Euphytica **72**: 171–175, 1994.

Endosperm starch properties in several wheat cultivars preferred for Japanese noodles

Hideho Miura & Sachiko Tanii
Department of Crop Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, 080, Japan

Received 19 July 1993; accepted 3 November 1993

Key words: amylose content, flour peak viscosity, Japanese noodle, *Triticum aestivum*, Wx protein

Abstract

To characterize superior genotypes for the white Japanese noodle, endosperm starch properties including amylose content, flour peak viscosity and starch-granule bound Waxy (Wx) proteins were compared using several cultivars preferred for noodle manufacture. Amylose contents from three seasons trials and flour peak viscosity from two seasons trials varied among cultivars. Low amylose content was a common property in the noodle cultivars, whereas a high peak viscosity was not always the case. When the Wx proteins were analyzed by sodium dodecylsulfate polyacrylamide gel electrophoresis, a clear reduction in the amount of low molecular weight protein or a lack of the high molecular weight protein occurred in the noodle cultivars. Segregation of Wx proteins was detectable in a B_1F_1 population, indicating that the Wx protein analysis has a potential as a surrogate of selecting low amylose genotypes in early generations.

Introduction

Wheat is processed into many different kinds of manufactured products. In eastern Asian countries such as Japan, Korea and China, the flour from soft wheats is often processed into noodles. An objective of wheat breeders and cereal chemists in Japan is to produce cultivars for the white Japanese noodle with high eating quality. Both quantitative and qualitative aspects of starch in the endosperm play important parts for processing and eating quality of the noodle (Moss, 1979; Oda et al., 1980; Toyokawa et al., 1989), whereas protein content of flours influences the chewiness of cooked noodles (Oh et al., 1983). The wheat endosperm starch is composed of amylose and amylopectin, and a low amylose content has been thought to be preferable for noodle manufacture.

The works of Moss (1979) and Oda et al. (1980) implied that selection for low amylose in early generations could prove fruitful. However, screening in the segregating generations is impracticable due mainly to difficulties in accurate measuring the content on a single grain basis. The most convenient method for assessing the noodle quality is by using a viscoamylograph to measure starch pasting properties. This method can also be restrictive because the amount of flour or starch required limits its application until the later generations of a breeding program (Crosbie, 1991; Panozzo & McCormick, 1993).

In maize and rice, it has been reported that amylose synthesis in the endosperm is controlled by the *Wx* loci (Echt & Schwarts, 1981; Sano, 1984). The *Wx* genes encode for the starch granule-bound protein known as Waxy (Wx) protein with a molecular weight of about 60 kDa. This type of Wx protein is also detected in wheat (Schofield & Greenwell, 1987) and cultivars with less Wx protein amount possess lower amylose content (Yamamori et al., 1992).

Therefore, a better understanding of the genetic relationships among amylose content, starch paste peak viscosity and amount of Wx proteins would contribute to characterize superior genotypes in early generations and consequent development of improved cultivars. In the present study, we compared endosperm starch properties of interest using several cultivars grown for noodle production.

Materials and methods

Source of grain and flour

Two Japanese cultivars, Chihokukomugi and Kanto 107, and one Australian cultivar, Halberd were used. The soft red winter wheat Chihokukomugi is a leading cultivar grown mainly for noodle production in northern Japan. The soft red spring wheat Kanto 107 is a breeding line with about the lowest amylose content among accessions of Japanese wheats (Kuroda et al., 1989) and is widely used in Japanese noodle breeding programs. Halberd, a soft white spring wheat, is a major component cultivar in Australian Standard White which is highly regarded in Japan for its suitability for the production of Japanese noodles. Spring and winter wheat cultivars, Chinese Spring (CS) and Cheyenne (CNN), respectively, were also included as checks.

To detect segregation of the Wx proteins in early generations, CS × Kanto 107 F_1 plants were developed and backcrossed with Kanto 107 to produce a B_1F_1 population.

Grain samples of the cultivars were obtained from three seasons trials conducted in the research field of Obihiro University during the 1990-1992 seasons. Kanto 107 and Halberd were grown together with CS in the spring sown trials. Chihokukomugi and CNN were grown in the autumn sown trials where CS was included again. The spring and autumn sowings were planted early-May and late-September, respectively, and both harvested in August. 100 g of grain were milled on a Brabender Quadrunt Junior Test mill to produce a 60% extraction flour.

Amylose content

Starch granules were separated from the resultant flours using conventional methods. 100 mg of the starch granules were weighed accurately and the percentage amylose content was determined as mg using the Auto Analyzer System II (Bran + Lubbe Co.). In this system, amylose content was estimated as compared to the amylose content of the artificially synthesized standards. The assessment was carried out at least twice.

Flour peak viscosity

A Rapid Visco Analyser (Newport Scientific) was used to measure the pasting property of flour samples obtained from the 1990 and 1992 trials. The peak viscosity of the 4 g flour sample was recorded as stirring number. The analysis was made at least twice.

Wx protein

The half endosperms were crushed and their starch granules were isolated according to the method described by Echt & Schwarts (1981). Two grains for 5 cultivars and a single grain for the B_1F_1 population were analyzed.

Wx proteins were separated into the high molecular weight and low molecular weight proteins by Laemmli's SDS-PAGE system (Laemmli, 1970) with a minor modification used by Nakamura et al. (1992). The proteins were stained with silver stain kits (Wako Pure Chemical Industry Ltd.).

Results

Amylose content measured in three seasons trials are presented in Table 1. The mean amylose contents of CS grown in the spring and autumn sowings were almost the same and yielded around 24% (24 mg amylose per 100 mg starch granules). Another check cultivar CNN showed a slightly higher content than autumn sown CS.

Variation in amylose content was clear among cultivars examined. The mean content of 21.33% in Kanto 107 was the lowest and 2.94% lower than that of CS. Chihokukomigi produced 22.72% amylose content grains which was 1.39% higher than Kanto 107 but 1.29% lower than autumn sown CS. Halberd showed a significantly lower content than CS in two seasons except 1992 when starch granules received damage due to preharvest sprouting.

For the peak viscosity measured in two seasons trials, significant differences among cultivars as well as between seasons were observed in autumn sown trials (Table 1). The highest peak viscosity was recorded in the flour from CNN and followed by Chihokukomugi and CS. In the spring sown trials, comparisons of Kanto 107 and Halberd with CS were limited in one season due to preharvest sprouting damage or insufficient amount of flour. Both cultivars showed a slightly

Table 1. Amylose content of cultivars grown in three seasons trials and flour peak viscosity in two seasons trials

Cultivars	Amylose content, % (mg amylose per 100 mg starch granule)				Flour peak viscosity (stirring number)		
	1990	1991	1992	Mean	1990	1992	Mean
Autumn sowing							
CS	23.93	24.12	23.99	24.01	283.0	246.2	264.6
CNN	23.97	25.30	24.10	24.46	384.0**	303.0*	345.5*
Chihokukomugi	22.18**	23.60*	22.39***	22.72***	322.3*	238.0	305.2
Spring sowing							
CS	24.50	24.75	23.56	24.27	214.7	275.5	245.1
Halberd	21.35***	23.00*	–[a]	22.18*	245.0	–	
Kanto 107	21.20***	20.81***	22.00***	21.33***	–	296.0	

[a] Not included due to damage by the preharvest sprouting or insufficient amount of flours.
*, **, ***; Significantly different from CS at the 5%, 1% and 0.1% levels, respectively.

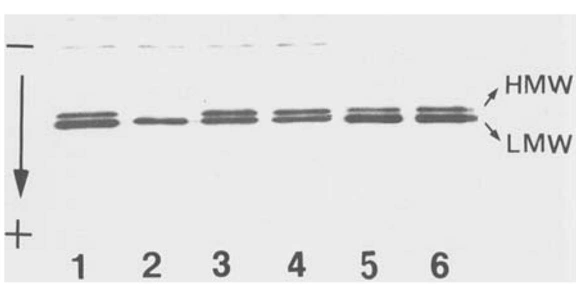

Fig. 1. SDS-polyacrylamide gel electrophoresis patterns of Wx proteins extracted from endosperm starch granules of five cultivars. 1; CS, 2; Kanto 107, 3; Halberd, 4; Chihokukomugi, 5; CNN, 6; CS.

higher peak viscosity than CS whilst the differences were not significant.

SDS-PAGE electrophotograms of starch granule Wx proteins from five cultivars are presented in Fig. 1. The Wx proteins of all cultivars except Kanto 107 were distinctly separated into two main bands, the high molecular weight (HMW) and low molecular weight (LMW) proteins. Wx protein bands detected in CS and CNN were nearly identical, having the LMW Wx protein band thicker than the HMW. Compared with these checks, Chihokukomugi and Halberd showed a clear decrease in the LMW protein levels, while differences in the HMW protein bands were somewhat vague. Kanto 107 exhibited a particular banding pattern, being characterized by a lack of the HMW protein and a great reduction in the LMW protein.

The banding patterns shown in Fig. 2 indicated that the segregation of the HMW and LMW proteins was detectable in a early generation when a B_1F_1 population from the cross between CS and Kanto 107 were analyzed on a single grain basis.

Discussion

Differences in amylose content of endosperm starch were clearly detected. Rankings for the content were consistent over seasons, with Kanto 107 < Halbert ≤ Chihokukomugi < CS ≤ CNN in order of lowness, indicating that the low amylose content was under genetic control and cultivar × season interactions were not large. These results confirm that low amylose content is a required factor for Japanese noodle production.

In a breeding program in which results from different seasons are compared, it is significant that relative rankings are held from season to season. Since cultivar × season interactions were small in this study, selection for low amylose content would be of benefit as a noodle breeding strategy. However, a relatively narrow range of the content among cultivars (Kuroda et al., 1989) and the difficulty in accurate measuring on a single grain basis may limit this strategy.

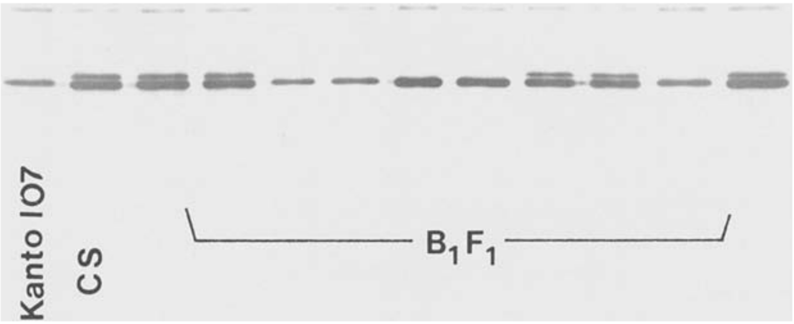

Fig. 2. Segregation of the HMW and LMW Wx proteins in a B_1F_1 population of a cross between CS and Kanto 107 where Kanto 107 was used as a recurrent parent. The Wx proteins were analyzed on a single grain basis.

A high peak viscosity is another important determinant for noodle production. Its measurements also require a relatively large sample size but have become the most widely accepted means of selecting for improved quality (Crosbie, 1991; Panozzo & McCormic, 1993). A negative correlation between amylose content and the peak viscosity has been detected. These characters, however, were not always correlated as CNN showed high scores for both characters in this study, and cultivars with only small differences in amylose content differed appreciably in starch peak viscosity (Crosbie, 1991). The viscosity can reflect the quality of resultant flours relevant to the degree of sprouting damage, rather than genetic property of grains itself. Thus it is thought that variation in the peak viscosity is affected by several factors apart from amylose content. Consequently, a high peak viscosity is not solely responsible for differences in amylose content.

There is a need for a more discriminating method which can be used for selecting low amylose genotypes in early generations. A correlation between low amylose content and reduced amounts of Wx proteins has been reported (Yamamori et al., 1992). In this study, although a limited number of cultivars was assessed, the ranking of amylose content corresponded with Wx protein amounts. These results are as expected, because the Wx proteins are involved in amylose synthesis. Detection of Wx protein bands using SDS-PAGE systems is less complicated and inexpensive compared to assessments of amylose content and peak viscosity. In addition, as demonstrated in Fig. 2, the Wx protein analysis in early segregating generations can be made readily using a half endosperm. This may permit screening of large populations on a single grain basis and then enable growing the remaining embryo of interest which will offer progenies in later generations for assessing the other starch properties such as amylose content and peak viscosity. Taking these points into consideration, it is expected that the Wx protein analysis has a potential as a surrogate of selecting low amylose genotypes in early generations.

Acknowledgements

We thank Drs. Y. Amano, N. Watanabe, T. Nakamura and Mr. K. Araki for their helpful discussion and assistance in amylose content analysis and SDS-PAGE analysis.

References

Crosbie, G.B., 1991. The relationship between starch swelling properties, paste viscosity and boiled noodle quality in wheat flours. J. Cereal Sci. 13: 145–150.

Echt, C. & D. Schwartz, 1981. Evidence for the inclusion of controlling elements within the structural gene at the waxy locus in maize. Genetics 99: 275–284.

Kuroda, A., S. Oda, S. Miyakawa & H. Seko, 1989. A method of measuring amylose content and its variation in Japanese wheat cultivars and Kanto breeding lines. Jpn. J. Breed. 39 (Suppl. 2): 142–143.

Laemmi, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

Moss, H.J., 1979. The pasting properties of some wheat starches free of sprout damage. p. 297–302. In: M.D. Gale & V. Stoy (Eds), Pro. 2nd Int. Symp. Preharvest Sprouting in Cereals, Edinburgh.

Nakamura, T., M. Yamamori, S. Hidaka & T. Hoshino, 1992. Expression of HMW Wx proteins in Japanese common wheat (*Triticum aestivum* L.) cultivars. Jpn. J. Breed. 42: 681–685.

Oda, M., Y. Yasuda, S. Okazaki, Y. Yamauchi & Y. Yokoyama, 1980. A method of flour quality assessment for Japanese noodles. Cereal Chem. 57: 253–254.

Oh, N.H., P.A. Seib, C.W. Deyoe & A.B. Ward, 1983. Noodles I. Measuring the textural characteristics of cooked noodles. Cereal Chem. 60: 433–438.

Panozzo, J.F. & K.M. McCormick, 1993. The rapid viscoanalyser as a method of testing for noodle quality in a wheat breeding programme. J. Cereal Sci. 17: 25–32.

Sano, Y., 1984. Differential regulation of *waxy* gene expression in rice endosperm. Theor. Appl. Genet. 68: 467–473.

Schofield, J.D. & P. Greenwell, 1987. Wheat starch granule proteins and their technological significance. p. 399–420. In: I.D. Morton (Ed), Cereals in European context, Ellis Horwood, Chichester.

Toyokawa, H., G.L. Rubenthaler, J.R. Powers & E.G. Schanu, 1989. Japanese noodle qualities. I. Flour components. Cereal Chem. 66: 382–386.

Yamamori, M., T. Nakamura & A. Kuroda, 1992. Variations in the content of starch-granule bound protein among several Japanese cultivars of common wheat (*Triticum aestivum* L.). Euphytica 64: 215–219.