BIOLOGIA PLANTARUM 46 (2): 275-279, 2003

Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid

H. NAYYAR and D.P. WALIA*

Department of Botany, Panjab University, Chandigarh 160014, India
*Department of Fruit Breeding and Genetic Resources, U.H.F., Nauni, Solan, India**

Abstract

Proline accumulation and mobilization in roots of 7-d-old seedlings of wheat genotypes varying in sensitivity towards water stress were compared. Water stress was induced by polyethylene glycol (PEG-6000; osmotic potential -1.5 MPa) in the presence of 0.1 mM abscisic acid (ABA), 1 mM calcium chloride, 0.5 mM verapamil (Ca^{2+} channel blocker), 0.5 mM fluridone (inhibitor of ABA biosynthesis). While both the genotypes did not differ in total proline accumulation, rate of proline accumulation and utilization was higher in tolerant genotype C 306 as compared to susceptible genotype HD 2380. The treatment with ABA and $CaCl_2$ caused further increase in proline accumulation during stress and reduced its mobilization during recovery. The membrane stability and elongation rate of roots was observed to be higher at ABA and calcium treatment in both the genotypes under stress. As was evident from inhibitor studies, the tolerant genotype was more responsive to ABA and the susceptible one to calcium.

Additional key words: apparent proline mobilization rate, fluridone, membrane stability index, root elongation rate, *Triticum aestivum*, verapamil.

Introduction

Proline is a compatible osmolyte and is known to accumulate in response to various kinds of abiotic stresses (Delauney and Verma 1993). Primarily, proline is accumulated in cytosol and contributes to osmotic adjustment in response to dehydration stress (Larher *et al.* 1993). Accumulation of proline in cytoplasm is accompanied by a reduction in the concentration of less compatible solutes and an increase in cytoplasmic water volume (Cayley *et al.* 1991). Proline might sustain protein synthesis (Steward *et al.* 1977) and could also be involved in the energetic metabolism (Lawlor 1995) by means of oxidative processes that take place via the activity of mitochondrial proline dehydrogenase and Δ^1-pyrroline-5-carboxylic acid dehydrogenase. It protects the membranes and proteins against the adverse effects of stresses (Santoro *et al.* 1992).

Water stress injury at various levels of plant organization has been reported to be alleviated by exogenous application of growth regulators (Kumar *et al.* 2001, Singh *et al.* 2001). ABA has been observed to increase with abiotic stresses and acts as a stress sensor and transducer (Cowan *et al.* 1997). Considerable evidence also indicate the existence of ABA-independent dehydration (Gosti *et al.* 1995) and cold (Nordin *et al.* 1991) induced signalling pathways and dissecting the interaction between ABA-dependent and ABA-independent signalling cascade is currently the focus of investigation (Shinozaki and Yamaguchi-Shinozaki 1997). ABA functioning during response to stress may involve calcium as a second messenger (Leung and Giraudat 1998). Proline content has been reported to increase along with ABA and calcium levels during stress (Knight *et al.* 1997, Trotel-Aziz *et al.* 2000, Yang *et al.* 2000). The regulation of proline accumulation under stress conditions is not sufficiently understood (Guerrier *et al.* 1997, Hare and Cress 1999). The contrasting

Received 13 November 2001, *accepted* 18 April 2002.
Abbreviations: ABA - abscisic acid; FL - fluridone; ProM - proline mobilization; PEG - polyethylene glycol; VP - verapamil.
Acknowledgement: We thank the Indian Council of Agricultural Research, New Delhi for providing financial assistance as research project.
E-mail: harshnayyar@hotmail.com

genotypes of various crops show variations in proline accumulation (Guerrier 1997, Ramanjulu and Sudhakar 2000). In the present study, we hypothesized that differences between the contrasting genotypes might be due to different regulation of proline accumulation during stress and recovery by calcium and ABA. Therefore, a study was planned to investigate the interaction of ABA and calcium in relation to proline accumulation during PEG-induced water stress and recovery in tolerant and susceptible wheat genotypes.

Materials and methods

The wheat (*Triticum aestivum* L.) seeds of genotypes C 306 (drought tolerant) and HD 2380 (drought susceptible) were surface sterilized with 0.1 % mercuric chloride for 2 min and thoroughly washed with sterilized distilled water. The seeds were germinated in autoclaved 10 cm Petri dishes lined with two layers of germination paper moistened with 3.5 cm^3 of sterilized distilled water in dark for 24 h at 23 °C in growth chamber (*SEW*, New Delhi, India). Thereafter, seedlings were grown at 16-h photoperiod (irradiance at the surface of the plants in growth chamber 200 μmol m^{-2} s^{-1}) at temperature of 23/20 °C for 7 d. 7-d-old seedlings were transferred to half strength Hoagland solution supplemented with 0.1 mM abscisic acid (ABA), 1 mM $CaCl_2$, 0.5 mM fluridone, 0.5 mM verapamil under the same experimental conditions for 24 h. The water stress was applied by keeping these pre-treated seedlings in the nutrient medium having polyethyleneglycol-6000 of -1.5 MPa osmotic potential for 72 h. Thereafter the seedlings were transferred to nutrient solution (without chemicals used for treatment) for recovery. The main roots were excised periodically for estimation of proline content according to Bates *et al.* (1972). The membrane stability index was measured with conductivity meter (*Elico*, Hyderabad, India) following the method of Premachandra *et al.* (1990). The apparent mean rate of proline mobilization (ProM) was calculated according to Trotel-Aziz (2000) as: ProM = ([ProS] - [ProR])/T, where [ProS] was the proline concentration at the end of stress treatment, [ProR] was the proline concentration at the end of recovery period and T is the duration of recovery period (h). The specific root elongation rate (RER) was calculated by following the equation: RER = ln (L_2 - L_0) × 100/d, where L_0 and L_2 are root lengths on day of recovery and 2 d after that, respectively, and d is the number of days beginning from day of recovery till final observation (2 in the present case). The experiment was set in 3 replications and data was statistically analyzed for standard error and analysis of variance using *Microsta* software.

Results

The proline content increased progressively with time of stress application in both the genotypes and reached maximum at 24 and 36 h in C 306 and HD 2380, respectively (Fig. 1). Faster rate of proline accumulation was observed in tolerant genotype than in susceptible one. No significant difference existed between the two genotypes for total proline accumulation after 36 h till the onset of recovery at 72 h. The decline in proline content during recovery was rapid in C 306 indicating higher apparent mean rate of proline mobilization (ProM) than in HD 2380 (Fig. 2). In calcium treated seedlings, an 6-fold and 4.5-fold increased proline content was observed in HD 2380 and C 306, respectively, at 72 h from the onset of stress (Fig. 1). Calcium caused decline in ProM relative to control (Fig. 2). The delay in ProM was significantly higher in HD 2380 than C 306. The membrane stability index (MSI) and root elongation rate (RER) were also significantly higher at $CaCl_2$-pretreated HD 2380 seedlings than in control ones (Fig. 2). Verapamil (VP, a calcium channel blocker) significantly reduced the proline accumulation in the two genotypes but HD 2380 showed more decrease (Fig. 1). In VP-pretreated seedlings, ProM was relatively higher in HD 2380 than C 306 (Fig. 2). ABA treatment caused 56.7 and 17.5 % increase in proline content over control in C 306 and HD2380, respectively (Fig. 1). ProM at ABA treatment was significantly lower in C 306 than HD 2380 (Fig. 2). ABA resulted in more increase in the MSI and RER in C 306 than HD 2380 (Fig. 2). In the presence of fluridone (FL), inhibitor of ABA biosynthesis, proline content declined in both the genotypes with C 306 having more pronounced effect than HD 2380 (Fig. 1). Fluridone increased ProM more in C 306 relative to HD 2380. MSI and RER declined with FL and C 306 was more influenced. In C 306, application of ABA along with VP partially mitigated the inhibitory effect of the later on proline accumulation and caused a significant increase in its content, while in HD 2380 the repressive effect of fluridone on proline accumulation was negated by simultaneous application of calcium (Fig. 1).

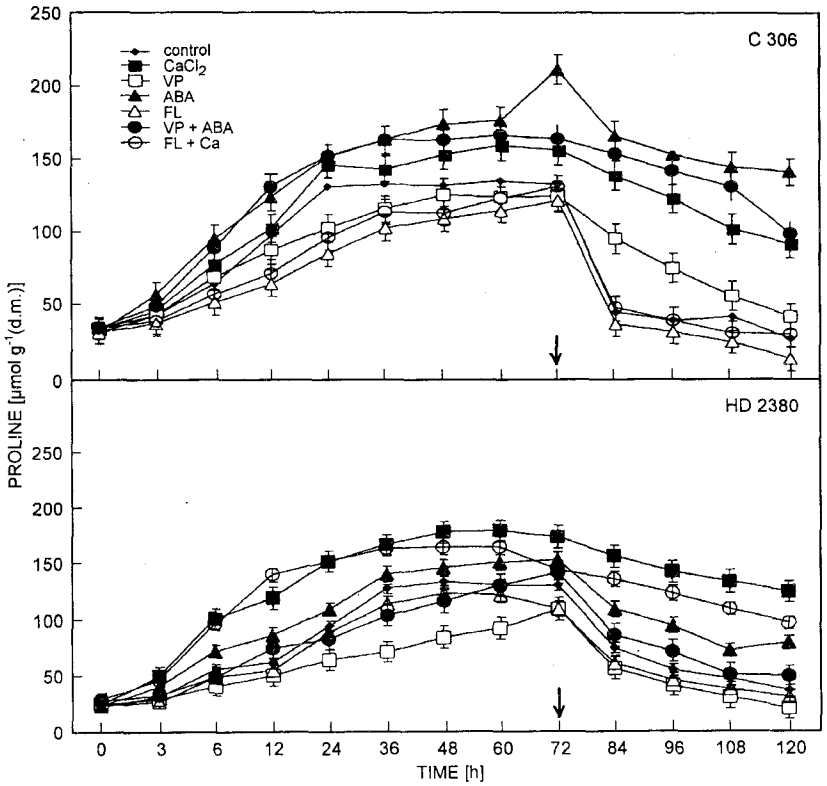

Fig. 1. Effect of calcium, abscisic acid and their inhibitors on proline accumulation during water stress and recovery in C 306 (tolerant) and HD 2380 (susceptible) genotypes of wheat. The 7-d old seedlings growing in half strength Hoagland solution were treated for 24 h with 1 mM $CaCl_2$, 0.5 mM verapamil (VP), 1 mM ABA, 0.5 mM fluridone (FL), and VP + ABA, FL + $CaCl_2$. The treated seedlings were water stressed using PEG-6000 of -1.5 MPa osmotic potential for 72 h and put to recovery (indicated by *arrow*) thereafter. Means ± SE, $n = 3$.

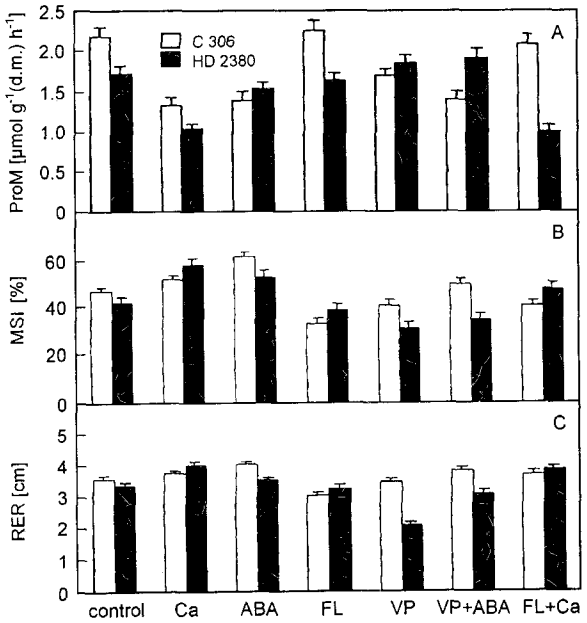

Fig. 2. Effect of calcium, abscisic acid and their inhibitors on apparent mean rate of proline mobilization (ProM), membrane stability index (MSI) and root elongation rate (RER) at 72 h of water stress in C 306 (tolerant) and HD 2380 (susceptible) genotypes of wheat. The 7-d old seedlings growing in half strength Hoagland solution were treated for 24 h with 1 mM $CaCl_2$, 0.5 mM verapamil (VP), 1 mM ABA, 0.5 mM fluridone (FL), VP + ABA, and FL + $CaCl_2$. The treated seedlings were water stressed using PEG-6000 of -1.5 MPa osmotic potential for 72 h and analysed. Means ± SE, $n = 3$.

Discussion

As the total proline content did not differ in two genotypes, the proline synthesis and its utilization rather than proline content per se may be of primary importance in adaptation to stresses (Hare and Cress 1997). ProM might be related to the capacity to repair some of the damage incurred during water stress when growth conditions are restored (Trotel-Aziz 2000) and in this context C 306 (tolerant genotype) appeared to be better adapted. Proline is involved in membrane protection and pressure potential stabilization (Santarius 1992) which seem to be more effective in tolerant genotype under stress as indicated by its higher MSI. ABA and calcium resulted in elevation of proline content and also delayed its mobilization in both the genotypes but a distinctive variation could be seen between the response of two genotypes to ABA and calcium indicating differential regulation of endogenous proline control. A reduction in ProM with ABA at the time of recovery from water stress has been observed in canola leaf discs by Trotel-Aziz (2000) and our observations also indicate a delay in proline utilization by ABA as well as by calcium. The biosynthesis of proline is influenced both by cellular calcium (Knight *et al.* 1997) and ABA (Dallmier and Stewart 1992). The regulation of proline biosynthesis may be dependent or independent of ABA as observed by Savoure *et al.* (1997) during their study on cold and osmotic stresses in *Arabidopsis thaliana*. Stress induced ABA may function with calcium as a signal transducer or acts independently of calcium (Leung and Giraudat 1998). In the present study, the participation of ABA and calcium in proline accumulation was evident from use of their respective inhibitors, fluridone and verapamil which could partially delay the proline rise during stress. Knight *et al.* (1997) noticed that calcium channel blockers could not completely stop the proline accumulation indicating the involvement of some additional intracellular calcium stores. Interestingly, in the present study, tolerant genotype (C 306) showed more sensitivity towards ABA for proline accumulation while the susceptible one appeared to calcium for the same. Also inhibitor studies supported these observations. Inhibition of ABA with FL reduced the elevation of proline content to more extent in tolerant genotype than in susceptible genotype, which exhibited higher inhibition by VP (calcium inhibitor). Simultaneously, when ABA was supplemented along with VP, C 306 produced more proline than HD 2380. Conversely, calcium application with FL resulted in higher proline content in HD 2380 than C 306. These observations indicate that the stress induced proline synthesis may be differentially regulated by ABA and calcium in contrasting wheat genotypes and the stress sensitivity may originate from variation in intracellular functioning of ABA and calcium. Our earlier studies also indicated differences in photosynthetic rate and activities of ribulose 1,5-bisphosphate and phosphoenolpyruvate carboxylase in these contrasting wheat genotypes in relation to ABA and calcium (Nayyar and Kaushal 2002). The contrasting maize genotypes differing in drought tolerance have been found to vary in ABA transport mechanisms (Jovanović *et al.* 2000) and it will be worthwhile to examine the presence of such mechanism(s) and to correlate them with proline functioning under stress conditions.

References

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water stress studies. - Plant Soil **39**: 205-207, 1973.

Cayley, S., Lewis, B.A., Guttman, H.J., Record, M.T., Jr.: Characterization of cytoplasm of *Escherichia coli* K-12 as a function of external osmolarity: implications for protein-DNA interactions *in vivo*. - J. mol. Biol. **222**: 281-300, 1991.

Cowan, A.K., Richardson, G.R., Maurel, J.C.G.: Stress-induced abscisic acid transients and stimulus-response coupling. - Physiol. Plant. **100**: 491-499, 1997.

Dallmier, K.A., Stewart, C.R.: Effeet of exogenous abscisic acid on proline dehydrogenase activity in maize (*Zea mays* L.). - Plant Physiol. **99**: 762-764, 1992.

Delauney, A.J., Verma, D.P.S.: Proline biosynthesis and osmoregulation in plants. - Plant J. **4**: 215-222, 1993.

Gosti, F., Bertauche, N., Vartaniam, N., Giraudat, J.: Abscisic acid-dependent and -independent regulation of gene expression by progressive drought in *Arabidopsis thaliana*. - Mol. gen. Genet. **246**: 10-18, 1995.

Guerrier, G.: Proline accumulation in leaves of NaCl-sensitive and NaCl-tolerant tomatoes. - Biol. Plant. **40**: 623-628, 1997.

Guerrier, G., Delumeau, O., Renard, M., Laval-Martin, D.: Proline metabolism and NAD-kinase activity in soybean calli during short and long term exposures to light and NaCl. - Biol. Plant. **40**: 1-9, 1997.

Hare, P.D., Cress, W.A.: Metabolic implications of stress-induced proline accumulation in plants. - Plant Growth Reg. **21**: 79-102, 1997.

Hare, P.D., Cress, W.A., Van Staden, J.: Proline synthesis and degradation: a model system for elucidating stress related signal transduction. - J. exp. Bot. **50**: 413-434, 1999.

Jovanović, Lj., Stikić, R., Hartung, W.: Effect of osmotic stress on abscisic acid efflux and compartmentation in the roots of two maize lines differing in drought susceptibility. - Biol. Plant. **43**: 407-411, 2000.

Knight, H., Trewavas, A.J., Knight, M.R.: Calcium signaling in

Arabidopsis thaliana responding to drought and salinity. - Plant J. **12**: 1067-1078, 1997.

Kumar, B., Pandey, D.M., Goswami, C.L., Jain, S.: Effect of growth regulators on photosynthesis, transpiration and related parameters in water stressed cotton. - Biol. Plant. **44**: 475-478, 2001.

Larher, F., Leport, L., Petrivalsky, M.: Effectors of osmoinduced proline response in higher piants. - Plant Physiol. Biochem. **31**: 911-922, 1993.

Lawlor, D.W.: The effects of water deficit on photosynthesis. - In: Smirnoff, N. (ed.): Environment and Plant Metabolism and Acclimation. Pp. 129-160. BIOS Scientific Publ., Oxford 1995.

Leung, J., Giraudat, J.: Abscisic acid signal transduction. - Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 199-122, 1998.

Nayyar, H., Kaushal, S.K.: Alleviation of negative effects of water stress in two contrasting wheat genotypes by calcium and abscisic acid. - Biol. Plant. **45**: 65-70, 2002.

Nordin, K., Heino, P., Palva, E.T.: Separate signal pathways regulate the expression of a low temperature-induced gene in *Arabidopsis thaliana* (L.) Heynh. - Plant mol. Biol. **16**: 1061-1071, 1991.

Premachandra, G.S., Saneoka, H., Ogata, S.: Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. - J. agr. Sci. **115**: 63-66, 1990.

Ramanjulu, S., Sudhakar, C.: Proline accumulation during dehydration in two mulberry genotypes with contrasting drought tolerance. - J. Plant. Physiol. **157**: 81-87, 2000.

Santarius, K.A.: Freezing of isolated thylakoid membranes in complex media. VIII. Differential cryoprotection by sucrose, proline and glycerol. - Physiol. Plant. **84**: 87-93, 1992.

Santoro, M.M., Liu, Y., Khan, S.M.A., Hou, L.X., Bolen, D.W.: Increased thermal stability of proteins in the presence of naturally occurring osmolytes. - Biochemistry **31**: 5278-5283, 1992.

Savoure, A., Hua, X.J., Bertauche, N., Montagu, V., Verbruggen, V.: Abscisic acid-independent and abscisic acid-dependent regulation of proline biosynthesis following cold and osmotic stresses in *Arabidopsis thaliana*. - Mol. gen. Genet. **254**: 104-109, 1997.

Shinozaki, K., Yamaguchi-Shinozaki, K.: Gene expression and signal transduction in water stress response. - Plant Physiol. **115**: 327-334, 1997.

Singh, D.V., Srivastava, G.C., Abdin, M.Z.: Amelioration of negative effect of water stress in *Cassia angustifolia* by benzyladenine and/or ascorbic acid. - Biol. Plant. **44**: 141-143, 2001.

Stewart, C.R., Boggess, S.F., Aspinall, D., Paleg, L.G.: Inhibition of proline oxidation by water stress. - Plant Physiol. **59**: 930-932, 1977.

Trotel-Aziz, P., Niogret, M.F., Larher, F.: Proline level is partly under the control of abscisic acid in canola leaf discs during recovery from hyper-osmotic stress. - Physiol. Plant. **110**: 376-383, 2000.

Yang, C.W., Wang, J.W., Kao, C.H.: The relation between accumulation of abscisic acid and proline in detached rice leaves. - Biol. Plant. **43**: 301-304, 2000.