Production of sweet wheat

Shimbata T[1], Saito M[1,3], Takiya T[1], Vrinten P[2], Nakamura T[3]

[1]Nippon Flour Mills Co., Ltd., Central Laboratory, 5-1-3 Midorigaoka, Atsugi, 243-0041, Japan, [2]Bioriginal Food & Science Corp., Melville Street, Saskatoon, SK, S7J 0R1, Canada, and [3]Tohoku National Agriculture Research Center, 4 Akahira, Shimo-kuriyagawa, Morioka 020-0198, Japan

ABSTRACT

Amylose and amylopectin are the major components of storage starch. An amylose-free mutant lacking granule-bound starch synthase I (GBSSI) and a high-amylose mutant lacking starch synthase IIa (SSIIa) have recently been produced in wheat. A double mutant lacking GBSSI and SSIIa was created from a cross of these two mutant lines. The absence of these two enzymes had pleiotropic effects on seed morphology and carbohydrate characteristics. Notably, the double mutant had high levels of maltose and sucrose and was therefore called "sweet wheat". The seed of the double mutant was severely shrunken and the seed weight reduced. The gelatinization properties of wholemeal analysed by a Rapid visco analyser also showed a unique profile with a much lower viscosity. Sweet wheat has many unique characteristics and is expected to serve as a new ingredient in food products.

INTRODUCTION

Plants with mutations in genes involved in starch synthesis have historically been used to create new varieties of crops, such as sweet corn. Now that more information is available regarding the genes involved in starch synthesis it has become possible to purposefully select lines with mutations in these genes. However, the phenotypes we observe in these mutants may not always match the phenotypes we would predict based on known gene functions. Therefore, the production of such mutants provides information on the process of starch synthesis.

Granule bound starch synthase I (GBSSI) is responsible for the synthesis of the largely linear α (1, 4)-linked glucan amylose, and starch in seeds of the wheat waxy (Wx) mutant, which lacks GBSSI, are amylose-free (Nakamura et al., 1995). The synthesis of amylopectin, which consists of α (1, 4)-linked, α (1, 6)-branched glucans, requires the co-operative action of several enzymes (Myers et al., 2000). One of these enzymes, starch synthase IIa (SSIIa) is thought to be involved in elongating the short chains of amylopectin (James et al., 2003). The wheat mutant lacking SSIIa, referred to here as the high amylose (HA) mutant, produces amylopectin with a higher level of short chains and a lower level of intermediate chains. The amylose level of the HA mutant is approximately 1.3-fold that of wild-type wheat (Yamamori et al., 2000). A double mutant which lacks both GBSSI and SSIIa was selected from a cross between Wx and HA. This line accumulates sugars at high levels, and we named the double mutant "sweet wheat" (SW) (Nakamura et al., 2006).

MATERIALS AND METHODS

Plant materials including the wild-type cultivar Chinese Spring (CS), the Wx common wheat cultivar Mochi-Otome and a HA mutant line developed in our previous work were grown in a green house.

Double mutants were selected by PCR-based screening of the F_2 and F_3 generations of a cross between the HA and Wx mutant lines with six primer sets (Nakamura et al, 2002; Shimbata et al., 2005; Nakamura et al., 2006). Seeds were harvested at 45-50 days post anthesis (DPA) for seed morphology observations and pasting analysis, while 25 DPA seed samples were used for sugar analysis. A PR-101 digital refractometer (ATAGO, Tokyo, Japan) was used to obtain Brix measurements from supernatant of crushed endosperm tissue. To measure sugar contents, endosperm tissue from 25 DPA seed samples was homogenized in dimethyl sulfoxide and centrifuged. The supernatant was removed and boiled for 10 minutes and cooled to room temperature. Samples were dried in a rotary evaporator and glucose, maltose and maltotriose levels were determined by the fluorescence assisted carbohydrate electrophoresis (FACE) method described by Morell et al. (1998) with some modifications. Fluorescent labelling of reducing residues was conducted using an eCAP N-linked oligosaccharide profiling kit (Beckman Coulter, CA) and separation of oligosaccharides was performed with the P/ACE system 5000 using P/ACE station software (Beckman Coulter, CA). To measure the level of sucrose, a HP capillary electrophoresis instrument with a bare-fused silica capillary (i.d. 50um, total length 112.5 cm, effective length 104 cm) was used with Agilent Basic Anion buffer (Agilent Technologies, CA) for separation, and HP Chemstation software (Agilent Technologies, CA) was used for quantification. Separation was carried out according to the application literature on the web site of Agilent Technologies (Soga and Serwe, 1999).

Wholemeal was prepared using an Ultra Centrifugal MILL ZM-200 (Retsch, Germany) at 14,000 rpm with a 0.75 mm screen. After determination of water content, the wholemeal samples were subjected to Rapid visco analysis (RVA; Newport Scientific, Australia) for measurement of pasting properties. 3.50 g of wholemeal (14% moisture basis) was added to 25.0 ml of 0.1 % of $AgNO_3$ in the test canister. After an initial agitation at 50 °C for 10 sec at 960 rpm, the suspension was held at 50 °C for 50 sec at 160 rpm, then heated to 95 °C at a rate of 3 °C /min, held at 95 °C for 10 min, cooled to 50 °C at a rate of 3 °C /min, and held at 50 °C for 2 min.

RESULTS AND DISCUSSION

A total of 728 F_2 plants were screened with DNA markers, and a plant which was heterozygous at the *SSIIa-D1* locus and homozygous null at all other *GBSSI* and *SSIIa* loci was identified. Out of 55 F_3 progeny from this plant, eight were the required *GBSSI* and *SSIIa* double-null mutants (Fig. 1) (Nakamura et al., 2006).

As expected, seed from the SW double mutant lacked amylose and cut kernels stained red-brown with iodine (data not shown). While mature seeds from the HA and Wx parents appeared basically similar to wild-type seeds, SW seeds were severely shrunken and shrivelled, and had reduced kernel weights (data not shown). Except for these differences in seed morphology, no differences in appearance between SW and the parental lines were observed during plant growth and development.

Interestingly, substantial amounts of both maltose and sucrose were present in the developing endosperm of the double mutant. We first detected the presence of soluble sugars as a sweet taste in immature kernels, similar to the taste of immature sweet corn.

The Brix measurement of juice from immature endosperm was 22, or about two times higher than regular wheat (Fig. 2). High levels of maltose were found in immature SW seeds, although it was barely detectable in wild-type, HA or Wx lines. Sucrose was detected in all lines, but levels were higher in SW (Nakamura et al., 2006).

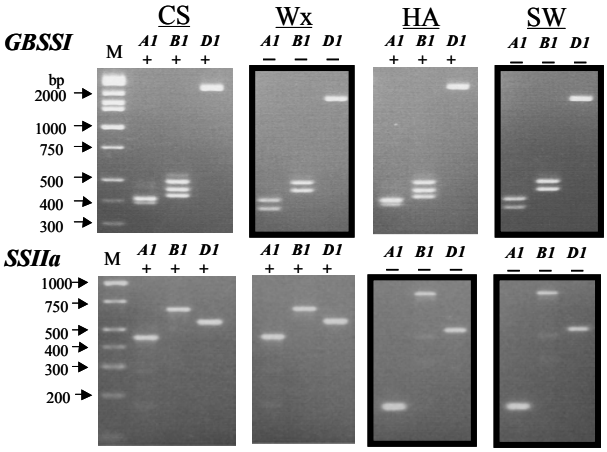

Figure 1. PCR-based selection of the double mutant SW. The presence (+) or absence (-) of functional *GBSSI* and *SSIIa* genes was determined by PCR analysis of wild-type wheat (CS), double-null mutant sweet wheat (SW), and the SW parental lines waxy (Wx) and high-amylose (HA). Common wheat is hexaploid and three homoeologous genes, designated *A1*, *B1* and *D1*, encode GBSSI and SSIIa proteins.

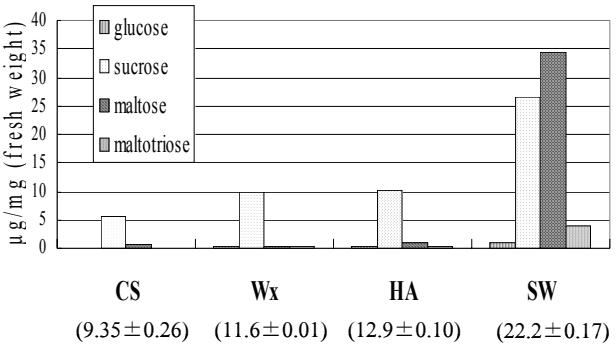

Figure 2. Sugar content of supernatant from 25 DPA immature endosperm tissue. The Brix values for each genotype are shown in parentheses. Glucose sucrose, maltose and maltotriose contents were determined by capillary electrophoresis.

A reduction in starch synthesis due to the lack of two starch synthesis enzymes in the double mutant could lead to the build-up of sucrose seen in SW. However, to our knowledge the high level of maltose in SW endosperm is quite unusual among plants carrying mutations in starch synthesis genes. Increased maltose levels have also been detected in a sweet corn line carrying a de-branching enzyme mutation (*su1*) along with *sugary enhancer*, a recessive modifier of *su1* (Ferguson et al., 1979). It will be interesting to determine what mechanism is responsible for the accumulation of maltose in SW.

The pasting profile of SW, as analysed by RVA (Fig. 3) was also dramatically different than wild-type or parental lines. Although a small peak was observed at 1.6 min, the viscosity of the SW sample was extremely low throughout the analysis. Generally, pasting profiles are affected by starch properties including amylose content and amylopectin structure. The unusual pasting profile seen here indicates that substantial changes in composition and structure occurred in SW storage carbohydrates.

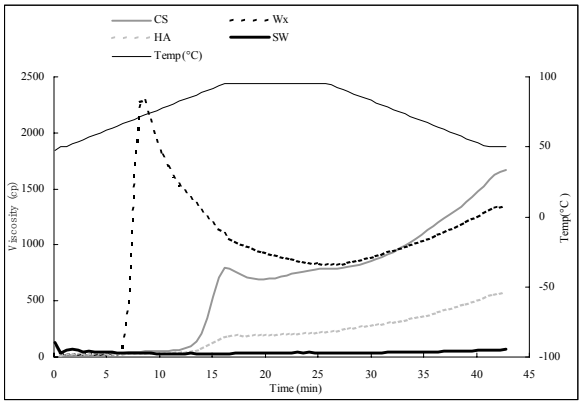

Figure 3. Pasting profiles of wholemeal samples from CS, Wx, HA, and SW measured using RVA. Each sample contains 10.8 % (w/w, dry weight base) wholemeal in 0.1 % $AgNO_3$ (total 28.5 g).

A *GBSSI*/*SSIIa* double mutant (*wx*/*su2*) of maize (Creech, 1965) possessed neither the altered seed morphology nor the increased sugar levels of SW (Shannon and Garwood, 1984). Wheat lines having only one active SSIIa protein and lacking all GBSSI proteins seem to bear a closer resemblance to the maize mutant than does SW. This suggests the possibility that the *su2* allele carried by the corn double mutant conditions only a reduction rather than an elimination of SSIIa activity. It will be interesting to determine if perfect null *GBSSI*/*SSIIa* double mutants in other plant species have characteristics resembling those of SW.

It also remains to be determined if the specific properties seen in SW obtained here are influenced by other aspects of genotype besides the *GBSSI* / *SSIIa* mutations. To clarify this, it will be necessary to produce *GBSSI*/*SSIIa* double mutants in various genetic backgrounds. The DNA marker sets used in this study are powerful tools for readily selecting other SW lines.

SW has unique properties and by developing applications using these properties, we hope to realize the potential of SW as a new food ingredient. To achieve this goal, further investigations of the starch structure and physicochemical properties of SW are under way.

ACKNOWLEDGEMENTS

We thank Dr. K. Komae, Y. Kurimoto and Y. Ishihara for useful suggestions and comments, and F. Saito for managing plant materials. This work was partially supported by a grant from Ministry of Agriculture, Forestry and Fisheries of Japan (Green Technology Project DM-1305).

REFERENCES

Creech, R. G. 1965. Genetic control of carbohydrate synthesis in maize endosperm. *Genetics*, 52: 1175-1186.

Ferguson, J. E., Dickinson, D. B., and Rhodes, A. M. 1979. Analysis of endosperm sugars in a sweet corn inbred (Illinois 677a) which contains the sugary enhancer (*se*) gene and comparison of *se* with other corn genotypes. *Plant physiol*., 63: 416-420.

James, M. G., Denyer, K., and Myers, A. M. 2003. Starch synthesis in the cereal endosperm. *Curr. Opin. Plant Biol*., 6: 215-222.

Myers, A. M., Morell, M. K., James, M. G., and Ball, S. G. 2000. Recent progress toward understanding biosynthesis of the amylopectin crystal. *Plant Physiol*., 122:989-997.

Morell, M. K., Samuel, M. S., and O'Shea, M. G. 1998. Analysis of starch structure using fluorephore-assisted carbohydrate electrophoresis. *Electrophoresis*, 19: 2603-2611.

Nakamura, T., Yamamori, M., Hirano, H., Hidaka, S., and Nagamine, T. 1995. Production of waxy (amylose-free) wheats. *Mol. Gen. Genet*., 248: 253-259.

Nakamura, T, Shimbata, T., Vrinten, P., Saito, M., Yonemaru, J., Seto, Y., Yasuda, H and Takahama, M. 2006. Sweet Wheat. *Genes Genet. Syst.*, 81: 361-365.

Nakamura, T., Vrinten, P., Saito, M., and Konda, M. 2002. Rapid classification of partial waxy wheats using PCR-based markers. *Genome*, 45: 1150-1156.

Shannon, J. C., and Garwood, D. L. 1984. Genetics and physiology of starch development. In: *Starch: Chemistry and Technology* (eds.: R. L. Whistler, J. N. BeMiller, and E. F. Paschall), pp. 25-86. Academic Press, Orland, FL.

Shimbata, T., Nakamura, T., Vrinten, P., Saito, M., Yonemaru, J., Seto, Y., and Yasuda, H. 2005. Mutations in wheat starch synthase II genes and PCR-based selection of a SGP-1 null line. *Theor. Appl. Genet*., 111: 1072-1079.

Soga, T., and Serwe, M. 1999. Rapid monitoring of carbohydrates in food with capillary electrophoresis. Publication number 5968-6985-E, library on the web site of Agilent Technologies (http://www.chem.agilent.com./scripts/Phome.asp).

Yamamori, M., Fujita, S., Hayakawa, K., Matsuki, J., and Yasui, T. 2000. Genetic elimination of a starch granule protein, SGP-1, of wheat generates an altered starch with apparent high amylose. *Theor. Appl. Genet*., 101: 21-29.