

## **S U M M A R Y**

of the doctoral thesis entitled:

### **RESEARCH ON THE RESPONSES OF PURPLE POTATO AND SWEET POTATO TO *IN VITRO* CULTIVATION**

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The doctoral thesis titled "Research on the responses of purple potato and sweet potato to *in vitro* cultivation" provides a comprehensive analysis of the *in vitro* responses of two purple potatoes cultivars, 'Purple Majesty' and 'Violet Queen', and two cultivars for sweet potatoes, 'RO-CH-M' and 'KSP-1' to different concentrations of synthetic hormones, namely auxins (NAA), cytokinins (BAP), and gibberellins (GA<sub>3</sub>). This research is significant in plant biotechnology and agricultural sciences, as it explores the physiological responses of these cultivars under controlled hormonal variations, aiming either to enhance breeding and develop new varieties with desirable traits, either to support commercial, free from viruses and disease tubers production, in the quantities requested by the market, at a specific time.

The research was structured around four scientific objectives, which included: (1) assessing the impact of hormonal variations on plant growth; (2) identifying cultivar-specific responses to hormonal changes; (3) evaluation of optimal hormone concentrations for best physiological responses, and (4) evaluating the complex roles of auxins, gibberellins, and cytokinins in micropropagation.

*In vitro* experimental studies were conducted at the Laboratory of Plant Micropropagation at the U.S.A.M.V. of Bucharest from 2021 to 2024 used biological material included the four varieties mentioned above, and the experimental designs included various hormonal treatments across a spectrum of concentrations, with Control setups maintaining no hormone additions.

The thesis contains 239 pages, divided into two main parts, further divided in chapters and subchapters: the bibliographical study, with two chapters and the personal research, with 7 chapters, and additional content, as the introduction, the summary, the final conclusions, the references, and annexes.

The first chapter, titled "Importance of purple potatoes and sweet potatoes at a global level. Economic and food perspectives", highlights the global significance of *S. tuberosum* (potato) and *I. batatas* (sweet potato) from economic and food security viewpoints, being an overview of the strategic importance and diverse benefits of cultivating these crops on a global scale. The chapter provides an in-depth look at their origins, highlighting their domestication, genetic diversity, and adaptability, which allowed widespread cultivation and staple status in diets worldwide. The chapter presents the nutritional value of these species, emphasizing their essential contributions to the human diet through key nutrients like carbohydrates, vitamins, and minerals, and specifically notes the enhanced antioxidant properties of purple potato varieties. It is presented the role these crops play in achieving SDG 2 ('Zero Hunger') by highlighting their potential to enhance food security, nutrition, and sustainable agricultural practices.

The second chapter, titled 'Current status of potato and sweet potato micropropagation research', presents the advancements and methodologies in potato and sweet potato's micropropagation. The chapter lists the principles and applications of micropropagation, presenting its role in the rapid, large-scale production of genetically uniform and disease-free plants. It emphasizes the importance of controlled environmental conditions, nutritional and hormonal regulation, and the selection of explant material for successful *in vitro* cultivation. The chapter also discusses the integration of micropropagation with other biotechnological approaches such as somaclonal variation, to enhance crop improvement and conservation efforts. Furthermore, it explores the significant role of potato and sweet potato technologies for micropropagation in the conservation of genetic resources through gene banks, ensuring the preservation and availability of diverse plant genotypes for future breeding and restoration projects.

The second part of the doctoral thesis is structured in 7 chapters, in which the personal research activities and their results are presented.

The third chapter, titled 'Materials and methods' presents the aim and objectives of the study, as well as the methodology used to achieve the proposed objectives, regarding the *in vitro* growth responses of the purple potato varieties 'Purple Majesty' and 'Violet Queen', along with the sweet potato varieties 'RO-CHM' and 'KSP-1' under various hormonal treatments. The methodology involved the preparation of biological material and the establishment of the experiments, utilizing a standard culture medium with addition of auxins, cytokinins and gibberellins, at different concentrations. Different hormonal treatments were chosen to understand their individual and combined effects on plant growth. Each hormone's concentration varied across a range of five concentrations to observe their influences distinctly. The study used statistical analysis to evaluate the data, ensuring the scientific validity of the findings. In summary,

Chapter IV, titled 'Results regarding the hormones influence on *in vitro* cultivation of 'Violet Queen' purple potato variety', discusses the effects of synthetic hormones on the growth of this variety. The chapter presents how different concentrations of auxins (NAA), along with fixed levels of cytokinins (BAP) and

gibberellins (GA<sub>3</sub>), influence growth parameters including roots and shoots growth, plant height, and leaves production. The main results of the chapter include: (1) on roots growth, Control plants (no hormones treatment) generally exhibited more roots compared to hormone-treated groups, NAA consistently initiated roots but inhibited growth at higher concentrations, while lower BAP concentrations (V<sub>2</sub> at 0.25 mg/L) promoted superior roots growth; (2) on shoots growth, the highest number of shoots was observed on NAA treatment on Y<sub>3</sub> (0.02 mg/L), as well on moderate concentrations of GA<sub>3</sub>, suggesting an optimal hormone balance at this concentrations for shoots growth; (3) for plant height, plants in Control were taller than those treated with hormones, suggesting that NAA may suppress stem elongation in addition with other two hormones at mentioned concentrations, as well as GA<sub>3</sub> showed a dose-dependent effect; (4) for leaves number, Y<sub>3</sub>, with a moderate NAA concentrations, increased leaves numbers at the end of time frame, highlighting NAA's role in enhancing leaves initiation, while moderate BAP level, like V<sub>2</sub> and V<sub>3</sub>, promote leaves production at the middle of time frame.

Chapter V, titled 'Results regarding the hormonal influence on *in vitro* cultivation of 'Purple Majesty' purple potato variety, examines the impact of synthetic hormones on the *in vitro* growth of the second purple potato variety, focusing on how varying concentrations of hormones affect roots and shoots formation, and overall plant growth. The main results include: (1) on roots growth: Y<sub>5</sub> (0.05 NAA mg/L) performed better than the Control, promoting the best roots number values, as well V<sub>2</sub> and V<sub>4</sub> on BAP experiment, which achieved the highest roots number; higher concentrations, such as 1 mg/L in variant X<sub>3</sub> (on GA<sub>3</sub>) significantly stimulated roots growth compared to Control; however, lower concentrations showed mixed results, with some variants displaying growth rates comparable to or lower than the Control; (2) auxins (NAA) showed significant increases in shoots numbers, with higher concentrations (Y<sub>5</sub>) being most effective; cytokinins (BAP), particularly at moderate concentrations (V<sub>2</sub>), promoted substantial shoots proliferation; GA<sub>3</sub> demonstrated varying effectiveness, with higher concentrations (X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>) consistently promoting shoots growth; (3) on plant height, even Control had best values, NAA higher concentrations, like in Y<sub>5</sub> perform with comparable shoots height; lower-medium concentration of BAP, V<sub>2</sub> promoted growth; taller explant were observed as well on higher concentrations of GA<sub>3</sub>, even growth rate were not consistently over time; (4) for leaves number, NAA demonstrates effectiveness at lower concentrations (Y<sub>2</sub>, Y<sub>3</sub>), while BAP had an optimal response in V<sub>2</sub> experiment; initially, GA<sub>3</sub> at a moderate concentration (1 mg/L) from X<sub>3</sub> provide most effective growth.

Chapter VI, titled 'Hormones action on purple potato growth', present the overview of purple potatoes conclusions about response to hormonal treatment though *in vitro* cultivation in specific experimental design.

Chapter VII, titled 'Results regarding the hormones influence on *in vitro* cultivation of 'RO-CH-M' sweet potato', explores the impact of synthetic hormones on the *in vitro* growth of the 'RO-CH-M' sweet potato variety, as it was done for the purple

potato. The main results include: (1) on root growth, higher concentrations (0.05 mg/L) from NAA (Y<sub>5</sub>) enhanced roots growth while BAP influenced roots growth with optimal concentrations around 0.25 mg/L; GA<sub>3</sub> suggest lower-moderate concentrations (1 mg/L) for roots growth, while higher concentrations have an inhibiting role; (2) on shoots growth, moderate concentrations of NAA (Y<sub>3</sub>) result in best results, while moderate BAP concentrations (V<sub>3</sub>) were most effective; optimal GA<sub>3</sub> concentration showed to be in X<sub>3</sub> variant; (3) on plant height, NAA had a negative impact, especially on Y<sub>5</sub>; higher BAP concentration marked an inhibitory effect compared to Control, while GA<sub>3</sub> s lower concentration appear to be more effective in promoting shoots height; (4) for leaves number, higher concentrations of NAA initially suppress leaves number but can stimulate growth over time, BAP in V<sub>3</sub> concentration was optimal for growth on early leaves growth, while low-medium GA<sub>3</sub> concentrations (X<sub>3</sub>) shows moderate effects.

Chapter VIII, titled 'Results regarding the hormones influence on *in vitro* cultivation of 'KSP-1' sweet potato hybrid', focuses on the impact of the same three hormone treatments on the *in vitro* growth of the second studied sweet potato variety. The main results include: (1) on roots growth, concentrations around 0.03 mg/L NAA perform better, and Control consistently showing fewer roots; V<sub>2</sub> and V<sub>3</sub> (BAP) promotes optimal roots growth, while higher concentrations do not rich such growth increases; especially at lower levels of GA<sub>3</sub> (0.5 mg/L), roots numbers enhancing growth; (2) on shoots growth, Y<sub>4</sub> and Y<sub>5</sub> (NAA) lead to more shoots numbers compared to Control, V<sub>2</sub> and V<sub>3</sub> (BAP) had superior values, and X<sub>3</sub> from GA<sub>3</sub> variant produce more shoots then Control; (3) NAA (Y<sub>3</sub> and Y<sub>4</sub>) inhibit growth compared to Control, while moderate BAP concentrations promoting better elongation than higher doses; for gibberellins, 2 mg/L was effective in growth; (4) for leaves number, high NNA concentration (Y<sub>5</sub>) outperforming Control, BAP with higher concentrations (V<sub>5</sub>) consistently reducing leaves numbers compared to moderate doses (V<sub>2</sub>) and Control, while GA<sub>3</sub> high doses (X<sub>5</sub>) showed optimal leaves growth, moderate levels (X<sub>2</sub> and X<sub>4</sub>) were less effective.

Chapter IX, titled 'Hormones action on sweet potato growth', presents the summary of the sweet potato results, listing all important conclusions about response of 'RO-CH-M' and 'KSP-1' to hormonal treatment though *in vitro* cultivation in specific experimental design.

Chapter X, titled 'General conclusions and recommendations', presents comprehensive conclusions and direct recommendations derived from the research findings presented in this thesis. The experiments present the varietal-specific responses of sweet potatoes and purple potatoes to hormone treatments. Purple potatoes showed distinct behaviors: 'Violet Queen' demonstrated steady growth and responsiveness to BAP and GA<sub>3</sub> treatments, while 'Purple Majesty' excelled in roots and shoots growth under NAA treatments. These findings emphasize the importance of refining hormone applications based on cultivar characteristics for optimizing growth in agricultural settings. On sweet potatoes, 'RO-CH-M' displayed a good growth across all measured parameters, highlighting its potential for high-performance agriculture

and tissue culture applications. In contrast, 'KSP-1' had moderate growth responses, suggesting its suitability for environments where steady growth rates are beneficial.

Based on the above-mentioned findings, the doctoral studies could have an impact on (1) enhanced use of synthetic hormones, as the research advances the knowledge about how different auxins, cytokinins and gibberellins concentrations influence the growth of sweet potato and purple potato studied varieties; (2) adjusting cultivation strategies by identifying variety-specific growth responses to hormonal treatments, which can enable more precise and efficient cultivation strategies that can be adapted to the specific needs of each potato variety, based on their unique physiological responses; (3) promoting agricultural diversification, as the results obtained from this research could support agricultural production, especially in regions where variable climatic conditions requires the development of new production systems, by providing adapted and high-performing seed material.

Following this thesis, future research directions could address the following areas: (1) hormonal optimization, thereby improving the efficiency of micropropagation techniques and plant health post-transplantation; (2) genetic studies, which could help elucidate the mechanisms by which these potato and sweet potato varieties respond differently to hormonal treatments, supporting breeding programs and the development of new varieties with increased yields and stress resistance; (3) in-depth analysis of qualitative parameters observed during experiments but not presented in the thesis (e.g., callus formation, anthocyanic callus, adventitious root proliferation on purple potatoes, secondary shoots, tuberization), as valuable data can be obtained regarding the physiological response of explants during their in vitro cultivation stage; (4) scaling and automation, as the large-scale application of research results could lead to new systems for optimizing hormone dosages and monitoring growth parameters in real-time; (5) extrapolating the findings to other economically important crops to contribute to global food security and crop resilience.