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RESEARCH ARTICLE

Differential response of wild and cultivated wheats to water deficits during grain development: changes in soluble carbohydrates and invertases

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Abstract Wheat, staple food crop of the world, is sensitive to drought, especially during the grain-filling period. Water soluble carbohydrates (WSCs), stem reserve mobilization and higher invertase activity in the developing grains are important biochemical traits for breeding wheat to enhance tolerance to terminal drought. These traits were studied for three accessions of *Triticum dicoccoides*(a tetraploid wheat progenitor species) - acc 7054 (EC 171812), acc 7079 (EC 171837) and acc 14004 (G-194-3 M-6 M) selected previously on the basis of grain filling characteristics. Check wheat cultivars- PBW-343 (a popular bread wheat cultivar for irrigated environments) and C-306 (widely adapted variety for rain-fed agriculture) were also included in this set. Analysis of variance revealed significant genotypic differences for the content of water soluble carbohydrates, activity of acid invertase and alkaline invertase. Acc 7079 was found to be a very efficient mobilizer of water soluble carbohydrates (236.43 mg g^{-1} peduncle DW) when averaged over irrigated and rain-fed conditions. Acid invertase activity revealed marked genotypic differences between wild and cultivated wheats. Alkaline invertase activity was highest in Acc 7079 when pooled across both the environments. On the whole, acc 7079 qualifies as a suitable donor for enhancing tolerance of bread wheat to terminal drought. The association of physio-biochemical differences observed with grain filling attributes on one hand and molecular markers on the other could be of use in improving wheat for water stress conditions.

Keywords Wild emmer · Stem reserve carbohydrates · Invertase · Grain-filling · Drought

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Introduction

Wheat is the most important food crop of the world in terms of harvested area, trade value and human nutrition, having more influence on global food security than any other crop (Reynolds et al. 2012). With current productivity increasing at a rate of about 1.1% per annum (Dixon et al. 2009), or even stagnating in some regions (Brisson et al. 2010), meeting the needs of a growing population with rising per capita consumption is a serious challenge. Of the various factors affecting wheat productivity and limiting its yield- biotic factors such as resistance to pests and diseases, and abiotic factors such as extremes of temperature, salinity, heavy metal toxicity, water deficit stress (drought) has been found to be most detrimental.

The sensitivity of wheat crop to soil drought is particularly important during reproductive and grain-filling period (Zrenner et al. 1995; Zinselmeier et al. 1999). Water deficit stress during reproductive development restricts the supply of sucrose to floral organs (anthers and young ovaries) and often leads to floral abortion, significantly affecting grain number. In biochemical terms, drought during grain-filling period affects efficient channeling of carbohydrates to sink organs (developing grains), severely impacting biochemical conversion, sucrose metabolism and starch accumulation. This adversely affects endosperm cell number, grain filling duration, resulting in smaller grains, reduced grain weight and an overall decreased grain yield.

Flag leaf is the primary photosynthetic organ, serving as the major source of carbon to the plant. Physiological studies of wheat have indicated that flag leaf contribution towards grain weight accounts for 41–43% of kernel dry matter at maturity (Amiri et al. 2013). However, the prevailing climatic conditions for spring wheat across large parts of the world during grain-filling period often coincide with rising air temperature and developing water stress due to increased soil evaporation which greatly affects grain growth and development. This terminal water deficit and concomitant heat stress generally result in stomatal closure (Chaves et al. 2002) and coordinated down-regulation of genes involved in Calvin cycle (Xue et al. 2008), causing rapid decline in photosynthesis after anthesis, that limits contribution of current assimilates to the grain (Johnson et al. 1981). Flag leaf photosynthesis alone cannot support both respiration and grain growth under such a condition (Rawson et al. 1983). Hence, an important source of carbon for grain-filling is the stem reserves (Blum 1998; Ehdaie et al. 2006).

Remobilization of stored WSCs plays a crucial role in grain-filling (Dreccer et al. 2009). The demand by the grain yield sink is a primary factor in determining stem reserve mobilization (Blum 1998). The reported contribution of WSCs to yield varies greatly with the environment, growing conditions and cultivar and can range from 10 to 20% under non-stressed conditions (Shearman et al. 2005) and up to 50% under severe stress (Blum 1998). For this reason, ability to store and mobilize large amounts of stem WSCs constitutes a seemingly desirable trait to incorporate in germplasm where terminal drought occurs frequently (Dreccer et al. 2009).

Once the sugars (sucrose from photosynthetic tissue and/or fructans from mobilized stem reserves) have reached those sinks, these must be degraded into hexoses or their derivatives for various metabolic and biosynthetic processes (Ruan et al. 2010). It is the cleavage of O-glycosidic bond between glucose and fructose that initiates sucrose utilization; and in plants this reaction is catalysed by two enzymes- invertase and sucrose synthase (cleavage). Plant invertases (β-D-fructofuranosidase EC 3.2.1.26) constitute a family of enzymes that irreversibly hydrolyse sucrose to glucose and fructose. Depending on their optimum pH, solubility and sub-cellular location, invertases are classified as vacuolar, apoplasmic (cell-wall) and cytoplasmic isoforms (Sturm 1999). Vacuolar and cell-wall invertases have acidic pH optima, i.e., 4.5–5.5, and are, therefore, referred to as acid invertases (soluble and insoluble acid invertase respectively). By contrast, cytoplasmic invertase has a neutral/alkaline optimal pH of 7.0–7.8 and hydrolyses sucrose in the cytosol (Sturm and Tang 1999). Unlike cell wall invertase that is bound to cell wall, soluble invertases- in the cytosol (alkaline) and vacuole (acid), are extractable in the crude supernatant after cell disruption. Invertases have roles in several plant physiological processes related to long-distance nutrient allocation as well as regulating developmental processes, hormone responses and biotic and abiotic interactions (Lalonde et al. 1997; Tymowska-Lalanne and Kreis 1998; Roitsch and Gonzalez 2004). Also, since invertase is actively involved in sucrose cleavage in sink tissue, its activity is regarded as biochemical marker of sink strength (Ranwala and Miller 1998).

The key to successful crop improvement is a continued supply of genetic variability and beneficial traits contained in this diversity (Dwivedi et al. 2008). In wheat crop, notable success in terms of improved yield, yield stability, increased disease resistance and input utilization efficiency has been obtained. Green revolution has immensely contributed to this success. However, much of the success was achieved at the cost of genetic diversity in the species (Warburton et al. 2006); average modified Roger's distances (MRD) within group of germplasm fell from 0.64 in the landraces to a low of 0.58 in the improved lines in the 1980's.

Wheat's tetraploid ancestor- Emmer wheat (*Triticum turgidum* ssp. dicoccon, BBAA) was domesticated about 9000 years ago, at the dawn of agriculture. Its direct progenitor- wild emmer (*Triticum turgidum* ssp. *dicoccoides*), despite having emerged through the bottleneck of amphiploidy, is highly variable genetically. Wild emmer, having existed millions of years, had time to accumulate variation through mutations and possible introgression from diploid relatives (Peng et al. 2011). *T. dicoccoides* possesses important beneficial traits, e.g., resistance to rust, powdery mildew, better amino acid composition, high photosynthetic yield, salt and drought tolerance (Nevo and Chen 2010), herbicide resistance, amylases and alpha amylase inhibitors (Wang et al. 2010) and higher micronutrients such as Zn and Fe in the grain (Cakmak et al. 2004; Uauy et al. 2006). Genes for higher grain weight (Kushnir and Halloran 1984) and genome region associated with high grain protein content (GPC) (Mesfin et al. 1999, 2000) have been transferred from *T. turgidum* var *dicoccoides* to common wheat.

Considering the narrow genetic base of the current cultivars, impact of global climate change on crop production has emerged as a major research concern. A critical role is envisaged for assessment of genetic variation in the available germplasm for drought tolerance traits thereby identifying donor lines and identification of metabolic alterations and genes controlling tolerance responses to abiotic stresses, especially drought. (Shanker et al. 2014).

Genetic variation exists for WSC accumulation in the stem at anthesis (Ruuska et al. 2006) and it has been suggested that breeding for high WSC should be possible due to its high heritability, though the trait appears to be controlled by complex polygenic regulation (Rebetzke et al. 2008). Stem reserves and their contribution to grain can be estimated by measuring post-anthesis changes in internode dry matter (Cruz-Aguado et al. 2000), changes in internode WSCs content (Shakiba et al. 1996) and/or estimated by determining sink activity (Gupta et al. 2011) during grain-filling period.

Keeping these points in mind, the present study investigated a chosen set of *T. dicoccoides* accessions and check wheat cultivars for content of water soluble carbohydrates, i.e., post-anthesis changes in peduncle WSCs and sink activity, in terms of post-anthesis changes in the activity of acid- and alkaline invertase in developing grains under water stress and non-stress environments.

Material and methods

Plant material

The present investigation was carried out on three accessions of *Triticum dicoccoides* (tetraploid, AABB genome) that originated in Israel. Two of these accessions- P.A.U. acc 7054 (EC 171812) and P.A.U. acc 7079 (EC No. 171837) were procured from National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The third *T. dicoccoides* accession, i.e., P.A.U. acc 14004 (G-194-3 M-6 M) was obtained from Centre for Plant Breeding Research, Wageningen, Netherlands. These accessions were chosen from a larger set of twenty six *T. dicoccoides* accessions that were initially evaluated for peduncle weight (at anthesis) and grain weight (at maturity). Two of these accessions- 7054 and 7079 were found to have higher peduncle dry weight and faster rate of grain-filling under rain-fed conditions, whereas acc 14004 had heavier peduncle under irrigated conditions. Additionally, all three of them were found to have higher grain weight than the average grain weight for the complete set. Along with these wild accessions, two wheat cultivars- PBW-343 and C-306 (hexaploid, AABBDD-genome) were also included in the current set. C-306 is a pre-green revolution variety, widely grown as a rain-fed cultivar. PBW-343, an Attila 'sib' which is a derivative of Veery group of wheats developed through spring wheat x winter wheat crosses at CIMMYT, Mexico, was released in 1995. It is one of the most widely cultivated wheat varieties in India.

Raising of *Triticum dicoccoides* plants in the field

Germinated seedlings of *T. dicoccoides* were transplanted in the experimental fields of Department of Plant Breeding and Genetics in two sets (later demarcated as irrigated and rain-fed) in the last week of October in 2011. Three replications per set were sown in randomized complete block design. The natural day length was supplemented with artificial light to ensure 14–15 hours of light per day. Additional light was given to over-ride a mild/ facultative winter habit and ensure timely flowering to arrive at an unbiased estimation of grain-filling related traits. Pre-sowing irrigation was given to both the sets and thereafter, one set was irrigated at periodic intervals, whereas, plants in the adjacent plot received water only available through rainfall and all irrigation was withheld from sowing to maturity. Soil moisture content was determined at different stages of crop development. The rain-fed set was found to have 70% less moisture content than irrigated set at anthesis. Although the crop season received a total rainfall of 108.8 mm (October 2011 to May 2012), the month of March (period of anthesis and active grain-filling) received no rainfall, which allowed sufficient building up of water deficit stress in crop.

Extraction and estimation of total water-soluble sugars

The content of water soluble sugars was evaluated from peduncle, uppermost internode of wheat stem, at anthesis, 10, 20, 30 and 40 days after anthesis (DAA). The dry flakes of crushed peduncle were extracted with water at 100°C for two hours. Sugars extract obtained upon centrifugation was used for the estimation of total sugars with phenol and sulphuric acid using the method of Dubois et al. (1956).

Extraction and assay of acid and alkaline invertase

Invertases were extracted from developing grains 10, 20 and 30 DAA according to Krishnan et al. (1985). The required tissue was crushed in chilled pestle and mortar with a pinch of PVP (polyvinyl pyrrolidine) and 20 mM HEPES Buffer (pH 7.5). The extracted material was centrifuged at 10,000×g. Activity of acid invertase was assayed from the supernatant using 200 mM sodium acetate buffer (pH 5.0) and 500 mM sucrose. The contents were incubated at 30°C for 60 min (Dey 1985). Assay of alkaline invertase was similar to acid invertase except that sodium acetate buffer was replaced with 100 mM sodium phosphate buffer (pH8.0). The reaction was stopped by adding 1 ml of alkaline copper tartrate reagent and reducing sugars were estimated by the method of Nelson (1944). In the blank, reaction was stopped at 0 min by adding alkaline copper tartrate reagent.

Statistical analysis

The results are expressed as means of three replicates. Data was analyzed using factorial analysis of variance due to genotypes, environments and the stages of development as well as their interactions, if any.

Results

Analysis of variance carried out on the set of lines (accessions of *T. dicoccoides* and cultivated wheat) grown under stress and non-stress environments revealed significant variation due to environment, genotypes and stages for all the three traits- content of WSCs, activity of acid invertase and activity of alkaline invertase (Table 1). Significant genotype x environment interaction for the content of WSCs; genotype x stages interaction for WSCs content and activity of acid invertase; and genotype x environment x stages interaction for the content of water soluble carbohydrates were observed in the present set.

Post-anthesis changes in content of water soluble carbohydrates

Content of WSCs and its mobilization was estimated at ten day intervals (starting from anthesis) in five accessions grown under irrigated and rain-fed environments. At anthesis, acc 14004 (217.05 mg g^{-1} peduncle DW) and acc 7079 (232.56 mgg^{-1} peduncle DW) had the maximum content of WSCs under irrigated and rain-fed conditions respectively (Table 2), indicating bulk accumulation of sugars in their stems. Level of total soluble sugars rose in all accessions under both environments at early grain-filling stage, i.e., ten days post anthesis, with acc 14004 accumulating maximum WSCs (379.84 mgg^{-1} peduncle DW) under irrigated and C-306 accumulating maximum amount (364.34 mgg^{-1} peduncle DW) under rain-fed conditions. Thereafter, all the accessions experienced a net decrease in WSCs content. The mass of fructans first increased and then decreased, indicating that fructans were hydrolysed at a much faster rate to allow for sufficient availability of readily metabolizable sugars. This further supports the idea of effective translocation of solutes from stem to the developing grain. Incidentally, PBW-343 was found to have lowest content of water soluble carbohydrates within the irrigated set during most of the stages of grain-filling. Towards the late phase of grain-filling (30 DAA), acc 7079 was observed to have the lowest WSCs within the rain-fed set (Table 2). Probably, it had exhausted its carbohydrate reserves in its quest to effectively translocate sugars for optimum grain development. Furthermore, enhanced mobilization of stem reserves in drought conditions is directly linked to an increased demand for these reserves by the ear (Wardlaw and Willenbrink 2000). C-306, on the other hand, was found to maintain highest WSC reserves under both the environment 30 days post-anthesis, indicating that C-306 might have a longer grain-filling period relative to other accessions. Shorter grain-filling duration may allow some avoidance of terminal stress, but longer duration allows greater utilization of stem reserves for grain-filling under stress. C-306 is a widely grown wheat variety in India, highly tolerant to terminal heat stress and well adapted to rain-fed agriculture. It is known for its stay green habit (allowing gradual loss of chlorophyll) and good grain-filling that contributes towards the formation of uniform- sized grains. The golden peduncle is its unique feature, i.e., green tissue does not turn to brown (senescence) but shines as a healthy, golden stem deep into the grain-filling stage. This characteristic morphological trait may be an outcome of gradual mobilization and utilization of water soluble carbohydrates from its peduncle.

Amount of mobilized WSCs was calculated for all the accessions under both irrigated and rain-fed conditions. Since the amount of WSCs mobilized varied from 62.01 mg g^{-1} peduncle DW (PBW-343) to 341.08 mg g^{-1} peduncle DW (acc 14004) under irrigated and 116.28 mg g^{-1} peduncle DW (acc 14004) to 263.57 mg g^{-1} peduncle DW (acc 7079 and C-306) under rain-fed conditions, greater genotypic variation could be noted for mobilized WSCs under irrigated than under rain-fed conditions. C-306 was at par with acc 7079 under rain-fed conditions as far as the extent of mobilization of WSCs is concerned (263 mg g^{-1} peduncle DW). However, when the data was pooled across the two environments, the net mobilization efficiency of acc 7079 was highest (236.43 mg g^{-1} peduncle DW) followed closely by acc

Table 1 Analysis of variance (ANOVA) for the traits studied on a set of *Triticum dicoccoides* accessions and cultivated wheats

Source of variation	WSCs		Acid invertase		Alkaline invertase	
	df	Mean Squares	df	Mean Squares	df	Mean Squares
Genotypes (G)	4	9,681.21*	4	878,168.71*	4	124,494.28*
Environment (E)	1	2,079.22	1	2,605.58	1	56,042.93*
G X E	4	7,176.64*	4	20,466.42	4	12,866.46
Stages (S)	3	132,815.23*	2	3,657,703.33*	2	986,984.98*
G X S	12	2,268.99*	8	59,570.43*	8	10,862.01
E X S	3	1,129.99	2	73,185.84*	2	2,666.28
G X E X S	12	4,181.99*	8	11,166.44	8	38,523.23
Error	39	939.28	29	17,337.92	29	72,231.27

*Indicates values significant at $P<0.05$

Table 2 Content of water soluble carbohydrates in peduncle of *Triticum dicoccoides* and check wheat cultivars under irrigated and rain-fed conditions during successive stages of grain development

Sr.No.	Accessions	Stages	Irrigated	Rain-fed	Mean (Genotypes)
1	**7054**	at anthesis	186.04	162.79	174.42
		10 DAA	263.56	271.31	267.44
		20 DAA	147.29	162.79	155.04
		30 DAA	69.77	54.26	62.01
		MOBILIZED WSCs	**193.79**	**217.05**	**205.42**
2	**7079**	at anthesis	147.29	232.56	189.92
		10 DAA	263.56	310.07	286.82
		20 DAA	186.04	201.55	193.80
		30 DAA	54.26	46.51	50.39
		MOBILIZED WSCs	**209.30**	**263.56**	**236.43**
3	**14004**	at anthesis	217.05	170.54	193.80
		10 DAA	379.84	201.55	290.69
		20 DAA	170.54	116.28	143.41
		30 DAA	38.76	85.27	62.01
		MOBILIZED WSCs	**341.08**	**116.28**	**228.68**
4	**PBW-343**	at anthesis	116.28	193.80	155.04
		10 DAA	131.78	217.05	174.42
		20 DAA	108.53	100.77	104.65
		30 DAA	69.77	54.26	62.01
		MOBILIZED WSCs	**62.01**	**162.79**	**112.40**
5	**C-306**	at anthesis	178.29	203.87	191.08
		10 DAA	224.80	364.34	294.57
		20 DAA	178.29	162.79	170.54
		30 DAA	77.52	100.77	89.15
		MOBILIZED WSCs	**147.28**	**263.57**	**205.43**
	MEAN (ENVIRONMENT)		**160.46**	**170.66**	

Least significant difference (0.05): Genotypes averaged over environments- 21.92

Environment averaged over genotypes - NS

Values are means of three replicates

Mobilized WSCs=Post anthesis maximum WSC content - post anthesis minimum WSC content

14004 (228.68 mg g^{-1} peduncle DW). Significant differences for the amount of soluble sugars mobilized could also be noted within cultivated wheats, i.e., between PBW-343 (112.40 mg g^{-1} peduncle DW) and C-306 (205.43 mg g^{-1} peduncle DW), justifying drought tolerant nature of C-306.

Post-anthesis changes in the activity of acid invertase

Activity of acid invertase was determined from the developing grains at an interval of ten days from the onset of anthesis-early (10 DAA), mid (20 DAA), late (30 DAA) and very late/pre-maturity (40 DAA) under both irrigated and rain-fed conditions. Phenological studies in wheat generally divide the event of grain development into four stages before it achieves complete physiological maturity. These are respectively termed as- watery ripe, milky ripe, soft dough and hard dough. During early phase of grain-filling, most of the accessions had higher activity of acid invertase under rain-fed conditions relative to their activity under irrigated conditions. In fact, acc14004 had the highest enzyme activity within both irrigated (1482.16 μmoles of reducing sugars formed min^{-1} g^{-1} FW) and rain-fed set (1689.28 μmoles of reducing sugars formed min^{-1} g^{-1} FW). During mid grain-filling phase (milky ripe stage), acc 7079 not only had the highest enzyme activity within the irrigated set, but also showed least decline (16%) in the enzyme activity with respect to preceding stage (Table 3), indicating better rate of sink filling relative to other accessions. Higher levels of enzyme involved in the breakdown of sucrose in the sink would increase sink capacity by lowering local concentration of sucrose, thereby generating a gradient that allows further unloading of sucrose (Wardlaw 1968; Liang et al. 2001). Under rain-fed conditions, however, acc 14004 had the highest enzyme activity (1005.43 μmoles of reducing sugars formed min^{-1} g^{-1} FW) among the studied accessions, though acc 7054 indicated least decline (32%) relative to early grain-filling phase (Table 3).

Almost 30 days after anthesis, when the activity of the enzyme was assayed (soft dough stage), relative to early grain-filling phase, an average 65% decline in acid invertase activity under irrigated and 75% decline under rain-fed conditions could be noticed. Usually, drought stress decreases the activities of both vacuolar and cell-wall bound invertase

Table 3 Activity of acid invertase in developing grains of *Triticum dicoccoides* and check wheat cultivars under irrigated and rain-fed conditions

Sr.No.	Accessions	Stages	Irrigated	Rain-fed	Mean (Genotypes)
1	**7054**	10 DAA	1402.78	1374.72	**913.32**
		20 DAA	887.60	937.82	
		30 DAA	528.20	348.82	
		MEAN	**939.53**	**887.12**	
2	**7079**	10 DAA	1244.29	1604.29	**963.72**
		20 DAA	1043.55	937.37	
		30 DAA	480.04	472.81	
		MEAN	**922.62**	**1004.82**	
3	**14004**	10 DAA	1482.16	1689.28	**1,023.52**
		20 DAA	964.40	1005.43	
		30 DAA	452.92	546.92	
		MEAN	**966.49**	**1080.54**	
4	**PBW-343**	10 DAA	645.25	712.90	**419.85**
		20 DAA	443.83	320.85	
		30 DAA	283.13	113.19	
		MEAN	**457.40**	**382.31**	
5	**C-306**	10 DAA	921.70	1078.91	**551.19**
		20 DAA	519.13	395.67	
		30 DAA	217.09	174.67	
		MEAN	**552.64**	**549.75**	
	MEAN (ENVIRONMENT)		**767.74**	**780.91**	

Least significant difference (0.05): Genotypes averaged over environments - 109.97

Environment averaged over genotypes – NS

Values are means of three replicates

Activity of acid invertase is represented as μmole of reducing sugars formed min^{-1} g^{-1} FW

during kernel development (Zrenner et al. 1995). Lower activity of invertase might contribute towards more efficient channelization of sucrose and other hexoses towards starch biosynthesis. Higher activity of the enzyme at late grain-filling phase may prove counter-productive as it would drive the reaction in backward direction and hinder effective accumulation of starch for grain formation. Accordingly, during late phase of grain-filling, starch accumulating enzymes predominate to allow for sufficient dry matter accumulation by converting received carbohydrates into starch reserves. Acc 7054 had the highest acid invertase activity under irrigated conditions during late grain filling phase. Acc 14004, just like early and mid-phase, retained highest acid invertase activity during late phase (546.92 μmoles of reducing sugars formed min^{-1} g^{-1} FW) under rain-fed conditions. Later, 40 DAA, when the crop had almost reached maturity and grain development was almost complete, acid invertase activity was assayed, but negligible activity could be determined in the studied accessions.

When a relative comparison for the activity of acid invertase was drawn between the studied wild accessions and cultivated bread wheats across two environments and three stages, significant genotypic difference was revealed in them. *T. dicoccoides* accessions displayed an average activity of 966.85 μmoles of reducing sugars formed min^{-1} g^{-1} FW as against cultivated wheats whose mean acid invertase activity was found to be 485.52 μmoles of reducing sugars formed min^{-1} g^{-1} FW.

Post-anthesis changes in the activity of alkaline invertase

Just like acid invertase, activity of alkaline invertase was evaluated during early-, mid-, late- and pre-maturity phase of grain-filling in all five accessions. During early phase of grain filling, that corresponded to watery ripe stage, acc 14004 expressed highest alkaline invertase activity (604.86 μmoles of reducing sugars formed min^{-1} g^{-1} FW) under irrigated conditions and acc 7079 (574.38 μmoles of reducing sugars formed min^{-1} g^{-1} FW) under rain-fed conditions (Table 4). During mid grain-filling phase (20 DAA), acc 7054 and acc 7079 did not appear to have experienced a significant decline in the enzyme activity under irrigated conditions in comparison to early phase (10 DAA). However, under rain-fed conditions, an average 32% decline in enzyme activity could be observed. Acc 7079 maintained highest alkaline invertase activity 20 days after anthesis just as it had displayed highest activity during early phase (10 DAA).

During the late phase of grain-filling (30 DAA), average alkaline invertase activity was found to be 200.88 μmoles of reducing sugars formed min^{-1} g^{-1} FW under irrigated conditions and 150.86 μmoles of reducing sugars formed min^{-1} g^{-1} FW under rain-fed conditions. Significant variation in the activity of the enzyme could be noticed across the stages of grain development as indicated by an average 61% decline in activity (relative to early grain-filling phase) under irrigated conditions and an average 66% decline (early grain-filling phase) under rain-fed conditions. Accordingly, negligible activity of alkaline invertase could be detected 40 days post anthesis.

It could be seen that among the five lines, acc 7079 had the highest alkaline invertase activity under rain-fed conditions and even when pooled for all the stages across both the environments, such that its net activity was 36% higher than that found for C-306 (Table 4).

Discussion

It has been found that many temperate plant species, particularly wheat, store a large portion of carbon in the form of water soluble carbohydrates (WSCs), primarily consisting of a range of fructo-oligosaccharides (fructans) in the stem (Xue et al. 2008). These reserves serve, in general, two important

Table 4 Activity of alkaline invertase in developing grains of *Triticum dicoccoides* and check wheat cultivars under irrigated and rain-fed conditions

Sr.No.	Accessions	Stages	Irrigated	Rain-Fed	Mean (genotypes)
1	**7054**	10 DAA	557.93	498.45	**373.84**
		20 DAA	455.10	341.29	
		30 DAA	224.28	166.03	
		MEAN	**412.43**	**335.26**	
2	**7079**	10 DAA	471.74	574.38	**387.69**
		20 DAA	449.06	392.40	
		30 DAA	272.90	165.69	
		MEAN	**397.90**	**377.49**	
3	**14004**	10 DAA	604.87	446.38	**369.09**
		20 DAA	421.17	362.60	
		30 DAA	233.37	146.15	
		MEAN	**419.80**	**318.37**	
4	**PBW-343**	10 DAA	472.83	392.62	**284.34**
		20 DAA	351.54	233.00	
		30 DAA	135.20	120.90	
		MEAN	**319.86**	**248.83**	
5	**C-306**	10 DAA	470.91	399.06	**285.13**
		20 DAA	299.23	247.44	
		30 DAA	138.63	155.52	
		MEAN	**302.92**	**267.34**	
	MEAN (ENVIRONMENT)		**370.58**	**309.46**	

Least significant difference (0.05): Genotypes averaged over environments - 41.68

Environment averaged over genotypes - 26.36

Values are means of three replicates

Activity of alkaline invertase is represented as μmole of reducing sugars formed min^{-1} g^{-1} FW

functions, (a) stored resources may give plants a competitive advantage (Heilmeier et al. 1986), (b) resources may bridge spatial and temporal gaps that exist between resource availability and resource demand (Kleijn et al. 2005), particularly under stress conditions. Further, fructans, the major carbohydrate portion of WSCs, might act as osmolytes enhancing water retention (Kawakami et al. 2008) and protect plants from drought and cold stress by stabilizing cellular membranes (Hincha et al. 2008).

Stem WSCs accumulation is influenced by the environmental factors (Ruuska et al. 2006). Considerable genotypic variation in stem WSCs has been observed in wheat and barley. However, in wheat crop with drought stress during grain-filling period, stem WSCs could potentially contribute to >50% of grain yield (Brooks et al. 1982). In our study, acc 14004 was found to mobilize maximum amount of water soluble carbohydrates (341.08 mg g^{-1} peduncle DW) from its peduncle under irrigated conditions (Table 2). Acc 7079 and C-306, on the other hand, mobilized maximum amount of these WSCs (263 mg g^{-1} peduncle DW) under rain-fed conditions. However, when the analysis was conducted across the two environments and the four stages, acc 7079 was found to be the most efficient mobilizer of soluble sugars (236.43 mg g^{-1} peduncle DW).

This is generally accepted that grain-filling rate in cereals is mainly determined by sink strength (Liang et al. 2001). During grain-filling period of wheat, kernels are very strong sinks for carbohydrates (Riffkin et al. 1995). The sink strength can be described as the product of sink size and sink activity (Venkateswarlu and Visperas 1987). Sink activity is a physiological restraint that includes multiple factors and key enzymes (invertase and sucrose synthase) involved in carbohydrate use and storage (Wang et al. 1993). Activity of acid and alkaline invertase investigated in the present study in the accessions of *T. dicoccoides* and cultivated wheats demonstrated significant genotypic variation and far higher activity of enzymes in *T. dicoccoides* relative to cultivated wheats. In fact, activity of acid invertase for each genotype averaged across the two environments clearly distinguished wild accessions which showed almost double the activity of this enzyme when compared with cultivated wheats (Table 3), encouraging us to opine that activity of acid invertase was capable of demonstrating acute differences between the two diverse groups of germplasm. Higher activity of the enzymes was in line with higher levels of WSCs identified in them- acc 14004 under irrigated and acc 7079 under rain-fed conditions. The observation of such a trend in these wild accessions may partly be due to the influence of environment where they originated and later evolved, i.e., eastern Mediterranean region, characterized by a long, hot dry summer and a short, mild wet winter with fluctuating amounts and distribution of rainfall (Loss and Siddique 1994; Peleg et al. 2005).

Physio-biochemical traits including water soluble carbohydrates and invertases are increasingly being integrated in breeding for stress tolerance. Delineation of prospective donor germplasm and underlying genetic mechanisms thus assumes significance. This is reflected in recent literature eg. identification of germplasm and relevant genomic regions for WSCs in relation to grain filling in winter wheat accessions (Zhang et al. 2014a). In another study, WSCs related cleaved amplified polymorphism (CAP) marker has been identified for use in breeding programmes (Zhang et al. 2014b). The present work is also oriented to similar research strategy but with relatively less explored wild wheat lines.

Thus, to conclude it can be said that *Triticum dicoccoides* accession 7079 (EC 171837), with its greater concentration of WSCs and higher enzymatic expression, i.e., higher activity of acid invertase and highest alkaline invertase activity, especially under rain-fed environment, looks promising to be involved as a donor accession in breeding programme for enhancing drought tolerance in cultivated wheat. Further, the use of such genetically divergent donors might open opportunities for exploring stress tolerance mechanisms that domestication and modern agriculture have missed out and also provide scope for discovery of new alleles as well as the use of this allelic diversity for functional analysis.

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