

## Granule-bound starch synthase and endosperm mealiness correlation in wheat (*Triticum aestivum* L.) with nonstandard colored caryopses

KLARA STIASNA<sup>1</sup>, MARIA PRESINSZKA<sup>1</sup>, TOMAS VYHNANEK<sup>1</sup>, VACLAV TROJAN<sup>1</sup>, EVA MRKVICOVA<sup>2</sup>, LUDEK HRIVNA<sup>3</sup>, LADISLAV HAVEL<sup>1</sup>

<sup>1</sup>Department of Plant Biology

<sup>2</sup>Department of Animal Nutrition and Forage Production

<sup>3</sup>Department of Food Technology

Faculty of Agronomy  
Mendel University in Brno  
Zemedelska 1, 613 00 Brno  
CZECH REPUBLIC

klara.stiasna@mendelu.cz

**Abstract:** Granule-bound starch synthase (GBSS) is a key enzyme in starch biosynthesis pathway. It is responsible for production of amylose, therefore low amylose level has been associated with GBSS deficiency. This enzyme, also known as waxy protein, is encoded by three homologous genes – *Wx-A1*, *Wx-B1* and *Wx-D1* and their dysfunction of any origin causes decrease of amylose content. Starch content and, therefore amylose content, is closely linked with structure of the cereal endosperm. It is known, that mealy endosperm contains more starch than vitreous, which contains more proteins. To identify null alleles of waxy genes in 25 genotypes of wheat with nonstandard colored caryopses, PCR markers were used. To determine the endosperm structure of these genotypes, the non-destructive Light Transflectance Meter (LTm) was used. Null alleles for *Wx-B1* locus were observed in 5 tested genotypes, loci *Wx-A1* and *Wx-D1* were amplified in all genotypes. Percentage of mealy caryopses per sample ranged from 0 to 100 % among tested genotypes. No significant correlation between these two parameters was observed.

**Key-Words:** wheat, *Triticum aestivum* L., mealy endosperm, waxy protein, GBSS

### Introduction

Starch, which accounts for 65 – 75% of wheat caryopsis dry weight, is one of the most significant elements of the cereal endosperm along with storage proteins [1]. It is a polymer composed of two glucose carbohydrates: amylose and amylopectin [2]. Amylose synthesis is regulated by enzyme granule-bound starch synthase (GBSS), also called waxy protein. In common wheat, because of its allohexaploidy ( $2n = 6x = BBAADD$ ), this enzyme is encoded by three homologous *waxy* genes located on the short arm of the chromosomes 7A (*Wx-A1* locus), 7D (*Wx-D1*), and on the long arm of the chromosome 4A (*Wx-B1*) which was translocated from its original short arm of 7B [3, 4, 5]. Products of these genes, the waxy proteins, have slightly different molecular weight around 59 – 60 kDa [6]. When only one or two *GBSS* genes are functional, the wheat is called partially waxy, while genotypes

with null allele at all three GBSS loci are called waxy (triple null), the starch has almost no amylose [7]. Waxy or partial waxy wheat types are desirable material for Asian noodles production [8] and has significant effect on bread making quality [9, 7]. Different null *Wx* alleles have different effect on amylose content in wheat.

Mealiness or vitreousness of wheat endosperm primary depends on starch content. Mealy endosperm contains more starch and less proteins and vice versa [10, 11]. This characteristic is essential for example in brewing industry in process of malting and other sectors of food industry and depends predominately on variety.

In this work, we wanted to verify whether there is a correlation between occurrence of waxy genes alleles and endosperm mealiness.

Table 1 Used genotypes overview, percentage of mealy grains and null allele incidence

Number	Genotype	Caryopsis color	Mealy grains [%]	Product size		
				<i>Wx-D1</i> 299 bp	<i>Wx-A1</i> 257 bp	<i>Wx-B1</i> 227 bp
1	Novosibirskaya 67	white	2	+	+	+
2	ANK-28A	purple	10	+	+	+
3	ANK-28B	purple	6	+	+	+
4	Abissinskaya Arraseita	purple	84	+	+	+
5	Konini	purple	30	+	+	-
6	Purple	purple	87	+	+	+
7	Purple Feed	purple	91	+	+	+
8	Indigo	purple	100	+	+	+
9	Rosso	purple	88	+	+	+
10	Citrus	yellow	0	+	+	+
11	Luteus	yellow	50	+	+	+
12	Bona Dea	yellow	89	+	+	+
13	TA 4024	yellow	3	+	+	-
14	UC66049	blue	99	+	+	+
15	Tschermaks Blaukörniger Sommerweizen	blue	98	+	+	-
16	Tschermaks Blaukörniger	blue	99	+	+	-
17	48M	blue	97	+	+	+
18	Skorpion (RU 440-6)	blue	76	+	+	+
19	RU 440-5	blue	96	+	+	+
20	Barevná 9	blue	23	+	+	-
21	Barevná 25	blue	74	+	+	+
22	Xiao Yian	blue	68	+	+	+
23	EF 02-54/9	blue	100	+	+	+
24	H 90-15-2	blue	85	+	+	+
25	Heroldo	white	34	+	+	+
Null allele incidence [%]				0	0	20

## Material and Methods

### Plant material

Total of 25 genotypes with nonstandard colored caryopses were analyzed. Sample collection was obtained from the Agricultural Research Institute Kroměříž, Ltd., Czech Republic and contained genotypes with white, purple, yellow and blue caryopses which are rich in natural pigments. The list of used genotypes is presented in Table 1.

### Identification of null *GBSS* alleles

Genomic DNA was isolated using DNeasy Plant Mini Kit (Qiagen, Germany) from 5 to 7 days old

wheat seedlings planted in controlled laboratory conditions. Primers #4F and #4R used in this study were developed by McLauchlan et al. [12]. Used primers were designed to amplify all three copies of *GBSS* genes on genome A, B and D. Primer details and PCR conditions are summarized in Table 2. The reaction took place in the volume of 25 µl. PCR products were separated on 1.5% agarose gel stained with ethidium bromide and visualized by UV exposure. The largest fragment (299 bp) belonged to the D genome, the intermediate one (257-bp) to the A genome and the smallest one (227-bp) to the B genome.

Table 2 Primers sequence and PCR conditions

Name	Sequence	Amplified loci	Cycling condition
#4F	AAGAGCAACTACCAGT	Wx-A1, Wx-B1	initial denaturation step of 2 min at 94 °C
#4R	TCGTACCCGTCGATGAAGTCGA	and Wx-D1	followed by 33 cycles of 94 °C 1 min, 54 °C 2 min, 72 °C 2 min

### Mealiness determination

Mealiness or vitreousness of wheat endosperm was examined according to Chandra et al. [13] using Light Transflectance Meter (LTm) originally developed for barley testing. This non-destructive

method is based on measurement of laser beam transfer through caryopses. For a single experiment, 97 caryopses are used from each genotype. Caryopses with mealy endosperm cause light scattering, LTm values are low. Vitreous caryopses

transmit more light thus LTm values are higher. Final number is an expression of mealy caryopses percentage in sample.

## Results and Discussion

### GBSS genes variability

In 25 tested genotypes variability occurred only in locus *Wx-B1*. Five genotypes (20%), namely Konini, TA 4024, Tschermaks Blaukörniger Sommerweizen, Tschermaks Blaukörniger and Barevná 9 were deficient in the 227 bp product. We can conclude that null allele is present in this locus. *Wx-A1* and *Wx-D1* alleles were present in the whole collection (Table 1, Figure 1). In a set of 103 Argentinian wheat cultivars [2], 24% samples lack

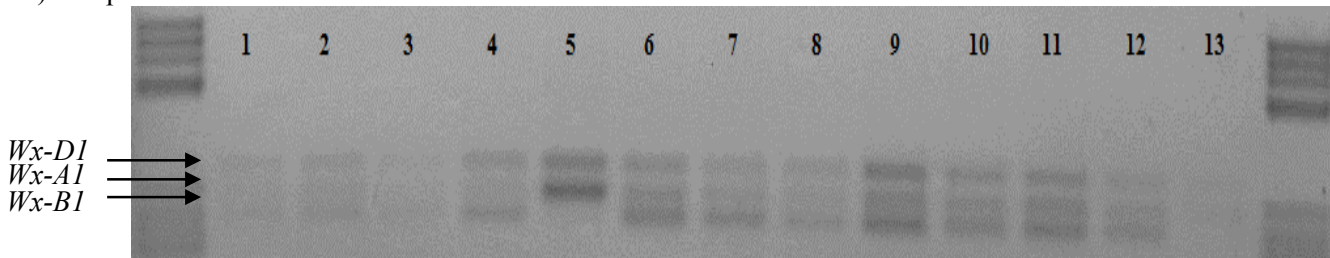
the PCR fragment for *Wx-A1*. Results for *Wx-B1* and *Wx-D1* were the same as in this paper. In Iranian cultivars the incidence of *Wx-B1* null allele was lower, 4.2% had no PCR product in this locus [1].

Distribution of the null alleles is the matter of geographical origin. Null allele for A genome is frequent in Turkish genotypes. On the other hand, Korean, Japanese and Australian wheat often carry the null allele *Wx-B1*. *Wx-D1* null alleles are very rare [14].

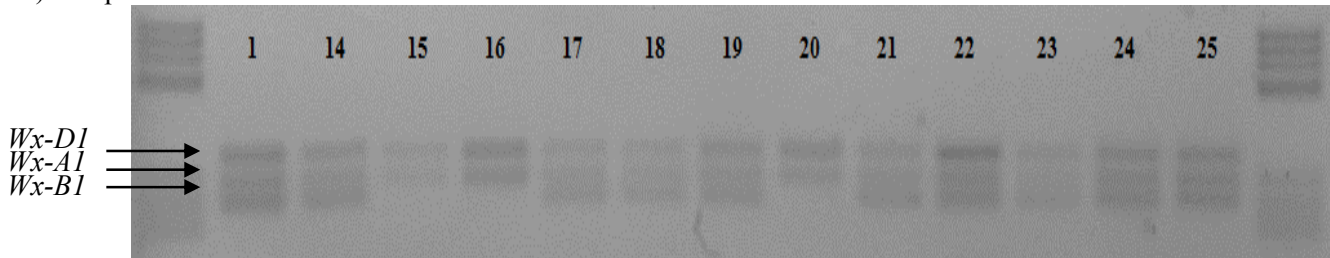
Starch from *Wx-B1* deficient wheat displayed some functional properties in starch pasting [15]. These partial waxy types may have the largest usage in Asian countries for production special noodle types, like udon or ramen [16].

Fig. 1 PCR products of *Wx-A1*, *Wx-B1* and *Wx-D1*

A) Samples 1 – 13



B) Samples 1 and 14 – 25



### Mealiness of wheat caryopses

Percentage of mealy caryopses present in samples ranged from 0 to 100%. Figure 2 shows examples of LTm values among 97 caryopses per sample. Two genotypes EF 02-54/9 and TA 4024 with extreme values are present. We can conclude, that 8 genotypes, namely Novosibirskaya 67, ANK-28A, ANK-28B, Konini, Citrus, TA 4024, Barevná 9 and Heroldo, had less than 50% of mealy caryopses in sample and the rest of 17 genotypes had 50% or more caryopses with mealy endosperm, even 8 of them had the percentage of mealy caryopses higher than 90%. Results are shown in Table 1. Genotypes with mealy endosperm may be interesting material for wheat beer brewing, because increasing mealiness causes increased malting yield [20].

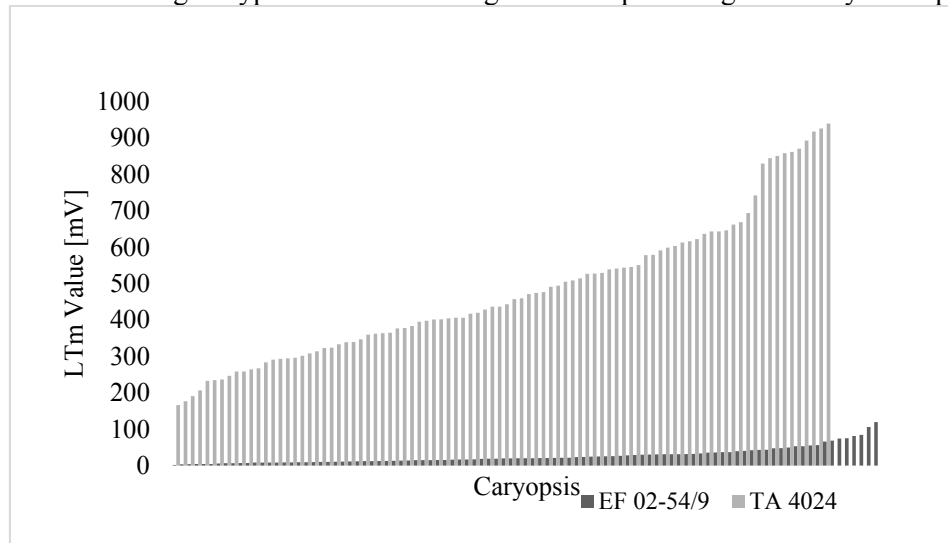
### Correlation between presence of *Wx-B1* null allele and endosperm mealiness

Even though many sources claim that *Wx-B1* has the largest effect on amylose content in wheat endosperm [15, 17, 18, 19] and its structure is closely linked to starch amount, no significant correlation was found between mealiness and occurrence *Wx-B1* allele in tested genotypes according to Pearson correlation coefficient.

Possible explanation could be the effect of the other two present *Wx-A1* and *Wx-D1* alleles which could substitute absence of *Wx-B1* product in genotypes with its deficiency.

Another reason could be that *GBSS* genes influence only one constituent of the starch, amylose. The second component, amylopectin, is controlled in other ways.

Fig. 2 Examples of two used genotypes with extreme high and low percentage of mealy endosperm



Legend: EF 02-54/9 has 100 % mealy caryopses, 3 % of TA 4024 caryopses are mealy, thus is considered vitreous.

## Conclusion

A set of 25 common wheat (*Triticum aestivum* L.) genotypes with white, purple, yellow and blue caryopses were examined in terms of *GBSS* or *waxy* genes variability. Five of them showed absence in *Wx-B1* locus and may therefore be considered as partially waxy and are potentially valuable in future breeding programs. Collection of wheat with nonstandard colored caryopses showed wide range of endosperm structure.

## Acknowledgement

This work was supported by IGA FA MENDELU No. 1/2014. Authors are grateful to Ing. Petr Martinek, CSc. for experimental material.

## References:

- [1] Khaniani BH, Darvish F, Bihamta MR, Hassani ME, Najafian G, Identification of a null allele at the *Wx-B1* locus in some of Iranian bread wheat genotypes, *Advances in Environmental Biology*, Vol.6, No.10, 2012, pp. 2586–2589.
- [2] Vanzetti LS, Pflüger LA, Rodríguez-Quijano M, Carrillo JM, Helguera M, Genetic variability for waxy genes in Argentinean bread wheat germplasm, *Electronic Journal of Biotechnology*, Vol.12, No.1, 2009, pp. 1–9.
- [3] Shure M, Wessler S, Fedoroff N, Molecular identification and isolation of the Waxy locus in maize, *Cell*, Vol. 35, No.1, 1983, pp. 225–233.
- [4] Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD, RFLP-based genetic maps of wheat homoeologous group 7 chromosomes, *Theoretical and Applied Genetics*, Vol.78, No.4, 1989, pp. 495–504.
- [5] Yamamori M, Nagamine T, Nakamura T, Endo TR, Waxy protein deficiency and chromosomal location of coding genes in common wheat, *Theoretical and Applied Genetics*, Vol.89, No.2–3, 1994, pp. 179–184.
- [6] Maryami Z, Fazeli A, Mehrabi A-A, Investigation of diversity of Waxy-A1 gene using amplification in different spices in A genome wheat's. *Advances in Environmental Biology*, Vol.8, No.7, 2014, pp. 2004–2007.
- [7] Ma H, Zhang X, Wang C, Gao D, Zhang B, Lv G, Wu R, Cheng X, Wang X, Bie T, Effect of *wx* genes on amylose content, physicochemical properties of wheat starch, and the suitability of waxy genotype for producing Chinese crisp sticks, *Journal of Cereal Science*, Vol.58, No.1, 2013, pp. 140–147.
- [8] Epstein J, Morris CF, Huber KC, Instrumental Texture of White Salted Noodles Prepared from Recombinant Inbred Lines of Wheat Differing in the Three Granule Bound Starch Synthase (Waxy) Genes, *Journal of Cereal Science*, Vol.35, No.1, 2002, pp. 51–63.
- [9] Lee MR, Swanson BG, Baik BK, Influence of amylose content on properties of wheat starch and breadmaking quality of starch and gluten blends, *Cereal Chemistry*, Vol.78, No.6, 2001, pp. 701–706.
- [10] Kosař K, Psota V, Havlová P, Šusta J, *Sladovnícký ječmen* In: Kosař K, Procházka S, *Technologie výroby sladu a piva*, Výzkumný ústav pivovarský a sladařský a.s., 2000.
- [11] Gamlath J, Milling energy of malting barley in relationship to endosperm hardness: a review,

- Master Brewers Association of the Americas Technical Quarterly*, Vol.44, No.1, 2007, pp. 8–14.
- [12] McLauchlan A, Ogbonnaya FC, Hollingsworth B, Carter M, Gale KR, Henry RJ, Holton TA, Morell MK, Rampling LR, Sharp, PJ Shariflou MR, Jones MGK, Appels R, Development of robust PCR-based DNA markers for each homoeo-allele of granule-bound starch synthase and their application in wheat breeding programs, *Australian Journal of Agriculture Research*, Vol.52, No.11-12, 2001, pp. 1409–1416.
- [13] Chandra S, Wheaton L, Schumacher K, Muller R, Assessment of barley quality by light transmission - The rapid LTM meter, *Journal of the Institute of Brewing*, Vol.107, No.1, 2001, pp. 39–47.
- [14] Urbano M, Margiotta B, Colaprico G, Lafianda D, Waxy proteins in diploid, tetraploid and hexaploid wheats, *Plant Breeding*, Vol.121, No.6, 2002, pp. 465–469.
- [15] Hansen LE, Jackson DS, Wehling RL, Wilson JD, Graybosch RA, Functionality of native tetraploid wheat starches: Effects of waxy loci alleles and amylose concentration in blends, *Journal of Cereal Science*, Vol.52, No.1, 2010, pp. 39–45.
- [16] Nagao S, *Processing Technology of Noodle Products in Japan* In: Kruger JE, Matsuo RB, Dick JW, *Pasta and Noodle Technology*, American Association of Cereal Chemists, 1996.
- [17] Miura H, Araki E, Tarui S, Amylose synthesis capacity of the three Wx genes of wheat cv. Chinese spring. *Euphytica*, Vol.108, No.2, 1999, pp. 91–95.
- [18] Yamamori M, Quynh NT, Differential effects of Wx-A1,-B1 and-D1 protein deficiencies on apparent amylose content and starch pasting properties in common wheat, *Theoretical and Applied Genetics*, Vol.100, No.1, 2000, pp. 32–38.
- [19] Kim W, Johnson JW, Graybosch RA, Gaines CS, Physicochemical properties and end-use quality of wheat starch as a function of waxy protein alleles, *Journal of Cereal Science*, Vol.37, No.2, 2003, pp. 195–204.
- [20] Psota V, Vejražka K, Hartmann J, Musilová M, Effect of the endosperm structure of barley caryopsis (*Hordeum vulgare* L.) on malt quality, *Kvasný Průmysl (Czech Republic)*, 2008