Induced mutagenesis in janeiro grass by ethyl-meta-sulfonate and its effect on spittlebug control

Mutagénesis inducida en pasto janeiro mediante etil- meta-sulfonato y su efecto sobre el control de salivazo

Mutagênese induzida em grama de janeiro por etil-meta-sulfonato e seu efeito no controle da cigarrinha

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Rev. Fac. Agron. (LUZ). 2022, 39(3): e223938
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v39.n3.04

Received: 16-11-2021
Accepted: 13-07-2022
Published: 03-08-2022

Animal Production
Associate editor: Dra. Rosa Razz
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

Keywords:
Pasture improvement
Variability
Resistance
Mutation induction

Abstract

In this research, the median lethal dose (LD_{50}) and resistance to spittlebug (*Mahanarva andigena*) were determined, using a mutagenizing agent ethyl meta-sulfonate (EMS) in Janeiro grass (*Eriochloa polystachya* Kunth) as a means to generate mutations. The study was carried out at the laboratory and greenhouse level, using a Complete Random Design (DCA) with ten treatments and three repetitions, which consisted of 5 doses of EMS (0.00%, 0.25%, 0.50%, 0.75% and 1.00%). for 2 impregnation times (24 and 48 hours). According to the results, the doses of ethyl meta-sulfonate (EMS) influenced the setting of the stolons of the janeiro grass, presenting a high rate of deflation in doses higher than 0.50%. Treatments with doses of 0.25% EMS at 48 hours achieved the best agronomic performance up to 95 days, where plant height had its best development with use close to that determined as DL_{50} (0.32%). The janeiro grass impregnated with EMS presented slight damage caused by the spittle.

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Resumen

En esta investigación se determinó la dosis letal media (DL_{50}) y la resistencia a salivazo (*Mahanarva andigena*), utilizando ethyl metano-sulfonato (EMS) agente mutagenizante en pasto Janeiro (*Eriochloa polystachya* Kunth) como medio para generar mutaciones. El estudio fue realizado a nivel de laboratorio e invernadero, usando para ello un Diseño Completo al Azar (DCA) con diez tratamientos y tres repeticiones, que consistieron en 5 dosis de EMS (0,00 %, 0,25 %, 0,50 %, 0,75 % y 1,00 %) por 2 tiempos de impregnación (24 y 48 horas). De acuerdo a los resultados, las dosis de ethyl metano-sulfonato (EMS) tuvieron efecto en el prendimiento de los estolones del pasto janeiro, presentándose un alto índice de deflación en dosis superiores a 0,50 %. Los tratamientos con dosis de 0,25 % de EMS a 48 horas, lograron el mejor comportamiento agronómico hasta los 95 días, donde la altura de planta tuvo su mejor desarrollo con el uso cercano a la determinada como DL_{50} (0,32 %). El pasto janeiro impregnado con EMS presentó daños leves causado por el salivazo.

Palabras clave: Mejoramiento de pastos, variabilidad, resistencia, inducción de mutaciones.

Resumo

Nesta pesquisa, a dose letal mediana (DL_{50}) e a resistência à cigarrinha (*Mahanarva andigena*) foram determinadas, utilizando-se um agente mutagenizante etil meta-sulfonato (EMS) em capim-janeiro (*Eriochloa polystachya* Kunth) como meio de gerar mutações. O estudo foi realizado em nível de laboratório e casa de vegetação, utilizando um Desenho Aleatório Completo (DCA) com dez tratamentos e três repetições, que consistiu em 5 doses de EMS (0,00 %, 0,25 %, 0,50 %, 0,75 % e 1,00 %) por 2 tempos de impregnação (24 e 48 horas). De acordo com os resultados, as doses de etil meta-sulfonato (EMS) tiveram efeito na fixação dos estolões do capim-janeiro, apresentando alto índice de deflação em doses superiores a 0,50%. Os tratamentos com doses de 0,25% EMS às 48 horas obtiveram o melhor desempenho agronômico até 95 dias, onde a altura das plantas teve seu melhor desenvolvimento com uso próximo ao determinado como LD_{50} (0,32%). O capim-janeiro impregnado com EMS apresentou danos leves causados pelo salivazo.

Palavras-chave: Melhoramento genético, variabilidade, melhoramento, mutações.

Introduction

In the early 1950s, improved cultivars were achieved through mutagenesis. At the beginning of the 20th century, mutagenesis techniques were applied to obtain rapidly mutant individuals and increase variability in plants (Rojas *et al*., 2016). FAO published in 1994 that 1.800 cultivars were obtained by mutagenesis. Currently, most of the crops exploited in the world come from mutated cultivars (González, 2014). For instance, 70% of the wheat (*Triticum aestivum* L.) sown in Italy came from mutated crops (González, 2014).

Despite the heavy reliance on chemical mutagenesis, traditional genetic screens do not readily reveal the underlying mutational process. This is because geneticists select phenotypes and, as a result, only a small minority of mutations within a target gene are examined (Greene *et al*., 2003).

Corrales *et al.* (2019) mentioned that the induction of mutations might be a viable option to create genetic improvement. This is because the practice is capable of inducing mutability with mutagenic agents.

The use of mutagenic agents, such as ethyl meta-sulfonate (EMS), has a fundamental role in the generation of variability (Porch *et al*., 2009). One of the most widely used chemical mutagen in breeding programs is EMS, which could produce tolerance related to phyto and entomopathogenic agents. This is because it creates many point mutations in almost all the studied genera. Moreover, the periodicity of induced mutations is independent of the genome volume (Greene *et al*., 2003; Rojas *et al*., 2016).

In janeiro grass (*Eriochloa polystachya* Kunth) it is difficult to find genetic variability to improve its productive potential and nutritional quality by conventional methods. This is because of its largely asexual reproductive system and its short evolutionary period. Despite of all those drawbacks, janeiro grass could increase forage production (Nobel, 1999).

The use of EMS in its genetic improvement for the specific case of janeiro grass has not been reported in the literature. Consequently, this study aims to determine the mean lethal dose (LD_{50}) to induce mutations with EMS in janeiro grass and evaluate resistance against spittlebug (*Mahanarva andigena*).

Materials and methods

Study location

The research, related to the collection and sowing of janeiro grass, was carried out in the rainy season (January to May) of the year 2020. It was developed at the laboratory and greenhouse level at the Faculty of Agricultural Sciences of the Technical University of Babahoyo, Ecuador, located 7.5 km from the Babahoyo-Montalvo road, geographically located at 79°32´ W longitude and 01°49´ S latitude, elevation of 8 m.a.s.l., temperature of 30.4 °C, relative humidity 65.5% (greenhouse), evaporation 1012.4 mm, heliophany 830.4 hours (Cobos *et al*., 2021).

Stolons management and experimental procedure

For this study, janeiro grass stolons of 8 cm in length were used, with a node where they emitted their root, which were rinsed and disinfected with sterile distilled water (three rinses) (Gómez e*t al*., 2020).

The methodology established by Pankhurst *et al*. (2004) was used for impregnation. The Biotechnology Laboratory at the Faculty of Agricultural Sciences applied a modified protocol to carry out the impregnation. The proposed protocol is briefly described as follows:

Approximately 2.000 stolons at different EMS concentrations (0.00%, 0.25%, 0.50%, 0.75%, 1.00%), were used in the present work. Once the dilutions were prepared, they were poured into bottles where the plant material was located to avoid splashing. Thereafter, 200 stolons, sterilized with ethanol at 70% (v/v) for 10 min and H_2O_2 at 5% (v/v) for 10 min were submerged for 24 hours and 48 hours. After this time, sterile deionized water (v/v) was added to the glasses with the stolons at room temperature. Finally, the solution was removed and the stolons treated with distilled water were rinsed 5 times at least 15 minutes each rinse.

The stolons treated with EMS and the untreated controls were sown in germinating trays located in a greenhouse. This was done to determine the mean lethal dose (LD_{50}). After 30 days, the stolons were transplanted into definitive covers to evaluate their vigor and resistance to the spittlebug attack.

The nymphs were collected as foam masses from areas affected by spittlebug. The transportation was done from the Bucay area to the

Babahoyo canton in containers with plant material; thus, the nymphs were kept during the transfer (Castro *et al.*, 2005).

400 plants.treatment^{-1} were transplanted from janeiro grass to the greenhouse and having completed 50 days of physiological development, they were inoculated with the nymphs of the spittlebug pest insect, which was done random. Each treatment contained a number of 5 nymphs. In other words, the study area was exposed to 50 spittlebug nymphs.

Variables evaluated

Percentage of germination and/or seizure**.** The germination percentage (take-off) was evaluated at 10, 20 and 30 days. The result was expressed as a percentage of germinated stolons.

The percentage of seizure was determined by applying the following equation:

$$\% \text{ take-up} = \frac{\text{No. of stolons sown - No. dead stolons x 100}}{\text{No. of stolons sown}}$$

Percentage of mortality

The percentage of mortality was determined at 10, 20 and 30 days, considering the stolons that were not alive.

$$\% \text{ mortality} = \frac{\text{No. of stolons sown - No. living stolons x 100}}{\text{No. of stolons sown}}$$

Growth vigor

It was based on a descriptor (table 1) with a scale established by CIAT's standard evaluation system for rice (Rodríguez *et al*., 2019). This variable was evaluated after 115 days in the presence of the spittlebug pest insect.

Table 1. Scale used to determine plant vigor.

Category	Scale
Very vigorous plant	1
Vigorous Plant	3
Intermediate or normal plants	5
Plants less vigorous than normal	7
Very weak and small plants	9

Number of spittlebugs per treatment

It was recorded by counting the number of spittlebugs in ten random plants present in each treatment at 60 days.

Pest severity

The damage in the janeiro grass was determined at 60 days, observing the damage caused in the plants and the presence of living individuals, according to Bhattarai (2019). This scale presented in table 2 is used to determine the severity of the pest in crops.

Table 2. Scale to determine severity.

Intensity of damage	Living individuals
No harm	0
Mild	1
Moderate	2
High	4
Strong	6
Very strong	>8

Experimental design and treatments

A Complete Random Design (DCA) was used for the statistical analysis, which consisted of ten treatments and three repetitions (Table 3). The factors under study included 5 doses of EMS (0.00%, 0.25%, 0.50%, 0.75% and 1.00%) and 2 impregnation times (24 and 48 hours), resulting in a total 10 treatments for three repetitions. The data was subjected to a variance analysis. Moreover, a regression analysis was performed to find a relationship between the impregnation dose and the variables with the statistical package Statgraphics Centurión XVI. II, with a linear probit model.

Table 3. Study treatments applied in janeiro grass.

Treatments	EMS concentrations (%)	Hours
T1	0.00%	24
T2	0.25%	24
T3	0.50%	24
T4	0.75%	24
T5	1.00%	24
T6	0.00%	48
T7	0.25%	48
T8	0.50%	48
T9	0.75%	48
T10	1.00%	48

Results and discussion

In the first phase of germination with different levels of EMS, mortality (figure 1) presented higher values in EMS concentrations from 0.6 to 1%. Similar values are reported by Mendoza (2020) in his research on mutagenesis induction in *Eustoma grandiflorum* cultivars by using EMS at concentrations of 0.75M and 1M, the death rate was greater than 60%. On the other hand, Yadav *et al*. (2016) in LD_{50} with EMS in mustard seeds reports that doses higher than 1% are highly lethal regardless of genotype and species, and that with a concentration of 0.50% EMS at three days for the variety RH-749, NRCHB-101 and *Sinapis alba*, germinated 33.88 and 25% respectively; while at 6 days was 62.94 and 57%, respectively.

LD_{50} in janeiro grass impregnated at 24 hours

Figure 1 show the regression obtained from the percentage of mortality against EMS doses. The resulting LD_{50} by Probit analysis was 0.49% of the EMS, in which there was a 50% mortality of janeiro grass. The most propitious dose was 0.50 at 24 hours of impregnation. The applied equation was y = -0.797923 + 1.60861 * LD_{50} EMS janeiro, with an R^2 of 58.61 percentage of deviation.

According to the LD_{50} results in janeiro grass impregnated at 24 hours, the mortality increases with the EMS doses. Those results are similar to a research developed by López (2011) who induced genetic variability by EMS in cultivation *in vitro* of *Cenchrus ciliaris* L. This author reported an LD_{50} of 0.5% and mentioned that the germination percentage decreases as the dose and time increases.

LD_{50} in janeiro grass impregnated at 48 hours

Figure 2 shows the linear regression fitted to a Probit regression model, being the equation of the fitted model Y = -0.952779 + 2.94161 * B. EMS dose (%) with an R^2 = 79.62. The obtained result indicates that LD_{50} was 0.32% EMS, in which the mortality of the janeiro grass was 50% with an impregnation time of 48 hours. In a study carried

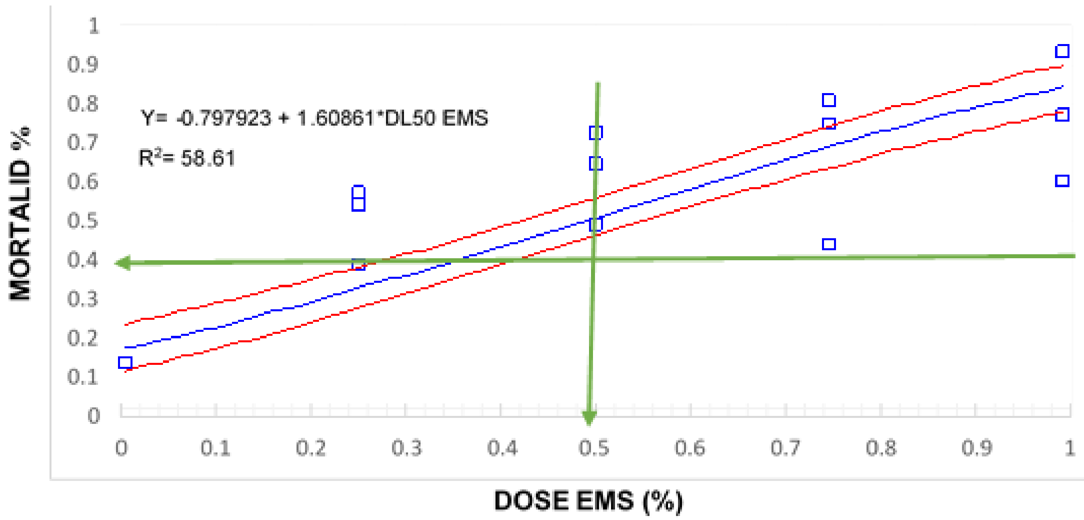

Figure 1. LD$_{50}$ in janeiro grass impregnated at 24 hours.

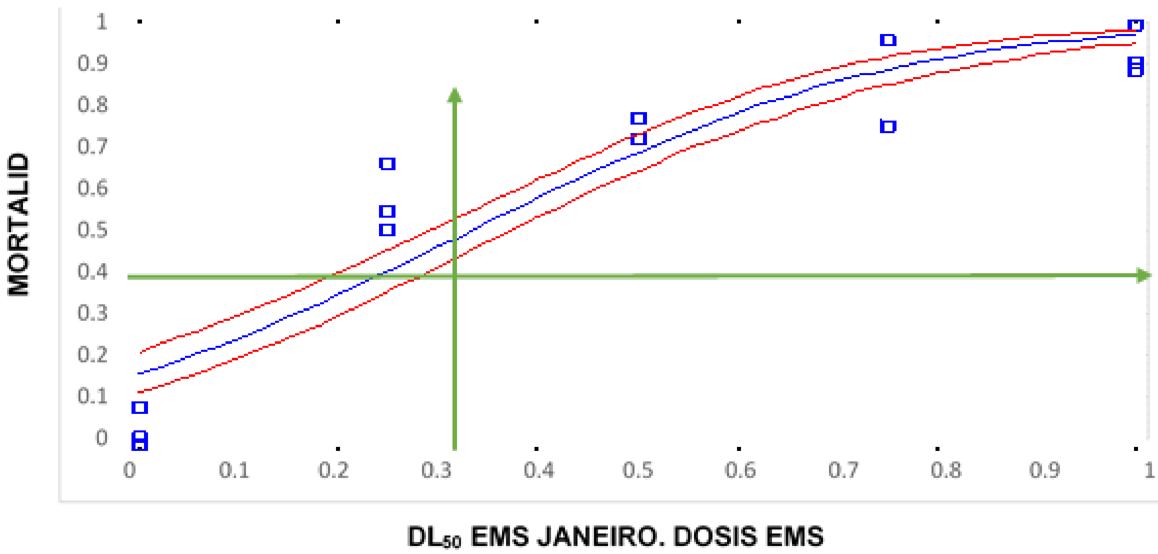

Figure 2. Dl$_{50}$ in janeiro grass at 48 hours of impregnation.

out by Costa (2017) which impregnated EMS *in vitro* vegetative material of artichoke (Romanesco) obtained an LD$_{50}$ of 0.37% of the EMS and observed that at high concentrations the explants become necrotic and die. Khalil *et al*. (2018) mentioned that a study carried out in two varieties of sugarcane (*Saccharum officinarum*) does not follow the obtained results in janeiro grass. This is because the LD$_{50}$ mutagenic treatment of ROC22 callus was 0.1% EMS for 17 h, while, for callus FN39, was 0.1% EMS for 14 h.

In the present investigation at 24 and 48 hours, the lowest concentrations of EMS showed higher values. This agrees with Sharma *et al*. (2005) who reported a higher mutagenic efficacy at low concentrations of EMS in beans. According to Konzak *et al*. (1965), the efficiency at low concentrations of a mutagenic agent is due to biological damage that increases with increasing dose at a faster rate than mutations.

Growth vigor

Table 4 displays the values of the evaluated characteristics. Those values show the growth and development processes of janeiro grass at 95 days. The data was used to make a comparison with a descriptive analysis and to determine the vigor of growth.

It can be evidenced that the dose of 0.50% EMS at 24 hours and 0.25% EMS at 48 hours of impregnation stood out statistically in all variables (height, stem diameter, number of leaves, length and width of the leaf); presenting better physiological development. On the other hand, the plants showed growth retardation at a higher dose of EMS.

The height of the plant varies depending on the doses of EMS. There were significant differences between treatments, the higher the dose the lower the growth. These results agree with what was expressed by Rojas *et al*. (2016) on optimal concentration of EMS in Bean (*Phaseolus vulgaris* L.) cv. 'DOR 364' whose high doses negatively influenced the variables related to growth and development. On the other hand, in coriander cultivation (*Coriandrum sativum* L) Kumar and Pandey (2020) mention that the height of the plant is reduced in high concentrations of EMS. However, the treatments with a lower concentration of EMS (0.1%

Table 4. Physiological development at 95 days in janeiro grass by EMS.

Treatments	Height (m)		Stem diameter (mm)		N° of sheets		Blade length (cm)		Blade width (cm)	
T7	1.54	a	4.70	a	11.60	a	38.9	a	1.99	a
T3	1.33	b	4.40	abc	9.90	ab	36.9	ab	1.93	a
T8	1.23	bc	4.60	ab	9.70	abc	36.7	ab	1.71	abc
T4	1.21	bc	3.90	bcd	9.80	abc	34.8	abc	1.80	ab
T2	1.18	bc	4.00	abcd	10.20	ab	34.8	abc	1.84	ab
T9	1.06	cd	3.60	de	8.70	bc	34.7	abc	1.49	c
T6	1.06	cd	2.80	abcd	8.80	bc	33.3	bc	1.88	ab
T1	1.05	cd	3.80	cd	8.40	bc	34.5	bc	1.87	ab
T10	0.94	d	3.80	cd	8.70	bc	32.2	c	1.59	bc
T5	0.91	d	4.00	de	7.60	c	32.3	c	1.47	c
Average	1.15		3.96		9.34		34.91		1.76	
Significance	**		**		**		**		**	
CV (%)	11.14		13.50		16.83		8.45		11.52	

ns = not important; **: Very significant (<0,0001)

and 0.3%) had a stimulating effect on plant height compared to the control. The reduction in the height of janeiro grass could be attributed to the inactivation of auxins in the plant by increasing exposure to EMS (Ashok *et al*., 2009). Mutagens can inhibit an energy supply system resulting in mitosis inhibition that may be associated with seedling growth depression (Emrani *et al*., 2011).

The diameter of the evaluated stem presented significant differences ($p<0.0001$), which shows that it increases as time passes and the dose of EMS decreases. Mostafa (2015) point out that in Mahogany (*Khaya senegalensis*) seedlings impregnated with dimethyl sulfate (DMS) the stem diameter is reduced from 1.70 cm to 0.70 cm when increasing the doses with respect to the control.

The foliar emission in the janeiro grass is affected by the doses and hours of impregnation of EMS applied, decreasing the number of leaves. This is compared with the study carried out by Akhtar (2014) who evaluated mutagens on the morphological behavior of tomato (*Solanum lycopersicum*); where the highest number of leaves was counted in doses of 8 mM (millimole) EMS (70 leaves) and 16 mM (61 leaves). As the dose level of EMS increased, number of leaves decreased from 29 to 20 at 24 mM and 32 mM, respectively.

Regarding the length of the leaf, it was evidenced that there was high significance (p=0000.1) obtained a better longitudinal development in T7 with respect to the control. A study carried out by Suthakar and Mullainathan (2015) in *Sorghum bicolor* L. mentioned that, in the case of leaf length, they obtained maximum development with EMS doses at 40 mM. In a study carried out by Hasang *et al*. (2020) in janeiro grass with gamma rays (60co), it was shown that some variables were higher with 52 Gy irradiation compared to the control treatment. What showed a stimulating effect of irradiation.

Table 5 shows the averages of the growth vigor variable for treatments T3 and T7 reached values of 1.80. Consequently, the plants are considered as very vigorous since they fit with the value 1 of the CIAT scale that was used to evaluate vegetative vigor. Note that those values were obtained from a descriptive category, where the doses for the treatments T3 and T7 were 0.50% EMS at 24 hours and 0.25% of EMS at 48 hours, respectively.

Table 5. Growth vigor at 115 days and in the presence of spittlebug, in janeiro grass by EMS.

Treatments	Growth vigor	Category
T1	3.70	Vigorous plants
T2	3.40	Vigorous plants
T3	1.80	Very vigorous plants
T4	5.00	Intermediate or normal plants
T5	7.80	Plants less vigorous than normal
T6	3.20	Vigorous plants
T7	1.80	Very vigorous plants
T8	5.20	Intermediate or normal plants
T9	8.00	Plants less vigorous than normal and very weak and small plants
T10	8.40	Plants less vigorous than normal and very weak and small plants

Number of spittlebugs per treatment at 60 days of inoculation

Table 6 shows the values of the number of spittlebugs at 60 days of inoculation in each treatment. For which we proceeded to extract the nymphs from each plant per study, which allowed us to define that the treatments T1 and T6 0.00% of EMS at 24 hours and 0.00% of EMS at 48 hours, respectively, presented a greater population of spittlebug. Followed by treatments T2 and T3 that showed between 15 and 17 nymphs. Finally, the treatments with the highest dose of EMS 1.00% at 24 and 48 hours showed a lower number of spittlebugs with 2.00 nymphs.

Table 6. Spittlebug number at 60 days after inoculation, in janeiro grass using EMS

Treatments	Initial Population	Population Final
T1	5 nymphs	19.00 nymphs
T2	5 nymphs	15.00 nymphs
T3	5 nymphs	13.00 nymphs
T4	5 nymphs	4.00 nymphs
T5	5 nymphs	2.00 nymphs
T6	5 nymphs	20.00 nymphs
T7	5 nymphs	17.00 nymphs
T8	5 nymphs	7.00 nymphs
T9	5 nymphs	3.00 nymphs
T10	5 nymphs	2.00 nymphs
Total	50.00 nymphs	102.00 nymphs

Regarding the number of nymphs, the results obtained at 60 days show that there was a total increase of 102.00 nymphs during the evaluation. During this period, the spittlebug nymphs went through the different stages (egg, nymph, adult).

Pest severity per plant

Table 7 shows the average severity of each treatment under study, for which the number of spittlebugs per plant was used. Using the severity scale, we can observe that treatments T4 and T5, with doses of 0.75 and 1.00% of EMS impregnated at 24 hours, and treatments T9 and T10, with doses of 0.75 and 1.00% of EMS impregnated at 48 hours, they presented values of 0 live individuals, considering those treatments without damage based on the severity scale used; while treatments T3 and T8 with doses of 0.50% EMS at 24 and 48 hours, presented values of 1 living individual and treatments T1, T2 with doses of 0.00% and 0.25% of EMS at 24 hours and treatments T6 and

T7 with doses of 0.00 % and 0.25 % of EMS at 48 hours presented values of 2 live individuals, causing slight and moderate damage, respectively, such as drying of the foliage, deterioration of its quality in the control treatments.

Table 7. Severity of the pest (spittlebug) by plants at 60 days after inoculation, in janeiro grass using EMS.

Treatments	EMS dose (%)	Hours of Impregnation	Nymph presence per plant / average	Crop damage intensity
T1	0.00	24	2	Moderate
T2	0.25	24	2	Moderate
T3	0.50	24	1	Mild
T4	0.75	24	0	No harm
T5	1.00	24	0	No harm
T6	0.00	48	2	Moderate
T7	0.25	48	2	Moderate
T8	0.50	48	1	Mild
T9	0.75	48	0	No harm
T10	1.00	48	0	No harm

The observed spittlebug damage agrees with the description made by Sotelo *et al*. (2003). Those entomologists of the CIAT, indicated that the intense attack of the Cercópids causes total drying of the foliage, whereas, slight attacks generate a growth retardation, and reduces the forage production. The damages of this pest have been registered in humid regions planted with susceptible grasses.

Conclusions

The treatments with doses of 0.25% of EMS at 48 hours, managed to maintain the best agronomic performance until 95 days, where the plant height had its best development similarly to the one determined as LD_{50} (0.32%).

The doses of ethyl-metha- sulfonate (EMS) had a direct effect on the seizure of the stolons of the janeiro grass, presenting a high deflation index in doses higher than 0.50%. Therefore, the best soak time was 48 hours.

The janeiro grass impregnated with EMS under greenhouse conditions showed slight damage caused by spittlebug. Thus, it cannot be stated that there was tolerance in this research work.

Literature cited

Akhtar, N. (2014). Effect of physical and chemical mutagens on morphological behavior of tomato (*Solanum lycopersicum*) cv. “Rio Grande” under conditions of heat stress. *Plant Breeding and Seed Science*, *70*(1), 69-79. https://doi: 10.1515/plass-2015-0014.

Ashok, V., Kumari, R., Amutha, R., Siva, T., Juliet, S. & Ananda, C. (2009). Effect of chemical mutagen on expression of characters in arid legume pulse–cowpea (*Vigna unguiculata* (L.) Walp.). *Research Journal of Agriculture and Biological Sciences*, *5*(6), 1115–1120. http://www.aensiweb.net/AENSIWEB/rjabs/rjabs/2009/1115-1120.pdf

Bhattarai, G., Schmid, R. & McCornack, B. (2019). Remote sensing data to detect Hessian fly infestation in commercial wheat fields. *Scientific Reports*, *9*, 6109. https://doi.org/10.1038/s41598-019-42620-0.

Castro, U., Morales, A. & Peck, D. (2005). Population dynamics and phenology of spittlebugs from *Zulia carbonaria* (Lallemand) pastures (*Homoptera: Cercopidae*) in the geographic valley of the Cauca River, Colombia. *Neotropical Entomology*, *34*(3), 459-470. https://doi.org/10.1590/S1519-566X2005000300015

Corrales-Lerma, R., Avendaño-Arrazate, C., Morales-Nieto, C., Santellano-Estrada, E., Villarreal-Guerrero, F., Melgoza-Castillo, A., Álvarez-Holguín, A. & Gómez-Simuta, Y. (2019). Radiación gamma para inducción de mutagénesis en pasto rosado (Melinis repens (Willd.) Zizka). *Acta Universitaria*, *29*, 1-10. https://doi.org/10.15174/au.2019.1847.

Cobos, F., Gómez, L., Reyes Borja, W., Hasang, E., Ruilova, M. & Duran, P. (2021). Effects of salinity levels in *Oryza sativa* in different phenological stages under greenhouse conditions. *Revista de la Facultad de Agronomía de la Universidad del Zulia*, *39*(1), e223905. https://doi.org/10.47280/RevFacAgron(LUZ).v39.n1.05.

Costa, A. (2017). Indución y evaluación de mutantes de alcachofa tolerantes a salinidad. Universitas Miguel Hernández. http:// http://dspace.umh.es/bitstream/11000/3445/1/Costa%20Ortiz%2C%20Aida%20TFGBiotec%202014-15.pdf.

Emrani, S., Arzani, A. & Saeidi, G. (2011). Seed viability, germination and seedling growth of canola (*Brassica napus* L.) as influenced by chemical mutagens. *African Journal of Biotechnology*, *10*(59), 12602-12613. https://doi.org/10.5897/AJB11.329.

Gómez, J., Aguirre, L., Gómez, L., Reyes, W., Rodríguez, J. & Arana, L. (2020). Dosis letal media para inducir mutaciones, con rayos gamma, en pastojaneiro (*Eriochloa polystachya* Kunth). *Revista de Producción Animal*, *32*(1), 73-83. https://revistas.reduc.edu.cu/index.php/rpa/article/view/e339.

González, M. (2014). Desarrollo de una plataforma de tilling en melón (*Cucumis melo* L.). Tesis Doctoral. Universitat Autónoma de Barcelona, Barcelona, España. 282 p. https://ddd.uab.cat/record/127025.

Greene, E., Codomo, C., Taylor, N., Henikoff, J., Till, B., Reynolds, S., Enns L., Burtner C., Johnson, J., Odden, A. & Steven, L. (2003). Spectrum of Chemically induced mutations from large-scale reverse genetic screening in Arabidopsis. *Genetics*, 164(2), 731–740. https://doi.org/10.1093/genetics/164.2.731.

Hasang, E., Gómez, J., Moreira, E. & Cobos, F. (2020). Variabilidad fenotípica, y desarrollo de estolones de pasto Janeiro (*Eriochloa polystachya* Kunth), irradiados a 52 gy de rayos gamma (60co). J*ournal of Science and Research*, *5*, 96-109. https://revistas.utb.edu.ec/index.php/sr/article/view/1000.

Khalil, F., Naiyan, X., Tayyab, M. & Pinghua, C. (2018). Screening of EMS-Induced Drought-Tolerant Sugarcane Mutants Employing Physiological, Molecular and Enzymatic Approaches. *Agronomy*, *8*(10), 226. doi:https://doi.org/10.3390/agronomy8100226.

Konzak, C., Nilan, R., Wagner, T. & Foster, R. (1965). Efficient chemical mutagenesis, the use of induced mutations in Plant Breeding (Rep. FAO/IAEA Tech. Meeting Rome, 1964). Porgamon Press, Oxford. p. 49–70.

Kumar, G. & Pandey, A. (2020). Ethyl metha- sulfonate-induced changes in cyto-morphological and biochemical aspects of *Coriandrum sativum* L. *Journal of the Saudi Society of Agricultural Sciences*, *18*(4), 469-475. https://doi.org/10.1016/j.jssas.2018.03.003.

López, E. (2011). Inducción de variabilidad genética para tolerancia a estreses abióticos mediante técnicas de cultivo *in vitro* en *Cenchrus ciliaris* L. Universidad Internacional de Andalucía, España. 113 p. https://core.ac.uk/download/pdf/72021262.pdf.

Mendoza, R. (2020). Inducción mutagénica en cultivares de *Eustoma grandiflorum* mediante el uso de metanosulfonato de etilo (EMS). Centro de Investigación y Asistencia en Tecnología y diseño del estado de Jalisco, A.C, México. https://ciatej.repositorioinstitucional.mx/jspui/bitstream/1023/746/1/Rafael%20Mendoza%20G%C3%B3mez%20Bio.pdf.

Mostafa, G. (2015). Effect of some chemical mutagens on growth, phytochemical composition and the induction of mutations in *Khaya senegalensis*. *International Journal of Plant Breeding and Genetics*, *9*(2), 57-67. https://doi:10.3923 / ijpbg.2015.57.67.

Nobel, P. (1999). Physicochemical & Environmental. Plant Physiology. Second Edition. Academic Press. San Diego, California, USA. 474 p. https://books.google.co.ve/books?id=yW_pptyiMHoC&dq=Nobel,+P.+(1999).&lr=&hl=es&source=gbs_navlinks_s.

Pankhurst, C, Blair, M & Broughton, W. (2004). Tilling the beans. In Proceedings of the 3rd International Scientific Meeting, Phaseomics III, pp. 13-15.

Porch, T., Blair, M., Lariguet, P., Galeano, C., Pankhurst, C. & Broughton, W. (2009). Generation of a Mutant Population for TILLING Common Bean Genotype BAT 93. *Journal of the American Society for Horticultural Science, 134*(3), 348-355. https://doi.org/10.21273/JASHS.134.3.348.

Rodríguez, N., Torres, C., Chaman, M. & Hidalgo J. (2019). Efecto del estrés salino en el crecimiento y contenido relativo del agua en las variedades IR-43 y amazonas de *Oryza sativa* "arroz" (Poaceae). *Arnaldoa, 26*(3), 931-942. https://dx.doi.org/10.22497/arnaldoa.263.26305.

Rojas, L., Collado, R., León, A., Rivero, L., Ocaña, B., Hernández, M., Veitía, N., Martirena, A., Torres, D. & García, L. (2016). Concentración óptima de metano sulfonato de etilo en *Phaseolus vulgaris* L. cv. ‘DOR 364’ para inducir variaciones fenotípicas. *Biotecnología Vegetal, 16*(3), 179-188. https://revista.ibp.co.cu/index.php/BV/article/view/526.

Sharma, S., Sood, R & Pandey, D. (2005). Studies on mutagen sensitivity, effectiveness and efficiency in urdbean [*Vigna mungo* (L.) Hepper]. *Indian Journal of Genetics and Plant Breeding, 65*(1), 20–22. https://www.isgpb.org/journal/index.php/IJGPB/article/view/1602.

Sotelo, G., Cardona, C & Miles, J. (2003). Desarrollo de híbridos de Brachiaria resistentes a cuatro especies de salivazo (Homoptera: Cercopidae). *Revista Colombiana de Entomología, 29*(2): 157-163. https://doi.org/10.25100/socolen.v29i2.9599.

Suthakar, V. & Mullainathan, L. (2015). Studies on Effect of Physical and Chemical Mutagens in Sorghum (Sorghum bicolor (L.) Moench) in M2 Generation". I*nternational Letters of Natural Sciences, 37*, 46-50. https://doi.org/10.18052/www.scipress.com/ILNS.37.46.

Yadav, P., Meena, H. S., Meena, P. D., Kumar, A., Gupta, R., Jambhulkar, S. & Singh, D. (2016). Determination of LD50 of ethyl methanesulfonate (EMS) for induction of mutations in rapeseed-mustard. *Journal of Oilseed Brassica, 1*(1), 77-82. http://srmr.org.in/ojs/index.php/job/article/download/33/32.