

# SOYBEAN (*GLYCINE MAX* (L.)) BREEDING AND MANAGEMENT FOR SOYBEAN RUST: A REVIEW

Meaza Tadesse\*

Kulumsa Agricultural Research Center, Assela, Ethiopia, Tel: +251910895861; Email: Tadme2121@gmail.com

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**Abstract:** Soybean is one of the most important economic crops as a source of protein and oil. Biotic stresses such as pathogens, insects and weeds can cause negative impacts on its production. Among the diseases, Asian soybean rust caused by *Phakopsora pachyrhizi* (Sydow. & Sydow.) is the most destructive, and over 80% losses are common when environmental conditions are conducive to disease development. To date, six race-specific resistance genes have been identified in plant introductions. However, races of *P. pachyrhizi* able to overcome the resistance conferred by these genes have evolved. Due to the limited availability of resistant varieties, fungicide application is the only management tool available for farmers, which significantly raises the production cost and the risk of environmental and human contamination. Thus, the transfer of resistance genes through classical breeding followed by marker-assisted selection allows the development of resistant varieties and their use as an efficient and cost-effective method for controlling soybean rust.

**Keywords:** Asian soybean rust; *Phakopsora pachyrhizi*; Soybean; *Glycine max*; Genetic resistance; *Rpp* gene.

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## 1. INTRODUCTION

Soybean (*Glycine max* (L.)) is a major source of vegetable oil and high quality protein in the world (Tukamuhabwa et al., 2012). It is ranked first in the international world market (55%) among other oil seed crops such as cotton seed, peanuts (groundnuts), sunflower seed, rape seed, palm and coconut (Chung and Singh, 2008). Currently, about 50 countries worldwide grow soybean (Boerma and Specht, 2004). The United States of America (USA) accounted for 40 to 45 % of the world's total soybean production in 2003 (Boerma and Specht, 2004). Records in 2008-2009 show Nigeria is the largest Africa's soybean producer (39 %), closely followed by South Africa (35 %) while Uganda is the third African producer (14 %).

Soybean breeding in Ethiopia was started in the 1950s on evaluation of introduced varieties with main emphasis of replacing soybean flour import with locally produced soybean flour and introducing the crop in to the existing crop production system and in the diet of the poor farmers (Hammer and Haraldson, 1975).

In Ethiopia, soybean is grown over wider agro-ecologies especially in low to mid altitude areas (1,300 to 1,700 masl) that have moderate annual rainfall (500-1500mm). soybean seed is highly valued for its high protein content (40%) that is used either for fresh green vegetables or processed products such as soy flour, soymilk, roasted soy beverage, fried soy nuts and soy meat for human consumption (Hartman et al., 2011). In addition, more than 90% of the soybean cake is used for livestock, poultry and aquaculture feeds due to its high protein content. Its high oil content (20%) is used for making processed food products (margarine and cooking oil) and industrial products such as cosmetics, plastics and paint removers, among others. Soybean is also an important source of isoflavines, which are used for reducing health risks associated with blood cholesterol and other diseases in human beings. Furthermore, soybean improves soil fertility through biological nitrogen fixation, thereby alleviating soil fertility problems, and when grown in rotation with maize, it reduces *Striga* infestations of maize (De Groote et al., 2010).

Soybean breeding in Ethiopia is characterized by plant introductions, which are being used as the only source of new genes. Currently the area covered under soybean production is 38072.70 ha with total production in quintal of 864,678.69. The productivity of the crop per hectare remains to be 22.71 (CSA, 2017/18). The low national yield could be attributed to various reasons. Some of these are related to low adoption of improved soybean production technologies; lack of improved varieties and poor cultural practice, a biotic and biotic stresses such as pathogens, insects and weeds can cause negative impacts on its production. Among the diseases, Asian soybean rust caused by *Phakopsora pachyrhizi* Syd. & P. Syd is the most destructive, and over 80% losses are common when environmental conditions are conducive to disease development (Calvo et al., 2008; Li et al., 2012). soybean rust was first observed in Ethiopia in October 2011 and again in 2015 and 2016.

## 2. LITERATURE REVIEW

Soybeans (*Glycine max* L.) serve as one of the most valuable crops in the world, not only as an oil seed crop and feed for livestock and aquaculture, but also as a good source of protein for the human diet and as a feedstock (Goldsmith and Masuda, 2009). The crop can be grown in tropical, sub-tropical as well as the temperate regions. It is a primary source of vegetable oil and protein concentrates. Soybean is an excellent source of major nutrients, about 40% of dry matter is protein and 20% fat (Lakshmeesha et al., 2013). Even if soybean is recently domesticated crop, it has a great potential for Ethiopia as it has been fully recognized by many researchers and organization for its economic importance and its domestic demands for various uses. Production of this crop is indispensable in the country to enrich the stable cereal crop with sufficient and high quality protein in order to overcome the problem of malnutrition. However, as other grain legumes, soybean, are attacked by a wide range of diseases (Lakshmeesha et al., 2013).

### 2.1 Soybean origin and distribution

Soybean (*G. max*.) originated from China and was domesticated from the wild soybean (*G. soja*) between 1500-1100 BC (Pathan and Sleper, 2008). It was introduced to European countries between the 16th and 17th centuries from China, Korea and Japan. In North America it was introduced in 1765 and was only introduced to Central and South America in the mid-1900s. In Africa, soybean was introduced by Chinese traders along the East coast in the nineteenth century (Giller and Dashiell, 2006). Today, soybean is grown throughout the world, in diverse climates ranging from temperate to tropical and subtropical regions (Tukamuhabwa et al., 2002). The main producers of soybeans are United States of America, Brazil, Argentina, China and India (Pathan and Sleper, 2008). In Ethiopia, soybean production is currently gaining prominence due to the increasing demand for its products.

### 2.2 Soybean taxonomy, cytology, floral and pollination system

Soybean belongs to the genus *Glycine* of Fabaceae (Leguminosae) family. Genus *Glycine* is further grouped into three subgenera: *Glycine* subgenus *Leptocytamus* (Benth.) F.J. Herm., *Glycine* (perennials), and *Soja* (Moench) annuals (Chung and Singh, 2008). *Glycine* subgenus *Leptocytamus* comprises of 6 species, including the most important species, *G. tabacina* (Labill.) Benth., *G. tomentella* Hayata and *G. canescens* F.J.Herm. The subgenus *Glycine* (perennials), on the other hand comprises of 2 species *G. petitiata* (A.Rich.) Schweinf. and *G. javanica* L. with several subspecies while the cultivated soybean (*Glycine max* (L.) Merr) and the wild soybean (*Glycine soja* Siebold and Zucc.) belong to subgenus *Soja*. Both the cultivated and the wild soybean are paleoploid with  $2n=40$  chromosomes and they are cross compatible (Singh and Hymowitz, 1999).

Soybean is a self pollinated crop with a typical papilionaceous flower. The flower is complete consisting of the calyx, corolla, pistil, and stamen. The calyx has five unequal sepals that surround the corolla, pistil and the stamen. The corolla consists of a standard (posterior and banner petal) with two wings and two keel petals that are not fused. The pistil has a single ovary with one to five ovules and a club shaped stigma. The flower has ten stamens that form a ring around the pistil. During anthesis, the filaments elongate pushing the anthers above the stigma thus ensuring self pollination (Singh, 2007). The receptivity of the stigma to pollen occurs one day before the pollen is shed.

### 2.3 Importance of soybeans

Soybean is an economically important leguminous crop that is grown for its oil and protein products (Tefera et al., 2009). The soybean seed contains an average of 40% protein and 20% oil that is used for making nutritious food products such as

soymilk, soy flour and soy sauce (Fabiya, 2006). It is also an important source of proteins in feed supplements for livestock. Besides its nutritive value, soybean has medicinal properties due to isoflavones content that reduces blood cancer, osteoporosis, blood cholesterol and heart diseases in human beings (Pathan and Slepser, 2008).

In addition, it improves soil fertility through biological nitrogen fixation, thus reducing the cost of purchasing inorganic fertilizers by resource constrained farmers (Misiko et al., 2008). In rotational system with cereals, soybean dual purpose varieties have shown its potential in reducing the levels of Striga (*Striga hermonthica* (Delile) Benth.) infestations (Chianu et al., 2006b). Furthermore, soybean is used as a raw material in industries for production of biodiesel, cosmetics, pesticides, hydraulic fluids, lubricants, paint removers and plastics; hence it can be utilized as a beneficial crop by small-holder farmers for income generation (Pathan and Slepser, 2008).

#### 2.4. Soybean production constraints in Ethiopia

Soybean production in Ethiopia is characterized by low yields. The low yields are as a result of several challenges which include a biotic, socioeconomic and biotic constraints (Kawuki et al., 2003b; Mohammad et al., 2003). The major a biotic constraints in soybean production are weather-related factors (e.g. extreme temperature, drought, water logging and frost), soil nutrient availability's and salinity (Slepser and Poehlman, 2006; Hartman et al., 2011).

A part from environmental factors, soybean production is constrained by several socio-economic factors including lack of high yielding varieties, poor agronomic practices, lack of awareness of soybean processing and utilization, lack of markets, lack of inputs, and lack of supportive policies by the government, among others. Biotic constraints such as pests (aphids and trips), diseases (Asian Soybean Rust, brown spot, soybean mosaic, downy mildew, frogeye leaf spot and bacterial blight), and weeds are also harmful to soybean production, reducing soybean yields. Among the biotic constraints, ASR is the major cause of low yields in many areas of the world (Hartman et al., 2011).

#### 2.5 Asian soybean rust

Asian soybean rust (ASR) is the most devastating foliar disease in soybean growing areas (Garcia et al., 2008; Li et al., 2012). It is caused by two obligate fungal species, *P. pachyrhizi* and *Phakopsora meibomia* (Bonde et al., 2006). The species *P. meibomia* is mainly found in the western hemisphere, is less aggressive and does not cause substantial yield losses in soybeans. On the other hand, *P. pachyrhizi* is more aggressive, and is responsible for significant yield reduction worldwide (Calvo et al., 2008).

This review focuses on *P. pachyrhizi*. The fungus that causes Asian soybean rust belongs to the *Fungi* Kingdom, *Basidiomycetes* Class, *Uredinales* Order, *Phakopsoraceae* Family, *Phakopsora pachyrhizi* specie. To date, the described fungus stages were uredinial, teleomorphic and basidial. The aecial stages have not been reported yet. Like all fungi of the group, *P. pachyrhizi* is a biotrophic pathogen that requires a living host to grow and survive. Naturally, *P. pachyrhizi* infects 31 species in 17 genera of legumes (Sinclair, J.B., Hartman, G.L. (1996)). The main hosts are *Glycine max*, *G. soja*, *Pachyrhizus erosus*, *Pueraria lobata* and *Vigna unguiculata*. According to Yeh et al. (1981) and Bromfield (1984) the fungus could attack up to 87 and 95 hosts, respectively.

Symptoms caused by soybean rust are different from those caused by other types of rusts. The uredinia are the fruiting bodies, which produce the uredinio spores released through an ostiole. The symptoms of soybean rust are characterized by small brownish to dark brown lesions, with one or more uredinia mainly on the bottom side of the plant leaflets. The lesions tend to be angular with 2 to 5 mm in diameter. May also occur on petioles, pods and stems. The color of the lesions varies with age and with the interaction between the host genotype and the pathogen isolate. The new lesions are initially light brown, becoming darker with the age (Reis, E.M., Reis, A.C., Carmona M. and Danelli A.L.D (2012).

The uredinio spores are the primary inoculum of soybean rust. These are asexual, small, lightweight spores, which are removed from uredinia when the infected leaf surface is dry. After removal, the air currents can transport the spores over long distances, which explain its spread from one field to another. In the presence of water and temperature between 21 and 25°C, the urediniospores deposited on the host leaf surface begins the germination process and infection (Reis, E.M., Reis, A.C., Carmona, M. and Danelli, A.L.D (2012). The penetration of the parasite occurs directly through the cuticle and epidermal cell wall of the host. The direct penetration rather than through the stomata, is a characteristic that differentiates *P. pachyrhizi* from other rusts fungi. The colonization begins shortly after penetration, the primary

branching hyphae gives rise to formation of a dense mycelium filling the intercellular spaces and inserting haustoria in the mesophyll and epidermal cells.

The fungus reproduction begins approximately at eight days after infection, and its first evidence is the hyphae aggregation forming the uredinia primordium. Uredinia have light brown to red brown appearance. In about 3 to 4 days, it starts the production of urediniospores. According to Alves et al. (2006), the latency period length varies with the temperature, being shorter for temperatures around 23°C, in agreement with previous studies of Melching et al. (1989).

### 2.5.1 Epidemiology of ASR

ASR infection process involves spore germination, formation of appressorium, penetration, development of urediniospores and finally sporulation (Li, 2009b). According to Bromfield (1984) urediniospores are the key primary source of inoculum of *P. pachyrhizi* that initiates ASR epidemics.

After the initial infection, a single germ tube germinates from each uredinio spore, and subsequently forming an appressorium that has a hyphal tube on its bottom part, enabling direct penetration of the epidermal cells through the leaf cuticle.

This explains why *P. pachyrhizi* has a wider range of hosts than most other rust pathogens, which penetrate the leaf via the stomata or wounds (Miles et al., 2003). Under favorable environmental conditions, new urediniospores are developed in pustules and the first urediniospores are released about 9 days after the initial infection (Kawuki et al., 2003b). Urediniospores may be released continuously for several weeks, depending on the initial inoculum, and the volume of spores produced within the first three weeks. This infection cycle is repeated on the same plant, neighboring plants or distant plants, of the same or many other legume species, as long as the environmental conditions are suitable and susceptible host plants are available.

Telia, basidiospores and teliospores have also been described in the life cycle of *P. pachyrhizi* but they are not the primary source of inoculum. The telial stage (sexual stage) is not very common but it has been induced under laboratory conditions to produce basidiospores. In the field, telia are sometimes observed towards the end of the growing cycle of soybean plants (Bromfield, 1984). However, importance of telia, teliospores and the basidial stage in the life cycle of *P. pachyrhizi*, and in epidemics of ASR, is not well understood. If there is an alternate host for the aecial stage of *P. pachyrhizi*, then it remains to be discovered.

### 2.5.2 Symptoms of ASR

The initial symptoms of ASR are small water-soaked lesions that develop either into grey, tan or reddish brown lesions mainly on the lower side of the leaves; but sometimes they may appear on the petioles, pods, cotyledons, and stems (Li, 2009b). In most cases, the lesion color varies depending on the lesion age, pathogen aggressiveness, host plant, and the interaction between the pathogen and the host (Li, 2009a). There are three major types of lesions and are described as tan, red brown and immune (Bromfield and Hartwig 1980; Bromfield, 1984). Tan-colored lesions (TAN) indicate a highly susceptible reaction with many urediniospores and high sporulation levels. Reddish brown lesion (RB) on the other hand, is a form of resistance that is characterized by small, irregular lesions without urediniospores, while an immune reaction is a complete resistance without visible symptoms. In some cases, an intermediate response with both TAN and RB lesions has been reported (Bonde et al., 2006). This reaction is described as mix (MIX) and is attributed to mixtures of *P. pachyrhizi* races in the inoculum.

### 2.5.3 Diagnosis of ASR

ASR is mainly diagnosed visually using a hand lens or a dissecting microscope. The key characteristic of ASR is the occurrence of uredinia that have urediniospores. In the field at early stages of ASR development, non-sporulating symptoms are easily confused with other fungal and bacterial diseases such as bacterial blight (*Pseudomonas syringae* Van Hall pathovar *glycinea*), brown spot (*Septoria glycines* Hemmi), frog-eye leaf spot (*Cercospora sojina* K.Hara), and downy mildew (*Peronospora manshurica* Naumov) Syd.) (Sinclair, 1982). It is, therefore, recommended that diseased leaf samples be incubated in a moist chamber and left overnight to enhance rust development and sporulation for accurate diagnosis. PCR tools (polymerase chain reaction) are also useful for ASR diagnosis when sporulating pustules are not visible (Frederick et al., 2002).

#### 2.5.4 Effect of ASR on soybean development stages and maturity

Soybean phenological stages and maturity duration play an important role in the development of ASR (Tschanz et al., 1985). According to Kawuki et al. (2004), soybean plants are susceptible to rust at all developmental stages. However, infections tend to be more severe during the reproductive stages (flowering and pod-filling stages) (Maria et al., 2007). With regard to maturity duration, early maturing varieties are heavily infected with higher rates of ASR incidences than late maturing varieties as reported by Tschanz et al. (1985). In contrast, a recent study conducted by Oloka et al. (2008) reported that late maturing varieties are heavily infested by ASR that result into substantial yield losses than early maturing varieties. These findings imply that breeders' choice of soybean maturity group and the use of a consistent maturity stage is crucial for the accurate evaluation of rust resistance.

#### 2.5.5 Effect of ASR on yield and seed quality

ASR spreads fast, causing severe crop damage, which leads to significant yield and quality losses. Soybean grain losses of 10-80% have been reported (Yorinori et al., 2005). The level of loss experienced depends upon prevailing weather patterns, the genotype and the maturity stage at the time of infection (Wang and Hartman, 1992). Kumudini et al. (2008) reported that yield losses are mainly attributed to premature leaf fall, reduced green leaf area in the canopy, reduced dry matter accumulation and reduced harvest index. Bennett (2005) also reported that heavily infected plants had significantly reduced pods/plant, seeds/pod, number of filled pods/plant, 1000 seed weight, seed germination and oil content. This information would be crucial for guiding proper planning of suitable control measures against ASR (Kawuki et al., 2003a).

#### 2.6 Control strategies for ASR

Since ASR affects vegetative growth, reproductive stage, yield and seed quality, effective control strategies are needed to enable soybean crop to withstand the devastating effects of the disease. Various control strategies have been suggested, including cultural practices, nutrition management, biological, fungicide application and host resistance but they are all limited options in one way or another.

##### 2.6.1 Cultural practices and nutrient management strategies

Cultural practices, such as controlling wild weed hosts, adjusting planting dates, planting early maturing varieties and site selection have been proposed to reduce ASR incidences and severity (Bromfield, 1984). The main challenge with cultural practices is that some of the alternative hosts are perennials, resulting into year-round spore production, thus making cultural practices unsuitable for ASR control. Fertilization with potassium, chloride, manganese, and phosphorous may affect the development of fungal diseases (Ebelhar et al., 2008). However, their effects on ASR have not been fully explored.

##### 2.6.2 Bio pesticide and biological control

Borges (2007) examined application of oils and plant extracts to control ASR infections. He reported that *Pelargonium* spp. and *Lavandula officinalis* Mill extracts had similar effects as fungicide applications in controlling ASR. These extracts also reduce environmental hazards, but they are expensive and may not be available in large quantities.

##### 2.6.3 Chemical control

Several systemic and protectant fungicides are used as the primary control measure for managing ASR globally (Levy, 2005). However, the appropriate application of fungicides is locally specific due to differences in the environmental factors (weather patterns), cropping systems and socio-economic factors (availability, equipment, labour, cost, etc.). In addition, the fungicide efficacy and timing of application is complicated by the maturity stage of the host and the disease progress. This makes fungicide control measures less applicable in most of the developing countries, including Ethiopia (Oloka et al., 2008).

##### 2.6.4 Host plant resistance

Host plant resistance is a more affordable method for managing ASR in agricultural systems of resource poor farmers (Twizeyimana et al., 2008). For this reason, considerable efforts have been directed towards screening soybean germplasm for resistance to *P. pachyrhizi*.



#### 2.6.4.1 STRATEGY AND SELECTION METHODS

Principal factors determining strategies and methods used for breeding for resistance to soybean rust and other desirable traits include (1) the genetic distance between the cultivar to be improved and resistant donor germplasm, (2) the direct and indirect screening methods available, (3) the genetics of resistance, and (4) the number of resistances and other traits to be improved (Singh et al., 2011). Various selection methods are used for identification of those progenies which possess the most useful combinations of the desired traits. The choice of the selection method depends on breeding objective as well as on other important factors such as the available variability, availability of agricultural machines and greenhouse, size and skill of breeding team, etc (Vishnyakova et al., 2013). The presence of multiple virulence genes in the pathogen population and the lack of multiple resistance genes in the host provides the soybean rust pathogen with a competitive advantage.

The deployment of specific single genes for resistance is thus unlikely to be a successful strategy (Antony, 2009). Some soybean genotypes initially identified as resistant to ASR have had this resistance broken, as has occurred with the genotypes carrying Rpp1 and Rpp3 when exposed to the *P. pachyrhizi* Taiwan-72-1 isolate (Hartwig, 1986) and to the new *P. pachyrhizi* isolate from the Brazilian state of Mato Grosso (MT, the Brazilian government abbreviation for this state), the *P. pachyrhizi* MT state isolate, described by Yorinori et al., (2004). Soybean resistance provided by Rpp1 and Rpp3 was defeated by the *P. pachyrhizi* MT isolate just two years after ASR was first detected in Brazil (Pierozzi et al., 2008).

Therefore, it is expected that breeding for polygenetic resistance may be more effective for obtaining durable resistance or tolerance (Ribeiro et al., 2009). The techniques used for resistance evaluations are selected based on reliability of the results and availability of resources i.e., time, labor, greenhouse space, and expertise (Hartman et al., 2013). Marker assisted selection (MAS) strategies known as forward selection have been used effectively in soybean since the mid 1990s to prescreen breeding populations for simply-inherited traits (Vishnyakova et al., 2013). But many complex traits have not been amenable to forward selection because quantitative trait loci (QTL) detected within one genetic context has not been sufficiently predictive of other genetic contexts (Bernardo, 2008). This has prompted us to investigate a Context Specific MAS (CSM) approach for complex traits (Sebastian et al., 2010).

Soybean currently has an SSR map that contains 1019 SSR markers distributed across 20 linkage groups (Song et al., 2004). These markers can be used to identify the genome location of SBR resistant genes and to help quickly integrate these genes into modern breeding lines through marker-assisted selection. Chakravarthi et al., (2006) reported the fundamental advantages of MAS compared to conventional phenotypic selection which are: (i) Simpler compared to phenotypic breeding (ii) Selection may be carried out at breeding stage and single plants may be selected with high reliability. microarray analysis is a useful technology for assaying transcriptional responses for biotic and abiotic plant stresses (Mentewab et al., 2005; Moy et al., 2004). This technology has been used for such studies including soybean rust caused by *Phakopsora pachyrhizi* (Panthee et al., 2007).

#### 2.6.4.2 Molecular Markers

The soybean genome sequence has provided a common reference frame for genomic features (genes, regulatory elements, other repeat sequences, markers, etc.) from both soybean and from related species (Steven et al., 2012). DNA-based molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, plant breeding, genetic engineering etc (Joshi et al., 2011). By using dense genetic marker maps, the contributions of separate regions of the genome on the trait values can be estimated once the mapping population is sufficiently large. In addition, agronomic important traits like nutritional quality, yield, flower time and durable resistance which appear to follow complex, polygenic inheritance patterns with multiple genes having small effects on the trait value can easily be analyzed using markers.

Marker Assisted Selection (MAS) refer to the use of DNA markers that are tight-linked to target loci as a substitute for or to assist phenotype screening. By determining the allele of a DNA marker, plants that possess particular genes or quantitative trait loci (QTL) may be identified based on their genotype rather than their phenotype (Jonah et al., 2011). MAS is in contrast to genetic engineering which involves the artificial insertion of such individuals genes from one organism into the genetic material of another (typically, but not exclusively from other unrelated species (Daniel et al., 2006).

### 2.6.4.3 Biotechnology

Diversity based on phenotypic and morphological characters, usually varies with environments and evaluation of traits requires growing the plants to full maturity prior to identification, but now the rapid development of biotechnology allows easy analysis of large number of loci distributed throughout the genome of the plants (Jonah et al., 2011). 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 (Morales et al., 2012). The availability of the soybean genome sequence has quickly enabled the identification of genes for numerous important traits (Steven et al., 2012). Microarray analysis has been performed to aid the development of resistant varieties by identifying resistance genes (DeMortel et al., 2007a). Transcriptional changes play a major role in plant defense process.

The coordinative up regulation genes lead to defense responses such as toxin production, cell wall structure changes, programmed cell death, and so on. It is, therefore, important to study differentially expressed genes of soybean in response to *P. pachyrhizi*, which should help to understand the mechanisms of rust development and aid towards the development of resistant soybean varieties for Asian soybean rust (Panthee et al., 2007). Panthee et al., (2007) found 112 genes with differential expression pattern in response to Asian soybean rust (ASR) caused by *P. pachyrhizi* in soybean. Those genes were not specific to the ASR rather were involved in general defense. Global gene expression analysis has emerged as an important tool to understand how plants respond to biotic and abiotic stresses in order to identify genes associated with specific traits (Panthee et al., 2009).

About 80 % of identified genes in this study shared no homology to previously described *Phakopsora* genes. This result demonstrates stage-specific gene expression in the development of uredinia. Small, non-coding RNAs have been characterized in plants as important factors involved in gene expression regulation in developmental processes (Mallory et al., 2006; Chen, 2005), as well as adaptation to biotic and abiotic stress conditions (Lu et al., Shukla et al., 2008). Kulcheski et al., (2011) found that Among the 11 miRNAs analyzed, all showed different expression profiles during biotic and abiotic stresses to soybean. The pattern of miRNAs expression was different for the distinct genotypes submitted to the pathogen stress. Most miRNAs were down regulated during the fungus infection in the susceptible genotype; however, in the resistant genotype, most miRNAs did not vary during rust attack (Kulcheski et al., 2011).

### 3. CONCLUSIONS

Development and use of cultivars resistant to soybean rust minimize yield losses, broaden adaptation and improve stability of performance of cultivars, reduce fungicide use and enhance fungicide use efficiency, minimize health hazards and adverse environmental impacts and reduce production costs. 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. 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. Molecular markers have been looked upon as a tool for a large number of applications ranging from localization of a gene to improvement of plant varieties by marker assisted selection, called genome analysis which has generated a vast amount of information and a number of databases are being generated to preserve and popularize it.

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