

Effects of silicon and drought stress on biochemical characteristics of leaves of upland rice cultivars¹

Características bioquímicas nas folhas de cultivares de arroz de terras altas em função de silício e estresse hídrico

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ABSTRACT - Silicon (Si) has beneficial effects on many crops, mainly under biotic and abiotic stress. Silicon can affect biochemical, physiological, and photosynthetic processes and, consequently, reduce drought stress. However, the effects of Si on rice (*Oryza sativa* L.) plants under drought stress are not well known. The objective of this study was to evaluate the effects of supplemental Si on proline content and peroxidase activity in upland rice plants in the presence and absence of drought stress. The experiment was conducted under greenhouse conditions and was arranged in completely randomized blocks in a 2 × 2 × 2 factorial scheme. Treatments comprised combinations of (1) presence or absence of Si supply (0 or 350 kg ha⁻¹ of Si), (2) presence or absence of a water deficit (−0.050 MPa or −0.025 MPa soil water potential values, respectively), and (3) two upland rice cultivars: Caiapo (traditional type) and Maravilha (modern type), with eight replications. Under water stress conditions, silicon fertilization reduced the proline content in the vegetative and reproductive phases of upland rice plants and increased peroxidase activity in the plants' reproductive phase, which could be indicative of stress tolerance.

Key words: *Oryza sativa*. Proline. Peroxidase. Enzyme. Hydric deficiency.

RESUMO - O silício tem efeitos benéficos sobre diversas culturas, principalmente sob estresses bióticos e abióticos. O silício pode afetar processos bioquímicos, fisiológicos e fotossintéticos e, conseqüentemente aliviar o estresse hídrico. No entanto, os efeitos do Si sobre plantas arroz (*Oryza sativa* L.) sob estresse hídrico não são muito bem conhecidos. O objetivo deste estudo foi avaliar o efeito do suprimento de Si no teor de prolina e na atividade da peroxidase em plantas de arroz de terras altas, expostos ou não ao estresse hídrico. O experimento foi realizado em casa de vegetação arranjados em um delineamento em blocos completos casualizados em esquema fatorial 2 x 2 x 2. Os tratamentos consistiram na ausência ou na presença de Si (0 e 350 kg ha⁻¹) com a ausência ou presença de déficit hídrico (- 0,025 MPa e - 0,050 MPa potencial de água no solo, respectivamente) e duas cultivares de arroz Caiapo (tipo tradicional) e Maravilha (tipo moderno), com oito repetições. Sob condições de estresse hídrico, a adubação com silício reduz o teor de prolina nas fases vegetativa e reprodutiva das plantas de arroz de terras altas e a atividade da peroxidase aumenta na fase reprodutiva do arroz, o que pode ser indicativo de tolerância ao stress.

Palavras-chave: *Oryza sativa*. Prolina. Peroxidase. Enzima. Deficiência hídrica.

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INTRODUCTION

Upland rice cultivation is increasing in importance worldwide as the water available for rice irrigation decreases, and is especially important in China due to urban and industrial growth (QU *et al.*, 2008; NASCENTE *et al.*, 2013). Therefore, a better understanding of the biochemical mechanisms of drought resistance will help to improve and stabilize rice yield under upland conditions and provide additional water savings (CARLIN; SANTOS, 2009).

Plant tolerance to unfavorable conditions, particularly water deficit, has been associated with proline (a non-protein amino acid formed in the leaf tissues of plants exposed to water stress) accumulation and increased antioxidative enzymes such as peroxidase (ASHRAF; FOOLAD, 2007). This enzyme is involved in many reactions inside plants, such as linking polysaccharides, oxidation of indole-3-acetic acid, monomer connections, lignification, oxidation of phenols, pathogen defense, regulation of cell elongation and others (KAO, 2003). Therefore, peroxidase may represent a regulatory mechanism for water loss by reducing cell water potential (CARLIN; SANTOS, 2009; CRUSCIOL *et al.*, 2009; FUMIS; PEDRAS, 2002; SHARMA; DUBEY, 2005). It may also be a biochemical marker of metabolic alterations generated by different types of stress, as reported by many authors (LIMA *et al.*, 2004; RAYMOND; SMIRNOFF, 2002; SHARMA; DUBEY, 2005).

However, the levels of proline in rice plants under stress are not well studied (CARLIN; SANTOS, 2009; DINGKUHN *et al.*, 1991; FUMIS; PEDRAS, 2002; LIMA *et al.*, 2004). Therefore, it is important to evaluate the behavior of new cultivars of upland rice under hydric deficiency (HEINEMANN; STONE; FAGERA, 2011).

Silicon is the second most abundant element in the Earth's crust (MA *et al.*, 2006), and several studies have demonstrated that Si plays an important role in plant tolerance to environmental stresses (GONG *et al.*, 2005; GUNES *et al.*, 2007a,b, 2008; MA, 2004; ZHU *et al.*, 2004;). In this sense, Gao *et al.* (2004), Gong *et al.* (2005), Gunes *et al.* (2007a) and Zhu *et al.* (2004) verified that application of Si induced higher proline concentrations under conditions of water deficit. According to the abovementioned authors, the effect of Si on the greater tolerance of higher plants to drought could be associated with an increase in the action of antioxidant defenses, a reduction in the oxidative damage to functional molecules and membranes, and maintenance of many physiological as well as photosynthetic processes under water deficit conditions. MING *et al.* (2012) stated that silicon application could enhance the resistance of upland rice

roots to water deficit stress by preventing the rapid decline of physiological and biochemical activities in roots.

Drought stress has been found to increase stomatal resistance, leaf hydrogen peroxide and proline concentrations, and leaf lipid peroxidation in chickpea (*Cicer arietinum* L.) (GUNES *et al.*, 2007b), sunflower (*Helianthus annuus* L.) (GUNES *et al.*, 2008) and rice (CHANDRU *et al.*, 2003). However, Si application decreased the levels of the above factors and alleviated membrane damage significantly by increasing leaf relative water content. Silicon fertilization reduced proline levels, indicating that Si reduces stress levels because proline is considered a biochemical indicator of stress and an osmotic regulator (GUNES *et al.*, 2008). Silicon is also known as an anti-stress agent and can reduce cuticle transpiration (MA *et al.*, 2006) or increase water efficiency (GAO *et al.*, 2004).

In this regard, there is not much information in the literature indicating whether Si application may have similar beneficial effects on rice under drought stress. This information will help scientists choose genotypes that are more tolerant to hydric deficiency for breeding (HEINEMANN; STONE; FAGERA, 2011). The reported work aimed to evaluate the effects of Si supply on leaf concentrations of Si, proline content and peroxidase activity in upland rice cultivars grown either with or without exposure to soil water deficits.

MATERIAL AND METHODS

The experiment was performed under greenhouse conditions in Botucatu, São Paulo, Brazil, in 40-L pots (0.40 x 0.40 x 0.25 m) with an effective depth of 0.30 m and a hole at the bottom to drain excess water. Pots contained 40 dm³ of a Typic Acrortox soil (33% clay, 4% silt, and 63% sand). The soil had the following properties: pH (1:2.5 soil/CaCl₂ suspension, 0.01 mol L⁻¹) 4.0, 8 g dm⁻³ organic matter, 3.0 mg dm⁻³ P, 11.0 mmol_c dm⁻³ Ca, 2.0 mmol_c dm⁻³ Mg, 0.6 mmol_c dm⁻³ K, 72.0 mmol_c dm⁻³ H+Al, and 16.0% base saturation. All of the soil chemical attributes were analyzed according to van Raij *et al.* (2001).

The experiment was arranged in completely randomized blocks in a 2 × 2 × 2 factorial scheme. Treatments comprised combinations between the presence or absence of supplied Si (0 or 350 kg ha⁻¹ of Si) and the presence or absence of a water deficit (−0.050 MPa or −0.025 MPa soil water potential values) applied to two upland rice cultivars: Caiapo (traditional type) and Maravilha (modern type).

Each pot was considered an experimental unit. Eight replicates of each combination were set up, totaling 64 experimental units ($2 \times 2 \times 2 = 8 \times 8 = 64$).

In order to establish the Si treatments, base saturation was increased to 60% by applying Ca and Mg silicate to 32 pots, while the other 32 pots received an application of dolomitic lime. Materials for correcting pH that had particles smaller than 0.30 mm (50 mesh) were used to enable the full soil reaction during the incubation period. Moreover, 150 mg dm⁻³ P (single superphosphate, 18% P₂O₅), 150 mg dm⁻³ K (potassium chloride, 60% K₂O), 5 mg dm⁻³ Zn, and 1 mg dm⁻³ B (fritted trace elements BR12, 9% Zn and 1.8% B) were added. The soil was then wetted to field capacity, covered with polyethylene film, and incubated for 30 d at 25 °C. Silicon was incorporated into the soil 2 days before sowing rice.

The rice was sown using 50 seeds in a 0.40 m row in each pot. Emergence occurred 5 d after sowing. At 9 d after emergence (DAE) mowing was done, leaving 30 plants per pot. Nitrogen and K were supplied (30 DAE) at concentrations of 30 mg dm⁻³ N (as urea) and 30 mg dm⁻³ K (as potassium chloride).

Soil water potential was monitored with conventional mercury tensiometers (13-mm in diameter, with a ceramic porous cup connected with tubing to a mercury manometer), which were constructed according to Richards (1949), and installed on the sowing date at a 15 cm depth, in four replications of each treatment (32 pots). After plant emergence and before the water deficit treatments were established, water additions were performed when the mean water potential in the soil reached -0.025 MPa. The treatments with soil water potentials of -0.025 and -0.050 MPa were established at 17 DAE and maintained until 70 DAE. Required water additions were performed according to recommendations by Crusciol *et al.* (2006) and the soil's water retention capacity curve. The soil's water retention capacity curve was determined in the laboratory according to the pressure plate methodology recommended by Richards (1949). The total water applied was 323 and 392 mm, respectively, in the treatments with and without water deficit.

When plants were at 40 DAE (vegetative stage) and 70 DAE (reproductive stage), four fresh leaves per pot (the first completely expanded leaves counting from the plant apex) were collected and immediately frozen in liquid nitrogen for use in biochemical determinations. The method of Torello and Rice (1986) was used for proline determination. Therefore, samples of plant tissue weighing 0.3 g were wetted and homogenized in 10 mL of a solution of sulfosalicylic acid (3%) in polyethylene tubes followed by centrifugation at 5000 rpm for 20 minutes. Afterward, one 2-ml aliquot of the supernatant was set aside and 2 ml of ninhydrin acid (BATES, WALDREN;

TEARE, 1973) and 2 ml of acetic acid were added. Then, the samples were placed in water to boil for 1 hour, after which they were cooled with ice cubes. Data was then collected on a spectrophotometer at a wavelength of 520 nm. Data are shown as micromoles of proline per g of fresh material.

To determine peroxidase enzyme activity, samples of fresh plant tissue weighing 0.3 g were wetted and homogenized in a 0.2 M phosphate solution at pH 6.7 and centrifuged at 10,000 rpm for 10 minutes. Peroxidase activity in the supernatant was determined by measuring the absorbance at 470 nm of tetraguaiacol (MACIEL *et al.*, 2007). The specific activity of peroxidase is shown as milligrams of protein per gram of fresh tissue.

Data were subjected to analysis of variance, and means were separated using the LSD test at the 0.05 probability level.

RESULTS AND DISCUSSIONS

Proline concentrations in the leaves were affected by the choice of cultivar, drought, Si, and the interaction of drought \times Si and drought \times cultivar \times Si (Table 1).

Under water stress, both of the rice cultivars tested produced higher concentrations of proline in their leaves (Table 2). Proline is an amino acid synthesized under stress conditions (CRUSCIOL *et al.*, 2009). According to Crusciol *et al.* (2006) the ideal water tension for a rice cultivar is -0.025 MPa, and when this tension is increased due to hydric deficiency, a reduction in rice yield results. Therefore, increasing the soil water tension produced stress in the rice plants and consequently induced them to produce proline.

Some authors suggest that proline is an indicator of water stress (BECKER; FOCK, 1986; CARLIN; SANTOS, 2009; CRUSCIOL *et al.*, 2009). In this regard, under low water tension (-0.025 MPa), the levels of proline were low also (Table 2). The data obtained in the present paper may indicate that, under water deficit conditions, the level of proline in rice leaves increases. Increased proline levels as a function of hydric deficiency have also been observed in rice (DINGKUN *et al.*, 1991, LIMA *et al.*, 2004) and wheat (FUMIS; PEDRAS, 2002).

Upon analysis of the interaction cultivar \times water tension, an effect was observed only for -0.050 MPa, in which the Caiapo cultivar showed the highest proline activity (Table 2). Caiapo is a traditional cultivar recommended for cultivation in upland areas due to its greater tolerance to drought, while Maravilha is suitable for cultivation under irrigation (CRUSCIOL *et al.*, 2006).

Therefore, we could infer that the content of proline in the cultivar Caiapo indicates greater drought tolerance.

Analysis of the interaction Si x water deficiency (Table 2) revealed that a water tension of -0.050 MPa was distinct from -0.025 MPa at both doses of silicon. These results are similar to those obtained by many authors (CARLIN; SANTOS, 2009; CRUSCIOL *et al.*, 2009; DINGKUHN *et al.*, 1991; GUNES *et al.*, 2007b; GUNES *et al.*, 2008; LIMA *et al.*, 2004), where increasing water stress was seen to increase the levels of proline in plants. For silicon application, the effect was observed only at -0.0050 MPa.

Silicon fertilization may have reduced water loss by the rice plants, so proline levels were lower in this situation (Table 2), because proline is described as an osmotic regulator (GUNES *et al.*, 2008). Under conditions ideal for upland rice development, -0.025 MPa (CRUSCIOL *et al.*, 2006), no significant effect of silicon fertilization on proline levels was observed, which may indicate that silicon fertilization is more effective under

stress conditions (MA, 2004). Si application could reduce levels of proline and, therefore, stress levels, given that proline is a biochemical indicator of stress (GUNES *et al.*, 2008).

When the effects of water stress x cultivar on levels of proline (Table 2) were analyzed, an effect was observed only for Maravilha at -0.050 MPa, where levels of proline were increased. Differences in proline levels as a function of cultivar were also observed by Dingkuhn *et al.* (1991) and Lima *et al.* (2004) in rice. This may be an intrinsic characteristic of each cultivar. For silicon fertilization x cultivar, an effect was observed only in Maravilha at 0 Si (Table 2), where no silicon fertilization promoted an increase in proline levels in the reproductive phase. Maravilha has less tolerance to water stress than Caiapo (CRUSCIOL *et al.*, 2006). In the interaction between water tension and silicon, proline levels were increased only for the condition of no silicon and higher stress (-0.050 MPa). This indicates that silicon reduced the levels of stress in the plants and, consequently, the levels of proline, as

Table 1 - Levels of proline in the leaves of rice plants in the vegetative and reproductive phases as a function of water tension, silicon fertilization and cultivars

Treatments	Proline content	
	Vegetative phase	Reproductive phase
Cultivar	$\mu\text{mol g}^{-1}$	
Caiapo	2.5357 a ⁽¹⁾	0.4465 b
Maravilha	0.7709 b	0.6136 a
Water tension (MPa)		
-0.025	0.32871 b	0.4022 b
-0.050	2.9789 a	0.6579 a
Silicon fertilization (kg ha ⁻¹)		
0	1.8048 a	0.6830 a
350	1.5019 b	0.3771 b
Factor	F values ⁽²⁾	
Blocks	0.31 ^{ns}	0.48 ^{ns}
Cultivar (C)	245.29**	7.04*
Water tension (WT)	552.80**	16.58**
Silicon (S)	7.22*	23.73**
C x WT	233.46**	10.45**
C x S	2.11 ^{ns}	4.74*
WT x S	8.50*	13.72*
C x WT x S	0.064 ^{ns}	7.92 ^{ns}
CV(%)	19.28	33.51

⁽¹⁾ Means followed by the same letter don't differ significantly by the LSD test at $p < 0.05$. ⁽²⁾ **, * and ns indicate: significant at 1% and 5%, and not significant, respectively

Table 2 - Significant interactions according to analysis of variance regarding proline activity ($\mu\text{mol g}^{-1}$) in the vegetative and reproductive phases

Interaction in vegetative phase		
Cultivar	Soil water tension	
	-0.025 MPa	-0.050 MPa
$(\mu\text{mol g}^{-1})$		
Caiapo	0.3502 a ⁽¹⁾ B	4.7212 a A
Maravilha	0.3071 a B	1.2347 b A
Silicon fertilization (kg ha^{-1})		
0	0.3158 a B	3.2937 a A
350	0.3415 a B	2.662 b A
Interaction in reproductive phase		
Cultivar		
Caiapo	0.4202 a A	0.4729 a A
Maravilha	0.04205 a B	0.8434 b A
Cultivar	Silicon fertilization	
	0 kg ha^{-1}	350 kg ha^{-1}
Caiapo	0.5311 a A	0.3620 a A
Maravilha	0.8349 b A	0.3923 a B
Silicon fertilization		Soil water tension
(Kg ha^{-1})	-0.025 MPa	-0.050 MPa
0	0.4388 a B	0.9272 a A
350	0.3656 a A	0.3886 b A

¹Means followed by the same letter (uppercase horizontally and lowercase vertically) don't differ by the LSD test at $p < 0.05$

observed by many authors (CARLIN; SANTOS, 2009; CRUSCIOL *et al.*, 2009). On the other hand, Gong *et al.* (2005) observed that application of Si reduced proline levels, as observed in this trial.

A notable difference in the data for peroxidase was that the cultivar Maravilha had higher peroxidase activity than the cultivar Caiapo (Table 3). In this context, water stress is also known to cause oxidative damage to plants, leading to increased production of reactive oxygen species (HEINEMANN; STONE; FAGERA, 2011; VERMA; DUBEY, 2003). Thus, the higher peroxidase activity in Maravilha is related to the genetic characteristics of the plant. However, it is noteworthy that this material has a low resistance to water deficit, which may lead to an increase in peroxidase as a protection against this type of stress. An effect of cultivar and the interaction water tension x Si was observed in the vegetative phase (Table 3). In addition, there were effects produced by the interactions cultivar x Si and water tension x Si in the reproductive phase.

Therefore, for the interaction water stress x silicon, there was an effect only at the zero dose, where a water tension of -0.050 MPa yielded the highest peroxidase activity (Table 4). One form of plant defense is to activate antioxidant enzymes, such as peroxidase, to defend against the deleterious effects of oxidative metabolism (LIMA *et al.*, 2004). It was observed that, when silicon was provided, there was no significant difference in peroxidase activity in the vegetative phase (Table 4). However, a slight tendency to lower activity was observed, which could indicate inhibition of this enzyme in the presence of silicon during this phase.

Increased activity of antioxidant enzymes in rice plants under stress conditions was observed by Verma and Dubey (2003) and Chandru *et al.* (2003) as a defense mechanism against stress. According to the authors, these enzymes break reactive oxygen species into water and molecular oxygen, preventing lipid peroxidation. In the interaction Si x water tension, an effect was noted only at -0.025 MPa, at which silicon fertilization increased peroxidase activity.

Table 3 - Peroxidase activity in the vegetative and reproductive phases as a function of water tension, silicon fertilization and cultivar choice

Treatments	Peroxidase activity	
	Vegetative phase	Reproductive phase
Cultivar	%	
Caiaipo	0.0731 b ⁽¹⁾	0.0755 a
Maravilha	0.0979 a	0.0818 a
Water tension (MPa)		
-0.025	0.0784 a	0.0634 b
-0.050	0.0926 a	0.0939 a
Silicon fertilization (kg ha ⁻¹)		
0	0.0785 a	0.0701 b
350	0.0925 a	0.0872 a
Factor	F values ⁽²⁾	
Blocks	1.13 ^{ns}	2.48 ^{ns}
Cultivar (C)	5.51*	1.65 ^{ns}
Water tension (WT)	1.18 ^{ns}	38.24**
Silicon (S)	1.75 ^{ns}	12.12**
C x WT	0.02 ^{ns}	0.38 ^{ns}
C x S	0.15 ^{ns}	28.53**
WT x S	8.71**	33.67**
C x WT x S	3.45 ^{ns}	2.53 ^{ns}
CV(%)	34.84	17.70

⁽¹⁾ Means followed by the same letter don't differ significantly by the LSD test at $p < 0.05$. ⁽²⁾ **, * and ns indicate: significant at 1% and 5%, and not significant, respectively

Table 4 - Significant interactions affecting peroxidase activity (%) according to analysis of variance in the vegetative and reproductive phases

Interaction in vegetative phase		
Silicon fertilization	Soil water tension	
	-0,025 MPa	-0.050 MPa
Kg ha ⁻¹	%	
0	0.0559 a B ⁽¹⁾	0.1011 a A
350	0.1010 b A	0.0840 a A
Interaction in reproductive phase		
Cultivar		
Caiaipo	0.0618 a B	0.0892 b A
Maravilha	0.0650 a B	0.0986 a A
Silicon fertilization Kg ha ⁻¹		
0	0.0691 a A	0.0710 b A
350	0.0577 a B	0.1167 a A

⁽¹⁾ Means followed by the same letter (uppercase horizontally and lowercase vertically) don't differ by the LSD test at $p < 0.05$

In the reproductive phase, the interaction water stress x cultivar (Table 4) had significant effects in both cultivars, observed as higher peroxidase activity with higher water tension. This increase may be related to the defense mechanism of action of oxidative enzymes. These results corroborate Lima *et al.* (2004), Verma and Dubey (2003) and Chandru *et al.* (2003).

When the interaction cultivar x soil water tension was analyzed, there was an effect at -0.050 MPa only, at which Maravilha had higher peroxidase activity than the cultivar Caiapo. Maravilha has less drought tolerance than Caiapo. The increased water tension in the soil above the recommended level (-0.025 MPa) creates a condition of water stress, causing the Maravilha cultivar to experience more stress than the Caiapo cultivar. Therefore, the activity of antioxidant enzymes is increased under water deficit conditions, as well as in less drought-tolerant cultivars, as a defense against oxidative metabolism.

In the interaction soil water tension x Si, there was an effect only at the 350 kg ha⁻¹ dose of silicon and a tension of -0.050 MPa, in which silicon fertilization increased the activity of peroxidase (Table 4). Several authors have stated that silicon is involved in increased tolerance to biotic and abiotic stress (CRUSCIOL *et al.*, 2009). Thus, under conditions of water stress, plants that were fertilized with silicon showed increased activity of peroxidase, an enzyme related to mechanisms of plant defense. The peroxidase prevents oxidation of lipids (VERMA; DUBEY, 2003). Similarly, the silicon would act on membrane stability, reducing membrane permeability under stress conditions (FAUTEUX *et al.*, 2006) and increasing phospholipid and glycolipid contents (WANG; GALLETTA, 1998). This action would contribute to the protective effect on the plant, resulting in increased activity of peroxidases (FAUTEUX *et al.*, 2006). Liang (1999) found a 24% increase in peroxidase activity in rice plants under stress conditions in the presence of silicon.

CONCLUSION

Under water stress conditions, silicon fertilization reduces the proline content of upland rice plants in both the vegetative and reproductive phases and increases peroxidase activity in the reproductive phase, which could be indicative of stress tolerance.

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