



INFLUENCE OF CALCIUM ON WATER RELATION OF TWO CULTIVARS OF WHEAT UNDER SALT STRESS

Nahid Akhtar^{1*}, F. Hossain² and A. Karim³

^{1,2}Plant Physiology and Biochemistry Laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Tel: 88-01754996573 and Fax: 88-02-7791052

³Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur

*Corresponding author: nahid_akhtar98@yahoo.com

Abstract

The purpose of the present investigation was to study the effects of Ca^{2+} on water relation of two wheat cultivars (Akbar and Kanchan) under salt stress. The two wheat cultivars were grown in pots with 0 and 150 mM NaCl salinity. Calcium was applied in the form of gypsum in 0.12, 0.24 and 0.36g pot⁻¹ (that is 20, 40 and 60 kg ha⁻¹) respectively. Salinity decreased RWC, WRC, exudation rate and ψ_{leaf} , while increased WSD and WUC. Application of increased levels of Ca improved the plant water status in both cultivars. The results obtained in the present study suggest that elevated Ca^{2+} increases salt tolerance by improving the plant water status.

Keywords: Calcium, Plant water relation, Salt stress, Wheat, Cultivar

Introduction

Salinity is an important environmental factor that can severely inhibit plant growth and agricultural productivity. Salt accumulation in irrigated soil is one of the main factors that diminish crop productivity, since most of the plants are not halophytic (Hoshida *et al.*, 2000). Salinity disturbs the plant's water relations due to decreased availability of water from soil solution as a result of lowered osmotic potential (Munns, 2005), thus making it difficult for roots to extract water from their surrounding media (Mittler, 2002).

Crop improvement for saline conditions requires an understanding of the mechanisms enabling salt tolerance. Mechanisms of salt tolerance, not yet clear, can be to some extent explained by stress-adaptation effectors that mediate ion homeostasis, osmolyte biosynthesis, toxic radical scavenging, water transport and long distance response coordination (Neill *et al.*, 2002). Chemical treatment and agronomical crop management practices have been tried to alleviate the salt stress, but the application of calcium to stressed plants attracted little attention. Supplementing the medium with Ca^{2+} alleviates growth inhibition by salt in plants (Imlay, 2003). Ca^{2+} sustains K^+ transport and K^+/Na^+ selectivity in Na^+ challenged plants. The interaction of Na^+ and Ca^{2+} on plant growth and ion relations is well established (Jaleel *et al.*, 2007). The purpose of this study was to investigate the ameliorative effects of supplemental Ca^{2+} on water relation of two cultivars of wheat under saline condition which is associated with salt tolerance.

Materials and methods

Plant material

Two wheat cultivars, namely AKBAR (tolerant) and KANCHAN (susceptible) were selected as plant materials. The seeds were obtained from Bangladesh Agricultural Research Institute, Gazipur.

Analysis of soil

The soil for the experiment was collected from the botanical garden of Jahangirnagar university, Savar, Dhaka. The soil samples used in the pots were dried, powdered and mixed thoroughly. The physical and chemical properties of the soil were analyzed in the laboratory of Soil Resources Development Institute (SRDI).

Treatments

Saline water was prepared artificially by dissolving calculated amount of commercially available salt (NaCl) with tap water to make 150 mM NaCl solution. Tap water was used as control.

Levels of calcium

Three levels of calcium were applied in the form of Gypsum.

Low (Ca1) = 20 kg ha⁻¹ (0.12g pot⁻¹), which is 50% of the recommended dose.

Optimum (Ca2) = 40 kg ha⁻¹ (0.24g pot⁻¹), which is 100% of the recommended does.

High (Ca3) = 60 kg ha⁻¹ (0.36g pot⁻¹), which is 150% of the recommended dose.

Nitrogen, potassium and phosphorus were applied in the form of urea, MP and TSP as their recommended does, 180, 110, 140 kg ha⁻¹, (1.08g pot⁻¹, 0.66 pot⁻¹ and 0.84g pot⁻¹), respectively.

Methods of cultivation

Each pot was filled up with 12kg air-dried soil. The respective amount of nutrients was incorporated with the soil before seed sowing. The compost was 1/4th of the soil by volume. The pots were kept under natural sunshine. The seeds were surface sterilized by soaking the seeds with 0.1% sodium hypochloride for three minutes followed by washing 7 to 8 times with tap water and three times with distilled water. Seeds of uniform size were directly sown on 12 November, 2006. Tap water was applied in all pots up to the emergence of seedling. After seedling establishment tap water in control pots and 12.5mM NaCl solution were applied in salt treatment. When the first leaf appeared i.e. ten days after emergence (DAE) actual amount of NaCl solutions were applied. Seedling in control group was irrigated with tap water.

Measurement of plant water status

Leaf (lamina) of same size and age of five seedlings from each treatment was collected and fresh weight of the leaf was taken immediately. The leaf was kept immersed in distilled water for 24 hour at room temperature in the dark. The turgid weight of the leaves was then measured. Afterwards the leaves were oven-dried at 80°C for 72 hour in order to take dry weight. The fresh, turgid and dry weights of the leaves were used to determine the following parameters (Sangakkara *et al.*, 1996):

$$\text{Relative Water Content (RWC)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Water Saturation Deficit (WSD) = 100 - RWC

$$\text{Water Retention Capacity (WRC)} = \frac{\text{Turgid weight}}{\text{Dry weight}}$$

$$\text{Water Uptake Capacity (WUC)} = \frac{\text{Turgid weight} - \text{Fresh weight}}{\text{Dry weight}}$$

Measurement of Exudation rate

Exudation rate was measured at 5 cm above from the base of the stem. At first, dry cotton was weighed. A slanting cut on stem was made with a sharp knife. Then the weighed cotton was placed on the cut surface. The exudation of sap was collected from the stem for 1 hour at normal temperature. The final weight of the cotton with sap was taken. The exudation rate was measured by deducting cotton weight from the sap containing cotton weight and expressed in mg per hour as follows:

$$\text{Exudation rate} = \frac{(\text{Weight of cotton + sap}) - \text{Weight of cotton}}{\text{Time (h)}}$$

Measurement of Leaf water potential

Leaf water potential was measured at 6AM by pressure-bomb technique (Tyree and Hammel, 1972).

Results

Relative water content: Salinity decreased RWC. RWC increased with the application of Ca from Ca₁ to Ca₃ levels both under control and saline conditions of the two varieties of wheat (Table 1).

Water saturation deficit: Salinity increased WSD at all levels of Ca (Ca₁, Ca₂ and Ca₃) compared to those of corresponding levels of Ca under control condition. The WSD showed a decreasing tendency due to application of higher level of Ca (Table 1).

Water retention capacity: Salinity reduced WRC significantly compared to that of control at all levels of calcium. However, application of Ca increased WRC both in control and salt treated plants of the two varieties of wheat (Table 1).

Water uptake capacity: Salinity increased WUC compared to that of control. Application of calcium decreased WUC in both control and saline condition (Table 1).

Exudation rate and leaf water potential: Salinity decreased exudation rate drastically. Application of increased level of Ca from Ca₁ to Ca₃ increased the exudation rate significantly both under control and saline condition (Table 2). Leaf water potential (ψ_{leaf}) decreased with the salinity. The ψ_{leaf} showed an increasing tendency with the increasing levels of Ca application (Table 2).

Discussion

The relative water content (RWC) signifies the water contents of plants. The relative water content was decreased by salinity (Table 1). Application of increased level of Ca from Ca₁ to Ca₃ increased the relative water content significantly both under control and saline condition (Table 1). Reduction in RWC due to salinity was reported in different crops by many workers (Ghoulam *et al.*, 2002 in sugar beet; Sayed and Gadallah, 2002 in sunflower). It is well known that salinity decreases soil water potential and thus reduces the water uptake by plant that reflect in the lower RWC. The results of this study imply that application of Ca exerted

beneficial effect of water uptake which was reflected with the higher RWC. Similar effect was reported by Francisco *et al.* (2004) in pepper plant.

Water saturation deficit (WSD) showed an inverse trend of RWC. Water saturation deficit indicates the degree of water deficit in plants. As expected salinity increased WSD at all levels of Ca (Ca₁, Ca₂ and Ca₃) compared to those of corresponding levels of Ca under control condition. The WSD showed a decreasing tendency due to application of higher level of Ca (Table 1). Under saline condition plants suffered from water deficit especially at high salt concentration (Orcutt and Nilsen, 2000), though information on Ca-induced change in WSD under saline condition is scarce. The result of the present study agrees well with that of Cheeseman (1988).

Water retention capacity (WRC) illustrates the capacity of plant cell to retain water. Water retention capacity is determined by cell structure. Plant grown under a high moisture regime maintains a higher ratio that could be due to the lower destruction of plant tissues by moisture deficit (Sangakkara *et al.*, 1996). Islam (2001b) showed in bushbean that plants grown under a high soil moisture regime had a higher ratio than that of the plants grown under mild stress and severe stress conditions. Salinity reduced the WRC significantly compared to that of control at all levels of calcium. However, application of Ca increased this ratio both in control and salt treated plants of the two cultivars of wheat (Table 1). Under saline condition the highest ratio was observed at the highest level of calcium indicating that the capacity of plant to absorb moisture increased with the increased level of calcium. Similar result was reported by Cramer *et al.* (1989) in barley.

The water uptake capacity (WUC) quantifies the ability of a plant to absorb water per unit dry weight in relation to turgid weight. A higher water uptake capacity under saline condition means a plant is subjected to water stress at a greater degree, because the plant would absorb more water to reach turgidity than a plant under control condition (Islam, 2001b). Salinity resulted in an increase in the water uptake capacity compared to that of control. Application of calcium decreased WUC in both control and saline condition. Therefore, calcium exerted a positive role to maintain better water relation under both control and saline condition. Similar effect was reported by Grieve and Fujiyama (1987) in rice and Francisco *et al.* (2004) in pepper plant.

Under normal condition exudation rate is higher than that of under stress condition. Exudation can thus be used as an indicator to measure the severity of stress. Salt stress decreased exudation rate drastically. Application of increased level of Ca from Ca₁ to Ca₃ increased the exudation rate significantly both under control and saline condition (Table 2). The highest exudation rate (264.301 mg/h in Akbar and 248.062 mg/h in Kanchan) was observed under control condition applied with Ca₃ and the lowest (83.521 mg/h in Akbar and 68.430 mg/h in Kanchan) was under saline conditions with Ca₁. Therefore, greater amount of Ca had a positive role on the maintenance of water relation of the two varieties of wheat under saline condition. A significant positive correlation ($r^2=0.9647$ in Akbar and $r^2=0.9983$ in Kanchan at control and $r^2=0.9603$ in Akbar and $r^2=0.9967$ in Kanchan at 150 mM; significant at 1% level) between levels of Ca and exudation rate was noticed (Fig.1 and 2). The lower exudation due to salinity indicated that the plant was subjected to water stress (Sangakkara *et al.*, 1996). Exudation rates directly related with the flow of transpiration stream. Increased exudation rate means a plant can absorb more water from the soil solution

than a plant with lower exudation rate. However, it is not clear from this study how higher levels of Ca increased the exudation rate. Further study is needed to elucidate the mechanisms of Ca induced enhancement of exudation in wheat.

Table 1: Effect of salinity and calcium levels on relative water content, water saturation deficit, water retention capacity and water uptake capacity of cv. Akbar and Kanchan

Salinity levels (mM)	Calcium levels (kg/ha)	Relative water content (%)		Water saturation deficit (%)		Water retention capacity (TW/DW)		Water uptake capacity	
		Akbar	Kanchan	Akbar	Kanchan	Akbar	Kanchan	Akbar	Kanchan
0	20	75.15	78.04	14.78	14.98	4.20	4.85	0.50	0.56
	40	77.30	79.08	13.54	13.88	4.98	5.10	0.36	0.52
	60	79.48	80.02	12.18	12.50	5.50	5.75	0.30	0.36
150	20	50.44	42.58	35.00	40.02	3.60	3.15	1.66	2.02
	40	52.72	44.20	25.45	28.08	4.06	4.06	1.38	1.84
	60	59.22	50.05	16.32	18.12	4.98	4.40	1.08	1.18
LSD (5%) CV (%)	-	1.78	1.36	0.83	1.82	0.92	0.56	0.07	0.05
	-	19.90	29.70	45.60	50.60	15.60	19.80	65.30	66.40

Table 2: Effect of salinity and calcium levels on exudation rate and leaf water potential of var. Akbar and Kanchan

Salinity levels (mM)	Calcium levels (kg/ha)	Exudation rate (mg/h)		Leaf water potential (MPa)	
		Akbar	Kanchan	Akbar	Kanchan
0	20	232.500	224.062	-0.564	-0.520
	40	243.135	235.203	-0.546	-0.500
	60	264.301	248.062	-0.504	-0.458
150	20	83.521	68.430	-0.815	-0.798
	40	89.527	79.520	-0.725	-0.710
	60	102.063	88.605	-0.720	-0.638
LSD (5%) CV (%)	-	10.19	8.23	0.042	0.051
	-	50.70	55.00	-47.80	-22.10

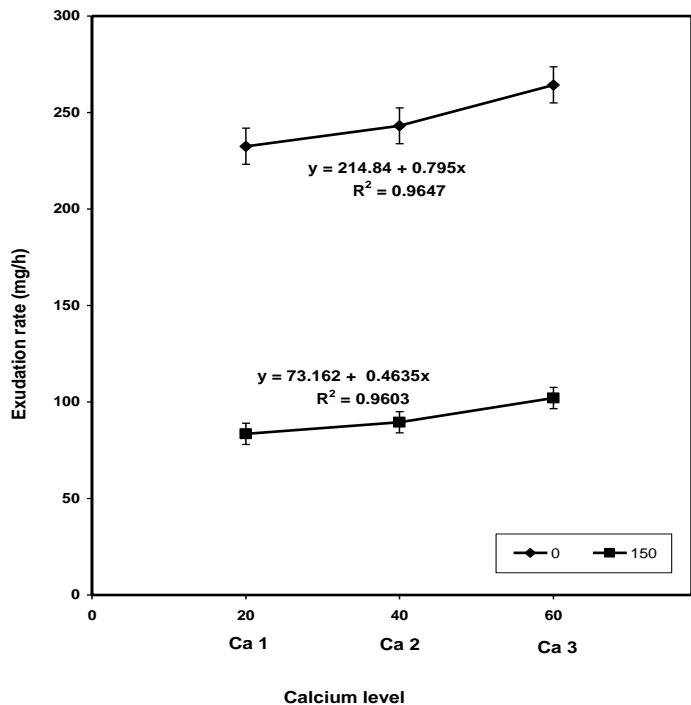


Fig. 1 Relationship between calcium levels and exudation rate of Akbar under saline conditions. Bars indicate \pm SE .

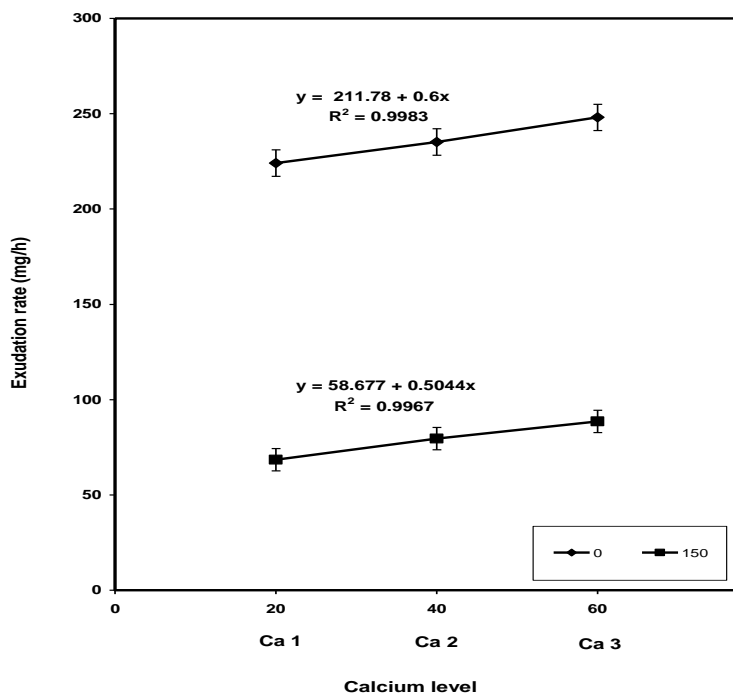


Fig. 2 Relationship between calcium levels and exudation rate of Kanchan under saline conditions. Bars indicate \pm SE .

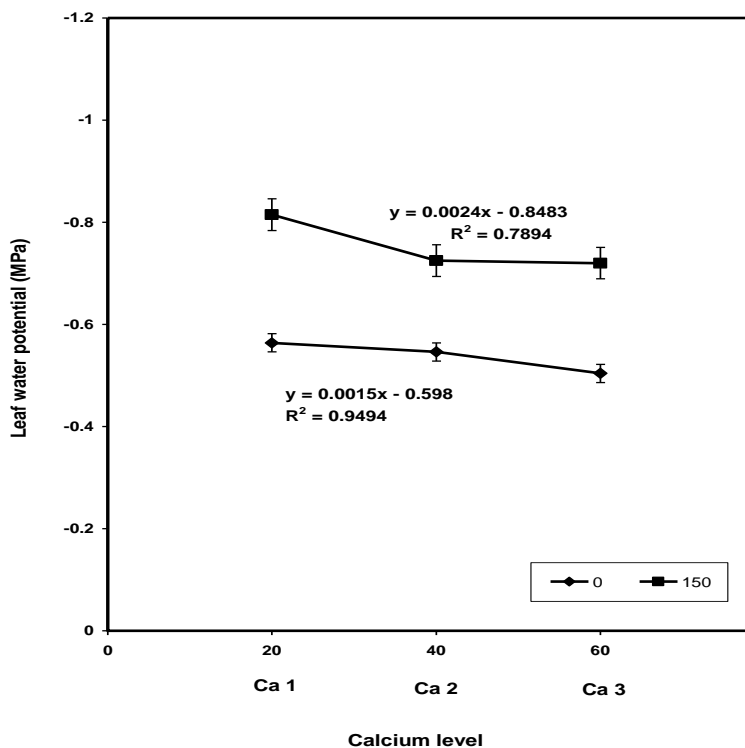


Fig. 3 Relationship between calcium levels and leaf water potential of Akbar under saline conditions. Bars indicate \pm SE.

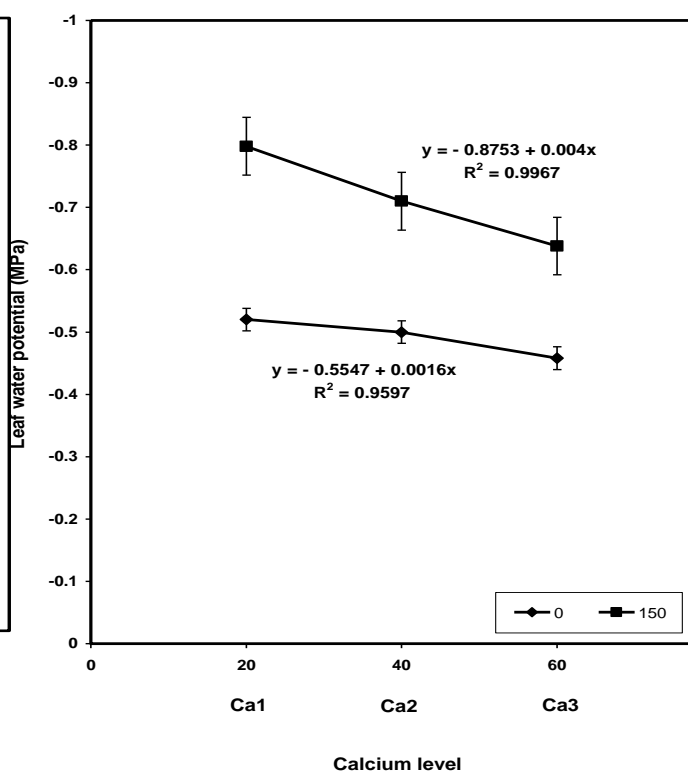


Fig. 4 Relationship between calcium levels and leaf water potential of Kanchan under saline conditions. Bars indicate \pm SE.

Leaf water potential (ψ_{leaf}) decreased with the salinity (Table 2). The ψ_{leaf} showed an

increasing tendency with the increasing levels of Ca application. The highest ψ_{leaf} (-0.504 in Akbar and -0.458 MPa in Kanchan) was recorded from Ca3 applied under control conditions and the lowest (-0.815 in Akbar and -0.798 MPa in Kanchan) was from Ca1 under saline conditions. A positive correlation ($r^2=0.9494$ in Akbar and $r^2=0.9597$ in Kanchan at control and $r^2=0.7894$ in Akbar and $r^2=0.9967$ in Kanchan at 150 mM; significant at 1% level) between ψ_{leaf} and levels of Ca was found (Fig.3 and 4). The decreased ψ_{leaf} due to salinity was reported by many workers for different crops (Nandwal et al., 2000; Carvajal et al., 1999). Kramer (1988) found that with the increase in Ca, ψ_{leaf} increased under saline condition in barley. Plant synthesizes different metabolites across the tonoplast to maintain turgor. However, the plants need to spend substantial energy to maintain turgor under water deficit conditions (Munns and Termaat, 1986). Higher ψ_{leaf} in optimal and high Ca salinized plant might suggest a mechanism of osmotic adjustment or an increase in cell wall elasticity.

Conclusion

From these results, it can be concluded that the addition of calcium to salt (NaCl) stressed wheat has a significant role in partial alleviation of salinity stress by improving the plant water status.

Acknowledgements

The authors wish to thank Jahangirnagar university for a research grant in support of this project.

References

- Carvajal, M., V. Martinez and A. Cerda. 1999. Influence of magnesium and salinity on tomato plant grown in hydroponics culture. *Journal of Plant Nutrition* 22, 177-190.
- Cheeseman, J. M. 1988. Mechanisms of salinity tolerance in plants. *Plant Physiology* 87, 547 – 550.
- Cramer, G.R., E. Epstein and A. Lauchli. 1989. Na-Ca interactions in barley seedlings: relationship to ion transport and growth. *Plant, Cell and Environment* 12, 551-558.
- Francisco, J. C., V. Martinez and M.Carvajal. 2004. Does calcium determine water uptake under saline conditions in pepper plants, or is it water flux which determines calcium uptake? *Plant Science* 166, 443-450.
- Ghoulam, C., A. Foursy and K. Fares. 2002. Effect of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany* 47, 39-50.
- Grieve, C.M. and Fujiyama H. 1987. The response of two rice cultivars to external Na/Ca ratio. *Plant and Soil* 103, 245-250.
- Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity, *Annual Review of Plant Physiology and Plant Molecular Biology* 51, 463-499.
- Hoshida, H., Y. Tanaka, T. Hibino, Y. Hayashi, A. Tanaka and T. Takabe, 2000. Enhanced tolerance to salt stress in transgenic rice that over expresses chloroplast glutamine synthetase. *Plant Molecular Biology* 43, 103-111.
- Imlay, J.A., 2003. Pathways of oxidative damage, *Annu. Rev. Microbiol.*, 57: 395-418.
- Islam, M.S. 2001b. Morpho-Physiology of blackgram and mungbean as influenced by salinity. An M.S. thesis. Dept. of Agronomy. Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur.

- Jaleel, C.A., R. Gopi, P. Manivannan and R. Panneerselvam, 2007. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) plants under salinity stress. Turkish Journal of Botany 31, 245-251.
- Kramer, P.J. 1988. Changing concepts regarding plant water relations. Plant, Cell and Environment 11, 565-568.
- Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7, 405-410.
- Munns, R. 2005. Genes and salt tolerance: bringing them together. New Phytologist 167, 645-663.
- Munns, S.R. and A. Termaat. 1986. Whole plant response to salinity. Australian Journal of Plant Physiology 13, 143-160.
- Nandwal, A.S., M. Godara, D.V. Kamboj, B.S. Kundu, A. Mann, B. Kumar and S.K. Sharma. 2000. Nodule functioning in trifoliate and pentafoliate mungbean genotypes as influenced by salinity. Biologia Plantarum 43, 459-462.
- Neill, S., R. Desikan, A. Clarke, R.D. Hurst and J.T. Hancock, 2002. Hydrogen peroxide and nitric oxide as signaling molecules in plants, Journal of Experimental Botany 53, 1237-1247.
- Orcutt, D. M. and E. T. Nilsen. 2000. The physiology of plants under stress. John wily & sons, Inc., 605 Third Avenue, New York, NY. 10158-0012, USA. pp. 177-235.
- Sangakkara, U.R., U.A. Hartwig and J. Nosberger. 1996. Responses of root branching and shoot water potentials of french bean (*Phaseolus vulgaris* L.) of soil moisture and fertilizer potassium. Journal of Agronomy and Crop Sciences 177, 165-173.
- Sayed, S.A. and M.A.A. Gadallah. 2002. Effects of shoot and root application of thiamin on salt-stressed sunflower plants. Plant Growth Regulators 36, 71-80.
- Tyree, M.T. and H. T. Hammel. 1972. The measurement of the turgor pressure and the water relation of plants by the pressure-bomb technique. Journal of Experimental Botany 23, 267-282.