

## Response of French bean cultivars to water deficits: Changes in endogenous hormones, proline and chlorophyll

K.K. UPRETI, G.S.R. MURTI and R.M. BHATT\*

*Plant Hormones Laboratory and Crop Physiology Laboratory\*, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560 0089, India*

### Abstract

Effects of water stress at different stages of plant growth on leaf relative water content (RWC), osmotic potential ( $\Psi_{os}$ ) and changes in contents of chlorophyll, abscisic acid (ABA), zeatin riboside (*t*-ZR), ethylene and proline in six cultivars of French bean (*Phaseolus vulgaris* L.) were studied. Under water stress,  $\Psi_{os}$  and RWC were highest in cv. Contender and lowest in cvs. IIHR-909 and Sel-2. The increase in contents of ABA and proline was marked in cv. Contender followed by cv. UPF-626. Decrease in *t*-ZR and chlorophyll contents was prominent in cv. IIHR-909. Ethylene production surged in all the cultivars under 4- and 8-d stress and declined under 12-d stress.

*Additional key words:* abscisic acid, drought tolerance, ethylene, osmotic potential, *Phaseolus vulgaris*, zeatin riboside.

### Introduction

French bean has high water requirement. Stansell and Smittle (1980) reported that low soil moisture at any stage of growth seriously hampers the productivity of beans. Our earlier studies have revealed that French bean cultivars show differential stress tolerance at various stages of plant growth. Attempts were made thus to investigate the effects of varied durations of water stress at different stages of plant growth on relative water content, osmotic potential and changes in chlorophyll, ABA, cytokinin (*t*-ZR), ethylene and proline contents in six promising cultivars of French bean with the aim to provide information on the physiological basis of differential sensitivity of the cultivars to water stress.

---

Received 27 January 1997, accepted 1 September 1997.

*Acknowledgements:* The authors are thankful to the Director of the institute for providing necessary facilities and to Mr. K.R. Earanna and Mr. M. Munivenkatappa for technical assistance. Thanks are also due to Dr. V.R. Sashidhar, Assoc. Professor, G.K.V.K., Bangalore for providing ABA and *t*-ZR antibodies. (IIHR contribution no. 4/97)

Fax: (+91) 80 8466291; e-mail: iihrr@400nicgw.nic.in

## Materials and methods

Seedlings of six French bean (*Phaseolus vulgaris* L.) cultivars (Arka Komal, Contender, IHR-909, Pusa Parvathi, Sel-2 and UPF-626) were raised in the plastic pots in a poly house. The pots were arranged in completely randomized design with three replications under each treatment. The plants were uniformly watered once a day. The average relative humidity was 72.4 - 75.7 % and the minimum and maximum temperatures varied between 19.4 and 28.6 °C.

Water stress treatments were imposed at vegetative (plant age 21 d), flowering (35 d) and fruiting (50 d) stages of development by withholding water supply for 4, 8 and 12 d. After the stress, the plants were irrigated sufficiently to bring back the soil moisture to saturation level. Osmotic potential ( $\Psi_{os}$ ) was measured with the *Wescor 5100C* vapour pressure osmometer. RWC was determined gravimetrically according to Barrs and Weatherley (1962). ABA in the crude extract and the cytokinin, zeatin riboside (*t*-ZR) from the butanol-soluble fraction were estimated following the *ELISA* procedures (Weiler 1982, Barthe and Stewart 1985). Free proline and chlorophyll contents were estimated according to Bates *et al.* (1973), and Hiscox and Israelstam (1979), respectively. Ethylene production was monitored according to Galliard and Grey (1969).

Table 1. Changes in osmotic potential (-MPa) in French bean under water stress.

Cultivars	Stress [d]	Vegetative stage		Flowering stage		Fruiting stage	
		control	treated	control	treated	control	treated
Pusa Parvathi	4	1.11	1.40	0.97	1.37	1.22	1.53
	8	0.99	1.39	1.15	1.86	1.43	1.99
	12	1.22	1.73	1.40	2.34	1.02	1.65
Arka Komal	4	1.32	1.60	1.16	1.61	1.02	1.33
	8	1.23	1.73	1.08	1.71	1.03	1.53
	12	1.01	1.69	1.20	2.21	1.15	1.90
UPF-626	4	0.80	0.96	0.86	1.06	1.04	1.24
	8	0.79	1.18	0.98	1.45	0.73	1.13
	12	0.64	1.08	1.10	1.74	1.12	1.54
Contender	4	0.79	0.92	0.60	0.82	0.95	1.13
	8	0.55	0.85	0.73	1.10	0.82	1.13
	12	0.72	1.11	1.09	1.69	0.95	1.41
Sel-2	4	1.26	1.61	1.43	1.94	1.35	1.75
	8	1.08	1.65	1.44	2.22	1.07	1.73
	12	1.32	2.00	0.93	-	1.45	2.27
IHR-909	4	1.21	1.61	1.61	2.15	1.24	1.69
	8	1.61	2.29	1.43	2.41	1.47	2.29
	12	1.38	2.08	1.29	-	1.39	-
		Cultivars (C)		Treatment (T)		C×T	
	SEM	0.024		0.017		0.042	
	CD 5 %	0.067		0.047		0.116	

## Results and discussion

**Osmotic potential ( $\Psi_{os}$ ):** Under sufficient moisture,  $\Psi_{os}$  in cv. Contender was highest and that of IIHR-909 was lowest at all the three stages of growth (Table 1). With increased duration of stress, there was decrease in  $\Psi_{os}$  at all the three growth stages, the effect being more pronounced at flowering stage in most of the cultivars (Table 1). The stressed plants of cv. IIHR-909 showed the lowest  $\Psi_{os}$  at the vegetative stage while in cvs. Arka Komal and Pusa Parvathi it was lower at flowering stage and in the cv. Sel-2 at fruiting stage. The cv. Contender had higher  $\Psi_{os}$  at all the three stages of growth than other cultivars and differences between the control and stressed plants in this cultivar were low.

**Relative water content (RWC):** Under irrigated conditions, the cv. Contender showed highest leaf RWC at all the stages followed by Arka Komal at vegetative and flowering stages and Pusa Parvathi at fruiting stage (Table 2). Leaf RWC was lowest in cvs. Sel-2 and IIHR-909. The impact of water stress on RWC was generally greater at flowering stage, followed by vegetative stage. The cv. Contender maintained high RWC under water stress at all the growth stages (Table 2). Thus, the better performance of cv. Contender under the conditions of water stress could be due to its higher  $\Psi_{os}$  and RWC.

Table 2. Changes in relative water content [%] in French bean under water stress.

Cultivars	Stress [d]	Vegetative stage		Flowering stage		Fruiting stage	
		control	treated	control	treated	control	treated
Pusa Parvathi	4	86.1	80.6	79.3	72.4	84.2	80.2
	8	85.2	70.3	84.1	63.8	94.1	84.9
	12	84.6	50.4	84.8	52.1	89.7	64.5
Arka Komal	4	90.2	80.2	88.7	72.6	84.0	76.5
	8	87.3	67.5	77.6	51.7	91.6	75.3
	12	87.4	61.5	90.6	43.4	84.3	57.3
UPF-626	4	90.6	82.6	85.8	72.4	86.9	76.6
	8	81.4	69.2	81.9	66.6	91.5	78.3
	12	90.9	70.8	80.3	49.6	86.4	63.3
Contender	4	88.0	83.4	89.0	83.2	93.3	89.4
	8	92.7	82.4	86.0	72.5	85.3	76.5
	12	84.9	68.2	90.7	65.7	90.1	73.7
Sel-2	4	79.7	68.0	74.1	56.3	81.5	69.0
	8	91.5	70.8	82.1	50.2	86.0	60.5
	12	76.8	45.1	80.9	-	80.6	41.2
IIHR-909	4	80.0	64.0	76.8	60.8	79.2	65.3
	8	84.4	58.0	83.8	45.1	85.2	51.0
	12	87.6	49.1	82.3	-	84.9	-
		Cultivars (C)		Treatment (T)		C×T	
	SEM	0.97		0.71		1.69	
	CD 5 %	1.98		1.44		3.93	

**ABA content:** Under irrigated conditions, ABA content in leaves was generally higher at vegetative stage than at flowering stage except in cv. IIHR-909 (Table 3). At both the growth stages, the irrigated plants of the cv. Contender recorded highest levels of ABA, followed by UPP-626. Water stress led to considerable ABA accumulation in the leaves and the effects were prominent at vegetative stage (Table 3). At both vegetative and flowering stages, the cv. Contender followed by UPP-626 recorded the highest concentration of ABA. ABA accumulation was low at vegetative stage and under 8 d stress at flowering stage in the cv. IIHR-909 and Sel-2. Pusa Parvathi showed lowest ABA accumulation at flowering stage under 12-d stress. As ABA accumulation in the stressed plants has been depicted as an important trait of drought adaptation (Quarrie 1980, Pierce and Raschke 1981), variations in growth and yield of the cultivars in response to stress observed earlier

Table 3. Changes in abscisic acid content [ $\text{ng g}^{-1}$ (f.m.)] in French bean under water stress.

Cultivars	Stress [d]	Vegetative stage		Flowering stage	
		control	treated	control	treated
Pusa Parvathi	8	4.81	10.73	3.54	7.45
	12	5.68	20.64	3.08	9.15
Arka Komal	8	2.05	8.90	4.84	9.88
	12	7.51	19.31	4.06	13.22
UPP-626	8	7.20	20.59	5.12	12.23
	12	8.30	40.85	4.64	18.59
Contender	8	7.88	30.59	8.12	18.29
	12	12.72	68.96	7.27	29.59
Sel-2	8	1.79	5.71	1.65	4.02
	12	3.05	16.25	2.43	-
IIHR-909	8	0.95	3.08	1.31	3.23
	12	1.92	11.58	1.76	-

Table 4. Changes in zeatin riboside content [ $\text{pg g}^{-1}$ (f.m.)] in French bean under water stress.

Cultivars	Stress [d]	Vegetative stage		Flowering stage	
		control	treated	control	treated
Pusa Parvathi	8	57.3	40.4	42.7	20.4
	12	51.7	22.1	45.3	10.7
Arka Komal	8	89.3	69.2	45.3	27.2
	12	71.5	44.9	57.7	22.3
UPP-626	8	59.8	47.2	35.8	15.5
	12	43.3	19.6	37.5	9.3
Contender	8	62.3	53.0	41.3	28.7
	12	58.6	40.0	48.7	20.6
Sel-2	8	46.5	23.8	44.5	12.8
	12	49.6	18.9	33.8	-
IIHR-909	8	56.3	29.0	51.6	13.4
	12	56.3	16.8	38.8	-

could be due to their differential ABA accumulation. Also there was a negative correlation between the osmotic potential and ABA increase in the stressed plants.

***t*-ZR content:** Under sufficient moisture, the concentration of *t*-ZR was highest in the vigorously growing cv. Arka Komal at both the stages of plant growth. Water stress led to considerable reduction in the levels of *t*-ZR at both vegetative and flowering stages (Table 4). Such a decline in cytokinin levels following water stress was also reported earlier (Itai and Vaadia 1971). Cvs. Arka Komal and Contender showed higher levels of *t*-ZR under water stress at both the stages than the other cultivars.

Table 5. Changes in ethylene production [ $\text{mm}^3 \text{g}^{-1}(\text{f.m.}) \text{h}^{-1}$ ] in French bean under water stress.

Cultivars	Stress [d]	Vegetative stage		Flowering stage		Fruiting stage	
		control	treated	control	treated	control	treated
Pusa Parvathi	4	24.7	28.2	37.4	48.3	7.2	9.4
	8	35.8	60.8	12.7	40.9	16.2	30.8
	12	37.4	52.9	20.4	45.5	7.8	16.1
Arka Komal	4	20.8	29.4	34.7	51.8	10.5	19.6
	8	33.7	61.7	16.0	48.8	7.2	23.2
	12	24.7	41.2	17.6	43.3	10.6	20.8
UPF-626	4	18.9	19.6	37.0	46.2	5.7	6.8
	8	20.9	32.9	25.9	56.1	6.5	18.8
	12	24.8	23.6	22.4	45.3	6.9	12.9
Contender	4	11.1	13.5	33.9	37.4	8.3	9.7
	8	20.1	29.9	22.4	36.1	12.1	23.4
	12	23.4	16.6	16.2	23.8	9.3	13.4
Sel-2	4	9.2	14.7	35.8	60.8	11.9	17.2
	8	13.3	37.7	30.9	78.3	11.2	28.8
	12	24.5	33.7	17.9	-	6.3	27.9
IIHR-909	4	19.9	22.7	39.7	54.3	12.7	23.8
	8	30.7	84.9	35.3	66.3	9.7	24.3
	12	32.3	78.6	27.0	-	10.4	-

**Ethylene production:** Ethylene production under sufficient moisture was high in the cvs. Pusa Parvathi followed by IIHR-909 at vegetative stage (Table 5). At flowering stage, it was highest in IIHR-909. Ethylene production in fruiting stage was low in all the cvs. and differences among cultivars were small. There was surge in ethylene production in most of the cultivars under water stress (Table 5). Under 12-d stress there was decline in ethylene production in most of the cultivars as compared to 8-d stressed plants, more prominently at vegetative stage. Decline in ethylene production at vegetative stage was more in the cv. Contender followed by UPF-626. ABA has been shown inhibitory to ethylene production in number of plants (Wright 1984). A higher level of ABA in the stressed plants of cvs. Contender and UPF-626 supports this observation.

**Proline content:** Under non-limiting water supply, the content of proline in the leaves was highest at vegetative stage in all the cultivars except Pusa Parvathi. Proline

content at vegetative and fruiting stages was highest in cv. Contender, and at flowering stage in the cv. UPF-626 (Table 6). There was an increase in proline content under water stress, the increase being more prominent with 12-d stress (Table 6). The magnitude of increase over control was highest in the cv. Contender, followed by UPF-626. The levels of proline under different stress treatments at fruiting stage were the highest in cv. UPF-626 followed by cv. Contender. At all the growth stages, the proline accumulation under stress was lowest in the cvs. IIHR-909 and Sel-2.

Table 6. Changes in proline content [ $\mu\text{mol g}^{-1}$ (f.m.)] in French bean under water stress.

Cultivars	Stress [d]	Vegetative stage		Flowering stage		Fruiting stage	
		control	treated	control	treated	control	treated
Pusa Parvathi	4	0.64	0.76	0.35	0.39	0.65	0.80
	8	0.42	0.78	0.38	0.53	0.79	1.00
	12	1.05	1.54	0.59	0.82	0.73	1.03
Arka Komal	4	0.97	1.08	0.46	0.55	0.66	0.77
	8	0.86	1.30	0.59	0.86	0.49	0.77
	12	0.48	0.99	0.69	1.01	0.74	1.13
UPF-626	4	0.88	1.12	0.50	0.68	0.56	0.73
	8	0.73	1.39	0.84	1.07	0.48	0.90
	12	1.23	2.06	0.93	1.23	0.75	1.24
Contender	4	0.71	1.06	0.37	0.59	0.71	0.86
	8	1.22	2.10	0.76	1.15	0.30	0.67
	12	1.09	2.12	0.99	1.62	0.99	1.47
Sel-2	4	0.58	0.67	0.20	0.27	0.58	0.67
	8	0.65	0.82	0.68	0.82	0.42	0.60
	12	0.83	1.24	0.61	-	0.69	0.89
IIHR-909	4	0.72	0.83	0.39	0.44	0.48	0.57
	8	0.67	0.83	0.49	0.60	0.39	0.54
	12	0.45	0.80	0.56	-	0.38	-
		Cultivars (C)		Treatment (T)		C×T	
	SEM	0.08		0.04		0.12	
	CD 5 %	0.23		0.15		0.37	

**Chlorophyll content:** The vigorously growing cv. Arka Komal recorded highest content of chlorophyll at vegetative and flowering stages under sufficient moisture and the cv. Contender at fruiting stage (Table 7). Water stress considerably lowered the chlorophyll content in the leaves of most of the cultivars with impact of stress being more prominent at flowering stage (Table 7). The stressed plants of the cvs. IIHR-909 and Sel-2 at vegetative stage recorded lower chlorophyll content as compared to other cultivars and the magnitude of its decrease under stress as compared to control was also greater in these cultivars (Table 7). The reduction in chlorophyll under stress was lowest in the cv. Contender

Table 7. Changes in chlorophyll content [ $\text{mg g}^{-1}(\text{f.m.})$ ] in French bean under water stress.

Cultivars	Stress [d]	Vegetative stage		Flowering stage		Fruiting stage	
		control	treated	control	treated	control	treated
Pusa Parvathi	4	1.24	0.97	1.04	0.68	1.65	1.38
	8	1.20	0.83	0.88	0.37	1.14	0.74
	12	1.38	0.85	1.71	1.06	1.55	1.07
Arka Komal	4	1.47	1.24	1.30	1.04	1.17	0.92
	8	1.60	1.22	1.87	1.28	1.75	1.20
	12	1.33	0.83	1.79	1.10	1.73	1.11
UPF-626	4	1.23	1.05	1.26	1.10	1.19	1.05
	8	1.18	0.90	1.04	0.68	1.34	1.04
	12	1.30	0.93	1.74	1.24	1.73	1.42
Contender	4	1.37	1.30	1.09	0.95	1.33	1.25
	8	1.60	1.39	1.56	1.24	1.95	1.71
	12	1.05	0.71	1.77	1.35	1.42	1.06
Sel-2	4	0.96	0.70	1.42	1.01	1.40	1.10
	8	1.44	0.94	1.08	0.40	1.09	0.65
	12	1.55	0.91	1.34	-	1.54	0.84
IIHR-909	4	0.92	0.75	1.36	0.91	1.47	1.14
	8	1.23	0.63	1.22	0.44	1.31	0.87
	12	1.28	0.55	1.33	-	1.32	-
		Cultivars (C)		Treatment (T)		C×T	
	SEM	0.09		0.04		0.13	
	CD 5 %	0.33		0.21		0.53	

French bean cultivars exhibited differences in water stress tolerance as evident from changes in  $\Psi_{os}$  and RWC under stress. The increase in ABA and proline was prominent in tolerant cv. Contender and decrease in *t*-ZR in susceptible cv. IIHR-909. The contents of ABA and proline under stress were higher at vegetative stage. The cultivars with higher potential of ABA and proline accumulation in the water stressed plants during the early phases of plant growth showed better stress tolerance and higher yield. Thus the levels of endogenous leaf ABA and proline would be promising indices for screening French bean genotypes for drought tolerance. Ethylene production appeared to be a weak parameter for drought resistance as its production declined under severe water stress.

## References

- Barrs, H.D., Weatherley, P.E.: A re-examination of the relative turgidity technique for the estimating of water deficits in leaves. - *Aust. J. biol. Sci.* **15**: 413-428, 1962.
- Barthe, G.A., Stewart, I.: Enzyme immunoassay (EIA) of endogenous cytokinins in citrus. - *J. agr. Food Chem.* **33**: 293-297, 1985.
- Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline in water stress studies. - *Plant Soil* **38**: 205, 1973.
- Galliard, T., Grey, T.C.: A rapid method for the determination of ethylene in the presence of other volatile natural products. - *J. Chromatogr.* **41**: 442-452, 1969.

- Hiscox, J.D., Israelstam, G.F.: A method for the extraction of chlorophyll from leaf tissue without maceration. - *Can. J. Bot.* **57**: 1332-1334, 1979.
- Itai, C., Vaadia, Y.: Cytokinin activity in the water stressed plants. - *Plant Physiol.* **47**: 87-90, 1971.
- Pierce, M., Raschke, K.: Synthesis and metabolism of abscisic acid in detached leaves of *Phaseolus vulgaris* L. after loss and recovery of turgor. - *Planta* **153**: 156-165, 1981.
- Quarrie, S.A.: Genotype differences in leaf water potential, abscisic acid and proline concentrations in spring wheat during drought stress. - *Ann. Bot.* **46**: 383-394, 1980.
- Stansell, J.R., Smittle, D.A.: Effect of irrigation regimes on yield and water use of snap bean. - *J. amer. Soc. hort. Sci.* **105**: 869-873, 1980.
- Weiler, E.W.: An enzyme-immunoassay for cis-(+)-abscisic acid. - *Physiol. Plant.* **54**: 510-514, 1982.
- Wright, S.T.C.: The effect of plant growth regulator treatments on the levels of ethylene emanating from excised turgid and wilted wheat leaves. - *Planta* **148**: 381-388, 1984.