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Silicate fertilization in non-conventional vegetables in the southern region of Minas Gerais in Brazil[1]

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ABSTRACT

Considering that the beneficial effects of silicon on human health and it´s accumulation in plants are little explored, studies of mineral nutrition in non-conventional vegetables are extremely relevant. Thus, the objective of this work was to identify non-conventional vegetables responsive to silica fertilization and evaluate the effect of fertilization on the content and accumulation of Si and macronutrients in these plants. The experiment followed a 4 x 5 factorial design, with four species of non-conventional vegetables (*Rumex acetosa* L., *Amaranthus retroflexus* L., *Sonchus oleraceus* L. and *Stachys byzantina*) and five doses of Si (0, 25, 50, 100 and 150 mg dm^{-3}). The relative chlorophyll index, the fresh and dry matter masses, the content and accumulation of Si and macronutrients were evaluated. The species *A. retroflexus*, *S. oleraceus*. and *S. byzantina* showed the highest contents and accumulation of Si. The application of doses above 100 mg dm^{-3} promoted the highest silicon content estimated for the species. The species *A. retroflexus*, *R. acetosa* and *S. byzantina* are non-silicon accumulators, whereas *S. oleraceus* is intermediate.

Keywords: *Amaranthus retroflexus* L.; *Rumex acetosa* L.; *Stachys byzantine*, *Sonchus oleraceus* L.; minerals.

INTRODUCTION

Silicon (Si) is the second most abundant element in the Earth's crust wich the average content in soil ranging from 25 to 35%; however, in soils in which the weathering process is very strong can contain less than 1% (Lima Filho, 2010). For plants, the main source of Si in soil is available in the form of silicic acid (H_4SiO_4) (Epstein & Bloom, 2006).

Although Si is not characterized as essential for plants, several studies show that their growing performance in environments with Si availability is favored despite their stress, and are superior in comparison to those grown in soils with a low availability of this mineral. This fact is mainly attributed to Si's capacity to promote tolerance to biotic and abiotic stresses in plants (Zanetti *et al.*, 2016; Peixoto & Junior, 2017).

Another important characteristic of Si which has aroused the curiosity of researchers concerns the essentiality of the mineral and its benefits to human health. It performs several functions in the human body, with the maintenance of bone health being undoubtedly the most relevant and of greater interest by scholars, especially due to the large incidence and diversity of diseases related to postmenopausal women and the elderly (Rodella *et al.*, 2014). Therefore, Si is an important mineral for both plants and humans. Vegetables can be favored by silicate fertilization, as well as becoming an excellent source of Si in human diets.

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In this context of healthy eating, non-conventional vegetables have been gaining space and becoming an option as a source of vitamins, minerals, proteins and fiber (Silva *et al.,* 2018c). These species include a series of plants that have been widely appreciated as part of family meals, but over the years have been forgotten or devalued, mainly by the rural exodus, which led people to consume more processed foods (Silva *et al.,* 2018a; Silva *et al.,* 2018b; Xavier *et al.,* 2019).

Despite the beneficial effects of Si on various crops and its importance to human health, little is known about its influence on the growth and mineral composition of plants, especially those characterized as non-conventional, which have an important contribution to diet, economy, and culture of traditional rural communities. The hypothesis is that plants may be favored with silicate fertilization and that non-conventional vegetables enriched with this element may supply the need for populations in situations of food insecurity. Thus, the objective of this work is to identify non-conventional vegetables responsive to silicone fertilization and evaluate the effect of fertilization on the content and accumulation of Si and macronutrients in these plants.

MATERIALS AND METHODS

The experiment was performed during the 2016 crop year, in Lavras, in the southern part of the State of Minas Gerais, Brazil, located at 21º14'S, 45º00'W and 918.8 m of altitude. The climate of the region is Cwa (mesothermal) with dry winter and rainy summer, according to Köppen classification (Álvares *et al.*, 2013).

The experiment followed a 4 x 5 factorial design, with four species of non-conventional vegetables, sorrel (*Rumex acetosa* L.) redroot amaranth (*Amaranthus retroflexus* L.), common sowthistle (*Sonchus oleraceus* L.) and lamb's ears (*Stachys byzantina*), being submitted to five levels of silicate fertilization (0, 25, 50, 100 and 150 mg dm^{-3}), totaling 20 treatments, with four replications and one plant per experimental plot. The tests were performed in a greenhouse using a completely randomized design, assuming homogeneous experimental conditions.

The Si source used in the experiment labeled a concentration of 98% SiO_2 and 6.5% soluble Si. The Si doses were applied only once directly to the substrate through surface incorporation, seven days before the transplanting seedlings.

The studied species derived from the germplasm collection of non-conventional vegetables of the Federal University of Lavras (UFLA). The seedlings of *A. retroflexus* and *S. oleraceus* were produced from seeds, in 200-cell plastic trays and commercial substrate based on pinus bark. Leaf fertilization was performed three times in the seedlings with leaf fertilizer, source of macro and micronutrients, in the dosage of 1 ml L^{-1} of water. The seedlings were transplanted to pots when they reached 8 cm in length. The *R. acetosa* and *S. byzantina* are commonly propagated vegetatively. Thus, the propagules were transplanted directly into the pots containing substrate, using vigorous and healthy offspring.

Plastic pots with a capacity of 3 dm^3 of soil were used, in which a single plant was conditioned. The used substrate consisted of an uncultivated soil collected in the 0 to 20 cm layer, classified as a dystrophic red latosol (Santos *et al.*, 2013) with the following characteristics: pH (in H_2O) = 5.4; exchangeable Al = 0.10 $cmol_c$ dm^{-3}; Ca^{2+} = 2.12 $cmol_c$ dm^{-3}; Mg^{2+} = 0.74 $cmol_c$ dm^{-3}; P-Mehlich = 2.91 mg dm^{-3}; K^+ = 70 mg dm^{-3}; Organic matter = 1.64 dag kg^{-1}; H + Al = 3.35 $cmol_c$ dm^{-3} ; V = 47.67%; Sum of bases = 3.04 $cmol_c$ dm^{-3}; t = 3.14 $cmol_c$ dm^{-3}; T = 6.39 $cmol_c$ dm^{-3}; m = 3.18%; Soil texture = sandy clay.

Correction of soil acidity was performed by applying 2.4 g of dolomitic limestone per pot to raise base saturation to 70%. The soil was incubated for 15 days, with moisture at 60% of water-holding capacity. There is no fertilizer recommendation for the species used in the study. Thus, it was decided that the fertilization of the species would be performed according to the recommended by Malavolta (1981) for the cultivation of potted plants. All plants were fertilized with 900 mg of nitrogen, 450 mg of potassium and 600 mg of phosphorus per pot. Nitrogen and potassium were applied in quotas (three times), being the first fertilization performed five days after transplanting seedlings, and the following fertilizations every 10 days in between one another. Phosphorus was applied in a single dose when the plants were established in the pots. The fertilizers' urea, potassium chloride, and monoammonium phosphate, respectively, were used as sources of nitrogen, potassium, and phosphorus.

Hand weeding was performed weekly in pots to prevent competition with weeds. Due to the plant's health throughout the experimental period, no intervention was necessary to control pests and diseases. Drip irrigation was performed according to the need of the plants.

The determination of the relative chlorophyll index (SPAD index) was performed using a SPAD-502 chlorophyll meter. The method is characterized as non-destructive and performed directly in the field. Measurements were made 35 days after transplanting on the fully developed leaves of the upper third of plants. The analysis was performed near the harvest time due to a longer accumulation time of said mineral in those plants, the moment in which it would be possible to identify any effect of Si in the parameter.

To measure fresh and dry matter masses, those plants produced from seeds were harvested when they reached a reproductive stage (flowering), a period of higher demand and accumulation of minerals, whereas the plants vegetatively propagated were harvested when the first senescence leaves emerged. Soon after the harvest, the shoot of plants (leaves and branches) was weighed for measuring of fresh matter mass. Subsequently, all plants were washed individually in running water, rinsed with distilled water and placed individually in properly identified paper bags. All materials were transferred to a convection oven at a temperature of 65 °C, where they remained until all plants reached constant weight. Afterward, the dry matter of each plant was determined.

For determination of the concentration of Si and macronutrients, the shoot of the dehydrated plants was milled individually in a Thomas Wiley mill. The Si contents of plants were determined through the colorimetric technique, a methodology described by Korndörfer *et al*. (2004). The Si accumulation was obtained by multiplying each plant's dry mass by its Si content. Through the methodology described by Silva (2009), the phosphorus content of the plants was determined by colorimetry, potassium by flame photometry, and calcium and magnesium by atomic absorption spectrophotometry.

For the variables Si content and Si accumulation that showed significance by the F test ($p<0.05$), regression equations were fitted. According to the equations, doses that provided higher content and Si accumulation in the evaluated species were estimated. Additionally, to identify the existing relationships between Si and other variables, an exploratory factorial analysis was performed. The statistical tests were performed through the development of scripts in the R (R Core Team) software for public use, through the package MVar.pt, version 1.9.6 (Ossani & Cirilo, 2017).

RESULTS AND DISCUSSIONS

Si content and accumulation in non-conventional vegetable species

Non-conventional vegetable species showed different Si content with the different applied doses of the mineral. The *S. oleraceus* showed a higher Si content at 0 mg dm^{-3} (3.95 ± 0.22 g kg^{-1}), followed by *R. acetosa* (3.32 ± 0.04 g kg^{-1}). At the higher doses, *S. oleraceus* kept standing out in relation to the Si content (dose 25 mg dm^{-3} at 4.60 ± 0.21 g kg^{-1} Si content; 50 mg dm^{-3} at 5.39 ± 0.25 g kg^{-1}; 100 mg dm^{-3} at 5.38 ± 0.03 g kg^{-1}; and 150 mg dm^{-3} at 5.06 ± 0.12). However, it varied according to the applied dose for the other species. The higher the applied doses of the material, the higher the Si contents; however, but very high doses showed an antagonistic effect, reducing the Si content in the plants (Figure 1).

Based on the derivatives of calculated equations, Si doses that would promote higher Si content in the species could be estimated. 240 mg dm^{-3} was determined as the dose responsible for the highest Si content estimated for *R. acetosa* (4.5 g kg^{-1} in the dry matter). For *A. retroflexus*, the application of 115 mg dm^{-3} promoted a Si content of 3.44 g kg^{-1} in dry matter. The application of 122.50 mg dm^{-3} Si in *S. byzantina* and 277.50 mg dm^{-3} in *S. oleraceus* promoted the maximum estimated levels of 4.52 and 5.43 g kg^{-1} Si in dry matter respectively.

The variation in the content and absorption capacity of Si within each species is influenced by genetics, and such variation is higher among different species due to the absorption mechanisms used by each (Hodson *et al.*, 2005; Liang *et al.*, 2015). There are three possible mechanisms of Si absorption by plants. Active absorption, in which Si absorption is fast, subsidized by Si transporters; passive absorption, which occurs in the same direction as the plant's transpiration stream, with Si absorption equal to water absorption; and Si-excluding plants, in which Si absorption is lower than water absorption by plants (Takahashi *et al.*,1990).

The presence or absence of Si transporters, as well as their higher or lower density in the plants, can explain the differences among species regarding the efficiency of Si absorption. The rate of transpiration of plants is another favorable factor for Si absorption; the higher the rate, the greater the absorption and accumulation efficiency of the mineral (Jones & Handreck, 1967).

A similar result was obtained by D'Imperio *et al*. (2016), which promoted an increase in Si contents in six species of leaf vegetables cultivated in a hydroponic system by applying 50 and 100 mg dm^{-3} of Si in the nutrient solution. The experiment found no significant difference in the mineral content among the studied plants. The bioavailability values quantified by the authors in an in vitro digestion process are much higher in plants that received Si via nutrient solution when compared to the control treatment (0 mg dm^{-3}).

The absorption of Si in rice plants varies depending on the applied doses of Si (Pati *et al.*, 2016). Although Si is an abundant element in the soil, the concentration of minerals available for plants is usually low, and its absorption depends on the external supply (Jawahar & Vaiyapuri, 2013).

The availability of silicic acid to plants may decrease as a function of the acid polycondensation, producing oligomeric silicic acid and colloidal particles of hydrated silica ($SiO_2.H_2O$), which are caused by high concentration of Si in soil. Another relevant aspect are adsorption and desorption processes of Si in soil reactive materials, mainly sesquioxides, reducing or increasing mineral availability in soil (Jones & Handreck, 1967).

The species are classified into three groups: Si accumulators, generally monocotyledons with Si leaf content, which can exceed 10 g kg^{-1} of mineral in dry matter; Si non-accumulators, generally leguminous, with Si content lower than 5 g kg^{-1}; and the intermediates, with Si content higher than 5 g kg^{-1}, but lower than 10 g kg^{-1} (Takahashi *et al.*, 1990).

With respect to the Si accumulation, *S. byzantina* stood out, showing better performance in all applied doses of Si. The *R. acetosa* species showed an accumulation capacity inferior to *S. byzantina*, but superior to the others (Figure 2). The *S. oleraceus* presented an intermediate result at all doses, whereas *A. retroflexus* was the species that showed the lowest accumulation of Si. Plants with higher leaf volume, such as *R. acetosa* and *S. byzantina* show greater capacity to accumulate Si. Similarly as to Si content, it was observed that Si accumulation is increasing until a determined dose, but reducing at high doses.

Based on the derivatives of equations calculated for the determination of doses that would provide the best estimated results for Si accumulation, it was indicated that, by applying 119 mg dm^{-3}, *R. acetosa* would reach a maximum accumulation of 96.94 mg Si plant^{-1}. The dose of 125.88 mg dm^{-3} would promote maximum accumulation of 73.17 mg Si plant^{-1} in *A. retroflexus*. Accumulation of up to 109.78 mg Si plant^{-1} in *S. byzantina* would be possible with the application of 249 mg dm^{-3} Si. The application of 462.5 mg dm^{-3} Si promoted a higher accumulation of Si in Si in *R. acetosa*. However, considering that it was a dose well outside the range of evaluated doses, the calculation was performed with a maximum dose of 150 mg dm^{-3}, with *S. oleraceus* plants showing an estimated maximum Si accumulation of 77 mg plant^{-1}.

Although all plants contain Si, they show great variation in their accumulation capacity of this mineral in their tissues. The difference in Si accumulation among species may be influenced by the root ability to intercept the mineral. The Si concentrations may range from 1 g kg^{-1} to over 100 g kg^{-1} of dry matter (Ma and Takahashi, 2002).

Doses that provided higher or lower content and accumulation of Si were different according to species. As the species in the present study are Si non-accumulators, it is assumed that mineral absorption occurs through a passive process that depends on the transpiration capacity of plants, besides root interception capacity of Si in each one. Si absorption is higher in the basal regions of roots, being the apical meristem and the root elongation zone less efficient in Si absorption (Yamaji and Ma, 2007).

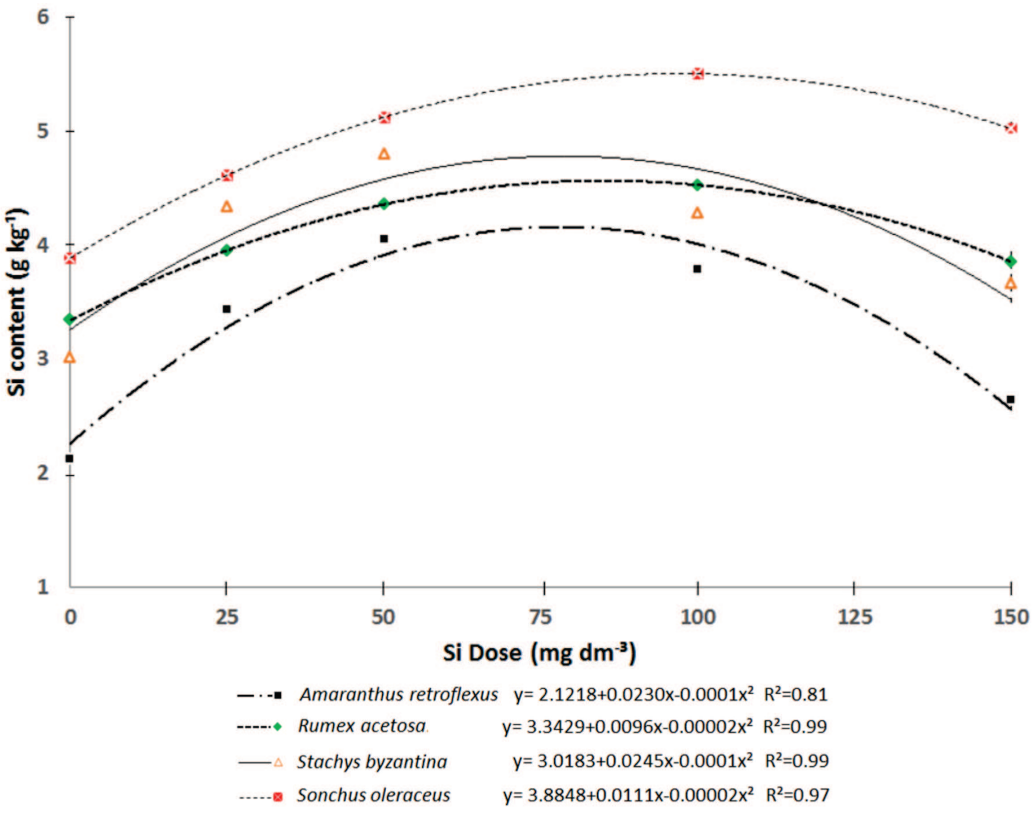

Figure 1: Average Si content in four non-conventional vegetable species as a function of silicon application.

Effects of Si on other minerals and on agronomic parameters

In order to verify the relationships and effects of Si on other minerals and on agronomic parameters, such as biomass production, the exploratory analysis of the data was performed.

According to the exploratory factorial analysis of the *R. acetosa* species (Figure 3A), except for variables K and Ca, which showed an inverse correlation with the others, Si positively correlated with P, Mg, fresh matter (FM), dry DM and SPAD index, i.e., the increase in Si content promotes gains in the mentioned variables and vice versa. The explanation of the total variance attributed to both factors was 84.53% (Table 1).

The variable least explained by the factorial model was phosphorus (P), whereas the most explained was DM. These results can be verified by taking the commonality of each variable. Commonality is how much of the variance of variables is measured or explained by the extracted “f” factors. These factors are non-observed variables intended to be measured base on the observed variables.

In the exploratory factor analysis in *A. retroflexus* (Figure 3B), it is possible to identify that Ca and FM are antagonistic to each other. P promotes gains in the SPAD index. Both, however, cause a reduction in K and DM variables, and vice versa. There is a strong positive influence of Si on the Mg content and vice versa. Si generally has a positive effect on the content of P, Ca, Mg and SPAD index in *A. retroflexus*. The explanation of the total variance attributed to both factors was 73.51% (Figure 1).

Ca in *S. byzantina* (Figure 3C) promotes increases in FM and DM. However, the correlation is inverse with Si, indicating its negative effect on the mentioned variables. Nevertheless, Si showed a positive effect on the contents of P and of the SPAD index. The explanation of the total variance attributed to both factors was 76.24% (Table 1).

For *S. oleraceus*, it was verified that Si increases the contents of K, P, Ca and Mg, but reduced FM and DM (Figure 3D). The explanation of the total variance attributed to both factors was 78.65% (Table 1).

The behavior and the relationships among the variables Si, P, K, Ca, Mg, FM and DM were different according to the species. The existing relationships, especially with respect to these minerals, go far beyond the chemical reactions occurring in soil, in detriment of fertilization with Si.

The application of Si, together with complete fertilizers in rice crop, provides a higher crop yield, besides increasing the absorption of macro and micronutrients (Pati *et al.*, 2016). In maize crop, fertilization with Si increases the absorption of minerals, increases the total soluble sugar, starch content and dry matter accumulation (Xu *et al.*, 2016).

Si is considered as a functional nutrient for several crops, especially for grasses, such as rice and sugarcane, favoring the growth and development of these species (Hodson *et al.*, 2005; Epstein & Bloom, 2006). Si has a synergistic effect on other minerals in soil, and may increase the availability of N and provides higher efficiency of phosphate fertilizers up to 10%, by reducing P retention in soil and increasing soluble P levels (Singh *et al.*, 2005; Pati *et al.*, 2016). Jongruk (2002) reported that the application of NPK fertilizers combined with Si significantly increased total N, P and K uptake of rice. Singh *et al.* (2005) state that the application of Si increases the uptake of K in rice.

In a study with lettuce culture, Kleiber *et al*. (2015) reported that Si nutrition did not alter the Mn content in the leaves of said culture. However, it caused a significant increase in relation to the control treatment in concentrations of N, P, Na, Fe and Si, and simultaneous decrease of Zn and Cu contents.

In contrast to the results obtained in the study, Lu *et al*. (2017) found that the biomass of the prince’s-feather (*A. hypochondriacus* L.) increased incrementally inasmuch as the doses of calcium silicate applied to soil also increased. However, there was no more positive effect on biomass at higher doses.

The positive effects of Si on yield and quality of several crops have been widely reported. The effect is more evident in grasses such as rice, maize, sugarcane, pearl millet and sorghum, among others (Liang *et al.*, 2015), since they are characterized as Si accumulators. The beneficial effects of this mineral on dicotyledons such as cotton, soybean, cucumber and some fruits, have been proven and are being increasingly investigated.

Wang *et al*. (2016) stated that the yield of apples was significantly increased in detriment of the application of Si. Chagas *et al*. (2016), in a study with rice and pearl millet cultivated in the Brazilian savanna, reported that the application of Si increases the yield of these crops. Costa *et al*. (2015) reported increased diameter of the Palmer mangoes associated with the application of Si. It is possible to state that the effects of Si on crops are positive and it is, therefore, a very promising mineral for increasing yield, quality, health and nutritional quality of several plant species.

In studies performed in the last decade, the positive effects of Si on chlorophyll biosynthesis were confirmed, especially in plants growing under stress conditions. For instance, the application of Si + Cd caused an increase in the content of chlorophyll a and b and carotenoids in relation to the treatment in which only Cd was applied in

Table 1: Exploratory factor analysis considering the variables phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), silicon (Si), silicon accumulation (plant), fresh mass (FM), dry mass (DM) and SPAD index, as a function of silicon fertilization in the species *R. acetosa*, *A. retroflexus*, *S. byzantina* and *S. oleraceus*

Factor					*R. acetosa*						
	Y	K	Ca	Mg	Si	Accumulation of Si	FM	DM	SPAD index	λ	%
1	0.190	**-0.708**	**-0.717**	0.287	**0.845**	**0.964**	**0.716**	**0.781**	0.550	4.200	64.69
2	-0.148	0.212	0.054	-0.244	-0.407	0.061	0.590	0.581	**-0.552**	1.288	19.84
Com.*	0.058	0.546	0.517	0.142	0.879	0.932	0.860	0.947	0.607	5.488	84.53
S.V.**	0.942	0.454	0.483	0.858	0.121	0.068	0.140	0.053	0.393	-	-
Factor					*A. retroflexus*						
	Y	K	Ca	Mg	Si	Accumulation of Si	FM	DM	SPAD index	λ	%
1	**0.467**	**-0.636**	**0.700**	0.185	0.653	0.535	**-0.730**	**-0.531**	**0.508**	2.933	40.82
2	0.029	-0.422	0.237	**-0.720**	**-0.727**	**-0.836**	-0.341	-0.268	0.425	2.349	32.69
Com.*	0.219	0.582	0.546	0.553	0.955	0.984	0.649	0.354	0.439	5.282	73.51
S.V.**	0.781	0.418	0.454	0.447	0.045	0.016	0.351	0.646	0.561	-	-
Factor					*S. byzantina*						
	Y	K	Ca	Mg	Si	Accumulation of Si	FM	DM	SPAD index	λ	%
1	0.073	-0.248	**-0.744**	**-0.681**	**0.911**	**0.737**	**-0.855**	**-0.769**	**0.483**	4.012	60.11
2	0.275	-0.279	-0.485	-0.546	-0.404	-0.046	0.019	0.463	-0.099	1.076	16.12
Com.*	0.081	0.139	0.789	0.762	0.993	0.545	0.732	0.806	0.243	5.089	76.24
S.V.**	0.919	0.861	0.211	0.238	0.007	0.455	0.268	0.194	0.757	-	-
Factor					*S. oleraceus*						
	Y	K	Ca	Mg	Si	Accumulation of Si	FM	DM	SPAD index	λ	%
1	**-0.656**	**-0.888**	**-0.669**	**-0.483**	**-0.873**	-0.484	**0.824**	**0.727**	0.169	4.133	57.37
2	-0.050	-0.058	-0.469	-0.290	-0.026	**-0.640**	-0.369	-0.665	**-0.485**	1.533	21.28
Com.*	0.433	0.792	0.668	0.317	0.762	0.643	0.816	0.971	0.264	5.666	78.65
S.V.**	0.567	0.208	0.332	0.683	0.238	0.357	0.184	0.029	0.736	-	-

* Commonality, ** Specific variances, Variance,

Coefficient of factor loading: < 0.3 (weak factor loading); 0.3 d" FL < 0.5 (low factor loading); 0.5 d" FL < 0.7 (moderate factor loading); 0.7 d" FL < 0.9 (high factor loading); 0.9 d" FL< 1.0 (strong factor loading).

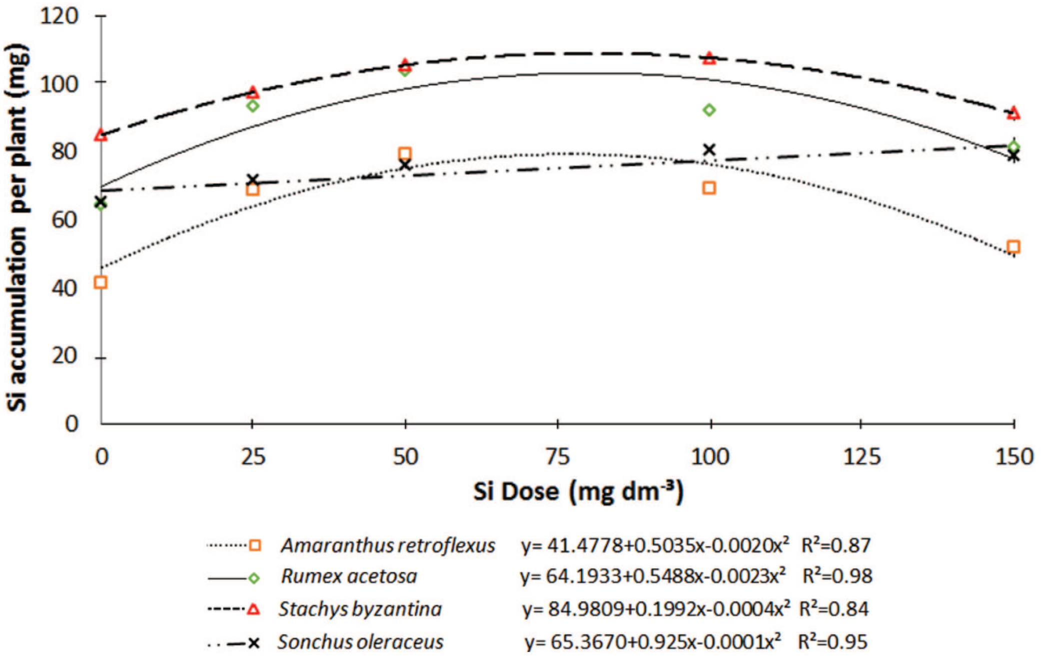

Figure 2: Accumulation of Si in four non-conventional vegetable species as a function of silicon application.

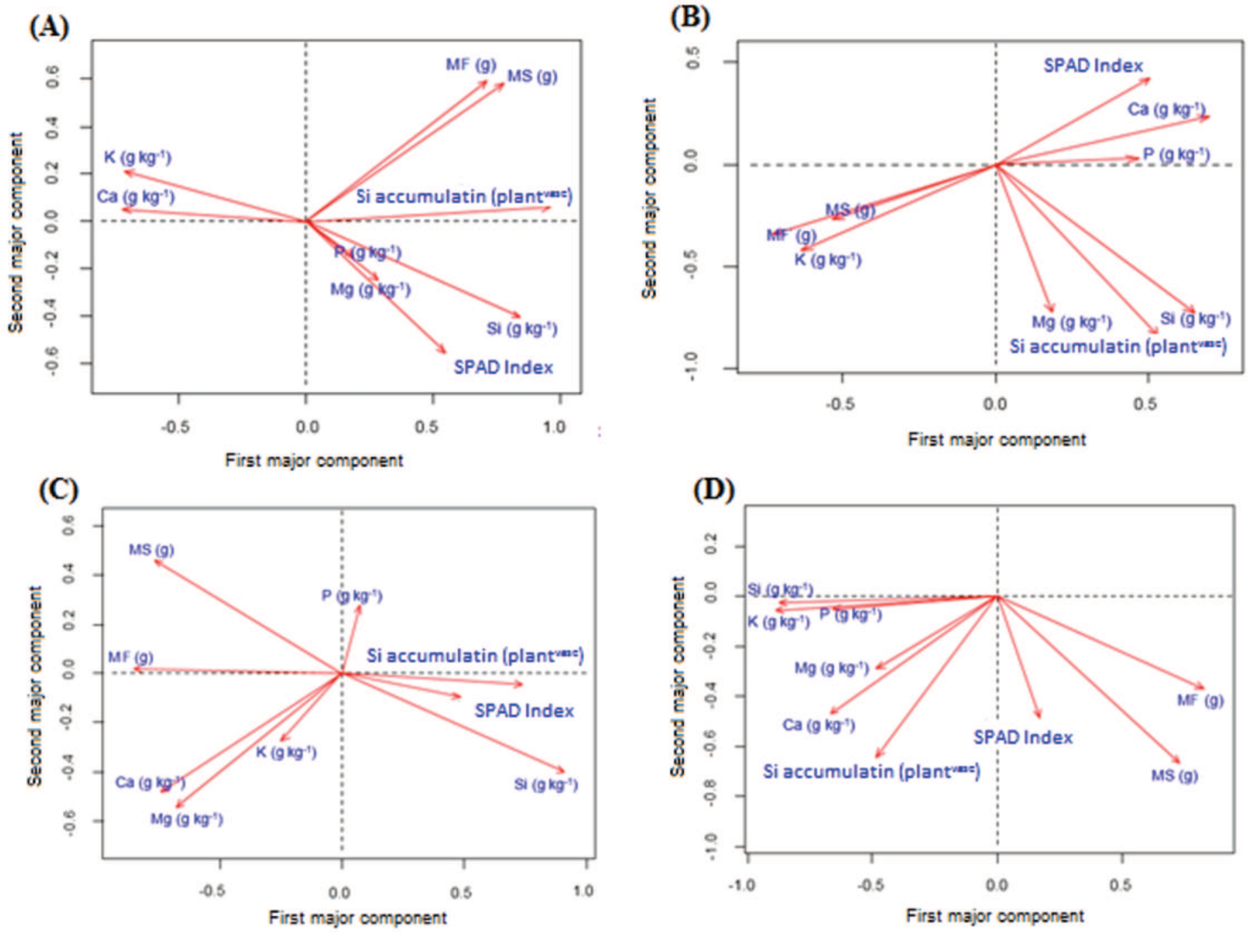

Figure 3: Exploratory factor analysis considering the variables, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), silicon (Si), silicon accumulation (plant-pot), fresh mass (FM), dry mass (DM) and SPAD index, as a function of silicon fertilization in the species (A) *R. acetosa*, (B) *A. retroflexus*, (C) *S. byzantina* and (D) *S. oleraceus*.

the maize crop (Mihalièová Malèovská *et al*. 2014). In the same culture, Xu *et al*. (2016) reported that Si fertilization increases grain yield by increasing photosynthesis and the activity of antioxidant enzymes in wheat (Hussain *et al*. 2015) and cucumber (Jianpeng *et al*. 2010).

Photosynthetic pigments are important properties regarding biomass production by plants. Stressed plants undergo chlorophyll reduction, which is attenuated by the application of Si, favoring the increase of chlorophyll. Although the species of the present study did not undergo any type of induced stress, there was a positive effect of silica fertilization on the chlorophyll content (Sattar *et al.*, 2016).

CONCLUSIONS

Silicate fertilization caused an effect on silicon content and accumulation in non-conventional vegetables. In general, Si content and accumulation were increasing with higher mineral doses, although very high doses had an antagonistic effect of reducing the response in plants. Thus, it is estimated that the application of 240; 115; 122.50; and 277.50 mg dm^{-3} provided the highest estimated silicon contents for R. acetosa, *A. retroflexus*, *S. byzantina* and *S. oleraceus*, respectively. Among these species only *S. oleraceus* is characterized as an intermediate accumulator of Si.

For the effects of Si on other minerals and agronomic parameters it was observed that silicon fertilization does not have a positive effect on green and dry matter of non-conventional vegetables. However, it generally improves phosphorus, calcium, potassium, magnesium and chlorophyll contents.

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