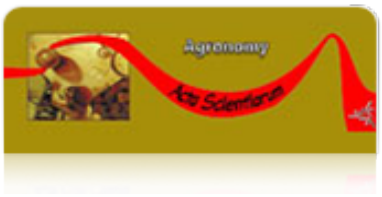

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Silicon and methyl jasmonate in the vegetative development and genetic stability of rice

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ABSTRACT. Given the high demand for food worldwide, options for maximizing food production with minimum environmental impact are needed. Therefore, the objective of the present study was to evaluate the effect of silicon (Si) and methyl jasmonate (MeJA) in the vegetative development and genetic stability of rice plants, BRSMG Caravera cultivar. The treatments used were T1 - Control group (without Si and MeJA); T2 - Tween foliar; and T3 - Si drench, T4 - MeJA foliar, and T5 - Si drench + MeJA foliar. The use of Si or Si + MeJA promoted an increase in the height, relative chlorophyll index (RCI), and fresh and dry masses, in addition to a greater Si accumulation in the plants. However, there was no difference in the quantity of DNA or in the coefficient of variation (CV) among the treatments, proving the use of silicon separately or in combination with methyl jasmonate contributed to the vegetative development and did not affect the genetic stability of the plants.

Keywords: silicic acid; jasmonates; *Oryza sativa* L.; quantification of DNA.

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Introduction

Rice (*Oryza sativa* L.) is one of the most produced and consumed cereal grains worldwide and is characterized as a main staple for over half of the world's population. The area under rice production exceeds 157.7 million hectares and yields 469.5 million tons. The main rice producing countries are China, India, and Indonesia (Aoki, Akai, & Ujiie, 2017). Brazil is eighth in the world ranking; however, it is also the first rice producing country outside of the Asian continent.

Proper management of fertilization is essential for increasing production and meeting the demand for this grain. Therefore, research on silicon sources and doses have been conducted to explore the potential of this element in the improvement of qualitative and quantitative attributes of rice crops (Chagas, Muraoka, Korndörfer, & Camargo, 2016).

Rice plants accumulate silicon and may present concentrations of this element that are higher than 10% of its dry mass (Yamamoto et al., 2012). In general, Poaceae use silicon in their tissues to increase vegetative growth and development, since this element improves the assimilation of nutrients (Yogendra et al., 2014), photosynthetic rate (Gautam et al., 2016), and the fresh and dry masses of their roots (Raza et al., 2016). In addition, it protects the plants against biotic stresses such as herbivory (Han, Lei, Wen, & Hou, 2015) and abiotic stresses, i.e., salinity (Liu et al., 2015), and promotes drought tolerance (Ahmed, Hassen, Qadeer, & Aslam, 2011).

Research on jasmonates has also been gaining increased attention. The jasmonic acid, which is derived from the linolenic acid, is a plant hormone of natural occurrence. The production of jasmonic acid depends on external stimulus and on gene expression (Wasternack, 2014). When applied in an exogenous manner, these compounds exhibit a crucial role in plant signaling responses to biotic and abiotic stresses (Fahad et al., 2014).

Jasmonates are also involved in several vegetative growth and development processes, such as the increase of fruit size and mass (Martínez-Esplá et al., 2014), chlorophyll pigment concentration, and the development of the root system (Awang, Ismail, Omar, & Islam, 2015). A recent study has shown

the interaction between methyl jasmonate and silicon in rice but only concerning the effects of plant defense against insects. Possible consequences on plant development were not examined in this study (Ye et al., 2013).

Some chemical treatments or stressful processes to which plants are subjected may alter the quantification of DNA. Flow cytometry is a fast and accurate technique used to estimate the quantity of DNA (Dolezel & Bartos, 2005) in several plant species such as *Passiflora edulis* Sims f. *flavicarpa* Deg. (Costa et al., 2016) and *Saccharum* spp. (Nogueira, Pio, Pasqual, Amaral, & Scherwinski-Pereira, 2015). Although the beneficial effects of both silicon and jasmonates in the development of plant species have been examined, studies on the effect of these substances, alone or in combination, in the quantification of Poaceae DNA are needed.

Therefore, the objective of the present study was to evaluate the effects of silicon and methyl jasmonate on the vegetative development and genetic stability of rice plants.

Material and methods

Location of the conduction of bioassays and cultivation of plant material

The rice was planted in 2 L polyethylene pots, containing 1.5 kg of soil (Dark Red Latosol), 0.23 g of NPK fertilizer (8-28-16) per pot, equivalent to 300 kg ha^{-1}, and 20 seeds of cv. BRSMG Caravera per pot were sown. Five days after germination, pruning was conducted, and only eight plants/pot remained. On the 30th and 60th day after the emergence of the plants, ammonium sulfate fertilizer was top-dressed at a rate of 0.045 g of N per pot, which is equivalent to 60 kg ha^{-1} (Soares et al., 2008). A phytosanitary control was not carried out.

Experimental design

A complete randomized design was conducted with five treatments and six replications, and each replication was composed of three plants. The treatments were as follows: T1 - Control (without silicon and methyl jasmonate), T2 - Tween foliar, T3 - Si drench, T4 - MeJA foliar, and T5 - Si drench + MeJA foliar.

Preparation and application of silicon, methyl jasmonate, and tween solutions

Silicon was applied through a silicic acid solution (SiO_2. XH_2O) (Vetec Química Fina, Duque de Caxias, Rio de Janeiro State, Brazil) at 1%, and an equivalent dosage of 1.0 t SiO_2 ha^{-1} was administered 30 days after emergence (Assis, Moraes, Auad, & Coelho, 2013); 0.75 g of silicic acid was diluted in 75 mL of water per pot, which was applied around the plant stem (drench). The pots of the other treatments (control, tween, and methyl jasmonate) received water in the same quantity.

The methyl jasmonate solution (Sigma-Aldrich, São Paulo, São Paulo State, Brazil) was prepared adding Tween®80 (polyoxyethylene sorbitan monostearate) (Sigma-Aldrich, São Paulo, São Paulo State, Brazil) as a solubilizing agent with concentration of 0.1 mL L^{-1} and 2.5 mL L^{-1} of ethanol, based on the methodology used by Moraes et al. (2009). The phytohormone concentration was altered to meet the 1.0 mM standard value, and the preparation of 1 L of the solution was divided in two stages. In the first, 2.5 mL L^{-1} of ethanol, 0.1 mL of Tween, 218 µL of methyl jasmonate, and 50 mL of distilled water were added to a 250 mL volumetric flask. This mixture was manually homogenized through agitation.

In the second stage, 300 mL of distilled water as well as the solution obtained in the first stage were placed in a 1 L test tube. After the mixture was manually homogenized by agitation, the test tube was filled with distilled water up to 1 L.

The foliar application of the phytohormone solution was conducted 39 days after emergence with the assistance of a 500 mL hand sprayer, using 25 mL of solution per plant, which was enough for a homogenous application.

In addition, a single tween foliar spray was conducted 39 days after the emergence of the plants to cancel out the effect of other substances in the results. The preparation of this solution was conducted as described previously in the first stage but without the use of methyl jasmonate.

Agronomic and physiological characteristics and silicon concentration

Three plants per pot were used to evaluate agronomic characteristics 80 days after emergence. The height of the plants was determined using a ruler. The fresh and dry masses of the aerial parts were measured with the assistance of a precision scale. To obtain the dry mass, the plants were cut off close to the soil surface, placed individually in paper bags (18 x 42 cm), and dried to a constant weight in an oven at 60°C.

Subsequently, the plants were ground in a Willey type knife mill (TECNAL, equipment for laboratories, Piracicaba, São Paulo State, Brazil), and the samples identified were wrapped in plastic bags (5 x 23 cm) and then were sent to the Laboratory of Mineral Nutrition of Plants at UFLA to determine the silicon concentration in the aerial part, based on the methodology of Korndorfer, Pereira, and Nolla (2004).

The SPAD-502 (Soil Plant Analysis Development) portable meter (Konica Minolta Sensing, Inc.) (TECNAL, equipment for laboratories, Piracicaba, São Paulo State, Brazil) was used to evaluate the relative chlorophyll index (RCI). The readings were conducted in the morning, between 8:00 am and 10:00 am, on three plants per pot, totaling 18 readings per treatment.

Genetic stability evaluated through flow cytometry

To determine the DNA content, three samples were used. Each sample was composed of 30 mg of young rice leaves (40 days after their emergence), along with the same quantity of tomato foliar mass (*Lycopersicum esculentum*), with a DNA quantity reference standard of 1.96 picograms (pg) (Doležel, Sgorbati, & Lucretti, 1992).

The leaves were cut on Petri dishes containing 1 mL of the Marie buffer comprising 50 mM glucose; 15 mM NaCl; 15 mM KCl; 5 mM Na_2.EDTA; 50 mM sodium citrate; 0.5% Tween 20; 50 mM HEPES (pH 7.2); and 1% (m/v) polyvinylpyrrolidone-10 (PVP-10) (Marie & Brown, 1993), to obtain a nuclear extract. This process was conducted under crushed ice to maintain the integrity of the nuclei. Subsequently, the material was suctioned using a Pasteur pipette and filtered in a 50 µm mesh. To the filtered material, 25 µg mL^{-1} of propidium iodide fluorochrome was added. After five minutes of adding fluorochrome, the samples were analyzed. Two readings were conducted in each sample, and 10,000 nuclei were read to estimate the DNA content (pg) and the CV.

The histograms were obtained using a FACSCalibur® cytometer (Becton Dickinson, Biosciences, San Jose, California), using the Cell Quest program. The nuclear DNA content of the plants was estimated through the ratio between the fluorescence intensity of the G1 nucleuses of the sample and the G1 nuclei of the reference standard, multiplying this ratio by the quantity of DNA of the reference standard. The histograms obtained were analyzed using a WinMDI 2.8 software.

Statistical analysis

The data were subjected to the analysis of variance and the means were compared through the Tukey test ($p < 0.05$), using the statistical software SAEG 9.0 (Ribeiro Júnior, 2001). Subsequently, the significant variables were obtained through Pearson's parametric linear correlation, and the data graphical analysis was conducted through the software SigmaPlot V.11.

Results

Agronomic and physiological characteristics and silicon concentration

There was an experimental accuracy regarding the CVs found in the statistical analysis. The treatments differ significantly between them ($p < 0.05$) for all agronomic and physiological characteristics evaluated. All plants subjected to treatments with silicon (Si) both separately or in combination with the methyl jasmonate (MeJA) presented an increase of 15.5% in their height in relation to the other treatments and an increase of 15.6% in the RCI compared with the control and tween groups. Therefore, the fresh and dry masses of these treatments were 36.4% and 36.9% higher, respectively, compared with the control, tween, and MeJA (Table 1).

Table 1. Height (cm), fresh and dry masses (g) of the aerial part and relative chlorophyll index (RCI) (SPAD) (mean±standard error) of rice plants (*Oryza sativa* L.) subjected to different treatments.

Treatments	Height*	Fresh mass*	Dry mass*	RCI*
Control	64.5±1.10 b	3.1±0.25 b	1.2±0.08 b	27.5±1.15 b
Tween	61.5±1.45 b	3.5±0.15 b	1.1±0.04 b	27.8±1.64 b
Si	73.4±2.52 a	5.2±0.48 a	1.7±0.15 a	32.7±1.08 a
MeJA	62.9±1.39 b	3.7±0.18 b	1.2±0.06 b	28.3±0.50 ab
Si + MeJA	75.6±1.71 a	5.6±0.29 a	2.0±0.05 a	32.8±0.97 a
Means	67.6	4.2	1.4	29.8
Test F	F = 14.32	F = 5.58	F = 14.53	F = 20.57
Valor *p*	*p* = 0.00000	*p* = 0.0024	*p* = 0.00000	*p* = 0.00000

*Means followed by the same letter in the same column are not significantly different according to Tukey's test ($p < 0.05$). Si: Silicon; MeJA: Methyl jasmonate.

Regarding the silicon concentration, the use of silicic acid both separately or in combination with MeJA provided a greater accumulation of this mineral in the leaves (Figure 1). The content of silicon found was 46.1% higher in the plants treated with Si + MeJA and 32.4% in the plants that received only silicic acid compared to the control group.

The relation between silicon content and rice agronomic and physiological characteristics was also examined, and this element correlated positively with all variables that were analyzed. Thus, the greater the silicon concentration in the plants was, the greater their height, RCI, and fresh and dry masses were (Table 2).

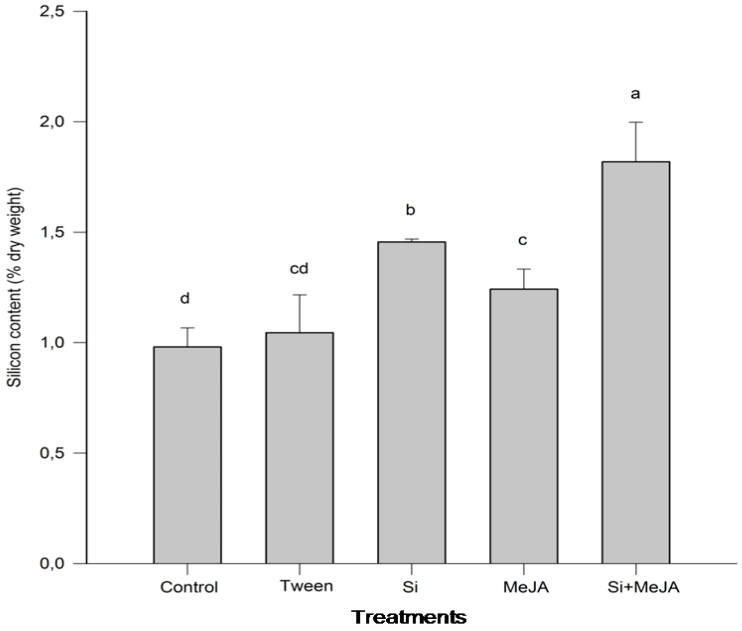

Figure 1. Silicon content (SiO_2) (%) (± standard error) in the dry mass of the aerial part of rice plants subjected to different treatments. Distinct letters in the columns show significant differences, based on the Tukey test ($p < 0.05$). The vertical bars show standard error (±). Si: Silicon; MeJA: Methyl jasmonate.

Table 2. Pearson's parametric linear correlation between silicon content and agronomic and physiological characteristics of rice plants.

Parameters	Height*	Fresh mass*	Dry mass*	RCI*
Silicon content	0.68	0.73	0.78	0.57
p < 0,05	0.0000338	0.00000557	0.000000295	0.00111

*Significant according to the t test ($p < 0.05$).

Genetic stability analyzed through flow cytometry

Regarding the quantification of DNA and the CV in the leaves, there was no significant difference ($p > 0.05$) between the treatments. The mean values obtained for DNA and the CV in the leaves were 0.97 pg and 0.71%, respectively (Table 3).

Table 3. DNA content and CV of rice leaves (*Oryza sativa* L.) subjected to different treatments and analyzed through flow cytometry.

Treatments	DNA content (pg) ns	CV (%) ns
Control	0.99	0.76
Tween	0.94	0.73
Si	0.98	0.66
MeJA	0.98	0.72
Si + MeJA	0.95	0.69
CV (%)	3.05	13.44

ns: Non-significant, based on the Tukey test ($p < 0.05$). Si: Silicon; MeJA: Methyl jasmonate.

Regarding the histograms, the choice of using tomato leaves as the reference standard, which presents DNA content of 1.96 pg, promoted the formation of its peak in a channel close to the one of the sample (*O. sativa*), without overlapping with the peak of the sample. The closer the peaks were, the lower the experimental error was. Therefore, the histograms show the formation of fine peaks, which confirms the reliability of the results obtained in the present study regarding the quantification of DNA, due to low CV values (Figure 2).

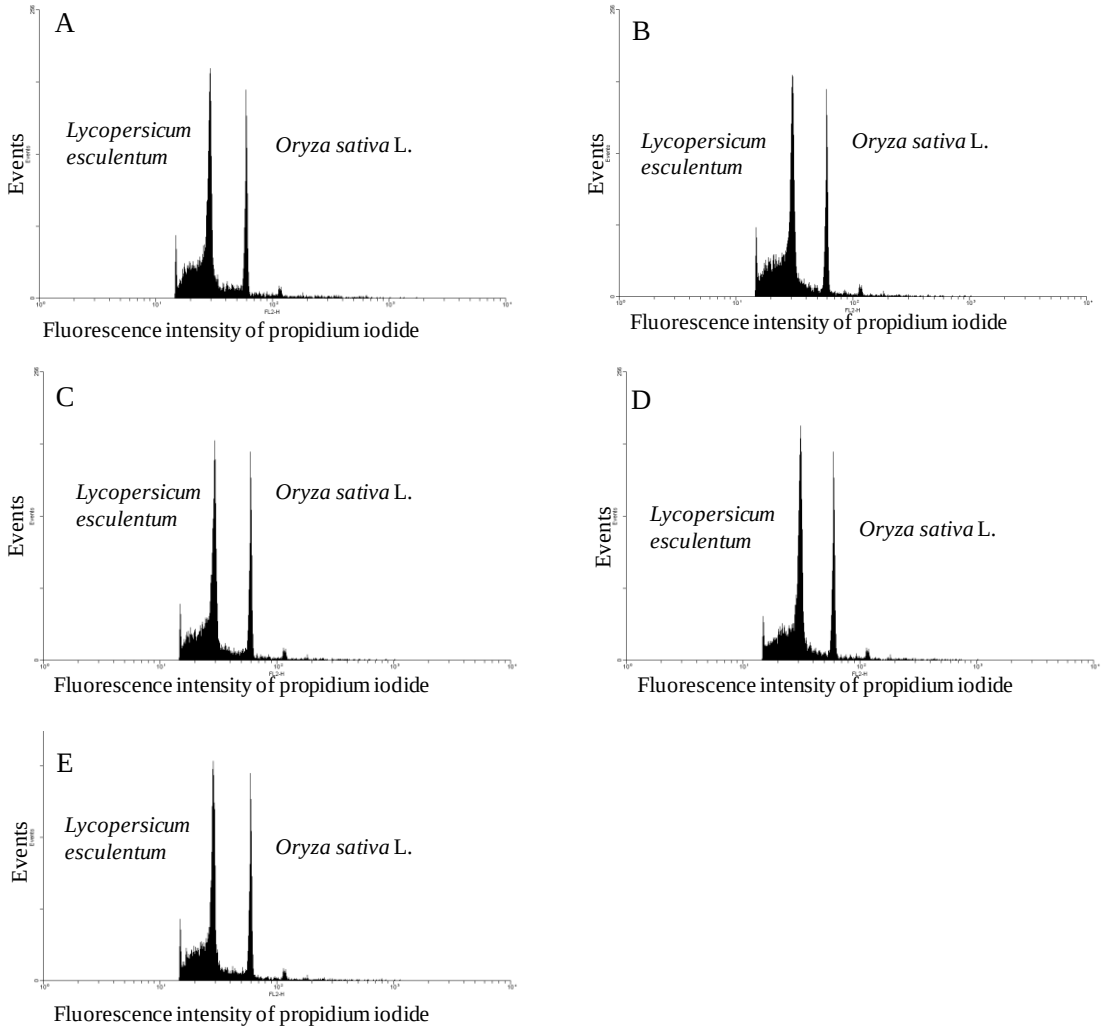

Figure 2. Histograms obtained through cytometric analysis of rice plants subjected to different treatments. (A) Control, (B) Tween, (C) Silicon, (D) Methyl jasmonate, and (E) Silicon and Methyl jasmonate.

Discussion

In the present study, higher concentrations of silicon were found in the treatments that received applications of silicon, and the highest value found was examined when silicon was used in combination with methyl jasmonate (Figure 1).

In general, the difference in the accumulation of silicon among different plant species has been explained by the specific ability of roots to absorb this element and by its availability in the soil solution (Ma & Yamaji, 2006; Henriet, Bodarwé, Dorel, Draye, & Delvaux, 2008).

Rice absorbs silicon in an active metabolic manner through specific carriers of Lsi1 influx and Lsi2 efflux. These two carriers are located in the roots and are responsible for the high capacity of rice to absorb silicon (Ma & Yamaji, 2006; Ma et al., 2007). This may explain the high levels of silicon found in plants, reaching values higher than 10% of the dry weight of the aerial part (Yamamoto et al., 2012). Therefore, in this study, it was confirmed that rice accumulates silicon, although the rates of this accumulation may vary within the same species (Ma & Yamaji, 2008).

Similar to what was observed in the present study (Figure 1), Ye et al. (2013) determined that the exogenous application of methyl jasmonate increased Lsi1, Lsi2, and the silicon content in rice leaves, showing that this element's effects are modulated by jasmonic acid. This study showed a strong relation between silicon and the jasmonic acid defense signaling pathway. This route is associated with plant defense responses to biotic stressors. Thus, rice and other plants that accumulate high levels of silicon may have developed defense mechanisms that were mediated by jasmonic acid, in which silicon is an integrated component (Ye et al., 2013).

The accumulation of silicon that was observed on the plants revealed possible positive correlations possible between the concentration of this element and agronomic and physiological characteristics, i.e., the greater the silicon concentration in the plants was, the greater their height, RCI, and fresh and dry masses were (Table 2). The same increase of agronomic characteristics that were examined in the present study was observed for wheat (*Triticum aestivum* L.), in which the increase in the foliar area and dry mass was positively correlated with the silicon concentration in the plants, although the chlorophyll concentration was not increased (Perez, Rodrigues, Moreira, & DaMatta, 2014). In contrast, Ávila, Baliza, Faquin, Araújo, and Ramos (2010) determined that rice plants subjected to the application of potassium silicate showed an increase in the RCI and in the chlorophyll contents a and b.

The use of silicon separately or in combination with methyl jasmonate positively affected all agronomic and physiological characteristics evaluated (Table 1). These results corroborate the findings of Ahmed et al. (2011), when they observed that the silicate fertilization increased the chlorophyll content, total dry mass, and *Sorghum bicolor* L. leaf area. Isa et al. (2010) also showed that the use of silicic acid increased the plant height and the chlorophyll index in different rice varieties.

After the absorption of the silicon available in the soil solution, this element moves through the flow of water transpiration in xylem to the aerial part and settles on the epidermal tissues as silica (Ma & Yamaji, 2006). The layer of silica that settled on the plant strengthens the plant wall, making the leaves more upright. Thus, there is an increase of light interception, which leads to an optimization of the photosynthetic apparatus (Tamai & Ma, 2008; Isa et al., 2010). In addition, the beneficial effects of silicon may also be attributed to the improvement in the water use efficiency and to the cell elongation, promoting the plant's growth and, consequently, the increase of biomass (Hossain et al., 2002; Isa et al., 2010).

Several studies have been conducted on the contribution of exogenously applied methyl jasmonate to the morphological/physiological characteristics of crops, including pepper *Capsicum annuum* L. (Awang et al., 2015), rice (Hsu, Chao, & Kao, 2013), and wheat (Anjum et al., 2016). However, in the present study, behavioral discrepancies were found since methyl jasmonate did not provide an increase in these characteristics when observed alone. Thus, the main contribution of methyl jasmonate is its ability to maximize the accumulation of silicon in plants. Silicon is responsible for the improvement of morphological/physiological characteristics.

Regarding the quantification of DNA and the CV, there was no significant difference for these attributes in the different treatments (Table 3). Therefore, the use of silicon and methyl jasmonate both separately or in combination, did not alter the plant DNA content, which confirms that, although these substances contribute for improving rice agronomic and physiological characteristics, they do not interfere in the genetic stability of rice plants. Another positive aspect is that the CVs ranged between 0.66% and 0.76% (Table 3), showing the reliability on estimating the plant DNA quantification obtained in the leaves. The results found in this study are considered safe, since the quality of cytometric analyses is based on their CVs (Galbraith, Lambert, Macas, & Dolezel, 2001).

The lower the CV was, the higher the quality of the analyses were and the more reliable the results obtained from flow cytometry are. Values for this parameter that are smaller than 5% are considered of high resolution to estimate the nuclear DNA content on plants (Dolezel & Bartos, 2005). In addition, the fine peak thickness obtained in the histograms was another indicator of the quality of the results found (Figure 2). Although this is in reference of another plant species, the results corroborate Costa et al. (2016), findings in which the use of silicic acid secured the genetic stability of yellow passion fruit vine (*P. edulis* Sims. f. *flavicarpa* Deg.), i.e., there was no alteration either in the quantification of DNA or in the CV of this fruit plant by this element.

Although the effect of the use of methyl jasmonate in the quantification of plant DNA was not observed in the present study, Pauwels et al. (2008) stated that this compost inhibited the cell cycle and positively correlated with the quantity of cells that was found in the G2 stage in the process of cell division, which promoted a lower growth of calluses Arabidopsis (*Arabidopsis thaliana*). In addition, Noir et al. (2013) determined that the presence of methyl jasmonate altered the nuclear DNA content in this same plant species, since it delays the start of the endoreduplication.

Therefore, as stated previously, plants under stressful conditions or subjected to chemical treatments are more susceptible to genetic instability. Some species presented an altered DNA content when evaluated through flow cytometry; these species include sugar cane, Saccharum spp. (Nogueira et al., 2015); red pitaya, Hylocereus undatus (Lopes et al., 2016; Menezes, Pio, Ramos, Pasqual, & Setotaw, 2016); and turmeric, Curcuma longa (Antoniazzi et al., 2016).

The results of the present study show a strong relation between silicon and methyl jasmonate that improves agronomic and physiological characteristics in plants, and these may be relevant elements in optimizing rice production.

Conclusion

The use of silicon separately or in combination with methyl jasmonate contributes to the vegetative development and does not affect the genetic stability of rice plants.

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