Pesquisa Agropecuária Brasileira

Phytopathology/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Phytophthora resistance behavior of soybean F1 populations with pyramided *Rps* genes

Abstract – The objective of this work was to identify the resistance behavior of soybean (*Glycine max*) F1 populations with pyramided *Rps* genes, when subjected to inoculation with pathotypes of *Phytophthora sojae*. In 2020 and 2021, hybridizations were performed considering the presence of *Rps* genes from different loci in the parents. To characterize the F1 generation, pathotypes PS2.4, PS14.4, PS34.1, PS36.1, and CMES1608 were inoculated into the lateral branches of adult plants. The reaction to the pathotypes was evaluated in a greenhouse experiment, through the percentage of infected, dead, and healthy lateral branches. The study allowed identifying the virulent reaction of parents and their inoculated progenies. Progenies from virulent combinations of the same pathotype did not present new resistances. Resistance is increased by the complementarity of different *Rps* genes from the genotypes used to obtain the F1 progenies. Soybean F1 populations with pyramided *Rps* genes are an efficient genetic tool to help control *Phytophthora sojae*.

Index terms: *Glycine max*, *Phytophthora sojae*, hybridizations, pathogen, resistance genes.

Guilherme dos Santos
Universidade Federal de Santa Maria, Frederico Westphalen, RS, Brazil.
E-mail: guilherme.agr.rs@gmail.com

Volmir Sergio Marchioro ✉
Universidade Federal de Santa Maria, Frederico Westphalen, RS, Brazil.
E-mail: volmir@marchioro.eng.br

Daniela Meira
Centro de Ensino Superior Riograndense, Sarandi, RS, Brazil.
E-mail: dmdanielameira94@gmail.com

Marcos Toebe
Universidade Federal de Santa Maria, Frederico Westphalen, RS, Brazil.
E-mail: m.toebe@gmail.com

Giovani Benin
Universidade Tecnológica Federal do Paraná, Pato Branco, PR, Brazil.
E-mail: giovani.bn@gmail.com

✉ Corresponding author

Received
March 14, 2025

Accepted
July 21, 2025

How to cite
SANTOS, G. dos; MARCHIORO, V.S.; MEIRA, D.; TOEBE, M.; BENIN, G. *Phytophthora* resistance behavior of soybean F1 populations with pyramided *Rps genes*. **Pesquisa Agropecuária Brasileira**, v.61, e04074, 2026. DOI: https://doi.org/10.1590/S1678-3921.pab2026.v61.04074.

Comportamento de resistência *à* fitóftora de populações F1 de soja com genes *Rps* piramidados

Resumo – O objetivo deste trabalho foi identificar o comportamento de resistência de populações F1 de soja (*Glycine max*) com genes *Rps* piramidados, quando submetidas à inoculação com patótipos de *Phytophthora sojae*. Em 2020 e 2021, foram realizadas hibridizações, tendo-se considerado a presença de genes *Rps* de diferentes loci nos genitores. Para caracterizar a geração F1, os patótipos PS2.4, PS14.4, PS34.1, PS36.1e CMES1608 foram inoculados em ramos laterais de plantas adultas. A reação aos patótipos foi avaliada em experimento em casa de vegetação, por meio da percentagem de ramos laterais infectados, mortos e sadios. O estudo permitiu identificar a reação de virulência dos genitores e de suas progênies inoculadas. Progênies de combinações virulentas de um mesmo patótipo não apresentaram novas resistências. A resistência é aumentada pela complementaridade de diferentes genes *Rps* dos genótipos utilizados para obter as progênies F1. Populações F1 de soja com genes *Rps* piramidais são uma ferramenta genética eficiente para auxiliar no controle de *Phytophthora sojae*.

Termos para indexação: *Glycine max*, *Phytophthora sojae*, hibridação, patógeno, genes de resistência.

Introduction

Phytophthora root rot, caused by *Phytophthora sojae*, is one of the main root diseases of soybean [*Glycine max* (L.) Merr.], which can cause significant losses in terms of grain productivity (Schmitthenner & Dorrance, 2022). The disease can result in plant death at different soybean phenological stages, and its aggressiveness can worsen when susceptible cultivars are associated with environments favorable for the development of the pathogen (Costamilan et al., 2011).

Genetic resistance is the most efficient strategy to control phytophthora root rot. This resistance can be divided into two types: vertical or qualitative, controlled by one or a few genes; and horizontal or quantitative, controlled by many genes and highly influenced by the environment (Lebreton et al., 2018).

According to Giachero et al. (2022), there are already more than 33 identified and mapped *Rps* genes and alleles, located in nine chromosomes (2, 3, 7, 10, 13, 16, 17, 18, and 19) that confer some type of resistance to specific pathotypes of *P. sojae* (Jiang et al., 2020). Pyramiding several resistance genes in a single cultivar can be a solution to increase the level and durability of resistance. Peng et al. (2023) and Zhao et al. (2024), for example, verified resistance gains with pyramided genes in creeping bentgrass (*Agrostis stolonifera* L.) and in rice (*Oryza sativa* L.), respectively.

The objective of this work was to identify the resistance behavior of soybean F1 populations with pyramided *Rps* genes, when subjected to inoculation with pathotypes of *P. sojae*.

Materials and Methods

The experiment was conducted in 2020 and 2021, in the municipality of Cambé, in the state of Paraná, Brazil (23º15'02"S, 51º14'53"W, at 895 m of altitude), specifically in a greenhouse with controlled temperature and humidity for soybean cultivation.

The used parents and the specific *Rps* genes that each of them contributed to the experiment were: 'Harlon' (*Rps1a*), L77-1863 (*Rps1b*), 'Beeson 80' (*Rps1c*), 'Williams 82' (*Rps1k*), L82-1449 (*Rps2*), 'Chapman' (*Rps3a*), L92-7857 (*Rps3c*), L85-2352 (*Rps4*), L85-3059 (*Rps5*), L85-1581 (*Rps6*), and PI399373 (Rps*8*).
The carried out crossings were: 'Harlon' with genotypes L82-1449, L85-2352, L85-3059, and L89-1581; L77-1863 with L82-1449, L92-7857, L85-2352, L85-3059, L89-1581, and PI399073; 'Beeson 80' with L82-1449, 'Chapman', L92-7857, L89-1581, and PI399073; 'Williams 82' with L92-7857, L85-2352, L85-3059, L89-1581, and PI399073; L82-1449 with 'Chapman', L85-3059, and PI399073; 'Chapman' with L85-2352 and L89-1581; L85-2352 with L85-3059 and PI399073; L85-3059 with L89-1581; and L85-2352 with PI399073.

Five *Phytophthora sojae* pathotypes were used to identify the virulence pattern of the parents, being inoculated into plants from their progenies. The pathotypes were obtained from Empresa Brasileira de Pesquisa Agropecuária (Brasília, DF), where they are stored in liquid nitrogen. The used pathotypes and their respective *Rps* gene virulence formula were: PS2.4 (*Rps1d*, *Rps2*, *Rps3b*, *Rps3c*, *Rps4*, *Rps6*, *Rps5*, and *Rps7*), PS14.4 (*Rps1d*, *Rps3a*, *Rps3b*, *Rps3c*, *Rps4*, *Rps5*, *Rps6*, *Rps7*, and *Rps8*), PS36.1 (*Rps1b*, *Rps1d*, *Rps2*, *Rps3a*, *Rps3b*, *Rps3c*, *Rps4*, *Rps5*, *Rps6*, *Rps7*, and *Rps8*), PS34.1 (*Rps1a*, *Rps1b*, *Rps1c*, *Rps1d*, *Rps1k*, *Rps2*, *Rps3a*, *Rps3b*, *Rps3c*, *Rps4*, *Rps5*, *Rps6*, and *Rps7*), and CMES1608 (*Rps1b*, *Rps1d*, *Rps1k*, *Rps3a*, *Rps3b*, *Rps3c*, *Rps5*, *Rps7*, and *Rps8*). In order to determine the parents' response to the isolates, their resistance patterns were previously characterized (Santos et al., 2025).

Each parent was sown in five 3.6 L pots filled with a mixture of three parts soil, one part substrate, and one part sieved and sterilized sand. Five seeds were sown in each pot at five different dates in October 2020. After the crosses were performed, at physiological maturity, the F1 seeds were harvested and sown under the same conditions, starting in April 2021.

Inoculation was performed using the toothpick technique described by Keeling (1982) and adapted by Yorinori (1996) for soybean stem canker inoculations. For the procedure, 1.2 cm toothpicks were cut into two pieces, with both pointed ends to reduce mechanical damage to the plants when inserted. All toothpicks were boiled three times in distilled water for 30 min in order to eliminate possible toxic agents and other inhibitors. Then, the toothpicks were placed in a Petri dish with a filter paper disk covering its internal surface, with their conical part upwards, spaced at 5.0 to 6.0 cm in diameter for the introduction of mycelium disks. The purification plate and toothpick assembly was sterilized at 120ºC for 20 min. After sterilization, the V8 culture medium (40 mL V8 juice, 0.6 g calcium carbonate, 1.0 g sucrose, 0.2 g yeast extract, 20 g agar, and 1,000 mL distilled water) was poured

into the Petri dishes, leaving 3.0 mm of the end of the toothpicks exposed. The pure isolates were prepared by collecting the hyphal tips of the isolates from the matrix and placing them in autoclaved Petri dishes that contained the V8 culture medium and would be sealed and wrapped in plastic film. The incubation period was four days in a biological oxygen demand (BOD) chamber, under an alternating photoperiod of 12 hours at 25–27°C. After incubation, five mycelium discs measuring 4.0 mm in diameter were transferred to new dishes containing the toothpicks in previously prepared solidified culture medium. The new dishes were incubated in the BOD for seven days, under a 12 hour photoperiod under fluorescent light and an average temperature of 25–27ºC. Up to two pathogens were inoculated on the lateral branches of each adult plant at stages V5 to R1 (Figure 1), by inserting contaminated twigs at 3.0 to 5.0 cm from the upper end of the branches. Then, the different reactions per tested individual were scored (Santos et al., 2023).

The evaluations were carried out 15 days after inoculation, following the methodology described by Yorinori (1996), which consists of counting the number of infected, dead, and healthy lateral branches of adult plants, as well as the final number of lateral branches. The results were then transformed into percentages based on the number of individuals tested in each population.

Results and Discussion

The adult plants of the F1 generation showed a lower percentage of dead lateral branches when obtained from the crosses aimed at pyramiding *Rps* genes than from those in which only one of parent had the disease (Table 1). Consequently, there was an addition of different levels of resistance in around 95% of the comparisons between individually tested parents and their progenies. These results are in accordance with the gene-by-gene theory described by Flor (1971): for each gene that conditions the reaction in the host, there is a corresponding gene in the causative agent that conditions the pathogenicity.

It should be noted that the pyramiding of two resistance genes susceptible to the tested pathotype did not generate resistance in the progeny. The percentage of dead lateral branches, for example, reached: 100% in the F1 progeny from the cross between 'Harlon' (*Rps1a*) and L82-1449 (*Rps2*), with 80 and 94% dead lateral branches, respectively, after inoculation with PS34.1; 100% in the F1 progeny from the cross between 'Beeson 80' (*Rps1c*) and 'Chapman' (*Rps3a*), with 43 and 88% of dead lateral branches, respectively, with the inoculation of CMES1608; and 88%, in the F1 progeny of 'Williams 82' (*Rps1k*) and L85-3059 (*Rps5*), with 83 and 86% dead lateral branches, respectively, with no added resistance.

Gene expression can be altered by several factors, such as epistasis, an interaction between alleles of resistance genes located in different loci that results in a reduced resistance or total susceptibility to resistance in hybrid plants (Carlborg & Haley, 2004; Phillips, 2008). A second factor concerns genetic incompatibility, when resistance genes from different genetic sources may not function correctly when pyramided (Lynch & Walsh, 1998).

A complementary relationship, which meets the objective of the addition of resistance, was observed in the cross between 'Harlon' (*Rps1a*) and L89-1581 (*Rps6*). The amount of dead lateral branches varied from 80 to 9% for 'Harlon' after inoculation with PS34.1 and PS36.1, respectively, but from 100 to 0% for L89-1581 after inoculation with PS36.1 and PS34.1, respectively. PS34.1 and PS36.1 were inoculated simultaneously into the lateral branches of adult plants from the F1 generation, and the presence of genes *Rps1a* and *Rps6* only caused 25% dead lateral branches.

The F1 progeny from L77-1863 (*Rps1b*) and L82-1449 (*Rps2*) was resistant to the PS2.4 and CMES1608 pathotypes, as no plant deaths were observed. For genotype L77-1863, no plant death was reported after inoculation with PS2.4, but 81% of plants died when individually inoculated with CMES1608. For the L82-1449 genotype, 81% of the plants died when individually inoculated with PS2.4, but only 17% died when inoculated with CMES1608, which is an indicative that complementarity of resistance occurred in the F1 plants due to the presence of the *Rps1b* and *Rps2* genes.

Based on the bilateral resistance gains obtained by the combination of *Rps* genes, the progenies from the crossings between 'Harlon' (*Rps1a*) and L89-1581 (*Rps6*), L77-1863 (*Rps1b*) and L85-2352 (*Rps4*), L77-1863 (*Rps1b*) and L89-1581 (*Rps6*), L77-1863 (*Rps1b*) and PI399073 (*Rps8*), 'Beeson 80' (*Rps1c*) and L89-1581 (*Rps6*), 'Beeson 80' (*Rps1c*) and PI399073 (*Rps8*), 'Williams 82' (*Rps1k*) and L85-2352 (*Rps4*), 'Williams 82' (*Rps1k*) and L89-1581 (*Rps6*), L82-1449

Figure 1. *Phytophthora sojae* inoculation into the lateral branches of soybean (*Glycine max*) adult plants (A), dead lateral branches of adult plants (B), infection reaction when susceptible (C), and hypersensitivity reaction when resistant (D).

Table 1. Characterization of the virulence of different *Phytophthora* root rot pathotypes to soybean (*Glycine max*) plants from parents and the F1 population[(1)].

Pathotype	FP (*Rps* gene)	MP (*Rps* gene)	F1 population				%DB			Resistance[(2)]
			NIB	NDB	NHB	FNB	FP	MP	F1	
PS2.4	'Harlon' (*Rps1a*)	L82-1449 (*Rps2*)	-	-	7	7	0	89	0	ADR
PS34.1	'Harlon' (*Rps1a*)	L82-1449 (*Rps2*)	-	4	-	4	80	94	100	NAR
PS36.1	'Harlon' (*Rps1a*)	L85-2352 (*Rps4*)	-	-	8	8	9	100	0	ADR
PS1608	'Harlon' (*Rps1a*)	L85-2352 (*Rps4*)	-	-	4	4	0	12	0	ADR
PS34.1	'Harlon' (*Rps1a*)	L85-3059 (*Rps5*)	2	2	4	8	80	100	50	ADR
PS36.1	'Harlon' (*Rps1a*)	L85-3059 (*Rps5*)	-	1	7	8	9	89	13	ADR
PS34.1	'Harlon' (*Rps1a*)	L89-1581 (*Rps6*)	2	-	6	8	80	0	25	ADR
PS36.1	'Harlon' (*Rps1a*)	L89-1581 (*Rps6*)	1	1	6	8	9	100	25	ADR
PS2.4	L77-1863 (*Rps1b*)	L82-1449 (*Rps2*)	-	-	9	9	0	89	0	ADR
PS1608	L77-1863 (*Rps1b*)	L82-1449 (*Rps2*)	-	-	8	8	81	17	0	ADR
PS14.4	L77-1863 (*Rps1b*)	L92-7857 (*Rps3c*)	-	-	8	8	0	87	0	ADR
PS1608	L77-1863 (*Rps1b*)	L92-7857 (*Rps3c*)	2	1	5	8	81	94	38	ADR
PS1608	L77-1863 (*Rps1b*)	L85-2352 (*Rps4*)	1	1	6	8	81	12	25	ADR
PS2.4	L77-1863 (*Rps1b*)	L85-2352 (*Rps4*)	-	-	8	8	0	29	0	ADR
PS2.4	L77-1863 (*Rps1b*)	L85-3059 (*Rps5*)	-	-	8	8	0	100	0	ADR
PS14.4	L77-1863 (*Rps1b*)	L85-3059 (*Rps5*)	-	-	7	7	0	87	0	ADR
PS2.4	L77-1863 (*Rps1b*)	L89-1581 (*Rps6*)	-	-	8	8	0	100	0	ADR
PS1608	L77-1863 (*Rps1b*)	L89-1581 (*Rps6*)	1	-	7	8	81	25	13	ADR
PS36.1	L77-1863 (*Rps1b*)	PI399073 (*Rps8*)	-	-	8	8	94	24	0	ADR
PS14.4	L77-1863 (*Rps1b*)	PI399073 (*Rps8*)	-	-	8	8	0	100	0	ADR
PS34.1	'Beeson 80' (*Rps1c*)	L82-1449 (*Rps2*)	4	-	4	8	100	94	50	ADR
PS36.1	'Beeson 80' (*Rps1c*)	L82-1449 (*Rps2*)	-	-	7	7	0	94	0	ADR
PS14.4	'Beeson 80' (*Rps1c*)	'Chapman' (*Rps3a*)	-	-	6	6	0	83	0	ADR
PS1608	'Beeson 80' (*Rps1c*)	'Chapman' (*Rps3a*)	3	5	-	8	43	88	100	NAR
PS36.1	'Beeson 80' (*Rps1c*)	L92-7857 (*Rps3c*)	-	-	9	9	0	89	0	ADR
PS14.4	'Beeson 80' (*Rps1c*)	L92-7857 (*Rps3c*)	-	-	8	8	0	87	0	ADR
PS34.1	'Beeson 80' (*Rps1c*)	L89-1581 (Rps6)	-	-	7	7	100	0	0	ADR
PS36.1	'Beeson 80' (*Rps1c*)	L89-1581 (Rps6)	-	-	8	8	0	100	0	ADR
PS14.4	'Beeson 80' (*Rps1c*)	PI399073 (Rps8)	-	-	8	8	0	100	0	ADR
PS34.1	'Beeson 80' (*Rps1c*)	PI399073 (Rps8)	-	-	7	7	100	6	0	ADR
PS1608	'Williams 82' (*Rps1k*)	L92-7857 (*Rps3c*)	2	-	6	8	83	94	25	ADR
PS34.1	'Williams 82' (*Rps1k*)	L92-7857 (*Rps3c*)	-	-	8	8	0	100	0	ADR
PS1608	'Williams 82' (*Rps1k*)	L85-2352 (*Rps4*)	1	1	5	7	83	12	29	ADR
PS36.1	'Williams 82' (*Rps1k*)	L85-2352 (*Rps4*)	-	-	8	8	0	100	0	ADR
PS1608	'Williams 82' (*Rps1k*)	L85-3059 (*Rps5*)	2	5	-	8	83	86	88	NAR
PS36.1	'Williams 82' (*Rps1k*)	L85-3059 (*Rps5*)	-	-	8	8	0	89	0	ADR
PS1608	'Williams 82' (*Rps1k*)	L89-1581 (*Rps6*)	1	-	6	7	83	25	14	ADR
PS36.1	'Williams 82' (*Rps1k*)	L89-1581 (*Rps6*)	-	-	9	9	0	100	0	ADR
PS14.4	'Williams 82' (*Rps1k*)	PI399073 (*Rps8*)	-	-	8	8	0	100	0	ADR
PS36.1	'Williams 82' (*Rps1k*)	PI399073 (*Rps8*)	-	-	8	8	0	24	0	ADR
PS2.4	L82-1449 (*Rps2*)	'Chapman' (*Rps3a*)	-	-	8	8	89	0	0	ADR
PS1608	L82-1449 (*Rps2*)	'Chapman' (*Rps3a*)	1	-	7	8	17	88	13	ADR
PS14.4	L82-1449 (*Rps2*)	L85-3059 (*Rps5*)	1	-	4	5	0	87	20	ADR
PS1608	L82-1449 (*Rps2*)	L85-3059 (*Rps5*)	1	-	5	6	17	86	17	ADR
PS2.4	L82-1449 (*Rps2*)	PI399073 (*Rps8*)	-	-	8	8	89	6	0	ADR
PS1608	L82-1449 (*Rps2*)	PI399073 (*Rps8*)	2	-	7	9	17	100	22	ADR

Continue...

Table 1. Continuation.

Pathotype	FP (*Rps* gene)	MP (*Rps* gene)	F1 population				%DB			Resistance[2]
			NIB	NDB	NHB	FNB	FP	MP	F1	
PS14.4	'Chapman' (*Rps3a*)	L85-2352 (*Rps4*)	-	-	7	7	83	13	0	ADR
PS1608	'Chapman' (*Rps3a*)	L85-2352 (*Rps4*)	2	-	6	8	88	12	25	ADR
PS14.4	'Chapman' (*Rps3a*)	L89-1581 (*Rps6*)	-	-	6	6	83	18	0	ADR
PS1608	'Chapman' (*Rps3a*)	L89-1581 (*Rps6*)	-	-	7	7	88	25	0	ADR
PS14.4	L85-2352 (*Rps4*)	L85-3059 (*Rps5*)	-	1	7	8	13	87	13	ADR
PS1608	L85-2352 (*Rps4*)	L85-3059 (*Rps5*)	1	1	6	8	12	86	25	ADR
PS14.4	L85-2352 (*Rps4*)	PI399073 (*Rps8*)	-	-	8	8	13	100	0	ADR
PS1608	L85-2352 (*Rps4*)	PI399073 (*Rps8*)	-	-	3	3	12	100	0	ADR
PS14.4	L85-3059 (*Rps5*)	L89-1581 (*Rps6*)	1	-	6	7	87	18	14	ADR
PS1608	L85-3059 (*Rps5*)	L89-1581 (*Rps6*)	1	-	4	5	86	25	20	ADR
PS14.4	L89-1581 (*Rps6*)	PI399073 (*Rps8*)	-	-	7	7	18	100	0	ADR
PS1608	L89-1581 (*Rps6*)	PI399073 (*Rps8*)	-	-	8	8	25	100	0	ADR

[1]FP, female parent; MP, male parent; NIB, number of infected lateral branches of adult plants; NDB, number of dead lateral branches of adult plants; NHB, number of healthy lateral branches of adult plants; FNB, final number of lateral branches of adult plants; and %DB, percentage of dead lateral branches of adult plants. [2]ADR, addition of resistance; and NAR, no addition of resistance.

(*Rps2*) and 'Chapman' (*Rps3a*), and L82-1449 (*Rps2*) and PI399073 (*Rps8*) may be recommended for introgression into commercial cultivars, as they showed important reductions in plant death compared with the individual performance of their parents when inoculated with different *P. sojae* pathotypes.

The main control of *P. sojae* has been based on the individual use of a few *Rps* genes in commercial cultivars. Despite the knowledge of the effects of reducing the infection rate of minor genes (Walker & Schmitthenner, 1984), the process of identification and selection at scale in soybean genetic improvement programs is still not applicable. However, genotype combinations generated the addition of resistance in the present study.

When inoculated individually with the CMES1608 pathotype, genotype L77-1863 (*Rps1b*) showed 94% dead lateral branches, which decreased to 0, 25, and 13% when combined with genotypes L82-1449 (*Rps2*), L85-2352 (*Rps4*), and L89-1581 (*Rps6*), respectively. When inoculated with the PS36.1 pathotype, the parental genotype L77-1863 (*Rps1b*) showed 81% dead lateral branches, which reduced to 0% when combined with PI399073 (*Rps8*).

Currently, in Brazil, commercial cultivars with resistance to *P. sojae* have *Rps* genes from allelic series 1, such as *Rps1a*, *Rps1c*, and *Rps1k* (Costamilan et al., 2021). Aiming at the rotation of *Rps* genes, together with the gene pyramiding strategy, it is important to perform the introgression of genes from genotypes L82-1449 (*Rps2*), L85-2352 (*Rps4*), L89-1581 (*Rps6*), and PI399073 (*Rps8*), which are not yet used commercially. In the present work, these combinations resulted in important reductions in the final number of dead lateral branches of the F1 progenies when compared with their parents. Costamilan et al. (2013) found that genes *Rps1a*, *Rps1b*, *Rps1c*, *Rps1k*, *Rps3a*, and *Rps8* conferred resistance to most *P. sojae* pathotypes, whereas Batista et al. (2022) concluded that *Rps1a* and *Rps1c* caused changes in virulence and *Rps3b* was effective.

The use of a single resistance gene is usually an efficient strategy for a complete or partial control of some pathotypes when inserted into susceptible lines of a given species. The specificity and durability of this resistance can vary due to several factors, such as the recombination plasticity of pathogens, the non-recognition of all strains by a given gene, and coevolution (Pink, 2002). Schmitthenner et al. (1994) highlighted that the effectiveness of resistance genes to *P. sojae* can vary according to the use intensity of the cultivar carrying the gene. According to the authors, in the United States, durability ranged from 8 to 20 years for the *Rps1a* and *Rps1k* genes, respectively.

By analyzing the percentage of plant death in the parents and their progenies (Figure 2), it is possible to observe the virulence of the tested pathotypes in a general context. The PS2.4, PS14.4, PS34.1, PS36.1,

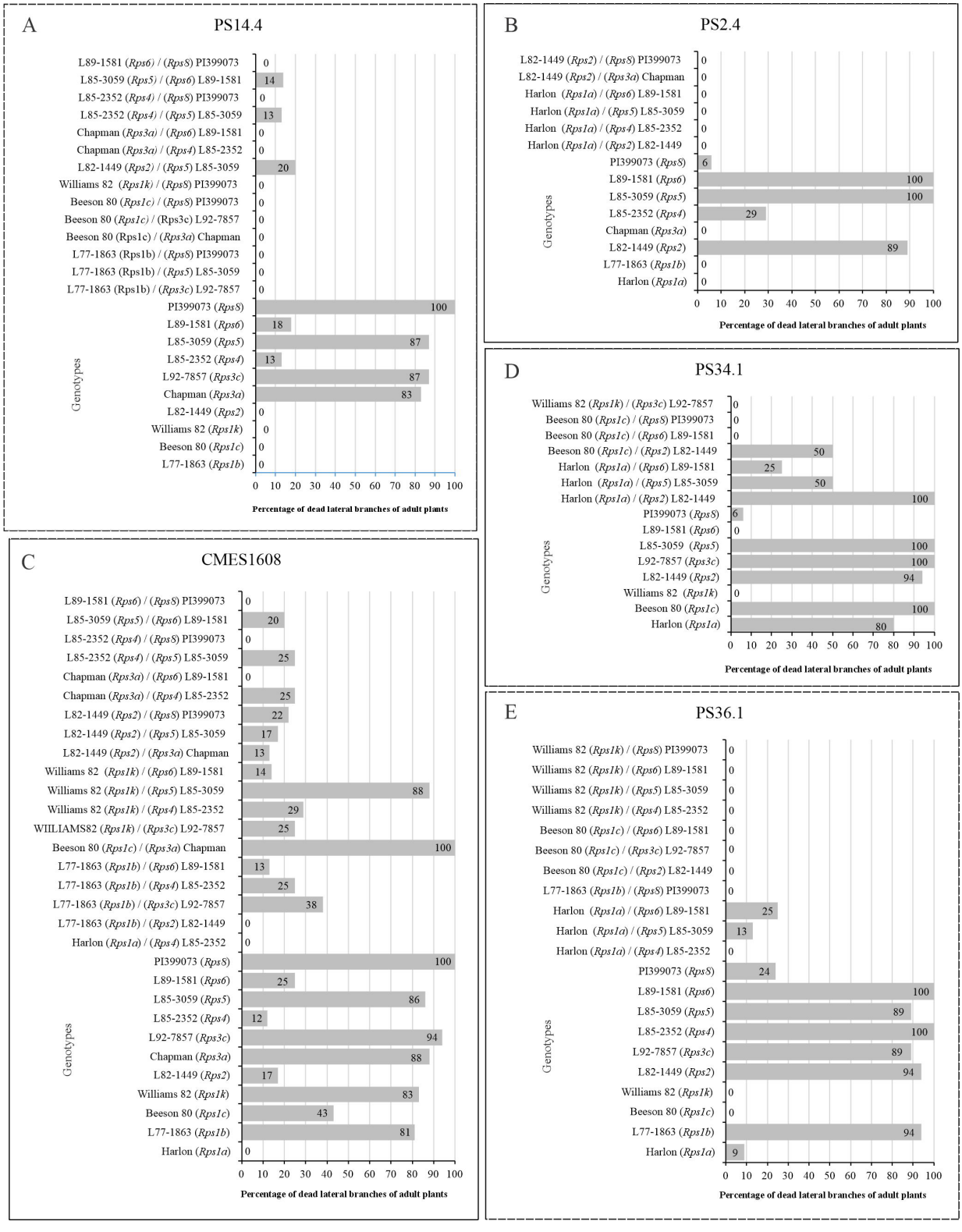

Figure 2. Percentage of dead lateral branches of adult soybean (*Glycine max*) plants from parents and F1 populations inoculated with pathotypes PS14.4 (A), PS2.4 (B), CMES1608 (C), PS34.1 (D), and PS36.1 (E).

and CMES1608 tested pathotypes showed different virulence in the evaluated genotypes, whose responses varied due to the different carried *Rps* genes.

The PS2.4 pathotype showed a high virulence in genotypes L85-3059 (*Rps5*), L89-1581 (*Rps6*), and L82-1449 (*Rps2*), but did not cause plant death among their descendants (Figure 2B), indicating that the combined use of *Rps* genes was effective in controlling the pathotype that occurs most frequently in soils in the Southern region of Brazil (Costamilan et al., 2013). Plants inoculated with pathotypes PS14.4 (Figure 2 A) and PS36.1 (Figure 2 E) presented a similar behavior, with the the number of dead lateral branches being drastically reduced in the inoculated progenies when compared with their parents. PS36.1 stood out since it caused more than 89% plant death among 6 of 10 tested parents, a value that was reduced to 25 and 13% in the progenies from the cross between 'Harlon' (*Rps1a*) and L89-1581 (*Rps6*) and between 'Harlon' (*Rps1a*) and L85-3059 (*Rps5*), respectively, reaching 0% in the other progenies.

The results showed a high virulence of the PS34.1 pathotype (Figure 2 D), presenting 80% plant death for 5 of 8 tested parents, specifically for L85-3059 (*Rps5*), L92-7857 (*Rps3c*), L82-1449 (*Rps2*), 'Beeson 80', (*Rps1c*), and 'Harlon' (*Rps1a*). The pathotype caused more than 50% dead lateral branhces even in progenies with combined genes, such as those from 'Beeson 80' (*Rps1c*) and L82-1449 (*Rps2*), 'Harlon' (*Rps1a*) and L85-3059 (*Rps5*), and 'Harlon' (*Rps1a*) and L82-1449 (*Rps2*). The combinations with the greatest reduction in the percentage of dead lateral branches were those between 'Williams 82' (*Rps1k*) and L92-7857 (*Rps3c*), 'Beeson 80' (*Rps1c*) and PI399073 (*Rps8*), and 'Beeson 80' (*Rps1c*) and L89-1581 (*Rps6*), which did not result in any dead plants, showing a great joint effect to control that pathotype.

Observing the virulence effect of the CMES1608 pathotype (Figure 2 C), it is possible to identify a more precise distribution in plant deaths for both parents and progenies with pyramided genes. Of 11 tested parents, only the following 4 showed plant death values below 25%: L89-1581 (*Rps6*), L85-2352 (*Rps4*), L82-1449 (*Rps2*), and 'Harlon' (*Rps1a*), meaning they could be classified as resistant according to Slaminko et al. (2010). Among the 19 progenies tested, the 5 that had no plant deaths were obtained from the crosses between: L89-1581 (*Rps6*) and PI399073 (*Rps8*), L85-2352 (*Rps4*) and PI399073 (*Rps8*), 'Chapman' (*Rps3a*) and L89-1581 (*Rps6*), L77-1863 (*Rps1b*) and L82-1449 (*Rps2*), and 'Harlon' (*Rps1a*) and L85-2352 (*Rps4*), proving to be good options for controlling that pathotype.

Conclusions

1. Soybean (*Glycine max*) F1 populations with pyramided *Rps* genes are an efficient genetic tool to help control *Phytophthora sojae*.
2. Progenies from virulent combinations of the same pathotype do not generate new resistance.
3. Resistance is added by complementarity in several F1 progenies obtained from genotypes with different *Rps* genes.

References

BATISTA, I.C.A.; SILVA, M.P.C.; SILVA JUNIOR, A.L.; ARRIGADA, M.P.G.; CAMARGO, M.P. de; FIGUEIREDO, A.; JUNIOR, B.T.H.; MIZUBUTI, E.S.G. A shift in pathotype diversity and complexity of *Phytophthora sojae* in Brazil. **Plant Disease**, v.107, p.1968-1972, 2023. DOI: https://doi.org/10.1094/PDIS-11-22-2558-SC.

CARLBORG, O.; HALEY, C.S. Epistasis: too often neglected in complex trait studies? **Nature Reviews Genetics**, v.5, p.618-625, 2004. DOI: https://doi.org/10.1038/nrg1407.

COSTAMILAN, L.M.; BERTAGNOLLI, P.F.; CARRÃO-PANIZZI, M.C.; STRIEDER, M.L. (Org.). **Soja**: resultados de pesquisa 2010/2011. Passo Fundo: Embrapa Trigo, 2011. 105p. (Embrapa Trigo. Documentos, 106). Available at: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1019642>. Accessed on: June 10 2022.

COSTAMILAN, L.M.; BERTAGNOLLI, P.F.; OLIVEIRA, A.C.B. de; MELO, C.L.P. de; SOARES, R.M.; CLEBSCH, C.C. **Resistência a *Phytophthora sojae* em linhagens de soja da Embrapa, em 2020**. Passo Fundo: Embrapa Trigo, 2021. 11p. (Embrapa Trigo. Circular técnica, 63). Available at: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1132543>. Accessed on: June 10 2022.

COSTAMILAN, L.M.; CLEBSCH, C.C.; SOARES, R.M.; SEIXAS, C.D.S.; GODOY, C.V.; DORRANCE, A.E. Pathogenic diversity of *Phytophthora Sojae* pathotypes from Brazil. **European Journal of Plant Pathology**, v.135, p.845-853, 2013. DOI: https://doi.org/10.1007/s10658-012-0128-9.

FLOR, H.H. Current status of the gene-for-gene concept. **Annual Review of Phytopathology**, v.9, p.275-296, 1971. DOI: https://doi.org/10.1146/annurev.py.09.090171.001423.

GIACHERO, M.L.; DECLERCK, S.; MARQUEZ, N. *Phytophthora* root rot: importance of the disease, current and novel methods of control. **Agronomy**, v.12, art.610, 2022. DOI: https://doi.org/10.3390/agronomy12030610.

JIANG, B.; CHENG, Y.; CAI, Z.; LI, M.; JIANG, Z.; MA, R.; YUAN, Y.; XIA, Q.; NIAN, H. Fine mapping of a *Phytophthora*-resistance locus *RpsGZ* in soybean using genotyping-by-sequencing. **BMC Genomics**, v.21, art.280, 2020. DOI: https://doi.org/10.1186/s12864-020-6668-z.

LEBRETON, A.; LABBÉ, C.; DE RONNE, M.; XUE, A.G.; MARCHAND, G.; BÉLANGER, R.R. Development of a simple hydroponic assay to study vertical and horizontal resistance of soybean and pathotypes of *Phytophthora sojae*. **Plant Disease**, v.102, p.114-123, 2018. DOI: https://doi.org/10.1094/PDIS-04-17-0586-RE.

LYNCH, M.; WALSH, B. **Genetics and analysis of quantitative traits**. Sunderland: Sinauer Associates, 1998. 980p.

PENG, P.; JIANG, H.; LUO, L.; YE, C.; XIAO, Y. Pyramiding of multiple genes to improve rice blast resistance of photo-thermo sensitive male sterile line, without yield penalty in hybrid rice production. **Plants**, v.12, art.1389, 2023. DOI: https://doi.org/10.3390/plants12061389.

PHILLIPS, P.C. Epistasis - the essential role of gene interactions in the structure and evolution of genetic systems. **Nature Reviews Genetics**, v.9, p.855-867, 2008. DOI: https://doi.org/10.1038/nrg2452.

PINK, D.A.C. Strategies using genes for non-durable resistance. **Euphytica**, v.124, p.227-236, 2002. DOI: https://doi.org/10.1023/A:1015638718242.

SANTOS, G.; MARCHIORO, V.S.; MEIRA, D.; TOEBE, M.; BENIN, G. Characterization of differentiating lines of phytophthora in soybean. **Crop Science**, v.65, e21451, 2025. DOI: https://doi.org/10.1002/csc2.21451.

SANTOS, G.; MARCHIORO, V.S.; MEIRA, D.; TOEBE, M.; BENIN, G.; KLEIN, L.A. Phytophthora root characterization in different phenological stages of soybean. **Physiological and Molecular Plant Pathology**, v.126, art.102039, 2023. DOI: https://doi.org/10.1016/j.pmpp.2023.102039.

SCHMITTHENNER, A.F.; DORRANCE, A.E. Phytophthora root and stem rot. In: Hartman, G.L. **Compendium of soybean diseases and pests**. Saint Paul: APS Press, p.73-76, 2015. Available at: <https://dr.lib.iastate.edu/server/api/core/bitstreams/f696b4c9-fad5-4b47-9a69-afd12855f4ea/content>. Accessed: Jun. 25 2022.

SCHMITTHENNER, A.F.; HOBE, M.; BHAT, R.G. *Phytophthora sojae* races in Ohio over a 10-year interval. **Plant Disease**, v.78, p.269-276, 1994. DOI: https://doi.org/10.1094/PD-78-0269.

SLAMINKO, T.L.; BOWEN, C.R.; HARTMAN G.L. Multi-year evaluation of commercial soybean cultivars for resistance to *Phytophthora sojae*. **Plant Disease**, v.94, p.368-371, 2010. DOI: https://doi.org/10.1094/PDIS-94-3-0368.

WALKER, A.K.; SCHMITTHENNER, A.F. Heritability of tolerance to Phytophthora rot in soybean. **Crop Science**, v.24, p.490-491, 1984. DOI: https://doi.org/10.2135/cropsci1984.0011183X002400030014x.

YORINORI, J.T. **Cancro da haste da soja**: epidemiologia e controle. Londrina: Embrapa Soja, 1996. 75p. (Embrapa-Soja. Circular técnica, 14). Available at: <http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/460380>. Accessed on: June 10 2022.

ZHAO, G.; LIU, Y.; LI, L.; CHE, R.; DOUGLASS, M.; BENZA, K.; ANGOVE, M.; LUO, K.; HU, Q.; CHEN, X.; HENRY, C.; LI, Z.; NING, G.; LUO, H. Gene pyramiding for boosted plant growth and broad abiotic stress tolerance. **Plant Biotechnology Journal**, v.22, p.678-697, 2024. DOI: https://doi.org/10.1111/pbi.14216.

Author contributions

Guilherme dos Santos: conceptualization, investigation, methodology, writing – original draft; **Volmir Sergio Marchioro**: curation, investigation, methodology, validation, writing – review & editing; **Daniela Meira**: conceptualization, data curation, investigation, methodology, validation, writing – original draft, writing – review & editing; **Marcos Toebe**: conceptualization, data curation, methodology, validation, writing – review & editing; **Giovani Benin**: conceptualization, methodology, validation, writing – review & editing.

Chief editor: Edemar Corazza
Edited by: Célia Tremacoldi

Data availability statement

Data in article: research data are available in the published article.

Declaration of use of AI technologies

No generative artificial intelligence (AI) was used in this study.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

To Tropical Melhoramento e Genética (TMG), for the location to carry out the study; and to Empresa Brasileira de Pesquisa Agropecuária (Embrapa), for support.

Disclaimer/Publisher's note: