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Response of Selected Wheat Cultivars to Calcium in Saline Condition

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ABSTRACT

An experiment in NaCl saline solution was conducted to study the effect of calcium on two selected wheat cultivars, one Chinese (Bao-119, sensitive) and one Pakistani (FSD-85, tolerant). Twenty days old 20 seedlings of each cultivar in ½ strength Hoagland nutrient solution were subjected to incremental salt stress until the required salinity levels (200 mol m^{-3} NaCl) were obtained in respective containers. Calcium concentrations were obtained by revising the Hoagland nutrient solution. Dry matter yield (DMY), Na^+, K^+, and Ca^{++} contents at 21 days, leaf area at 23 days, dehydrogenase activity of roots at 27 days and membrane permeability (Release of K^+) at 29 days were determined after imposing the salt stress. Salinity decreased, DMY, K^+ contents, leaf area and dehydrogenase activity of roots; whereas, Na^+ contents and membrane permeability increased. The reverse was also true with the addition of 8 mol m^{-3} calcium to the root medium. Generally, sensitive cultivar (Bao-119) was found more responsive to calcium as compared to tolerant cultivar (FSD-85). Further this calcium concentration was not enough to ameliorate the adverse effect of Na^+. More work is needed to determine the most suitable quantity of calcium to ameliorate the adverse effect of salinity and other side's affect of calcium on crops. Since these effects of calcium tantamount to high yield, it's wise, therefore to apply Ca^{++} as gypsum to saline-sodic soils in order to improve their productivity.

Key Words: Salinity; Ca^{2+}; Na^+; K^+; Leaf area; Membrane permeability

INTRODUCTION

A considerable portion of irrigated land resources in arid and semiarid regions of the world including Pakistan where a large area has been rendered agriculture unproductive due to high concentration of salts in the root zone. According to Khan (1998), 6.67 million hectares (M ha) area of Pakistan is salt affected. Salt affected soils can be managed by reclamation, but due to less availability of good quality water, low soil permeability and high cost of management, this approach is not feasible on a large scale (Qureshi *et al.*, 1990). Saline agriculture technology is an alternative approach for effective utilization of salt affected soils, which involves the cultivation of salt tolerant species/crop. This technology give economic return from salt affected soils and provide vegetative cover to soil which reduces evaporation and hence the rate of salinization (Qureshi & Barret-Lennard, 1998). Study of response of plants/crops to salinity under natural saline conditions is not feasible due to extreme variability in soil salinity both spatially and temporally (Hajrasuliha *et al.*, 1980; Richards, 1983). To avoid this problem comparative differences for salt tolerance among crops / varieties can be study under artificially salinized controlled conditions where sodium chloride has been added. Selection then is frequently based on ability to survive often under extreme sodium chloride concentration (Epstein, 1980; McGuire & Devorak, 1981). Many research workers (Shannon, 1978; Epstein, 1980; Ponamperma, 1984) have tried to develop rapid mass screening method for salt tolerance of agriculture crops at the early seedling growth stage, although this may not essentially correlate with the effects at latter stages of growth. However, it need less time, energy and space than screening mature plants and thus provides the opportunity to screen a wide range of genotypes.

In saline environment plants take up excessive amount of sodium at the cost of potassium and calcium (Kuiper, 1984). The accumulation of chloride parallels that of sodium (Yeo & Flowers, 1985). Calcium is required in the external medium to maintain the selectivity and integrity of cell membrane (Fageria, 1983). The role of calcium becomes even more important in saline environment (Rain, 1972). Therefore, high Na^+/Ca^{++} ratio in saline environment may impair the selectivity of root membrane and result impassive accumulation of sodium in the shoot and root (Kramer *et al.*, 1977). The ratio of Na^+/Ca^{++} in leaves may be a better indicator of salt tolerance of plants instead of sodium and calcium concentrations. Calcium requirement of plants are generally very small. However, the requirement of calcium in the external solution increases under adverse conditions (Clarkson & Hanson, 1980). At 50 mM NaCl in the external solution, growth of beans decreased and concentration of Na^+ in leaves increased only when Na^+/Ca^{++} ratio exceed 17 (La Haye & Epstein, 1969). Increasing concentration of Ca^{++} in the external solution (50 mM) depressed not only the uptake of Na^+ by roots of beans (*Phaseous vulgaris*) plants, but also its translocation to leaves in the presence of 10 mM $CaSO_4$ (Na^+/Ca^{++} =5), sodium did not enter the beans leaves (La Haye & Epstein, 1971). Decreased in Na^+/Ca^{++} ratio through the addition of Ca^{++} to the root

environment (with 40 mM salinity & Na^+/K^{++} ratio of 20), slightly increased the growth and K^+ uptake by rice (Kawasaki & Moritsugu, 1978). Yeo and Flower (1985) found no effect of Na^+/Ca^{++} ratio in culture solution on the growth or Na^+ concentration in rice shoot, when salinity treatments were imposed for 7 days on 14 days old seedling. Poor root growth in saline environment (Ponnamperuma, 1984) may also be associated with calcium deficiency (Wyn Jones & Lunt, 1967). In this study an attempt has been made to assess the extent of response of selected salt tolerant and salt sensitive (at seedling stage) wheat cultivars to calcium in saline solution.

MATERIALS AND METHODS

Experiment was conducted in wire house of the Department of Soil Science and Agricultural Chemistry, Zhejiang Agricultural University, Hangzhou, P.R.China during 18th November to 4th January with natural daylight and day/night temperature of $21/8^0$C respectively. Sufficient healthy seeds of two wheat cultivars {1 Chinese (Ba0-119) and I Pakistani (FSD-85)} were soaked in 0.2% fungicide solution for 18 h. After draining fungicide solution, the seeds were washed thrice with tap water. Then seeds were sown in quartz sand in iron trays. The condition in trays kept moist with water and trays remained covered until the spout came out and waited for nine days. Thirteen days old 20 seeding of each cultivar were transferred to 1cm plugged holes in wooden covers over 32 liters of ½ st6rength Hoagland nutrient solution (Hoagland & Amon, 1950) in plastic containers. Ten holes were used for each cultivars and each holes having two seedlings. Twenty days old seedlings were subjected to incremental stall stress in full strength Hoagland solution. Salt concentrations were increased by 25 mol m^{-3} after every 12 h by adding NaCl to nutrient solution until the required salinity levels (Nutrient solution, 200 NaCl mol m^{-3}) were obtained in respective containers. Calcium concentrations were obtained by modifying the full strength Hoagland nutrient solution (Pervaiz, 1993).Three treatments used in the experiments were as under.

T1= Nutrient solution T2= 200 NaCl + 0.4 Ca^{++} (mol m^{-3})
T3= 200 NaCl + 8.0 Ca^{++} (mol m^{-3})

Solution were renewed after every seven days in the beginning but latterly changed after three days due to higher growth of plants and pH 6.0- 6.5 was daily maintained and any loss of water was made regularly. Solution was aerated for 9 h everyday with air pump by splitting in to three equal parts and intervals. Twelve plants were harvested 21 days after salinization. The plants were washed with running tap water followed by a quick rinse in distilled water. The plants tissue dried at 70^0C were weight and ground in mortar with pestle and stored in polyethyene bags and redried at the time of weighing for analysis .The ground materials were digested with 1 N HCl for 24 h at 40^0C, then the required volume was shaked for one and half hour and filtered. In the digest sodium, potassium and calcium were determined by "ICP" model. Jarrel Ash, ICPA-9000. Leaf area was measured after 23 days of salinization by portable area meter (model, LI 3000. Li-COR, U.S.A.).

Dehydrogenase activity of the roots was determined after 27 days of salinization using the method as: One-gram root sample was taken in test tube. Added 5 mL 0.4% TTC and 5 mL 0.1 M phosphate buffer solution. Then the sample was incubated for 3 h at 37^0C. After 3 h samples were immediately taken out from incubator and added 2 mL 2N H_2SO_4 for stopping the enzyme reaction. Then ground the roots in pestle mortar with ethyl acetate up to no color and made volume up to 50 mL. Then reading was taken at 485 nm wavelengths on spectrophotometer and calculated as:

Reduced TTC ($g.g^{-1}$ fresh weight h^{-1}) = 50 x reading of sample / weight of sample x time (h)
Where TTC= 2.3.5-triphenyl tetrazolium chloride.

Membrane permeability was determined after 27 days of salinization using the method as given below: Two separate sets are needed to measure the membrane permeability. Half gram fresh leaves (small pieces) were taken in 50 mL glass beaker and 20 mL glass tube for set first and set 2nd respectively. In the first set, 10 mL redistilled water was added and deleted the air with injector from the leaves. Then allowed it to stand for 3 h at $20\text{-}30^0$C and finally supernatant was transferred to 20 mL glass tube for the determination of K^+. In set 2nd, 10 mL redistilled water was added. Then led the test tube and boiled for 10 to 15 minutes. After boiling, cooled it and supernatant solution was transferred to 20 mL clean glass test tube for K^+ determination. Potassium contents were determined with flamephotometer and calculated as

Release of K%= Reading of set I x 100 / Reading of set II

Statistical analysis was done by the method given by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Addition of salinity decreased, DMY of shoot and root, K^+ contents, leaf area and dehydrogenase activity of root whereas Na^+ contents and membrane permeability increased. Addition of Ca^{++} in salinized root medium, increased DMY, K^+ content, leaf area and dehydrogenase activity of root whereas Na^+ content and membrane permeability decreased. Data in Table I revealed that salinity (T2) decreased the DMY both of shoot and root. These results are in agreement with those of Khan *et al.* (1998), Saqib *et al.* (2000) and Shafqat *et al.* (2002). In saline treatment (T2), average DMY of shoot and root of both the cultivars was 42 and 52% of control, respectively.

The addition of calcium in T3 increased the average DMY of shoot and root up to 142 and 133% of T2, respectively. These results are in agreement with those of Khan *et al.* (1998) and Sadiq *et al.* (2002). The increase of DMY of shoot due to addition of calcium to saline treatment was more pronounced in sensitive cultivar (Bao-119) which was 157% with coefficient of variation 54% as compared to FSD-85 (130%) with coefficient of variation 26%. The increase of DMY of root was also more pronounced in Bao-119. The addition of calcium indicated the role of calcium in root development by retaining more K^+ and Ca^{++} as compared to saline treatment (Pervaiz, 1993). Ponnamperuma (1984) reported that the decrease in root growth is associated with salinity but Wyn Jones and Lunt (1967) observed that is associated with calcium deficiency.

The data of ionic composition is given in Table II to IV. The data in Table II revealed that salinity (T2) increased Na^+ contents both in shoot and root. The increase of Na^+ contents is in agreement with those of Saqib *et al.* (2000), Akhtar *et al.* (2001) and Shafqat *et al.* (2002). In saline treatment (T2), FSD-85 accumulated more Na^+ contents (1689 m mol kg^{-1} D.W.) than Bao-119 (1615 m mol kg^{-1} D.W.). Accumulation of more Na^+ in the shoot of FSD-85 and given higher DMY is its evidence to salt tolerance. The addition of calcium in T3 gave average Na^+ contents of shoot and root up to 55 and 92% of T2, respectively. The extent of decrease of Na^+ contents of shoot due to addition of calcium to saline treatment was more pronounced in sensitive cultivar Bao-119 which was 50% with coefficient of variation 99% as compared to FSD-85 (41%) with coefficient of variation 77%. In case of roots, the extent of decrease of Na^+ due to the addition of calcium was also more pronounced in sensitive cultivar Bao-119. This support the role of calcium for reducing the permeability of root membrane to Na^+, resulting decrease in uptake of Na^+ by wheat. La Haye and Epstein (1969, 1971) observed this in beans.

Data in Table III revealed that salinity (T2) decreased K^+ contents both in shoot and root. Kawasaki and Martisugu (1978), Saqib *et al.* (2000) and Shafqat *et al.* (2002) have reported similar results. In saline treatment (T2), FSD-85 accumulated more K^+ contents (1076 m mol kg^{-1} D.W.) as

Table I. Response of selected wheat cultivars to calcium in saline condition regarding dry matter yield of shoot and root

	Cultivars					
	Bao-119	FSD-85	Average	Bao-119	FSD-85	Average
Treatments	Dry weight of shoot (g)			Dry weight of root (g)		
T1	2.75	2.34	2.53	0.55	0.70	0.63
T2	0.94 (34)	1.15 (50)	1.05 (42)	0.31 (56)	0.34 (49)	0.33 (52)
T3	1.48 (157)	1.50 (130)	1.49 (142)	0.43 (139)	0.44 (129)	0.44 (133)
Average	1.72	1.65		0.43	0.49	
Coefficient of variation (%)	54	26		28	38	

T2 and T3= Figures in parentheses are percentage of their respective T1 and T2 respectively

Table II. Response of selected wheat cultivars to calcium in saline condition regarding Na^+ contents of shoot and root

	Cultivars					
	Bao-119	FSD-85	Average	Bao-119	FSD-85	Average
Treatments	Na^+ contents of shoot (m mol kg^{-1} dry weight)			Na^+ contents of root (m mol kg^{-1} dry weight)		
T1	9	9	9	34	34	34
T2	1615	1689	1652	998	1032	1015
T3	808 (50)	993 (59)	901 (55)	877 (88)	999 (97)	938 (92)
Average	811	897		636	688	
Coefficient of variation (%)	99	77		83	82	

T3= Figures in parentheses are percentage of T2

Table III. Response of selected wheat cultivars to calcium in saline condition regarding K^+ contents of shoot and root

	Cultivars					
	Bao-119	FSD-85	Average	Bao-119	FSD-85	Average
Treatments	K^+ contents of shoot (m mol kg^{-1} dry weight)			K^+ contents of root (m mol kg^+ dry weight)		
T1	1681	1740	1711	975	1142	1059
T2	973 (58)	1076 (62)	1025 (60)	286 (29)	275 (24)	281 (27)
T3	1327 (136)	1340 (125)	1334 (130)	516 (180)	505 (184)	511 (182)
Average	1327	1385		592	641	
Coefficient of variation (%)	27	24		59	70	

T2 and T3= Figures in parentheses are percentage of their respective T1 and T2 respectively

Table IV. Response of selected wheat cultivars to calcium in saline condition regarding leaf area and Na^+:Ca^{++} rato of shoot

	Cultivars					
	Bao-119	FSD-85	Average	Bao-119	FSD-85	Average
Treatments	Leaf area (cm^2)			Na^+ and Ca^{++} ratio		
T1	43	38	41	0.1676	0.1946	0.1811
T2	14 (33)	16 (42)	15 (37)	35.11	40.21	37.66
T3	21 (150)	23 (144)	22 (147)	7.15	8.01	7.58
Average	26	26		14	16	
Coefficient of variation (%)	58	43		132	133	

T2 and T3= Figures in parentheses are percentage of their respective T1 and T2 , respectively

Table V. Response of selected wheat cultivars to calcium in saline condition regarding membrane permeability of leaves and dehydrogenase activity of roots

	Cultivars					
	Bao-119	FSD-85	Average	Bao-119	FSD-85	Average
Treatments	Membrane permeability (Release of k%)			Dehydrogenase activity (TTC ($g.g^{-1}$ fresh wfight h^{-1})		
T1	7	6	7	152	149	147
T2	60 (857)	73 (1217)	67 (957)	61 (40)	78 (52)	74 (50)
T3	26 (43)	37 (51)	32 (48)	198 (325)	235 (301)	206 (278)
Average	31	39		137	154	
Coefficient of variation (%)	87	86		51	51	

T2 and T3= Figures in parentheses are percentage of their respective T1 and T2, respectively

compared to Bao-119 (973 m mol kg^{-1} D.W.). The addition of calcium in T3 gave K^+ contents of shoot and root up to 130 and 182% of T2, respectively. The extent of increase of K^+ contents of shoot due to addition of calcium to saline treatment was more pronounced in sensitive cultivar Bao-119 which was (136%) of T2 with coefficient of variation 27% as compared to FSD-85 (125%) with coefficient of variation 24%. In case of roots, the extent of increase of K^+ due to the addition of calcium was more pronounced in tolerant cultivar FSD-85. Generally shoot accumulated more K^+ contents than roots. From the ionic composition, it is observed that FSD-85 accumulated more K^+ and Na^+ contents and showed maximum relative DMY in saline treatment whereas sensitive cultivars Bao-119 showed more response to the addition of calcium in saline medium. It means that productivity of sensitive cultivar can be increased by the addition of calcium in saline-sodic soil.

Data in Table IV revealed that with the addition of calcium, sensitive cultivar Bao-119 gave low Na^+:Ca^{++} ratio (7.15) as compared to FSD-85 which is an other indicator of more response of Bao-119 and showing its tolerance under saline condition. Further leaf area was decreased due to salinity and saline treatment (T2) gave only 37% average leaf area. Zammurad *et al.* (1977) and Manzoor (1999) and Qadir and Shamas (1999) reported similar result. The addition of calcium (T3) increases the leaf area of Bao-119 to 150% of T2 and FSD-85 to 144%. The coefficient of variation for Bao-119 was 58% and 43% for FSD-85.

Data in Table V revealed that average membrane permeability was increased (957% of control) due to salinity. The addition of calcium (T3) decreased the membrane permeability and gave average membrane permeability only 48% of T2. Bao-119 gave only 43% whereas FSD-85, 51% of T2 membrane permeability with coefficient of variation 87 and 86%, respectively. Greenway and Munns (1980) also reported the reduction in membrane permeability. This indicated the role of calcium to reduce the rate of release of K^+ from the leaves of cultivars and more response of Bap-119 to calcium as compared to FSD-85. From the Table V it was also observed that salinity decrease the dehydrogenase activity of roots up to 50% which is the cause of low DMY. Murumkar and Chavan (1987) and Mattioni *et al.* (1987) also reported reduction in root dehydrogenase activity. The addition of calcium increased the dehydrogenase activity up to 278% of T2. The extent of increase was more pronounced in Bao-119 (325% of control) indicating it's more response to calcium as compared to tolerant cultivar FSD-85. From this study it was concluded that sensitive cultivar Bao-119 gave more response to calcium as compared to tolerant cultivars FSD-85. Further this Ca^{++} concentration was not enough to ameliorate the adverse effect of Na^+. Since these effects of added Ca^{++} tantamount to highly yield, it wise, therefore to apply Ca^{++} as gypsum to saline-sodic soils in order to improve their productivity. More work is needed in this area in relation to other side's affect of Ca^{++} on crops.

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