



## **Potassium Silicate in the Acclimatization of Arabica Coffee Seedlings under Shaded and Full Sun Conditions**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors PFMCF and DCB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SJF and PCS managed the analyses of the study. Authors WSS, SSB and SPF managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** The objective of this study was to evaluate the effect of the application of potassium silicate ( $K_2SiO_3$ ) on the acclimatization of arabica coffee seedlings, under shaded and full sun conditions.

**Study Design:** Randomized block design, with the treatments arranged in the subdivided portions scheme, with 13 replicates.

**Place and Duration of Study:** The experiment was conducted from November 2016 to February 2017 in the greenhouse, at the State University of Norte Fluminense Darcy Ribeiro - UENF, in the municipality of Campos dos Goytacazes - RJ, Brazil.

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**Methodology:** The experiment was conducted in randomized blocks, with the treatments arranged in the subdivided portions scheme, with 13 replicates. The plots referred to two forms of acclimatization (shading and full sun), and the subplots were arranged to two nutrition conditions (application of  $K_2SiO_3$  or not). The biometric, physiological and nutritional parameters of the plants were evaluated. The means of the collected data were submitted to analysis of variance and compared by the Tukey test at 5% of probability.

**Results:** A significant interaction between the acclimatization and application of  $K_2SiO_3$  was observed in the SPAD index and potassium contents in the leaves. The SPAD index was higher when  $K_2SiO_3$  was applied in both the acclimatization methods; and  $K_2SiO_3$  application resulted in higher potassium contents in the shaded seedlings. Regarding the biometric variables, an increase in the fresh and dry matter of the aerial part was observed, when the  $K_2SiO_3$  was used. In relation to the physiology, there was a beneficial effect in relation to the gradual acclimatization of the seedlings for all the parameters evaluated; while  $K_2SiO_3$  increased only the chlorophyll contents in the leaves.

**Conclusion:** The application of  $K_2SiO_3$  might be effective in making the seedlings rustic, thereby increasing the tolerance against insolation.

**Keywords:** *Coffea arabica*; silicon; propagation.

## 1. INTRODUCTION

The production of coffee seedlings in nursery is usually done in a shaded condition, in which the seedlings, before going to the field, require a gradual acclimatization process, which facilitates the ambiance and the bonding. However, the acclimatization of the seedlings is a process that takes about 30 days, a fact that increases the time of permanence of the seedlings in the nursery. It demands resources and labor, contributing to the increase of the cost of production. It is believed that adequate nutrient management may reduce the need for acclimatization, since some nutrients have the potential to increase the rusticity of the seedlings before being taken to the field.

Silicon (Si) is a beneficial mineral element that has been studied in agriculture in different plant species, aiming at resistance to plagues, diseases and in post-harvest, always promoting a greater rusticity of plants or fruits [1,2,3]. In Brazil, Si has recently been included in the legislation for the production and sale of fertilizers and correctives, as a beneficial micronutrient for plants. Once absorbed, the nutrient accumulates in the walls of the epidermis and contributes to the strengthening of the foliar structures, increasing tolerance to biotic and abiotic stresses, and in plant protection against pathogenic attack [4].

In contrast, Potassium (K) is an essential element of great importance in plants, since it stimulates the development of the root, controls the turgidity of cells, transport sugars, and helps

in the formation of enzymes and proteins [5]. Moreover, K acts on the regulation of opening and closing the stomas, with an effect on guard cells, in which the accumulation of this nutrient allows these cells to accumulate water and to swell, promoting the opening of the stomas. When the water supply is low, K is pumped out of the guard cells, so the pores close and loss of water is avoided [5].

Some researchers have been studying the potential of using  $K_2SiO_3$ , in the management of diseases [3,6] fruit quality [7] or even in the plants acclimatization [2]. Unifying the benefits of Si and K, it is believed that these elements can increase the tolerance of arabica coffee seedlings to insolation, assisting in the reduction of acclimatization time and reducing its permanence in the nursery. In this context, this study was undertaken to evaluate the biometric, physiological and nutritional aspects of arabica coffee seedlings submitted to the application of  $K_2SiO_3$  associated to management in the acclimatization of arabica coffee seedlings.

## 2. MATERIALS AND METHODS

The experiment was conducted from November 2016 to February 2017 in a greenhouse, in the State University of Northern Rio de Janeiro - UENF, in the city of Campos dos Goytacazes - RJ, located at 21°48' latitude, 41°20' longitude and an altitude of 11 m. According to the Köppem climatic classification, the weather in the region is classified as Aw (wet tropical), with a rainy summer and dry winter. The annual

average temperature is 24°C, with annual average rainfall of 1.023 mm.

The experimental design was randomized block design, with treatments arranged in subdivided plots containing 4 treatments and 13 replicates, with 3 experimental units in each block. Hence, there were 39 seedlings per treatment, resulting in a total of 156 seedlings. The plots referred to two forms of acclimatization (shading and full sun), and the subplots were subjected to two nutrient conditions (presence and absence of  $K_2SiO_3$ ) as follows: T1 (Shading +  $K_2SiO_3$ ); T2 (Full sun +  $K_2SiO_3$ ); T3 (Shading + absence  $K_2SiO_3$ ); T4 (Full sun + absence  $K_2SiO_3$ ).

Coffee seedlings arising from cultivar Red Catuaí Coffea IAC 99 were cultivated in plastic bags of 10 x 20 cm. The substrates, irrigation and cultural treatments were carried out according to the standard recommendations [8]. A commercial product, Aminoagro Silício Foliar Fertilizer® ( $K_2SiO_3$  – 168 g of  $SiO_2$  L<sup>-1</sup> and 210g of  $K_2O$  L<sup>-1</sup>) was used as Si source. Six applications each consisting of 5 ml of  $K_2SiO_3$  L<sup>-1</sup> totalizing 0.84 g of  $SiO_2$  and 1.05 g of  $K_2O$  was done. The first application was done on the seedlings with two pairs of fully developed leaves. The applications continued until the acclimatization period, which began when 4 pairs of leaves emerged from the seedlings.

A total of 156 seedlings were subjected to the acclimatization processes according to each treatment. 78 seedlings of T1 and T3 treatments were treated to acclimatization with gradual shading (50% shading for 14 days, 30% shading for 14 days and after 28 days the seedlings were grown in full sun); the remaining seedlings of treatments T2 and T4 were grown under full sun during the whole acclimatization stage. The process of acclimatization of the seedlings lasted for 35 days.

Before the acclimatization, the following variables were evaluated:

- (a) Biometrics: plants height (H), measured from the base to the plant apex; lap diameter (DC) measured with digital pachymeter, in the lap plants base, at 1 cm of the soil; dry matter weight of the aerial part (PMSPA) and dry matter weight of roots (PMSR), measured on a analytical digital balance, after the material was dried in an oven at 70°C, for 72 hours. Statistical analysis was done for all the biometric

variables The Dickson quality index (IQD) was calculated by the formula:

$$IQD = MST/[(H/DC) + (PMSPA/PMSR)];$$

- (b) Physiological variables (done between 8:00 and 10:00 in the morning): nitrogen balance index (NBI-G and NBI-R), total chlorophyll (SFR-G and SFR-R), anthocyanins (ANT-RG and ANT-RB), and flavonoids (FLAV), were estimated by a fluorometer model Multiplex (Force-A) with multiplex excitation light sources (ultraviolet, blue, green, and red). The equipment was approached 1 cm from the seedling, directing the light chamber to the whole plant in the direction from the apex to the base. The green color intensity of the leaves was also measured using a Portable Chlorophyll Meter, [model SPAD-502 "Soil Plant Analyzer Development" (Minolta, Japan)], where three readings were recorded, and one leaf was used in each third sample of the plant. All the physiological variables were analyzed statistically.
- (c) Nutritional variables: nitrogen (N), phosphorus (P), K and Si contents present in the aerial part of the seedlings. To measure the nutritional contents, all the leaves of the seedlings were dried at 70°C in a forced ventilation oven for 72 hours and then milled with Wiley-type knife mills. Each sample corresponded to 1 replicate, containing material of 3 experimental units. For the determination of N contents, the plant material was digested by  $H_2SO_4$ , and later determined by the Nessler method [9]. The contents of P and K were determined using Shimadzu brand plasma (ICPE-9000), after digestion with  $HNO_3$  and  $H_2O_2$ , in an open digestion system. The process of Si extraction in the plant was determined by the yellow method. The process of silicon extraction in the plant is through oxidation of the organic matter, by the removal of the carbon of the vegetal tissue with hydrogen peroxide (digestion). Sodium hydroxide added to the digestion solution has the purpose of improving the efficiency of the oxidant ( $H_2O_2$ ) and increasing the pH of the solution in order to maintain the silicon of the vegetal tissue in solution [10].

All the collected data were submitted to analysis of variance, in which the average of the

treatments were analyzed by the Tukey test ( $P = .05$ ) with the aid of the statistical program SISVAR 5.6.

### 3. RESULTS AND DISCUSSION

It was observed that there was a significant interaction between acclimatization and application of  $K_2SiO_3$  in the variables, SPAD index and K contents in leaves. It was verified that the variables, namely, height of plants; anthocyanin index (ANT\_RB and ANT\_RG) and flavonoids (FLAV); nitrogen balance (NBI\_R and NBI\_G) and total chlorophyll (SFR\_G and SFR\_R) were influenced by the acclimatization factors. The  $K_2SiO_3$  application had an effect on the fresh and dry mass of the aerial parts and on the K and Si contents in the leaves.

Regarding the variables that showed a significant interaction between the factors (acclimatization x  $K_2SiO_3$ ), it was found that, in the shaded seedlings with  $K_2SiO_3$  application, the SPAD index was higher as compared to the full sun seedlings with absence of silicon. This indicates that Si and/or K may have contributed to the increase of chlorophyll content in shaded seedlings. In addition, it was observed that the application of  $K_2SiO_3$  increased the K content in the seedlings in full sun, as shown in Table 1.

**Table 1. Test of averages for the sources of variation that presented significant interaction between the tested factors**

FV	SPAD	
	With $K_2SiO_3$	Without $K_2SiO_3$
Shaded	29.63 aA	26.08 aB
Full sun	23.39 bA	14.15 bB

*Averages followed by the same letters do not differ between themselves by Tukey test ( $P = .05$ ). Lowercase letters represent the columns and uppercase represent the lines*

Several species of plants presented a physiological adaptation to the level of shading and it is expected that with the increase of the

availability of incident light, there is also a decrease in the total chlorophyll content, the reverse occurs with a lower light availability [11]. This fact stems from the need to increase the photosynthetic apparatus to capture more light in conditions of decrease in the amount of available light, notably increasing the amount of chlorophyll b of the photosystems I and II [5]. Similar result was found by [12], who observed an increase in the SPAD index when evaluating the influence of light on the coffee trees under nursery conditions.

It was verified that the application of  $K_2SiO_3$  raised the SPAD index of the seedlings. Considering what has been discussed in the literature with the use of silicon in plants, it is possible that this result occurred due to the accumulation of Si in the leaves, creating a mechanical barrier, thereby limiting the loss of water by the leaves [13]. This layer of silica can then act in the action of irradiation in the processes of photo inhibition and photo-oxidation of chlorophyll. In rice culture [14], it was observed that the silicate also increased the SPAD values, in addition to chlorophyll a and b, corroborating with the results found in the present study.

It was found that the content of K was higher in the seedlings in full sun that received the application of  $K_2SiO_3$ . Pozza et al. also observed the effect of the silicon application on the increment of K levels in arabica coffee plants, a fact that may have occurred in the present work [15].

For the variables that presented significant variance among the treatments in the acclimatization factor of the seedlings, the averages test was performed, in which it was possible to verify the effect of this factor isolated in the behavior of these variables, as shown in Table 2.

**Table 2. Test of averages for the significant variables in the plots (acclimatization factor)**

FV	Plants height (cm)	ANTH_RB	ANTH_RG	FLAV
Shaded	17.793 a	-0.813 b	-0.095 b	0.036 b
Full sun	16.870 b	-0.721 a	-0.031 a	0.823 a
FV	NBI_G	NBI_R	SFR_G	SFR_R
Shaded	1.006 a	1.218 a	1.278 a	1.223 a
Full sun	0.156 b	0.160 b	1.044 b	0.994 b

*Averages followed by the same letters do not differ between themselves by the Tukey test ( $P = .05$ )*

The seedlings grown in shaded condition showed higher growth than those of full sun, and this result was corroborated by [16], who reported the higher growth of coffee plants under shaded conditions. It is reasonable to consider that a species such as, arabica coffee, well adapted to conditions of low temperatures and high altitudes, showed some degree of photo inhibition in full sun acclimatization, negatively influencing the SPAD index, with the degradation of chlorophylls as well as influencing the increase of secondary compounds, such as flavonoids and anthocyanins.

The seedlings grown in full sun showed higher indexes of flavonoids and anthocyanins. These compounds are secondary metabolites that play a fundamental role in plant protection, acting to protect against oxidizing agents, such as ultraviolet rays or oxidative agents of undesired metabolic processes [17]. In this way, the production and accumulation of these compounds can be detected by the photo-oxidative damages caused in the seedlings due to the high irradiance or by the potential photo inhibition of the region where the experiment was conducted. Similar results were observed while studying the accumulation of anthocyanins and flavonoids in two varieties of lettuce cultivated in greenhouses and in full sun, and attributed the increase of these compounds to the intensification of their biosynthesis, which is normally stimulated by UV-B radiation from the sun [18].

Nitrogen balance (NBI\_G and NBI\_R) and chlorophyll indexes (SPAD, SFR\_G and SFR\_R) were higher in the shaded seedlings. The seedlings of this experiment acclimatized to full sun, possibly presented greater difficulties in the evapotranspiration and consequently in the absorption of nutrients, including the available nitrogen in the substrate, which may explain in part, the lower nitrogen balance indices observed in the environmentally acclimated seedlings protected.

The potassium silicate application showed an increase in the fresh and dry matter, and the Si contents of the aerial part (Table 3).

The results obtained do not corroborate with the data observed by [19], in which silicon application did not show a difference in the fresh and dry matter of the coffee plants. However, the results found in the present work may be related to K. [20] examined the influence of auxins and K on the sprouting of conilon coffee, and found that K has the potential to regulate the action of auxins, which is considered as an element that contributes to plant growth by favoring apical dominance. This fact may be related to the increment in fresh and dry matter of coffee plants, since in  $K_2SiO_3$  there is presence of  $K_2O$ , which is soluble in water and readily available to the seedlings.

Some evaluated variables were not influenced by any of the treatments tested (Table 4). It was observed that the treatments did not cause any differences on the average diameter of the lap of the coffee seedlings. Tatagiba et al. [16] studied the effects of shading on the arabica coffee seedlings and reported that the lap diameter of the seedlings was not affected by different degrees of luminosity. Probably the short period of acclimatization of the seedlings was not enough to interfere with this variable.

The Dickson quality index did not show a significant difference between the treatments, evidencing that there was no difference in the morphological quality of the seedlings. The values of the index varied between 0.358 and 0.404 indicating that the seedlings, regardless of the treatment, presented good quality, since the values were above 0.20, which is considered as the minimum established value [21].

In relation to the content of nutrients in the leaf, it was observed that the K and Si contents were influenced by the treatments. However [15] observed that the absorption efficiency of P increased in arabica coffee plants when fertilized with calcium silicate, evidencing that the Si can potentiate the absorption of other elements. In addition, the authors found that the silicate fertilizer did not increase the N content of the aerial part, corroborating with the results found in the work.

**Table 3. Average test for the significant variables in the subplots (factor  $K_2SiO_3$ )**

FV	Fresh matter of the aerial part (g)	Dry matter of the aerial part (g)	Si (g Kg <sup>-1</sup> )	K (g Kg <sup>-1</sup> )
With $K_2SiO_3$	7.079 a	2.020 a	0.245 a	22.08333 a
Without $K_2SiO_3$	6.563 b	1.904 b	0.217 b	17.10417 b

*Averages followed by the same letters do not differ between themselves by the Tukey test ( $P = .05$ )*

**Table 4. Analysis of variance, coefficient of variation and general average of the variables studied in each isolated factors and in the interaction of factors**

Variation source (FV)	Lap diameter (mm)	Dry matter of the root (g)	Dicks on quality index	Nitrogen (g Kg <sup>-1</sup> )	Phosphorus (g Kg <sup>-1</sup> )
Acclimatization	1.001 <sup>ns</sup>	2.366 <sup>ns</sup>	3.0760 <sup>ns</sup>	2.4276 <sup>ns</sup>	4.108 <sup>ns</sup>
CV (%)	5.97	31.74	23.88	13.50	13.77
K <sub>2</sub> SiO <sub>3</sub>	1.033 <sup>ns</sup>	1.6355 <sup>ns</sup>	0.0203 <sup>ns</sup>	1.8149 <sup>ns</sup>	0.864 <sup>ns</sup>
CV (%)	6.30	15.28	14.12	12.23	15.97
Acclim.x K <sub>2</sub> SiO <sub>3</sub>	0.061 <sup>ns</sup>	13.4316 <sup>ns</sup>	3.1670 <sup>ns</sup>	0.1556 <sup>ns</sup>	0.034 <sup>ns</sup>
Overall Average	2.92	0.37318	0.37318	15.85625	2.43125

\*significant at the 5% probability and \*\*significant at the 1% probability level by the F test. <sup>ns</sup>No significant

#### 4. CONCLUSION

It has been noticed in the present study that the potassium silicate increased the silicon and potassium contents in the leaves and provided an increase in fresh and dry mass in the aerial part of arabica coffee seedlings. The potassium silicate did not interfere with the production of anthocyanins and flavonoids and increased the chlorophyll content in leaves of the seedlings. The acclimatization under shading increased the total chlorophyll index, the nitrogen balance, and decreases the anthocyanin and flavonoid contents of the seedlings. Therefore, it can be mentioned that the application of K<sub>2</sub>SiO<sub>3</sub> might be effective in making the seedlings rustic, thereby increasing the tolerance against insolation.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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