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Effect of water deficit on carbohydrate status and enzymes of carbohydrate metabolism in seedlings of wheat cultivars

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The effect of water deficit on carbohydrate status and enzymes of carbohydrate metabolism (α and β amylases, sucrose phosphate synthase, sucrose synthase, acid and alkaline invertases) in wheat (*Triticum aestivum* L.) was investigated in the seedlings of drought-sensitive (PBW 343) and drought-tolerant (C 306) cultivars. The water deficit was induced by adding 6% mannitol (water potential -0.815 Mpa) in the growth medium. The water deficit reduced starch content in the shoots of tolerant seedlings as compared to the sensitive ones, but increased sucrose content in the shoots and roots of tolerant seedlings, indicating their protective role during stress conditions. It also decreased the α-amylase activity in the endosperm of seedlings of both the cultivars, but increased α and β amylase activities in the shoots of tolerant ones. Sucrose phosphate synthase (SPS) activity showed a significant increase at 6 days of seedling growth (DSG) in the shoots of stressed seedlings of tolerant cultivar. However, SPS activity in the roots of stressed seedlings of sensitive cultivar was very low at 4 DSG and appeared significantly only at day 6. Sucrose synthase (SS) activity was lower in the shoots and roots of stressed seedlings of tolerant cultivar than sensitive ones at early stage of seedling growth. Higher acid invertase activity in the shoots of seedlings of tolerant cultivar appeared to be a unique characteristic of this cultivar for stress tolerance. Alkaline invertase activity, although affected under water deficit conditions, but was too low as compared to acid invertase activity to cause any significant affect on sucrose hydrolysis. In conclusion, higher sucrose content with high SPS and low acid invertase and SS activities in the roots under water deficit conditions could be responsible for drought tolerance of C 306.

Keywords: *Triticum aestivum* L., Water deficit, Acid invertase, Alkaline invertase, α and β-Amylase, Sucrose phosphate synthase, Sucrose synthase, Sucrose, Starch

Water deficit is a major abiotic factor, which adversely affects plant growth and productivity. It is becoming a greatest limitation factor even in those regions, where the problem was negligible because of the changing scenario of global climate[1]. Approximately, 70% of the global available water is employed in agriculture and 40% of the world food is produced in irrigated soils[2]. Some irrigation (around 10%) uses water from aquifers, leading to many underground water tables being exploited unsustainably[3]. There is need to develop crop varieties, which can grow in sub-optimal environments. Therefore, in recent years, biochemical and molecular basis for plant responses to water deficit stress has been the subject of intense research[4].

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Abbreviations: DSG, days of seedling growth; SPS, sucrose phosphate synthase; SS, sucrose synthase

Wheat (*Triticum aestivum* L.) is an important cereal, whose productivity depends upon the production, translocation, storage and utilization of carbohydrates. Carbohydrates serve as a source of energy and also act as signalling molecules in regulation of metabolic pathways under normal and stressed conditions[5]. During germination, starch present in the cereal endosperm is hydrolyzed to glucose by amylases and is then converted to sucrose by the sucrose phosphate synthase. Sucrose, thus formed is then transported to the growing embryonic axis, where it is hydrolyzed and the products so formed are used as energy source for the growth of seedlings[6].

Plants employ numerous strategies to control water status and resist drought. Water deficit has been reported to increase the accumulation of total water-soluble carbohydrates in the stems of drought-tolerant wheat genotypes[7]. Changes in carbohydrate composition are of particular importance because of their

direct relationship with such physiological processes as photosynthesis, translocation and respiration. Soluble sugar content, therefore, has been proved to be a better marker for selecting improvement of drought tolerance in durum wheat (*Triticum durum* Desf.)[8]. Sugars interact with the sensor proteins and initiate a signal transduction cascade that results in cellular responses such as altered gene expression and enzymatic activities[5].

The expression of a large number of stress responsive genes corresponding to enzymes of carbohydrate metabolism are down or upregulated by the sugar status of the cell, indicating the role of sugars during abiotic stresses[9,10]. Drought-tolerant durum wheat cultivar has shown higher expression of a number of drought related genes as compared to the drought-sensitive cultivar, particularly under water deficit conditions, indicating that they have a different ability to induce the drought molecular response[11]. Studies on carbohydrate metabolism could therefore, provide information about the differential response of drought tolerance in wheat cultivars (sensitive and tolerant) to water deficit.

The determination of expression pattern of enzymes of carbohydrate metabolism in response to drought and an improved understanding of their roles in stress adaptation may provide the basis of effective engineering strategies, leading to greater stress tolerance. Thus, in the present study, we have investigated the changes in carbohydrate status (starch and sucrose content) and activities of carbohydrate metabolizing enzymes (α- and β-amylases, sucrose phosphate synthase, sucrose synthase and acid and alkaline invertases) in the growing seedlings under control and water deficit condition in drought-sensitive (PBW 343) and drought-tolerant (C 306) wheat varieties.

Materials and Methods

Germination of seeds

Wheat (*Triticum aestivum* L.) grains of cv. PBW 343 (drought-sensitive) and C 306 (drought-tolerant) were washed with water, sterilized with 0.1% $HgCl_2$ and cultured aseptically in 250 cm^3 conical flasks on 0.7% agar medium. The flasks were then kept in an incubator at 25±1°C in the dark as described previously[12]. Water deficit was provided by adding 6% mannitol (water potential -0.815 Mpa) in the medium.

Extraction and estimation of starch and sucrose

The shoots, roots and endosperm of control and stressed seedlings were taken at 2, 4 and 6 days of seedling growth (DSG). Starch was estimated from the sugar-free residue left after extraction of sugars after hydrolyzing it to glucose with amylo-glucosidase[13] and starch content was determined by multiplying the amount of glucose formed by 0.9[14]. Sugars were quantitatively extracted with 80 and 70% ethanol as described previously[15]. From the aqueous extract obtained after evaporating ethanol at 50°C under vacuum, sucrose was estimated after hydrolyzing with excess of acid invertase[16].

Extraction and determination of enzyme activities

Activities of enzymes of carbohydrate metabolism viz., α and β-amylases, sucrose phosphate synthase (SPS), sucrose synthase (SS) and acid and alkaline invertases were determined in shoots, roots and endosperm of control and water stressed seedlings at 2, 4 and 6 DSG. α- and β-Amylases were extracted by the method described earlier[17]. Activity of α-amylase was determined by incubating 0.6 ml of starch solution (prepared by dissolving 67 mg of starch in 100 ml of 0.06 *M* KH_2PO_4), 1.2 ml of 0.2% calcium acetate and 0.2 ml of enzyme for 20 min at 30°C. The reaction was stopped by adding 5 ml of iodine solution (6 mg I_2 + 60 mg KI in 100 ml of 0.05 *M* HCl) and the colour developed was read at 610 nm. The assay for β-amylase was the same as that for α-amylase, except that 0.2% calcium acetate was replaced by 100 m*M* sodium acetate buffer (*p*H 3.6) containing 1 m*M* EDTA.

SPS was extracted and assayed by the method described earlier[13]. SS was also extracted by the same procedure and assayed by taking 100 μmoles of HEPES buffer (*p*H 6.5), 2 μmoles of UDP and 50 μmoles of sucrose in a total volume of 0.4 ml. Reaction was initiated by adding 0.1 ml of enzyme, incubated at 30°C for 30 min and stopped by adding 0.5 ml of alkaline copper tartrate reagent and fructose released was estimated by measuring nmoles sucrose cleaved $min^{-1}mg^{-1}$ protein[18]. Acid and alkaline invertases were extracted by crushing the tissue with 50 m*M* sodium phosphate buffer (*p*H 7.0)[19] and their activities were determined[15]. Protein content of all the enzyme extracts was determined[20] for measuring the specific activity.

Results

The starch and sucrose content and the enzymes of carbohydrate metabolism were studied in the endosperm, shoots and roots of control and stressed wheat seedlings of PBW 343 (drought-sensitive) and C 306 (drought-tolerant) cultivars at 2, 4 and 6 DSG. Starch content was maximum in the endosperm and decreased with the progress of seedling growth. It was higher in the endosperm of stressed seedlings and was lower in the shoots and roots of stressed seedlings of tolerant cultivar than the sensitive one. The sucrose content in shoots of control and stressed seedlings of two cultivars was comparable at 2 and 4 DSG, but by day 6, it increased in the stressed seedlings, increase being higher in the tolerant cultivar (Table 1). Of all the tissues, roots had the lowest sucrose content. Water stress resulted in higher sucrose content in the roots of tolerant seedlings, whereas it was negligible (<0.1 mg g^{-1} fresh wt.) in the sensitive one.

α-Amylase activity was higher in the endosperm, as compared to shoots and roots in both the cultivars and was highest at 4 DSG. In general, α and β-amylase activities were higher in the endosperm of seedlings of PBW 343 than the tolerant cultivar. Stress induced by 6% mannitol decreased the α-amylase activity in the endosperm of seedlings of both the cultivars. α-Amylase activity was very low in the roots and was negligible in the shoots at 2 DSG,

Table 1—Effect of water deficit induced by 6% mannitol on starch and sucrose content (mg g^{-1} fresh wt.) of PBW 343 and C 306 seedlings at different days of seedling growth

[Values were mean ± SD of three replicates]

Starch content	Treatment	Days of seedling growth		
		2	4	6
Shoot				
PBW 343	Control	4.7 ± 0.6	2.6 ± 0.1	3.2 ± 0.5
	Stress	-	3.6 ± 0.7	3.2 ± 1.5
C 306	Control	5.9 ± 0.5	4.4[c] ± 0.1	4.9[c] ± 0.2
	Stress	-	3.2[a] ± 0.01	2.9[a] ± 0.2
Root				
PBW 343	Control	2.8 ± 0.0	1.6 ± 0.01	1.0 ± 0.0
	Stress	-	1.7 ± 0.01	1.6 ± 0.1
C 306	Control	2.6 ± 0.1	1.9 ± 0.01	0.5[c] ± 0.2
	Stress	-	1.5 ± 0.2	0.8[b] ± 0.2
Endosperm				
PBW 343	Control	233.9 ± 4.5	152.6 ± 8.6	64.3 ± 0.9
	Stress	253.2 ± 4.0	176.1 ± 2.3	129.5[a] ± 9.2
C 306	Control	212.7 ± 7.2	151.8 ± 3.4	47.7 ± 1.2
	Stress	238.2 ± 1.1	221.1[ab] ± 7.7	137.5[a] ± 6.0
Sucrose content				
Shoot				
PBW 343	Control	23.4 ± 1.9	32.2 ± 1.5	9.0 ± 3.9
	Stress	-	34.4 ± 6.1	17.6 ± 5.2
C 306	Control	20.3 ± 0.3	32.0 ± 3.2	14.8 ± 0.8
	Stress	-	32.2 ± 2.7	37.7[ab] ± 3.8
Root				
PBW 343	Control	0.5 ± 0.0	1.3 ± 0.0	0.08 ± 0.01
	Stress	-	0.05 ± 0.01	0.05 ± 0.01
C 306	Control	± 0.0	2.7 ± 0.0	0.3 ± 0.04
	Stress	-	3.1[b] ± 0.5	1.3[ab] ± 0.02
Endosperm				
PBW 343	Control	23.9 ± 6.4	39.3 ± 5.8	31.1 ± 3.2
	Stress	17.0[a] ± 0.0	34.1 ± 3.8	24.6 ± 3.9
C 306	Control	22.4 ± 0.9	32.8 ± 6.2	57.3 ± 12.7
	Stress	13.3[a] ± 1.2	32.1 ± 1.2	27.9[a] ± 4.2

-, Data could not be taken due to insufficient development of shoots and roots. Differences significant in comparison with respective controls at [a] —P<0.01; in comparison with stressed seedlings of PBW 343 at [b] —P< 0.01; in comparison with control seedlings of PBW 343 at [c] —P<0.01 (student's t-test)

but developed in the control seedlings of both the cultivars by day 4. Under water deficit condition, α-amylase activity increased in the shoots of tolerant cultivar and was significantly higher as compared with the sensitive cultivar. β-Amylase activity was maximum at 2 DSG, declined thereafter and became negligible by day 6. It was also higher in the endosperm of sensitive as compared to tolerant seedlings. Stress resulted in decline in β-amylase activity from the endosperm of both the cultivars, except at 2 DSG in C 306. β-Amylase activity was observed up to 4 DSG in the shoots and roots of control seedlings of tolerant cultivar, but became almost negligible after 2 DSG in the control seedlings of sensitive one. Under water deficit condition, β-amylase activity remained higher in the shoots of tolerant seedlings as compared to sensitive ones (Table 2).

The tolerant cultivar (C 306) exhibited a higher SPS activity as compared with the sensitive one in all the tissues of control seedlings at 2 DSG. SPS activity was higher in the roots of control seedlings of tolerant cultivar than the sensitive one during different DSG. Under water deficit condition, SPS activity was reduced to almost negligible levels up to 4 DSG in the roots of sensitive cultivar, but the roots of tolerant seedlings exhibited a significantly higher SPS activity even at 4 DSG. SPS activity was comparable in the

Table 2—Effect of water deficit induced by 6% mannitol on α and β amylase activities (μg starch hydrolyzed $min^{-1}mg^{-1}$ protein) of PBW 343 and C 306 seedlings at different days of seedling growth

[Values were mean ± SD of three replicates]

α-Amylase activity	Treatment	Days of seedling growth		
		2	4	6
Shoot				
PBW 343	Control	Negligible	25.1 ± 3.1	0.03 ± 0.01
	Stress	-	12.3^{a} ± 2.1	0.04 ± 0.02
C 306	Control	Negligible	17.3 ± 2.0	0.03 ± 0.01
	Stress	-	32.5^{ab} ± 2.3	0.14^{ab} ± 0.01
Root				
PBW 343	Control	Negligible	11.5 ± 1.5	0.04 ± 0.01
	Stress	-	3.5^{a} ± 0.6	0.04 ± 0.01
C 306	Control	Negligible	2.1^{c} ± 0.5	0.05 ± 0.01
	Stress	-	0.4^{b} ± 0.02	0.05 ± 0.01
Endosperm				
PBW 343	Control	468 ± 28	7283 ± 202	2040 ± 203
	Stress	234^{a} ± 60	5025^{a} ± 450	2005 ±176
C 306	Control	280^{c} ± 15	4123^{c} ± 545	4023^{c} ± 151
	Stress	136^{a} ± 25	2348^{ab} ± 312	1943^{a} ± 421
β-Amylase activity				
Shoot				
PBW 343	Control	14.2 ± 0.9	0.2 ± 0.05	Negligible
	Stress	-	4.7^{a} ± 0.6	Negligible
C 306	Control	15.4 ± 0.8	5.9^{c} ± 0.2	Negligible
	Stress	-	11.7^{ab} ± 0.9	Negligible
Root				
PBW 343	Control	17.2 ± 2.2	0.05 ± 0.01	Negligible
	Stress	-	0.50 ± 0.06	Negligible
C 306	Control	12.2 ± 3.2	4.6^{c} ± 0.2	Negligible
	Stress	-	0.2^{a} ± 0.01	Negligible
Endosperm				
PBW 343	Control	993 ± 70	77 ± 10	Negligible
	Stress	683^{a} ± 10	40 ± 10	Negligible
C 306	Control	279^{c} ± 42	98 ± 14	Negligible
	Stress	483 ± 58	38^{a} ±11	Negligible

-, Data could not be taken due to insufficient development of shoots and roots. Differences significant in comparison with respective controls at [a]—$P<0.01$; in comparison with stressed seedlings of PBW 343 at [b] —$P< 0.01$; in comparison with control seedlings of PBW 343 at [c] —$P<0.01$ (student's t-test).

shoots of control and stressed seedlings of tolerant cultivar at 4 DSG, but as the germination progressed, it increased to a higher level in the stressed seedlings at 6 DSG. The shoots of stressed seedlings of tolerant cultivar had relatively higher SPS activity, as compared with the sensitive one at 6 DSG (Table 3).

Sucrose synthase (SS) activity, in general was low in all the tissues of control and stressed seedlings of both the cultivars. In the endosperm, the activity was hardly detectable during germination and was negligible in the shoots and roots at 2 DSG. Maximum SS activity was observed in the shoots and roots of control seedlings at 4 DSG in the sensitive cultivar, as compared with tolerant one. Under water deficit condition, SS activity was low in the shoots and roots of C 306 at 4 DSG, as compared to the sensitive one. With further seedling growth, SS activity reached at comparable levels in the shoots of control and stressed seedlings of tolerant cultivar. SS activity was less in the roots of stressed seedlings of tolerant cultivar as compared with the sensitive one (Table 3).

Acid invertase activity was higher in shoots and roots, but minimum in the endosperm. In shoots of both the cultivars acid invertase activity was very low at 2 DSG (specific activity in the range of 10-14 nmoles of sucrose hydrolyzed $min^{-1}mg^{-1}$ protein) and increased remarkably at day 4. Under water deficit condition, the shoots of tolerant cultivar had higher acid invertase activity, but their roots had lower enzyme activity as compared to the sensitive one (Table 4). Alkaline invertase activity in shoots and roots was observed to be lower in comparison with acid invertase activity (Table 4). The endosperm

Table 3—Effect of water deficit induced by 6% mannitol on sucrose phosphate synthase activity (nmoles sucrose formed $min^{-1}mg^{-1}$ protein) and sucrose synthase activity (nmoles sucrose cleaved min^{-1} mg^{-1} protein) of PBW 343 and C 306 seedlings at 2, 4 and 6 days of seedling growth

[Values were mean ± SD of three replicates]

SPS activity	Treatment	Days of seedling growth		
		2	4	6
Shoot				
PBW 343	Control	41.7 ± 3.96	79.4 ± 5.0	46.6 ± 4.1
	Stress	-	40.8^{a} ± 3.8	63.4 ± 4.0
C 306	Control	94.9^{c} ± 3.2	30.8 ± 5.7	35.9 ± 0.3
	Stress	-	31.2 ± 5.9	84.8^{ab} ± 3.3
Root				
PBW 343	Control	54.3 ± 1.2	2.5 ± 0.3	2.2 ± 0.3
	Stress	-	0.5^{a} ± 0.01	46.1^{a} ± 6.1
C 306	Control	61.9^{c} ± 1.5	24.6^{c} ± 1.5	58.2^{c} ± 3.8
	Stress	-	16.9^{b} ± 1.1	49.6 ± 0.9
Endosperm				
PBW 343	Control	63.6 ± 2.0	51.6 ± 1.7	59.4 ± 3.1
	Stress	84.6^{a} ± 2.9	30.7^{a} ± 5.8	122.8^{a} ± 13.3
C 306	Control	80.5 ± 4.5	33.4^{c} ± 1.6	54.6 ± 3.1
	Stress	54.8^{ab} ± 4.4	21.7^{a} ± 2.1	64.0^{b} ± 3.9
SS Activity				
Shoot				
PBW 343	Control	Negligible	22.1 ± 0.9	4.5 ± 0.3
	Stress	-	9.5^{a} ± 1.0	6.2 ± 1.1
C 306	Control	Negligible	7.1^{c} ± 1.2	5.7 ± 1.6
	Stress	-	2.9^{ab} ± 0.5	6.3 ± 1.5
Root				
PBW 343	Control	Negligible	10.9 ± 1.5	1.8 ± 0.1
	Stress	-	1.5^{a} ± 0.4	5.1^{a} ± 0.2
C 306	Control	Negligible	2.5^{c} ± 0.3	0.8^{c} ± 0.1
	Stress	-	0.2^{ab} ± 0.01	2.6^{ab} ± 0.2

-, Data could not be taken due to insufficient development of shoots and roots. SS activity was negligible in endosperm at all stages of seedling growth. Differences significant in comparison with respective controls at [a] —P<0.01; in comparison with stressed seedlings of PBW 343 at [b] —P< 0.01; in comparison with control seedlings of PBW 343 at [c] —P<0.01 (student's t-test).

Table 4—Effect of water deficit induced by 6% mannitol on acid and alkaline invertase activities (nmoles sucrose hydrolyzed min^{-1} mg^{-1} protein) of PBW 343 and C 306 seedlings at different days of seedling growth

[Values were mean ± SD of three replicates]

Acid invertase activity	Treatment	Days of seedling growth		
		2	4	6
Shoot				
PBW 343	Control	10.4 ± 1.4	399.7 ± 4.4	342.4 ± 5.4
	Stress	-	181.5[a] ± 21.6	310.2 ± 8.3
C 306	Control	14.1 ± 2.8	573.1[c] ± 8.5	438.6[c] ± 6.2
	Stress	-	329.4[ab] ± 4.5	473.2[b] ± 4.0
Root				
PBW 343	Control	380.2 ± 4.2	639.6 ± 9.1	463.8 ± 2.9
	Stress	-	376.9[a] ± 4.9	423.3 ± 7.8
C 306	Control	274.1 ± 3.4	501.9[c] ± 3.5	429.4[c] ± 2.9
	Stress	-	291.6[ab] ± 6.2	116.8[ab] ± 1.3
Endosperm				
PBW 343	Control	8.6 ± 0.4	8.8 ± 0.3	18.8 ± 1.6
	Stress	5.8 ± 1.6	14.7 ± 1.5	9.1[a] ± 0.9
C 306	Control	6.5 ± 0.4	7.8 ± 1.8	16.1 ± 2.3
	Stress	4.8 ± 0.6	5.3[b] ± 0.9	6.5[a] ± 1.4
Alkaline invertase activity				
Shoot				
PBW343	Control	32.0 ± 1.2	11.7 ± 3.5	12.3 ± 2.1
	Stress	-	14.3 ± 3.0	11.3 ± 1.9
C306	Control	25.0 ± 1.4	6.1 ± 1.7	13.8 ± 4.8
	Stress	-	9.9 ± 3.4	17.7 ± 1.8
Root				
PBW343	Control	66.0 ± 1.2	12.4 ± 4.4	12.4 ± 3.2
	Stress	-	18.3 ± 3.7	19.2 ± 1.8
C306	Control	47.0[c] ± 1.1	10.2 ± 3.0	26.5[c] ± 2.2
	Stress	-	25.9[a] ± 1.2	16.5 ± 1.9
Endosperm				
PBW343	Control	10.0 ± 2.0	Negligible	Negligible
	Stress	17.0 ± 3.7	Negligible	Negligible
C306	Control	17.2 ± 4.5	Negligible	Negligible
	Stress	20.7 ± 2.8	Negligible	Negligible

Alkaline invertase activity was negligible in endosperm after 2 DSG-, data could not be taken due to insufficient development of shoots and roots. Differences significant in comparison with respective controls at [a] —P<0.01; in comparison with stressed seedlings of PBW343 at [b] —P< 0.01 ; in comparison with control seedlings of PBW 343 at [c] —P<0.01 (student's t-test).

contained negligible alkaline invertase activity after 2 DSG.

Discussion

Starch, the major reserve component of wheat grains during germination is hydrolyzed to glucose, maltose and low molecular weight oligosaccharides by α- and β-amylases. With progress of seedling growth, β-amylase activity decreased from 2 to 6 DSG and α-amylase increased from 2 to 4 DSG in the shoots, roots and endosperm of germinating seedlings (Table 2). A comparative study on kinetic properties of partially purified amylases from the endosperm of PBW 343 and C 306 seedlings was reported earlier[21]. Water deficit reduced the mobilization of starch from endosperm to embryonic axis, as indicated by the higher starch content and reduced activities of α and β-amylases in the endosperm of stressed seedlings of both the cultivars (Tables 1 and 2). β-Amylase activity, though higher at 2 DSG in the stressed seedlings of tolerant cultivar could not account for the faster hydrolysis of starch, because it was accompanied by the decreased α-amylase activity (Tables 1 and 2). The decreased amylase activity, leading to accumulation of starch under water deficit condition was also reported earlier[22]. In sensitive cultivar, α-amylase activity was higher in the shoots of control seedlings and reduced significantly under water

deficit condition. However, in tolerant cultivar, stress resulted in a significant increase in α-amylase activity in shoots, as compared with stressed seedlings of sensitive cultivar (Table 2). β-Amylase activity was higher in the shoots and roots of control as well as water deficit seedlings of tolerant cultivar as compared to the sensitive one (Table 2). This indicated a cumulative increase in total amylase activity in shoots of stressed seedlings of tolerant cultivar, resulting in the rapid hydrolysis of transitory starch of shoots, leading to more availability of glucose for shoot growth. A high negative correlation between amylase activity and starch content in shoots was also reported earlier[15]. Drought survival was enhanced by faster hydrolysis of starch in shoots due to stimulation of amylase activity, so as to maintain or increase the concentration of low molecular weight carbohydrates, which helped plants to retain turgidity and protect protolplasmic constituents[23]. The higher amylase activity observed in the shoots of stressed seedlings of tolerant cultivar might be associated with the *de novo* synthesis of α-amylase as observed in the leaves of water stressed barley seedlings[24].

Sucrose helps in osmotic adjustment[25,26]. Consistent differences in osmotic adjustment among wheat cultivars differing in drought tolerance were also reported and under drought, the high osmotic adjustment cultivar was found to yield better than low osmotic adjustment cultivar[27,28]. Sugars are known to stabilize proteins, thus protect them against different stresses[29]. Sucrose substitutes for water in maintaining hydrophilic structures in their hydrated orientation and also protects the cell during desiccation by the formation of an intracellular glass which prevents cellular collapse and functional damage[30]. High sugar accumulation during early seedling development might reflect undesirable growth conditions at a crucial developmental period, resulting in reversible developmental arrest that acts as a protection mechanism[5]. Higher sucrose content in shoots and roots of stressed seedlings of tolerant cultivar was observed at 6 DSG.

SPS activity was remarkably high at 2 DSG in all the tissues of control seedlings of tolerant cultivar (Table 3), suggesting the naturally higher tendency of this cultivar to synthesize sucrose during initial phase of seedling growth. However, excess sucrose formed during this phase might be utilized for the growth of seedlings of tolerant cultivar, basically a tall variety[21], bringing sucrose levels comparable to the seedlings of sensitive cultivar (PBW 343), a double dwarf cultivar[21], at 2 DSG (Table 1). Under water deficit condition, SPS activity was higher in the shoots of tolerant cultivar at 6 DSG. However, under such stress conditions, SS activity was very low in the shoots of tolerant cultivar, at 4 DSG and became comparable to the control seedlings at day 6 (Table 3). The combined effect of SPS and SS activities resulted in an increase in the sucrose content of shoots of stressed seedlings of tolerant cultivar at 6 DSG (Table 1). Earlier, higher levels of sucrose in the stems of drought-tolerant wheat seedlings as compared with sensitive one were also reported[7]. The stimulation of sucrose synthesis by activation of sucrose synthesizing enzymes was observed in stressed seedlings[15,31]. Accumulation of sucrose in stressed seedlings due to diminished activities of enzymes of sucrose breakdown was also reported[32].

A significantly higher SPS activity and reduced SS in the roots of control and stressed seedlings of tolerant cultivar as compared with the sensitive cultivar could be responsible for the higher sucrose content in the roots of tolerant seedlings (Tables 1 and 3) and appeared to be a unique characteristic of stressed tolerant cultivar.

The acid and alkaline invertases and SS activities were almost negligible in the endosperm of seedlings of both the cultivars, suggesting that sucrose synthesized from glucose formed from breakdown of starch was transported to the growing sinks without significant hydrolysis. Acid invertase activity was higher in shoots and roots as compared with endosperm (Table 4). It was lower in the roots of stressed seedlings of tolerant cultivar, as compared with the sensitive one (Table 4), which could account for reduced sucrose hydrolysis, leading to its accumulation in the roots (Table 1).

Sucrose accumulation is controlled by the low levels of acid invertase activity[33]. However, such inverse relationship between sucrose content and acid invertase activity in the shoots of stressed seedlings of tolerant cultivar was not observed. Acid invertase activity was significantly higher in the shoots of tolerant seedlings both under control and water deficit conditions, as compared to the sensitive ones. Besides, sucrose content was also higher in the stressed shoots of tolerant seedlings as compared to the sensitive ones at 6 DSG. The correlation between sucrose content and acid invertase activity is a hot topic, yet under research. Acid invertase activity had

no role in the mobilization of stored sucrose in the leaves of *Lolium temulentum*[34]. Therefore, higher sucrose content in the shoots and roots of tolerant seedlings under water deficit conditions could possibly be due to higher SPS activity in these seedlings, which might enable it to adapt to water deficit condition in a better manner as compared with the sensitive one. Nevertheless, the higher acid invertase activity in the shoots of seedlings of tolerant cultivar appeared to be a unique characteristic for stress tolerance. Alkaline invertase activity, although affected under water deficit condition (Table 4) was too low as compared to acid invertase activity to cause any significant effect on sucrose hydrolysis.

In conclusion, the present study indicated that under water deficit condition the stress tolerant nature of C 306 could be due to higher sucrose content, high SPS activity and low acid invertase and SS activities in the roots (the first organs, which face water deficit condition during growth). The better growth of this cultivar under stress condition could be due to high acid invertase and α- and β-amylase activities in the shoots.

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