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REVIEW

Advances on molecular studies of the interaction soybean - Asian rust

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Abstract - *Effective management practices are essential for controlling rust outbreaks. The main control method used is the application of fungicides, which increases substantially the cost of production and is harmful to the environment. Prevention is still the best way to avoid more significant losses in soybean yields. Alternatives, such as planting resistant varieties to the fungus, are also important. The use of resistant or tolerant varieties is the most promising method for controlling Asian soybean rust. Recently, five dominant genes resistant to soybean rust were described:* ***Rpp1, Rpp2, Rpp3, Rpp4*** and ***Rpp5****. However, little is known about the molecular interaction among soybean plant and soybean rust and on the molecular pathway triggered by pathogen recognition. Understanding the molecular mechanisms involved in defense responses is of primary importance for planning strategies to control stress and, consequently, to increase plant adaptation to limiting conditions.*

Key words: Phakopsora pachyrhizi, *resistance, plant-pathogen, molecular biology.*

ASIAN SOYBEAN RUST

Asian Soybean Rust (ASR) is caused by *Phakopsora pachyrhizi* Syd. and Syd; uredial anamorph; *Malupa sojae* (syn. *Uredo sojae*); Domain Eukaryota; Kingdom Fungi; Phylum Basidiomycota; Order Uredinales; Class Urediniomycetes; Family Phakopsoraceae; Genus *Phakopsora* (Index Fungorum 2010). Rust is considered a polycyclic disease. The fungus is able to complete several generations in a single cycle of the host. Temperatures and humidity that favor the growth and development of soybean plants also favor the development of rust (Zambolin 2006). According to Freire et al. (2008) the South and North American continents were free of *P. pachyrhizi* until 2001. Then *P. pachyrhizi* was reported in Paraguay (Morel and Yorinori 2002), and became established in Bolivia, Argentina (Rossi 2003) and Brazil (Yorinori et al. 2005) in 2002/2003. In 2004, ASR was reported for the first time in the USA (Schneider et al. 2005). These authors estimated that the disease caused yield losses from 10 to 80 %.

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According to Ono et al. (1992) *P. pachyrhizi* and *Phakopsora meibomiae* have wide host ranges and are able to sporulate on 31 species in 17 genera of leguminous plants. Rust samples taken from wild host plants are able to infect a broad range of plant species in greenhouse environments (Jarvie 2009). Recently, new host species from 25 genera were identified in evaluations made in greenhouses, including 12 genera that had not been reported previously (Slaminko et al. 2008). The presence of a susceptible host, viable pathogen spores and suitable environmental conditions are prerequisites for the development of a soybean rust epidemic. The optimum temperature for urediniospore germination ranges between 12 and 27 °C. Urediniospore germination is greater in darkness and requires a period of leaf moisture. Germination takes about 6 hours in optimum temperature and moisture conditions (Kochman 1977).

First symptoms of ASR could be described as small water-soaked lesions that develop into grey, tan to dark brown or reddish brown (RB) lesions (uredinia) particularly on abaxial leaf surfaces (Sinclair 1989). The color of the lesion depends on lesion age and interaction with the host genotype. RB lesions with little sporulation indicate a resistant reaction, whereas tan lesions with much sporulation indicate a susceptible reaction. The number of pustules per lesion increases with age of the lesion and groups of spores (urediniospores) are expelled from each pustule (uredinia) through a central pore (Sinclair 1989).

The disease destroys leaf tissue resulting in lower photosynthetic activity, premature defoliation and reduced life cycle. In addition, the premature leaf abscission prevents grain maturation (Sinclair 1989), and the rust infection during pod formation or seed fill can cause embryo abortion and pod abscission (Yorinori et al. 2005). The cumulative effect of rust on the production leads to a decrease in the number of pods and seed and a reduction in seed weight (Sinclair 1989).

P. pachyrhizi forms asexual uredospores on short stalks within a uredium 5-8 days after inoculation on colonized leaves. Uredospores are released from uredia through an ostiole and dispersed by wind. Under appropriate conditions, uredospores germinate a single germ tube and the penetration occurs directly through epidermis, but can also occur through stomatal openings (Zambolin 2006). Penetration by *P. pachyrhizi* starts with the formation of a funnel-shaped structure, termed appressorial cone, within the appressorium. This cone is contiguous with the cell wall of the penetration hypha, which is also called transepidermal vesicle. During penetration, the epidermal cells collapse become disorganized and show signs of cell death (Panstruga 2003, Mendgen et al. 2006). After penetration, the hypha grows through the epidermal cell and reaches the intercellular space. The primary hypha may branch to form a secondary one and finally, haustorium mother cells differentiate in close contact with mesophyll cells. The haustorium provides a wide contact surface inside the host cell for acquisition of sugars and amino acids through a symport proton gradient (Mendgen et al. 2006).

A recent study depicted a two-year field trial in Brazil where soybean rust was responsible for 37-67 % of soybean seed yield losses (Kumudini et al. 2008). This study coincided with yield losses observed in Asia up to 80 % (Miles et al. 2003). Effective management practices are essential for controlling rust outbreaks. The main control method used is the application of fungicides, which substantially increases the cost of production and is harmful to the environment (Zambolim 2006). The chemical group most often used for rust control is a mixture of strobilurin and triazole.

Prevention is still the best way to avoid more significant losses in soybean production. The method is to offset the timing of soybean production and pods maturation in condition that does not favor *P. pachyrhizi*. In addition, lowering inoculum levels by implementation of a soybean-free period is important. Alternatives such as using resistant varieties to the fungus are also important. However, resistance does not mean that the disease does not occur, but allows greater stability and efficiency of chemical control (Anuário Brasileiro de Soja 2009). Recently, soybean cultivars resistant to the fungus were released in Brazil. These varieties boast characteristics that curb fungal growth and ensure higher production stability, reducing the losses induced by the disease, and the environmental impacts caused by repeated fungicide applications.

MOLECULAR INTERACTION BETWEEN SOYBEAN AND ASR

Immunity to *P. pachyrhizi* occurs when no visual lesions are produced by the soybean plant. A resistant response leads to the formation of RB lesions indicating a hypersensitive reaction. A susceptible response occurs when tan lesions develop, indicating fungal

growth and development. The genetics of resistance of the only five dominant genes to specific soybean rust isolates has been described: *Rpp1, Rpp2, Rpp3, Rpp4* and *Rpp5* (Bromfield and Hartwig 1980, Mclean and Byth 1980, Hartwig and Bromfield 1983, Hartwig 1986, Garcia et al. 2008).

In order to identify new sources of resistance in soybean, Miles et al. (2006) evaluated the entire germplasm collection (16,000 accessions) of the United States Department of Agriculture (USDA) against a mixture of five *P. pachyrhizi* isolates. After two rounds of evaluation, only 850 accessions were identified with partial tolerance or resistance reactions to *P. pachyrhizi*, which correlates to less than 5 % of USDA germplasm collection.

Resistance alleles different from those already described in the literature were also identified in several other genotypes (Laperuta et al. 2008, Pierozzi et al. 2008). When the disease was first detected in Brazil, all the described resistance genes were effective against the fungus. However, in 2003, a new race of *P. pachyrhizi* broke the resistance conferred by genes *Rpp1* and *Rpp3* while *Rpp2, Rpp4* and *Rpp5* remained resistant.

Although *Rpp2*- and *Rpp4*-mediated resistances have been stable in Brazil (Hartman et al. 2005), only dominantly inherited *R* gene-mediated resistance against *P. pachyrhizi* has been overcome in nature several times because of the great capacity of the fungus to develop new race. Generally, this scenario of breakdown of *R* gene-mediated resistance is known as the 'boom and bust' syndrome. In addition to pyramiding known *Rpp* resistance genes into modern cultivars to create a more durable and broad-spectrum disease resistance, the selection of novel sources of resistance to *P. pachyrhizi* is desirable (Goellner et al. 2010).

Along with single gene resistance, partial resistance to soybean rust has been described (Hartman et al. 2005). This kind of resistance may be controlled by minor genes and may be expressed as reduced uredinial number and size, a longer latent period and other components related to fungal reproduction. Recently, the average number of uredinia per lesion and the average uredinial diameter were reported to be components of partial resistance in soybean rust and were a reflection of fungal growth in the host tissue (Bonde et al. 2006).

All described *Rpp* genes have already been mapped on soybean chromosomes (Chr), *Rpp1* was mapped on chromosome 18, *Rpp2* on Chr 16, *Rpp3* on Chr 6, *Rpp4* on Chr 18 and *Rpp5* on Chr 3 (Hyten et al. 2007, Garcia et al. 2008, Silva et al. 2008, Hyten et al. 2009). Additionally, some alleles was mapped on the same chromosome, for exemple, *Rpp1b* was also mapped on Chr 18, while *Rpp Hyuuga* was also mapped on Chr 6 (Monteros et al. 2007, Chakraborty et al. 2009)

Despite the physical location of the *Rpp* genes and the recent release of the soybean genome (Schmutz et al. 2010), none of them was cloned yet. However, significant progress has been made towards cloning *Rpp4*, which remained the most stable gene when challenged against isolates of fungus from different parts of the world (Yamaoka et al. 2002, Bonde et al. 2006). Sequencing of the mapped region in the susceptible genotype Williams 82 identified a cluster of three resistance genes, CC-NBS-LRR (coiled-coil, nucleotide-binding site, leucine-rich repeats), within a 2cM region on chromosome 18, which shows sequence similarly to the lettuce RGC2 family of resistance genes (Meyer et al. 2009). In addition, Virus-induced gene silencing (VIGS) demonstrated that silencing of the *Rpp4* candidate genes diminished resistance in PI459025B (that carries *Rpp4* resistance allele), confirming that one of the genes in the cluster is responsible for resistance.

There is a clear evidence of evolutionary forces acting on the *Rpp4* locus. Differences in gene number between Wm82 and PI459025B are likely due to duplication or unequal recombination. In addition, given the similarity of all *Rpp4* candidate genes between genotypes, it is possible that small amino acids differences may play a key role in resistance (Meyer et al. 2009).

Little is known about the molecular interaction among soybean and *P. pachyrhizi* and the defense pathways triggered by pathogen recognition. Understanding the molecular mechanisms involved in defense responses is of primary importance to plan strategies for controlling stress and consequently to increase plant adaptation in limiting conditions. The development of sequencing techniques and gene expression analysis on a large scale, combined with new bioinformatics tools for data analysis have facilitated the structuring of extremely valuable databases to create strategies for genetic engineering.

Given the rarity of Asian rust resistance in soybean, few genomic tools are available for examining the resistance of *P. pachyrhizi* in immune (*R)* genomes. Therefore, rust resistance research has focused on genotype independent platforms like microarray analyses

to identify genes involved in resistance and susceptibility. Van de Mortel et al. (2007) used the soybean affymetrix gene chip to study changes in gene expression in resistant and susceptible genotypes when inoculated with ASR. A biphasic gene response to *P. pachyrhizi* infection was seen in both genotypes. Differences in gene expression between inoculated and mock plants peaked at 12 hours post inoculation (hpi) and returned to almost basal levels by 24 hpi, in both resistant and susceptible genotype. At 72 hpi a second larger wave of defense gene expression could be observed, which was earlier in the resistant than in the susceptible interaction. The early transcriptional response observed in susceptible and resistant plants might represent a general response of soybean to the nonspeciûc recognition of any pathogen, presumably by interaction with microbe-associated or microbe-induced molecular patterns (MAMPs and MIMPs) (Mackey and Mcfall 2006). By contrast, the second likely response relates to R-gene detection of *P. pachyrhizi* (Posada-Buitrago and Frederick 2005, Tremblay et al. 2009).

In a similar approach, Panthee et al. (2007) identified genes, which might be involved in a defense response against *P. pachyrhizi* by susceptible soybean cv.5601 plants 72h after infection (hai) using microarray. Most of the induced genes had defense and stress related functions such as genes encoding an SA-related protein, heat shock protein (HSP), leaf senescence-associated receptor like kinase, and chalcone synthase.

Recently, Pandey et al. (2011) combined the work of Van de Mortel et al. (2007) with VIGS, to screen 140 candidate genes that might play a role in *Rpp2*-mediated resistance toward *P. pachyrhizi*. This study identified 11 genes that compromised *Rpp2*-mediated resistance when silenced, including *GmEDS1*, *GmNPR1*, *GmPAD4*, *GmPAL1*, five predicted transcription factors, an O-methyl transferase, and a cytochrome P450 monooxygenase. Additionally, a large scale transcript profiling approach conducted with soybean plants (accession PI200492) has revealed an up-regulation in gene expression for lipoxygenases and peroxidases in an incompatible interaction, suggesting an important function for these genes in *Rpp1*-mediated resistance (Choi et al. 2008).

Using laser capture microdissection, Tremblay et al. (2010) isolated susceptible soybean palisade and mesophyll cells showing signs of infection, extracted the RNA and performed transcriptome profiling. A total of 2,982 genes were differentially expressed, from which 685 were up-regulated and 2,297 were down regulated. Complementary to transcriptional analyses in the host, gene transcript profiling has also been performed with the fungus (Posada-Buitrago and Frederick 2005, Tremblay et al. 2009). A recent study of gene expression within *P. pachyrhizi* germinating spores allowed the identification of 488 unique expressed sequence tags (ESTs). One hundred eighty nine of these ESTs showed significant similarly (E-value < 10^{-5}) to sequences deposited in the NCBI non-redundant protein database. These genes were assigned putative roles in primary metabolism, gene and protein expression, cell structure and growth, cell division, cell signaling and cell communication (Posada-Buitrago and Frederick 2005). Recently, a cDNA library was constructed based on the separation of uredinia from host tissue by using laser-captured microdissection (Tremblay et al. 2009). About 80 % of identified genes in this study shared no homology to previously described *Phakopsora* genes. This result demonstrates stage-speciûc gene expression in the development of uredinia.

While the techniques have proven effective at looking at genes downstream of *Rpp* genes, more research is needed to identify potential candidate genes that could be used to engineer sustainable resistance into soybean against *P. pachyrhizi.*

CONCLUDING REMARKS

The use of resistant varieties is the optimal control strategy due to the value of a technology already being aggregated to the seed. The rust resistance genes have been incorporated in breeding programs in Brazil and the United States. Resistant varieties with genes of greater resistance were released; however, the resistance stability is uncertain, once the large number of races of this fungus already described proves the great variability of the pathogen. As a result, the use of fungicides is still necessary to ensure the survival of resistant varieties. Recently, research on Asian soybean rust has mainly focused on host range studies, epidemiology and the evaluation of yield loss and control measures. These studies have predominantly centered on the diseased plant. However, as soybean cultivars with resistance to all known races of *P. pachyrhizi* are not available yet, more basic research on this pathogen is also necessary. Elements in the infection process of the fungus and possibilities of interference to adapt new plant protection strategies need to be identified.

Avanços dos estudos moleculares da interação da soja - ferrugem asiática

Resumo - *Práticas efetivas são necessárias para o controle da ferrugem. O principal método de controle utilizado é a aplicação de fungicidas, o que aumentará substancialmente o custo de produção e são prejudiciais ao meio ambiente. A prevenção ainda é a melhor maneira de evitar mais perdas significativas na produção de soja. Alternativas, como o plantio de variedades resistentes ao fungo, também são importantes. O uso de variedades resistentes ou tolerantes é o método mais promissor para o controle da ferrugem asiática da soja. Recentemente, cinco genes de resistência a ferrugem da soja foram descritos* **Rpp1, Rpp2, Rpp3, Rpp4** e **Rpp5**. *No entanto, pouco se sabe sobre a interação molecular entre a planta e ferrugem da soja e as rotas desencadeadas na planta pelo reconhecimento do patógeno. Compreender os mecanismos moleculares envolvidos nas respostas de defesa é de primordial importância no planejamento de estratégias para controle do estresse e, consequentemente, para aumentar a adaptação das plantas a condições limitantes.*

Palavras chave: Phakopsora pachyrhizi, *resistência, planta-patógeno, biologia molecular*.

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