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Activities of sucrose to starch metabolizing enzymes during grain filling in late sown wheat under water stress

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Abstract: Tolerance to water deficit in relation to activities of sucrose-to-starch metabolizing enzymes and starch accumulation was studied in the grains of contrasting wheat (*Triticum aestivum* L.) genotypes (WH1021 and WH1080; tolerant) and (WH711 and HD2687; susceptible) under late planting conditions. The activities of starch metabolizing enzymes i.e. sucrose synthase (SuSase), ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS) and starch branching enzymes (SBE) were substantially enhanced by water deficit in all genotypes at early to mid-grain filling stage showing peaks at 14 to 21 days after anthesis (DAA); while decreased significantly at mid-late grain filling stage with maximum decline at 35 DAA. Activities of all the enzymes under study showed maximum decline in activity (28.4–60%) in susceptible genotype WH711; whereas WH1021 proved to be most tolerant one with minimum decline in enzyme activity (14.9–32.8%). Starch content was also markedly reduced (21%) in WH711 due to drought while WH1021 reported 12% decline corresponding well with enzyme activity. A faster pre-mature cessation of starch deposition occurred in susceptible wheat genotypes compared to tolerant ones. A significant and positive correlation of the enzyme activities with starch accumulation ($r = 0.491–0.555$ at $P_{0.05}$ for SuSase, AGPase, SSS and $r = 0.638$ at $P_{0.01}$ for SBE) under well watered conditions indicated that enhancing the activities of the enzymes would lead to increase in starch accumulation and thus faster grain filling. Genotype WH1021 proved to be most efficient based on comparatively higher enzyme activity and least yield penalty under late planting conditions combined with water scarcity.

Keywords: Drought, Enzymes, High temperature, Starch, Wheat, Yield

INTRODUCTION

Water scarcity and high temperature (>30°C) at the time of grain filling is one of the major constraints in increasing productivity of wheat in tropical countries like India. Wheat is sensitive to soil drought as important stages of wheat development (stem elongation, heading-flowering, grain filling) occur during the time when the water deficit in the soil increases in rain-fed regions (Allahverdiyev *et al.,* 2015). Enzymes involved in starch synthesis, including sucrose synthase (SuSase), ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS) and starch-branching enzyme (SBE) play key roles in starch synthesis in grain. Reduced starch content resulting from decreased activity of sucrose-to-starch metabolizing enzyme accounts for most of the reduction in grain dry matter and thus yield under conditions of post-anthesis drought stress (Gao *et al.,* 2003; Yi *et al.,* 2014). Sucrose synthase catalyses the cleavage of sucrose, the main transported form of assimilates in wheat plants to form UDP-glucose and fructose, which is thought to be the first step in the sucrose-to-starch conversion. AGPase produces ADP-Glucose, the primer of the starch chain (Smith and Denyer, 1992), and is regarded as the rate-limiting enzyme in starch biosynthesis (Preiss, 1988). SSS elongates the amylose and amylopectin chains (De´jardin *et al.,* 1997). The activity of SSS is reported to be positively correlated with the rate of starch synthesis in wheat grains (Keeling *et al.,* 1993). SBE is the sole enzyme capable of forming α-1,6 linked branches on already synthesized and/or elongating amylose molecules (Preiss *et al.,* 1991) and thereby plays a key role in starch production in wheat endosperm.

Extensive studies have been done on the effects of heat and drought stress on the activities of enzymes involved in sucrose-to-starch metabolism in cereals (Gao *et al.,* 2003; Jianchang *et al.,* 2004; Yan *et al.,* 2008). Ahmadi and Baker (2001) reported that reduction in grain growth rate of water stressed wheat plants resulted from a reduced SSS activity, whereas growth cessation was mainly due to the inactivation of AGPase. Grain filling in cereals is mainly determined

by sink strength which can be described as the product of sink size and sink activity (Liang *et al.,* 2001). Sink activity is a physiological restraint that includes multiple factors and key enzymes involved in carbohydrate utilization and storage. As information is scarce on changes in activities of key enzymes in sucrose-to-starch catalytic pathway in water stressed wheat grains during the filling period, more work was needed to achieve a better understanding of these important aspects of the regulation of starch synthesis and to apply this knowledge for crop improvement. The present investigation was, therefore, carried out to study the changes in activities of SuSase, AGPase, SSS, SBE and their relationship with the accumulation of starch, amylose and amylopectin in the grains of wheat plants subjected to water deficit under late sown conditions.

MATERIALS AND METHODS

Plant materials and growing conditions: The experiment was conducted in the fields of Wheat Section of the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. Two tolerant (WH1021, WH1080) and two susceptible (WH711, HD2687) wheat genotypes were tested under late planting conditions for differential response to water stress tolerance at 7, 14, 21, 28 and 35 days after anthesis (DAA) for enzymatic studies and starch accumulation. Water deficit was created by withholding the irrigation at anthesis stage till maturity. Average gravimetric soil moisture content at anthesis was recorded to be 13.1% (0–15 cm) and 17.9% (15–45 cm); while 10.1% (at 0–15 cm) and 14.7% (15–45 cm) at maturity. In late sown (16th December) conditions, sowing was delayed by about one month than normal sowing which exposed the plants to mean maximum temperatures of up to 4.23°C and mean minimum temperatures of up to 2.93°C higher during grain growth duration. The experiment was laid out in randomized block design (RBD) with three replications. Plot dimensions were 3 m x 1.2 m with inter-plant and inter-row spacing of 5 cm and 22.5 cm, respectively. Plants were tagged at the time of anthesis and labeled ears of each plot were sampled at weekly interval from 7-35 DAA and stored in liquid nitrogen for estimation of enzyme activities. Grain yield from plot (excluding the border plants) was recorded in grams at maturity. Weight and number of total grains per spike from five spikes was taken and average was recorded.

Extraction and assay of starch metabolizing enzymes: Nearly 15-20 developing grains amounting to 0.5 g of the grains were removed from middle portion of spikes at 7, 14, 21, 28 and 35 DAA and were homogenized in a pre-chilled pestle and mortar at 4°C with 2 ml of extraction buffer [50 mM of 3-(N-morpholino) propane sulphonic acid (MOPS) pH 7.4, 2 mM of $MgCl_2$, 1 mM of EDTA and 2 mM of Dithiothritol (DTT)]. The homogenate was centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge at 4° C. Protein in the enzyme extract was measured (Lowry, 1951) using bovine serum albumin as standard and the enzyme activities were expressed on the basis of nmol min^{-1} mg $protein^{-1}$.

Enzyme assays

Sucrose synthase (EC 2.4.2.13): Sucrose synthase was assayed by the method of Shannon and Dougherty (1972). One ml of reaction mixture containing UDP (1 µmol), sucrose (25 µmol) and MES buffer (pH 6.5, 60 µmol) was incubated with 0.2 ml of enzyme preparation at 37°C for 15 min. The reaction was terminated by placing the reaction mixture in boiling water bath for 2 min. Released fructose was measured by method of Somogyi (1945).

ADP glucose pyrophosphorylase (EC 2.7.7.27): AGPase was assay in the reverse direction by modified method of Kleczkowski *et al.* (1993). One ml of reaction mixture [50 mM MOPS (pH 7.4), 7.5 µmol $MgSO_4.7H_2O$, 3 µmol 3-PGA, 0.5 µmol $NADP^+$, 0.5 µmol ADP-glucose, 2 units Phosphoglucomutase and 2 units glucose-6-phosphate dehydrogenase] was incubated with 100 µl of enzyme extract. The reaction was

Table 1. Grain yield and yield components of wheat genotypes under irrigated and drought stress conditions at physiological maturity.

Parameter	Genotype	Irrigated	Drought	Percent reduction
Grain yield/plot (g)	WH1021(T)	952.0^{a}	835.0^{c}	12.29
	WH1080(T)	862.5^{b}	719.0^{de}	16.64
	WH711(S)	700.0^{e}	471.5^{g}	32.64
	HD2687(S)	737.5^{d}	512.5^{f}	30.51
Grain weight/spike (g)	WH1021(T)	2.02^{a}	1.79^{b}	11.39
	WH1080(T)	1.65^{c}	1.43^{e}	13.33
	WH711(S)	1.49^{de}	1.18^{f}	20.81
	HD2687(S)	1.54^{d}	1.27^{f}	17.53
Grain number/spike	WH1021(T)	65^{a}	57^{c}	12.31
	WH1080(T)	61^{b}	49^{d}	19.67
	WH711(S)	49^{d}	35^{f}	28.57
	HD2687(S)	58^{c}	44^{e}	24.14

Means with different letter are statistically significant at P0.05 using Fisher's Least Significant Difference. T- Tolerant; S- Susceptible

Table 2. Effect of drought stress on the activities of sucrose-to-starch metabolizing enzymes at different days after anthesis (DAA) in the grains of late sown wheat genotypes.

Genotype	Enzyme Activities (nmol min^{-1} mg $protein^{-1}$)	7 DAA		14 DAA		21 DAA		28 DAA		35 DAA	
		IR	D	IR	D	IR	D	IR	D	IR	D
WH1021(T)		29.10^{c}	32.58^{a}	33.90^{b}	42.15^{a}	41.23^{a}	40.25^{b}	35.36^{a}	30.21^{c}	31.76^{a}	26.65^{c}
WH1080(T)	Sucrose	28.29^{d}	31.65^{b}	32.11^{b}	39.85^{a}	39.24^{c}	37.95^{d}	32.99^{b}	27.96^{d}	30.09^{b}	24.25^{d}
WH711(S)	Synthase	19.28^{g}	20.58^{f}	23.50^{d}	28.85^{c}	30.25^{ef}	29.15^{g}	25.36^{e}	19.15^{f}	21.01^{e}	15.05^{f}
HD2687(S)		20.27^{f}	21.99^{e}	23.66^{d}	29.25^{c}	30.57^{e}	29.55^{fg}	25.49^{e}	19.56^{f}	21.46^{e}	15.58^{f}
WH1021(T)		1.59^{bc}	1.92^{a}	3.35^{c}	4.35^{a}	4.93^{a}	4.60^{a}	3.50^{a}	2.65^{b}	2.40^{a}	1.72^{b}
WH1080(T)	Soluble Starch	1.35^{d}	1.61^{b}	2.95^{d}	3.81^{b}	3.90^{b}	3.68^{b}	2.60^{b}	2.05^{c}	1.75^{b}	1.15^{c}
WH711(S)	Synthase	1.09^{e}	1.27^{de}	1.78^{f}	2.45^{e}	2.73^{c}	2.42^{c}	1.70^{cd}	1.19^{e}	1.13^{c}	0.55^{d}
HD2687(S)		1.39^{cd}	1.61^{b}	1.85^{f}	2.49^{e}	2.80^{c}	2.60^{c}	1.95^{c}	1.42^{de}	1.02^{c}	0.56^{d}
WH1021(T)	ADP-	9.70^{b}	13.10^{a}	16.50^{c}	23.50^{a}	23.20^{b}	25.20^{a}	25.20^{a}	17.20^{c}	12.50^{a}	8.40^{c}
WH1080(T)	glucose	8.20^{c}	10.90^{b}	13.30^{d}	18.40^{b}	19.20^{d}	21.20^{c}	22.50^{b}	14.80^{d}	10.50^{b}	6.90^{d}
WH711(S)	pyrophos-	5.20^{de}	6.10^{d}	8.90^{f}	11.90^{e}	15.10^{f}	12.90^{h}	9.70^{e}	4.90^{g}	4.40^{e}	2.10^{fg}
HD2687(S)	phorylase	3.90^{e}	4.90^{de}	9.20^{f}	12.50^{de}	16.20^{e}	14.20^{g}	7.90^{f}	4.80^{g}	2.90^{f}	1.70^{g}
WH1021(T)	Starch	18.70^{b}	22.30^{a}	33.90^{c}	45.20^{a}	55.60^{a}	51.90^{b}	43.60^{a}	35.60^{c}	33.01^{a}	28.10^{b}
WH1080(T)	Branch-	16.20^{c}	22.10^{a}	26.70^{e}	37.20^{b}	45.60^{c}	41.60^{d}	39.10^{b}	30.50^{e}	25.20^{c}	19.90^{d}
WH711(S)	ing En-	14.20^{d}	18.90^{b}	25.60^{f}	34.50^{c}	40.30^{e}	36.50^{f}	31.80^{d}	21.50^{f}	18.40^{e}	7.50^{f}
HD2687(S)	zyme	12.20^{e}	16.50^{c}	22.10^{g}	32.60^{d}	40.30^{e}	36.50^{f}	32.50^{d}	21.50^{f}	19.20^{de}	7.50^{f}

Statistical comparison was among wheat genotypes within each sampling date separately. Means were tested by LSD at $P_{0.05}$ level ($LSD_{0.05}$). Means with different letter are statistically significant while atleast one letter common shows they are statistically insignificant using Fisher's Least Significant Difference. T- Tolerant; S- Susceptible; IR- Irrigated; D- Drought.

started by the addition of 200 µl of sodium pyrophosphate (2.5 µmol). The pyrophosphorolytic activity of AGPase was assayed spectro-photometrically by monitoring the increase in absorbance due to conversion of NADP to NADPH at 340 nm.

Soluble starch synthase (EC 2.4.1.21 UDPG (ADPG): α -1, 4-glucan α -4-glucosyl transferase): ADP-glucose and UDP-glucose starch synthase were assayed following the colorimetric method (Leloir *et al.,* 1961) using amylopectin as the primer. Reaction mixture (0.4 ml) containing 20 µmol of glycine buffer (pH 8.3); 0.15 µmol EDTA; 4 mg amylopectin; 1.5 mg glutathione, and 0.5 µmol ADP-glucose or UDP-glucose was incubated with 0.1 ml of enzyme preparation in shaking water bath at 37°C for 1 h. Reaction was stopped by dipping the reaction mixture in a boiling water bath for 2 minutes. The ADP (or UDP) formed was measured by the pyruvate kinase procedure. After the reaction was stopped, 20 µl each of phosphoenol pyruvate (0.02M in 0.4 M KCl) and pyruvate kinase (25 unit, freshly diluted with 0.1 M $MgSO_4$) were added and the contents were incubated for another 30 minutes at 37°C followed by the addition of 0.2 ml of 2, 4-dinitrophenyl hydrazine (0.1% in 2N HCl), 10N NaOH (0.2 ml) and 95% ethanol (4 ml), at an interval of 5 min each. The contents were mixed and centrifuged. The absorbance of the brown colored supernatant was measured at 520 nm and compared with control in which the reaction mixture was boiled immediately after the addition of enzyme extract.

Starch branching enzyme (EC 2.4.1.18 α -1, 4-glucan-6-glucosyl transferase): Decrease in the absorbance of iodine-amylose complex after incubation of enzyme extract with amylose, provided a measure of the activity of starch branching enzyme (Hawker and Downton, 1974). The reaction mixture (1.5 ml) containing sodium citrate (100 µmol), amylose (300 µg) and enzyme extract (0.1 ml) was incubated at 30°C for 15 min. The reaction was stopped by the addition of 0.5 ml of 2 N HCl followed by the addition of 1 ml of iodine reagent [0.5 ml of iodine stock solution (6 g KI and 600 mg I_2 in 100 ml of water) added to 50 ml of 0.05N HCl]. Water was added to the reaction mixture to give a final volume of 5 ml. The absorbance of the amylose-iodine complex was read at 590 nm. Amylases have been taken care by giving heat treatment at 70°C for 20 min and $HgCl_2$ treatment as α-amylases are heat labile whereas, $HgCl_2$ is a potent inhibitor of β -amylase activity at concentration of 10 µmol.

Estimation of starch, **amylose and amylopectin content:** Starch was estimated following method of Clegg (1956). Amylose was estimated by the method of Williams *et al.* (1970). The content of amylopectin was calculated from the difference between total starch and amylose content.

Statistical analysis: Data were analyzed according to randomized block design, performing analysis of variance (ANOVA) using Statistical Analysis Package (SAS, version 9.2) for each sampling date separately. Means were tested by LSD at $P_{0.05}$ level ($LSD_{0.05}$) and significance of differences among treatments and genotypes were determined**.**

Table 3. Starch, Amylose and Amylopectin content at different days after anthesis (DAA) in the grains of late sown wheat genotypes under irrigated and drought stress conditions.

Wheat Genotypes	mg g^{-1}DW	7 DAA		14 DAA		21 DAA		28 DAA		35 DAA	
		IR	D	IR	D	IR	D	IR	D	IR	D
WH1021(T)	Starch	60.00^{b}	67.00^{a}	201.00^{c}	293.00^{a}	400.67^{c}	464.00^{a}	697.00^{b}	619.00^{d}	745.00^{a}	655.00^{e}
WH1080(T)		58.00^{b}	66.00^{a}	199.00^{c}	265.00^{b}	386.00^{d}	442.00^{b}	702.00^{a}	611.00^{e}	739.00^{b}	639.00^{f}
WH711(S)		31.00^{f}	34.00^{e}	122.00^{g}	147.00^{e}	264.00^{h}	325.00^{f}	689.00^{c}	559.00^{g}	709.00^{d}	561.00^{h}
HD2687(S)		47.00^{d}	53.00^{c}	136.00^{f}	170.00^{d}	273.00^{g}	335.00^{e}	698.00^{ab}	585.00^{f}	719.00^{c}	591.00^{g}
WH1021(T)	Amylose	15.00^{bc}	18.00^{a}	54.00^{d}	76.00^{a}	116.00^{a}	100.00^{b}	169.00^{a}	151.00^{b}	184.00^{a}	163.00^{d}
WH1080(T)		12.00^{e}	13.00^{de}	49.00^{ef}	64.00^{b}	101.00^{b}	97.00^{c}	153.00^{b}	131.00^{e}	172.00^{b}	149.00^{f}
WH711(S)		13.00^{de}	14.00^{cd}	48.00^{f}	58.00^{c}	85.00^{e}	66.00^{g}	137.00^{d}	111.00^{g}	156.00^{e}	121.00^{h}
HD2687(S)		14.00^{cd}	16.00^{b}	51.00^{e}	63.00^{b}	93.00^{d}	72.00^{f}	141.00^{c}	115.00^{f}	168.00^{c}	139.00^{g}
WH1021(T)	Amylopec-tin	45.00^{c}	49.00^{a}	147.00^{d}	217.00^{a}	284.67^{c}	364.00^{a}	528.00^{c}	468.00^{e}	561.00^{b}	492.00^{d}
WH1080(T)		46.00^{c}	51.00^{a}	150.00^{d}	201.00^{b}	285.00^{c}	345.00^{b}	549.00^{b}	480.00^{d}	565.00^{b}	490.00^{d}
WH711(S)		18.00^{f}	20.00^{f}	74.00^{h}	89.00^{f}	179.00^{f}	259.00^{e}	552.00^{ab}	448.00^{f}	553.00^{c}	440.00^{f}
HD2687(S)		33.00^{e}	37.00^{d}	85.00^{g}	107.00^{e}	180.00^{f}	263.00^{d}	557.00^{a}	470.00^{e}	551.00^{c}	452.00^{e}

Statistical comparison was among wheat genotypes within each sampling date separately. Means were tested by LSD at $P_{0.05}$ level ($LSD_{0.05}$). Means with different letter are statistically significant while atleast one letter common shows they are statistically insignificant using Fisher's Least Significant Difference. T- Tolerant; S- Susceptible; IR- Irrigated; D- Drought.

RESULTS AND DISCUSSION

Yield and yield attributes: Water scarcity combined with late sowing significantly decreased yield and its components in wheat genotypes (Table 1). Tolerant genotypes WH1021 and WH1080 maintained their yield under drought stress conditions as indicated by less reduction (12.29 and 16.64%) in comparison to susceptible genotypes, WH711and HD2687. Grain weight and grain number per spike were also significantly reduced under drought stress conditions showing more than twice reduction in susceptible genotypes over tolerant ones. Genotype WH1021 was found to be superior in retaining grain number under both irrigated and drought stress conditions as compared to other genotypes. Earlier study by Gao *et al.* (2003) also confirmed the activities of SuSase, AGPase, SSS and SBE peaked at 15-20 DAA; while starch accumulation peaked at 25-30 DAA. In addition Yan *et al.* (2008) reported marked reduction in starch accumulation due to high temperature stress resulting in reduced grain number and yield also supports our results.

Changes in activities of enzymes: Table 2 shows changes in activities of enzymes under irrigated and drought stress conditions at different days after anthesis. Under drought stress, SuSase activity increased within 7-18 DAA and reached its peak within 16-18 DAA, thereafter it declined to values lower than those in irrigated conditions after 21 DAA. The study by Jianchang *et al.* (2004) also reported substantially enhanced SuSase activity in drought stressed wheat grains peaked at 18-21 DAA with a sharp decline thereafter. These results suggest that water stress enhanced the capacity of plants to break down and take up of sucrose during early grain filling. Since SuSase is involved in sucrose cleavage in sink tissue, its activities are regarded as biochemical markers of sink strength (Ibrahim *et al.,* 2013). AGPase activity in WH1021 and WH1080 peaked at 28 DAA under irrigated condition and at 21 DAA under drought stress conditions, whereas WH711 and HD2687 showed their peaks at 21 DAA under irrigated condition and within 16-18 DAA under drought conditions. Earlier Jianchang *et al.* (2004) had also reported AGPase activity exhibited a peak at 21-27 DAA in well watered grains and 15-18 DAA in water deficit wheat grains. Cao *et al.* (2012) also reported AGPase, SSS and SBE activities each showed a single peak curve during grain filling and peaked at 20-25 DAA in accordance with findings of our study. These results indicated that water deficit contributed to the enhancement of AGPase activity during early-mid grain filling, whereas water supply increased AGPase significantly during mid-late grain filling, for the all genotypes. SSS and SBE activities in irrigated grains increased from 7-21 DAA with grain development. It was substantially enhanced by drought stress within 7-18 DAA and then declined as compared to irrigated grains. The peak exhibited by enzyme activities in wheat grains under drought stress were at lesser days after anthesis in comparison to peak exhibited under control conditions. This implies that activity in water deficit grains was greater initially and declined faster after reaching a maximum when compared to well-watered grains.

Starch, **amylose and amylopectin content:** Starch content (Table 3) shows a rapid accumulation period over 7-28 DAA. Tolerant genotypes (WH1021 and WH 1080) had nearly same amount of starch content up to 14 DAA and they behaved almost the same. The water deficit greatly accelerated starch accumulation in the grains of susceptible (WH711and HD2687) genotypes up to 21 DAA while it slowed down later at mid-late grain filling in comparison to well watered control. Significant reduction in starch content of susceptible genotypes suggested a reduction in active grain filling period under the influence of drought. On 14 DAA, amylose content of both control as well as stressed

Table 4. Relationship of starch content in wheat grains with the activities of enzymes involved in sucrose-to-starch metabolism during the rapid starch accumulation period (7-28th DAA) under irrigated and drought stress conditions.

Correlations (n=16)	Starch content	
	Irrigated	Drought
Sucrose Synthase (SuSase)	0.504*	0.211
ADP-glucose pyrophosphorylase (AGPase)	0.555*	0.234
Soluble starch synthase (SSS)	0.491*	0.178
Starch branching Enzyme (SBE)	0.638**	0.341

* and ** indicate correlation significance at the $P_{0.05}$ and $P_{0.01}$ levels of probability.

grains was significantly increased by about 4 times of the initial value (7 DAA); afterwards it showed a steady increase up to 28 DAA. Both susceptible genotypes significantly differed in amylopectin content at all sampling stages from 7-35 DAA following the same pattern as starch. Earlier Kaur *et al.* (2011) had also reported that high temperature and drought resulted in reduced grain sink potential and mature grain mass caused by reduction in endosperm size and starch accumulation. Further study by Yi *et al.* (2014) stating decrease in total starch, amylase and amylopectin all at mid-late stage of grain filling under drought is in accordance with the results presented in this study regarding slowed down starch accumulation at mid-late grain filling. Since the partitioning of carbon into starch reserves, depends on assimilate supply as well as demand, the decreased starch level could have been due to either photo-assimilate supply during the period of intense reserve accumulation or to a direct impairment of the starch synthesis machinery as a result of sink dehydration (Bhargava and Sawant, 2013).

Correlations of enzyme activities with starch accumulation: The correlations of activities of all the four enzymes with starch content were analyzed (Table 4). Activities of SuSase, SSS and AGPase were positively and significantly correlated with starch accumulation (r = 0.491* to 0.555*, P = 0.05), while that of SBE was positively and more significant correlated with starch accumulation (r = 0.638**, P = 0.01) under control conditions but not so under water deficit. A close correlation of enzymatic activity with starch accumulation rate has been observed in rice and wheat grains (Nakamura and Yuki, 1992; Lu *et al.,* 2008; Cao *et al.,* 2012) which suggest that accelerated grain filling under water deficit is mainly associated with enhanced sucrose to starch metabolizing enzyme activity in wheat grains. Also a significant correlation of these enzyme activities with starch accumulation under well watered conditions indicated that enhancing the activities of the enzymes would lead to increase in starch accumulation and thus grain growth. This may lead to a faster grain filling and thus help in avoiding the unfavorable environmental conditions during grain filling of wheat.

Conclusion

Water stress initially enhanced activities of SuSase, AGPase, SSS and SBE at early to mid-grain filling stages while it led to significant decrease (28.4-60% in susceptible; 14.9-32.8% in tolerant genotypes compared to well-watered control) in the activity of these enzymes at mid-late grain filling stage. These results suggest that a mild water deficit during early grain filling could prove beneficial by enhancing remobilization of stored reserves from the stems to grains and accelerate accumulation of starch and the grain-filling rate. The activities of these enzymes were positively correlated with starch accumulation suggesting that decrease in enzyme activity led to the reduction in grain weight and thus yield. A faster pre-mature cessation of starch deposition was noticed in susceptible wheat genotypes compared to tolerant ones. It was found that the comparatively higher enzyme activities in grains induced by water deficit are responsible for yield stability in cultivar (WH1021) which proved to be the most tolerant one for late sowing.

Future perspectives: Synthesis of starch during grain filling of wheat is a very dynamic and complicated process. Present study and future efforts made in this direction might help in understanding the control mechanisms associated with the pathway of starch biosynthesis and thus provide chemical means to manipulate starch content vis-à-vis grain yield under heat and drought. Recent advances in genomics technologies combined with knowledge of the mechanism by which sugars regulate grain set under drought and high temperature conditions could be exploited for generating germplasm that can be used in wheat breeding for abiotic stress tolerance and thus prove beneficial for food security under changing climatic scenarios.

REFERENCES

Ahmadi, A. and Baker, D.A. (2001). The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. *Plant Growth Reg*., 35: 81–91

Allahverdiyev, T.I., Talai, J.M., Huseynova, I.M. and Aliyev, J.A. (2015). Effect of drought stress on some physiological parameters, yield, yıeld components of durum (*Triticum durum* desf.) and bread (*Triticum aestivum* L.) wheat genotypes. *Ekin J. Crop Breed and Gen.,* 1: 50-62

Bhargava, S. and Sawant, K. (2013). Drought stress adaptation: metabolic adjustment and regulation of gene expression. *Plant Breed*., 132: 21–32

Cao, Y.N., Hu, W.H. and Wang, C.S. (2012). Relationship among the key enzymatic activities involved in starch synthesis and amylopectin chain distributions in developing wheat grain. *African J. Biotech*., 11: 805–814

Clegg, K.M. (1956). The application of anthrone reagent to the estimation of starch in cereals. *J. Sci. Food Agric*., 7: 40–44

De´jardin, A., Rochat, C.,Wuille´me, S. and Boutin, J.P. (1997). Contribution of sucrose synthase, ADP -glucose pyrophosphorylase and starch synthase to starch synthesis in developing pea seeds. *Plant Cell Environ*., 20: 1421–1430

Gao, S.J, Guo, T.C., Wang, W.J. and Han, J.F. (2003). Changes in the activities of enzymes involved in starch synthesis in the kernel during grain filling in winter wheat cultivars of different spike types. *Sci. Agric. Sinica*., 36: 1373–1377

Hawker, J.S. and Downton, W.J.S. (1974). Starch synthetases from *Vitis vinifera* and *Zea mays*. *Phytochem*., 13: 893–900

Ibrahim, A.H., Shahaby, O.A., Hamed, S.A. and Younis, M.E. (2013). Parental drought and defoliation effect on yield, grains biochemical aspects and drought performance of sorghum progeny. *J. Stress Physiol. Biochem*., 9: 258–272

Jianchang, Y., Jianhua, Z., Zhiqing, W., Guowei, X. and Qingsen, Z. (2004). Activities of key enzymes in sucrose-to-starch conversion in wheat grains subjected to water deficit during grain filling. *Plant Physiol*., 135: 1621–1629

Kaur, V., Behl, R.K., Singh, S. and Madaan, S. (2011). Endosperm and pericarp size in wheat (*Triticum aestivum L*.) grains developed under high temperature and drought stress conditions. *Cereal Res. Commun.,* 39: 515–524

Keeling, P.L., Bacon, P.J. and Holt, D.C. (1993). Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. *Planta,* 191:342–348

Kleczkowski, L.A., Villand, P., Luthi, E., Olsen, O. and Priers, J. (1993). Insensitivity of barley endosperm AGPase to 3-phosphoglycerate and orthophosphate regulation. *Plant Physiol*., 101:179-186

Leloir, L.F., Fekete, M.A.R. and Cardini, C.E. (1961). Starch and oligosaccharide synthesis from uridine diphosphate glucose. *J. Biol. Chem.,* 236: 636–641

Liang, J.S., Zhang, J.H. and Cao, X.Z. (2001). Grain sink strength may be related to the poor grain filling of indica-japonica rice (*Oryza sativa*) hybrids. *Plant Physiol*., 112: 470–477

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, J.L. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chemis*., 193: 265–275

Lu, B., Guo, Z. and Liang, J. (2008). Effects of the activities of key enzymes involved in starch biosynthesis on the fine structure of amylopectin in developing rice (*Oryza sativa* L.) endosperms. *Sci China Ser C: Life Sci.,* 51: 863–871

Nakamura, Y. and Yuki, K. (1992). Changes in enzyme activities associated with carbohydrate metabolism during development of rice endosperm. *Plant Sci.,* 82: 15–20

Preiss, J. (1988). Biosynthesis of starch and its regulation. In Preiss, J. (ed.), The Biochemistry of Plants, Academic Press, New York, 14: 184–249

Preiss, J., Ball, K., Smith-White, B., Iglesias, A., Kakefuda, G. and Li, L. (1991). Starch biosynthesis and its regulation. *Biochem. Society Trans*., 19: 539–547

Shannon, J.C. and Dougherty, C.T. (1972). Movement of ^{14}C labeled assimilates into kernels of *Zea mays* L. II. Invertase activity of the pedicel and placento-chalazal tissues. *Plant Physiol*., 49: 203–206

Smith, A.M. and Denyer, K. (1992). Starch synthesis in developing pea embryos. *New Phytol.,* 122: 21–33

Somogyi, M. (1945). A new reagent for the determination of sugar. *J Biol Chem.,* 160: 61-69

Williams, P.G., Kazima, F.D. and Hlynka, I. (1970). A rapid colorimetric procedure for estimating amylose content of starches. *Cereal Chem*., 47: 411–420

Yan, S.H., Yin, Y.P., Li, W.Y., Li, Y., Liang, T.B., Wu, Y.H., Geng, Q.H. and Wang, Z.L. (2008). Effect of high temperature after anthesis on starch formation of two wheat cultivars differing in heat tolerance. *Acta Ecologica Sinica*., 28: 6138–6147

Yi, B., Zhou, Z.F., Gao, M., Zhang, Z., Han, Y., Yang, G., Xu, W. and Huang, R. (2014). Effect of drought stress during flowering stages on starch accumulation and starch synthesis enzymes in sorghum grains. *J. Integrative Agric.,* 13: 2399-2406