International Journal of Research Studies in Zoology
Volume 4, Issue 3, 2018, PP 12-16
ISSN No. 2454-941X
DOI: http://dx.doi.org/10.20431/2454-941X.0403002
www.arcjournals.org

Phenylalanine Ammonia Lyase Activities in Groundnut (*Arachis Hypogaea* L.) in Response to Root and Foliar Application of Two Sources of Silicon

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Abstract: *Phenylalanine ammonia lyase content in leaves of groundnut ranged from 30.40 to 42.14 and 25.07 to 34.71 units when sodium meta silicate was applied as foliar spray (@ 0.5, 1.0, 2.0, 3.5 and 5.0%), and soil application (@ 5.0, 7.5, 10.0, 15 and 20%) and 33.78 to 52.89 and 30.04 to 41.66 units when calcium silicate was applied through leaves and soil at the same doses, respectively in contrast to 22.62 units in plants without silica fertilizer applications. Among the two sources of silica fertilizers tested, foliar spray of calcium silicate at 5 per cent was effective in facilitating the increasing the activities of Phenylalanine ammonia lyase (PAL) in groundnut leaves.*

Keywords: *Calcium silicate, Dicot, Phenylalanine ammonia lyase, Sodium meta silicate.*

1. Introduction

Silicon is considered as a beneficial element for plant growth. It helps the plants to overcome multiple stresses including biotic and abiotic stresses. The deposition of silica on epidermal layer offers a physical barrier to pests [1]. Si also plays an active role in the biochemical processes of a plant and may play a role in the intracellular synthesis of organic compounds [2]. The amount of silicon in plant is almost equivalent to macronutrients like magnesium (Mg), phosphorus (P) and calcium (Ca) [3].

Relative to monocots, dicot plants such as tomato (*Lycopersicon esculentum* Mill), cucumber (*Cucumis sativa* L.) and soybean (Glycine max Merril) are poor accumulators of Si with values of less than 0.1% of their biomass. Dryland grasses such as wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), rye (Secale cereale L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* L.), corn (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) contain about 1% Si in their biomass, while aquatic grasses have up to 5% Si content. Si nutrition not only increases the lignin content but also enhances the activities of peroxides, polyphenol oxidase, and phenylalanine ammonialyase (PAL) in rice leaves [4].

Though several sources of silicon are available in the market or industry (Na_2SiO_3, K_2SiO_3, $CaSiO_3$, $MgSiO_3$, slag, fly ash, ceramics waste, paddy straw and husk etc.) they are reported to vary in their efficiency, uptake by plants, cost-effectiveness, extent of protection to plants from biotic and abiotic stresses, from crop (monocot) to crop (dicot), soil to soil and tropical to temperate regions [5]. Silica solubilizing bacteria could not work satisfactorily, as soil silica is low in tropical region soils [6]. Though the silica content in the earth crust is around 25 per cent, plants could not absorb the silica, as it is in unavailable form. Moreover, tropical region soils are having comparatively less silica than temperate region silica. Another problem with silica is that work on the influence of silica on induced resistance of dicot plants is in infancy, as they are less responsive to silica. In order to overcome certain inherent problems, screening and identification of efficient source of silica, method of application and dose for dicot plants, especially in groundnut need to be attempted, for which, suitable research should be formulated and executed.

2. MATERIALS AND METHODS

Pot culture experiments were conducted at Insectary, Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai. The design adopted was completely randomized block design (CRD). The groundnut (Variety: VRI II) was used with twenty one treatments with 3 replicate each. Two sources of silicon *viz*., sodium meta silicate and calcium silicate were applied in two methods of application a) Control b) Soil application at five doses of 5, 7.5, 10, 15 and 20 % @ 15 ml/500 g of soil on 20 days after sowing c) Foliar application was done on 0.5, 1.0, 2.0, 3.5 and 5.0% @ 5 ml/seedling on 25, 30 and 35 days after sowing. Each pot was maintained with two plants. These plants were used to assay the biochemical characters of leaf samples such as enzyme activity and silica content as per the standard procedures.

The leaf samples were collected from the pots during 30, 45, 60 and 75 days after sowing in all treatments and utilized for analyzing certain bio chemical factors. Laboratory experiments were carried out at Bio-chemistry laboratory, Department of Biotechnology, Agricultural College and Research Institute, Madurai to study Phenylalanine ammonia lyase activities in leaf tissues of groundnut.

2.1. Estimation of Phenyl Alanine Ammonia Lyase (PAL)

Leaf tissues (300 mg) from each of the three replicates for each treatment were homogenized in the ice-cold 0.25 M borate buffer (pH 8.7) in an ice bath. The homogenate was centrifuged at 5000 rpm for 15 minutes at 4°C. The supernatant was centrifuged at 15000 rpm for 15 minutes at 4°C. As such, the resultant clear yellowish green supernatant was used as a crude enzyme extract. The reaction mixture contained 1 ml of enzyme extract, 0.5 ml of 0.2 M borate buffer (pH 8.7), 1.3 ml of distilled water and 0.2 ml of 1 M L-phenylalanine. Changes in absorbance at 290 nm was observed using spectrophotometer (Spectronic model) [7]. Enzyme activity was expressed on fresh weight basis as µ mol min-1ml-1gm-1.

2.2. Statistical Analysis

Data obtained from the biochemical analysis were subjected to ANOVA (Analysis of Variance). Before analysis, data on percentage were subjected to arcsine transformation. In order to know the interaction between treatments, data were subject to factorial CRD analysis and the means obtained were separated by LSD (Least Significant Difference) [8].

3. RESULTS AND DISCUSSION

Table1. *Phenylalanine ammonia lyase content in groundnut as induced by difference sources of silica nutrition under pot culture (Lab experiment)*

Treatments		Phenylalanine ammonia lyase (Units)*				Mean
		30 DAS**	45 DAS	60 DAS	75 DAS	
T_1	Foliar spray of sodium meta silicate @ 0.5 %	21.77 $(4.67)^{k}$	28.00 $(5.29)^{k}$	33.22 $(5.76)^{l}$	38.59 $(6.21)^{j}$	30.40 $(5.51)^{k}$
T_2	Foliar spray of sodium meta silicate @ 1.0 %	23.12 $(4.81)^{j}$	29.24 $(5.41)^{j}$	34.58 $(5.88)^{k}$	38.72 $(6.22)^{j}$	31.42 $(5.61)^{j}$
T_3	Foliar spray of sodium meta silicate @ 2.0 %	25.89 $(5.09)^{h}$	32.83 $(5.73)^{g}$	38.64 $(6.22)^{g}$	43.35 $(6.58)^{g}$	35.18 $(5.93)^{g}$
T_4	Foliar spray of sodium meta silicate @ 3.5 %	28.05 $(5.30)^{f}$	35.06 $(5.92)^{ef}$	40.80 $(6.39)^{ef}$	45.83 $(6.77)^{f}$	37.44 $(6.12)^{f}$
T_5	Foliar spray of sodium meta silicate @ 5.0 %	32.29 $(5.68)^{d}$	39.51 $(6.29)^{d}$	45.79 $(6.77)^{d}$	50.97 $(7.14)^{d}$	42.14 $(6.49)^{d}$
T_6	Drenching of sodium meta silicate @ 5.0 %	19.24 $(4.39)^{m}$	23.37 $(4.83)^{m}$	27.26 $(5.22)^{o}$	30.42 $(5.52)^{n}$	25.07 $(5.01)^{n}$
T_7	Drenching of sodium meta silicate @ 7.5 %	20.53 $(4.53)^{l}$	24.97 $(5.00)^{l}$	28.89 $(5.37)^{n}$	32.40 $(5.69)^{m}$	26.70 $(5.17)^{m}$
T_8	Drenching of sodium meta silicate @ 10.0 %	22.64 $(4.76)^{j}$	27.33 $(5.23)^{k}$	31.59 $(5.62)^{m}$	35.51 $(5.96)^{l}$	29.27 $(5.41)^{l}$
T_9	Drenching of sodium meta silicate @ 15.0 %	24.77 $(4.98)^{i}$	29.79 $(5.46)^{ij}$	34.43 $(5.87)^{k}$	38.55 $(6.21)^{j}$	31.89 $(5.65)^{ij}$

T_{10}	Drenching of sodium meta silicate @ 20.0 %	26.92 (5.19)^{g}	32.31 (5.68)^{g}	37.60 (6.13)^{hi}	42.01 (6.48)^{h}	34.71 (5.89)^{g}
T_{11}	Foliar spray of calcium silicate @ 0.5 %	25.16 (5.02)^{hi}	31.14 (5.58)^{h}	36.94 (6.08)^{i}	41.86 (6.47)^{h}	33.78 (5.81)^{h}
T_{12}	Foliar spray of calcium silicate @ 1.0 %	29.16 (5.40)^{e}	35.48 (5.96)^{e}	41.66 (6.45)^{e}	47.40 (6.88)^{e}	38.43 (6.20)^{e}
T_{13}	Foliar spray of calcium silicate @ 2.0 %	33.75 (5.81)^{c}	40.74 (6.38)^{c}	47.32 (6.88)^{c}	53.24 (7.30)^{c}	43.76 (6.62)^{c}
T_{14}	Foliar spray of calcium silicate @ 3.5 %	38.46 (6.20)^{b}	46.30 (6.80)^{b}	53.38 (7.31)^{b}	59.72 (7.73)^{b}	49.47 (7.03)^{b}
T_{15}	Foliar spray of calcium silicate @ 5.0 %	41.79 (6.46)^{a}	49.51 (7.04)^{a}	56.93 (7.55)^{a}	63.31 (7.96)^{a}	52.89 (7.27)^{a}
T_{16}	Drenching of calcium silicate @ 5.0 %	22.32 (4.72)^{jk}	27.71 (5.26)^{k}	33.00 (5.74)^{l}	37.14 (6.09)^{k}	30.04 (5.48)^{kl}
T_{17}	Drenching of calcium silicate @ 7.5 %	24.44 (4.94)^{i}	30.22 (5.50)^{i}	35.54 (5.96)^{j}	40.21 (6.34)^{i}	32.60 (5.71)^{i}
T_{18}	Drenching of calcium silicate @ 10.0 %	26.81 (5.18)^{g}	32.82 (5.73)^{g}	38.46 (6.20)^{gh}	43.50 (6.60)^{g}	35.40 (5.95)^{g}
T_{19}	Drenching of calcium silicate @ 15.0 %	28.12 (5.30)^{f}	34.48 (5.87)^{f}	40.43 (6.36)^{f}	45.82 (6.77)^{f}	37.21 (6.10)^{f}
T_{20}	Drenching of calcium silicate @ 20.0 %	31.76 (5.64)^{d}	38.61 (6.21)^{d}	45.42 (6.74)^{d}	50.84 (7.13)^{d}	41.66 (6.45)^{d}
T_{21}	Untreated check	17.92 (4.23)^{n}	20.51 (4.53)^{n}	24.19 (4.92)^{p}	27.84 (5.28)^{o}	22.62 (4.76)^{o}
SEd		0.0423	0.0383	0.0354	0.0333	0.0369
CD (P=0.05)		0.0853	0.0773	0.0715	0.0673	0.0744

**Each value is the mean of three replications; **DAS: Days after sowing*

Figures in parentheses are square root transformed values

In a column, means followed by common letter(s) are not significantly different by LSD (P= 0.05)

A pot culture experiment was conducted to investigate the Phenylalanine ammonia lyase activities in groundnut plant, applied with different sources of silica nutrition revealed that the phenylalanine ammonia lyase activity of groundnut leaves ranged from 22.62 (control) to 52.89 units (foliar spray of calcium silicate 5%) (Table 1). The highest mean phenylalanine ammonia lyase content was in leaves treated with foliar spray of calcium silicate @ 5.0 per cent (52.89 units) followed by foliar spray of calcium silicate @ 3.5 per cent (49.47 units), foliar spray of calcium silicate @ 2.0 per cent (43.76 units), foliar spray of sodium meta silicate @ 5.0 per cent (42.14 units), drenching of calcium silicate @ 20.0 per cent (41.66 units), foliar spray of calcium silicate @ 1.0 per cent (38.43 units), drenching of calcium silicate @ 15.0 per cent (37.21 units), drenching of calcium silicate @ 10.0 per cent (35.40 units), drenching of sodium meta silicate @ 20.0 per cent (34.71 units), foliar spray of calcium silicate @ 0.5 per cent (33.78 units), drenching of calcium silicate @ 7.5 per cent (32.60 units), drenching of sodium meta silicate @ 15.0 per cent (31.89 units), drenching of calcium silicate @ 5.0 per cent (30.04 units), drenching of sodium meta silicate @ 7.5 per cent (26.70 units), and drenching of sodium meta silicate @ 5.0 per cent (25.07 units) when compared to control (22.62 units). Same trend was noticed in 30, 45, 60 and 75 DAS.

Silicon is considered as an important beneficial element. Silicon may act directly on insect herbivores leading to reduction in insect performance and plant damage. Various indirect effects may also be caused, for example, by delaying herbivore establishment and thus an increased chance of exposure to natural enemies, adverse weather events or control measures that target exposed insects. Silicon is widely considered as activator by stimulating the expression of natural defense reaction through the production of phenolic compounds.

From the present study, among the two sources of silica fertilizers tested, foliar spray of calcium silicate at 5 per cent was effective in facilitating the increasing the activities of phenylalanine ammonia lyase (PAL) in groundnut leaves. Work on the influence of various sources of fertilizers on the deposition rate of silica in leaves of dicot plants are in infancy in the Country, however, Rezende *et al.* [9] opined that foliar application of silicon was able to help the rice crop to deposit more silica.This result is conformity with the findings of Correa *et al.* [10] and In bar *et al.* [11] who also noticed that application of silica based fertilizers could increase certain plant defense substances *viz.*, chitinase, peroxidase, 1,3-glucanase, phenylalanine ammonia lyase, proteinase and lipoxygenase contents in tomato, cotton and wheat plants. Cherif *et al.* [12] assiduously assessed that, silica based fertilizers increased the synthesis of enzymes peroxidase, β-1, 3-glucanase and chitinase in cucumber plants which conferred the pest and disease resistance. Roncatto and Pascholati [13] stated that, silicon increased the peroxidase activity, apart triggers the defense activity *via* act as an elicitor [14].

PAL is the first enzyme of phenylpropanoid metabolism in higher plants and it plays significant role in regulating the accumulation of phenolics, phytoalexins, and lignins. These three factors increases the resistance against herbivore [15] [16]. Thus silica based fertilizers drawing resistance against insect pests and disease causing pathogens. Apart these enzymes interfere with the phenolic compound metabolism and reduce the nutritional quality of leaves and increase indigestibility of cells which primarily confers resistance against insect pests [17]. Therefore, an increasing phenylalanine ammonia lyase enzyme activities initiate and produce defensive phytoalexins against insect pests and disease causing pathogens.

4. Acknowledgement

The authors are thankful to Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625 104, Tamil Nadu, India for providing infrastructural facilities to carry out the project work.

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Citation: *P Parthiban, et al., "Phenylalanine Ammonia Lyase Activities in Groundnut (Arachis Hypogaea L.) in Response to Root and Foliar Application of Two Sources of Silicon", International Journal of Research Studies in Zoology, vol. 4, no. 3, p. 12-16, 2018. http://dx.doi.org/ 10.20431/2454-941X.0403002*