultivars cultivated in Switzerland. *Journal of Berry Research*. **2017**, 7, 43-59. https://doi.org/10.3233/JBR-160144.

Kulczyński, B.; Gramza-Michałowska, A. Goji berry (*Lycium barbarum*): composition and health effects–a review. *Polish Journal of Food and Nutrition Sciences*. **2016**, 66, 67-76. https://doi.org/10.1515/pjfns-2015-0040.

Levin, R.A.; Miller, J.S. Relationships within tribe Lycieae (Solanaceae): paraphyly of Lycium and multiple origins of gender dimorphism. *American Journal of Botany*. **2005**, 92, 2044-2053. https://doi.org/10.3732/ajb.92.12.2044.

Li, Q.; Yu, X.; Xu, L.; Gao, J.M. Novel method for the producing area identification of Zhongning Goji berries by electronic nose. *Food Chemistry*. **2017**, 221, 1113-1119. https://doi.org/10.1016/j.foodchem.2016.11.049.

Liu, Y.; Zeng, S.; Sun, W.; Wu, M.; Hu, W.; Shen, X.; Wang, Y. Comparative analysis of carotenoid accumulation in two goji (*Lycium barbarum* L. and L. ruthenicum Murr.) fruits. *BMC plant biology*. **2014**, 14, 1-14.

Liu, B.; Xu, Q.; Sun, Y. Black goji berry (Lycium ruthenicum) tea has higher phytochemical contents and in vitro antioxidant properties than red goji berry (*Lycium barbarum*) tea. *Food quality and safety*. **2020**, 4, 193-201. https://doi.org/10.1093/fqsafe/fyaa022.

Llorent-Martínez, E.J.; Fernández-de Córdova, M.L.; Ortega-Barrales, P.; Ruiz-Medina, A. Characterization and comparison of the chemical composition of exotic superfoods. *Microchemical Journal*. **2013**, 110, 444-451. https://doi.org/10.1016/j.microc.2013.05.016.

Lu, J.; Li, H.; Quan, J.; An, W.; Zhao, J.; Xi, W. Identification of characteristic aroma volatiles of Ningxia goji berries (*Lycium barbarum* L.) and their developmental changes. *International journal of food properties*. **2017**, 20, S214-S227. https://doi.org/10.1080/10942912.2017.1295254.

Lu, Y.; Guo, S.; Zhang, F.; Yan, H.; Qian, D.W.; Wang, H.Q.; Jin, L.; Duan, J.A. Comparison of functional components and antioxidant activity of *Lycium barbarum* L. fruits from different regions in China. *Molecules*. **2019**, 24, 2228. https://doi.org/10.3390/molecules24122228.

Ma, Z.F.; Zhang, H.; Teh, S.S.; Wang, C.W.; Zhang, Y.; Hayford, F.; Yang, L.; Ma, T.; Dong, Z.; Zhang, Y.; Zhu, Y. Goji berries as a potential natural antioxidant medicine: An insight into their molecular mechanisms of action. *Oxidative Medicine and Cellular Longevity*. **2019**, 9, 2437397. https://doi.org/10.1155/2019/2437397.

Mikulic-Petkovsek, M.; Schmitzer, V.; Slatnar, A.; Stampar, F.; Veberic, R. Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *Journal of food science*. **2012**, 77, C1064-C1070. https://doi.org/10.1111/j.1750-3841.2012.02896.x.

Miller, J.S. Phylogenetic relationships and the evolution of gender dimorphism in Lycium (Solanaceae). *Systematic Botany*. **2002**, 27, 416-428. https://doi.org/10.1043/0363-6445-27.2.416.

Miller, J.S.; Kamath, A.; Damashek, J.; Levin, R.A. Out of America to Africa or Asia: inference of dispersal histories using nuclear and plastid DNA and the S-RNase self-incompatibility locus. *Molecular Biology and Evolution*. **2011**, 28, 793-801. https://doi.org/10.1093/molbev/msq253.

Ming, M.; Guanhua, L.; Zhanhai, Y.; Guang, C.; Xuan, Z. Effect of the *Lycium barbarum* polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. *Food Chemistry*. **2009**, 113, 872-877. https://doi.org/10.1016/j.foodchem.2008.03.064.

Mocan, A.; Moldovan, C.; Zengin, G.; Bender, O.; Locatelli, M.; Simirgiotis, M.; Atalay, A.; Vodnar D.C.; Rohn, S.; Crișan, G. UHPLC-QTOF-MS analysis of bioactive constituents from two Romanian Goji (*Lycium barbarum* L.) berries cultivars and their antioxidant, enzyme inhibitory, and real-time cytotoxicological evaluation. *Food and chemical toxicology*. **2018**, 115, 414-424. https://doi.org/10.1016/j.fct.2018.01.054

Mocan, A.; Cairone, F.; Locatelli, M.; Cacciagrano, F.; Carradori, S.; Vodnar, D.C.; Crișan, G.; Simonetti, G.; Cesa, S. Polyphenols from *Lycium barbarum* (Goji) fruit European cultivars at different maturation steps: Extraction, HPLC-DAD analyses, and biological evaluation. *Antioxidants*. **2019**, 8, 562. https://doi.org/10.3390/antiox8110562.

Niro, S.; Fratianni, A.; Panfili, G.; Falasca, L.; Cinquanta, L.; Alam, M.R. Nutritional evaluation of fresh and dried goji berries cultivated in Italy. *Italian Journal of Food Science*. **2017**, 29, 398-408. https://doi.org/10.14674/1120-1770/ijfs.v649.

Oğuz, H.I.; Erdoğan, O. A study on the development performances of goji berry (*Lycium barbarum* L.) Varieties. *Fresenius Environmental Bulletin*. **2016**, 25, 5581-5586.

Ozkan, E.E.; Ozden, T.Y.; Toplan, G.G.; Mat, A. Phenolic content and biological activities of *Lycium barbarum* L (Solanaceae) fruits (Goji berries) cultivated in Konya, Turkey. *Tropical Journal of Pharmaceutical Research.* **2018**, 17, 2047-2053. http://dx.doi.org/10.4314/tjpr.v17i10.22.

Pedro, A.C.; Maurer, J.B.B.; Zawadzki-Baggio, S.F.; Ávila, S.; Maciel, G.M.; Haminiuk, C.W.I. Bioactive compounds of organic goji berry (*Lycium barbarum* L.) prevents oxidative deterioration of soybean oil. *Industrial crops and products*. **2018**, 112, 90-97. https://doi.org/10.1016/j.indcrop.2017.10.052.

Peng, Y.; Ma, C.; Li, Y.; Leung, K.S.Y.; Jiang, Z.H.; Zhao, Z. Quantification of zeaxanthin dipalmitate and total carotenoids in *Lycium* fruits (Fructus Lycii). *Plant Foods for Human Nutrition*. **2005**, 60, 161-164. https://doi.org/10.1007/s11130-005-9550-5.

Pires, T.C.S.P.; Dias, M.I.; Barros, L.; Calhelha, R.C.; Alves, M.J.; Santos-Buelga, C.; Ferreira, I.C.F.R. Phenolic compounds profile, nutritional compounds and bioactive properties of *Lycium barbarum* L: A comparative study with stems and fruits. *Industrial Crops & Products*. **2018**, 122, 574-581. https://doi.org/10.1016/j.indcrop.2018.06.046.

Potterat, O.G. Goji (*Lycium barbarum* and L. chinense): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. *Planta Medicinal*. **2010**, 76, 7-19. https://doi.org/10.1055/s-0029-1186218.

Potterat, O.; Hamburger, M. Goji juice: a novel miraculous cure for longevity and well-being? A review on composition, pharmacology, health-related claims and benefits. *Schweizerische Zeitschrift für Ganzheitsmedizin*. **2008**, 20, 399-405.

Protti, M.; Gualandi, I.; Mandrioli, R.; Zappoli, S.; Tonelli, D.; Mercolini, L. Analytical profiling of selected antioxidants and total antioxidant capacity of goji (*Lycium* spp.) berries. *Journal of pharmaceutical and biomedical analysis*. **2017**, 143, 252-260. https://doi.org/10.1016/j.jpba.2017.05.048.

Qian, D.; Zhao, Y.; Yang, G.; Huang, L. Systematic review of chemical constituents in the genus *Lycium* (Solanaceae). *Molecules*. **2017a**, 22, 1-33. https://doi.org/10.3390/molecules22060911.

Qian, D.; Yang, J.; Kang, L.P.; Ji, R.F.; Huang, L.Q. Variation of sweet chemicals in different ripening stages of wolfberry fruits. *Chinese Herbal Medicines*. **2017b**, 9, 329-334. https://doi.org/10.1016/S1674-6384(17)60112-6.

Rotar, A.M.; Vodnar, D.C.; Bunghez, F.; Căunescu, G.M.; Jimborean, M.; Semeniuc, C.A. Effect of Goji Berries and Honey on Lactic Acid Bacteria Viability and Shelf Life Stability of Yoghurt. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. **2015**, 43, 196-203. https://doi.org/10.15835/nbha4319814.

Rybicka, I.; Kiewlicz, J.; Kowalczewski, P.Ł.; Gliszczyńska-Świgło, A. Selected dried fruits as a source of nutrients. *European Food Research and Technology*. **2021**, 247, 2409-2419. https://doi.org/10.1007/s00217-021-03802-1.

Shahrajabian, M.H.; Sun, W.; Cheng, Q. A review of goji berry (Lycium barbarum) in traditional Chinese medicine as a promising organic superfood and superfruit in modern industry. *Academia Journal of Medicinal Plant*s. **2018**, 6, 437-445. http://dx.doi.org/10.15413/ajmp.2018.0186.

Shen, T.; Zou, X.; Shi, J.; Li, Z; Huang, X.; Xu, Y.; Chen, W. Determination geographical origin and flavonoids content of goji berry using near-infrared spectroscopy and chemometrics. *Food Analytical Methods*. **2016**, 9, 68-79.

https://doi.org/10.1007/s12161-015-0175-x.

Skenderidis, P.; Kerasioti, E.; Karkanta, E.; Stagos, D.; Kouretas, D.; Petrotos, K.; Hadjichristodoulou, C.; Tsakalof, A. Assessment of the antioxidant and antimutagenic activity of extracts from goji berry of Greek cultivation. *Toxicology reports*. **2018**, 5, 251-257. https://doi.org/10.1016/j.toxrep.2018.02.001.

Skenderidis, P.; Lampakis, D.; Giavasis, I.; Leontopoulos, S.; Petrotos, K.; Hadjichristodoulou, C.; Tsakalof, A. Chemical properties, fatty-acid composition, and antioxidant activity of goji berry (*Lycium barbarum* L. and *Lycium chinense* Mill.) fruits. *Antioxidants*. **2019**, 8, 60. https://doi.org/10.3390/antiox8030060.

Spano, M.; Maccelli, A.; Di Matteo, G.; Ingallina, C.; Biava, M.; Crestoni, M.E.; Bardaud, J-X.; Giusti, A.M.; Mariano, A.; Scotto D'Abusco, A.; Sobolev, A.P.; Lasalvia, A.; Fornarini, S.; Mannina, L. Metabolomic Profiling of Fresh Goji (*Lycium barbarum* L.) Berries from Two Cultivars Grown in Central Italy: A Multi-Methodological Approach. *Molecules*. **2021**, 26, 5412. https://doi.org/10.3390/molecules26175412.

Stavrescu-Bedivan, M.M.; Pelcaru, C.F.; Croitoru, C.M.; Mihai, C.D.; Ciceoi, R. Preliminary survey for mapping the distribution of spontaneous goji berry shrubs in Romania. *Scientific Papers. Series B, Horticulture*. **2022**, LXV, 907-912.

Sun, Y.; Rukeya, J.; Tao, W.; Sun, P.; Ye, X. Bioactive compounds and antioxidant activity of wolfberry infusion. *Scientific reports*. **2017**, 7, 1-8. https://doi.org/10.1038/srep40605.

Vidović, B.B.; Milinčić, D.D.; Marčetić, M.D.; Djuriš, J.D.; Ilić, T.D.; Kostić, A Ž.; Pešić, M.B. Health benefits and applications of goji berries in functional food products development: A review. *Antioxidants.* **2022**, 11, 248. https://doi.org/10.3390/antiox11020248.

Wang, B.; Han, L.; Liu, J.-M.; Zhang, J.; Wang, W.; Li, B.-G.; Dong, C.-X.; Bai, C.-C. *Lycium* Genus Polysaccharide: An Overview of its Extraction, Structures, Pharmacological Activities and Biological Applications. *Separations.* **2022**, 9, 1-41. https://doi.org/10.3390/separations9080197.

Wang, C.C.; Chang, S.C.; Inbaraj, B.S.; Chen, B.H. Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum* L. and evaluation of antioxidant activity. *Food chemistry*. **2010**, 120, 184-192. https://doi.org/10.1016/j.foodchem.2009.10.005.

Wang, Y.; Chen, H.; Wu, M.; Zeng, S.; Liu, Y.; Dong, J. Chemical and genetic diversity of wolfberry. In *Lycium barbarum and Human Health*. Springer, Berlin, Germany, 2015, pp. 1–26.

Wang, Y.; Jin, H.; Dong, X.; Yang , S.; Ma, S.; Ni, J. Quality evaluation of *Lycium barbarum* (wolfberry) from diferent regions in China based on polysaccharide structure, yield and bioactivities. *Chinese Medicine*. **2019**, 14, 1-10. https://doi.org/10.1186/s13020-019-0273-6.

Wenli, S.; Shahrajabian, M.H.; Qi, C. Health benefits of wolfberry (Gou Qi Zi, *Fructus barbarum* L.) on the basis of ancient Chinese herbalism and Western modern medicine. *Avicenna Journal Phytomedicine*. **2021**, 11, 109-119. https://doi.org/10.22038/ajp.2020.17147.

Wetters, S.; Horn, T.; Nick, P. Goji who? Morphological and DNA based authentication of a "superfood". *Frontiers in plant science*. **2018**, 9, 1-14. https://doi.org/10.3389/fpls.2018.01859.

Wojdyło, A.; Nowicka, P.; Bąbelewski, P. Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties. *Journal of functional foods*. **2018**, 48, 632-642. https://doi.org/10.1016/j.jff.2018.07.061.

Xia, Q.; Niu, M.; Wu, C.; Zhou, R. Formation of ethyl carbamate in Goji wines: Effect

of Goji fruit composition. *Food science and biotechnology*. **2016**, 25, 921-927. https://doi.org/10.1007/s10068-016-0151-2.

Van Straten, M.; Griggs B. Superfoods: Nutrient-dense foods to protect your health. London: DK Publishing, **2006**.

Yang, L.; Liang, Q.; Zhang, Y.; Wang, S.; Yuan, F.; Wang, J. Variation of phytochemical composition of Lycium chinense leaves as an endemic high-value healthy resource. *Scientia Horticulturae*. **2021**, 281, 109910. https://doi.org/10.1016/j.scienta.2021.109910.

Yao, R.; Heinrich, M.; Weckerle, C.S. The genus Lycium as food and medicine: A botanical, ethnobotanical and historical review. *Journal of ethnopharmacology*. **2018a**., 212, 50-66. https://doi.org/10.1016/j.jep.2017.10.010.

Yao, R.; Heinrich, M.; Zou, Y.; Reich, E.; Zhang, X.; Chen, Y.; Weckerle, C.S. Quality Variation of Goji (Fruits of *Lycium* spp.) in China: A Comparative Morphological and Metabolomic Analysis. *Frontiers in Pharmacology*. **2018b**, 9, 151. https://doi.org/10.3389/fphar.2018.00151.

Yao, R. The globalization of goji (fruits of *Lycium barbarum* L. and *L. chinense* Mill.): What are the drivers?SC2| Short Communication 2-Regulation/ Globalization. 19th International Congress of the International Society for Ethnopharmacology. 13 June, 2019. Dresden, Germany.

Yao, R.; Heinrich, M.; Zhao, X.; Wang, Q.; Wei, J.; Xiao, P. What's the choice for goji: Lycium barbarum L. or L. chinense Mill.? *Journal of Ethnopharmacology*. **2021**, 10, 114185. https://doi.org/10.1016/j.jep.2021.114185.

Yin, G.; Dang, Y. Optimization of extraction technology of the Lycium barbarum polysaccharides by Box–Behnken statistical design. *Carbohydrate Polymers*. **2008**, 74, 603-610. https://doi.org/10.1016/j.carbpol.2008.04.025.

Yossa Nzeuwa, I.B.; Guo, B.; Zhang, T.; Wang, L.; Ji, Q.; Xia, H.; Sun, G. Comparative Metabolic Profiling of Lycium Fruits (*Lycium barbarum* and *Lycium chinense*) from Different Areas in China and from Nepal. *Journal of Food Quality*. **2019**, 1-6. https://doi.org/10.1155/2019/4396027.

Zhang, L.; Zheng, G.; Teng, Y.; Wang J. Comparison research on fruit quality of *Lycium barbarum* L. in different regions. *Northwest Pharmaceutical Journal*. **2012**, 27, 195-197.

Zhang, D.; Xia, T.; Dang, S.; Fan, G.; Wang, Z. Investigation of Chinese wolfberry (*Lycium* spp.) germplasm by restriction site-associated DNA sequencing (RAD-seq). *Biochemical genetics*. **2018**, 5, 575-585. https://doi.org/10.1007/s10528-018-9861-x.

Zhang, Q.; Chen, W.; Zhao, J.; Xi, W. Functional constituents and antioxidant activities of eight Chinese native goji genotypes. *Food Chemistry*. **2016**, 200, 230-236. https://doi.org/10.1016/j.foodchem.2016.01.046.

Zhao, R.; Jin, R.; Chen, Y.; Han, F.M. Hypoglycemic and hypolipidemic effects of *Lycium barbarum* polysaccharide in diabetic rats. *Chinese herbal medicines*. **2015a**, 7, 310-315. https://doi.org/10.1016/S1674-6384(15)60057-0.

Zhao, J.; Li, H.; Xi, W.; An, W.; Niu, L.; Cao, Y.; Wang, H.; Wang, Y.; Yin, Y. Changes in sugars and organic acids in wolfberry (*Lycium barbarum* L.) fruit during development and maturation. *Food Chemistry*. **2015b**, 173, 718-724. https://doi.org/10.1016/j.foodchem.2014.10.082.

Zheng, G.Q.; Zheng, Z.Y.; Xu, X.; Hu, Z.H. Variation in fruit sugar composition of *Lycium barbarum* L. and *Lycium chinense* Mill. of different regions and varieties. *Biochemical Systematics and Ecology*. **2010**, 38, 275-284. https://doi.org/10.1016/j.bse.2010.01.008.

Zhou, Z.; Jing, L.; Cui, G.; Feng, Q.; Xiao, Y. Effects of polysaccharide from

Lycium barbarum in alloxan-induced diabetic mice. *African Journal of Biotechnology*. **2009**, 8, 6634-6637. https://doi.org/10.5897/AJB09.484.

Academic Editor: Dr. Isabela Maria Simion

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2020; 25(6): 2180-2187
doi: 10.25083/rbl/25.6/2180.2187

Received for publication, July, 28, 2020
Accepted, August, 28, 2020

Original paper

Research on the growth and development of some varieties of Lavandula Angustifolia (Mill.) in the South-East of Romania

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Abstract The purpose of this paper was to analyze the canopy volume of lavender shrubs as a morphologic trait influenced by variety and also to evaluate the development of the root system on a Chromic Luvisol with an argic B horizon. The crop was established in 2017 at Belciugatele Didactic Station/Moara Domnească Farm (44°30′ North, 26°15′ East) using the following lavender varieties: Sevstopolis, Vera, Hidcote, and Buena Vista. During 2017-2019, plants' height and diameters were measured at harvest, and these observations were used to determine lavender shrubs' canopy volume, by applying the derived ellipsoid volume formula. Varieties (*A*) obtained different values in terms of canopy volumes, and these values also varied under the influence of the different doses and combinations of mineral and organic fertilizers (*B*). The highest canopy volume was registered by Vera, with values ranging from 0.0557 m^3 (1^{st} year, Control) and 0.0944 m^3 (3^{rd} year, organic fertilization with manure at 30 t/ha). The evaluation of the root system distribution and development was performed after carrying out a soil profile between two plants, along the row, for each researched variety. Measurements were conducted using a frame of 50/50 cm, and data sampling was performed for every 10 cm layer, by counting and measuring the roots. Based on these observations the root section area (RSA) was determined. The values of this indicator ranged between 283.06 mm^2 (Buena Vista) and 378.29 mm^2 (Vera).

Keywords *Lavandula angustifolia* (Mill.), volume, ellipsoid formula, RSA.

To cite this article: MIHALAȘCU C, BOLOHAN C, TUDOR V, MIHALACHE M, TEODORESCU RI. Research on the growth and development of some varieties of *Lavandula Angustifolia* (*Mill.*) in the South-East of Romania. *Rom Biotechnol Lett*. 2020; 25(6): 2180-2187. DOI: 10.25083/rbl/25.6/2180.2187

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Introduction

Lavandula angustifolia (Mill.) is part of the *Lavandula* genus, a genus with 47 useful plant species across the Globe. Along with other 233 genera, comprising 5500 species, they form the *Labiatae* (*Lamiaceae*) family GÜNER, 2000 [5]; KORKUNC et al, 2018 [8]; ŁYCZKO, 2020 [10].

Lavender originates in the Mediterranean area and is found in a range of ecologic and geographic areas, from the Balkan countries, North America, North Africa, and India to the Arabic area, South Asia and Australia (REID, 2000 [17]; UPSON and ANDREWS, 2004 [27]; MOJA et al, 2016 [14]; LOPES and BARATA, 2017 [9]; KORKUNC et al, 2018 [8]; ŁYCZKO et al, 2020 [10].

Fertilization is an important factor in growing *Lavandula angustifolia* (Mill.), and regardless of the fertilizers' type (organic or mineral), it has a positive impact on the species yield (MAGANGA, 2004 [11]; ȘEKEROĞLU and ÖZGÜVEN 2008 [22]; SEIDLER-LOŻYKOWSKA et al, 2014 [21]; SKOUFOGIANNI et al, 2017 [24]). Organo-mineral fertilizers contribute to a higher yield of the lavender crop due to a slow release of nutrients around the root system (SILVA et al, 2017 [23]), and also help improving soil physical and chemical properties (SAKR et al, 2015 [19]), playing an important role in restoring soil fertility (USLGA, 2019 [30]).

It is preferable to grow lavender in farming conditions rather than using and capitalizing wild lavender plants, because lavender crops have a positive effect on preserving soil water content (between 5 and 20 cm depth) and on soil erosion phenomena, reducing the mechanical impact of raindrops on soil particles, diminishing soil erosion and reducing the risk of rain washing away soil (PLEGUEZUELO et al, 2009 [16].

Results obtained by different researchers show that organic fertilization has a high impact on lavender plants, especially on young plants, or seedlings, because it assures better development of the root system, stimulating roots growing (JELAČIĆ et al, 2008 [6]; BEATOVIĆ et al, 2012 [1]; RYAN, 2016 [18]; NAJAR et al, 2019 [15]).

Irrigation is another factor that helps young plants' roots development, mainly drip irrigation is recommended, in the first two years, in areas with high risks of hydric stress (MAGANGA, 2004 [11]; KIMBROUGH and SWIFT, 2009 [4]; DAFF, 2012 [28]; ERNST, 2017 [4]; SALEHI et al., 2018 [20]); MIHALAȘCU et al, 2020 [13]. But it must be considered that, excess moisture in addition to poor soil drainage increases the risk of developing pathogens (MASON, 2014 [12]).

Materials and Methods

Researches were carried out at Belciugatele Didactic Station/Moara Domnească Farm, Ilfov county (44°3' Nord, 26°15' Est), during 2017-2019, on a Chromic Luvisol with an argic B horizon. The crop was established in 2017, under moderate drip irrigation, with the factors ***A*** – lavender varieties: Sevstopolis (a_1), Vera (a_2), Hidcote (a_3) and Buena Vista (a_4) and ***B*** – doses and combinations of mineral and organic fertilizers $N_0P_0K_0$-Ct (b_1), $N_{60}P_{60}K_{60}$ (b_2) CAN: calcium nitrate $N_{60}CaO_{15,5}Mg_{11,1}$ (b_3), $N_{30}P_{30}K_{30+}$Manure 15 t/ha^{-1} (b_4), Manure 30 t/ha^{-1} (b_5).

Upon crop establishment (March 2017) the soil was characterized by a 7.64 pH and a humus content of 1.92 in the soil layer 0-40 cm. In terms of soil's physical parameters, bulk density (BD) was 1.63 g/cm^3, total porosity (TP) had a value of 33,21% and the degree of compaction was 34.91%.

During 2017-2019, climatic conditions of the vegetation period (March – October) differed compared to the multiannual values (1961-2007), both in terms of temperatures and precipitations (Table 1). Thus, temperatures recorded higher values than the normal multiannual values of the area, by 3.7°C in 2017, 2.3°C in 2018 and 1,9°C in 2019. In terms of rainfall, except for 2018 (Table 1), the amount was slightly over the growing season average (449.5 mm).

Table 1. Climatic conditions during the research period (2017-2019)

Month	Average monthly temperature (°C)				Rainfall (mm)			
	2017	**2018**	**2019**	**1961-2007**	**2017**	**2018**	**2019**	**1961-2007**
March	15,0	3,7	9,5	4,8	44,5	0,2	31,2	40,0
April	16,5	16,4	11,3	11,1	90,0	0,0	78,4	46,9
May	18,6	19,7	17,0	16,7	47,3	0,0	148,2	66,0
June	22,4	22,5	23,1	20,4	46,8	53,2	109,4	77,0
July	26,4	23,0	22,6	22,3	105,2	107,6	76,0	67,7
August	24,2	24,1	24,3	21,4	37,1	2,0	2,4	57,4
September	19,1	19,2	19,1	16,6	37,0	28,9	4,8	52,9
October	11,5	13,8	12,6	10,7	70,9	10,4	41,1	41,6
Average / Sum	**19,2**	**17,8**	**17,4**	**15,5**	**478,8**	**202,3**	**491,5**	**449,5**

a. Canopy volume measurements (m^3)

During 2017-2019, at harvest, lavender plants' diameter and height were measured for each variant. Using these biometrical parameters, the derived ellipsoid volume formula proposed by THORNE et al, 2002 [24] was used to calculate plants canopy volume, in cubic meters (m^3), as follows:

$$CV = 2/3\pi H(A/2xB/2)$$

where: CV = canopy volume (m^3), H = plant height, A = major canopy axis, and B = minor canopy axis.

b. Root system measurements

To evaluate root system distribution and development a soil profile was carried out between two plants, along the row, for each researched variety, applying 1987 ICPA's methodology ([29]) used by other researchers for perennial species like vegetable crops and fruit trees (DUDU et al, 2015 [3]; BOLOHAN, 2018 [2]; TUDOR et al, 2019 [26]. The soil profile was conducted in the 3rd year of the crop (November 2019) for the experimental variant with the highest impact on plants' development, Manure 30 t/ha^{-1}. Root system measurements were carried out using a frame of 50/50 cm (Figure 1), and data sampling was performed for every 10 cm layer, counting and measuring the roots with the digital calliper (Figure 1). Based on the data collected, the root section area (RSA) was determined for each variety, first in each soil layer (10 cm) and then total RSA (0-50 cm).

Figure 1. Soil profile and root systems measurement with metrical frame in *Lavandula* varieties, 2019.

c. Statistical analysis

A two-way analysis of variance (ANOVA), followed by Fisher's LSD test, when ANOVA resulted in $p<0.05$, was used to determine and compare the differences of means between the factors (*A* and *B*).

Pearson correlation was conducted to analyse to which extent canopy volume and root system development are related.

Results and Discussions

a. Canopy growth and development

Each year, the influence of the variety (A) on lavender plants' canopy volume is noticeable. For the experimental factor B, varieties obtained uniform statistical differences compared to the mean of the four varieties, starting with the second year of the crop (Table 2).

Every year, the highest canopy volume (m^3) was obtained by plants of Vera variety, while plants belonging to Buena Vista variety had the smallest canopy volume.

After the first year (1st crop year), the canopy volume of Vera plants had values ranging from 0.0557 m^3 to 0.0682 m^3 (Table 1), and very significant differences ($p<0.001$) compared to the average of the four varieties (Table 2). For Sevstopolis, the canopy volume was also higher than Control, with differences between 0.0037 m^3 and 0.0041 m^3, during the first year, but only for the mineral fertilization $N_{60}P_{60}K_{60}$ the difference was statistically assured. Buena Vista and Hidcote obtained lower values of the canopy volume compared to the average of the four varieties, with differences statistically significant, ranging from -0.0076 (Hidcote, Control) and -0.0196 (Buena Vista, $N_{60}P_{60}K_{60}$).

In the second year of the crop (2nd year), the average canopy volume of lavender varieties had values from 0.0489 m^3 to 0.0587 m^3 (Table 2), and varieties Vera and Sevstopolis obtained higher volumes than average, with statistically assured differences, while Hidcote and Buena Vista had lower values than Control, with very significant negative differences. The pattern and significance of canopy volume differences of each variety compared to the average volume maintained in the third year of the crop (3rd year), having higher values for Vera and Sevstopolis and lower values for Buena Vista and Hidcote.

Considering that canopy volume as a morphological trait in lavender plants is influenced by variety is supported by the differences obtained among each of the four lavender varieties (Tabel 3). Volume differences distribution highlights their emphasis based on crop maturity. Pronounced differences were obtained after the third year of the crop (3rd year).

Table 2. Variety influence on canopy volume (m^3) of lavender varieties for each year of crop development

Crop year	Fertilization rate	Sevstopolis (a_1)		Vera (a_2)		Hidcote (a_3)		Buena Vista (a_4)		Avg. varieties (Ct)
		Volume (m^3)	Diff. (m^3)	Volume (m^3)	Diff. (m^3)	Volume (m^3)	Diff. (m^3)	Volume (m^3)	Diff. (m^3)	Volume (m^3)
	$N_0P_0K_0$	0.0396	0.0037 ^{ns}	0.0557	0.0199***	0.0283	-0.0076^{oo}	0.0199	-0.0160^{ooo}	**0.0359**
1st year	$N_{60}P_{60}K_{60}$	0.0465	0.0033 ^{ns}	0.0682	0.0250***	0.0346	-0.0086^{ooo}	0.0236	-0.0196^{ooo}	**0.0432**
	CAN	0.0418	0.0041*	0.0580	0.0203***	0.0298	-0.0079^{ooo}	0.0211	-0.0166^{ooo}	**0.0377**
(2017)	$N_{30}P_{30}K_{30+}$**M15**	0.0440	0.0038 ^{ns}	0.0624	0.0223***	0.0322	-0.0080^{ooo}	0.0220	-0.0181^{ooo}	**0.0402**
	Manure30	0.0410	0.0036 ^{ns}	0.0588	0.0214***	0.0293	-0.0081^{ooo}	0.0205	-0.0169^{ooo}	**0.0374**
LSD 5%: 0.0040 m^3; LSD 1%: 0.0056 m^3; LSD 0.1%: 0.0078 m^3										
	$N_0P_0K_0$	0.0580	0.0091***	0.0711	0.0221***	0.0403	-0.0086^{ooo}	0.0264	-0.0226^{ooo}	**0.0489**
2nd year	$N_{60}P_{60}K_{60}$	0.0651	0.0096***	0.0801	0.0246***	0.0472	-0.0084^{ooo}	0.0298	-0.0258^{ooo}	**0.0555**
	CAN	0.0634	0.0107***	0.0742	0.0216***	0.0443	-0.0084^{ooo}	0.0288	-0.0239^{ooo}	**0.0527**
(2018)	$N_{30}P_{30}K_{30+}$**M15**	0.0668	0.0107***	0.0781	0.0221***	0.0479	-0.0081^{ooo}	0.0312	-0.0248^{ooo}	**0.0560**
	Manure30	0.0687	0.0100***	0.0820	0.0233***	0.0502	-0.0085^{ooo}	0.0339	-0.0248^{ooo}	**0.0587**
LSD 5%: 0.0031 m^3; LSD 1%: 0.0044 m^3; LSD 0.1%: 0.0063 m^3										
	$N_0P_0K_0$	0.0653	0.0109***	0.0802	0.0258***	0.0431	-0.0113^{ooo}	0.0291	-0.0253^{ooo}	**0.0544**
3rd year	$N_{60}P_{60}K_{60}$	0.0722	0.0123***	0.0866	0.0267***	0.0477	-0.0121^{ooo}	0.0330	-0.0269^{ooo}	**0.0599**
	CAN	0.0706	0.0128***	0.0834	0.0257***	0.0459	-0.0119^{ooo}	0.0311	-0.0266^{ooo}	**0.0577**
(2019)	$N_{30}P_{30}K_{30+}$**M15**	0.0786	0.0149***	0.0899	0.0262***	0.0512	-0.0125^{ooo}	0.0351	-0.0286^{ooo}	**0.0637**
	Manure30	0.0822	0.0149***	0.0944	0.0271***	0.0554	-0.0119^{ooo}	0.0372	-0.0301^{ooo}	**0.0673**
LSD 5%: 0.0037 m^3; LSD 1%: 0.0052 m^3; LSD 0.1%: 0.0075 m^3										

Ct = control

Analysing the differences among varieties every year (Table 3), for each level of the experimental factor *B*, it is observed that the highest values were obtained between Buena Vista (a_4) and Vera (a_2), ranging from -0.0359 m^3 (1st year) to -0.0573 m^3 (3rd year). The smallest differences among varieties were obtained between Buena Vista and Hidcote (a_3), after the first crop year (2017) and between Sevstopolis (a_2) and Vera in the following years (2018 and 2019). All volume differences among varieties were statistically assured.

The average canopy volume of the four lavender varieties ranged in the first year (1st year) between 0.0359 m^3 (Control) and 0.0432 m^3 ($N_{60}P_{60}K_{60}$) (Figure 2).

Mineral fertilization with $N_{60}P_{60}K_{60}$ and organo-mineral treatment $N_{60}P_{60}K_{60}$+ Manure15 t/ha^{-1} generated statistically assured volume growths, compared to the unfertilized variant (Ct). Ammonium nitrate (CAN) and organic fertilization (Manure 30 t/ha^{-1}) also brought canopy volume increases, but the differences were not significant in statistical terms ($p>0.05$) (Figure 2).

Table 3. Canopy volume (m^3) differences among varieties

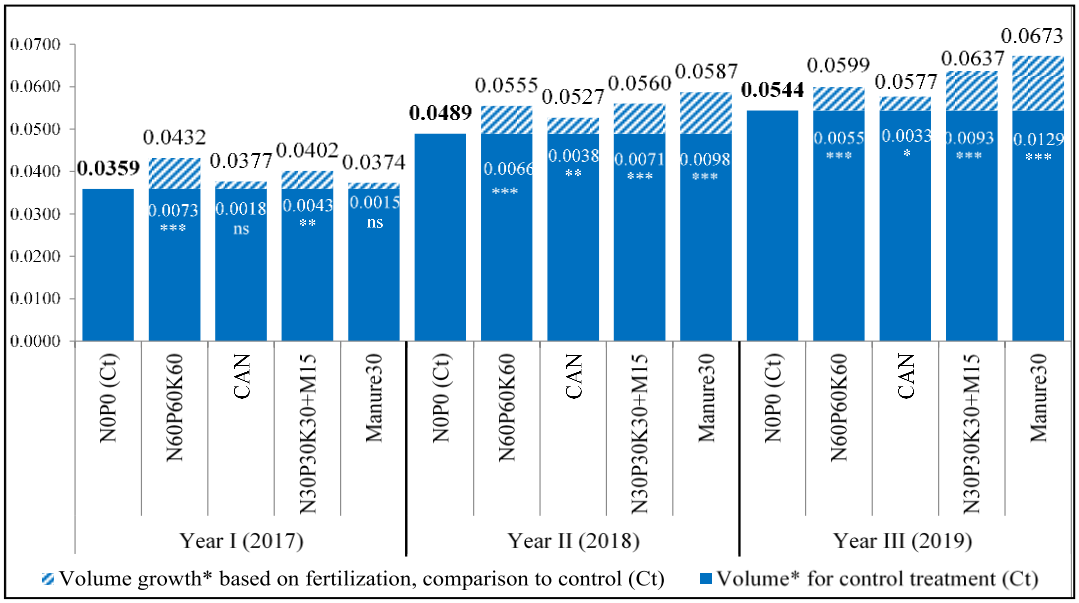
Crop year	Fertilization	Differences (m^3) among varieties*					
		a_2-a_1	a_3-a_1	a_4-a_1	a_3-a_2	a_4-a_2	a_4-a_3
	$N_0P_0K_0$	0.0162	-0.0113	-0.0197	-0.0275	-0.0359	-0.0084
1st year	$N_{60}P_{60}K_{60}$	0.0217	-0.0119	-0.0229	-0.0336	-0.0446	-0.0110
	CAN	0.0161	-0.0120	-0.0207	-0.0281	-0.0368	-0.0087
(2017)	$N_{30}P_{30}K_{30+}$**M15**	0.0184	-0.0118	-0.0220	-0.0302	-0.0404	-0.0102
	Manure30	0.0148	-0.0147	-0.0235	-0.0295	-0.0383	-0.0088
LSD 5%: 0.0040 m^3; LSD 1%: 0.0056 m^3; LSD 0.1%: 0.0078 m^3							
	$N_0P_0K_0$	0.0131	-0.0177	-0.0316	-0.0308	-0.0447	-0.0139
2nd year	$N_{60}P_{60}K_{60}$	0.0150	-0.0179	-0.0353	-0.0329	-0.0503	-0.0174
	CAN	0.0109	-0.0191	-0.0346	-0.0299	-0.0455	-0.0155
(2018)	$N_{30}P_{30}K_{30+}$**M15**	0.0114	-0.0189	-0.0355	-0.0302	-0.0469	-0.0167
	Manure30	0.0153	-0.0166	-0.0329	-0.0318	-0.0482	-0.0163
LSD 5%: 0.0031 m^3; LSD 1%: 0.0044 m^3; LSD 0.1%: 0.0063 m^3							
	$N_0P_0K_0$	0.0148	-0.0222	-0.0363	-0.0371	-0.0511	-0.0140
3rd year	$N_{60}P_{60}K_{60}$	0.0143	-0.0245	-0.0393	-0.0388	-0.0536	-0.0148
	CAN	0.0128	-0.0247	-0.0394	-0.0376	-0.0523	-0.0147
(2019)	$N_{30}P_{30}K_{30+}$**M15**	0.0113	-0.0274	-0.0435	-0.0387	-0.0548	-0.0161
	Manure30	0.0158	-0.0232	-0.0414	-0.0390	-0.0573	-0.0183
LSD 5%: 0.0037 m^3; LSD 1%: 0.0052 m^3; LSD 0.1%: 0.0075 m^3							

a_1 – Sevstopolis, a_2 – Vera, a_3 – Hidcote, a_4 – Buena Vista

Influenced by fertilization (Figure 2), plants canopy volume had statistically assured increases between 0.0033 m^3 (CAN) and 0.0098 m^3 (Manure30 t/ha^{-1}) (2nd year), and between 0.0544 m^3 (Ct) to 0.0673 m^3 (Manure30 t/ha^{-1}) (3rd year).

b. Root system distribution and development

Analysing data in Figure 3 and Table 4 it is shown that, with the highest RSA (mm^2) values for all four researched lavender varieties, the root mass was mainly distributed at 0-10 cm depth in the soil profile.



LSD 5%: 0.0024 m^3; LSD 1%: 0.0034 m^3; LSD 0.1%: 0.0047 m^3

Figure 2. Canopy volume (m^3) as influenced by fertilization.

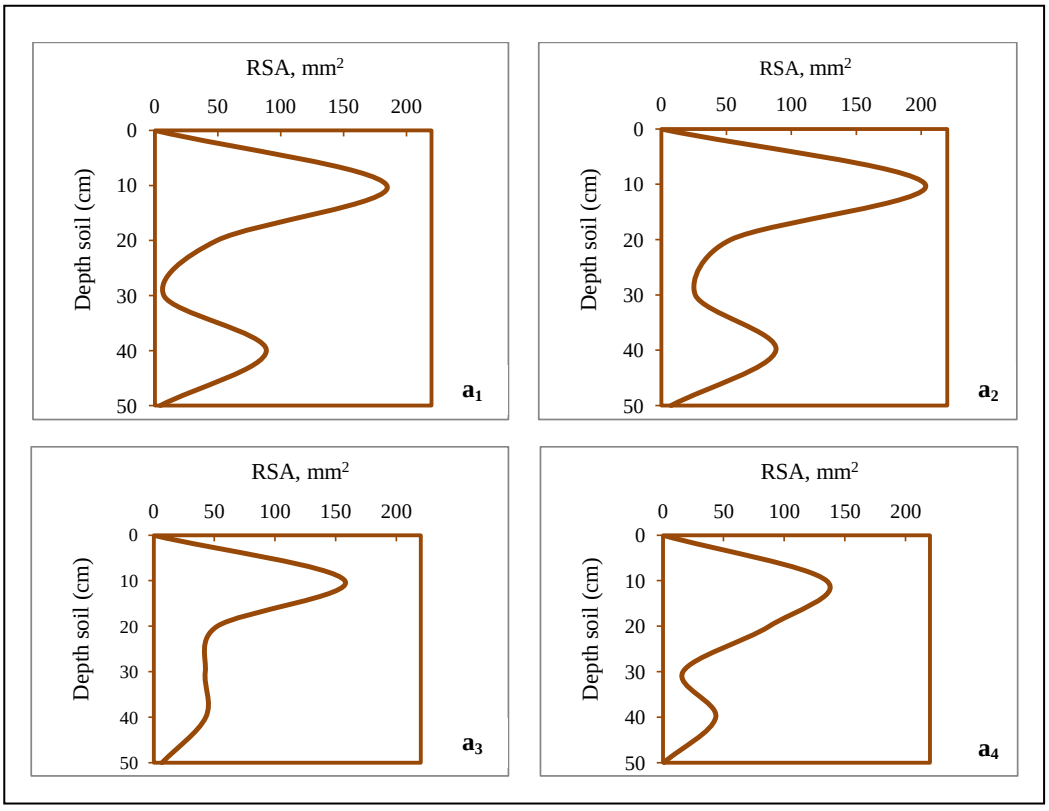


Figure 3. Root Section Area (RSA) from *Lavandula* variety:
a_1 - Sevstopolis; a_2 - Vera; a_3 - Hidcote; a_4 - Buena Vista.

The average RSA of the lavender varieties in this soil layer (Table 4) was 169.99 mm^2, with Vera obtaining the highest value (203.10 mm^2), and Buena Vista having the lowest value (134.97 mm^2). At 30-40 cm depth, Sevstopolis (88.56 mm^2) and Vera (88.16 mm^2) had an increase in RSA compared to the previous soil layer (20-30 cm), correlated to better development of the canopy volume. Hidcote (42.81 mm^2) and Buena Vista (43.51 mm^2) had lower RSA values and moderate canopy development.

Table 4. Root section area (RSA) of lavender varieties at different soil depth (0-50 cm)

Variety	RSA (mm^2) - Soil Depth (cm)					Total RSA (mm^2) 0-50 cm	Diff.
	0-10	10-20	20-30	30-40	40-50		%
Sevstopolis	184,33	49,29	3,94	88,56	4,34	330,46	102,2
Vera	203,10	53,43	26,12	88,16	7,48	378,29	117,0
Hidcote	157,55	52,26	42,41	42,81	6,78	301,81	93,3
Buena Vista	134,97	87,62	16,46	43,51	0,50	283,06	87,5
Average	169,99	60,65	22,23	65,76	4,78	**323,41**	**100 %**

The total root section area (0-50 cm) ranged as influenced by variety between 283.06 mm^2 for Buena Vista and 378.29 mm^2 for Vera. The average root section area of the four lavender varieties had a value of 323.41 mm^2 (Table 4). Vera and Sevstopolis varieties obtained positive differences compared to the average of 17.0% and 2.2%, respectively. Hidcote and Buena Vista had smaller values compared to the average RSA (0-50 cm) by -6.7% and -12.5%, respectively.

c. Canopy volume correlation to root system area

Lavender plants canopy volume is closely related to the root section area (Figure 4). Pearson correlation coefficient of 0.9851 highlights a strong dependency between the two variables, and the value of the regression coefficient indicates that plants' canopy volume is strongly influenced (97.32%) by the root section area (RSA).

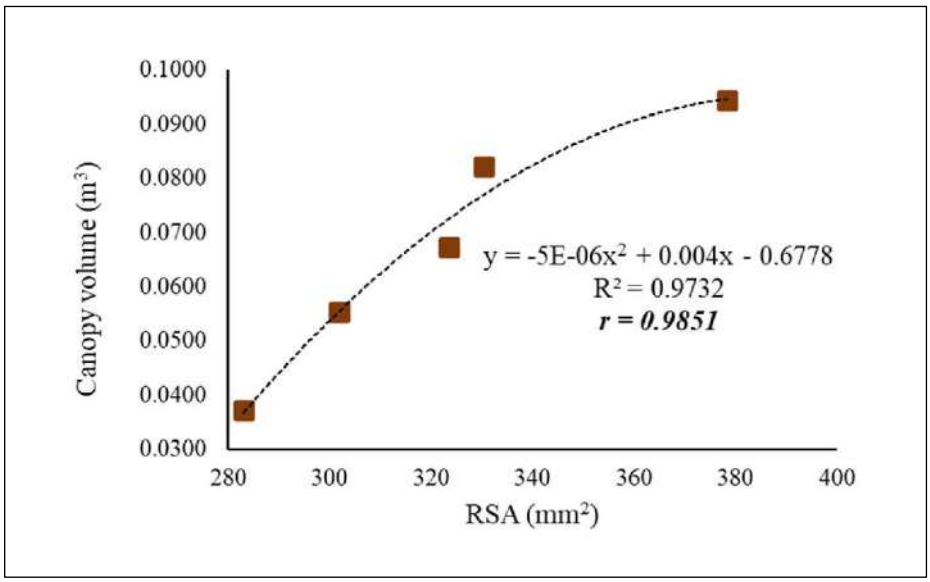


Figure 4. Scatterplot between lavender canopy volume and root section area (RSA), Manure30 t/ha^{-1}, 3rd year (polynomial regression equation).

Conclusions

Vera variety had the highest canopy volume for all doses and combinations of mineral and organic fertilizers (*B*), ranging from 0.0557 m^3 for control (unfertilized variant), in the 1st year, and 0.0944 m^3 for Manure30 t/ha^{-1} (organic fertilizer), in the 3rd year. Volume differences obtained by this variety compared to the average value and the differences it had compared to the other varieties were statistically significant.

Beyond the variety, fertilization also influenced the canopy volume. In the 1st year, mineral and organo-mineral fertilization treatments generated statistically assured volume growths, and in the following years (2nd and 3rd year), volume differences compared to Control (unfertilized variant) were also statistically significant for all doses and combinations of mineral and organic fertilizers (*B*).

Lavender plants' canopy volume is closely related to the development of the root system, varieties with a better vigour (higher canopy volume) have higher values of the root system mass.

Varieties rooting was normal. RSA was distributed mainly within 0-10 cm depth (mean value of 169.99 mm^2). Variety influenced the roots section area within 0-50 cm depth. Thus, Vera had the highest values (378.29 mm^2) and Buena Vista had the lowest values (283.06 mm^2).

References

1. BEATOVIĆ D., JELAČIĆ S., KIŠGECI J., MORAVČEVIĆ Đ., MILOŠEVIĆ D.K., ZARIĆ V., FILIPOVIĆ N. Application of local peat in the lavander nursery production (*Lavandula angustifolia* Mill.). In Proceedings of the Seventh Conference on Medicinal and Aromatic Plants of Southeast European Countries, (Proceedings of the 7th CMAPSEEC), Subotica, Serbia, 27-31 May, 2012, 273-278.
2. BOLOHAN C. Cercetări privind ecologia, biologia și tehnologia de cultură a speciei *Cynara cardunculus* L. – cardon, în zona de sud-est a României (Moara Domnească – Ilfov). Teză de doctorat, USAMV București, 2018, 42-49. https://rei.gov.ro/teza-doctorat/F-CA-14198-18-07-2018
3. DUDU G., ASĂNICĂ A., MIHALACHE M., RĂDUCU R., TEODORESCU R.I., TUDOR V. The root development patterns of 'Stella' grafted on mahaleb seedlings in an orchard with incompatibility symptoms. *Romanian Biotechnological Letters*, 2015; vol. 20, no. 5, 10850-10862.
4. ERNST M. Lavender. CCD-CP-127. Lexington, KY: Center for Crop Diversification, University of Kentucky – College of Agriculture, *Food and Environment*, 2017, CCD-CP-127. https://www.uky.edu/ccd/production/crop-resources/nursery-ornamental/lavender
5. GÜNER A., ÖZHATAY N., EKIM T., BASER K.H.C. Flora of Turkey and East Aegean Islands. Supplement II. Edinburgh University Press, vol. 11, 2000, 618-619. https://www.researchgate.net/publication/273136401_Flora_of_Turkey_and_the_East_Aegean_Islands
6. JELAČIĆ S., KIŠGECI J., BEATOVIĆ D., MORAVČEVIĆ Đ., Bjelić V. The use of biostimulators and slow decomposing fertilizer with different ways of lavander seedlings production. In Proceedings of the Fifth Conference on Medicinal and Aromatic Plants of Southeast European Countries (5th CMAPSEEC), Brno, Czech Republic, 2008, 2-5 September, 103.
7. KIMBROUGH K.A., SWIFT C.E. Growing lavender in Colorado. Gardening Series. Flowers, Colarado State University – Extensions, 2009, no. 7, 245.
8. KORKUNÇ M. Research of Lavender Plant Propagation in the Province of Diyarbakir. *Middle East Journal of Science*, 2018, 4(2), 58-65. https://doi.org/10.23884/mejs.2018.4.2.01
9. LOPES V., BARATA A.M. Ex situ morphological assessment of wild Lavandula populations in Portugal. Arabian Journal of Medicinal & Aromatic Plants, 2017, vol. 3(1) 87-100.
10. ŁYCZKO J., PIOTROWSKI K., KOLASA K., GALEK R., SZUMNY A. *Mentha piperita* L. Micropropagation and the Potential Influence of Plant Growth Regulators on Volatile Organic Compound Composition. Molecules 2020; 25(11), 1-15. https://doi.org/10.3390/molecules24162900
11. MAGANGA A. Influence of variety, organic cultural practices on yield, essential oil content of lavender, rosemary in interior BC. South Thompson Organic Producers Association (STOPA), 2004, 3-23. http://www.certifiedorganic.bc.ca/programs/osdp/OSDP-FinalReport_I-016.pdf
12. MASON J. Growing and knowing lavender. Book ACS Distance Education, Queensland, Australia, 2014, 14-26.
13. MIHALAȘCU C., TUDOR V., BOLOHAN C., MIHALACHE M., TEODORESCU R. The effect of different fertilization upon the growth and yield of some *Lavandula angustifolia* (Mill.) varieties grown in South East Romania. *Scientific Papers*. Series B, Horticulture, vol. LXIV, Issue 1, Print ISSN 2285-5653.
14. MOJA S., GUITTON Y., NICOLÈ F., LEGENDRE L., PASQUIER B., UPSON T., JULLIEN F. Genome size and plastid trnK-matK markers give new insights into the evolutionary history of the genus *Lavandula L.* Plant Biosystems – *An International Journal Dealing with all Aspects of Plant Biology*, 2015, vol 150(6), 1216-1224. https://doi.org/10.1080/11263504.2015.1014006
15. NAJAR B., DEMASI S., CASER M., GAINO W., CIONI P.L., PISTELLI L., SCARIOT V., 2019. Cultivation Substrate Composition Influences Morphology, Volatilome and Essential Oil of *Lavandula Angustifolia* Mill. *Agronomy*, Special Issue

"Sustainability and Quality in Ornamental Horticulture: Challenges and Perspectives" 2019, 9(8), 1-19. https://doi.org/10.3390/agronomy9080411

16. PLEGUEZUELO C.R.R., ZUAZO V.H.D., RAYA A.M., MARTÍNEZ J.R.F., RODRÍGUEZ B.C. High reduction of erosion, nutrient losses by decreasing harvest intensity of lavender grown on slopes. *Agronomy for sustainable development*, 2009, 29(2), 363-370.
17. REID A. Growing lavender in Western Australia. Department of Agriculture and Food, Western Australia, 2000, *Perth. Bulletin*, 4454, 1-20. https://researchlibrary.agric.wa.gov.au/bulletins/45/
18. RYAN M.D. Revaluing residues: effects of composts, vermicomposts from corn, fig, citrus residues on the development of *Rosmarinus officinalis* L., *Lavandula angustifolia* L. *Master's thesis*, Norwegian University of Life Sciences, Ås, 2016.
19. SAKR W.R., ELBAGOURY H.M., REFAAY M.S. Can Sheep Manure, Yeast Substitute Conventional N for *Lavandula angustifolia* Production in Sandy Soils?. *American-Eurasian J. Agric. & Environ. Sci*., 2015. Vol. 15(4), 612-622. DOI: 10.5829/idosi.aejaes.2015.15.4.12597
20. SALEHI B., MNAYER D., ÖZÇELIK B., ALTIN G., KASAPOGLU K.N., DASKAYA-DIKMEN C., SHARIFI-RADF M., SELAMOGLUG Z., ACHARYAH K., SENH S., MATTHEWSJ K.R., FOKOUK P.V.T., SHAROPOVL F., SETZERM W.N., MARTORELLO M., SHARIFI-RAD J. Plants of the Genus *Lavandula*: From Farm to Fharmacy. *Natural Product Communications*, 2018, vol. 13(10), 1385-1402. https://doi.org/10.1177%2F1934578X1801301037
21. SEIDLER-LOŻYKOWSKA K., MORDALSKI R., KUCHARSKI W., KĆDZIA B., BOCIANOWSKI J. Yielding and quality of lavender flowers (*Lavandula angustifolia* Mill.) from organic cultivation. *Acta Sci. Pol., Hortorum Cultus*, 2014 13(6), 173-183.
22. ȘEKEROĞLU N., ÖZGÜVEN M. Determination of optimum phosphorus doses for high flower yield, essential oil content in common Lavender (*Lavandula angustifolia* Mill.). In Proceedings of the Fifth Conference on Medicinal, Aromatic Plants of Southeast European Countries (5th CMAPSEEC), Brno, Czech Republic, 2008, 2-5 September, 91.
23. SILVA S.M., LUZ J.M.Q., NOGUEIRA P.A.M., BLANK A.F., SAMPAIO T.S., PINTO J.A.O., JUNIOR A.W. Organo-mineral fertilization effects on biomass, essential oil of lavender (*Lavandula dentata* L.). *Industrial Crops and Products*, 2017, vol. 103, 133-140. https://doi.org/10.1016/j.indcrop.2017.04.004
24. SKOUFOGIANNI E., GIANNOULIS K.D., BARTZIALIS D., TZOUVARA D., DANALATOS N.G. The effect of fertilization on *Lavandula angustifolia* L. yield in North Greece. In VIII International Scientific Agriculture Symposium, "Agrosym 2017", Jahorina, Bosnia, Herzegovina, October 2017. *Book of Proceedings*, Faculty of Agriculture, University of East Sarajevo, 1037-1043.
25. THORNE M.S, SKINNER Q.D., SMITH M.A., RODGERS J.D., LAYCOCK W.A., CEREKCI S.A., Evaluation of a technique for measuring canopy volume of shrubs. *Jornal of Range Management*, 2002, vol. 55(3), 235-241.
26. TUDOR V., GEORGE D., TEODORESCU R., MIHALASCU C., RADUCU D., ASANICA A., GÎDEA M., ROSCULETE C., TIU J.V., MIHALACHE M. Determination of the interrelations between the apple root system and the physico-chemical soil properties. *Rom Biotechnol Lett.* 2019; 24 (3): 432-439. http://doi: 10.25083/rbl/24.3/432.439
27. UPSON T., ANDREWS S. The genus *Lavandula*. *Botanical Magazine Monograph* (Royal Botanic Garden) Kew, 2004, 1-442.
28. *** DAFF, 2012. Department of Agriculture, Forestry and Fisheries. Directorate: Plant Production, Division Industrial Crops, Pretoria, South Africa, 8-14. https://www.daff.gov.za/Daffweb3/Portals/0/Brochures%20and%20Production%20guidelines/Production%20Guidelines%20Lavender.pdf
29. *** ICPA Methodology, Metodologia elaborării studiilor pedologice *PARTEA a II-a* – Elaborarea studiilor pedologice în diferite scopuri, 1987. *Redacția de propagandă și tehnică agricolă*, București, 115-131.
30. *** USLGA, United States Lavander Growers Association. https://www.uslavender.org/lavender-101

Scientific Papers. Series B, Horticulture. Vol. LXV, No. 2, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

START OF THE BLUEBERRY BREEDING PROGRAM AT THE UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY MEDICINE OF BUCHAREST

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Abstract

Blueberry varieties are one of the central decision and part of the orchard success when it comes to the investor's choice. Therefore, even it is remarked a steadily increase of fruits demand from the global blueberry market, the consumer and producers claim new varieties of blueberries with new and better quality traits such as firmness, flavor, shelf life, storability, mechanical harvest, resistance to biotic and abiotic stress and even decorative values enhancement. In this trend, our endeavor at the Faculty of Horticulture Bucharest is focused to start breeding cultivars for the next blueberry generation. Beside the general breeding goals set for blueberries, at the University of Agronomic Sciences and Veterinary Medicine of Bucharest we are targeting additional objectives such as earliness in ripening, fruit color variability and decorative appearance. For the spring of 2021, we choose the following blueberry varieties as genitors: 'Simultan', Hannah's choice', 'Early blue', 'Duke', 'Chandler', 'Pink lemonade', 'Spartan', 'Blue ribbon', 'Hortbleu petite', 'Peach sorbet', V. angustfolium. First hybridization results are promising and new crossing and evaluations are about to be done in the next years.

Key words*: Vaccinium corymbosum, Vaccinium angustifolium, crossing, variety.*

INTRODUCTION

In the range of the berry crops, blueberries are ones of the most appreciated and desired fruits by consumers. Beside the well-known nutraceutical proprieties (Min S et al., 2017) and benefits for human consumption (Kalt W. et al., 2019), the blueberries are expecting to continue grow in interest for farmers and investors worldwide.

In this respect, the breeders must develop new blueberry varieties with superior traits (Pluta S. & Zurawicz E., 2014) that match both the actual and future consumer preferences and growers' expectations (Gilbert J. et al, 2014). The difficulties in choosing the right traits for the new blueberry cultivars come from the relevance of these traits in different parts of the world including adaptation, resilience (Lobos G. & Hancock J.F., 2015) and industry priorities among consumers demands (Gilbert J., 2016). So, some of the plant and fruit characteristics represent common goal for many breeders to achieve but some of them still define the local or regional particularities.

Nevertheless, fruit quality remains a strong target in the business. For instance, firmness, sweetness (Gilbert J. et al., 2015), flavour (Sater H., 2020; Farneti B. et al., 2017), shelf life and overall appearance are most relevant traits to be addressed (Gallardo K. et al., 2018).

On the other side, for the large-scale production, the emerging blueberry varieties need to be ready for mechanical harvesting and in this regard, additional traits of plants and fruits are needed such as plant architecture, compact ripening period, excellent fruit firmness, easy detachment from stalks etc.

Modern techniques (Cappai F. et al., 2020) as marker-assisted breeding method (Mengist, M.F. et al., 2021) are developed and extended to be predictable and to have a better selection efficiency in the breeding activity. For this, special logistics and knowledge is required.

Not for long time ago, few breeding companies aimed to create blueberry varieties with

ornamental value or mixt valorisation of plants, opening a new direction for blueberry breeding programmes (Kobelt M., 2020) and enlarging the genetic datasets.

Famous breeding companies as Fall Creek started specific breeding programme for northern highbush blueberries in Europe (Fresh Plaza, 2020) and many of the latest cultivars became already well-known and appreciated.

For the producers it is very important to start a new plantation with a high value genetic material, and to influence the market and trends for fresh blueberry consumption. This is one of the reasons to increase and speed the breeding work for the upcoming period.

For Romania, the Research Institute for Fruit Growing Pitesti is the single institution that own a breeding programme for blueberry and in more than 30 years of activity in this field, fifteen great Romanian blueberry varieties have been bred (Mladin P. et al., 2012) and are available for growers (Ancu I. et al., 2013).

At the Faculty of Horticulture in Bucharest, a great number of blueberry varieties have been collected and studied in the past 10 years. Also, since 2016, in the frame of the Laboratory for sensorial analyses of the Research Centre for Studies of Food Quality and Agricultural Products, we organized yearly tasting sessions with more than 60 blueberry varieties.

The great interest of the consumers and farmers for the national and international blueberry assortment indicate us the opportunity to start a new breeding programme and bring our contribution to the next blueberry generation.

The current paper is presenting the first steps of our effort in developing a long lasting and fruitful breeding programme in the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

MATERIALS AND METHODS

For the first year of breeding activity at the Faculty of Horticulture Bucharest, beside the general breeding goals set for blueberries, at the University of Agronomic Sciences and Veterinary Medicine of Bucharest we are looking for additional objectives such as earliness in ripening, fruit size, colour variability and decorative plant traits.

In the spring of 2021, we choose the following blueberry varieties to work with as genitors: 'Simultan' (RO), 'Hannah's choice', 'Early blue', 'Duke', 'Chandler', 'Blue ribbon', 'Hortbleu petite', 'Peach sorbet' and *V. angustifolium*.

From the nine blueberry varieties, 16 cross combinations have been done between 17.04.2021 and 6.05.2021 (Table 1).

Table 1. Dates of hybridizations made in 2021 and genitors used in crossings

No	Pollination date	Cross combination
1	20.04.2021	Simultan x Duke
2	20.04.2021	Simultan x Hannah's choice
3	20.04.2021	Simultan x Hortbleu petite
4	6.05.2021	Simultan x Blue Ribbon
5	20.04.2021	Duke x Simultan
6	17.04.2021	Duke x Early blue
7	20.04.2021	Duke x Hannah's choice
8	20.04.2021	Duke x Chandler
9	17.04.2021	Hannah's choice x Duke
10	20.04.2021	Hannah's choice x Simultan
11	17.04.2021	Early blue x Duke
12	1.05.2021	Blue ribbon x Simultan
13	20.04.2021	Chandler x Duke
14	6.05.2021	V. ang x Hortbleu petite
15	6.05.2021	V. ang x Peach sorbet
16	6.05.2021	Peach sorbet x Hortbleu petite

We aim to harvest the seed from other varieties that cannot be used this year for controlled hybridization such as: 'Pink lemonade', 'Spartan', 'Toro', 'Pink breeze', 'Legacy'.

Each plant container was utilized for only one cross combination (Figure 1).

Figure 1. Blueberry hybridization plot in the experimental field of the Faculty of Horticulture Bucharest

The mother plants have been prepared in advance (Figure 2):

- selecting the right flower clusters,
- flowers were protected by special paper to avoid accidental or foreign pollination.
- emasculation of flowers
- reintroducing the flower clusters into the paper bags

The father plants were used to harvest the pollen from the suitable flowers and moment. Pollen drops were captured in the Petri vessels and regularly shacked about 24h-36h until the full release of the pollen. After 1-2 days, the paper bags were opened, and the pollen was gentle placed with the brush on the top of the stigma. Then the number of pollinated pistils were counted and bag resealed.

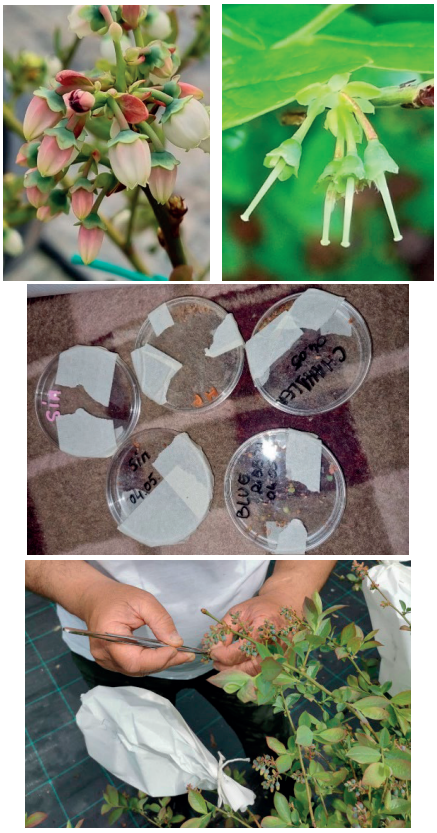

Figure 2. Blueberry breeding steps

After three weeks, the control was made for each bag and cross combination. The fruits set was observed (Figure 3), and the hybrid fruits counted.

Figure 3. Hybrid fruits set (Early blue x Duke)

RESULTS AND DISCUSSIONS

From the 16 cross combinations (Figure 4), 756 flowers were pollinated in 2021 and 653 hybrid fruits were set up. In this respect, the percentage of 86.38% of fruit set is considered a promising one.

Some examples of different hybrid fruits obtained are in Figures 5, 6, 7 and 8.

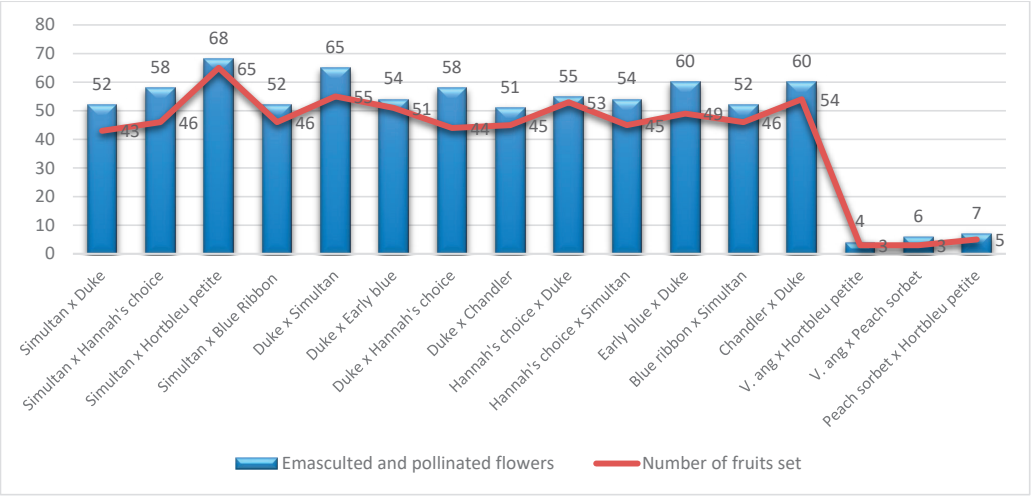

Figure 4. The results of blueberry varieties cross combinations made in 2021

Figure 5. 'Duke' x 'Simultan'

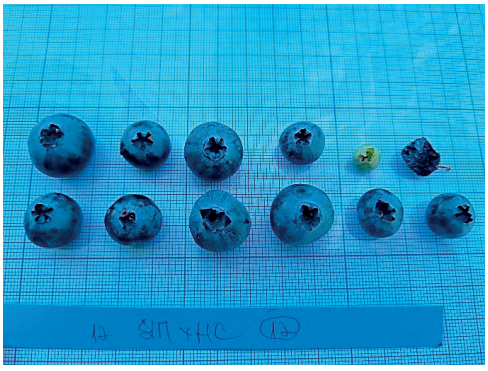

Figure 6. 'Simultan' x 'Hannah's choice'

Figure 7. 'Simultan' x 'Duke'

Figure 8. 'Chanticleer' x 'Duke'

Analysing in depth each cross combination (Figure 9), we can observe that most of the varieties exceed 80% of fruits set. The highest percentage of 90.00% was achieved by 'Chandler' followed by 'Hannah's choice' and 'Blue ribbon'. The lowest share of fruit sets (60%) was remarked at *Vaccinium angustifolium*.

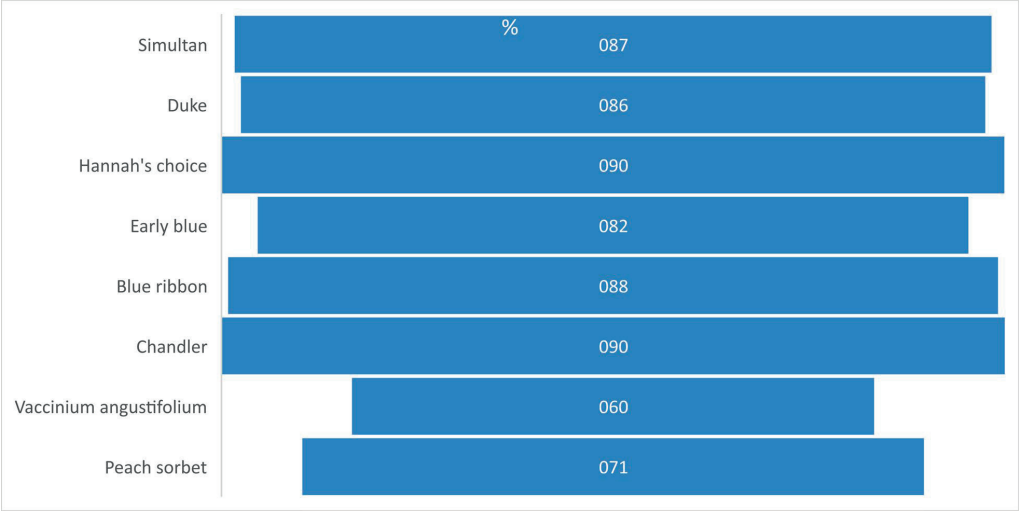

Figure 9. The fruit set percentage of the mother blueberry varieties regardless the male genitor

The *V. angustifolium* and 'Peach sorbet' variety choose for the decorative purpose has encountered lower percentages. Although, the fruits set up from the interspecific combinations (*V. angustifolium* x *V. corymbosum*) allow us to follow the hybrid seeds in further breeding process.

CONCLUSIONS

First hybridization results are promising, and new crossing and evaluations are about to be done in the next years.

Interspecific hybridization results in a lower number of fruits set than intraspecific crossings. The highest number of fruit sets percentage (90%) was calculated at 'Chandler' x 'Duke' combination.

Early varieties combinations range between 81.67% and 88.46% fruit set.

REFERENCES

Ancu, I., Nicola, C., Sturzeanu, M. (2013). Fruit quality characteristics of some blueberry genotypes. Fruit Growing Research, Vol. XXIX, 1-7

Brazel Berry. 2021 https://www.brazelberry.de/en/

Cappai, F., Amadeu, R. R., Benevenuto, J., Cullen, R., Garcia, A., Grossman, A., Ferrão, L., & Munoz, P. (2020). High-Resolution Linkage Map and QTL Analyses of Fruit Firmness in Autotetraploid Blueberry. Frontiers in plant science, 11, 562171. https://doi.org/10.3389/fpls.2020.562171.

Farneti, B., Khomenko, I., Grisenti, M., Ajelli, M., Betta, E., Algarra, A. A., Cappellin, L., Aprea, E., Gasperi, F., Biasioli, F., & Giongo, L. (2017). Exploring Blueberry Aroma Complexity by Chromatographic and Direct-Injection Spectrometric Techniques. Frontiers in plant science, 8, 617. https://doi.org/10.3389/fpls.2017.00617

Fresh Plaza (2020). Fall Creek introduces five new blueberry varieties

Gallardo, K., Zhang, Qi., Dossett, M., Polashock, J., Rodriguez-Saona, C., Vorsa, N., Edger, P., Ashrafi, H., Babiker, E., Finn, C., Iorizzo, M., (2018). Breeding Trait Priorities of the Blueberry Industry in the United States and Canada. HORTSCIENCE 53(7):1021–1028. https://doi.org/10.21273/HORTSCI12964-18

Gilbert, J., Olmstead J.W. (2016). Breeding for the Ideal Fresh Blueberry. Growing Produce, March 2016.

Gilbert, J.L., Guthart, M.J., Gezan, S.A., Pisaroglo de Carvalho, M., Schwieterman, M.L., Colquhoun, T.A., Bartoshuk, L.M., Sims, C.A., Clark, D.G., Olmstead, J.W. (2015). Identifying Breeding Priorities for Blueberry Flavor Using Biochemical, Sensory, and Genotype by Environment Analyses. PLoS One. Sep 17; 10(9):e0138494. doi: 10.1371/journal.pone. 0138494. PMID: 26378911; PMCID: PMC4574478.

Gilbert, J., Olmstead, J., Colquhoun, T., Levin, L., Clark, D., Moskowitz, H. (2014). Consumer-assisted Selection of Blueberry Fruit Quality Traits.

HortScience. 49. 864-873. 10.21273/HORTSCI. 49.7.864.

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2019). Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Advances in Nutrition*. https://doi.org/10.1093/advances/nmz065

Kobelt M., 2020. Lubera Edibles. https://www.luberaedibles.com/ en/blueberries-for-the-home-garden-8211-the-lubera-edibles-assortment-p67

Lobos, G., Hancock, J.F. (2015). Breeding blueberries for a changing global environment: a review, Front Plant Sci., 6, 782 doi: 10.3389/fpls.2015.00782

Mengist, M.F., Bostan, H., Young, E. et al. High-density linkage map construction and identification of loci regulating fruit quality traits in blueberry. Hortic Res 8, 169 (2021). https://doi.org/10.1038/s41438-021-00605-z

Min, S, Hayley, L, Andrew J., Xiao, Q.S. (2017). Blueberry as a source of bioactive compounds for the treatment of obesity, type 2 diabetes and chronic inflammation, Journal of Functional Foods, Volume 30, 16-29, ISSN 1756-4646, https://doi.org/10.1016/j.jff.2016.12.036.

Mladin, P., Mladin, G., Coman, M., Chițu, E., Chițu, V. (2012). Recent progress in berries breeding in Romania. Acta Hortic. 926, 47-51 DOI: 10.17660/ActaHortic.2012.926.4

Pluta, S. and Zurawicz, E. (2014). The high-bush blueberry (*Vaccinium corymbosum* L.) breeding programme in Poland. Acta Hortic. 1017, 177-180 DOI: 10.17660/ActaHortic.2014.1017.20

Sater, H. (2020). Understanding blueberry aroma origin, inheritance, and its sensory perception by consumers. Dissertation thesis, University of Florida.

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UNIVERSITY OF CRAIOVA

Vol. XIX (LV) - 2014

SWEET CHERRY BREEDING PROGRAMME AT ISTRITA FARM, USAMV BUCHAREST

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Keywords: *breeding, cross pollinated, hybrids, genitor, P. avium*

ABSTRACT

Breeding sweet cherry (Prunus avium L.) takes long time and in order to obtain new cultivars, there are mandatory specially steps to follow. The big number of sweet cherry cultivars established in the 12 ha orchard of the Istrita Farm become a good opportunity and challenge for hybridization and new cultivar creation. The main traits taken into consideration were: ripening time, size of the fruits, fruit appearance, organoleptic quality, resistance or tolerance to specific pests and diseases, self-fertility, blossoming time, vigor, type of the fruiting branches and the productivity. In this paper are presented the results obtained during the last two years since the Breeding Programme at USAMV Bucharest started. Now we have two series of sweet cherry hybrids (414 hybrids from 12 combinations/2013 and 447 hybrid stones from 24 combinations/2014 and 1000 stones from open pollination).

INTRODUCTION

In the last decade 120 new varieties have been released by breeders from Europe and Asia. Most of these varieties have one or more outstanding traits that make them noteworthy (Sansavini S., 2005).

The main goal of breeders is to create varieties resistant to unfavorable environmental factors, but possess a greater capacity to exploit the optimal climate conditions, soil and culture that lead to increased production (Botu I., 1994; Cociu et al.,1999).

Aims to improve the cherry worldwide concern: self fertility, fruit size, compactness trees (spur), extend the season of consumption by staggering the time of ripening fruit, early ripening, quality of fruit, flesh firmness, mechanical harvesting possibility, disease resistance, late flowering. The achievement of these objectives dealing large research centers, such as Davis - California and Geneva - New York in USA, Summerland and Vineland in Canada, Agen and Bordeaux in France, Rome and Verona in Italy, Gembloux in Belgium, Gissen and Oppenheim in Germany, Holovousy in Czech, Skiernewice in Poland, Kiev in Ukraine, Chisinau in Moldova, Pitesti, Bistrita and Iasi in Romania, etc.

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Genetic cherry improvement Canadian program on auto restore fertility species ended with approval of autofertile first commercially valuable cultivars such as 'Stella' (1970), 'Lapins' & 'Sunburst' (1984), 'Newstar' (1988). These cultivars have opened a new perspective of the cherry culture, representing cultural and technical advantages: reducing the height of the trunk, proper summer or winter cutting, treatment with growth inhibitory substances, etc. Enhancers were obtained by mutagenesis "compact" form cultivas of "standard" ones such as 'Lambert Compact', 'Compact Stella', Early Compact' , 'Van Compact' in Canada, 'Van Compact' and 'Compact Napoleon' in Czech, 'Big Burlat', 'Duron Compact' and 'Compact' in Italy (Lapins, 1976; Fideghelli, 1988; Vondraceck J., 1986, cited by Budan S., 2000). USA program to improve the cherry in 1995 were released potentially high agrobiologic cultivars (earliness, yield, fruit size and quality): 'Early Red', 'Giant Red' and 'Firm Red'.

Currently, cherry genetic improvement program in Romania, is one of the highest rated programs compared to other European countries. Ideal cultivar that satisfy to the interests of growers, technologists, traders and consumers alike and the whole, it was created and continues to be the desire of all genetic improvement programs (Zwintscher, 1973; Cociu V., 1990, cited by Budan S., 1992; Tudor V.et al, 2010; Asănică A., 2013).
Achieving in short term of any goal depends largely on the existence and proper use of sources of genes. And in this respect, there is sufficient information available to breeders.
Given to this, the current program envisages improvement Romanian complex objectives that can be achieved in successive stages and refers to both trees and fruit. The trees must meet the following conditions:

- have low vigor and many spur formations (type spur or semispur);
- precocity of fruiting and high productivity each year regulary;
- resistance to frost, white frost and drought or a better ability to adapt into new ecological conditions of culture;
- resistance to virus diseases, hard managed by conventional means.

MATERIAL AND METHODS

Fruit Growing Istrita Farm and Nursery is located on the parallel 43° 29 'north latitude and the meridian 25° 34' east longitude, at an altitude of 134 m, in the municipality Sahateni, Buzau. Distances that separate the major urban centers are 105 km from Bucharest and 25 km from the city of Buzău. Nursery area falls within the plain area starts at the foot of Carpathian hills (Dealu Mare), just below the hill Istrita and connects this area Bărăgan Plain. The climate is semi-humid, very warm. The average annual temperature is around 11,3°C summer and winter around 23°C around 0°C, average monthly temperatures recorded negative values only in the months of January to March. Average annual rainfall amounts is about 500 mm. Groundwater is a continues canvas to a depth of about 6 m. Climatic conditions were favourable for hybridization works in the spring of 2013, in exchange spring of 2014 was difficult, registering over two consecutive frost mornings (31.03 and 1.04), -4°C and -2°C, causing serious damage material to be worked up to that time. And in the immediate future minimum temperatures were within 2 to 7°C, which persisted until noon, accompanied by strong winds, which thus affecting the pollination of flowers and fruits binding. Area planted with cherry is over 12 ha, with young orchards established since 2008, intensive, with a density of 1000 trees/ha. The cultivars grown are: 'Van', 'Celeste', 'Lapins', 'Kordia', 'Giant Red', 'Ferrovia', 'Early Red', 'Firm Red', Skeena, 'New Star', 'Regina', 'Durona, 'Sweethart' grafted on different rootstocks: PHLC, CAB6P, CAB11E, COLT and Gisela 6.

Breeding sweet cherry takes long time and in order to obtain new cultivars, there are mandatory especially steps to follow (Table 1). Standard breeding techniques (i.e. self pollination, open pollination, crossing by emasculation and hand pollination) have been employed (Cociu V., Oprea Şt., 1989). Selection of suitable parents from geographically distant groups (USA and Canada) were chosen in association with from the European Group cultivars.

Table 1

Scheme of obtaining new cherry cultivars

Year I	Observations specification. The choice of the parents. Establish the work plan and supply with the necessary materials. Diallel hybridization and self pollination as planned. Obtaining hybrid seeds.
Year II	Sowing hybrid seeds in pots. Planting the hybrids in the selection orchard.
Year III	Grafting on low vigour rootstocks.
Years IV–VI	Observations in selection orchard for resistance to frost, drought, disease, plant habitus, type of branching. Selective fruit quality and other criteria established by the program. Grafting on rootstocks with low vigour of the most valuable hybrids (20 plants each), in order to organize the competition microculture.
Year VII	Establishment of the competition microculture with hybrids grafted last year (series of 10 trees) and whose value was confirmed.
Year IX	First observations on yield and fruit quality in microculture grafted hybrids.
Years XII–XV	Submission documentation for preomologation hybrids tested during 3-5 years of fruiting. Establish the competition culture in two or three different areas with the preomologated hybrids.

The main characters of interest in sweet cherry breeding program are: age of maturation, fruit size, fruit appearance, taste quality, resistance or tolerance to diseases and pests, self fertility, the flowering time, growth vigour, fruit type formations and productivity. In this sense for the period 2013-2014 we have chosen sources of genes for the characteristics set forth in Table 2.

Table 2

Sources of genes for goal achievements

Earliness	'B. Burlat', 'Celeste', 'Early Red', 'Spectral', 'Sublim', 'Ponoare'
Lateness	'Skeena', 'Regina', 'Amar Galata', 'Amar Maxut'
Productivity	'New Star', 'Early Red', 'Giant Red'
High quality of fruits (size, taste)	'Giant Red', 'Firm Red', 'Regina', 'Celeste', 'Van', 'Sublim', 'Kordia'
Bitter fruit	'Amar Galata', 'Amar Maxut', 'Margo'

RESULTS AND DISCUSSIONS

In order to execute controlled hybridization performed in the spring of 2013, it were selected maternal genitors ♀: 'Early Red', 'New Star', 'Van', 'Giant Red' and 'Firm Red' and as paternal genitors ♂ were selected: 'B. Burlat', 'New Star', 'Giant Red', 'Kordia', 'Early Red' and 'Van'.

Cherry breeding program at the Istrita Farm and Nursery started in 2013 with a favourable development conditions for hybridization, obtaining 414 hybrid plants of 12 hybrid combinations (Figure 1); in 2014 resulting hybrids from 24 combinations: 447 hybrid stones and 1000 stones from open-pollination.

Figure 1. Results, 2013
(left – image of orchard, right – hybrid population (♀N.Star x ♂E.Red)

Using 'Early Red' cultivar as maternal genitor in combination with: 'B. Burlat', 'New Star', 'Kordia', 'Giant Red' and 'Van' results fruit hybrid percentages between 1.4 to 5.9%, absorbable fruit occur because that they did not reach harvest maturity (Figure 2).
The same cultivar used as the paternal genitor, performs well in combination with the good compatibility of the 'Giant Red', resulting 77 hybrid fruits (43.5%), which were matured 49 hybrid fruits and stones (Figure 5). From the interbreeding with 'New Star', it resulted 98 hybrid fruits (20.8%) at fruit set time, and a number of 50 hybrid stones finally (Figure 3).

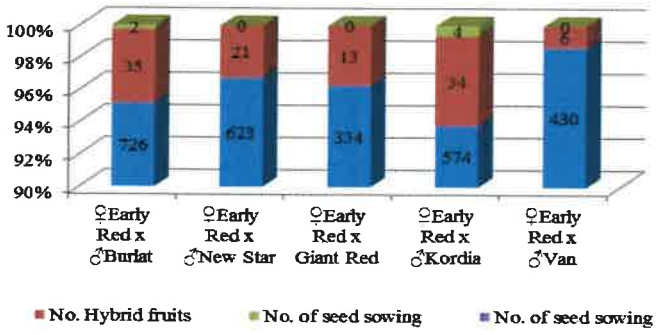

Figure 2. Behaviour of 'Early Red' cultivar as a maternal genitor (♀), 2013

From hybrid combination ♀'New Star' x ♂'B. Burlat' resulted the largest number of hybrid seeds (64) from a number of 456 self-pollinated flowers and 94 hybrid fruits recorded after fruits sett (Figure 3). 'New Star' used as the maternal genital (♀) showed very good compatibility with all cultivars were crossed yielding the percentage of fruits with 'Early Red' of 27.6%, 26.4%

with 'Kordia', 20.8% with 'B. Burlat', 19.5% with 'Van' and 13.5% with 'Giant Red'. Significant hybrid progeny resulting from a combination with 'B. Burlat' – 64, with 'Early Red' 50, 36 of the cross with 'Van', 27 with 'Kordia' and 16 with 'Giant Red' (Figure 3).

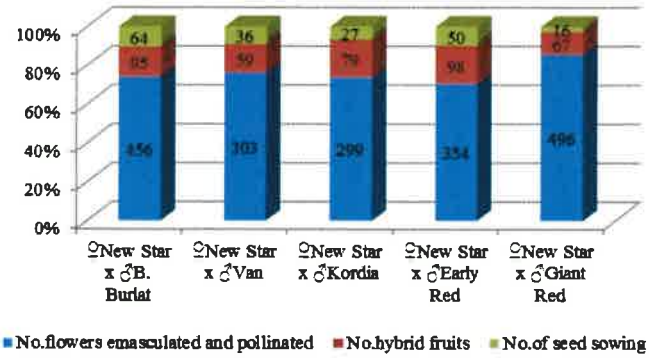

Figure 3. Behaviour of 'New Star' as a maternal plant (♀), 2013

'Van' maternal genitor performed well in cross with 'Early Red', setting 75 hybrid fruits, but they have stopped growing and fell (Figure 4). Good compatibility has recorded 'New Star', about 55 (50.9%) hybrid fruit were obtained and 52 hybrid seeds. Also, 'B. Burlat' sett 27 hybrid fruits and finally 24hybrid stones from the fruits carried by the harvest maturity time (Figure 4).

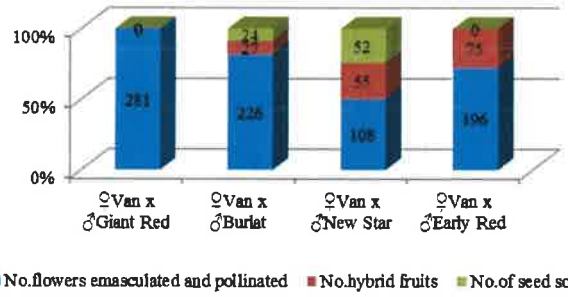

Figure 4. Behaviour of 'Van' as a maternal plant (♀), 2013

From combination ♀'Giant Red' x ♂'Early Red' resulted in total 49 hybrid stones and lower hybrid populations from crosses ♀'Giant Red' x ♂'New Star' with only 7 stones (Figure 5).

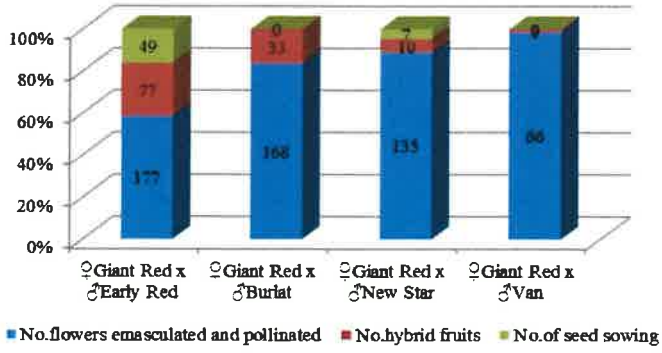

Figure 5. Behaviour of 'Giant Red' as a maternal plant (♀), 2013

'Firm Red' (Large Red x Garnet) crossed with 'Giant Red' (Large Red x Ruby) had encounter poor results, possibly due to the existence in their pedigree of the 'Large Red' cultivar as a maternal genitor (Figure 6).

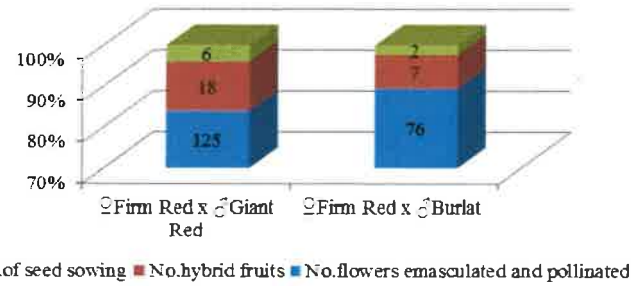

Figure 6. Behaviour of 'Firm Red' as a maternal plant (♀), 2013

Self fertile ability of New Star and 2013 satisfactory results prompted us to use it in the next hybridization year.

In 2014, difficult hybridization climatic conditions registered between 31.03-7.04, have reduced the number of flowers (Figure 7), this could be observed from the number of initial castrated flowers and the pollinated flower number with percentages ranging from 56.3 to 79.6% (Table 3). The lowest percentage of viable pistils pollinated associated with a lower resistance at low temperatures is observed at 'Giant Red' cultivar (from 14.6 to 29.3%) compared with 'Skeena' (60.7 to 73.9%) and 'Celeste' with high resistance (61.0 to 90.0%) (Asănică et all, 2012). Controlled hybridization works were performed and the Regina cultivar too, with somewhat late flowering pbenophase (11.04) but the bags were destroyed due to strong winds (Figure 7).

Figure 7. Damage to biological material, 2014 (left-frozen pistils, right-bags destroyed by wind)

Table 3

Resistance of cherry flowers during the controlled hybridization operation, 2014

Hybrid combination	No. flowers emasculated	No. flowers pollinated	%
♀Giant Red x ♂Burlat	988	259	26.2
♀Giant Red x ♂Amar Maxut	1160	361	31
♀Giant Red x ♂Spectral	630	185	29.3
♀Giant Red x ♂Ponoare	1066	156	14,6
Total	3844	961	14.6 – 29.3
♀New Star x ♂Giant red	558	364	65.2
♀New Star x ♂Burlat	500	355	71
♀New Star x ♂Early red	885	657	74.2
♀New Star x ♂Skeena	428	337	78
♀New Star x ♂Celeste	310	212	68
♀New Star x ♂Amar Galata	569	453	79.6
♀New Star x ♂Amar Maxut	701	520	74.1
♀New Star x ♂Spectral	548	382	69.7
♀New Star x ♂Sublim	797	449	56.3
♀New Star x ♂Ponoare	987	702	71.1
Total	6283	4431	56.3 -79.6
♀Celeste x ♂Skeena	456	412	90.0
♀Celeste x ♂New Star	376	230	61.0
♀Celeste x ♂Giant Red	317	200	63.0
♀Celeste x ♂Amar Galata	331	245	74.0
♀Celeste x ♂Sublim	374	242	64.7
♀Celeste x ♂Spectral	295	225	76.2
♀Celeste x ♂Ponoare	525	349	66.4
Total	2674	1903	61.0-90.0
♀Skeena x ♂New Star	312	224	71.7
♀Skeena x ♂Early Red	353	261	73.9
♀Skeena x Giant Red	415	252	60.7
Total	1080	737	60.7-73.9

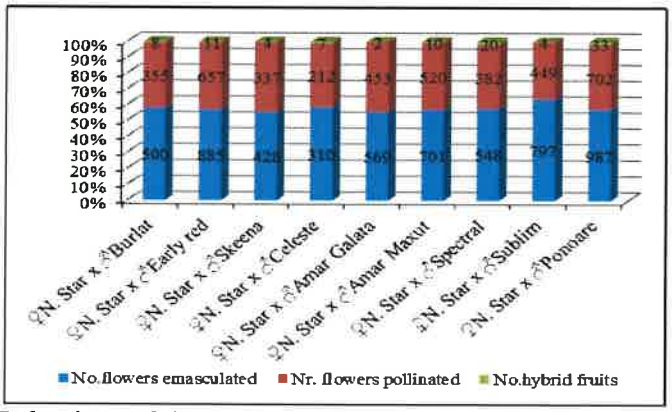

Figure 8. Behaviour of 'New Star' cultivar as a maternal genitor (♀), 2014

'Ponoare' cultivar used as paternal genitor (♂) in all crosses made, showed very good compatibility, resulting in the largest hybrid progeny: 64 stones with 'Celeste' (Figure

10), 45 with 'Giant Red' (Figure 9) and 33 with 'New Star' (Figure 8). A population of 20 stones resulted from crossing 'New Star' (♀) x 'Spectral' (♂), the other crossings resulting in lower hybrid progenies.

Although large losses of floral organs ready for pollination were registered, 'Giant Red' proved pretty good compatibility both in crossing with 'Ponoare' (45 hybrid stones) and 'Spectral' (27 stones) even with 'B. Burlat' (26 stones) (Figure 9).

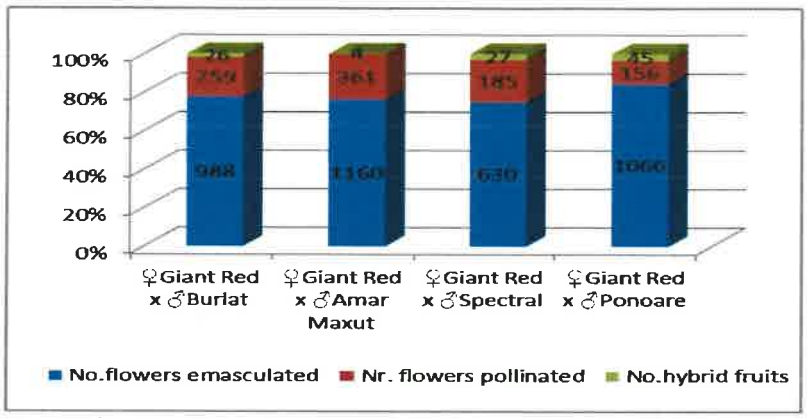

Figure 9. Behaviour of 'Giant Red' cultivar as a maternal genitor (♀), 2014

'Celeste' had good cross compatibility with 'Ponoare' (64 stones) and 'Amar Galata' (31 stones) and moderate to 'New Star' (15 stones) and 'Spectral' (11 stones) (Figure 10).

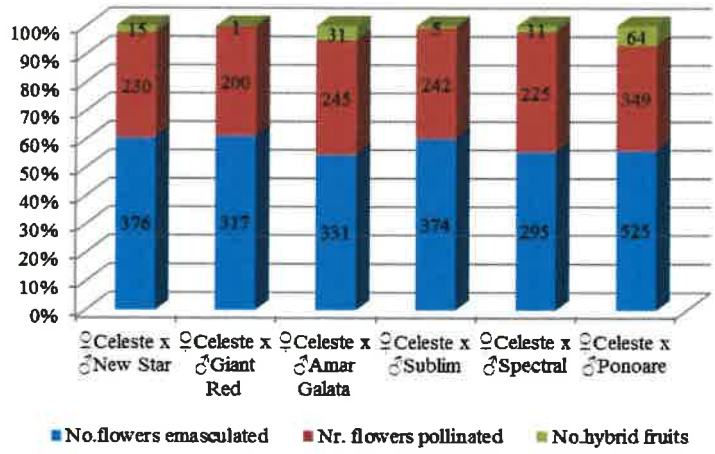

Figure 10. Behaviour of 'Celeste' cultivar as a maternal genitor (♀), 2014

From early cultivars crossings with 'Early Red' and 'Giant Red', late one 'Skeena' had a reduced compatibility, obtaining only 9, respectively 10 stones (2.8-3.5%) (Figure 11).

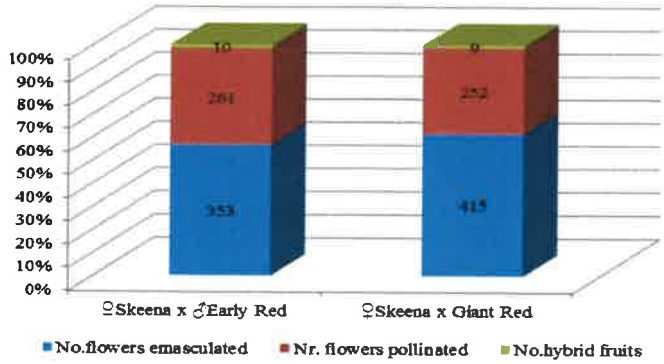

Figure 11. Behaviour of 'Skeena' cultivar as a maternal genitor (♀), 2014

The data presented in Figure 12 confirms that 'New Star' is a self fertile cultivar, being the only one who set up to 12.8%, resulting in 23 hybrid stones (Figure 12) and in 2014 resulted from 'Skeena' a surprising number of 34 fruits. This requires to be repeated to become concluding by initiated studies (Figure 13).

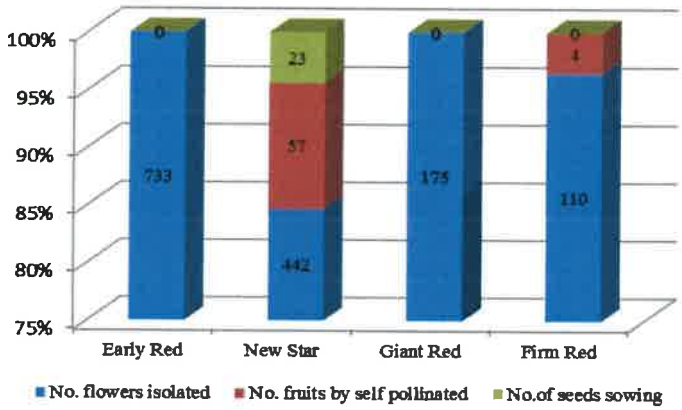

Figure 12. Self pollination made in 2013

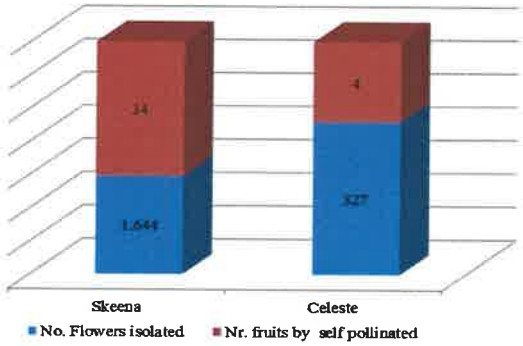

Figure 13. Self pollination made in 2014

CONCLUSIONS

The climate is of major importance in the development of successful hybridization operations.

For getting significant hybrid populations have to be pollinated around 1000 flowers/combination, in order to eliminate any possible damages occurred by climatic accidents.

During the two years after the initiation of cherry breeding program at Istrita Farm and Nursery (UASVM Bucharest) were already obtained two hybrid cherry series (414 hybrid plants of 12 combinations / 2013, of these 6 combinations with higher hybrid progeny than 25 individuals/population and 447 hybrid stones of 24 combinations/2014 and 1000 stones from open-pollination).

During the past two years of experience, significant results had emphasized by 'New Star' used both as maternal and paternal genitor.

'Ponoare', 'Early Red', 'Spectral' and 'Amar of Galata' can be used as paternal genitors (♂).

ACKNOWLEDGMENT

We are gratefully to Mrs. Sorina Sîrbu from SCDP Iasi and Mr. Sergiu Budan from ICDP Pitesti Mărăcineni for paternal biological material made available for use in crossbreeding scheme.

This work was supported by the strategic grant POSDRU/159/1.5/S/133255, Project ID 133255 (2014), co-financed by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007-2013.

REFERENCES

Asănică A., Hoza D., Tudor V., Temocico G., 2012, Evaluation of some sweet cherry cultivars to winter freeze in different areas of romania, Scientific Papers, Series B, Horticulture, Vol. LVI, 2012, ISSN Online 2286-1580, ISSN-L 2285-5653.

Asănică A., Hoza D., 2013, Pomologie, Editura Ceres, ISBN 978-973-40-0984-8.

Botu Ion, 1994, Ameliorarea plantelor horticole. Reprografia Universitatii din Craiova.

Budan S., 1992, Rezultate parţiale privind crearea soiurilor autofertile de cireş. Cercetări de Genetică Vegetală, ICCPT Fundulea, Vol.II.

Budan S., Grădinariu G. 2000, Cireşul. Ed. Ion Ionescu de la Brad, ISBN 973-8014-11-5.

Cociu V., Botu I., Serboiu L., 1999, Progrese in ameliorarea plantelor horticole din Romania, Vol.1, Ed. Ceres, ISBN 973-40-0464-6.

Cociu V., Oprea Şt., 1989, Metode de cercetare în ameliorarea plantelor pomicole, Editura Dacia, Cluj-Napoca, ISBN 973-35-0077-1.

Sansavini S., and S. Lugli. 2005, Trends in sweet cherry cultivars and breeding in Europe and Asia. Pp. 1. In: Proceedings of the 5th International Cherry Symposium. Bursa, Turkey.

Tudor V., Temocico G., Asănică A., Alecu I., Alecu E., 2010, Catalogul speciilor şi soiurilor de pomi fructiferi şi căpşun cultivate la Ferma Didactică a U.S.A.M.V. Bucureşti, Editura Ceres, ISBN978-973-40-0890-2.

*** Pepiniera Pomicola Istrita. 100 ani de activitate, Editura Tehnică Agricolă, 1993.

Romanian Biotechnological Letters *Vol. 21, No. 4, 2016*

ORIGINAL PAPER

The Effect of Drip Irrigation on Several Physical and Chemical Features of Soil

Received for publication, July 4, 2015
Accepted, April 18, 2016

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Abstract

Anthropic influences resulted in the alteration of soil fertility, as the main limiting factors, such as compaction, acidification, structure destruction, etc., had a negative influence on the physical and chemical features of soil. The most important changes in several physical and chemical features of soils resulted from the action of irrigation, drainage, fertilization and improvement of agricultural soils. Research carried out between 2011 and 2012 aimed to determine the effect of drip irrigation on several physical features of soil, such as bulk density (BD), total porosity (TP) and compaction degree (CD), as well as several chemical features such as the soil soluble salts content mg/100g soil, the content in mobile ($N\text{-}NH_4$ + $N\text{-}NO_3$) (ppm), P and K. The experience was one bifactorial: factor A=variety (apricot: a1–'Dacia', a2–'Comandor', a3–Tudor'; apple: a1-'Romus 3', a2-'Generos', a3-'Ionathan'), factor B = hydric regime (b1-unirrigated Control-Ct, b2-drip irrigation 4l/h), placed after the linear method with 5 trees/variant. In both fruit-tree species, compaction degree increased with depth experimental variants. The soil soluble salts content (mg/100g soil) varied between 32 and 36 mg/100g soil, was uniformly distributed in all the experimental variants under study, and correspond to the non-saline soil. The N amount available to the plants ($N\text{-}NH_4$ + $N\text{-}NO_3$) was very low in all variants. The comparison between the variants of the water regime showed that the N amount was slightly higher in b2 (irrigated), which was specific to each soil type.

Keywords: *drip irrigation, soil, physical and chemical properties, apple, apricot*

1. Introduction

In Romania, the data recorded by the National System of Soil Quality Monitoring showed that about 7.5 mil ha of the arable lands were affected by one or several limiting factors. Moisture deficit was recorded on 7.1 mil ha, i.e. 48.2% of the degraded area, followed by water erosion – 4.3 mil ha, i.e. 29.5% of the total degraded area. Other types of degradation account for less: 8.4% chemical damage, 4.2% complex deterioration, 2.6% by wind erosion. In recent years a considerable area, more than 1 million hectares (6.9% of the total), has been removed from crop production circuit (MUNTEANU & al. [18]; MIHALACHE & al. [15]; CÎMPEANU & al. [11]). More important changes of some physical and chemical soils properties are noted under the influence of irrigation, drainage, fertilization and amendment (CANARACHE [3], [4], [5]). Thus, for example, under the influence of irrigation water, soil structure undergoes predominantly negative changes. At the sudden contact with irrigation water, the air from the dry soil aggregates is 'trapped' within the latter. Air pressure increases inside the aggregate, based on the capillary tension caused by water intrusion. At some point, the air pressure inside the aggregate, defeat is higher than its cohesion and causes its 'explosion'(PLEȘA & al. [19]; PLEȘA & al. [20]; DUMITRU & al. [11]). Fertilization and good quality water irrigation led to an increase in organic matter, particularly at 20-40 cm depth in the chernozem of the Mărculeşti area, and in the alluvial soils. Irrigation resulted in

the removal of $CaCO_3$ from the surface to a depth of 25-30 cm. This increase of the humus content in the ploughed layer for 20 years and the proper application of technological components resulted in the very good conservation of these soils characterized by a high level of fertility (MIHALACHE & al.[14]; MIHALACHE [17]). The cambic chernozems of Fundulea recorded a reduction of the humus content, mobile potassium, mobile phosphorus, as well as an increased reaction under the influence of the irrigation rate, especially in the unfertilized variants, due to the irrigation that used less satisfactory water. Structural hydraulic conductivity showed a slight decrease under the influence of irrigation, as the fertilized variants were the most visible. In such cases, the content in water stable aggregates was reduced from 15 to 11% while the structural instability index increased from 0.42 to 0.54. The bulk density determined on chernozem decreased slightly from 1.25 g/cm^3 to 1.22 under the influence of irrigation; the same trend was also recorded in alluvial soil and cambic chernozem (MIHALACHE & al. [14]; MIHALACHE [17]).The other agro-physical features, such as penetration resistance, macro-porosity and water permeability of the soils under study, indicated no statistically significant differences between the experimental factors (MIHALACHE & al. [14]; MIHALACHE, 2014 [17]). The combined effect of hydrological, pedological improvement work performed on the gleyic mollic soils of the Radauti Depression 24 years ago, showed an increase in bulk density and porosity, together with the significant decrease in the number of macropores. The high values of bulk density in the ploughed layer showed that soil compaction was caused by the secondary soil tillage, not by the direct effect of the plough (FILIPOV & al. [12]; AILINCĂI & al.[1]).Generally, more intense leaching of soluble salts is noted on the irrigated soils, when compared with the non-irrigated soil in the area. Leaching intensity depends on soil permeability, irrigation method and system, irrigation water composition, the presence of a drainage system. These issues are of particular interest for saline soils. Irrigation based on high amounts of water (submersion irrigation and surface drainage) favours the leaching of soluble salts; otherwise the effect is the opposite, i.e. salinisation. Sprinkling irrigation uses small amounts of water and results in washing of the soluble salts from the upper horizons to the lower ones, even in the absence of drainage. The experiments conducted on saline and alkaline soils demonstrate the effect of irrigation on soil desalination. It should be noted, however, that irrigation washes off not only the harmful soluble salts but also the fertilizing elements (nitric nitrogen, phosphorus, etc.).The application of the foliar fertilizer can be more efficient than the one applied to the soil. However, the combination between soil treatments and foliar treatments is recommended for nutrient management in apple tree (AMIRI & al.[2]). It is known that irrigation water, regardless of its origin, has higher mineral content than meteoric water. Therefore, its effect on salts accumulation will differ essentially from that of rainwater. It is understandable that the intensity of salts accumulation in the soil will increase at the same time with the mineralization level of the irrigation water and the volume of water taken (total irrigation time) (CÎMPEANU & al. [6], [7], [8]). By irrigating with slightly mineralized waters (1 g/l), the high salinization of the soil occurs after about 20 years of irrigation, while the use of strongly mineralized irrigation water (>5 g/l) results in the actual salinization of soils after 1-2 years of irrigation (HANAN [13]). In addition to salts, irrigation water also brings large

quantities of silt on the irrigated land (CÎMPEANU & al. [9]). The amount of deposited silt depends on the turbidity of the irrigation water and the irrigation standard. Low turbidity (0.5 g/l) and a moderate watering rate (3000 m^3/ha) leads to 1500 kg/ha silt accumulation. The materials held in suspension by the rivers have a significant influence on the quality of the irrigation water. The richest in nutrients are fine silts (ø 0.005 mm), but they lead to lower

soil permeability. Silt intake may be an important factor for improving saline soils and those with sandy texture. Research carried out between 2011 and 2012 aimed to determine the effect of drip irrigation on several physical features of soil, such as bulk density (BD), total porosity (TP) and compaction degree (CD), as well as several chemical features such as the soil soluble salts content mg/100g soil, the content in mobile ($N\text{-}NH_4$ + $N\text{-}NO_3$) (ppm), P and K.

2. Materials and Methods

The experimental plots were located at the Belciugatele, Moara Domnească Teaching Farm, and included the study of two species: apricot grafted on Mirobolan and apple grafted on M9. The places belong to the Romanian Plain relief subdivision Vlăsiei, in the transition from steppe to forest area. Mostly, the relief is flat, with small depressions of different shapes and sizes. The groundwater is located at 6 to 10 m depths. The annually average rainfalls were 318.21 l/m^2 in 2011 and 401 l/m^2 in 2012. Both values are under the multiannual average of the 1960-2010 period (610.91 l/m^2). The soil under the experiments was typical reddish preluvosoil (according to SRTS-2012) or Chrome Luvisol (according to WRB-ST-1998). Table 1 presents soil chemical analysis (according to ICPA METHODOLOGY, 1987) [21], and show a weak acidic soil reaction with values ranging from 5.82 to 6.19 (pH units). Humus content was low (from 1.20 to 2.10%) in topsoil (0-72 cm, corresponding to the sequence of horizons Ap-AB), and very low (0.36 to 0.60%) at the bottom of the soil profile (72-150 cm).

Table 1. The main chemical properties of the soil - Moara Domnească

Level	Depth cm	pH_{H2O} units pH	Humus (Corg x 1.72) %	SB me/100 g soil	Ah %	*T=**SB+***Ah	****V%
Ap	0-16	6.12	2.10	13.61	6.57	20.18	67
Apt	16-29	5.82	1.92	13.39	6.33	19.72	68
Am	29-40	6.19	1.80	15.98	4.30	20.28	79
AB	40-72	6.00	1.20	20.09	2.60	22.69	89
Bt_1	72-93	6.02	0.60	21.09	2.53	23.62	89
Bt_2	93-130	6.04	0.36	22.03	1.70	23.73	93
Bt_3	130-150	6.18	0.36	22.03	1.70	23.73	93

*T = Cation exchange capacity; **SB=Sum of exchangeable cations ***Ah = Hydrolitic acidity; V%= Percentage of base saturation

The orchards were founded in 2004 and the tree planting distances were: 5 x 4 m for apricot and 4 x 3.5 m for apple. The experiment was bifactorial: factor A = variety (apricot: a1 – ´Dacia´, a2 – ´Comandor´, a3 – ´Tudor´; apple: a1-´Romus 3´, a2-´Generos´, a3-´Ionathan´), factor B = hydric regime (b1-non-irrigated Control - Ct, b2- drip irrigation 4l/h, placed after the linear method with 5 trees/variant). The soil physical properties considered were: bulk density (BD – g/cm^3), total porosity (TP - %v/v), compaction degree (CD - %v/v) and were analyzed according to the ICPA METHODOLOGY, 1987 [21]. Soil samples were collected in metal cylinders (Figure 1) from two depths (0-20 and 20-40 cm), from the water regime variants: b1 (non-irrigated) and b2 (irrigated) (Figure 2), and from the three varieties: Romus 3 - a1, Generos - a2 and Ionathan - a3 in case of apple orchard, and Dacia – a1, Comandor – a2 and Tudor – a3 in case of apricot orchard (Figure 3). The data was interpreted according to the ICPA METHODOLOGY, 1987 [21]). There were determined also several chemical characteristics of the soil, as follows: the soluble salts content (mg/100g soil) determined in watery extract 1:5; the nitric nitrogen ($N\text{-}NO_3$) (ppm) and the ammonium nitrogen ($N\text{-}NH_4$) (ppm) determined by the ammonium acetate-lactate method; the mineral nitrogen determined by summing of $N\text{-}NO_3$ (ppm) and $N\text{-}NH_4$ (ppm); the contents of mobile phosphorus and mobile potassium that can be

extracted from acetate lactate. All the chemical characteristics were analyzed according to the ICPA METHODOLOGY, 1987 [21].

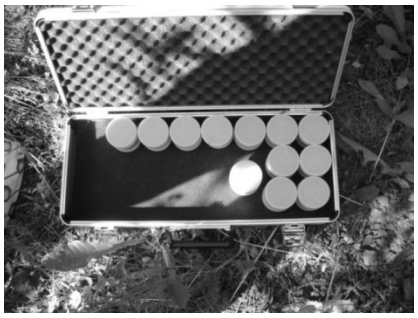

Figure 1. Metal cylinders used in soil sampling

Figure 2. Soil sampling from the water regime variants: b1-non-irrigated (left) and b2 - irrigated (right) in apple orchard

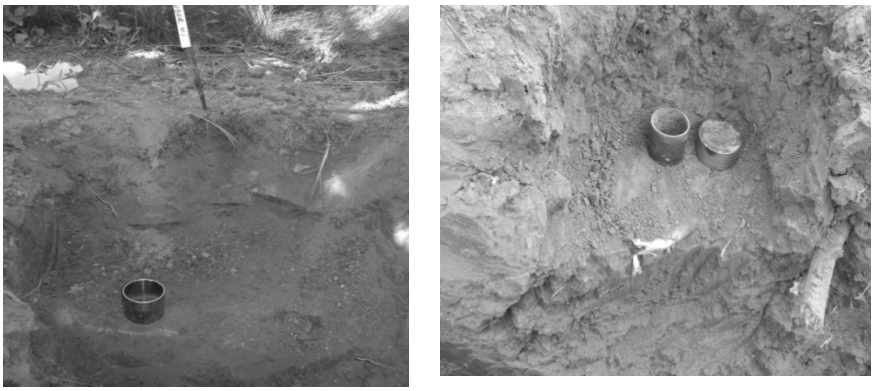

Figure 3. Soil sampling from the water regime variants: b1-non-irrigated (left) and b2 - irrigated (right) in apricot orchard

3. Results and discussions

The effect of drip irrigation on the soil physical properties

The research carried out between 2011 and 2012 aimed to observe the effect of drip irrigation on several physical properties of soil, such as: bulk density (BD), total porosity (TP) and compaction degree (CD) (Table 2). The values of bulk density (BD) indicating the soil loosening or compaction in the non-irrigated variant (b1) at 0-20 cm depth, ranged between the limits, i.e. 1.24 (moderately loose) – 1.45 g/cm^3 (slightly compact). As for the 20-40 cm

depth, the bd values varied within a narrow interval, i.e. between 1.57 (moderately compact) and 1.62 g/cm^3 (moderately compact), in the irrigated variant (b2), bulk density (BD) values ranged between 1.36 (slightly aerated, not compact) and 1.45 g/cm^3 (slightly compact) at 0-20 cm depth, and between 1.60 (moderately compact) and 1.64 g/cm^3 (moderately compact) at 20-40 cm depth. This shows that the compaction level increases with depth in both experimental variants (table 2). The bulk density increase at 20-40 cm depth is closely determined by the increase of clay content within the soil profile (Table 2). The arrangement of the solid soil particles, together with bulk density, can be expressed by the total soil porosity (TP). This feature provides information about the loosening and water movement potential within the soil (e.g. Higher values of TP means that the soil has high water retention capacity, high permeability and good loosening). As for soil characterization in terms of total porosity (TP), the values of this parameter were inversely proportional to bulk density. It is noteworthy that in variant b1 (non-irrigated) at 0-20 cm depth, the porosity was high, i.e. between 49.5 and 52.5 % v/v, when compared to variant b2 (irrigated), which had medium values, between 44.5 and 47.5% v/v. In both experimental variants (b1 and b2), at 20-40 cm depth and at the same water rate, the TP values ranged between 38-39% v/v in case of b1 variant, and from 37.5 to 39% v/v in case of b2 variant (Table 2). These values correspond to a low total porosity and thus to a system lacking loosening and water movement. In conclusion, we can say that irrigation can influence the state of soil settlement. As concerns the soil compaction degree (CD), the table from below indicates that the apple-tree orchard is located on a non-compacted soil. In b1 variant (non-irrigated), at 0-20 cm depth, the CD limits varied between -1.16 (non-compaction) and 4.83%v/v (slightly compaction), whereas in b2 variant (irrigated) the values ranged between -9.14 and -3.15%v/v, which correspond to a non-compacted soil (Table 2). The CD values corresponding to the compacted layer were recorded at 20-40 cm depth and were found in both experimental water regime variants, b1 (-24.81..-21.84%v/v) and b2 (-25.795..-22.83%v/v) (Table 2).

Table 2. The main soil physical properties of the apple orchard

	Experimental variant	**Depth (cm)**	**BD (g/cm^3)**	**TP (% v/v)**	**CD (% v/v)**
Romus 3 (a1)	b1 Ct	0-20	1.325	49.5	-1.1545
		20-40	1.595	39	-22.83
	b2	0-20	1.435	45.5	-9.145
		20-40	1.64	37.5	-25.795
Generos (a2)	b1 Ct	0-20	1.31	49.5	-1.16
		20-40	1.575	39.5	-21.84
	b2	0-20	1.455	44.5	-3.155
		20-40	1.615	38	-24.81
Ionathan (a3)	b1 Ct	0-20	1.24	52.5	4.83
		20-40	1.62	38	-24.81
	b2	0-20	1.365	47.5	-5.15
		20-40	1.6	39	-22.83

The values of bulk density (BD), reflecting the soil loosening or compaction in the non-irrigated variant (b1) at 0-20 cm depth, varied between the limits of 1.2 (moderately loose) - 1.45 g/cm^3 (slightly compact), and between a relatively narrow interval of 1.56 (moderately loose) - 1.65 g/cm^3 (moderately compact) at 20-40 cm depth. In case of the irrigated variant (b2), bulk density (BD) values ranged between 1.28 (slightly loose non-compacted) and 1.37 g/cm^3 (slightly loose non-compacted) at 0-20 cm depth, and between 1.61 (moderately loose) and 1.63 g/cm^3 (moderately loose) at 20-40 cm depth. It results that the compaction level increases with depth in both experimental variants on the hydrological regime (Table 3). In variant b1 (irrigated) at 0-20 cm depth, the porosity values ranged between 44.5% v/v

(medium) and 54.5% v/v (high). These values were close to those obtained in variant b2 (irrigated), which recorded values between 46 (medium) and 51% v/v (high) (Table 3). In both experimental variants (b1 and b2) at the 20-40 cm depth, the values varied between 21-40% v/v (b1) and from 37.5 to 38.5% v/v (b2) (Table 3). These values correspond to a very low total porosity, indicating that the systems lack of proper aeration and water movement. In conclusion, it can be noticed a decrease of the total soil porosity within the soil profile due to soil compaction by applying irrigation. Analysis of the results on soil compaction degree (CD) from the table below showed that the soil from the apricot orchard was non-compacted. In variant b1 (non-irrigated), at 0-20 cm depth, the values ranged between -11.11% v/v (loose) and 8.82% v/v (slightly compacted), whereas in b2 variant (irrigated) the values correspond to a non-compacted and slightly compacted soil, ranging from -8.15% v/v and 1.83% v/v (Table 3). At the 20-40 cm depth, there was noticed that the compacted soil shifted to non-compacted soil in both the experimental variants of the water regime, i.e. from -27.77% v/v to -2.85% v/v in b1 (non-irrigated) and from -25.8% v/v to 0.25% v/v in b2 (irrigated) (Table 3).

Table 3. The main soil physical properties of the apricot orchard

Variety (a)	Experimental variant	Depth (cm)	BD (g/cm^3)	TP (% v/v)	CD (% v/v)
Dacia (a1)	b1 Ct	0-20	1.45	44.50	-11.11
		20-40	1.56	40	-2.85
	b2	0-20	1.37	46.00	-8.15
		20-40	1.63	37.50	-25.80
Comandor (a2)	b1 Ct	0-20	1.2	54.5	8.82
		20-40	1.59	21.5	-21.83
	b2	0-20	1.37	47.5	-5.15
		20-40	1.61	38.5	-23.82
Tudor (a3)	b1 Ct	0-20	1.25	50.5	0.84
		20-40	1.65	36.5	-27.77
	b2	0-20	1.28	51	1.835
		20-40	1.62	37.5	-0.25

The effect of drip irrigation on the soil chemical properties

The research carried out between 2011-2012 aimed to measure the following soil chemical properties: the content in soluble salts as mg/100g soil, the N content ($N\text{-}NH_4$ + $N\text{-}NO_3$) (as ppm), mobile P and K contents (Tables 4, 5). Analysis of the soil soluble salts content (mg/100g soil) showed that the values ranged between 32 and 36 mg/100g soil, were uniformly distributed in all the experimental variants studied, and correspond to non-saline soils (Table 4). The nitrogen sources for plant nutrition are both ammonia and nitric forms, and depend on the species and plant age, soil reaction, the soil buffer capacity and the presence or absence of certain cations and anions. Based on a formula used by the ICPA METHODOLOGY, 1987 [21], we calculated the amount of nitrogen available in plants ($N\text{-}NH_4$ + $N\text{-}NO_3$). The obtained result was a very low N content in all variants studied, significantly below the standard limit of 40 ppm (Table 4). Thus, in the case of Romus 3 and Ionathan varieties, in the irrigated variant (b2), the nitrogen amount at 0-20 cm depth was higher, reaching values of 27.55 ppm (Romus 3) and 28.5 ppm (Ionathan). As for the Generos variety at the same depth, the N content was only 8.55 ppm, and higher (18.05 ppm) in the irrigated variant (b2) at 20-40 cm depth (Table 4). The values of accessible (mobile) phosphorus indicated a medium content in the first 20 cm depth in b2 variant (irrigated), i.e. 25.26 ppm for Romus 3 variety and 30.79 ppm for Generos variety, and a high content (37.37 ppm) for Ionathan variety. At the other depth (20-40cm), the b2 variant (irrigated) recorded a low content in phosphorus; in b1 variant (non-irrigated) only in Ionathan variety the content

was medium (28.37 ppm) at 0-20 cm depth and low at 20-40 cm depth (13.15-15.22 ppm) (Table 4). The evaluation of accessible (mobile) K^+ was based on the values calculated for 0-40 cm depth, and ranged between the limits of 25-40 ppm, which can be classified as low contents (Table 4).

Table 4. The main soil chemical properties of the apple orchard

Variety (a1)	Experimental variant	Depth (cm)	Soluble salts mg/100g soil	N (N-NH_4 + N-NO_3) (ppm)	P_{AL}(ppm)	K_{AL}(ppm)
Romus 3 (a1)	b1 Ct	0-20	35	17.1	28.37	40
		20-40	35	15.2	15.22	25
	b2	0-20	35	27.55	25.26	35
		20-40	35	21.85	14.53	25
Generos (a2)	b1 Ct	0-20	35	8.55	9.72	40
		20-40	35	9.5	13.15	25
	b2	0-20	35	8.55	30.79	40
		20-40	35	18.05	16.95	25
Ionathan (a3)	b1 Ct	0-20	32	10.45	26.98	25
		20-40	36	13.3	15.22	25
	b2	0-20	36	28.5	37.37	35
		20-40	35	19.0	15.57	25

The soil soluble salts content mg/100g soil) varied between 32 mg/100g soil and 42 mg/100g soil, were uniformly distributed in all the experimental variants under studied and correspond to non-saline soil (Table 5). Also in case of the apricot varieties, the N content was very low in all the variants, significantly below the standard limit of 40 ppm (Table 5). Comparing the variants of the water regime, were noted differences both between the depths and the varieties. Thus, the N content values were higher (i.e. 21.85 ppm) in b1 variant (non-irrigated) at 20-40 cm depth for the Tudor variety, followed by 17.1 ppm, at 0-20 cm depth in b2 (irrigated) for Dacia and also Tudor varieties in the same experimental variants. The very low values of nitrogen, between 4.75 ppm and 21.85 ppm, indicate the low N content in soil and as a result is recommendations for N-based fertilizer application (Table 5). The values of the accessible (mobile) phosphorus content were low and very low for the two depths in both experimental variants (b1 and b2). Only the Tudor variety recorded a medium level, i.e. 22.84 ppm (b1) and 20.07 ppm (b2) at 0-20 cm depth in both experimental variants (b1 and b2) (Table 5). The evaluation of accessible (mobile) K^+ content at 0-40cm depth, showed that the values ranged between the limits (25-35ppm), which indicate an extremely low K content (Table 5).

Tabel 5. The main soil chemical properties of the apricot orchard

Variety (a)	Experimental value	Depth (cm)	Soluble salts mg/100g sol	N-(NH_4 + N-NO_3) (ppm)	P_{AL} (ppm)	K_{AL} (ppm)
Dacia (a1)	b1 Ct	0-20	35	15.2	13.15	35
		20-40	32	9.5	12.46	25
	b2	0-20	32	17.1	13.15	25
		20-40	35	12.35	6.69	30
Comandor (a2)	b1 Ct	0-20	30	4.75	12.46	35
		20-40	30	6.65	6.95	30
	b2	0-20	42	5.7	13.15	30
		20-40	36	15.2	9.69	35
Tudor (a3)	b1 Ct	0-20	33	14.3	22.84	30
		20-40	35	21.85	14.88	35
	b2	0-20	36	17.1	20.07	30
		20-40	36	14.25	14.19	25

4. Conclusions

The soil compaction increases with depth in both experimental variants for both studied species. Bulk density at 20-40 cm depth is higher because of the higher clay content within the soil profile.

Application of irrigation has resulted in a decrease of total soil porosity at deeper layer due to compaction.

The soil compaction degree (CD) values have shown that the upper layer is not compacted.

The soil soluble salts content varied between 32 and 35 mg/100 g soil, in both apple and apricot, without significant differences between variants.

The nitrogen amount ($N\text{-}NH_4$ + $N\text{-}NO_3$) was low in all experimental variants, significantly under the standard of 40 ppm for both species. The b2 variant (irrigated) recorded a slight increase of N content for each variety, when the water regime variants were compared.

The very low values of nitrogen content obtained for both apple and apricot, resulted in recommendations for n-based fertilizer application.

The values of accessible phosphorus content, in b2 variant (irrigated) from apple orchard, varied between medium at 0-20 cm depth and low at 20-40 cm depth. In b1 variant (non-irrigated) the P content was low and medium at 0-20 cm depth, and low at 20-40 cm depth. In case of apricot orchard, the P content was low and medium in both hydric regime variants (b1, b2).

The evaluation of accessible (mobile) K^+ content for both studied species, at 0-40 cm depth, showed that the values ranged between the limits of 25-40 ppm, which indicate a low K content in soil.

References

1. C. AILINCĂI, D. BUCUR, S. CÎMPEANU, D. AILINCĂI, Investigations on the influence of cropping systems and erosion on yield and soil chemical characteristics in the Moldavia plain (North-Eastern Romania), Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Vol.61/2005, Editura Academic Press, ISSN 1454-2382,pp. 298-303 (2005)
2. M. E. AMIRI, E. FALLAHI, A. GOLCHIN, Influence of foliar and ground fertilization on yield, fruit quality, and soil, leaf, and fruit mineral nutrients in apple, Journal of Plant Nutrition, Vol.31, Issue 3, pp515-525 (2008)
3. A. CANARACHE, Procedeu de apreciere a variației rezistenței solului la arat în raport cu umiditatea. Analele Secț. Ped., XXXII, București, pp 99-103 (1968)
4. A. CANARACHE, Modifcarea însuşirilor fizice ale solului prin afânare adâncă, Prod. Vegetală, cereale şi plante tehnice, nr.11, I.C.C.P.T. Fundulea (1978)
5. A. CANARACHE, Fizica solurilor agricole, Editura Ceres, Bucureşti (1992)
6. S. CÎMPEANU S., I. JINGA, I. PLEŞA, C. IGNAT, Cercetări privind influența irigării cu ape de spălare provenite din complexele avicole precum şi a fertilizării organice asupra producțiilor de porumb şi soia în condițiile agriculturii ecologice. Lucrările sesiunii ştiințifice U.S.A.M.V. Bucuresti(1995)
7. S. CÎMPEANU, I. JINGA, I. PLEŞA, Cercetări privind influența irigației cu apă uzată provenită de la „ROMSUINTEST" Periş, şi a fertilizării organo-minerale asupra producției de porumb boabe. Lucrările sesiunii ştiințifice U.S.A.M.V. – Bucureşti (1996)
8. S. CÎMPEANU, I. JINGA, Cercetări privind influența asupra solului şi producției agricole a irigării cu ape uzate provenite din complexul Romsuintest Periş - Lucrarile Sesiunii ştiințifice USAMV, Bucureşti (2000)
9. S. CÎMPEANU, G. MĂRGĂRIT, Influența fertilizării cu nămol de păsări şi a irigării cu apă convențional curată asupra producției de grâu, Lucrarile Sesiunii ştiințifice USAMV, Bucureşti (2001)
10. S.M. CÎMPEANU, E. MANOLE, V. OLARU, M. VOICULESCU, F. ISRAEL-ROMING, T. APOSTOL,Tehnologii de reabilitare si reconstructie ecologica a solurilor contaminate din zona depozitelor de deseuri urbane si industriale, Editura Noua, ISBN 978-606-596-003-9, Bucureşti (2011)
11. E. DUMITRU, P. GUŞ, R. ENACHE, M. DUMITRU, Efecte remanente ale unor practici agricole asupra stării fizice a solului. Editura Risoprint, Cluj-Napoca (1999)

12. F. FILIPOV, D. LUPAŞCU, A. RĂDUCU, B.E. BELDIMAN, Influenţa măsurilor ameliorative asupra însuşirilor morfologice, fizice şi chimice ale solurilor cu exces de umiditate din Podişul Sucevei, Editura Junimea, ISBN 973-37-0679-5 (2001)
13. J. HANAN. Denver water quality. Colorado Flower Growers Association Bulletin, nr.280, Denver, Colorado, USA (1981)
14. M. MIHALACHE, G.V. ROMAN, D.I. MARIN, Effects of irrigation on chemical and physical properties of some soils located in Southern part of Romania. Reuninon IP Agriculture durable et ressources hydriques comparaison europeenne, Thessalonique, Grecia (2004)
15. M. MIHALACHE, L. ILIE, Pedologie - Solurile României. Ed. Dominor, Bucureşti, ISBN 978-973-1838-71-7 (2008)
16. M. MIHALACHE, L. ILIE, Bonitarea terenurilor agricole. Ed. Dominor, Bucureşti, ISBN 973-0-03902-X (2009)
17. M. MIHALACHE, Pedologie. Editura Estfalia, ISBN 978-606-8284-88-0 (2014)
18. I. MUNTEANU şi colab., Status of Soil Mapping, Monitoring, and Database Compilation in Romania at the beginning of the 21st century, European soil bureau (2000)
19. I. PLEŞA, I. JINGA, A. ENE, Irigaţii, desecări şi combaterea eroziunii solului, Curs litografiat, I.A.N.B.Bucuresti (1990)
20. I. PLEŞA, I. JINGA, A. ENE, S. CÎMPEANU, Îmbunătăţiri funciare şi irigarea culturilor. Îndrumător de lucrări practice, Editura AMC, USAMV Bucureşti (2000)
21. *** METODOLOGIA ELABORĂRII STUDIILOR PEDOLOGICE, ICPA (1987).

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Agriculture and Agricultural Science Procedia 10 (2016) 172 – 177

Agriculture and Agricultural Science Procedia

5th International Conference "Agriculture for Life, Life for Agriculture"

In Vitro Embryo Culture of Some Sweet Cherry Genotypes

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Abstract

Due to a very low germination percentage of some sweet cherry's hybrids seeds in the breeding process, a new research using embryo rescue method was started. The goal of this work was to establish an efficient propagation protocol using the embryo culture in vitro. The sweet cherry seeds comes from eight hybrid genotypes as follows: 13.3.M (Giant Red x Early Red), 13.4.M (Giant Red x New Star), 13.8.M (New Star x Kordia), 13.9.M (New Star x Van), 13.11.M (New Star x Burlat), 13.12.M (New Star x Early Red), 13.18.M (Van x New Star), 13.19.M (Van x Early Red). The biological material was sterilised using alcohol 96% (w/v) and $Ca(OCl)_2$ in different concentrations. Four culture mediums with macro and microelements were tested (based on Lee & Fossard and Murashige & Skoog) reacting differently according to the genotype. The LF medium (V1) offers superior nutrition for biological material in terms of macro and microelements and vitamins, comparing to V3 culture medium, constituted as MS medium. The germination period in vitro conditions indicate V1 medium with the lowest infection rate 26.3%. The most vigorous plants obtained in vitro were registered on LF medium.

Peer-review under responsibility of the University of Agronomic Sciences and Veterinary Medicine Bucharest

Keywords: sweet cherry; hybrids; embryo culture; germination; in vitro culture medium

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Peer-review under responsibility of the University of Agronomic Sciences and Veterinary Medicine Bucharest
doi:10.1016/j.aaspro.2016.09.049

1. Introduction

In vitro embryo culture is utilised in fruit growing to obtain viable plants from some varieties with low germination of seeds, those who comes from distant hybridisations or crossings between early varieties (Stanys V., 1998; Dulic J. et al., 2016) especially in stone fruits (Turkey H.B., 1933, 1934; Balla et al., 1996). To solve this problem, often is required the embryo rescue method in aseptic culture medium conditions (Sanders et al., 1963; Raghavan, 1980; Bridgen, 1994).

Modern solutions are always taken into account in order to efficient propagate valuable biological material (Standardi A., 2012). The plant regeneration through embryo culture is in some author's opinion also influenced by environmental conditions of seed production (Chiriacula M. et al., 1985).

But, a decisive factor remains *in vitro* culture medium. More mediums were tested including Murashige & Skoog (1962), Gamborg's (1968) B5 (Rizzo et al., 1998) White and M&S modified (Stanys V., 1998). The evolution of seed germination and plant regeneration differed depending on their components, formulation and of course experienced genotypes.

In this work, we proposed to establish an optimal culture medium for rescue the sweet cherry hybrids by embryo culture technique as a solution for hybrid seeds germination.

2. Materials and methods

The biological material was represented by sweet cherry seeds of eight hybrid combinations as follows: Giant Red x Early Red (13.3.M), Giant Red x New Star (13.4.M), New Star x Kordia (13.8.M), New Star x Van (13.9.M), (13.11.M) New Star x Burlat, 13.12.M (New Star x Early Red), 13.18.M (Van x New Star), 13.19.M (Van x Early Red). They have been obtained in 2013 by cross pollination in the experimental sweet cherry orchard of Istrita, Buzau Nursery of the University of Agronomic Sciences and Veterinary Medicine (UASVM), Bucharest. At the ripening time, the seeds were collected from hybrid fruits and stored in the Breeding Laboratory of UASVM Bucharest. All the biological material was preserved according to Cociu V and Oprea S (1989) method. Precisely, the sweet cherry hybrid seeds were rinsed, disinfected with 0.1% Derosal solution for 10-15 minute, placed in polyethylene bags together with a suitable amount of sand humidified with sterile water. Stone bags were kept in refrigerated temperatures of 4°C.

In March 2014, all the seed were sown individually, in peat substrate, using two liter pots. The most part of the seeds had germinate (92.25%) and start growing, later being planted in the field. The rest of ungerminated seeds (7.75%) were extracted from pots and sent on the 22th of January, 2015 to Micropropagation Laboratory of the Research Institute of Fruit Growing Pitesti Maracineni for embryo culture.

2.1. Culture mediums

Four variants of culture mediums have been set up based on Lee Fossard (1977) and Murashige & Skoog (1962) (Table 1).

Table 1. Culture medium variants used in embryo culture for sweet cherry hybrid seeds

Components	V1	V2	V3	V4
Macroelements	L F (1977)	L F (1977)	MS (1962)	MS (1962)
Microelements	L F (1977)	L F (1977)	MS (1962)	MS (1962)
Vitamins	L F (1977)	L F (1977)	MS (1962)	MS (1962)
Chelates (NaFeEDTA)	32 mg/l	32 mg/l	32 mg/l	32 mg/l
Phytohormons:				
-IBA	5 mg/l	-	5 mg/l	-
-BAP	20 mg/l	-	20 mg/l	-
Sucrose	20 g/ l	20 g/ l	20 g/ l	20 g/ l
Agar	6 g/l	6 g/l	6 g/l	6 g/l

2.2. Disinfection of biological material

Seeds without endocarp were disinfected by rinsing with the following steps: Rinsing with water and detergent TWEEN 20, Rinse in water, Immersion in alcohol 96% (w/v) = 2 min, Immersion in Ca(OCl)2 = 3 min, Three rinsing x 5 min with distillated and sterilised water.

2.3. Growth chamber conditions

T=21^{0}C, Photoperiod: 10 days dark and then 8 hours dark and 16 hours light

3. RESULTS AND DISCUSSIONS

The first assessments were made at three weeks of culture. A first observation was that, after inoculation of the seeds on aseptic culture medium (Figure 1), targeting the processes of growth and development, it often occurs loses a significant amount of biological material due to infections (Table 2).

Fig. 1. The seeds after inoculation on aseptic culture medium

Although the embryo is protected by endocarp, the sensitivity of tissues that come in contact with the sterilization agents is high. Therefore, an impediment to achieving high rates of vitro plants represents or necrosis embryos after sterilization or infections (Table 2).

Table 2. Losses of biological material due to infections

Culture medium	Biologic material type	Giant Red		New Star				Van		Total
		E. Red	N.Star	Kordia	Van	Burlat	E. Red	N. Star	E. Red	
		13.3. M	13.4. M	13.8.M	13.9.M	13.11.M	13.12.M	13.18.M	13.19.M	
V1	Nr.of seeds inoculated	20	30	25	30	25	30	30	30	220
	Nr.of seeds noninfected	18	27	18	23	10	23	25	18	162
	Nr.seeds ungerminated	5	0	9	0	7	0	2	8	31
	Nr.of explants	10	16	5	15	8	15	15	7	91
V2	Nr.of seeds inoculated	20	30	25	30	25	20	30	30	210
	Nr.of seeds noninfected	12	9	1	5	8	8	8	7	58
	Nr.seeds ungerminated	11	8	13	14	13	7	9	9	84
	Nr.of explants	5	7	3	3	2	7	6	6	39
V3	Nr.of seeds inoculated	20	20	20	20	20	20	20	20	160
	Nr.of seeds noninfected	10	8	7	6	4	8	9	4	56
	Nr.seeds ungerminated	9	7	9	6	13	7	7	13	70
	Nr.of explants	7	8	6	8	4	8	9	4	24
V4	Nr.of seeds inoculated	20	20	20	20	20	20	20	20	160
	Nr.of seeds noninfected	5	5	4	4	2	5	6	2	33
	Nr.seeds ungerminated	13	10	13	10	13	10	9	16	94
	Nr.of explants	5	6	4	7	2	6	6	2	38

The results obtained showed that under the given circumstances, the embryo culture had been influenced by two factors: the genotype and the culture medium. Analysing the behaviour of the genotypes on V1 culture medium (Figure 2), it shows that here was recorded the lowest infection rate of 26.3% and at other variants (V2, V3 and V4) it was located between 65 to 79.3%. Also on this culture medium (V1) was obtained the highest number of explants from all hybrid combination (91 explants) with 5 explants at 13.8.M and 16 explants at13.4.M (Figure 3).

The number of ungerminated seeds varied between 0 (13.4.M, 13.9.M and 13.12.M) to 9 ungerminated seeds (13.8.M) (Figure 2).

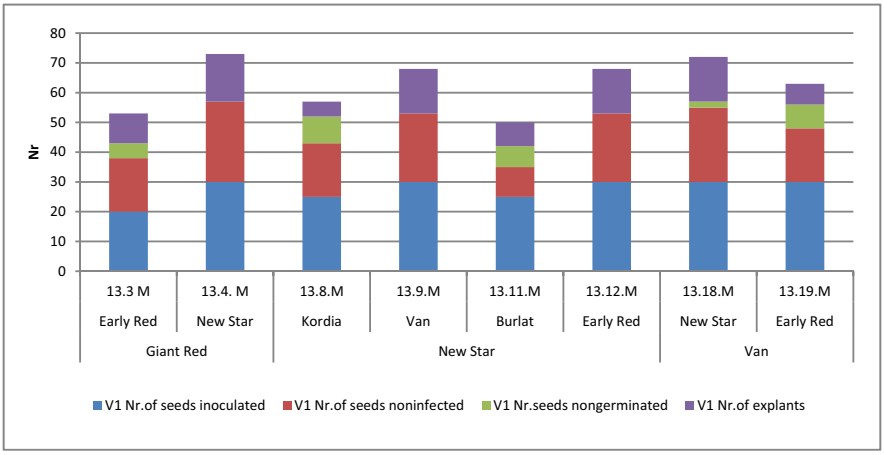

Fig. 2. Sweet cherry genotypes evolution on V1 culture medium

On variant V2 (Figure 3), good results were recorded also by 13.4.M, 13.12.M, 13.18.M and 13.19.M genotypes with a 6-7 explants. Genotypes 13.8.M, 13.11.M and 13.9.M recorded between 13 and 14 embryos that not started.

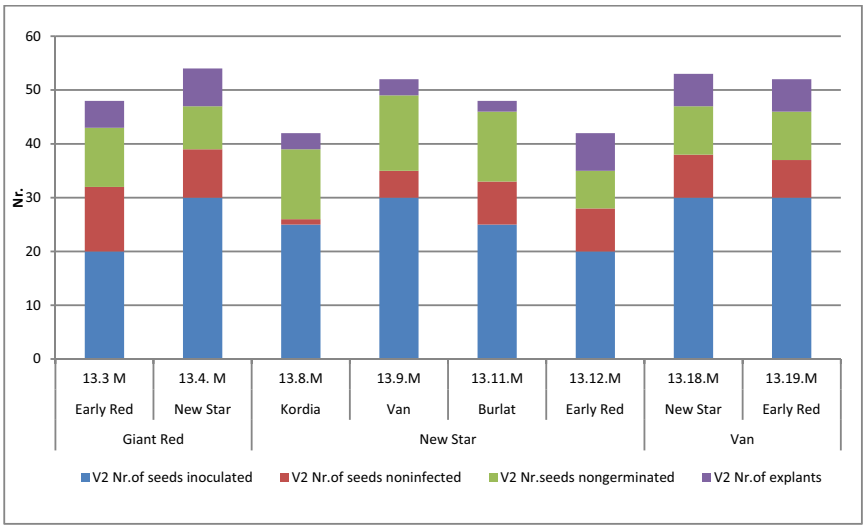

Fig. 3. Sweet cherry genotypes evolution on V2 culture medium

Evolution of sweet cherry genotypes on V3 medium confirms also that genotype 13.4.M, 13.18.M, 13.9,M and 13.12.M, record a higher number of explants (8 and 9 explants) (Figure 5). Total number of ungerminated seeds is 70 on the present culture medium, the worst situation have been noticed in the case of 13.11.M and 13.19.M genotypes which recorded each 13 ungerminated seeds (Figure 4).

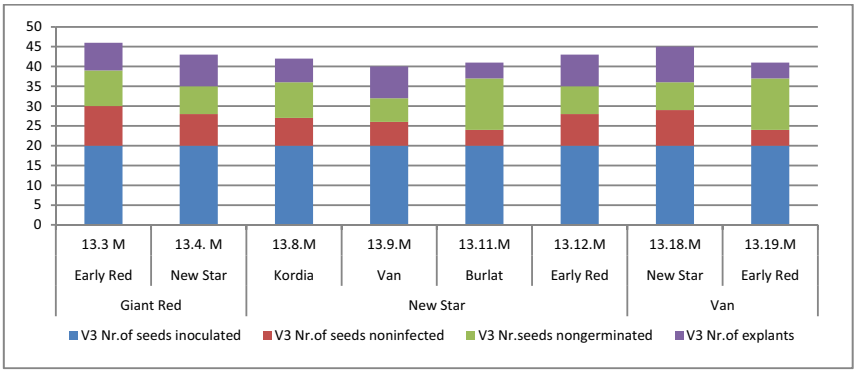

Fig. 4. Sweet cherry genotypes evolution on V3 culture medium

Analysing the same factor - genotype (Figure 5) it is revealed that the number of explants is 2 at 13.11 M, 13.19 and 7 at 13.9 genotype. The total number of ungerminated embryos on this culture medium is 94, with 9 at 13.18.M genotype and 16 at 13.19.M genotype (Figure 5).

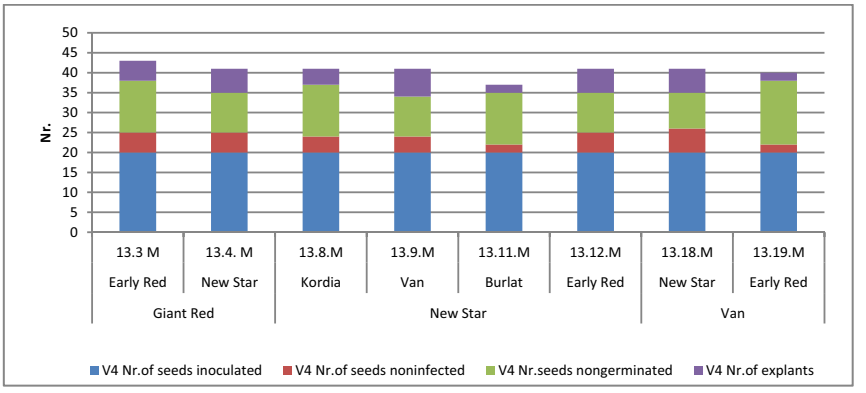

Fig. 5. Sweet cherry genotypes evolution on V4 culture medium

The culture mediums by content of the macroelements, microelements, vitamins and hormonal balance, had an important influence on embryo culture. The LF medium (V1) offers superior nutrition for biological material in terms of macro and microelements and vitamins, comparing to V3 culture medium, constituted as MS medium (Figure 6).

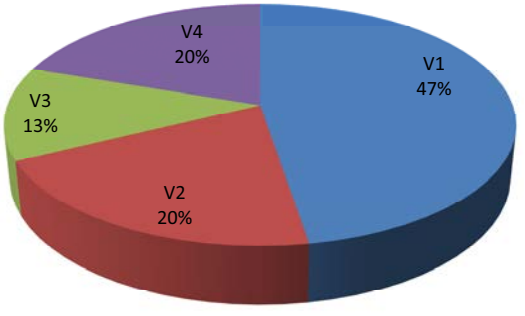

Fig. 6. Efficiency of culture mediums on sweet cherry genotypes embryo rescue

The germination period *in vitro* conditions indicate V1 medium with the shortest infection rate 26,3%. The most vigorous plants obtained *in vitro* were registered on V1 medium (Figure 7).

Fig. 7. Vigor of plants on V1 medium

4. CONCLUSIONS

The culture mediums by content of the macroelements, microelements, vitamins and hormonal balance, had an important influence on embryo culture. The MS medium (V1) offers superior nutrition for biological material in terms of macro and microelements and vitamins, comparing to V3 culture medium, constituted as MS medium.

The germination period *in vitro* conditions indicate LF medium (V1) with the lowest infection rate 26.3%.

The most vigorous plants obtained *in vitro* were registered on LF medium (V1).

References

Balla, I., Brozik, S., 1996. Embryo Culture of Sweet Cherry Hybrids. Acta Hortic. 410, DOI: 10.17660, 385-386.

Bridgen, M.P., 1994. A Review of Plant Embryo Culture. Hortscience. 29, 1243-1246.

Chiriacula, M., Rudi, E., Popescu O., Popescu, A., 1985, Rezultate și perspective în obținerea soiurilor extratimpurii și timpurii la cireș și vișin prin embriocultură, Lucrările celui de al III-lea Simpozion național de culturi de celule și țesuturi vegetale. EXTRAS.

Cociu, V., Oprea, S., 1989, Metode de cercetare in ameliorarea plantelor pomicole (in Romanian), Ed. Dacia, Cluj-Napoca.

De Fossard, R.A., 1977. Tissue culture in horticulture – a perspectives Acta Hortic. 78, 455-459.

Dulić, J., Ognjanov, V., Ercisli, S., Miodragović, M., Barać, G., Ljubojević, M., Dorić, D., 2016. In vitro Germination of Early Ripening Sweet Cherry Varieties (*Prunus avium* L.) at Different Fruit Ripening Stages, Erwerbs-Obstbau Journal, DOI 10.1007/s10341-016-0265-y, online ISSN 1439-0302, Springer Berlin Heidelberg, 1-6.

Gamborg, O. L., Miller, R.A., Ojima, K., 1968. Nutrient requirements of suspension cultures of soybean root cells, Expt. Cell Res., 50, 151–158

Murashige, T., Skoog, F., 1962. A revised for rapid growth and bioassay with tabacco tissue cultures; Physiol Plant., 15, 473–497.

Raghavan, V., 1980. Perspectives in plant cell and tissue culture. Rev. Cytol., Suppl. 11B, 209–240.

Rizzo, M., Bassi, D., Byrne, D., Porter, K., 1998. Growth of Immature Peach [Prunus persica (L.) Batsch.] Embryos on Different Media, DOI: 10.17660/ActaHortic.1998.465.15, Acta Hortic. 465, 141-144.

Sanders, M.E., Ziebur, N.K., 1963, Recent advances in the embryology of angiosperms, Intl. Soc. Plant Morphol, Delhi: 297- 326

Standardi A., 2012, Encapsulation: promising technology for nurseries and plant tissue laboratories. AgroLife Scientific Journal, Vol 1, Issue 1, ISSN-L 2285-5718, 48-54.

Stanys, V., 1998. In vitro techniques to increase the output of cherry seedlings from early-ripening parents, Acta Hortic. 468, 203-208

Turkey, H. B., 1933. Artificial culture of sweet cherry embryo. Jour. Hered. 24, 7-12.

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Agriculture and Agricultural Science Procedia 6 (2015) 157 – 164

Agriculture and Agricultural Science Procedia

"ST26733", International Conference "Agriculture for Life, Life for Agriculture"

Analysis of Some Phenolic Compounds and Free Radical Scavenging Activity of Strawberry Fruits During Storage Period

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Abstract

The objective of this study was to evaluate the quantity of some phenolic compounds and free radical scavenging activity of strawberries (*Fragaria x ananassa* Duch.) during storage period at different temperatures. Freshly harvested of three strawberry cultivars fruits ('San Andreas', 'Benicia' and 'Albion') were placed in polyethylene bags and kept in refrigerated (4°C) and frozen (-85°C) conditions for 7 days. Therefore, the analyses performed were applied on the fruits in stages: fresh, refrigerated and frozen. With regard to the bio compounds analysis, we mention: total phenolic content (TPC) expressed as g gallic acid equivalents (GAE), total flavonoid content (TFC) expressed as g of rutin equivalents (RE), and free radical scavenging activity expressed as 50% effective concentration (EC50) (mg/ml). With regard to the TPC in all analyzed storage stages have revealed 'San Andreas' cultivar with a maximum content of 0.326 g GAE / 100 g fresh weight (FW). Also, the same cultivar recorded high levels of total flavonoid content of 0.424 g RE / 100 g FW, all at the end of refrigeration period. In terms of free radical scavenging activity, 'Benicia' cultivar highlighted the best results EC50 = 3.094 mg/ml.

Peer-review under responsibility of the University of Agronomic Sciences and Veterinary Medicine Bucharest

Keywords: bioactive compounds; biochemical changes; strawberry cultivar.

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Peer-review under responsibility of the University of Agronomic Sciences and Veterinary Medicine Bucharest
doi:10.1016/j.aaspro.2015.08.053

1. Introduction

In recent years, many research studies highlight the role and the importance of fruit and vegetables consumption (Wang et al., 2014). Despite this fact large proportions of population do not meet the World Health Organization recommendations (Ness and Powles, 1997; Krølner et al., 2011). Fruits and vegetables consumption is important because prevents the occurrence of the chronic diseases, including type 2 diabetes (Muraki et al., 2013), obesity Tetens and Alinia (2009) and overall hence the quality of life (Patthamakanokporn et al., 2008). This is due to the abundance in composition of fiber, antioxidants, and other bioactive compounds with beneficial health effects (Muraki et al., 2013). With regard to the fruits, they represent a valuable source of polyphenols which contribute to the nutritive quality, and also giving some organoleptic properties. Their composition differs from one cultivar to another, also being influenced by biotic and abiotic factors (Garcia-Salas et al., 2010). One such example is represented by the berries. Lately, they have gained much attention due to their potential source of valuable bioactive compounds (Mahmood et al., 2012). Among them, strawberries are the most studied berries (Amaro et al., 2012). Their fresh consumption brings real contribution to oxidative status through their high content of phenolic compounds. One of the real problems of this fruits is that they are highly perishable, and suffer post harvest changes both fresh and during storage conditions (Peano et al., 2014). In the same trend, the storage conditions directly affect their nutritional properties including phenolic compounds and free radical scavenging activity (Cordenunsi et al., 2005). Therefore, in this paper we looked at how the different storage conditions influenced the phenolic compounds and free radical scavenging activity for three strawberries cultivars 'San Andreas', 'Benicia' and 'Albion' after harvesting.

2. Research methods

The biological material was represented by the fruits provided from the three cultivars of strawberry (*Fragaria* x *ananasa* Duch.). Fruits provided from the 'San Andreas', 'Benicia' and 'Albion' cultivars were harvested until the end of May and were kept in 3 different storage conditions according to Table 1.

The fruits were milled and were subjected to the extraction method adapted after Cheel et al. (2006) with clorhidric acid (HCl) 1% in methanol (MeOH), for 30 minutes on ice bath. The raport of the extraction was 1:5. The extracts were shaken and left at room temperature for 48 hours. The extracts were then filtered through Whatman paper, the supernatant was then subjected to the following analyses.

Table 1. Storage conditions of strawberries

Cultivars	Fresh	Refrigerated	Freezer(-85°C)
San Andreas	3 days (t_0)	t_0 + 7 days	t_0 + 7 days
Benicia			
Albion			

The total phenolic content from fruits was determined using a method adapted after Singleton et al. (1999). The proper diluted extracts were oxidized with the Folin–Ciocâlteu reactive and neutralized with sodium carbonate 30%. After 45 minutes, the samples absorption was recorded at the wavelength (λ) of 750 nm. Quantifying the results were based on the sample curve of the gallic acid, based on the equation:

$$Abs = 0.00968 + 0.000167857 \times C_{\text{gallic acid}}, R = 0.996, p < 0.05.$$

The results were then shown as g of gallic acid equivalents (GAE) / 100 g fresh weigh (FW).

The flavonoid content was determined using a method adapted after Tuker et al. (2012) having as reference the rutin. The diluted extracts were then mixed with a sodium nitrite ($NaNO_2$) 5%. After 5 minutes was added aluminium chloride ($AlCl_3$) 10%, following that after another 6 minutes NaOH of 1M concentration and water were added too.

After 45 minutes, the samples absorption was measured at the wavelength (λ) of 510 nm. The results were then obtained based on the sample curve of the rutin:

$Abs=-0.0068 + 0.000627455 \times C$ rutin, $R = 0.999$, $p < 0.05$.

Results were then shown as g of rutin equivalents (RE) /100 g fresh weigh (FW).

The free radical scavenging activity of the extracts was determined using stabile radical 2,2 diphenyl-1-picrylhydrazyl (DPPH•), after a method adapted after Fen Shyur et al. (2005). The inhibitory effect of DPPH was calculated using to the following formula:

$Abs=-0.0068 + 0.000627455 \times C$ rutin

IC50 (EC50) represents the level where 50% of the radicals were scavenged by strawberries extracts.

A general linear model, Bonferoni and Tukey tests were used for the comparison of means for the content of bio compounds between groups, using Statistical Package for Social Science (SPSS version 21.0). The statistical significance was considered for the probability value of difference $p < 0.05$. The obtained results were expressed as mean values ± standard error. Microcal Origin version 6.0 software was used for the charts design.

3. Results and Discussions

The results of the determination of total phenolic content for the cultivars in all three analysed stages are shown in Table 2. With regard to the fresh strawberries, the maximum value was recorded by *'San Andreas'* cultivar with the highest value of 0.289 g GAE / 100g FW, followed by *'Albion'* (0.230 g GAE / 100g FW), and *'Benicia'* (0.210 RE / 100g FW). Statistical data showed that the calculated F value (2, 6) = 27.863 was significantly higher than the one of critical F (theoretical) (2, 6) = 5.14 (significant differences at $p < 0.05$).

Table 2. Total phenolic content – fresh strawberries

Cultivars	Mean g GAE / 100 g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.289	0.007	0.259	0.319
Benicia	0.210	0.001	0.207	0.213
Albion	0.235	0.011	0.187	0.283

According to the Table 3, the total phenolic content in the case of refrigerated strawberries shows that there is an increase for all three cultivars with almost 0.100 g. Also, in this case, 'San Andreas' has revealed the highest phenolic content of 0.424 g GAE / 100 g FW, similar with those obtained by Rekika et al. (2005). This was demonstrated statistically, the value of calculated F (2, 6) = 16.926, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 for $p = 0.003$, being significantly strong positive.

As in the case of flavonoid content, the 'Benicia' cultivar recorded higher values than the same cultivar recorded in the fresh fruits case.

Table 3. Total phenolic content – refrigerated strawberries

Cultivars	Mean g GAE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.424	0.012	0.370	0.477
Benicia	0.375	0.008	0.340	0.410
Albion	0.343	0.009	0.306	0.380

In the case of frozen strawberries, total phenolic content is presented in Table 4 and highlights the high values of 0.336 g GAE / 100g FW, also for *'*San Andreas*'* cultivar. As we compared with the fresh fruits stage, in this case *'*Benicia*'* was the one who had not significant differences, while *'*San Andreas*'* and *'*Albion*'* had significantly higher values. The results obtained in this case are similar to those highlighted by Van de Viewing et al. (2013) analysing other strawberry cultivars.

The statistical processing showed that between cultivars calculated F value (2, 6) = 76.894, is significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences $p < 0.01$).

Table 4. Total phenolic content – frozen strawberries

Cultivars	Mean g GAE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.336	0.003	0.321	0.350
Benicia	0.274	0.003	0.259	0.289
Albion	0.331	0.005	0.311	0.352

Following the determination of total phenolic content for *'*San Andreas*'*, *'*Benicia*'* and *'*Albion*'* cultivars in fresh, refrigerated and frozen stages, has been observed that in all cases 'San Andreas' cultivar registered the maximum phenolic content. With regard to the influence of temperature on phenolic compounds, we can say that refrigerated case (4 ºC) was reached the maximum quantity of total phenols (as can be seen in Figure 1). The results are similar to the literature, according to Ayala - Zavala et al. (2004) total phenolic compounds are increasing continuously in berries during the storage period.

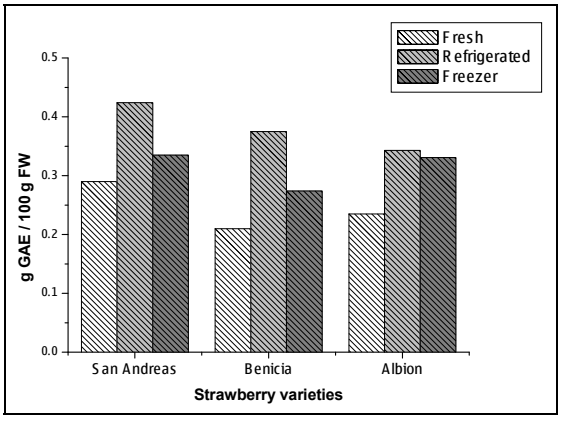

Figure 1. Total phenolic content during all three analysed stages

The results of the determination of flavonoid content for the cultivars in all three analyzed stages are shown in Tables 5, 6 and 7. With regard to the fresh strawberries, the maximum value was recorded in cultivar 'San Andreas' with the highest value of 0.264 g RE / 100g FW, followed by 'Albion' and 'Benicia'.

The value of calculated F (2, 6) = 15.981, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences $p < 0.05$). According to the statistical calculation performed it was shown a significant positive difference between cultivars.

Table 5. Determination of flavonoid content – fresh strawberries

Cultivars	Mean g RE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.264	0.006	0.239	0.289
Benicia	0.163	0.002	0.156	0.171
Albion	0.210	0.008	0.177	0.243

According to the Table 6, the flavonoid content in the case of refrigerated strawberries shows an increase recorded by all three cultivars. Also, in this case, 'San Andreas' has revealed the highest flavonoid content of 0.326 g RE / 100 g FW. This was demonstrated statistically, the value of calculated F (2, 6) = 333.04, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences $p < 0.05$).

It should be noted that in this case the *'*Benicia*'* cultivar recorded higher values than the cultivar *'*Albion*'*, without to follow the order registered in the case of fresh strawberries.

Table 6. Determination of flavonoid content – refrigerated strawberries

Cultivars	Mean g RE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.326	0.006	0.301	0.351
Benicia	0.282	0.008	0.250	0.315
Albion	0.240	0.009	0.202	0.278

In the case of frozen strawberries, flavonoid content is presented in Table 7 and highlights the high values of 0.238 g RE / 100g FW for *'*San Andreas*'* cultivar. As the size of values, they are close to those registered in the case of the fresh ones. Statistical, also in this case there were significant differences at $p < 0.01$, calculated value of F (2, 6) = 110.99, significantly higher than the critical F value (theoretical) (2, 6) = 5.14.

Table 7. Determination of flavonoid content – frozen strawberries

Cultivars	Mean g RE / 100g FW	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.238	0.005	0.218	0.259
Benicia	0.217	0.012	0.167	0.267
Albion	0.229	0.006	0.201	0.256

As is shown in Figure 2, it can be stated that in terms of flavonoid content in all three cases analyzed (fresh, chilled and frozen strawberries) stood out *'*San Andreas*'* cultivar. Concerning the maximum amount of flavonoid accumulation, it has to be highlighted refrigeration stage. On the one hand, this can show that strawberries, regardless of cultivar, can be harvested 10 days later than the initial period.

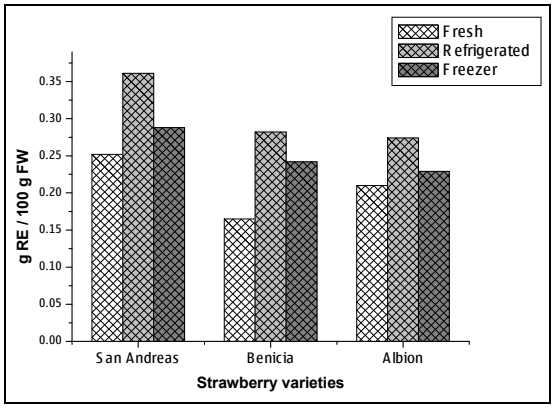

Figure 2. Flavonoid content during all three analysed stages

The free radical scavenging activity of the strawberries extracts is inversely proportional to the EC_{50}.

Thus, according to Table 8, EC_{50} of the extracts shows an important rate of inhibition of *'San Andreas'* cultivar against DPPH free radical. The recorded values are similar, or rather are correlated with the high values obtained in fresh fruits stage. In the support of the obtained results, statistical data realized for this stage shows a high level of significance, calculated F value (2, 6) = 83.259, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 for the probability $p < 0.05$.

Table 8. EC 50 – fresh strawberries

Varieties	Mean mg/ml	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	2.011	0.093	1.613	2.409
Benicia	3.094	0.023	2.994	3.193
Albion	2.596	0.038	2.431	2.762

With regard to the refrigerated strawberries, we can observe from Table 9 a slight decrease of the free radical scavenging activity of the extracts. Nevertheless, the EC_{50} values for the extracts keep the previous decreasing order: *'San Andreas'*, *'Albion'* and *'Benicia'*. In this case calculated F value (2, 6) = 45.961, significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant difference).

Table 9. EC_{50} – refrigerated strawberries

Cultivars	Mean mg/ml	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.593	0.030	0.463	0.723
Benicia	0.998	0.030	0.871	1.125
Albion	0.848	0.031	0.715	0.981

Analyzing the results from the Table 10, we can say that in the case of the extracts obtained from frozen strawberries, *'San Andreas'* showed the best antiradical activity with a value of EC_{50} = 0.580 mg / ml. The calculated F value (2, 6) = 49.365, showed a significantly higher than the critical F value (theoretical) (2, 6) = 5.14 (highly significant differences for $p < 0.05$).

Table 10. EC_{50} – frozen strawberries

Cultivars	Mean mg/ml	Std. Error	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
San Andreas	0.580	0.118	0.071	1.089
Benicia	1.643	0.050	1.429	1.858
Albion	1.055	0.027	0.938	1.173

Observing overall, antiradical activity of strawberry extracts analyzed in fresh, refrigerated and frozen stages (Figure 3), we can say that *'*San Andreas*'* cultivar had the best activity in all analyzed stages, followed by the *'*Albion*'* cultivar.

Considering the storage methods, high values were reported for the refrigeration, a sign that after harvesting some biochemical changes occurred in the fruits structure. In all storage conditions, we see that they keep a particular order, different from that mentioned above. This reveals that the free radical scavenging activity is not necessarily closely related to or influenced only by these two analysed features. According to Pérez-Jiménez and Saura-Calixto (2006), the presence of other compounds (amino and uronic acids) in the test solutions may produce higher antioxidant activity to that produced by the polyphenols alone.

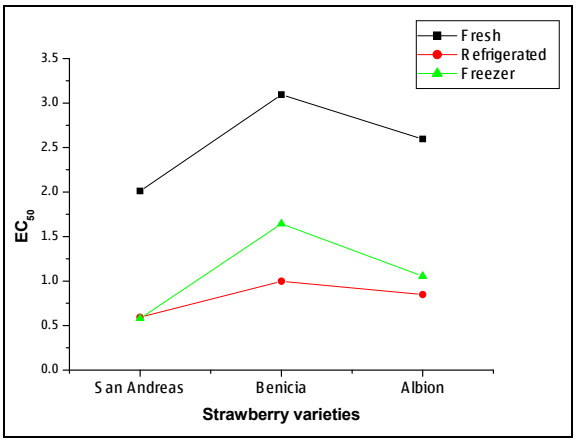

Figure 3. EC_{50} values during all three analysed stages

4. Conclusions and Recommendations

Biochemical analysis of strawberries showed that: in all analyzed stages high phenols and flavonoids content registered at variety 'San Andreas'; regarding the modification of certain biochemical compounds at different temperatures, we conclude that the maximum of bioaccumulation was recorded in refrigerated stage; and last but not the least the antioxidant capacity of the strawberry extracts highlighted *'*San Andreas*'*, and yet we cannot say that in this case phenols were those that caused it.

References

Amaro, L. F., Soares, M. T., Pinho, C., Almeida, I. F., Ferreira, V. O., Pinho, O., 2012. Influence of Cultivar and Storage Conditions in Anthocyanin Content and Radical-Scavenging Activity of Strawberry Jams, International Journal of Biological, Food, Veterinary and Agricultural Engineering 6, 112-116.

Cheel, J., Theoduloz, C., Rodriguez, J. A., Caligari, P. D. S., Schmeda-Hirschmann, G., 2006, Free Radical Scavenging Activity and Phenolic Content in Achenes and Thalamus from Fragaria chiloensis spp. Chiloensis, F. vesca and F. x ananasa cv. Chandler, Food Chemistry 102, p. 36-44.

Cordenunsi, B. R, Genovese, M. I., Oliveira do Nascimento., J. R., Hassimotto N. M. A., Santos, R. J., Lajolo F. M., 2005. Effects of Temperature on the Chemical Composition and Antioxidant Activity of Three Strawberry Cultivars, Food Chemistry 91, p. 113–121.

Garcia–Salas, P., Morales-Soto, A., Segura-Carretero, A., and Fernández-Gutiérrez, A., 2010. Phenolic-Compound-Extraction Systems for Fruit and Vegetable Samples, Molecules 15, p. 8813-8826.

Krølner, R., Rasmussen, M., Brug, J., Klepp, K. I., Wind, M., and Due P., 2011. Determinants of Fruit and Vegetable Consumption Among Children and Adolescents: A Review of the Literature. Part II: Qualitative Studies, International Journal of Behavioral Nutrition and Physical Activity 8, p. 1 – 38.

Mahmood, T., Abas, A. M., and Saari, N., 2012. Effect of Maturity on Phenolics (Phenolic Acids and Flavonoids) Profile of Strawberry Cultivars and Mulberry Species from Pakistan, Int J Mol Sci. 13, p. 4591–4607.

Muraki, I., Imamura, F., Manson, J. E., Hu, F. B., Willett, W. C., Van Dam, R. M., Sun, Q., 2013. Fruit Consumption and Risk of Type 2 Diabetes: Results from Three Prospective Longitudinal Cohort Studies, BMJ, p. 1-15.

Ness, A. R. and Powles, J. W., 1997. Fruit and Vegetables, and Cardiovascular Disease: A Review, International Journal of Epidemiology 26, p. 1–13.

Patthamakanokporn, O., Puwastien, P., Nitithamyong, A., Sirichakwal, P. P., 2008. Changes of Antioxidant Activity and Total Phenolic Compounds During Storage of Selected Fruits. Journal of Food Composition and Analysis 21, p. 241–248.

Peano, C., Giuggioli, N. R. and Girgenti,V., 2014. Effect of Different Packaging Materials on Postharvest Quality of Cv. Envie2 Strawberry, International Food Research Journal 21, p. 1165-1170.

Perez-Jimenez ,J., Saura-Calixto, F., 2006. Effect of Solvent and Certain Food Constituents on Different Antioxidant Capacity Assays, Food Res Int 39, p. 791-800.

Rekika, D., Khanizadeh, S., Dechenes, M., Levasseur, A., and Charles A. T., 2005. Antioxidant Capacity and Phenolic Content of Selected Strawberry Genotypes, HortScience 40, p. 1777-1781.

Shyura, L. F., Tsunga, J. H,. Chenb, J. H, Chiua, C. Y., and Loa, C. P., 2005. Antioxidant Properties of Extracts from Medicinal Plants Popularly Used in Taiwan, International Journal of Applied Science and Engineering 3, p. 195-202.

Singleton, V. L., Orthofer, R., and Lamuela – Raventos, R. M., 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin – Ciocalteu Reagent, Methods in Enzimology 299, p. 152-178.

Tetens, I. and Alinia, S., 2009. The Role of Fruit Consumption in The Prevention of Obesity, Journal of Horticultural Science & Biotechnology ISAFRUIT, Special Issue: 47–51.

Turker, G., Kizilkaya, B., Cevik, N. and Gonuz, A., 2012. Free Radical Scavenging Activity and Phenolic Content of Edible Wild Fruits from Kazdagi (Ida Mountains), Turkey, Journal of Medicinal Plants Research 6, p. 4989-4994.

Van De Velde, F., Tarola, A. M., Güemes, D. and Pirovani, M. E., 2013. Bioactive Compounds and Antioxidant Capacity of *Camarosa* and *Selva* Strawberries (*Fragaria x ananassa* Duch.), Foods 2, p. 120-131.

Xia, W., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Wei, B., Hu, F. B., 2014. Fruit and Vegetable Consumption and Mortality from All Causes, Cardiovascular Disease, and Cancer: Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies, BMJ 1 – 14.

The European Journal of Plant Science and Biotechnology ©2007 Global Science Books

Apricot Genetics and Biotechnology in Romania

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ABSTRACT

The apricot genetic improvement programme performed in Romania between 1986-2006 included clearly formulated steps such as: wide species information, gene bank development based on worldwide exchange of the biological material, widened genetic variability through intra and interspecific hybrids, *in vitro* immature embryo research of interspecific hybrids from the *Prunus* genus, mutagenesis, genetic study of different valuable combinations in F_1, F_2, backcross generation, pollen grain study of various apricot phenotypes, molecular biology investigation, and tissue culture trials. Romania conserves a rich gene bank, including 655 apricot phenotypes originating from North America, Australia, Asia, and Europe. The genetic variability induced by conventional methods demonstrates that apricot-tree species still have genetic reserves for evolution. The reproductive barriers were overcome by the biotechnological methods applied after the hybridisation of ♀*Prunus armeniaca* × ♂*Prunus persica*, or ♀*Prunus persica* × ♂*Prunus armeniaca*, saving immature embryos often aborted in interspecific hybridisation due to nutritional non-compatibility. The genetic progress of the fruit quality characteristics resulted from backcross methods and physical mutagenesis by the mutagenic agent ^{60}Co 3000R. The electrophoretic investigation revealed peroxidase cryoresistance in the Comandor genitor. Strong shoots were obtained from MS culture medium supplied with 2.0 μM indole-3-butyric acid (IBA) + 2.0 μM benzylaminopurine (BAP) + 0.1 μM gibberellic acid (GA_3), using meristem tips as initial explants. Shoot elongation and caulogenesis were significantly improved in a medium prepared with depleted deuterium water instead of distilled water. Heterosis was independent of the culture medium composition. The 1983-2006 breeding programme validated the following varieties: 'Rareş', 'Valeria', 'Carmela', 'Viorica', 'Nicuşor', 'Adina', 'Alexandru', 'Bucovina', 'Siret', 'Atractiv', 'Dacia', 'Excelsior', 'Favorit', 'Comandor', 'Olimp', 'Tudor', 'Traian', 'Cristal', 'Danubiu', 'Auraş', 'Fortuna', 'Orizont', 'Amiral', 'Augustin'.

Keywords: *Armeniaca vulgaris*, genetic variability, heredity

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Received: 22 February, 2007. Accepted: 20 July, 2007.

Invited Review

INTRODUCTION

Background to apricot culture and research

Known since ancient times, the apricot tree has been appreciated and grown, without particular attention, on mountain slopes in the temperate areas of Central Asia and China.

Apricot-tree culture is favourable in the latitudes of 35° and 43° North, and heights of 700-1,500 m, with rainfalls under 500 mm/year, and lowest temperatures of -33°C in the cold areas. The continental climate records no temperature fluctuations. In Central Asia and Asia Minor, the apricot tree grows at altitudes of 1,000-2,500 m (Lilleland 1935, Loschnig and Passecker 1954).

Owing to its characteristics, the apricot tree is paramount in fruit-tree growing, and is renowned for the high food value of its fruit.

The apricot tree is particularly interesting from a fruit-tree growing viewpoint as it grows fast and yields fruit 3-5 years after planting; under favourable ecological conditions and adequate culture technologies, it can reach high yields each and every year (Bordeianu *et al*. 1967; Bălan 1991).

The studies performed on important European and American genetic apricot-tree collections, such as Yalta-Ukraine, Montpellier and Avignon-France, Bucharest and Constanța-Romania, Cacak-Yugoslavia, and Davis-USA, have emphasised a large range of phenotypes and genotypes originating from various geographic areas and recognizable, among others, by their ecological adaptability (Couranjou 1975; Bailey and Hough 1977; Layne 1980; Bălan 1991).

Many of the world cultivars come from local populations that are well adapted to their original places, although their pedigree is unknown. In his 1989 apricot-tree monography, F. Monastra reported that they held 15.7% of the total described cultivars (Strada *et al.* 1989).

All these apricot-tree phenotypes are a significant genetic inheritance of the entire world despite their impossibility of preservation in only one location and the requirements of registration and patenting which limit free access to genetic material of all the countries interested in developing apricot-tree growing.

Only 30 (23%) of the cultivars registered by the International Board for Plant Genetic Resources (IBPGR) are available in the commercial plantations of the main apricot-tree growing countries, i.e. France, Spain, Greece, Morocco (Audergon 1995).

Apricot culture and research in Romania

Romania has long been concerned with apricot-tree growing. In 1871, I. Henţescu published his work, "Pomology", providing recommendations for the growing of four apricot-tree varieties: ‘Ananas’, ‘Nancy’, ‘Muscatello’, ‘Violeta’ (the first two varieties still exist in the present fruit-tree collections).

By the end of the 19th century, there were several *ex-situ* apricot-tree collections in the nurseries of Bistriţa Pietroasele and Vişani; some varieties are still grown today in some orchards in the apricot-tree growing areas: ‘De Breda’, ‘Luiset’, ‘Rozal’, ‘Cea mai bună de Ungaria’/‘Best of Hungary’/.

In 1921, Nicola Krier created the first Romanian apricot-tree varieties, still sparsely grown: ‘Dulci de Vişani’ /‘Sweet of Vişani’/, ‘Târzii de Bucureşti’/‘Early of Bucharest’/ (Bordeianu *et al*. 1964).

After 1950, Nicolae Constantinescu and his collaborators, Vasile Cociu, Mircea Botez, Ecaterina Bumbac, set the basis of the first systematic research for the creation of apricot-tree varieties, implicitly concerned with the development of the genetic resources (Cociu 1981).

Vasile Cociu continued these studies on an international level between 1960-1980, his collaboration with Prof. Hough from Rutgers University being among the most memorable for the exchange of biological material and the development of the apricot-tree genetic resources.

After 1980, the activity was carried out even further by Viorica Bălan and her collaborators at SCDP Băneasa Bucureşti, and Elena Topor and her collaborators at SCDP Valul lui Traian Constanța–Dobrogea, reaching from 62 apricot-tree phenotypes in 1980 to 518 phenotypes in 1990 and 655 phenotypes in 2003 (Cociu 1990; Bălan *et al.* 1993, 2006; Cociu 2006).

The preservation of the genetic resources resulted in studies on the apricot-tree genetic variability with particular focus on adaptability and quality, as well as the transmission of such hereditary traits as: flowering period and fruit maturation, fruit shape, soluble dry matter content, vitamin C content, fruit acidity, skin and pulp colour, resistance to frost and diseases, as well as the substantiation of some genetic-based improvement strategies (Bălan 1993).

The improvement methods were diversified, resulting in complex hybridisation between the best hybrids F_1, SIB and backcross hybridisation. Thus, a very large genetic diversity was created, allowing gene combinations and recombinations according to the aims of the studies.

The improvement strategies were based on the objectives specific to every new phase, in close relation with the objectives set in the most important fruit-tree research centres in: France (Couranjou J, Audergon JM), Italy (Bassi D, Guerierro R), Greece (Karayannis I), Spain (Egea J, Burgos L and Dicenta F), Turkey (Gülcan R).

Summary of world apricot biotechnology advances

The *in vitro* micropropagation of the apricot can be an efficient method of obtaining healthy, pathogen-free – mainly to Sharka – uniform plantlets, in a controlled environment, as well as of *in vitro* conservation of the genetic resources. The review of apricot genetics and biotechnology in Romania presents the most significant results obtained by different Romanian research teams in comparison with other authors’ relevant works.

In 1989, G. Marino obtained plant regeneration in *P. armeniaca* and, by continuing his research, in 1993 he reported the importance of the carbon source on the multiplication rate, concluding that sorbitol (6.0%) gave better results than sucrose (3.0%). Balla and Vertesy (1999) studied the micropropagation of some Hungarian apricot genotypes, in order to obtain virus-free plants and noticed the importance of IAA (indole acetic acid) in the shoot elongation process. Remarkable results in apricot micropropagation were obtained by Pérez-Tornero *et al*. (1999, 2000), who successfully induced culture initiation starting from apical meristems, cultivated on the basal medium QL (Quoirin and Lepoivre 1977) or modified WPM (Lloyd and McCown 1981) of different phytohormone concentrations, obtaining the best results on the mediums supplied with BA (N^6-benzyladenine) (1.78-3.11 μM). They also noticed the frequent phenomenon of apical necrosis of the *in vitro* cultivated *Prunus*. In the same year, Pérez-Tornero *et al*. (2000b) communicated the effects of BA (2.22–2.66 μM) on improving caulogenesis and the stimulatory effects of IBA (indole-3-butyric acid) (29.53 μM) and NAA (α-naphthaleneacetic acid) (10.74 μM) on rhizogenesis. Pérez-Tornero *et al.* (2006) also studied the effect of the explant origin on the *in vitro* culture establishment in four cultivars, pointing out that even the axillary shoots were significantly more contaminated than the meristems, the time to produce elongated shoots was shorter and there were no differences in rooting ability between the shoots micropropagated from meristems and from axillary shoots. Koubouris and Vasilakakis (2006) studied the factors that affect the rapid proliferation and rooting in an apricot cultivar representative for Greece. In order to obtain healthy plants, they used Na-cefotaxime, an antibiotic, in the culture medium. To improve shoot induction, every two weeks they used subcultures in a medium supplemented with 2.2 μM BA + 5.71 μM IAA, and for the optimum root induction they used 19.6 μM IBA. Studying the acclimatisation phase of *Prunus* sp. rootstocks, Rogalski

et al. (2003) pointed out the significant effect of the IBA treatment, genotype and genotype x IBA concentration interaction, on the survival rate in *P. armeniaca* 'Capdeboscq'.

In Romania, research on the *in vitro* culture of the apricot developed in the last ten years, focusing on cultivar and hybrid micropropagation (Butic-Keul *et al*. 2004; Corneanu *et al*. 2006), interspecific (♀*P. armeniaca* x ♂*P. persicum*, ♀*P. persicum* x ♂*P. armeniaca*) immature embryo germination (Bălan *et al*. 1999), and rootstock micropropagation (Popa *et al*. 2005). Different culture mediums and phytohormone balances, the effects of some unconventional bioactive substances (deuterium-depleted water, magnetic fluids) were tested in order to increase micropropagation or rooting rate.

Deuterium-depleted water (DDW) is defined as having the deuterium content under 80 ppm, which, compared with natural water, has 144 ppm. Recent research points out that there is a correlation between the deuterium content in water and DNA degradation. Cell ageing is connected to the gradual accumulation of errors in DNA or the dysfunctional DNA repairing mechanisms (Goodall 2003). In *Robinia pseudoacacia* var. *oltenica*, the DDW concentration in the culture medium had a significant effect on the shoot elongation, as well as on the organogenesis processes (Corneanu *et al*. 2006). Similar results were obtained by Butnaru *et al*. (2001, 2004) in *Chrysanthemum indicum* and Radovet-Salinski *et al*. (2004) in *Coleus blumei*.

Magnetic fluids (MF) are ultrastable colloidal suspensions of ferro- and ferrimagnetic particles in different liquids (water, petroleum, oleic acid, vitamins, a.o.). They were initially used in physics and technics (aerospatial industry) and, after the year 1990, they were tested in biology and medicine. The stimulative effect of magnetic fluids on the development and almost on the *in vitro* rooting process was reported by Corneanu *et al*. (2000, 2004, 2006), Butnaru *et al*. (1999) and Minea *et al*. (2003) in many species of horticultural interest and, in the last years, also in woody species (*Robinia pseudoacacia*, *Prunus avium*).

This review is structured into two distinct sections: genetics of apricot tree in Romania and biotechnology of apricot tree in Romania.

GENETICS OF APRICOT TREES IN ROMANIA

This section presents the research methods employed by the authors of this review, as well as the results obtained from the studies conducted by the corresponding author and the interdisciplinary team, between 1980 and 2006, in accordance with the internationally acknowledged scientists and specialist literature in the field of apricot genetics

The apricot genetics and breeding programme in Romania included: cytogenetics, wide information on the species, adaptability, genetic, physiological and biochemical characteristics and traits, productivity, resistance to stable diseases, initial genetic variability, preservation of genetic resources, selection of genitors, widening genetic variability through intra- and interspecificities, mutagenesis, cryoprotein studies and embryo collection, genetic studies on heredity types and the genetic mechanisms referring to cytoplasmic heredity, genetic transgression, heterosis effect, dominance of some characteristics, segregation in F_1, F_2 progenies, backcross and V_2 mutants, study on microcultures and competitive cultures of the most valuable apricot elites, and the validation as varieties of the apricot trees that corresponded to the breeding objectives.

Biological material for the study of initial genetic variability, preservation of genetic resources, selection of genitors

Devoted to the principle of evolution and preservation of biodiversity, Romania conserves a rich gene bank in the main areas: The Research Station for fruit tree growing Băneasa-Bucharest, with 655 phenotypes, and The Research Station for fruit tree growing Constanța with 471 phenotypes.

The apricot-tree phenotypes preserved derive from the following geographic areas: North America (New Jersey-65, Canada-58, California-74), Australia-6, Asia (China-23, Middle Asia-2, Armenia-2, Iran-2), Europe (Romania-257, France-25, Germany-4, Yugoslavia-10, Greece-3, The Czech Republic and Slovenia-14, CSI-3, Italy-32, Ukraine-10, Macedonia-52, Spain-2, Holland-2, Hungary-12, the Republic of Moldova-5, Bulgaria-2).

Biological material for the study of induced variability and various genetic mechanisms

The biological material for the study of induced variability and various genetic mechanisms, and for the selection of new phenotypes was obtained from intra- and interspecific hybridisation, auto-fecundation, backcrossing, physical and chemical mutagenesis, and *in vitro* cultures.

Hybridisation resulted in 9,000 intraspecific and 1,100 interspecific hybrids.

The genitors used were: 'Comandor' standard, V2- 56 'Comandor', 'Excelsior', 'Dacia', 'Sirena', 'Selena', 'Olimp', 'Sulina', 'Litoral', 77.3.52BV, 'Valeria', 'Rareş', 'Carmela', 'Viorica', 'Favorit', 'Mamaia', 'Roşii de Băneasa', 'Red of Băneasa', 'Sirena', 'Sulina', 'Patriarca temprano', 'Early Orange', 'Goldrich', 'Harcot', 'HW 407', 'Skaha', 'Sungold', 'Sunglo', 'Vivagold', 630203, 'Wenatchee', 'Blenryl', 'Harogem', 'Sundrop', 'Stella', 'Riland', 'Kinred', NJA 58, NJA 25, 'Goldrich', 'Ksongady Magyar Kayszi', 'Salah', B12/6, NJA13, 'Farmingdale', NJA 20, 'Steaua Roşie', 'Red Star', 'Cais trandafiriu', 'Rosy Apricot-tree', Marculesti 19, B 28/2, CR 2-63, 'ReUmberto' F_2, 83.15.23 BI The auto-fecundation of the phenotypes 'Comandor', 'Olimp', 'Selena', 'Sulina', 'Litoral' resulted in 15-82 C_1 descendant. Within hybrid families, 15-230 descendants F_2 were obtained. Backcrossing resulted in 120 descendants. Physical mutagenesis used the mutagenic agent $^{60}C_O$3000R, and resulted in 140 V_2 'Comandor' mutants. Chemical mutagenesis used the mutagenic agent hydroxyl amine, and resulted in 20 M_1 'Olimp' individuals. 1100 hybrids resulted from the *in vitro* saving of immature embryos.

Statistical data processing

The data resulted from the studies conducted below were statistically processed for the following characteristics: tree height, crown height, crown diameter on the row and perpendicular to the row, trunk diameter on the row and perpendicular to the row, trunk-section surface, the ramification angle of the mother branches, the length and number of the annual shootlets per one metre of mother branch, the number of foreseen shootlets per fruit tree, the length of the two-year old branches per one metre of mother branch, fruit cluster formation, leaf surface per offshoot, respiration and photosynthesis intensity, the attack degree of some specific diseases, the free and bound water content, the carbon hydrates content of one-year old shootlets (both in the dormant and vegetative stages), the percentage of damaged and dead buds, the biometric elements of the fruit, the soluble dry matter content, the vitamin C content.

As the studied quantitative characteristics were expected to have the highest phenotypical value, sampling (average samples) was performed on more than 10 and less than 50 individuals from each tested population. Research in the quantitative genetics and statistical analysis of fruit trees in the US (West Lafayette), cherry tree in California, apricot tree in France and Romania, peach tree in France, genetically resistant plants in France and Romania, and horticultural plants in Romania, had previously reported that this type of sampling was sufficient to provide objective proof: Crăciun (1981), Lapins (1983), Hansche *et al*. (1975), Ceapoiu (1968,1980), Couranjou (1975, 1989), Monet (1967, 1977), Monet and Bastard (1971, 1982), Pena (1986), Cociu and

Oprea (1989), Bălan (1991); Bălan *et al*. (2006).

The measurement tools and devices were unchanged for all the sampled individuals and samples.

To analyse the variability of the studied traits and characteristics, it was agreed, according to the genetic experimental techniques, that the *s%* value of 0-10 refers to low variability, the interval 10-20 refers to average variability, and values over 20 refer to high variability. Environmental variance (V_E), calculated according to the Nokorinthop formula, was used to emphasise the influence of the environment on the phenotype.

$V_E = 1/3(s^2P_1 + s^2P_2 + s^2F_1)$ is the average between the parents and the generation F_1.

Genetic variance is the genotype variance, and was calculated according to the formula:

$V_G = V_P - V_E$, where V_P is total variance, or the F_2 variance.

Heritability was calculated according to the formula $h^2 = V_E/V_P$.

The difference between the parental arithmetic means and the arithmetic means of the descendants F_1, F_2, C_1, V_2 and M_1 was calculated.

Genetic studies conducted on apricot in Romania

Apricot-tree cytogenetics

The chromosomal set known until the present is 2n = 16 (Darlington 1945; Bălan 1991). **Fig. 1** shows the chromosomal set present in the mitotic metaphase of active apricot-tree rootlets of *Armeniaca vulgaris*, hybrid '88.4.11 BI'.

The meiotic divisions observed in several apricot-tree varieties and hybrids showed no anomaly, which reflects the safe descendant transmission of some parental traits, i.e. their genetic continuity. **Fig. 2** shows that the meiotic divisions (diakinesis to prophase 1, metaphase 1, anaphase 1, telophase 1 and metaphase 2, anatelophase 2) were normal in the descendant hybrid '88.4.11 BI'. The display of chromosomes followed the Salesses method (1988).

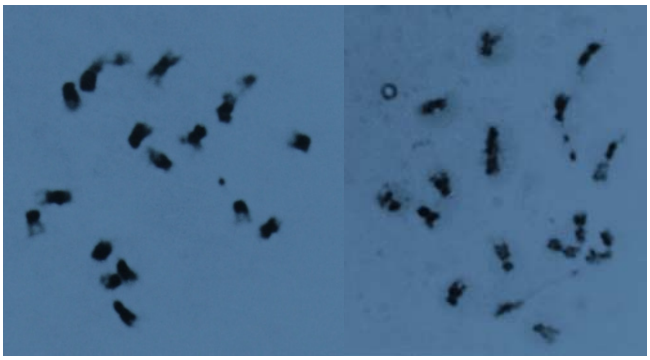

Fig. 1 Chromosomes in mitotic metaphase in apricot.

Initial and induced variability, heredity type and genetic mechanisms of descendant trait transmission

The research methods and results refer to the initial and induced variability, the heredity type and the genetic mechanisms of descendant trait transmission: vegetative growth, morphological characteristics, certain physiological characteristics, frost resistance and wintering, behaviour under the main pathogen attacks – as elements of apricot-tree adaptability in the studied areas, production traits and fruit quality.

Variability of vegetative growth

The methods included in this sub-section had been previously established and used by the authors: Crăciun et al (1978), Cociu (1969), Couranjou (1975), McLean and Rasmussen (1980), Renaud (1980), Bassi (1990), Bassi *et al*. (1995), Bălan (1991), Dejampour and Zeinalabedini (2006).

The following characteristics were recorded in the collection-preserved material, parents, hybrid descendants, CI crossbred descendants, backcross descendants, V_2 and M_1 mutants, both in the juvenile and maturity stages: tree height, crown height, crown diametre on the row, crown diameter perpendicular to the row, trunk diameter perpendicular to the row, trunk diameter on the row, trunk cross-section surface.

Tree height, crown height, crown diameter and crown volume

The traits – tree height, crown height, and crown diameter on the row and perpendicular to the row – were calculated with respect to their heritability.

The crown volume is characteristic for each phenotype, as the result of crown diameter and its height, calculated under conditions of free crown growth..The crown volume, measured in m^3, was calculated based on the Sarger formula: $V = ½(D+d) \times H \times 0.416$, where D = crown diameter on the row, d = crown diameter perpendicular to the row, and H = crown height. Calculations also included the initial and induced variability of the crown volume, and the correlation between its components; genetic analysis was performed on the crown shape, as well as the assessment of descendant selection of the apricot-trees that had a low crown volume in their juvenile phase and were likely to maintain it during their entire life.

The trunk diameters on the row and perpendicular to the

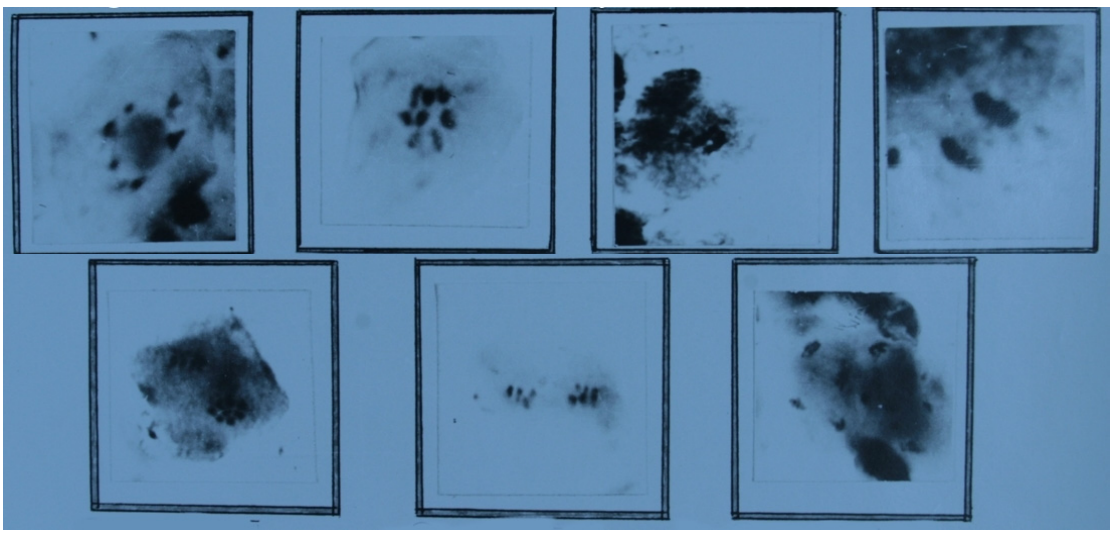

Fig. 2 Meiotic divisions in hybrid descendant 88.4.11.BI. Diakinesis to prophase 1, metaphase 1, anaphase 1, telophase 1 (top); Metaphase 2, anaphase 2, anatelophase 2 (bottom).

Table 1 Heritability of tree height in apricot tree.

Hybrid family	No. of descendants	V_P M	V_E m/%	V_G m/%	H^2
Comandor × Excelsior	15	1.91	1.01/53	0.91/47	0.48
Excelsior × Comandor	18	2.05	1.06/52	0.99/48	0.48
Comandor × Dacia	22	3.43	1.73/51	1.70/49	0.49
Comandor × 77.3.52BV	12	1.66	0.94/57	0.72/43	0.43
Excelsior × Goldrich	50	1.91	1.0/53	0.91/47	0.48

row, measured in cm, were calculated in all fruit-trees, 50 cm above the soil surface.

The trunk-section surface was calculated using the following formula: $\pi \times 1/4(D+d)^2$ The growth rate of the trunk surface, i.e. the trunk thickness as a component of the fruit-tree vigour, was a selection criterion for the low-vigour phenotypes, together with the crown volume.

The following studies were conducted by Viorica Bălan, Elena Topor, Valerica Tudor and collaborators (1986-2006). They resulted in collection-preserved phenotypes in which the tree height varied between 3.0 m in the elite 77.3.52. BV-Romania, and 5.6 m in the following varieties: 'Cea mai bună de Ungaria'/'Best of Hungary'/-Hungary, 'Sulina'-Romania, and 'Sungold'-America. The amplitude of variation was 2.6 m, whereas the average height of all phenotypes was 4.8 m. 6% of the phenotypes were shorter than the average of all the preserved phenotypes. In the maturity period (seven years after planting), the amplitudine of the initial variation in tree height allowed the selection of short genitors which were employed for the genetic study of this trait. The height variability of the hybrid descendants belonging to ♀Excelsior × ♂Goldrich, ♀Excelsior × ♂Comandor, ♀Comandor × ♂Dacia, ♀Comandor × ♂77.3.52 BV was average, as the variability coefficient s% varied between limits (10.6-12.2) during the intense growth of the descendants, i.e. between years 2 and 4 when fruition started. The variation limits of the tree height in the fourth year of growth were 1.90 m in the descendant 83.8.16 BI (parents: ♀Comandor and ♂Dacia), and 2.15 m in the descendant 83.4.3 BI (parents: ♀Comandor and ♂ 77.3.52 BV). In the phenotypical illustration of the tree height, out of the total variance V_P, genetic variance V_G was between 43% and 49%, while environmental variance V_E was somewhat higher (between 51% and 57%). The heritability h^2 of the tree height varied between 0.43 and 0.49, which reflects the percentage of hybrid descendants inheriting the height of their genitors (**Table 1**). 30-50% were short-height hybrid descendants, and the maintenance of this trait during their ontogenetic evolution of growth and development (years 2-8), and the high value of the genetic variance V_G allowed the phenotype-based selection of apricot-tree elites that were shorter than their parents. On average, the hybrid generation was distinctly taller than the parents. Cytoplasmic heredity was identified in the tree height trait where the 'Excelsior' variety was used as maternal genitor. In the crossbred descendants C_1, the tree height variability was low in 'Sirena' C_1 (s% = 6.32) and 'Selena' C_1 (s% = 6.53), and average in 'Sulina' C_1 (s% = 12.26) and 'Litoral' C_1 (s% = 17.44). At a similar age, the crossbred descendants were shorter than the hybrid descendants, with the following limits: 0.40-1.45 m in year 2 compared with 1.20-1.90 m in the hybrid descendants, and 0.75-1.50 m in year 3 compared with 1.60-2.50 m in the same descendants. At a similar age (year 4), the average height of the mutants V_2 'Comandor' ($\bar{x}$ = 2.3 m) and M_1 'Olimp' ($\bar{x}$ = 2.55 m) was very significantly distinct from the standard phenotypes 'Comandor' ($\bar{x}$ = 3.7 m) and 'Olimp' ($\bar{x}$ = 3.80 m). Crossbreeding, physical (^{60}Co) and chemical (hydroxylamine) mutagenesis resulted in descendants shorter than their common ancestor, either 'Comandor' or 'Olimp'. There were insignificant differences in the average between the crossbred descendants C_1 'Comandor' and V_2 'Comandor', as well as between C_1 'Olimp' and M_1 'Olimp'.

In the collection-preserved phenotypes, the crown heights varied between 2.4 and 4.8 m, the large diameter of the crown between 3.2 and 5.5 m, and the crown volume between 2.3 and 5.7 m^3. In Romania, the shortest height (2.4 m) and width (3.2 m) of the crown was recorded in 77.3.52 BV. Also, narrow widths of the crowns were recorded in the following phenotypes: 'Dacia' (3.5 m) in Romania, 'Don Gaetano' (4.2 m) in Spain, and 'Canada 60012' (4.2 m), 'CR-2-63' (4.0 m), 'Manitoba' (4.2 m), 'Vivagold' (3.9 m), and 'Canada 6001' (4.2 m) in the US. In the hybrid descendants, the variations in crown height were low (s% = 9.61) in the ♀Comandor × ♂Excelsior family, or moderate (s% = 14-16.5) in the other hybrid families. In year 4, the crown height reached up to 2.3 m in 50-85% of descendants of the studied hybrid families, excepting those of the ♀Comandor × ♂77.3.52 BV family in which only 10% had this height while the rest were taller than 2.3 m. The crown volume ranged between narrow and average (10.8%) in the ♀Comandor × ♂Excelsior family, and large in the other families. Cytoplasmic heredity occurred for crown size and volume even if 'Excelsior' was the maternal genitor. The genetic variance V_G for crown height, large crown diametre, and narrow crown diametre was lower than the environmental variance V_E in all the hybrid families under study, i.e. between 28% and 48% of the total variance. The improvement value of crown height, large crown diameter, and narrow crown diameter was foreseen in 21-47% of cases, as heritability varied between 0.27-0.47 for crown height, 0.21-0.40 for large crown diameter, and 0.25-0.33 for narrow crown diameter. In the crossbred descendants C_1, the smallest crown size and volume were recorded in 'Sirena' C_1 while the highest were recorded in 'Olimp' C_1. The variation in crown height was low in 'Selena' C_1 (s% - 8.26), very high in 'Olimp' C_1 (s% = 98.4), and average in the following descendants: 'Sirena' C_1 (s% = 18.8), 'Comandor' C_1 (s% = 17.6), 'Litoral' C_1 (s% = 17.6).The large diameter of the crown recorded low variations in 'Selena' C_1 (s% = 6.45), and very high variations in 'Sirena' C_1 (s% = 34.4) and 'Sulina' C_1 (s% = 38.18). The narrow diameter of the crown showed high variability, as the variability coefficient s% was 25.2 in 'Sirena' C_1, and 45% in 'Comandor' C_1. For the narrow crown diametre, distinctly significant differences were determined between the mean of the crossbred descendants 'Olimp' C_1 and 'Sulina' C_1, and the standard phenotypes 'Olimp' and 'Sulina'. For the crown volume, the differences were distinctly significant between the crossbred phenotype 'Olimp' and the standard phenotype 'Olimp', and very distinctly significant between the crossbred descendants C_1 and the standard phenotypes 'Sirena', 'Selena', 'Comandor' and 'Litoral'. There was a large variation range in crown size and volume in the mutants V_2 'Comandor' and M_1 'Olimp'. In the relation between the standard phenotype 'Comandor' and the mutant phenotype V_2 'Comandor', there were distinctly significant differences between the average crown heights, and very distinctly significant differences between the small diameters and volumes of the crown. The significant differences of the average crown size and volume between the standard phenotypes 'Comandor' and 'Olimp', and the crossbred phenotypes C_1 'Comandor' and 'Olimp', as well as between the same standard phenotypes and the mutant phenotypes V_2 'Comandor' and M_1 'Olimp', proved the efficient crossbreeding and physical and chemical mutagenesis methods, resulting in the creation of new low-vigour phenotypes. The multiple regressions of the simultaneous influence on the

Table 2 Partial correlations between the crown height, large crown diametre, narrow crown diametre, and crown volume in the hybrid and crossbred descendants C_1 of apricot tree

Grade-2 correlations	Partial correlation coefficients (*r*)	Correlation signification highest *r*
rxyzw (H × D)	-0.97	000
rxzyw (H × d)	-0.81	0
rgzxw (D and d)	-1.10	000
rxwzy (H and V)	0.81	X
rywzx (D and V)	0.27	
rzwxy (d and V)	0.11	

x = crown height (H); y = large diametre of crown (D); z = narrow diametre of crown (d); w = crown volume (v) r (highest) 5% = 0.75; 1% = 0.87; 0.1% = 0.95.

three crown sizes of the hybrid, crossbred and mutant descendants emphasise the existence of a significant positive correlation between the crown height and the crown volume $r = 0.81$, and an insignificant correlation between the large diameter and the crown volume $r = 0.27$, and between the narrow diameter and the crown volume $r = 0.11$. A very significant negative correlation was evident between the crown height (H) and its large diametre (D), and the large crown diameter (D) and the narrow diameter (d) (**Table 2**).

Conclusions reveal that:

- For the height characteristics, VG=1/2VP (genetic variance represents ½ of total variance) the methods employed were: controlled hybridisation, inbreed by cross breeding, and mutations by physical and chemical gene mutation.

- For the crown shape characteristics, genetic recombination was involved by controlled hybridisation.

Genetic analysis of crown shape

The dialelic hybridisation between the oval-shaped and flat-shaped crown phenotypes resulted in descendants with three types of crown shape: oval, flat, and round. The analysis of the crown shape frequency in the hybrid descendants F_1 pointed that no segregation rapport can be established for this trait; consequently, the occurring non-parental flat shape was not the result of new gene combinations but of genetic recombinations. Thus, determinations regarding the recombination probability (p), the gametes (Os, as, Oa, ss) probability, phenotype frequency and, in the end, total crossingovers and non crossingovers were performed on the descendants from the following hybrid families: ♀Excelsior (oval) × ♂Goldrich (flat), ♀Excelsior (oval) × ♂Comandor (flat), ♀Comandor (flat) × ♂Excelsior (oval), ♀Comandor (flat) × ♂Dacia (oval) ♀Comandor (flat) × ♂77.3.52 BV (oval) (**Table 3**). The analysis of the above data shows that the non crossover-determined parental phenotypes can occur with a frequency between 87% (♀Excelsior × ♂Comandor) and 97% (♀Excelsior × ♂Goldrich and ♀Comandor × ♂Dacia). The frequency of the non crossover-determined non-parental phenotypes can range from 3% (♀Excelsior × ♂Goldrich and ♀Comandor × ♂Dacia) to 13% (♀Excelsior × ♂Comandor).

Trunk diametre and trunk section surface

The trunk diametres on the row and perpendicular to the row, measured in cms, were calculated in all fruit-trees, 50 cm above the soil surface.

The trunk-section surface was calculated by using the follwing formula: $\pi \times 1/4(D+d)^2$ The growth rate of the trunk surface, i.e. trunk thickness as a component of the fruit-tree vigour, was a selection criterion for the low-vigour phenotypes, together with the crown volume

The results revealed that, in the collection-preserved phenotypes, the variation amplitude of the trunk section surface was higher in the Romanian varieties, compared with varieties from USA, Italy, France, Spain, and Asia Minor. The selection 77.3.52 BV recorded the smallest surface of the trunk section (69.4 cm^2) whereas the Romanian variety 'Litoral' had the largest surface (147.3 cm^2). The frequency polygon of the trunk surface variation represents a plurimodal distribution with a slightly negative asymmetry, which points to the heterogeneity of the biological material under study. In the four-year old hybrid descendants, the trunk section surface varied between 10.20-55.37 cm^2. The variation range was average for the trunks of the hybrid descendants of ♀Excelsior × ♂Comandor (s% = 10.7), and ♀Comandor × ♂Excelsior (s% = 15), and high in the descendants of ♀Excelsior × ♂Goldrich (s% = 29.09), ♀Comandor × ♂77.3.52 BV (s% = 33.9), and ♀Comandor × ♂Dacia (s% = 39.3). The configuration of the diagram and the regression line pointed that there was no actual correlation between the surface of the trunk section and the crown volume, as the correlation coefficient was $r = 0.58$. The crossbred descendants were characterised by significant differences in the average surface of the trunk cross-section compared with their ancestors: 'Sirena', 'Selena', 'Olimp', 'Sulina', 'Comandor', 'Litoral'. The trunk section surface of the phenotype C_1 'Comandor' was significantly different

Table 3 Genetic analysis of crown shape. (Viorica Bălan)

Hybrid family	Oval %	Round %	Flat %	p	Gametes (Os, as Oa, ss) probability	Phenotype frequency (Os, Oa, as, ss) %	Total cross overs %	Total non cross overs %
♀Excelsior oval × ♂Goldrich flat	77	19	4	0.04	Os/0.48 as/0.48 Oa/0.02 ss/0.02	Os/73 as/24 Oa/2 ss/1	3	97
♀Excelsior oval × ♂Comandor flat	28	56	16	0.16	Os/0.42 as/0.42 Oa/0.08 ss/0.08	Os/66 as/21 Oa/8.6 ss/4.4	13	87
♀Comandor flat × ♂Excelsior oval	50	40	10	0.1	Os/0.45 as/0.45 Oa/0.05 ss/0.05	Os/69 as/22 Oa/5 ss/4	9	91
♀Comandor flat × ♂Dacia oval	50	45.5	4.5	0.04	Os/0.48 as/0.48 Oa/0.02 ss/0.02	Os/73 as/24 Oa/2 ss/1	3	97
♀Comandor flat × ♂77.3.52 BV oval	71.4	14.3	14.3	0.14	Os/0.43 as/0.43 Oa/0.07 ss/0.07	Os/67.5 as/21.6 Oa/7.5 ss/3.4	11	89

from V_2 'Comandor', i.e. it had lower vigour.

In conclusion, physical gene mutation and crossbreeding, induced inbreed and mutation were involved for fruit-tree vigour (crown shape and size, trunk-section surface).

Variability of some morphological characteristics

In this sub-chapter, we included methods that had been previously used by the authors: Decourtye (1967), Couranjou (1975), Bailey and Hough (1977), Barlow (1980), Hansche and Beres (1980), Sjujka-Lacza (1985), Bassi (1990), Bălan (1991), Bassi *et al*. (1995), Bălan (1999), Audergon *et al*. (2006), Bassi and Audergon (2006), Costes *et al*. (2006), Legave *et al*. (2006).

The research conducted by Viorica Bălan and collaborators in Romania is in accordance with the above-mentioned literature.

The morphological characteristics that may provide new phenotypes of higher values were studied with the aim of contributing to further practical and scientific information necessary to create new apricot-tree varieties that use solar energy more efficiently in order to obtain high top-quality yields and better adaptability to weather conditions.

The morphological characteristics studied by Viorica Bălan and collaborators were: the ramification angle of the mother branches, the length and number of the annual shootlets per one metre of mother branch, the number of foreseen shootlets per fruit-tree, the length of the two-year old branches per one metre of mother branch, fruit cluster formation, leaf surface per offshoot. Measurements were performed on groups of three fruit-trees belonging to each phenotype preserved in the collection, and on each individual and family in the case of the hybrid, mutant, C_1, F_2, and backcross populations.

The ramification angle of mother branches recorded high variations in the hybrid descendants, as the variability coefficient s% varied between 25.9% in the ♀Comandor × ♂77.3.52 BV family, and 57.5% in the ♀Excelsior × ♂Goldrich family. The adequate insertion angle of the mother branches (40-50°) was recorded in 25% of the ♀Excelsior × ♂Goldrich descendants, 35% of the ♀Comandor × ♂Dacia descendants, and in 40% of the ♀Comandor × ♂77.3.52 BV descendants.

The number of annual shootlets per one metre of mother branch varied widely in the hybrid descendants, the variation classes ranging from 10-95 in the first crown level, 12-99 in the second, and 12-108 in the third. Compared with the genitors, some of the descendants recorded less shootlets grown on one metre of mother branch, e.g. ♀Excelsior × ♂Goldrich and ♀Excelsior × ♂Comandor, while others had the same number as their genitors, e.g. in 40% of ♀Comandor × ♂Excelsior and in 15% of ♀Comandor × ♂77.3.52.BV; however, no descendant had more shootlets per one meter of mother branch. The length of the annual shootlets per one metre recorded average variations, as illustrated by the lowest limits of the variability coefficient, s% = 14 in the ♀Comandor × ♂77.3.52.BV descendants, and s% = 33 in the ♀Excelsior × ♂Comandor descendants. The number of foreseen shootlets (shootlet/shootlet) varied within 1-35 (in ♀Comandor × ♂Dacia) and 1-22 (in ♀Comandor × ♂77.3.52.BV). The induced variability of the foreseen shootles number varied widely, irrespective of the level: lowest in the F_1 ♀Excelsior × ♂Comandor descendants (s% = 39), medium in the F_1 ♀Comandor × ♂Dacia descendants (s% = 31), or highest, in the F_1 ♀Excelsior × ♂Dacia descendants (s% = 39).

The flowering branches per one metre of mother branch recorded wide variations in number and length, both within the same family and between families. The variation classes for the number of flowering branches per one metre of mother branch ranged between 1 and 50, while the branch length was between 1 and 60 cm. The descendant transmission of the flowering branch type was studied, pointing to the transgressive heredity of the characteristic, which allowed the identification of some spur-branched hybrids F_1 (3-6 cm), particularly in the ♀Excelsior × ♂Goldrich and ♀Excelsior × ♂Comandor families. The cytoplasmic heredity pf the maternal genitor 'Excelsior' was also emphasised for the descendant transmission of the flowering branch type.

The distance of the fruting branch insertion on the multiannual branches varied between 1 and 50. The heredity of the branch framework was transgressive, as the distance between the fruiting branches was either longer or shorter in the descendants, compared with their parents. The standard distance between the branches was considered to be 5-10 cm.

The leaf surface per offshoot unit varied within 15-40 cm^2 in the hybrid descendants, whereas the variability coefficient ranged between 26.7 in the F_1 ♀Excelsior × ♂Goldrich descendants and 34.8 in the F_1 ♀Comandor × ♂Excelsior descendants.

The conclusions of this study reveal that:

- Very distinctly significant differences of the average leaf surface were recorded between the 'Excelsior' and 'Goldrich' parents, as well as between these parents and their F_1 descendants.
- Similar to the parents, the angle between the leaves and the horizontal line in the descendants F_1 of the 'Excelsior', 'Goldrich', 'Dacia', 77.3.52BV phenotypes was either 30-45° or 60-90°, without exceeding 90°, which proved optimum for the best capture of solar energy.
- The 'Excelsior' phenotype showed its cytoplasmic heredity in the descendant transmission of the leaf surface trait.
- Genetic transgresion and cytoplasmic heredity were involved by controlled hybridisation in the May-bunch and spur-form fruit branches.

Apricot-tree adaptability

Flowering time, fruit maturity, and photosynthesis-respiration

The methods included in this sub-chapter followed the specific methods for this field, presented in specialist literature, such as: Couranjou (1975), Kuebemen *et al*. (1979), Hansen and Ryngo(1979), (Bailey *et al*. (1983), Chang (1984), Suranyi (1985), Dorobanțu *et al*. (1986), Cociu at al (1985), Loukas and Pontikis (1985), Audergon *et al*. (1988), Manganaris (1989), Bassi (1990, 1999), Bălan (1991), Badenes *et al*. (2006), Burgos (1995), Geuna et al (2006), Muleo *et al*. (2006), Paydas *et al*. (2006), Poessel *et al*. (2006), Struss *et al*. (2006), Topor and Burtoiu (2006).

Adaptability is the capacity of the body to provide normal functioning of the vital and reproductible processes under the action of the weather factors. In order to emphasise the adaptability level of the apricot-tree phenotypes, observations and determinations were performed at the beginning and end of both the flowering and fruit maturation phases, in order to measure: the intensity of respiration and photosynthesis, the two simultaneous and opposed metabolic chains, the behaviour under conditions of frost and wintering, and the attack of some specific diseases.

Flowering and fruit maturation. The temperature necessary for the optimum fruition phenophases was calculated by summing up the active temperatures over the biological threshold (+6.5°C). It was considered that flowering began when 10% of the flowers bloomed, and ended when 90% of the flowers senesced, whereas fruit maturation was recorded when 90% of the fruit had ripened. Determinations referred to the amplitude of variability, the heredity of flowering, and the maturation of the fruit.

Respiration and photosynthesis intensity were determined in the phenotypes selected as genitors and the hybrid descendants in 100 g plant material/hour, in two important periods of time: 15 May-15 June, the time of the most intense physiological processes; and 15-29 September, when these processes slowed down.

The intensity of photosynthesis was determined by the

Ivanov-Kosovici method based on the determination of the cabon dioxide amount absorbed by the plants during photosynthesis, given a certain air volume. The CO_2 amount was calculated according to the following formula: if = [(a-b) × f × 0.9 × 60]/(g × t). The intensity of respiration was determined by the Boysen-Jensen formula based on the amount of CO_2 released through respiration by the plant material. The variation amplitude of these physiological characteristics was determined in the parental phenotypes and their hybrid descendants.

In this field, the results obtained by Viorica Bălan and collaborators in Romania showed that controlled hybridisation resulted in descendants F_1 that exceeded the variation limits of the parental phenotypes, reflecting the transgressive heredity of the flowering trait. The differences between the descendants F_1 resulted from the same parents (alternatively used as mother and father), which pointed to the cytoplasmic heredity of the standard phenotypes 'Comandor' and 'Excelsior'. The heredity of fruit maturation was illustrated by three cases: transgressive (7%) to very early (5-25 June), in the combination of ♀late maturation × ♂semi-early maturation in ♀Comandor × ♂Dacia; dominant for the early maturation of the father genitor, in the combination of ♀late maturation (August) × ♂very early maturation (June) in ♀Comandor × ♂77.3.52 BV; and intermediary, in the combination of ♀average maturation × ♂late maturation in ♀Comandor × ♂Excelsior and ♀Excelsior × ♂Comandor.

The photosynthesis-respiration balance, both during the intensive growth of the shootlets and fruit, 15 May-15 June, and during the inactive phase (quiescence), pointed to the normal metabolism of the accumulated organic matter available for the vital activities, which reflected the physiological adaptation of the phenotypes selected as genitors, as well as their descendants, in the areas under study, i.e. the Romanian Plain and Dobrogea.

The results led to the following conclusions:

- Genetic transgressions and cytoplasmic heredity were involved for the blooming time of apricot F_1 descendants.
- For fruit maturation, genetic transgressions, dominant and intermediary heredity was involved by controlled hybridisation and induced gene mutations by physical mutagenesis.

Behaviour under conditions of frost and wintering

These traits were analysed according to the methods employed by: Hatch and Walker (1969), Hansche *et al*. (1972), Richardson *et al*. (1974), Erez *et al*. (1979), Raming (1980), Gilreath and Bouchanam (1981), Giulivo *et al*. (1983), Guerriero *et al*. (1985), Bălan and Ivaşcu (1989), Guerriero *et al*. (2006), Pedryc *et al*. (2006), Rodrigo *et al*. (2006), Ruiz *et al*. (2006).

There are numerous elements illustrating the resistance or susceptibility of the collection-preserved apricot-tree phenotypes, genitors and descendants in relation to low winter temperatures and fluctuating temperatures recorded at the end of winter and in spring time. Out of these elements, the determinations referred to: the free and bound water content and the carbon hydrate content, both in the dormant and vegetation stages, cryosusceptibility of malate dehydrogenase and peroxydase in the buds that were naturally exposed to frost during winter, and the percentage of dead flower buds.

Initial and induced variabilities, the heredity and genetic mechanisms of this characteristic were emphasised.

The carbon hydrate content of one-year old shootlets, both in the dormant and vegetation stages, was based on the extraction of sugars by ethylic alcohol and perchloric acid, respectively, under established conditions; the sugars were subsequently treated with anthrone (a tricyclic aromatic hydrocarbon). The starch extract was measured by photocolorimetry.

The cryosusceptibility of malate dehydrogenase and the peroxidase isosimic spectrums were analysed by using phenotype buds that had been naturally exposed to frost (-16°C) and temperature fluctuations (-6°C +16°C).

Acellular homogenates were prepared by manual grinding with quartz sand in 1 Nm Tris- dithiothreitol buffer to protect enzymatic activity. The supernatants centrifugated with 2.6 mg/ml total protein were extracted by vertical discontinuous electrophoresis on polyacrylamide gels. The enzymatic activities were emphasised by the formazan reaction for malate dehydrogenase using nitrotetrazolin-blue and oxidized nicotin aminodimelcatide (NAD) while a buffer mixture of benzenidyn 1 mg/ml and perchydrol 0.03% was used for peroxidase.

The results revealed that the starch content of the annual shootlets varied widely, as the variation coefficient s% ranged between 21.7 in the F_1 ♀Comandor × ♂Excelsior descendants, and 78.9 in the F_1 ♀Comandor × ♂77.3.52BV descendants in their dormant stage, and between 45.5 and 60.7 in their vegetation period. The variation limits of the starch amount were recorded in December-February, between 0.17-4.32 mg/100 g dry matter in the F_1 ♀Comandor × ♂Dacia descendants, and 0.30-6.0 in the F_1 ♀Comandor × ♂77.3.52.BV descendants, whereas in April-May the variations were 0.30-1.45 mg/100 g in the former, and 0.39-6.10 in the latter. The soluble carbon hydrate content varied widely, recording higher values than starch, i.e. 1.35-13.85 mg glucose/100 g dry matter, compared with 0.17-4.32 mg starch/100 g in the F_1 ♀Comandor × ♂Dacia descendants in December-February, and 4.36-11.95 mg glucose/100 g, compared with 0.30-1.45 mg starch/100 g in April-May. The correlation between the starch-carbon hydrate rapport and the frost resistance of the flowering buds was observed in the F_1 ♀Excelsior × ♂Goldrich descendants, where 8% recorded losses of only 20-30%, while the starch amount was 1.54 mg/100 g higher than the amount of soluble carbon hydrates. The bound water content was higher in the hybrid descendants F_1 than in their parents, e.g. 0.4-5.5 mg/100 g dry matter in the descendants F_1 of ♀Comandor × ♂Dacia, 0.3-3.50 mg/100 g in the genitor ♀Comandor, and 0.26-4.1 mg/100 g in the genitor ♂Dacia. The heterosis of the bound water content was determined in the descendants F_1, compared with their parents. The free water content of the descendants F_1 recorded low or average variations, both in the dormant stage and the vegetation period.

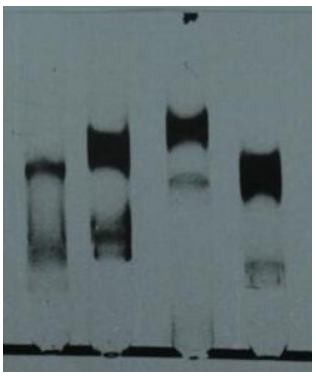

Fig. 3 Cryosusceptibility of cytoplasmic and mitochondrial dehydrogenase in genitor Comandor. Isosimic spectrums indicating decreasing activity in mitochondrial and cytoplasmic malate dehydrogenase.

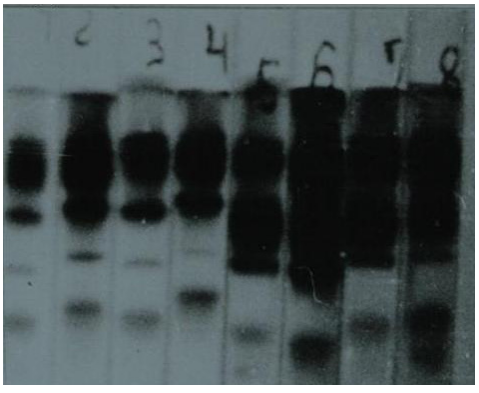

Fig. 4 Peroxidase cryoresistance in genitor Comandor. Isosimic spectrums indicating peroxidase activation.

The free water-bound water balance varied inversely, as the bound water level was higher than the free water level in the dormant stage, and lower in the vegetation period. The cytoplasmic and mitochondrial malate dehydrogenase had evident lower activity in the dead buds, compared with the living buds, in the genitors 'Comandor' and 77.3.52 BV, which emphasised their cryosusceptibility (**Fig. 3**). However, peroxidase manifested cryoresistance (**Fig. 4**), as the peroxidasic isosimic spectrum indicated no differences reflecting cryoinactivation, both in the living and the dead buds. Peroxidase cryoresistance and malate dehydrogenase cryosusceptibility were employed as control indicators for cryoresistance variation in the descendants F_1.

The conclusions resulted from the research data refer to the folowing:

- Heterosis of the content in carbon hydrates, free water and bound water present in the early shoots can be expected in descendants F_1.
- Transgressive heredity was revealed for the frost resistance traits of descendants F_1.
- There was a correlation between the peroxidase cryoresistance of the genitors and the frost resistance of descendants F_1.

Death of flower buds

Frost resistance of the flower buds is a biological characteristic influenced by such factors as: absolute temperature, temperature evolution from the end of the winter dormant stage to the flowering stage, and the physiological condition of the fruit-tree.

Specialist literature (Hatch and Walker 1969; Couvillon and Hendershott 1974; Erez and Lavee 1971; Spiegel-Roy and Alston 1979; Snir 1983; Paunovic and Plazinic 1976, 1983; Paunovic *et al*. 1983, 1985; Paunovic 1986; Scalabrelli and Couvillon 1985; Guerriero *et al*. 2006; Szalay *et al*. 2006) and personal research (Bălan and Ivaşcu 1989, 1995) show that sometimes the flower buds belonging to the same phenotype resist up to -25°C, while some other times they can die at -10°C.

The measurement of the damage caused to the flowering buds started in mid-January, after the temperatures decreased below -15°C, then followed in February-March, when temperatures fluctuated from (+) to (-), sometimes between +16°C and -16°C.

The percentage of damaged buds was determined, and the evaluation of resistance was based on marks ranging from 1 to 5 (Bălan 1991), as follows: 1 = highly resistant phenotypes (0-20% damaged buds), 2 = resistant phenotypes (20-25% damaged buds), 3 = slighly resistant phenotypes (51-70% damaged buds), 4 = sensitive phenotypes (71-80% damaged buds), 5 = highly sensitive phenotypes (81-90% damaged buds).

The results obtained by Viorica Bălan and collaborators emphasise that the flowering buds resistance to frost and wintering (-18°C) and to the temperature fluctuations recorded in February and March (-16°C to +16°C) was transgressively transmitted in descendance, as illustrated not only by the more resistant, but also by 10-25% more susceptible descendants than the genitors, e.g. in ♀Comandor × ♂Excelsior, ♀Excelsior × ♂Goldrich.

In conclusion, heterosis was found for the resistance of fruit buds F_1 to low winter temperatures (-18°C).

Behaviour under the attack of the main diseases

Research on the stable resistant diseases of the apricot tree is specific to various growing areas, as mentioned in specialist literature: Rishbeth (1964), Mănescu *et al*. (1975), Vanderzwet *et al*. (1975), Carter and Moller (1977), Klement (1977), Garrett (1979), Mircetich (1981), Ceapoiu and Negulescu (1983), Okie (1983), Rozsnyay (1983), Bălan *et al*. (1985), Morvan *et al*. (1985), Niyujto *et al*. (1985), Reffatti (1985), El-Kady *et al*. (1986), Baicu and Săvescu (1986), Nemeth (1986), Dosba *et al*. (1988), Morvan (1988), Karayannis (1988), Bălan *et al*. (1989), Crăciun and Crăciun (1989), Bassi (1990), Ionică *et al*. (1990), Bălan *et al*. (1995), Bălan and Stoian (1995), Bassi (1999), Bălan *et al*. (1999), Dicenta *et al*. (1999, 2006), Egea *et al*. (1999), Şesan and Oprea (1999), Karayannis *et al*. (2006), Krska *et al*. (2006), Nicotra *et al*. (2006), Oztűrk *et al*. (2006), Romero *et al*. (2006), Trandafirescu and Teodorescu (2006).

Although literature mentions several apricot-damaging fungi, the most frequent in Romania are: *Monilinia laxa* (Aderh *et* Ruhl) *Honey*, *Stigmina carpophila* (lev) M. B. Ellis, and *Cytospora cincta* Sacc. producing important damage to all the apricot-tree growing areas .

Between 1982 and 2006, a team coordinated by Viorica Bălan conducted a research programme with respect to the genetics of resistance to stable diseases, aiming to create some phenotypes that are genetically resistant to high-frequency pathogen attack.

The particularity of this programme consisted in the absence of resistant genitors belonging to the wild species, spontaneous populations, or cultivated forms.

Therefore, the programme was started by providing natural infection conditions, i.e. plant treatments were stopped for three consecutive years. The selection lot consisted of 1600 F_1 descendants belonging to 64 combinations resulting from dialelic hybridisations of ancestral Romanian biotypes, complex hybridisation, backcrossing, hybridisation between geographically distant fruit trees, crossbreeding. Under strong infection conditions, selection was performed on the phenotypes resistant to the attack of the following pathogens: *Monilinia laxa* (Aderh *et* Ruhl) *Honey*, *Stigmina carpophila* (Lev) M. B. Ellis, and *Cytospora cincta* Sacc., mycoplasmas, bacteriosis, *Pseudomonas siringae* p.v. *siringae* van Hall, and viral diseases, particularly *Plum-pox*.

The selected phenotypes were vegetatively multiplied (20 trees from each phenotype), and then introduced into protected areas and screened by artificial inoculation.

Moreover, three apricot-trees from each collection-preserved phenotype, as well as each individual from the mutant, crossbred C_1 and F_2, and backcross populations, were artificially inoculated with the fungi *Monilinia laxa* and *Cytospora cincta* under orchard conditions, with minimum treatment applied to the collection and no other phytosanitary treatment. The phenotype variability of the reaction to pathogen attack was determined, and the resistant phenotypes were selected as genitors.

Dialelic hybridisation was performed between the phenotypes selected as resistant genitors, as well as between the resistant genitors and top-quality genitors. Determinations were performed on the descendants: induced genetic variability, genetic mechanisms, and the heredity types of resistance to stable diseases.

The attack produced by *Monilinia laxa* was observed on the branches after flowering, and the observations included the percentage of damaged branches out of 100 (F%) and the intensity (I%) of the disease on each branch, according to the assessment scale of 0-4 (Săvulescu and Săvulescu 1959). The degree of the attack was calculated according to the following formula: $GA = (F \times I)/100$. Artificial inoculation was made by the virulent ML_{27} strain of *Monilinia laxa* by spraying the sack-covered flowers with a spore suspension in sterile distilled water (concentration 10^6 spores/ml).

The attack produced by the fungus *Cytospora cincta* was evaluated according to the drying level of the branches that had been artificially inoculated with fragments of fungus colonies by using two virulent strains: C_{36} and C_{41} grown on a CGA. medium. The inoculum was applied to scalpel-cut wounds made in October, and the evaluation of resistance to the action of the fungus was made by measuring the necrosis length starting from the infection point, as follows: 0-3 cm-resistant (R), 3.5-18 cm-medium resistant (MR), over 18 cm-susceptible (S) (Rozsnyai 1977).

The attack produced by the fungus *Stigmina carpophila* on leaves and fruit was determined by observing the frequency of the damaged organs out of a total of 100, and the attack intensity for each damaged organ by using the scale

of 0-6 (Baicu and Săvescu 1986). The degree of the attack was subsequently calculated according to the folowing formula: GA = (FxI)/100, where F = attack frequency and I = attack intensity.

The biological material was also tested for infection with such pathogens as *Apricot chlorotic leaf roll*, *Plum pox* (Sharka) and *Pseudomonas siringae* p.v. *siringae* van Hall; as the biotic factors were involved in apricot-tree damage, especially in complex actions together with micoses.

Micoplasmic infections were observed by optical microscopy, using the Diens method. Resistance to the attack of the bacterium *Pseudomonas siringae* p.v. *siringae* van Hall. was tested *in vitro*. Ten shootlets were sampled from each fruit tree belonging to a phenotype, as well as from each descendant, and subsequently inoculated with bacterial *Pseudomonas* suspension, a mixture of three strains: syringomicin-producing T_{96}, strong ice-nucleating T_{1328}, and medium syringomicin-producing and medium ice-nucleating T_{1318} of 10°UFC titre.

To evaluate the attack of the virus *Plum pox*, samples of 150 leaves and 25 fruit were collected from each tree under study, and determinations were made for the intensity of the attack, the frequency of the attack, and the degree of the attack was calculated. For a better interpretation of the results, the entire biological material was subject to screening according to the degree of the attack and the results of the ELISA test. The frequency of the Sharka attack was calculated by using the formula f% = (n × 100)/N, where n = the number of attacked leaves or fruit, and N = the number of observed leaves and fruit. The intensity of the Sharka attack was calculated in order to analyse the spreading degree of the attack as the ratio between the area of the attacked leaves or fruit and the total area of the observed organs. The interpretation of the results was based on the scale of 0 to 6 classes equivalent to some percentage of the attack intensity (attack surface%/attack intensity class): (1-3)/1, (4-10)/2, (11-25)/3, (26-50)/4, (51-75)/5, (76-100)/6. The relative expression of the intensity of the *Plum pox* attack was calculated by the formula I = [F(i × f)]/n, where F = attack frequency, i = class or percentage of the attacked area, f = number of attacked cases within the same class, and n = total number of attacked cases. The degree of the attack (GA%) illustrated the seriousness of the attack, and was determined according to the relation: GA% = (F × I)/100.

The results show that the consecutive three-year absence of treatments and the conditions provided by the Bucharest area, where *Monilinia laxa* Aderh *et*. Ruhl Honey and *Stigmina carpophila* M. B. Ellisse may produce over 70-80% damage, created favourable conditions for screening. Thus, out of 1600 descendants of 64 genomic families resulted from dialelic breeding between geographically distant partners, complex hybridisation, and backcrossing, only 13 descendants of nine genomic families were selected according to their field resistance to the two pathogens. The degree of attack (DA%) of the 13 descendants was limited to 2.5-7.5 in *Monilinia laxa*, and 1.8-7.5 in *Stigmina carpophila*. 'Marculesti 19' was the maternal genitor in three of the nine genomic families, and 'Re Umberto' was the maternal genitor in five families and the paternal genitor in one of the selected families. The selected elites were retested by artificial inoculation under glasshouse conditions, and no difference was observed, compared with their behaviour under natural infection conditions. The selection 83.15.23 (♀Re Umberto × ♂Timpurii de Chişinău/Early of Chişinău) was also resistant to inoculation based on fragments of *Cytospora cincta* colonies whereas selections 83.29.4 B1 and 83.29.3 B1 (♀Mr.19 × ♂CR5-180) showed average resistance. The selection 83.29.4B1 was highly resistant to pathogens and temperature fluctuations, and produced top-quality fruit; therefore, it was homologated in 1984 under the name of 'Dacia', and was protected by a patent. The phenotypes that resisted the attack of stable pathogens were the basis for the genetic study of resistance to diseases, as well as genitors in the genetic breeding programme for apricot trees. The study of the F_1 descendants resultant from ♀resistant ('Dacia', 83.15.23B1, 77.3.52 BV) × ♂intermediary ('Comandor', 'Excelsior', 'Goldrich') showed high variability to the attack of the pathogens under study. The transgressive heredity of the reaction to the pathogenic action of the fungus *Stigmina carpophila* was determined in the following descendants F1: ♀Comandor × ♂Excelsior, ♀Excelsior × ♂Goldrich, ♀Excelsior × ♂Comandor, ♀Comandor × ♂Excelsior, ♀Early Orange × ♂Don Gaetano. The hybrid descendants F_1 ♀Excelsior × ♂Goldrich, ♀Comandor × ♂Dacia, of the ♀susceptible × ♂resistant type, and ♀Comandor × ♂77.3.52.BV of the ♀susceptible × ♂average resistant type, reacted to *Cytospora cincta* Sacc., manifested heterosis under conditions of inoculation with the virulent strains C_{36} and C_{41}. The $^{60}C_O$ physical mutagenesis, in a rate of 3000R, induced resistance to the fungus *Stigmina carpophila* in 33% of the total mutants V_2 Comandor.

The conclusions of the above results indicate that:

- Intermediate resistance to *Monilinia laxa* was involved by controlled hybridisation and physical gene mutation.
- Genetic transgression was induced by controlled hybridisation for resistance to *Stigmina carpophila*.
- Heterosis of *Cytospora cincta* resistance was induced by controlled hybridisation (VG>VP). Induced gene mutation was found for *Cytospora cincta* resistance and for the biochemical traits quality.
- Resistance to Sharka was analysed, resulting in the selection of resistant genitors, i.e. 'Stella', 'Early Orange', and NJA 42.

Yield characteristics

Yield level and regularity

Literature emphasises that apricot yield is still deficitary worldwide; therefore, yield characteristics are an important objective in the research programmes of the developing countries: Sansavini *et al*. (1974, 1988), Moore (1979), Friundlund (1979), Lespinasse *et al*. (1985), Crăciun (1987), Bălan (1991), Smith and Mollendorf (1999), Bertazzoli and Rivaroli (2006), Brunton *et al*. (2006), Ghelfi and Lucchi (2006), Mittermayer (2006), Olgun and Adanacioglu (2006), Albuquerque *et al*. (2006).

In Romania, Viorica Bălan and the research team analysed the fruit yield as a biological characteristic and, at the same time, as an indicator for the behaviour of the varieties under the given environmental conditions. Early fruiting, i.e. the fruiting time of the studied variants, was recorded for each tree belonging to the collection-preserved phenotypes, as well as for each descendant, and was genetically analysed.

High yields, i.e. marked 3 on a scale of 1-3, were recorded in 10-30% descendants F_1 of the following families: ♀Excelsior × ♂Goldrich, ♀Excelsior × ♂Comandor, ♀Comandor × ♂77.3.52.BV, ♀Comandor × ♂Dacia, ♀Olimp × ♂$CR_{2\text{-}65}$. 30% mutants V_2 'Comandor' were similar to the standard phenotype 'Comandor'.

Fruit quality

The complex physical and chemical characteristics of the apricots, such as the biometric elements and the biochemical components of quality, have been an important field of research for numerous specialists worldwide, among which: Couranjou (1975), Giulivo *et al*. (1983), Guerrriero (1984), Cociu and Hough (1985), Margarian *et al*. (1986), Mehlenbacker and Hough (1986), Hansche(1986), Bassi (1990, 1999), Bassi and Negri (1991), Bălan (1995), Guerriero *et al*. (1995), Bălan *et al*. (1999), Bassi and Audergon (2006), Bălan *et al*. (2006a, 2006b), Benedikova (2006), Bureau *et al*. (2006), Ham (2006), Lachkar and Mlika (2006), Mencarelli *et al*. (2006), Pennone and Abbate (2006), Petrişor *et al*. (2006), Tzoneva and Tzonev (1999), Wang and Liu (2006).

The biometric elements and biochemical components of the fruit quality were determined for each individual studied,

and the data were statistically processed and analysed for: initial variability and the variability induced by hybridisation, crossbreeding, mutagenesis, F_2, backcrossing; the genetic mechanisms, and the heredity of the quality traits and characteristics; phenotypical correlations of some features referring to the fruit quality. A number of 30 fruit were sampled from each variant in order to determine its physical characteristics, and 2-3 kg were collected for the biochemical determination of the quality. The basic colour of the skin was evaluated by using the apricot-tree colour code devised by Ctifl France (Centre technique interprofessionnel des fruits et légumes) and adopted by the Romanian specialists.

Height (H), dorso-ventral diametre (D), and lateral diameter (d) were measured by the vernier caliper. The data obtained were statistically interpreted to emphasise variability and to calculate the shape index $I.F = H^2/D \times d$, When the index was higher than 1.0, the fruit was elongated whereas, when lower than 1.0, the fruit was either round or flat round. The average weight of the fruit was determined on 30 fruit sampled from each variant, and the results were statistically processed to obtain the initial and induced variabilities, and the descendant transmission of the respective trait. The pulp/stone ratio was determined by weighing the latter separately and relating the value to the total fruit weight, which resulted in pulp productivity.

The soluble dry matter content provided an overview of the content in carbon hydrates, organic acids, and mineral salts, determined by the Zeiss scaled refractometre. To avoid determination errors, fruit were sampled from all the variants after reaching the consumption maturity, the gathering time being based on the colour code and the Hunter lab measurements, originally correlated with the penetrometre-based determinations. The reducing sugar content was achieved by the Fehling-Soxhlet method, i.e. the polysugars and protein removal without converting saccharose into glucose and fructose. Vitamin C ascorbic acid, considered one of the most important vitamins for its catalytic functions and direct intervention in metabolism, was determined by the Thillmans method modified by 2-6 dichlorinephenolindophenol. Titre was established by using ascorbic acid. Measurement was made in mg% of 100g fresh matter by the following formula: ascorbic acid (vitamin C) = (V × F × 1000 m)/m × mp. The mean of the three determinations performed for each sample was statistically processed in order to analyse the genetic variability and heredity of this important biochemical component of apricot quality.

The results point out that,genetic variability was induced for the following fruit quality traits: average fruit weight (g) in the backcross descendants ♀Comandor × ♂F_1 ('Excelsior' × 'Comandor') s% = 25.42, and F_2 'Olimp' s% = 21.80; titratable acidity-malic acid (g%) in the mutants V_2 'Comandor' s% = 29 and F_2 'Olimp' s% = 20.11; soluble dry matter (DM%) s% = 24.02, titratable acidity-malic acid (g%) s% = 46.06, ascorbic acid-vitamin C (mg.%) s% = 26.60 in the F_1 ♀Olimp × ♂NJA19 descendants.

The alternative use of the variety 'Comandor' as maternal and paternal genitor, in combination with the variety 'Excelsior', resulted in low variability in the former case, and average variability in the latter, which points to the cytoplasmic heredity of both partners. The study of the genetic progress achieved in the descendants F_1, F_2, backcross, mutants V_2, pointed to the statistically assured increase in the fruit quality traits, compared with one or both parents in the following cases (**Table 4**): a) soluble dry matter in the backcross generation ♀Comandor × ♂F_1 ('Excelsior' × 'Comandor'), mutants V_2 'Comandor', and descendants F_1 ♀Olimp × ♂NJA_{19}; b) ascorbic acid-vitamin C in the descendants F_2 'Excelsior' × 'Comandor', mutants V_2 'Comandor', and descendants F_2 'Olimp'; c) average fruit weight in mutants V_2 Comandor. The values of soluble dry matter (DM%) of the standard phenotype 'Comandor' were compared with the backcross descendants 'Comandor' and mutants V_2 'Comandor', emphasizing an increase from 15.6% in the standard phenotype to 18.46% in the backcross descendants, and 19.56% in the mutants V_2. An increase in soluble dry matter, compared with the standard phenotype 'Comandor', was also recorded in the descendants F_1 ♀Comandor × ♂Dacia, i.e. 16.29% on average. The mutants V_2 'Comandor' recorded an increased content in ascorbic acid-vitamin C, as well as increased average fruit weight, which showed that physical mutagenesis was an efficient method of improving some fruit quality traits.

The analysis on the genetic mechanism of the fruit shape heredity in the descendants F_1 of five genomic families emphasised three types of gene actions: no descendent segregation, 3:1 segregation, and 1:1 segregation. The non-segregated monohybrid descendants were ♀Comandor × ♂Dacia elongated fruit Aa × round fruit aa, and ♀Comandor × ♂77.3.52 BV elongated fruit Aa × elongated fruit Aa.

Table 4 Genetic variability induction of some quality characteristics in the descendants F_1, F_2, backcross, mutants V_2, and signification of differences from parents or standard phenotype. (Viorica Bălan and Valerica Tudor)

Genitors/Descendants	Dry matter (DM%)	Malic acid (g/%)	Vitamin C (mg/%)	Fruit weight (g)
F_1 ♀Excelsior x ♂Comandor	15.8	1.67	15.5	43.4
F_1-P_1xP_2	-1.89	-0.04	2.54/**	11.16/000
F_1-P_1	-4.0/000	0.45	1.58	11.16/000
F_1-P_2	+0.21	-0.53	3.5/***	11.16/000
F_1 ♀Comandor x ♂Excelsior	14.1	1.58	12.74	40.07
F_1-P_1xP_2	-3.59/000	-0.13	0.22	14.93/000
F_1-P_1	-1.49	-0.62	0.74	14.93/000
F_1-P_2	-5.7/000	0.36	-1.18	14.93/000
F_1 ♀Comandor x ♀Dacia	16.29	1.98	11.80	44.9
F_1-P_1xP_2	+0.98	0.16	-1.40	26.1/000
F_1-P_1	+1.68	0.22	-0.20	10.1/000
F_1-P_2	+0.27	0.54	-2.6/000	42.1/000
Backcross (B_2) Comandor x F_1 (♀Excelsior x ♀Comandor)	18.46	1.06	10.66	55.94
B_2-P_2 Comandor	+2.87/**	1.14/00	-1.34	+0.97
Mutants V_2 Comandor	19.56	0.99	19.56	58.37
Standard Comandor V_0	15.6	2.20	12.00	55
V_2-V_0	3.97/***	-1.21	7.56/***	3.37/***
F_1Olimp x NJA19	19.56	1.10	8.74	69.50
F_1-P_1 x P_2	+5.38/***	-0.81	-0.79	6.07/000
F_1-P_1	+3.0/***	0.10	-1.42	3.36/* * *
F_1-P_2	+7.76/***	-1.52	-0.16	15.5/000
F_2 Olimp	17.78	1.06	12.25	62.26
F_2Olimp-P_1Olimp	+1.22	0.04	2.09/*	3.88/000

The 1:1 segregation of the fruit shapes pointed to the descendant 'Goldrich' acting as a double recessive tester, and confirmed the heterozigotic nature of the parent 'Excelsior' as illustrated by the mutual monohybridisations ♀ExcelsiorAa × ♂ComandorAa and ♀ComandorAa × ♂ExcelsiorAa.

Based on these results, the following conclusions can be summed up:

- Controlled hybridisation, backcross, and physical gene mutation were efficient methods to improve fruit quality.
- Transgressions of dry substance and vitamin C were revealed in apricot descendants F_1.
- There were positive non-linear genetic correlations between the content in soluble dry matter and vitamin C.
- The heredity of the fruit shape was dominant, with segregation 3:1 in the combination of ♀elongated fruit (Aa) x ♂elongated fruit (Aa), and segregation 1:1 in the combination of ♀elongated fruit (Aa) x ♂round fruit (aa).

BIOTECHNOLOGY OF APRICOT TREE IN ROMANIA

Micropropagation

Culture initiation

The reaction to micropropagation is genotype and explant type dependent, according to many research teams results (Butic-Keul *et al*. 2004; Corneanu *et al*. 2006; Pérez-Tornero *et al*. 1999; Pérez-Tornero *et al*. 2006; Rogalski *et al*. 2003).

In Romania, young shoots from the following cultivars were used for micropropagation: 'Best of Hungary', 'Favorit', 'Mamaia', 'Excelsior' and 'Comandor', hybrids: progeny F_2 'Excelsior' × 'Comandor' and rootstocks: 'Stella' and 'Goldcot', from which apex, meristems, leaves and microcuttings were sampled (**Table 5**). The aseptic method of the biological material differed according to genotype and laboratory. The general scheme of sterilization was: washing in water, dipping into ethanol 70-95%, 10-60 sec; dipping into sterilizing agent (Domestos 20%, NaOCl 0.8-4%, $HgCl_2$ 0.3%), 10-20 min, three washes in sterile distilled water (**Table 1**). For culture initiation, MS (Murashige and Skoog 1962) and QL (Quoirin and Lepoivre 1977) media were tested. The culture mediums were supplied with different hormone balances and bioactive unconventional substances: DDW (30 ppm deuterium content) and magnetic fluids (**Table 2**). A multiple emulsion was used as a magnetic fluid. In multiple emulsions, the magnetic part was formed by iron oxides FeO and Fe_2O_3, in a ratio of 1:1 (magnetite), which was obtained by coprecipitation in an alcaline medium and stabilised with oleic acid (Minea *et al*. 2003). The medium was solidified with agar (7%), pH = 5.6-5.8. The culture vessels were maintained at 24-26°C, 16 h photoperiod, white light (Osram L58 w/30) at a PPFD of 35 μmol m^{-2} s^{-1}.

The explants that gave the best results concerning viability, as well as future evolution, were the apices and apical meristems. Similar results were obtained by Pérez-Tornero *et al*. (2006) who also observed that the survival of the explants was higher when axillary shoots instead of meristems were used for the culture introduction. In the case of young leaves and microcuttings, the shoots viability was low (under 20%), and total necrosis of the explants was observed 20 days after culture initiation. Shoot development and the elongation process were dependent on the genotype, as well as on the phytohormone balance of the culture medium (**Table 6**).

In the cultivars 'Best of Hungary', 'Favorit' and 'Mamaia' (Butic-Keul *et al*. 2004), the best results were obtained on the MS medium + 14.9 μM/l 2 iP (N^6-2-isopentenyl adenine) + 0.5 μM/l IBA + 0.26 μM/l GA_3 (gibberelic acid) + 56.8 μM/l vitamin C + 54.3 μM/l adenine sulphate, the length of the obtained shoots being 10.6-19.6 mm. In the rootstocks 'Stella' and 'Goldcot', better development was noticed on the basal QL medium supplied with 2.2-4.4 μM BA + 0.5 μM NAA + 0.02 μM GA_3, in comparison with the basal medium MS with the same phytohormone supplement, the regeneration percentage being 19.7-21.6% (Popa *et al*. 2005). By comparing the reaction to micropropagation of the cultivars 'Excelsior' and 'Comandor' with their hybrid progeny F_2, Corneanu *et al*. (2006) pointed out that the heterosis effect was also manifested *in vitro*, while the hybrid had the best results as far as the main shoot elongation and the basal caulogenesis process were concerned. The authors emphasised the specificity of the reaction, depending on the genotype × culture medium interaction, observation sustained also by Pérez-Tornero and Burgos (2000a) in their studies on the micropropagation of the cultivars 'Bulida', 'Helena', 'Canino', 'Lorna'. Corneanu *et al*. (2006) observed improved results when the culture medium was prepared with DDW, as the shoot elongation process and the foliar organogenesis were significantly increased.

According to Pérez-Tornero and Burgos (2000a), MS is the worst medium for some sensitive genotypes (e.g. all plants died within six weeks in the cultivar 'Bulida', while in 'Currot' and 'Helena' explants showed hyperhydricity symptoms, due to the excessive ammonium content of the MS medium) in comparison with QL or modified WPM media. In opposition, the cultivars tested in Romania: 'Best of Hungary', 'Favorit', 'Mamaia', 'Excelsior', 'Comandor' and their F_2 progeny reacted very well on the MS medium and no vitrification processes were observed.

Secondary basal shooting process was dependent on the genotype, as most authors emphasised (Butic-Keul *et al*. 2004; Corneanu *et al*. 2006). In some cultivars on the initiation medium mentioned above, 1-2 basal shoots ('Best of Hungary', 'Favorit', 'Mamaia') were obtained, in others ('Excelsior', 'Comandor') no caulogenesis took place while, in the hybrid F_2 ♀Excelsior × ♂Comandor), 3-6 basal shoots/explant were obtained on the medium prepared with

Table 5 Asepsization methods in *P. armeniaca*. (Mihaela Corneanu and Viorica Bălan)

Genotype	Biological Material	Aseptization methods	Laboratory	Reference
H_1	Immature seeds	Ethanol 95%	Research Station for Fruit-Tree Growing Baneasa	Bălan *et al.* 1999
H_2				
H_3				
H_4				
H_5				
H_6				
'Best of Hungary'	Shoots	Ethanol 70% -10 sec.	University Babes-Bolyai Cluj Napoca	Butic-Keul *et al.* 2004
'Favorit'		Domestos 20% -15 min.		
'Mamaia'				
'Stella'	Shoots	Ethanol 70% -60 sec.	Research Station for Fruit-Tree Growing Băneasa	Popa *et al.* 2005
'Goldcot'		NaOCl 0.8% -20 min		
'Excelsior'	Shoots	NaOCl 4.0% -10 min.	University of Craiova	Corneanu *et al.* 2006
'Comandor'		$HgCl_2$ 0.3% 10 min.		
♀Excelsior x ♂Comandor				

Table 6 Results obtained in *in vitro* culture initiation in *Prunus armeniaca* (cultivars, hybrids) in Romania. (Mihaela Corneanu)

Genotype	Explant	Optimum culture medium	Results	References
H_1	IM	H basal; MS modified; LP modified	G	Bălan *et al.* 1999
H_2				
H_3				
H_4		LP modified		
H_5				
H_6				
'Best of Hungary'	A	MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA_3 + 10 mg/l vitamine C + 10.0 mg/l adenine sulphate	C, E, R	Butic-Keul *et al.* 2004
'Favorit'		MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA_3 + 10 mg/l vitamin C + 10.0 mg/l adenine sulphate	C, E, R	
'Mamaia'		MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA_3 + 10 mg/l vitamine C + 10.0 mg/l adenine sulphate	C, E	
		MS + 1.0 mg/l iP + 0.1 mg/l IBA + 0.1 mg/l GA_3 + 10 mg/l vitamine C	R	
'Stella'	AM	LP + 2.2 – 4.4 µM/l BAP + 0.5 µM/l NAA + 0.02 µM/l GA_3	C	Popa *et al.* 2005
'Goldcot'				
'Excelsior'	AM	MS + 1.0 mg/l BAP + 0.1 mg/l NAA	C, E	Corneanu *et al.* 2006
'Comandor'		MS + 2.0 mg/l BAP + 2.0 mg/l IBA + 0.1 mg/l GA_3		
♀Excelsior x ♂Comandor		MS + 1.0 mg/l BAP + 0.1 mg/l NAA (prepared with DDW)		

Explant type: IM – immature embryo; A- apex; AM – apical meristem
Culture medium: MS modified; LP modified: ½ macroelements and iron; double microelements
Results: G – embryo germination; C – caulogenesis; E – shoot elongation; R - rhizogenesis

Table 7 The effect of the culture medium, deuterium-depleted water (DDW) and explant size on the main biometrical characters in hybrid progeny F_2 ♀Excelsior x ♂Comandor (Fisher's Test) (Corneanu *et al.* 2006).

Character	Fisher's Test 1- subculture medium; 2- DDW; 3- explant size					
	1	2	3	1 x 2	2 x 3	1 x 2 x 3
Shoot length	22.90***	0.60	356.97***	21.85***	6.89*	8.29***
No of leaves/explant	1.93	7.38**	87.34***	3.75**	5.03*	3.12**
Secondary shoot number	2.34*	21.51***	20.34***	1.52	5.13*	2.55*

Table 8 Results obtained in *in vitro* subculture in *Prunus armeniaca* (inter- and intraspecific hybrids) in Romania. (Mihaela Corneanu)

Genotype	Explant	Optimum culture medium*	Results	References
H_1	Germinated embryos	H basal; MS modified; LP modified	P; LS	Bălan *et al.* 1999
H_2		H basal; MS modified; LP modified	P; LS	
H_3		H basal; MS modified; LP modified	P; LS	
H_4		MS modified; LP modified	P	
H_5		MS modified; LP modified	P	
H_6		LP modified	P	
♀Excelsior x	Neoformed shoots 1 mm	MS + 2.0 mg/l BAP + 2.0 mg/l IBA + 0.1 mg/l GA_3	E, CCE	Corneanu *et al.* 2006
♂Comandor	Neoformed shoots 3 mm		E, C	
(F_2)		MS + 0.1 mg/l BAP + 0.1 mg/l IBA + 0.1 mg/l GA_3 + 60 mg/l FM	E, R	

* Culture medium with best results
MS modified; LP modified: ½ macroelements and iron; double microelements
Results: R – rhizogenesis ; P – plantlets; LS – lateral shoots; E – shoot elongation; CCE – caulogenesis via embryogenic calli; C – caulogenesis

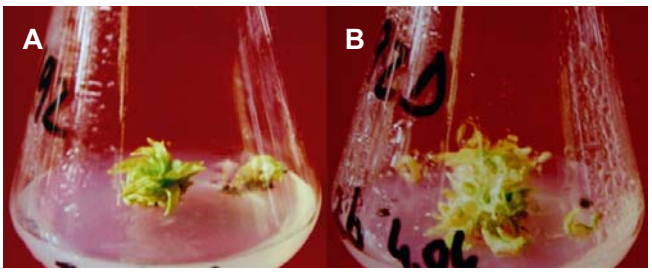

Fig. 5 The effect of DDW on caulogenesis process on *P. armeniaca*, F_2 hybrid ♀'Excelsior' × ♂'Comandor'. (**A**) MS + 1 mg/l BAP + 0.1 mg/l NAA. (**B**) MS + 1 mg/l BAP + 0.1 mg/l NAA prepared with DDW.

DDW (**Fig. 5**). DDW exercised an action of protection, both at the DNA and telomere level, which assured the chromosome integrity. But the telomer was partly lost with each cell division, therefore the division number characteristic to a cell was different (Hayfick, cited by Goodall 2003), depending on the genotype and probably on the tissue. The increase in the cell multiplication rate was limited by the telomer integrity. When the value (the division number characteristic to the genotype) was surpassed, cell divisions became abnormal, either resulting in aneuploid cells or cell division stopped. The knowledge of this concept, as well as the use of DDW in culture medium preparation, can be a way of improving the *in vitro* multiplication rate of the explants.

Subculture

The neoformed shoots must be transferred (subculture) on a fresh medium after 30-45 days; after this period, apex necrosis occurs at a high rate, a fact noticed by other authors in the *Prunus* species (Scaltsoyiannis *et al*. 1998); Balla and Vértesy 1999; Pérez-Tornero *et al*. 2000a).

In the experiments performed by Corneanu *et al*. (2006), the *in vitro* neoformed shoots of different size (3 mm, 4 leaves; 1 mm, 2 leaves) were transferred on eight variants of MS basal medium supplied with 0.44-8.9 µM BA + 0.5-9.8 µM IBA + 0.3 µM GA_3. The analysis of the effect of the subculture medium, initiation medium and explant type, pointed out the significance of each factor, as well as their interaction (Fisher's test) on the growth, differentiation and dedifferentiation processes. The main shoot elongation was very significantly influenced by the phytohormone balance, as well as by the explant size, a fact proved both by the Fisher's test and a very significantly positive correlation

between the shoot length and IBA quantity (r = +0.3374). The best results (shoot length = 20.5 ± 0.2 mm, 45 days) were obtained on the MS medium + 8.9 μM BA + 9.8 μM IBA + 0.3 μM GA_3. Organogenesis in the leaves was significantly stimulated in the medium variants where the auxin: cytokinin ratio favoured the latter. Caulogenesis at the shoot base was significantly influenced both by the phytohormone balance and the presence of DDW in the initiation culture medium, as well as by the explant size (**Table 7**). The highest number of shoots/explant was recorded in small shoots (1 mm) (8-12 shoots/explant), on the culture medium MS 8.9 μM BA + 9.8 μM IBA + 0.3 μM GA_3, originating from a medium prepared with DDW. This finding proves that DDW has a long term effect, having an important role in tissue rejuvenation. Knowing the effect of different controllable factors on the explant development allows their modellation in order to obtain the best results (**Table 8**).

Rhizogenesis

Obtaining strong rooted plantlets is a condition to carry out a succesful acclimatisation process. Most authors observed that a reduced concentration to 2/3 -1/2 basal medium salts improved the rhisogenesis process (Balla and Vértesy 1999; Kamali *et al*. 2006). Some authors recomended an induction period in the darkness for certain genotypes (Pérez-Tornero *et al*. 2000a, 2006); however, this might produce chlorosis and shoot necrosis, fact confirmed by Koubouris and Vasilakakis (2006) in the cultivar 'Babecou'.

In some genotypes, the rhisogenesis process can be obtained even in the initiation culture. Butic-Keul *et al*. (2004) obtained rooted plantlets on the same medium used for culture initiation, but the reaction to the culture medium was genotype-specific. The highest number of roots was obtained in the cultivars 'Best of Hungary' and 'Favorit' on MS + 4.9 μM 2 iP + 0.5 μM IBA + 0.26 μM GA_3 + 56.8 μM vitamin C + 54.3 μM adenine sulphate, while the cultivar 'Mamaia' rooted better in the absence of adenine sulphate. The number of neoformed roots in all the three genotypes was low (1.1-1.6 roots/explant) due to the low auxin content, as the culture medium was originally conceived to promote caulogenesis. Rhizogenesis occurred as a secondary process.

In order to induce rhizogenesis in the hybrid progeny F_2 ♀Excelsior × ♂Comandor, Corneanu (2006) supplemented the culture medium (80% MS) with phytohormones (0.44 μM BA + 0.5 μM IBA + 0.3 μM GA_3) and different concentrations of magnetic fluid (0-200 mg/l MF). Roots were obtained on all the medium variants supplied with magnetic fluid, but in a concentration of 60 mg/l the number of roots/explant was maximum (4.6 ± 0.2), while shoot elongation showed a correspondingly positive reaction (20-24 mm growth of the transferred shoots in 45 days). In the control variant (non-MF medium), no rooting process was observed and the shoots length presented a significant negative difference in comparison with the variant MF (7-10 mm). No caulo- or callogenesis at the shoot base was recorded in any experimental variant. These findings are in accordance with the previous ones obtained by Corneanu *et al*. in other species: *Aloe arborescens* (1994), *Fragaria ×annanasa* (1995), *Aztekium riitteri* (1996a), *Mammillaria duwei* (1996b), *Drosera rotundifolia* (1998), *Coryphantha elephantidens* (2000), *Prunus avium* (2004a), *Robinia pseudoacacia* (2004b) as a result of culture medium supplementation with magnetic fluid.

Immature embryo culture and seedling acclimatisation

The *in vitro* culture of immature embryos is an efficient method of rescuing the embryos resulting from interspecific hybridisation, as they frequently abort due to the genetic incompatibility between the embryo and endosperm.

In order to assure viable progeny of some interspecific hybrids, namely *P. armeniaca* × *P. persicum* (H_3) and *P. persicum* × *P. armeniaca* (H_1, H_2, H_4, H_5 and H_6), Bălan *et al*. (1999) used culture immature embryos *in vitro* and obtained acclimatized plantlets (**Table 8**).

To initiate the *in vitro* embryo culture, immature embryos (1-10 mm) were used as explants, harvested 6-7 weeks after pollination, interspecific hybridation progenies ♀*P. armeniaca* (*P.a*) *x* ♂*P. persica* (*P.p*), ♀*P.p* × ♂*P.a* (Bălan *et al*. 1999): H_1 – ♀*P.p* × ♂*P.a* (♀Garden Delight × ♂B19/20); H_2 – ♀*P.p* × ♂*P.a* (♀Prios Magdalena × ♂Dacia); H_3 – ♀*P.a* × ♂*P.p* (♀Olimp × ♂PI 132030); H_4 – ♀*P.p* × ♂*P.a* (♀Michigan × ♂B4/73); H_5 – ♀*P.p* × ♂*P.a* (♀Sweet Gold × ♂Favorit); H_6 - *P.p* × ♂*P.a* (♀Platycarpa × ♂Dacia). To rescue immature embryos, three variants of hormone-free culture media were tested: H basal (Theobald and Hough 1960), MS modified and QL modified (double microelement quantity, half macroelements and iron quantities). In the embryo culture, the test tubes were maintained at 16°C in the darkness, until germination started, then transferred to 22-25°C with 16 h photoperiod.

As with neoformed shoot subculture, the explant size was as important as the culture medium composition. For the successful germination of embryos used as explants, they should be >4 mm in size, otherwise an *in ovulo* culture for 1-2 weeks is recommended before excision. This observation is in accordance with Burgos and Ledbetter (1993) who found that embryos between 5 and 9 mm germinated and developed into plants in a significantly higher percentage that the ones in more mature stages.

The hybrids H_1, H_2, H_3 reacted favourably in all the three variants of the culture mediums, resulting in 100% germination, while the hybrids H_4, H_5, H_6 recorded modest results (16-18%) on LP medium; H and MS mediums were inadequate, inducing germination inhibition. The mediums used for culture initiation were not always the best for plantlet development. A reduction to half the macroelements and iron, and the doubling of microelements stimulated embryo germination and plant development. The lower salts concentration improved rhizogenesis and subsequent plant growth after acclimatisation. The resulting plantlets were acclimatised in three steps: uncovering the test tube for 5 days (in the culture room), maintaining them for 10 days in the test tube with distilled water and then transferring them into pots and providing high humidity. 54% of the hybrid plantlets H_1, H_2, H_3 developed lateral shoots, with high number of leaves on short stems, proving the success of acclimatisation.

CONCLUDING REMARKS

In order to develop future breeding strategies, genetic engineering and the DNA technology may merge with other non-conventional methods, such as biotechnology (somaclonal variation and stress resistance). For successful research, several things must be taken into consideration, among which increasing knowledge of the apricot genotype at the molecular level and the high expressions of inserted genes.

In the future, the studies based on molecular genetics and biotechnologies will be associated with biodiversity, and will employ combined research methods, such as: traditional hybridisation, backcross, mutagenesis, inbreeding, and unconventional genetic transformation. It is noteworthy that this strategy is already applied in some European countries: France, Italy, and Spain.

ACKNOWLEDGEMENTS

For the long-term support of our research, we would like to express our gratitude to the following: Prof. Dr. Doc. Teofil Crăciun (Professor of Genetics, University of Agronomic Sciences and Veterinary Medicine Bucharest); Prof. Dr. Doc.Vasile Cociu ("Gheorghe Ionescu-Şişeşti" Romanian Academy of Agricultural and Forestry Sciences); Prof. Dr. Petre Raicu (Professor of Genetics, University of Agronomic Sciences and Veterinary Medicine Bucharest); Prof. Dr. Ioan Florea Dumitru (Professor of Molecular

Genetics, University of Agronomic Sciences and Veterinary Medicine Bucharest); Prof. Dr. Lucian Gavrilă (Professor of Genetics, University of Agronomic Sciences and Veterinary Medicine Bucharest); Ms. Cristina Florea (biologist, National Programme Director in the Romanian Ministry of Research); Ms. Elena Nistor (teaching assistant, Foreign Languages Department of the University of Agronomic Sciences and Veterinary Medicine Bucharest).

Many thanks to: Dr. Maria Oprea (Senior Researcher, plant pathology and plant protection, Plant Protection Research Institute, Bucharest); Mrs. Elena Popa (biochemistry researcher, Research and Development Station for Fruit-Tree Growing Băneasa, Bucharest); Dr. Nicolae Branişte (Senior Researcher, genetics and genetic resources preservation, Research Institute for Fruit-Tree Growing Piteşti).

Also, many thanks to: Prof. Dr. L.F. Hough (Rutgers University, USA); Senior Researcher J.M. Couranjou (INRA France); Senior Researcher R. Monet (Research Centre -Bordeaux, France); Senior researcher J.M. Audergon (INRA Montfavet, France; Coordinator of the ISHS apricot-tree working group); Prof. Dr. D. Bassi (Milan University, Italy); Prof. Dr. R. Guerriero (University of Pisa, Italy); Senior Researcher C. Fideghelli (Director of the Fruit-tree Growing Research Institute - Rome, Italy); Senior Researcher L. Burgos (CEBAS-CSIC Murcia, Spain); Senior Researcher J. Egea (CEBAS-CSIC Murcia, Spain).

And, last but not least, our deepest gratitude to our patient families.

REFERENCES

Albuquerque N, Burgos L, Egea J (2006) Variability in cultivar characteristics as factors influencing productivity in apricot. *Acta Horticulturae* **701**, 267-269

Audergon JM, Castelain C, Morvan G, Chastelliere MG (1988) Apricot varietal sensibility and genetic variability to apricot chlorotic leaf roll disease. *Acta Horticulturae* **235**, 205-215

Audergon JM (1995) Variety and breeding, apricot culture. *Acta Horticulturae* **384**, 35-44

Audergon JM, Asins MJ, Guerierro R, Vitti R, Monteleone P, Bartolini S, Laghezali MD, Mamouni A, Mlika MD, Hellali R, Ben Mimoun M, Krichen L, Gulcan R, Paydas S, Albagnac G, Bureau S, Gouble B, Grotte M, Reich M, Legave JM, Audergon JM, Lambert P, Blanc A, Richard JC, Clauzel G (2006) Prospection, characterisation and assessment of apricot genetic resources in the Mediterranean region for the production in arid and semi-arid areas. *Acta Horticulturae* **701**, 263-266

Audergon JM, Giard A, Lambert P, Blanc A, Gilles F, Signoret V, Richard JC, Albagnac G, Bureau S, Gouble B, Grotte M, Reich M, Legave JM, Clauzel G, Dicenta F, Scortichini M, Simeone AM, Guerierro R, Vitti R, Monteleone P, Bartolini S, Martins JMS, Tsiantos J, Psallidas P (2006) Optimisation of apricot breeding by a joint conventional and molecular approach applied to the main agronomic traits. *Acta Horticulturae* **701**, 317-320

Badenes ML, Moustafa TA, Martinez-Calvo J, Llacer G (2006) Resistance to Sharka trait in a family of self-pollination of 'Lito' apricot cultivar. *Acta Horticulturae* **701**, 381-384

Baicu T, Săvescu A (1986) Sisteme de combatere integrată a bolilor şi dăunatorilor pe culture. Integrated pest and disease control systems according to crops. Ceres Publishing House, Bucharest, 264 pp

Bailey C, Hough LF (1977) Apricots. In: Janick J, Moore JN (Eds) *Advances in Fruit Breeding*, Purdue University Press, West Lafayette, Indiana, pp 367-383

Bailey CH, Kotowski S, Hough LF (1983) Estimate of chilling requirements of apricot selections. *Acta Horticulturae* **121**, 99-102

Balla I, Vertesy J (1999) *In vitro* culture of Hungarian apricot (*Prunus armeniaca L*) varieties. *Acta Horticulturae* **560**, 395-398

Barlow NWB (1980) The relationship between leaf size and shoot length in apple. *Journal of the American Society of Horticultural Science* **3**, 279-283

Bassi D (1990) *Germoplasma di albicocco* – Viagio di studio effetuato nella Republica Populare Cinese dal 2 al 26 juglio 1990 nell ambito agli accordi di cooperatione scientifica tra il CNR e la CAAS (personal report)

Bassi D, Negri P (1991) Ripening date and fruit traits in apricot progenies. *Acta Horticulturae* **293**, 133-140

Bassi D, Bellini E, Guerriero R, Monastra F, Pennone F (1995) Apricot breeding in Italy, Apricot Culture. *Acta Horticulturae* **384**, 47-54

Bassi D (1999) Apricot culture: present and future. *Acta Horticulturae* **488**, 35-42

Bassi D, Audergon JM (2006) Apricot breeding: update and perspectives. *Acta Horticulturae* **701**, 279-294

Bassi D, Bartolini S, Viti R (2006) Recent advances on environmental and physiological challenges in apricot growing. *Acta Horticulturae* **717**, 23-32

Bălan V, Oprea M, Drăgoescu E, Ivaşcu A (1985) Resistance of some apricot varieties hybrids to *Altemaria tenuissima*. Acta *Horticulturae* **192**, 231-238

Bălan V, Oprea M, Ivaşcu A (1989) The variability of field resistance against pathogens agents *Monilia laxa*, *Stigmina carpophillum* and *Cytospora cincta* in some hybrids. *Acta Horticulturae* **293**, 203-210

Bălan V, Ivaşcu A (1989) Resistance to frost and wintering of some apricot varieties with different geographical origin under Romanian plain conditions. *Acta Horticulturae* **293**, 331-340

Bălan V (1991) Cercetări privind variabilitatea genetică la cais cu privire specială la adaptabilitate şi calitate. Research on the genetic variability of the apricot-tree with special focus on adaptability and quality, PhD Thesis, University of Agronomic Sciences and Veterinary Medicine Bucharest, Romania, 247 pp

Bălan V, Stoian E, Stancu T, Ivaşcu A (1995) Breeding for diseases resistance in apricot problems and prospects. *Acta Horticulturae* **384**, 103-110

Bălan V (1995) Utilization of induced mutations in apricot breeding. *Acta Horticulturae* **384**, 231-236

Bălan V, Stoian E (1995) Susceptibility of apricot cultivars to Plum-pox virus in Romania. *Acta Horticulturae* **384**, 384-388

Bălan V, Ivaşcu A, Popa E (1995) Aptitude of several genitors to transmit chemical fruit traits and the vigour degree in apricot breeding programme. *Acta Horticulturae* **384**, 73-78

Bălan V, Ivaşcu A (1995) Genetic resources at apricot and peach in Romania. In: Schmidt H, Kellerhals M (Eds) *Progress in Temperate Fruit Breeding*, Eucarpia Fruit Breeding Section. Meeting Held at Wadenswil/Einsiedeln, Switzerland, August 3, 1993, Kluver Academic Publishers, Boston, pp 439-447

Bălan V (1999) Cumulus of positive characteristics of different apricot parental phenotypes and the way to transmitting in apricot hybrids descendants. *Acta Horticulturae* **488**, 257-266

Bălan V, Ivaşcu A, Toma S, Costea G (1999) Studies on immature embryos in *in vitro* culture of interspecific hybrids from the genus *Prunus*. *Acta Horticulturae* **488**, 557-559

Bălan V, Popa E, Bădescu V, Mihai R, Cerbu E (1999) Biochemical changes detected in different apricot varieties artificially inoculated with *Cytospora cincta* Sacc. *Acta Horticulturae* **488**, 661-666

Bălan V, Oprea M, Toma S (1999) Reaction of apricot cultivar from national collection to *Monilinia laxa* Lev. (Aderh.) inoculum. *Acta Horticulturae* **488**, 667-670

Bălan V, Tudor V, Petrişor C (2006a) Providing the quality features variability of apricot descendants: F_1, F_2, back-cross and V_2. *Acta Horticulturae* **701**, 175-178

Bălan V, Tudor V, Petrişor C (2006b) Maintenance of biodiversity of apricot tree phenotypes in Romania. *Acta Horticulturae* **701**, 199-206

Bălan V, Petrişor C, Lazăr V, Popescu M, Vlaicu I (2006) Chromaticity differences in evaluation of fruit quality of some apricot genotypes for fresh consumption. *Acta Horticulturae* **701**, 565-570

Benedikova D (2006) Apricot breeding in Slovak Republic for fruit quality and resistance to disease – new Slovak apricot varieties created by RBS Vesele. *Acta Horticulturae* **701**, 377-380

Bertazzoli A, Rivaroli S (2006) A trade flows analysis of fresh apricots: The European Markets. *Acta Horticulturae* **717**, 281-286

Bordeianu T, Constantinescu N, Stefan N (1967) *Pomologia Republicii Populare Romîne*. *Fruit-tree Growing in the Popular Republic of Romania* (Vols 1-3), Romanian Academy Publishing House, Bucharest, 756 pp

Brunton JG, Carillo A, Cos J, Sanchez PG, Montiel (2006) The apricot situation in the region in Murcia. *Acta Horticulturae* **717**, 193-196

Bureau S, Gouble B, Grotte M, Audergon JM, Albagnac G (2006) Variability of volatile aroma composition in apricot fruits. *Acta Horticulturae* **717**, 327-332

Bureau S, Reich M, Marfisi C, Audergon JM, Albagnac G (2006) Application of Fourier-transform infrared (FT-IR) spectroscopy for the evaluation of quality traits in apricot fruits. *Acta Horticulturae* **717**, 347-350

Burgos L, Ledbetter CA (1993) Improved efficiency in apricot breeding: Effects of embryo development and nutrient media on in vitro germination and seedlings establishment. *Plant Cell, Tissue and Organ Culture* **35**, 217-222

Burgos L (1995) Preliminary results of an inheritance study of genetic incompatibility in apricot, Apricot Culture. *Acta Horticulturae* **192**, 85-89

Butic-Keul A, Zapartan M, Deliu C (2004) Regenerarea *in vitro* si micropropagarea la cais. *In vitro* regeneration and micropropagation in apricot. *Proceedings of 15th National Symposium of Plant Tissue Culture: Physiopathology of the plant cell in vitro culture*, Daya, Satu Mare, pp 128-135

Butnaru G, Terteac D, Potentz I (1999) Magnetic fluids improving effect in *in vitro* regenerates in hipogravity. *Journal of Magnetism and Magnetic Materials* **201**, 435-439

Butnaru G, Ştefănescu I, Rogobete S, Titescu G (2001) Deuterium depleted water effects on plants behaviour. Proceedings of 2nd European Meeting on Environmental Chemistry, Dijon, France, 203-208

Butnaru G, Baciu A, Ştefănescu I, Titescu G, Împuşcătoiu D, Sărac I (2004) The action of different types of water on *in vitro* organogenesis in *Chrysanthemum indicum* L. *Proceedings of 3rd Congress "Water – a miracle"*, Constanţa, Vol **1**, 55-56

Carter MV, Moller WJ (1977) Eutypa canker and dieback of apricot, *OEPP Bulletin* **7**, 85-93

Ceapoiu N (1968) *Metode Statistice Aplicate în Experienţele Agricole şi Biolo-*

gice. *Statistical Methods Applied to Agricultural and Biological Experiments*, Agro-forestry Publishing House, Bucharest, 550 pp

Ceapoiu N (1980) *Evoluţia Speciilor*. *Evolution of Species*, Romanian Academy Publishing House, Bucharest, 480 pp

Ceapoiu N, Negulescu F (1983) *Genetica şi Ameliorarea Rezistenţei Plantelor*. *Disease Resistance Genetics and Improvement of Plants*, Romanian Academy Publishing House, Bucharest, 538 pp

Chang WF (1984) Relationship of seed germination and respiration during stratification with cultivar chilling requirements in peach. *Journal of the American Society of Horticultural Science* **109**, 42-45

Cociu V (1969) Breeding and study programme for fruit trees, strawberry and other berries between 1970 and 1980. *Theoretic and Applied Genetics* **6**, 470-492

Cociu V (1981) Cîteva aspecte ale zonării şi microzonării la pomi fructiferi şi arbuşti. Some issues of fruit-tree and bush variety zoning and microzoning. *Scientific Papers* 9, ICPP Mărăcineni, Piteşti, pp 9-14

Cociu V, Hough LF (1985) Germplasm of apricots and some suggestions for its use. Apricot culture and decline. *Acta Horticulturae* **192**, 291-296

Cociu V, Bumbac E, Bălan V, Hough LF (1985) Promising apricots hybrids with early and very early ripening. *Acta Horticulturae* **192**, 313-322

Cociu V, Oprea S (1989) *Metode de Cercetare în Pomicultură*. *Research Methods in Fruit-Tree Breeding*, Dacia Publishing House, Cluj-Napoca, 122 pp

Cociu V (1990) *Soiurile Noi, un Factor de Progress in Pomicultură*. *New Varieties, a Factor of Progress in Fruit-Tree Growing*, Ceres Publishing House, Bucharest, 188 pp

Cociu V (2006) 50 years of apricot genetic breeding in Romania. *Acta Horticulturae* **701**, 355-358

Corneanu GC (1989) *Radio-Susceptibilitatea Comparativă a Plantelor*. *Elemente de Radio-Biologie a Plantelor*. *Compared Radio-Susceptibility in Plants*. *Elements of Plant Radio-Biology*, Ceres Publishing House, Bucharest, 225 pp

Corneanu M, Corneanu G, Bădică C, Minea R, Bică D, Vékas L (1994) *In vitro* organogenesis at *Aloe arborescens* (Liliaceae). *Revue Roumain de Biologie, Biologie Végétale* **39** (1), 45 - 52

Corneanu M, Corneanu GC, Bădică C, Bică D, Minea R, Cosmulescu SN, Neculae L (1995) *In vitro* regeneration of the senescent or partial vitrified plants on the culture media supplied with magnetic fluids. *Genetics and Evolutionism* **2**, 51-57

Corneanu M, Maiuru G, Minea R (1996a) *In vitro* multiplication and acclimatisation in *Aztekium ritteri* (Fam. *Cactaceae*). *Acta Botanica Horti Bucurestiensis 1994-1995*, 175-180

Corneanu M, Corneanu G, Ebata J, Furukawa H., Bronzetti G, Bică D, Minea R, Gabor L (1996b) Organogenesis process in *Mammillaria duwei* (Cactaceae) depending on culture media composition. IPPS in Bulgaria – Second Scientific Conference "Propagation of Decorative Plants" (Razmnegenesis na Dekorativni Rasteniia), Sofia, Lesotehniceski Universitet Ed., 35 – 41

Corneanu M, Corneanu GC, Glodeanu E, Gabor L, Minea R, Bică D, Morariu VV, Bădică C, Hănescu V (1998) Medium culture composition and organogenesis processes in *Drosera rotundifolia*. *Acta Horticulturae* **457**: 109-114.

Corneanu M, Corneanu GC, Hănescu V, Gabor L, Minea R, Frangopol C (2000) The effect of the interaction magnetic fluids – X-rays on the *in vitro* micropropagation at *Coryphantha elephantidens* (Lem.) Lem. *Propagation of Ornamental Plants* **4**, 50-57

Corneanu M, Mîndrilă G, Corneanu GC, Cristea C, Atyim P, Minea R (2004a) The influence of humic acids and magnetic fluids on shoot elongation and *in vitro* rooting processes in *Prunus avium* L. *USAMV-CN Bulletin, Series Animal Science and Biotehnology* **60**, 313-318

Corneanu M, Mîndrilă G, Corneanu GC, Cristea C, Atyim P, Neţoiu C, Bică D (2004b) *In vitro* micropropagation in *Robinia pseudoacacia* var. *Oltenica*. *Proceedings XXXIV ESNA Annual Meeting*, Mondograf, Novi Sad, pp 231-234

Corneanu M, Tudor V, Corneanu CG, Gamaneci G, Ştefănescu I (2006a) The effect of genotype and depleted deuterium water on *in vitro* organogenesis in apricot (*Prunus armeniaca* L.). *Proceedings of 15th National Symposium of Plant Tissue Culture: The Conservation of Plant Vitroculture*, Alma Mater, Sibiu, pp 115-130

Corneanu M, Corneanu CG, Cristea C, Băbeanu C, Roşca A, Ştefănescu I, Mîndrilă G (2006b) The effects of the depleted deuterium water on *in vitro* organogenesis processes in *Robinia pseudoacacia* var. *oltenica*. *Revue de Cytologie et Biologie Végétales - Le Botaniste* **28**, 236-245

Costes E, Fournier D, Audergon JM, Legave JM, Clauzel G (2006) Architectural diversity of apricot trees: which morphological characters can be used to classify cultivars? *Acta Horticulturae* **701**, 105-111

Couranjou J (1975) L'amelioration genetique de l'abricotier. *INRA Publications* **387**, **388**, **407**

Couranjou J (1989) Main genetic results from a study of an apricot semidiallel. *Acta Horticulturae* **293**, 87-94

Couvillon CA, Hendershott CH (1974) A characterisation of the "after rest" period of flower buds from two peach cultivars of different chilling requirements. *Journal of the American Society of Horticultural Science* **99**, 25-26

Crăciun T, Tomozei I, Coles N, Nasta A (1978) *Genetica*. *Genetics*, Didactic and Pedagogic Publishing House, Bucharest, 623 pp

Crăciun T (1981) *Genetica Plantelor Horticole*. *Genetics of Horticultural Plants*, Ceres Publishing House, Bucharest, 537 pp

Crăciun T (1987) *Geniul Genetic şi Cultura Plantelor*. *Genetic Genius and Plant Breeding*, Ceres Publishing House, Bucharest, 265 pp

Crăciun T, Crăciun LL (1989) *Dicţionar de Biologie*. *Dictionary of Biology*, Albatros Publishing House, Bucharest, 467 pp

Decourtye L (1967) Etude de quelques caracters a controle genetique simple chez le pommier (*Malus* sp.) et le poirier (*Pyrus communis*). *Annales de Amelioration des Plantes* **17**, 243-265

Dejampour J, Zeinalabedini M (2006) Determination of some vegetative and bloom characteristics of some local apricots in Azarbaijan (Iran) ecological conditions. *Acta Horticulturae* **717**, 63-66

Dicenta F, Pérez-Campoy PJ, Martínez-Gómez P, García-Brunton J, Botella MA (1999) Natural spread of Sharka disease in fruit-tree orchards in Murcia (Spain). *Acta Horticulturae* **488**, 775-778

Dicenta F, Rubio M, Martinez-Gomez P (2006) Susceptibility to Sharka (Plum-pox potyvirus) in *Prunus mandshurica* × *Prunus armeniaca* seedlings. *Acta Horticulturae* **701**, 223-225

Dorobanţu N, Molea I, Voican V (1986) *Lucrări Practice de Fiziologia Plantelor*. *Practical Works in Plant Physiology*, Nicolae Bălcescu Agronomic Institute Publishing House, Faculty of Horticulture, 136 pp

Dosba F, Lansac M, Audergon JM, Maison P, Massonie G (1988) Tolerance to Plum-pox virus in apricot. *Acta Horticulturae* **235**, 275-281

Egea J, Burgos L, Martinez-Gomez P, Dicenta F (1999) Apricot breeding for sharka resistance at CEBAS-CSIC, Murcia (Spain). *Acta Horticultu-rae* **488**, 153-157

El-Kady S, Hevesi M, Sule S, Klement Z (1986) Pathological and serological separation of some *Pseudomonas syringae* p.v. *syringae* strains isolated from apricot, sour cherry and pepper hosts. *Acta Horticulturae* **192**, 193-204

Erez A, Lavee S (1971) The effect of climatic conditions on dormancy development of peach buds temperature. *Journal of the American Society of Horticultural Science* **96**, 711-714

Erez A, Couvillon GA, Hendershott CH (1979) The effect of cycle length on chilling negation by high temperatures in dormant peach leaf buds. *Journal of the American Society of Horticultural Science* **104**, 573-576

Friundlund PR (1979) Incompatibility between apricot and *Prunus tomentosa* seedlings. *Fruit Varieties Journal of the American Pomological Society* **33**, 90

Garrett CME (1979) Screening *Prunus* rootstocks for resistance to bacterial canker caused by *Pseudomonas mors prunorum*. *Journal of the American Society of Horticultural Science* **3**, 189-193

Geuna F, Bassi D, Failla O, Mignani I (2006) Use of morphological and AFLP markers assess possible synonymy between apricot accessions. *Acta Horticulturae* **701**, 215-218

Ghelfi R, Lucchi M (2006) Apricot production in Italy: Value chain in the domestic and international markets. *Acta Horticulturae* **717**, 265-270

Gilreath PR, Bouchanam DW (1981) Rest prediction for low chilling "Sungold" nectarine. *Journal of the American Society of Horticultural Science* **106**, 189-193

Giulivo G, Nicotra A, Roselli G (1983) Ruolo ed obiectivi del miglioramento genetico in frutticolutra. Nuove aequisizioni del miglioramento genetico italiano e scelte varietali in frutticoltura 2. *Quaderno ISOI* **83**, Centro operativo ortofrutticolo Ferrara

Goodall KB (2003) In search of the fountain of youth - preliminary analysis of deuterium's role in DNA degradation. Available online: www.slwater.com/Fof4-DDWpdf, 15 pp

Guerriero R (1984) ICAPI promettenti selezioni di albicocco. Informatore Agrario. Nuove aquisizioni del miglioramente genetico italiano e scelte varietali in frutticoltura 2, *Guaderno IFSOI* **83**, Centro operativo ortofrutticolo Ferrara, 5 pp

Guerriero R, Indiogine S, Scalabrelli G (1985) Influence on relations between bud and mother plant on changes in peach bud dormancy. *Acta Horticulturae* **173**, 113-122

Guerriero R, Indiogine SE, Scalabrelli G (1985) The effect of cyclic and constant temperatures in fulfilling the chilling requirement of two apricot cultivars. *Acta Horticulturae* **192**, 41-48

Guerriero R, Monteleone R, Marocco F (1995) Distribution of main fruit and trees traits in some apricot progenies, Apricot Culture and Decline. *Acta Horticulturae* **192**, 79-83

Guerriero R, Monteleone P, Viti R (2006) Evaluation of end of dormancy in several apricot cultivars according to different methodological approaches. *Acta Horticulturae* **701**, 99-103

Guerriero R, Monteleone P, Iacona C (2006) Apricot breeding in Pisa: some new selections for Italian growers. *Acta Horticulturae* **701**, 341-346

Guerriero R, Lomi F, D'Onofrio C (2006) Influence of some agronomic and ecological factors on the constancy of expression of some descriptive characters included in the UPOV apricot descriptor list. *Acta Horticulturae* **717**, 51-54

Guerriero R, Viti R, Bartolini S, Iacona C (2006) Parents for spring frost tolerance in apricot. *Acta Horticulturae* **717**, 153-156

Ham H (2006) Preliminary study on the effect of climate on colour development of two apricot (*Prunus armeniaca*) cultivars. *Acta Horticulturae* **717**,

381-384
Hansche P, Hesse C, Beres V (1972) Estimates of genetic environmental effects on several traits in peach. *Journal of the American Society of Horticultural Science* **97**, 76-79
Hansche P, Beres W (1980) Genetic remodeling offrait and nut trees to facilitate cultivars improvement. *Horticultural Sciences* **15**, 710-715
Hansche P (1986) Heritability offrait quality traits in peach and nectarine breeding stocks dwarfed by the dw gene. *Horticultural Sciences* **21**, 1193-1195
Hansche PE, Hesse GO, Beres V (1975) Inheritance of fruit size, soluble solids and ripening date in *Prunus domestica* cv. *Journal of the American Society of Horticultural Science* **5**, 522-524
Hansen P, Ryngo K (1979) Translocation and metabolism of carbohydrate fraction of ^{14}C–photosynthesis in "Frendi" prune, *Prunus domestica*. *Journal of the American Society of Horticultural Science* **104**, 622-625
Hatch AH, Walker DR (1969) Rest intensity of dormant peach and apricot leaf buds as influenced by temperature cold hardiness and respiration. *Journal of the American Society of Horticultural Science* **94**, 304-307
Ionică M, Bălan V, Stoian E (1990) Cercetări privind agentul de tip micoplasmă, izolat în Romania din pomi de cais în decline. Research on the mycoplasma-type agent isolated from declining apricot. *Annals of Cereals and Technical Plants Research Institute Fundulea* **XXIII**, SC Agris - Redacția revistelor agricole SA Fundulea, pp 29-33
Kamali K, Majidi E, Zarghami R, Arvin MJ (2006) Differences in micropropagation of vegetative rootstock (GF677) and other almond seed genotypes. *Acta Horticulturae* **726**, 199-200
Karayannis I (1988) Susceptibility of apricot cultivars to Plum-pox virus in Greece. *Acta Horticulturae* **235**, 271-275
Karayannis I, Mainou A, Stylianidis D, Thonidis T, Karayannis NI, Tsaftaris A (2006) Resistant to Sharka disease (PPV) apricot hybrids of high quality, selected in Greece. *Acta Horticulturae* **701**, 337-340
Klement K (1977) Apricot apoplexy. *EPPO Bulletin* **7**, 7-9
Koubouris G, Vasilakakis M (2006) Improvement of *in vitro* propagation of apricot cultivar 'Bebecou'. *Plant Cell, Tissue and Organ Culture* **85**, 173-180
Krska B, Oukropek I, Audergon JM, Polak J, Kominek P (2006) Evaluation of resistance of apricot progeny (VestarxSEO) to Plum-pox virus. *Acta Horticulturae* **701**, 313-316
Kuebemen EA, Wallace DH, Ludford PM (1979) Photosynthetic measurements of field grown dry beans and their relation to selection for yield. *Journal of the American Society of Horticultural Science* **104**, 480-482
Lachkar A, Mlika M (2006) New apricot varieties selected from the Tunisian breeding programme. *Acta Horticulturae* **717**, 189-192
Lapins KO (1983) Mutation breeding. In Moore JN, Janick J (Eds) *Methods in Fruit Breeding*, Purdue University Press, West Lafayette, 74-99
Layne REC (1980) Harogem apricot. *Fruit Varieties Journal of the American Pomological Society* **34**, 20
Legave JM, Fournier D, Costes E (2006) Genotype × location interactions on architectural and floral traits in apricot tree. *Acta Horticulturae* **717**, 33-36
Lespinasse Y, Lespinasse JM, Ganne B (1985) Inheritance of two agronomical characters in the apple tree (*Malus pumila* Mill): Compact type habit and fruit colour. *Acta Horticulturae* **159**, 34-47
Lilleland O (1935) Growth study of the apricot fruit. *Journal of the American Society of Horticultural Science* **33**, 237-245
Lilleland O (1935) Growth study of the apricot tree II – The effect of temperature. *Journal of the American Society of Horticultural Science* **33**, 269-279
Lloyd G, McCown B (1981) Commercially –feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. *Proceedings International Plant Propagation Society* **30**, 421-427
Loschnig J, Passecker F (1954) *Die Marille (Aprikose) und thre Kultur*, Osterreichischer Agrarverlag, Wien, 398 pp
Loukas M, Pontikis CA (1985) Pollen isozyme polymorphism in types of *Pistacia vera* and related species. *Journal of the American Society of Horticultural Science* **54**, 95-102
Manganaris AG, Alston FH (1989) Glutamate oxaloacetate transminase izoenzymes in apple cultivars and rootstocks. *Journal of the American Society of Horticultural Science* **64**, 9-15
Margarian AA, Marutian SA, Michaelian VM (1986) Nitrogen metabolism in apricot as a function of mineral nutrition. *Acta Horticulturae* **192**, 35-40
Marino G (1989) Isolation and culture of apricot (*Prunus armeniaca* L) protoplasts: initial results. *Acta Horticulturae* **293**, 391-394
Marino G, Bertazza G, Magnanini E, Altan DA (1993) Comparative effects of sorbitol and sucrose as main carbon energy sources in micropropagation of apricot. *Plant Cell Tissue and Organ Culture* **34**, 235-244
Mănescu C, Baciu E, Cosmin S (1975) *Combaterea Biologică în Pomicultură. Biological Control in Pomology*, Ceres Publishing House, Bucharest, 227 pp
McLean IB, Rasmussen HP (1980) Graphic display of quantitative and qualitative characters association in roses. *Journal of the American Society of Horticultural Science* **5**, 625-628
Mehlenbacker SA, Hough LP (1986) Progress in apricot breeding in New Jersey. *Acta Horticulturae* **192**, 167-175
Mencarelli F, Bellincontro A, Forniti R, Vizovitis K, Botondi R, Valentini M, Sequi P, DiNatale C, Basile B, Romano R (2006) Factors affecting the apricot quality for the consumer with special attention to the use of 1-MCP and of NDT for detection of bruising. *Acta Horticulturae* **717**, 315-320
Minea R, Tamaș A, Corneanu M, Corneanu CG (2003) The utilisation of multiple emulsion with magnetic fluid content in biological cultures. *Annals of West University of Timisoara, Series Chemistry* **12**, 17-21
Mircetich MS (1981) Phytophora root and crown rot of apricot trees. *Acta Horticulturae* **121**, 385-395
Mittermayer F (2006) The apricots market - An EU perspective. *Acta Horticulturae* **717**, 261-264
Monet R (1967) Contribution a l'etude genetique du pecher. *Annales de l'Amelioration des Plantes* **17**, 5-11
Monet R, Bastard Y (1971) Contribution a l'etude du controle genetique du quelques caracteres morfologique chez le pecher. *Annales de l'Amelioration des Plantes* **22**, 399-403
Monet R, Bastard Y (1971) Resistance au gel et evolution physiologique des bourgeons floreaux du pecher. *Annales de l'Amelioration des Plantes* **27**, 717-728
Monet R, Bastard Y (1971) Physiologie vegetale. *C.R. Academie des Science Paris Serie S*, 2317-2319
Monet R (1977) Amélioration du pecher par la voie sexuée; exposé d'une méthode. *Annales d'Amelioration des Plantes* **27**, 223-234
Monet R, Bastard Y (1982) Estimation du coefficient de régression enfant/parent de quelques caractères du pêcher dans le cas de familles issues d'autofécondations. *Agronomie* **2**, 347-358
Moore JN (1979) Pomology research and production in Romania and Poland. Fruit varieties. *Journal of the American Pomological Society* **33**, 70-73
Morvan G, Castelain C, Arnoux M (1985) Prospective for the control of apricot chlorotic leaf roll, a mycoplasma disease by cross protection. *Acta Horticulturae* **192**, 167-175
Morvan G (1988) The epidemiology of Plum-pox virus in the apricot orchards of southern France: Description of computer tools and main observation. *Acta Horticulturae* **235**, 263-271
Muleo R, Remorini D, Iacona C, Guerriero R, Massai R (2006) Cultivar-specific adaptation of growth and photosynthetic activity in developing apricot dense canopies. *Acta Horticulturae* **717**, 55-58
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497
Németh M (1986) *Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees*, Martinus Nijhoff Publishers, The Netherlands, 840 pp
Nicotra A, Conte L, Moser L, Fantechi P, Barbagiovanni I, Corazza ML, Vitale S, Magnotta A (2006) Breeding programme for *Monilinia laxa* resistance on apricot. *Acta Horticulturae* **701**, 307-311
Nyujto F, Németh M, Koller M (1985) Investigation on the resistance to Plum-pox virus disease of the apricot variety "Cegledi Bibor Kajszi C 244". *Acta Horticulturae* **192**, 159-166
Okie WR (1983) Rootstocks for cherry, plum and apricot, present and future. *Fruit Varieties Journal of the American Pomological Society* **37**, 15-23
Olgun A, Adanacioglu H (2006) Production and marketing of organic dried apricot and the tendencies of apricot producers for the future in Turkey: Case study of Malatya. *Acta Horticulturae* **717**, 271-280
Ozturk K, Olmez HA, Guloglu U, Kuden A (2006) Evaluation of the resistance of some apricot varieties growing in Malatya to winter hardiness and late spring frost. *Acta Horticulturae* **701**, 247-251
Paunovic SA, Plazinic R (1976) Ispitanje nasledivanje u Kaisije (*Prunus armeniaca*) u F1 generaciji. *Prestampano iz iugoslavensky vocarstava* **37-38**, Cacak
Paunovic SA, Plazinic R, Orgasonovic D, Lyufic M (1983) The influence of the parent cultivars on obtaining late blooming apricot hybrids. *Acta Horticulturae* **121**, 275-282
Paunovic SA, Plazinic R (1983) The breeding and introduction of new apricot selections. *Acta Horticulturae* **121**, 235-244
Paunovic SA, Padzarevic P, Kojonic R (1985) Knlonska seleksja I izbor najboljih autohtonic kajsija injenik podloga. *Prestampano iz iugoslavensky vocarstava* **71-72**, Cacak
Paunovic SA (1986) Thirty-five years of apricot breeding and clonal selections of apricot cvs and rootstocks. *Acta Horticulturae* **192**, 299-306
Paydas S, Eti S, Gülcan R, Derin K, Yilmaz KU (2006) *In vitro* investigations on pollen quality, production and self incompatibility of some apricot varieties in Malatya – Turkey. *Acta Horticulturae* **701**, 75-80
Pedryc A, Korbuly J, Sardi E (2006) Relationship between sugar composition of the buds and frost tolerance in apricot cultivars. *Acta Horticulturae* **701**, 57-62
Pena A (1986) *Genetică Horticolă – Manual de Procedură Pentru Lucrări de Laborator*. *Horticultural Genetics – Procedure Manual for Laboratory Works*, Nicolae Bălcescu Agronomic Institute Publishing House, Bucharest, 160 pp
Pennone F, Abbate V (2006) Apricot breeding in Caserta: new perspectives of apricot growing in Southern Italy. *Acta Horticulturae* **717**, 157-162
Pérez-Tornero O, Burgos L, Egea J (1999) Introduction and establishment of apricot *in vitro* through regeneration of shoots from meristem tips. *In Vitro Cell. The International Journal of Developmental Biology* **35**, 249-253
Pérez-Tornero O, Burgos L (2000a) Different mediums requirements for micropropagation of apricot cultivars. *Plant Cell, Tissue and Organ Culture* **63**, 133-141
Pérez-Tornero O, Lopez JM, Egea J, Burgos L (2000b) Effect of basal me-

dia and growth regulators on the *in vitro* propagation of apricot (*Prunus armeniaca* L) cv. Canino. *Journal of Horticultural Science and Biotechnology* **75**, 283-286

Pérez-Tornero O, Lopez JM, Burgos L (2006) Influence of explant type (meristem vs. axillary shoots on the introduction and establishment *in vitro* of four apricot cultivars. *Acta Horticulturae* **717**, 229-232

Petrişor C, Bălan V, Lazăr V, Tudor V (2006) Evolution of colour during maturation of different Romanian apricot cultivars. *Acta Horticulturae* **717**, 369-372

Poessel JL, Faurobert M, Loonis M, Corre MN, Olivier G, Restier V, Audergon JM, Masse M (2006) Physiological and genetic studies on apricot/ *Prunus* rootstocks graft compatibility. *Acta Horticulturae* **701**, 87-93

Popa G, Bălan V, Ivaşcu A, Plocon C (2005) Studies on the suitable *in vitro* culture conditions to micropropagation of some apricot and peach rootstock /in Romanian/ Proceedings of 14th National Symposium of Plant Tissue Culture: Vitrocultures in cormophytae, experimental models in biology research, Bion, Satu Mare, pp 133-137

Quoirin M, Lepoivre P (1977) Etude de milieux adaptes aux cultures *in vitro* de *Prunus*. *Acta Horticulturae* **78**, 437-442

Radovet-Salinski D, Cachita-Cosma D, Blidar CF, Beles D (2004) The influence of low deuterium water (87.5 ppm D) on hyperhidric Coleus blumei Benth vitrocultures. Proceedings of 3rd Congress "Water – a miracle". Constanţa **1**, 59-60

Ramming DW (1980) Apricot cultivar situation in North America. *Fruit Varieties Journal of the American Pomological Society* **34**, 70-74

Reffatti E, Osler R, Loi N, Carraro L, Benetti V, Tomasi A, Vindimiari ME (1985) An attempt in progress to eradicate Plum-pox. *Acta Horticulturae* **235**, 291-297

Renaud R (1980) Mutagenese artificielle sur le prunier d'ente. Caracteres agronomiques de deux mutants selections. *INRA Publication* **546**

Richardson A, Seeley S, Walker D (1974) A model for estimating the completion of rest for "Redhaven" and "Elberta" peach trees. *Journal of the American Society of Horticultural Science* **9**, 331-332

Rishbeth Y (1964) Stump infection by basidiospores of *Armillaria mellea*. *British Mycological Society* **47**, 460

Rodrigo J, Julian C, Herrero M (2006) Spring frost damage in buds, flowers and developing fruits in apricot. *Acta Horticulturae* **717**, 87-88

Rogalski M, Antunes de Moraes LK, Felisbino C, Crestani L, Guerra MP, Lima da Silva A (2003) Acclimatization of micropropagated *Prunus* sp. rootstock. *Revista Brasileira de Fruticultura* **25**, 279-281

Romero C, Llacer G, Badenes ML, Pedryc A (2006) Relationship among apricot cultivars from Hungary and a South European pool determined by SSR markers. *Acta Horticulturae* **701**, 233-239

Rozsnyay ZD (1977) Cytospora, canker and decline dieback of apricots. *OEPP Bulletin* **17**, 69-80

Rozsnyay ZD (1983) The appearance of *Eutypa armeniacae* (Hansford) carter on apricot and grape in Hungary. *Acta Horticulturae* **121**, 397-400

Ruiz D, Campoy JA, Egea J (2006) Chilling requirements of apricot varieties. *Acta Horticulturae* **717**, 67-70

Salesses G, Bonnet A (1988) Cytogenetic study of hybrids between hazelnut varieties carrying a translocation in heterozygous state. *Cytologia* **5**, 407-413 (in French)

Sansavini S, Costa G, Marangoni B, Bordini R (1974) Sei anni di osservazioni su due cultivar e dodici portinnesti di albicocco, Instituto Coltivazioni di Arboree, Universita di Pisa. *Rivista di Ortoflorofrutticultura Italiana* **4**, 286-303

Sansavini S, Bassi D, Grandi M, Pirazzini P, Montevecchi P, Lunati U, Cavicchi C (1988) Le cultivar di albicocco in Emilia-Romagna. III contributo. *Rivista di Frutticoltura* **6**, 41-47

Săvulescu T, Săvulescu O (1959) *Tratat de Patologie Vegetală. Treatise on Plant Pathology* (Vol 1), Romanian Academy Publishing House, Bucharest, 485 pp

Scalabrelli G, Couvillon GA (1985) Influenza della temperatura e del tipo di gemme sul termine della dormienza nel pesco as "Redhaven". *Acta Horticulturae* **192**, 157-165

Scaltsoyiannis A, Iliev I, Moulalis D, Passayiannes G, Tsoulpha P, Tsaksira M (1998) Rejuvenation and micropropagation of wild cherry trees (*Prunus avium* L.). Plant Breeding and Genetics for Agriculture that is Friendly to the Environment. Proceedings of the 7th PanHellenic Congress, Heraklion, Crete, pp 272-280

Sesan T, Oprea M (1999) Influence of antagonistic micromyceta from phyllosphere on the main pathogens of apricot-tree. *Acta Horticulturae* **488**, 705-710

Sjujka-Lacza J (1985) Data on the morphology and anatomy of *Prunus armeniaca* L. *Acta Horticulturae* **192**, 9-19

Smith C, Mollendorf L (1999) Apricot (*Prunus armeniaca* L.) production and breeding in South Africa. *Acta Horticulturae* **488**, 119-124

Snir I (1983) Chemical dormancy breaking of red raspberry. *Journal of the American Society of Horticultural Science* **18**, 710-713

Spiegel-Roy P, Alston FH (1979) Chilling and post dormant heat requirement as selection criteria for late flowering pears. *Journal of the American Society of Horticultural Science* **54**, 115-120

Strada GD, Pennone F, Fideghelli C, Monastra F, Cobianchi D (1989) Monografia di cultivar di albicocco. Istituto Sperimentale per la Frutticoltura, Roma, 257 pp

Struss D, Ahmad R, Aman M, Southwick SM (2006) Identifying cultivars and genetic diversity of apricot by molecular markers. *Acta Horticulturae* **701**, 253-256

Suranyi D (1985) The role of ecological factors affecting the fruit thinning of apricot. *Acta Horticulturae* **192**, 73-81

Szalay L, Papp J, Pedryc A, Szabo Z (2006) Diversity of apricot varieties based on traits determining winter hardiness and early spring frost tolerance of floral buds. *Acta Horticulturae* **701**, 131-134

Theobald WL, Hough LF (1960) The relationship between stages of peach embryo development and seedling growth and survival. *Proceedings of the American Society for Horticultural Science* **75**, 163-170

Topor E, Burtoiu MC (2006) New apricot selections with very early ripening maturity obtained under Romanian conditions. *Acta Horticulturae* **701**, 367-369

Trandafirescu M, Teodorescu G (2006) Behaviour of some apricot and hybrids from national collection to *Monilinia laxa* (Aderh et Ruhl) Honey infection. *Acta Horticulturae* **701**, 371-375

Tzoneva E, Tzonev R (1999) Pomological peculiarities of native Japanese Ansu and Mume cultivars and some interspecific hybrids. *Acta Horticulturae* **488**, 233-235

Vanderzwet T, Otto WA, Westwood MN (1975) Variability in degree of fire blight resistance within and between pyrus species, interspecific hybrids and seedling progenies. *Euphytica* **23**, 295-304

Wang Yu-Zhu, Liu Qi-Zhi (2006) Apricot germplasm resources in China. *Acta Horticulturae* **701**, 181-189