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=== REVIEW ===

New insights into the interaction between cultivated potato and *Phytophthora infestans*

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SUMMARY. Late blight is the most destructive disease of potato. Due to the sexual and asexual reproduction, late blight has the capacity to evolve rapidly, progression that makes breeding for resistance very challenging. The favourable characteristics of vertical and horizontal resistance might be a good source for breeding resistant cultivars. Understanding the infection steps and defence response of plants is important for the next breeding programs. The goal of this review is to discuss some new insights into the interaction between pathogen and host and to point out new ways of transferring durable resistance genes to *Phytophthora infestans* into potato gene pool.

Keywords: effectors, late blight, resistance genes, *Solanum*

Introduction

Potato (*Solanum tuberosum* L.) is one of the most prominent cultivated plant of humanity, ranking third as a food crop after rice and wheat. Due to increasing consumption of potato, the importance of this crop intensified in the last years. The origin and the first domestication area of potato was the Andes Mountains, Peruvian region of South America (Pérez *et al*., 2001). Taking into account European evaluation data, Romania occupies the third place in the extension of potato cultivation area and the sixth place if we choose the volume of potato production as criteria. Romanian annual potato consumption shows a decrease tendency, in 2012 it was 98.3 kg /capita, which is 6.4% less than in 2006 (Vlad and Done, 2014). Romanians often called potato crop as the second bread, which proves its important role in alimentation (Baciu *et al*., 2009).

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Nowadays potato is one of the most chemically protected crop in the world. Every year more and more million dollars are spent for different treatments against fungi and herbivores.

In case of late blight disease the annual financial loss due to different crop protection methods and crop losses can be 3 billion dollars. With chemical control and targeted breeding, it is possible to reduce the annual yield loss to 16%, which is still insufficient (Fry, 2008).

Cultivated potato is a tuber-bearing autotetraploid (2n=48) species, which is a result of multiple successive hybridization of diploid species (Thermoshuizen, 2007). Potato has got a large number of related wild species, around 230, that all carry resistance genes for different diseases and pests (Spooner *et al*., 2014).

Potato breeders are always looking for new possibilities to obtain more resistant cultivars against different diseases. Wild *Solanum* species represent a rich reservoir of resistance genes that might be used in breeding programs, but the majority of them cannot be crossed with potato crop.

Two different kinds of resistance have been described against late blight and other diseases, vertical and horizontal, both interesting to increase crop protection by using resistant cultivars. Vertical resistance has a monogenic nature, is specific to pathogen strains, but isn't durable. The operating mechanism of vertical resistance is based on the gene for gene model, the pathogen avirulence genes are detected by matching resistance genes, the detection being either direct or indirect. In the case of incompatible interaction, the plant remains resistant. This type of resistance provide a protection just for a short period, because the pathogen, in our case the oomycete *P.infestans* evolves rapidly and overcomes the respective resistance gene. To date there are resistance genes discovered and characterized but most likely there are more to be discovered (Pérez *et al.*, 2001).

The hexaploid *Solanum demissum* was for the first time used as a source of vertical resistance against late blight, due to the identified eleven resistance genes (R1-R11). These genes confer a race specific resistance since the virulent new strains of *P.infestans* rapidly overcome this type of resistance (Jo *et al*., 2011; Saldana *et al*., 2011). Nowadays more than 50% of world potato cultivars contain *S. demissum* germplasm (Pérez *et al*., 2001).

Another solution could be the accumulation of resistance genes in *Solanum tuberosum*, the multiple R genes can confer a broad spectrum resistance to various diseases (Tan *et al*., 2010; Hajianfar *et al*., 2014). The breeders recognizing the unfavourable characteristics of vertical resistance, for example the non-durability, try to identify the genes involved in the horizontal resistance and to include this type of resistance in the new cultivars.

Horizontal resistance provides a general protection against pathogens, more durable than vertical resistance, but isn't specific and involves more genes in the defense response (Saldana *et al*., 2011). Introducing a horizontal resistance mechanism

instead of R genes into cultivated potato could be a promising method. Difficulties for breeders represent the polygenic nature and the linkage with other characteristics of plants, for example the late maturity of the host plant. The above mentioned new type of resistance confers a general protection against all races of *P.infestans*.

Screening after new resistance sources from wild species continues even current days, in the latest study researchers found some race nonspecific resistance genes in *Solanum chiquidenum* and *S. multiinterruptum*. The newly tested wild species show another or unknown type of response again *P. infestans*, probably in these cases the host-pathogen interaction is different than that with *S. demissum* (Pérez *et al.*, 2001).

Late blight of potato

Late blight of potato caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is recognized worldwide as the most devastating disease of potato. This pathogen left his mark in human history by the great Irish Potato Famine in XIX century. The huge loss of potato yield caused one million people death and more than one million people emigration from Ireland (Goss *et al.*, 2014). *P. infestans* is a hemibiotrophic oomycete, which mean that in the early stage of infection requires a living tissue, this period could be for couple of days to weeks (Termorshuizen, 2007). This oomycete has a capacity to develop resistance to new fungicides or resistant potatoes, quickly alters genetically in consequence increases the virulence capacity.

P. infestans is a heterothallic oomycete, with two mating types, respective A1 and A2, therefore it is able to reproduce both asexually and sexually. Oospores resulting after sexual reproduction are more resistant to abiotic stresses than asexual reproductive forms, they remain infectious even four years (Turkensteen *et al.*, 2000). Sexual reproduction increases the genetic variation of oomycete, which could generate new strains, new genotypes resulting in quicker adaptation and more powerful attacks (Sujkowski *et al.*, 1994).

In *Stramenipiles* regnum *P. infestans* has the biggest genome, 240Mb, which can be divided into two parts, based on gene contents. Approximately, 25% of the genome represents the gene dense region, and the other 75% the gene-sparse region. In the first region the housekeeping genes are present and the second region contains the effector or other genes, which play an important role in the virulence of the oomycete. Another difference between above mentioned two regions is: in contrast with gene dense region, the gene–sparse region includes large number of repetitive sequences, which are dynamic and serve as site for evolutionary processes (Jiang and Tayler 2012). This region promotes an increasing genetic variety of genes, which has a role in pathogenicity and host specificity (Vleeshouwers, 2011).

Life cycle *of P. infestans*

Since the appearance of A2 mating type, the sexual life cycle near the asexual life cycle of *P. infestans* has been revealed. The features of the spores represent a cornerstone for the success of oomycete, this survival structures allowing space and time dispersion to *P.infestans* (Judelson and Blanco 2005).

The asexual life cycle begins with the landed sporangia on the leaf surface, and can germinate in two different modes, adapting to the weather conditions. In dry and hot conditions the sporangium directly germinates via germ tube, otherwise in humidity and low temperature the sporangium releases biflagellate zoospores. These motile zoospores swim and encyst in the host surface, and in order to penetrate they secrete enzymes to digest the cuticle (i.e. pectate lyases), the cell wall components (cellulases) and additionally produce suppressors, which keep down the plant defence response (proteinase inhibitors) (Judelson and Blanco, 2005; Danies *et al*. 2014).

For the sexual reproduction, the presence of both mating types in the same location is required. This life cycle begins with the formation of oogonium and antheridium. The formed oospore can germinate either hyphae tube or sporangium. Important traits of oospores in contrast with zoospores are: they are more resistant to environmental changes, they can survive until next year and can infect the new culture of potato. The development of these two different germination modes of spores in both asexual and sexual reproduction, represent an evolutionary advantage, ensuring the possibility of colonization in any environmental conditions.

Interaction of late blight with the host plant

Plant pathogens use diver's strategies to penetrate into the plant via water pores, intercellular spaces or trough wounds, depending on the level of perceive system development of host plants. Oomycete can invaginate haustoria into the plasma membrane, which form closer interface for the next interaction steps with plant tissue (Han *et al.*, 2013).

At the beginning of the infection all pathogens confront with the first layer of plant defence, with the basal immunity system. Transmembrane pattern recognition receptors (PRRs) of plants detect the highly conserved microbial molecules, called PAMPs, which could be peptides, derived from bacteria or polysaccharides (chitin or beta-glucans) in the case of fungi or oomycete. As a result of the confrontation the PAMP triggered immunity (PTI) is switching on (Rouxel and Balesdent, 2010).

The successful pathogens have a well-developed ability to suppress the PTI by the effector molecules, which are products of oomycete avirulence (*avr*) genes. These effectors manipulate the host cell structure and function, in this way facilitating the infection and triggering defence responses (Kamoun, 2006). In this defence level the MAP kinase signalling will activate the pathogen-responsive genes transcription, the strengthening of cell wall and the Reactive Oxygen Species (ROS) production (Chisholm *et al*., 2006).

If an effector is recognized by a host cell resistance protein (R), the effector triggered resistance (ETI) will be activated, this is more rapid and vigorous compared with PTI (Jiang and Tyler, 2012). This type of interaction complies with the 'gene for gene' theory and lead to the hypersensitive response (HR), programmed cell death (PCD) at the site of infection. The collapse of the infected tissue creates a physical barrier to prevent the proliferation and spread of the pathogen.

Resistance and avirulence proteins

Resistance proteins are part of the second layer defence system, the majority of them contain Nucleotide Binding Site domain (NBS) and Leucine-Rich Repeat (LRR) domain. There are two subgroups of these proteins based on the amino terminal, either Coiled Coil (CC) or toll/interleukin receptor (TIR) domains.

In the *Solanum* species all resistance genes encode CC-LRR-NBS intracellular proteins against *P. infestans*.

In the construction of NBS domain, protein motifs kinase 1a or P loop, kinase 2a and kinase 3a, which are involved in the binding and hydrolysis of ATP or GTP take part. Each domain has a specific role in the interaction. NBS domain works as a molecular switch regulating the signal transduction (Tameling *et al.*, 2006). The LRR domain is involved in a specific recognition of effector molecules and represents a platform for upstream activators (Belkhadir *et al*., 2004; Tameling and Takken, 2008).

Another classification criterion of resistance genes is the evolution pattern, two types are known: the fast evolving (Type I) and the slowly evolving resistance genes (Type II). The only difference is attributed to the frequency of sequence changes.

Up to 2013 in total 68 functional resistance genes against *P. infestans* were identified in *Solanum* species (Rodewald and Trognitz, 2013).

Solanum resistance proteins are classified into seven families based on the different resistance specificities to *P. infestans* (Vleeshouwers *et al.*, 2011).

Effector proteins

Oomycetes, like *P. infestans* secrete hundreds of effector molecules, which target the host cells. Their primary role is the virulence, the secondary role is to elicit innate immunity in plant. In function of acting site, the effectors are grouped in cytoplasmic and apoplastic effectors. Effectors also could act as elicitors or toxins.

Oomycete apoplastic effectors interact in the extracellular space with plant cell wall, host's proteases and defence–response networks. Based on their activity they can be divided into: extracellular toxins, hydrolytic enzymes and enzyme inhibitors. Many apoplastic effectors were identified by biochemical isolation or bioinformatics methods (Oh *et al*., 2009).

Hydrolytic enzymes can digest the carbohydrates from apoplast and the cell wall degrading enzymes promote the oomycete penetration into host plant. Degradation

of pectin and β-glucan is realized by endo-polygalacturonase and β-glucanases secreted by oomycete (Nowicki *et al*., 2012; Jiang and Tyler, 2012).

When potatoes are attacked by *P.infestans*, as a response, they begin to excrete pathogenesis related proteins (PR) like glucanases, proteases and chitinases, therefore the oomycete secrete enzyme inhibitors like serine protease inhibitors (EPI1 and EPI10), cysteine protease inhibitors (EPIC1 EPIC2), etc. A set of elicitins (INF1, INF1A and INF2B) genes were identified in *P. infestans* genome, which encode some small, cysteine rich proteins and have the role to induce a hypersensitive response of the host plant.

The stability of effector molecules in the apoplast is due to disulphide bridges in their structure. Another member of this group is represented by PcF-like SCR74 and SCR91 toxins, which are secreted in the early stage of infection. They have two identified biological activity, the elicitation of the phenylalanine ammonia-lyase (PAL) activity and the promotion of leaf whitening (Liu *et al.*, 2004; Kamoun, 2006; Orsomando *et al*., 2011). PiNPP1 takes part in Nep1-like family, it is induced in the later stages of infection, during the necrotrophic phase (Kamoun, 2006; Kelley *et al*., 2010).

After translocation into cytoplasm, the cytoplasmic effectors of *P. infestans* target the different subcellular compartments of host cell, entering into host cell by lift draft mediated endocytosis (Nowicki *et al.,* 2012)

Similarly with resistance genes, avirulence genes are rapidly evolving and highly diverse genes, which encodes modular proteins. Based on protein domain characteristics, two types of cytoplasmic effectors, RXLR and crinkler (CRN) effectors were distinct (Jiang and Tyler, 2012).

RXLR effectors are products of *avr* genes, their name derived from conserved amino terminal motif, which have a role in the translocation into host cell. The amino terminal part of effector represents the sign for secretions and the carboxyl terminal part plays the effector side of protein (Morgan and Kamoun, 2007). Two additional protein motifs are important to be present, the dEER motif, which is required to entry into host cell, and W, Y, L motifs in the carboxy-terminal of effector with a role in the PCD suppression. Crinkler effectors belong to modular proteins family, like RXLR effectors, are rapidly evolving and can produce plant necrosis if they overexpress. The crinklers can enter into the nucleus and inhibit the PCD (Kamoun, 2006; Jiang and Tyler, 2012).

Other cytoplasmic effectors targets molecules, which have a key role in the recognition of effectors (Saunder *et al*., 2012).

Pseudogenesis and mutation lead to allelic variation of these genes. They could differ in the expression time, *Avr1* express in the early stage while *Avr3a* in the later stages of infection (Vleeshouwers *et al*., 2011). Some effectors could be important for potato breeders, for example the Avr3a, which could suppress the PCD induced by INF1 (Vleeshouwers *et al*., 2011).

These examples demonstrate, that effectors could act at a different level in plant, gene expression level, protein modification level or regulation level, for promoting plant susceptibility. Understanding their acting mechanism, could be helpful to design new, more targeted breeding programs.

Interaction between oomycete and plant cell is a very complex process, each part of this pathosystem develop their "attacker arm" and their defensive system (Fig. 1). This figure represents schematically the main steps of interaction. Oomycete secrete two types of effectors: intracellular effectors (IE) and cytoplasmic effectors (CE), which enter in the plant cell by endocytosis (1-3). After recognition of pathogen associated molecules (PAMPs) by pattern recognition receptors (PRR), will activate a PAMP triggered immune response (PTI), which will turn on other defensive responses, like Reactive Oxygen Species (ROS) production (4). Infected plants cells secrete proteases (P) to interact with intracellular effectors (5), after that as a response the oomycete secrete protease inhibitors (6). Cytoplasmic effectors, or RXLR effectors, are recognized by resistance proteins (R) and will activate the Effector Triggered Response (ETI) (7). RXLR effectors also can inhibit the PTI, or the ETI (10). If the interaction is incompatible, the Programmed Cell Death (PCD) will be triggered, that means a hypersensitive response (HR) and the infection keep back. Another type of cytoplasmic effectors, the crinklers (CRN) can enter into nucleus and inhibit the PCD (9).

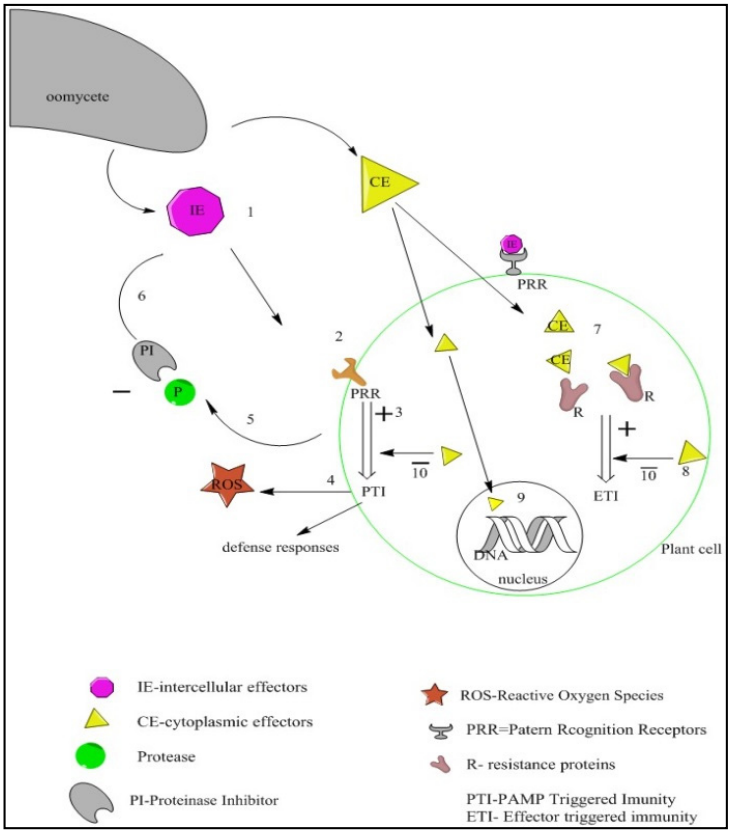

Figure 1. Schematic representation of the oocycete – plant cell interaction

Theories explaining the interaction between pathogen and host

Vertical resistance is based on the 'gene for gene' model. The pathogen avirulence genes are detected by matching resistance genes. In the case of incompatible interaction, the plant remains resistant. This type of resistance provides protection just for a short period, because the pathogen, in our case the oomycete *P.infestans,* rapidly evolves and overcomes the respective resistance gene. Several breeding programs obtained resistant potato cultivars with one or more resistance genes from *Solanum demissum*, but the "super" races of *P. infestans* rapidly overcomes this resistance.

A total of four different theories are known, which explain the interaction between resistance gene and *avr* gene products. The first theory considers a classical receptor-ligand interaction, which predicts direct interaction of effectors with receptor proteins. The second model assumes on indirect interaction, effectors interact with specific receptors, which can activate the resistance proteins and initiate a defence reaction.

Nowadays the most accepted model is 'guard' model, the third and fourth models, which presupposes the association of resistance proteins with the target of effector molecules. There are two theories for this type of interaction, the first is based on the activation of resistance proteins by conformation changes of the effector target molecule, which are physically connected. The second theory is based on the affinity changes of the effector target molecules. Direct interaction with resistance gene products only occurred after the effectors interact and change the affinity of targeted molecules (van der Hoorn and Kamoun, 2008; Maekawa *et al.*, 2011).

Conclusion

Late blight of potato is still remaining one of the greatest damage causing disease of potato. To prevent or to control these pathogen more insights into the biology and life cycle of the *Phytophthora infestans* are needed.

The most used methods to transfer resistance into cultivated potato, represent the crossing with wild species, they representing the source of resistance genes.

In some cases when it is not possible to obtain viable hybrids, in natural way or this hybrids, besides the resistance to late blight, have another unfavorable trait, breeders used other methods, for example identifying and cloning broad spectrum resistance genes (Song *et al.,* 2003; Brylińska *et al.,* 2015).

Knowing that *P. infestans*, is a rapidly evolving species, hybrids which contain, just one resistance genes will be rapidly overcome by the pathogen. Pyramiding of resistance genes, could be a better way to obtain durable resistance to late blight (Tan *et al*., 2010).

Based on the current knowledge, about the interaction of *Phytophthora infestans* and potato, the best method for obtaining durable resistance could be transferring both type of resistance, resistance genes and quantitative trait loci, which take part in horizontal resistance, and confer race-nonspecific resistance (Bormann *et al*., 2004).

A faster solution for breeders could be somatic hybridization combined to new tools of genomics, proteomics and next generation sequencing, which have the advantage to combine characteristics of wild *Solanum* species with beneficial proprieties of cultivated potato.

Via somatic hybridization more genes, for example quantitative trait loci, part of horizontal resistance, can be transferred without the short comes of transgenesis which may transfer fewer genes and is yet not accepted by consumers

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