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Field screening of Paraguayan soybean germplasm for resistance to charcoal rot

Fernández-Gamarra, M. A., Mohan-Kohli, M., Scholz-Drodowski, R., Vargas, M. J., Agüero, R., Riveros, M., Sanabria-Velázquez, A. D. and Enciso-Maldonado, G. A.

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SUMMARY

The aim of this work was to identify genetic resistance to charcoal rot (*Macrophomina phaseolina*) in soybean germplasm from the National Breeding Program of the Instituto Paraguayo de Tecnología Agraria (Paraguayan Institute of Agricultural Technology). During two seasons, 51 commercial and experimental lines from the local breeding program were field evaluated in Itapúa-Paraguay. The lines were planted in single rows previously infested, using a completely randomized block design with four repetitions. The charcoal rot severity was evaluated in the stems and roots at the physiological maturity stage. On a Root and Stem Severity index scale of 1-5, the median severity for the 34 early maturity genotypes was 1.5 and 1 in 2017/2018 and 2018/2019, respectively. Nine genotypes (AG-6525 xi, SP14041, SP14222, SP14583, SP15013, SP15133, SP15218, SP16020, and SPB-14146) were rated as resistant (1) in both evaluations. The median severity for the 15 semi-early genotypes was 2 and 1 in 2017/2018 and 2018/2019, respectively. This study allowed us to identify previously unreported sources of resistance to charcoal rot in maturity group IV, V and VI. We believe that germplasm screening under field conditions is a viable alternative to identify breeding lines which are less sensitive to charcoal rot.

Keywords: *Macrophomina phaseolina*, plant genetic resources, genetic resistance, soilborne diseases

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RESUMEN

El objetivo de este trabajo fue identificar resistencia a la pudrición carbonosa (*Macrophomina phaseolina*) en germoplasma de soja del Programa Nacional de Mejoramiento del Instituto Paraguayo de Tecnología Agropecuaria (IPTA). Durante dos temporadas, se evaluaron 51 líneas comerciales y experimentales del programa de mejoramiento local en Itapúa-Paraguay. Las líneas se sembraron en hileras individuales previamente infestadas, utilizando un diseño de bloques completamente al azar con cuatro repeticiones. Se evaluó la severidad de la pudrición carbonosa en los tallos y raíces en la etapa de madurez fisiológica. En una escala de índice de severidad de raíz y tallo de 1 a 5, la severidad media de los 34 genotipos de madurez temprana fue de 1,5 y 1 en 2017/2018 y 2018/2019, respectivamente. Nueve genotipos (AG-6525 xi, SP14041, SP14222, SP14583, SP15013, SP15133, SP15218, SP16020 y SPB-14146) fueron resistentes (1) en ambos períodos. La severidad media de los 15 genotipos semi-precoces fue de 2 y 1 en 2017/2018 y 2018/2019, respectivamente. Se identificaron fuentes de resistencia a la pudrición carbonosa no reportadas previamente en los grupos de madurez IV, V y VI. El cribado de germoplasma en el campo es una alternativa viable para identificar líneas menos sensibles a la pudrición carbonosa.

Palabras clave: *Macrophomina phaseolina*, recursos fitogenéticos, resistencia genética, patógenos de suelo

Fernández-Gamarra , M. A. (ORCID: 0000-0003-3895-4945) y Scholz-DrodowskI, R. (ORCID: 0000-0002-0212-4432): Instituto Paraguayo de Tecnología Agraria, Centro de Investigación Capitán Miranda, Ruta VI, Km 16, Capitán Miranda, C. P. 6990, Paraguay. Mohan-Kohli, M.: Cámara Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas, Avenida Brasilia 840, Asunción, C. P. 1204, Paraguay. Vargas, M. J. (ORCID: 0000-0001-7645-316X) y Riveros, R. (ORCID: 0000-0003-2149-2385): Instituto de Biotecnología Agrícola, Avda. Brasilia 939, Asunción, C. P. 1204, Paraguay. Agüero, R. (ORCID: 0000-0001-7535-8769): Universidad San Carlos Filial Encarnación, Carrera de Agronomía, Avenida Carlos Antonio López y Jorge Memmel, Encarnación, C. P. 6000, Paraguay. Sanabria-Velázquez, A. D. (ORCID: 0000-0002-2038-5388) y Enciso-Maldonado, G. A. (ORCID: 0000-0002-9528-7627): Centro de Desarrollo e Innovación Tecnológica de Itapúa, Ruta VI, Km 38, Hohenau, C. P. 6290, Paraguay.

Correspondence to: gui77eenciso@gmail.com

INTRODUCTION

Soybean is the most important agricultural crop in Paraguay. Currently, 10 million tons of grains are produced on 3.5 million hectares, positioning Paraguay as the sixth-largest producer and fourth-largest exporter in the world. Over the past two decades, the crop has become extremely important to the national economy, generating foreign currency and sources of employment in the industry, transportation, and other sectors (http://capeco.org.py/).

Charcoal rot, caused by *Macrophomina phaseolina*, is a destructive disease that threatens soybean cultivation worldwide, causing yield losses of approximately 2.5 million tons in the main soybean producing countries (Argentina, Bolivia, Brazil, Canada, China, India, Paraguay, and the United States); in Paraguay, the losses reach 1600 tons (Wrather et al., 2010). This pathogen can infect soybeans at any phenological stage; however, symptoms are commonly observed after flowering at the primary root and lower stem between the beginning of maturity (R7) and completed plant maturity (R8) (Mengistu, Smith et al., 2011; Gupta et al., 2012). Severe epidemics of the disease have been observed in Paraguay during the years 1996, 2001, 2005, and 2006, coinciding with the drought and high temperature conditions during the reproductive stage of the crop and thereby resulting in significant yield losses. In 2006, charcoal rot ranked sixth among the most economically significant plant diseases for soy-producing countries (Argentina, Bolivia, Brazil, Canada, China, India, Paraguay, and the United States) (Wrather et al., 2010). In Paraguay, the disease was observed in all the 48 locations evaluated between April and August 2008 (Orrego-Fuente et al., 2009) and in 2017 and 2019, soybeans production areas with high populations of *M. phaseolina* were identified in the Department of Itapúa, Paraguay (Maidana-Ojeda et al., 2020).

Some strategies proposed for the management of this disease are early planting, crop rotation, use of irrigation, seed treatment and fungicide applications, and biological control (Gupta et al., 2012). However, the most feasible method to prevent yield losses in soybean crops relies on promoting the resistance to the disease of the host plant (Romero-Luna et al., 2017). Genetic resistance is a crucial strategy that minimizes the use of fungicides, reduces crop loss, and promotes sustainable production management (Romero-Luna et al., 2017).

The identification of charcoal rot-resistant soybean genotypes is valuable for the breeding programs to select parental lines in the development of well adapted and high yielding cultivars that benefit farmers (Mengistu, Smith et al., 2011). To date, no soybean genotypes with high levels of resistance have been identified (Mengistu, Arelli et al., 2011). However, moderate resistance to charcoal rot has been observed in soybean genotypes with different levels of maturity (Paris et al., 2006; Mengistu et al., 2007; Mengistu, Arelli et al., 2011; Mengistu et al., 2013). The best method for evaluating host resistance to charcoal rot is based on the assessment of root and stem severity (RSS) and an index of colony-forming units (microsclerotia), under field conditions at the physiological maturity stage.

The level of resistance of the commercial cultivars and advanced lines in the breeding program in Paraguay is currently unknown. Therefore, the present study was undertaken to identify the level of resistance to charcoal rot in the experimental lines as well as the commercial soybean cultivars under field conditions.

MATERIALS AND METHODS

Field location

Field trials were established during two crop cycles, 2017/2018 and 2018/2019, at the Captain Miranda Research Center of the Paraguayan Institute of Agricultural Technology (IPTA, for its initials in Spanish), Captain Miranda, Itapúa (27° 12’ 03.6”S and 55° 47’ 28.1” W). The experiments were seeded on November 14th 2017, and November 27th 2018.

Plant material

A collection of 51 soybean varieties and breeding lines, representing Maturity Groups (MG) from IV to VI (early to semi-early maturity), was obtained from the National Breeding Program of the IPTA. These materials were selected based on their high yield and good agronomic performance over the years. Thirty-four (34) of the lines and varieties were classified as early maturity group, MG 4.5 – 5.5, while other 15 were classified as semi-early, MG 5.6 – 6.5. A charcoal rot susceptible cultivar A-4910 (MG IV) was used as a susceptible control and an advanced line DT97-4290 (GMV) with moderate resistance, used as a resistant control.

Macrophomina phaseolina isolation and inoculum production

An isolate of *M. phaseolina* collected from a soybean field in Choré (San Pedro region, Paraguay) in 2015, was identified as the most virulent compared with other isolates from different parts of the country (unpublished data). A mycelial transfer was made from the mycelial plate to create inoculum in the potato-dextrose-agar culture medium (PDA).

The method for inoculum production was adapted from Mengistu et al. (2007) where 400 ml (by volume) of millet grains (*Pennisetum glaucum*), were soaked in 4 liters of distilled water for 24-48 hours. The liquid was discarded, and the grains were divided equally in two parts and placed in autoclavable bags. The bags with millet grains were autoclaved twice (121 °C for 30 min). Sterilized millet grains were placed in bags (1.8 kg), together with the *M. phaseolina* mycelia from a pure culture plate. The bags were closed and incubated at 30 °C for two weeks. After this period, the fungus colonized the millet grains covering them with microsclerotia. These infected grains were removed from the sealed bags to allow air drying and then stored in plastic containers at 4 °C, until use.

Field experiment

The cultivars and breeding lines under study were distributed on the field using a completely randomized block design with four repetitions. Each line was planted in a single row of 1 m long and separated to 0.45 m from other rows. The field where the study was carried out had no history of occurrence of soybean charcoal rot; therefore, the inoculation of the plants was carried out during planting: 3 g of infected millet grains were evenly distributed in each row throughout the experimental unit (Mengistu et al., 2007). Subsequently, 20 soybean seeds were sown in each row, 2 cm deep, for each experimental unit. The plot area was kept free of weeds with pre and post-emergence herbicide applications. Irrigation was not applied during the test. Precipitation and maximum daily air and ground temperature data were obtained from the IPTA-Captain Miranda weather station during the growing season of the crop.

Disease evaluation

The stem and root severity assessment of *M. phaseolina* was evaluated at the physiological maturity stage (R7) (Fehr et al., 1971). Four soybean plants of each genotype were randomly selected and gently uprooted. The stem and main roots of each plant were cut longitudinally and visually rated for the intensity of discoloration and microsclerotia load covering vascular and cortical tissue by the Root and Stem Severity index (RSS) (Mengistu et al., 2007). The RSS index visually classifies the genotypes into five categories as follows: 1) no visible microsclerotia on the tissue; 2) very few microsclerotia visible in the marrow, vascular tissue or under the epidermis, or the vascular tissue is not discolored; 3) vascular tissue is partially discolored, and the microsclerotia have partially covered the tissue; 4) the vascular tissue is discolored with numerous microsclerotia embedded in the tissue, the microsclerotia are also visible under the outer epidermis in the stem and root sections; and 5) vascular tissue obscured due to a large amount of microsclerotia both inside and outside the stem and root tissues. Subsequently, these values were categorized as resistant (R) (values = 1), moderately resistant (MR) (values from > 1 to 2), moderately susceptible (MS) (values from > 2 to 3), and susceptible (S) (values from > 3 to 5) (Paris et al., 2006).

Statistical analyses

Because stem and root severity were evaluated using an ordinal scale, disease ratings were rank transformed using PROC RANK and they were analyzed using nonparametric methods. The effect of genotypes was evaluated using Wald type statistics employing ANOVAF option of the PROC MIXED of SAS (SAS Institute Inc., Cary, NC). The data for each season (2017/2018 and 2018/2019) were not pooled because there was a significant interaction between the effect of genotypes and season in a preliminary ANOVA. Genotypes within each maturity group and the year were analyzed separately.

RESULTS AND DISCUSSION

The weather conditions, average daily air temperatures and precipitations, were similar in both crop seasons (Figure 1A and 1B) and considered moderate for charcoal rot development in the field. Both seasons were relatively warm and wet with an average temperature of 24.9 °C, precipitation about 893.5 mm, and 76 % of relative humidity during the 2017/2018 season compared with an average temperature of 25.5 °C, precipitation about 1078

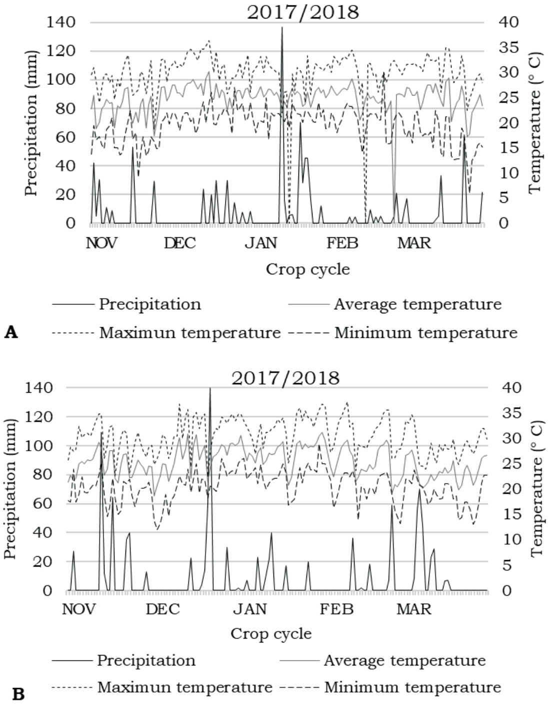

Figure 1. Weather conditions recorded during the soybean crop cycles 2017/2018 (A) and 2018/2019 (B)

Table 1. Average soil temperature at 20, 10 and 5 cm depth, and relative humidity of air (RH) during the 2017/2018 and 2018/2019 soybean crop cycles

Period	Soil temperature			RH (%)
	20 cm	10 cm	5 cm	
Nov-17	22.8	19.9	20.7	68.7
Dec-17	26.6	24.5	25.0	73.7
Jan-18	26.2	23.8	24.3	83.2
Feb-18	26.2	27.3	24.2	75.4
Mar-18	25.9	26.9	26.8	78.4
Nov-18	24.2	25.9	26.1	66.5
Dec-18	25.9	27.7	28.1	63.0
Jan-19	27.9	29.7	29.7	70.6
Feb-19	26.9	28.5	28.7	68.5
Mar-19	24.4	25.3	25.5	70.9

mm and 68 % of relative humidity in the 2018/2019 season. Average soil temperatures, at depths of 5 and 10 cm, were 3.4 and 2.9 °C higher in 2018/2019 than in 2017/2018 (Table 1). In January 2019, the highest average soil temperatures were recorded at 5 and 10 cm. During both years, there was enough rainfall to favor the normal growth and development of the crop.

Resistance to charcoal rot in early maturing soybean genotypes (MG 4.5 to 5.5)

The disease intensity based on the scale of Root and Stem Severity index (RSS) (Mengistu et al., 2007) for the 34 early maturity genotypes was 1.5 (minimum 1 and maximum 3) and 1 (minimum 1 and maximum 2) in 2017/2018 and 2018/2019, respectively. Based on the RSS scale, the susceptible control A4910, reached a value of 4 in 2017/2018 crop cycle and 3 in the 2018/2019 crop season, indicating thereby a moderate level of expression of the disease in the experiment (Table 2). Overall, the Charcoal Rot infection index in 34 early genotypes ranged from 1 to 4 in the first year and from 1 to 3 in the second year. While significant variation of infection was seen among the genotypes within the year and over the two years, nine genotypes were rated R in both evaluation periods (AG-6525 xi, SP14041, SP14222, SP14583, SP15013, SP15133, SP15218, SP16020 and SPB-14146). In addition, genotypes DT974290CR, EXP-PA101 and SP16003 were considered MR in both periods. Only the genotype SP14002 maintained moderately susceptible (MS) reaction in both crop cycles. All other genotypes showed a variable response in each season, with the genotypes generally scoring lower severity values during the second year (Table 2).

Resistance to charcoal rot in semi early maturing soybean genotypes (MG 5.6 to 6.5)

The median severity for the 15 semi-early genotypes was 2 (minimum 1 and maximum 3) and 1 (minimum 1 and maximum 1) in 2017/2018 and 2018/2019, respectively (Table 3). Much less variation for disease reaction was observed among 15 semi early genotypes, especially in the second year of the test (2018/2019). While disease index among these genotypes ranged from 1 to 4 in the first year and from 1 to 3 in the second year, the susceptible control genotype (A4910RG) demonstrated the highest infection in both crop cycles. Despite the lower disease spread in this group, none of the genotypes showed R reaction for both years of evaluation, except for SP14018. The genotypes SP14544 and DT974290CR (resistant control) were observed to be MR both years.

Although environmental conditions were conducive to the development of the disease during both years, there was a significant difference in the soil temperature, precipitation, and relative humidity between the years. The lower precipitation during November and December in 2017/2018, coinciding with pod filling stage (R5-R6), was most likely responsible for the higher disease infection in this year compared with 2018/2019, when rainfall was higher. The inadequate quantity or distribution of rainfall are factors that reduce the relative humidity of the air, which favors the stress of soybean plants and increases the probability of *M. phaseolina* infection, restricting the development of the roots, therefore, the greatest RSS disease scores during the first year could be due to environmental conditions favoring pathogen infection. (Mengistu, Arelli et al., 2011). Unlike the 2017/18 planting season, in 2018/2019 the average air and soil temperature was higher; however, a better distribution of rainfall was observed between November and December, which could have reduced the infecting ability of the pathogen.

Given that higher levels of *M. phaseolina* are observed during the dry years compared to wet years (Mengistu, Arelli et al., 2011), the lower amount of water in the soil and lower relative humidity seemed to have contributed to a higher disease colony forming units in the root and stem tissues during the first year. Also, the level of microsclerotia in stems and roots is higher in soybean plants under water stress conditions in the soil (Kendig et al., 2000), coinciding with our observations during season 2017/2018.

The charcoal rot fungus, *M. phaseolina,* is a monocyclic pathogen and natural inhabitant of the soil (Mihail and Taylor, 1995). Therefore, its

Table 2. Reactions of soybean genotypes with early maturation (group 4.5 – 5.5) to *M. phaseolina* in 2017/2018 and 2018/2019

Genotype	2017/2018						2018/2019					
	Median	Min.	Max.	Mean Rank	Letter Group	**Reaction**[y]	Median	Min.	Max.	Mean Rank	Letter Group	**Reaction**[y]
A-4910RG (control)	4	3	5	182.06	A	S	3	2	3	190.97	AB	MS
A-5909RG	2.5	1	3	119.6	ABCDEFGH	MS	1	1	1	70.4692	I	R
AG-6525 xi	1	1	2	57.4989	HI	R	1	1	1	70.4692	I	R
AG-6565 xi	3	2	5	155.7	ABC	MS	2	1	2	152.6	BCDE	MR
DT974290CR	1.5	1	2	76.2593	FGHI	MR	2	1	2	132.67	DEFG	MR
EXP-PA 101	2	2	3	119.21	ABCDEFGH	MR	1.5	1	2	102.8	FGHI	MR
J-955-1-1-1-4	1.5	1	3	86.2688	DEFGHI	MR	1	1	1	70.4692	I	R
M-6211 Ipro	1	1	1	43.6964	I	R	2	2	2	165.47	ABCD	MR
NS-4903 RG	3	1	4	133.5	ABCDEF	MS	1	1	1	70.4692	I	R
SJ 11014	1	1	1	43.6964	I	R	2	1	4	150.96	BCDE	MR
SJ13397	3	2	3	144.2	ABCDE	MS	1.5	1	2	119.8	EFGH	MR
SP14002	2.5	1	3	111.83	BCDEFGH	MS	2.5	2	4	180.6	ABC	MS
SP14041	1	1	2	62.4568	HI	R	1	1	2	100.35	GHI	R
SP14042	2.5	2	3	130.59	ABCDEF	MS	1	1	1	70.4692	I	R
SP14222	1	1	4	74.0408	FGHI	R	1	1	2	89.9261	HI	R
SP14530	1	1	1	42.1543	I	R	1.5	1	2	113.22	EFGH	MR
SP14534	1	1	3	70.7878	FGHI	R	1.5	1	2	133.14	DEFG	MR
SP14583	1	1	3	81.2614	DEFGHI	R	1	1	1	70.4692	I	R
SP14584	1	1	2	59.3998	HI	R	3	3	4	195.92	A	MS
SP15013	1	1	2	59.3998	HI	R	1	1	1	70.4692	I	R
SP15022	2.5	1	4	111.93	BCDEFGH	MS	2	2	2	165.47	ABCD	MR
SP15113	2.5	1	4	119.27	ABCDEFGH	MS	1	1	1	70.4692	I	R
SP15133	1	1	1	43.6964	I	R	1	1	1	70.4692	I	R
SP15218	1	1	1	43.6964	I	R	1	1	1	70.4692	I	R
SP15220	2.5	1	3	113.36	BCDEFGH	MS	2	2	2	165.47	ABCD	MR
SP16003	2	1	2	93.493	CDEFGHI	MR	2	1	2	152.6	BCDE	MR
SP16010	3.5	2	5	165.96	AB	S	1.5	1	3	120.13	EFGH	MR
SP16011	1.5	1	3	91.7651	CDEFGHI	MR	1	1	2	100.35	GHI	R
SP16016	2.5	1	3	116	BCDEFGH	MS	1	1	1	70.4692	I	R
SP16017	3	2	5	153.6	ABCD	MS	1	1	1	70.4692	I	R
SP16020	1	1	2	60.3094	GHI	R	1	1	2	83.3392	HI	R
SP16023	3	2	3	146.69	ABCDE	MS	1	1	1	70.4692	I	R
SP16024	1.5	1	4	97.2875	CDEFGHI	MR	1	1	1	70.4692	I	R
SPB-14146	1	1	2	57.4989	HI	R	1	1	1	70.4692	I	R
SPB-14151	1	1	1	43.6964	I	R	2	1	3	150.01	CDE	MR
TEC 5936 Ipro	1	1	2	64.8226	FGHI	R	2	1	2	152.6	BCDE	MR

[y] The severity values of 1 were strong (R); the values between > 1 and 2 were moderately resistant (MR); values between > 2 and 3 were moderately susceptible (MS); values greater than 3 were susceptible (S).

[Z] Mean ranks with the same letter are not significantly different according to the LSD test with probability of α=0.05.

Table 3. Reactions of soybean genotypes with semi-early maturation (group 5.6 – 6.5) to *M. phaseolina* in 2017/2018 and 2018/2019

Genotype	2017/2018						2018/2019					
	Median	Min.	Max.	Mean Rank	Letter Group	**Reaction**[y]	Median	Min.	Max.	Mean Rank	Letter Group	**Reaction**[y]
A-4910RG (control)	4	3	5	182.06	A	S	3	2	3	190.97	A	MS
BMX- POTENCIA RR	2	1	4	130.59	ABCDEF	MR	1	1	1	70.4692	D	R
DM-6262 RSF ipro	2	1	3	83.2118	EF	MR	1	1	1	70.4692	D	R
DT974290CR (control)	1.5	1	2	129.27	ABCDEF	MR	2	1	2	132.67	BC	MR
SOJAPAR R-24 RR	2	1	3	93.2953	CDEF	MR	1	1	1	70.4692	D	R
SP14018	1	1	2	144.2	ABCDE	R	1	1	1	162.78	AB	R
SP14080	3	2	4	108.75	BCDEF	MS	1	1	1	70.4692	D	R
SP14266	2.5	2	3	99.8736	CDEF	MS	1	1	1	70.4692	D	R
SP14544	1.5	1	2	145.31	ABCDE	MR	2	1	3	70.4692	D	MR
SP14546	1.5	1	3	100.52	CDEF	MR	1	1	1	70.4692	D	R
SP15030	2	1	3	154.19	ABC	MR	1	1	1	100.35	CD	R
SP15106	1.5	1	3	108.2	BCDEF	MR	1	1	1	70.4692	D	R
SP15116	2	1	5	79.6905	F	MR	1	1	2	70.4692	D	R
SP15201	2	1	3	109	BCDEF	MR	1	1	1	70.4692	D	R
SP15202	3	2	3	106.78	BCDEF	MS	1	1	1	143.33	B	R
SP15203	2.5	1	4	64.8226	F	MS	1.5	1	3	70.4692	D	MR
SP15219	3	2	3	76.2593	F	MS	1	1	1	70.4692	D	R

[y] The severity values of 1 were strong (R); the values between > 1 and < 2 were moderately resistant (MR); values between > 2 and < 3 were moderately susceptible (MS); values greater than 3 were susceptible (S).

[Z] Mean ranks with the same letter are not significantly different according to the LSD test with probability of α=0.05.

transmission to the plants occurs partly through the soil. Epidemics caused by this type of pathogen is the result of an accumulation of the inoculum over several seasons (Vale et al., 2001). In any year with a disease conducive environment, a high percentage of incidence and severity can be guaranteed. The annual rainfall during the soybean crop season in Paraguay is generally sufficient for the farmers to obtain economically high yields. However, those years when the rain is late for planting in time, or it does not occur in the middle of the growth cycle, the crops are subjected to water stress, which results in an increase in the severity of charcoal rot and an imminent loss of production (Orrego-Fuente et al., 2009). Under such situations, the genotypes categorized as MR or R in this study save the farmers from losing on their investment. It is also important to mention that these genotypes probably adapt better to the drier areas of the country. Despite this, because we worked in a soil with no history of the disease, the inoculum pressure was artificially increased by inoculation. In addition, the controls presented conspicuous symptoms and the objective of this work was related to the identification of resistant germplasm, focusing on the response of the plant, and not on the density of inoculum in the soil, which we assumed was uniform in the field due to artificial inoculation.

The selection of cultivars for resistance is one of the most important decisions that farmers make to maximize productivity. However, it has been frequently observed that decisions made on the disease performance of a cultivar in one year may not work another year, as seen in this research. Disease severity data obtained in this study demonstrates the importance of evaluating soybean genotypes over several years. The results of this two-year study showed that there are two early genotypes classified as R, and at least six early genotypes and two semi-early genotypes with MR rating during both years. While it is essential to keep testing these lines in the future, they can be valuable sources of resistance to charcoal rot in the local soybean breeding program.

It must be mentioned that both R genotypes

(SP15133 and SP15218) identified in this study derive their charcoal rot resistance from the line DT974290 (resistant control), rated as MR here. In other words, there must be additional factors for charcoal rot resistance in the same parent not being identified at present or others contributed by the second parent of the cross. In any case, this work opens a new avenue to upgrade the level of resistance to charcoal rot by exploiting the new lines in various combinations or even identifying the minor contributions of the second parents in the future research.

The resistance to *M. phaseolina* in soybeans is polygenic and quantitative, mediated by genes that encode universal stress protein family like leucine-rich repeat receptor-like protein kinase which responds to: 1) stress factors, the cyclophilin that responds to oxidative stress; 2) multi antimicrobial extrusion proteins, 3) glutathione S-transferase C-terminal domain enzyme that responds to cell defense and protection from oxidative stress (Coser et al., 2017), and 4) the activation of genes such as HARPIN INDUCED1 that encodes a coiled-coil nucleotide binding leucine rich repeat (Lawaju et al., 2018), a defense signaling protein that ends in a systemic acquired resistance pathway which is mediated through salicylic acid (Aljaafri et al., 2017).

Therefore, the greater the complexity of the resistance in a genotype, the slower the decomposition of the resistance by the pathogen (Vale et al., 2001). We believe that any future study must include genome association component to identify genes which influence resistance in the newly identified genotypes. Genomic selection can improve estimates of genetic value and selection accuracy for improving charcoal rot resistance in soybeans (Coser et al., 2017).

Charcoal rot in soybeans is a disease of economic importance in Paraguay. Given that the information on resistance of early and semi-early germplasm is absent, our field study not only demonstrated the validity of the screening methodology but also identified superior sources of charcoal rot resistance in the local germplasm. Finally, it is important to develop relevant information on the response of these lines against *M. phaseolina* in the different production regions of the country, as well as to use them to develop additional resistant cultivars.

CONCLUSION

The authors report nine early maturing soybean genotypes (AG-6525 xi, SP14041, SP14222, SP14583, SP15013, SP15133, SP15218, SP16020 and SPB-14146) as a source of resistance to charcoal rot in Paraguayan soybean germplasm.

REFERENCES

Aljaafri, W. A., McNeece, B. T., Lawaju, B. R., Sharma, K., Niruala, P. M., Pant, S. R., and Klink, V. P. (2017). A harpin elicitor induces the expression of a coiled-coil nucleotide binding leucine rich repeat (CC-NB-LRR) defense signaling gene and others functioning during defense to parasitic nematodes. *Plant Physiology and Biochemistry*, *121*, 161-175. https://doi.org/10.1016/j.plaphy.2017.10.004

Coser, S. M., Chowda Reddy, R. V., Zhang, J., Mueller, D. S., Mengistu, A., Wise, K. A., Allen, T. W., Singh, A. and Singh, A. K. (2017). Genetic Architecture of Charcoal Rot (*Macrophomina phaseolina*) Resistance in Soybean Revealed Using a Diverse Panel. *Frontiers in Plant Science*, *8*, 1626. https://doi.org/10.3389/fpls.2017.01626

Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. (1971). Stage of Development dDscriptions for Soybeans, *Glycine Max* (L.) Merrill 1. *Crop Science*, *11*(6), 929-931. https://doi.org/10.2135/cropsci1971.0011183X001100060051x

Gupta, G. K., Sharma, S. K., and Ramteke, R. (2012). Biology, Epidemiology and Management of the Pathogenic Fungus *Macrophomina phaseolina* (Tassi) Goid with Special Reference to Charcoal Rot of Soybean (*Glycine max* (L.) Merrill). *Journal of Phytopathology*, *160*(4), 167-180. https://doi.org/10.1111/j.1439-0434.2012.01884.x

Kendig, S. R., Rupe, J. C., and Scott, H. D. (2000). Effect of Irrigation and Soil Water Stress on Densities of *Macrophomina phaseolina* in Soil and Roots of Two Soybean Cultivars. *Plant Disease*, *84*(8), 895-900. https://doi.org/10.1094/PDIS.2000.84.8.895

Lawaju, B. R., Lawrence, K. S., Lawrence, G. W., and Klink, V. P. (2018). Harpin-inducible defense signaling components impair infection by the ascomycete Macrophomina phaseolina. *Plant Physiology and Biochemistry*, *129*, 331-348. https://doi.org/10.1016/j.plaphy.2018.06.020

Maidana-Ojeda, M., Sosa-Gerke, J. C., Bogado-González, C. E., Fernández-Gamarra, M. A., Sanabria-Velázquez, A. D., Lopez-Nicora, H. D., and Enciso-Maldonado, G. A. (2020). Cuantificación de inóculo de *Macrophomina phaseolina* (Tassi) Goid en parcelas de soja del Departamento de Itapúa-Paraguay. *Investigaciones y Estudios-UNA*, *11*(2), 69-77. https://revistascientificas.una.py/ojs/index.php/rdgic/article/view/194

Mengistu, A., Ray, J. D., Smith, J. R., and Paris, R. L. (2007). Charcoal Rot Disease Assessment of Soybean

Genotypes Using a Colony Forming Unit Index. *Crop Science*, *47*(6), 2453-2461. https://doi.org/10.2135/cropsci2007.04.0186

Mengistu, A., Smith, J. R., Ray, J. D., and Bellaloui, N. (2011).Seasonal Progress of Charcoal Rot and Its Impact on Soybean Productivity. *Plant Disease*, *95*(9), 1159-1166. https://doi.org/10.1094/PDIS-02-11-0100

Mengistu, A., Arelli, P. A., Bond, J. P., Shannon, G. J., Wrather, A. J., Rupe, J. B., Chen, P., Little, C. R., Canaday, C. H., Newman, M. A., and Pantalone, V. R. (2011). Evaluation of Soybean Genotypes for Resistance to Charcoal Rot. *Plant Health Progress*, *12*(1), 6. https://doi.org/10.1094/PHP-2010-0926-01-RS

Mengistu, A., Arelli, P., Bond, J., Nelson, R., Rupe, J., Shannon, G., and Wrather, A. (2013). Identification of Soybean Accessions Resistant to *Macrophomina phaseolina* by Field Screening and Laboratory Validation. *Plant Health Progress*, *14*(1), 25. https://doi.org/10.1094/PHP-2013-0318-01-RS

Mihail, J. D. and Taylor, S. J. (1995). Interpreting variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production, and chlorate utilization. *Canadian Journal of Botany*, *73*(10), 1596-1603. https://doi.org/10.1139/b95-172

Orrego-Fuente, A. L., Grabowski, C., Soilan, L., Ferreira, L., and Del Valle, C. (2009). *Distribución geográfica de* Macrophomina phaseolina *en los cultivos de soja, sésamo y maní.* In A. L. Orrego-Fuente (Ed.), Macrophomina phaseolina, *hongo causante de la pudrición carbonosa del tallo* (pp. 27-34). UNA/INBIO, San Lorenzo, Paraguay.

Paris, R. L., Mengistu, A., Tyler, J. M., and Smith, J. R. (2006). Registration of Soybean Germplasm Line DT97–4290 with Moderate Resistance to Charcoal Rot. *Crop Science*, *46*(5), 2324-2325. https://doi.org/10.2135/cropsci2005.09.0297

Romero-Luna, M. P., Mueller, D., Mengistu, A., Singh, A. K., Hartman, G. L., and Wise, K. A. (2017). Advancing Our Understanding of Charcoal Rot in Soybeans. *Journal of Integrated Pest Management*, *8*(1), 8. https://doi.org/10.1093/jipm/pmw020

Vale, F. X. R., Parlevliet, J. E., and Zambolim, L. (2001). Concepts in plant disease resistance. *Fitopatologia Brasileira*, *26*(3), 577-589. http://dx.doi.org/10.1590/S0100-41582001000300001

Wrather, A., Shannon, G., Balardin, R., Carregal, L., Escobar, R., Gupta, G. K.; Morel, W., Ploper, D., and Tenuta, A. (2010). Effect of Diseases on Soybean Yield in the Top Eight Producing Countries in 2006. *Plant Health Progress*, *11*(1), 29. https://doi.org/10.1094/PHP-2010-0102-01-RS