

Evaluation of soybean mutant lines for resistance to stink bugs (*Hemiptera: Pentatomidae*)

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ABSTRACT

Soybean is one of the main oilseed crops in the world, and stink bugs complex can cause an important reduction in seed yield and quality. The primary control strategy against this pest consists in the use of insecticides according to the economic damage thresholds established for each species. Additionally, genetic resistance allows a more friendly agriculture, reducing the applications of insecticides, contamination, and production costs. To date, only a few genotypes with polygenic resistance are known, and they were introduced in soybean breeding programs. However, there are no records of the use of induced mutation techniques to obtain a new variability for resistance to stink bugs. The aim of this study was to evaluate mutant soybean lines for resistance to stink bug complex, generated by chemical mutation with ethyl methane sulfonate (EMS). The field experiments with six replications were conducted in 2020/21 and 2021/22 in Oro Verde, Argentina. Forty-one genotypes, including mutant lines, experimental lines and resistant and susceptible cultivars were evaluated. Variations among years, genotypes and their interaction were observed for the duration of the phenological cycle, height, lodging, 100 seed weight (100-wt), yield and percentage of damaged seeds. The mutant lines showed variability in the percentage of damaged seeds between 36.8–57.8%. The mutant line Mut12 showed the best performance with an average of 38.8% of seed damage, and was located among the six genotypes with less seed damage, including the wild-type line LAE9758803-B. We confirm that induced mutant lines could present variability for resistance to stink bugs that may complement the study and development of new sources of resistance. The mutant line Mut12 could be used as breeding material in different breeding programs, and future studies should be done to understand the type of resistance that it has.

Keywords: soybean, mutants, stink bugs.

RESUMEN

La soja es uno de los principales cultivos oleaginosos en el mundo. En la Argentina, el complejo de chinches fitófagas causa reducción del rendimiento y de la calidad de sus semillas. El control químico, basado en niveles de daño económico, es la principal estrategia de manejo. Sin embargo, un sistema productivo sustentable requiere un menor número de aplicaciones de insecticidas, lo que permite reducir costos y la consecuente contaminación. La resistencia genética constituye otra estrategia del Manejo Integrado de Plagas, menos explorada para este grupo insectil. Se conocen pocos genotipos con resistencia que han sido introducidos en programas de mejoramiento, pero no existe registro del uso de técnicas de mutación inducida para obtener nueva variabilidad de resistencia a chinches fitófagas. El objetivo de este trabajo fue evaluar líneas de soja mutantes generadas por mutación química con etilmetanosulfonato para determinar su resistencia al complejo de chinches fitófagas. Se realizaron experimentos de campo con seis repeticiones en los años 2020/21 y 2021/22 en Oro Verde, Argentina. Se evaluaron 41 genotipos, incluyendo líneas mutantes y testigos. Se observó variación entre años, genotipos y de interacción en la duración de ciclo, altura, vuelco, peso de 100 semillas, rendimiento y porcentaje de semilla dañada. Las líneas mutantes mostraron una variabilidad en

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el porcentaje de semilla dañada de entre 36,8 y 57,8%. Mut12 tuvo el mejor comportamiento con un promedio de 38,8% de daño en semilla, y se ubicó entre los seis genotipos con menor daño. Esta investigación confirma que las líneas mutantes inducidas presentaron variabilidad para el rasgo de resistencia a chinches y que podrían complementar el estudio y desarrollo de nuevas fuentes de resistencia. Mut12 podría usarse como material parental en diferentes programas de mejoramiento y se deberían realizar futuros estudios para comprender el tipo de resistencia que posee.

Palabras clave: soja, mutantes, chinches fitófagas.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr. (Fabales: Fabaceae)] is an annual plant widely cultivated in the world and native to Southeast Asia (Pathan and Sleper, 2008). In Argentina, it is one of the main crops, with a total planted area of 16.84 million hectares during the 2023/24 year. Additionally, it has an average yield of 3.1 t/ha and an estimated production of 50 million tons (BCR-GEA, 2024). The importance of this crop lies in the use of its grains for human and animal nutrition and for the development of biofuels and industry due to its high protein and oil content and nutritional quality (Espina *et al.*, 2018; Liu, 2004).

Among the pests that affect soybean crops, the phytophagous stink bug complex (Hemiptera: Pentatomidae) is particularly notable for damaging developing seeds, leading to yield losses and reduced grain quality. Stink bugs use their piercing-sucking mouthparts to inject digestive enzymes into the plant tissue, which dissolve cell walls and result in loss of cellular content. This damage can cause abortion or deformation of grains and pods (Depieri and Panizzi, 2011). Additionally, feeding sites are entry routes for different pathogenic microorganisms (Todd, 1981). In Argentina, the most frequent species of pentatomids that affect this crop are the southern green stink bug *Nezara viridula* L. and the redbanded stink bug *Piezodorus guildinii* Westwood. To a lesser extent, other species such as the brown-winged stink bug *Edessa mediotubunda* Fabricius 1794, and the green belly stink bug *Diceraeus furcatus* Fabricius 1794 also impact soybean crops (Saluso *et al.*, 2011).

The primary management strategy for phytophagous stink bugs involves field monitoring and the application of insecticides based on established economic damage thresholds (Gamundi and Sosa, 2008). Additionally, soybean crops exhibit genetic resistance, which serves as an essential tool to reduce insecticide use. Depending on the type of resistance, it can also minimize harm to beneficial insects such as pollinators and natural predators of pest species (Marquez *et al.*, 2018; Gatehouse *et al.*, 2011; Smith, 2005).

Painter (1951) proposed three main mechanisms of resistance: antibiosis, where the plant negatively affects the insect's biology by prolonging the immature stages, reducing fecundity or causing lethal effects; antixenosis (non-preference), where morphological or chemical traits in the plant deter pests from feeding or encourage them to select alternative hosts; and tolerance, which refers to the plant's ability to withstand or recover from insect damage by producing new shoots, leaves, or fruits.

Later, Stout (2013) introduced a dichotomous framework that categorized plant responses into resistance and tolerance. He defined resistance as "plant traits that reduce the extent of in-

jury done to a plant by an herbivore" and further divided it into constitutive resistance (expressed regardless of prior injury) and inducible resistance (activated or enhanced following injury). Resistance was also classified as direct (traits with immediate effects on herbivore behavior or biology) or indirect (traits that rely on natural enemies for their effect). Tolerance, on the other hand, was defined as "traits or physiological processes that lessen the impact of damage on yield loss."

For instance, the development of the IAC 100 cultivar in Brazil is notable, along with several lines that exhibit favorable traits against stink bug damage in Brazil (Lucini *et al.*, 2021; Rossetto *et al.*, 1995; 1986), the United States (McPherson *et al.*, 2007), and Argentina (Vicentin and Saluso, 2017; Vicentin and Saluso, 2011; Tomasini, Saluso, and Vicentin, 2012). However, incorporating resistance to stink bugs into breeding programs is challenging because it is a quantitative trait controlled by two or more genes with additive, dominant, and epistatic effects.

The study and incorporation of resistance have primarily relied on a few genotypes such as PI171451, PI227687, PI229358, and PI229321 as genetic donors for resistance (Bansal *et al.*, 2013).

On the other hand, recent advances in molecular approaches, such as association mapping (Ghione *et al.*, 2021; Sanches Martins, 2021; Chang and Hartman, 2017), enable significant progress in identifying candidate genes for use in the marker-assisted selection of genotypes resistant to various insects, including stink bugs. As a complement to known resistance genotypes, induced mutagenesis using physical or chemical mutagens can be employed to generate additional variability. Induced mutagenesis is a methodology widely applied across numerous crops that facilitates the creation and acceleration of new variability in traits of interest (Khan and Tyagi, 2013). This approach can enhance breeding programs by providing novel genetic resources for improving pest resistance.

Genome editing, commonly known as gene editing, is a new generation technology that includes systems like the clustered regularly interspaced short palindromic repeats (CRISPR-Cas9 system). Genome editing involves the induction of targeted mutagenesis, allowing the generation of crops and insects varieties, without adding foreign DNA to the genome. This enhances consumer-preferred commercial traits in crops and supports effective insect pest management (Komal *et al.*, 2023; Verma *et al.*, 2023).

Several traits have been improved in soybean using physical, chemical and gene editing mutagenesis. This includes yield, early maturity, protein and oil content in grain, amino acid and fatty acid content, resistance to viruses, diseases and nematodes, lodging resistance and improvements in plant structure.

Other enhancements include drought resistance, nodulation and biological nitrogen fixation, absence of lipoxigenases, reduction of allergens, low content of trypsin inhibitors in grain, tolerance to aluminum, sulfonylureas resistance, regulation of the pigmentation of leaves by altering the anthocyanin pathway (Komal *et al.*, 2023; Verma *et al.*, 2023; Ayan *et al.*, 2022; Khan and Tyagi, 2013).

To date, Cagliari *et al.* (2020) have studied CRISP/Cas targeted mutagenesis on three genes with resulting phenotypes observed in stink bugs *Euschistus heros* (F) (Hemiptera: Pentatomidae). However, there are no known studies of soybean mutant lines developed for stink bug resistance.

The aim of this work was to evaluate stink bug resistance among mutant lines derived from the soybean line LAE9758803-B.

MATERIALS AND METHODS

Forty-one soybean genotypes were evaluated, including 26 mutant lines and the wild-type line LAE9758803-B (A 5634 RG x FAINTA 760), known for its resistance to stink bugs (Jacobi, 2013). The mutant lines were developed by chemical mutation with ethyl methane sulfonate (EMS) in 2017 at the biotechnology laboratory of the experimental station Marcos Juárez (INTA) in Argentina. These mutants were advanced and increased individually at the experimental station of INTA in Paraná until the trials took place. The other genotypes tested included experimental lines with potential insect resistance: LAE1112474 (DM 6200 x Crocket), LAE1112371 (LAE9649001 x Crocket), LAE1112263 (LAE9649001 x Crocket), LAE10125243 (PI227687 x BRM9926600) and LAE10125244 (PI227687 x BRM9926600). Additionally, resistance genotypes such as IAC 100, BR79-15.149, Cerrito FAINTA (MC NAIR 800 X PI 227687), FAINTA 760 (CURTIS X PI 227687), BR82-12.462 were evaluated along with three susceptible genotypes: cultivars INTA Paraná 6200, Cultivar 1, and Cultivar 2.

The trials were conducted at the experimental station of INTA in Paraná (31°50'51"S, 60°32'16"W) during the years 2020/21 and 2021/22. The sowing dates were March 11, 2020 and November 19, 2021. A no-tillage system was used for both trials. Seed inoculation was performed in the rows at sowing time. In both years, wheat served as a previous cover crop, and adequate fertility conditions were maintained. The rainfall and temperature data were collected from the agrometeorological observatory, located approximately 600 meters away from each trial site.

To prevent defoliation by lepidopterans without affecting the development of phytophagous stink bug populations, applications of lufenuron (5%) were made as needed. Weed control was implemented throughout the growing seasons according to the species present using herbicides, following their specifications and manual removal.

The trials consisted of plots with four rows 6 meters long, spaced 0.52 meters apart. A randomized complete block design was used with six repetitions. In both years, the evaluation focused on the population of phytophagous stink bugs that developed naturally in the trials from the beginning of fruiting stage to physiological maturity of the crop. Only the species present during this period were recorded. The stink bug populations were monitored using the drop-cloth method (McPherson *et al.*, 2007) with three random samples taken each year at two stages: at the beginning of fruiting stage (R3) and at the beginning of seed fill stage (R5). This approach allowed us to determine whether economic damage thresholds had been reached but did not enable quantification of stink bug numbers or species distribution across each evaluated genotype.

The phenology was recorded weekly from one block using the Fehr and Caviness (1977) scale to determine the cycle duration of genotypes from emergence to harvest maturity. Additionally, plant height in centimeters and lodging percentage (visual scale from 0 to 100% of total plot area) were measured prior to harvest. The weight of 100 seeds (100-wt) was recorded in grams after harvest. From each plot, 5 meter lengths of the two central rows were harvested with an experimental plot combine Wintersteiger® Classic Plus to determine the yield in kilograms per hectare, adjusted for commercial humidity at 13.5% (SAG-PyA, 2008). The resistance to stink bugs was evaluated based on the percentage of damaged seeds. This assessment involved visually determining the proportion of damaged seeds in a 150 g sample of each repetition (modified from Mc Pherson *et al.*, 2007; Hoffmann-Campo *et al.*, 2005). The statistical program Infostat version 2020 (Di Rienzo *et al.*, 2020) was used to verify assumptions regarding normality of residuals and homoscedasticity of variances before statistical analyses. Significant treatment means were separated using the Scott and Knott test.

For yield and percentage of damaged seeds, an analysis of variance with an AMMI model was conducted to study potential interactions between sources of variation. Furthermore, damage data were analyzed by quartiles: the data of each year were classified according to quartile values as shown in table 1. Cases grouped by genotype were counted regardless of year and represented graphically through a bar chart.

Quartile	Year	
	2020/21	2021/22
1	< Q1 = 27.13	< Q1 = 45.54
2	Q1 = 27.13 - Q2 = 32.64	Q1 = 45.54 - Q2 = 54.40
3	Q2 = 32.64 - Q3 = 41.35	Q2 = 50.40 - Q3 = 62.28
4	> Q3 = 41.35	> Q3 = 62.28

Table 1. Classification of the percentage of damaged seeds data in quartiles, years 2020/21 and 2021/22.

Genotype	Cycle duration* (days)		Plant height* (cm)		Lodging* (%)		100-wt* (g)	
	2021	2022	2021	2022	2021	2022	2021	2022
Cultivar 1 (susceptible)	174	170	120	125	100	0	17.1	18.8
Cultivar 2 (susceptible)	174	164	100	125	2	0	19.8	21.1
INTA Paraná 6200 (susceptible)	174	164	75	85	0	0	15.3	17.7
LAE1112474	162	164	95	100	0	0	15.1	14.7
BR 79-15.149	174	164	85	70	100	100	10.0	10.0
Cerrito FAINTA	174	164	95	110	100	30	15.5	17.9
Fainta 760	174	170	90	110	100	30	16.3	19.3
LAE10125243	174	164	120	80	100	100	11.1	11.6
LAE1112263	174	164	95	80	0	0	15.2	17.4
LAE1112371	174	164	100	95	0	0	16.9	15.8
LAE9758803-B (wild type)	174	164	100	115	0	0	15.6	16.8
Mut1	174	164	95	100	100	20	19.2	18.4
Mut2	174	164	95	105	0	0	17.2	18.9
Mut3	174	164	150	115	100	100	19.7	18.9
Mut4	174	164	115	95	2	0	19.4	19.5
Mut5	174	164	130	95	100	0	19.6	18.5
Mut6	174	164	115	90	100	0	19.9	19.2
Mut7	174	164	110	85	100	0	19.7	19.0
Mut8	174	164	115	100	100	0	15.2	16.8
Mut9	174	164	105	115	10	0	14.9	17.1
Mut10	174	164	95	100	10	0	16.1	17.1
Mut11	174	164	95	105	100	0	20.0	20.5
Mut12	174	164	115	115	0	0	14.5	15.7
Mut13	174	164	120	110	20	0	13.8	15.1
Mut14	174	164	115	110	20	0	16.5	17.6
Mut15	174	164	80	95	0	0	15.6	16.4
Mut16	174	164	95	105	50	0	16.3	17.3
Mut17	174	164	115	115	100	0	14.6	16.5
Mut18	174	164	105	90	10	0	13.4	15.3
Mut19	174	164	95	105	0	0	12.8	16.0
Mut20	174	164	100	75	0	0	17.0	18.2
Mut21	174	164	120	110	40	30	16.6	16.2
Mut22	174	164	85	115	40	20	15.7	15.9
Mut24	174	164	110	95	100	100	17.7	19.2
Mut25	174	164	100	100	40	0	15.8	16.9
Mut26	174	164	130	115	10	0	14.9	16.0
BR 82-12.462	178	164	70	75	100	100	9.0	9.9
Crocket	178	170	85	115	100	100	14.6	14.6
LAE10125244	178	170	95	80	100	100	12.2	11.5
IAC 100	181	170	110	70	100	100	10.8	11.3
Mut23	185	170	90	110	100	0	15.4	16.0
Mean	174	165	103	100	53	23	15.7	16.6

Variables that were registered in only one block of the trials in both years that could not be analyzed statistically.

Table 2. Cycle duration, plant height, lodging and 100 seed weight (100-wt) of the genotypes evaluated for resistance to stink bugs. Oro Verde. Years 2020/21 y 2021/22.

Source	Square Sum	df	Mean Square	F Value	P Value	(Error)
Environment	33159325.9	1	33159325.9	14.15	0.0037	*** (Environment>Rep)
Environment>Rep	23441753.8	10	2344175.38	3.95	<0.0001	***
Genotype	199708878	40	4992721.94	8.4	<0.0001	***
Environment*Genotype	53168392.4	40	1329209.81	2.24	0.0001	***
Error	234081226	394	594114.79			
Total	543559575	485				

Note: Significances: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 '.' 1.

Table 3. Analysis of variance for the AMMI model of yield in the genotypes evaluated for resistance to stink bugs. Oro Verde. Years 2020/21 and 2021/22.

RESULTS

During both years and trials, the predominant stink bug species were *N. viridula* and *P. guildinii*, particularly during the reproductive period of the crop. The population densities for each species exceeded one individual per linear meter at the beginning of fruiting stage (R3) and more than two individuals per linear meter at the beginning of the seed fill stage (R5), surpassing economic thresholds (Gamundi and Sosa, 2008). Additionally, some individuals of *D. furcatus* and *E. meditabunda* were present. At harvest time, individuals of *E. heros* (F.) were also observed.

Both years presented favorable temperature and rainfall conditions for the development of the trials without any significant abiotic stress. Regarding the temperatures, there were no major differences between years. However, notable differences were observed in total rainfall and its distribution: during the year 2020/21, it was more irregular and abundant with a total of 845.5 mm compared to 721.6 mm in 2021/22. The latter year was distinguished by higher rainfall records in February and March, coinciding with the reproductive period of the genotypes.

A marked difference was observed in the behavior of the soybean genotypes evaluated regarding the studied variables over both years. As mentioned earlier, variables such as cycle duration, plant height, lodging, and 100-seed weight were recorded from a single block and, since delays in maturity can occur along with reduced 100-seed weight due to stink bug damage, these variables were not subjected to a statistical analysis but are described below (table 2).

The cycle duration from emergence to maturity at harvest was 174 ± 3 days for all the genotypes in 2020/21 year and 165 ± 2 days in 2021/22 year. Some genotypes presented important differences between years; for instance, mutant line Mut5 exhibited a difference of 35 days. Most mutant lines had a difference of about 10 days between years, similar to the original wild-type line LAE9758803-B. The experimental line LAE1112474 was notably stable with only a two-day difference.

In general, the mutant lines displayed greater variability compared to the wild type-line LAE9758803-B across variables such as plant height and lodging. The plant heights averaged 103 ± 16 cm in the first year and 100 ± 15 cm in the second year. For lodging resistance, some genotypes remained stable across both years, while others showed high variability. The average 100-wt was 16 ± 3 g during the first year and 17 ± 3 g in the second year.

A highly significant genotype x environment interaction was observed for yield ($P < 0.05$). The genotypes exhibited unequal performances in their yields across the evaluated years (table 3).

The yields were 3708.64 ± 701.73 kg/ha (P value < 0.0001, CV 22.7%) in 2020/21 year and 3590.84 ± 756.59 kg/ha (P value < 0.0001, CV 23.3%) in 2021/22 year. On average, there was a difference of approximately 512 kg/ha between both years.

The mutant soybean lines presented high variability among themselves and generally had lower yields compared to the wild-type line LAE9758803-B (table 4).

The stink bug densities could not be registered for each genotype and repetition; instead, the population was monitored throughout both trials each year. As previously mentioned, the infestation observed in both years exceeded the economic thresholds established by Gamundi and Sosa (2008), considering the predominant species *N. viridula* and *P. guildinii*. This level of infestation was deemed sufficient to detect differential responses in seed damage for each year and genotype. However, it did not allow for quantification of stink bug number or species developed on each evaluated genotype.

The seed damage was observed as feeding punctures, discolorations, shriveling, and seeds with deformations without completing their filling. An important variation was observed between both years and among genotypes in each year, but the analysis of the interaction between environment and genotype was non-significant, indicating that the differences in damaged seeds were similar for each genotype throughout the evaluated years (table 5).

The average percentage of damaged seeds was significantly lower in the year 2020/21 (34.3%) compared to the year 2021/22 (53.7%) (P value < 0.0001, CV: 22.85%). A notable variability was observed among the evaluated mutant lines, with percentages of damaged seeds ranging from 36.8% to 57.8%. Considering this variable, Mut12 averaged at 36.8%, and ranked among the seven genotypes with least damage, including the wild-type line LAE9758803-B (table 6).

The experimental lines LAE1112474, LAE1112371, LAE1112263, LAE10125243 and LAE10125244 exhibited percentages of damaged seeds ranging from 40.1 to 45.3%. These values were higher than those observed in genotypes considered resistant and also exceeded those of two out of the three susceptible cultivars (INTA Paraná 6200 and Cultivar 2). Notably, Cultivar 2 was among the six genotypes with least

Genotype	Mean Yield (kg/ha $\pm$ SD)			
	2020/21		2021/22	
Cultivar 1 (susceptible)	4492.5 $\pm$ 692	a	4870.8 $\pm$ 1436	a
Cultivar 2 (susceptible)	4352.7 $\pm$ 758	a	5713.7 $\pm$ 1091	a
INTA Paraná 6200 (Susceptible)	3501.8 $\pm$ 919	b	4057.5 $\pm$ 1251	b
LAE9758803-B (Wild Type)	4264.8 $\pm$ 640	a	4710.7 $\pm$ 1032	a
Fainta 760	4593.2 $\pm$ 698	a	4064.0 $\pm$ 1107	b
Mut22	4079.8 $\pm$ 234	a	4536.8 $\pm$ 1019	a
IAC 100	4103.3 $\pm$ 869	a	3888.7 $\pm$ 685	b
LAE1112474	2908.5 $\pm$ 721	c	4771.3 $\pm$ 611	a
Mut12	3113.5 $\pm$ 788	c	4203.8 $\pm$ 333	b
Mut16	3663.7 $\pm$ 792	b	3556.2 $\pm$ 567	c
Mut18	3326.0 $\pm$ 415	c	3788.7 $\pm$ 770	b
BR 79-15.149	2531.8 $\pm$ 622	d	4540.7 $\pm$ 1328	a
LAE1112371	2966.5 $\pm$ 673	c	4063.5 $\pm$ 1065	b
Crocket	3164.3 $\pm$ 666	c	3847.8 $\pm$ 833	b
LAE10125244	2658.3 $\pm$ 843	c	4276.2 $\pm$ 1024	b
Mut24	3631.8 $\pm$ 1538	b	3171.3 $\pm$ 872	c
LAE1112263	2764.8 $\pm$ 1054	c	4017.2 $\pm$ 1158	b
Mut14	3329.0 $\pm$ 556	c	3389.2 $\pm$ 788	c
Mut25	3186.2 $\pm$ 999	c	3504.0 $\pm$ 705	c
BR 82-12.462	3000.2 $\pm$ 827	c	3551.3 $\pm$ 811	c
Mut4	3105.8 $\pm$ 615	c	3421.5 $\pm$ 546	c
Mut10	2958.8 $\pm$ 635	c	3540.7 $\pm$ 713	c
Mut13	3276.5 $\pm$ 591	c	3195.0 $\pm$ 1027	c
Mut1	2937.2 $\pm$ 537	c	3490.8 $\pm$ 1173	c
Mut8	2484.8 $\pm$ 355	d	3859.5 $\pm$ 750	b
Mut2	3368.5 $\pm$ 518	c	2920.8 $\pm$ 569	c
Mut7	3144.0 $\pm$ 450	c	2994.5 $\pm$ 286	c
Mut26	3422.1 $\pm$ 846	b	2660.3 $\pm$ 695	c
LAE10125243	2200.2 $\pm$ 1035	d	3881.7 $\pm$ 895	b
Mut19	2661.8 $\pm$ 782	c	3396.5 $\pm$ 736	c
Mut5	2908.7 $\pm$ 691	c	3139.2 $\pm$ 656	c
Mut9	3151.3 $\pm$ 577	c	2848.3 $\pm$ 751	c
Mut21	3033.7 $\pm$ 348	c	2948.2 $\pm$ 436	c
Cerrito FAINTA	2421.7 $\pm$ 636	d	3531.7 $\pm$ 785	c
Mut3	2735.8 $\pm$ 514	c	2917.5 $\pm$ 123	c
Mut15	2234.5 $\pm$ 492	d	3311.5 $\pm$ 321	c
Mut11	2643.8 $\pm$ 703	c	2778.7 $\pm$ 971	c
Mut6	2545.3 $\pm$ 337	d	2823.3 $\pm$ 1232	c
Mut17	2263.7 $\pm$ 920	d	2905.8 $\pm$ 957	c
Mut23	1649.7 $\pm$ 610	e	2255.8 $\pm$ 633	c
Mut20	1443.8 $\pm$ 643	e	1879.7 $\pm$ 576	c
Mean	3078.6		3590.8	
C.V.	22.7		23.3	

Means with a common letter are not significantly different ($p > 0.05$).

Table 4. Mean yield and standard deviation (SD) of the genotypes evaluated for resistance to stink bugs. Oro Verde. Years 2020/21 and 2021/22.

Source	Square Sum	df	Mean Square	F Value	P Value	(Error)
Environment	46498.55	1	46498.55	168.39	<0.0001	*** (Environment>Rep)
Environment>Rep	2761.37	10	276.14	2.73	0.0029	***
Genotype	20679.21	40	516.98	5.11	<0.0001	***
Environment*Genotype	4280.09	40	107	1.06	0.3796	
Error	40243.23	398	101.11			
Total	114462.45	489				

Note: Significances: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ' 1.

Table 5. Analysis of variance for the AMMI model of damaged seeds in the soybean genotypes evaluated for resistance to stink bugs. Oro Verde. Years 2020/21 and 2021/22.

damaged seeds. This might be attributed to its higher yield, which could have led to a dilution effect on damage.

The analysis of damaged seeds from both years, categorized into quartiles (figure 1), revealed differences among the genotypes. The control genotypes with known resistance IAC 100, BR 79-15.149, Cerrito Fainta and the mutant line Mut12 were notable for having a higher proportion of cases within Q1 (0-25%) and fewer or no cases in Q4 (26-50%). Specifically, Mut12 had 50% of its cases within Q1 and no cases in Q4 across both years. In comparison, the wild-type LAE9758803-B had 25% of its cases in Q1 and no cases in Q4.

At the other extreme were lines Mut9, Mut8, Mut14, and Mut11, which consistently showed more instances of damage values above those in Q1 over both years.

DISCUSSION

The search for genetic resistance to phytophagous stink bugs in soybeans has been conducted by several researchers, including Lucini *et al.* (2021), Vicentin and Saluso (2017) and Mc Pherson *et al.* (2007). This research is driven by the benefits of genetic resistance, such as reduced or eliminated use of insecticides, cost savings, and the promotion of more environmentally friendly agricultural practices.

Over time, a few naturally occurring genotypes have been utilized as sources of resistance for hybridization purposes (Bansal *et al.*, 2013).

For traits like leaf morphology, plant architecture, seed composition, and yield, Espina *et al.* (2018) successfully generated and observed phenotypic variation in an EMS-mutagenized soybean population.

In this study, we were the first to investigate and identify variability generated by chemical mutation with EMS for stink bug resistance in a group of soybean mutant lines.

An important environmental effect was observed for each variable studied. Generally, across both years, genotypes were similar in terms of cycle duration and plant height but exhibited wide variation in lodging and 100-wt. Future studies spanning two or three years with repetitions could provide more precise data.

Yield was another variable that showed a strong interaction

with the environment. Higher yields were observed in year 2021/22 compared to year 2020/21 for all the evaluated genotypes. This can be primarily attributed to increased rainfall during February and March, a period when most of the evaluated genotypes were in their reproductive stages, a critical time when pod and grain numbers are determined.

Important differences in the seed damage percentages were observed between the mutant lines and the wild-type line LAE9758803-B. Notably, mutant line mut12 performed best, surpassing the wild type and ranking among the genotypes considered resistant. Additionally, Mut12 displayed stable plant height values and no lodging across both years but was more variable and inferior in yield compared to the wild-type line.

Future studies will be necessary to identify the genes associated with Mut12's superior response to stink bug damage.

CONCLUSIONS

Genetic resistance against stink bugs could serve as a valuable complement in soybean cultivation to reduce the number of insecticides applications. This approach would favor lower production costs, reduced environmental contamination and more environmentally friendly practices.

A population of artificially induced mutant lines exhibited variability in resistance to the stink bug complex based on the percentage of damaged seeds. The mutant line Mut12 was identified among the top genotypes with fewer damaged seeds. This line could be utilized in crossbreeding within various breeding programs to develop elite soybean cultivars resistant to stink bugs. Future research should focus on evaluating Mut12 further to determine the type of insect resistance it possesses.

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Genotype	Damaged seeds (%) $\pm$ SD		Damaged seeds (%) Mean $\pm$ SD	
	2020/21	2021/22		
Cultivar 1 (susceptible)	38.1 $\pm$ 7	54.9 $\pm$ 13	46.5 $\pm$ 13	c
Cultivar 2 (susceptible)	27.7 $\pm$ 9	42.1 $\pm$ 13	34.9 $\pm$ 13	a
INTA Paraná 6200 (susceptible)	27.9 $\pm$ 7	50.4 $\pm$ 12	39.2 $\pm$ 15	b
IAC 100	18.6 $\pm$ 7	42.8 $\pm$ 6	30.7 $\pm$ 14	a
BR 79-15.149	23.7 $\pm$ 5	41.3 $\pm$ 22	32.5 $\pm$ 18	a
Cerrito FAINTA	24.6 $\pm$ 8	42.2 $\pm$ 9	33.4 $\pm$ 12	a
BR 82-12.462	27.1 $\pm$ 10	45.3 $\pm$ 10	36.2 $\pm$ 13	a
Mut12	29.2 $\pm$ 7	44.3 $\pm$ 10	36.8 $\pm$ 11	a
LAE9758803-B (wild type)	30.4 $\pm$ 2	44.4 $\pm$ 6	37.4 $\pm$ 8	a
Fainta 760	26.0 $\pm$ 6	51.9 $\pm$ 16	38.9 $\pm$ 18	b
Crocket	29.8 $\pm$ 9	48.5 $\pm$ 13	39.1 $\pm$ 14	b
LAE1112263	36.2 $\pm$ 7	44.0 $\pm$ 11	40.1 $\pm$ 10	b
Mut16	31.9 $\pm$ 10	50.9 $\pm$ 7	40.5 $\pm$ 13	b
Mut21	30.3 $\pm$ 6	51.5 $\pm$ 5	40.9 $\pm$ 12	b
Mut18	31.5 $\pm$ 5	50.6 $\pm$ 9	41.0 $\pm$ 12	b
LAE1112474	39.9 $\pm$ 13	42.8 $\pm$ 13	41.3 $\pm$ 13	b
Mut13	29.7 $\pm$ 7	53.2 $\pm$ 6	41.4 $\pm$ 14	b
LAE1112371	35.7 $\pm$ 10	47.8 $\pm$ 9	41.7 $\pm$ 11	b
Mut3	26.9 $\pm$ 7	57.1 $\pm$ 20	42.0 $\pm$ 21	b
Mut19	30.1 $\pm$ 11	54.7 $\pm$ 10	42.4 $\pm$ 16	b
LAE10125243	39.6 $\pm$ 6	45.9 $\pm$ 15	42.7 $\pm$ 12	b
Mut4	33.4 $\pm$ 5	52.2 $\pm$ 6	42.8 $\pm$ 11	b
Mut15	33.3 $\pm$ 11	53.8 $\pm$ 4	43.5 $\pm$ 13	b
Mut22	34.0 $\pm$ 7	53.3 $\pm$ 11	43.6 $\pm$ 13	b
Mut24	33.6 $\pm$ 9	56.7 $\pm$ 5	45.2 $\pm$ 14	c
LAE10125244	37.4 $\pm$ 7	53.2 $\pm$ 11	45.3 $\pm$ 12	c
Mut1	36.6 $\pm$ 12	55.7 $\pm$ 11	46.1 $\pm$ 16	c
Mut25	34.3 $\pm$ 6	58.6 $\pm$ 10	46.4 $\pm$ 15	c
Mut10	36.1 $\pm$ 12	58.4 $\pm$ 6	47.3 $\pm$ 15	c
Mut2	32.8 $\pm$ 12	62.7 $\pm$ 12	47.8 $\pm$ 20	c
Mut17	34.1 $\pm$ 8	62.4 $\pm$ 14	48.2 $\pm$ 18	c
Mut6	38.3 $\pm$ 11	58.8 $\pm$ 15	48.5 $\pm$ 17	c
Mut5	37.4 $\pm$ 8	61.8 $\pm$ 11	49.6 $\pm$ 16	c
Mut7	36.4 $\pm$ 9	63.2 $\pm$ 11	49.8 $\pm$ 17	c
Mut11	39.2 $\pm$ 6	60.6 $\pm$ 12	49.9 $\pm$ 14	c
Mut14	42.4 $\pm$ 8	62.1 $\pm$ 9	52.3 $\pm$ 13	d
Mut26	44.3 $\pm$ 14	62.5 $\pm$ 6	53.4 $\pm$ 14	d
Mut9	42.0 $\pm$ 12	67.0 $\pm$ 11	54.5 $\pm$ 17	d
Mut8	48.2 $\pm$ 9	63.3 $\pm$ 9	55.8 $\pm$ 12	d
Mut20	46.3 $\pm$ 9	67.0 $\pm$ 15	56.6 $\pm$ 16	d
Mut23	52.5 $\pm$ 13	63.1 $\pm$ 19	57.8 $\pm$ 17	d
Mean	34.3	53.7	44.0	
C.V.	24.7	21.3	22.9	

Means with a common letter are not significantly different ($p>0.05$).

Table 6. Percentage of damaged seeds and standard deviation (SD) in the soybean genotypes evaluated for resistance to stink bugs. Oro Verde. Years 2020/21 and 2021/22.

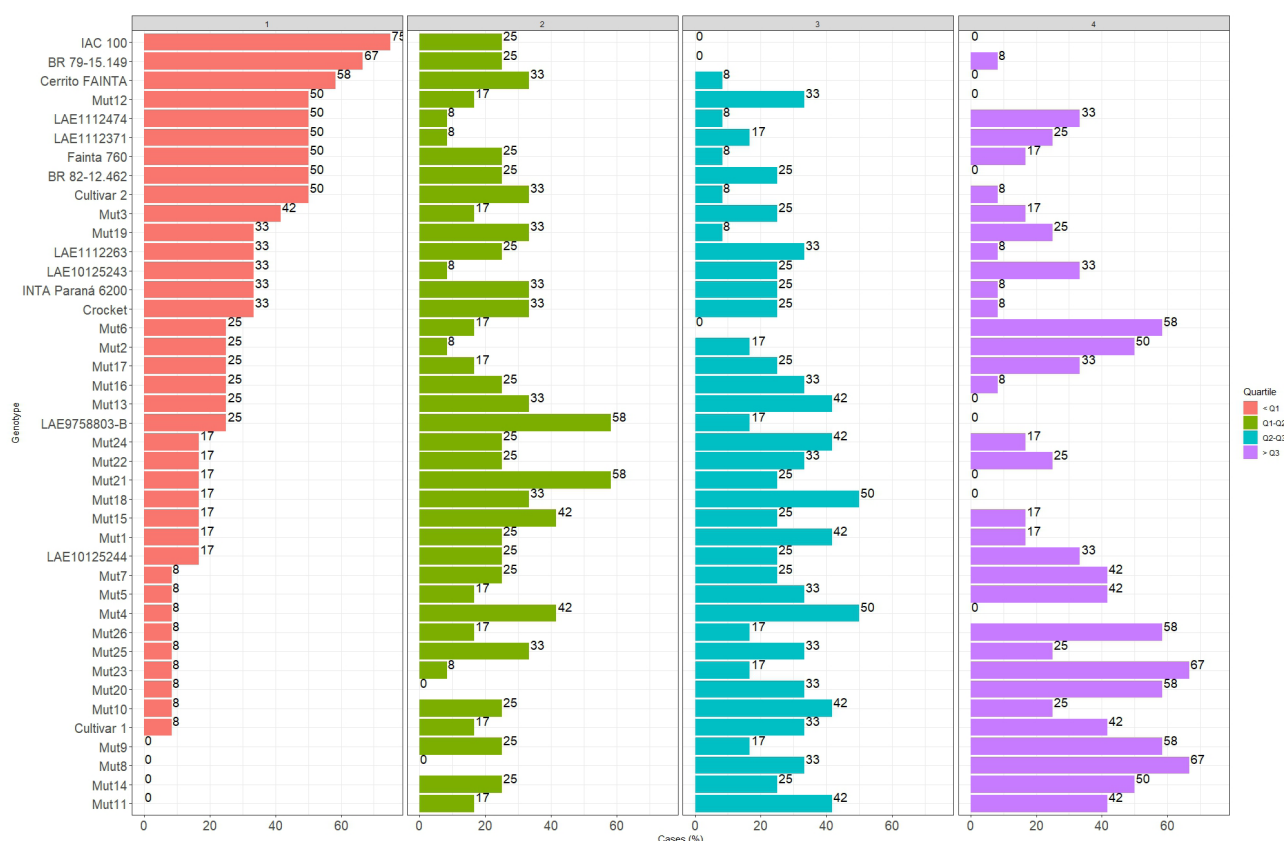


Figure 1. Categorization by quartiles of the cases with damaged seeds by stink bugs for each genotype evaluated for resistance to stink bugs in the years 2020/21 and 2021/22.

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