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Silicon as a mitigator of water deficit stress in radish crop

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ABSTRACT

Silicon is the second most abundant element in the Earth's crust and has several beneficial effects on plants, such as increase photosynthetic capacity and improve the architecture of the aerial parts. Water stress negatively affects plant development and, specifically in horticultural species, impacts metabolic processes with consequences on quality and productivity. The aim of this research was to investigate through physiological and biochemical parameters if the use of silicate in place of dolomitic limestone can contribute to the tolerance of radish plants submitted to water deficiency. The experiment was carried out in a greenhouse in Botucatu, Brazil, with radishes of the Apolo variety, distributed in randomized blocks in *a* 3 × 2 factorial scheme, being three irrigation depths, with high soil moisture at tensions corresponding to 10 kPa (control, FC - field capacity), 20 kPa (MWD - moderate water deficiency), and 30 kPa (SWD - several water deficiency), and two soil correctives, limestone and silicate, totaling six treatments and three repetitions. Irrigation management was performed with tensiometers. The parameters evaluated included biometrics, biomass, growth rates, photosynthesis, enzymatic activity, soluble protein, and proline. Through discussion of the results, it was possible to conclude that the silicate provided tolerance to water stress, reduced the percentage of cracked roots, and increased yield in the radish crop. Therefore the use of silicate in arid areas with low water availability is of great importance.

1. Introduction

Radish (*Raphanus sativus* L.) belongs to the Brassicaceae family, and is characterized by yield of globular roots, bright scarlet in color with white pulp, and for having a short cycle, between 25 and 35 days, cultivated in fertile soils with a pH of around 5.5–6.8 (Cardoso and Hiraki, 2001; de Paiva et al., 2013).

Although silicon is not considered an essential element for plant growth, it has numerous positive effects, such as increase photosynthetic capacity, and improve the architecture of aerial parts, tolerance to water stress, mitigates ammonium toxicity and attack by pests and pathogens (Viciedo et al., 2019; Ma and Yamaji, 2006; Bakhat et al., 2018). This element is the second most abundant in the Earth's crust, surpassed only by oxygen and found in practically all rocks and soil types, in various forms of pure or impure silica (Russell, 1994; Sommer et al., 2006).

Fertilization using silicate, containing silicon in the chemical composition, has been gaining ground in agriculture mainly due to the benefits of its application coupled with low acquisition costs. It is necessary that these silicon sources demonstrate characteristics that are favorable for their use, which aim to improve soil conditions, such as a high concentration of this element in soluble form and low concentration of heavy metals (Korndorfer and Oliveira, 2010).

Silicon presents several beneficial effects in plants by improving soil chemical conditions, such as increasing pH, acting as a corrective, decreasing Al^{3+} levels, increasing base saturation, acting in the conversion of toxic elements from soluble to insoluble fractions, and acting in the polymerization and precipitation of these toxic elements with silicate compounds (Korndorfer and Oliveira, 2010; Majumdar and Prakash, 2020).

The liming technique is performed by applying limestone to the soil to correct acidity, neutralizing the protons in the soil solution, however, silicates can be a viable alternative to neutralize acidity as a replacement for conventional liming (Galindo et al., 2021a). Silicate can reduce the time associated with liming reactions in the soil profile due to its greater solubility and alkalinity compared to limestone (Crusciol et al., 2016; Galindo et al., 2021b).

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Available to the plant, silicon is absorbed in the form of monosilicic acid, by mass flow, accumulating mainly in the middle lamella of the leaves, the region of maximum transpiration, and assimilated in the form of polymerized silicic acid (Ma and Yamaji, 2006) .

Water stress negatively affects the accumulation of radish plant biomass (Stagnari et al., 2018) and also of other species whose agronomic interest is the roots and tubers, such as sugar beet and red beet (*Beta vulgaris* L.), and potatoes (*Solanum tuberosum* L.) (Li et al., 2016; Shaw et al., 2002; Stagnari et al., 2014a, 2014b). However, there are no conclusive studies on the use of silicates to mitigate the effects of water stress on the development of radish culture, such as growth rates and biochemical behavior in response to oxidative stress.

The central hypothesis of the experiment is that with the application of silicate in the soil, in the form of calcium and magnesium silicate, radish plants should develop anatomical and physiological mechanisms capable of protecting the tissues from the impact of hydric deficiency compared to the plants grown with dolomitic limestone.

The aim of this research was to demonstrate through physiological and biochemical parameters that the use of calcium and magnesium silicate in place of dolomitic limestone could contribute to the tolerance of radish plants when subjected to hydric deficiency.

2. Material and methods

The experiment was carried out in a 40 m^2 agricultural greenhouse in the Horticulture Department of the Faculty of Agricultural Sciences of the São Paulo State University-UNESP, Botucatu Campus, São Paulo-Brazil, located to 22° 52′ 55″ S, 48° 26′ 22″ W. Biochemical analyses were performed at the Department of Chemical and Biological Sciences-Biosciences Institute/UNESP. The city's climate, according to Koppen, is classified as warm temperate (mesothermal), with an average annual rainfall of 1428 mm. The average annual temperature in this region is 20.5 °C and relative humidity between 70 and 20% (Alvares et al., 2013; Cunha and Martins, 2009) .

2.1. Soil data

To carry out the experiment, a previous soil analysis was carried out using a sample composed of nine removal points, the analyzed depth was from zero to 20 cm deep, and the soil classified as dystrophic red latosol, medium texture (Embrapa 2006). The characteristics obtained by chemical analysis they are described in Table 1.

According to the analysis of the soil, soil corrections with dolomitic limestone and with silicate and basic fertilization, were carried out 60 days before sowing and followed the recommendations of Raij et al. (1997). In both soil preparations, the base saturation (BS: $Ca^{2+} + Mg^{2+} + K^{+}/Ca^{2+} + Mg^{2+} + K^{+} + H^{+} + Al^{3+} \times 100$) was raised to 80%, which corresponded to the dose of 2,9 g/dm^3 of dolomitic limestone and 3,65 g dm^3 of silicate. Subsequently, each corrective was properly mixed with the soil. The total relative neutralization power (TRNP) of dolomitic limestone was 90%. The basic fertilization which corresponded to the dose of 10 mg dm^3 of nitrogen, 180 mg dm^3 of P_2O_5, 60 g dm^3 of K_2O, 1.5 mg dm^3 of zinc and 0.75 mg dm^3 of boron. Polyethylene plastic pots containing 10 dm^3 of soil were used. Crop was carried out during the months of November and December 2018.

According to the manufacturer, the composition of calcium and magnesium silicate (Silifértil ambiental LTDA®), which was used as a soil corrective to replace limestone, contained: CaO (36–40%), MgO (6–9%), SiO_2 (22%), Fe (1.6%), Mn (2.9%), K (0.9%), P (2.5%), S (0.9%), Zn (0.6%), Cu (0.3%), B (0.8%), Mo (0.001%), Co (0.007%), and TRNP (63%).

2.2. Treatments and experimental design

Irrigation management was carried out using two tensiometers per experimental plot, installed at a depth of 15 cm in the soil and five cm away from the plant. Monitoring of water tension in the soil (kPa) was performed daily with the aid of a digital tensimeter and the values converted to volumetric humidity, based on the equation for adjusting the soil water retention curve proposed by Van Genuchten, 1980. From the calculated volumes, the replacement of water to adjust the depths was performed daily.

The experimental design was in randomized blocks in a 3 × 2 factorial scheme, being three irrigation depths, with high soil moisture at tensions corresponding to 10 kPa (control), 20 kPa (MWD-Moderate water deficiency), and 30 kPa (SWD-Severe water deficiency), and two soil correctives, limestone and silicate, totaling six treatments. Only one type of soil was used, that is, the same soil for both correctives. Three repetitions ($n = 3$) were used, composed of two pots (10 dm^3 of soil) in each repetition, each pot with four plants, simulating the spacing used commercially (eight centimeters between plants), totaling eight plants per repetition. Therefore, each treatment consisted of six pots, totaling 24 plants.

2.3. Crop management and irrigation

Sowing was carried out with eight seeds per pot, 1.5 cm deep and irrigated daily at 10 kPa. The variety used was the Apollo radish. Fourteen days after sowing, when the seedlings had five definitive leaves, thinning was carried out, leaving four plants per pot. On the same day, equally in all pots, the covering fertilization was also carried out, according to Raij et al. (1997), which corresponded to the dose of 3.3 mg dm^3 of nitrogen and 20 mg dm^3 of K_2O.

The radish cycle can be divided into five phenological phases: phase I-from zero to 7 days; phase II-from 8 to 14 days; phase III-from 15 to 21 days; phase IV-from 22 to 28 days; phase V-from 29 to 35 days after sowing (DAS) (Marouelli et al., 2008) with adaptations. The different irrigation depths were applied from the last day of phase II (14 DAS) until harvest day. In 14 DAS, the plants were already stabilized, with guaranteed survival and ending the vegetative period, for later, under different levels of water deficit, to start the phases of greater water demand in the formation of the globular root. On the same day (14 DAS) the height I (cm) of the plants was measured. The height of the plants was measured with the aid of a ruler, expressed in centimeters (cm), from the surface of the soil to the apex of the plants.

2.4. Gas-exchange parameter

Gas exchange analysis was performed with the aid of a portable infrared gas analyzer, model LI-COR 6400 IRGA (LI-COR, Lincoln, NE, USA), at 33 DAS in the morning period, being measured the middle third of the fully expanded leaves. The rate of assimilation of CO_2 (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), the internal CO_2 concentration in the leaf (Ci, μmol mol^{-1}), and transpiration rate (E, mmol m^{-2} s^{-1}) were determined. Instantaneous water-use efficiency (iWUE, in μmol CO_2 $mmol^{-1}$ H_2O) was calculated by dividing A by E.

Table 1
Physicochemical characteristics of soil samples used for the experiment.

Depth	pH	OM	P_{resin}	K	Ca	Mg	Al^{3+}	H+Al	BS	CEC	V	S	B	Cu	Fe	Mn	Zn	Si
cm	$CaCl_2$	g dm^{-3}	mg dm^{-3}	mmolc dm^{-3}							%	mg dm^{-3}						
0–20	4.1	14.0	4.0	0.2	5.0	2.0	11.0	60.0	6.0	66.0	10.0	10.0	0.42	1.3	26.0	0.4	0.1	2.3

OM: organic matter, BS: base sum, CEC: cation exchange capacity, V: base saturation.

2.5. Antioxidant enzymes assay and L-proline content

At the time of harvest, leaf samples were frozen in liquid nitrogen for further biochemical analysis. The content of total soluble protein content (mg g^{-1} FW) was analyzed with bovine serum albumin (BSA) as standard protein (Bradford, 1976), superoxide dismutase enzyme activity (SOD, UI µg $protein^{-1}$), peroxidase (POD, µmol H_2O_2 decomposed min^{-1} g^{-1} FW), and the L-proline content in µmol g^{-1} FW (Bates et al., 1973; Giannopolitis and Ries, 1977; Peixoto et al., 1999) .

2.6. Yield, biometric parameters and growth indices

Harvest occurred 35 days after sowing (DAS), when the yield and biometric parameters were evaluated: height II (cm), number of leaves, leaf area-LA (cm^2), fresh leaf weight-FLW (g), dry leaf wight-DLW (g), longitudinal and transverse diameter of globular roots (mm), fresh and dry weight of globular roots (g), percent of cracked globular roots (%), and total dry weight of the plant-TDW (g).

The height II of the plants was measured in the same way as the height I. The number of leaves was obtained by separating and counting each leaf of a plant. Leaf area was measured with the aid of the integrative model Li-COR LI-3000 m and the results were expressed in cm^2. Fresh weight of the leaf and root was performed immediately after harvesting, by obtaining the weight on a scale precision with the values expressed in grams (g). Dry weight of the leaf and root was determined by placing the samples in an oven with forced air circulation at $60\,^\circ C \pm 5$ until constant weight (g) and, later, they were weighed on a precision scale.

The determination of the longitudinal and transverse diameter of the globular roots were performed with the aid of a digital caliper and the values were expressed in millimeters (mm). The number of cracked globular roots was obtained by separating and counting the cracked roots for each treatment is calculated the percentage (%).

From the LA, DLW, and TDW values, the following growth indices were calculated: SLA (specific leaf area), LWR (leaf weight ratio, or inverse of the SLA), and LAR (leaf area ratio), according to Atwell et al. (1999) and Wilson et al. (1999) as follows: SLA (cm^2 g^{-1}) = LA/DLW; LWR (g^{-1}) = DLW/TDW; LAR (cm^2 g^{-1}) = LA/TDW. Where: DLW-dry leaf weight; TDW-total dry weight of the plant; LA-leaf area.

2.7. Statistical analysis

To perform the statistical analysis, the data obtained were initially submitted to the Kolmogorov-Smirnov normality test with the aid of the GraphPad Prism software, v. 8.0.1 (San Diego, CA, USA). When the data presented normality, they were subjected to analysis of variance (Two-Way ANOVA), and when significant, the LSD test was applied to compare the means $p < 0.05$ using Sisvar software, v. 5.6 (Lavras, MG, Brazil). The results are presented as mean ± standard deviation values ($n = 3$). For the 12 yield parameters (leaf and root), principal component analysis was performed using the statistical software JMP 10 (SAS Institute Inc., USA).

3. Results and discussion

The results of the ANOVA with the agricultural correctives (silicate and limestone), irrigation depths (control-10 kPa, moderate water deficiency MWD-20 kPa, and severe water deficiency SWD-30 kPa) and the interaction between factors in all parameters evaluated in radish plants are presented in Table 2. Parameters showed a significant difference for correctives and depths, with the exception of height I, leaf area ratio-LAR, and internal CO_2 concentration, and also transpiration rate for the correctives. There was an interaction in 16 of the 24 evaluated parameters.

Table 2
Results of the analysis of variance (ANOVA) of the effect of agricultural correctives, irrigation depths, and the interaction of these factors in the parameters studied in radish plants. ns represents not significant; * represents $P \leq 0.05$ and ** represents $P \leq 0.01$.

	Correctives (C)	Depths (D)	C x D
Plant height I	0.375 ns	0.284 ns	0.273 ns
Plant height II	0.000 **	0.000 **	0.017 *
Number of leaf	0.024 *	0.008 **	0.584 ns
Leaf area	0.000 **	0.000 **	0.023 *
Fresh weight leaf	0.000 **	0.000 **	0.021 *
Dry weight leaf	0.000 **	0.000 **	0.150 ns
Root longitudinal diameter	0.000 **	0.000 **	0.031 *
Root transverse diameter	0.004 **	0.002 **	0.038 *
Fresh weight root	0.000 **	0.000 **	0.014 *
Dry weight root	0.000 **	0.000 **	0.009 **
Total dry weight of plant	0.000 **	0.000 **	0.009 **
Cracked globular roots	0.000 **	0.044 *	0.015 *
Specific leaf area - SLA	0.016 *	0.000 **	0.004 **
Leaf weight ratio - LWR	0.045 *	0.029 *	0.030 *
Leaf area ratio - LAR	0.639 ns	0.554 ns	0.568 ns
CO_2 assimilation rate	0.039 *	0.000 **	0.010 **
Stomatal conductance	0.000 **	0.000 **	0.122 ns
Internal CO_2 concentration	0.098 ns	0.220 ns	0.600 ns
Transpiration rate	0.057 ns	0.000 **	0.187 ns
Instantaneous water use efficiency	0.000 **	0,3742 ns	0.002 **
Total soluble proteins	0.007 **	0.000 **	0.025 *
Superoxide dismutase activity - SOD	0.000 **	0.000 **	0.000 **
Peroxidase activity - POD	0.000 **	0.000 **	0.187 ns
L-proline content	0.000 **	0.000 **	0.001 **

3.1. Gas-exchange parameter

The plants presented a decrease in the CO_2 assimilation rate (A) due to the increase in hydric deficiency, when compared to plants that were grown in a 10 kPa depth (Fig. 1A). However, plants that received limestone showed a decrease in photosynthetic rate only for 30 kPa. On the other hand, plants cultivated with silicate showed less effect of hydric deficiency, indicating that silicate can mitigate the effect of this physical stress. This can be proven by the non-difference in the internal concentration of CO_2 in the leaf (Ci) (Fig. 1C), even with lower stomatal conductance (g_s) than the treatments with silicate in lower irrigation depths (Fig. 1B).

In 20 kPa depth, the transpiration (E) of the plants cultivated with silicate did not differ from the 10 kPa depth, on the other hand there was a difference for these depths in the plants cultivated with limestone (Fig. 1D). Greater sensitivity in stomatal control is essential in situations of hydric deficit, which in this case allowed the assimilation rate to stabilize, even with the decrease in water availability.

Presence of silicon in the leaves helps to maintain gas exchange, acting on stomatal sensitivity, in addition to improving water uptake and transport (Yavaş and Ünay, 2017) , demonstrated in the present experiment. In other crops, performance occurs in the same way, such as in rice (*Oryza sativa* L.) and carnations (*Dianthus caryophyllus* L.), where this response was associated with an expression of proteins related to photosynthesis and hormonal signaling (Agarie et al., 1998; Soundararajan et al., 2017) . In barley, the performance of this element was also shown to be similar, as well as helping to increase the rate of CO_2 assimilation in plants under salt stress (Liang et al., 1996).

Similar to what was observed in the CO_2 assimilation rate and also to gs measurements, the instantaneous water use efficiency-iWUE (Fig. 1E) of plants cultivated in soil treated with silicon was reduced due to the water deficit, while for plants cultivated in soil that receive limestone, no significant difference between soil water tensions was observed. On the other hand, when comparing the action of these two neutralizing agents in each hydric condition, it was found that the use of limestone contributed to the increase in iWUE in control plants, as well as in those subjected to 20 kPa water tension, while the stress of 30 kPa showed no difference.

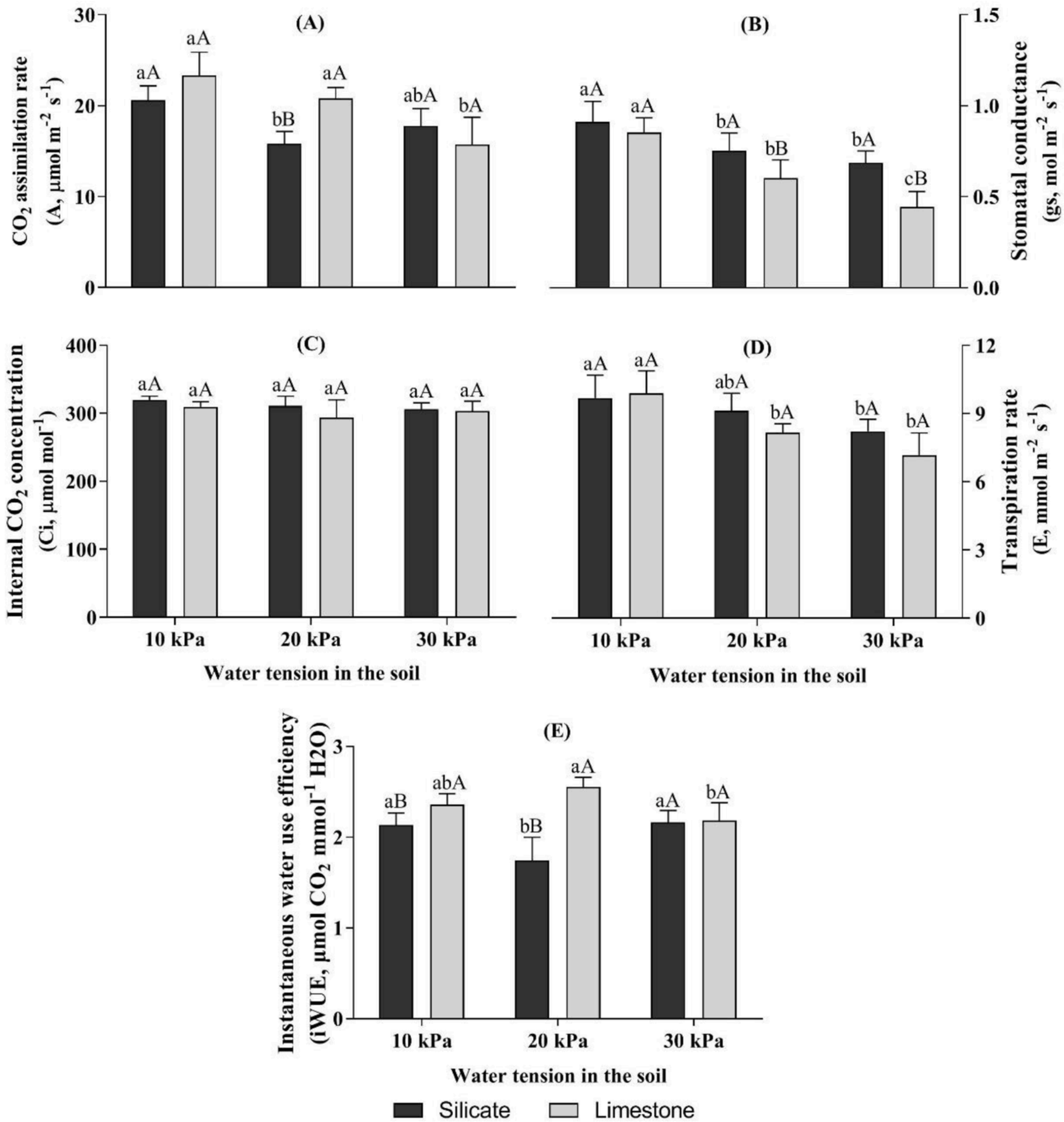

Fig. 1. Photosynthetic parameters of radish plants at 33 days after sowing (DAS), submitted to different soil acidity correctives (silicate and limestone) and irrigation depths (water tension in the soil). (A) CO_2 assimilation rate (A, µmol m^{-2} s^{-1}), (B) stomatal conductance (g_s, mol m^{-2} s^{-1}), (C) internal CO_2 concentration in the leaf (Ci, µmol mol^{-1}), (D) transpiration (E, mmol m^{-2} s^{-1}) and (E) Instantaneous water use efficiency (iWUE, µmol CO_2 $mmol^{-1}$ H_2O)
Means followed by lowercase letters (different) on the bars differ for each depth and means followed by uppercase letters (different) differ for each agricultural corrective by the LSD test ($p < 0.05$).

The reduction of iWUE in plants under 20 kPa treated with silicon probably induce the increase in gs, compared to plants that receive limestone under the same water conditions, suggesting an increase of E rates, and consequently, lower iWUE. Many researches show a divergent effect, where silicon acts to improve water savings in plants due to its influence on stomatal movement (Gao et al., 2006; Viciedo et al., 2019, 2020).

The water use efficiency (iWUE) is an important parameter related to plant production, as it ensures higher productivity in unfavorable environments (Silva et al., 2021). Thus, high iWUE allows the plant to maintain the metabolic processes essential for its development (Flexas, 2016). However, the iWUE response at the leaf level is directly associated with the various physiological processes that control CO_2 and water gradients, such as, for example, leaf vapor pressure deficits, that is, the air between the leaf and its surroundings (Hatfield and Dold, 2019). Rocha et al. (2021) found that fertigation with silicon attenuated the effects of water deficit and favored the physiological aspects in hybrid forages (*Brachiaria* spp.).

3.2. Antioxidant enzymes assay and L-proline content

Total soluble protein content (Fig. 2A) was higher in plants submitted to the 10 kPa depth in both corrective materials, and there was no significant difference between the results of the other depths. With the 10 kPa depth and limestone there was a higher content of soluble protein compared to silicate. According to Zuccarini (2008), under conditions of abiotic stresses, such as hydric stress, silicon helps to maintain the integrity of the cell membrane, acting as a mitigator of stress.

Activity of the superoxide dismutase (SOD) enzyme (Fig. 2B) presented a significant difference between the two corrective agents at all

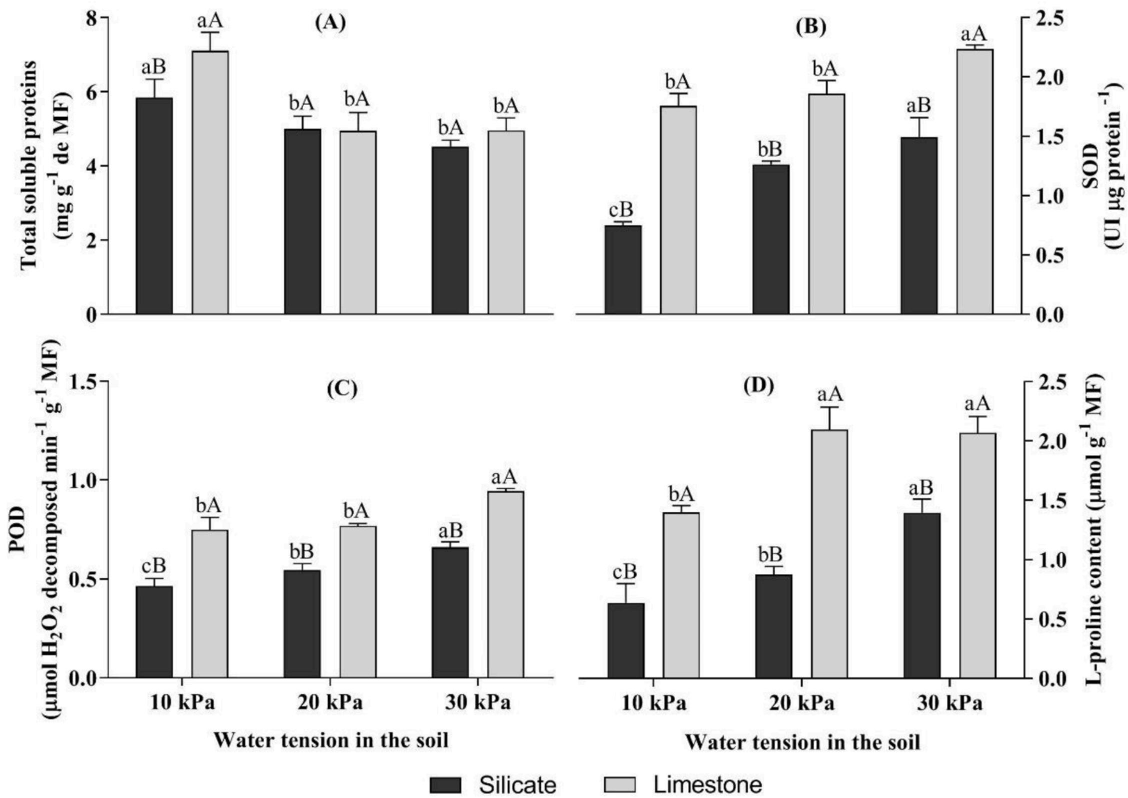

Fig. 2. Biochemical markers recorded on radish plants at 35 days after sowing (DAS), submitted to different soil acidity correctives (silicate and limestone) and irrigation depths (water tension in the soil). (A) total soluble protein content (mg g^{-1} MF), (B) specific activity of the superoxide dismutase enzyme (SOD, UI µg $protein^{-1}$), (C) peroxidase (POD, µmol H_2O_2 decomposed min^{-1} g^{-1} MF, and (D) L-proline content (µmol g^{-1} MF)
Means followed by lowercase letters (different) on the bars differ for each depth and means followed by uppercase letters (different) differ for each agricultural corrective by the LSD test ($p < 0.05$).

depths, being higher in the limestone treatments. For limestone, the 10 kPa depth generated higher activity than the others and for silicate there was a linear increase according to the decrease in water availability.

The decomposition of the superoxide anion (O_2^-) to H_2O_2 by means of the SOD enzyme prevents lipid peroxidation, this being the first enzyme with activity related to the effect of oxidative stress on biochemical and physiological alterations in plants. SOD activity is accompanied by the overproduction of H_2O_2, therefore, the plants have a system of enzymes involved in the dismutation of the H_2O_2. Peroxidase (POD) and catalase (CAT) are enzymatic systems capable of degrading the H_2O_2 directly into H_2O and O_2 (Gill and Tuteja, 2010).

Biczak et al. (2016) concluded that the increase in SOD activity observed in low concentrations of chemical compounds demonstrates the defense mechanism of plants to control oxidative stress. These compounds in high concentrations generate oxidative damage to plant cells, which prevents the mobilization of antioxidative enzymes, inducing an even more significant increase in the levels of reactive oxygen species (ROS) in cells, reducing the possibility of defense and leading to cell death.

The sharp increase in peroxidase activity (POD) indicated that the activity of this enzyme in radish leaves continued to increase with the extent of hydric deficit (Fig. 2C). According to the experiment results, compared to limestone, plants cultivated with silicate in all depths, showed less POD activity.

Peroxidase acts mainly to dismute H_2O_2 from the plant cell, being an indicator of oxidative stress in plant cells (Noctor and Mhamdi, 2017). The silicate demonstrated the potential to increase tolerance to water stress, reducing the biosynthesis of excess reactive oxygen species or modulating the activities of the main ROS elimination enzymes, such as peroxidase and catalase, which catalyze the conversion of hydrogen peroxide into H_2O (Zaid et al., 2018).

Proline content presented a significant difference between plants cultivated with silicate and limestone in all irrigation depths, showing a greater accumulation of this amino acid at 30 kPa for silicate and 20 and 30 kPa for limestone (Fig. 2D). Proline is an amino acid able to significantly reduce lipid peroxidation in terms of malondialdehyde, stabilizing membranes, DNA, and proteins, in addition to acting in osmotic adjustment which helps to maintain the potential in plants exposed to conditions of physical stress (Ashraf et al., 2018; Li et al., 2016).

Result is in agreement with those found by Mahdavikia et al. (2017) , showing that proline content increases in radish crop under abiotic stress conditions, to minimize damage induced by ROS, as well as in the cells of the photosystems, membranes, and proteins. Therefore, as there was less proline content in plants cultivated with silicate, this corrective apparently mitigated the effects of water stress in the radishes.

3.3. Yield and biometric parameters

Plant height I at 14 DAS showed no significant difference between limestone and silicate (Fig. 3A). In this initial phase, all experimental plots received the same irrigation depth (10 kPa), since the application of the different depths was performed from the same day as the evaluation of this parameter (14 DAS). In the germination and initial growth of the seedlings, the plant used the energy reserves of the seeds, therefore, they probably had not yet assimilated the elements of the silicate or limestone and presented similar results in height I.

The height II of the plants at 35 DAS, presented a difference between the irrigation depths and also between the limestone and silicate correctives (Fig. 3B). Compared to the control depth (10 kPa), the 30 kPa depth (SWD) reduced the height II of plants grown with silicon by 31.2

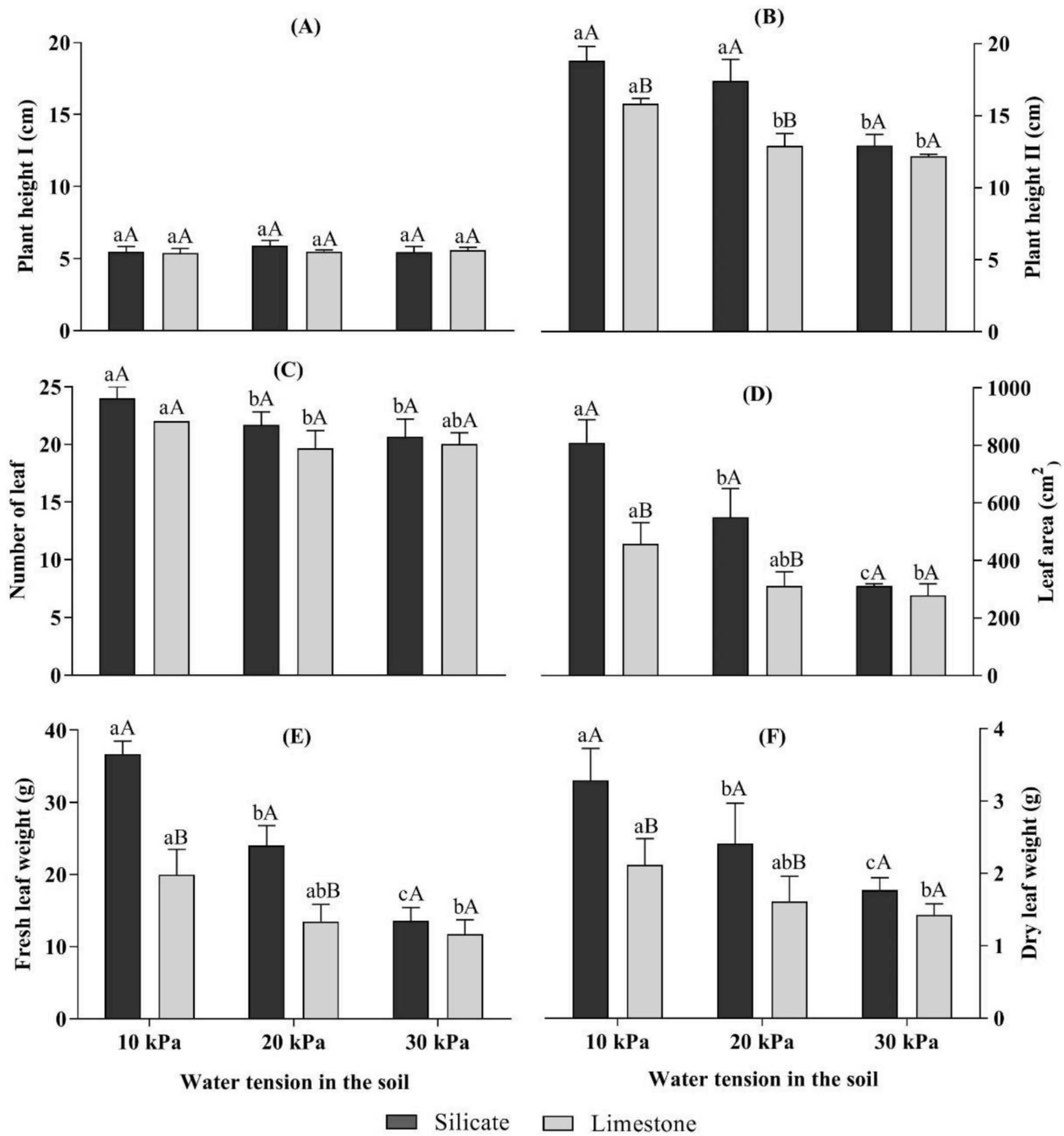

Fig. 3. Plant biometric characterization of radish at 35 days after sowing (DAS), subjected to different soil acidity correctives (silicate and limestone) and irrigation depths (water tension in the soil).(A) plant height I (cm) at 14 DAS; (B) plant height II (cm) at 35 DAS; (C) number of leaves; (D) total leaf area (cm^2); (E) fresh leaf weight (g); and (F) dry leaf weight (g)

Means followed by lowercase letters (different) on the bars differ for each depth, and means followed by uppercase letters (different) differ for each agricultural corrective by the LSD test ($p < 0.05$).

and limestone by 23.2%. Among the correctives, with silicate there was an increase of 15.7% in relation to limestone in the 10 kPa depth, 25.9% in the 20 kPa depth (MWD), and there was no significant difference in the 30 kPa depth. According to Taiz and Zeiger (2013), one of the main and most serious consequences of hydric restriction on vegetables is reduced plant growth.

For the number of leaves, there was no significant difference between silicate and limestone (Fig. 3C). With respect to the different irrigation depths, in the treatment with limestone, a difference was observed, with a 10.6% decrease in the number of leaves in the 20 kPa (MWD) depth compared to the control (10 kPa). Treatment with silicate the number of leaves was 9.7% lower in the plants of the 20 kPa (MWD) depth compared to the control (10 kPa), and there was no significant difference between the 20 kPa (MWD) and 30 kPa (SWD) depths.

Leaf area presented a difference in the treatments with limestone and silicate, and in the irrigation depths applied (Fig. 3D). Mean values decreased due to the increase in water stress. With respect to the treatments with limestone and silicate, silicate promoted increases in leaf areas of 43.3% and 43.4% compared to limestone, at the depths 10 and 20 kPa, respectively. Corroborating this result, Zhao et al. (2010), working with sugarcane, found that under water deficiency, the plants suffered a reduction in the number, length, and width of leaves, consequently resulting in less leaf area.

Reduction in the leaf area of plants under hydric stress is a morphological strategy to decrease the transpiration area, consequently minimizing the loss of water to the atmosphere. In this way, there is a reduction in the area of light interception and photosynthesis, and when the stress is very severe and prolonged, the plants cannot maintain or recover their leaf area (Sahoo et al., 2013; Taiz and Zeiger, 2013).

Mean values of fresh and dry leaf weight demonstrated that there was an interaction between the treatments where limestone or silicate was applied, with the different irrigation depths (Fig. 3E and 3F). The values decreased due to the increase in water stress. The plants in the silicate areas in depths 10 and 20 kPa had a higher fresh weight (45.6 and 43.8%) and dry weight (35.8 and 33.1%) when compared to the plants in the limestone areas in the respective depths. For these parameters, there was no significant difference between the correctives in the 30 kPa (SWD) depth.

Rodrigues et al. (2013), observed similar results in radish plants subjected to 80% water depth available in the soil. (Léles et al., 2008) studying the radish culture submitted to different doses of silicon, observed an increase in the fresh and dry weight of the aerial parts with the presence of silicon.

Longitudinal diameter and transversal diameter of the radish root grown with silicate did not differ statistically between the 10 and 20 kPa depths, however, comparing the 30 kPa depth with the 10 kPa depth, reductions of 37.4 and 37.6%, respectively were observed in the variables mentioned above (Fig. 4A and 4B). With respect to both root diameters of plants cultivated with limestone, no significant difference was observed between the depths, with reductions of 18.4 and 12.2%, respectively comparing depths 10 and 30 kPa.

Comparing the longitudinal and transversal diameters of the root in both correctives (limestone and silicate) in the different irrigation depths applied, it was possible to observe that for the irrigation depth at 30 kPa there was no statistical difference. For the other depths (10 and 20 kPa) the treatments using silicate demonstrated increases of 24.6 and 28.2% at 10 kPa, and 28.4 and 17.4% at 20 kPa, respectively, when compared to those obtained with the limestone application.

Rodrigues et al. (2013) mention that the radish root diameter can be compromised as water restriction occurs in the soil, observing that plants maintained on soil water availability at 80% of the field capacity demonstrated greater gains in root diameter compared to plants under hydric restriction of 20%, as observed by Pimentel (2004) , who reported that the root system of plants is affected under hydric deficit.

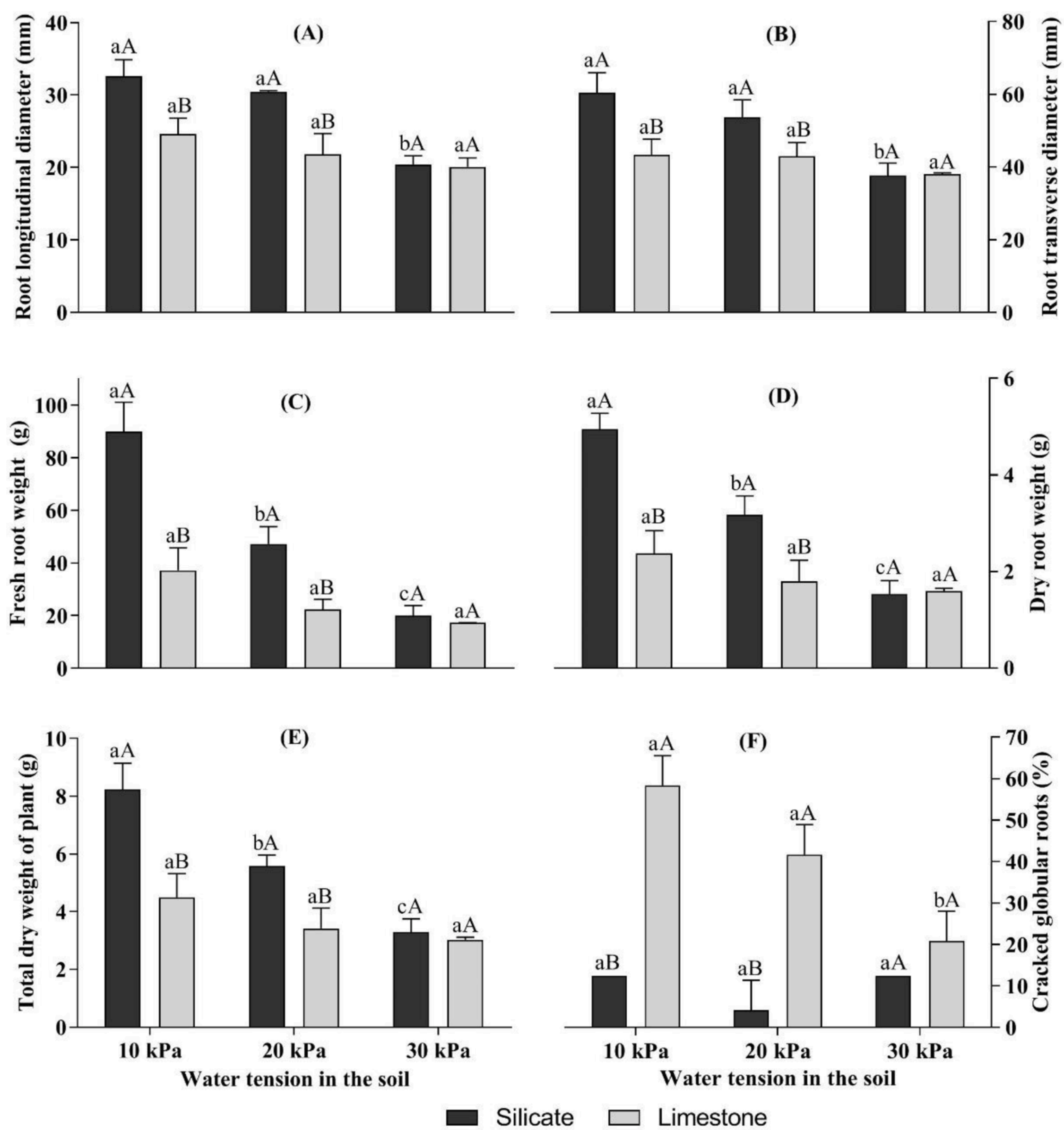

Fig. 4. Plant production components of radish plants at 35 days after sowing (DAS), subjected to different soil acidity correctives (silicate and limestone) and irrigation depths (water tension in the soil).(A) longitudinal diameter of the globular root (mm); (B) transverse diameter of the globular root (mm); (C) fresh globular root weight (g); (D) dry globular root weight (g); (E) total dry weight of plants (g); and (F) cracked globular roots (%).
Means followed by lowercase letters (different) on the bars differ for each depth, and means followed by uppercase letters (different) differ for each agricultural corrective by the LSD test ($p < 0.05$).

These results partially corroborate those obtained in the present research.

Hameed et al. (2013) reported that the use of silicon as a supplement can increase root growth under conditions of hydric stress. In agreement with the current work, Hattori et al., al.(2003) mentioned that silicon provides stimulation to the elongation and extensibility of the cell wall of sorghum roots.

For the fresh weight and dry weight of the root, as well as for the total dry weight of the plant (Fig. 4C–E), similar results were observed in all levels of irrigation depth, in all treatments. For these variables, there was no significant difference between the depths for the limestone, and between the silicate and the limestone in the 30 kPa depth. However, for the soil corrective with silicate, between the depths, there was a linear decrease in these variables due to the reduction in the depths. Furthermore, when simultaneously analyzing the two correctives in each irrigation depth, it was found that the application of silicate increased the fresh root weight (58.5 and 52.8%) and dry root weight (51.9 and 43.2%) in addition to the total dry weight of the plant (45.4 and 38.9%), in the irrigation depths 10 and 20 kPa, respectively.

Brum et al. (2006) evaluated the development of the radish root system in a condition of hydric deficit, observed that water management can significantly affect the fresh weight in different phenological phases of the crop, especially at 21 days of development, consequently negatively affecting the production of biomass in function of the aerial part, which is the first region affected by stress.

Studies performed by Wang et al. (2015) in cucumber crop, demonstrated that plants supplemented with silicon can present increased root/shoot ratio, leading to changes in root morphology and promoting greater water absorption capacity. In addition, Sonobe et al. (2010) reported that the application of silicon can increase the absorption of water by plants in the crop of sorghum, although it did not induce root development under stress conditions.

With the increase in water stress, there was a reduction in the percentage of cracked roots of plants cultivated with limestone in the soil (Fig. 4F). When silicate was used, there was no difference in any of the depths used. It was also found that under conditions of greater water supply (10 and 20 kPa), soil correction with silicate reduced the percentage of cracked roots compared to the application of limestone, with no difference under severe hydric deficiency (30 kPa).

Silicon is often deposited on the cell wall, strengthening it, so the cells are tolerant to the internal pressure of the turgor, reducing cell lysis, and consequently reducing cracks. This result agrees with Cunha et al. (2017), who evaluated radish cultivars under different hydric conditions and planting times. The authors observed that, regardless of the cultivar, high soil moisture could decrease the quality of the radish roots. Thus, in some moments of the development of the culture, the regular application of water can prevent the appearance of cracks.

3.4. Principal component analysis

Principal component analysis (PCA) of 12 characteristics of leaves and roots in 6 combinations of agricultural correctives and water stress levels, allowed the general observation of data in a smaller dimension (Table 3). The total variability was explained by five principal components (PC). However, two of these were considered the most important, as they presented eigenvalues > 1.0. Of these five PCs, the first two (PC1 and PC2) represented 95.91% of the total variation.

PC1 was responsible for 83.04% of the total variation, being effective in separating plants cultivated with silicate 10 and silicate 20 kPa from the others, mainly limestone 20, limestone 30, and silicate 30 kPa (Fig. 5A). The analysis of PC1 loads (Fig. 5B) suggests that this separation is due to the parameters: plant height II (PhII), number of leaf (Nl), leaf area (La), fresh leaf weight (FLW), dry leaf weight (DLW), root longitudinal diameter (Rld), root transverse diameter (Rtd), fresh root weight (Frw), dry root weight (Drw), and total dry weight of plant (TDW), since all these characteristics demonstrated high positive loads

Table 3
Load matrix, eigenvalues, and proportion of variation associated with two principal components (PC) of the PCA of 12 leaf and root characteristics in 6 combinations of agricultural correctives and water deficit stress levels in the radish crop.

Characteristic	PC1	PC2
Plant height I (PhI)	0.467	− 0.838
Plant height II (PhII)	0.955	0.177
Number of leaf (Nl)	0.935	0.224
Leaf area (La)	0.996	0.080
Fresh leaf weight (Flw)	0.995	0.079
Dry leaf weight (Dlw)	0.989	0.084
Root longitudinal diameter (Rld)	0.967	0.010
Root transverse diameter (Rtd)	0.976	− 0.019
Cracked globular roots (Cgr)	− 0.387	0.856
Fresh root weight (Frw)	0.988	0.062
Dry root weight (Drw)	0.994	0.012
Total dry weight of plant (Tdw)	0.997	0.037
Eigenvalue	9.965	1.544
Percent (%)	83.043	12.867
Cum Percent (%)	83.043	95.910

(> 0.90) in the PCA, in addition to the plant height I (PhI) that presented an intermediate positive load. PC1 scores and loads revealed that 10 kPa silicate provided greater yield in the radish culture due to the strong positive correlations between PhII, Nl, La, FLW, DLW, Rld, Rtd, Frw, Drw, and TDW.

PC2 represented 12.86% of the total variation and was mainly related to Cgr (cracked globular roots) with a positive load and PhI with a negative load. PC2 in the score graph was important to separate the 10 kPa limestone and 20 kPa limestone treatments from the others, since these treatments presented positive loads, and mainly the limestone treatment 10 kPa, which is related to the highest percentage of cracked roots, according to the load graph, corroborating with Fig. 4F. The negative load of PhI reflects the still insignificant values of plant height, which until the day of this evaluation received the same amount of water. Therefore, the analysis of the main components was efficient in confirming the results presented here.

3.5. Growth indices

Analysis of growth indices there was an interaction between the corrective and the depths in specific leaf area (SLA) and leaf weight ratio (LWR), whereas for LAR (leaf area ratio) there was no interaction. Compared to limestone, the presence of silicate provided higher values of SLA in the 10 and 20 kPa depths and lower LWR values in the 10 kPa depth (Table 4). As the depths increased, increasing values of SLA and decreasing values of LWR were observed.

Plant growth indices are fundamental for determining the accumulation and distribution of biomass in the plant's organs during its life cycle. SLA represents the useful area for plant growth and photosynthesis, which consequently results in the volume of carbohydrates assimilated by plants. Therefore, a plant under hydric stress has a smaller specific leaf area (Taiz and Zeiger, 2004), as observed in radish crop by Brum et al. (2006). The increase in SLA provided by silicate compared to limestone must be related to the deposition of silicon on the cell wall, strengthening the cells against water loss and consequently reducing the impact of hydric stress.

LWR is higher when the aerial part of the plant overlaps the weight of the root system (Magalhães, 1979). LWR was lower for the silicate in the 10 kPa depth, in comparison with the limestone in the same depth, because the silicate provided larger roots, an organ of commercial interest, even though the aerial part was also larger compared to the limestone. In the 30 kPa depth, the silicate did not present proportional results, probably because it has high amounts of ions, which significantly reduce the osmotic potential of the soil when there is high water tension in the soil (Raviv and Blom, 2001).

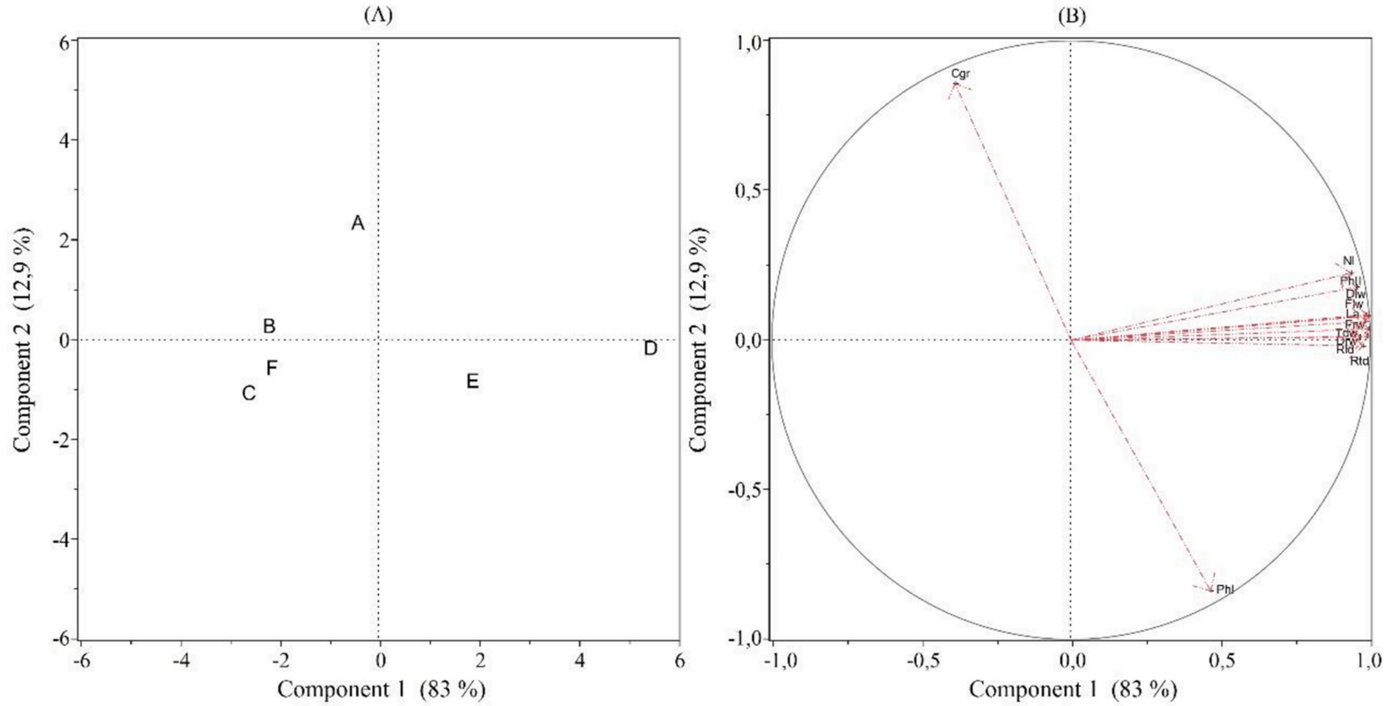

Fig. 5. Score graph (A) and load graph (B) of the main component analysis of 12 leaf and root characteristics in 6 combinations of agricultural soil correctives and water stress levels in the radish crop. Score graph legend: limestone 10 kPa (A), limestone 20 kPa (B), limestone 30 kPa (C), silicate 10 kPa (D), silicate 20 kPa (E), and silicate 30 kPa (F). See Table 3 for legend of the load chart characteristics.

Table 4
Growth indices: specific leaf area - SLA (leaf area/dry leaf weight); leaf weight ratio - LWR (dry leaf weight/total dry weight); and leaf area ratio – LAR (leaf area/total dry weight) of radish plants at 35 days after sowing (DAS), subjected to different soil acidity correctives (silicate and limestone) and irrigation depths (water tension in the soil).

		Depths (Water tension in the soil)		
		10 kPa	20 kPa	30 kPa
SLA (cm^2 g^{-1})	Limestone	217.10 ± 13.79 aB	194.82 ± 9.11 bB	194.63 ± 6.72 bA
	Silicate	244.34 ± 13.45 aA	230.06 ± 10.01 aA	177.21 ± 10.59 bA
LWR (g^{-1})	Limestone	0.47 ± 0.01 aA	0.47 ± 0.04 aA	0.47 ± 0.03 aA
	Silicate	0.40 ± 0.04 bB	0.43 ± 0.03 bA	0.53 ± 0.02 aA
LAR (cm^2 g^{-1})	Limestone	102.20 ± 5.14 aA	92.00 ± 7.25 aA	92.00 ± 8.68 aA
	Silicate	97.75 ± 9.33 aA	99.80 ± 6.87 aA	95.42 ± 8.77 aA

Different lowercase letters in the same row and different uppercase letters in the same column indicate statistical difference from the means by the LSD test ($p < 0.05$).

4. Conclusions

It was concluded that plants treated with silicate presented better photosynthetic efficiency, including the other components of photosynthesis in the crop of radish. In metabolic terms, silicon improved aspects of stress mitigation, notably the activity of antioxidative enzymes and L-proline accumulation. In this sense, the application of silicate as a neutralizing mineral could be an interesting strategy for growing radish and other vegetables in areas with less water availability.

The correction of the soil with silicate provided tolerance to water stress at moderate level, reduced the percentage of cracked roots, and increased yield of the radish crop. Thus, the importance of using silicate in this crop and its interface with hydric availability was evidenced.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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