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Calcium Accumulation in Grasses in Relation to their Root Cation Exchange Capacity

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Abstract: In order to assess the role of root CEC on the accumulation of Calcium in roots or shoots, pot-culture experiments with wild grasses was carried out. The seven species of grasses used were *Sporobolus diander* (L.), *Eleusine indica* (L.) Gaertn., *Heteropogon contortus* (L.) P. Beauv. Ex Roem and Schult, *Cynodon dactylon* (L.) Pers., *Panicum repens* Jacq, *Stenotaphrum dimidiatum* (L.) Brongn. and *Chloris barbeta* Sw. These grasses were significantly different in their root cation exchange capacity (4.6 to 12.3 C mol (P^+) kg^{-1}; $p<0.05$) as well as in their average root biomass (10 to 121 g per plant; $p<0.05$)/shoot biomass (4 to 60 g per plant; $p<0.05$) while growing in same environments. Four treatments of calcium (0.20, 0.25, 0.30 and 0.37 g kg^{-1} of soil) were given to all these species against a control; each treatment was given in four split doses with a gap of about 10 days in between. Calcium in the roots and shoots of these grasses was assessed at the end of the experimental cultures. Negative correlations were found between average Ca accumulations in plant tissues and root cation exchange capacity at all treatment levels. The correlation patterns between root cation exchange capacity and Ca in roots and that in shoots were quite distinct. Experiments of these kinds can reveal exact relationships between root characteristics and mineral accumulations in plants, which will have applications in agriculture involving mixed crops.

Key words: Calcium, root-biomass, shoot-biomass root CEC grasses, metal accumulation

INTRODUCTION

Plants in general have high amount of Ca in their tissues (Marschner, 1995) and Ca is a universally known protective agent in plants against adverse soil factors (Plieth, 2005). Addition of mineral elements such as Ca into soils to improve plant growth is a standard agricultural practice (Hirschi, 2004). Increase of calcium concentration in nutrient solution has positive effects on the contents and yields of effective components in medicinal plants (Supanjani *et al*., 2005). Calcium has a significant effect on the reduction the effect of stress condition during seed germination (Amini Dehaghi *et al*., 2007).

The reproducibility and species specificity of the Cation Exchange Capacity (CEC) of plant roots (Haynes, 1980), its specific role in the selective adsorption of different mineral ions to root surfaces (Kirkby and Pilbeam, 1984) or shoot cation content (Wacquant, 1977) are significant observations, requiring further experimental inquiries.

The rate of root uptake and also the physiotype of plants are significant to the amount of Ca in plant shoots (Broadley *et al*., 2003). Whether, Ca accumulation in shoots of different species is a phylogenetically fixed characteristic (Northupa *et al*., 2005), controlled by inherent root characteristic such as root CEC or a strongly environmentally dependent characteristic (Józefaciuk and Szatanik-Kloc, 2004) is an unsettled issue. Moreover, knowledge of all inherent and environmental factors affecting root environment relations is essential to minimize the deleterious effects of limited nutrient availability in plants (Belesky *et al*., 2008).

Role of root CEC as one of the inherent plant factors that affect plant environment relations requires sufficient evidence. Hence, there is high scope in testing the role of root CEC on the accumulation of Ca in plant roots and shoots, especially in relation to varied amounts of Ca to soils.

Grasses are considered ideal for experimental analysis on root surface characteristics such as root CEC, because they in general have the potentials to accumulate moderate to high amount of metals in their shoots (Ebbs and Kochian, 1998; Sattelmacher, 2001); grasses have finer network of intensive root system to provide large root surface with high volume of root-soil contact (Pivetz, 2001). The major objective of the current experiments was to assess the role of root CEC on the accumulation of Ca in roots or shoots in relation to small changes in concentration of the metal in the soil medium. The grasses selected were all significantly different in their root/shoot biomass and root CEC.

The study is highly relevant because, knowledge of all the morphological and physiological species-specific

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characteristics in mineral absorption from soils have very high role in the selection of suitable combination of diverse species in mixed cropping patterns towards the development of sustainable agriculture. Moreover, information of species specific features on metal accumulation pattern in plants has advantages in the identification of hyper accumulator species useful in phytoremediations.

MATERIALS AND METHODS

Seven species of wild grasses such as *Sporobolus diander* (L.), *Eleusine indica* (L.) Gaertn., *Heteropogon contortus* (L.) P. Beauv. Ex Roem and Schult, *Cynodon dactylon* (L.) Pers., *Panicum repens* Jacq, *Stenotaphrum dimidiatum* (L.) Brongn and *Chloris barbeta* Sw., with distinct root CEC (4.59 to 12.33 cmol (+) kg^{-1}; F = 96.7; $p<0.05$) and significantly different biomass (f = 79.5 with $p< 0.05$; Table 1), selected from among the 30 grasses identified in a field survey (March 2005 to January 2007) of *Thrissur* District (10.31-10.52° N; 76.13- 76.21° E; 47 m altitude), Kerala, South India were used. Root-stalks of grasses were dug out directly from the fields (various places of the *Thrissur* district) and planted out in plastic bags (35×25 cm) on 1 January (2007) filled with top soil (sandy clay loam: 20.1% coarse sand, 40.2% fine sand, 8.9% silt and 30.8% clay), river sand and dried cow-dung in the 1: 1: 1 ratio; pH 5.34; 2100 μg N g^{-1}, 140 μg P g^{-1}, 500 μg K g^{-1} and 210 μg Ca g^{-1}. The bags were filled up to 2/3rd of the volume with 2.5 kg potting mixture. The study was conducted in the open ground (experimental station) in the college campus at *Kottayam* District, Kerala; 9.15-10.21° N; 76.22- 77.25°E, 3 m altitude. In order to minimize the growth of weeds, the soil surface was covered with a plastic sheet. One root-stalk (2.5 cm shoot base and 2.5 cm root) was planted in a bag and three replications maintained.

One month after the planting, four Ca treatments (0.20, 0.25, 0.30 and 0.37 g kg^{-1} of soil) were given to all the seven species as aqueous solutions of Ca $(NO_3)_2$ in four split doses of 50 mL each (9-10 days apart) on 31st Jan., 10th Feb., 19th Feb and 28th Feb., year 2007. Doses were divided into four to minimize loss by seepage of the solution added. The treatments were administered between 07:00 to 08:00 h around the plant on the soil surface. Plants were harvested on 30 March 2007, about one month after the final treatment. Low doses were used to avoid serious positive or negative impact of the metal if any on the growth of the plants.

The experimental design was restricted Cluster Random Sampling Design (CRD). No blocking was applied. In the control bags, 50 mL of distilled water was applied every time when Ca was applied to treatment bags. All the cultured plants were watered daily and uniformly in the evening using well water (neutral pH, negligible nutrients and no detectable amount of toxic metals; approximately 250 mL per bag per day). The mean temperature of the air during period of growth was 27°C; maximum 36°C and the minimum 22°C; relative humidity during the period was 73 to 83%.

Root CEC of all species was measured from the triplicate controls at about 90 days of growth on 30 March 2007. Three fresh root samples (2 g each) of each plant from all the three bags were measured. Average root CEC was measured as by Drake *et al.* (1951).

For the final measurement of biomass and Ca in tissues, the harvested plant roots and shoots were carefully washed in running tap water and finally in deionised water. The aboveground portions were separated from their roots after air-drying (for 7 days), then dried in a hot air oven at 70°C for 72 h to constant weights and weighed.

After the measurement of total biomass of oven dried roots and shoots, about 10 g each of the root and shoot samples were separately powdered in an agate mortar and pestle and HNO_3 extracts prepared as by Zarcinas *et al.* (1987) using 1 g of the sample. Concentration of Ca was determined using an Atomic Absorption Spectrophotometer (Perkin Elmer, 200). Total biomass and that of the root and shoot biomass at the control and the different treatments were compared and analyzed using three-way ANOVA and Fisher's Least Significant Difference method of MRT was used for mean separation (George and Mallery, 2007).

RESULTS

Root and shoot biomass of each species after the treatment: All the grasses were significantly different in their root, shoot and average biomass per plant (Table 1). Comparison of the average root and shoot biomass in individual species revealed that both were significantly different (at the controls as well as in the treatments) in *Eleusine indica*, *Heteropogon contortus*, *Cynodon dactylon*, *Stenotaphrum dimidiatum* and *Chloris barbeta*, but were not significantly different in *Panicum repens* and *Sporobolus diander*. However, in all grasses there were no significant differences in the biomass of the shoot/root over different treatments.

Ca accumulation in roots and shoots: Ca per unit weight of plant biomass (μg g^{-1}) in shoots or roots (at the controls as well as in the treatments) over different grasses and that between shoots and roots in all grasses were significantly different (Table 2). Ca increased

Table 1: Total root and shoot biomass of seven tropical grass species with distinct root CEC, at the control and the treatments of four graded doses of Ca (g Ca kg^{-1} of soil)

		Average shoot Biomass (g) per plant						Average root Biomass (g) per plant					
		Ca treatment levels						Ca treatment levels					
Name of species	Root CEC (cmol(+)•kg^{-1})	Control	0.20	0.25	0.30	0.37	SE	Control	0.20	0.25	0.30	0.37	SE
		(g kg^{-1})						(g kg^{-1})					
Sporobolus diander	12.33[a]	17[i]	23[i]	20[i]	17[i]	25[i]	1.61	13[i]	22[i]	19[i]	14[i]	14[i]	1.58
Eleusine indica	9.10[b]	28[j]	24[j]	33[j]	21[j]	28[j]	1.57	09[k]	7[k]	10[k]	6[k]	8[k]	0.5
Heteropogon contortus	8.08[c]	121[k]	111[k]	112[k]	79[k]	86[k]	6.33	60[h]	65[h]	66[h]	42[h]	47[h]	3.9
Cynodon dactylon	7.53[d]	26[l]	28[l]	33[l]	26[l]	32[l]	1.34	13[n]	13[n]	16[n]	20[n]	23[n]	1.46
Panicum repens	6.27[e]	65[m]	41[m]	42[m]	58[m]	59[m]	3.42	60[m]	34[m]	40[m]	48[m]	54[m]	3.40
Stenotaphrum dimidiatum	4.69[f]	10[n]	29[n]	50[n]	42[n]	31[n]	4.6	07[q]	9[q]	19[q]	21[q]	16[q]	1.94
Chloris barbeta	4.59[g]	18[p]	21[p]	16[p]	16[p]	13[p]	0.96	04[r]	4[r]	4[r]	4[r]	5[r]	0.15

Means with the same superscripts within a column or row do not differ significantly

Table 2: Ca accumulation per unit biomass (µg g^{-1}) in roots and shoots of seven species of grasses at the control and the different Ca treatments*

	Control		C1 (0.20 g kg^{-1})		C2 (0.25 g kg^{-1})		C3 (0.30 g kg^{-1})		C4 (0.37 g kg^{-1})	
Name of species	R	S	R	S	R	S	R	S	R	S
Sporobolus diander	595	389	1009	1978	1619	2839	2291	2947	2650	3031
Eleusine indica	520	918	676	3156	1085	4459	1760	4710	2311	4935
Heteropogon contortus	376	811	1495	1560	1903	1961	2896	2251	4396	2568
Cynodon dactylon	865	237	2211	1220	2236	2272	2336	2500	2534	2607
Panicum repens	670	467	1458	1525	1476	1642	2668	1713	3630	2301
Stenotaphrum dimidiatum	61	162	82	1500	102	1565	126	1615	316	1638
Chloris barbeta	102	912	212	3951	322	4062	334	4218	350	4748

R = Ca in root biomass (µg g^{-1}); S = Ca in shoot biomass (µg g^{-1}). *Means within all columns/ rows are significantly different

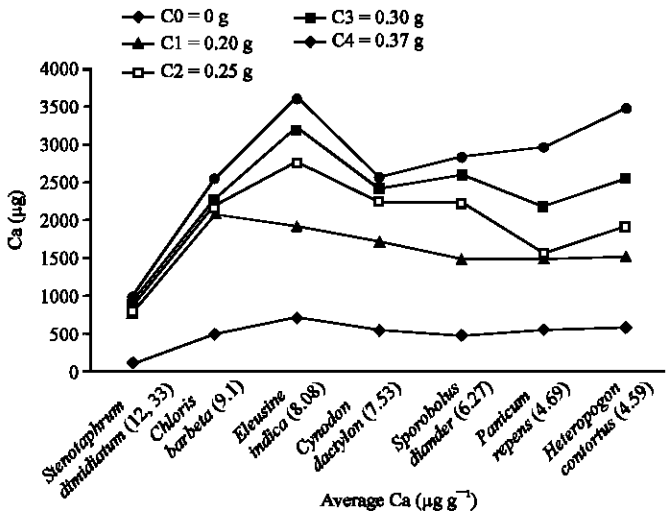

Fig. 1: Negative interrelationships between root CEC and average Ca (µg g^{-1}) in seven grasses (root CEC given in brackets after the species name) treated with five graded treatments (C0 to C4; Ca g kg^{-1} of soil)

gradually from the control to the higher treatment concentrations; the increase in amount of Ca was found higher in the shoots than that in the roots.

Correlation between root CEC and Ca in plants: Root CEC and the amount of Ca in plants (Fig. 1) were negatively correlated with each other at all treatment levels (C0 to C4). However, distinct patterns of correlations between Ca in shoot/shoot and root CEC were observed in these grasses; correlations between root CEC and Ca in roots was negative and the strength of the correlations was found increasing, as the treatment concentration increased. It appeared that the increase in root CEC had no positive influence on Ca in root tissues. The general average (of shoot and root together) of Ca in tissues was also found negatively correlated with that of root CEC. But the correlation between root CEC and Ca in shoots showed positive relationships at different

treatment concentrations. Moreover, the correlation of root CEC and Ca in plant shoots was found decreasing as the amount of addition of Ca in soil increased.

DISCUSSION

Unlike the biomass distribution of perennial grasses (Ray, 1993) shoot biomass was significantly greater than the root biomass in five of the grasses studied. Since there were no significant differences in the shoot/root biomass of a particular species over the various treatments, it became evident that the experiments could assess the interrelationship of the rates of Ca accumulation and root CEC, independent of any stimulatory influence of the nutrient on the growth of the plants. Compared to Ca in temperate grasses, Ca in shoots of these tropical grasses (Table 2) was found comparatively low (0.13 to 0.36% of dry matter). The reason may be due to the comparatively low amount of Ca in the soils and due to low pH of the soils; soil pH in general plays a significant role in the amount of Ca accumulation in plant shoots (Epstein, 1972).

In general, rate of accumulation of Ca in all the seven species was quite linear, increasing with the increase in treatment concentration. Onweremadu (2007) observed similar trends of increase in Ca in gmelina. However, increasing patterns were not similar in the seven species. There were no significant correlation between Ca concentration in shoots and roots of these different grasses ($r<0.20$) along the different treatments. These observations did not agree with that of Plieth (2005) that Ca from soils flows continuously in to plants through roots to shoots and the continuous accumulation of Ca become excessive and toxic to plants; on the contrary Ca accumulation per unit biomass, both in shoots and roots of these grasses was found mostly species specific. This was in conformity with the view of Broadley *et al.* (2003) that Ca in plant shoots depends on rate of root uptake and also the physiotype of the plant. It was interesting to note that at the control stage, two species (*Sporobolus diander* and *Panicum repens*) were found to have more calcium in their roots than in their shoots. But as the Ca availability increased in the soil, the response of both of these species towards Ca in shoot and root was not similar. In *Sporobolus diander*, *Stenotaphrum dimidiatum* and *Chloris barbeta* the Ca accumulation patterns along the different treatments were quite different.

It may also be noted that the two species with very high shoot Ca, *Eleusine indica* and *Chloris barbeta* were the plants with comparatively low root Ca as well. The trend was that, as the Ca in shoots increases, that in the root decreases. However, there was no correlation between Ca in root and shoots. Ca accumulation in plant shoots may be controlled not only by species specific absorption process (Marschner, 1995), but also the translocation process. Moreover, Ca accumulation process in grasses shall be through both the *symplast* and *apoplast,* as is suggested by White (2001); because, if the absorption was through the *apoplast* alone, which is quite a passive process, increase of Ca in roots should have shown a corresponding increase in the shoot as well. The cosmopolitan grass species *Eleusine indica*, which is an already identified alternative forage grass (Regmi *et al.*, 2004), may be further explored as to its Ca accumulation potential as a Ca rich forage crop.

In general, strong positive correlation between the Ca addition and average Ca concentration in grasses was observed. It may be noted that this trend was available with the quite mild additions of Ca in the present experiment. According to Kirkby and Pilbeam (1984), dicotyledons with high root CEC accumulate more Ca than monocotyledons, which have low root CEC. However, only negative correlations were observed between root CEC and average Ca in roots of these grasses. The general average (of shoot and root together) of Ca in tissues was also found negatively correlated with that of root CEC. But positive correlations were observed between root CEC and Ca in shoots at all the treatment levels. Strength of correlation of root CEC and Ca in plant shoots was found decreasing as the amount of addition of Ca in soil increased.

Overall, the current studies revealed that Ca accumulation in grasses is species specific, not related to the plant biomass, but correlated to root CEC to a certain extent. Moreover, the amount of Ca per unit weight of roots and shoots are not correlated. These observations provide more insight into the inherent as well as environmental factors determining mineral accumulation process in plants. Therefore, these kinds of studies are essential to resolve complex ecological relations of mineral absorptions as well as species specific properties, like root CEC, on mineral accumulation process in plants. Clarity in these mechamisms shall be highly useful to attain maximum productivity of field systems through multi-crop ecological farming, while keeping the soil fertility sustainable.

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