PHYSIOLOGICAL RESPONSES OF SOME STRAINS OF LENTIL (*LENS CULINARIS* MEDIC.) TO WATER DEFICIT

S.N. Chishti & M. Ashraf
Institute of Pure and Applied Biology,
Bahauddin Zakariya University, Multan

The effect of drought stress on the germination and growth of nine cultivars/lines of lentil, ILL 5845, ILL 6451, ILL 6788, ILL 6793, ILL 6796, ILL 6439, ILL 6778, Local Masoor and Masoor 18-10 was assessed. Treatments comprised three and six drought cycles. The plants were grown in pots.

Increasing drought stress intensity (-0.4 MPa and -0.8 MPa of polyethylene glycol 8000) had no significant effect on total germination of all the cultivars/lines, but there was significant effect on rate of germination. ILL 6439, Local Masoor and Masoor 18-10 had considerably higher speed of germination compared with the other cultivars/lines at all the polyethylene glycol treatments. ILL 6439 and ILL 6451 produced significantly greater biomass compared with the other cultivars/lines. There was no significant effect of drought on total soluble proteins and soluble carbohydrates but total free amino acids increased significantly in tolerant accession, ILL 6439. The remaining cultivars/lines did not show any consistent difference in their drought resistance.

INTRODUCTION

Although the lentil crop is known to be adapted to drought conditions, there seems little information on the physiology of this crop, particularly on its osmoregulation (Anonymous, 1989). There is also a need for the determination of a phyiological/biochemical parameter that could act as a specific indicator of drought tolerance than yield itself. This criterion may be useful in measuring the response of the plant integrated over a substantial part of its cycle and can be used for testing large number of plants.

Soluble sugars and soluble proteins along with other osmotica such as free amino acids, proline and glycine betaine are known to play various roles in the metabolic and physiological responses of plants to drought stress (Hsiao, 1973). Eaton (1955) observed the accumulation of carbohydrates in cotton under moisture stress. He concluded that this occurred due to decreased carbohydrate utilisation by the cotton plants. Similarly, Ackerson (1981) studied the role of leaf carbohydrates in osmoregulation in cotton in response to drought stress. He found that water stress acclimated plants had more glucose than non-acclimated plants. Hsiao (1973) tentatively deduced that different physiological and metabolic processes of plants are affected differently at various degrees of water stress.

The present study was undertaken to draw parallels between drought tolerance and different physiological parameters. In addition a part of the objective was to assess the drought tolerance at the germination stage in simulated drought conditions and to draw correlations, if any, between tolerance at two different stages of plant life cycle.

MATERIALS AND METHODS

Seeds of seven accessions of lentil (ILL 5845, ILL 6451, ILL 6788, ILL 6793, ILL 6796, ILL 6439, ILL 6778) were obtained from ICARDA (International Centre for Agricultural Research in Dry Areas), Aleppo, Syria and two accessions (Local Masoor and Masoor 18-10) were obtained from NIAB (Nuclear Institute for Agriculture and Biology), Faisalabad, Pakistan.

Germination experiment: Seed samples of nine cultivars/lines of lentil were surface sterilized in 5% solution of sodium hypochlorite for five minutes before experimentation. Plastic Petri dishes with internal diameter of 6 cm were used. The drought treatments used were different concentrations of polyethylene glycol (mol. wt. 8000) i.e. control, -0.4 MPa and -0.8 MPa, in full strength nutrient solution (Rorison, in Hewitt, 1966). Germination experiment was conducted in a growth room at 32 ± 6°C with 12 hours day length and relative humidity of 66-84%.

Plastic Petri dishes were arranged in a randomised complete block design with three blocks. Each block contained three treatments and nine accessions for each drought treatment. Ten surface sterilized seeds of each accession were placed on filter paper in each Petri dish. Five ml of appropriate treatment solution was applied daily to each Petri dish after rinsing out the previous solution.

Number of seeds germinated were counted daily and data were recorded for 15 days. A seed was considered germinated when both plumule and radicle had emerged $\geq$ 0.5 cm. Data were arscine transformed for the statistical analysis. Rate of germination was determined on the basis of days to 50% germination, calculated from the untransformed data.

Growth experiment: Ordinary river sand was washed thoroughly first with tap water, then 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.

Four, 6 day-old seedlings of comparable size of each accession were transplanted equidistant from each other into each plastic pot. A known weight of fine gravel was placed on the soil surface of each pot to minimise evaporation. The experiment had four blocks in a randomised complete block design. Each block contained 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 begun 21 days after the start of the experiment and the drying treatments continued for further 35 days.

The watering treatments were as follows:

T_0 = Watering each day to field capacity throughout the experiment.

T_1 = Plants were subjected to drought three times until wilting occurred and rewatered to field capacity. (One cycle of drought is one time wilting and rewatering).

T_2 = Plants were subjected to drought six times (as in T_1).

The treatment T_1 was begun when three cycles of T_2 had been completed. Plants were considered wilted when 2-3 leaves of a plant were wilted. After the drought plants had begun wilting, these plants and the corresponding control plants were rehydrated by watering the soil to field capacity. After the completion of drought cycle, measurements for the following physiological parameters were made. Plant

Table 1. Rate of germination (days to 50% germination) and germination percentage of nine accessions of lentil grown under different concentrations of polyethylene glycol (8000)

Accession number/name	Rate of germination			Germination percentage		
	Polyethylene glycol concentrations (MPa)			Polyethylene glycol concentrations (MPa)		
	Control	-0.4	-0.8	Control	-0.4	-0.8
ILL 5845	3.72 a ± 0.14	5.44 ac ± 0.55	7.83 ab ± 0.58	83.33 ab ± 12.14	90.00 ad ± 10.10	83.33 a ± 8.90
ILL 6451	4.66 a ± 0.19	5.16 ac ± 0.17	8.16 a ± 0.25	76.66 a ± 27.56	73.33 b ± 6.74	70.00 b ± 10.10
ILL 6788	3.84 a ± 0.15	4.54 b ± 0.16	7.01 bc ± 0.33	100.00 b ± 0.00	100.00 c ± 0.00	100.00 c ± 0.00
ILL 6793	3.82 a ± 0.11	4.48 b ± 0.10	7.29 abc ± 0.43	96.66 bc ± 3.36	100.00 c ± 0.00	93.33 c ± 6.74
ILL 6796	3.62 a ± 0.23	5.72 d ± 0.28	8.31 cd ± 0.07	100.00 bc ± 0.00	96.66 acd ± 3.36	96.66 c ± 3.36
ILL 6439	3.62 a ± 10.0	4.37 b ± 0.07	6.46 cd ± 0.11	93.33 bc ± 6.74	93.33 acd ± 6.74	96.66 c ± 3.36
ILL 6778	4.32 a ± 0.08	5.34 ac ± 0.22	8.32 a ± 0.56	96.66 bc ± 3.36	93.33 acd ± 6.74	83.33 a ± 3.36
Local Masoor	4.43 a ± 0.08	4.51 bc ± 0.67	6.55 cd ± 0.29	76.66 a ± 3.36	86.66 d ± 3.36	70.00 b ± 0.00
Masoor 18-10	3.97 a ± 0.14	4.61 b ± 0.22 LSD = 1.12	5 72 d ± 0.37	90.00 cd ± 5.83	76.66 b ± 8.90 LSD = 8.1	93.33 c ± 6.74

samples were taken at 9.00 hours in the morning.

Soluble proteins: Total soluble proteins were determined as described by Lowry *et al.* (1951).

Total free amino acids: One ml of each sample extract for the soluble proteins was taken in culture tube and 1 ml of 10% pyridine and 1 ml of 2% ninhydrin solution were added into each tube. The optical densities of these coloured solutions were read at 570 nm using spectrophotometer (Hitachi, U2000) and free amino acids were calculated following Hamilton and Van Slyke (1943).

Total soluble sugars: Total soluble sugars were extracted and determined as described by Smith (1981). One ml of the extract was taken and 10 ml of anthrone solution was added to it. The test tube was shaken and heated in boiling water for 12 minutes, cooled and absorbance was read at 625 nm in a spectrophotometer (Hitachi, U2000).

Statistical analysis of data: The results of all the parameters were subjected to an analysis of variance and Least Significant Difference (LSD) was calculated following Snedecor and Cochran (1974) for comparing treatment means.

RESULTS AND DISCUSSION

Data regarding germination percentage and rate of germination (days to 50% germination) are presented in Table 1. Data for germination percentage (Table 2) showed that lower osmotic potential values of the culture solution containing polyethylene glycol (PEG 8000) had non-significant effect on total germination percentage, whereas accessions differed significantly in total germination percentage and the interaction accession x treatment was also highly significant. The large difference between accessions was due to the difference in germination percentage at controls.

Increasing polyethylene glycol concentration in the growth medium significantly reduced the rate of germination in all the accessions (Tables 1 & 2). At -0.4 MPa of

Table 2. Analysis of variance summaries (mean squares) of days to 50% germination (rate of germination) and germination percentage of nine accessions of lentil grown in control and in different treatments of polyethylene glycol 8000 in full strength Rorison nutrient solution

Source of variance	Degree of freedom	Rate of germination	Germination percentage
Blocks	2	4.4^{NS}	41.9^{NS}
Accessions (Acc.)	8	7.19***	204.7***
Treatments (T)	2	14.16***	43.2^{NS}
Acc. x T	16	4.35***	126.1***
Residual	52	0.47	24.6

*** = Significant at 0.001 level.
NS = Non-significant.

Table 3. Total soluble proteins (mg/g fresh weight), total free amino acids (mg/g fresh weight) and soluble carbohydrates (mg/g dry tissue) of nine accessions of lentil grown in control, three or six drought cycles

Accession number/name	Soluble proteins			Free amino acids			Soluble carbohydrates		
	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles	Control	3 cycles	6 cycles
ILL	1.02 ± 0.15	0.91 ± 0.5	1.04 ± 0.007	23.75 a ± 8.75	27.50 a ± 10.75	73.00 a ± 0.00	42.27 ab ± 8.19	40.45 a ± 5.00	39.77 ab ± 11.60
ILL 6451	1.04 ± 0.11	1.08 ± 0.09	0.98 ± 0.19	64.75 b ± 4.25	67.50 ab ± 13.5	61.25 a ± 3.75	50.91 ac ± 10.00	42.05 a ± 1.6	49.55 b ± 0.91
ILL 6788	1.11 ± 0.05	1.11 ± 0.09	1.10 ± 0.16	42.25 cde ± 11.25	29.50 a ± 0.00	38.75 b ± 7.75	31.36 b ± 0.00	51.14 d ± 23.41	49.55 b ± 12.74
ILL 6793	1.25 ± 0.11	0.96 ± 0.08	0.97 ± 0.12	61.00 bd ± 3.00	33.25 a ± 8.75	28.75 b ± 5.25	50.68 ac ± 9.78	47.05 a ± 4.78	45.00 ab ± 6.82
ILL 6796	1.02 ± 0.11	0.91 ± 0.11	1.20 ± 0.06	51.25 de ± 6.25	32.00 a ± 8.00	66.25 a ± 11.25	45.00 a ± 2.73	34.55 a ± 1.82	43.64 ab ± 0.46
ILL 6439	1.08 ± 0.01	1.19 ± 0.09	1.03 ± 0.00	26.00 a ± 3.50	49.75 b ± 18.75	85.13 c ± 26.13	47.50 ac ± 2.50	44.09 a ± 7.27	41.82 ab ± 4.55
ILL 6778	1.07 ± 0.06	1.04 ± 0.01	0.89 ± 0.08	36.5 ac ± 12.50	39.50 ab ± 8.50	33.25 b ± 1.75	60.22 c ± 3.42	42.05 a ± 1.14	45.46 ab ± 0.00
Local Masoor	1.06 ± 0.11	1.03 ± 0.02	1.08 ± 0.02	66.25 b ± 11.25	47.50 b ± 7.50	30.50 b ± 0.00	50.67 ac ± 3.86	61.82 b ± 20.00	34.55 a ± 0.00
Masoor 18-10	1.27 ± 0.06	0.96 ± 0.05 LSD = NS	0.97 ± 0.21	40.25 e ± 2.25	30.50 a ± 6.5 LSD = 13.04	35.25 b ± 5.75	41.14 ab ± 4.78	59.32 b ± 12.50 LSD 13.00	44.55 ab ± 0.00

polyethylene glycol ILL 5845, ILL 6451, ILL 6796 and ILL 6778 had lower rate of germination (greater number of days to 50% germination) than the other accessions, whereas at -0.8 MPa, ILL 6439, Local Masoor and Masoor 18-10 had significantly higher speed of germination than that of other accessions.

The results from the germination experiment clearly show that even with small number of accessions examined, variation in speed of germination in response to drought exists, although total germination percentage in all accessions was not significantly affected by simulated drought conditions. Some accessions such as ILL 6439, Local Masoor and Masoor 18-10 had considerably higher speed of germination compared with the other accessions of lentil.

Analysis of variance of the data from mean leaf soluble proteins (Table 3) showed that there was no significant effect of repeated cycles of drought on any accessions. Mean free amino acids (Table 3) showed that increasing drought intensity increased free amino acids in ILL 5845, and ILL 6439 and decreased in ILL 6793 and Local Masoor whereas free amino acids concentration in the remaining accessions remained almost unchanged at different drought cycles.

There was no significant effect of drought on soluble carbohydrates but the response of accessions to increasing drought cycle was significantly different (Table 3). At the first drought treatment, Local Masoor and Masoor 18-10 had significantly higher soluble carbohydrates than the other accessions, whereas at 6 drought cycles accessions did not differ significantly.

There are, however, few relationships between accessions performance measured at different growth stages, and using these different parameters. Thus the measure of drought resistance obtained at different growth stages varies, as previously reported for different crops in relation to salinity tolerance (Kingsbury and Epstein, 1984; Ashraf and McNeilly, 1988). This is well illustrated by the data for Local Masoor and Masoor 18-10. In germination experiment, they had higher rate of germination, but were lowest in biomass production of all the accessions. By contrast, ILL 6439 and ILL 6451 were the highest in biomass production but ILL 6451 had lowest speed of germination of all accessions.

Analysis of leaf soluble proteins, soluble carbohydrates and free amino acids show that only leaf free amino acids were increased significantly in two drought tolerant accessions, ILL 6439 and ILL 6451. The increase in the free amino acids as a result of increased drying cycles might have contributed to lower the osmotic potential of the tolerant accessions, since it is crucial for a plant to reduce the cell osmotic potential to a level to provide high turgor potential for maintaining growth (Hsiao, 1973).

The detection of variation in response to drought stress in very small sample of lentil accessions examined here suggests that advancement of drought tolerance through selection and breeding methods is possible.

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