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

RUIQIONG LI1, LIJIANG HOU1, AIHUA ZHANG1, YUNZE LU1, WEINING SONG1, WULETAW TADESSE2, XIAOMING WANG1, MANSHUANG LIU1, WEIJUN ZHENG3 and SHENGBAO XU4

1State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, 712100, Yangling, Shaanxi, China
2International Centers for Agricultural Research in the Dry Areas, Rabat, Morocco
3Corresponding author’s email: zhengweijun@nwsuaf.edu.cn
4These authors contributed equally to this work.

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 amylopectins is 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 amylose 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.

[Image 56x110 to 290x290]

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, more medium granules existed 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.
Heat stress affects the amylose/amylopectin ratio in wheat grain: Under heat stress, the amylose/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). 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>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Origin</th>
<th>The whole growth period (day)</th>
<th>The grain filling stage (day)</th>
<th>T&lt;sub&gt;mean&lt;/sub&gt; (°C)</th>
<th>Mean T&lt;sub&gt;max&lt;/sub&gt; (°C)</th>
<th>Mean T&lt;sub&gt;min&lt;/sub&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>SAFI-1/ZEMAMRA-1</td>
<td>ICARDA</td>
<td>203</td>
<td>42</td>
<td>20.36</td>
<td>21.60</td>
<td>26.02</td>
</tr>
<tr>
<td>32</td>
<td>SAFI-1/ZEMAMRA-1</td>
<td>ICARDA</td>
<td>203</td>
<td>41</td>
<td>20.35</td>
<td>21.27</td>
<td>25.98</td>
</tr>
<tr>
<td>228</td>
<td>HUBARA-3*2/SHUHA-4</td>
<td>ICARDA</td>
<td>202</td>
<td>40</td>
<td>20.18</td>
<td>21.30</td>
<td>25.78</td>
</tr>
<tr>
<td>94</td>
<td>TINAMOU-2/TEVEE-1/SHUHA-6</td>
<td>ICARDA</td>
<td>203</td>
<td>41</td>
<td>20.35</td>
<td>21.30</td>
<td>25.98</td>
</tr>
<tr>
<td>95</td>
<td>TINAMOU-2/TEVEE-1/SHUHA-6</td>
<td>ICARDA</td>
<td>203</td>
<td>41</td>
<td>20.35</td>
<td>21.30</td>
<td>25.98</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Grain weight (g/100-grains)</th>
<th>Amylose/Amylopectin</th>
<th>Amylose weight (g/100-grains)</th>
<th>Amylopectin weight (g/100-grains)</th>
<th>The change rate of grain weight</th>
<th>The change rate of amylose weight</th>
<th>The change rate of amylopectin weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TS</td>
<td>LS</td>
<td>TS</td>
<td>LS</td>
<td>TS</td>
<td>LS</td>
<td>LS</td>
</tr>
<tr>
<td>44</td>
<td>40.06 ± 1.16</td>
<td>40.74 ± 3.15</td>
<td>0.31 ± 0.01</td>
<td>0.26 ± 0.02</td>
<td>9.4</td>
<td>8.42</td>
<td>30.64</td>
</tr>
<tr>
<td>32</td>
<td>39.50 ± 1.07</td>
<td>39.54 ± 0.93</td>
<td>0.27 ± 0.02</td>
<td>0.25 ± 0.01</td>
<td>8.5</td>
<td>8.02</td>
<td>30.99</td>
</tr>
<tr>
<td>228</td>
<td>45.15 ± 0.91</td>
<td>42.94 ± 1.13</td>
<td>0.22 ± 0.00</td>
<td>0.20 ± 0.00</td>
<td>8.2</td>
<td>7.07</td>
<td>36.93</td>
</tr>
<tr>
<td>94</td>
<td>44.79 ± 1.79</td>
<td>37.82 ± 4.90</td>
<td>0.30 ± 0.01</td>
<td>0.35 ± 0.01</td>
<td>10</td>
<td>9.85</td>
<td>34.33</td>
</tr>
<tr>
<td>132</td>
<td>42.74 ± 1.61</td>
<td>35.91 ± 3.73</td>
<td>0.23 ± 0.01</td>
<td>0.26 ± 0.02</td>
<td>8.1</td>
<td>7.37</td>
<td>34.65</td>
</tr>
<tr>
<td>95</td>
<td>47.39 ± 2.21</td>
<td>39.10 ± 2.69</td>
<td>0.24 ± 0.00</td>
<td>0.30 ± 0.02</td>
<td>9.2</td>
<td>8.9</td>
<td>38.16</td>
</tr>
<tr>
<td>CS</td>
<td>29.16 ± 1.00</td>
<td>22.93 ± 1.17</td>
<td>0.33 ± 0.02</td>
<td>0.29 ± 0.00</td>
<td>7.2</td>
<td>5.12</td>
<td>21.97</td>
</tr>
</tbody>
</table>

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.
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 GW5 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.

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-type granules 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
reduction in 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 alterations in response to heat stress.

Acknowledgement

We acknowledge the financial support of National Key R&D Program of China (2017YFD0300202-2) and Cyrus Tang Breeding Foundation.

References


Hence, 197: 430-441.


