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Interactive Effect of Foliar Application of 24-Epibrassinolide and Root Zone Salinity on Morpho-physiological Attributes of Wheat (*Triticum aestivum* L.)

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ABSTRACT

Among various environmental stresses, salt stress is an extensively damaging to major crops all over the world. An experiment was conducted to examine the role of 24-epibrassinolide on growth, chlorophyll pigments and gas exchange characteristics of salt tolerant wheat cultivar S-24 under control or saline conditions i.e., 0 or 150 mM of NaCl. The leaves of 56 day old plants were sprayed with 0, 0.0125 or 0.025 mg L^{-1} of 24-epibrassinolide (24-epiBL) and studied the effect after three weeks of spray. Plant growth decreased on imposition of salt stress. Salinity had non-significant effect on chlorophyll contents and photosynthetic rate. While reduction in water use efficiency and increase in transpiration rate was observed under saline conditions, non-significant effect of 24-epibrassinolide was observed on plant biomass, total chlorophyll, net CO_2 assimilation rate, sub-stomatal CO_2 conc. C_i/C_a ratio and water use efficiency.

Key Words: Brassinosteroids; Wheat; Salinity; Gas exchange characteristics; Chlorophyll pigments

INTRODUCTION

Soil salinity causes harmful effects on different physiological attributes, which negatively affect the plant growth and development. Imposition of salinity in the rooting medium is responsible for reduction in leaf area (Neuman *et al.,* 1988), or decrease in growth (Fung *et al.,* 1998) causing the disturbances in the photosynthetic process (Singh *et al.,* 1996). Salinity has deleterious effects on plant growth, because plants may suffer due to osmotic stress, specific ion toxicity, ion imbalance and oxidative stress (Tester & Davenport, 2003). The effect of salinity is very harmful for wheat. Although wheat is grown under both irrigated and rain-fed conditions, both types of agriculture are threatened by salinization (Mujeeb-Kazi & Diaz de Leon, 2002).

Brassinosteroids (BRs) are the first steroidal hormones reported in plants, with significant growth-promoting activity. In addition to growth promotion, they also play important roles in other developmental processes like seed germination, rhizogenesis, flowering, senescence, abscission and maturation. BRs are effective in reducing abiotic stress induced inhibitory effects (Rao *et al*., 2002) and regarded as essential for growth and development of plants (Li & Chory, 1999). Their exogenous application improves the potential productivity of crops by activating cell elongation, vascular differentiation and/or proton pump (Hayat & Ahmad, 2003). Generally, gymnosperms, monocotyledonous and dicotyledonous plants contain brassinosteroids (Clouse & Sasse, 1998). Brassinolide for the first time was extracted from pollen of *Brassica napus* by Grove *et al*. (1979) but up till now approximately 60 related compounds have been identified (Haubrick & Assmann, 2006). Among natural brassinosteroids are the biologically most active groups. Brassinolide are assumed as an active form of brassinosteroids playing roles in specific developmental processes in plants (Choi *et al*., 1993).

Problem of salinity is increasing day by day (Qayyum & Malik, 1988). One of the best solutions is to use saline soils effectively for improved salt tolerance in crops. For this purpose different approaches, were adopted, among those one is the exogenous application of plant growth regulators (Ashraf & Foolad, 2007). The objective of this study was to observe the effect of exogenous application of 24-epiBL as foliar spray on growth and photosynthetic capacity of wheat under saline and non-saline conditions.

MATERIALS AND METHODS

A pot experiment was conducted to assess the interactive effect of foliar application of 24-epibrassinolide (24-epiBL) and salinity on morpho-physiological attributes of wheat cultivar S-24 during the 2005-06. Seeds were obtained from Department of Botany, University of Agriculture, Faisalabad. Two salinity levels were 0 (control) and 150 mmol L^{-1} of NaCl. 24-epibrassinolide levels were 0 (water spray), 0.0125 and 0.025 mg L^{-1}, which were foliarly applied. Tween-20 was used as surfactant.

Fifteen seeds were sown in each plastic pot (20 cm diameter and 24 cm depth) containing about 8 kg dry river sand. Each pot was supplied with 1.5 L full strength Hoagland's nutrient solution. After 10 days of germination, the plants were thinned to four plants per pot. The plants were allowed to grow for 56 days after germination before the application of salinity (using NaCl) in increments of 50 mm per day along with full strength Hoagland's nutrient solution until the desired (150 mM) was obtained. The control plants were irrigated only with full strength Hoagland's nutrient solution. After three weeks of treatment application, the data were recorded. Two plants form each pot were up-rooted carefully, washed with distilled water and fresh weight of shoots and roots were recorded. The samples were oven dried at 65^oC for one week and then dry weights were recorded.

Chlorophyll contents. The chlorophyll *a*, *b* and total chlorophyll were estimated adopting the method of Arnon (1949).

Gas exchange characteristics. Measurements of net CO_2 assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) were made on fully expanded youngest leaf of eleven weeks old plants using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These measurements were made from 12:00 to 1:30 with the following specifications/ adjustments: leaf surface area 11.35 cm^2, ambient CO_2 concentration (Cref) 354.4 μmol mol^{-1}, temperature of leaf chamber varied from 9 to 24^oC, leaf chamber gas flow rate (v) 392.8 mL min^{-1}, Molar flow of air per unit leaf area (Us) 404.84 mol m^{-2} s^{-1}, ambient pressure (P) 99.2 kPa, water vapor pressure (e_{ref}) into chamber ranged from 20.5 to 23.1 mbar, PAR (Q_{leaf}) at leaf surface was maximum up-to 1048 μmol m^{-2} s^{-1}.

Statistical analysis. Analysis of variance (ANOVA) was employed for carrying out statistical analysis of data (Steel & Torrie, 1980). The mean values were compared with least significance difference test (LSD).

RESULTS

Exogenous application of different levels of 24-epibrassinolide to 56-days old plants did not cause increasing or decreasing effects on growth parameters including shoots, roots fresh as well as dry weights of wheat under control or saline conditions, whereas salinity had significantly ($p \leq 0.05$) reducing effect on shoot fresh and dry weights (Table I; Fig. 1).

Interpretations for chlorophyll *a* and *b* showed that chlorophyll *a* was more pronounced at 0.0125 mg L^{-1} level of 24-epiBL under control condition and at 0.025 mg L^{-1} level under 150 mm of NaCl. Chlorophyll *b* was high at 0.025 mg L^{-1} level under control of salinity while under saline it was high at control level of 24-epiBL. Effect of 24-epiBL and salinity on total chlorophyll was consistent under all treatments (Table I; Fig. 1).

There was no considerable effect of 24- epiBL and salinity on net CO_2 assimilation rate (*A*), sub-stomatal CO_2 concentration (C_i), C_i/C_a ratio and water use efficiency (WUE). However, transpiration rate (*E*) and stomatal conductance (g_s) reduced under both levels of 24-epiBL under control or saline conditions. Under saline condition, g_s was low at 0.025 mg L^{-1} level, whereas *E* was better at all levels of 24-epiBL. Salinity had an increasing ($p \leq 0.05$) effect on g_s and *E* ($p \leq 0.01$). Data on sub-stomatal CO_2 concentration (*Ci*) indicated a significant increase under salt stress. There was reduction in WUE of wheat with the application of salinity (Table I; Fig. 2).

DISCUSSION

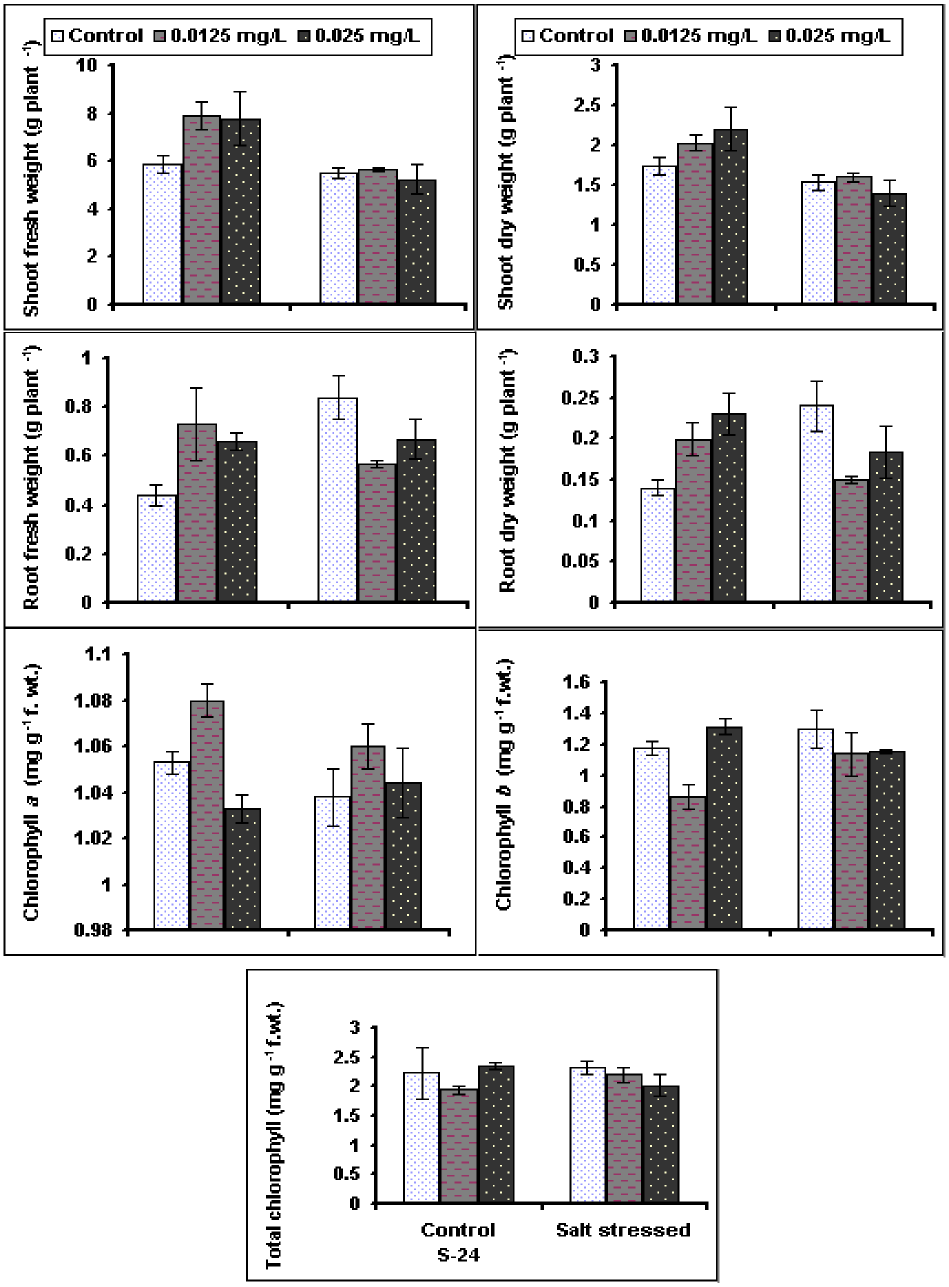
Imposition of salinity to rooting medium had inhibitory effect on growth of wheat. However, application

Table I. Mean squares from analyses of variance of data for growth, chlorophyll contents and gas exchange characteristics of wheat when 56-days old plants were subjected to varying levels of 24-epibrassinolide under control or saline conditions

Source of variation	Degrees of freedom	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
24-epiBL	2	2.702ns	0.074ns	0.014ns	0.002ns
Salinity (S)	1	8.75*	0.666*	0.043ns	8.802ns
24-epiBL x S	2	1.36ns	0.092ns	0.079*	0.007*
Error	18	1.89	0.11	0.017	0.002
		Chlorophyll a	Chlorophyll b	Total chlorophyll	
24-epiBL	2	0.002*	0.143*	0.088ns	
Salinity (S)	1	1.455ns	0.0182ns	8.003ns	
24-epiBL x S	2	2.5ns	0.052ns	0.093ns	
Error	18	3.36	0.0365	0.054	
		A	E	g_s	
24-epiBL	2	6.133ns	0.309**	14809.6**	
Salinity (S)	1	4.5ns	0.240**	6752.1*	
24-epiBL x S	2	1.3ns	0.047ns	1066.9ns	
Error	18	5.63	0.031	1340.4	
		C_i	C_i/C_a	WUE	
24-epiBL	2	1156.9ns	0.009ns	8.363ns	
Salinity (S)	1	11687.5**	0.0943**	71.13**	
24-epiBL x S	2	2115.3ns	0.017ns	17.51ns	
Error	18	1409.6	0.011	7.5	

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = non-significant. 24-epiBL= 24-epibrassinolide

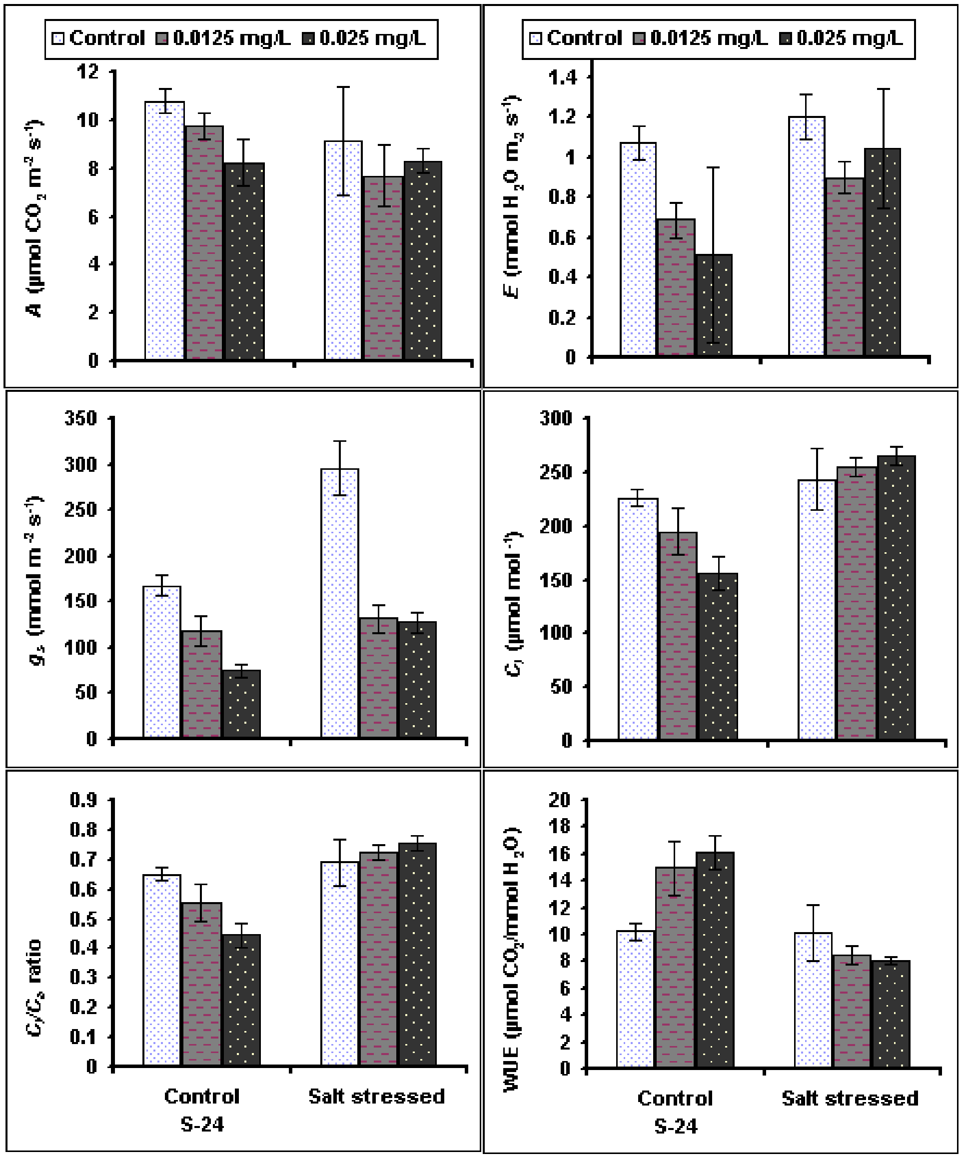
Fig. 1. Growth attributes and chlorophyll contents of wheat cultivar when 56-days old plants were subjected for different levels of foliar application of 24-epibrassinolide under control or saline conditions



of 24-epiBL did not ameliorate the adverse effects of salinity. In wheat reduction in biomass is sometime up to 50% (Maas & Hoffman, 1997). Salinity also reduced the leaf area, leaf length, root and shoot dry weights of wheat (Ashrafuzzaman *et al.*, 2002). Reduction due to salinity was also observed in shoots and roots growth of maize (Khodary, 2004) and soybean (Shalhevet *et al.*, 1995). Brassinosteroids had profound effect on rice at germination and seedling stage (Anuradha & Rao, 2001). Similarly exogenous application of brassinosteroids also had positive effect in increasing the resistance of pine and rice against abiotic stresses (Pullman *et al.*, 2003). However, such effectiveness of 24-epiBL on growth of salt stressed plants depends on type of plant species, growth stage, concentration of brassinosteroids and application mode (Amzallag, 2002). For example, exogenous application of 100 μM of 24-epiBL as a foliar spray was better for the growth and yield of rice (Krishnan *et al.*, 1999), while for tomato it was 3 μM (Vardhini & Rao, 2001).

Roots are in direct contact with salts, but their growth

Fig. 2. Gas exchange characteristics of wheat cultivar when 56-days old plants were subjected for different levels of foliar application of 24-epibrassinolide under control or saline conditions



rate is less affected by salts as compared to shoots. However, results about the effects of brassinosteroids on root growth are contradictory i.e., in some cases brassinosteroids promote root growth as observed in soybean (Sathiyamoorthy & Nakamura, 1990) and maize (Romani *et al*., 1983). But in others, epibrassinolide had an inhibitory effect on root growth of mungbean and wheat (Roddick & Ikekawa, 1992). However, in present study effect of 24-epibrassinolide was not prominent on root fresh and dry biomass (Fig. 1). These contrasting reports are probably due to the involvement of brassinosteroids in various biochemical reactions or regulation of concentrations of other hormones like auxin, GA and cytokinins, which are involved in different growth attributes. According to Mussig *et al.* (2003) brassinosteroids had positive role on growth of roots if its concentration is greater than its threshold value and this concentration is genotype specific.

Exogenous application of 24-epiBL particularly @ 0.0125 mg L^{-1} increased the chlorophyll pigments (chl. *a*) under both non-saline and saline conditions (Fig. 1). Increase in chlorophyll contents by brassinolide are in accordance with the findings of Fariduddin *et al*. (2003) for *Vigna radiata*. Similarly this increasing trend is also supported by the findings of Sairam (1994), Bhatia and Kaur (1997), and Hayat *et al*. (2000).

Photosynthesis plays an important role in the crop growth. Environmental factors are also involved in the disturbance of this characteristic. Green leaves and stem are the sites for photosynthesis and its rate also depends on chlorophyll bearing surface area (Edwards & Walkers, 1983). As photosynthesis is a fundamental physiological processes for plant growth, in many plant species reduction in growth is often associated with reduction in photosynthetic capacity under stress conditions e.g., under saline conditions net CO_2 assimilation reduced in many plant species such as in tomato and turnip rape (Makela *et al*., 1998, 99), *Brassica* species (Nazir *et al*., 2001) and wheat (Ashraf & Bashir, 2003). However according to Downton *et al.* (1985), in spinach NaCl concentrations up to 200 or even 350 mm did not inhibit the rate of photosynthesis. Similar pattern was observed in our study in which salinity did not reduce net CO_2 assimilation rate (Fig. 2). Application of epiBL had increasing effect on photosynthetic rate of wheat and mustard (Braun & Wild, 1984). But our results are contrary to these. Decrease in photosynthetic rate is associated with stomatal or non-stomatal limitations/factors (Dubey, 2005). In our study there was no decrease in stomatal conductance and due to this reason photosynthetic rate also remained un-affected (Fig. 2).

Overall, in present study, foliar application of 24-epiBL had no any prominent effect on growth and photosynthetic characteristics of wheat under control or saline conditions. However, due to 24-epiBL application transpiration rate decreased under saline conditions, which eventually improved the water use efficiency.

REFERENCES

Amzallag, G.N., 2002. The adaptive potential of plant development: evidence from the response to salinity. *In*: Lauchli, A. and U. Luttage (eds.), *Salinity: Environment-Plant-Molecules,* pp: 291–312. The Netherlands: Kluwer

Anuradha, S. and S.S.R. Rao, 2001. Effect of brassinosteroids on salinity stress induced inhibition of germination and seedling growth of rice (*Oryza sativa* L.). *Pl. Growth Regul*., 33: 151–3

Arnon, D.T., 1949. Copper enzyme in isolated chloroplasts polyphenoloxidase in Beta vulgaris. *Pl. Physiol*., 24: 1–15

Ashraf, M. and M.R. Foolad, 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot*., 59: 206–16

Ashraf, M. and A. Bashir, 2003. Salt stress induced changes in some organic metabolites and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. *Flora*, 198: 486–98

Ashrafuzzaman, M., M.A.H. Khan and S.M. Shahidullah, 2002. Vegetative growth of maize (Zea mays) as affected by a range of salinity. *Crop Res. Hisar*., 24: 286–91

Bhatia, D.S. and J. Kaur. 1997. Effects of homobrassinolide and humicil on chlorophyll content, Hill activity and yield component in mungbean [*Vigna radiata* (L.) Wilczek]. *Phytomorphology*, 47:421-426.

Braun, P. and A. Wild, 1984. The influence of brassinosteroid on growth and parameters of wheat and mustard plants. *J. Pl. Physiol*., 116: 189–96

Choi, Y.H., T. Inoue, S. Fujioka, H. Saimoto and A. Sakurai, 1993. Identification of brassinosteroids like active substances in plant cell cultures. *Biosci. Biotech*., 57: 860–1

Clouse, S.D. and J.M. Sasse, 1998. Brassinosteroids: Essential regulators of plant growth and development. *Annu. Rev. Pl. Physiol. Pl. Mol. Biol*., 49: 427–51

Downton, W.J.S., W.J.R. Grant and S.P. Robinson, 1985. Photosynthetic and stomatal responses of spinach leaves to salt stress. *Pl. Physiol*., 78: 85–8

Dubey, R.S., 2005. Photosynthesis in plants under stressful conditions. *In*: Pessarakli, M. (ed.), *Hand Book Photosynthesis*, 2nd edition, pp: 717–8. C.R.C. Press, New York, USA

Edwards, G.E. and D.A. Walkers, 1983. *C3, C4 Mechanism of Cellular and Environmental Regulation of Photosynthesis,* pp: 78–85. Black well scientific Publication, Oxford, U.K

Fariduddin, Q., A. Ahmad and S. Hayat. 2003. Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide. Photosynthetica., 41: 307-310.

Fung, L.E., S.E. Wang, A. Altman and A. Hutterman, 1998. Effect of NaCl on growth, photosynthesis, ion and water relations of four poplar genotypes. *For. Ecol. Manag*., 107: 135–46

Grove, M.D., G.F. Spencer, W.K. Rohwwedder, N. Mandava, J.F. Worley, J.D. Warthen, G.L. Stiffens, J.L. Flippen-Anderson and J.C. Look, 1979. Brassinolide, a plant growth promoting steroid isolated from Brassica napus pollen. *Nature*, 281: 216–7

Haubrick, L.L. and S.M. Assmann, 2006. Brassinosteroids and plant function: some clues, more puzzles. *Pl. Cell Environ*., 29: 446–57

Hayat, S., A. Ahmad, A. Hussain and Q. Fariduddin. 2000. Photosynthetic rate, growth and yield of mustard plants sprayed with 28-homobrassinolide. *Photosynthetica*, 38: 469-471.

Hayat, S. and A. Ahmad, 2003. Soaking seeds of Lens culinaris with 28-homobrassinolide increased nitrate reductase activity and grain yield in the field in India. *Annu. Appl. Biol*., 143: 121–4

Khodary, S.E.A., 2004. Effect of salicylic acid on growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. *Int. J. Agric. Biol*., 6: 439–63

Krishnan, S., K. Azhakanandam, G.A.I. Ebenezer, N.P. Samson and P. Dayanandan, 1999. Brassinosteroids and Benzylaminopurine increase yield in IR 50 Indica Rice. *Current Sci*., 76: 145–7

Li, J. and J. Chory, 1999. Brassinosteroid actions in plants. *J. Exp. Bot*., 50: 275–82

Maas, E.V. and G.J. Hoffman, 1997. Crop salt tolerance-current assessment. *J. Irrig. Drain Div. Amer. Soc. Civ. Eng*., 103: 115–34

Makela, P., J. Mantila, R. Hinkkanen, E. Pehu and P. Peltonen-Sainio, 1998. Effect of foliar applications of glycinebetaine on stress tolerance, growth and yield of spring cereals and summer turnip rape in Finland. *J. Agron. Crop Sci*., 176: 223–34

Makela, P., M. Kontturi, E. Pehu and S. Somersalo, 1999. Photosynthetic response of drought and salt-stressed tomato and turnip rape plants to foliar applied glycine betaine. *Physiol. Pl*., 105: 45–50

Mujeeb-Kazi, A. and J.L. Diaz de Leon, 2002. Conventional and alien genetic diversity for salt tolerant wheats: focus on current status and new germplasm development. *In*: Ahmad, R. and K.A. Malik (eds.), *Prospects for Saline Agriculture,* Vol. 37, pp: 69–82. Dordrecht: Kluver Academic Publishers

Mussig, C., G.H. Shine and T. Altman, 2003. Brassinosteroids promote root growth in Arabidopsis. *Pl. Physiol*., 133: 1261–71

Nazir, N., M. Ashraf and R. Ejaz, 2001. Genomic relationships in oilseed Brassica with respect to salt tolerance-photosynthetic capacity and ion relations. *Pakistan J. Bot*., 33: 483–501

Neuman, D.S., M. Wagner, J.H. Braatne and J. Howe, 1988. Stress physiology-abiotic. *In*: *Biology of Populus and its Implications for Management and Conservation,* pp: 423–53. National Research Press, Ottawa

Pullman, G.S., Y. Zhang and B.H. Phan, 2003. Brassinolide improves embryogenic tissue initiation in conifers and rice. *Plant Physiol*., 22: 96–104

Qayyum, M.A. and M.D. Malik. 1988. Farm production losses in salt-affected soils. Proc. 1st Nat. Congr. Soil Sci. Lahore, Oct., 1985: 356-364.

Rao, S.S.R., B.V.V. Vardhini, E. Sujatha and S. Anuradha, 2002. Brassinosteroids-a new class of phytohormones. *Curr. Sci*., 82: 1239–45

Roddick, J.G. and N. Ikekawa, 1992. Modification of root and shoot development in monocotyledon and dicotyledon seedlings by 24-epibrassinolide. *J. Pl. Physiol*., 140: 70–4

Romani, G., M.T. Marre, A. Bonetti, R. Cerana, P. Lado and E. Marre, 1983. Effects of brassinosteroids on growth and electogenic proton extrusion in maize root segments. *Physiol. Pl*., 59: 528–32

Sairam, R.K. 1994. Effects of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. *Plant Growth Regul*., 14: 173-181.

Sathiyamoorthy, M. and S. Nakamura, 1990. In vitro root induction by 24-epibrassinolide on hypocotyls segments of soybean [*Glycine max* (L.) Merr.]. *Pl. Growth Regul*., 9: 73–6

Shalhevet, J., M.G. Huck and V.P. Schroeder, 1995. Root and shoot growth responses to salinity in maize and soybean. *Agron. J.*, 87: 512–6

Singh, M., R. Chaturvedi and P.V. Sane, 1996. Diurnal and seasonal photosynthetic characteristics of Populus deltoides Marsh leaves. *Photosynthetica*, 32: 11–21

Steel, R.G.D. and J.H. Torrie, 1980. *Principles and Procedures of Statistics: A Biomaterical Approach, with special reference to Biological Science*. McGraw Hill Book Co., New York

Tester, M. and R.J. Davenport, 2003. Na+ transport and Na+ tolerance in higher plants. *Ann. Bot*., 91: 503–27

Vardhini, B.V. and S.S.R. Rao, 2001. Effect of brassinosteroids on growth and yield of tomato (*Lycopersicum esculentum* Mill.) under field conditions. *Indian J. Pl. Physiol*., 6: 326–8

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