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ALPHA-AMYLASE, PROTEASE ACTIVITIES AND ASSOCIATED CHANGES UNDER WATER STRESS CONDITIONS IN WHEAT SEEDLING

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Alpha-amylase, protease, reducing sugars, non-reducing sugars, protein and total amino acids were studied in seedlings of 4 wheat genotypes, for a period of 96 hrs. after sowing, under stressed and non-stressed conditions. It was observed that α-amylase, protease, reducing and non-reducing sugars and total mino acids increased in all the genotypes subjected to -0.6 MPa water stress. The genotypes Chakwal-86 and DS-4 showed the highest α-amylase activity, sugars and total amino acids. On the other hand DS-17 and Pavon showed higher protease activity than the others. The highest reduction in protein content was recorded in DS-17 and Pavon. The significance of these findings is discussed.

Key words: Alpha-amylase, Sugars, Protease, Protein, Amino acids.

Introduction

Water stress is a major constraint for cropping in the arid and semi-arid regions of the world. For better cropping, highest plant population is required, which is only possible if seeds germination is satisfactory under these environments. Three stages can be distinguished during seed germination: (i) inhibition of water, (ii) induction of enzymatic activities and initiation of meristematic activity in the axis and (iii) the protrusion of the radicle through the seed coat [1]. Our earlier findings [2,3] showed that germination percentage is reduced due to water stress both in tolerant as well as in susceptible genotypes of wheat.

Depending upon the species, duration and intensity of stress, the metabolism of plants is modified [4]. Alpha-amylase is an important enzyme which has an active role in the hydrolysis of starch just before a seed germinates. It may also be indirectly responsible for the maintenance of requisite water potential, by providing solute sugars during the seed germination phase. Todd [5] reported an increase in amylase activity when he subjected leaves to stress conditions. However, Stewart [6] (using bean leaves provided a circumstantial evidence by showing an increase in sugars and decrease in starch) suggests an increase in α-amylase activity. In contrast to the increase in α-amylase induced in leaves by stress, the enzyme formation in germinating seeds is depressed [7].

Protein and amino acid metabolism has been associated with the adaptation of plants to environmental changes and stress. Water stress is thought to affect protein synthesis [8]. There are very few reports which show effect of water stress on protease activity.

The present study was therefore planned to investigate the changes in α-amylase, protease activities, protein, amino-acids and sugars during water stress in germinating seed and seedlings of wheat.

Materials and Methods

The experiments were conducted in growth cabinet maintained at 30/25°C day/night temperature and with two treatments 0.0 and -0.6 MPa (PEG-6000). Seeds of four wheat genotypes i.e. Chakwal-86 and DS-4 (Tolerant) DS-17 and Pavon (susceptible) were surface-sterilized with 10% of sodium hypochlorite for 10 mins., washed thoroughly in sterile distilled water and planted in glass bowls containing 50 ml of the treatment solutions [9]. Three replicates were kept in a completely randomized block design, in the growth cabinet. The seeds were allowed to germinate in the dark for 72 hrs. and then exposed to 12 hrs. photoperiod (41.7 u mol m^2 s^{-1}). Samples were collected randomly every 24 hrs. after sowing for the assay of α-amylase, protease, protein and total amino acids. At the same time separate samples were taken out and dried at 70°C in an oven for the estimation of sugars.

Alpha-Amylase. Alpha-amylase activity was determined according to the method of Jones and Varner [10]. Ten seedlings were extracted in 0.2M citrate buffer (pH 5.5), centrifuged at 10,000 g and the supernatant was used for the assay of enzymes.

Alpha-amylase was assayed using suitable volumes (0.02-

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TABLE 1. MEAN SQUARES FOR SOME BIOCHEMICAL PARAMETERS

Parameters	Time	Variety	Time X Variety	Treatment	Treatment X Time	Treatment X Variety	Time X Treatment X Variety	Error
Degree of freedom	3	3	9	1	3	3	9	64
α-amylase	89852.344**	126.344**	35.677*	810.844**	1019.344**	348.344**	97.177*	5.219
Reducing sugar	702.557**	4.516N.S.	3.068*	47.419*	41.694*	12.289*	3.162*	0.738
Non-reducing sugar	24396.229**	296.752**	61.422*	1920.223**	1093.04**	285.169**	99.332*	2.173
Protease activity	5.075**	0.009*	0.010*	0.886**	0.041*	0.018*	0.006*	0.001
Protein	36.084**	1.206*	0.072N.S.	6.391*	1.935*	0.580*	0.770*	0.102
Total Amino acids	3972.102**	114.467*	17.917*	457.539*	26.151*	32.940*	9.718	1.776

N.S. = Non significant. * = Significant. ** = Highly significant

0.2 ml) of the supernatant and made to 1.0 ml with distilled water. The reaction was started by adding to the medium 1.0 ml of the starch substrate and allowed to continue for a period of 1 hour. The reaction was stopped by adding 1.0 ml of the iodine reagent. To this reaction mixture, 5.0 ml of distilled water was added, mixed and read at 620 nm in a spectrophotometer (Hitachi 150-20). The α-amylase activity was calculated as the amount of starch hydrolysed per gram fresh weight per hour.

Starch substrate. One hundred and fifty mg native potato starch (non solublized) was added in 100 ml of a solution containing 600 mg KH_2PO_4 and 200 µmol $CaCl_2$, boiled for 1 min and centrifuged for 10 min at 3000 g. The clear supernatant was used as the substrate.

Iodine reagent. Six g of potassium iodide and 600 mg of iodine in 100 ml of water. Before use 1.0 ml of the stock solution was added to 0.05 N HCl and made to 100 ml. This iodine solution was used to stop the α-amylase reaction.

Protease. Ten seedlings were homogenized, using a mortar and pestle, extracted with cold 1% NaCl in 0.2 M phosphate buffer (pH 7.5) and centrifuged at 12,000 g for 30 min. One ml of the supernatant was incubated at 50°C with 5 ml of 1% casein solution in 0.2 M sodium phosphate buffer (pH 6.0). The reaction was terminated after 60 mins with 1 ml of 40% TCA [11]. The proteolytic activity was measured at 570 nm in the TCA soluble fraction after reacting with Folin phenol reagent [12].

Reducing and non-reducing sugars. Following Somogyi (13) the reducing sugars were determined in an aliquot of 80% alcoholic extract of the dry samples and the non-reducing sugars were calculated according to the method of Loomis and Shull [14].

Protein and total amino acid. Ten seedlings were ground in 0.1 M NaCl solution [11] in the ratio of 1:10 (w/v), using a mortar and pestle and filtered through nylon cloth. The filtrate was precipitated with an equal volume of 10% TCA and centrifuged at 1,000 g for 5 min. The pellet was re-suspended in 0.1 N NaOH and the protein was then estimated by the method of Lowry *et al.* [12]. The supernatant was assayed for total amino acid by the method of Moor & Stein [15].

Results and Discussion

Alpha-amylase activity. The α-amylase activity was slightly lower in stressed plants than in the control after 24 hrs. of incubation in all genotypes. After this time period the activity in stressed plants started increasing more rapidly, surpassed the control in all genotypes after 72 hrs. and continued to increase further (Fig. 1). At the time of the termination of the experiment (96 hrs.) the two genotypes, Chakwal-86 and DS-4, not only had the highest amounts of α-amylase activity but the percentage increase in the stressed seedlings, compared to the control, was also higher (Chakwal-86 24%, DS-4 30%, DS-17 and Pavon 6%). The rate of increase in α-amylase activity was higher in Chakwal-86 and DS-4 compared with DS-17 and Pavon.

Reducing and non-reducing sugars. Reducing and non-reducing sugars also showed a trend similar to that of α-amylase activity (Fig. 1). In the case of reducing sugars the average for stressed seedlings, of all the genotypes, was 25% higher than the controls and the respective data for non-reducing sugar was 27%. The genotypes Chakwal-86 and DS-4 (tolerant) accumulated more sugar than DS-17 and Pavon (non-tolerant). The accumulation of both types of sug-

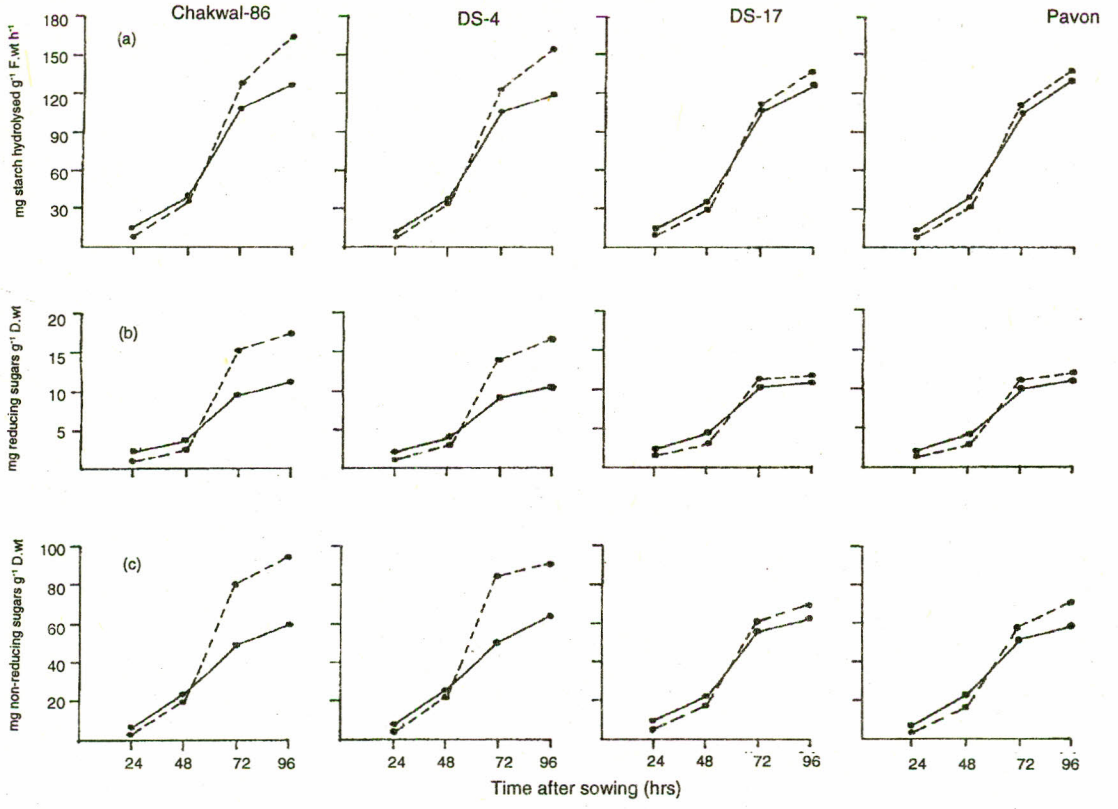

Fig. 1. α-Amylase activity (a), Reducing sugars (b), Non-reducing sugars (c) Four wheat genotypes grown under normal (—) and water stress (---) conditions.

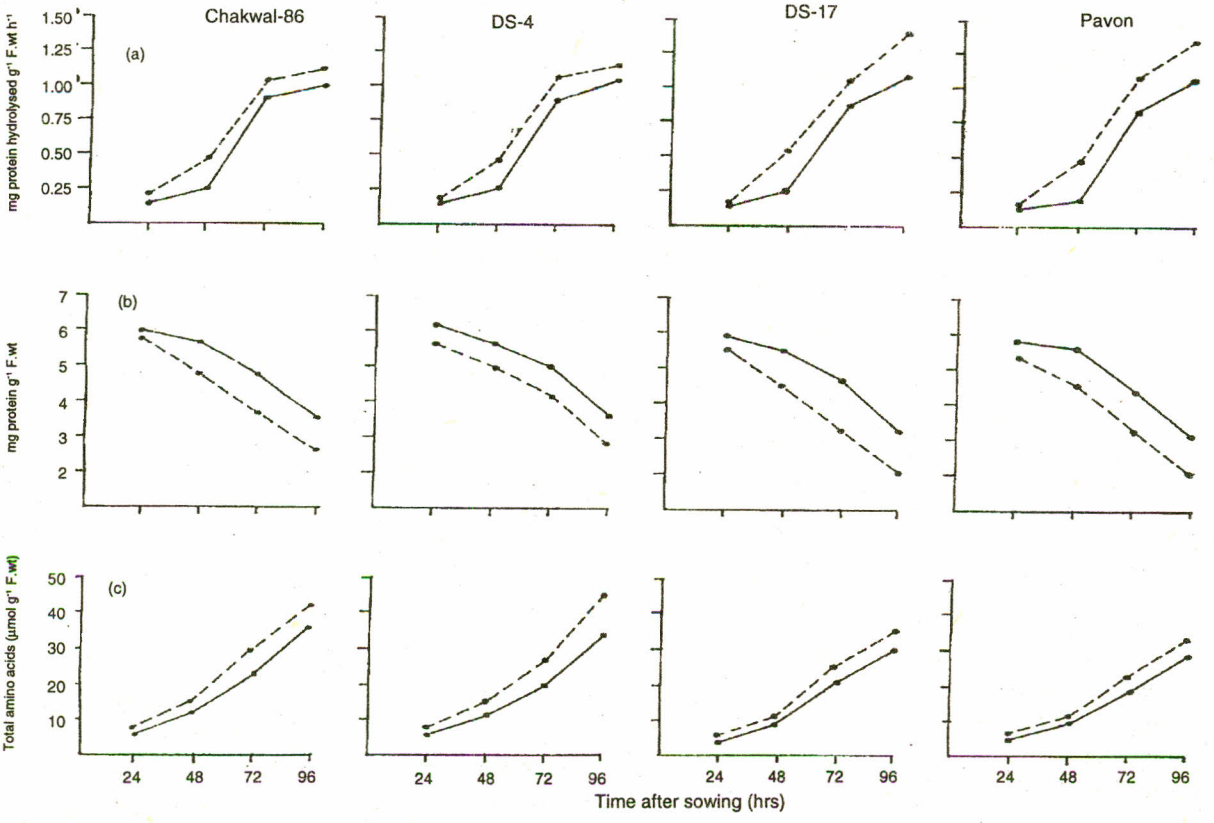

Fig. 2. Protease activity (a), Protein (b) Total amino acids (c) Four wheat genotypes grown under normal (—) and water stress (---) conditions.

ars increased significantly with the passage of time but the accumulation of non-reducing sugars was higher than the reducing sugars.

Protease activity. At 24 hrs. protease activity was similar in control and stressed seedlings (Fig. 2). Beyond this time period the activity increased sharply, more so in stressed seedlings than in the controls. The activity was highest in the non-tolerant DS-17 and Pavon, where values of 1.4 and 1.3 mg protein hydrolysed g^{-1} F.Wt. h^{-1} respectively were found as against 1.12 and 1.01 (mg protein hydrolysed g^{-1} F.Wt. h^{-1}) for both Chakwal-86 and DS-4. The value of the controls never exceeded 1.1.

Protein and amino acids. Total protein decreased in stressed seedlings to a greater extent than in the control (Fig. 2). The highest reduction was recorded in DS-17 (from 5.6 mg to 2.0 mg) and the lowest in DS-4 (from 5.7 mg to 2.8 mg). There was a concomitant increase in total amino acids, the highest content was in DS-4 under drought (45 µmol g^{-1} F.Wt.). In the case of the controls, total amino acids were lower than in their respective stressed seedlings.

Germination is critical phase in plant establishment, especially under drought conditions. Chances of survival are higher in plants capable of rapid root and shoot development, as the root provides capacity to extract water and the shoot ground cover and energy and prevents evaporative loss of water from soil. During germination, biochemical changes take place which provide the basic framework for subsequent growth and development. The initial metabolic changes that occur immediately after the inhibition of water increase in hydrolytic enzymes such as α-amylase and protease [16,24].

It was observed, in the present study, that after 24 hrs. the activity of α-amylase, in all the genotypes, was higher under stress and increased with the passage of time. However, compared with tolerant genotypes (Chakwal-86 and DS-4) the increase in α-amylase activity was less in the susceptible genotypes (D-17 and Pavon). Vieira da Silva [16] found an increase in sugars due to α-amylase activity in drought resistant *Gossypium anomcilum.* The increase in sugar accumulation has also been reported by Premachandra *et al.* [17] and Ashraf *et al.* [18] under water stress conditions. Amongst organic solutes, sugars are considered to play a major role in osmotic adjustment [19]. The higher accumulation of sugars (reducing and non-reducing) in drought tolerant genotypes (DS-4 and Chakwal-86) further supports the above findings.

Proteases play an important role, especially during germination, in the mobilization of storage proteins for the *de novo* synthesis of enzymes and cell membrane. They may have a role in drought tolerance, as accumulation of amino acids allows plants in many cases to overcome water stress through osmotic adjustment [19-20]. What contribution they make towards osmotic adjustment has not been elucidated. The contribution of amino acids may be marginal as most of the studies assign the major role to sugars and organic acids and even to K^+ [17,18]. The consistent increase of protease activity under water stress, in all the genotypes, is significant. However, its larger increase in susceptible genotypes (DS-17 and Pavon) casts doubt on its role in drought tolerance. This is further compounded by the fact that the amino acid content is not consistent with the protease activity actually found. The susceptible genotypes have higher protease activity but lower amino acid content. The genotypes DS-4 and Chakwal-86 had lesser increase in protease activity but had higher amino acid content (Fig. 2). This may be due to (i) stimulation of amino acid synthesis or (ii) inhibition of oxidation of amino acids. The work on protease activities under drought stress is scanty. However, Mothes [22] reported that drought enhances protease activity. Other stresses do not seem to have similar effect. Thus Sheoran and Grag [23] and Khan *et al.* [24] reported that salinity stress reduced protease activity. Comparison with salinity stress may not be valid as Jones and Armstrong [7] have shown that under salinity stress the ion effect also plays a role in addition to the osmotic effect. Whether the increase in amino acids is a response to drought or a result of their non utilization or of the break down in the protein synthesis mechanism, unfortunately could not be studied. However, circumstantial evidence from the present study (increase in protease activity) suggests the last to be the most plausible.

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