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Effect of water stress at different stages of grain development on the characteristics of starch and protein of different wheat varieties

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Abstract

The effect of water stress (WS) at 8 and 15 days post anthesis (DPA) on the characteristics of starch and protein separated from C-306, HD-2329, PBW-175, PBW-343 and NI-5439 wheat varieties was studied. WS-induced changes in A-, B- and C-type granules distribution were variety- and stage-dependent. A-type granules increased in response to WS at both stages in all varieties, the extent of increase being greater at 15 DPA. The proportion of B-type granules decreased in all the varieties, except C-306, in response to WS at 15 DPA. C-type granules also decreased in response to 15 DPA in all varieties, except HD-2329. The starch from wheat exposed to WS at 15 DPA showed lower amylose content, lipids content and pasting temperature, and higher peak viscosity, final viscosity and setback. DSC analysis of starches showed two endotherms (associated with the melting of crystallites and amylose–lipid [AML] complexes) during heating, and an exotherm (associated with reforming of AML) during cooling. Transition temperatures (T_o, T_p and T_c) of AML dissociation and association were lower for starch from wheat exposed to WS, the effect being more at 15 DPA. The changes in pasting and thermal properties of starch caused by WS were observed to be related to lipids, amylose content and distribution of granules. The effect of WS on accumulation of different dimethyl formamide-soluble and insoluble proteins was significant and variety dependent.

Keywords: Wheat starch; Protein; Water stress; Thermal; Morphology; Pasting properties

1. Introduction

The quality of wheat grain is dependent on the characteristics of starch and protein present. Evers (1971, 1974), Simmonds and O'Brien (1981), Dengate and Meredith (1984), Morrison and Scott (1986) and Stoddard (1999) reported bimodal distribution of granules in wheat starch, while Bechtel, Zayas, Kaleikau, and Pomeranz (1990) and Raeker, Gaines, Finney, and Donelson (1998) found a trimodal distribution. The largest sized granules are called the A-type granules and are thought to form soon after anthesis and may continue to grow throughout grain filling. The intermediate-sized granules (B-type) and the smallest granules (C-type) are perhaps initiated at specific times after anthesis, depending on cultivar, growing location, and isolation method. Amylose content, branched chain-length distribution of amylopectin (Jane et al., 1999), phosphate monoester, phospholipid, and lipids content instead of lipid contents (Lim, Kasemsuwan, & Jane, 1994; Lin & Czuchajowska 1997; Morrison 1989; Soulaka & Morrison 1985; Tester & Morrison 1990), starch granule size distribution (Raeker et al., 1998; Sahlstrom, Brathen, Lea, & Autio, 1998), crystalline structures (Hizukuri et al., 1997), and granular architecture (Tester, Morrison, Gidley, Kirkland, & Karkalas, 1994) all affect the functional properties of starch.

Wheat is one of the most important food crops. Wheat is grown under irrigated as well as rain-fed conditions worldwide. Under rain-fed conditions the developing grains are frequently exposed to mild to severe stress at different stages of grain development. Although the effect of drought stress on grain development and its yield in wheat

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is well documented (Ahmadi & Baker, 2001; Barlow, Lee, Munns, & Smart, 1980; Khanna-Chopra, Rao, Maheswari, Xiaobing, & Shivshanker, 1994; Wardlaw, 2002), the studies on changes in characteristics of starch and protein of the mature grains, in response to water stress (WS) are, however, scanty. It is, therefore, imperative that a comprehensive analysis of the drought stress-induced changes in the starch and protein characteristics of wheat grains be carried out in cultivars categorised as drought-tolerant (recommended for rain-fed conditions) and drought-susceptible (recommended for irrigated conditions). In the present study, starch and protein separated from the mature grains of different wheat varieties, which were subjected to single drought stress treatment at 8 and 15 days post anthesis (DPA), were evaluated for stress-induced changes.

2. Materials and methods

2.1. Materials

Wheat seeds of drought-tolerant (C-306, PBW-175, NI-5439) and susceptible (HD-2329, PBW-343) varieties were collected from Punjab Agricultural University, Ludhiana (Punjab) and the Directorate of Wheat Research, Karnal (Haryana). Plants were raised in 5 l pots in the net house at Guru Nanak Dev University, Amritsar. Clay loam soil and farm manure (2:1 ratio) was mixed with 5 g per pot of N:P:K (5:2.5:1.2) and used for raising the plants. The sowing was done on 20th November and the grains were harvested on 13th April. After thinning, three plants were maintained in each pot. The plants were irrigated daily and the quantity of water applied was always in excess, up to full drainage. For imposition of WS at 8 and 15 DPA stages, water supply was withheld for 5 days followed by resumption of irrigation and the grain samples were harvested at maturity in three replicates, with each replicate comprising of plants from 8 to 10 different pots. Grains of each cultivar were finally milled by using Super Mill-1500 (Newport Scientific, Warriewood, Australia) to obtain whole wheat meal.

2.2. Starch isolation

Stiff dough was prepared by mixing (100 g) meal with distilled water (45–55 ml) in a laboratory pin mixer (National Mfg Co., Lincoln, NE) for 3 min at slow speed. The dough ball was kept covered with moist cheese cloth at 30 °C for 1 h. Starch was washed from the dough ball, by kneading with hand under a stream of distilled water, over a sieve with mesh openings of 70 μm, fitted over a 2 l vessel. The starch slurry was wet-sieved twice through the bolting cloth, to remove bran and endosperm cell-wall impurities. The material retained on the cloth was discarded. Starch slurry was then centrifuged at 2500*g* for 10 min. The upper pigmented layer (tailings) was carefully removed, using a small spatula, mixed with water (20 ml) and decanted from any starch which had settled after 30 min. The starch fraction plus starch from the decanting step were purified by resuspending in distilled water and centrifugation (Wolf, 1964). Four such purification cycles were carried out to obtain pure starch. The starch was finally dried at 40 °C in a forced-air drier. The starch recovery was between 45% and 48%.

2.3. Granule size analysis

Granule size distribution of starches was determined by the method described by Kaur, Singh, Sandhu, and Guraya (2004).

2.4. Amylose and lipids content

Apparent amylose content of the starches isolated from different varieties was determined using the method of Williams, Kuzina, and Hlynka (1970). The quantity of amylose was determined from a standard curve plotted using blends of amylose and amylopectin. Total lipids were extracted by the method of Hoover and Manuel (1996).

2.5. Pasting properties

Pasting properties of isolated wheat starches were studied using a Rapid Visco Analyzer (Newport Scientific, Australia). Viscosity profiles of starches from different wheat varieties were recorded using starch suspensions (6% w/w; 14% moisture basis, 28 g total weight). The temperature–time conditions included a heating phase from 50 to 95 °C at 6 °C/min, a holding phase at 95 °C for 1.5 min, a cooling phase from 95 to 50 °C at the rate of 6 °C/min and a holding phase at 50 °C for 2 min. A constant rotating paddle (160 rpm) was used. Pasting temperature, peak viscosity, trough viscosity, final viscosity and setback were obtained.

2.6. Thermal properties

Thermal properties were analysed using differential scanning calorimeter DSC-822 (Mettler Toledo, Greifense, Switzerland) equipped with a thermal analysis data station. Starch sample (3.5 mg dwb) was weighed into a 40 μl capacity aluminum pan (Mettler, ME-27331), and distilled water was added by Hamilton micro-syringe, to obtain a starch–water suspension containing 70% water. Pans were sealed and allowed to stand for 1 h at room temperature before heating in the DSC. The analyser was calibrated using indium and an empty aluminum pan was used as reference. Sample pans were heated at a rate of 10 °C/min from 40 to 110 °C and cooled at the same rate to 40 °C. Onset temperature (T_o); peak temperature (T_p); conclusion temperature (T_c) and enthalpy of gelatinisation (ΔH_{gel}) were calculated for the exotherm and the endotherms.

2.7. Protein isolation and quantification

The gliadin and glutenin fractions from the grains were extracted using the protocol as described (Abdemeshani & Najafian, 1994; Masouleh, 2005). Grains (3–5 grains, 200–300 mg tissue) from each control and stress sample were ground. Finely ground flour (150 mg) was mixed with 1.5 M dimethyl formamide in 1:5 ratio. The samples were homogenised thoroughly for 30 min at room temperature with intermittent vortexing. The samples were centrifuged at 6000 g and the supernatant (gliadin fraction) was stored at −20 °C till further analysis. The pellet was suspended in 1 ml of buffer, containing 0.125 M Tris–HCl (pH 6.8) and 1% SDS, and mixed thoroughly for 30 min. After centrifugation at 6000*g*, the pellet was re-suspended in 750 μl of buffer, containing 0.5 M Tris–HCl (pH 6.8), 2.75% SDS and 2% DTT. The pellet was dissolved and incubated at 60 °C for 1 h with intermittent vortexing. The samples were centrifuged at 6000*g* for 15 min at 4 °C and the supernatant (glutenin fraction) was stored at −20 °C till further analysis. Quantification of the protein samples were carried out using the Bradford method (Bradford, 1976), with BSA as the standard, and the samples were resolved on 12% SDS-PAGE.

2.8. Statistical analysis

The data reported are average of three replications and were subjected to analysis of variance using Minitab Statistical Software (State College, PA).

3. Results and discussion

Imposition of WS at both the stages resulted in decrease in grain water potential, thus implying that the plants were under stress. The inter-variety differences in the grain water potential were not significant (data communicated elsewhere).

3.1. Starch characteristics

3.1.1. Granules characteristics

Starches from all the varieties showed the presence of A-, B- and C-type granules. A-, B- and C-type granules with size of >15, 5–15 and <5 μm, respectively, have been reported previously (Bechtel et al., 1990). The change in proportion of A-, B- and C-type granules in response to WS was stage- and variety-dependent. The proportion of B-type granules decreased significantly in all varieties except C-306, in response to WS at 15 DPA (Table 1). Similarly C-type granules also decreased in response to stress at 15 DPA in all varieties except HD-2329. The stress-induced increase in A-type granules was observed at 8 DPA in HD-2329 and NI-5439, contrary to 15 DPA in PBW-175 and PBW-343. NI-5439 however, did not show any difference in proportion of A-type granules between stress at 8 and 15 DPA. The proportion of A-type granules was not significantly affected by stress at either of the stress stages in C-306. WS-induced increase in A-type granules was the highest in PBW-175 (from 76.57% in control to 83.63% at 15 DPA). Since formation of starch granules has been reported to be initiated 4–5 DPA (Briarty, Hughes, & Evers, 1979), granule size distribution was affected at both stages of grain development in all varieties except NI-5439. The variation in size and shape of starch granules observed in the study may be due to difference in genotype, as observed by Raeker et al. (1998). The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as physiology of the plant, as stated by Badenhuizen (1969).

Table 1
Effect of water stress on granule size distribution in starch separated from different wheat varieties

Variety	Treatment	A-type		B-type		C-type	
		Proportion (%)	Average diameter (μm)	Proportion (%)	Average diameter (μm)	Proportion (%)	Average diameter (μm)
C-306	Control	79.10^{e}	23.84^{a}	12.13^{b}	12.78^{c}	8.79^{cd}	2.63^{b}
	8 DPA	79.07^{e}	25.98^{b}	12.83^{b}	12.35^{bc}	8.09^{c}	2.22^{ab}
	15 DPA	79.81^{ef}	27.55^{de}	12.46^{b}	12.31^{bc}	7.73^{ab}	2.22^{ab}
HD-2329	Control	75.68^{c}	29.61^{f}	16.73^{f}	11.55^{a}	7.61^{ab}	2.28^{ab}
	8 DPA	80.90^{f}	27.06^{d}	11.33^{b}	12.37^{bc}	7.79^{b}	2.28^{ab}
	15 DPA	77.88^{d}	28.55^{ef}	13.83^{c}	11.97^{a}	8.29^{c}	2.29^{ab}
PBW-175	Control	76.57^{c}	32.15^{g}	14.74^{d}	11.25^{a}	8.69^{cd}	2.42^{ab}
	8 DPA	75.86^{c}	26.52^{c}	15.50^{e}	12.03^{b}	8.65^{cd}	2.32^{ab}
	15 DPA	83.63^{g}	27.66^{de}	9.14^{a}	12.53^{bc}	7.23^{a}	2.18^{a}
PBW-343	Control	71.36^{a}	24.96^{ab}	19.70^{g}	11.96^{a}	8.96^{de}	2.26^{ab}
	8 DPA	71.71^{a}	25.83^{bcd}	19.24^{g}	11.86^{a}	9.04^{e}	2.67^{b}
	15 DPA	76.82^{cd}	26.08^{c}	15.21^{e}	12.23^{b}	7.96^{bc}	2.24^{ab}
NI-5439	Control	73.70^{b}	25.54^{bc}	16.90^{f}	11.92^{a}	9.40^{e}	2.25^{ab}
	8 DPA	77.10^{cd}	25.50^{bc}	14.87^{de}	12.13^{b}	8.49^{cd}	2.20^{a}
	15 DPA	77.16^{cd}	25.80^{bcd}	14.37^{cd}	12.23^{b}	8.47^{cd}	2.19^{a}

Values with the same superscripts in a column do not differ significantly ($p < 0.05$).

3.1.2. Amylose and lipids content

Apparent amylose content (AAC) of starches from wheat exposed to WS at 15 DPA was significantly lower than their counterpart starches from unstressed or 8 DPA-stressed wheat (Table 2). It is likely that WS conditions at 15 DPA might have reduced specifically granule-bound starch synthase (GBSS) activity, which is involved in amylose biosynthesis (Robyt, 1984). AAC was observed to be positively correlated to B-type ($r = 0.79$, $p \leqslant 0.005$) and C-type ($r = 0.66$, $p \leqslant 0.05$) and negatively to A-type ($r = -0.80$, $p \leqslant 0.005$) granules. Lipids content was also lower in starches from wheat exposed to WS conditions. Total lipids content for starches from wheat exposed to WS at 15 DPA ranged between 0.38% and 0.45%, against 0.67% and 0.78% for starches from unstressed wheat. A reduction in lipids content on exposure to WS conditions has also been reported earlier in lupin seeds (Carvalho, Chaves, & Ricardo, 2005) and chickpea genotypes (Nayyar, Kaur, Singh, & Upadhyaya, 2006).

3.1.3. Pasting properties

The pasting temperature of starch separated from different wheat varieties varied between 80.6 and 87.2 °C, with the highest value being observed for PBW-343 starch. C-306, HD-2329 and PBW-175 starches showed significantly lower pasting temperature, when compared to starches from PBW-343 and NI-5439 (Table 2). Starch from wheat exposed to WS at 15 DPA showed significantly lower pasting temperature and higher peak viscosity, final viscosity and setback than that from unstressed wheat (Fig. 1). Peak viscosity of starches increased with exposure of wheat to WS at 8 and 15 DPA. PBW-343 starch showed significantly different setback, compared to starches from other varieties. Higher peak viscosity, final viscosity and setback in starches from wheat exposed to WS conditions may be attributed to a decrease in lipids content. Lipids inhibit

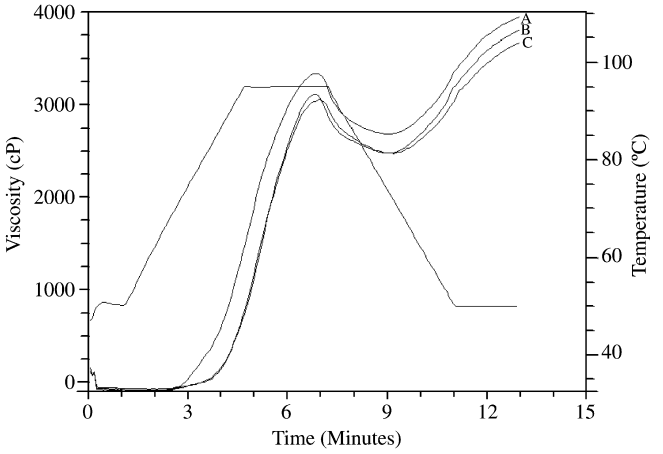

Fig. 1. Pasting curves of starches separated from PBW-175 ((A) 15 DPA, (B) 8 DPA and (C) Control).

starch swelling and amylose leaching during heating, which results in a decrease in hot paste viscosity. The effect of change in the proportion of A-, B- and C-type granules on pasting properties could not be ruled out also. Peak ($r = 0.66$; $p \leqslant 0.05$) and final viscosity ($r = 0.76$; $p \leqslant 0.005$) showed positive correlations with A-type and negative correlations with B-types granules. Pearson correlation coefficients of peak and final viscosity with B-type granules was -0.68 ($p \leqslant 0.005$) and -0.78 ($p \leqslant 0.005$), respectively. During cooling of heated starch pastes, viscosity increases due to aggregation of amylose (Miles, Morris, Orford, & Ring 1985). The final viscosity and setback were negatively correlated with AAC ($r = -0.71$ and -0.74, respectively; $p \leqslant 0.005$). Blazek and Copeland (in press) also observed a negative correlation of peak viscosity, final viscosity and setback of wheat starch with amylose content. The higher setback and final viscosity in starches separated from stressed wheat may be attributed to a decrease in lip-

Table 2
Effect of water stress on apparent amylose content and pasting properties of starch separated from different wheat varieties

Variety	Treatment	Apparent amylose content (%)	Pasting temperature (°C)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
C-306	Control	31.0^{e}	82.5^{c}	2970^{bc}	694^{f}	3500^{c}	1217^{c}
	8 DPA	30.7^{e}	81.7^{b}	3015^{c}	685^{f}	3720^{e}	1220^{c}
	15 DPA	26.8^{a}	80.2^{a}	3196^{d}	587^{cd}	3895^{ef}	1470^{e}
HD-2329	Control	30.1^{d}	84.6^{d}	2640^{a}	659^{e}	3175^{b}	1180^{c}
	8 DPA	28.3^{b}	82.3^{c}	2750^{d}	533^{bc}	3500^{c}	1197^{c}
	15 DPA	27.8^{b}	81.4^{b}	2870^{b}	725^{fg}	3800^{e}	1370^{d}
PBW-175	Control	30.2^{d}	82.2^{c}	3050^{c}	584^{cd}	3660^{d}	1200^{c}
	8 DPA	29.2^{c}	82.1^{c}	3106^{cd}	637de	3800^{e}	1262^{c}
	15 DPA	26.8^{a}	81.8^{bc}	3334^{f}	656^{e}	3940^{f}	1330^{d}
PBW-343	Control	34.7^{f}	87.2^{e}	2580^{a}	458^{a}	2830^{a}	710^{a}
	8 DPA	34.0^{g}	87.0^{e}	2990^{c}	766^{g}	3260^{b}	1023^{b}
	15 DPA	32.3^{f}	83.9^{d}	3000^{c}	610^{d}	3570^{c}	1185^{c}
NI-5439	Control	32.2^{e}	83.9^{d}	2866^{b}	487^{ab}	3575^{c}	1195^{c}
	8 DPA	31.3^{b}	82.2^{c}	3026^{c}	802^{g}	3653^{d}	1237^{c}
	15 DPA	28.1^{b}	80.6^{a}	3230^{e}	610^{d}	3795^{e}	1360^{d}

Values with the same superscripts in a column do not differ significantly ($p < 0.05$).

ids content rather than the change in amylose content. Lipids have been reported to reduce the reassociation (retrogradation) of amylose during cooling of heated pastes, due to formation of amylose–lipid complexes (AMLs). Therefore, in the starches from wheat exposed to WS conditions, a higher peak viscosity, final viscosity and setback were observed, due to a decrease in lipids. The changes in rheological properties of starches are usually dependent upon the proportion of small/large size granules, and amylose content, as well as AMLs (Singh & Kaur, 2004).

3.1.4. Thermal properties

The transition temperatures (T_o; T_p; T_c) and enthalpies of gelatinisation (ΔH_{gel}) of the starches from different wheat varieties are shown in Table 3. Starches separated from all the wheat varieties showed two endotherms during heating; the first and second endotherm is associated with the melting of crystallites and amylose–lipid complexes, respectively (Fig. 2). T_o, T_p, and T_c of the starches from all wheat varieties ranged between 56.1–59.3 °C, 61.3–63.7 °C and 66.2–69.9 °C, respectively. ΔH_{gel} of starches from different wheat varieties ranged from 9.3 to 11.3 J/g. ΔH_{gel} of the starches from all varieties, except NI-5439 and PBW-175, decreased with WS, however, the effect at 15 DPA was more pronounced. Double-helical and crystalline structures are disrupted in starches during gelatinisation. This order–disorder phase transition caused melting of crystals, which was illustrated by DSC endotherms in the range of 60–85 °C for various native starches (Jacobs, Eelingen, Clauwaert, & Delcour, 1995). ΔH_{gel} reflected primarily the loss of molecular (double-helical) order (Cooke & Gidley, 1992). High transition temperatures have been reported to result from a high degree of crystallinity, which provides structural stability and makes the granule more resistant to gelatinisation (Barichello, Yada, Coffin, & Stanley, 1990). T_o, T_p, and T_c for the second endotherm were in between 92.7 and 96.8 °C, 98.3 and 101 °C and 103 and 104 °C, respectively. PBW-343 starch showed the highest T_o, T_p, and T_c values for the second endotherm, whereas C-306 starch showed the lowest. Enthalpies of AML dissociation varied from 0.57 to 1.45 J/g. T_p and T_c for the second endotherm were negatively correlated with A-type granules ($r = 0.59$ and -0.69, respectively; $p \leqslant 0.05$), while a positive correlation of T_p and T_c with B- and C-type granules was observed. Pearson correlation coefficients of T_p and T_c of the second endotherm with B-type granules were 0.53 ($p \leqslant 0.05$) and 0.64 ($p \leqslant 0.05$), respectively, against 0.68 ($p \leqslant 0.005$) and 0.76 ($p \leqslant 0.05$), respectively, with C-type granules.

Upon cooling, all starches showed an exotherm, which had a peak temperature between 85.6 and 87.9 °C (Fig. 3). The appearance of an exotherm during cooling has been attributed to the reforming and possible crystallisation of AML (Ottenhof, Hill, & Farhat, 2005). Enthalpies of AML reassociation ranged between 0.91 and 1.52 J/g. Ottenhof et al. (2005) observed a second endotherm at 97 °C during heating and an exotherm at a peak temperature of ~86 °C during cooling in wheat starch. Similar melting and reforming temperatures have been reported in wheat flour by Jovanovich, Zamponi, Lupano, and Anon (1992). These complexes have been reported to be present in some native cereal starches (Morrison, Law, & Snape 1993) and more are formed during heating (Le Bail et al., 1999). T_o, T_p and T_c of the exotherm for the starch separated from wheat exposed to WS at 8 and 15 DPA were lower than those observed for their

Table 3
Effects of water stress on thermal properties of starch separated from different wheat varieties

Variety	Treatment	1st Endotherm				2nd Endotherm				Exotherm			
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)
C-306	Control	56.5^{a}	61.4^{c}	67.1^{bc}	10.1^{c}	93.0^{a}	99.8^{b}	104^{bc}	1.01^{ab}	88.2^{ab}	86.6^{ab}	83.7^{ab}	1.11^{ab}
	8 DPA	56.9^{a}	61.9^{c}	67.5^{c}	9.6^{b}	92.9^{a}	98.6^{a}	103^{a}	1.00^{ab}	87.7^{a}	86.1^{a}	83.0^{a}	1.16^{ab}
	15 DPA	58.0^{c}	63.0^{e}	68.6^{d}	9.0^{a}	92.7^{a}	98.3^{a}	103^{a}	1.03^{ab}	87.5^{a}	85.9^{a}	82.9^{a}	1.12^{ab}
HD-2329	Control	57.8^{bc}	62.8^{e}	69.2^{de}	11.3^{d}	93.0^{ab}	99.1^{ab}	103^{a}	0.97^{a}	88.5^{b}	86.7^{ab}	84.0^{b}	0.92^{a}
	8 DPA	57.2^{b}	61.8^{c}	66.8^{b}	10.3^{c}	93.0^{ab}	98.7^{a}	103^{a}	0.80^{a}	88.3^{ab}	86.6^{ab}	83.8^{ab}	0.95^{a}
	15 DPA	56.1^{a}	61.3^{a}	66.7^{ab}	9.3^{a}	92.7^{a}	98.5^{a}	103^{a}	0.97^{a}	88.0^{ab}	85.9^{a}	83.5^{ab}	1.22^{a}
PBW-175	Control	57.8^{b}	62.6^{d}	66.8^{b}	10.4^{c}	93.8^{ab}	99.8^{b}	104^{bc}	1.09^{ab}	88.5^{b}	86.9^{ab}	84.1^{bc}	0.73^{a}
	8 DPA	57.0^{b}	62.1^{d}	68.4^{d}	10.2^{c}	93.3^{ab}	98.7^{a}	103^{ab}	1.06^{ab}	88.0^{ab}	86.4^{ab}	82.9^{a}	1.27^{a}
	15 DPA	57.9^{bc}	61.9^{c}	67.8^{cd}	10.1^{c}	93.0^{ab}	98.4^{a}	102^{a}	0.73^{a}	87.1^{a}	85.6^{a}	82.7^{a}	0.91^{a}
PBW-343	Control	56.3^{a}	61.4^{bc}	66.2^{a}	10.4^{c}	96.8^{c}	101.1^{b}	105^{c}	0.57^{a}	90.7^{c}	89.1^{c}	86.3^{d}	1.10^{ab}
	8 DPA	56.3^{a}	60.9^{ab}	66.5^{c}	$9.7b^{c}$	93.8^{ab}	99.2^{b}	104^{bc}	1.38^{b}	88.3^{ab}	86.4^{ab}	83.8^{ab}	1.35^{b}
	15 DPA	56.4^{a}	60.5^{a}	66.2^{a}	9.4^{ab}	93.2^{ab}	98.4^{a}	102^{a}	1.04^{ab}	87.8^{a}	86.3^{a}	83.5^{ab}	1.12^{ab}
NI-5439	Control	59.0^{d}	63.7^{e}	69.4^{e}	10.6^{cd}	94.9^{b}	99.9^{b}	104^{bc}	0.91^{a}	89.5^{bc}	87.9^{bc}	85.1^{c}	1.19^{ab}
	8 DPA	58.9^{ab}	63.5^{e}	69.9^{e}	10.5^{c}	93.7^{ab}	98.8^{a}	104^{bc}	1.45^{b}	88.1^{ab}	86.4^{ab}	83.6^{ab}	1.52^{b}
	15 DPA	59.3^{d}	63.1^{e}	69.3^{e}	10.3^{c}	92.8^{a}	98.7^{a}	103^{ab}	0.95^{a}	87.7^{a}	86.2^{a}	83.4^{ab}	1.26^{b}

T_o = Onset temperature, T_p = Peak temperature, T_c = Endset temperature, ΔH_{gel} = Enthalpy of gelatinisation (dwb, based on starch weight). Values with the same superscripts in a column do not differ significantly ($p < 0.05$).

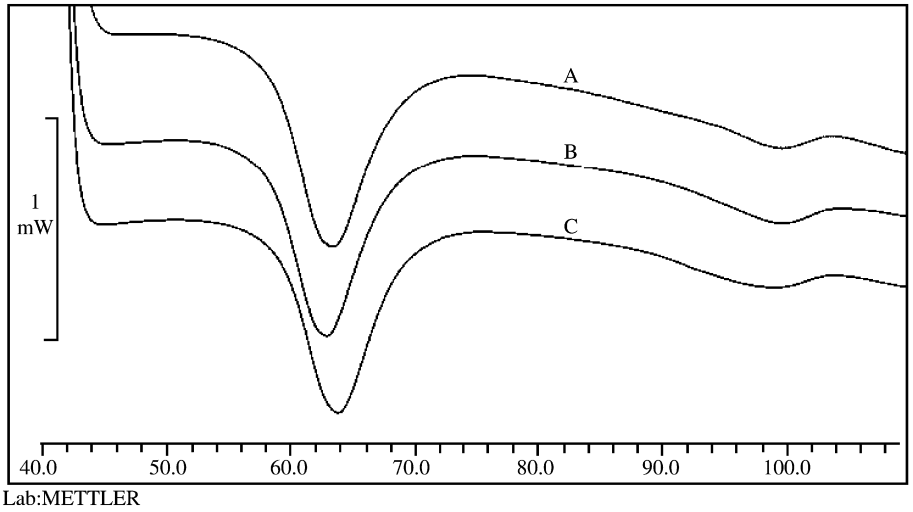

Fig. 2. DSC endotherms of starches separated from NI-5439 ((A) Control, (B) 8 DPA and (C) 15 DPA).

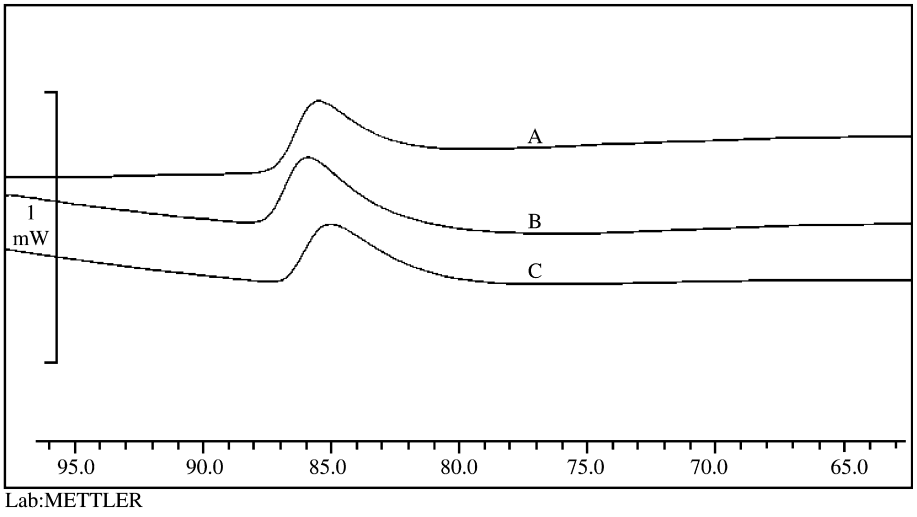

Fig. 3. DSC exotherms of starches separated from NI-5439 ((A) Control, (B) 8 DPA and (C) 15 DPA).

counterpart control starches. The effect of WS at 15 DPA was greater on T_o, T_p and T_c of the exotherm than at 8 DPA. Among the starches from different varieties, PBW-343 starch showed the highest T_o, T_p and T_c of the exotherm. A correlation between amount of lipids and AML content in wheat starches has been reported previously (Nebesny, Rosicka, & Tkaczyk, 2005). The starches separated from stressed wheat had a lower temperature of reassociation (exotherm), as compared to starch from unstressed wheat. This indicates that less stable AML were generated in the starches from stressed wheat. This may be attributed to lower amylose and lipids content in starch from stressed wheat. A decrease in fat content up to 39% has been reported in chickpea because of stress (Nayyar et al., 2006). T_p and T_c of the exotherm showed similar correlations with A-, B- and C-type granules as described for T_p and T_c of second endotherm.

3.2. Protein characteristics

3.2.1. Dimethylformamide (DMF) soluble proteins

The DMF-soluble fraction of wheat grain proteins consists predominantly of gliadins (Mecham, Fullington, & Green, 1981). The gliadin fraction comprises multiple proteins, which on the basis of molecular mass are characterized as α, β, γ and ω (Payne, Holt, Lawrence, & Law, 1982; Ram, Jain, Dawar, Singh, & Shoran, 2005). Although gliadins are resolved better by acid-PAGE, in order to resolve other DMF-soluble proteins also, electrophoresis was carried out on SDS-PAGE. Analysis of gliadin and glutenin in the present study, was carried out on the mature grains; the synthesis of these proteins begins at 12 DPA. SDS-PAGE analysis of the DMF-soluble fraction (Fig. 4), as reported previously (Mecham et al., 1981), revealed the presence of multiple bands. A protein subunit

of 48,100 Da, that was prominent in the control and 8 DPA-stressed grains of C-306, was completely inhibited in response to WS at 15 DPA. However, in the grains of other varieties this protein subunit was very lightly stained and was more or less unaffected by WS. The accumulation of a subunit of 40,100 Da was observed in PBW-175 and PBW-343 only under control conditions. The expression of proteins corresponding to 43,500 Da, 41,400 Da and 29,800 Da decreased in response to WS, at both 8 and 15 DPA, in all the varieties except C-306, which showed that the accumulation of this protein was sensitive to WS at the latter stage.

The expression pattern of a 38,100 Da protein subunit was also similar, except that in PBW-343 the expression of this band was enhanced in response to WS at 8 DPA. WS imposition resulted in a detectable enhancement in accumulation of three protein subunits of 28,600 Da, 27,100 Da and 14,700 Da in all varieties, albeit in a stage-dependent manner. Interestingly a triplet of 23,400 Da, 24,200 Da and 25,200 Da bands, which were weakly stained, was down-regulated in all varieties by WS at both stages of development. On the contrary, the effect of WS on the accumulation of a 13,900 Da band was observed to be variety-dependent.

3.2.2. DMF-insoluble proteins

SDS-PAGE analysis of DMF-insoluble proteins (glutenins) also revealed differential banding patterns in the mature seeds of different varieties of wheat (Fig. 5). On the basis of molecular mass the glutenin subunits are designated as high molecular weight (HMW) and low molecular weight (LMW) (Payne et al., 1982). The accumulation of a glutenin subunit of 149,100 Da was enhanced in response to WS at 8 DPA in C-306 and HD-2329, whereas WS at 15 DPA had no detectable effect. On the contrary, the accumulation of this subunit was inhibited in mature grains in response to WS at both stages in NI-5439. The effect of WS on a 121,000 Da subunit was also variety-dependent. Whereas the accumulation of this protein increased in the grains in response to WS at 8 DPA in C-306, this subunit in the grains of PBW-343 and NI-5439 was inhibited under WS conditions. The stage-dependent induction in response to WS was also observed for an 84,200 Da protein, which was present only in the 8 DPA-stressed grains of C-306 and under control conditions in HD-2329.

The accumulation of three glutenin subunits (appearing as a triplet) of 63,400 Da, 61,700 Da and 60,400 Da was higher in C-306 and HD-2329 when WS was imposed at

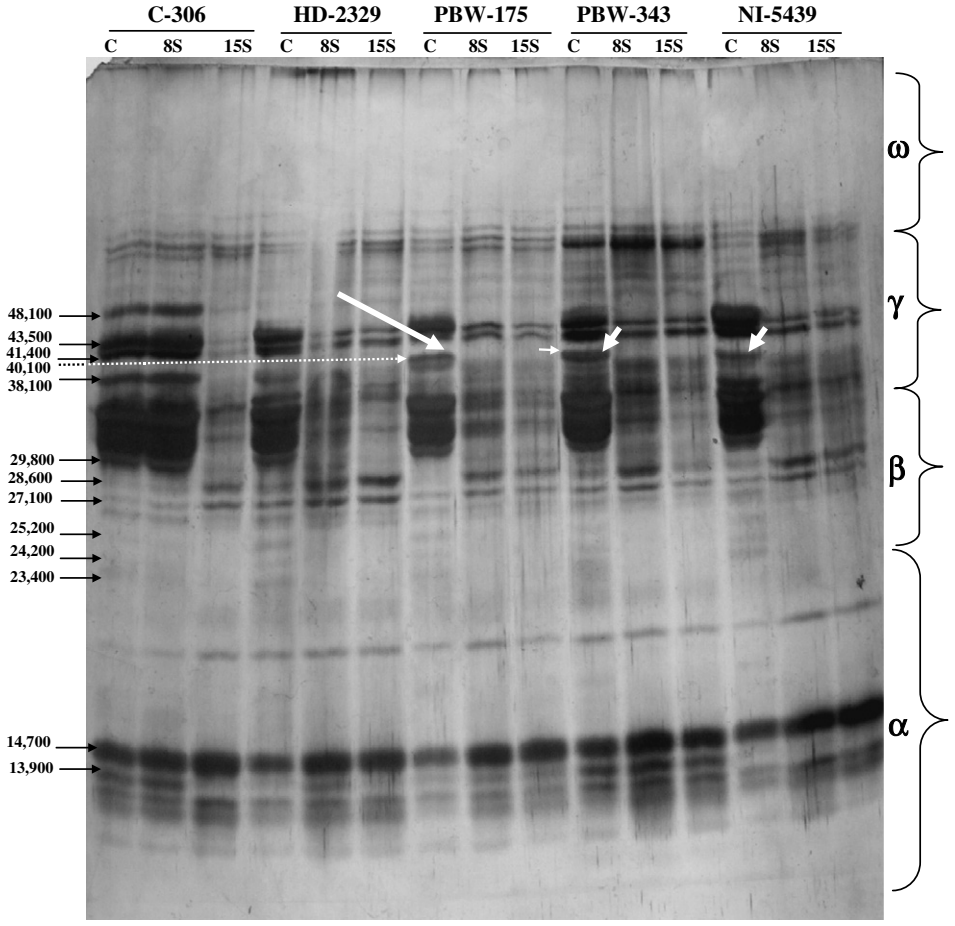

Fig. 4. SDS-PAGE analysis of the DMF-soluble proteins isolated from the mature grains of different wheat cultivars subjected to water stress at 8 and 15 DPA.

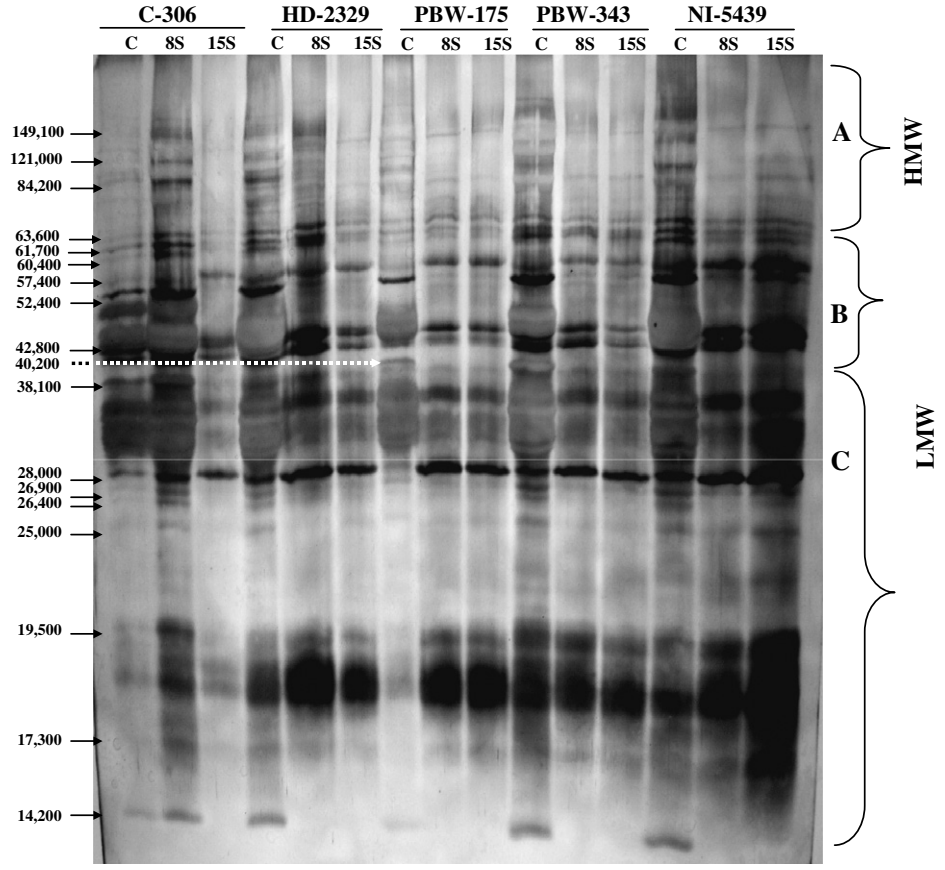

Fig. 5. SDS-PAGE analysis of the DMF-insoluble proteins isolated from the mature grains of different wheat cultivars subjected to water stress at 8 and 15 DPA.

8 DPA, whereas the opposite effect was observed in PBW-343 and NI-5439. The glutenin subunit corresponding to 52,400 Da was observed to be down-regulated in response to WS at both 8 and 15 DPA in all the varieties except C-306. The accumulation of this protein in C-306 increased in response to WS at 8 DPA, whereas WS at 15 DPA inhibited the expression of this polypeptide. The effect of WS on the accumulation of LMW proteins was also variety- and stage-dependent.

It is evident from our results that the varieties differing in their sensitivity to WS showed variability in the accumulation of different DMF-soluble and insoluble proteins in the mature grains of wheat. Furthermore the effect of WS was observed to be stage- and variety-dependent. The stage-dependent affect of WS on different protein subunits, e.g., the inhibition of 48,100 Da, 43,500 Da, 41,400 Da, 38,100 Da and 29,800 Da proteins in the grains of C-306 in response to WS at 15 DPA, may be because these proteins are synthesised at specific developmental stages (at 15 DPA and not at 8 DPA in this case). The differential regulation of accumulation of proteins (especially gliadins and glutenins) in the mature grains of wheat by WS may have important implications in the processing qualities, and thus warrants further investigation.

4. Conclusions

The study showed a pronounced effect of exposure of wheat to WS conditions on the characteristics of starch and proteins. The effect of WS on A-, B- and C-type granules varied with the developmental stage and variety. The differences in amylose content, lipid content, thermal and pasting properties of starches were observed to be affected by WS, more on exposure to 15 DPA. The difference in pasting and thermal properties brought about by WS conditions seem to be attributed to a change in lipids content, amylose content and granular size distribution. Wheat produced on exposure to WS conditions will vary in its suitability for different food products.

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