24 (1) : 7-12, 1982

Responses of Two Differentially Sensitive Cicer arietinum L. Cultivars to Water Stress: Protein Content and Drought Resistance

G. SINGH and V. K. RAI

Department of Bio Sciences, H. P. University Simla - 171005, India

Abstract. Two cultivars of *Cicer arietinum* L. with contrasting sensitivities to water stress, cv. C 214 (relatively resistant) and cv. 130 (susceptible) were compared for their protein content and soluble nitrogen (sol-N) under water stress. During water stress shoots of the cv. C 214 showed an increase (over the control) in both protein and sol-N content. The total nitrogen (total-N) also increased in the cv. C 214 while remaining almost unaffected in the cv. G 130. Cultivar C 214 shoots recorded lower protein/sol-N ratios. The results have been discussed in relation to resistance to water stress.

Water stress affects the protein levels of the biological systems. SHAH and LOOMIS (1965) in sugar beet, SAUNIER *et al.* (1968) in creosote bush and DWI-VEDI *et al.* (1979) in excised rice leaves showed decrease in protein levels under water stress. However, GHAZALEH and HANDERSHOTT (1967) and TODD and BASLER (1965) could not find any deleterious effects of drought on soluble protein levels of sweet orange and wheat, respectively. An apparent increase in protein has also been reported (WADLEIGH and TEARE 1945 and SUBBOTINA 1962). Thus large variations in protein levels have been noticed depending upon the experimental materials and conditions.

Characterization of drought resistance at metabolic level from comparative studies on differentially sensitive cultivars of the same crop under water stress has been attempted, *e.g.* MALI and MEHTA (1977a, b) and HANSON *et al.* (1977) but such studies are lacking in legumes. Therefore, the present communication compares two differentially sensitive cultivars of *Cicer arietinum* L. under identical water stress conditions.

MATERIALS AND METHODS

The seedlings of *Cicer arietinum* cv. G 130 (susceptible) and cv. C 214 (resistant) were raised as described previously (SINGH and RAI 1980). The water stress was provided to the 10 day old seedlings by dissolving PEG-6000 in nutrient media and samples were collected after 8 days of stress. After complete removal of pigments from the samples with 80% ethanol, the pro-

Received July 24, 1980; accepted May 4, 1981

teins were precipitated with 15% TCA at 0 °C for 24 h, then centrifuged and washed with ethanol and then defatted with boiling ethanol : ether (3 : 1) mixture. The residue was dissolved in 5 ml of 0.3 N NaOH at 30 °C for 18 h, centrifuged and residue washed once with 4 ml of 0.3 M NaOH. The supernatants were combined and final volume made 10 ml with 0.3 M NaOH. Of



Figs. 1 and 2. Changes in protein content (Fig. 1) and soluble nitrogen content (Fig. 2) of cv. C 214 (full line) and cv. G 130 (dashed line) in shoot and root at various osmotic potentials in substrate.

the above extract 0.5 ml was used to determine protein in samples after the method of LOWRY *et al.* (1951). The soluble nitrogen (sol-N) was determined by traditional microkjeldahl method as described by PIRIE (1955).

RESULTS

In shoots of both the cultivars protein levels show phasic changes (Fig. 1). At milder stress (*i.e.* -3×10^5 Pa) protein levels showed an increase which decreases at -6×10^5 Pa and again showed an increase beyond -8×10^5 Pa. A distinct cultivarial difference is also evident, where resistant cv. C 214 showed a higher increase over control in protein levels as compared to susceptible cv. G 130. It is further noticed that in the susceptible cv. G 130 the level of proteins mostly remained lower than the control.

However, the absolute protein content [mg protein g^{-1} dry m.] was higher in the susceptible cv. G 130 as compared to resistant cv. C 214 (Table 1).

Also in roots with increasing stress levels in both the cultivars protein changes show a phasic nature. The percent inhibition was much lower in resistant cv. C 214 than that in susceptible cv. G. 130 (Fig. 1).

In cv. C 214 shoots there is a sharp rise in the sol-N fraction with increasing stress levels while in cv. G 130 an increase of only ca. 30% is noticed. Thus cv. C 214 showed higher increase in both protein as well as sol-N as

TABLE	1
-------	---

The effect of different levels of water stress on proteins $[mg g^{-1} dry m.]$ of shoot and root of cultivars 0 214 and G 130 on 8th day

<u></u>	Osmotic potential in substrate [×10 ⁵ Pa]						
Snoot	0	-1	3	-6	-8	-10	Mean
Cv. C 214	66.06	68.41	105.72	68.98	74.06	89.83	78.84
Cv. G 130	136.18	125.97	158.53	109.56	128.82	125.78	130.38
Mean	99. 82	97.19	132.12	89.27	101.44	107.80	

C.D. (0.05) (i) To compare effect of stress = 11.50; (ii) to compare cultivars = 6.64.

Post	Osmotic potential in substrate $[\times 10^5 \text{ Pa}]$						
	0	-1	-3	-6	8	-10	Mean
Cv. C 214	60.80	58.86	63.88	41.70	61.85	68.65	57.07
Cv. G 130	84.16	104.85	64. 10	4 7.22	78.92	81.80	76.40
Mean	72.47	80.24	64.29	44.46	63.72	70.23	

C.D. (0.05) (i) To compare effect of stress = 10.75; (ii) to compare cultivars = 6.20.

compared to cv. G 130. In roots sol-N decreases initially but shows recovery beyond -6×10^5 Pa. The pattern of changes is similar to that observed for proteins and again relative values are higher in cv. C 214 as compared to cv. G 130.

In shoots absolute sol-N values [mg g⁻¹ dry m.] were much higher in cv. C 214 than those in cv. G 130 while in roots the values mostly remained comparable (Table 2). Thus it seems that cv. C 214 maintained low protein/sol-N ratios while cv. G 130 maintained higher protein/sol-N ratios. A look at the balance sheet shows that with increasing stress levels there was an increase even in total-N of cv. C 214 while in cv. G 130 it remained almost unaffected. Thus the better performance of cv. C 214 under stress may be related to its raised-N levels.

DISCUSSION

An earlier experiment on the growth of *Cicer arietinum* L. cultivars established a higher susceptibility of cv. G 130 to water stress as compared to cv. C 214 in the early vegetative stage (SINGH and RAI 1980). Thus changes in proteins were undertaken to check if the cultivarial difference is related in any way to their protein levels and metabolic patterns under stress.

It has been observed that in resistant cv. C 214 with increasing stress levels there is a distinct increase in protein and sol-N while in cv. G 130

		Osmotic potential in substrate ($\times 10^5$ Pa)						
		0	-1	-3	6	-8	10	
Cv. C 214								
Shoot	Pro-N	10.57	10.95	16.92	11.04	11.85	14.3	
	Sol-N	12.96	25.36	26.32	27.59	32.38	42.5	
Root	Pro-N	9.73	9.42	10.22	6.67	9.90	10.9	
	Sol-N	6.08	5.89	4.05	4.29	7.79	6.8	
Cv. G 130								
Shoot	Pro-N	21.79	20.16	25.36	17.53	20.61	20.1	
	Sol-N	11.84	12.59	15.85	15.43	12.88	12,5	
Root	Pro-N	13.47	16.78	10.10	7.56	12.63	13.1	
	Sol-N	8.68	10.16	6.47	3.85	7.89	8.1	
Total-N cv. C 214		18.67	25.81	28.76	24.80	34.96	37.3	
Shoot + Root ev. G 130		27.89	29.85	28.97	22.19	27.01	27.1	

TABLE 2

A balance sheet of protein-N, soluble-N and total-N as affected by water stress levels in cv. C 214 and cv. G 130 (nitrogen in mg g^{-1} dry m.)

these changes were minimal and a decrease in their level was indicated. Further it is interesting to note that in both the cultivars protein changes are phasic. Thus our results confirm the pattern of protein changes shown by CHEN et al. (1964) in soil grown Citrus seedlings where three distinct phases were observed. The first phase was characterized by an increase in protein level of seedlings. This was interpreted in terms of some 'factor' produced under milder stress which promotes protein synthesis. The second phase was characterized by a decrease in protein levels explained as resulting from the degradation of macromolecules making them more susceptible to proteolysis. The third phase showed again an increase in protein levels and this was interpreted as due to resynthesis from the increased soluble pool of smaller molecular weight proteins. Increase in the protein levels under drought stress could be explained on the basis of findings of KESSLER (1961) who showed an increase in RNA content during dehydration which may result in increased protein synthesis. CHEN et al. (1968) showed intensification of RNA synthesis in wheat during germination under stress. WAGNER (1954) suggested that protein synthesis may be stimulated by a shift in equilibria due te amino acid accumulation. However, this was discarded by CHEN et al. (1964) on the basis that protein synthesis was far beyond that expected from equilibria shifts only.

On the other hand decreased protein synthesis rate under drought stress has repeatedly been confirmed using labelled amino acid incorporation studies (BEN ZIONI et al. 1967, DHINDSA and CLELAND 1975). Reduction in protein synthesis under drought has been correlated to increased mono/polysome ratios (HSIAO 1970, BEWLEY 1972) and also that rewatering results to quick recovery to polymeric forms (HSIAO 1970).

A distinct increase in total-N and protein in resistant cv. C 214 has been recorded over control. This increase in total-N levels must help the plants in sustaining higher protein levels. A distinct increase in total-N has also been noted by CHEN *et al.* (1964) in *Citrus* seedlings. An improvement in nitrogen metabolism has been reported under salt stress also (KESSLER and SNIR 1969, LANGDALE *et al.* 1973).

A distinct cultivarial response has been recorded. Though the total protein content [mg g⁻¹ dry m.] is larger in the sensitive cv. G 130 but under stress conditions cv. C 214 showed an increase as compared to control plants, in cv. G 130 there is a decreasing trend. Total-N under stress also increased in cv. C 214 while in cv. G 130 there is no appreciable change. Thus the better performance of cv. C 214 as compared to cv. G 130 under stress seems to be related to its capacity to increase specific proteins under drought conditions. STUTTE and TODD (1967) have shown that resistant wheat cultivars under drought maintain a higher percentage of large molecular mass proteins as compared to non-resistant cultivars.

Acknowledgement

The authors thank the Head of Bio-Sciences, H. P. University Simla for providing laboratory facilities and Dr. Manjit Singh Virk, of CPRI Simla for helping in statistical analysis. The senior author thanks CSIR, New Delhi for senior Research Fellowship.

REFERENCES

- BEN ZIONI, A. C., ITAI, C., VAADIA, Y.: Water and salt stresses. Kinetin and protein synthesis in tobacco leaves. Plant Physiol. 41: 361-365, 1967.
- **BEWLEY, J.** D.: The conservation of polyribosomes in the moss *Tortula ruralis* during total desiccation. J. exp. Bot. 23: 692-698, 1972.
- CHEN, D., KESSLER, B., MONSELISE, S. P.: Studies on water regime and nitrogen metabolism of citrus seedlings grown under water stress. Plant. Physiol. 39: 379-386, 1964.
- CHEN, D., SARID, S., KATCHALSKI, E.: The role of water stress in the inactivation of m RNA of germinating wheat embryos. Proc. nat. Acad. Sci. USA 61 : 1378-1383, 1968.
- DHINDSA, R. S., CLELAND, R. E.: Water stress and protein synthesis: Differential inhibition of protein synthesis. — Plant Physiol. 55: 778-781, 1975.
- DWIVEDI, S., KAR, M., MISHRA, D.: Biochemical changes in excised leaves of Oryza sativa subjected to water stress. Physiol. Plant. 45: 35-40, 1979.
- GHAZALEH, M. Z. S., HANDERSHOTT, C. H.: Effect of drought and low temperature on leaf freezing points and water soluble proteins and nucleic acid content of sweet orange plants. — Proc. amer. Soc. hort. Sci. 90: 93-102, 1967.
- HANSON, A. D., NELSON, C. E., EVERSON, E. H.: Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. — Crop Sci. 17:720 to 726, 1977.
- HSIAO, T. C.: Rapid changes in levels of polyribosomes in Zea mays in response to water stress. - Plant Physiol. 46: 281-285, 1970.
- KESSLER, B.: Nucleic acids as factors in drought resistance of higher plants. Rep. Adv. Bot. 2: 1153-1159, 1961.
- KESSLER, B., SNIR, I.: Salt effects on **m**cleic acids and protein metabolism in citru wedlings. --Proc. first int. Citrus Symp. 1 : 381-386, 1969.
- LANGDALE, J., THOMAS, R., LITTLETON, T. G.: Nitrogen metabolism of star grosses as effected by nitrogen and soil salinity. - Agron. J. 65: 468-470, 1973.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J.: Protein m resurement with Folin-phenol reagent. - J. biol. Chem. 193: 265-275, 1951.
- MALI, P. C., MEHTA, S. L.: Effect of drought on protein and isozymes in rice durint germaination.
 Phytochemistry 16: 643-646, 1977a.
- MALI, P. C., MEHTA, S. L.: Effect of drought on enzymes and free proline in rice varieties. -Phytochemistry 16: 1355-1358, 1977b.
- PIRTE, N. W.: Proteins. In: PEACH, K., TRACEY, M. V. (ed.): Modern Methods of Plant Analysis. Vol. IV. Pp. 23-65. Springer Verlag, Berlin 1955.
- SAUNIER, R. E., ĤULL, H. M., EHRENREICH, J. H.: Aspects of the drought toleran e in creosote bush. — Plant Physiol. 43: 401-404, 1968.

- SHAH, C. B., LOOMIS, R. S.: Ribonucleic acid and protein metabolism in sugar beet during drought. Physiol. Plant. 18: 240-241, 1965.
- SINGH, G., RAI, V. K.: Responses of two *Cicer arietinum* L. varieties to water stress. Ind. J. Ecol. 7: 246-253, 1980.
- STUTTE, C. A., TODD. G. W.: Effects of water stress on soluble protein in *Triticum aestivum* L. Phyton 24: 67-75, 1967.
- SUBBOTINA, N. V.: Sov. Plant Physiol. 9: 62-65. 1962. In: HSLAO, C. T.: Plant responses to water stress. - Annu Rev. Plant Physiol. 24: 569, 1973.
- TODD, G. W., BASLEE, E.: Fate of various protoplasmic constituents in droughted wheat plants. - Phyton 22 : 79-85, 1965.
- WADLENGH, R. P., TEARE, I. D.: Growth and biochemical composition of bean plants as conditioned by soil moisture tension and salt concentration. — Plant Physiol. 20: 106-132, 1945.
- WAGNER, W. G.: The effect of artificial wilting on the sugar content and respiration rate of maturing green peas. New Phytol. 50: 335-358, 1954.

BOOK REVIEW

FLEIG-HARBAUER, G.: DER JAPANISCHE GARTEN – WEGE ZU MODERNER GESTALTUNG. – BLV Verlagsgesellschaft, München-Wien-Zürich 1981. 143 pp. Hard cover DM 49,80, ÖS. 410,-, Sfr. 49,80.

In the present world with a steadily increasing technical development and urbanization the Man becomes more and more estranged from nature. The technique facilitates and rationalizes human activity in many fields but, on other hand, it changes the configuration of the living environment — a high level of decibels disturbe a previously quiet landscape, the verdure is replaced by concrete, iron and synthetic substances, and monotous grey predominates over a vivid disk any of colours. The concentration of inhabitants in towns makes the interhuman relations easier but simultaneously leads to a sense of estrangement, lonesomeness and stress situations in the life of individuals. No wonder that there is an ever increasing number of those who seek rest, composure and equilibrium in nature which, regrettably, is on the wane nowadays. This stimulates the effort to use every however small area to preserve or revive the beauty of the living nature and contribute to harmonious relations between its parts and between it, as a whole, and the urban environs. A prefigurement of this effort — even though issuing from other historical and social conditions — are the Japanese gardens and parks. They are distinguished by simplicity and beauty, using natural materials in their original form and colour to a maximum extent.

The book reviewed acquaints the reader with the different types of gardens, pointing out the differences in their architectonic style and in the function of individual components, and informs about the aesth tie effect of plants, water sheets and natural materials — wood, sand and stones. A collection of black-and-white and colour photographies accompanies the reader through ancient temple and palace gardens (Kyôto, Tokyo) and through parks of modern eities (e.g. Ósaka). The comparison of the architecture of a Japanese garden with the architectonic lay-out of large Europern gardens and parks (palace parks in West Germany) is instructive particularly for an expert. An amateur horticulturist will appreciate the closing part of the book, which brings ideas how to apply the functional and aesthetic elements typical of the Japanese gardens in both large public parks and miniature private gardens of other countries. The book will delight not only a nature-fancier but also an expert-biologist, as it shows the importance of his vocation in helping people to find spiritual recreation.

DANUŠE HODÁŇOVÁ (Prah a)