

WATER STRESS INDUCED CHANGES IN COMMON POLYAMINES AND ABSCISIC ACID IN FRENCH BEAN

K.K. UPRETI* AND G.S.R. MURTI

Plant Hormones Laboratory, Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore-560 089

Received on 29 May, 2004, Revised on 2 April, 2005

SUMMARY

The effects of water stress imposed at flowering stage on the leaf relative water content (RWC), electrolytic conductivity and levels of polyamines and abscisic acid (ABA) in three cultivars of french bean differing in drought tolerance were studied. Amongst the cultivars, tolerant cv. Contender showed lesser decline in RWC and maintained stable electrolyte leakage than the susceptible cv. Arka Suvidha. The effect of stress on polyamines content depended upon cultivars and stress severity. Putrescine content increased under 3 and 6 days stress condition but declined under 9 days. Increase in putrescine was more prominent in susceptible cv. Arka Suvidha under 3 days stress and in the tolerant cv. Contender under 6 days stress. In the 9 days stressed plants, the decline in this polyamine was marked in the tolerant cultivar. The spermidine content showed a declining trend in all the cultivars following increased duration of stress, with tolerant cv. Contender showing lesser changes. In contrast, the spermine content increased progressively with stress in all the cultivars, and the tolerant cv. Contender maintained high levels in the stressed plants. The abscisic acid (ABA) content that increased gradually with increasing durations of stress showed a pattern similar to spermine. Putrescine and spermidine contents in stressed plants did not show any relationship with electrolyte leakage/RWC while spermine content was positively related to ABA content. The possible role of spermine in water stress tolerance has been discussed.

Key words: Abscisic acid, electrolyte leakage, french bean, polyamines, water stress.

INTRODUCTION

Polyamines are important growth regulatory polycationic molecules known to be involved in wide range of plant developmental processes including embryogenesis, root development, flowering and senescence (Galston and Sawhney 1990, Tiburcio *et al.* 1993) and also in plant responses to abiotic stress tolerance (Flores 1991, Galston *et al.* 1997). Investigations have revealed an increase in polyamine biosynthesis in plants experiencing water stress (Turner and Stewart 1986, Tiburcio *et al.* 1993, Aziz and Larher 1995, de Mejia *et al.* 2003). This metabolic adjustment of polyamine is of adaptive significance because of

association of polyamines in the regulation of cellular ionic environments, maintenance of membrane integrity, prevention of chlorophyll loss and stimulation of synthesis of proteins, nucleic acids and protective alkaloids (Evans and Malmberg 1989, Kumar *et al.* 1997). However, precise mechanism by which polyamines are associated with stress tolerance is still unclear. Besides increase in polyamine pool under stress, a shift in hormonal balance characterized by increase in abscisic acid (ABA) is a well established physiological event. This hormone has been shown as an integral component of signal transduction pathway in plants subjected to water stress (Munns and Sharp 1993, Popova *et al.* 2000). There is hardly any information on the relationship between

* Corresponding author, E-mail: kku@ihr.ernet.in

polyamines and ABA under stress conditions. Our earlier study showing increase in ABA and cytokinin levels in the water stressed plants following polyamine application (Upreti and Murti 1999) depicts probable relationship between polyamines and ABA in plant response to stress. In the present investigation, changes in the levels of polyamines and their relationship with relative water content (RWC), electrolyte leakage and ABA under conditions of water stress in three french bean cultivars with varied drought tolerance were examined with an objective to identify component polyamines better related with ABA and stress tolerance.

MATERIALS AND METHODS

Seeds of three cultivars of french bean namely, Contender (relatively tolerant to water stress), Arka Komal (moderately tolerant) and Arka Suvidha (susceptible) were sown in plastic pots (12" height) containing garden soil and farm yard manure (2:1) under natural day length in a polyhouse of Indian Institute of Horticultural Research, Hessaraghatta. After emergence, the seedling were thinned to one per pot and the pots were arranged in completely randomised design with four replications. After 28 days of seedling emergence (stage coinciding flowering stage), water stress treatments of 3, 6 and 9 days durations were imposed by withholding water supply. Control plants were uniformly irrigated daily throughout the experimentation period. Soil moisture content as measured gravimetrically were 32.4% in the control and 26.3, 23.1 and 18.8% in the 3, 6 and 9 days stressed plants. After stress treatments, the plants were irrigated to saturation level. During the experiment, the average relative humidity was between 63.2-69.1% and the maximum and minimum temperatures varied between 20.3-29.7°C.

Samples of young and fully developed leaves were taken from the control and stressed plants, washed thoroughly with distilled water and dried over filter paper. Leaf samples were analysed for RWC, electrolyte leakage and levels of free polyamines and ABA. For determining the RWC and electrolyte leakage, the leaf discs (1.0 cm diameter) were uniformly cut. RWC (%) was determined gravimetrically according to Barrs and Weatherly (1962) after recording fresh, dry and saturated weights of 10 leaf discs. For measuring electrolytic

conductivity, 30 leaf discs were floated in 25.0 ml of distilled water for 24 hours. The conductivity of the solution was measured using a Conductivity meter (ELICO, India). The electrolytic leakage was expressed as relative injury index calculated after boiling the samples in their effusate at 80 °C for 2.0 hr, and measuring the total conductivity of solution (Campus and Pham-Thi 1997).

Free leaf polyamine levels were estimated following the HPLC procedure (Flores and Galston 1982). Leaf sample (5g) was homogenised with 20 ml of chilled 5% (v/v) perchloric acid in a mortar. The homogenate was clarified by centrifugation at 4°C for 15 min (4000 rpm). For benzylation, 1 ml of 2N NaOH and 75 µl of benzoyl chloride were added to 1 ml of supernatant. The reaction mixture was vortexed briskly for 1 min and incubated further for 1 hr at room temperature. After adding 2 ml of saturated NaCl, the benzoylated polyamines were extracted in 3 ml of chilled diethyl ether. The ether phase separated was dried under nitrogen. The residue was suspended in 2 ml methanol and passed through 0.45 µm filter paper (Millipore, USA). The methanolic sample after drying over nitrogen at 40 °C was dissolved in 50 µl of methanol for HPLC analysis. A 5 µl of methanolic benzoylated polyamine sample was injected into the HPLC system (Waters, USA, Model 244) fitted with µ Bondapak C18 column and UV/Visible detector (Waters, USA Model M486) adjusted to 282 nm. An isocratic solvent system of methanol (62 %, v/v) containing 1% acetic acid at 1 ml flow rate was employed for resolving the benzoylated polyamines. The quantification of free polyamines, putrescine, spermidine and spermine (Sigma, USA) in the sample was performed using these as external standards.

For ABA estimation, the leaf sample (1 g) was extracted in ice cold 80% (v/v) methanol. The methanolic extract after evaporating *in vacuo* at 40 °C, was dissolved in water and pH was adjusted to 3. The aqueous acidic extract was partitioned 3 times against diethyl ether, and ether phase separated and condensed under nitrogen. The residue was dissolved in 1 ml of Tris buffer (20 mM, pH 7.8) and the ABA levels were estimated by ELISA procedure (Weiler 1982) using laboratory raised polyclonal antibodies.

RESULTS AND DISCUSSION

Water stress treatments led to gradual decline in leaf RWC and increase in the electrolytic leakage in all the cultivars (Table 1). Among the cultivars, the responses to stress in terms of RWC and electrolyte leakage were varied with susceptible cv. Arka Suvidha recording greater changes. The stressed plants of tolerant cv. Contender maintained relatively balanced RWC and electrolyte leakage values. The percentage injury in the leaves under stress was also higher in the cv. Arka Suvidha (17.4-70.4%), moderate in the Arka Komal (12.8-52.8%) and least in the cv. Contender (1.8-40.8%) (Table 1). Leaf RWC and electrolyte leakage under stress condition have been demonstrated as widely accepted selectable markers for cultivar indexing for stress tolerance. A high values of leaf RWC and low

electrolyte leakage have been associated with the drought tolerance behaviour of cultivars. The changes in RWC and electrolyte leakage under stress in cultivars conform to earlier observed differential stress reponse of the cultivars (Upreti *et al.* 1998).

Total polyamines increased in all the cultivars with the durations of stress, the only exception being susceptible cv. Arka Suvidha under 9 days stress (Table 2). Amongst the cultivars, the total polyamine changes varied with the duration of stress. While in tolerant cv. Contender, the total polyamine content showed progressive increase with the duration of stress, in susceptible cv. Arka Suvidha it showed an increase under 3 and 6 days and a decline under 9 days stress. In the cv. Arka Komal, the increase in total polyamines was higher in 6 days stressed plants as compared to those with 3 and 9 days stress (Table 2).

Table 1. Leaf relative water content and electrolyte leakage in french bean cultivars under water stress

Cultivars	Stress duration (d)	RWC (%)	Electrolyte leakage (µS/g fw)	% injury
Arka Komal	0	87.4	-	-
	3	86.6	78.8	12.8
	6	83.2	63.7	39.6
	9	82.4	47.3	52.8
Contender	0	84.2	-	-
	3	89.7	84.6	1.8
	6	86.3	69.8	27.0
	9	83.2	57.2	40.8
Arka Suvidha	0	84.0	-	-
	3	84.1	71.0	17.4
	6	82.2	58.6	58.3
	9	80.9	41.2	70.4
		Control (C)	Treatment (T)	CxT
RWC	CD 5%	2.42	4.19	6.62
	SEM	1.21	2.06	3.26
Electrolyte leakage	CD5%	12.18	17.22	29.40
	SEM	8.09	8.60	14.71

Among the component polyamines, the putrescine content showed an increase under 3 and 6 days but registered a decline under 9 days stress in the cultivars (Table 2). The susceptible cv. Arka Suvidha showed greater increase under 3 days (62.4%) and the cv. Contender under 6 days stress (135.2%) in this polyamine as compared with unstressed plants. In the 9 days stressed plants, the decline in putrescine was more prominent in the susceptible cv. Arka Suvidha (68.3%) and less in tolerant cv. Contender (6.5%). An initial rise in putrescine followed by decline under stress has been reported in *Brassica napus* (Geay *et al.* 1984) and

barley (Turner and Stewart 1986). No relationship between putrescine and RWC or electrolyte leakage was found in the water stressed plants. This indicated that putrescine may not have any significant role to play in stress tolerance in french bean.

On the other hand, the spermidine content declined gradually with the increasing duration of stress in the cultivars (Table 2). At all the durations of stress, the decline in spermidine was high in susceptible cv. Arka Suvidha (18.8-53.4%) and low in tolerant cv. Contender (10.9-31.6%). The stress induced decline in spermidine

Table 2. Free and total polyamines (nmol g⁻¹ fw) levels in french bean cultivars under water stress

Cultivars	Stress duration (d)	Putrescine		Spermidine		Spermine		Total polyamines	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress
Arka Komal	0	188.8 (±5.62)	-	62.4 (±1.65)	-	122.4 (±4.34)	-	373.6 (±11.57)	-
	3	235.0 (±6.91)	286.4 (±4.16)	57.6 (±0.85)	50.1 (±1.69)	160.8 (±3.32)	190.6 (±3.30)	453.4 (±11.01)	527.1 (±9.08)
	6	147.7 (±2.48)	292.1 (±3.82)	42.1 (±3.96)	28.8 (±2.42)	142.2 (±3.76)	226.7 (±2.77)	332.0 (±10.32)	547.6 (±8.93)
	9	175.5 (±3.92)	116.9 (±2.41)	62.8 (±2.52)	37.2 (±1.07)	138.5 (±2.20)	277.2 (±4.06)	376.8 (±8.56)	431.3 (±7.59)
Contender	0	115.1 (±1.82)	-	67.6 (±2.08)	-	220.1 (±5.11)	-	402.8 (±9.13)	-
	3	129.2 (±4.62)	181.1 (±7.52)	79.2 (±1.48)	70.6 (±4.24)	199.6 (±5.78)	250.8 (±3.88)	408.0 (±12.01)	502.5 (±15.76)
	6	84.1 (±1.51)	197.8 (±5.25)	89.5 (±3.69)	65.2 (±3.61)	294.1 (±7.79)	505.1 (±8.06)	467.7 (±13.08)	768.1 (±16.97)
	9	137.8 (±2.26)	128.8 (±3.19)	92.6 (±4.18)	63.3 (±1.35)	158.3 (±5.16)	626.2 (±9.57)	388.7 (±11.52)	818.3 (±14.13)
Arka Suvidha	0	188.9 (±1.87)	-	85.1 (±5.18)	-	145.3 (±3.02)	-	419.3 (±10.14)	-
	3	135.2 (±0.98)	219.6 (±2.62)	116.2 (±1.24)	94.4 (±5.56)	106.6 (±4.09)	127.4 (±3.69)	358.0 (±6.28)	441.4 (±11.92)
	6	221.0 (±6.93)	299.0 (±4.14)	84.8 (±1.86)	51.3 (±3.34)	103.9 (±2.63)	129.0 (±5.27)	409.7 (±11.43)	479.3 (±12.82)
	9	231.2 (±8.67)	73.2 (±2.97)	65.4 (±2.33)	30.5 (±4.13)	104.2 (±4.75)	145.0 (±6.68)	400.8 (±15.77)	248.7 (±13.66)

Data in parentheses are the standard error mean of 4 replications

has been reported earlier (Turner and Stewart 1986). In the stressed plants, the content of spermidine was more in cv. Arka Suvidha under 3 days as against cv. Contender under 6 and 9 days stress. No relationship was evident between spermidine and RWC or electrolyte leakage in the cultivars under water stress conditions.

In contrast to spermidine, the spermine content showed progressive increase in the cultivars with the durations of stress and cultivars showed differential response. The magnitude of increase in spermine content under stress was high in the cv. Contender (25.7-295.6%), moderate in cv. Arka Komal (18.5-100.1%) and low in cv. Arka Suvidha (19.5-39.2%) as compared to unstressed control plants (Table 2). A decrease in spermidine with increase in spermine under water stress conditions has also been reported earlier in barley (Turner and Stewart 1986). The mechanism by which such changes in spermidine and spermine occur under stress conditions needs investigation.

With imposition of water stress, there was accumulation of ABA in the leaves of cultivars (Fig. 1). At all the stress levels, the ABA accumulation was more in the cv. Contender, followed by cv. Arka Komal. In the 9 days water stressed plants, a 7.4, 2.7 and 2.2 fold accumulation in ABA was recorded in the cvs Contender, Arka Komal and Arka Suvidha, respectively. Similar trends in ABA levels in the cultivars of wheat (Quarrie 1980), Sorghum (Kannangara *et al.* 1982) and french bean (Upreti *et al.* 1998) under water stress have been reported earlier. A positive relationship between ABA

and spermine ($r=0.914$ at 1%) in the stressed plants was observed.

Putrescine, spermidine and spermine are the commonly occurring free polyamines responsible for most of the polyamine dependent responses in plants. The levels of these polyamines are reported to alter significantly under wide range of stress. The trends in these polyamines have been shown species, cultivars, and stress severity dependent (Kumar *et al.* 1997). In our study also we observed the changes in individual polyamines dependent upon cultivar and stress severity. It appears that not all the polyamines have similar role in stress response mechanism. The polyamine, spermine that consistently increased under stress and showed good relationship with ABA in the cultivars appeared to be associated with cultivar tolerance to water stress. This association may be the result of better maintenance of turgidity and cell membrane stability by increased levels of spermine as evident by lesser changes in RWC and electrolyte leakage under stress in the tolerant cv. Contender. Spermine has been shown to counter the stress induced changes in electrolyte leakage (Basra *et al.* 1997) and RWC (Upreti and Murti 1999) in plants. The effect of polyamine especially of spermine to enhance ABA levels in stressed plants of peas was reported earlier (Upreti and Murti 1999). Aurisano *et al.* (1993) also showed that exogenous ABA application leads to polyamine changes in wheat seedlings. Further, there are reports that spermine and spermidine exert response similar to that of ABA in the closure of stomata (Liuk *et al.* 2000). The similarity in trends of spermine and ABA under stress and positive relationship between them in stressed plants indicate that spermine is vital polyamine associated with stress tolerance in french bean.

ACKNOWLEDGEMENTS

The authors are thankful to the Director of the institute for providing the necessary facilities, and to Shri H.L. Jayaram and Shri K.R. Earanna for technical help.

REFERENCES

Aurisano, N., Bertani, A., Mattana, M. and Reggiani, R. (1993). Abscisic acid induced stress-like polyamine pattern in

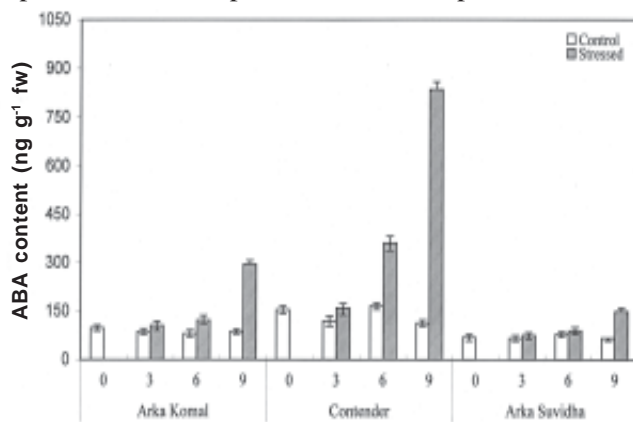


Fig. 1. Leaf abscisic acid content in the french bean cultivars under water stress

- wheat seedlings, and its reversal by potassium ions. *Physiol. Plant.* **89**: 687-692.
- Aziz, A. and Larher, F. (1995). Changes in polyamine titers associated with proline response and osmotic adjustment of rape leaf discs submitted to osmotic stresses. *Plant Sci.* **112**: 175-186.
- Barrs, H.D. and Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for the estimating water deficits in leaves. *Aust. J. Biol. Sci.* **15**: 413-428.
- Basra, R.K., Basra, A.S., Malik, C.P. and Grover, I.S. (1997). Are polyamines involved in the heat shock protection of mung bean seedlings? *Bot. Bull. Acad. Sin.* **38**: 165-169.
- Campus P.S. and Pham-Thi A.T. (1997). Effects of abscisic acid pretreatment on membrane leakage and lipid composition of *Vigna unguiculata* leaf discs subjected to osmotic stress. *Plant Sci.* **130**: 11-18.
- Evans, P.T. and Malmberg, R.C. (1989). Do polyamines have role in plant development? *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **40**: 235-269.
- Flores, H.E. and Galston, A.W. (1982). Analysis of polyamines in higher plants by High Performance Liquid Chromatography. *Plant Physiol.* **69**: 701-706.
- Flores, H.E. (1991). Changes in polyamine metabolism in response to abiotic stress. In: R.D. Slocum and H.E. Flores (eds.), *Biochemistry and Physiology of Polyamines in Plants*, pp. 214-228. CRC Press, Boca Raton.
- Geay, A., Vartanian, N. and Queiroz, O. (1984). Variations des teneurs en polyamines et leurs précurseurs au cours de l'adaptation morphogénétique du colza. *Brassica napus* L. var. *Oleifera*. *M. a la Société Botanique Française* **131**: 99-111.
- Galston, A.W. and Sawhney, R.K. (1990). Polyamines in plant physiology. *Plant Physiol.* **94**: 406-410.
- Galston, A.W., Kaur-Sawhney, R., Altabella, T. and Tiburcio, A.F. (1997). Plant polyamines in reproductive activity and response to abiotic stress. *Bot. Acta* **110**: 197-207.
- Kannangara, T., Durley, D.C., Simpson, G.M., Stout, D.G. (1982). Drought resistance in sorghum bicolor: IV. Hormonal changes in relation to drought stressed field grown plants. *Can. J. Plant Sci.* **62**: 317-330.
- Kumar, A., Altabella, T., Taylor, M.A. and Tiburcio, A.F. (1997). Recent advances in polyamine research. *Trends in Plant Sci.* **2**: 124-130.
- Liuk, K., Fu, H., Bei, Q. and Luan S. (2000). Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. *Plant Physiol.* **124**: 1315-1325.
- de Mejia, E.G., Martinez, R.V., Castano, T.E. and Loarca, P.G. (2003). Effect of drought on polyamine metabolism, yield, proline content and *in vitro* protein digestibility in tepary (*Phaseolus acutifolius*) and common (*Phaseolus vulgaris*) bean seeds. *J. Sci. Food Agric.* **83**: 1022-1030.
- Munns, R. and Sharp, R.E. (1993). Involvement of abscisic acid in controlling plant growth in soils of low water potential. *Aust. J. Plant Physiol.* **20**: 425-437.
- Popova, L.P., Outlaw Jr, W.H., Aghoram, K. and Hite, D.R.C. (2000). Abscisic acid-an intraleaf water stress signal. *Physiol. Plant.* **108**: 376-381.
- Quarrie, S.A. (1980). Genotypic differences in leaf water potential, abscisic acid and proline concentrations in spring wheat during drought stress. *Ann. Bot.* **46**: 383-394.
- Tiburcio, A.F., Campus, J.L., Figueras, X. and Besford, R.T. (1993). Recent advances in the understanding of polyamine functions during plant development. *Plant Growth Regul.* **12**: 331-340.
- Turner, L.B. and Stewart, G.R. (1986). The effect of water stress upon polyamine levels in barley (*Hordeum vulgare* L.) leaves. *J. Exp. Bot.* **37**: 170-177.
- Tiburcio, A.F., Kaur-Sawhney, R. and Galston, A.W. (1993). Spermidine biosynthesis as affected by osmotic stress in oat leaves. *Plant Growth Regul.* **13**: 103-109.
- Upreti, K.K. and Murti, G.S.R. (1999). Effect of polyamines on the changes in endogenous hormones in pea under water stress conditions. *Indian J. Plant Physiol.* **4**: 1-5.
- Upreti, K.K., Murti, G.S.R. and Bhatt, R.M. (1998). Response of french bean cultivars to water deficits: Changes in endogenous hormones, proline and chlorophyll. *Biol. Plant.* **40**: 381-388.
- Weiler, E.W. (1982). An enzyme-immunoassay for cis (+)-abscisic acid. *Physiol. Plant.* **54**: 510-514.