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Effect of silicon on the activity of peroxidase, polyphenol oxidase and nitrate reductase in cowpea and wheat

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ABSTRACT

Nutrient culture experiments were conducted to study the effect of various doses (25, 50, 100, 200, 400 and 800 μg g^{-1}) of silicon on the activity of certain enzymes (peroxidase, polyphenol oxidase and nitrate reductase) in the leaves of wheat and cowpea. Lower doses of silicon (25-200 μg g^{-1}) significantly decreased the activity of peroxidase and polyphenol oxidase enzymes. The increasing concentrations of silicon (400-800 μg g^{-1}) resulted in a concomitant increase in the activity of peroxidase and polyphenol oxidase in both the test crops. Nitrate reductase showed a different trend from that of peroxidase and polyphenol oxidase in both the test crops. A significant increase in nitrate reductase activity was observed at lower additions of silicon whereas higher doses of silicon (400-800 μg g^{-1}) resulted in a decrease in nitrate reductase activity in both the test crops suggesting the involvement of Si in the enzymatic activities of plants.

KEYWORDS

Silicon;
Peroxidase;
Polyphenol oxidase;
Nitrate reductase;
Cowpea;
Wheat.

INTRODUCTION

Silicon (Si) is considered a plant nutrient "anomaly" because it is not considered to be essential for plant growth and development, there being no evidence that it is a part of plant constituents or enzymes[1]. However, beneficial effects of Si have been observed in various plant species[2,3] at low silicon levels. Liang *et al.*[4] showed that added Si decreased the electrolytic leakage from leaves of salt stressed barley. This indicates that Si may affect the structure and integrity of plasma membranes by influencing the stress-dependent peroxidation of membrane lipids and strongly suggested[5] that Si may be involved in metabolic or physiological and/or structural activity in higher plants exposed to biotic and abiotic stresses.

Keeping the above facts in view a nutrient culture study was undertaken to analyze the effect of various concentrations of Si on the activity of peroxidase, polyphenol oxidase and nitrate reductase in cowpea and wheat.

EXPERIMENTAL

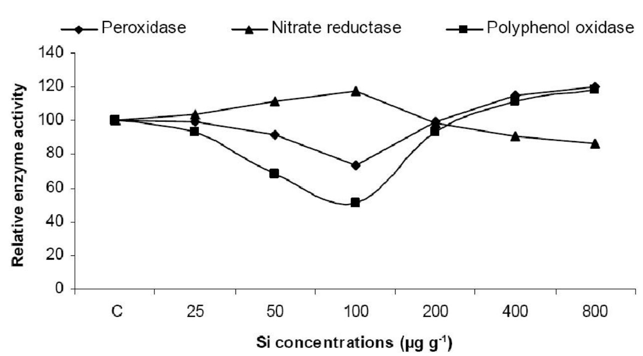
Silicon as sodium metasilicate was applied at doses of 25, 50, 100, 200, 400 and 800 μg g^{-1}. Different concentrations of silicon were prepared separately by taking corresponding amounts (calculated on the basis of molecular weight) of the chemical per 100 g of silica. Pots without any added Si constituted the control. Each pot contained 100 g silica.

Experiments were set during the month of July for cowpea and December for wheat. Fifteen certified seeds of *Vigna unguiculata* (L.) Walp. Var. Pusa Komal and *Triticum aestivum* L. Var. Lok-1 were sown equidistantly at 2 cm. depth in each replicate. Hoagland solution was provided as a source of water. After establishment, seedlings were thinned to ten in number in each replicate. Sets in triplicate were prepared to record observations.

The activities of nitrate reductase, peroxidase and polyphenol oxidase enzymes and total phenol contents were estimated by methods described by Aery[6].

RESULTS AND DISCUSSION

Decrease in the activity of peroxidase and polyphenol oxidase was observed up to 200 µg g^{-1} dose of Si in both the test crops, beyond which a gradual enhancement in the activity was observed. The magnitude of increase varied with both the test crops. The maximum activities of peroxidase and polyphenol oxidase were observed in cowpea at 800 µg g^{-1} dose of Si and were 119.80% and 118.05% higher, respectively, over the control (Figure 1). Enhancement in peroxidase and polyphenol oxidase activities was also observed in wheat at 800 µg g^{-1} concentration of silicon and was 171.01% and 116.66%, respectively, over the control, for wheat (TABLES 1 and 2).



Units: Peroxidase and Polyphenol oxidase: Change in absorbance/ minute/ g fresh weight of leaf, Nitrate reductase: µmoles of NO_2^-/ 30 minutes/ g fresh weight of leaf

Figure 1 : Showing the effect of silicon as sodium metasilicate on relative enzyme activity in the leaves of *Vigna unguiculata* (L.) Walp.

TABLE 1 : Effect of different Si concentrations on the activity of enzymes in cowpea (Relative enzymatic activity given in parenthesis)

Si concentrations (µg g^{-1})	Peroxidase	Polyphenol oxidase	Nitrate reductase
C	27.4889	1.6000	0.0174
	(100)	(100)	(100)
25	27.2555	1.4889	0.0180
	(99.15)	(93.05)	(103.44)
50	25.1222	1.0889	0.0194
	(91.39)	(68.05)	(111.49)
100	20.2777	0.8222	0.0204
	(73.76)	(51.38)	(117.24)
200	27.3222	1.5000	0.0172
	(99.39)	(93.75)	(98.85)
400	31.5999	1.7889	0.0158
	(114.95)	(111.80)	(90.80)
800	31.9331	1.8888	0.0151
	(119.80)	(118.05)	(86.78)

Units: Peroxidase and Polyphenol oxidase: Change in absorbance/ minute/ g fresh weight of leaf; Nitrate reductase: µmoles of NO_2^-/ 30 minutes/ g fresh weight of leaf

TABLE 2 : Effect of different Si concentrations on the activity of enzymes in wheat (Relative enzymatic activity given in parenthesis)

Si concentrations (µg g^{-1})	Peroxidase	Polyphenol oxidase	Nitrate reductase
C	1.5333	2.9333	0.0429
	(100)	(100)	(100)
25	1.4667	2.9111	0.0437
	(95.65)	(99.24)	(101.86)
50	1.3333	2.8667	0.0573
	(86.95)	(97.92)	(133.56)
100	1.3111	2.5333	0.0629
	(85.50)	(86.36)	(146.62)
200	1.5333	2.5667	0.0472
	(100)	(87.50)	(110.02)
400	2.1555	3.1107	0.0356
	(140.57)	(106.04)	(82.98)
800	2.6222	3.4222	0.0318
	(171.01)	(116.66)	(74.12)

Units: Peroxidase and Polyphenol oxidase: Change in absorbance/ minute/ g fresh weight of leaf; Nitrate reductase: µmoles of NO_2^-/ 30 minutes/ g fresh weight of leaf

Peroxidase participates in lignin biosynthesis, indole acetic acid degradation and converts H_2O_2 to water. It is also related to lignin and suberin synthesis, which increase the hardness of tissues and to the production of quinones and active oxygen, which possess antibiotic properties[7]. An increase in peroxidase activity may be used as an indicator of heavy metal stress[8].

In the present investigation, higher concentrations of Si have led to a substantial increase in peroxidase activity in both the test crops whereas lower additions of silicon (25 to 200 μg g^{-1}) significantly decreased the peroxidase activity in both the test crops (TABLES 1, 2 and 3). Increase in peroxidase activity was also observed in tolerant genotype of lentil (*Lens culinaris*) during salt stress[9]. Under stressed conditions the increase in the amount of phenols (Figure 2) appears to be accompanied by an enhancement in the peroxidase activity causing the destruction of auxins[10].

TABLE 3 : Relationship between applied concentrations of Si as sodium metasilicate and activity of certain enzymes.

Parameters	Regression equation (Y)	Correlation coefficient (r)
Cowpea		
1. Peroxidase	Y = 25.075 + 0.0105x	0.724 (PS)
2. Polyphenol oxidase	Y = 1.269 + 0.0008x	0.627 (NS)
3. Nitrate reductase	Y = 0.019 - 0.0000x	-0.757 (S)
Wheat		
1. Peroxidase	Y = 1.342 + 0.0016x	0.953 (S**)
2. Polyphenol oxidase	Y = 2.732 + 0.0008x	0.730 (S)
3. Nitrate reductase	Y = 0.052 - 0.0000x	-0.673 (NS)

x = Applied sodium metasilicate concentrations; y = Parameters studied; S = Significant at 0.1 % level; S = Significant at 2 % level; PS = Significant at 5 % level; NS = Significant at 10 % level**

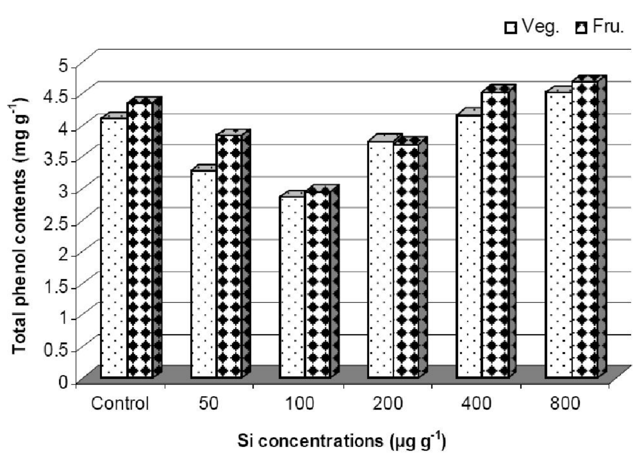


Figure 2 : Showing the effect of silicon as sodium metasilicate on total phenol contents in the leaves of *Vigna unguiculata* (L.) Walp.

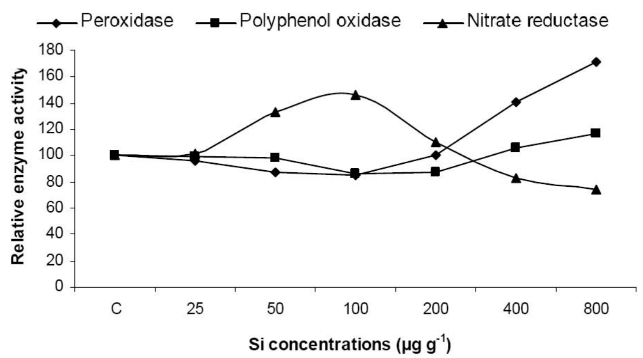
Liang *et al.*[5] observed that exogenous silicon significantly increased the activities of SOD, POD, CAT and GR (glutathione reductase) in the roots of salt-stressed barley. The induction of these enzymes coincided with a decrease in concentration of MDA (malondialdehyde). They suggested that oxidation damage induced by salt can be alleviated by the addition of silicon. Jio-Jing *et al.*[11] observed that exogenous Si could reduce withering of cucumber leaves and increased the antioxidant activities and reduced lipid peroxidation induced by chilling. Similarly, Wang *et al.*[12] observed that Si alleviates the stress caused by NaCl and suggested that exogenous Si increases antioxidative enzyme activities under NaCl stress. Ahmad and Haddad[13] investigated the effect of Si on major antioxidative enzymes including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), relative water content (RWC), chlorophyll and soluble protein contents, proline (Pro) and glycine betaine (GB) accumulation in wheat plants under drought stress. Si has been shown to partially change the negative effects of drought stress and increased the tolerance of wheat by increasing proline and glycine betain contents. Activities of SOD, CAT, APX and POD also increased. They concluded that Si alleviates water deficit of wheat by preventing the oxidative membrane damage and may be associated with plant osmotic adjustment.

Polyphenol oxidase is essentially a copper-dependent enzyme[14] and is involved in the biosynthesis of lignin and alkaloid and catalyzing the phenol oxidation into quinones in the presence of atmospheric oxygen. In the present study, lower doses of Si significantly decreased the polyphenol oxidase activity whereas higher doses (400 and 800 μg g^{-1}) resulted in an enhancement in PPO activity. It is possible that the mechanism of increasing polyphenol oxidase activity occurs in a 'latent form' in plants. Increased PPO activity in the plant tissues after silicon stress could have resulted from activation of 'latent phenolase' in cells[15].

Silicon could increase the activities of peroxidase and polyphenol oxidase because it triggers defence processes in plants, acting as an elicitor. Increased PPO activity also might be due to *de novo* synthesis of PPO under silicon stress[16].

Cherif *et al.*[17] also reported that amendment of nutrient solutions with soluble Si resulted in a marked increase in stimulation of chitinase activity and in a more intense and rapid activation of peroxidase and polyphenol oxidases in cucumber after infection with *Pythium* spp. Si also has an effect on the activity of enzyme such as H^+-ATPase on plants submitted to saline stress, suggesting that it can somehow affect the structure and the integrity of the plasma membrane[18].

Nitrate reductase (NR) showed a different trend from that of peroxidase and polyphenol oxidase in both the test crops. The maximum activity of nitrate reductase was observed at 100 µg g^{-1} dose of Si which showed an increase of 117.24% and 146.62% in cowpea and wheat, respectively. A slight increase in nitrate reductase activity was also observed at 200 µg g^{-1} concentration of Si in case of wheat (Figure 3). Beyond 100 and 200 µg g^{-1} dose of Si a gradual reduction in nitrate reductase activity was observed in cowpea and wheat, respectively (TABLES 1 and 2).



Units: Peroxidase and Polyphenol oxidase: Change in absorbance/ minute/ g fresh weight of leaf, Nitrate reductase: µmoles of NO_2^-/ 30 minutes/ g fresh weight of leaf

Figure 3 : Showing the effect of silicon as sodium metasilicate on relative enzyme activity in the leaves of *Triticum aestivum* L.

Nitrate metabolism is important for providing the organic nitrogen essential for plant growth and nitrate reductase (NR) is the key enzyme in this process[19]. In green algae, fungi and higher plants, reduction of nitrate to nitrite is catalysed by nitrate reductase, an enzyme complex of higher molecular weight which contain FAD, cytochrome and molybdenum as prosthetic group. Nitrate reductase is an inducible enzyme in plants and it is induced by nitrate[20].

Mo as a cofactor is required for the transfer of electrons from $NADH_2$ to nitrate. The source of the reductant is either from mitochondrial electron transport in the dark or photosynthetically produced reductant in the light[20]. Nitrate reductase enzyme is very sensitive to heavy metals because they have an affinity for sulfhydryl groups.

In the present study, lower doses of Si significantly increased the NR activity in both the test crops namely, cowpea and wheat. Higher concentrations of Si (400-800 µg g^{-1}) led to a substantial decrease in NR activity in both the test crops (TABLES 1 and 2). Choudhary[21], Madhwani[22] and Surana[23] also observed a reduction in the activity of nitrate reductase in crop plants at higher levels of Cr, Se and Li, respectively. In the present studies, the reduction in the NR activity at higher concentrations of Si might be due to inadequacy of the reducing power, binding of SH groups of enzyme and replacement of molybdenum from the enzyme[24].

According to Ghosh *et al*.[25] the decreased yield of potato caused by salt stress was due to nutritional imbalance resulting in the inactivation of enzyme such as nitrate reductase (NR). NR contains bound iron and its activity has been reported to decrease in iron deficiency[26].

Si has positive benefits to cellular protection, especially under salt stress[27]. Mali and Aery[28] observed that Si plays an important role in plant physiology and induce the dry matter production, nodulation and uptake of nitrogen in cowpea plants. Kumar and Singh[29] observed high positive correlation among NR activity, grain protein concentration and grain yield per plant in maize leaves. They suggested that leaf NR activity could be useful as a marker enzyme for N content in plants, grain protein concentration and grain yield.

Results of present investigation indicated that higher concentrations of Si were positively correlated with peroxidase and polyphenol oxidase activity and negatively correlated with nitrate reductase activity (TABLE 3). These results seem to suggest that Si is involved in the metabolic or physiological activity in plants exposed to abiotic stresses.

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