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COMPARATIVE PERFORMANCE OF TWO METHODS FOR PROLINE ESTIMATION IN WHEAT

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Abstract

Wheat leaf extraction, with 3% sulfosalicylic acid and 0.5% toluene was compared for proline determination. It was observed that toluene extracted 50-60% more proline than sulfosalicylic acid. Accumulation of proline in the leaf, under salinity stress, is further substantiated.

Introduction

Plants experiencing salinity/water stress in their root zone respond physiologically by regulating their metabolism to adjust to the adverse conditions. As a consequence, a number of low molecular weight products such as proline, betaine, polyols, polyamines, sugars etc., accumulate (Morgan, 1984; Wyn Jones, 1985; Naqvi *et al.*, 1994). A number of workers have assigned the role of proline as an osmotic effector (Barnett & Naylor, 1966; Palfi & Juhas, 1970; Morgan, 1984; Voetberg & Stewart, 1984) or substrate for energy and nitrogen immediately after recovery from stress (Sivaramakrishnan *et al.*, 1988). However, in other studies, it has not been unequivocally supported (Hanson *et al.*, 1977; Aloni & Rosenshtein, 1984).

Bates *et al.*, (1973) extracted leaf segments with 3% sulfosalycilic acid and reacted the filterate with acid-ninhydrin solution to develop chromophore. Weimberg *et al.*, (1981) employed 0.5 % aqueous toluene, used by microbiologists for a long time, and reported that the technique was rapid and simple for quantitative extraction of water soluble low molecular weight solutes from plant cells. We, therefore, compared the tissue extraction technique of Bates *et al.*, (1973) with that of Weimberg *et al.*, (1981) for estimation of proline in wheat leaf segments.

Materials and Methods

Samples: Healthy wheat (*Triticum aestivum* L. cv. Pavon) caryopses were soaked for 2 h in distilled water and planted with embryo side up in 250 ml wide mouthed glass bottles containing 0.3 (control), 9.5 and 13.7 dSm^{-1} of NaCl in 1/10 Hoagland nutrient solution solidified with 0.8% agar. Seedlings were raised under near saturation moisture condition at 25/20 ± 2^0C day/night temperature and stressed to allow proline accumulation. Three days after planting, the seedlings were exposed to 12 h photoperiod (22 Wm^{-2}) and harvested after 10 days.

Extractions: Leaves were cut into small pieces, mixed thoroughly and randomly divided into two lots. From one lot, 0.5 g sample was taken from each treatment separately and placed in test tubes containing 10.0 ml of aqueous 3% sulfosalicylic acid and homogenised in an electric homogenizer. The homogenate was filtered through Whatman # 2 filter paper and designated as Filterate A (Bates *et al.*, 1973).

From the other lot of the chopped leaves, 0.5 g sample was transferred to test tubes containing 10.0 ml of 0.5% aqueous toluene. The test tubes were then shaken on a reciprocal shaker as recommended by Weimberg *et al.*, (1981). After 60 min., the extract was filtered through Whatman # 2 filter paper and designated as Filterate B.

Choromophore development: Following Bates *et al.*, (1973), 2.0 ml of the filterate A or B was reacted with 2.0 ml acid - ninhydrin and 2.0 ml of glacial acetic acid in a test tube. The mixture was heated for 1 h at 100^0C in a water bath and the reaction was terminated in an ice bath. The reaction mixture was then extracted with 4.0 ml toluene and vortexed in a mixer for 10 – 15 seconds. The toluene layer containing chromophore was aspirated from the aqueous phase, warmed to room temperature (25^0C) and the absorbance read at 520 nm in a Hitachi Spectrophotometer (150 – 20) using toluene for a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight (FW) basis as follows: [(ug proline/ml X 4 ml toluene)/115.5 ug/umol]/ [(0.5 g sample/5] = u moles proline / g of fresh weight material.

Proline determinations are means of three replicates and repeated twice with similar results. Data from one experiment was analysed statistically using Duncan's multiple range test.

Results and Discussion

Extraction with 3% aqueous sulfosalicylic acid (Filtrate A) showed less yield of proline per g FW of the leaf (Table 1) compared to extraction with 0.5 % aqueous toluene (Filtrate B). The efficiency of proline extraction with 0.5 % aqueous toluene was thus 50 – 60 % higher in all the three stress levels tested.

Table 1. Comparison of extraction methods for proline determination under salinity stress.

Salinity levels ($dS\ m^{-1}$)	Proline [umoles $(g\ FW)^{-1}$] Filterate A	Filterate B	Means
0.3 (control)	0.39	0.62	0.51^{C}
9.5	5.08	7.56	6.32^{b}
13.7	6.39	9.96	8.17^{a}
Means	3.95^{b}	6.05^{a}	

LSD (0.05) Filterates 0.60, Salinity level 0.24, Salinity level 1.03, within filterate

These data further substantiates the earlier reports of proline accumulation with increasing salinity/water stress. Proline contents of the wheat leaves significantly increased from 0.51 (control) to 6.32 (9.5 dS m^{-1}) and to 8.17 (13.7 dS m^{-1}) umoles $(g\ FW)^{-1}$. Stress at 9.5 dS m^{-1} increased the proline content 12.4 folds, while stress of 13.7 dS m^{-1} further increased it to 16.0 folds. Compared to other free amino acids, the accumulation of proline is unique (Aspinall & Paleg, 1981; Handa *et al.*, 1983), but similar to other low moleculr weight solutes such as organic acids and carbohydrates (Ford, 1984; Newton *et al.*, 1986).

Therefore, the aqueous toluene extraction, used by Wiemberg *et al.*, (1981) for leaf extraction of low molecular weight solutes seem to be superior than the aqueous sulfosalicylic acid used by Bates *et al.*, (1973) and others (Khanzada *et al.*, 1986; Sivaramakrishnan *et al.*, 1988; Reddy & Veeranijaneyulu, 1991). The present method eliminates the process of grinding which is laborious, time consuming and may cause variability between samples. Besides aqueous toluene extract can also be used to determine other low molecular weight solutes such as betaine, carbohydrates and other amino acids etc.

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