Proline Concentrations in Water Stressed Grasses

U.G. BOKHARI AND J.D. TRENT

Abstract

This study was conducted to screen several warm- and cool-season grasses for their proline-accumulating ability under water stressed conditions in the growth chamber. Plants of Old World bluestems (*Bothriochloa* spp.), tall fescue *(Festuca arundinacea)*, western wheatgrass *(Agropyron smithii)* and weeping lovegrass *(Eragrostis curvula)* were subjected to water stress conditions at the vegetative stage. Water stressed plants exhibited a significantly greater ($P<.05$) increase in proline concentration than the non-stressed and the stress relieved plants. There was also a significant difference ($P<.01$) in the proline-accumulating ability of various species. An interdependency was observed between leaf water potential and proline concentration in all the species under water-stressed conditions.

Free proline accumulates in many plant species during periods of water stress (Chu et al. 1976, Barnett and Naylor 1966, Kemble and McPherson 1974, Singh et al. 1973, Stewart et al. 1966, Waldren and Teare 1974). The exact role of proline in water stressed plants is not known. Singh et al. (1973) suggested that the degree of proline accumulation in water stressed barley corresponded to varietal drought resistance. Hanson et al. (1977) cautioned against using proline-accumulating potential in plants as an index of drought resistance in screening methods for cereal breeding programs. Barnett and Naylor (1966) suggested that water stressed plants use proline as a source of storage for carbon and nitrogen while Stewart and Lee (1974) reported that some halophytes use proline to adjust themselves osmotically. In turgid leaves, oxidation of proline occurs to maintain low levels of cellular proline, but in water stressed plants proline concentration increases because proline oxidation is inhibited or the rate of proline biosynthesis is increased (Boggess et al. 1976, Morris et al. 1969, Stewart et al. 1977). Our study was undertaken to (1) screen 4 different grass species for free proline accumulation, and (2) determine if a relationship exists between proline accumulation and periods of water stress under environmentally controlled growth chamber conditions.

Authors are research agronomist and agricultural research technican, respectively, USDA, Agricultural Research Service, Southwestern Livestock and Forage Research Station, Route 3, El Reno, Okla. 73036.

This article is a contribution of the USDA, ARS, Southern Region in cooperation with the Oklahoma Agr. Exp. Sta., El Reno, Okla. 73036.

Manuscript accepted April 16, 1984.

Materials and Methods

Seven-year old plants of Old World bluestems (*Bothriochloa* spp.) and 30-day old seedlings of weeping lovegrass (*Eragrostis curvula*, cv. 'Morpa'), western wheatgrass *(Agropyron smithii)*, and tall fescue (*Festuca arundinacea*, cv. 'Kenhy') were transplanted into 26-cm diameter × 30-cm deep buckets filled with a local soil. The OWBS grasses included the varieties: Plains *(B. ischaemum)* and Caucasian *(B. caucasican)* and 3 experimental blends of *B. intermedia*, var. indica designated as "B," "L," and "T." There were 4 plants/pot in each of the 4 replicates of weeping lovegrass, tall fescue and western wheatgrass while the OWBS plants consisted of a single sod of 15-cm diameter in each pot.

Growth chamber conditions for the OWBS and weeping lovegrass plants were 35/25°C, day/night temperature, alternating with 14h photoperiod at 400 micro Einstein cm^{-2} sec^{-1} (400–700 Nm) photoflux density. Conditions for the other grasses were 20/15°C, day/night temperature, 12h photoperiod and the same light intensity as for the other grasses.

The grasses were acclimatized to their respective growth chamber conditions for 45 days during which time they were watered regularly to maintain a –80 kPa soil water potential. On day 45, vegetative tissues were sampled from 4 plants in each of 4 replications, watering was then discontinued for 7 days and vegetative tissue again sampled from 4 plants of each species. The soil was watered and after 4 to 6 hours of light, vegetative tissues were again harvested.

Proline was determined according to the method described by Bates et al. (1973). Approximately 0.5g of fresh or frozen plant material was homogenized in 10 ml of 3% aqueous sulfosalicyclic acid and filtered through Whatman's No. 2 filter paper. Two ml of filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for

1 hr at 100°C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a Bausch and Lomb Spectrometer 710[1]. Appropriate proline standards were included for calculation of proline in the sample.

Water potentials of stressed and nonstressed plants were determined at mid-day with a Wescor L-51 leaf hygrometer/psychrometer[1] and soil water potentials were measured with a Wescor thermocouple psychrometer in conjunction with a Wescor HR-33T dew point microvoltmeter.[1]

Results and Discussion

Water stressed plants of all the species accumulated greater amounts ($P<.05$) of proline than the nonstressed and the stress-relieved plants (Table 1), except for Caucasian, which accumulated slightly more proline under stress-relieved conditions. Water stressed plants of cool-season grasses (tall fescue and WWG) accumulated relatively more proline than the water-stressed plants of warm-season grasses, except for the variety Plains which accumulated greater amounts of proline. The total amounts of proline in water-stressed plants of lovegrass, WWG, plains and tall fescue was significantly greater ($P<.01$) than the nonstressed plants of the same species.

Table 1. Proline concentration (mg/g dry wt) in different species under growth chamber experiment.

Species	Nonstressed	Stressed	Stress-related
Plains	3.30	9.72**	0.43
Caucasian	0.44	0.72*	1.06
"B"	2.86	4.39**	4.23
"L"	3.15	5.14**	4.80
"T"	3.33	4.26**	2.32
Tall fescue	3.94	7.48**	0.30
Western wheatgrass	1.05	16.25**	6.25
Love grass	0.49	2.66**	0.60

*,**Significantly different from nonstressed plants at 5% and 1% level, respectively, using L.S.D.

The increased proline concentration of water-stressed plants was accompanied by lower leaf water potential. For example the water potential in stressed plants ranged from −1.8 to 2.7 MPa compared with −.7 to −1.3 MPa bars in nonstressed plants (Table 2). There is an interdependent relationship between proline concentration and water potential (Table 1 and 2). It is obvious that stressed plants with a high proline concentration exhibited lower water potential, but maintained a greater gradient (more negative) than the nonstressed plants. For example the water potential of water-stressed OWBS dropped from −.6 to −2.7 MPa and the proline concentration increased from 0.44 to 5.14 mg/g dry wt. Similarly in water-stressed lovegrass the water potential dropped from −1.3 MPa to −2.3 MPa, but proline increased from 0.49 to 2.66 mg/g dry wt. The decrease in water potential of stressed plants cannot be entirely attributed to the increase in proline concentration, because the increase in proline concentration will bring about small changes in osmotic potential (Chu et al. 1976). The role of proline as an osmoticum in water-stressed plants is not clear. Proline might an indirect effect on solute accumulation, which in turn increases the osmotic potential.

The results of this study confirm the reports of others (Barnett and Naylor 1966, Singh et al. 1973) that there is an increase in the proline concentration of water stressed plants.

Increased proline concentration in water stressed plants is due either to the inhibition of proline oxidation or to the breakdown of proteins. In some plants proline concentrations increase because of more rapid biosynthesis of proline from its precursors (Barnett and Naylor 1966, Morris et al. 1969). Boggess et al. (1976) using ^{14}C-glutamate showed that proline biosynthesis was predominant in water stressed barley plants.

[1]Mention of a trade name or specific equipment does not imply its approval by the U.S. Department of Agriculture to the exclusion of other products that may also be suitable.

Table 2. Leaf water potentials (-MPa) in different species at vegetative stage under stressed and nonstressed conditions. (10 bar tension is equivalent to 1 MPa).

Species	Stressed	Nonstressed
Plains	2.35**	1.10
Caucasian	2.75**	1.20
"B"	1.95**	.90
"L"	1.85**	.60
"T"	1.95**	1.05
Love grass	2.35**	1.35
Western wheatgrass	2.60**	1.00
Tall fescue	1.95**	7.0

**Significantly different from June nonstressed plants at 1% using L.S.D.

Plants subjected to salinity stress also accumulate more proline (Stewart and Lee 1970, Chu et al. 1976). Increase in proline concentration of water stressed plants cannot be used as an index of drought resistance; however, it is a good indicator of the extent of water stress exerted on the plants under drought conditions.

Warm-season grasses are usually efficient utilizers of water under water stressed conditions by maintaining lower water potential (Downes 1969, Slayter 1970, Hsiao and Acevedo 1974). Whether proline plays any role in imparting drought tolerance by these plants cannot be substantiated from these data. The fact that proline concentration decreased in plants when water stress was relieved indicates some important role in the adjustment of plants to water stress conditions. This phenomenon was not limited to the warm-season grasses. Cool-season grasses exhibited similar trends in proline concentration under water stressed and stress relieved conditions.

Literature Cited

Barnett, N.M., and A.W. Naylor. 1966. Amino acid and protein metabolism in bermuda grass during water stress. Plant Physiol. 41:1222-1230.

Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline water stress studies. Plant Soil. 39:205-207.

Boggess, S.F., C.R. Stewart, D. Aspinall, and L.G. Paleg. 1976. Effect of water stress on proline synthesis from radioactive precursors. Plant Physiol. 58:398-401.

Chu, T.M., D. Aspinall, and L.G. Paleg. 1976. Stress metabolism. VII. Salinity and proline accumulation in barley. Aust. J. Plant Physiol. 3:219-28.

Downes, R.W. 1969. Differences in transpiration rates between tropical and temperate grasses under controlled conditions. Planta 88:261-273.

Hanson, A.D., C.E. Nelsen, and E.H. Everson. 1977. Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. Crop Sci. 17:720-726.

Hsiao, T.C., and E. Acevedo. 1974. Plant responses to water deficits, water-use efficiency and drought resistance. Agr. Meterol. 14:59-84.

Kemble, A.R., and H.T. McPherson. 1974. Liberation of amino acids in perennial rye grass during wilting. Biochem. J. 58:46-49.

Morris, C.J., J.F. Thompson, and C.M. Johnson. 1969. Metabolism of glutamic acid and N-acetylglutamic acid in leaf discs and cell free extracts of higher plants. Plant Physiol. 44:1023-1026.

Singh, T.N., L.G. Paleg, and D. Aspinall. 1973. Stress metabolism I. Nitrogen metabolism and growth in the barley plant during water stress. Aust. J. Biol. Sci. 26:45-46.

Slayter, R.O. 1970. Comparative photosynthesis, growth and transpiration of two species of Atriplex. Planta 93:175-189.

Stewart, C.R., S.F. Boggess, D. Aspinall, and L.G. Paleg. 1977. Inhibition of proline oxidation by water stress. Plant Physiol. 59:930-932.

Stewart, C.R., and J.A. Lee. 1974. The role of proline accumulation in halophytes. Planta 120:279-289.

Stewart, C.R., C.J. Morris, and J.F. Thompson. 1966. Changes in amino acid content of excised leaves during incubation. II. Role of sugar in the accumulation of proline in wilted leaves. Plant Physiol. 41:1585-1590.

Waldren, R.P., and I.E. Teare. 1974. Free proline accumulation in drought-stressed plants under laboratory conditions. Plant Soil. 40:689-692.0