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Pineapple plantlets 'Vitória' fertilized with potassium chloride and silicic acid: growth and nutritional composition

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ABSTRACT

The objective of this study was to evaluate the effect of silicon and potassium fertilization on the growth and nutrient content of pineapple plantlets 'Vitória' obtained from in vitro cultivation. The experiment was conducted in a greenhouse from November 2013 to April 2014, totaling 150 days. The experimental design was randomized blocks, in a 4 x 2 factorial scheme, with four doses of potassium chloride (0, 1.25, 2.5 and 3.75 g L^{-1}) with or without the application of silicic acid, with five replications of 12 plants each. The silicon concentration in pineapple plants ranged from 11.57 to 16.97 g kg^{-1}, and an increase of 33% in the nutrient levels in plants fertilized with the lowest dose of potassium chloride was observed. It was observed that increasing doses of potassium chloride together with silicic acid resulted in lower root volumes in pineapple plantlets. Potassium accumulation of pineapple plantlets fertilized with silicic acid was lower than that of non-fertilized plantlets.

Key words: acclimatization; *Ananas comosus* var. *comosus*; fertilization; plantlets production; potassium; silicon

Mudas de abacaxizeiro 'Vitória' fertilizadas com cloreto de potássio e ácido silícico: crescimento e composição nutricional

RESUMO

O objetivo deste trabalho foi avaliar o efeito da fertilização de silício e de potássio no crescimento e no conteúdo nutricional de mudas de abacaxizeiro 'Vitória' oriundas de cultivo in vitro. O experimento foi realizado em casa de vegetação no período de novembro de 2013 a abril de 2014, totalizando 150 dias. O delineamento experimental foi em blocos ao acaso, em esquema fatorial 4 x 2, com quatro doses de cloreto de potássio (0, 1,25, 2,5 e 3,75 g L^{-1}) com ou sem aplicação de ácido silícico e com cinco repetições. A unidade experimental foi composta por 12 plantas. A concentração de silício em plantas de abacaxizeiro variou de 11,57 a 16,97 g kg^{-1} e observou-se um aumento de 33% nos níveis de nutrientes em plantas fertilizadas com a menor dose de cloreto de potássio. Observou-se que as doses crescentes de cloreto de potássio em conjunto com o ácido silícico resultaram em menores volumes radiculares nas mudas. O acúmulo de potássio nas mudas de abacaxizeiro fertilizado com ácido silícico foi menor que o das mudas não fertilizadas.

Palavras-chave: aclimatização; *Ananas comosus* var. *comosus*; fertilização; produção de mudas; potássio; silício

Introduction

Pineapple is a tropical plant of significant economic importance in Brazil, mainly for generating employment in agriculture and for its high consumer demand, since the fruit can be consumed *in natura* and/or processed. Among the pineapple-producing regions, the Rio de Janeiro State stands out as the sixth largest one, with average productivity of 22 thousand fruits per hectare (IBGE, 2015). This low productivity is influenced mainly by the occurrence of the major fungal disease affecting pineapple, fusariosis, caused by the etiologic agent *Fusarium subglutinans*. Thus, the use of cultivars resistant to this disease, such as ‘Vitoria’, can be a more viable alternative for producers of this culture (Ventura et al., 2009).

Another factor that also contributes to the low productivity of pineapple is the attack of the mealy bug (*Dysmicoccus brevipes*), which is harmful to the development of the plant by sucking sap. This animal is also a vector of a complex disease of viral origin known as pineapple wilt. The most effective way to prevent the spread of this pest is the use of quality plantlets (Sanches, 2005).

For this purpose, the first step is the implantation of new crops with plantlets that present high morphological, physiological and plant health quality standard. The spread of pineapple through plant tissue culture allows the producer to obtain thousands of plantlets from a single bud in a shorter length of time. However, it is noteworthy that these plantlets need to go through a long acclimatization process and subsequent acclimatization after coming out of *in vitro* condition (Baldotto et al., 2010), putting up the plantlets price and making it not economically feasible for pineapple producers.

Advances in plant propagation methods are increasingly present in agriculture, in the pursuit of alternatives to increase the quality and quantity of plantlets (Baldotto et al., 2010; Freitas et al., 2012; Santos et al., 2011a, b). The addition of appropriate doses of nutrients such as potassium and/or the application of silicon (Asmar et al., 2013) can provide promising results in the propagation of pineapple, since success has been reported when these nutrients are applied in isolation to other fruits (Barnes et al., 2009).

Silicon is considered an agriculturally beneficial element for many Liliopsidas and some Magnoliopsidas (Guntzer et al., 2012). After being accumulated, silicon provides anatomical changes in their tissues, such as the emergence of thick epidermal cells (Sujatha et al., 2013), as a consequence of silica deposition (Asmar et al., 2011), resulting in the formation of a mechanical barrier capable of hindering the attack of sucking (Gomes et al., 2005) and chewing (Hunt et al., 2008) insects besides preventing fungi penetration (Epstein, 1999). It can also act *as an activator* of *plant* defense and resistance *reactions* via production of phenolic and tannins (Ma & Yamaji, 2006), which can act as inhibitory substances to pathogens.

Unlike silicon, potassium is essential for the development of plants (Ramos et al., 2011) and, in the pineapple plant, it is the most absorbed mineral nutrient. It takes part directly or indirectly in numerous biochemical processes involved in the metabolism of carbohydrates, such as photosynthesis and respiration, and its lack is reflected in a low growth rate (Römheld et al., 2010). In many cases, potassium deficient plants tend to be more susceptible to attack by pests (Sarwar, 2012) and diseases (Wang et al., 2013). However, the effects of the application of combined doses of potassium and silicon, aiming the production of fruit quality plantlets, need to be searched.

In this sense, the objective of this work was to evaluate the effect of potassium chloride and silicic acid on growth and nutritional composition of pineapple plantlets ‘Vitória’ from *in vitro* cultivation.

Materials and Methods

The experiment was conducted from November 2013 to April 2014, totaling 150 days in the greenhouse with black shade cloth (50%) protected with transparent plastic of 150 microns at its top.

The experimental design was randomized blocks, in factorial scheme 4x2, four doses of potassium chloride (0; 1.25; 2.5 and 3.75 g L^{-1}) with or without the use of silicic acid, with five replications and 12 plants per experimental unit.

During the experiment period, minimum temperature ranged from 19.69^{o}C to 24.55^{o}C, average temperature was 22.03^{o}C and maximum temperature ranged from 24.02^{o}C to 45.17^{o}C with an average of 34.58^{o}C.

Plantlets of the pineapple cv. ‘Vitória’ used for the experiment deployment were produced by Biomudas Laboratory located in the municipality of Venda Nova do Imigrante - ES. They were transplanted to 128-cell polystyrene trays previously filled up with quartz sand, and set up for 15 days under intermittent mist. After that period they were conducted with sprinkler system for 135 days with deionized water for acclimatization.

The fertilization with potassium chloride was initiated at 20 days after transplanting of plantlets and in intervals of 30 days during the experiment. Very pure silicic acid was used for the supply of silicon, which was applied to at 30 days after transplanting of plantlets and in intervals of 30 days during the experiment. Both the fertilization with silicic acid and the potassium chloride were performed in the substrate, and deionized water was applied to control.

At 150 days after transplanting, pineapple plantlets were collected to carry out the following measurements: plantlets height (measured with graduated scale, from the base to the end of the larger leave); leaf area (*bench-top leaf area meter Li-3100*, *LICOR*, Lincoln, NE, USA); root surface area, root average diameter and root volume (obtained after scanning and analysis in software Winrhizo); shoot and root dry mass (obtained after drying in a forced ventilation over at 70°C for three days).

After drying, shoot samples were ground in a Wiley mill to pass a 20-mesh sieve and stored in hermetically sealed bottles, for subsequent assessment of the levels of silicon (Si), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S).

N concentrations were determined by sulphur digestion of the plant material. Next, analyses were carried out by Nessler method (Jackson, 1965). The levels of P, K, Ca, Mg and S were determined using a Shimadzu ICPE-9000, after $HClO_4$-*HNO_3 digestion* in open digestion system (Peters, 2005). The silicon (Si) extraction process in the plant was determined by the yellow method, according to the methodology described by Elliott & Snyder (1991).

Data were subjected to analysis of variance (F-test). Averages obtained for the factor silicic acid were compared by F-test ($p = 0.05$), while the averages obtained for the dose of potassium chloride were subjected to regression analysis ($p = 0.05$).

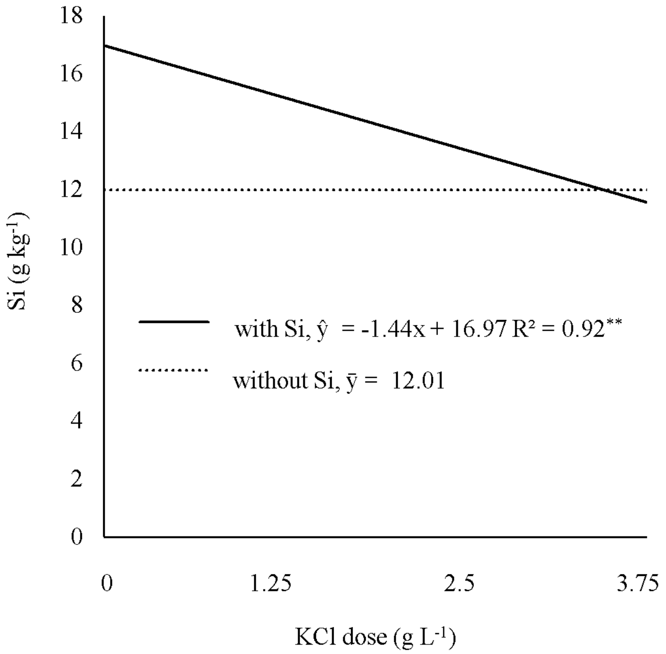
Results and Discussions

Table 1 shows the ANOVA summary with F values for each variable measured. It is observed that, with the exception of variable contents of nitrogen, phosphorus, calcium, magnesium and sulphur, there was significant interaction between the factors studied.

Silicon levels in pineapple plantlets ranged from 11.57 to 16.97 g kg^{-1}, and an increase of 33% in content of the plantlets with silicic acid and non-fertilized with potassium chloride was found (Figure 1). This result shows that despite the silicon source studied in this work is slightly soluble in water, the increase in Si content in pineapple plantlets is effective in providing Si to the pineapple plantlets ‘Vitória’ at this stage.

The presence of silicon in pineapple plantlets cv. ‘Vitória’ that had not been supplemented with silicon can be explained by the fact that silicon is the second most abundant element in nature (Ma & Yamaji, 2006) and, thus, is present everywhere, including in irrigation water.

Superficial area, average diameter, and dry matter weight of pineapple plants were not affected by silicic acid and potassium chloride (KCl) doses application. Nevertheless, the plants fertilized with of 3.75 KCl had higher values of superficial area and diameter of roots, while the highest root dry matter weight value was obtained without application of KCl (Figure 2).



* significant at $p < 0.05$

Figure 1. Silicon content in pineapple plantlets ‘Vitória’ at 150 days after transplanting, in relation to potassium chloride doses in the with or without of silicic acid.

It was observed that an increase in the doses of KCl in pineapple plantlets ‘Vitória’ fertilized with silicic acid has provided smaller root volume values. While in pineapple plantlets ‘Vitória’ that did not receive fertilization with silicic acid, the increase in the doses of KCl provided greater values of root volume (Figure 2C).

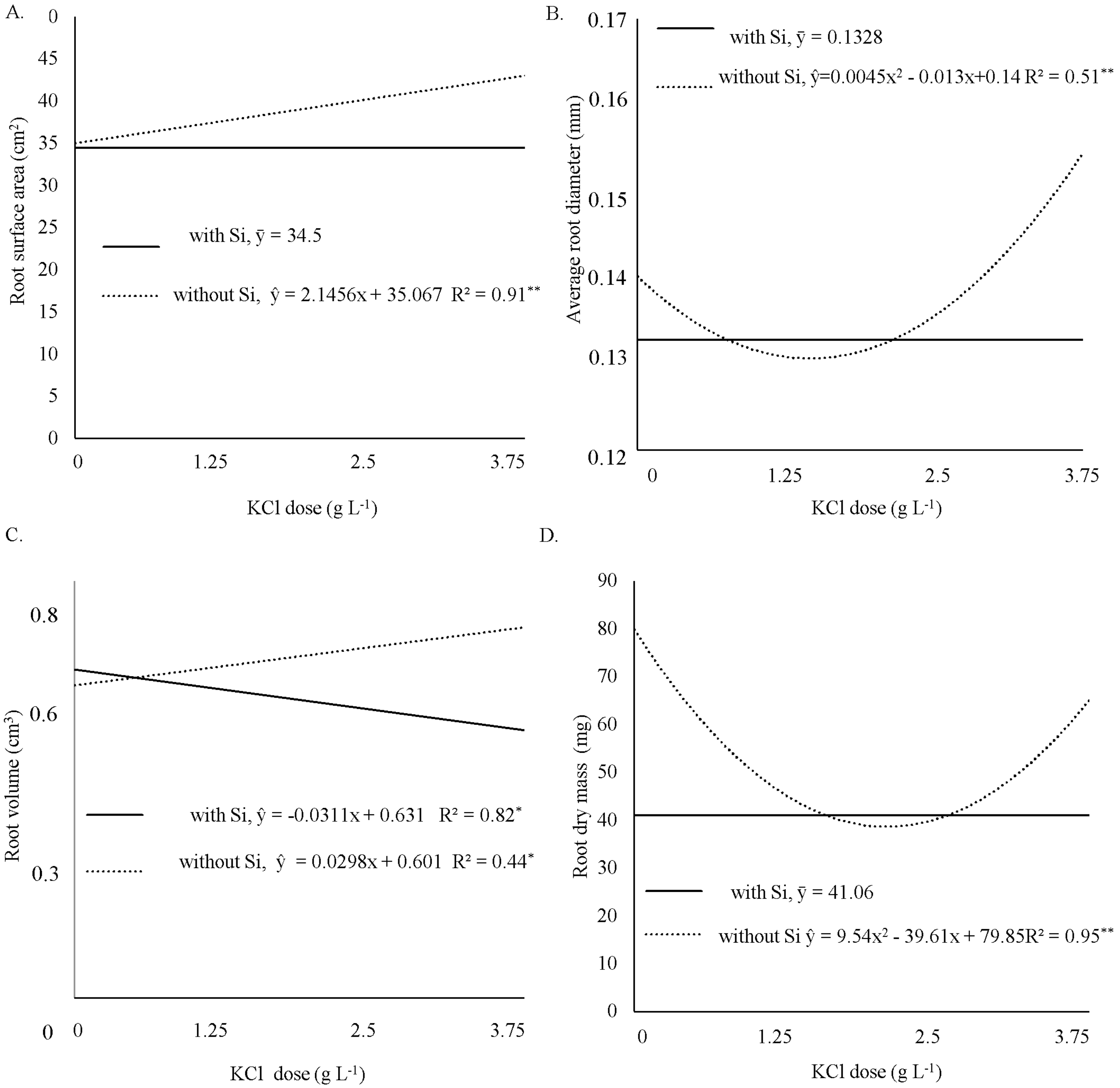
By evaluating the shoot area, it was found that an increase in KCl doses reduced the plantlets height of pineapple ‘Vitória’ when fertilized with silicic acid, and it was found a 16.07% reduction in fertilized plantlets at the highest dose of KCl (Figure 3).

Pineapple plantlets ‘Vitória’ also presented reduction of 12.25% and 17.55% in leaf area and dry mass, respectively, regardless the KCl dose (Table 2).

Table 1. Summary of the analysis of variance for plantlets height, leaf area, shoot dry mass, root dry mass, root surface area, average root diameter, root volume, and contents of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur and silicon in pineapple plantlets ‘Vitória’ at 150 days after transplanting, in relation to potassium chloride doses in the with or without of silicic acid.

Variation causes	F values						
	Plantlets height (cm)	Leaf area (cm^2)	Shoot dry mass (mg)	Root dry mass (mg)	Root surface area (cm^2)	Average root diameter (mm)	Root volume (cm^3)
Silicic acid	12.4183**	5.4197**	13.7147**	21.4205**	7.7417**	2.9642^{ns}	8.4191**
KCl doses	1.5444^{ns}	1.704^{ns}	1.0831^{ns}	5.6868**	0.3559^{ns}	2.5787^{ns}	1.624^{ns}
Silicic acid x KCl doses	4.3789**	1.1561^{ns}	2.7197^{ns}	8.6571**	3.2661**	3.2730**	3.2715**
Overall Average	8.37	50.00	77.95	49.4	36.79	0.137	0.614
CV (%)	7.72	15.85	14.72	20.64	12.69	9.1	13.39
Variation causes	F values						
	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulphur	Silicic
	mg $plant^{-1}$						
Silicic acid	6.4063**	11.5279**	46.3618**	10.5998**	17.1038**	10.5089**	17.58**
KCl doses	0.2958^{ns}	1.4199^{ns}	67.0802**	1.6813^{ns}	0.8116^{ns}	0.5910^{ns}	8.1376**
Silicic acid x KCl doses	0.4109^{ns}	0.8290^{ns}	5.3539**	0.9863^{ns}	0.8051^{ns}	0.7976^{ns}	4.1329**
Overall Average	0.881	0.0417	1.43	0.341	0.109	0.0618	13.15
CV (%)	17.92	24.34	12.64	20.22	16.22	19.24	11.61

^{ns}not significant; * significant at $p < 0.05$; **significant at $p < 0.01$ by the F test



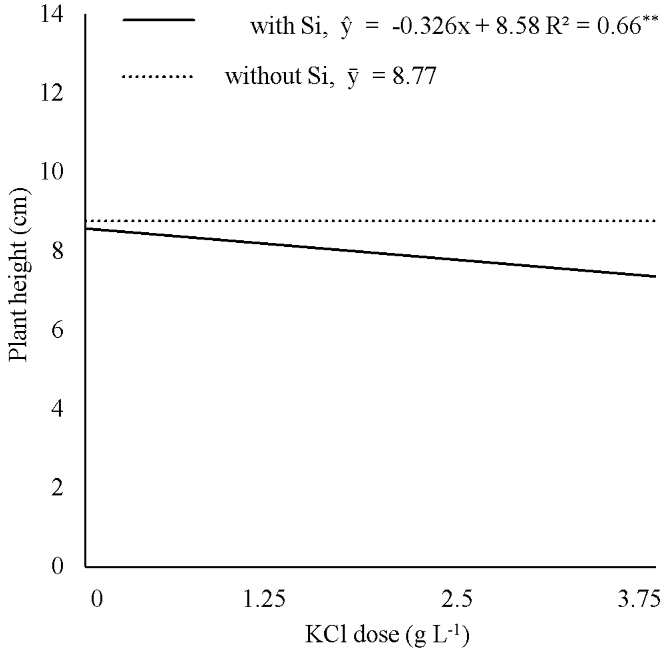
*significant at $p < 0.05$; **significant at $p < 0.01$ by the F test

Figure 2. (A.) Root surface area, (B.) root average diameter, (C.) root volume (D.) root dry mass of pineapple plantlets 'Vitória' at 150 days after transplanting, in relation to potassium chloride doses in the with or without of silicic acid application.

Silicon can be provided to plants through diverse sources, the most researched and used one is the silicate. It's mainly delivered through the ground, through the use of steel slag in the form of calcium and magnesium silicates (Sousa et al., 2007). The more the plant absorbs silicon the greater are its beneficial effects, and no toxic effect on plants has been verified, without any researches related to limits for the application of that element (Korndörfer et al., 2004). However, this study indicates that phytotoxicity may have occurred caused by application of silicic acid in pineapple plantlets 'Vitória'. Silicate fertilizing presented lower growth of pineapple plantlets 'Vitória' in addition to less accumulation of nitrogen, phosphorus, calcium, magnesium and sulphur (Table 2).

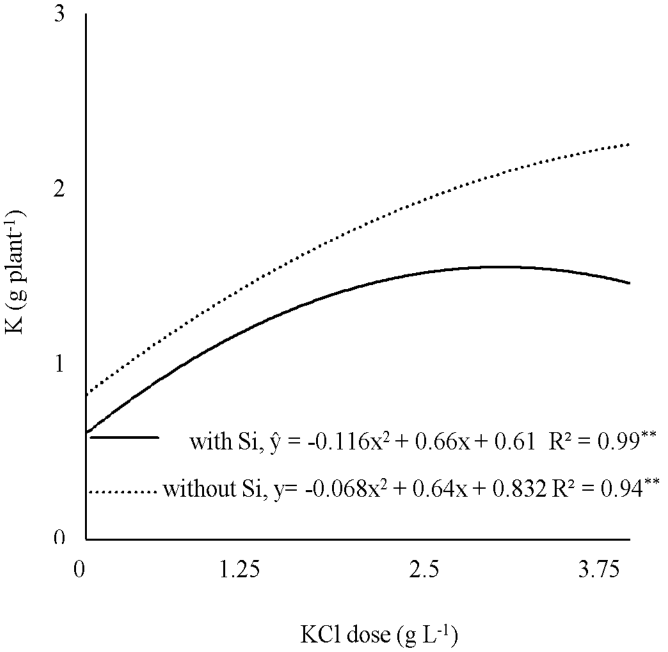
Silicon accumulated in the epidermal cells and stomata walls is in the H_4SiO_4 (monosilicic acid) form, when silicon polymerizes itself, the flexibility of stomata walls decreases and they tend to remain closed. Thus, transpiration decreases as well as the loss of water (Luz et al., 2006). As a result, the photosynthesis decreases and this can lead to lower growth of pineapple plantlets 'Vitória' grown with silicic acid (Figures 3 and 4).

Another factor that may have led to negative effects on plantlets growth of pineapple 'Vitória' was the physical impediment formed after interaction between plantlets cultivation substrate and silicic acid fertilization, which was worsening as more fertilization were carried out. This may



*significant at $p < 0.05$; **significant at $p < 0.01$ by the F test

Figure 3. Height of pineapple plantlets 'Vitória', at 150 days after transplanting, in relation to potassium chloride doses in the with or without of silicic acid application.



*significant at $p>0.05$; **significant at $p<0.01$ by the F test

Figure 4. Potassium content in pineapple plantlets 'Vitória' at 150 days after transplanting, in relation to potassium chloride doses in the with or without of silicic acid application.

Table 2. Leaf area, shoot dry mass, contents of nitrogen, phosphorus, calcium, magnesium and sulphur in pineapple plantlets 'Vitória' at 150 days after transplanting in relation to the with or without of silicic acid application.

Silicic acid	Leaf area (cm^2)	Shoot dry mass (mg)	Nutrient Contents				
			Nitrogen	Phosphorus	Calcium	Magnesium	Sulphur
			mg plant^{-1}				
with	46.74 b	70.44 b	0.810 b	0.0356 b	0.3016 b	0.0962 b	0.05375 b
without	53.27 a	85.44 a	0.952 a	0.0478 a	0.3811 a	0.1220 a	0.06975 a

Averages followed by the same letter in the columns do not differ from each other, based on the F-test ($p < 0.05$).

have compromised root expansion of pineapple plantlets 'Vitória', since that may have diminished the flow of nutrients and water to the shoot of the plantlets. In addition, the plantlets from *in vitro* cultivation are more sensitive and have less ability to tolerate nutritional stress.

Luz et al. (2006), in order to evaluate the effect of silicate fertilizer (silica gel source) on the performance of nine varieties of lettuce grown in nutrient solution on hydroponic system, found that these showed smaller shoot diameter, less fresh and dry mass of shoot and root. According to the authors, this probably occurred because silicon interfered in the absorption of an essential nutrient.

Regarding potassium content, it turns out that pineapple plantlets 'Vitória' that have been fertilized with silicic acid had smaller amounts of this nutrient in relation to non-fertilized ones (Figure 4).

Crusciol et al. (2013) verified that silicon application has provided less potassium leaf content in bean crops, when evaluating the effect of foliar silicon application, in stabilized silicic acid form, on nutrition and productivity of crops of soybeans, beans and peanuts. However, there was no effect of the treatments on foliar levels of nitrogen, phosphorus, calcium, magnesium and sulphur.

Pei et al. (2010) applied sodium silicate in *Triticum aestivum* and found that potassium levels were lower when the plants received silicate fertilization.

In the literature, there are no ranges of silicon levels recommended for soils specifying their disability, suitability or excess regarding pineapple crops. However, with the results of this study, it was possible to verify that the silicic acid dose used promoted negative effect on the crop. It is suggested that further work should be carried out, evaluating de effects of different sources and dosages of silicon on pineapple plantlets growth, as well as monitoring irrigation water in order to recognize the influence on silicon quantity provided by this method.

Conclusions

Fertilization with potassium chloride increases surface area, root diameter and volume of pineapple plantlets 'Vitória' in the acclimatization phase, which contributes to greater nutritional absorption.

Fertilization with silicic acid reduces growth, accumulation of nitrogen, phosphorus, potassium, calcium, magnesium and sulphur on pineapple plantlets 'Vitória' at acclimatization phase, thus the use of the dosage applied in this study is not recommended.

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