# Variation in osmotic adjustment of accessions of lentil (*Lens culinaris* Medic.) in response to drought stress

# M. ASHRAF, M. H. BOKHARI and S. N. CHISHTI

Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan

# SUMMARY

The effect of drought stress on the growth of nine accessions of lentil, ILL 5845, ILL 6451, ILL 6788, ILL 6793, ILL 6796, ILL 6439, ILL 6778, Local Masoor and Masoor 18–10 was assessed in a pot experiment, using control and drought cycles.

Accessions ILL 6439 and ILL 6451 produced significantly greater biomass, had highest osmotic adjustment, a high wax content, a high leaf resistance, a high relative water-content and high leaf elasticity  $(\Delta \Psi_w/\Delta R = \text{gradient of water potential/gradient of relative water}$ content) compared with the other accessions.

From this study, it is established that the drought tolerance of the lentil accessions examined here is highly related to their capacity for osmotic adjustment. Thus osmotic adjustment could be a selection criterion for breeding for drought resistance in lentil. The detection of variation in the response to drought stress in a very small sample of lentil accessions examined here suggests that the advancement of drought tolerance through selection and breeding methods is possible.

Key-words: drought, lentil, osmotic adjustment, relative water content, succulence.

# INTRODUCTION

Water deficit is a major problem to plants grown in warm, arid and semiarid climates, because of low rainfall and a high rate of evapotranspiration. However, water deficiency causes many changes in the morphology, physiology and yield of crops (Atmon 1973; Hurd 1976; Quisenberry 1982).

Several strategies have been devised to overcome the problem of drought stress. Many scientists are of the view that the conventional canal irrigation system should be replaced with closed metal, concrete or plastic conduits, because it will reduce water losses caused by evaporation and seepage. In addition, there are many such means of saving irrigation water but they are all very expensive to be contemplated in most of the underdeveloped countries.

Selection of plant species with considerable drought resistance has been considered to be an economic and efficient means of alleviating agricultural problems in dry areas.

Correspondence: Dr M. Ashraf, 210/B Satellite Town, Jhang Saddar, PC 35206, Pakistan.

It has been reported that tolerance to drought may depend upon a capacity for osmotic adjustment in plants (Blum 1974; Turner & Jones 1980).

The main reason for undertaking the present study, was to investigate the intraspecific variation in osmotic adjustment in response to drought in lentil. Existence of appropriate genetic variation is a prerequisite for the improvement of any character, through selection and breeding (Atsmon 1973; Hurd 1976; Quisenberry 1982).

As there is little information available on the water relations of lentil, different leaf water-relation parameters with regard to drought stress were studied in a greenhouse. In addition to water potential and osmotic potential, leaf elasticity was also measured, because changes in tissue elasticity in many plant species in response to drought stress can lead to lower leaf water-potentials caused by maintained turgor (Turner & Jones 1980; Turner *et al.* 1987).

Many plant scientists are of the view that there must be a single physiological/ biochemical selection criterion for selecting drought tolerant plants (Atsmon 1973; Hsiao 1973; Blum 1974; Hurd 1976). With this in mind, an attempt was also made to explore an appropriate physiological/biochemical parameter, which may prove to be a criterion for breeding for drought tolerance in lentil.

## MATERIALS AND METHODS

Seeds of seven accessions of lentil, i.e., ILL 5845, ILL 6451, ILL 6788, ILL 6793, ILL 6439, ILL 6778, were obtained from ICARDA (International Centre for Agricultural Research in Dry Areas) Aleppo, Syria, and two accessions i.e., Local Masoor and Masoor 18–10 were obtained from NIAB (Nuclear Institute for Agriculture and Biology) Faisalabad, Pakistan. The latter two are approved commercial cultivars.

The growth of the lentil crop is severely affected by soil texture. It has been observed that this crop grows well on sandy loam soil (Z. A. Cheema, pers. comm.). As the soil available in the Botanic Gardens of the Bahauddin Zakariya University Multan is clay loam, thoroughly washed river sand was mixed in appropriate proportion with the Gardens' soil to get sandy loam soil.

Ordinary river sand was collected from the Chenab river bed near Muzaffargarh. Before mixing with the soil it was washed thoroughly with tap water, with distilled water and finally with full strength nutrient solution (Rorison in Hewitt 1966). Pots of 18 cm size were filled with 3.5 kg oven dried sandy loam soil (Electrical conductivity, 1.4 dS/m; pH, 7.4).

Four, 6-day-old seedlings of comparable size, of each accession, were transplanted at an equal distance from each other in each plastic pot. A known weight of fine gravel was placed on the soil surface of each pot to minimize evaporation. The experiment had four blocks in a randomized complete block design. Each block contained nine accessions and three drought treatments. The experiment was conducted in a wire-netting house during the winter 1989–90.

The pots were irrigated every week with half strength nutrient solution (Rorison in Hewitt 1966), for 21 days. The watering treatments were started 21 days after the start of the experiment and the drying treatments continued for further 35 days.

The watering treatments were as follows:

1.  $T_0$  = watering each day to field capacity throughout the experiment.

2.  $T_1$  = the plants were subjected to three consecutive drought cycles. (One drought

cycle was started by withholding water and continued until wilting occurred. The plants were then rewatered to field capacity.)

3.  $T_2$  = The plants were subjected to six drought cycles as in  $T_1$ .

The treatment  $T_1$  was begun when three cycles of  $T_2$  had been completed. The plants were considered wilted when 2–3 leaves of a plant had slightly curled leaflets. After the plants exposed to drought had begun wilting, these plants and the corresponding control plants were rehydrated by watering the soil to field capacity. One day after the rehydration of plants all measurements for growth, water relations and physiological parameters were made.

After the completion of drought treatments, data for the parameters listed below were recorded.

## Leaf resistance

Leaf resistance was measured with an automatic porometer (Mk<sub>3</sub>, Delta-T Device). Pump rate of the instrument was adjusted at the pump-down time 2 seconds. Then RH range was adjusted to 40–50%. A fully expanded leaf from each plant was inserted in the cup with sensor head to take counts of both sides of the leaf. Leaf resistance (s/cm) values were obtained from the standard curve. Leaf resistance data were taken three times a day, i.e. at 0900, 1200 and 1700 hours and pooled to calculate mean leaf resistance per day.

## Osmotic potential $(\psi_s)$

After the completion of three or six drought cycles, 1-2g of the fully expanded youngest leaves were excised from each plant on the following morning at 0900 hours. The leaf material was frozen into 2 cm<sup>3</sup> polypropylene tubes for 2 weeks, thawed and the frozen sap was extracted by crushing the material with a metal rod. After centrifugation (8000 g) for 4 minutes, the sap was used directly for the osmotic potential determination in an osmometer TP 10B (Camlab Limited).

## Leaf water potential $(\psi_w)$

A fully expanded leaf (leaf size ranged from  $10-12 \text{ cm}^2$  and petiole size from 1.2 to 1.5 cm) was excised from each plant at 0900 hours and immediately wrapped in aluminium foil. The petiole of the wrapped leaf was inserted in the chamber of a pressure bomb with the cut end of the petiole protruding from the chamber (Chas, W. Cook and Sons, Birmingham, U.K.). The leaf water potential measurements were made following Turner (1981).

### Estimation of elasticity

The leaf was excised from each plant at 0900 hours, weighed  $(W_1)$  and then inserted in the pressure bomb, and its water potential was  $(\psi_{w2})$  measured. The leaf was over-pressured by 0.5 MPa over the water-potential value of each leaf for 90 s to express a small volume of xylem sap. The pressure was released and the new water potential  $(\psi_{w2})$  was measured. Then this leaf was weighed again  $(W_2)$ , and dried at 85°C for 24 h and weighed  $(W_d)$ . The elasticity (E) was estimated following Thomas (1987) because  $\psi_w$  versus relative water content was rectilinear. The symbol, E should not be confused with  $\varepsilon$  which represents bulk modulus of elasticity.

W ref = 
$$W_1 - W_d + (\psi_{w1} - 0.5) (W_1 - W_2) / (\psi_{w2} - \psi_{w1})$$

W ref = weight of water in a leaf at the reference  $\psi_w = 0.5$  MPa. Change in relative water content

$$\Delta \mathbf{R} = (\mathbf{W}_1 - \mathbf{W}_2) / \mathbf{W} \text{ ref } \mathbf{E} (\mathbf{MPa}) = (\psi_{w2} - \psi_{w1}) / \Delta \mathbf{R}.$$

#### Degree of hydration

Leaf hydration (H) is the ratio of water content in a turgid leaf to its dry weight.

$$H_{(g \text{ water})(g^{-1} d \text{ wt}) = W \text{ ref}/W_d}$$

#### Succulence

Leaves were randomly taken from each plant, their fresh weight was taken and their area was measured using a graphic method. Then the leaves were dried at 70°C for 1 week and their dry weight taken. The succulence was estimated by the following formula:

Succulence = 
$$\frac{\text{Fresh wt} - \text{Dry wt (g H_2 O)}}{\text{Leaf area (m^2)}}$$

#### Relative water content

Fresh leaf material was randomly collected from each plant. Their fresh weight was taken and leaves were dipped in 10 ml distilled water in test tubes. These test tubes were left for 24 h under the fluorescent tube lights (light intensity 50 W/m<sup>2</sup>). After 24 h, the leaves were blotted and their turgid weight was recorded. The leaf material was dried at 70°C for a week and the dry weight was recorded. The relative water content was calculated by the following formula:

$$R.W.C. = \frac{Fresh wt - Dry wt}{Turgid wt - Dry wt} \times 100$$

#### Estimation of epicuticular wax

Epicuticular wax content was determined following Silva Fernandes *et al.* (1964). Leaves (0.5 g) were randomly taken from each plant and their area was measured using a graphic method. The leaf samples were washed three times in 10 ml cold carbon tetrachloride for 30 s/wash. The extract thus obtained was filtered, evaporated to dryness and the remaining wax was weighed. The wax content was expressed on the basis of leaf area only, i.e. wax content g/m<sup>2</sup>.

One plant from each pot was harvested after three or six drought cycles. Plant roots were removed carefully from the soil, and then were washed in distilled, deionized water. Fresh weight of roots and shoots was taken. The shoot and root material was dried at 70°C for 1 week and dry weight of roots and shoots was recorded.

#### Statistical Analysis

The data were subjected to two-way analysis of variance and least significant differences (LSD) used to detect differences between accessions and treatments following Snedecor & Cochran (1980).

#### RESULTS

The shoot dry weight data of nine accessions of lentil grown in three or six drought cycles and their analysis of variance are presented in Figure 1 and Table 1, respectively.



Fig. 1. Mean shoot dry weight (g/plant) of nine accessions of lentil grown in control, three or six drought cycles.

Table 1. Analyses of variance (mean squares) of shoot and root dry weights, and number of leaves per plant of nine accessions of lentil grown in control, three or six drought cycles

Source of variance	Degrees of freedom	Shoot dry weight	Root dry weight	Number of leaves per plant
Blocks	3	0·36 NS	0.001 NS	885.2*
Accessions	8	3.01**	0.016**	8927-4**
Treatments	2	3.82**	0.018**	10 582.3**
Accessions × treatments	16	1.27**	0.012**	3195-2**
Residual	78	0·21	0.002	345.8

\*, \*\*, Significant at 0.05 and 0.001 levels, respectively.

NS = Not significant.

Increasing drought intensity caused a significant reduction (P < 0.001) in shoot dry weight in all the accessions. Accessions differed significantly (P < 0.001), and accessions × treatment interaction was also highly significant (P < 0.001). After three drought cycles, ILL 6451 and ILL 6439 had a significantly greater shoot dry matter than the other accessions. After the same drought treatment shoot dry matter of ILL 6788 and Masoor 18–10 reduced significantly, whereas all the other accessions had intermediate shoot dry biomass. After six drought cycles, ILL 6451, ILL 6439, ILL 6778 and ILL 6788 produced greater shoot dry biomass than the other accessions. ILL 6796, ILL 5845 and Local Masoor were the lowest in shoot dry weight of all the accessions.

Summaries of analysis of variance of root dry weight are given in Table 1 and indicate that drought stress had caused a significant (P < 0.001) reduction in the dry weight of roots in all accessions. Accessions and accession × treatment interaction terms were highly significant (P < 0.001). After both drought treatments, there was no significant difference

<b>.</b> .	R	oot dry weig	ht	Numbe	er of leaves p	er plant
Accession number/name	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles
ILL 5845	0·24a	0·12ab	0·08a	79·1ad	71·8a	45.5ad
ILL 6451	x	y	у	x	x	у
	0·21abd	0·15ab	0·12а	75·7ad	65·1ac	56·3а
ILL 6788	x	xy	у	x	x	x
	0·17abcd	0·15ab	0·13а	75∙1ad	56·1ac	51∙7ad
ILL 6793	x	x	x	x	x	x
	0·16bcd	0·12ab	0·11a	85·4ad	58·1ac	50∙8ad
ILL 6796	x	x	x	x	у	у
	0·17abcd	0·09a	0·08a	119·3ad	116∙0Ъ	96∙6bc
ILL 6439	x	y	у	x	х	x
	0·13cd	0·16ab	0·10а	124·5bc	110-0b	83∙0b
ILL 6778	x	х	x	x	х	у
	0·19ad	0·17Ь	0·09a	145·7c	116·7b	112∙6с
Local Masoor	x	x	у	x	y	y
	0·10cd	0·15ab	0·15а	73·3a	39∙5c	37·4ad
Masoor 18–10	x	x	x	x	y	y
	0·14d	0·14ab	0·08a	102·0bd	45·5a	28∙8d
	x	x	x	x	у	У

Table 2. Mean dry weights of roots (g/plant) and number of leaves per plant of nine accessions of lentil grown in control, three or six drought cycles

Means with the same letters in each column and each row do not differ significantly at 5% level.

between accessions, but accessions differed significantly due to the greater difference in dry matter of the controls.

Increasing drought stress intensity had markedly reduced (P < 0.001) the number of leaves per plant (Table 2) in all accessions. Accessions ILL 6796, ILL 6778 and ILL 6439 had the highest and Local Masoor and Masoor 18–10 had the lowest number of leaves per plant of all accessions after the completion of both the drought treatments.

Increasing drought cycles significantly decreased (P < 0.001) leaf osmotic-potential (Table 3) of all accessions. The accessions differed significantly (P < 0.001) in the response to drought stress. Accession ILL 5845 had significantly the highest and ILL 6788 the lowest osmotic potential after the completion of three drought cycles, whereas the remaining seven accessions did not differ significantly. After the second drought treatment, ILL 6793 and Local Masoor had significantly (P < 0.05) higher leaf osmotic-potential than the other accessions, whereas all the remaining accessions did not differ significantly.

After the first drought treatment, osmotic adjustment was high in ILL 6451, ILL 6788, ILL 6439 and ILL 6778 and low in ILL 5845 and ILL 6796 compared with the other accessions. After the completion of six drought cycles ILL 5845, ILL 6439, ILL 6451 and ILL 6778 had higher osmotic adjustment than the other accessions.

Leaf water-potential decreased significantly (P < 0.001) in all the accessions as a result of repeated drought cycles (Table 3). Accessions ILL 5845, ILL 6788 and ILL 6796 had significantly higher leaf water-potential than the other accessions after the completion of three drought cycles. After six drought cycles ILL 5845 and ILL 6788 maintained a

Table 3. Mear control, three	1 osmotic potential (-MPa), water potential or six drought cycles	(-MPa), e	lasticity (MP	a), leaf hydr	ation (g wat	er g <sup>-1</sup> d wi	) and rela	tive water co	ntent (%)	of nine	accessions of ler	ıtil grown in	
Accession	Osmotic potential		Water pot	ential	Relative	e water coi	ntent	Ek	sticity		Leaf hydr	ation	
number/ name	Δw <sub>5</sub> * Δ Control 3 cycles (MPa) 6 cycles (M	∉,* [Pa) Cor	atrol 3 cycle	s 6 cycles	Control	3 cycles	6 cycles	Control 3	cycles 6 c	cycles (	Control 3 cycle	s 6 cycles	

		Osm	otic pote	ntial		Wa	iter potent	tial	Relativ	e water co	ntent	I	Elasticity		Le	af hydratic	u
Accession number/ name	Control	3 cycles	Δψ,* (MPa)	6 cycles	∆ψ,* (MPa)	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles
ILL 5845	1-58ab	1-82a	0-24	2-7a	1·12	1-2a	1.57acd	1·73a	68-88acdef	73-67ac	67-90ad	2-63ad	4-25ad	6-49abc	3-09ac	4-91a	3-99ac
ILL 6451	х 1-44а	х 2.63b	1.19	y 2·72a	1·28	1·70ab	xy 2·00abde	y 2·3bc	x 58-18bcde	A 63-58bcd	70-88ad	5-67b	y 5-54ac	2 7-49a	4∙21bd	y 5-61ac	ху 3-97ас
ILL 6788	x 1·7lab v	3.5c	1.79	y 2·39a 7	0-68	x 1-4ab	xy 1-5acd	y 2-0ab v	x 50-28bd	xy 63·16bcd v	y 71-88ad v	x 3-18ad	x 4·67acd v	y 7-22a 7	x 4-14abd X	y 4·10abde	x 3·18ab x
ILL 6793	1-49a	2.34b	0.85	ء 1-82b ء	0-33	1.7ab	2·1bcdf	2-0ab	58-46bcde	65-89cd	51-96bc	5·29b	5.52ac	5-69bc	3-5ab	4∙24ab ¥	3∙37ab ¥
ILL 6796	1-85b	2.45b	9-0	2.66a	0.81	1-3a	1-37cd	2·17abc	64-38cde	71-64c	65-62be	2-96ad	5.68bc	5-80bc	4·29b	4·15abd	3-68abc
ILL 6439	1.78b X	2·78b	1-00	2-94a v	1-16	1-2a ×	1-63d x	2·13ab v	59-90be *	72·30c v	76-11ab v	4-35abc x	5-62ba x	7-86a v	2-06c x	3-42bde v	3·31ab v
ILL 6778	1∙55ab ×	2.64b	1-09	2-65a v	1·10	l∙67ab ×	1.80be xv	1-97ab	59-81be v	61-56d *	50-52c xv	3-09acd	3-92d	5·14b v	4-58bd x	6-43c v	3-82ac *
Local Masoor	1-44a x	2·39b v	0-95	2·15c	0-71	1-63ab X	2-27ef v	2-45bc v	61·17e x	29-06d	64-39ae v	2:75d *	5.51ac v	6-87ac v	4-00ab X	3·12de xv	2-62b v
Masoor 18-10	1-62ab	2-42b	0·8	2·4a	0-78	1-9b	2-53f	2.7c	78-52f	47.67e	56·14ce	2·78d	1.73e	6.00abc	3-94ab	3-04e	4-50c
	×	y		y		×	Y	Y	×	y	y	x	Y	2	xy	×	y

Means with the same letters in each column and each row do not differ significantly at 5% level.  $\Delta \psi_1^* = Differences$  between osmotic potential value of rehydrated plants and control plants.

**Table 4.** Analyses of variance (mean squares) of leaf water potential, osmotic potential, elasticity, leaf hydration, and relative water content of nine accessions of lentil grown in control, three or six drought cycles

Source of variance	Degrees of freedom	Water potential	Osmotic potential	Relative water content	Elasticity	Leaf hydration
Blocks	3	0·021 NS	0·012 NS	28·8 NS	1·21 NS	0·23 NS
Accessions	8	0.861***	0.236***	298.9***	6.78***	1.67**
Treatments	2	0.612**	0.351***	258·3*	9.03***	1.04 NS
Accessions × Treatments	16	0.510***	0.097*	204.8***	4.57***	1.28*
Residual	78	0.098	0.038	48·1	0.98	0.6

\*, \*\*, \*\*\*, Significant at 0.05, 0.01 and 0.001 levels respectively.

NS = Not significant.

significantly higher leaf water-potential than the other accessions. However, Masoor 18–10 had the lowest leaf water-potential after both drought treatments.

Increasing drought stress intensity had significant (P < 0.05) effect on the relative water content (Table 3) of all accessions, ILL 6451, ILL 6439 and Local Masoor had the highest and ILL 6778 the lowest relative water content of all accessions. In general, the relative leaf water-content of ILL 6451, ILL 6788 and ILL 6439 increased and that of Masoor 18–10 decreased with the increase in drought cycles. By contrast, relative leaf water-content of the remaining accessions remained unchanged after all drought treatments.

Analysis of variance of the data (Table 4) for tissue elasticity (the gradient  $\psi_w/\Delta R$ ) showed that elasticity increased significantly (P < 0.001) in all the accessions with increasing water deficit intensity. The response of the accessions to drought was also highly significant. After the first drought treatment, accessions ILL 5845, ILL 6788 and ILL 6778 had a significantly lower and Masoor 18–10 a higher elasticity than the other accessions (Table 3). After experiencing six drought cycles ILL 6793, ILL 6796 and ILL 6778 had a significantly lower elasticity than the other accessions.

Leaf hydration increased due to repeated cycles in ILL 5845 and ILL 6439 and decreased in Local Masoor (Table 3), whereas leaf hydration in the remaining accessions remained unaffected. After the completion of three drought cycles ILL 6778 had a significantly (P < 0.05) higher and ILL 6439, Local Masoor and Masoor 18–10 a lower leaf hydration than the other accessions. After the second drought treatment, Local Masoor had the least and Masoor 18–10 the greatest leaf hydration of all accessions.

The mean data for epicuticular wax (Table 5) show that increasing drought stress intensity significantly (P < 0.001) increased the leaf epicuticular wax in all accessions. After three drought cycles ILL 6788, ILL 6796, ILL 6439, ILL 6778 and Local Masoor had significantly greater epicuticular wax than the other accessions, whereas after the second drought treatment ILL 5845, ILL 6451, ILL 6439 and Local Masoor had the highest and ILL 6778 the lowest epicuticular wax deposition of all accessions.

The repeated drought cycles had significant (P < 0.05) effect on succulence in all accessions and the response of accessions (Table 5) was different (P < 0.01). In general, succulence increased in ILL 5845, ILL 6451 and ILL 6788 and decreased in ILL 6439 with

# OSMOTIC ADJUSTMENTS AND DROUGHT STRESS

Table 5. Mean epicuticular wax, succulence and leaf resistance of nine accessions of lentil, in control, three or six drought cycles

	Γ	eaf wax (μg/cm	( <sup>1</sup> )	Succ	ulence (g H <sub>2</sub> O	/m²)	Leaf	resistance (s/	cm)
Accession number/name	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles
ILL 5845	107·23ab	115-5a	344-49ace	110-11a	136-38ab	145-95ae	5-1a	9-5a	9.1a
ILL 6451	x 75-55a	x 139-00abc	y 406·77a	x 131-59abc	xy 151-05a	y 192-21bdf	x 8-0abc	x 10-6a	x 14-6b
ILL 6788	x 117-12ab	x 182-97abcd	y 156-54b	x 130-61abc	x 103-47b	y 206-0b :	x 5.3a 2	xy 12-9a	y 9-2a
ILL 6793	x 68·31a 	x 125·17ab	x 249-52bce	x 125·16ab	x 113-93b	y 136·49a	x 6.9abc 	y 10-1a ,	xy 10-lab č
ILL 6796	x 179-26b	209-51bcd	y 295·79cde	148.63bcd	135·20ab	147-04ae	y 6.3abc	9.6a	10.8ab
ILL 6439	х 85·74а х	xy 224-54cd	y 361·02ade 2	x 146·76bcd	x 128·88ab	74-03c	x 8·labc ř	x 12·3a č	х 9.8а С
ILL 6778	179-74b	y 204·13abcd	310-25e	137-31abc	109-51b	y 136-69a	10.5c	13-6a	10-0ab
Local Masoor	178-51b	247-91d	y 364-92ade	159-68cd	123-09ab	162·87ade	9.6abc	م 11-8a ٽ	9.6a
Masoor 18-10	x 109-14ab x	x 148∙5abc x	y 245·11bce y	x 172-96d x	y 152-92a x	x 170-21ef x	10-3bc x	х 10-9а х	11-6ab x

Means with the same letters in each column and each row do not differ significantly at 5% level.

Source of variance	Degrees of freedom	Leaf resistance	Epicuticular wax	Succulence
Blocks	3	18·0 NS	2814.6 NS	376·8 NS
Accessions	8	110.6***	56 789·2***	9682.7***
Treatment	2	91.5***	69 882·6***	134.5*
Accessions × Treatments	16	56.3**	32 322 - 2***	1736-6**
Residual	78	11.1	3924.9	531.4

Table 6. Analyses of variance (mean squares) of leaf resistance, epicuticular wax and succulence of nine accessions of lentil grown in control, three or six drought cycles

\*, \*\*, \*\*\*, Significant at 0.05, 0.01 and 0.001 levels, respectively.

NS = Not significant.

the increase in the intensity of drought stress, whereas in the remaining accessions, leaf succulence was almost uniform after both the treatments.

Leaf resistance generally increased in all accessions due to drought stress (Table 5). The accessions did not differ significantly for leaf resistance after the first drought treatment. However, after the second treatment, ILL 6451 was the highest in leaf resistance of all accessions.

#### DISCUSSION

The results for the dry matter of shoots and roots clearly show that there is a great variation in the response of the accessions to increasing drought stress. Accessions, ILL 6439 and ILL 6451 were not affected by three drought cycles and produced significantly more shoot dry matter than the other accessions examined.

Osmotic adjustment is considered as an important phenomenon responsible for drought tolerance in plants, because it can assist in maintaining physiological activity by adequate uptake of water from the growth medium (Turner & Jones 1980; Turner 1981; Morgan 1984). It is interesting to note that the high drought-tolerant accessions ILL 6439 and ILL 6451 had relatively lower leaf osmotic-potentials, whereas leaf osmotic-potentials were relatively higher in the two sensitive accessions, Local Masoor and Masoor 18–10. This clearly reflects that synthesis of organic solutes must have occurred in the former two accessions in response to water deficit. A correlation between leaf hydration and osmotic potential was not found. This result is in contrast to the early findings of Thomas (1986) who found a correlation between the two variables in *Dactylis glomerata*.

Deposition of wax on the leaf surface of the two drought-tolerant accessions, ILL 6439 and ILL 6451 was higher than that of the two sensitive Local Masoor and Masoor 18–10. The results for epicuticular wax content of the accessions differing in tolerance can be related to the data for leaf diffusive resistance, as the tolerant accessions had greater leaf resistance compared with the drought-sensitive accessions. Epicuticular wax content on the leaf surface plays a pivotal role in minimizing evaporative loss (Johnson *et al.* 1983; Jordan *et al.* 1984). Reduction in evaporative loss in the two drought-tolerant accessions can be associated with their capacity to maintain high relative water-content. In contrast, the low deposition of epicuticular wax, low leaf resistance and low relative water-content in Local Masoor and Masoor 18–10 may have been major factors responsible for their sensitivity to water deficit.

The crucial role of leaf diffusive resistance has long been recognized in influencing gas exchange through its regulation of water vapours and  $CO_2$  diffusion (Baker 1984). It is now well established that severe plant water-deficits are associated with the increase in stomatal resistance. But the correlation of leaf resistance with leaf water potential and turgor potential has been questioned. For example, Osnubi (1985) has observed in cowpea that the increase in leaf resistance was independent of leaf water potential. Similarly, Black *et al.* (1985) observed an independence of leaf resistance with leaf water and turgor potentials in peanut (*Arachis hypogea*) plants experiencing drought conditions. By contrast, Sinclair & Ludlow (1985) suggested that relative water content might have a close relationship with leaf resistance. However, in the present study a close relationship was observed between leaf diffuse resistance and relative water content in accessions differing in drought tolerance but there was no correlation between leaf resistance and water potentials of all the accessions. These results contradict those of Bennett *et al.* (1987) who did not find any relationship between stomatal conductance and relative water content in maize and soybean.

The elasticity of the cell wall is a characteristic of plant cells which allows volume changes to occur over a range of hydrostatic pressures (Baker 1984). The elasticity is an important parameter in cell-water relations, controlling the way in which the cell water-potential changes as the cell volume changes (Dainty 1976). In the present study there was a considerable increase in leaf elasticity in the two drought tolerant accessions. Therefore, leaf elasticity can be related to the drought tolerance of the accessions which modifies the argument of Turner (1979) that sometimes large differences in elasticity may have only a small effect on drought tolerance.

The identification of germplasm of any sort within lentil which has enhanced drought tolerance is clearly of great potential value. Such material may be exploited for direct use in soils which experience mild drought conditions. The detection of variation in response to drought stress in a very small sample of lentil accessions examined here suggests that advancement of drought tolerance through selection and breeding methods is possible. In addition to osmotic adjustment, variables such as relative water content, leaf diffusive resistance, epicuticular wax content and tissue elasticity could be selection criteria for drought tolerance in lentil.

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