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POTASSIUM-SODIUM SELECTIVITY OF SUNFLOWER AS INFLUENCED BY CALCIUM SUPPLY UNDER SALINE CONDITIONS

Arshad Ali*, Badr-u-Zaman, M. Salim and Rahmatullah

Soil Salinity Lab., Land Resources Research Institute, National Agricultural Research Center, Park Road, Islamabad-45500, Pakistan

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SUMMARY

A hydroponic study was conducted to investigate the influence of Ca supply on K^+/Na^+ selectivity of sunflower at 0, 75 and 150 mM NaCl in root medium. Sunflower (*Helianthus annuus* L. cv. Hysun-33) seeds were germinated in moist quartz sand and twelve-day old seedlings were foam-plugged in lids of plastic pots each containing 2.5 l of continuously aerated half strength Hoagland's nutrient solution without calcium salts. The solution pH was adjusted to 5.9 by adding HCl and NaOH. There were three salinity levels (0, 75 and 150 mM NaCl) and two calcium levels (5 and 10 mM $CaCl_2$). The experiment was organized in a randomized complete block design (RCBD) in three replicates. At low concentration of calcium (5 mM), sunflower growth decreased drastically with increasing concentration of NaCl in nutrient solution. On the other hand, plants grown in high concentration of NaCl experienced less damage with relatively higher concentration of calcium. At relatively higher concentration of calcium (10 mM Ca), sunflower plants absorbed and translocated relatively more potassium and less sodium, than at low concentration of calcium (5 mM Ca), demonstrating the positive role of calcium in alleviating the hazardous effects of salinity on sunflower growth.

Key words: Ca alleviation effects, K:Na selectivity, NaCl salinity, sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is becoming an increasingly important source of edible vegetable oil throughout the world because of its high polyunsaturated fatty acid content and no cholesterol. The increasing demand for edible oil in the country increases the hectarage of sunflower, where some soils are salt affected. Sunflower is a moderately salt tolerant crop therefore it has a wide margin to be grown successfully on most salt-affected soils as long as ECe does not exceed 4.8 dS

* Corresponding author: Tel.: 92-51-9255060,
e-mail: salim@pnut-narc.isb.sdnpk.org.

m^{-1}. Moreover, the oil content of seed, for which the crop is grown, shows little response to salinity (Francois, 1996). This places sunflower in the same tolerance category as soybean (Maas and Hoffamn, 1997) and safflower (Francois and Bernstein, 1964), the other two major edible oil crops. Although the threshold for all the three species is nearly identical, the rate of yield decline above the threshold is lesser for sunflower as compared with soybean and safflower (Francois, 1996). Supplementation of a medium with a high level of Ca^{2+} alleviates growth inhibition (LaHaye and Epstein, 1969; Hanson, 1984; Kinraide and Parker, 1987; Yan *et al.*, 1992; Yermiyahu *et al.*, 1997a) by inhibiting the absorption of Na^+ and K^+ by plant root (LaHaye and Epstein, 1971). Kinraide (1998) clearly stated that calcium could completely relieve Na^+ toxicity as long as NaCl stays below 135 mM. Under the prevailing conditions, the alleviating effect of calcium along with salinity stress needs to be investigated with respect to the plant growth and ionic uptake of sunflower. Moreover, selective absorption of sodium and potassium by root is involved in salt tolerance of plants. K^+/Na^+ selectivity of plant is therefore used as one of the indicators. There is little information available on the response of most commonly grown sunflower cultivars to salt stress. This paper describes the growth response of sunflower to varied NaCl salinity levels and the alleviation effect of Ca^{2+} on NaCl. Total biomass production, leaf area and the uptakes of Na^+, K^+ and Ca^{2+} by sunflower grown at low and high levels of calcium supply in a saline root medium are also discussed.

MATERIALS AND METHODS

The experiment was conducted in solution culture. Sunflower (*Helianthus annuus* L. CV. Hysun-33) seeds were geminated in moist quartz sand. Twelve-day-old seedlings were foam-plugged in lids of plastic pots each containing 2.5 l of continuously aerated half strength Hoagland's nutrient solution (Epstein, 1972), without calcium. The nutrient solution was changed at ten-day intervals. The light intensity was 450 μmol $m^{-2}s^{-1}$. The photoperiod was adjusted to 14 h light period. The temperature was maintained at 30±2°C. The solution pH was adjusted to 5.9 with HCl and NaOH and monitored regularly. There were three salinity levels (0, 75 and 150 mM NaCl) and two calcium levels (5 and 10 mM $CaCl_2$). The salinity levels were imposed one week after transplantation by incremental addition of 25 mM NaCl on alternate days. Calcium levels were supplied as $CaCl_2$ two days after transplanting. The pots were arranged according to a randomized complete block design (RCBD) in three replicates. The plants were harvested 36 days after transplanting. Immediately after harvest, the plants were separated into root and shoot, rinsed with deionized water and oven dried at 60°C to constant weight. The dried plant samples were ground to pass a 40-mesh Wiley mill. Ground sub-samples of root and shoot were digested in 2:1 perchloric-nitric di-acid mixture to estimate Na^+, K^+ and Ca^{2+} in the digest by atomic absorption spectroscopy. The data were statistically analyzed according to RCBD and treatment means were compared using Duncan's multiple range (DMR) test (Gomez & Gomez, 1984). Data regarding leaf area were gathered by taking a fully developed leaf from each plant. Potassium-sodium selectivity was calculated using the following formula (Yeo and Flowers, 1984) K:Na selectivity = (K^+/Na^+ in plant tissue) / (K^+/Na^+ in root medium).

RESULTS AND DISCUSSION

There was a significant interactive ($P<0.05$) effect of NaCl salinity and calcium supply on dry matter yield of sunflower (Table 1). Decrease in total biomass production with increasing NaCl salinity at both levels of calcium supply was linear and significant ($P<0.05$). Adverse effect of root medium salinity on plant growth was reported earlier by Hussain and Elahi (1995) and Ali *et al.* (1999). It was more pronounced at the lower level of calcium supply (5 mM Ca) than at the higher level of calcium supply (10 mM Ca). In other words, Ca^{2+} supply significantly alleviated the adverse effect of NaCl salinity on total biomass production. Relative reductions in total biomass production at 5 mM calcium supply level were 8.66% and 32.20% at 75 and 150 mM NaCl, respectively. At 10 mM calcium supply level, 52% and 12% increases in total biomass production were registered at 0 and 75 mM NaCl, respectively. When comparing the biomass production at the low calcium supply level without salinity, 26% and 42% reductions were recorded at 75 and 150 mM NaCl, respectively. Application of external calcium has long been known to ameliorate salinity stress symptoms in many species. Although this effect is based on several phenomena (Kinraide, 1998), a clear physiological basis is now available from many studies on the impact of Ca^{2+} on ion channel. Kinraide also reported that calcium could completely relieve Na^+ toxicity as long as the NaCl concentration was less than 135 mM. Furthermore, high Na^+ can impose an osmotic stress that calcium is unable to relieve. The results of this study are in accordance with these findings. Fifty-two percent increase in total biomass production at 10 mM calcium level + 0 mM NaCl as against 5 mM calcium supply + 0 mM NaCl is indicative of a high calcium requirement of sunflower (Hysun-33).

A significant ($P<0.05$) interactive effect of NaCl salinity and calcium supply was observed on leaf area of sunflower (Table 1). Reduction in leaf area was noticed with increasing NaCl salinity levels. At 5 mM Ca^{2+}supply level, there occurred 13% and 38% reductions in leaf area at 75 and 150 mM NaCl levels, respectively. At 10 mM Ca^{2+} supply level, 2% and 32% reductions in leaf area were recorded at 75 and 150 mM NaCl, respectively. However, at 0 mM NaCl salinity level, 63% increase in leaf area was registered at the higher calcium level (10 mM) as compared with the lower calcium level (5 mM). The relative increase in leaf area at the elevated calcium level is indicative of better plant performance in the presence of high calcium in root medium.

Table 1: Effect of calcium on dry matter yield and leaf area of sunflower under saline conditions

Treatment	Dry matter yield (g/pot)		Leaf area (cm^2)	
	5 mM Ca	10 mM Ca	5 mM Ca	10 mM Ca
0 mM NaCl	5.31 b	8.11 a	201. 83 b	329. 14 a
75 mM NaCl	4.85 c	5.95b	174.35 b	189.19b
150 mM NaCl	3.60d	4.73 c	125.71 c	135. 06 c

Means sharing same letters are statistically non-significant at $P<0.05$

Sodium chloride salinity and calcium levels affected significantly ($P<0.05$) the calcium uptake by plants (Table 2). With increasing NaCl concentration, there were significant decreases in Ca^{2+} uptake at both calcium levels. However, calcium uptake was relatively higher at 10 mM calcium level than at 5 mM Ca^{2+} level. At 10 mM Ca^{2+} supply level, 49%, 33% and 16% increases in calcium uptake were recorded at 0, 75 and 150 mM NaCl, respectively, as against 5 mM Ca^{2+} supply level.

Table 2: Effect of calcium levels on net calcium, sodium and potassium uptakes (mg/pot) by sunflower under saline conditions

Treatment	Calcium		Sodium		Potassium	
	5 mM Ca	10 mM Ca	5 mM Ca	10 mM Ca	5 mM Ca	10 mM Ca
0 mM NaCl	80.90 b	130.303 a	20.94 d	36.24 c	354.57 b	586.93 a
75 mM NaCl	60.40 c	80.60 b	82. 10 a	56.60 b	281.41 c	378.50 b
150 mM NaCl	44.20 d	51.90 c	66.10b	56.53 b	208. 16 d	275.07 c

Means sharing same letters are statistically non-significant at $P<0.05$.

By increasing NaCl concentration, significant ($P<0.05$) increases in sodium uptake were recorded at both calcium levels. At 5 mM Ca^{2+}supply level, maximum Na^+ uptake (82.10 mg/pot) was recorded at 75 mM NaCl. In contrast, at 10 mM Ca^{2+} supply level, 31% less Na^+ uptake was recorded. However, at 150 mM NaCl, reductions in sodium uptake were recorded at both calcium levels. An explanation for the reduction in sodium uptake is the reduced dry matter yield at 150 mM NaCl.

Kinraide (1998) reported that the main effect of Ca^{2+}on Na^+ uptake at high Ca^{2+} concentration was the electrostatic displacement of Na^+ from the plasma membrane surface. Maathuis and Amtmann (1999) concluded that the direct effect of Ca^{2+} on the movement of Na^+ is manifested in voltage-independent channels (VICs). VIC-mediated Na^+ currents in wheat and maize root protoplasts were strongly reduced by external Ca^{2+}. Robert and Tester (1997) and Tyerman *et al.* (1997) reported Na^+ inhibition by Ca^{2+} in intact wheat roots. Similarly, Jacobson *et al.* (1961), Rains and Epstein (1967), Elzam and Epstein (1969) and LaHye and Epstein (1969) demonstrated that calcium in the root medium depresses the absorption of sodium.

Significant ($P<0.05$) interactive effect of NaCl salinity and calcium levels was observed on K^+ uptake (Table 2). By increasing NaCl concentration in the root medium, decline in K^+ uptake was observed at both calcium levels. However, the magnitude of K^+ uptake reduction was significantly lower at 10 mM Ca^{2+} supply level than at 5 mM Ca^{2+} supply level. The highest K^+ uptake was recorded when plants were grown in non-saline medium at 10 mM Ca^{2+} supply level. At 10 mM Ca^{2+} supply level, 65% and 7% higher K^+ uptakes were recorded at 0 and 75 mM NaCl salinity levels, respectively, as against 0 mM NaCl and 5 mm Ca^{2+} supply levels. At 150 mM NaCl level, K^+ uptake was 32% higher at the higher level of Ca^{2+} supply (10 mM) that at the lower level of Ca^{2+}supply (5 mM). At 10 mM Ca^{2+} supply level, plants absorbed and translocated relatively more K^+ and less Na^+ than at the low concentration of calcium (5 mM). Pitman (1966) reported that calcium may alter the selectivity of K^+ uptake in certain plants. A part of the calcium may have a simple physical effect on root permeability. In addition, it seems reasonable to think that calcium acts on metabolism, in view of its own effect on enzyme systems and in particular on certain ATPase systems. Moreover, Kinraide (1999) in his study concluded that Ca^{2+} prevents the leakiness of intercellular K^+.

Data in Figure 1 indicate a significant positive correlation (r=0.812) between dry matter yield (g/pot) and Ca^{2+} concentration in shoot. It indicates higher Ca^{2+} accumulation with the increasing dry matter yield (g/pot). However, data in Figure 2 exhibit negative correlation (r=-0.82) between dry matter yield (g/pot) and sodium concentration in shoot, which is indicative of growth inhibition due to increasing sodium concentration in shoot. The accumulation of Na^+ in shoot decreases plant growth as explained by Salim and Pitman (1983).

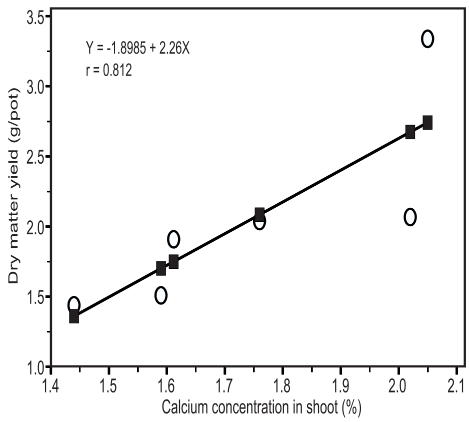

Figure 1: Relationship of calcium concentration in shoot on dry matter yield

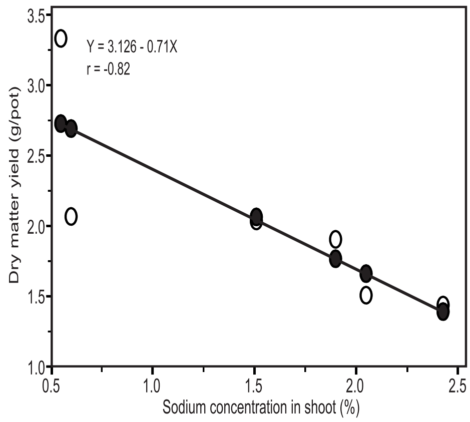

Figure 2: Relationship of Na^+ concentration in shoot with dry matter yield

Data in Figure 3 indicate a significant positive correlation (r=0.82) between dry matter yield (g/pot) and K concentration in shoot. It indicates higher K accumulation with the increasing shoot dry weight. The analysis showed that K^+ was transported preferentially to Na^+ in the presence of 10 mM Ca^{2+} supply and selectivity became more pronounced in the presence of high calcium concentration in the root medium.

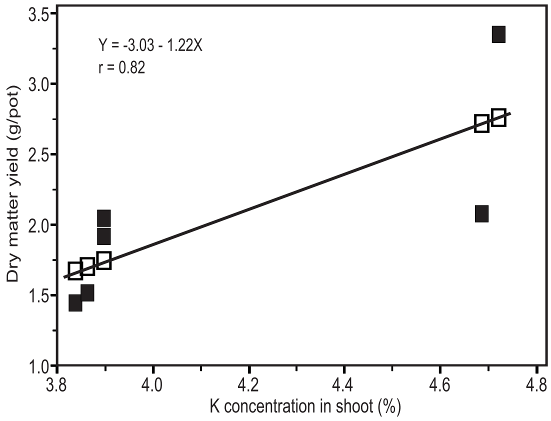

Figure 3: Relationship of K concentration in shoot with dry matter yield

K:Na selectivity in root and shoot was significantly affected by salinity levels (Figures 4 and 5). With increasing salinity levels, K concentrations in root and shoot were drastically decreased. The calcium levels significantly affected K:Na selectivity. However, K:Na selectivity significantly improved in plants supplied with 10 mM Ca as compared with those supplied with 5 mM Ca. The capacity of plants to maintain a high cytosolic K:Na selectivity is likely to be one of the key determinants of plant salt tolerance. Maathuis and Amtmann (1999) concluded that K^+ uptake at the root/soil boundary is via highly K^+ selective pathways whereas Na^+, at least in part, appears to move through a less selective system which in some cases is blocked by Ca^{2+}. They also mentioned the direct effect of Ca^{2+} on the movement of Na^+ which is manifested by voltage-independent channels. Such findings provide an explanation of how increased Ca^{2+} leads to the lower sodium uptake and therefore helps to establish high K^+ / Na^+ selectivity.

CONCLUSIONS

At the low concentration of calcium (5 mM), sunflower growth decreased drastically with increasing concentration of NaCl in nutrient solution. On the other hand, plants grown in the high concentration of NaCl experienced less damage with the higher concentration of calcium. At the higher concentration of calcium (10 mM Ca), sunflower plants absorbed and translocated relatively more potassium and less sodium, than at the low concentration of calcium (5 mM Ca) demonstrating the positive role of calcium in alleviating the hazardous effects of salinity on sunflower growth.

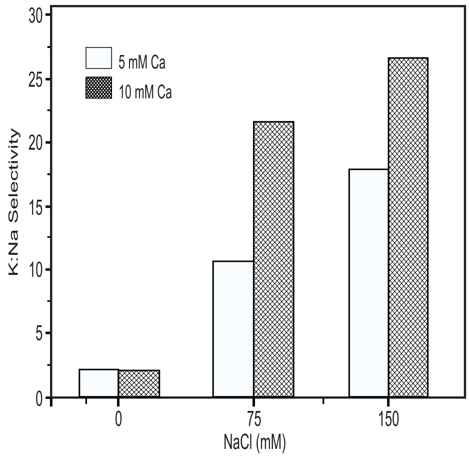

Figure 4: Effect of calcium on K:Na selectivity in root

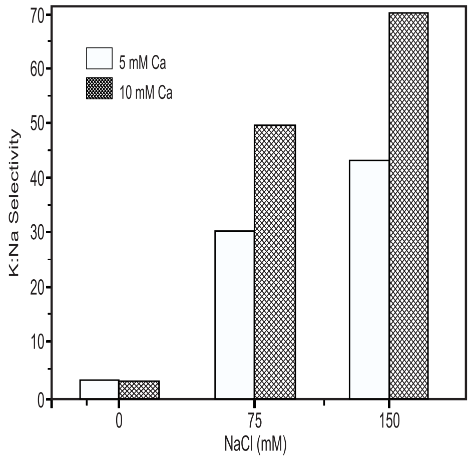

Figure 5: Effect of calcium on K:Na selectivity in shoot

Results of this investigation highlight the utility of using gypsum ($CaSO_4$ $2H_2O$) as soil amendment for soil reclamation. Gypsum not only improves soil health but it is also directly involved in physiological processes of ion uptake by plants, contributing to their improved growth.

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SELECTIVIDAD DEL GIRASOL RESPECTO AL POTASIO Y SODIO BAJO LA INFLUENCIA DE LA PROVISION DEL POTASIO Y LAS CONDITIONES DE SALINIDAD

RESUMEN

La investigacion hidroponica fue efectuada para hacer constar la influencia de la provision de Ca sobre la selectividad K^{+}/Na^{+} de girasol en las concentraciones de NaCl de 0, 75 y 150 en el medio para el desarrollo de la raiz. Las semillas del girasol (*Helianthus annuus* L., variedad Hysun-33) germinaban en la arena de cuarzo humeda y las plantulas de 12 dias eran fijadas a las tapas de vasijas plasticas de las cuales cada una contenia 2,5 litres de media concentracion de la solucion de Hoagland sin sales de calcio. La solucion era constantemente aireada. El valor de la solucion de pH era regulado a 5,9 por la adicion de HCl y NaOH. Eran utilizados tres niveles de salinidad (0, 75 y 150 mM NaCl) y dos nivelas de calcio (5 y 10 mM $CaCl_2$). El experimento fue efectuado según el sistema de bloques completos casuales (RCBD), con tres repeticiones. En caso de mas baja concentracion de calcio (5 mM), el crecimiento del girasol se reducio drasticamente con el aumento de la concentracion de NaCl en la solucion nutritiva. De otro lado, las plantas cultivadas con la grande concentracion de NaCl eran menos dañadas en relativamente mas altas concentraciones de calcio. Con relativamente mas grande concentracion de calcio (10 mM Ca) la plantas de girasol absorbian y trasladaban relativamente mas grandes cantidades de potasio y menos de sodio que con mas baja concentracion de calcio (5 mM Ca). Eso indica que el calcio tiene la funcion positiva en la reduccion de efectos negativos de salinidad sobre al crecimiento del girasol.

SÉLECTIVITÉ DU TOURNESOL EN CE QUI CONCERNE LE POTASSIUM ET LE SODIUM SOUS L'EFFET DE L'APPPORT DE CALCIUM DANS DES CONDITIONS SALINES

RÉSUMÉ

Une étude hydroponique a été menée pour établir l'effet de l'apport de Ca sur la sélectivité K^+/Na^+ dans des concentrations de NaCl de 0, 75 et 150 dans le médium de la racine. Les graines de tournesol (*Helianthus annuus* L.) ont été mises à germer dans du sable quartzifère et les germes de 12 jours ont été fixés sur des couvercles de pots de plastique qui contenaient chacun 2.5 litres de solution Hoagland de demi-concentration sans sels de calcium. La solution était constamment aérée. La solution pH a été ajustée à 5.9 par l'addition de HCl et de NaOH. Il y avait trois niveaux de salinité (0, 75 et 150 mM NaCl) et deux niveaux de calcium (5 et 10 mM $CaCl_2$). L'expérience a été menée selon le système de blocs complets choisis au hasard (RCBD) à trois reprises. Quand la concentration de calcium était peu élevée (5 mM), la croissance du tournesol a diminué de façon drastique avec l'augmentation de la concentration de NaCl dans la solution nutritive. D'un autre côté, les plantes cultivées dans de grandes concentrations de NaCl ont subi moins de dommages quand la concentration de calcium était relativement plus grande. Avec une concentration de calcium relativement plus grande (10 mM Ca), les plantes de tournesol ont absorbé et déplacé une quantité relativement plus grande de potassium et moindre de sodium qu'avec une concentration moins grande de calcium (5 mM Ca). Ceci démontre le rôle positif du calcium dans la diminution des effets négatifs de la salinité sur la croissance du tournesol.