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SALINITY STRESS AND THE SENESCENCE PROCESS IN WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Salinity/Water stress commonly cause leaf yellowing due to changes in chlorophyll, reduction in endogenous cytokinins as well as an enhancement in abscisic acid (AbA) contents. Since cytokinin (CK) and abscisic acid (AbA) levels are known to differentially influence the senescence process, their effect(s) on chlorophyll contents of salinity stressed wheat (*Triticum aestvium* L.) seedlings showed that chlorophyll degradation was reduced by CK, benzylamino purine, BAP, but not by other treatments.

Introduction

Senescence has been defined as endogenously controlled deteriorative changes which are natural causes of death of cells, tissues, organs or organisms (Leopold, 1980). The most general characteristics of senescence include breakdown of selected macromolecules as well as specialized complex metabolites e.g., chlorophyll, progressive deterioration and loss of functions of membranes, and at the final stage degeneration of internal structure of cell (Penarrubia & Mareno, 1995). Salinity/water stress commonly cause leaf yellowing due to changes in chlorophyll, faster maturation and often other symptoms of leaf senescence in intact plants (Prisco & O'Leary, 1972; Aharoni *et al.*, 1977; Maas & Grieve, 1990; McCree *et al.*, 1990). Plants exposed to salinity/water stress are also known to exhibit reduction in endogenous cytokinins and an enhancement in abscisic acid (AbA) contents (Hartung & Davies, 1994; Naqvi, 1994, 1995; Poljakoff-Mayber & Lerner, 1994). Besides, AbA accelerates the breakdown of membrane integrity, including the chloroplast envelop in wheat leaves (Wittenbach, 1977) and cytokinins reduce this impairment in sunflower (Itai & Benzioni, 1974). Since chlorophyll disappearance has been the principal criterion of senescence (Leopold, 1980), experiments were carried out to determine whether salinity stressed wheat seedlings do exhibit early symptoms of senescence.

Material and Methods

Planting Protocol: Healthy wheat seeds (*Triticum aestivum* L. cv. Mehran 89), after surface sterilization for 20 minutes with 1% Na-hypochlorite, were thoroughly washed with distilled water. The seeds were then imbibed overnight in distilled water. Thirty seeds were planted on moulded plastic sieves in a glass-jar with sufficient growth solution to touch the net (Naqvi *et al.*, 1994). After 72 h of seed germination in darkness in

a growth cabinet programmed at 25/20°C day/night temperature the seedlings were exposed to 12 h photoperiod (22 wm^{-2}). Since NaCl effect is comparable to a mixture of salts (Maas & Grieve, 1990), therefore, it was used to induce salinity.

Experiments: Seedlings raised in 0.5 $x10^{-6}$M $CaSO_4$ (control) or $130x10^{-6}$M NaCl (control) for 6 days were transferred to these two freshly prepared solutions which were either used as control or supplemented with 10^{-6}M abscisic acid (AbA), 10^{-6}M benzylaminopurine (BAP), $130x10^{-6}$M NaCl, NaCl+AbA. In an other set the control treatment ($CaSO_4$+NaCl) was supplemented with AbA, BAP and AbA+BAP. The seedlings were kept in a growth cabinet in a randomized manner for another 4 days after which the experiment was terminated to determine shoot and root lengths and the chlorophyll contents.

In another set of experiments, fully expanded leaves were excised from the base of 6 day old seedlings and the cut end was immediately dipped in glass vials containing 15.0 ml of 0.5 mM $CaSO_4$ as base solution (excised control). These excised leaves were then transferred to solutions which were supplemented with 130 mM NaCl, 10^{-6}M AbA, 10^{-6}M BAP, NaCl + AbA, NaCl + BAP, NaCl + AbA + BAP. Each vial contained 10 leaves and the whole set up was transferred to the growth chamber where they were maintained in a randomized manner for further 4 days (total 10 days) after which the experiment was terminated. Fully expanded leaves were harvested from 10 day old seedlings (intact control) for chlorophyll (Chl) estimation.

Chlorophyll Extraction: Excised leaves, from both the experiments, were separately chopped and mixed to make a homogeneous sample. From each treatment, 0.1 gm sample was carefully weighed and transferred to vials containing 10.0 ml of 80% acetone (v/v) and left at room temperature in complete darkness. After overnight extraction, the solution was decanted made upto 10.0 ml (80% acetone) and absorbance was recorded at 663.2 and 646.8 wavelengths in a Hitachi spectrophotometer (150-20). The formula derived by Lichtenthaler (1987) was used to quantify (mg/g fresh weight) chlorophylls a ($12.25\ A_{663.2}$-$2.79\ A_{645.8}$) and b ($21.5\ A_{646.8}$-$5.10\ A_{663.2}$). Chlorophyll (Chl) stability was also calculated (Sarkar, 1993).

The experiments were performed in triplicate and repeated once with qualitatively similar results. Data were subjected to Duncan's Multiple Range Test to get indices of significance.

Results and Discussion

Wheat seedlings raised in $0.5x10^{-6}$M $CaSO_4$ (control) for 6 days when transferred to base solution supplemented with $130x10^{-6}$M NaCl, 10^{-6}M AbA, 10^{-6}M BAP, AbA+NaCl, BAP+NaCl without affecting shoot significantly reduced the growth of root (Table 1). The growth reduction observed followed the order AbA+NaCl > BAP+NaCl and AbA+BAP+NaCl > NaCl > AbA and BAP. Seedlings raised under combined salinity stress (control) of $CaSO_4$ and NaCl for 6 days when transferred to saline solution supplemented with 10^{-6}M AbA, and AbA+BAP significantly reduced the shoot and root growth (Table 1). However, treatments with 10^{-6}M BAP enhanced the shoot and root growth while AbA improved only the root growth. Chl$_a$ contents did not show significant reduction under $CaSO_4$ (base solution) or $CaSO_4$ + NaCl treat-

Table 1. Effect of abscisic acid (AbA) and benzylaminopurine (BAP) on chlorophylls a and b content of intact seedlings under salinity stress.

Treatment	Seedling length (cm)					
Shoot	Root	Chl_a	Chl_b	Chl Stability		Chl_a:Chl_b
			$0.5x10^{-6}$M $CaSO_4$			
Control ($CaSO_4$)	14.38^a	13.75^a	9.01^a	3.72^{ab}	100.00	2.46
NaCl	14.23^a	11.48^c	9.13^a	3.55^{ab}	101.95^a	2.60
AbA	14.15^a	12.66^b	8.77^a	3.72^{ab}	100.29^a	2.44
BAP	14.40^a	12.33^b	8.35^a	3.90^a	99.43^a	2.30
AbA+NaCl	13.67^a	9.25^e	7.93^a	3.20^{bc}	91.62^b	2.52
BAP+NaCl	13.97^a	10.17^d	9.37^a	3.69^{ab}	97.89^a	2.66
NaCl+AbA+BAP	13.79^a	10.80^{cd}	8.64^a	3.35^{abc}	94.50^{ab}	2.58
			$0.5x10^{-6}$M $CaSO_4$ + $130x10^{-6}$M NaCl			
Control	9.59^c	3.48^g	9.51	3.29^{bc}	102.35^a	2.92
AbA	8.23^c	3.68^g	7.23	2.85^c	82.42^c	2.60
BAP	11.11^b	5.06^f	9.04	3.26^{bc}	100.68^a	2.87
AbA+BAP	9.48^c	3.90^g	8.78	3.19^{bc}	95.96^{ab}	2.79

Similar postscript indicates nonsignificant different ($p>0.05$)

ments. Treatments with AbA reduced to a certain extent while BAP did not show any reduction in the Chl_b content under our experimental conditions. Additionally AbA made the chlorophyll unstable without affecting the Chl_a/Chl_b ratio.

In another set, where excised leaves were infilterated with treatment solutions for 24 h, the results showed that even under control the excised leaves contained less Chl_a and b than those of the seedling leaves (Table 2). However, the Chl_a:Chl_b ratio was not affected indicating that both the chlorophylls were equally degraded. This was further indicated by a 25% reduction in the chlorophyll stability between the two controls. Calculating the chlorophyll stability within the excised leaves treatment, it was found that infilteration with BAP alone or in combination with AbA and NaCl significantly increased the chlorophyll stability as compared with the NaCl, AbA or their combination which reduced it.

Varshney & Baijal (1977), working with four grasses viz., *Panicum antidotale*, *Setaria sphacelata*, *Chloris gayana* and *Pennisetum pedicellatum*, observed that the values of Chl_a and total chlorophylls were higher with the increase of salinity and Chl_a:Chl_b ratio was remarkably constant. They further observed that the increase in Chl_b was more than that of Chl_a indicating that the ratio, contrary to the conclusion that it increased, actually decreased. They in fact determined Chl_b:Chl_a rather than Chl_a:Chl_b ratio.

Abdullah (1986) concluded that higher ratio of Chl_a:Chl_b, with micronutrient treatments under salinity stress was an indicator of salt tolerance of wheat. Critical evaluation of the data actually indicates a decrease from 3.76 (salinity control) to 1.0-

Table 2. Effect of abscisic acid (AbA) and benzylaminopurine (BAP) on chlorophylls a and b contents of detached wheat leaves under salinity stress ($130x10^{-6}$M NaCl).

Treatment	Chl_a	Chl_b	Chl	Stability	Chl_a:Chl_b
C (Seedling leaves)	10.52^a	3.89^a	---	---	2.70
C (excised leaves)	7.98^c	2.80^b	74.81	---	2.85
NaCl	7.62^c	2.53^b	---	94.15^{bc}	3.01
ABA	7.19^d	2.50^b	---	90.00^c	2.88
BAP	8.96^b	3.64^a	---	117.00^a	2.46
NaCl + AbA	7.09^d	2.54^b	---	90.00^c	2.79
NaCl + BAP	8.92^b	3.17^{ab}	---	119.10^a	2.81
AbA + BAP	8.58^b	3.48^a	---	112.00^a	2.47
NaCl + AbA + BAP	8.18^c	2.74^b	---	101.30^b	2.99

Similar postscript indicates nonsignificant difference ($P>0.05$).

2.3 (salinity control + micronutrients) indicating a proportionately higher decrease in Chl_a than in the Chl_b content. Chl_a:Chl_b ratios have been found to decrease slightly during leaf senescence, with few exceptions, suggesting a somewhat faster degradation of Chl_a (Goldschmidt, 1980).

Richards (1992) observed that as salinity increased the duration of growth increased in the field grown *Medicago, Atriplex, Thinopyrum, Puccinnellia, Hordeum, Trifolium, Triticum, Helianthus* and *Amaranthus*. These observations indirectly suggest that chlorophyll content was not adversely affected by increase in salinity and thus senescence of the plants tested was delayed. However, Maas & Poss (1989) observed that salinity hastened wheat maturity indicating acceleration in senescence process.

Our results, showing that salinity stress did not affect the Chl_a:Chl_b ratio and their content, is supported by the observation that chlorophyll content remained relatively unaffected by water stress (Prabha *et al.*, 1985). We suggest that based on the solvent(s) used, relevant formulae needs to be applied to calculate Chl_a and Chl_b or the total chlorophylls content (Lichtenthaller, 1987), otherwise the interpretation of the results may be misleading.

References

Abdullah, Z. 1986. Growth response and reproductive yield of wheat grown on saline soils of Pakistan ammended with Zn, Cu, Mn and Fe. In: *Environmental stress and Plant Growth*. (Eds.) S.S.M.Naqvi and R. Ansari, Atomic Energy Agricultural Research Centre, Tando Jam, Pakistan. pp. 43-65.

Aharoni, N., A. Blumenfelds and A.E. Richmond. 1977. Hormonal activity in detached lettuce leaves as affected by leaf water content. *Plant Physiol.*, 59: 1169-1173.

Goldschmidt, E.E. 1980. Pigment changes associated with fruit maturation and their control. In: *Senescence in Plants*. (Ed.) K.V. Thimann, CRC Press, Boca Raton, Florida, USA. pp. 207-218.

Hartung, W. and W.J. Davies. 1994. Abscisic acid under drought and salt stress. In: *Handbook of Plant and*

Crop Stress. (Ed.) M. Pessarakli, Marcel Dekker, Inc., New York, USA. pp. 401-414.

Itai, C. and A. Benzioni. 1974. Regulation of senescence and related metabolic processes. In: *Plant Growth Substances*, 1973. Hirokawa Publishing, Tokyo, Japan. pp. 1022-1026.

Leopold, A.C. 1980. Aging and senescence in plant development. In: *Senescence in Plants*. (Ed.) K.V. Thimann, CRC Press, Boca Raton, Florida, USA. pp. 1-12.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.*, 148: 350-382.

Maas, E.V. and C.M. Grieve. 1990. Spike and leaf development in salt-stressed wheat. *Crop Sci.*, 30: 1309-1313.

Maas, E.V. and J.A. Poss. 1989. Salt sensitivity of wheat at various growth stages. *Irrig. Sci.*, 10: 29-40.

McCree, K.J., C.J. Fernandez and R.F. de Oliveira. 1990. Visualizing interactions of water stress responses with a whole-plant simulation model. *Crop Sci.*, 30: 294-300.

Naqvi, S.S.M. 1994. Plant hormones and stress phenomena. In: *Handbook of Plant and Crop Stress*. (Ed.) M. Pessarakli, Marcel Dekker, Inc., New York, USA. pp. 383- 400.

Naqvi, S.S.M. 1995. Plant/Crop hormones under stressful conditions. In: *Handbook of Plant and Crop Physiology*. (Ed.) M. Pessarakli, Marcel Dekker, Inc., New York, USA. pp. 645-660.

Naqvi, S.S.M., R. Ansari and A.N. Khanzada. 1982. Response of salt stressed wheat seedlings to kinetin. *Pl. Sci. Lett.*, 26: 279-283.

Naqvi, S.S.M., S. Mumtaz, S.A. Ali, A. Shereen, A.H. Khan, M.Y. Ashraf and M.A. Khan. 1994. Proline accumulation under salinity stress. Is abscisic acid involved? *Acta Physiol. Plant.*, 16: 117-122.

Penarrubia, L. and J. Moreno. 1995. Senescence in plants and crops. In: *Handbook of Plant and Crop Physiology*. (Ed.) M. Pessarakli, Marcel Dekker, Inc., New York, USA. pp. 461-481.

Poljakoff - Mayber, A. and H. R. Lerner. 1994. Plants in saline environment. In: *Handbook of Plant and Crop STress* (Ed.) M. Pessarakli, Marcel Dekker, Inc., New York, USA. pp. 65-96.

Prabha, C., Y.K. Arora and W.S. Wagle. 1985. Phospholipids of wheat chloroplasts and its membranes under water stress. *Plant Sci.*, 38: 13-16.

Prisco, J.T. and J.W. O'Leary. 1972. Enhancement of bean leaf senescence by NaCl salinity. *Physiol. Plant.*, 27: 95-100.

Richards, R.A. 1992. Increasing salinity tolerance to grain crops: Is it worthwhile? *Plant Soil*, 146: 89-98.

Sarkar, R.K. 1993. Effect of water stress on proline accumulation and its association with certain biochemical characters in soybean. *Ind. J. Plant Physiol.*, 36: 184-186.

Varshney, K.A. and B.D. Baijal. 1977. Effect of salt-stress on chlorophyll contents of some grasses. *Ind. J. Plant Physiol.*, 20: 161-163.

Wittenbach, V.A. 1977. Induced senescence of intact wheat seedlings and its reversibility. *Plant Physiol.*, 59: 1039-1042.

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