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REGULAR ARTICLE

Genetic resistance and silicon in the control of stem rot in *Capsicum spp*.

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JEABJ: Conceptualization and design, Data custody and Data analysis, Writing the manuscript, Manuscript Review, Supervision, Responsible for funding; BAS: Experimental data collection, Data custody and Data analysis, Literature review, Writing the manuscript; LLP: Experimental data collection.

Abstract

Pepper stem rot is a disease caused by *Sclerotium delphinii*, a necrotrophic pathogen and a natural soil inhabitant. Identifying genotypes of *Capsicum* resistant to the pathogen and applying silicon (Si) can be effective management measures. The objective of the study was to identify sources of resistance in 24 accessions of *Capsicum* spp. against *S. delphinii*, and to evaluate the potential of sodium silicate (Si) to induce resistance. Two experiments were conducted: In Experiment I, the resistance reaction of *Capsicum* in a greenhouse was evaluated. The experiment was conducted in two periods of the year (July and November 2019). In Experiment II, the effect of Si on *Capsicum* resistance was evaluated. The experimental design used in Experiment I employed randomized blocks in a factorial design of 2 (isolates) x 24 (accessions), with five replications. For Experiment II, six accessions were selected with contrasting resistance responses observed in Experiment I, in a factorial design of 1 (isolate) x 6 (accessions) x 4 (doses: 0.0, 0.025, 0.05, and 0.1 mL per vase). Accessions BGH 71 and BAGC 134 showed greater resistance to the pathogen. Accession BAGC 134 demonstrated high resistance stability in both periods and against the two isolates tested. Si doses had no significant effect on the resistance reaction. Therefore, the genotypes BGH 71 and BAGC 134 have the potential to be used in breeding programs for *Capsicum* for resistance to *S. delphinii* for control of stem rot.

Keywords

Capsicum; Genetic Control; *Sclerotium delphinii*; Sodium Silicate

Introduction

Peppers include all species and varieties of the genus *Capsicum* that typically bear fruits smaller than bell peppers, with varying shapes and often pungent taste (Carvalho et al., 2003). *Capsicum* cultivation takes place in all regions of Brazil, with the states of Pará and Espírito Santo being the largest producers (IBGE, 2020). In this culture, various fungal diseases cause a reduction in production. Among these diseases, collar rot has been occurring frequently during periods of high rainfall in the western region of the State of Pará (personal communication).

Plants affected by collar rot may also show leaves with symptoms of necrotic spots in concentric patterns. Both diseases were traditionally associated with *Sclerotium rolfsii* Sacc (Tremacoldi, 2010). However, through phylogenetic analysis of concatenated genes, the causal agent of collar rot and leaf spot in black pepper was identified as *Sclerotium delphini* (syn: *Sclerotium rolfsii* var. *delphini*) (Severo et al., 2021). *S. delphini* is an important pathogen reported in *Ajuga* sp., peanut, *Hosta* sp., *Iris* sp., and apple (Xu et al., 2010). Although it has a limited number of hosts compared to *S. rolfsii*, *S. delphinii*, as *S. rolfsii*, does not produce spores and survives in the soil through the formation of sclerotia. *S. delphinii* differs from *S. rolfsii* in the morphology of its sclerotia, as *S. delphinii* has larger sclerotia with reddish-brown coloration, primarily formed towards the periphery of the colony (Stevens, 1931).

Collar rot can cause significant production losses in various crops (Bedendo, 2018). It is a challenging disease to control because the pathogens have a high capacity for saprophytic competition and produce resistant structures (sclerotia) that can survive for many years under conditions of high humidity and temperature (Duarte et al., 2006; Bergamin Filho; Amorim, 2018). The fungus can spread through the transportation of contaminated materials by humans, agricultural implements, wind, water, and animals, with disease development favored during the rainy season, where the presence of patches in infected fields is noticed (Padua et al., 2007; Wang et al., 2017).

Collar rot is a problem for pepper production in Brazil, as there are no products registered with the Ministry of Agriculture, Livestock and Supply (in portuguese Ministério da Agricultura, Pecuária e Abastecimento - MAPA) capable of controlling the pathogen. The use of resistant varieties and the application of silicate fertilizers emerge as options in the integrated management of the disease, as they can reduce

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production costs and environmental impacts. The supply of silicon, whether through soil, foliar, or nutrient solution, contributes to reducing the intensity of economically important diseases in *Capsicum*, grasses, beans, and wheat (Jayawardana et al., 2014; Datnoff et al., 2007; Moraes et al., 2006; Bélanger et al., 2003). *Capsicum* genotypes with moderate resistance to *S. rolfsii* have already been identified (Soares et al., 2017; Sahana et al., 2020), although genetic resistances against *S. delphinii* are not yet known.

Given the above, the objectives of this study were: (i) to identify sources of resistance in *Capsicum* spp. accessions against *S. delphinii*; (ii) to evaluate the effect of sodium silicate on the control of *S. delphinii* in *Capsicum* spp.

Materials and methods

Plant material and pathogen isolates

The study was conducted in the Phytopathology Laboratory of the Department of Plant Science at the Federal University of Piauí, Teresina, PI. The isolates COUFPI 206 and COUFPI 249 of *S. delphinii* were used, both previously identified by concatenated phylogeny of ITS regions and the large subunit of rDNA (LSU) (Severo et al., 2021) and deposited in the phytopathogenic fungi collection of the UFPI Phytopathology Laboratory (COUFPI). The isolates were obtained from black pepper (*Piper nigrum* L.) plants with leaf rot in the city of Santarém, Pará. Twenty-four accessions of peppers (19 accessions) and bell peppers (5 accessions) from the genus *Capsicum* were used, selected according to seed availability and germination percentage, of which 10 accessions belong to the Active Germplasm Bank of *Capsicum* (BAGC) of the Seed and Genetic Resources Laboratory of the Department of Plant Science, UFPI, and 14 accessions belong to the Vegetable Germplasm Bank (BGH) of the Federal University of Viçosa, Minas Gerais.

Experiment I: Evaluation of Capsicum accession resistance against Sclerotium delphinii

The isolates were cultured on potato dextrose agar (PDA) culture medium, incubated at 26 ± 2 °C in an incubator with a 12-hour photoperiod for 10 days. 250 mL Erlenmeyer flasks containing 100 g of sterilized and autoclaved rice at 121 °C for 20 minutes were inoculated with three 5 mm diameter culture disks containing fungal mycelium from the isolates and incubated at 26 ± 2 °C until complete substrate colonization. The flasks were shaken daily.

Capsicum accessions were sown in 288-cell polystyrene trays containing sterilized substrate (sand + soil + organic compost, 1:1:1). Fifteen days after emergence (DAE), seedlings were transplanted into black plastic bags for seedlings (1.5 L) containing the substrate mentioned above, autoclaved at 121 °C for one hour, twice on consecutive days. One plant was transplanted per bag. The experiment was in a randomized complete block design (RCBD) with a factorial arrangement (2 isolates x 24 accessions), with five replications, and was conducted in two periods (July and November 2019).

Fungal inoculation was performed at 30 DAE by depositing 5 g of inoculum at the plant collar, near the stem-soil interface. The plants were kept in a humid chamber for 48 hours (Soares et al., 2017; Mahadevakumar et al., 2018). Control plants consisted of one plant from each accession inoculated with non-colonized rice with the fungus. Evaluations were conducted daily until all plants died, using a descriptive scale of Schoonhoven and Pastor-Corrales (1987) ranging from 1 to 9, where: 1- Plant without visible symptoms; 3- Plant with 10% of wilted and chlorotic leaves; 5- Plant with 25% of wilted and chlorotic leaves; 7- Plant with 50% of wilted and chlorotic leaves; 9- Dead plant or severely infected with 100% of the foliage wilted, chlorosis, necrosis, and/or premature defoliation. Subsequently, the disease index (DI) of McKinney (1923) was calculated:

$$ID = (\sum [\![f*v]\!])/(n*x) *100 \quad (1)$$

Where: f = number of plants in each category; v = scale score; n = total number of plants; x = maximum infection degree.

After obtaining the DI data, resistance reaction classes were defined, where they were divided into Resistant (R) (DI = 0 – 33.33%), Tolerant (T) (DI = 34.44 – 66.67%), and Susceptible (S) (DI = 67.68 – 100%). The data obtained were subjected to analysis of variance, and the means were compared by the Scott-Knott test at a 5% probability level, using R software version 3.5.1. The Shapiro-Wilk test was applied to determine if there was normality of data regarding the comparison between study periods. Afterward, factorial analysis of each of the isolates separately was performed, and the means were compared by the Scott-Knott test at a 5% probability level.

Experiment II: Effect of sodium silicate on Capsicum accession resistance against Sclerotium delphinii

Accessions that showed contrasting resistance reactions in Experiment I were selected for this experiment. Only isolate COUFPI 249 was used in this experiment, as it was the most aggressive in Experiment I. The experimental design adopted was a RCBD in a factorial scheme (4 doses x 1 isolate x 6 accessions), with five replications. Plants received applications of sodium silicate (commercial product Armurox©: total silicon content 4.6%) at doses of 0.0; 0.025; 0.05, and 0.1 mL per pot, at 5, 10, 15, 20, and 25 DAE, in the form of a 25 mL solution in the substrate. Fungal inoculation was performed at 45 DAE, where 5 g of inoculum was deposited at the plant collar, near the stem-soil interface. Control plants were inoculated with the pathogen and were not treated with the product. Plants were kept in a humid chamber for 48 hours after inoculation (Soares et al., 2017; Mahadevakumar et al., 2018). Evaluations were conducted daily for 15 days after inoculation (DAI) using the previously mentioned descriptive scale. The data obtained were subjected to analysis of variance and regression analysis when significant, evaluating trend lines and "R^2" using R software version 3.5.1.

Results and discussion

Experiment I: Evaluation of Capsicum accession resistance against Sclerotium delphinii

All accessions showed disease symptoms after inoculation with the fungi in both periods of the experiment, revealing the absence of immunity (Table 1). For statistical analysis, values observed at six DAI (20% of dead plants) were adopted because below this value, the results would be underestimated,

while above it, most plants would be dead or with a score of seven, i.e., the plant showing more than 50% wilt.

Considering the first period (July/2019), there was no statistically significant difference ($p < 0.05$) between fungal isolates (Table 1). Control plants showed no disease symptoms. Accessions BGH 71 and BGH 134 were those that expressed tolerance/resistance against both fungal isolates in both periods evaluated (Table 1), demonstrating high stability of resistance. Stability of resistance is an important characteristic when seeking to identify plant genotypes resistant to pathogens. Therefore, both accessions are interesting for future studies of inheritance of resistance within breeding programs. Other accessions (BAGC 197, BAGC 206, BGH 80, and BGH 143) showed tolerance against only isolate COUFPI 206. Furthermore, tolerance/resistance reactions were more pronounced in the experiment during the first period of the year (July/19) than in the second period (November/19), as there were 14 resistance reactions in July, compared to 29 in November (Figure 1). Together, these results reveal instability of resistance, whether against the pathogen isolate or due to environmental conditions.

The resistance reactions expressed were relatively variable for some accessions, where there was a change in category depending on the period, showing genotype x environment interaction present in this study, as in the cases of accessions BGH 84 and BGH 178, which expressed different resistance reactions depending on the period, for both isolates, being classified as susceptible (S) to both isolates in period 1 (July) and tolerant (T) to both isolates in period 2 (November) (Table 1).

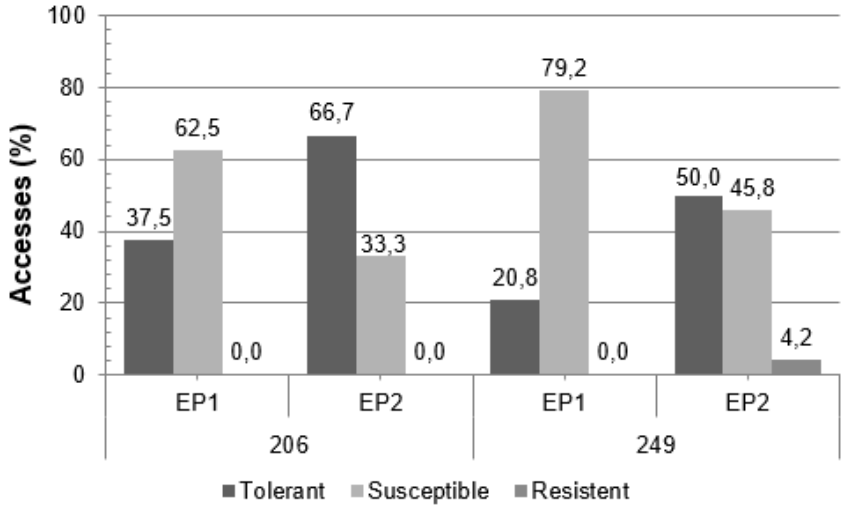

Figure 1. Classification of *Capsicum* spp. accessions into three levels of resistance (Resistant (R), Tolerant (T), and Susceptible (S)) to infection by two isolates (COUFPI 206 and COUFPI 249) of *Sclerotium delphinii*, in two periods (EP1 - July 2019 and EP2 - November 2019).

Table 1. Note, average severity (SEV), and resistance reaction of *Capsicum* spp. accessions in two evaluation periods (July and November/2019) after inoculation with *Sclerotium delphinii* (isolates COUFPI 206 and COUFPI 249).

Access	COUFPI 206						COUFPI 249					
	Grade		SEV (%)		Resistance Reaction		Grade		SEV (%)		Resistance Reaction	
	Jul	Nov	Jul	Nov	Jul	Nov	Jul	Nov	Jul	Nov	Jul	Nov
BAGC 221	$5,7^{bA}$	$7,6^{aA}$	64,4	77,7	T	S	$7,4^{aA}$	$4,0^{cB}$	82,2	37,7	S	T
BAGC 208	$6,2^{bA}$	$4,9^{cA}$	68,8	46,6	S	T	$5,8^{bA}$	$3,8^{cB}$	64,4	42,2	T	T
BAGC 206	$5,0^{bA}$	$4,7^{cA}$	55,5	60,0	T	T	$6,2^{bA}$	$6,2^{bA}$	68,8	60,0	S	T
BAGC 205	$7,8^{aA}$	$6,6^{bA}$	82,2	73,3	S	S	$8,6^{aA}$	$7,8^{aA}$	95,5	86,6	S	S
BAGC 197	$5,8^{bA}$	$5,0^{cA}$	64,4	55,5	T	T	$7,4^{aA}$	$7,7^{aA}$	82,2	77,7	S	S
Laranja	$7,4^{aA}$	$5,7^{bA}$	82,2	55,5	S	T	$7,8^{aA}$	$6,6^{bA}$	86,6	73,3	S	S
Amarela B	$7,8^{aA}$	$5,4^{cB}$	86,6	60,0	S	T	$7,4^{aA}$	$6,2^{bA}$	82,2	68,8	S	S
Amarela G	$7,0^{aA}$	$6,2^{bA}$	77,7	68,8	S	S	$5,8^{bA}$	$7,4^{aA}$	64,4	82,2	T	S
Vermelha P	$5,0^{bA}$	$5,1^{cA}$	55,5	64,4	T	T	$7,8^{aA}$	$7,4^{aA}$	86,6	73,3	S	S
Vermelha G	$5,8^{bA}$	$5,8^{bA}$	64,4	64,4	T	T	$7,2^{aA}$	$5,4^{cB}$	73,3	60,0	S	T
BGH 1486	$7,8^{aA}$	$7,1^{aA}$	86,6	68,8	S	S	$7,8^{aA}$	$6,4^{bA}$	86,6	64,4	S	T
BGH 178	$7,8^{aA}$	$3,4^{cB}$	86,6	37,7	S	T	$8,2^{aA}$	$6,5^{bA}$	82,2	64,4	S	T
BGH 568	$7,4^{aA}$	$8,4^{aA}$	82,2	86,6	S	S	$8,2^{aA}$	$7,0^{bA}$	91,1	77,7	S	S
BGH 957	$7,0^{aA}$	$6,9^{bA}$	77,7	68,8	S	S	$7,0^{aA}$	$5,8^{bA}$	77,7	64,4	S	T
BGH 959	$8,2^{aA}$	$5,4^{cB}$	82,2	60,0	S	T	$8,2^{aA}$	$8,6^{aA}$	91,1	95,5	S	S
BGH 143	$5,8^{bA}$	$5,8^{bA}$	64,4	64,4	T	T	$8,2^{aA}$	$6,2^{bB}$	91,1	68,8	S	S
BGH 138	$7,8^{aA}$	$8,2^{aA}$	86,6	91,1	S	S	$8,6^{aA}$	$8,6^{aA}$	95,5	95,5	S	S
BGH 135	$6,8^{aA}$	$5,9^{bA}$	68,8	60,0	S	T	$7,8^{aA}$	$8,2^{aA}$	86,6	91,1	S	S
BGH 134	$3,5^{bA}$	$3,4^{cA}$	46,6	37,7	T	T	$4,6^{bA}$	$3,0^{cA}$	51,1	33,3	T	R
BGH 84	$7,0^{aA}$	$5,8^{bA}$	77,7	64,4	S	T	$7,3^{aA}$	$3,8^{cB}$	73,3	42,2	S	T
BGH 80	$5,4^{bA}$	$5,0^{cA}$	60,0	55,5	T	T	$6,2^{bA}$	$3,8^{cB}$	68,8	42,2	S	T
BGH 72	$9,0^{aA}$	$6,2^{bB}$	100,0	68,8	S	S	$9,0^{aA}$	$5,0^{cB}$	100,0	55,5	S	T
BGH 71	$5,8^{bA}$	$3,4^{cB}$	64,4	37,7	T	T	$5,1^{bA}$	$4,0^{cA}$	64,4	37,7	T	T
BGH 181	$6,6^{aA}$	$4,2^{cB}$	73,3	46,6	S	T	$4,2^{bA}$	$3,4^{cA}$	46,7	37,7	T	T
C.V. (%)	**26,11**		-	-	-	-	**21,54**		-	-	-	-

An important factor to consider is the optimal conditions provided for the development of the pathogen. Since it is a soil-inhabiting pathogen, the use of sterilized soil eliminated the selection pressure that naturally occurs, favoring infection. The inoculum used (colonized rice grains) on the surface near the stem allowed the pathogen to come into contact more quickly with the host, as the pathogen naturally survives in the soil in the form of resistant structures (sclerotia), i.e., the inoculum pressure is lower in the natural environment. Thus, even with these optimal development conditions provided, some accessions still showed resistance stability in both seasons and for the two isolates tested. Obtaining *Capsicum* genotypes resistant to *S. rolfsii* was also achieved through a similar inoculation and evaluation methodology, where 15 resistant accessions were observed (Soares et al., 2017). Variable levels of resistance of *Capsicum* spp. genotypes against *S. rolfsii* or *Sclerotinia sclerotiorum* were also observed in other studies (Yanar & Miller, 2003; Sahana et al., 2020), revealing that the existence of resistance genes, although partial, in commercial genotypes or non-commercial *Capsicum* accessions.

Experiment II: Effect of sodium silicate on Capsicum accession resistance against Sclerotium delphinii

In this study, six accessions were selected according to the reactions observed in experiment I, of which three were classified as susceptible (BAGC 205, Amarela G, and BGH 72) and three as tolerant (BAGC 208, BAGC 206, and BGH 134) (Table 1). Regression analysis was used to observe differences between the tested doses, with the model with the highest coefficient of determination (R^2) being used (Figure 2). There was a cubic regression adjustment for three susceptible accessions; for the others, the models were not significant, showing that the doses of sodium silicate did not influence the resistance response of the accessions, except for accession BGH 72 (doses 0.025; 0.05 and 0.1 mL per pot) (Table 2).

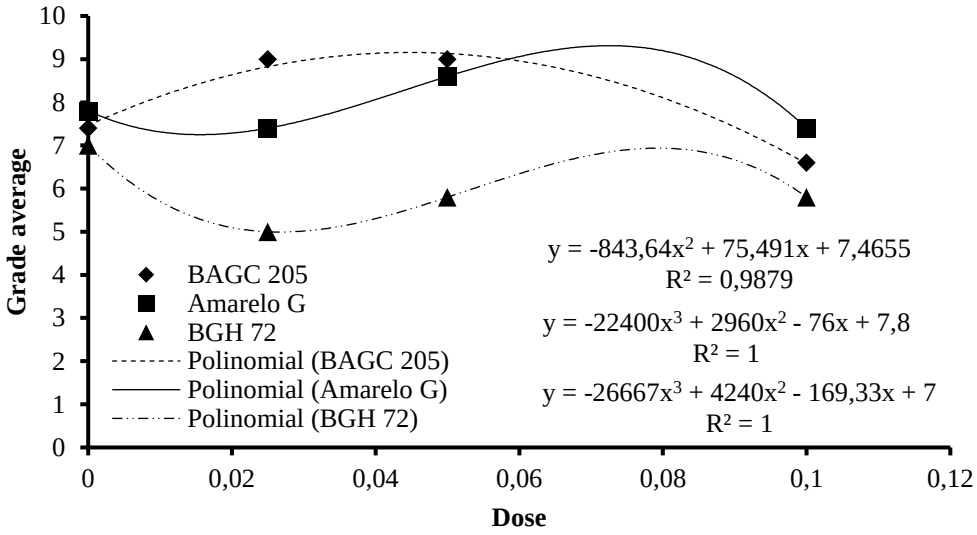

Figure 2. Regression for the infection level of isolate COUFPI 249 in accessions BAGC 205, Amarela G, and BGH 72, on the influence of applying different doses of sodium silicate.

In experiment I, accessions BAGC 208 and BGH 134 (second period) were classified as tolerant and resistant, respectively. Although there was no influence of Si application for these accessions, both showed stability regarding resistance classification according to experiment I. On the other hand, accession BGH 72, which was classified as susceptible in experiment I, remained susceptible at the 0.0 mL per pot dose, but for the other doses, it was classified as tolerant, showing that silicon application may have influenced the resistance response for this accession (Table 2). Silicon application in grasses has a more pronounced effect on resistance against pathogens (Wordell Filho et al., 2013). However, in dicotyledons, silicon supply is not always efficient, and this effect seems to be dependent on the botanical species/pathogen combination. For example, soil silicon supplementation was effective in *Capsicum annum* against the development of anthracnose caused by *Colletotrichum gloeosporioides*, with silicon acting on the induction of acquired systemic resistance, and consequent disease development delay (Jayawardana et al., 2014). Several studies in cucurbits show the efficiency of silicon application in controlling fungal and oomycete phytopathogens, such as *Oidium* sp. (Samuels et al., 1991), *Pythium aphanidermatum*, and *Fusarium solani* (El-Samman, 2000), downy mildew (*Pseudoperonospora cubensis*) (Yu et al., 2010), and *Fusarium oxysporum* f. sp. *cucumerinum* (Zhou et al., 2018). In *Capsicum* spp., the potential of silicon application to reduce symptoms of *Phytophthora capsici* wilt (French-Monar et al., 2010) and anthracnose (Jayawardana et al., 2015) has also been confirmed.

Table 2. Note, severity (%), and resistance reaction of six *Capsicum* spp. accessions after Si application and inoculation with *Sclerotium delphinii* (isolate COUFPI 249).

Dose	**Access / Grade**					
(mL vase^{-1})	**BAGC 208**	**BAGC 206**	**BAGC 205**	**Amarela G**	**BGH 134**	**BGH 72**
0	5,0^{B}	7,0^{A}	7,8^{A}	7,8^{A}	5,0^{B}	7,0^{A}
0,025	5,0^{C}	7,0^{B}	9,0^{A}	7,4^{B}	5,0^{C}	5,0^{C}
0,05	5,0^{C}	7,0^{B}	9,0^{A}	8,6^{A}	5,0^{C}	5,8^{BC}
0,1	5,0^{CD}	7,0^{AB}	6,6^{AB}	7,4^{A}	4,2^{D}	5,8^{BC}
CV (%)	**11,44**					
	Severity (%) / Resistance reaction					
0	55,56 T	77,78 S	82,22 S	86,67 S	55,56 T	77,78 S
0,025	55,56 T	77,78 S	100,00 S	82,22 S	55,56 T	55,56 T
0,05	55,56 T	77,78 S	100,00 S	95,56 S	55,56 T	64,44 T
0,1	55,56 T	77,78 S	73,33 S	82,22 S	46,67 T	64,44 T

Conclusions

Capsicum accessions did not express immunity against *S. delphinii* isolates.

Accessions BGH 71 and BGH 134 showed the best resistance response against both *S. delphinii* isolates in the two periods evaluated.

The application of sodium silicate had no significant effect on the resistance response of *Capsicum* accessions against *S. delphinii*.

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