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Inheritance of Waxy Endosperm Character in a Common Wheat Lacking Three Wx Proteins

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Summary

The endosperm of a common wheat produced by genetic elimination of waxy (Wx) protein turned red-brown by iodine staining in F₃ and F₄ generations, indicating the presence of a waxy phenotype. Measurement with an auto-analyzer revealed that the F₃ and F₄ generations of variant wheat contained 0.6 - 0.7 % amylose in the endosperm starch, values comparable to those of a waxy rice variety (0 % amylose), suggesting that the waxy phenotype is inherited in a stable manner. In the waxy common wheat, both $\lambda_{\max}$ and the blue value of endosperm starch indicated a marked reduction in the content of amylose. These results confirmed that wheat deficient in the Wx proteins lacked amylose. The production of a waxy wheat may be a significant step toward genetic modification of starch composition in wheat flour.

Key Words : Common wheat, waxy endosperm, amylose, waxy (Wx) protein.

Introduction

In common wheat (*Triticum aestivum* L.), waxy phenotype lacking amylose in grain starch has not yet been detected (Kihara 1979). The waxy (Wx) protein, which is the product of a *waxy* gene, is a granule-bound starch synthase (GBSS) responsible for amylose production in storage starch (Preiss 1991). Combination of null alleles for the three Wx proteins (Wx-A1, Wx-B1 and Wx-D1 proteins) of hexaploid common wheat enabled us to select a F₂ progeny lacking the Wx proteins (Nakamura *et al.* 1995). Iodine staining of endosperms of these F₂ seeds revealed the presence of a waxy phenotype (red-brown endosperm) and endosperm starch contained 0.7 % amylose (Nakamura *et al.* 1995). However, the endosperm character of waxy common wheat has not been studied in successive generations.

In this report, we will describe the mode of inheritance of the waxy phenotype and characterize the endosperm in successive F₃ and F₄ generations of a common wheat deficient in three Wx proteins.

Materials and Methods

Three wheat cultivars, 'Kanto 107' and 'Saikai 173' lacking both the Wx-A1 and Wx-B1 proteins and 'Bai Huo' lacking the Wx-D1 protein (Yamamori *et al.* 1994 b), were used as parents. Among 720 F₂ seeds from two crosses, 'Bai Huo'/'Kanto 107' and 'Bai Huo'/'Saikai 173', 14 F₂ seeds showed a waxy character (red-brwon endosperm by iodine staining) and lacked the Wx proteins (Yamamori *et al.* 1994a). From these waxy F₂ seeds, ten F₃ and F₄ lines (six from 'Bai Huo'/'Kanto 107' and four from 'Bai Huo'/'Saikai 173') were produced for starch analysis of waxy wheat in the present study.

Starches from the distal half of grains were extracted according to the method of Echt and Schwartz (1981) and used for Wx protein analysis, iodine staining tests and amylose analysis.

A solubilized Wx protein produced by boiling starch granules in a SDS buffer (Echt and Schwartz 1981) was subjected to modified SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Nakamura *et al.* (1992) and detected by silver staining. Cross sections of endosperms were stained with an iodine solution (0.2 % I₂ - 2 % KI). For staining mature pollen grains, a 0.067 % I₂ - 0.67 % KI solution was used.

The maximum absorbance ($\lambda_{\max}$) and absorbance at 680 nm (blue value) of the iodine-starch complex, which indicate the presence of differences in amylose content, were determined according to the method of Konishi *et al.* (1985) using 5 mg starch swollen in 1 N NaOH. Amylose content was measured with an autoanalyzer (Technicon Autoanalyzer, Bran + Lubbe Co.) (Kuroda *et al.* 1989). As controls for the measurement of the amylose content, starch of a waxy rice cv 'Hidekomochi' was assumed to contain 0.0 % amylose and wheat starch purchased from Wako Pure Chemical Industries Ltd. to contain 31.2 % amylose. The amylose content of this wheat starch (31.2 %) was determined using potato amylose and amylopectin (Sigma) as controls.

Results and Discussion

All (more than 100) of the F_3 and F_4 seeds examined became red-brown by iodine staining or showed a waxy phenotype. In these seeds, the Wx protein could not be detected by electrophoretic analysis (Table 1). These results are consistent with those obtained in the F_2 seeds exhibiting a waxy phenotype. The endosperm starch granules from this waxy wheat also turned red-brown by iodine staining. In addition, large starch granules were dark brown at the hilum and some showed dark rings. In contrast, normal starch granules from non-waxy cv 'Norin 61' were blue-black.

The starch-iodine complex of the waxy wheat (F_3) yielded a different spectrum from those of 'Norin 61' and 'Kanto 107'. The $\lambda_{\max}$ and blue value (B. V.) of starches from the F_3 and F_4 generations of 10 waxy lines were significantly lower than those of the three parental cultivars and 'Norin 61'. This remarkable difference was observed in starches from the non-waxy rice cv 'Nipponbare' versus waxy rice cv 'Hidekomochi' (Table 1). These observations suggest that the amylose content of waxy wheat was reduced because both $\lambda_{\max}$ and B. V. also decreased in starch with a low content of amylose in rice (Uematsu *et al.* 1988) and *Amaranthus hypochondriacus* (Konishi *et al.* 1985). The amylose content, determined with an autoanalyzer, was 0.6 % in F_3 seeds of 10 waxy lines and 0.7 % in F_4 seeds, values comparable to those of the waxy rice cv 'Hidekomochi' (0.0 %) (Table 1). The negligible differences in the amylose content, $\lambda_{\max}$ and B. V. between waxy wheat and waxy rice may be attributed to the fact that waxy wheat contains only a small amount of amylose, or does not produce amylose whereas the properties of the other starch component (amylopectin) are different between wheat and rice. The amylose content of the waxy wheat should be determined more precisely by the use of other methods, e.g. gel filtration or potentiometric titration.

Pollen grains from F_2 and F_3 waxy plants of wheat

Table 1. $\lambda_{\max}$ (maximum absorbance), blue value (absorbance at 680 nm) and amylose content of variant wheat

Cultivars/Lines	$\lambda_{\max}$ (nm)	Blue value	Amylose (%)	Wx protein ³⁾
Norin 61	588	0.475	30.1	+
Variant (F_3) ¹⁾	537	0.059	0.6	—
Variant (F_4) ²⁾	537	0.064	0.7	—
Kanto 107	579	0.403	24.6	+
Saikai 173	577	0.426	25.1	+
Bai Huo	582	0.491	30.3	+
Nipponbare (non-waxy rice)	567	0.271	16.7	+
Hidekomochi (waxy rice)	533	0.040	0.0 ⁴⁾	—

¹⁾ Average of F_3 seeds set on 10 F_2 variants.

²⁾ Average of F_4 seeds set on 10 F_3 variants.

³⁾ + and — indicate presence and absence, respectively.

⁴⁾ 'Hidekomochi' was assumed to contain 0 % amylose.

became red-brown with 0.067 % I_2 - 0.67 % KI solution while normal (non-waxy) pollen grains turned blue-black. However, staining with 0.2 % I_2 - 2 % KI sometimes generated dark red-brown pollen grains, which were not distinguishable from the non-waxy pollen grains (blue-black in color). Thus, 0.067 % I_2 - 0.67 % KI was suitable for discriminating waxy from non-waxy pollen grains. Among pollen grains collected from the parental F_1 hybrid, the red-brown and blue-black staining reactions by this iodine solution have been reported to occur in a 7 : 1 ratio (blue-black : red-brown) (Yamamori *et al.* 1994a).

The red-brown coloration of endosperm, starch granules, pollen grains and the marked reduction of the amylose content or absence of amylose clearly confirmed the inheritance of the waxy character in two successive generations, F_3 and F_4 . In the F_2 seeds, Yamamori *et al.* (1994a) reported that the waxy character (red-brown endosperm) coincided with the absence of the Wx protein, or non-waxy character with the presence of the Wx protein. Furthermore, this study revealed that the lack of Wx protein in waxy wheat was inherited. Thus, evidence was presented that a genotype free of Wx proteins became a waxy phenotype in common wheat, which had already been inferred based on studies on rice (Sano 1984) and maize (Echt and Schwartz 1981).

In spite of the relatively narrow range of amylose content (21 - 30 %) in common wheat compared to rice (0 - 30 %) (Nakagahra *et al.* 1986 ; Yamamori *et al.* 1992), wheat flour is processed into various foods, such as bread, noodles and biscuits. Soulaka and Morrison (1985) pointed out that a wider range of wheat starch composition would be desirable for determining the effect of starch on bread-baking quality. Using flours containing 21.9 - 25.0 % amylose, Oda *et al.* (1980) showed that a lower content of amylose flour enhanced the glutinosity of Japanese noodles and improved their eating quality. The use of waxy wheat should enable to clarify the effect of the amylose content on flour or dough properties. Mixing of non-waxy (30 % amylose) wheat and waxy (0 %) wheat in different ratios could produce flours with an amylose content ranging from 0 to 30 %. Such materials may enable to examine the effect of a reduced amylose content on dough properties.

Visser and Jacobsen (1993) suggested that the amylose-free potato they produced may be used in microwave-ready foods because it yields a clear paste which does not retrograde. When wheat starch became swollen in heated SDS buffer for Wx protein extraction, the waxy starch yielded clear paste whereas non-waxy starch became cloudy or produced a dull paste. The formation of a clear paste of waxy wheat was presumably caused by the lack of amylose because waxy 'Hidekomochi' rice also showed a clear paste in heated SDS buffer while non-waxy 'Nipponbare' became cloudy. In addition, waxy corn starch shows a clear paste while non-waxy corn produces an opaque paste (Swinkels

1985). The production of a waxy common wheat with novel starch composition or properties may be a significant step toward the genetic modification of flour starch which has not been studied as extensively in wheat as in rice (Satoh 1985).

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