

THE INVOLVEMENT OF PROLINE AND SOME METABOLITES IN WATER STRESS AND THEIR IMPORTANCE AS DROUGHT RESISTANCE INDICATORS

Andreas J. Karamanos

Agricultural University of Athens, Laboratory of Crop Production, 75 Iera Odos, 118 55 Athens, Greece

Introduction

Water shortage induces a deviation of plant metabolism from its normal pattern (Hsiao, 1973). Total soluble nitrogen tends to increase in water-stressed tissues because of an enhanced proteolysis, a depressed protein synthesis, a *de novo* enhanced synthesis of amino acids and a reduced incorporation of them to proteins (Petrie and Wood, 1938; Kemble and McPherson, 1954; Barnett and Naylor, 1966). Sugar metabolism is also affected: an increase in soluble sugar concentration, especially that of disaccharides, has been reported for a number of crop plants subjected to drought (Cutler et al., 1977; Turner et al. 1978; Ford and Wilson, 1981).

The accumulation of the amino acid proline in tissues of several plant species is regarded as a general response to water and other kinds of stresses (Chen et al., 1964; Barnett and Naylor, 1966; Chu et al., 1974, 1976). In addition, some other amino compounds (betaine, glutamate, glutamine, asparagine, etc.; for a review, see Drosopoulos et al., 1985) were also found at higher concentrations in stressed tissues.

The deviations from the normal metabolism mentioned above are either "passive", namely the simple result of stress-induced changes in some enzymatic systems, or "adaptive" i.e. oriented towards enabling plants to withstand the imposed stress.

In the latter case, a certain physiological role should be ascribed to each of the accumulating substances. Most of them are regarded as "compatible solutes" or "compatible osmotica" (Yancey et al., 1982), namely solutes accumulating in the cytoplasm without denaturing the enzymes essential for the metabolic processes of life. Proline is also regarded as a source of energy, carbon, and nitrogen for the recovering tissues (Singh et al., 1973b; Blum and Ebercon, 1976), whereas other roles have been ascribed to other substances.

Despite the close association between free proline accumulation and water shortage found in numerous works, proline concentration has not been considered yet as a safe parameter for describing the plant ability to withstand stress. The same applies to a much greater extent for the other substances. The purpose of this work is to reveal the possible problems and peculiarities involved in the association of plant water status with the accumulation of proline and some other metabolites. The ultimate target is to present some reasons for the ambiguity of the experimental results and to suggest ways of overcoming it.

Both published and unpublished experimental results obtained from several field experiments at the Agricultural University of Athens (Laboratory of Crop Production) in combination with results from other works will be used. The description of the field experiments and the related methodology has been given elsewhere (Karamanos et al., 1983; Drossopoulos et al., 1985, 1987; Karamanos et al., 1995). The aspects to be covered here refer: (i) to the kinds of substances accumulating under stress in an effort to search for the most effective potential stress indicators. (ii) To possible differences among plant organs in the levels of the accumulating substance. (iii) To the effects of plant development, and (iv) to possible differences among plant genotypes.

Results and Discussion

1. The type of substance

Water shortage brings about a net increase in the total soluble nitrogen of the stressed tissues (Karamanos et al., 1986). Free proline is the substance most readily accumulating: it accounts for up to 70 % of the total amino compounds during the periods of more severe stress (Table 1).

Table 1. The range of the values for proline and for the sum of the other amino compounds ($\mu\text{mol.g}^{-1}$ f.wt) observed throughout growth in the organs of the wheat cultivars Yecora and Generoso grown in the field under rainfed (D) and irrigated (W) conditions (Drossopoulos et al., 1985)

	Leaves		Stems and sheaths		Roots		Ears	
	Proline	Others	Proline	Others	Proline	Others	Proline	Others
Generoso								
W	1.1–17.6	4.4–11.9	0.4–3.9	1.5–13.6	0.3–2.1	0.9–9.8	0.6–10.5	2.1–14.1
D	1.2–30.8	2.9–13.5	0.6–57.6	2.2–15.6	0.7–34.7	4.1–16.2	2.9–17.8	2.7–16.3
Yecora								
W	1.0–18.6	2.3–16.3	0.6–14.2	1.4–18.1	0.3–6.9	2.4–15.8	2.8–9.1	7.1–21.2
D	1.2–19.5	8.1–13.8	1.0–23.7	4.7–19.2	0.6–9.5	1.5–14.6	2.1–12.2	4.6–18.9

With the exception of betaine, which was found to accumulate in water – and salt stressed barley and tropical grasses (Hanson and Nelson, 1978; Stewart and Larher, 1980; Ford and Wilson, 1981), the accumulation of other amino-compounds in water stressed tissues appears to be of minor importance. There is a tendency, however, for serine, glutamine and alanine to accumulate in the laminae of water-stressed wheat plants (Fig. 1).

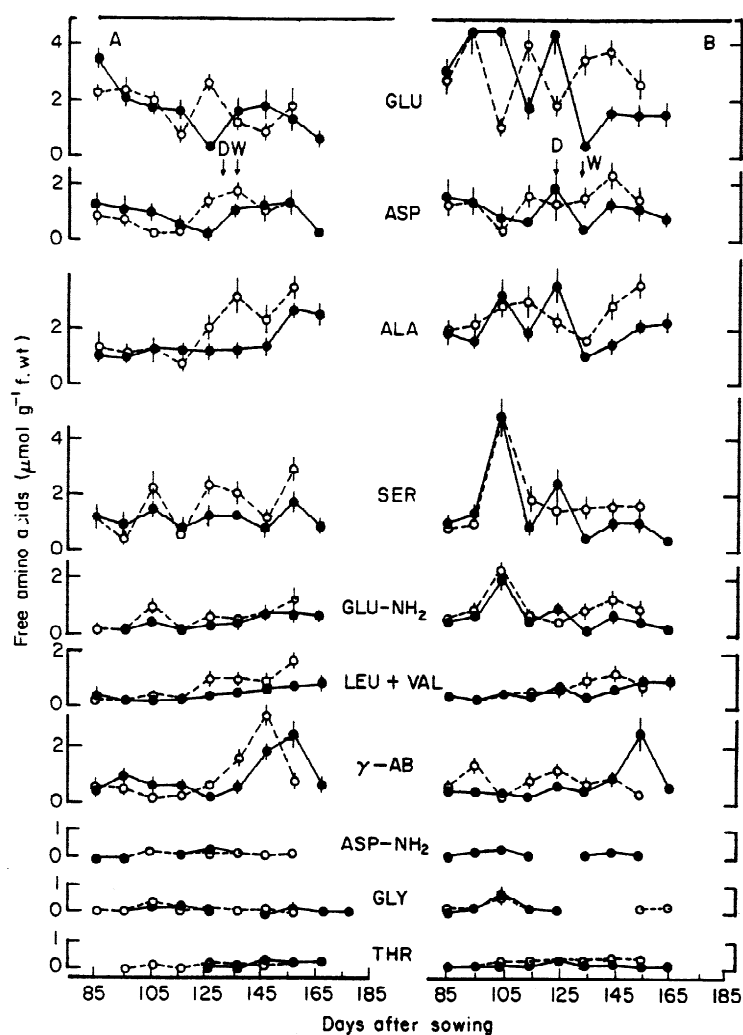


Fig. 1. The time courses of the most abundant amino compounds other than proline in the leaf laminae of the wheat cultivars Generoso, A, and Yecora, B, grown in the field under rainfed (○-○) and irrigated (●-●) conditions. Abbreviations: Glu, glutamate; Asp, aspartate; Ala, alanine; Ser, serine; Glu-NH₂, glutamine; Leu + Val, leucine plus valine; γ -AB, γ -aminobutyric acid; Asp-NH₂, asparagine; Gly, glycine; Thr, threonine (Drossopoulos et al., 1985)

From the ethanol soluble sugars, sucrose was the substance more closely following the fluctuations in Ψ_e (Fig. 2), although no significant difference between treatments in its concentration was evident. Glucose and fructose were maintained at lower levels with no obvious increase in the leaves under drought.

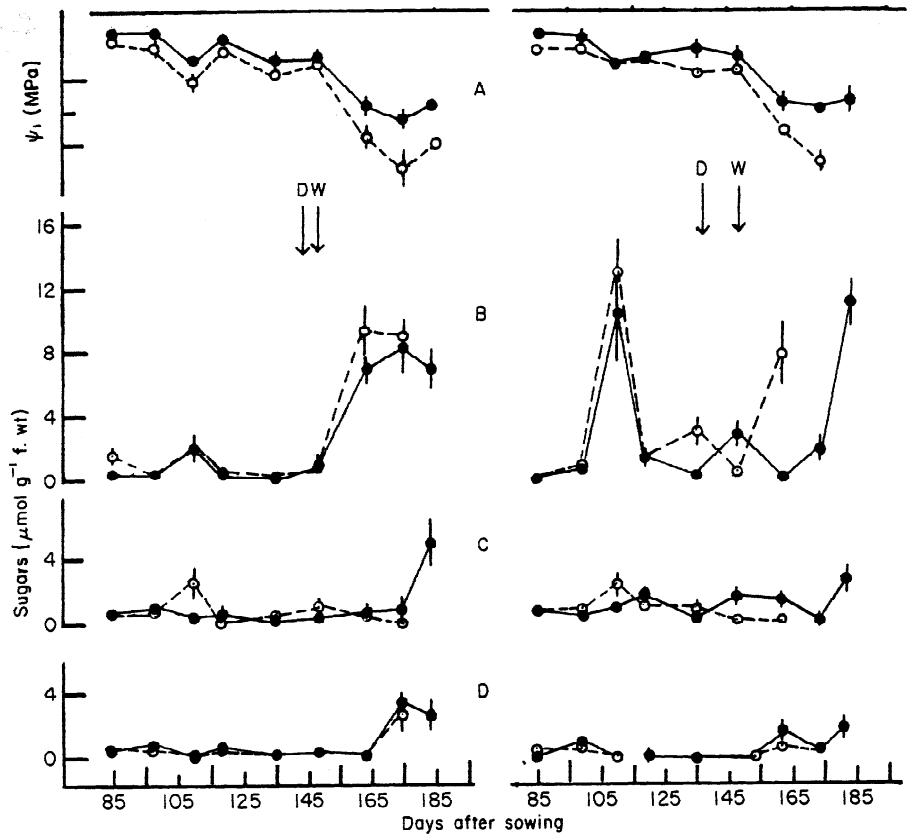


Fig. 2. The time courses of the leaf water potential at midday (Ψ_e) and of the concentrations of sucrose (B), glucose (C) and fructose (D) in the leaves of the wheat cultivars Generoso (left) and Yecora (right). ●—●, irrigated treatment; ○-○, rainfed treatment. The vertical bars show the standard errors of the means (Drossopoulos et al., 1987)

2. The variation among organs

Substantial differences in the type of the accumulating substances and their concentration have been detected among the organs of the same plant. Proline was more readily accumulating in the stems (including sheaths) and roots than in the leaves of water-stressed wheat plants (Fig. 3). An accumulation in the reproductive organs was also observed.

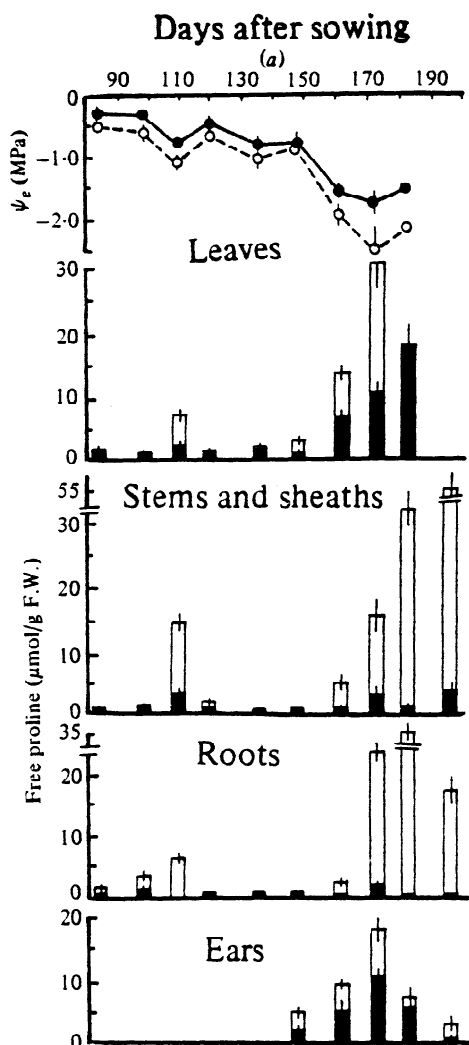


Fig. 3. The time courses of proline accumulation in the leaves, stems, roots, and ears of the wheat cultivar Generoso grown in the field with the corresponding pattern of leaf water potential (Ψ_e). Black areas, irrigated treatment; white areas, rainfed treatment. The vertical bars show the standard errors of the means (Karamanos et al., 1983)

well have been affected by plant development and/or stress history. Singh et al. (1973b) found that barley plants not previously exposed to several cycles of water stress accumulated proline more readily and in higher amounts than plants which were

Singh et al. (1973a), however, found that the accumulation rate was greatest in laminae, lowest in roots and intermediate in leaf sheaths and shoot apices of barley plants. At any time, the levels of the substance in a given organ result from either an *in situ* biosynthesis or/and a translocation from other sources. It is postulated that all the above-ground parts of the plant are able to synthesize proline, whereas roots are considered as net proline importers (Oaks, 1966; Singh et al., 1973). The fact that stems and sheaths of water-stressed wheat plants are able to accumulate large amounts of proline after the death of the foliage (Fig. 3) strongly supports this view.

As regards the other amino compounds, clear differences are visible among the various organs (Fig. 4) exhibiting strong interactions with plant development.

Ethanol-soluble sugars also show a different pattern of accumulation among organs (Drossopoulos et al., 1987). The highest concentrations are detected in the stems and sheaths during the reproductive stage. Very high levels of glucose and fructose were found only in the stems of water-stressed wheat plants (*ibid.*).

3. The effects of development

There is evidence that plants exhibit a change in their water balance with their stage of development (Fisher, 1973; Connor, 1975; Morgan, 1977). The possible developmental effects could also be associated with the previous stress history of the plants. Accordingly, substances accumulating under stress conditions might

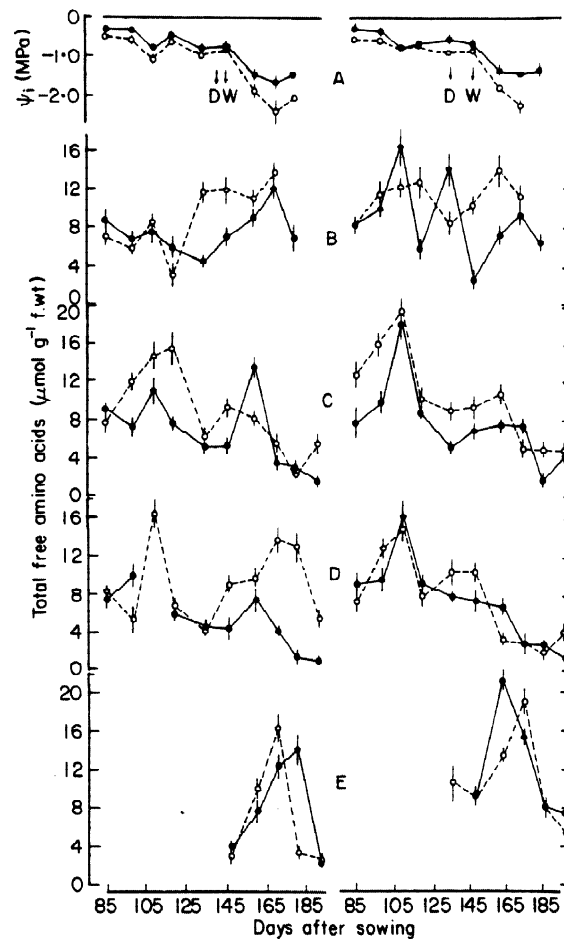


Fig. 4. The time courses of the leaf water potential at midday (A), and of the total amino compounds except proline in the leaves (B), stems and sheaths (C), roots (D), and ears (E) of the wheat cultivars Generoso (left) and Yecora (right). ●—●, irrigated treatment, ○- -○, rainfed treatment. The vertical bars show the standard errors of the means (Drossopoulos et al., 1985)

preconditioned to water stress. However, results from our field experiments do not agree with these findings.

An easy way to detect possible effects of plant development or stress history, is to plot the concentrations of the accumulated substance against the corresponding values of Ψ_e and look for possible systematic deviations in the graphs. In no case did we find any consistent difference between points obtained from different irrigation treatments for any accumulating substance (Fig. 5).

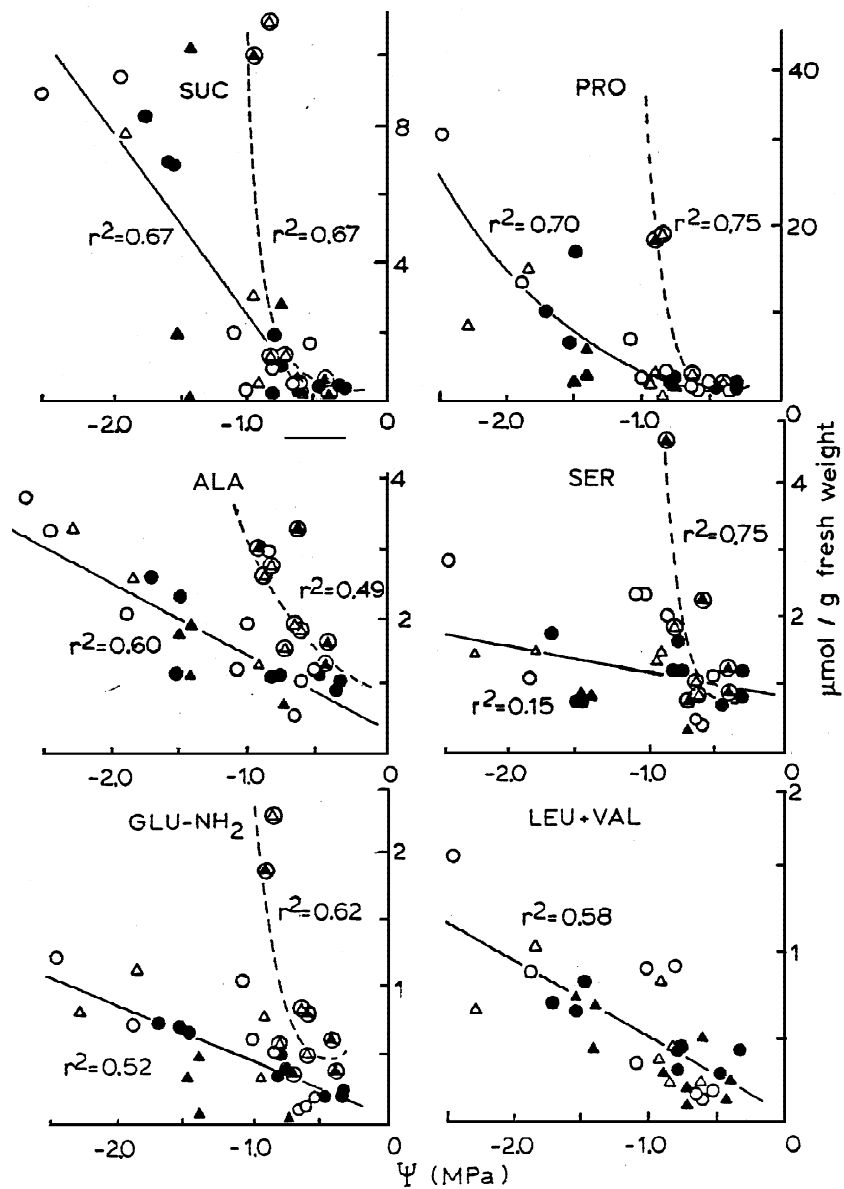


Fig. 5. The relations between the concentrations of various substances in the leaves and the leaf water potential (Ψ_e) in the wheat cultivars Generoso (circles) and Yecora (triangles) grown under irrigated (filled symbols) and rainfed conditions (open symbols). The values from Yecora in the vegetative phase are enclosed in circles. Suc, sucrose; Pro, proline; Ala, alanine; Ser, serine; Glu-NH₂, glutamine; Leu + val, leucine + valine. The lines indicate the regressions of best fit with their coefficients of determination (r^2) (Karamanos et al., 1986)

On the other hand, some obvious departures from the overall trends for different stages of development can be seen in the same figure: higher levels of proline at a given Ψ_e were accumulated in the vegetative than in the reproductive phase of wheat (Fig. 5). In faba beans (Fig. 6), plants at the latest stages of development tend to accumulate lower levels than younger plants at the same values of Ψ_e (see also Venekamp et al., 1987).

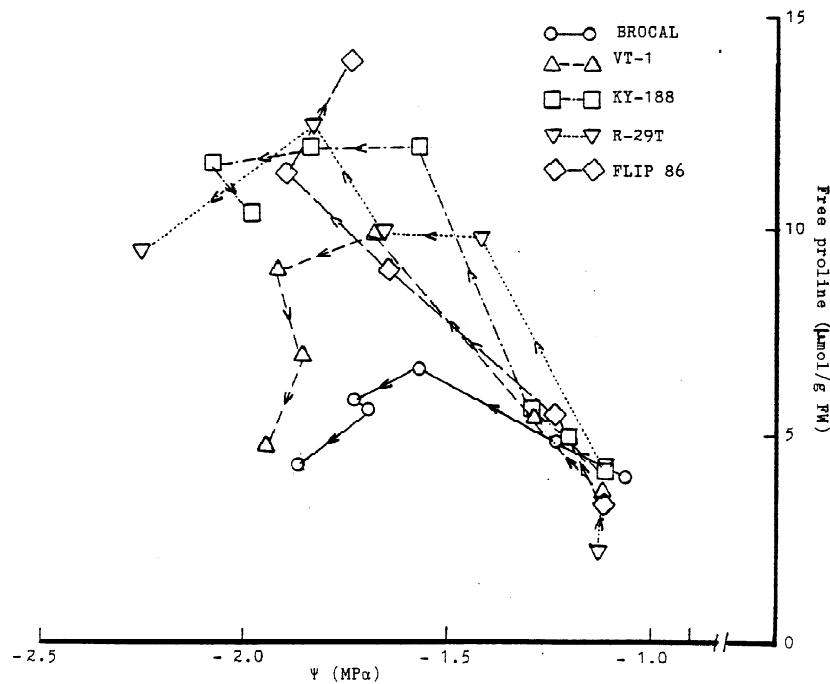


Fig. 6. The relations between free proline accumulation and leaf water potential (Ψ_e) for five faba bean cultivars. The lines and arrows indicate the sequence of data points obtained for each cultivar

These results can be interpreted by means of the suggested role of proline as an energy, carbon and nitrogen source which enhances tissue recovery on the relief of stress (Singh et al., 1973; Blum and Ebercon, 1976). Thus, the younger more actively growing plants are endowed with a greater ability to recover after stress (i.e. a greater potential for proline accumulation) than plants at later growth stages. Alternatively, the high proline levels in wheat plants at the vegetative phase could also be explained in terms of the occasional and rapid decline in Ψ_e observed in one day (Waldren and Teare, 1974; Waldren et al., 1974; Venekamp et al., 1987). This latter approach may also explain the lack of major developmental effects in the graphs relating proline with Ψ_e in cases where Ψ_e fell gradually over a long time (Fig. 7).

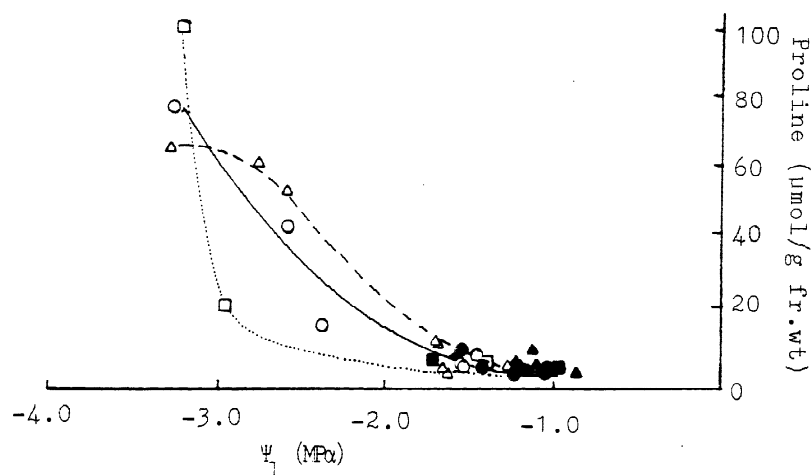


Fig. 7. The relations between free proline accumulation and the leaf water potential (Ψ_e) after anthesis for three wheat cultivars. Circles (—○—), Generoso; triangles (- Δ -), Yecora; squares (...□...), Siete Cerros (Papatheohari, 1995)

4. The effects of genotype

Proline accumulation was found to vary among and within plant species, as, for example, in barley cultivars (Singh et al., 1972), bermuda grass ecotypes (Barnett and Naylor, 1966), and genotypes of sorghum (Blum and Ebercon, 1976), rice (O'Toole and Chang, 1979), and faba beans (Venekamp et al., 1987).

In our field work with the wheat cultivars, a different behaviour is clearly visible (Fig. 7). The differences refer to the threshold values for rapid accumulation (approx. more negative than -2.3 MPa in Generoso and -3.0 MPa in Siete Cerros), the overall shape of the relationships, and the highest values obtained (65 , 75 , and 120 $\mu\text{mol/g}$ DW for Yecora, Generoso, and Siete Cerros, respectively). In another field experiment with the same cultivars, the threshold values of Ψ_e were much higher (-1.4 to -1.5 MPa), a fact that clearly shows that growth conditions (among them, temperature: Chu et al., 1974; Venekamp et al., 1987) affect the response of the plants to water shortage.

A different behaviour among faba bean cultivars was also detected in another of our field experiments. There were striking differences in the sensitivity of accumulation, as well as in the highest concentrations observed for each cultivar (Fig. 6). The two late cultivars KY-188 (K) and R-29T (R) tended to accumulate more proline than the early ones Brocal (B) and VT-1 (V). It was also obvious that the sensitivity of accumulation fell as the plants grew older. Finally, the overall relationship was quite different from that detected in wheat showing no apparent threshold Ψ_e -value.

As regards other substances, possible genotype effects (see, e.g., sucrose – glutamine – serine – and glutamine – accumulation in the leaves of two wheat cultivars) (Fig. 1) are bound with differences in the growth and development of the cultivars. Early cultivars grow faster in the vegetative phase and appear to be more responsive to changes of their plant water status in this phase.

Conclusions

The information presented above can be summarized as follows:

1. It is important to choose the plant organ in which proline and other related substances accumulate more readily, since accumulation was found to vary among plant organs. There is strong evidence in cereals that the accumulation is more intense in stems, especially when sheaths are included, than in leaves.

2. Proline is clearly the substance most readily accumulating in water-stressed plants. Other amino compounds and sugars, with the exception of betaine for some species, are of minor importance in terms of the sensitivity of the stress-induced concentration although they may exhibit significant correlations with Ψ_e .

3. Plant development is a factor which has to be seriously taken into account although its effects may be affected or even confused by those arising from the previous stress history of plants. It is suggested to sample and examine plants at comparable growth stages, or, at least, not to mix results from contrasting stages (e.g. too early and too late stages), in order to avoid misleading interpretations.

4. The effect of genotypes on the accumulation intensity is clear and may arise from their growth habit. It appears that the higher the growth rate at a given stage, the higher the potential for accumulation and *vice versa*. The length of the biological cycle may also be another important factor, with the late genotypes tending to exhibit higher concentrations than the earlier ones.

Threshold Ψ_e -values for accumulation may or may not exist, depending on the species examined. Nevertheless, whenever a threshold Ψ_e exists, its value is by no means “universal” and changes with growth conditions. The interaction of water shortage with other kinds of stresses may play here an important role.

A possible association of proline accumulation with the drought resistance of different genotypes will obviously have a major practical importance: it would provide an easy screening criterion to plant breeders and help agronomists to assess the field performance of genotypes. The main problem, however, lies in the ambiguity of the term “drought resistance” for field crops. The criteria change among investigators: Singh et al. (1972) used the yield stability index over a range of water stress to associate proline accumulation with the drought resistance of barley varieties. The same authors (1973b) examined additionally the ability for leaf survival under stress,

as well as the leaf relative growth rate upon the relief of stress. The speed of plant recovery after the alleviation of water stress was associated with higher proline concentrations in sorghum leaves by Blum and Ebercon (1976). We tried to examine proline accumulation in two wheat cultivars using a number of parameters related to drought resistance. First, the value of Ψ_e at which leaves started rolling, an indication of turgor less point, was less negative in Yecora than in Generoso (Karamanos et al., 1983). Secondly, stomatal closure, an indication of drought avoidance, was more abrupt in Generoso than in Yecora at values of Ψ_e more negative than -1.5 MPa (*ibid.*). Thirdly, the relationship between grain yield and the average Ψ_e after anthesis was less steep in Generoso than in Yecora indicating a higher yield stability in the former for a given range of Ψ_e -values (Fig. 8). All three criteria point to cv. Generoso as being more drought resistant than Yecora. Bearing in mind that Generoso accumulates proline more readily than Yecora, we have an indication for a positive association between proline accumulation and drought resistance.

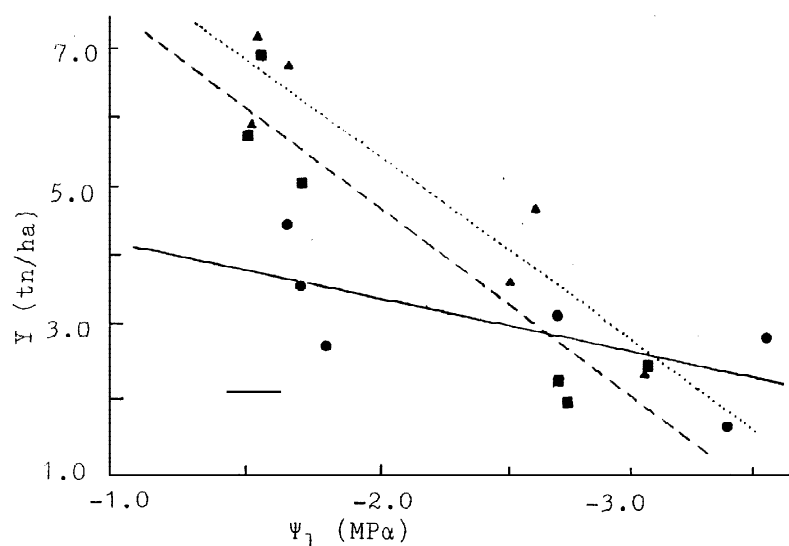


Fig. 8. The relations between seed yields (Y) and the average leaf water potential after anthesis (Ψ_l) for the wheat cultivars Yecora, Generoso, and Siete Cerros. For symbols, see Fig. 7 (Papatheohari, 1995)

Similarly, in faba bean, the cultivars exhibiting a greater yield stability to water shortage (FLIP-86, KY-188, and R-29T: Karamanos et al., 1995) showed the highest potential for proline accumulation (Fig. 6). Thus, proline accumulation may become a useful tool as a criterion for drought resistance assessment of crop genotypes, provided that the points mentioned above will be taken into account.

References

- Barnett, N. M., A. W. Naylor, 1966. Amino acid and protein metabolism in Bermuda grass during water stress. *Pl. Physiol.*, 41, 1222-1230.
- Blum, A., A. Ebercon, 1976. Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. *Crop Sci.*, 16, 428-431.
- Chen, D., B. Kessler, S. P. Monselise, 1964. Studies on water regime and nitrogen metabolism of citrus seedlings grown under water stress. *Pl. Physiol.*, 39, 379-386.
- Chu, T. M., D. Aspinall, L. G. Paleg, 1974. Stress metabolism. VI. Temperature stress and the accumulation of proline in barley and radish. *Aust. J. Pl. Physiol.*, 1, 87-97.
- Chu, T. M., D. Aspinall, L. G. Paleg, 1976. Stress metabolism. VII. Salinity and proline accumulation in barley. *Aust. J. Pl. Physiol.*, 3, 219-228.
- Connor, D. J., 1975. Growth, water relations, and yield of wheat. *Aust. J. Pl. Physiol.*, 2, 353-366.
- Cutler, J. M., D. W. Rains, R. S. Loomis, 1977. Role of changes in solute concentration in maintaining favourable water balance in field-grown cotton. *Agron. J.*, 69, 773-779.
- Drossopoulos, J. B., A. J. Karamanos, C. A. Niavis, 1985. Changes in free amino compounds during the development of two wheat cultivars subjected to different degrees of water stress. *Ann. Bot.*, 56, 291-305.
- Drossopoulos, J. B., A. J. Karamanos, C. A. Niavis, 1987. Changes in ethanol soluble carbohydrates during the development of two wheat cultivars subjected to different degrees of water stress. *Ann. Bot.*, 59, 173-180.
- Fisher, R. A., 1973. The effect of water stress at various stages of development on yield processes in wheat. In: *Plant Response to Climatic Factors*, UNESCO-Paris, 233-241.
- Hsiao, T.C., 1973. Plant responses to water stress. *A. Rev. Pl. Physiol.*, 24, 519-570.
- Karamanos, A. J., J. B. Drossopoulos, C. A. Niavis, 1983. Free proline accumulation during development of two wheat cultivars with water stress. *J. Agric. Sci., Camb.* 100, 429-439.
- Karamanos, A. J., J. B. Drossopoulos, C. A. Niavis, 1986. Water stress-induced alterations in nitrogen and carbohydrate metabolism of cereals and their possible ecological implications. In: *Plant Metabolism Regulation*, Bulg. Academy of Sciences, "M. Popov" Institute of Plant Physiology, Sofia, 24-36.
- Karamanos, A. J., A. Peppas, C. E. Avgoulas, 1995. A comparative study of the water relations and productivity of contrasting faba bean (*Vicia faba* L.) cultivars. In: *Improving Production and Utilisation of Grain Legumes (Proc. 2nd European Conference on Grain Legumes)*, AEP-Copenhagen, 124-125.
- Kemble, A. R., H. T. McPherson, 1954. Liberation of amino acids in perennial ryegrass during wilting. *Bioch. J.*, 58, 46-50.
- Morgan, J. M., 1977. Changes in diffusive conductance and water potential of wheat plants before and after anthesis. *Aust. J. Pl. Physiol.*, 4, 75-86.

- Oaks, A., 1966. Transport of amino acids to the maize root. *Pl. Physiol.*, 41, 173-180.
- O' Toole, J. C., T. T. Chang, 1979. Drought resistance in cereals. Rice: A case study. In: *Stress Physiology in Crop Plants*, Eds H. Mussell and R. C. Staples, Wiley-New York, 373-405.
- Papatheohari, A. Y., 1995. A study of the responses of three bread wheat cultivars (*Triticum aestivum* L. em. Thell, cvs. Yecora, Generoso, and Siete Cerros) to water shortage. Doctorate Thesis, Agricultural University of Athens.
- Petrie, A. H. K., J. G. Wood, 1938. Studies on the nitrogen metabolism of plants. III. On the effect of water content on the relationship between proteins and amino acids. *Ann. Bot.*, 2, 887-898.
- Singh, T. N., D. Aspinall, L. G. Paleg, 1972. Proline accumulation and varietal adaptability to drought in barley: a potential metabolic measure of drought resistance. *Nature*, 236, 188-190.
- Singh, T. N., D. Aspinall, L. G. Paleg, S. F. Boggess, 1973. Stress metabolism. II. Changes in proline concentration in excised plant tissues. *Aust. J. Biol. Sci.*, 26, 57-63.
- Singh, T. N., L. G. Paleg, D. Aspinall, 1973a. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. *Aust. J. Biol. Sci.*, 26, 45-56.
- Singh, T. N., L. G. Paleg, D. Aspinall, 1973b. Stress metabolism. III. Variations in response to water deficit in the barley plant. *Aust. J. Biol. Sci.*, 26, 65-76.
- Turner, N. C., J. E. Begg, M. L. Tonnet, 1978. Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence on the water potential at which stomata close. *Aust. J. Pl. Physiol.*, 5, 597-608.
- Venekamp, J. H., C. Grashoff, J. T. M. Koot, 1987. An analysis of conditions determining decline in water potential and concurrent proline accumulation in leaves of four cultivars of *Vicia faba* L. *J. Agron. Crop Sci.*, 158, 304-316.
- Waldren, R. P., S. D. Teare, 1974. Free proline accumulation in drought-stressed plants under laboratory conditions. *Plant and Soil*, 40, 689-692.
- Waldren, R. P., S. D. Teare, S. W. Ehrler, 1974. Changes in free proline concentration in sorghum and soybean plants under field conditions. *Crop Sci.*, 14, 447-450.
- Yancey, P. H., M. E. Clark, S.C. Hand, R. D. Bowlus, G. N. Somero, 1982. Living with water stress: Evolution of osmolyte systems. *Science*, 217, 1214-1222.