



Obtaining of Interspecific Hybrids between Durum Wheat (2n=28) and Triticale (2n=42) and Molecular Evidence of Alien Introgressions in Advanced Backcross Line

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Abstract

The hexaploid triticale is an amphidiploid obtained from the cross between durum wheat (2n=28) and rye (2n=14). It is characterized with high protein content, resistance to biotic- (powdery mildew and rust) and abiotic (cold and drought) stress factors. The hybridization between durum wheat and triticale is complicated and is rarely applied in durum wheat breeding improvement. Here, we report the results of long-standing experiments for obtaining of interspecific hybrids between Bulgarian durum wheat genotypes as mother plants and triticale (2n=42) lines from CIMMYT – Mexico. Although the crossability between two species was relatively high, hybrid plants were obtained only by means of embryo rescue method, due to endosperm degeneration. All regenerated F₁ hybrids were with low fertility and produced seeds with reduced endosperm and low viability. The F₁ hybrids were backcrossed to the recurrent durum wheat genotypes. Strict and repeated selection of plants with durum wheat phenotype was performed in the segregation populations. Thirty-three SSRs mapped on the A, B and D genomes and chromosomes of common wheat were used for molecular characterization of one of the obtained advanced backcross lines. The SSR markers showed introgression of 10 chromatin segments from triticale in the studied backcross line in loci on chromosomes of several groups - 1 (*Xwmc 24-1AS*, *Xgwm 136-1AS*, *Xgwm 268-1BL*), 2 (*Xgwm 95-2AS*), 3 (*Xgwm 5-3AS*), 4 (*Xgwm 165-4AS*, *Xgwm 165-4BL*) and 7 (*Xwmc 9-7AL*, *Xwmc 83-7AS* и *Xgwm 400-7BS*). In addition to introgressions, new recombinant alleles, which did not correspond correctly to the alleles of both parents, were detected in loci on chromosomes of 7 group (*Xgwm 282-7AL*, *Xwmc 83-7AS*, *Xgwm 46-7BS*, *Xgwm400-7BS*) and in loci on chromosomes 2AL (*Xgwm 312*), 5AL (*Xwmc 327*) and 6BL (*Xgwm644*).

Key words: durum wheat, triticale, interspecific hybridization, embryo rescue method, SSR markers, introgressions

Introduction

The interspecific hybridization is a reliable method for the transfer of useful genetic information from one species to another, which can lead to significant changes in the productivity or distribution of cereals (Mujeeb-Kazi and Kimber, 1985). One of the main tasks in the breeding improvement of durum wheat are connected with increasing resistance to abiotic (frost and drought) and biotic (diseases) stress factors. The potential of rye donor genes for wheat improvement has been recognized long since by many researchers and the efforts were made to incorporate a rye chromosome segments into wheat germplasm (Merker, 1984, Reynolds et al, 2011). It is well known that rye is characterized by high tolerance

to biotic and abiotic stresses, large number of spikelets, better nutritive quality and root system among the cereals (Kumar et al., 2014).

Triticale has many advantages and has been used as a bridge for transferring useful genes from the rye genome to wheat (Sethi, 1989, Saulescu et al., 2011). The hexaploid triticale is an amphidiploid produced from cross between durum wheat (2n=28) and rye (2n=14) and is distinguished with high protein content, higher resistance to diseases and cold in comparison with the common- and durum wheat and has been successfully used to enhance the genetic variability in the common wheat (Nikongolo et al., 1991; Bizimungu et al., 1998; Gupta et al., 2007). It is considered that amphidiploids could be constant

source of discovery and transfer of new traits (Jang et al., 1994). According to Bizimungu et al.(1998) the introgression of genetic variability from triticale to the wheat is theoretically possible because of homoeology between rye and wheat genomes (Merker,1984) and the proven from Lukaszewski and Gustafson (1983) spontaneously occurring translocations between the chromosomes of both species. Many authors reported for positive effects of several wheat/rye translocations particularly those involving the short arm of rye chromosome 1R on yield and nitrogen-use efficiency related to root characters, stress tolerance and adaptation in bread wheat (Villareal ET AL., 1998; Ehdiae ET AL., 2003; Hysing ET AL., 2007).

The hybridisation between durum wheat and triticale is complicated and was used seldom in durum wheat breeding improvement up to now, despite the presence of effective in vitro protocols for obtaining of hybrid embryos and molecular methods for detecting of alien transfers. Recent advances in molecular biology, principally the development of PCR-based DNA marker systems have provided powerful tools for detection of alien germplasm introgression that can facilitate the breeding work in transferring valuable genes of closely related species to durum wheat. Among the markers elaborated, microsatellites (simple sequence repeats - SSRs) can be used in wide hybridization to monitor and map desirable alien genes in segregating populations as they are locus-specific and are inherited in a codominant manner (Shen et al., 2004). The flanking regions of microsatellites show sufficient homology between closely related species, allowing the primers of wheat SSR markers to be successfully used in studies of other cereals (Zhang et al., 2005, Todorovska et al., 2013)

We reported results of long-standing experiments for obtaining of interspecific hybrids between Bulgarian durum wheat genotypes as mother plant and triticale (2n=42) lines from CIMMYT – Mexico using a backcross strategy. The gene introgressions from triticale in one of the obtained advanced backcross lines (*Tr. durum* (D-7192) x *Triticosecale*) F₁ x *Tr. durum* (Vazhod) BC₁ F₁ x *Tr. durum*) BC₂F₁ were identified via SSR markers.

Materials and Methods

Plant material and interspecific crosses

Three Bulgarian durum wheat (*Triticum durum* Desf.) breeding lines developed in the Field Crops Institute (FCI, Chirpan) were crossed to hexaploid triticale (*x Triticosecale* Wittmack), line № 837 mex/06 kindly provided from CIMMYT –

Mexico (Table 1). Durum wheat genotypes were used as maternal parents in the hybridization. The parents were grown in the experimental field of FCI, while earlier generations of hybrid plants and the backcross progenies - in the greenhouse. The hybrid seeds were removed from the spikelets 16 to 20 days after pollination and embryo rescue methods were applied according to the protocols described by Hadzhiivanova et al. (2012). The *in vitro* regenerated F₁ plants were cultivated in greenhouse conditions to maturity. All F₁ hybrids were single backcrossed to recurrent durum wheat genotypes used as male parents. The advanced backcross line BL (*Tr. durum* (D-7192) x *Triticosecale*)F₁ x *Tr. durum* (Vazhod) BC₁ F₁ x *Tr. durum*) BC₂F₁ was obtained through two rounds of backcrossing. Strict and repeated selection of plants with durum wheat phenotype was performed in the segregation populations. The selection was carried out using the bulk-population method.

Table 1. Crossability between durum wheat and triticale

Cross combination	Florets pollinated Nr.	Seeds obtained Nr.	Crossability %
1.6189D x Triticale	846	146	17.3
2.7189D x Triticale	192	44	22.9
3.7192D x Triticale	330	27	8.2
<i>Total</i>	1368	217	15.9
Backcross progenies BC1			
1.6189D x Triticale	270	1	0.37
2.7189D x Triticale	240	2	0.83
3.7192D x Triticale	220	3	1.4
<i>Total</i>	730	6	0.82

Table 2 . Plants regeneration after embryo rescue

Cross combination	Cultivated embryos	Regenerated Plants		Adapted plants	
	Nr.	Nr.	%	Nr.	%
1.7189 x Triticale	30	10	33	3	30
2.6189 x Triticale	40	19	48	5	26
3.7192 x Triticale	10	3	30	1	33
<i>Total</i>	80	32	40%	9	28

Table 3. Means of some characteristics of F1 hybrid plants

Cross combination	Plant grown	Spikelets	Grain per spike	Fertility	Grain weight
	to maturity	per spike	Nr.	%	per spike
	Nr	Nr			g
6189 x Triticale	3	28	2	7.1	0.5
7189 x Triticale	3	30	2.5	8.3	0.7
7192 x Triticale	1	28	12	42.9	0,39
Means of all cross combinations	7	28.7	5.5	19.4	0.53

Microsatellite marker assay

DNA was extracted from leaves of bulked samples (5 plants per sample) from the durum wheat and triticale parents and the selected BL grown in a greenhouse, using a modified CTAB method according to Murray and Thompson (1980). Twenty-nine microsatellite primer pairs specific for genome of common wheat, amplifying 33 SSR total loci (without chromosomes 2B and 6A) were used. PCR reactions were performed in a final volume of 10 µL in a Gene Amp 2900 thermocycler. The reaction mixture contained 250 nmol/L of each primer, 0.2 mmol/L of each deoxynucleotide, 1.5 mmol/L of MgCl₂, and 0.8 U Taq polymerase. The development of the microsatellite markers, primer sequences, chromosome location and the annealing temperature are presented by Röder et al. (1998). Fragment sizes were calculated with Fragment Manager (Pharmacia) software by comparing with internal size standards added to each lane in the loading buffer. The repeat number of alleles was calculated according to the fragment sizes and number of repeat units at the corresponding locus in cv. Opata or Chinese spring. Peaks of SSR markers were scored as 1 (present) and 0 (absent). The alien genome introgression levels in the progeny from SSR data equaled the number of loci showing alien alleles in the introgression line divided by the total number of polymorphic SSR loci.

Results and Discussion

Three durum wheat breeding lines were hybridized to one triticale line. The crossability between them expressed as a percent of pollinated florets produced caryopsis with embryo is presented in Table 1. From 1368 pollinated flowers 217 seeds were obtained, i.e. the main crossability for all hybrid combinations was 15.9 %. Variation in crossability depending on durum

wheat genotype as female parent was also found. The lowest seed set was detected in breeding line D-7192 – 8.2 % and the highest – in breeding line D – 7189 – 22.9 %.

The various durum wheat genotypes differed not only in the number of received caryopsis but also in the number of caryopsis containing embryo and their degree of differentiation. Even within the same genotype there were embryos with different levels of differentiation. The endosperm of all hybrid combinations was watery or missing. There were caryopses consisting only of seed coat without embryos, probably died at early stage of development due to the rapid degeneration of the endosperm or its absence.

The above presented results confirm that success of the alien hybridization is highly genotype dependent (Jiang et al., 1994). The average percentage of seed set received of all hybrid combinations is relatively good and agree with the data reported by other authors about crossability between these two species (Baeva et al., 1981, Hills et al., 2007). Data for the vitality of the hybrid seeds are contradictory. Baeva et al. (1981) have obtained viable hybrid seeds without using embryo rescue, while Hills et al. (2007) - non-viable hybrid seeds. Merker (1984) noted that when common wheat is used as mother parent in cross with hexaploid triticale higher seed set can be received, but at the expense of the disturbed endosperm development and subsequent embryos death if they are not rescued *in vitro*. In experiments including hybridization between durum wheat and rye, Hede et al. (2000) reported incompatibility between the genomes of both species occurring at post-gametic phase of fertilization. The lack of normal endosperm in the hybrid caryopses can be explained by the presence of the R genome of rye which is more distinct from the A and B genomes of durum wheat. Our results

show that the incompatibility between the genomes of rye and durum wheat is maintained even after backcrossing of the stable amphidiploid triticale with durum wheat as recurrent parent.

We were able to obtain hybrid plants between durum wheat and triticale when using durum wheat as mother plants only by means of embryo rescue method. From 80 isolated embryos 32 plant were regenerated and 9 were successfully adapted (Table 2). The percent of regenerated plants (40 %) and especially the percent of adapted plants (28.3 %) were satisfactory. The lose of most of the regenerated plants at different development stages was due to weak vitality of hybrid plants and not controlled conditions during acclimation and further cultivation. The highest number of regenerated and adapted plants was achieved for hybrid combination 6189 x Tcl., while the percent

of adapted plants was highest in the hybrid combination 7192 x Tcl. The ability for *in vitro* regeneration was still independent from crossability of used durum wheat genotypes. The small number of regenerated plants resulting from the rescue of immature hybrid embryos is wanted and has been observed in a number of previous studies, too. Hede et al.(2000) reported that at *in vitro* cultivation of embryos from cross between *Tr. durum* x *S. cereale* only 4.57% of all rescued embryos were developed to normal plants. The failure of many of the rescued hybrid embryos to germinate and reduced viability of the hybrid seedlings is common in interspecific crosses, possibly due to activation of postzygotic incompatibility mechanisms (Bajaj, 1990).

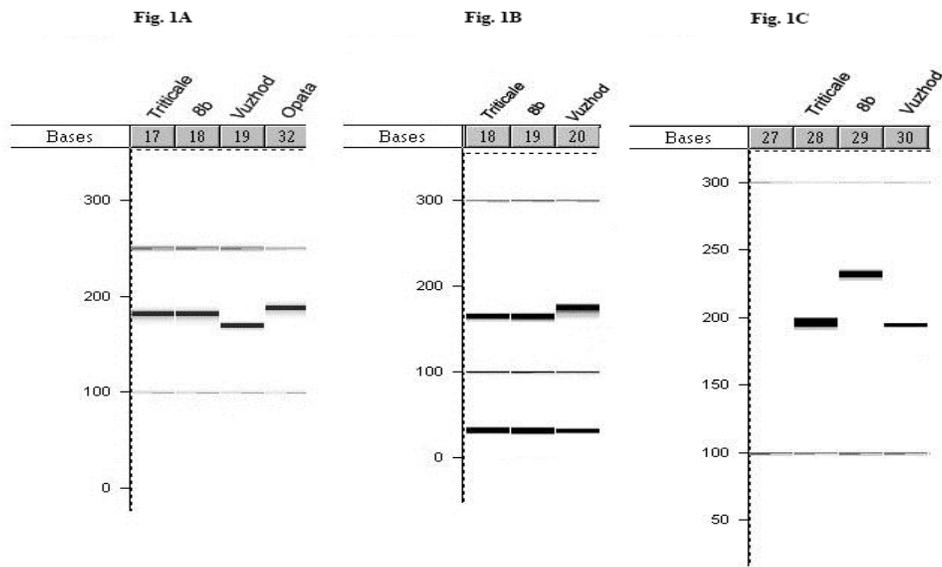


Figure 1A. Introgression of genomic DNA from Triticale in backcross line (BL) in microsatellite locus Xgwm 268-1BL. **Figure 1B.** Introgression of genomic DNA from Triticale in backcross line (BL) in microsatellite locus Xwmc 9-7AL. **Figure 1C.** Homeological recombination between Triticale and Durum wheat cv. Vazhod in locus Xgwm 282-7AL.

A part of the spikes of regenerated F₁ hybrid plants were selfed and the rest were backcrossed with pollen from durum wheat. All F₁ hybrid plants were identical and looked more like father parent – the triticale line. They had higher stem, longer and loose spike, anthocyan color of anthers and open flowering. The mean values of some characteristics of F₁ hybrids are presented in Table 3. All F₁ hybrid plants were with low fertility, the majority of received hybrid caryopsis died due to endosperm degeneration resulting in a formation of relatively few seeds. The mean number of seeds per spike of F₁ hybrid plants varied from 2 for cross combination 1 to 12.5 for cross combination 3. The

resulting hybrid seeds were significantly longer than those of the durum wheat and were with reduced and shrivel endosperm and low viability and germination rate.

BC₁ seeds were obtained after backcrossing of F₁ hybrids to the durum wheat parent. The average crossability rate from all combinations (0.82 %) was significantly lower, in comparison than that accounted for F₁ hybrids – 15.9 % (Table 1.) The germination rate of seeds from the backcrossed progeny was higher compared to selfed F₁ hybrids. The fertility and vitality of hybrid plants increased after each subsequent generation.

Table 4. Amplification in microsatellite loci at parent, introgressions and recombinations in backcross line (BL)

Backcross line and parent	Monomorphic loci	Lack of PCR amplification in the parent (null loci)	Polymorphic loci	Introgressions	Recombinations
Triticale (AABBRR) Backcross line Durum wheat cv. Vuzhod (AABB)	Xgwm 642-1D	Xgwm 484-2D	xw24-1A, xg136-1A, xg99-1A,	Xwmc 24-1A	Xwmc 327-5A
		Xgwm 261-2D	xg268-1B, xg95-2A, xg312-2A,	Xgwm 136-1A	Xgwm 644 6B
		Xgwm 456-3D	xg5-3A, xg285-3B, xg165-4A,	Xgwm 268-1B	Xgwm 282-7A
		Xgwm 165-4D	xg165-4B, xw327-5A, xg639-5A,	Xgwm 95-2A	Xgwm 46- 7B
		Xgwm 190-5D	xg639-5B, xg639-5D, xg408-5B,	Xgwm 5-3A	Xwmc 83-7A
		Xgwm 469-6D	xg234-5B, xg644-6B, xw9-7A,	Xgwm 165-4A	Xgwm 312-2A
		Xgwm 325-6D	xw83-7A, xg282-7A, xg233-7A,	Xgwm 165-4B	Xgwm 400-7B
		Xgwm 437-7D	xg400-7B, xg302-7B, xg46-7B	Xwmc 9-7A	Xwmc 327-5A
		Xgwm 484-2D	xw24-1A, xg136-1A, xg99-1A,	Xwmc 83-7A	Xgwm 644 6B
	Xgwm 261-2D	xg268-1B, xg95-2A, xg312-2A,	Xgwm 400-7B	Xgwm 282-7A	
TOTAL	1	8	24	10	7
%			72.72	41.66	29.16

The backcross strategy has been widely applied in the breeding programs aiming at introduction of alien germplasm into cultivated wheat. The strategy based on single backcrossing with selected bulk breeding (SBBS's) has advantages in retaining or improving the adaptation of the recurrent parents, and at the same time transferring most of the desired donor genes in a wide range of scenarios and has been successfully used in wheat improvement at CIMMYT breeding strategy (Wang et al., 2009).

Using this strategy we developed an almost uniform advanced backcross line (*Tr. durum* (D-7192) x *Triticosecale*)F₁ x *Tr. durum* (Vazhod) BC₁F₁ x *Tr. durum*) BC₂F₄ after two rounds of backcrossing. Strict and repeated selection of plants with durum wheat phenotype was performed in the segregation populations. The selection was carried out using the bulk-population method.

This line was further characterized with microsatellite markers to follow the transmission

of some introgressed segments from A and B genome of *Triticosecale*. Twenty-nine microsatellite primer pairs specific for genome of common wheat (Röder, et al., 1998), amplifying 33 SSR total loci mapped on all 14 chromosomes of the A and B genomes, excluding 2B and 6A and 10 SSR markers on the D genome were employed for the genotyping of hybrid lines and parents – durum wheat and triticale involved in this interspecific hybridization. The applied 33 SSRs markers amplified 24 loci in the genome of triticale (*x Triticosecale* Wittmack), ($2n = AABBRR$) and the same number in the genome of durum wheat cv. Vazhod, used as recurrent parent in the backcrossing of hybrid line (Table 4). Introgression of 10 segments of chromatin from triticale was recorded in hybrid line at loci on the chromosome group first (Xwmc 24-1AS, Xgwm 136-1AS, Xgwm 268-1BL), second (Xgwm 95-2AS), third (Xgwm 5-3AS), fourth (Xgwm 165-4AS, Xgwm 165-4BL) and on the seventh one (Xwmc 9-7AL, Xwmc 83-7AS and Xgwm 400-7BS) (Fig. 1A, 1B). The detected introgression in the loci on the short arms of chromosomes 1AS (*Xwmc 24* и *Xgwm 136*) and 7AS (*Xwmc 83* and *Xwmc 9*) were localized at a distance between them of about 35-38 cM and 20 cM, respectively. Additional investigations are needed to determine the length of the introgressed segments and the contained in them potential genes of agronomical value.

In addition to introgressions, new alleles which did not correspond correctly to the alleles of both parents, were detected in the backcross line. Recombination (new) alleles of a length different from that of either parents was found at loci on the seventh group of chromosomes (Xgwm 282-7AL, Xwmc 83-7AS, Xgwm 46-7BS, Xgwm400-7VS) and at the loci of chromosomes 2AL (Xgwm 312), 5AL (Xwmc 327) and 6BL (Xgwm644) (Fig. 1C). The percentage of introgressions from A and B genomes of triticale in the studied backcross line is high (41.7), while those of recombinations is lower (29.2).

These results reveal the chromatin introgressions of triticale from the homologous of durum wheat A and B genomes and recombination between chromosomes of these genomes in studied hybrid line. Additional investigations including R-genome specific marker for detection of potential introgressions of segments of R genome or recombination between homoeologues chromosomes (inter-genomic) of both species involved in hybridisation are in progress.

Conclusions

The present study demonstrates the possibility interspecific hybrids between durum

wheat as mother plants and hexaploid triticale (as a bridge for transferring useful rye genes) to be obtained. Although the crossability between both species was relatively high, hybrid plants were obtained only by means of embryo rescue method, due to endosperm degeneration. The observed low fertility and viability of F_1 hybrids plant was overcome by backcrossing and increased with each subsequent generation. The gene introgressions from triticale in one of the obtained advanced backcross line (*Tr. durum* (D-7192) \times *Triticosecale*) $F_1 \times Tr. durum$ (Vazhod) $BC_1 F_1 \times Tr. durum$) $BC_2 F_1$ were identified via SSR markers. The results reveal the chromatin introgressions from triticale only in the homologous A and B genomes of durum wheat. Recombination events between chromosomes of both species were identified, too. Additional molecular investigations for detection of likely introgressed chromosome segments of R genome and phenotypic characterization of advanced backcross line for agronomical important traits are in progress.

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