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ROLE OF SULPHUR FOR POTASSIUM/SODIUM RATIO IN SUNFLOWER UNDER SALINE CONDITIONS

Badr-uz-Zaman*, Arshad Ali, M. Salim and B.H. Niazi

Soil Salinity Laboratory, Land Resources Research Institute, National Agricultural Research Centre, Islamabad, Pakistan

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SUMMARY

A hydroponic study was conducted to investigate the role of sulphur application on K/Na selectivity by sunflower and alleviation effect of sulphur on plant growth under saline conditions. Three salinity levels (0, 75, and 150 mM sodium chloride) and two sulphur levels (1 and 4 mM magnesium sulphate) were maintained in the root medium. The treatments were replicated thrice and arranged on growth chamber bench according to a complete randomized design (CRD). Seeds of *Helianthus annuus* (cv. Hysun-33) were germinated in quartz sand under standard conditions. One-week old seedlings were transferred to pots containing 2.5 l continuously aerated half strength modified Hoagland's nutrient solution. The plants were provided optimum growth conditions. First harvest on 25th and second harvest on 30th day were taken after germination. Plants grown with 4 mM sulphur level showed significant growth whereas interaction between sulphur and salinity was highly significant ($P<0.01$) for growth parameters like fresh weight, dry matter yield of shoot and root, diameter and length of stem and root. The positive correlation of dry matter yield with sulfur ($r=0.93$), potassium ($r=0.99$) and calcium ($r=0.83$) were observed, whereas sodium had negative relationship ($r=-0.99$) with dry matter yield. Potassium/sodium selectivity in plants was significantly higher ($P< 0.01$) at 4 mM sulphur level than at 1 mM.

Key words: **sunflower, salinity, sulphur, potassium selectivity**

INTRODUCTION

Sunflower is an important oilseed crop being grown in Pakistan at an area of 144 thousand hectares with an annual production of 195 thousand tons (Anonymous, 1999). To meet the national requirement, Pakistan had imported 1.9 million tons of edible oil, spending US $ 788 million during the year 1999-2000 (Anony-

* Corresponding author, Phone: 92-51-9255060, Fax: 92-51-9505034, e-mail: salinity@isb.sdnpk.org

mous, 2000). Sunflower is a "medium salt tolerant" crop that can successfully be grown on marginal saline soils. Root medium salinity significantly interferes with plant growth functions. Potassium/sodium selectivity ultimately affects crop yield (Hoo *et al.*, 1999). Sunflower oil contains important sulphur containing compounds such as methionine and cysteine that play important roles in the structure and function of many enzymes (Torchinsky, 1981). Sulphur applied from various sources improved the growth of sunflower (Zaman *et al.*, 1996) whereas potassium in shoot resulted in favorable K/Na ratio (Bohra and Doerffling, 1993). Accumulation of sodium ions in its tissues creates metabolic problems such as cell injuries, nutrients imbalance and abnormal water potentials. As potassium sodium selectivity is considered a yardstick of crop salt tolerance (Yeo and Flower, 1984), this study was carried out to investigate K/Na selectivity on growth parameters at elevated sulphur supply under saline conditions.

MATERIALS AND METHODS

Viable seeds of *Helianthus annuus* L. (cv. Hysun-33) were germinated in moist quartz sand. One-week-old plants were transferred to pots containing half strength continuously aerated modified nutrient solution (Hoagland and Arnon, 1950). Two levels of sulphur i.e. 1 and 4 mM were maintained in the root medium using magnesium sulphate and three levels of sodium chloride i.e., 0, 75 and 150 mM were induced by increments of 25 mM sodium chloride on alternate days. The pots were arranged according to two-factor completely randomized design (CRD) in triplicate. The plants were grown at 30 ± 2°C. The light intensity was around 450 μ mol^{-1} s^{-1} and pH was maintained at 5.5 ± 0.2. The first harvest (H_1) was taken on the 25^{th} day and the 2^{nd} (H_2) on the 30^{th} day after germination. The plant roots were rinsed with deionized water and excess water was immediately blotted. The length and diameter of the stem and root were recorded. The plants were separated into shoot and root portions. After recording fresh weight (FW), the plant parts were dried at 65°C to constant dry weight. The amount of water content was calculated by difference of fresh weight and dry matter yield. Dried plant samples were ground to pass through 40 mesh using Wiley Mill and were digested using di-acid nitric acid and perchloric acid (2:1) mixture. Potassium, calcium and sodium contents were determined using atomic absorption spectrophotoscopy. Sulphur as sulphate was determined colorimetrically as given by Verma *et al.* (1977). Relative growth rates (RGR) were calculated according to Hunt (1978). The results were statistically analyzed using the F- test as given by Gomez and Gomez (1976).

RESULTS AND DISCUSSION

Salinity tolerance was enhanced significantly ($P<0.01$) due to sulphur application in the root medium. Fresh weight was significantly ($p<0.01$) increased at higher level (4 mM) of sulphur application. At 4 mM sulphur application, FW of shoot (H_1) increased by 25, 42 and 5 percent at 0, 75 and 150 mM sodium chloride, respectively, as compared with 1 mM sulphur application. Shoot FW (H_2) was increased by 24, 97 and 12 percent at 0, 75, and 150 mM sodium chloride, respectively, with 4 mM than at 1 mM of sulphur application (Table 1).

Table 1: Effect of sulphur application under saline conditions on fresh weight (g plant^{-1}) of sunflower

NaCl (mM)	H_1				H_2			
	Magnesium sulphate applied (mM)							
	1	4	1	4	1	4	1	4
	Shoot		Root		Shoot		Root	
0	7.38b	9.23a	0.95d	1.81a	17.79ab	22.13a	3.30b	4.06a
75	3.88d	5.51c	1.11c	1.56b	6.82d	13.45c	2.18c	4.31a
150	3.37d	3.55d	0.58e	0.75d	4.45e	4.97de	1.31d	1.59d

Means sharing same letters are statistically non-significant (P<0.01) according to LSD test

Table 2: Effect of sulphur application on dry matter yield (g plant^{-1}) under saline conditions

NaCl (mM)	S applied (mM)			
	1	4	1	4
	H_1		H_2	
0	0.87 b	1.07 a	2.09 b	2.92a
75	0.55 d	0.65 c	1.14 c	1.76b
150	0.39 e	044 d	0.75 d	0.98c

Means sharing same letters are statistically non-significant (P<0.01) according to LSD test

Sulphur application increased the tolerance of sunflower to salinity indicating an increase in fresh and dry weight of plants. As sulphur and potassium have synergistic relationship and potassium plays a significant role in maintaining water content of tissues, therefore, such positive results could be seen especially at 75 mM of applied sodium chloride along with 4 mM of sulphur. Apparently the leaves were fully expanded indicating that they had maintained their turgor pressure by increasing either their inorganic or organic ion content by regulating the osmotic potential comparing with the external environment, as observed by Leland (1996).

Dry matter yield of shoot significantly ($P<0.01$) increased with sulphur application by 23, 18 and 13 percent at 0 ,75 and 150 mM sodium chloride, respectively, at 4 mM sulphur application (H_1). The shoot dry matter at H_2 was increased by 40, 80 and 52 percent at 0, 75 and 150 mM of sodium chloride at 4 mM sulphur application respectively (Table 2). This is in accordance with the results reported by Katerji *et al.* (1996). Ouerghi *et al.* (1991) reported that salinity reduces biomass.

Diameter and length of root and stem were also significantly ($P<0.01$) affected by sulphur levels. The diameter and length of the root were increased by 142 and 34 percent, respectively, at 75 mM of sodium chloride application when the concentration of sulphur was increased from 1 to 4 mM at H_2. The diameter and length of the stem were increased by 22 and 17 percent, respectively, at 75 mM of sodium chloride application when the concentration of sulphur was increased from 1 to 4 mM of H_2 (Table 3).

Table 3: Effect of sulphur application under saline conditions on root and shoot diameter (mm) and length (cm) of sunflower

NaCl (mM)	Root				Stem			
	Magnesium sulphate applied (mM)							
	1	4	1	4	1	4	1	4
	Diameter (mm)		Length (cm)		Diameter (mm)		Length (cm)	
0	3.17ab	3.25ab	21.50b	23.00ab	4.92ab	5.42a	63.16b	64.17a
75	1.50c	3.63a	21.83ab	29.17a	3.58cd	4.38bc	49.67c	58.00b
150	2.33bc	2.50b	17.17b	24.33ab	2.67e	3.42de	44.33cd	45.67c

Means sharing same letters are statistically non-significant ($P<0.01$) according to LSD test

Table 4: Effect of sulphur application in sunflower under saline conditions on RGR (g g^{-1} dry wt. d^{-1})

NaCl (mM)	Sulphur applied (mM)		Means
	1	4	
0	0.15 c	0.16 b	0.16 B
75	0.14 c	0.17 a	017A
150	0.07 d	0.12 c	0.09 C
Means	0.12 B	0.16A	

Means sharing same letters are statistically non-significant ($P<0.01$) according to LSD test

There was a significant ($P<0.01$) interaction between NaCl applied with sulphur application on relative growth rate. At 75 and 150 mM of NaCl applied, RGR was decreased by 6 and 44 percent, respectively, as compared with the control. There was an improvement in RGR by 33 percent at 4 mM than at 1 mM of sulphur applied (Table 4). There was a highly significant effect of elevated level of sulphur application on K/Na selectivity by sunflower under saline conditions (Table 5).

Table 5: Effect of sulphur application on K/Na ratio by sunflower under saline conditions

NaCl (mM)	S applied (mM)			
	1	4	1	4
	H_1		H_2	
0	0.89 ab	1.83 a	1.62 ab	2.29 a
75	0.75 ab	0.92 ab	1.09 bc	1.53 ab
150	0.68 b	0.71 b	0.57 c	1.05 bc

Means sharing same letters are statistically non-significant ($P<0.01$) according to LSD test

In H_1, K/Na was increased by 106, 23 and 4 percent at 0, 75 and 150 mM of NaCl application in the root medium, respectively. With the increasing level of NaCl, this ratio was decreased by 16 and 24 percent; 50 and 61 percent at 1 and 4 mM of sulphur applied, respectively. In H_2, K/Na selectivity was increased by 41, 40 and 4 percent at 0, 75 and 150 mM of NaCl application in the root medium, respectively. With the increasing level of NaCl, this ratio was decreased by 33 and 65 percent; 33 and 54 percent at 1 and 4 mM of sulphur application, respectively. It has been reported that high concentration of potassium in the cytoplasm is related to salt tolerance in plants (Hajibagheri *et al.*, 1989). Potassium has a synergistic relation with sulphur. Being an essential metabolic requirement, the entry of potassium ions was improved at higher level of sulphur application indicating an increase in K/Na ratio by shoot system over root system in sunflower.

There was a significant positive correlation (r=0.93) between DMY and sulphur applied in the shoot (H_2). For growth of shoot, the application of sulphur cannot be ignored when Na^+ in the system is operating. Sulphur has an important physiological significance during the early growth of plants (Onkar, 1984). The S-H group of cysteine is very important because it can be oxidized to form a disulfide (S-S) bond when the S-H group from two cysteine molecules interact. The disulfide bond is an important feature in stabilizing the three-dimensional structure of many enzymes since it can link chains of amino acids together to form the three-dimensional structure necessary for enzyme activity. Among the oil seed crops, sunflower requires relatively higher amounts of sulphur for oil production (Nabi *et al.*, 1989) (Figure 1). Similarly, potassium also shared a positive correlation with dry matter yield (r=0.99) (Figure 2). Sulphur increases uptake of potassium in sunflower (Saqure *et al.*, 1990) (Figure 2). The existence of a positive interaction between sulphur and magnesium has also been suggested by Blenkhorn (1974).

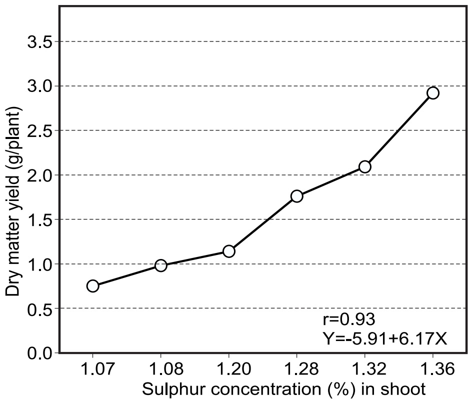

Figure 1: Relation between sulphur concentration and dry matter yield in H_2 by sulphur application under saline conditions

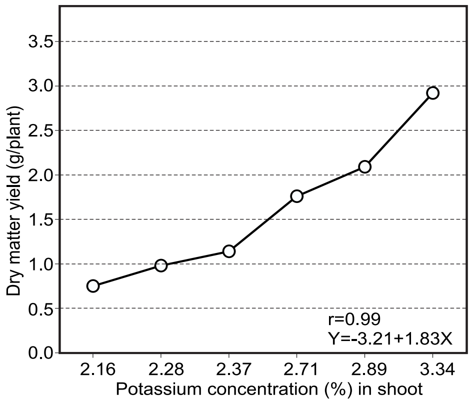

Figure 2: Relation between potassium concentration and dry matter yield in H_2 by sulphur application under saline conditions

Significantly positive correlation (r=0.83) was found between dry matter yield and calcium concentration in shoot (Figure 3). Sodium ion toxicity is very clearly depicted in Figure 4 where DMY and Na are negatively correlated (r=-0.99). Salinity reduces biomass besides decreasing length of root and stem (Ouerghi *et al.*, 1991).

Under such conditions, volume of chloroplast may decrease inhibiting photosynthetic rate (Younis *et al.*, 1983).

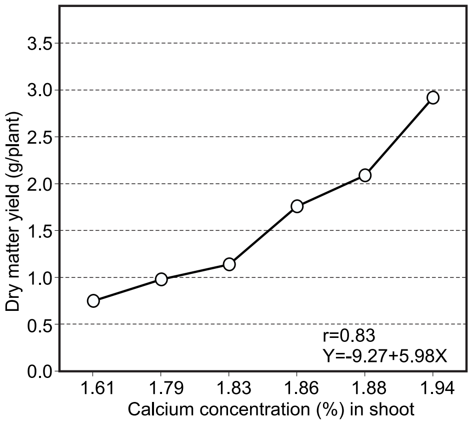

Figure 3: Relation between calcium concentration and dry matter yield in H_2 by sulphur application under saline conditions

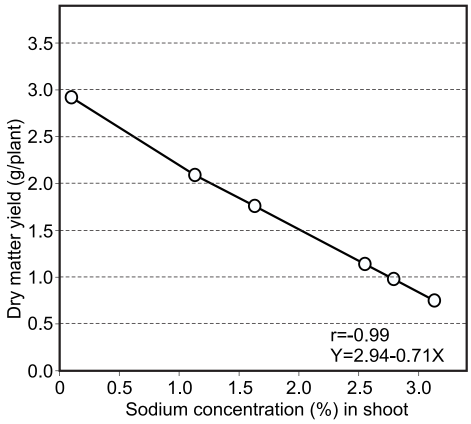

Figure 4: Relation between sodium concentration and dry matter yield in H_2 by sulphur under saline conditions

Sulphur application significantly affected K/Na ratio in sunflower plants (H_2) under saline conditions (Figures 5 and 6). Taleisnik and Grunberg (1994) have obsvered that with the increase in salinity, K/Na ratio is lowered, but in this study, this ratio was improved significantly ($P<0.01$) in the presence of sulphur in the root medium indicating a positive effect of sulphur application on sunflower growth. A greater K/Na ratio reduces the deleterious effects of sodium ion on plant growth (Prakash *et al.*, 1996). An increased K/Na ratio is an indication of increased potassium and reduced sodium ion uptake (Joshi, 1984).

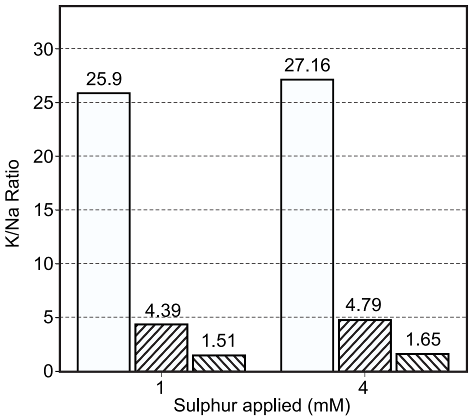

Figure 5: Potassium-sodium ratio in shoot (H_2) as affected by the application of sulphur under saline conditions

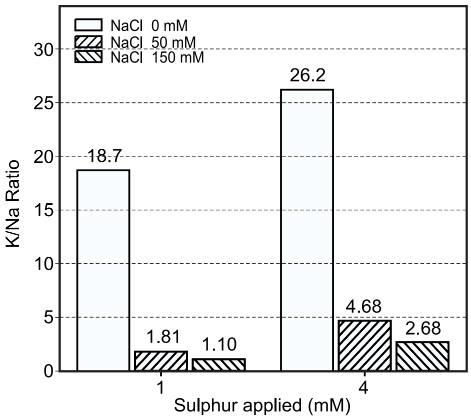

Figure 6: Potassium-sodium ratio in root (H_2) as affected by sulphur application under saline conditions

This increased uptake of potassium may be the result of direct competition between potassium and sodium ion at the plasmalemma (Epstein, 1966). It has been reported that the main salt interference to nutrient uptake is by K-Na competition (Silverbush and Ben- Ashir, 1987). Higher K/Na ratio improves leaf water potential (Devitt *et al.*, 1981). For plants exposed to salinity, the ability to adjust rapid changes in water potential involves accumulation of potassium ions (Weinberg *et al.*, 1982).

CONCLUSIONS

Sulphur as sulphate cannot be underestimated regarding its pivotal role in improving K/Na selectivity and increasing the capability of calcium ions to decrease the induced injurious effects of sodium ions in sunflower growth. To a marginal saline-sodic system, with S application, the cultivation of this important oil seed crop can be more productive.

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INFLUENCIA DE AZUFRE EN RELACIÓN ENTRE POTASIO Y SODIO EN LAS CONDICIONES DE SALINIDAD EN GIRASOL

RESUMEN

El ensayo hidropónico se ha efectuado con el fin de investigar el papel de aplicación de azufre en la selectividad de girasol hacia K/Na, tanto como el efecto aliviante de azufre en el crecimiento de las plantas en las condiciones de salinidad. El medio de raíz tenía tres grados de salinidad (0, 75 y 150 mM de cloruro de sodio) y dos dosis de azufre (1 y 4 mM de sulfato de magnesio). Las variantes del ensayo se han efectuado en tres repeticiones y colocadas en una cámara por el sistema bloques al azar. La semilla de girasol (*Helianthus annuus*) de variedad Hysun-33 se ha germinado en la arena de cuarzo en las condiciones estándar. Las plántulas de una semana de edad, se han desplazado en los recipientes con 2,5 l de solución nutritiva de Hoagland 50%, modificada bajo continua aeración. Las plantas han sido cultivadas en las óptimas condiciones de crecimiento. La primera cosecha se ha llevado a cabo 25 y la segunda 30 días después de la germinación. Las plantas cultivadas con 4 mM de azufre, han tenido un crecimiento significante, mientras que la interacción entre azufre y salinidad, ha sido altamente significante ($P<0,01$) para los parámetros, como son el peso fresco, rendimiento de materia seca del tallo y de la raíz, y diámetro y longitud del tallo y de la raíz. Se ha establecido una correlación positiva entre el rendimiento de la materia seca y el azufre ($r=0,93$), potasio ($r=0,99$) y calcio ($r=0,83$), mientras que sodio con esta característica, estuvo en correlación negativa ($r=-0,99$). La selectividad de las plantas hacia potasio/sodio, era significativamente más alta ($P<0,01$) en la dosis de 4 mM azufre, que en 1 mM.

RÔLE DU SOUFRE SUR LE RAPPORT DE POTASSIUM ET DE SODIUM DANS LE TOURNESOL DANS DES CONDITIONS SALINES

RÉSUMÉ

Une étude hydroponique a été faite pour examiner le rôle d'une application de soufre sur la sélectivité du tournesol et l'effet tempérant du soufre sur la croissance des plantes dans des conditions salines. Trois degrés de salinité (0, 75 et 150 mM de chlorure de sodium) et deux doses de souffre (1 et 4 mM de sulfate de magnésium) ont été maintenus à la base de la racine. Les variantes ont été traitées trois fois et distribués dans les cases de croissance selon le système de bloc aléatoire. Les semences de tournesol (*Helianthus annuus)* (cv. Hysun-33) ont été mises à germer dans du sable quartzeux dans des conditions standard. Les semis d'une semaine ont été transférés dans des pots contenant 2.5 l de solution nutritive Hoagland modifiée à 50% sous aération constante. Les plantes étaient cultivées dans des conditions de croissance optimale. La première récolte a été faite 25 jours et la seconde, 30 jours après la germination. Les plantes cultivées avec 4 mM de soufre ont montré une croissance significative tandis que l'interaction entre le soufre et la salinité était hautement significative ($P<0.01$) pour des paramètres comme la masse fraîche, le rendement en matières sèches de la tige et de la racine, le diamètre et la longueur de la tige et de la racine. Une corrélation positive entre le rendement en matière sèche et soufre ($r=0.93$), potassium ($r=0.99$) et calcium ($r=0.83$) a été constatée tandis que le sodium était en corrélation négative ($r=-0.99$) avec le rendement en matière sèche. La sélectivité des plantes envers le potassium et le sodium était significativement plus élevée ($P<0.01$) pour la dose de soufre de 4 mM que pour la dose de l mM.