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Physiological Mechanisms of Poor Grain Growth in Abnormally Early Ripening Wheat Grown in West Japan

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Abstract : We found a symptom of abnormally early ripening in a farmer's field in Natajima, Yamaguchi Prefecture, Japan, in 2004/2005, and examined its physiological mechanisms for two weeks until maturity. In the following two seasons, 2005/2006 and 2006/2007, we examined the mechanisms throughout the grain filling period at another farmer's field where the symptoms appeared in the preceding four seasons. The grain yield was lower in abnormally early ripening (AER) than in the normal because of lighter grain weight in 2004/2005. The grain weight and water soluble carbohydrate, WSC, in culm were similar at the beginning of symptom, two weeks before maturity, then the grain weight increased and WSC in culm decreased more sharply in the normal than in the AER. So the grain weight was poorer and more WSC in culm remained unutilized at maturity in the AER. Another field showed the symptom of AER in both seasons. The spike dry weight and WSC in culm were the similar between the treatments from anthesis to milk ripe stage in 2005/2006, then they showed almost similar pattern in their change as in 2004/2005 until maturity. It was thought that the slower grain growth in later phase might be due to limited current assimilation and poor remobilization of culm reserves to the grains in AER.

Key words : Abnormally early ripening, Culm reserves, Poor grain filling, Remobilization, Starch granules, Wheat.

The wheat production has been impaired due to abnormally early ripening, a physiological disorder in Kyushu region of west Japan since the late 1980's (Ishikawa et al., 1993; Taniguchi et al., 1996). The symptom of the disorder appears first as the senescence of lower leaves after heading which extends to upper leaves and finally the spike withers up. As a result, the grain growth is inhibited resulting in lighter grains and ultimately lower grain yield. The abnormally early ripening (AER) plants can be distinguished from the normal plants by their red-colored spikes (Taniguchi et al., 1996).

The symptom of abnormally early ripening appears almost every year in some specific fields causing yield losses in different degrees depending on the prevailing weather conditions (Ishikawa et al., 1993). The reduced root activities due to water damage around heading are considered to induce abnormally early ripening phenomenon (Kira et al., 1993; Ikeda et al., 1994; Sano et al., 1994). However, the mechanisms of poor grain filling resulting from the abnormally early ripening are still unknown.

Natajima district is the most important area for wheat production in Yamaguchi prefecture of west Japan. Abnormally early ripening has been a problem for the farmers in this area. However, it was difficult to arrange the experimental fields because we can not predict which field or which part of a field will show the symptoms. There are several patterns in the symptom appearance of abnormally early ripening: the senescence in the whole field at once, the spotty senescence in a field, and the beginning of senescence from an edge of a field spreading to the opposite side. Even though the above area is known to have frequent appearance of the symptoms, never do all the fields in the area show the symptoms.

However, we found the typical symptoms of abnormally early ripening at two weeks before maturity in 2004/2005. We sampled the plants starting at that time until maturity to investigate the mechanism of poor grain filling incuded by the abnormally early ripening.

In addition, another field where the symptoms appeared only in the northern half in the previous seasons, was examined in 2005/2006 and 2006/2007. We sampled the plants there starting from anthesis to investigate the mechanisms prior to the symptom appearance.

Materials and Methods

1. Field experimentation

The experiments were conducted at the farmer's fields in Natajima district in Yamaguchi, west part of Japan. The farmers used Norin 61, a spring cultivar bred in Kyushu.

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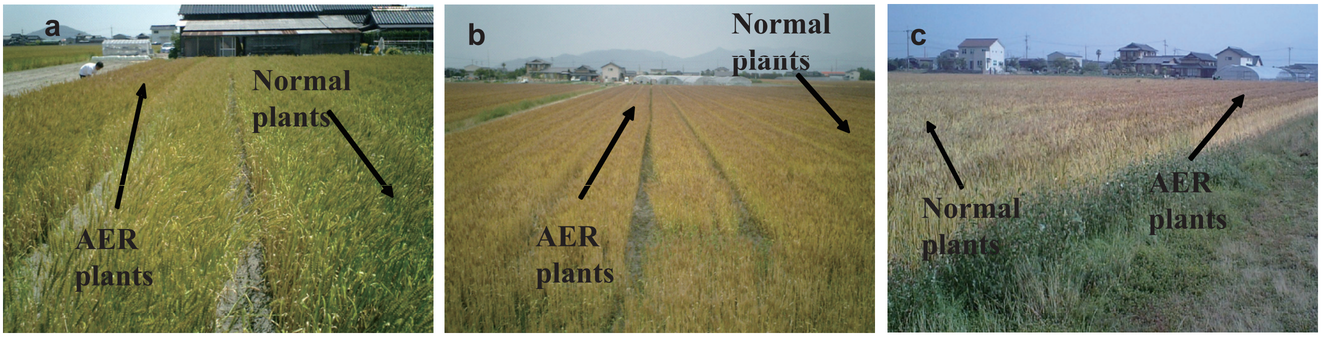

Fig. 1. Wheat fields showing abnormal early ripening (AER) symptoms. a: field A in 2004/2005, b: field B in 2005/2006, and c: field B in 2006/2007.

Exp. 1 was conducted in field A where we found the symptom by chance in the season 2004/2005. On 18 May 2005 we found the symptom of abnormally early ripening in two rows at the east edge of the field (Fig. 1a). With the permission of the farmer, we started the sampling on 19 May. The plants with symptoms were distinguished from the normal plant by their red colored spikes while the normal plants had still green colored spikes at that time. The experiment consisted of two treatments, normal and abnormally early ripening with three replicates.

Exp. 2 was conducted in another field B starting from anthesis, prior to the appearance of the symptoms in 2005/2006 and 2006/2007. Field B was chosen because we predicted the appearance of symptoms in the field from the history of the appearance. The farmer informed us that all the plants in the northern half area of the field showed the symptoms while the plants in the southern half area did not show any symptoms for the previous four successive seasons, 2001/2002–2004/2005. Actually, this field showed the symptoms of abnormally early ripening in its northern half in both seasons studied. The AER plants were distinguished from the normal ones by their red spikes in both seasons (Fig. 1b, c). The normal and AER plants were used with three replicates in each season.

2. Sampling

(1) Experiment 1

We started sampling from 2 wk before maturity in field A in 2004/2005. Thirty tillers of normal and AER plants were sampled on 19, 23, 26, 30 May, and 3 June in 2005. Twenty tillers were selected from each sample for uniformity and used to determine dry weight. The plants were separated into culm with sheaths, leaves, and spikes, then oven dried at 70°C for 48 hr and weighed. The grains were separated from the spikes with tweezers and weighed. In addition, the normal and AER plants each in 1.05 m^2 (0.7 m×1.5 m) were sampled at maturity with 3 replicates to examine the yield and yield components.

The number of spikes per m^2 was counted. Then, all the plants were dried for 48 hr at 70°C and weighed to determine the biomass yield. All spikes were hand-threshed and weighed for grain yield. Thousand grains were counted and weighed for 1000-grain weight. The harvest index was calculated from the grain yield divided by biomass yield. The number of grains per spike was calculated as the grain yield, spike number and 1000 grain weight. The culm was milled to determine the content of water-soluble carbohydrate (WSC) by the anthrone method. The content of structural materials in the culm was determined through subtracting WSC from culm dry weight.

(2) Experiment 2

We started sampling in field B from the time of anthesis in the seasons, 2005/2006 and 2006/2007. Thirty tillers were sampled from the northern half (AER plants) and southern half (normal plants) on 5, 19, 22, 26, 29 May and 3, 5 June in 2005/2006 then measured for dry weight of different plant parts and WSC content in culm as in Exp. 1. In addition, all the plants in the 1.05 m^2 sampling plot (0.7 m×1.5 m) were sampled from the normal and AER plants with 3 replicates at anthesis (5 May), milk ripe stage (19 May), and maturity (5 June) to determine dry weight per m^2 in 2005/2006. In 2006/2007, 20 grains were sampled at 7 d after anthesis (3 May), 14 d after anthesis (10 May), 19 d after anthesis (15 May), and at maturity (8 June) in 2006/2007. The grains were sampled from the first and second floret of the seventh spikelet (from bottom) of a spike, and stored in a fixative containing 80% ethanol for starch granule counting.

Yield and yield components at maturity were also measured in 2005/2006 and 2006/2007 as in Exp. 1.

3. Estimation of WSC content of culm

The milled culm was weighed and extracted once with 80% ethanol at 60°C for 15 min followed by two successive extractions with distilled water at 80°C for 30 min each. The extracts were combined and evaporated to dryness. The dried carbohydrates were resolved in 1 mL distilled water and centrifuged at 5000 rpm for 5 min followed by the addition of charcoal to make clear

Table 1. Grain yield, biomass yield, harvest index and yield components in normal and abnormally early ripening (AER) plants of wheat cultivar Norin 61 in field A in Natajima, Yamaguchi in 2004/2005.

Treatments	Grain yield ($g\ m^{-2}$)	Biomass yield ($g\ m^{-2}$)	Harvest index (%)	Spike m^{-2}	Grain $spike^{-1}$	1000-grain weight (g)
Normal plants	405	1026	39.5	425	25.7	37.1
AER plants	289	980	29.4	415	24.7	28.0
Significance	*	NS	**	NS	NS	**

* and **, significant at 5%, 1% level of significance, respectively. NS, non significant.

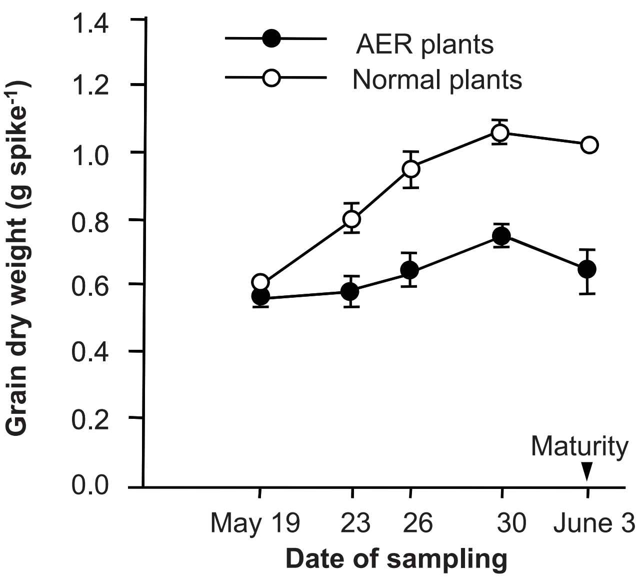

Fig. 2. Changes in grain dry weight of wheat in abnormally early ripening (AER) and normal plants in field A in 2004/2005. The vertical bars are the standard errors of the means.

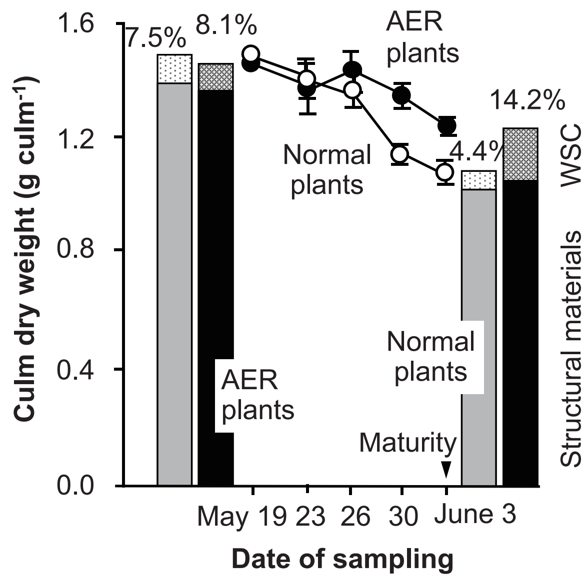

Fig. 3. Changes in culm dry weight (lines with circles), water soluble carbohydrate, WSC (upper segments of the bars) and structural materials in culm (lower segments of the bars) of abnormally early ripening (AER) and normal wheat plants in field A in 2004/2005. The numbers at the top of the bars indicate the culm WSC in percentage. The vertical bars are the standard errors of the means. DAA : days after anthesis.

solution. Twenty μL of the clear solution were added to 10 mL anthrone reagent (Yemm and Willis, 1954) and heated for 10 min in a boiling water bath and then cooled with ice water. The WSC of the reacted solution was measured using a spectrophotometer at 620 nm wave length.

4. Counting the starch granules

Three middle sized grains, which were selected from 20 grains, were homogenized with 2 mL of iodine (0.2% I_2, 2% KI) solution in a mortar with a pestle and suspended in 8 mL of 80% glycerin solution. The starch granules were counted using a Hematocytometer and Image analysis software, Photoshop and PopImagines. Three types of starch granules were distinguished according to their diameter, A type (>16 μm), B type (5 to 16 μm), and C type (<5 μm) (Bechtel et al, 1990).

5. Statistical Analysis

The data collected from the Exp.1 were subjected to one way (treatment) analysis of variance in a model of completely randomized design (CRD) and those from the Exp. 2 were subjected to two way (treatment and season) analysis of variance in the same model.

Results

1. Exp. 1 in field A in 2004/2005

Table 1 shows the grain yield, biomass yield, harvest index and yield components of normal and AER plants in field A in 2004/2005. Grain yield showed significantly higher in normal plants than in AER plants. Biomass yield did not show significant difference between the treatments. Harvest index showed significantly higher in normal plants than in AER plants. Spike number per m^2 and grain number per spike did not show difference between the treatments while 1000-grain weight was significantly heavier in normal plants than in AER plants.

Fig. 2 shows the changes in grain dry weight. The grain dry weight in AER plants was similar to that in normal plants on 19 May. It increased rapidly in

Table 2. Grain yield, biomass yield, harvest index and yield components in normal and abnormally early ripening (AER) plants of wheat cultivar Norin 61 in field B in Natajima, Yamaguchi in 2005/2006 and 2006/2007.

Growing seasons and treatments	Grain yield ($g\ m^{-2}$)	Biomass yield ($g\ m^{-2}$)	Harvest index (%)	Spike m^{-2}	Grain $spike^{-1}$	1000-grain weight (g)
Season 2005/2006						
Normal plants	349	817	43.9	372	27.0	34.9
AER plants	199	613	32.4	273	28.1	25.9
Season 2006/2007						
Normal plants	676	1867	36.3	506	31.2	42.9
AER plants	573	1689	34.0	460	29.5	42.2
Significance						
Treatments	**	NS	NS	**	NS	*
Growing seasons	**	**	NS	**	**	**
Interaction	NS	NS	NS	NS	NS	*

* and **, significant at 5%, 1% level of significance, respectively. NS, non significant.

normal plants up to on 30 May reaching peak (1.05 g $spike^{-1}$) with a slight decline till maturity. In AER plants, however, it increased very slowly reaching peak (0.74 g $spike^{-1}$) on 30 May with a decline till maturity.

Fig. 3 shows the changes in culm dry weight and its components, WSC and structural materials in culm in 2004/2005. Culm dry weight was almost similar in both normal and AER plants on 19 May. It decreased rapidly in normal plants up to maturity on 3 June, while it decreased hardly until 26 May followed by a slight decline till 3 June. The WSC and structural materials in culm were also almost similar in both normal and AER plants on 19 May, while only the structural materials were similar at maturity on 3 June. The WSC in the culm was much higher, 14.2%, in AER plants than 4.4% in normal plants at maturity on 3 June.

2. Exp. 2 at field B in 2005/2006 and 2006/2007

Table 2 shows grain yield, biomass yield, harvest index and yield components of the plants in field B in 2005/2006 and 2006/2007. Grain yield was significantly higher in 2006/2007 than in 2005/2006. It was significantly higher in normal plants than in AER plants as in field A. Biomass yield was significantly heavier in 2006/2007 than in 2005/2006, but it did not differ significantly between normal and AER plants as in field A. Harvest index did not show any difference between the seasons and also between the treatments. Spike number per m^2 was significantly more in 2006/2007 than in 2005/2006, and was significantly more in normal plants than in AER plants. Grain number per spike was significantly higher in 2006/2007 than in 2005/2006, but it did not differ between normal and AER plants. The 1000-grain weight was significantly heavier in 2006/2007 than in 2005/2006 and significantly heavier in normal plants than in AER plants. However, it showed significant

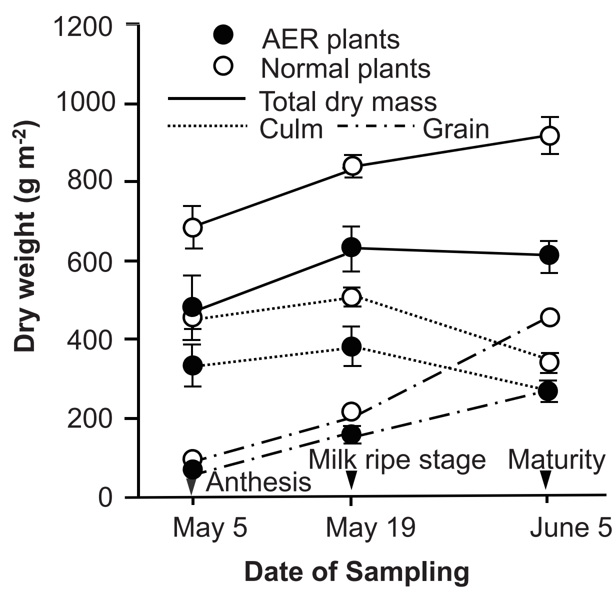

Fig. 4. Changes in dry weight of total biomass, culm and grain of wheat of abnormal early ripening (AER) and normal plants in field B in 2005/2006. The vertical bars are the standard errors of the means. DAA : days after anthesis.

interaction indicating the significant difference between normal and AER plants only in 2005/2006 as in Exp. 1.

Fig. 4 shows the changes in dry weight of total biomass, culm and grains per m^2 in 2005/2006. Total dry weight was heavier in normal plants than in AER plants. It increased throughout the grain filling period in normal plants while it increased only from anthesis to milk ripe stage, and it did not increase until maturity in AER plants. Culm dry weight was heavier in normal plants than in AER plants. It increased from anthesis to milk ripe stage, and then decreased until maturity in both treatments. However, it declined

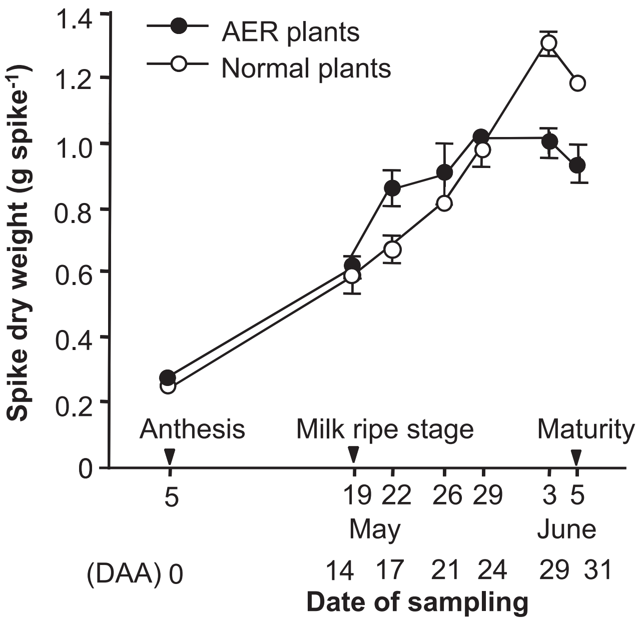

Fig. 5. Changes in spike dry weight of wheat in abnormal early ripening (AER) and normal plants in field B in 2005/2006. The vertical bars are the standard errors of the means. DAA is the days after anthesis. DAA : days after anthesis.

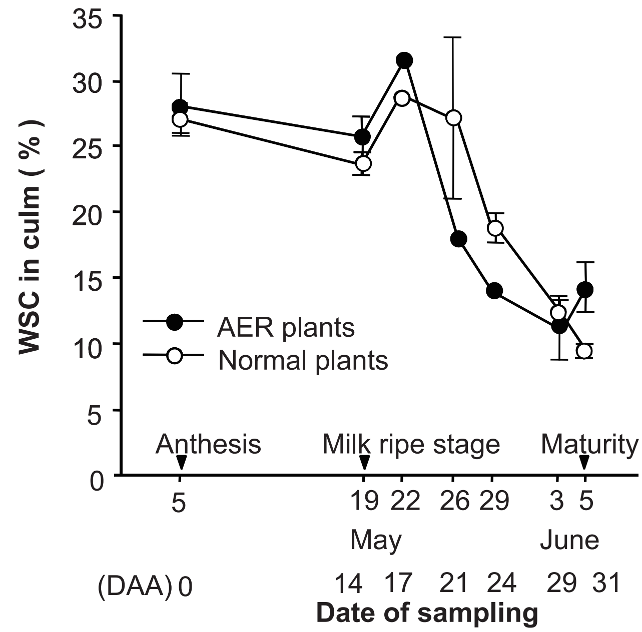

Fig. 6. Changes in WSC content of culm in abnormal early ripening (AER) and normal plants in field B in 2005/2006. The vertical bars are the standard errors of the means. DAA : days after anthesis.

Table 3. Number of starch granules ($\times 10^6$) in a grain of wheat cultivar Norin 61 at different days after anthesis for normal and abnormally early ripening (AER) plants in field B in Natajima, Yamaguchi in 2006/2007.

Treatments	Grains sampled at 7 DAA(3 May)			Grains sampled at 14 DAA (10 May)			Grains sampled at 19 DAA (15 May)			Grains sampled at maturity (8 June)		
	A Type	B Type	C Type	A Type	B Type	C Type	A Type	B Type	C Type	A Type	B Type	C Type
Normal pplants	0	1.03	10.7	1.36	6.42	6.85	3.6	12.7	33.1	6.4	54.2	103.3
AER plants	0	3.43	13.8	2.26	7.09	8.63	4.2	13.7	34.5	6.3	51.6	87.7
Significance	NS	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS

*, significant at 5% level of significance. NS, non significant.

more sharply in normal plants than in AER plants. Grain dry weight was almost zero at anthesis in both treatments. It increased more sharply in normal plants than in AER plants particularly from milk ripe stage to maturity.

Fig. 5 shows the changes in spike dry weight in 2005/2006. Spike dry weight was almost similar in both normal and AER plants from anthesis to milk ripe stage. It increased more rapidly in AER plants than in normal plants temporarily for a few days from milk ripe stage. Then it increased slightly in AER plants, but linearly in normal plants until 3 June. It was heavier in normal plants than in AER plants for the final several days before maturity.

Fig. 6 shows the changes in the WSC content of culm in 2005/2006. The WSC content in AER plants was similar to that in normal plants on 5 May at anthesis, and thereafter it hardly changed for 17 d until 22 May. After 22 May, it decreased rapidly until 29 May, a few days before maturity in AER plants, but it hardly decreased for 4 d and then decreased rapidly until maturity in normal plants. At maturity, it was higher in AER plants than in normal plants.

Table 3 shows the number of starch granules in a grain at different days after anthesis (26 April) in 2006/2007. The number of B-type granules at 7d after anthesis and A-type granule at 14 d after anthesis were more in AER plants than in normal plants. However, the numbers of all types of granules did not differ significantly between normal and AER plants at any days except for the above two cases.

Discussion

Abnormally early ripening appears as the senescence of leaves after a short period of anthesis and as the reddening of spikes (Ishikawa et al., 1993; Taniguchi et al., 1996). The wheat field, field A, in Exp. 1 showed the typical symptoms of abnormally early ripening at the east edge at 2 wk before maturity in 2004/2005 (Fig. 1a). The abnormally early ripening (AER) plants did not show any differences from the normal plants in growth parameters before the appearance of the symptoms. Another field, field B, also showed the visual symptoms of abnormally early ripening only at the northern half, however, and they had fewer spikes before the appearance of the symptoms in both seasons studied (Table 2). This special phenomenon in the field B may be due to the difference in soil fertility between the northern and southern halves of the field as only the southern half, where the symptom never appeared, was reclaimed with the fertile soil once upon a time.

The early ripening imposes interference in grain growth producing lighter grains (Taniguchi et al., 1996). In Exp. 1, the grain growth was studied after the symptoms appeared 2 wk before maturity. The grain growth was hampered greatly from the time of symptom appearance until lighter grains were produced at maturity (Fig. 2, Table 1). In Exp. 2, however, the grain growth was studied from anthesis until symptom appearance in 2005/2006. The grain growth in AER plants was similar to that in normal plants from anthesis to milk ripe stage but the former increased more rapidly than the latter for a few days after the milk ripe stage and stopped grain growth before maturity resulting in lighter grains than in the normal plants (Fig. 5).

Grain growth starts with the increase in the number of endosperm cells in wheat grains followed by the increase in cell volume through accumulation of organic substances (Singh and Jenner, 1984). The organic substances come from the assimilate stored temporarily in vegetative organs during the grain filling period (Schnyder, 1993). The grain growth is supported only by current assimilation in initial and early grain filling phases, but by both current assimilation and culm reserves in late grain filling phase and only by culm reserves in final phase of grain filling (Takahashi et al., 1993).

The grain growth in AER plants was similar to that in normal plants until the milk ripe stage, i.e., initial and early grain filling phases but it was greatly hampered after the milk ripe stage i.e., late and final phase of grain filling in AER plants. Grain filling was slower in AER plants than in normal plants in the last two phases of grain filling (Figs. 2, 4). The slower grain growth in the late and final phases may be due to the shorter current assimilation and /or poor remobilization of culm reserves to grains. The AER plants senesced earlier compared to normal plants which reduced the assimilate production for grain growth through current assimilation (Fig. 1). Moreover, TDM did not increase after milk ripe stage (Fig. 4) which indicates the cessation of photosynthesis and dry matter production after milk ripe stage when the plants induced by abnormal early ripening phenomenon. The senescence of leaves and cessation of current assimilation may be due to the problem in root absorption due to water logging condition or restricted root growth. The lower root activity due to high water status around heading induces abnormal early ripening phenomenon (Ikeda et al., 1994; Sano et al., 1994). Ishikawa et al. (1993) also reported that many abnormal early ripening phenomena appeared with wet damage phenomenon. On the contrary, Nishida et al. (1993) reported that low water status after heading is the cause for abnormal early ripening.

The culm reserves play a vital role in buffering grain yield when canopy photosynthesis is restricted by senescence (Austine et al., 1980; Gaunt and Wright, 1992; Takahashi and Kanazawa, 1996). The decline of culm dry weight indicates the remobilization of reserved materials from stem to grains. In AER plants, the amount of reserve material stored from anthesis to milk ripe stage (initial and early grain filling phases) was similar to that in normal plants (Fig. 4). However, the culm dry weight at anthesis was heavier in the normal plants than in the AER plants due to a larger number of effective tillers (Table 2). On the other hand, the culm dry weight of AER plants declined more slowly than that of normal plants indicating poor remobilization of culm reserves to the grains leaving more reserves unutilized at maturity in AER plants (Figs. 3, 4). Moreover, the WSC content of culm in AER, was similar to that in normal plants at 2 wk before maturity, but it was much higher in AER plants (14.2%) than in normal plants (4.4%) at maturity (Fig. 3). Although, the WSC remobilized more rapidly in AER plants than in normal plants, it ceased to remobilize before maturity resulting in much unutilized WSC remained in the culm at maturity (Fig. 6). Thus, the grain of AER plants receives very little or no culm reserves at the final phase of grain filling even though the culm still had a lot of reserve materials at maturity. The remobilization and utilization of reserve materials for grain filling largely depends on the sink activity, i.e., starch biosynthesis in endosperm. Enhanced sink activity usually results in increased remobilization and grain filling rate (Yang et al., 2004). Therefore, the poor remobilization and poor utilization of culm reserves in AER plants at later phase of grain filling may be due to less sink demand, i.e., poor sink activity. Abnormally early ripening disorder is associated with high water status in the soil (Ikeda et al., 1994; Sano et al., 1994; Ishikawa et al., 1993) which may contribute to the poor sink activity by regulating the keys enzymes involved in sucrose-to starch conversion in endosperm (Yang et al., 2004).

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