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Free Proline Accumulation and Drought Resistance in *Cicer arietinum* L.

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Abstract. Two cultivars of *Cicer arietinum* L. with different sensitivity to drought in the early vegetative stage were compared for their capacity to accumulate proline when subjected to water stress. Both shoots and roots of the two selected cultivars accumulated proline under water stress and this effect increased with increase of water stress level. A high positive correlation between free proline and water saturation deficit (WSD) was recorded while these two factors were negatively correlated to the dry weight of the shoot. Also effects of duration of stress on proline accumulation patterns in relation to drought resistance have been shown.

Increased accumulation of free proline during water stress has been reported by many workers in different plants (BARNETT and NAYLOR 1966, PALFI *et al.* 1974, SINGH *et al.* 1972, STEWART 1972, 1973 and STEWART *et al.* 1966). SINGH *et al.* (1972, 1973) expressed a relationship between the magnitude of water stress and proline accumulation and suggested proline accumulation as an index of drought resistance in barley. However, when HANSON *et al.*, (1977) and MALI and MEHTA (1977) compared the resistant and susceptible cultivars of barley and rice respectively, proline as an index of drought resistance seemed unreliable. Therefore, the present investigation screens two *Cicer arietinum* L. cultivars having differential sensitivities to water stress, for accumulation of proline under water stress.

MATERIAL AND METHODS

Seeds of *Cicer arietinum* L. cultivars *viz.* C-214 and G-130 procured from Panjab Agricultural University, Ludhiana were germinated on moist filter papers and four day old seedlings were transferred to water culture in Arnon and Hoagland medium. The seedlings were allowed to adapt towards water culture for 5 days. Ten day old seedlings were subjected to water stress (osmotic potentials ψ_s 0 to -10×10^5 Pa) by dissolving PEG 6000 (ZUR 1966) and the sampling was done after 8 days or after, 1, 2, 4, 6 and 8 days at ψ_s 0, -3×10^5 and -8×10^5 Pa for time course studies. Free proline

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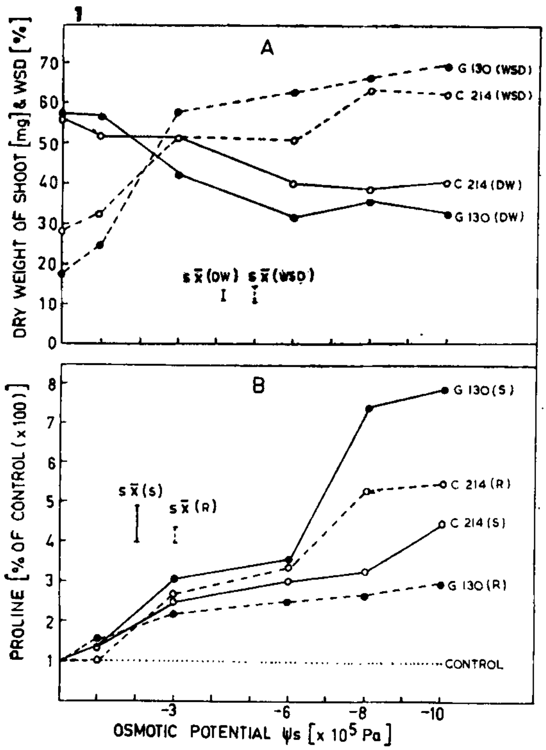

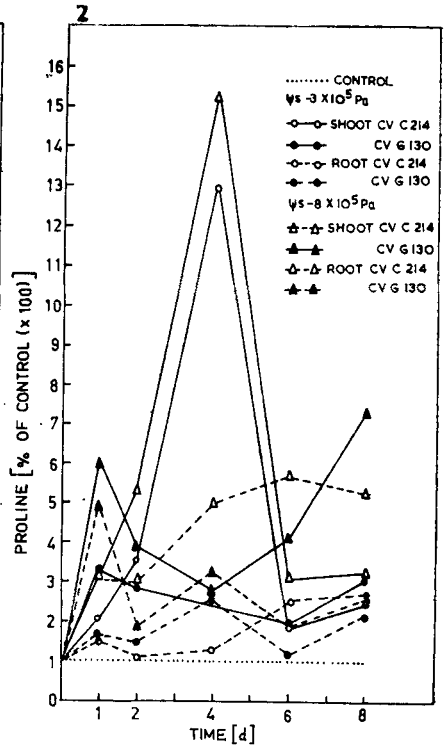

Fig. 1. A. Dry weight of shoot (DW) and water saturation deficit of third leaf (WSD) of cv. C-214 and cv. G-130 at various levels of water stress. — B. Accumulation of free proline at various levels of water stress in the shoots (S) and roots (R) of cv. C-215 and cv. G-130.
Fig. 2. Effect of duration of water stress (ψ_s — 3×10^5 and -8×10^5 Pa) on accumulation of free proline in shoots and roots of cv. C-214 and cv. G-130.

content was estimated in shoots and roots separately according to SINGH *et al.* 1973 and water saturation deficit of the third leaf from the top was measured (WEATHERLEY and SLATYER 1957).

RESULTS

Increasing water stress level resulted in increased inhibition of growth in both the cultivars (Fig. 1 A). Significantly more inhibition was in cv. G-130 as compared to cv. C-214 due to decreased ψ_s of the substrate from -3×10^5 to -10×10^5 Pa. That this inhibition is a direct effect of water stress is borne out by the measurement of WSD in the third leaf; the relatively more sensitive cultivar cv. G-130 shows higher water saturation deficit than cv. C-214 (Fig. 1 A). There is an inverse relationship between the dry weight per shoot and the WSD ($R = -0.8605$).

When proline accumulation was measured on the 8th day of stress (Fig. 1 B) it was noticed that in the shoot proline accumulation was significantly higher in the sensitive cv. G-130 (from ψ_s -3×10^5 to -10×10^5 Pa). At $\psi_s - 10 \times 10^5$ Pa there was a four fold increase in resistant cv. C-214 while sensitive cv. G-130 recorded about eight fold increase. However, in the roots more proline accumulation was in resistant cv. C-214. This accumulation of more proline in the shoots of sensitive cv. G-130 was contrary to the general reports available. Therefore, to check the validity of this relationship a time course experiment was set, where proline accumulation as a result of stress was studied. Proline accumulation occurred both at low stress level (ψ_s -3×10^5 Pa) or at higher stress level (-8×10^5 Pa) in shoots of both the

TABLE 1
Total free proline content ($\mu g\ g^{-1}$ dry weight of plant tissue)

Days	Cultivar C-214					
	0	−1	−3	−6	−8	−10
1	1060	–	2030	–	3000	–
2	780	–	1930	–	3000	–
4	630	–	5240	–	7040	–
6	1790	–	3500	–	5880	–
8	1410	1630	3560	4200	4960	6490

cultivars (Fig. 2). However, the resistant cv. C-214 accumulated higher proline between 2–6 days of stress and beyond 6 days; proline accumulation was higher in the sensitive cv. G-130. In roots at lower stress (ψ_s -3×10^5 Pa) during the first four days sensitive cv. G-130 showed more proline accumulation but after that the trend was reversed. At higher stress (ψ_s -8×10^5 Pa) there was more proline in resistant cv. C-214 from the second day onwards (Fig. 2). Therefore, duration of stress plays an important role in proline *versus* resistance relationships. The free proline content was markedly higher in the sensitive sv. G-130 at all the levels of stress treatments (Table 1).

DISCUSSION

Earlier works showed that accumulation of proline could serve as an index of magnitude of stress in Bermuda grass (BARNETT and NAYLOR 1966), spinach and clover grass (PALFI and JUHASZ 1969, 1970), and barley (SINGH *et al.* 1973 and STEWART *et al.* 1966). In an earlier study *Cicer arietinum* L. cv. C-214 was assumed to be relatively more drought resistant than the cv. G-130 during early vegetative growth (SINGH 1978). In the present experiment cultivars C-214 and G-130 accumulated proline as a result of water stress and a positive correlation was observed between WSD of the leaf and the proline content after 8 days of stress ($R = 0.8122$). When the difference between the sensitive and resistant cultivars was studied, it was noticed that between 2–6 days more proline (in % of unstressed plants) accumulated in the resistant cv. C-214 while after 6 days proline accumulation was distinctly higher in the sensitive cv. G-130.

SINGH *et al.* (1972, 1973) and PALFI and JUHASZ (1969 and 1970) suggested proline accumulation also as an index of drought resistance. Our study with *Cicer arietinum* confirmed this during 2–6 days of stress only, but later on as the stress duration is extended the relationship is reversed *i.e.*, from 6 days onwards the sensitive cv. G-130 accumulated more proline than the resistant cv. C-214 in the shoot. Therefore, the duration of stress plays a significant role in proline accumulation patterns to the given stress. MALI and MEHTA (1977) showed higher accumulation of proline (relative to unstressed plants) in resistant rice cultivar whereas the total proline was higher in the susceptible cultivar. BLUM and ADELINA (1976) and HANSON *et al.*,

Cultivar G-130						
0	−1	−3	−6	−8	−10	($\times 10^5$ Pa)
1580	—	4090	—	8060	—	
2030	—	5090	—	6770	—	
2430	—	—	—	6900	—	
2050	—	3260	—	6670	—	
1660	2160	4710	5350	9850	10 430	

(1977) showed that in sorghum and barley during the first 4 days of stress the respective sensitive cultivars accumulated more proline and thus negated any relationship between drought resistance and proline accumulation. Our results suggest that the duration of stress must be taken into account when considering the relationship between drought resistance and proline accumulation since the results are reversed in the case of *Cicer arietinum* during the first 6 days and afterwards.

The higher values of total proline content in the sensitive cv. G-130 at all stress levels as compared to the resistant cv. C-214 also show that proline itself may not be important factor in drought resistance. The same has been shown in the case of rice cultivars by MALI and MEHTA (1977).

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BOOK REVIEW

FENCHEL, T., BLACKBURN, T. H.: BACTERIA AND MINERAL CYCLING. — Academic Press, London—New York—San Francisco 1979. 225 pp. US $ 34.00.

This book is a general review describing the relationships between bacterial metabolism (including blue-green algae) and the chemical environment of the biosphere, setting a magnificent example to reviewers how to find an equilibrium between the depth and width of information.

The book divided into nine chapters discusses the matter from three main aspects: energetics of bacterial processes, a prokaryote and biosphere chemical evolution, and interconnection between cycling of different elements in natural ecosystems.

The first chapter is devoted to the basic information on energy yielding processes (chemotrophic and phototrophic reactions) showing different typical electron donor/acceptor systems, and to the carbon, nitrogen, sulfur, and phosphorus assimilation. In Chapter 2 a biological and geological evidence for parallel evolution of the prokaryotes and for corresponding changes in the composition of the atmosphere, lithosphere and biosphere is discussed. A "palhirrotrophic"-G/+/-organism having a simple photosystem based on a carotene-type pigment has been, for example, postulated as a hypothetical prokaryote which had appeared $3.6-3.9 \times 10^9$ years ago. In Chapter 3 the dominating role of bacteria in detritus food chains in different ecosystems has been shown (*e.g.* the kinetics of uptake, turnover and mineralization of dissolved organics in natural waters, decomposition of particulate detritus and so on).

The next three chapters (4 to 6) concerning the microbial transformations of the carbon, nitrogen and sulfur compounds form the central part of the book. In the Carbon Cycle Chapter dissimilative reactions and the autotrophic reduction of carbon dioxide are the two main subchapters; the ammonia liberation (nitrogen mineralization), nitrification and dissimilative and assimilative nitrate reduction are discussed in detail in the Nitrogen Cycle Chapter. In Chapter 6 the dissimilative sulfate reduction dependent on the linked oxidation of reduced carbon compounds or hydrogen (*e.g.* in *Desulfovibrio*), chemotrophic sulfur oxidations (*e.g.* in *Thiobacillus*), phototrophic sulfur oxidations (*e.g.* in *Chloroflexis*), and a sulfur turnover are stressed. A heterogeneous collection of chemical reactions of Fe, Mn, Mo, Se, Hg, and P mediated by microorganisms in nature is presented in Chapter 7, while in the next chapter (8) a few cases of symbiosis which involve prokaryotes and which are important in microbial element cycling (such as the rumen system in the ruminant mammalia, the symbiotic nitrogen fixation, and the prokaryotic endosymbionts of eukaryote cells) are pointed out. The last chapter (9) is devoted to quantitative estimates of pool sizes and transfer rates of the global cycling of C, N and S presented as the compartment and illustrative models.

Concluding, let me recommend this book as a very advisable textbook for students and graduate courses and for all those interested in ecology, soil and water microbiology, and environmental engineering.

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