

## Haplotype Analysis of *Wx* and *Alk* Genes and Amylopectin Chain-Length Distribution among Kazakhstan Glutinous Rice Lines

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### Abstract

Although there is low precipitation in Kazakhstan, rice is grown in regions serviced by irrigation canals. And breeding has begun in order to reduce the cost of importing glutinous rice. To develop glutinous rice cultivars efficiently, we need information on the haplotypes of *Wx* and *Alk* genes that affect eating quality. We investigated the haplotypes and amylopectin chain-length distribution in the first glutinous rice breeding lines developed in Kazakhstan. Such information could be useful to other countries starting glutinous rice breeding using parents possessing variation in *Wx* and *Alk* haplotypes.

**Discipline:** Plant breeding

**Additional key words:** amylose content, *SSIIa*, waxy protein

### Introduction

Agriculture is Kazakhstan's most important industry. More than 80% of the nation's land area (approx. 2.7 million km<sup>2</sup>) is occupied by farmland, with pastures accounting for 80%, arable land for 18%, and meadows for 2% (FAO 1995). Kazakhstan has a continental climate, with cold dry winters and hot dry summers. The lowest monthly average air temperature is -18.7°C in the north and -3°C in the south; the highest monthly average air temperature is 18.8°C in the north and 28.8°C in the south. The short cropping season is limited to the period between April and September in the north, and between March and October in the south. Most of the land is classified as steppe or desert, with an estimated average annual precipitation of 150-320 mm. Yet rice is grown in arid or semi-arid areas serviced by irrigation canals, such as along the lower Syr Darya River (South Kazakhstan Region and Kyzylorda Region) and the Ili River (Almaty Region), where rice is grown from May to September. The total rice growing area

in these regions is about 113 000 ha, or about 17% of the total irrigated area in Kazakhstan (UNDP 2015). The annual consumption of rice in Kazakhstan is 132 600 t (7.5 kg per capita).

In Kazakhstan, rice is usually consumed as pilaf, a side dish, soup, porridge with milk, or a fermented beverage, all prepared from non-glutinous rice. Glutinous rice is mainly consumed by Kazakhstan's Asian citizens, and can also be used as a component of baby foods (powdered and liquid infant formulas). Glutinous rice porridge can reportedly protect against gastric mucosa irritation from chemical factors, and is thus recommended as a dietary food for patients with impaired digestive tract function (Kovalev 2004). Given its special qualities and useful properties, glutinous rice could become more popular worldwide including Kazakhstan with high marketing value. As of 2015, 28 non-glutinous rice cultivars were being grown commercially in Kazakhstan. Of those cultivars, 14 were developed in Kazakhstan. However, virtually all glutinous rice is imported, at three to four times the cost of domestic

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non-glutinous rice. As growing it domestically will lower the price in Kazakhstan and contribute to food security, local rice breeders began developing new cultivars adapted to Kazakhstan's irrigated land.

The difference in eating quality between glutinous and non-glutinous rice is mainly due to the amylose content. Amylose synthesis in the rice grain is catalyzed by granule-bound starch synthase I (GBSSI), which is encoded by the *Wx* gene on chromosome 6 (Smith et al. 1997, Sano 1984, Mikami et al. 2008). Further, the *Alk* (*SSIIa*) gene also on chromosome 6, the locus apart from *wx* locus with 28.8cM, affects eating quality through variation in the amylopectin chain-length distribution (Umemoto et al. 2008). As glutinous rice has not been previously bred in Kazakhstan, there is no information on the *Wx* and *Alk* haplotypes of Kazakhstan rice cultivars and donor cultivars, or on the interactions between these genes in the genetic background of Kazakhstan cultivars. Here, we describe the *Wx* and *Alk* haplotypes and amylopectin chain-length distribution in the first glutinous rice breeding lines developed in Kazakhstan. The approach to developing glutinous rice cultivars and monitoring the *Alk* haplotype could be applicable to other countries where glutinous rice breeding is performed with none-glutinous rice possessing functional *Alk* as the crossing parents.

## Materials and Methods

### 1. Plant materials

#### (1) Crossing parents

Russian cultivars Viola and Violetta (both glutinous; *wx*), developed by the All-Russian Rice Research Institute, Krasnodar, were used as female donors for the waxy (*wx*) trait. Kazakhstan cultivars Bakanasski, Marzhan, and Akdala (non-glutinous; *Wx*) were used as pollen parents with preferable agronomic traits. Bakanasski and Akdala were developed by the Institute of Plant Biology and Biotechnology (IPBB), Almaty, Kazakhstan; Bakanasski was released in 2008 for the Almaty Region, while Akdala is not an officially registered cultivar. Marzhan was developed by the I. Zhahaev Kyzylorda Rice Research Institute, Kyzylorda, Kazakhstan; it was released in 1987 for the Kyzylorda Region.

#### (2) Glutinous lines

Glutinous lines were bred in a glasshouse in 2013 from the crosses Viola × Akdala, Viola × Marzhan, Viola × Bakanasski, Violetta × Akdala, and Violetta × Marzhan. Two generations of progeny were grown each year until F<sub>4</sub>. During the breeding, individual plants producing opaque grains typical of glutinous rice were selected, resulting in 21 lines (Table).

#### (3) Field cultivation

The F<sub>4</sub> lines were grown in an experimental field of

IPBB, Balkhash, Almaty Region, in 2015 for an evaluation of waxy and amylopectin traits without replication. The field management of these lines followed conventional agricultural practices in the region.

#### (4) Control samples

As controls for amylose content, the existence of *Wx* protein, and *Wx* and *Alk* haplotype analysis, the following Japanese cultivars were grown in an experimental field at the NARO Hokkaido Agricultural Research Center, Sapporo, Japan in 2014: *japonica* cultivars Kitamizuho (*Wx*-a, *alk*), Yukigozen (*Wx*-b, *alk*), and Hakuchou-mochi (*wx*, *alk*).

## 2. Evaluation of waxy and amylopectin traits

Grain of the field grown plants was evaluated for amylose content, the existence of *Wx* protein, *Wx* and *Alk* haplotypes, and amylopectin chain-length distribution. Rice flour was prepared using a hammer till the flour became a fine powder.

#### (1) Determination of amylose content

The apparent amylose content in the flour was determined by using an Auto Analyzer II (Bran+Luebbe Co. Ltd., Norderstedt, Germany) as described by Ando et al. (2010). This colorimetric method was based on the starch - iodine reaction.

#### (2) Identification of *Wx* protein in seed storage protein

The *Wx* protein in the crude protein extracted from rice flour was identified by SDS-polyacrylamide gel electrophoresis (PAGE) according to Ashida et al. (2011) in 7.5% polyacrylamide gel.

#### (3) DNA extraction and PCR amplification

Genomic DNA was extracted for PCR amplification from the rice flour by using DNA Sui Sui reagent (Rizo Co. Ltd., Tsukuba, Japan) as per the manufacturer's instructions. To identify the *wx* allele, we performed PCR using marker Glu23 (Wanchana et al. 2003). To identify *Wx*-a and *Wx*-b, we conducted derived cleaved amplified polymorphic sequence (dCAPS) analysis according to Yamanaka et al. (2004). To discriminate apparent high- and intermediate-amylose *Wx*-a types, we used markers *Wx*Ex6 and *Wx*Ex10 (Chen et al. 2010). To identify *Alk* haplotypes, we used markers SNP3 and SNP4 according to Hiratsuka et al. (2009).

#### (4) Determination of amylopectin structure

The amylopectin chain length was analyzed by fluorescence-assisted carbohydrate capillary electrophoresis after the gelatinized starch was debranched by isoamylase (Fujita et al. 2001). Results are the means of two measurements.

## Results

### 1. Waxy traits

Both glutinous parents (Viola and Violetta) had an apparent amylose content of 1.9%. The non-glutinous parents

(Bakanasski, Marzhan, and Akdala) had a content of around 20% (Table). All F<sub>4:5</sub> lines had content similar to that of the glutinous parents, from -0.2% to 3.0%. SDS-PAGE analysis showed the existence of Wx protein in the three Kazakhstan non-glutinous parents, and its absence in introduced glutinous cultivars (Fig. 1) and in glutinous lines (Table). Each glutinous parent had the *wx* allele (Fig. 2). All Kazakhstan

non-glutinous parents had *Wx-a*, while the glutinous parents had the *Wx-b*-derived *wx* allele. The non-glutinous parents harbored the ‘C-C’ SNP combination (Chen et al. 2010), indicating that the *Wx-a* allele of the non-glutinous parents gives an intermediate amylose content. As expected from the haplotype of the glutinous parents, each glutinous line had the *Wx-b*-derived *wx* allele (Table).

**Table. Waxy and amylopectin traits of glutinous lines and their parents**

No.	Line/cultivar	Cross combination	Generation	Wx Protein <sup>1)</sup>	Amylose content (%)	Haplotype	
						<i>Wx</i> locus <sup>2)</sup>	<i>Alk</i> locus
1	F <sub>4:5</sub> -1	Viola × Akdala	F <sub>5</sub>	nd	1.2	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
2	F <sub>4:5</sub> -2	do.	F <sub>5</sub>	nd	0.8	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
3	F <sub>4:5</sub> -3	do.	F <sub>5</sub>	nd	-0.2	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
4	F <sub>4:5</sub> -4	do.	F <sub>5</sub>	nd	0.5	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
5	F <sub>4:5</sub> -5	do.	F <sub>5</sub>	nd	0.6	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
6	F <sub>4:5</sub> -6	do.	F <sub>5</sub>	nd	1.6	<i>wx</i> ( <i>Wx-b</i> derived)	<i>Alk/alk</i>
7	F <sub>4:5</sub> -7	Viola × Marzhan	F <sub>5</sub>	nd	1.6	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
8	F <sub>4:5</sub> -8	do.	F <sub>5</sub>	nd	1.8	<i>wx</i> ( <i>Wx-b</i> derived)	<i>Alk/alk</i>
9	F <sub>4:5</sub> -9	do.	F <sub>5</sub>	nd	1.4	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
10	F <sub>4:5</sub> -10	do.	F <sub>5</sub>	nd	1.9	<i>wx</i> ( <i>Wx-b</i> derived)	<i>Alk/alk</i>
11	F <sub>4:5</sub> -11	Viola × Bakanasski	F <sub>5</sub>	nd	1.4	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
12	F <sub>4:5</sub> -12	do.	F <sub>5</sub>	nd	0.6	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
13	F <sub>4:5</sub> -13	do.	F <sub>5</sub>	nd	0.4	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
14	F <sub>4:5</sub> -14	do.	F <sub>5</sub>	nd	0.5	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
15	F <sub>4:5</sub> -15	do.	F <sub>5</sub>	nd	0.3	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
16	F <sub>4:5</sub> -16	do.	F <sub>5</sub>	nd	1.2	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
17	F <sub>4:5</sub> -17	Violetta × Akdala	F <sub>5</sub>	nd	1.0	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
18	F <sub>4:5</sub> -18	do.	F <sub>5</sub>	nd	3.0	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
19	F <sub>4:5</sub> -19	do.	F <sub>5</sub>	nd	0.9	<i>wx</i> ( <i>Wx-b</i> derived)	<i>Alk/alk</i>
20	F <sub>4:5</sub> -20	Violetta × Marzhan	F <sub>5</sub>	nd	0.8	<i>wx</i> ( <i>Wx-b</i> derived)	<i>Alk/alk</i>
21	F <sub>4:5</sub> -21	do.	F <sub>5</sub>	nd	1.4	<i>wx</i> ( <i>Wx-b</i> derived)	<i>Alk/alk</i>
22	Parental cultivars	Viola	-	nd	1.9	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
23		Violetta	-	nd	1.9	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>
24		Bakanasski	-	d	20.2	<i>Wx-a</i> (intermediate AC)	<i>Alk</i>
25		Marzhan	-	d	21.7	<i>Wx-a</i> (intermediate AC)	<i>Alk</i>
26		Akdala	-	d	18.2	<i>Wx-a</i> (intermediate AC)	<i>Alk</i>
27	Control cultivars	Kitamizuho	-	d	26.9	<i>Wx-a</i> (high AC)	<i>alk</i>
28		Yukigozen	-	d	19.6	<i>Wx-b</i>	<i>alk</i>
29		Hakuchou-mochi	-	nd	-0.3	<i>wx</i> ( <i>Wx-b</i> derived)	<i>alk</i>

<sup>1)</sup> nd: not detected; d: detected.

<sup>2)</sup> *wx* haplotype was identified according to Wanchana et al. (2003); identification of *Wx-a* type and *Wx-b* type was according to Yamanaka et al. (2004); *Wx-a* haplotype (high or intermediate AC) was identified according to Chen et al. (2010).

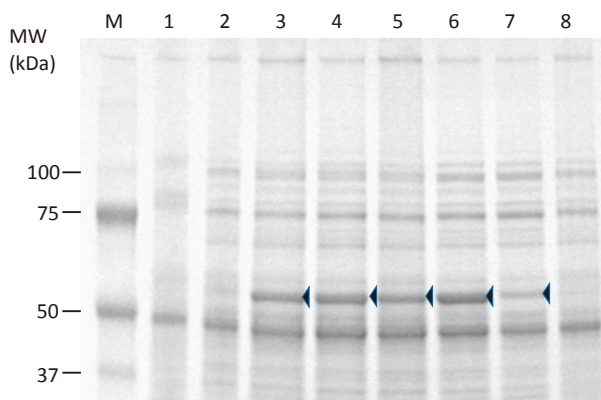
## 2. Amylopectin traits

The glutinous parents had ‘A’ at SNP3 and ‘GC’ at SNP4, indicating that they had the non-functional *alk* allele (Umemoto & Aoki 2005) (Fig. 3). The non-glutinous parents had ‘G’ at SNP3 and GC at SNP4, indicating that they had the functional *Alk* allele. All control cultivars (Kitamizuho, Yukigozen, and Hakuchou-mochi) had *alk*. Among all the F<sub>4:5</sub> lines investigated, 15 had the *alk* haplotype, six were heterogeneous *Alk/alk*, and none had the *Alk* haplotype (Table).

## 3. Amylopectin chain-length distribution analysis

Figure 4 shows a comparison of the chain-length distribution of amylopectin among the five cultivars used as crossing parents. The ratios of short chains’ degree of polymerization (DP) (DP7 to DP9) were highest with Viola, and chains with DP10 to DP13 were highest with Marzhan. Between the two glutinous rice possessing the same non-functional *alk* allele, Viola had higher ratios in relatively short chains DP6 to DP13. And among the three non-glutinous rice cultivars with functional *Alk*, Marzhan showed highest ratios in chains from DP6 to DP13.

When comparing the averaged chain ratios of the two glutinous rice with *alk* and the three non-glutinous rice with *Alk* by subtraction, non-glutinous cultivars had less short chains with DP7 to DP8 and more medium-length chains with DP11 to DP18 (Fig. 5A). These differences in the chain-length distribution of amylopectin between cultivars with *alk* and *Alk* are the same as previously reported

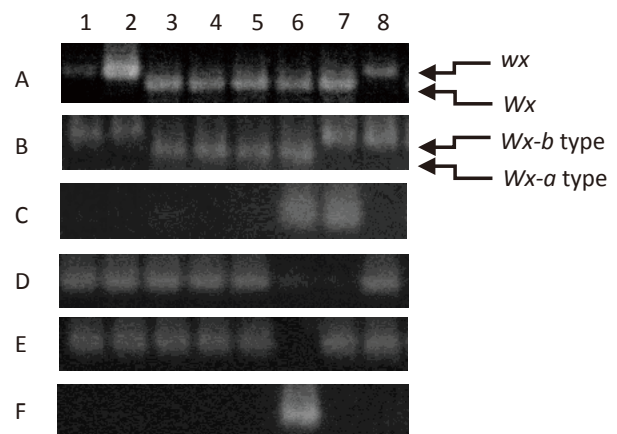


**Fig. 1. Detection of *Wx* protein by SDS-PAGE analysis among crossing parents and controls**

M: Marker; 1: Viola (glutinous, Kazakhstan); 2: Violetta (glut, Kazakhstan); 3: Bakanasski (non-glut, Kazakhstan); 4: Marzhan (non-glut, Kazakhstan); 5: Akdala (non-glut, Kazakhstan); 6: Kitamizuho (non-glut, Japan); 7: Yukigozen (non-glut, Japan); 8: Hakuchou-mochi (glut, Japan). Arrowheads indicate the 60-kD *Wx* protein band.

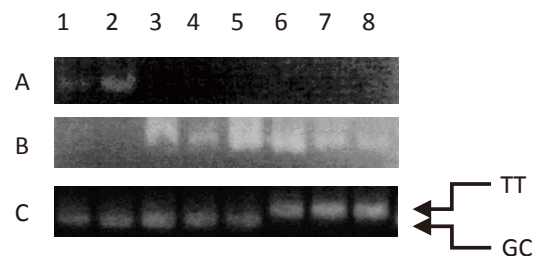
(Umemoto et al. 2008).

Among the six progenies derived from Viola × Akdala (Fig. 5 B, Table), F<sub>4:5-6</sub> harboring *Alk/alk* had the most less chains with DP7 to DP11 and more chains with DP17 to around DP20 as compared to the glutinous parent Viola with *alk*. These characteristics were basically similar to the chain-length profile of non-glutinous rice harboring *Alk*



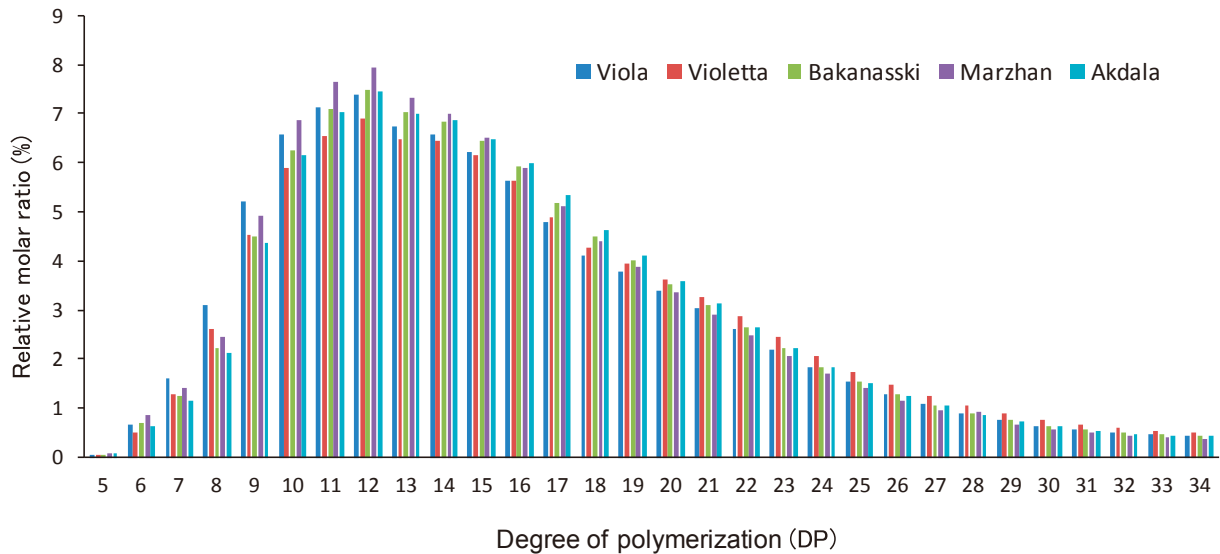
**Fig. 2. Identification of *Wx* gene haplotypes by PCR assay of crossing parents**

A: Glu23 (23-bp insertion in exon 2; Wanchana et al. 2003); B: dCAPS marker (SNP in intron 1; Yamanaka et al. 2004); C, D: *WxEx6* (SNP in exon 6: C, ‘A’; D, ‘C’); (E, F) *WxEx10* (SNP in exon 10; E: ‘C’; F, ‘T’; Chen et al. 2010). 1: Viola (glutinous, Kazakhstan); 2: Violetta (glut, Kazakhstan); 3: Bakanasski (non-glut, Kazakhstan); 4: Marzhan (non-glut, Kazakhstan); 5: Akdala (non-glut, Kazakhstan); 6: Kitamizuho (non-glut, Japan); 7: Yukigozen (non-glut, Japan); 8: Hakuchou-mochi (glut, Japan).



**Fig. 3. Identification of *Alk* haplotype by PCR assay of crossing parents**

A: SNP3(‘A’) marker; B: SNP3(‘G’) marker; C: SNP4 marker. SNP3 and SNP4 identify SNPs located at bases 2209 (A/G) and 2340 (GC/TT), respectively, from the translation start point of *SSIa* of Nipponbare cDNA (AB115915) (Umemoto et al. 2005). 1: Viola; 2: Violetta; 3: Bakanasski; 4: Marzhan; 5: Akdala; 6: Kitamizuho; 7: Yukigozen; 8: Hakuchou-mochi.



**Fig. 4. Comparison of chain-length distribution of amylopectin among five cultivars used as crossing parents**

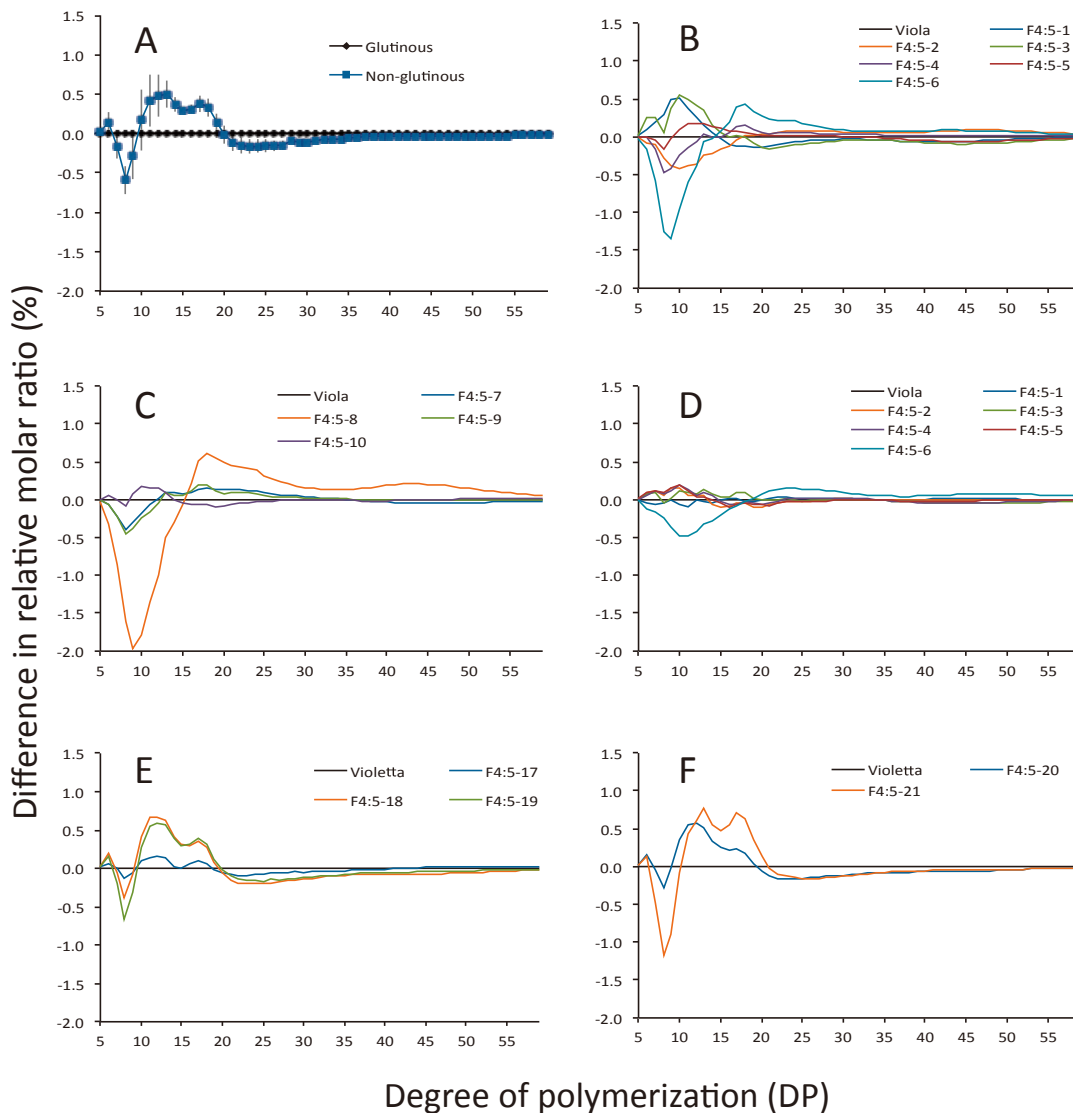
Viola and Violetta are the glutinous parents with *alk* (non-functional) allele, while Bakanasski, Marzhan, and Akdala are the non-glutinous parents with *Alk* (functional) allele.

as compared to the glutinous rice harboring *alk* (Fig. 5A), although the magnitude of difference relative to the corresponding *alk* cultivar varied. The same holds true for the one of the progenies derived from Viola × Marzhan (Fig. 5C, Table). F<sub>4:5-8</sub> harboring *Alk/alk* showed less short chains compared to the two progenies with *alk* (F<sub>5-7</sub> and F<sub>5-9</sub>). However, another progeny with *Alk/alk* (F<sub>5-10</sub>) had a very similar chain-length distribution to that of the parent (Viola) with *alk* and there is a clear discrepancy between the genotype and phenotype. All progenies derived from Viola × Bakanasski had *alk*, and none showed a chain-length profile similar to that of non-glutinous rice with *Alk* when compared to glutinous rice with *alk* (Fig. 5D, Table). Among the three progenies derived from Violetta × Akdala (Fig. 5E, Table), F<sub>3-17</sub> harboring *alk* and F<sub>5-19</sub> harboring *Alk/alk* had a chain-length distribution similar to that of the glutinous parent Violetta harboring *alk* and non-glutinous rice harboring *Alk* in Fig. 5A, respectively. However, another progeny (F<sub>5-18</sub>) harboring *alk* showed a very similar chain-length distribution to that of the above mentioned F<sub>5-19</sub> with *Alk/alk*, for which we lack further data to explain this discrepancy between the genotype and haplotype. Both progenies derived from Violetta × Marzhan had *Alk/alk*, among which F<sub>5-20</sub> had a very similar profile of chain distribution to that of the non-glutinous rice with *Alk* in Fig. 5A when compared to glutinous rice harboring *alk* (Fig. 5F). The other progeny (F<sub>5-21</sub>) also basically showed a chain-length distribution similar to that of F<sub>5-20</sub>, although the extent of the difference with the glutinous parent Violetta was larger than that of F<sub>5-20</sub>.

## Discussion

Amylose content in rice grains is controlled primarily by the *Wx* locus, with the known alleles *Wx-a*, *Wx-b*, *Wx-op*, *Wx-in*, and *wx* (Sano 1984, Mikami et al. 2008). *Wx-a* and *Wx-b* occur widely, and rice harboring *Wx-a* produces more waxy proteins (GBSSI), resulting in a higher amylose content than *Wx-b*. The non-functional *wx* does not produce amylose, thus contributing to the highly sticky texture of cooked rice. *Wx-a* is classified into two main haplotypes, giving intermediate- and high-amylose contents (Chen et al. 2008). Among our materials, all non-glutinous parents had the intermediate-amylose form (Fig. 2). Because the amylose content is a major determinant of the eating quality of rice (Mikami et al. 2008), we assume that the *Wx-a* haplotype reflects the preferred taste of cooked rice in Kazakhstan. The *wx* genes of the glutinous parents were derived from *Wx-b* (Fig. 2), indicating that the parents were originally developed from germplasms introduced from the “Glutinous Rice Zone” of Thailand (Yamanaka et al. 2004), and not derived from an independent natural variation causing the *wx* (glutinous) phenotype.

The *SSIIa* (*Alk*) gene affects the eating quality of rice as functional *Alk* contributes to firmer, less sticky cooked rice after storage, especially at low temperature, better than non-functional *alk* (Umemoto et al. 2008). SNP markers showed that glutinous parents had the non-functional *alk/alk* and that non-glutinous parents had the functional *Alk/Alk* (Fig. 3). *Alk* of the non-glutinous parents might also



**Fig. 5. Comparison of chain-length distribution of amylopectin**

A: Subtraction of each chain ratio (in average) of glutinous parents from non-glutinous parents; B: Subtraction of Viola × Akdala progenies from Viola; C: Subtraction of Viola × Marzhan progenies from Viola; D: Subtraction of Viola × Bakanasski progenies from Viola; E: Subtraction of Violetta × Akdala progenies from Violetta; F: Subtraction of Violetta × Marzhan progenies from Violetta. Vertical bars in A represents standard deviation of each chain ratio of the three non-glutinous cultivars.

reflect the preferred eating quality of rice in Kazakhstan. These hypotheses about the relationship between *Wx* and *Alk* haplotypes, and the preference of Kazakhs to cooked rice should be further investigated using more cultivars consumed in Kazakhstan. Among the  $F_5$  glutinous lines, we found progenies heterozygous at the *Alk* locus (Table), and therefore they could be used in breeding glutinous cultivars with *Alk* and *alk* haplotypes, thus having different cooking properties.

The characteristics of differences in the chain-length

distribution of amylopectin between the cultivars harboring *alk* (glutinous parents) and *Alk* (non-glutinous parents) reflected those of previous studies, in spite of rather large variation within the same *Alk* haplotype (Fig. 4 and Fig. 5A) (Umemoto et al. 2008, Okamoto et al. 2002). Based on the above phenotypic differences between *Alk* and *alk*, we have evaluated the chain-length distribution of 21 glutinous progenies derived from the cross between glutinous Viola or Violetta and non-glutinous Akdala, Marzhan, or Bakanasski (Fig. 5B to 5F). Although we did not

obtain a glutinous line homozygous for *Alk*, there were six lines harboring *Alk/alk* and 15 lines harboring *alk*. All the *Alk/alk* progenies except one (F<sub>5</sub>-10) had less amylopectin short chains with DP around 7 to 10 as compared to their glutinous parents with *alk*. All the *alk* progenies except one (F<sub>5</sub>-18) had chain-length distribution similar to that of the glutinous parents with *alk* or lacked the clear characteristics we observed with *Alk/alk* lines (i.e. less short chains, DP7 to DP10; more chains, DP11 to DP18). These results tell us that the *Alk* allele has a dominant effect on amylopectin chain-length distribution compared to the *alk* allele, and that the *Alk* haplotype is the chief determinant of characteristics in chain-length distribution among the progenies evaluated in this study. There were two progenies (F<sub>5</sub>-10 and F<sub>5</sub>-18) showing a discrepancy between the genotype and phenotype, which should be reevaluated using descending lines of these progenies. It is possible that other genetic factors affect amylopectin chain length, such as variations in the function of starch branching enzyme I, starch branching enzyme IIb, starch synthase IIIa, or starch phosphorylase could exist between the parents we used for crossing (Okamoto et al. 2002, Nishi et al. 2001, Fujita et al. 2007, Satoh et al. 2008). It is well known that the temperature during grain-filling also affects the chain-length distribution of amylopectin (Umamoto et al. 1999, Inouchi et al. 2000). However, this is probably not the case in the present study as the heading period among the progenies was within 10 days by observation, and apparently did not affect the grain-filling.

The processing quality of a glutinous progeny harboring *Alk* is different from that of the glutinous parents harboring *alk* (Okamoto et al. 2002). Thus, we need to clarify the effects of the *Alk* haplotype on processing and eating quality for further breeding of glutinous rice in Kazakhstan. We also need to further determine the *Wx* and *Alk* haplotypes of rice cultivars grown in Kazakhstan that are intended for use as crossing parents, in order to advance breeding efficiently.

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