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HEAT STRESS IN FILLING STAGE CONFERS DISTINCT EFFECT ON STARCH GRANULES FORMATION IN DIFFERENT THERMOTOLERANT WHEAT ACCESSIONS

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Abstract

Heat stress affects the accumulation of starch, which is responsible for the major loss in wheat (*Triticum aestivum* L.) yield. Clarifying the changes of starch composition and granule particles in wheat grain under heat stress are required for understanding the heat adaptation of wheat. The investigation of starch granule morphology and the amylose/amylopectin ratio in seven spring wheat lines of varying levels of thermotolerance under heat stress revealed that A-type starch granules generally decreased and medium granules (between A-type and B-type granules) dramatically increased in the heat-sensitive wheat lines after heat stress compared with the heat-resistant lines. A remarkable decrease in amylopectin was also observed in the heat-sensitive wheat lines. Alterations in A-type starch granules and amylopectin deposition were found to be responsible for the reduction in grain width and weight following heat stress in the heat-sensitive wheat lines. These results provide new insights into the heat effect on wheat starch formation.

Key words: Wheat, Filling grain, Heat stress, Starch granules, Thermotolerance.

Introduction

Wheat (*Triticum aestivum* L.) is an important staple crop but is sensitive to high temperature. It has been reported that a 1°C increase in temperature cause around a 10% decline in yield (Lesk *et al.,* 2016). In most wheat growing areas, the highest temperatures usually occur during the wheat grain filling stage (Farooq *et al*., 2011; Pradhan & Prasad, 2015), which affects the grain filling process (Prasad *et al.*, 2008; Ristic *et al*., 2008; Wang *et al.*, 2011; Lobell *et al.*, 2012), thereby reducing grain weight (Prasad *et al.*, 2011; Prasad &Djanaguiraman, 2014) and resulting in yield loss. With global climate change, more frequent and severe heat stress will be expected in the future and poses great risk to the sustainable supply of wheat products (Semenov *et al.*, 2008).

Mature wheat grains mainly consist of starch (>70%) and storage proteins (Shewry, 2007; Sestili *et al.*, 2010; Slade *et al.,* 2012). Wheat starch is synthesized as discrete granules in amyloplasts, and consist of amylose and amylopectin. The amylose is a linear polymer of glucose which comprises 25% content of wheat grain starch,whereas amylopectinis a highly branched polymer that comprises 75% content of wheat grain starch (Hurkman *et al.*, 2003; Wang *et al.*, 2012; Zhang *et al.*, 2010).Two distinct starch granules (A-type and B-type) are formed during wheat filling stage, and deposit in wheat grain (Bancel *et al.*, 2010; Zhang *et al.*, 2010; Zheng *et al*., 2014). Compared to storage proteins, starch is more vulnerable to heat (Farooq *et al.*, 2011). Accumulating studies suggested that the process of starch deposition is severely inhibited under heat stress (Hurkman *et al.*, 2003; Liu *et al.*, 2011; Wang *et al.*, 2012). However, conflicting results have been obtained in the alterations of amylase and amylopectin deposition, as well as in alterations in starch granules formation following heat stress (Liu *et al.*, 2011; Thitisaksakul *et al.*, 2012; Zhang *et al.*, 2010), suggesting that the heat effect on wheat starch deposition need to further clarify. In this study, seven spring wheat lines with different thermotolerance were investigated in starch granule morphology and the amylose/amylopectin ratio with heat stress. Our results showed that the starch granule formation and starch content were dramatically altered by the heat stress, and this severe alteration was typically observed in the heat-sensitive wheat lines, suggesting that the heat effect on starch deposition is depended on the thermotolerance of wheat varieties, and provided a new perspective to evaluate thermotolerance of wheat germplasms.

Materials and Methods

Experimental design: Seven spring wheat lines (Table 1), which showed different thermotolerance in previous heat tolerant screening, were selected to evaluate the starch deposition alteration with heat stress. To create the heat stress in natural condition, late sowing approach was applied in the field at Northwest A&F University in Yangling, China (34°17′N, 108°04′E). Selected wheat lines were sown at a late sowing date (February 18, 2016), and at a timely sowing date (November 20, 2015) as the normal temperature control. The experiment was conducted according to a randomized block design in triplicate. Each block contained seven rows (2m in length, 0.25 m apart) with 40 kernels planted per row. The flowering date was recorded when the main stems of 50% of the plants flowered in a row. The maturity date was recorded when

95% of spike lost the green color. For each wheat line, 20 uniform main spikes were randomly harvested from each line and each sown bulk for observation ingrain length, grain width, and the grain weight using a Wanshen SC-G automatic grain test instrument (Wanshen SC-G, China) and for starch evaluation. The temperature data can be obtained from the available online weather database (http://www.tianqihoubao.com/lishi/yangling.html).

To confirm the alteration of starch deposition at filling stage in the field, an experiment was conducted in temperature fine-controlled condition. In details, wheat plants (*T. aestivum* L. cv. Chinese Spring) were cultured in pots (26cm in diameter and 20cm in high) in a greenhouse at Northwest A&F University. The pots were filled with 2.6kg of clay soil, which contained organic matter. The pots were watered once a week to avoid water deficiency. At 12 days after flowering, the plants were transferred to a growth chamber (24/17°C, 14/10 h diurnal cycle). After two days of environmental adaptation, the plants were divided into two groups. One group of plants was subjected to heat conditions (37/17°C, 14/10h) in a new growth chamber. The other group of plants remained under the same conditions (24/17°C, 14/10 h) as a control.

Starch granule observations and starch particle size measurements: At least three mature grains from the middle part of the spike in the main stem were randomly selected from each wheat line. The transverse section of the middle of the grain was evenly divided into three layers (Fig. 1). At least three sections in each layer were randomly selected for granule size observations, which were examined with a JSM-6360LV scanning electron microscope (SEM) (JEOL, Tokyo, Japan). The particle size of the starch granules that could be separated from each other was measured using Image J (1.48v) software to assess the longest diameter of each starch granule. In the field experiment, about 1,000 starch granules (from 27 pictures) per wheat line from the different sowing dates were counted; in temperature controlled condition, about 2,000 starch granules from 50 images were counted.

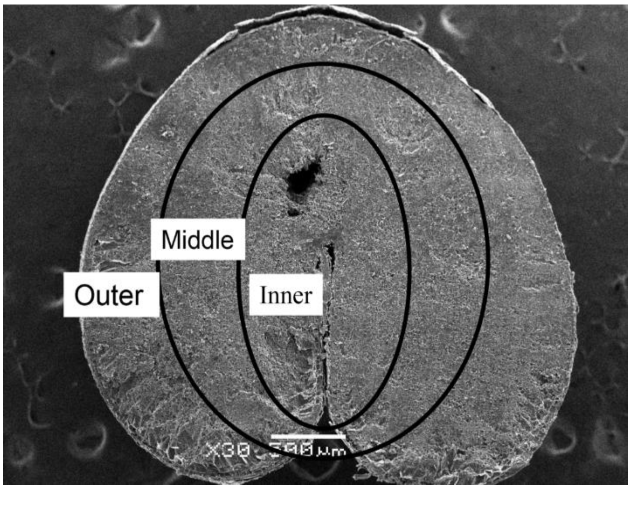


Fig. 1. Schematic diagram of the stratified observations of wheat grain starch granules.

Determination of amylose content in total starch: Grains were ground into a fine powder and fitted with a 0.18-mm screen. The amylose content of the total starch was determined in triplicate using an Amylose/ Amylopectin Assay Kit (Megazyme, Wicklow, Ireland) following the manufacturer's protocol.

Statistical analysis: Correlation analysis was conducted by IBM SPSS 19.0 (SPSS, Chicago, IL, USA). To determine the significant difference in grain characters and starch deposition between heat stress and control, the Student's t-test (two tails) was applied in Excel 2013.

Results

Effects of late sowing on wheat grain weight and size: Late sowing, mimicking heat stress, was performed to evaluate the thermotolerance of seven wheat lines. The average temperature for late sowing was about 1°C higher than that of normal sowing during the grain filling stage (Fig. 2A, Table 1). Four lines (132, 95, CS, 94) showed a significant decrease in grain weight of more than 14% (Fig. 2B) and thus were confirmed as heat-sensitive lines, whereas the other three lines (44, 32, 228) displayed a slight decline in grain weight of less than 5% and thus were assigned as heat-resistant lines. The analysis of grain size showed that the width was reduced and the length was increased in all seven wheat lines. However, the width was reduced less in the heat-resistant lines compared with the heat-sensitive lines. By contrast, the length increased more in the heat-resistant lines than in the heat-sensitive lines (Fig. 3A). Correlation analysis showed that the decrease in grain width was significantly correlated with the reduction in grain weight, and could explain the 65.3% grain weight variation under heat stress (Fig. 3B).

Heat stress affected the size of A-type starch granules: Wheat lines of different thermotolerance exhibited distinct starch granule deposition under normal growth conditions. Under normal growth conditions, A-type and B-type granules could be classified clearly based on their diameter (>14 μm and <8 μm) for the heat-sensitive wheat lines (132, 95, CS, 94), with few medium granules (8–14 μm) being observed (Figs. 4 and 5). By contrast, moremedium granulesexisted in the heat-resistant lines (44, 32 and 228).

Under heat stress (late sowing treatment), the particle size of A-type granules showed a remarkable decrease in the heat-sensitive lines compared with the heat-resistant lines (Fig. 4). Furthermore, more medium granules (8–14 μm) were observed in the heat-sensitive lines under heat stress (Fig. 4), indicating that the formation of A-type and medium granules was heat sensitive in the heat-sensitive wheat. In the heat-resistant lines, granule distribution showed a similar pattern under normal and heat stress conditions, which was also similar to the distribution pattern in the heat-sensitive lines under heat stress. This finding indicated that the distribution pattern of starch granules observed in the heat-resistant lines was a heat-adapted distribution and may be related to their thermotolerance.

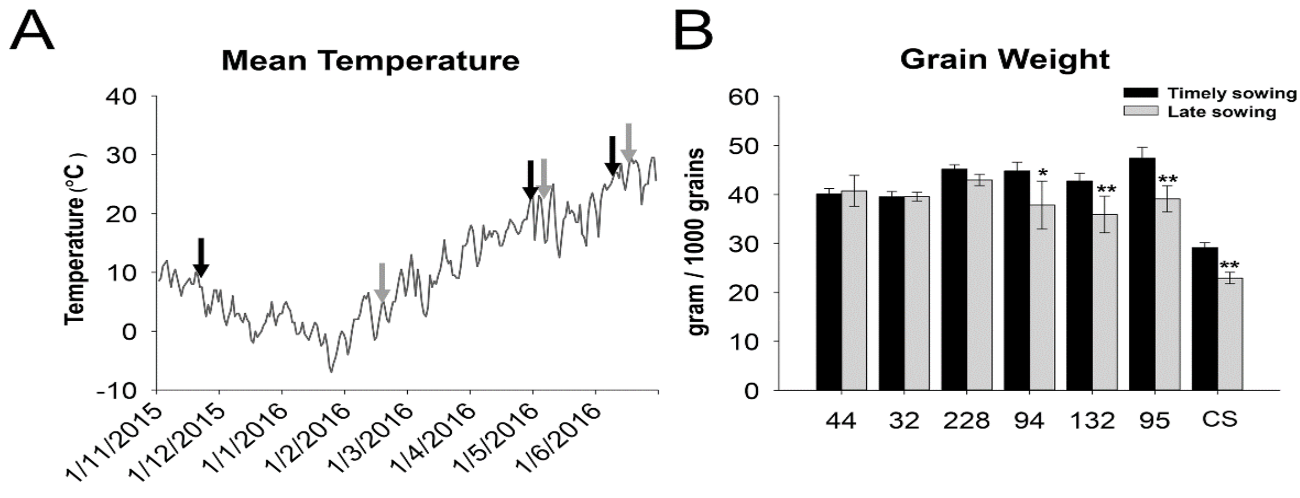


Fig. 2. Effects of late sowing on wheat grain weight.
A. The average daily temperature during the whole wheat growth period. Black and gray arrows indicate the sowing date, flowering date, and maturation date (in sequence) of wheat lines under timely sowing conditions and late sowing conditions, respectively. The flowering and maturation dates are shown as the mode date for all lines.
B. Effects of heat on grain weight. The black bar represents timely sowing, the gray bar represents late sowing. Data represent the mean ±SE (n=3). * p<0.05, ** p<0.01.

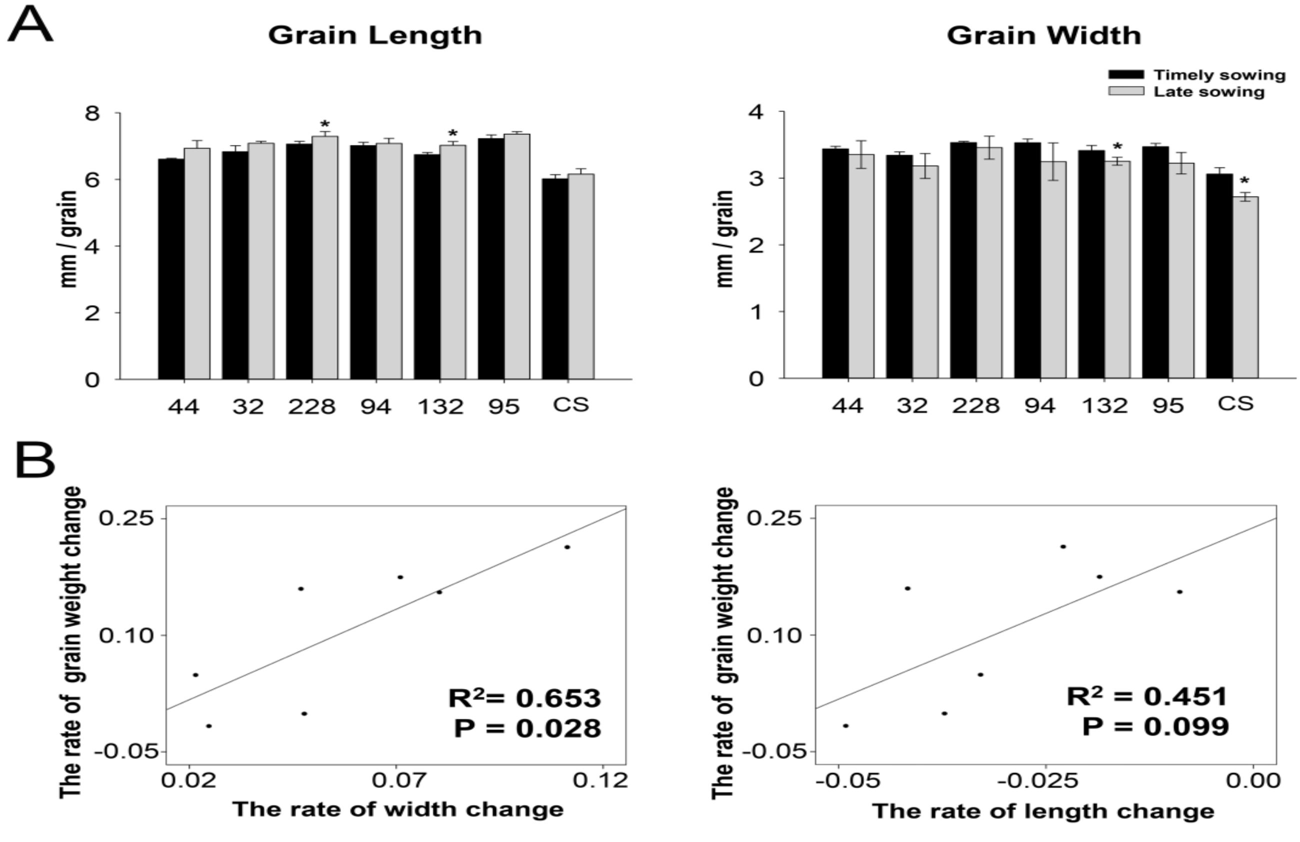


Fig. 3. Effects of late sowing on wheat grain shape.
A. Changes in grain width and length for timely seeded and late seeded wheat. The black bar represents normal sowing date, the gray bar represents late sowing date. Data represent the mean ±SE (n=3).* p<0.05.

Heat stress affects the amylose/amylopectin ratio in wheat grain: Under heat stress, the amylase/ amylopectin ratio decreased in all the heat-tolerant lines, whereas the reverse trend was observed in most of the heat-sensitive lines (Fig. 6A, Table 2). The content of amylose varied amongthe seven wheat lines, whereas the content of amylopectin remained relatively consistent among the heat-resistant lines but was remarkably reduced in the heat-sensitive lines (Fig. 6B, Table 2). Further analysis showed that the decrease in amylopectin content was significantly related to the reduction in grain weight (Fig. 6C), indicating that amylopectin deposition is the main determining factor for the loss of grain weight under heat stress.

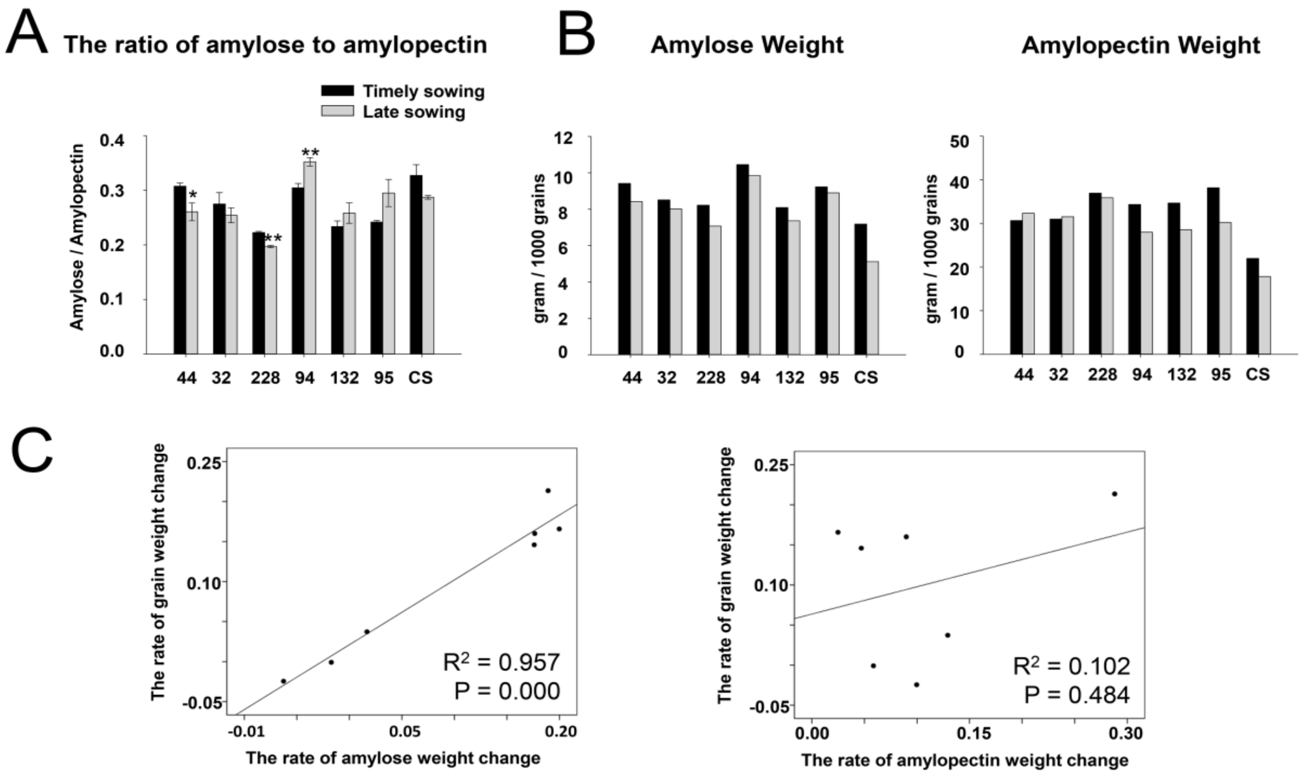
Table 1. Grain filling duration, mean temperature (Tmean), mean minimum (Tmin) and maximum (Tmax) temperatures during grain-filling periods for timely and late seeded wheat in Yangling Shaanxi, 2016.

No.	Name	Origin	The whole growth period (day)		The grain filling stage (day)		T_{mean} (°C)		Mean T_{max} (°C)		Mean T_{min} (°C)	
			Timely seeded	Late seeded	Timely seeded	Late seeded	Timely seeded	Late seeded	Timely seeded	Late seeded	Timely seeded	Late seeded
44	SAFI-1/ZEMAMRA-1	ICARDA	203	120	42	39	20.36	21.60	26.02	27.33	14.70	15.88
32	SAFI-1/ZEMAMRA-1	ICARDA	203	120	41	43	20.35	21.27	25.98	26.93	14.71	15.61
228	HUBARA-3*2/SHUHA-4	ICARDA	202	120	40	41	20.18	21.30	25.78	26.95	14.59	15.64
94	TINAMOU-2//TEVEE-1/SHUHA-6	ICARDA	203	120	41	41	20.35	21.30	25.98	26.95	14.71	15.64
132	KARAWAN-1/TALLO 3//JADIDA-2	ICARDA	202	120	40	41	20.18	21.30	25.78	26.95	14.59	15.64
95	TINAMOU-2//TEVEE-1/SHUHA-6	ICARDA	203	120	41	41	20.35	21.30	25.98	26.95	14.71	15.64
CS	Chinese Spring		206	121	43	39	20.76	21.83	26.41	27.53	15.11	16.13

Table 2. Changes in starch composition for timely and late seeded wheat.

Varieties	Grain weight (g/100-grains)		Amylose/ Amylopectin		Amylose weight (g/100-grains)		Amylopectin weight (g/100-grains)		The change rate of grain weight	The change rate of amylose weight	The change rate of amylopectin weight
	TS	LS	TS	LS	TS	LS	TS	LS			
44	40.06 ± 1.16	40.74 ± 3.15	0.31 ± 0.01	0.26 ± 0.02	9.4	8.42	30.64	32	-0.02	0.11	-0.05
32	39.50 ± 1.07	39.54 ± 0.93	0.27 ± 0.02	0.25 ± 0.01	8.5	8.02	30.99	32	0	0.06	-0.02
228	45.15 ± 0.91	42.94 ± 1.13	0.22 ± 0.00	0.20 ± 0.00	8.2	7.07	36.93	36	0.05	0.14	0.03
94	44.79 ± 1.79	37.82 ± 4.90	0.30 ± 0.01	0.35 ± 0.01	10	9.85	34.33	28	0.16	0.06	0.19
132	42.74 ± 1.61	35.91 ± 3.73	0.23 ± 0.01	0.26 ± 0.02	8.1	7.37	34.65	29	0.16	0.09	0.18
95	47.39 ± 2.21	39.10 ± 2.69	0.24 ± 0.00	0.30 ± 0.02	9.2	8.9	38.16	30	0.17	0.04	0.21
CS	29.16 ± 1.00	22.93 ± 1.17	0.33 ± 0.02	0.29 ± 0.00	7.2	5.12	21.97	18	0.21	0.29	0.19

Note: TS: Timely seeded; LS: Late seeded; The amylose weight is estimated by multiplying the 1000-grain weight with amylose content, and the amylopectin weight is estimated by multiplying the 1000-grain weight with amylopectin content



B. Correlation analysis between the rate of change for grain weight, and grain width and length.
Fig. 4. Alterations in grain starch granules in late sown wheat
A. Starch granule deposition for timely and late sowing conditions. The red arrow indicates medium granules. Scale bar = 20μm.
B. Granule size distribution for each wheat line under different sowing conditions. Curves in black represent timely sowing, curves in gray represent late sowing. Black arrows indicate A-type starch and B-type starch granules under timely sowing conditions. Gray arrows indicate medium-sized starch granules under late sowing conditions.

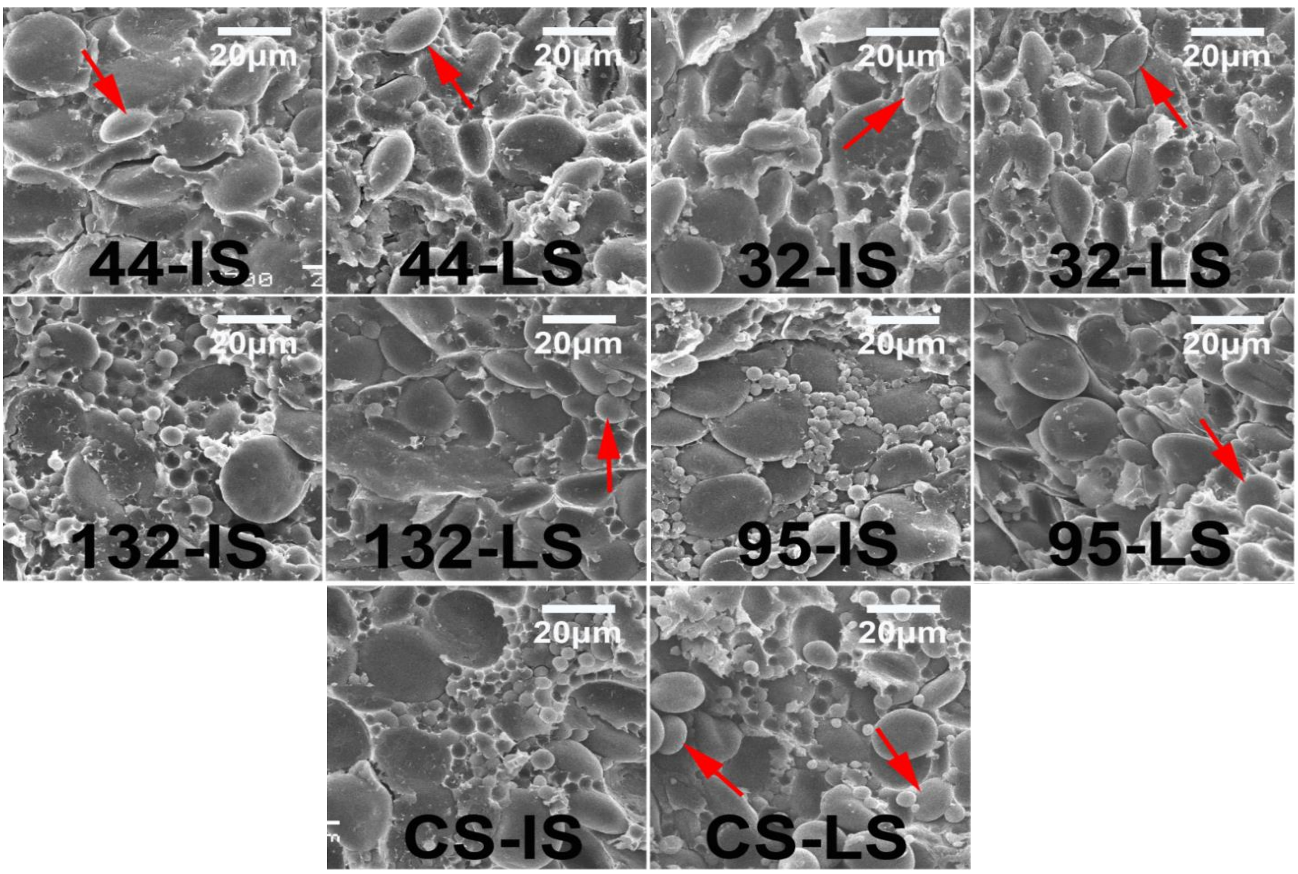


Fig. 5. Starch granule deposition status of wheat lines 32, 228, 132, 95, and CS under timely and late sowing conditions. The red arrow indicates medium granules. Scale bar = 20μm.

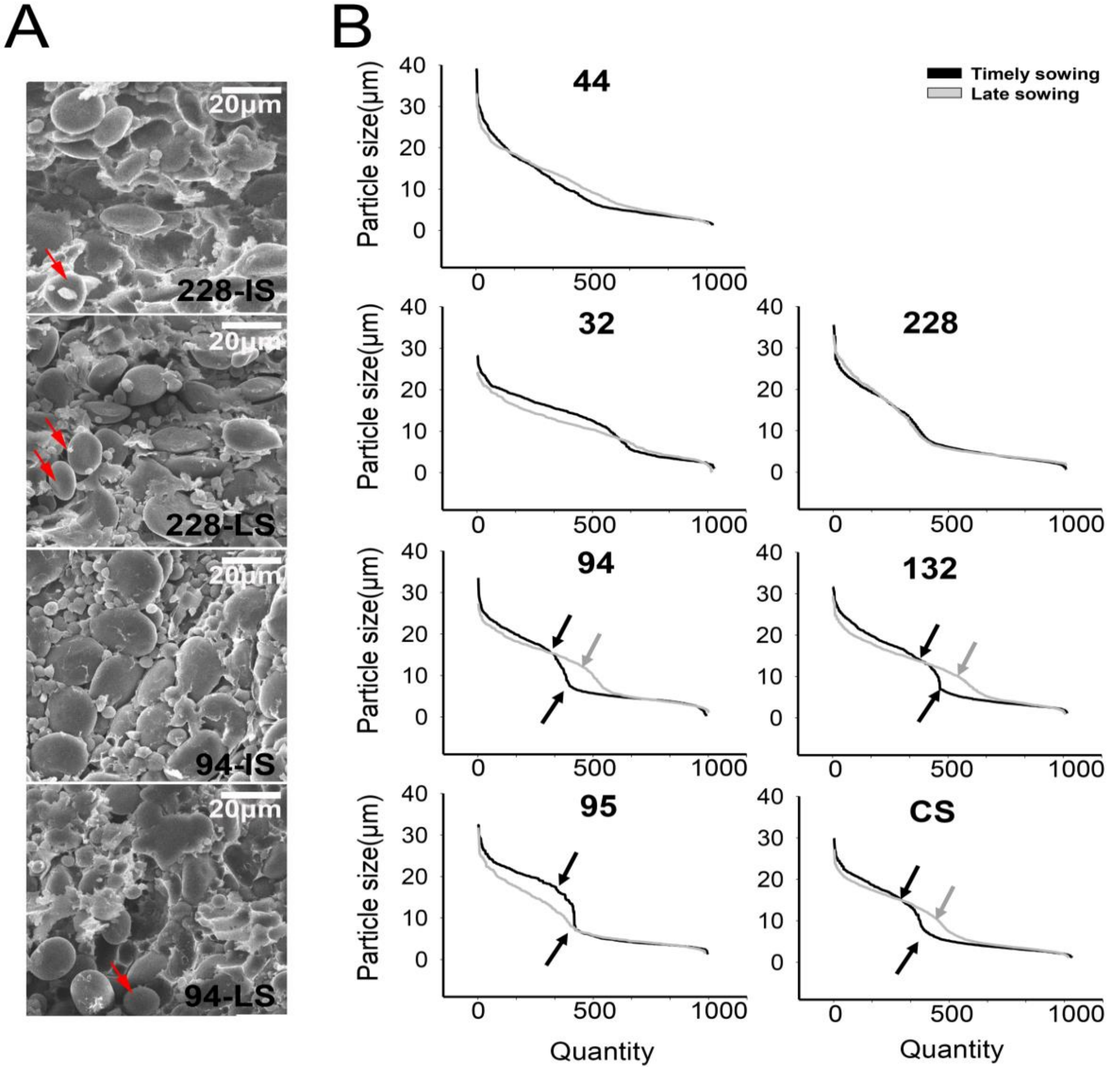


Fig. 6. Alterations in amylose/amylopect in in late sown wheat

A. The amylose content of starch in all wheat lines. The black bar represents timely sowing date, the gray bar represents late sowing date. Data represent the mean ±SE (n=3).* $p<0.05$, ** $p<0.01$.

B. The amylose weight was determined by multiplying the 1000-grain weight by the amylose content. The amylopectin weight was determined by subtracting the amylose weight of 1000 grains from the1000-grain weight.

C. Correlation analysis of the rate of change in grain weight, and the amylose and amylopectin weight.

Alterations in grain starch granules following the finely controlled heat stress treatment: To confirm that the alterations in starch granules in the heat-sensitive wheat varieties were caused by heat stress, wheat line CS was further tested in a growth chamber, as described in the Materials and Methods. Briefly, severe heat stress was applied to filling grains at 15 days after flowering (Fig. 7A) when starch granules had already formed and granule size was assessed (Hurkman & Wood, 2011; Thitisaksakul *et al.*, 2012). SEM observations revealed that the size of A-type granules was decreased under heat stress, along with a dramatic increase in medium granules (Fig. 7B), as shown in the heat-sensitive lines subjected to the late sowing treatment (Fig. 4B). This finding strongly suggested that alterations in starch granules produced after late sowing resulted from the increase in temperature.

Discussion

Late sowing mimicking heat stress has been demonstrated to be an effective approach to distinguish heat-resistant wheat lines from wheat germplasms (Nahar *et al.*, 2010; Sharma *et al.*, 2008; Ubaidullah *et al.*, 2006). In this study, seven wheat lines were clearly divided into two types based on changes in grain weight by the late sowing method, indicating that the late sowing treatment effectively mimicked heat stress in this study. Further, grain width was decreased more in the heat-sensitive wheat lines than in the heat-resistant wheat lines under heat stress, whereas no significant difference was observed in grain length between the heat-sensitive and heat-resistant wheat lines. Statistical analysis revealed that the decrease in grain width was significantly related to the reduction in grain weight. This result also confirmed the findings of previous studies. For example, Dholakia *et al.,* (2003) reported that grain width was significantly related to grain weight. Studies of *GW*5 and *GW2* also demonstrated that grain width was closely related to grain weight in rice and wheat (Weng *et al.,* 2008; Hong *et al.,* 2014). Our results suggest that maintaining or decreasing grain width under heat stress is an important indirect parameter for screening heat-resistant wheat lines.

Accumulated dry mass in grain is another crucial factor determining wheat grain weight as well as grain size. Under heat stress, the reduction in starch accumulation in grain is the main reason for the loss in wheat yield (Jenner, 1991). In this study, the content of starch in mature grains reduced was under heat stress, and also this confirming the findings of previous studies (Hurkman *et al.,* 2003; Zhao *et al.,* 2006; Yan, 2008). However, the effect of heat on amylopectin was distinct for the heat-resistant and heat-sensitive wheat lines. Correlation analysis showed a significant correlation between the content of amylopectin and grain weight under heat stress, suggesting that the deposition of amylopectin is the main determining factor for the loss in grain weight under heat stress.

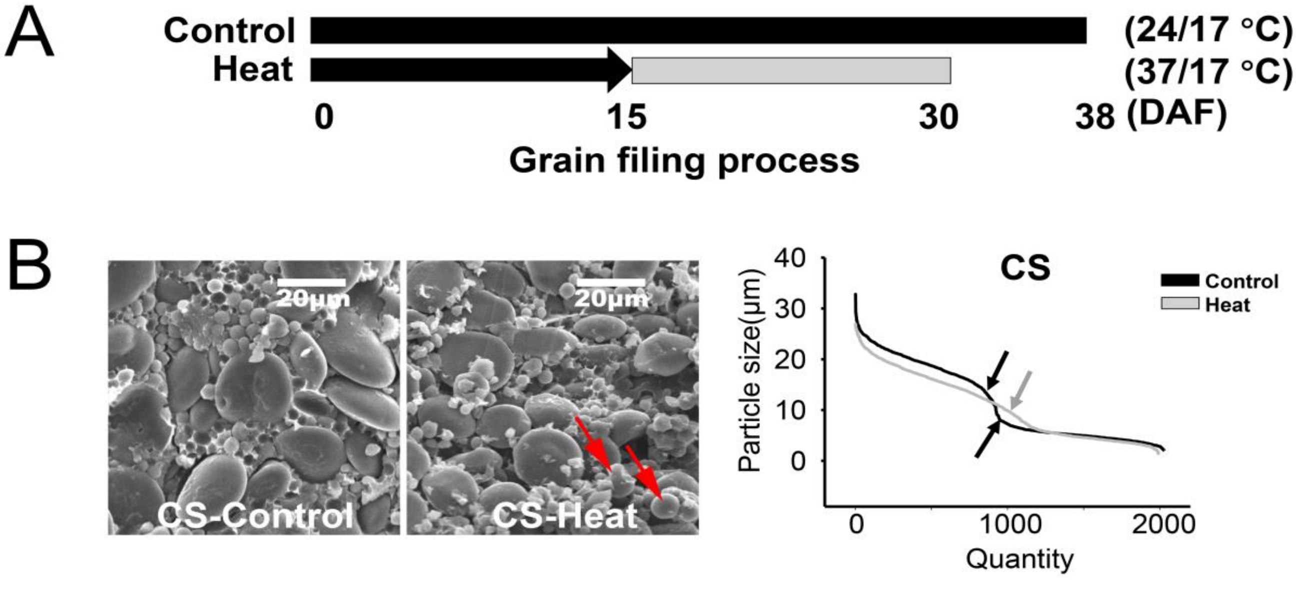


Fig. 7. Starch granule alterations under heat stress.
A. Schematic diagram of the wheat growth conditions and heat treatment. Heat stress was applied to filling grains from 15 days after flowering in growth chambers.
B. Starch granules were observed by SEM. The two images on the left show starch granule deposition under heat and control conditions. Red arrows indicate medium granules. Scale bar = 20μm. The last image shows granule distribution under heat and normal conditions. Black arrows indicate A-type and B-type starch granules under timely sowing conditions. Gray arrows indicate medium-sized starch granules under late sowing conditions.

Large number of research workers have demonstrated that heat stress not only affects starch deposition, but also influences starch granule size (Liu *et al.,* 2011; Lu *et al.,* 2013). Under heat stress, A-type starch granules was decreased in number and size, and B-type starch granules became smaller in size and increased in number in mature grains (Hurkman *et al.,* 2003; Hurkman & Wood, 2011; Wang *et al.,* 2012). In this study, we noted a general decrease in A-type granules under heat stress. Furthermore, we observed a remarkable increase in the quantity of medium starch granules (8–14μm) in the heat-sensitive wheat lines. The synthesis of A-type granules began four days after anthesis, with granule growth and development continuing over the next 20 days. By contrast, the initiation of B-type granule synthesis occurred 10 days after anthesis, with significant granule growth beginning 20 days after anthesis (Shinde *et al.,* 2003). Medium starch granules are predominantly A-type, and these granules fail to enlarge because of the inhibition caused by heat stress. Interestingly, under normal conditions, more medium starch granules existed in the heat-resistant lines compared with the heat-sensitive lines, and no remarkable alterations occurred in these medium granules following heat stress (Fig. 4B), suggesting a different heat adaptation strategy in the heat-resistant lines. This characteristic may be exploited when screening for thermotolerant wheat germplasms in the future. Furthermore, B-typegranules do not appear to be affected by heat stress, indicating that varieties with a high proportion of B-type granules may also exhibit better thermotolerance; however, further investigations are needed to confirm this.

Conclusions

Decreased grain width and reduced starch contents resulted in the loss of grain weight under heat stress. Alterations in A-type starch granules and amylopectin deposition were found to be responsible for the reductionin grain width and weight following heat stress in the heat-sensitive wheat lines. This finding provides fundamental data for understanding grain weight loss that may aid future screening for heat-resistant germplasms. Further investigations are required to clarify the mechanisms underlying these wheat starch alterationsin response to heat stress.

Acknowledgement

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References

Bancel, E., H. Rogniaux, C. Debiton, C. Chambon and G. Branlard. 2010. Extraction and proteome analysis of starch granule-associated proteins in mature wheat kernel (*Triticum aestivum* L.). *J. Proteome Res.*, 9: 3299-3310.

Dholakia, B.B., A. Jss, H. Singh, M.D. Lagu, M.S. Roder, V.S. Rao, H.S. Dhaliwal, P.K. Ranjekar and V.S. Gupta. 2003. Molecular marker analysis of kernel size and shape in bread wheat. *Plant Breed.*, 122: 392-395.

Farooq, M., H. Bramley, J.A. Palta and K.H.M. Siddique. 2011. Heat stress in wheat during reproductive and grain-Filling phases. *Critical Rev. In Plant Sci.*, 30: 491-507.

Hong, Y., L. Chen, L.P. Du, Z. Su, J. Wang, X. Ye, L. Qi and Z. Zhang. 2014. Transcript suppression of TaGW2 increased grain width and weight in bread wheat. *Func. & Integ. Genom.*, 14: 341-349.

Hurkman, W.J., K.F. McCue, S.B. Altenbach, A. Korn, C.K. Tanaka, K.M. Kothari, E.L. Johnson, D.B. Bechtel, J.D. Wilson and O.D. Anderson. 2003. Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. *Plant Sci.*, 164: 873-881.

Hurkman, W.J. and D.F. Wood. 2011. High Temperature during grain fill alters the morphology of protein and starch deposits in the starchy endosperm cells of developing Wheat (*Triticum aestivum* L.) Grain. *J. Agri. & Food Chem.*, 59: 4938.

Jenner, C.F., T.D. Ugalde and D. Aspinall. 1991. The Physiology of Starch and Protein Deposition in the Endosperm of Wheat.*Aust. J. Plant Physiol.*, 18: 211-226.

Lesk, C., P. Rowhani and N. Ramankutty. 2016. Influence of extreme weather disasters on global crop production. *Nature*, 529: 84-87.

Liu, P., W. Guo, Z. Jiang, C. Feng, X. Zhu, Y. Peng, A. Kuang and C.R. Little. 2011. Effects of high temperature after anthesis on starch granules in grains of wheat (*Triticum aestivum* L.). *J. Agri. Sci.*, 149: 159-169.

Lobell, D.B., A. Sibley and O.M.J. Ivan. 2012. Extreme heat effects on wheat senescence in India. *Nat. Climate Chan.*, 2: 186-189.

Lu, H., C. Wang, T. Guo, Y. Xie, W. Feng and S. Li. 2013. Starch composition and its granules distribution in wheat grains in relation to post-anthesis high temperature and drought stress treatments. *Starch - Stärke*, 66: 419-428.

Nahar, K., K.U. Ahamed, M. Fujita and M. Hasanuzzaman. 2010. Phenological variation and its relation with yield in several wheat (*Triticum aestivum* L.) cultivars under normal and late sowing mediated heat stress condition. *Notul. Scientia Biologi.*, 2: 51-56.

Pradhan, G.P. and P.V.V. Prasad. 2015. Evaluation of wheat chromosome translocation lines for high temperature stress tolerance at grain filling stage. *PLoS ONE*, 10: e0116620.

Prasad, P., S. Pisipati, Z. Ristic, U. Bukovnik and A. Fritz. 2008. Impact of nighttime temperature on physiology and growth of spring wheat. *Crop Sci.*, 48: 2372-2380.

Prasad, P.V.V. and M. Djanaguiraman. 2014. Response of floret fertility and individual grain weight of wheat to high temperature stress: sensitive stages and thresholds for temperature and duration. *Funct. Plant Biol.*, 41: 1261-1269.

Prasad, P.V.V., S.R. Pisipati, I. Momčilović and Z. Ristic. 2011. Independent and Combined Effects of High Temperature and Drought Stress During Grain Filling on Plant Yield and Chloroplast EF-Tu Expression in Spring Wheat. *J. Agron. & Crop Sci.*, 197: 430-441.

Ristic, Z., U. Bukovnik, I. Momčilović, J. Fu and P.V. Prasad. 2008. Heat-induced accumulation of chloroplast protein synthesis elongation factor, EF-Tu, in winter wheat. *J. Physiol.*, 165: 192-202.

Semenov, M.A., N.G. Halford and N.G. Halford. 2008. Identifying target traits and molecular mechanisms for wheat breeding under a changing climate. *J. Exp. Bot.*, 60: 2791-2804.

Sestili, F., M. Janni, A. Doherty, E. Botticella, R. D'Ovidio, S. Masci, H.D. Jones and D. Lafiandra. 2010. Increasing the amylose content of durum wheat through silencing of the SBEIIa genes. *BMC Plant Biol.*, 10: 144.

Sharma, R., A. Tiwary and G. Ortiz-Ferrara. 2008. Reduction in kernel weight as a potential indirect selection criterion for wheat grain yield under terminal heat stress. *Plant Breed.*, 127: 241-248.

Shewry, P.R. 2007. Improving the protein content and composition of cereal grain. *J. Cereal Sci.*, 46: 239-250.

Shinde, S.V., J.E. Nelson and K.C. Huber. 2003. Soft wheat starch pasting behavior in relation to A- and B-type granule content and composition 1. *Cereal Chem.*, 80: 91-98.

Slade, A.J., C. McGuire, D. Loeffler, J. Mullenberg, W. Skinner, G. Fazio, A. Holm, K.M. Brandt, M.N. Steine and J.F. Goodstal. 2012. Development of high amylose wheat through TILLING. *BMC Plant Biol.*, 12: 69.

Thitisaksakul, M., R.C. Jiménez, M.C. Arias and D.M. Beckles. 2012. Effects of environmental factors on cereal starch biosynthesis and composition. *J. Cereal Sci.*, 56: 67-80.

Ubaidullah, R., T. Mohammad and A.S. Hafeezullah. 2006. Screening of wheat (*Triticum aestivium* L.) genotypes for some important traits against natural terminal heat stress. *Pak. J. Biol. Sci.*, 9: 2069-2075.

Wang, X., J. Cai, D. Jiang, F. Liu, T. Dai and W. Cao. 2011. Pre-anthesis high-temperature acclimation alleviates damage to the flag leaf caused by post-anthesis heat stress in wheat. *J. Plant Physiol.*, 168: 585-593.

Wang, X., J. Cai, F. Liu, M. Jin, H. Yu, D. Jiang, B. Wollenweber, T. Dai and W. Cao. 2012. Pre-anthesis high temperature acclimation alleviates the negative effects of post-anthesis heat stress on stem stored carbohydrates remobilization and grain starch accumulation in wheat. *J. Cereal Sci.*, 55: 331-336.

Weng, J., S. Gu, X. Wan, H. Gao, T. Guo, N. Su, C. Lei, X. Zhang, Z. Cheng and J. Wang. 2008. Isolation and initial characterization of GW5, a major QTL associated with rice kernel width and weight.*Cell Res.*, 18: 1199-1209.

Yan, S.H. 2008. Effect of high temperature during grain filling on starch accumulation, starch granule distribution, and activities of related enzymes in wheat grains: Effect of high temperature during grain filling on starch accumulation, starch granule distribution, and. *Acta Agronomica Sinica*, 34: 1092-1096.

Zhang, C., D. Jiang, F. Liu, J. Cai, T. Dai and W. Cao. 2010. Starch granules size distribution in superior and inferior grains of wheat is related to enzyme activities and their gene expressions during grain filling. *J. Cereal Sci.*, 51: 226-233.

Zhao, H., T. Dai, Q. Jing, D. Jiang, W. Cao, W. Lu and X. Tian. 2006. Effects of high temperature during grain filling on key enzymes involved in starch synthesis in two wheat cultivars with different quality types. *Acta Agron. Sinic.*, 32: 423-429.

Zheng, K., Q.T. Jiang, X.W. Zhang, X.J. Lan, S.F. Dai, Y.M. Wei, A. Laroche, Z.X. Lu and Y.L Zheng. 2014. Differences in starch composition and physiochemical properties are influenced by granule types in wheat and its relatives. *Pak. J. Bot.*, 46(6): 2239-2248.

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