USE OF MOLECULAR MARKERS IN IMPROVING RESISTANCE TO BIOTIC STRESS IN SOLANACEAE – A REVIEW

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Abstract

Solanaceae family comprises tens of genera and thousands of species, including numerous cultivated plants such as tomato, potato, eggplant, tobacco, deadly nightshade and petunia, used for human diet, ornamental and pharmaceutical purpose as well as biological model systems. Plants must continuously defend against attacks from pest, viruses, bacteria and fungi for improving biotic stress tolerance. Molecular markers linked to specific genes responsible for resistance / tolerance to pathogens or pests can be used to accelerate the breeding process in order to create new varieties that are not only desirable for the quality of the end product but are also resistant to biotic stress. This review brings together data referring to molecular markers linked to various phenotypic traits related to plant biotic stress resistance and the benefits of resistance versus chemical protection.

Key words*: biotic stress, molecular markers, SNP, SSR, resistance, Solanaceae.*

INTRODUCTION

The Solanaceae family comprises species present on all continents except Antarctica, species with various durations of life cycle and adapted to a wide range of life environments. Members of this family are used not only for human diet or ornamental purposes, but also in the pharmaceutical field, since some of the substances they metabolize have medicinal properties, and last but not least they are useful as model plants in scientific research. As the gene content of the different species remains constant despite their different phenotypes, Solanaceae family represents an excellent model for studying plant adaptation to natural or agricultural environments (Mueller et al., 2009).

During their life cycle, plants must continuously defend against attacks from pests, viruses, bacteria and fungi. Traditional cultivated tomato lack genetic diversity. Therefore, it has been suggested to transfer the desired resistance traits from their wild type relatives (Rick & Chetelat, 1995). Plant breeders are looking for novel techniques to hasten the breeding process with the aim of creating new varieties of plants tolerant/resistant to biotic stress. In the past three decades, with the advent of large scale DNA sequencing, new ways to improve the classic breeding techniques have been discovered. More and more genes putatively responsible for specific or broad resistance to biotic stress are being revealed every year, and changes in the DNA composition, either in the coding regions or in the regulatory regions, led to the identification of numerous molecular markers that are linked to tolerance/resistance to biotic stress. For instance, Paterson et al. (1988) used for the first time a complete Restriction Fragment Length Polymorphism (RFLP) linkage map in tomato to identify quantitative trait loci (QTLs).

QTL mapping can be used to further understand the genetic basis of various traits,

including resistance to biotic stress (Barone et al., 2008). Once QTLs for a specific trait (e.g. resistance/susceptibility to a certain pathogen or pest) have been identified, there are two avenues of follow-up research. In case the genome of the species under study is not sequenced, the identified QTL region can be sequenced. Then, the putative functions of the genes found within that region can be assessed by sequence similarity comparisons with homologue genes from other organisms. In case the genome of the species under study has been sequenced, and the genes from that particular QTL region already identified, the focus of next studies will be on the gene/genes connected to the trait of interest, and what are the differences at DNA level between cultivars resistant and susceptible to a certain biotic stress. At this point, molecular markers are extremely helpful.

Molecular markers point out variations in DNA and can appear due to DNA mutations, such as substitutions (point mutations), rearrangements (deletions or insertions) and repeated DNA sequences. Markers localized within DNA close to the genes of interest are called gene tags (Collard et al., 2005). Plant breeding that uses molecular markers - marker-assisted selection (MAS) - has numerous advantages: selection of genotypes at seedling stage (hence time saving compared to traditional methods of selection), gene pyramiding (combining multiple genes responsible for a particular trait in a single genotype), avoidance of the transfer of undesirable genes, selection of traits with low heritability, etc. (Devi et al., 2017). For example, gene pyramiding is extremely useful tool when resistance to some biotic stress factors is controlled in small measure by several genes, rather than strong resistance due to only one or two main genes, as is the case of resistance to late blight (Adhikari et al., 2017).

The present review aims to catalogue data related to the use of molecular markers in species belonging to Solanaceae family, to point out the progress made using them for developing resistance to different pathogens and pests that affect species belonging to this family, as well as the benefits of resistance versus chemical protection.

An overview on molecular markers used for Solanaceae family studies

Some of the molecular markers employed to help the breeding process of cultivated plants from Solanaceae family are Single Nucleotide Polymorphism (SNP), Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Cleaved Amplified Polymorphic Sequence (CAPS), Sequence-Related Amplified Polymorphism (SRAP), Single-Nucleotide Amplified Polymorphism (SNAP), Conserved Orthologs Set (COS), Sequence Characterized Amplified Region (SCAR) (see Table 1).

Once the markers have been mapped to a specific place in the genome, they can be linked to various genes responsible to desirable/undesirable traits and can be used for selection in the breeding process.

Table 1. Molecular markers used for Solanaceae species

Marker type	Species	Reference
SNP	eggplant	Barchi et al., 2011 Acquadro et al., 2017
	potato	Hamilton et al., 2011 Draffehn et al., 2013 Mosquera et al., 2016 Berdugo-Cely et al., 2017 Enciso-Rodriguez et al., 2018 Santa et al., 2018 Tagliotti et al., 2018
	tomato	Jimenez-Gomez and Maloof, 2009 Sim et al., 2011 Hamilton et al., 2012 Sim et al., 2012 Iquebal et al., 2013 Viquez-Zamora et al., 2013 Kevei et al., 2015
	Solanum pimpinellifolium L.	Celik et al., 2017
RAPD	eggplant	Demir et al., 2010 Toppino et al., 2008
	pepper	Ilbi, 2003
	okra	Aladele et al., 2008 Nwangburuka et al., 2011 Prakash et al., 2011 Ikram-ul-Haq et al., 2013 Kaur et al, 2013
CAPS	eggplant	Toppino et al., 2008
	potato	van der Voort et al., 2000
	tomato	Yang et al, 2014
SSR	tomato	He et al., 2003; Ruiz et al., 2005; Grushetskaya et al., 2007; Sim et al., 2011; Ning et al., 2012; Todorovska et al., 2014
	eggplant	Stàgel et al., 2008; Tümbilen et al., 2011; Demir et al., 2010; Barchi et al., 2011

	potato	Feingold et al., 2005; Ghislain et al., 2001; Ghislain et al., 2009
	pepper	Kim et al, 2008; Ibarra-Torres et al, 2015
	russian box thorn	Chen et al., 2017
	cape gooseberry	Garzon-Martinez et al., 2012
AFLP	potato	van der Voort et al., 2000; Ghislain et al., 2001; Isidore et al., 2003; van Os et al., 2006
	tomato	Ning et al., 2012
	pepper	Caranta et al., 1999
	okra	Akash et al., 2013; Kyriakopoulou et al., 2014
RFLP	pepper	Kim et al, 2008
	tomato	Tanksley et al., 1992
	potato	Tanksley et al., 1992
	Solanum pimpinellifolium L.	Sharma et al., 2008
ISSR	Pepper	Ibarra-Torres et al., 2015
SRAP	tomato	Ruiz et al., 2005; Shaye et al., 2018
	eggplant	Li et al., 2010
SNAP	pepper	Kim et al., 2008
COS	potato	Lindqvist-Kreuze et al, 2013
SCAR	tomato	Yang et al., 2014

Molecular markers application to improve the resistance of Solanaceae species to some biotic stress factors

Molecular markers application for viruses resistance

Resistance to multiple viruses (tomato mosaic virus, ToMV; tomato spotted wilt virus, TSWV; tomato yellow leaf curl virus, TY-LCV) and additional resistance to *Verticillium* and *Fusarium oxysporum* Schlecht. present in "Anastasia" tomato variety can be detected using both SSR and SRAP marker systems (Ruiz et al., 2005). SSR markers used in the study could be used to discriminate among the three main cultivar types used in the study, but not among cultivars of the same type that had different phenotypes. Nevertheless, all cultivars studied could be differentiated using SRAP markers.

Potato virus Y (PVY) resistance can be classified into two main types of resistance: extreme resistance (ER), which shows either no symptoms or limited necrosis, and hypersensitive resistance (HR), that manifests either local necrotic lesions or systemic necrosis (Solomon-Blackburn & Barker, 2001). Kasai et al (2000) developed SCAR markers linked to Ry_{adg} gene in potato and PCR - based DNA markers linked to two ER genes ($Ry_{sto}$and Ry_{adg}) were used for MAS in potato by Heldak et al., (2007). In pepper resistance gene *Pvr4* was tagged using AFLP markers (Caranta et al., 1999). In the same study, one marker (the closest to *Pvr4* gene) was transformed to CAPS marker in order to aid MAS, especially *pvr* gene pyramiding in a single cultivar.

Yellow leaf curl virus, transmitted by the white fly, causes severe loss of production in tomatoes (Cohen and Lapidot, 2007). Resistance to this virus has been mapped to chromosome 11, to the *Ty-2* gene (Yang et al., 2014).

Molecular markers application for bacteria resistance

Ralstonia pseudosolanacearum causes the bacterial wilt in numerous Solanaceae species (Heyward, 1991). One QTL marker related to bacterial wilt resistance has been identified in tomato on chromosomes 6 using SSR markers (Geethanjali et al., 2010). This QTL has been confirmed and an additional QTL has been identified on chromosome 12 by Wang et al., (2013) using recombinant inbred lines (RILs). Recently, Kim et al. (2018) identified 265 SNPs located in these two QTLs that are responsible for resistance/susceptibility to bacterial wilt. One of these SNP markers located within a functional gene on chromosome 12, *Solyc12g009690.1*, may be used to select varieties resistant to bacterial wilt. In eggplant, resistance QTLs were identified on chromosomes 3 and 6 (Salgon et al., 2017).

Streptomyces scabiae Thaxter causes scarring of the potato tubers surface, reducing their quality and marketability. Enciso-Rodriguez et al. (2018) have identified a novel SNP locus for common scab resistance, mapped in a WRKY transcription factor region, on chromosome IX.WRKY transcription factors are known for their role in controlling systemic and acquired resistance, as they are either activating or repressing genes responsible for defence-related proteins synthesis.

Molecular markers application for fungi resistance

Early blight is caused by the bacterium *Alternaria solani* Sorauer. Resistance to this

disease is controlled by multiple genes, out of which none is conferring a major resistance (Adhikari et al., 2017). Furthermore, even if some wild species are resistant to early blight, the cultivated varieties that are moderately resistant have undesired traits as low yield and late maturity. Combining the QTLs from different species by gene pyramiding may produce a variety resistant to early blight (Adhikari et al., 2017).

Arafa et al (2017) identified two genes on tomato chromosome 6 that could confer resistance to tomato late blight produced by *Phytophtora infestans* Mont., result validated by SSR analysis. For potato, numerous studies on QTL mapping have been performed. QTLs for *late blight* resistance are present on chromosomes I, III, V, VII, VIII, IX, XII (Ghislain et al., 2001; Visker et al., 2003; Mosquera et al., 2016; Santa et al., 2018). The conclusion of the QTL studies was that polygenic resistance is more efficient and durable as opposed to resistance conferred by Rpi-genes (R genes to *P. infestans*), which was less effective. However the drawback of polygenic resistance in potato is the late maturity associated with the resistance, trait that is not desired by the breeders and growers (Danan et al, 2011). Nevertheless, using SNP markers, Draffehn et al. (2013) were able to select plants that had improved quantitative resistance to late blight, but were not compromised by late maturity. Furthermore, Mosquera et al. (2016) identified SNP markers in potato associated with quantitative resistance to late blight but not linked to late maturity. One of the R genes to *P. infestans* in *Solanum bulbocastanum* Dunal, a wild *Solanum* species, is the *RB* gene, mapped to chromosome 8, using RFLP and RAPD molecular markers (Naess et al., 2000). In a subsequent study, Colton et al. (2006) developed a PCR-based DNA marker for tracking the *RB* gene throughout the breeding process, while transferring this R-gene from *S. bulbocastanum* to *S. tuberosum*.

Root rot and seedling damping off is caused by *Phytophthora capsici* Leonian in pepper. Kim et al (2008) constructed a linkage map using RFLP and SSR markers. They developed SNAP, SSR and CAPS markers to QTL loci responsible to resistance to *P.capsici*.

Toppino et al., (2008) designed CAPS markers to be used for indirect selection of *Fusarium* resistance in eggplant. They initially performed an initially study of resistance inheritance using *Solanum aethiopicum* L. as a resistance donor, and discovered that one gene, *Rfo-sa1*, was responsible for the *Fusarium* resistance. Subsequent Bulk Segregant Analysis with RAPD markers led to the identification of three RAPD markers linked to the resistance trait, that were subsequently transformed to CAPS markers to be used for selection in future studies.

Molecular markers application for pest resistance

Even though potato cyst nematode broad-spectrum resistance in potato was considered to be a complex inherited trait. Van der Voort et al., (2000) demonstrated that actually two loci are responsible for *Globodera pallida* resistance: *Gpa5* and *Gpa6*. Both loci were mapped to chromosomes 5 and 9 using initially an AFLP marker online catalogue. Thereafter, the loci were identified using CAPS markers based on RFLP insert sequences. *Gpa 5* locus is located on chromosome 5 in a region that is also responsible for fungal and viral resistance. *Gpa 6* appears to be is also located in a resistance cluster on chromosome 9, which contains a virus resistance gene in a homologous tomato genome.

Santa et al., (2018) identified 15 QTLs linked to resistance to *Tecia solanivora*, guatemalan potato moth tuber, out of which 10 were identified in the phenotypic field trial and 10 in the storage conditions. Furthermore, seven QTLs out of the total fifteen QTLs identified were related to resistance and eight QTLs were related to susceptibility to *T. solanivora*.

Avila et al (2019) have identified QTLs for resistance to tomato-potato psyllid (TPP) in tomato wild relatives. Some of the accessions that have shown good resistance to TPP, also shown resistance to the bacterium *Candidatus* Liberibacter solanacearum (Lso).

Sun et al. (2020), using SNP markers, mapped resistance to aphid, *Myzus persicae*, to three QTL loci, two on chromosome 2, and one on chromosome 4. The QTL loci on chromosome 2 are affecting aphid survival and reproduction, whereas the locus on chromosome 4 is affecting aphid reproduction. The fine mapping of the locus on chromosome 2 affecting reproduction identified a DNA region containing resistance genes of the receptor-like kinase family containing a leucine-rich repeat domain (LRR-RLKs).

CONCLUSIONS

During their development, plants must continuously defend against attacks from viruses, bacteria, fungi and pests. Wild plants have developed in time resistance against such attacks, but cultivated plants, since they have been selected through domestication for different traits (yield, colour, taste, size, etc.) have lost resistance/tolerance to pathogens and pests. For instance, compared to the wild species of tomato, that have a large genetic diversity, cultivated varieties of tomato have much less diversity, less than 5% of the genetic variation of the wild species (Bai & Lindhaut, 2007).

Wild plants, relatives of the cultivated ones can be used a reservoir for genetic resistance. The molecular markers can be employed to verify the transfer of the desired DNA regions that control useful traits including resistance/tolerance to biotic stress during the breeding process. Genetic resistance, along with other desirable traits (taste, shape, size, etc.), can be transferred as well by marker-assisted selection from some established traditional cultivars. In order to make this possible, numerous studies to detect which of the cultivars are resistant to one or more pathogens /pests have been performed (Mihnea et al., 2019; Mîndru et al., 2019). Once the cultivars with the desired trait are determined, the breeders may proceed to marker-assisted selection. The marker-assisted selection is not only accelerating the breeding process, but is also making it more efficient and is changing the breeding focus from phenotype selection to gene selection (Bai & Lindhaut, 2007).

Chemical protection of cultivated plants is used successfully against a wide range if organisms (bacteria, fungi, insects, etc.). However, their use has several strong disadvantages: development of pesticide resistance, water, soil and air pollution, disappearance of pollinators, etc. Developing plants with genetic resistance negates the need for chemical protection and subsequently eliminates its negative effects. As novel resistance genes are identified, they should be tagged and used in the creation of new varieties of plants resistant to common pathogen and pests.

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