

AGRICULTURAL ACADEMY – SOFIA
FIELD CROPS INSTITUTE – CHIRPAN

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**Studies on the use of distant hybridization in durum
wheat breeding (*Triticum turgidum* L. subsp. *durum*
(Desf.))**

ABSTRACT

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1. INTRODUCTION

Breeding progress in cultivated plants is becoming increasingly difficult to achieve, not only because of the higher requirements placed on new cultivars, but also because of the continuous decline in genetic diversity in modern agricultural systems.

The need to increase and stabilize the yield potential of durum wheat, as well as the possibilities for its further improvement, depend to a considerable extent on finding an appropriate solution to the problem of declining genetic variability in modern cultivars. A higher level of genetic diversity would enable the development of cultivars with increased productivity, better quality and greater adaptability, which in turn would reduce the risk of disease epidemics and yield losses resulting from climatic anomalies.

According to current concepts, the principal methods for generating initial genetic diversity are mutational and hybrid variability, their interaction with environmental conditions, and selection of valuable recombinant forms. In recent decades, with the accumulation of sufficient experimental data and the improvement of technologies, new methods for inducing genetic diversity have been used increasingly frequently.

Distant hybridization is one of the methods used to create new plant forms or to transfer useful genetic information from one species to another. In intergeneric and interspecific crosses, hybrid populations with increased genetic diversity are obtained. The extensive segregation and formation of new genotypic and phenotypic combinations in the F₂ and subsequent generations provide a source of new genetic diversity that would be difficult to generate through intervarietal hybridization.

Distant relatives of durum wheat possess a number of agronomically valuable traits that may serve as important sources of genes for improved grain quality and resistance to various pests, diseases and abiotic stresses. Despite the considerable opportunities offered by interspecific and intergeneric hybridization for broadening the genetic base of durum wheat, their application has so far been hindered by evolutionarily established mechanisms that restrict crossing between distantly related species. The rapid development of *in vitro* culture methods and their application not only to model organisms but also to economically important species provide opportunities to overcome interspecific crossing barriers.

In recent years, there has been increasing interest in incorporating genetic diversity from wild and cultivated species into breeding programs. This has been facilitated by the development and improvement of methods that enable rapid screening of large numbers of individuals for various physiological and agronomic traits. Rapid advances in biochemical, molecular and molecular-cytogenetic methods enable precise identification of genes and provide molecular markers associated with agronomically important traits.

The need to enrich genetic diversity for important agronomic traits in order to ensure further breeding progress in durum wheat, together with the potential but insufficiently exploited opportunities of distant hybridization for the transfer of valuable genetic information, led to the formulation of the main objective of the present study:

To investigate the potential for producing interspecific and intergeneric hybrids between durum wheat (*Triticum durum*) and distantly related species of the family Gramineae, and to identify the transfer of alien genetic material using phenotypic, cytological and molecular approaches.

The transfer of alien genetic material into the durum wheat genome is a long-term process based on research in various fields. The main research tasks through which the study was carried out were formulated as follows:

1. To determine the crossability between distantly related species of the genera *Aegilops* and *Triticum*, and the synthetic species *Triticale*, and different cultivars and breeding lines of durum wheat (*Triticum durum*);
2. To optimize *in vitro* techniques for overcoming embryo incompatibility in interspecific and intergeneric hybridization;
3. To study the development of F₁ hybrid plants and obtain subsequent backcross generations;
4. To identify the transfer of genetic material in advanced backcross lines derived from distant hybridization of durum wheat (*Triticum durum*) with species of the genera *Triticum* and *Aegilops*, and *Triticale*;
5. To conduct a phenotypic evaluation of advanced backcross lines obtained through distant hybridization of *Triticum durum* with species of the genera *Triticum* and *Aegilops*;
6. To evaluate the yield and quality of advanced durum wheat breeding lines obtained through distant hybridization with species of the genera *Triticum* and *Aegilops*.

2. MATERIALS AND METHODS

The experimental work on the dissertation topic was carried out at the Field Crops Institute, Agricultural Academy – Chirpan, and the Agrobiointitute, Agricultural Academy – Sofia, in two stages. The first stage was conducted during the 2005/06 (2006), 2006/07 (2007), 2007/08 (2008), 2008/09 (2009), 2009/10 (2010) and 2010/11 (2011) growing seasons. The second stage was conducted during the 2019/20 (2020), 2020/21 (2021) and 2021/22 (2022) growing seasons, under the different meteorological conditions characteristic of each season.

2.1. Materials for the study

The study included accessions of distantly related species of the genera *Aegilops*, *Triticum* and *Triticale*, presented in Table 1. The accessions were provided by the National Plant Genebank at the Institute of Plant Genetic Resources (IPGR), Sadovo, Bulgaria, and were used as male parents in the interspecific and intergeneric crosses. Different Bulgarian and foreign durum wheat cultivars and advanced breeding lines were included as female parents. The cultivars included in the study were: Apulicum 233, Gergana, Progress, Beloslava, Vazhod and Victoria – Bulgarian breeding; Iride and Meridiano – Italy; and Superdur – Austria. The Bulgarian breeding lines were: 6189, 6467, 6948, 7110, 7121, 7192, 7208, 7216, 7281, 7307, 7346, 7351, 7383, 7416, 7505, 7538, 7544, 7552, 7647, 7767, 7793 and 7794. The Bulgarian cultivars and breeding lines were developed through conventional breeding methods in the experimental field of the Field Crops Institute – Chirpan.

Table 1. Origin and genome formula of the distantly related species used in the study.

Distantly related species	Genome formula/ 2n	Origin / Accession No.
<i>Aegilops tauschii</i> Coss.	14 DD	IPGR – Sadovo BGR30421
<i>Aegilops umbellulata</i> Zhunk.	14 UU	IPGR – Sadovo BGR35357
<i>Aegilops cylindrica</i> Host	28 CCDD	IPGR – Sadovo BGR19082
<i>Aegilops crassa</i> Boiss.	28 DDM	IPGR – Sadovo BGR35355
<i>Aegilops ventricosa</i> Tausch.	28 DDUU	IPGR – Sadovo BGR34143
<i>Aegilops kotschy</i> Boiss.	28 SSUU	IPGR – Sadovo BGR36756
<i>Aegilops juvenalis</i> (Thell.)Eig	42 DDMU	IPGR – Sadovo BGR 35356
<i>Triticum monococcum</i> L.	14 A ^b A ^b	IPGR – Sadovo
<i>Triticum dicoccum</i> (Schränk.) Schubl.	28 BBA ^u A ^u	IPGR – Sadovo BGR32749
<i>Triticum dicoccoides</i> (Koern. ex Aschers. et Graebn.) Schweif.	28 BBA ^u A ^u	IPGR – Sadovo BGR22616
<i>Triticum carthlicum</i> Nevski	28 BBA ^u A ^u	IPGR – Sadovo BGR31349
<i>Triticum polonicum</i> L.	28 BBA ^u A ^u	IPGR – Sadovo BGR33820
<i>Triticum timopheevii</i> (Zhuk.) Zhuk.	28 A ^b A ^b GG	IPGR – Sadovo BGR 31249
<i>Triticum macha</i> (Dekapr. and Menabde) MacKey	42 BBA ^u A ^u DD	IPGR – Sadovo BGR31242
<i>Triticum spelta</i> (L.) Thell	42 BBA ^u A ^u DD	IPGR – Sadovo BGR33818
<i>Triticale</i>	42 AABBRR	CIMMYT – Mexico

Classification of species of the genus *Aegilops* (van Slageren, 1994).

Classification of species of the genus *Triticum* (Goncharov, 2002, with additions according to Goncharov et al., 2009).

2.2. Methods of analysis

2.2.1. Hybridization methods and determination of crossability

The study was conducted using durum wheat genotypes grown in the experimental field of the Field Crops Institute – Chirpan, as well as distantly related species of the genera *Triticum* and *Aegilops* and the synthetic species *Triticale*, grown under greenhouse conditions to synchronize flowering. Hybridization was performed by manual emasculation and pollination of spikes according to the established methodology at the Field Crops Institute – Chirpan. Crossability was calculated as a percentage by dividing the number of ovaries containing embryos by the number of pollinated florets and multiplying by 100.

2.2.2. Embryo rescue

In some of the cross combinations, *in vitro* embryo rescue was applied. Immature hybrid embryos were isolated 16–20 days after pollination and cultured on P8 medium and modified MS medium. **Variation 1.** P8 medium (Novolotsky et al., 1985), supplemented with Staba Modified Vitamins and the growth regulators kinetin – 0.1 mg/L and IAA – 0.1 mg/L.

Variation 2. Modified MS medium (Murashige and Skoog, 1962), with the following composition: B5 macronutrients (Gamborg et al., 1968), MS micronutrients, vitamins according to Chu et al. (1990), asparagine – 200 mg/L, casein hydrolysate – 200 mg/L, kinetin – 0.1 mg/L, IAA – 0.1 mg/L and ABA – 0.1 mg/L.

The regenerated plants were acclimatized in a sterile peat–soil mixture and subsequently grown in a greenhouse. The number of embryos cultured, the degree of endosperm

development, and the number and percentage of regenerated and acclimatized plants were recorded.

2.2.3. Methods for harvesting F₁ hybrid progenies and obtaining backcross generations

The F₁ hybrid plants obtained were monitored through phenological and morphological observations. Some of them were left to self-pollinate, while hybrids exhibiting pronounced traits of the distantly related species were backcrossed with different durum wheat cultivars used as recurrent parents. The resulting seeds were harvested individually and used to establish F₂ and BC₁ generations.

2.2.4. Methods for identifying the transfer of genetic material from distantly related species into the durum wheat genome

Morphological, cytological and molecular methods were applied to detect the transfer of genetic material from distantly related species. The morphological analysis included the evaluation of major traits such as plant height, spike length, tillering, leaf parameters, pubescence, culm angle and plant form. Cytological investigations were conducted using temporary acetocarmine preparations of anthers from F₁ and BC₁ plants to detect abnormalities in meiosis and microsporogenesis and to assess pollen fertility.

Molecular analysis was performed using SSR and ISSR markers. SSR markers with known chromosomal locations in the A, B and D genomes of wheat were used, together with ISSR primers to detect fragments specific to the distantly related species. Genomic DNA was isolated from leaf material, and the amplification products were analyzed by fragment analysis and electrophoretic separation. Data from the SSR and ISSR profiles were scored as the presence or absence of alleles/fragments specific to the parental forms and were used to assess introgression events in the hybrid and backcross progenies.

2.2.5. Morphological (phenotypic) and agronomic methods for trait analysis in advanced backcross and breeding lines derived from distant hybridization

The advanced backcross and breeding lines were evaluated for phenotypic, agronomic and quality traits. In a breeding nursery, heading date, plant height, flag leaf and main spike parameters, number of grains per spike, grain weight per spike, thousand-kernel weight, and protein and gluten content were recorded. Promising lines were tested in a comparative variety trial using a randomized complete block design with four replications. Grain yield and quality were evaluated using traits such as grain yield, thousand-kernel weight, test weight, vitreousness, protein and wet gluten content, SDS sedimentation and yellow color b*. Gliadin fractions were analyzed by acid polyacrylamide gel electrophoresis.

2.2.6. Statistical analysis of the results

The obtained results were statistically analyzed using descriptive statistics and analysis of variance (ANOVA). Two-way analysis of variance (ANOVA) was applied to assess the effects of genotype, environment and their interaction, and differences between mean values were tested using Duncan's multiple range test. Statistical analysis was performed using the Statistica 13.0 software package.

3. RESULTS AND DISCUSSION

3.1. Determination of crossability and compatibility between distantly related species and different durum wheat cultivars and breeding lines

A necessary condition for the introduction of desirable traits into a cultivated species is the existence of a certain degree of crossability between the species (Fedak, 1999). For this reason, the evaluation of crossability between distantly related and cultivated species is the first step in the process of using alien genetic material in breeding programs.

Mean crossability data for durum wheat with seven species of the genus *Aegilops*, eight species of the genus *Triticum* and hexaploid triticale over a five-year research period are presented in Table 2. Over the five growing seasons, the mean crossability of durum wheat with species of the genus *Aegilops* was 4.5%, with species of the genus *Triticum* – 25.1%, and with the synthetic species *Triticale* – 22.2%. As expected, crossability in hybridization of durum wheat with species of the genus *Triticum* was higher than that in hybridization with species of the genus *Aegilops*.

Table 2. Mean crossability (%) of *Triticum durum* with species of the genus *Aegilops*, the genus *Triticum* and the synthetic species *Triticale* for the period 2005–2010.

Cross	Pollinated florets, No.	Ovaries containing embryos, No	Crossability, %
<i>Tr. durum</i> × <i>Ae. tauschii</i>	928	42	4,5
<i>Tr. durum</i> × <i>Ae. umbellulata</i>	2 186	67	3,1
<i>Tr. durum</i> × <i>Ae. cylindrica</i>	2 341	227	9,7
<i>Tr. durum</i> × <i>Ae. crassa</i>	1 391	37	2,7
<i>Tr. durum</i> × <i>Ae. ventricosa</i>	1 062	33	3,1
<i>Tr. durum</i> × <i>Ae. kotschy</i>	2 081	68	3,3
<i>Tr. durum</i> × <i>Ae. juvenalis</i>	1 806	57	3,2
	11 795	531	4,5
<i>Tr. durum</i> × <i>Tr. monococcum</i>	153	23	15,0
<i>Tr. durum</i> × <i>Tr. dicoccoides</i>	462	230	50,0
<i>Tr. durum</i> × <i>Tr. dicoccum</i>	440	84	19,1
<i>Tr. durum</i> × <i>Tr. carthlicum</i>	262	52	19,8
<i>Tr. durum</i> × <i>Tr. polonicum</i>	224	93	41,5
<i>Tr. durum</i> × <i>Tr. timopheevii</i>	373	37	9,9
<i>Tr. durum</i> × <i>Tr. macha</i>	252	31	12,3
<i>Tr. durum</i> × <i>Tr. spelta</i>	348	81	23,3
	2 514	631	25,1
	4 597	1 020	22,2
<i>Tr. durum</i> × <i>Triticale</i>			

The low crossability between durum wheat and species of the genus *Aegilops* observed in the present study was expected due to the genetic distance between the two genera, the lack of homology between their chromosomes, and the presence of postzygotic reproductive barriers. Despite the low values, the ovaries obtained demonstrate the possibility of using these distantly related species as sources of genetic diversity.

When comparing the crossability of durum wheat with species of the genus *Triticum*, a relationship was observed with the presence of homologous genomes in the species involved in the crosses. Crossability between the species was higher when a greater number of homologous genomes were present in the hybridized genotypes. Crosses of durum wheat with tetraploid species possessing the AB genome showed a significantly higher mean crossability (33.1%) compared with hexaploid species possessing the ABD genome (18.7%), diploid

species possessing the A genome (15.0%), and tetraploid species possessing the G genome (9.9%) (Figure 1).

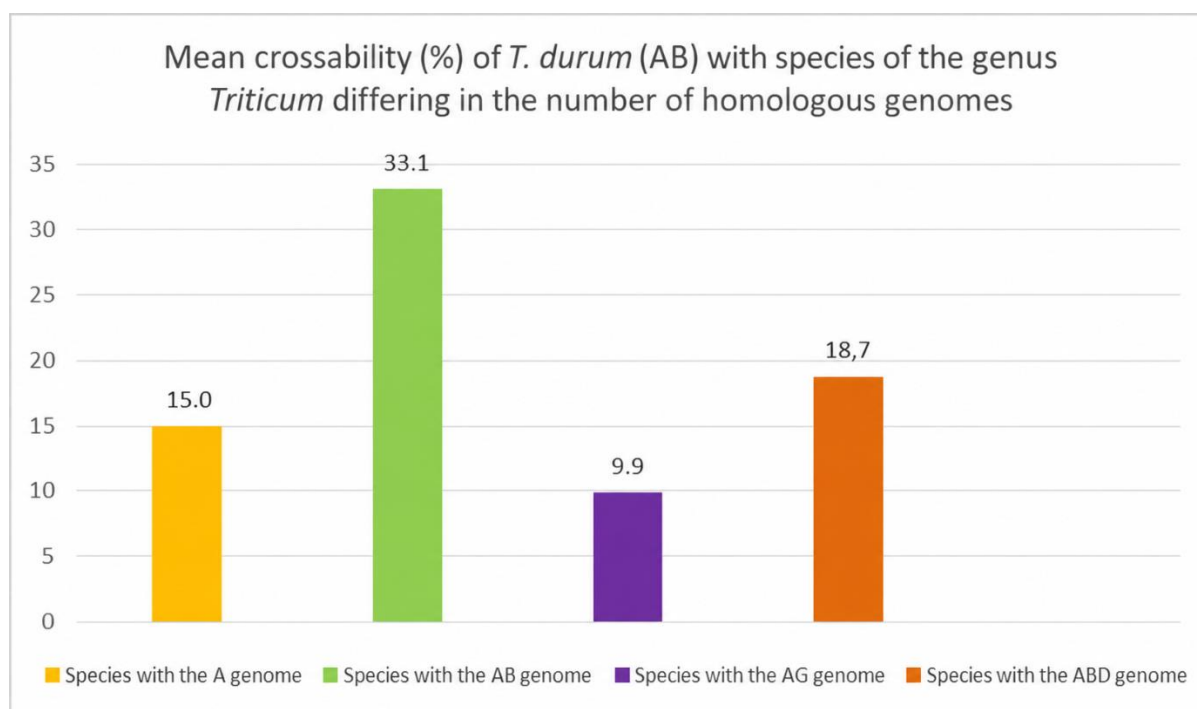


Figure 1. Mean crossability (%) of *Triticum durum* with species of the genus *Triticum* differing in the number of homologous genomes

Genotypic differences in crossability attributable to the female parent, *Triticum durum*, were observed in the hybrid combinations studied. Different durum wheat cultivars and lines were used as female parents in crosses with distantly related species of the genera *Aegilops* and *Triticum*, and with the synthetic species *Triticale*, as presented in Tables 3 and 4. In the hybridization of durum wheat with different species of the genus *Aegilops*, the greatest variation in crossability attributable to the female parent was recorded in crosses with *Aegilops cylindrica* – from 1.4 % for breeding line 7208 to 39.2 % for line D-7767, and with *Aegilops tauschii* – from 0 % for line 7416 to 20 % for line 7351.

In the hybridization of durum wheat with different species of the genus *Triticum*, the greatest variation in crossability attributable to the female parent was recorded in crosses with *Triticum monococcum* – from 5.7 to 20 %, with *Triticum dicoccum* – from 7.3 % to 44.3 %, with *Triticum timopheevii* – from 5.4 % to 34.5 %, and with *Triticum spelta* – from 9.8 % to 70 %. In crosses of different durum wheat genotypes with *Triticale*, crossability varied within a comparatively narrower range, from 2.5 to 28.1 %.

Comparison of the data on the effect of the durum wheat genotype as the female parent shows that the cultivar Victoria was characterized by higher crossability than the other genotypes used in hybridization with almost all species of the genus *Aegilops* and with two species of the genus *Triticum* – *Triticum dicoccum* and *Triticum polonicum* (Tables 3 and 4).

Table 3. Effect of the *Triticum durum* genotype on crossability in distant crosses with species of the genus *Aegilops*.

Durum wheat, <i>female parent</i>		Distantly related species <i>male parent</i>	Genome	Pollinated florets, No.	Ovaries obtained, No.	Crossability, %
Vazhod				568	19	3,3
7416	×	<i>Ae. tauschii</i>	D	186	0	0
7351				94	19	20,2
F ₁ (Gergana × Vazhod)				80	4	5,0
Vazhod				142	2	1,4
Victoria	×	<i>Ae. umbellulata</i>	U	568	30	5,3
Beloslava				626	11	1,6
6467				176	12	6,8
7208				154	2	1,3
7281				282	8	2,8
7383				238	2	0,8
Vazhod				503	43	8,5
Victoria	×	<i>Ae. cylindrica</i>	DC	319	66	20,7
D-6467				126	3	2,4
D-7767				125	49	39,2
7281				126	4	3,2
7208				96	1	1,04
7121				1044	61	5,8
Victoria				424	8	1,9
Progress	×	<i>Ae. crassa</i>	DM	262	4	1,5
7110				156	0	0
7383				102	0	0
D-7538				115	5	4,3
F ₁ (Vazhod × 6189)				200	18	9
F ₁ (Gergana × 6189)				52	2	3,8
F ₁ (A-233 × Beloslava)				80	0	0
Beloslava				148	0	0
Vazhod	×	<i>Ae. kotschy</i>	SU	294	5	1,7
Victoria				456	31	6,8
Iride				233	20	8,6
Meridiano				356	7	2,0
7307				118	0	0
7346				138	3	2,2
D-7208				166	1	0,6
D-7505				172	1	0,6
Vazhod				228	14	6,1
Victoria	×	<i>Ae. juvenalis</i>	DMU	196	12	6,1
Beloslava				106	1	0,9
7110				152	3	1,9
D-7208				496	8	1,6
D-7281				290	2	0,7
D-7505				154	6	3,9
7351				30	0	0
7416				154	11	7,1

Table 4. Effect of the *Triticum durum* genotype on crossability in distant crosses with species of the genus *Triticum* and *Triticale*.

Durum wheat, <i>female parent</i>	Distantly related species <i>male parent</i>	Genome	Pollinated florets, No.	Ovaries obtained, No.	Crossability, %
F ₁ (Vazhod × 6189)			53	3	5,7
Superdur ×	<i>Tr. monococcum</i>	A ^b	100	20	20,0
Vazhod			96	43	44,8
D-6189 ×	<i>Tr. dicoccoides</i>	A ^u B	39	19	48,7
D-7552			183	105	57,4
7307			80	40	50,0
7346			64	23	35,9
Progress			80	30	38,0
Victoria ×	<i>Tr. dicoccum</i>	A ^u B	70	31	44,3
7544			290	23	7,3
7383			120	35	29,2
7416 ×	<i>Tr. carthlicum</i>	A ^u B	74	12	16,2
D-7505			68	5	7,4
Victoria			34	19	55,9
Vazhod ×	<i>Tr. polonicum</i>	A ^u B	106	63	59,4
7416			84	11	13,1
F ₁ (6189× Beloslava)			80	2	2,5
F ₁ (A 233× × Gergana)	<i>Tr.timopheevii</i>	A ^b G	62	5	8,1
F ₁ (Vazhod × Victoria)			120	8	6,7
F ₁ (Gergana × Vazhod)			56	3	5,4
Гергана			55	19	34,5
(233 × × Superdur)	<i>Tr. macha</i>	A ^u BD	132	10	7,6
масов посев			120	21	17,5
Beloslava			80	15	18,8
7346 ×	<i>Tr. spelta</i>	A ^u BD	20	14	70,0
7307			54	14	25,9
D-7505			102	29	28,4
F ₁ (Progress × Beloslava)			92	9	9,8
Vazhod			2618	653	24,9
Progress ×	<i>Triticale</i>	ABR	190	65	34,2
D-7647			298	122	40,9
D-7793			302	4	1,3
D-7794			461	51	11,1

The results obtained show that crossing between durum wheat and distantly related species is possible, but its success depends on the genetic relatedness of the parental forms, ploidy level, genotypic characteristics of the durum wheat cultivars used and, to a certain extent, environmental conditions.

3.2. Optimization of *in vitro* techniques for overcoming embryo incompatibility in interspecific and intergeneric hybridization

Incompatibility in crosses of durum wheat with distantly related species of the genera *Triticum* and *Aegilops* and the synthetic species *Triticale* is also manifested during hybrid embryogenesis, through the failure to form embryos or their degeneration at different stages of embryonic development. Various techniques have been developed to overcome these abnormalities, with the embryo culture method being characterized by high efficiency (Valkoun et al., 1990).

3.2.1. Optimization of the culture medium composition for embryo rescue in distant hybrids obtained from crosses of *Triticum durum* with species of the genera *Triticum* and *Aegilops* and the synthetic species *Triticale*

The composition of the culture medium can be considered a determining factor for the successful development of hybrid embryos under *in vitro* conditions. In a preliminary experiment, we investigated the effect of the growth regulator ABA (abscisic acid) on the precocious germination of immature embryos from distant crosses of durum wheat with species of the genera *Aegilops* and *Triticum*, as well as with the synthetic species *Triticale*. Equal numbers of immature embryos from crosses of *Triticum durum* with the wild species *Aegilops cylindrica*, *Aegilops kotschy*, *Triticum macha*, *Triticum spelta* and *Triticale* were cultured on two modified culture media: P8 without added ABA (variant 1) and MS supplemented with abscisic acid (variant 2) (Table 5). A total of 60 plants were regenerated from the 160 hybrid embryos cultured. In variant 2, 41 plants were regenerated, whereas in variant 1, approximately two times fewer plants, 19, were regenerated (Figure 2). No substantial genotype $\times$ medium interaction was observed, i.e. embryos from all hybrid combinations responded similarly to the different culture medium variants.

A greater number of plants were regenerated on our modified culture medium, variant 2. This is due to the fact that a greater proportion of the undifferentiated embryos were able to undergo differentiation, germinate and develop into plants compared with those cultured on variant 1.

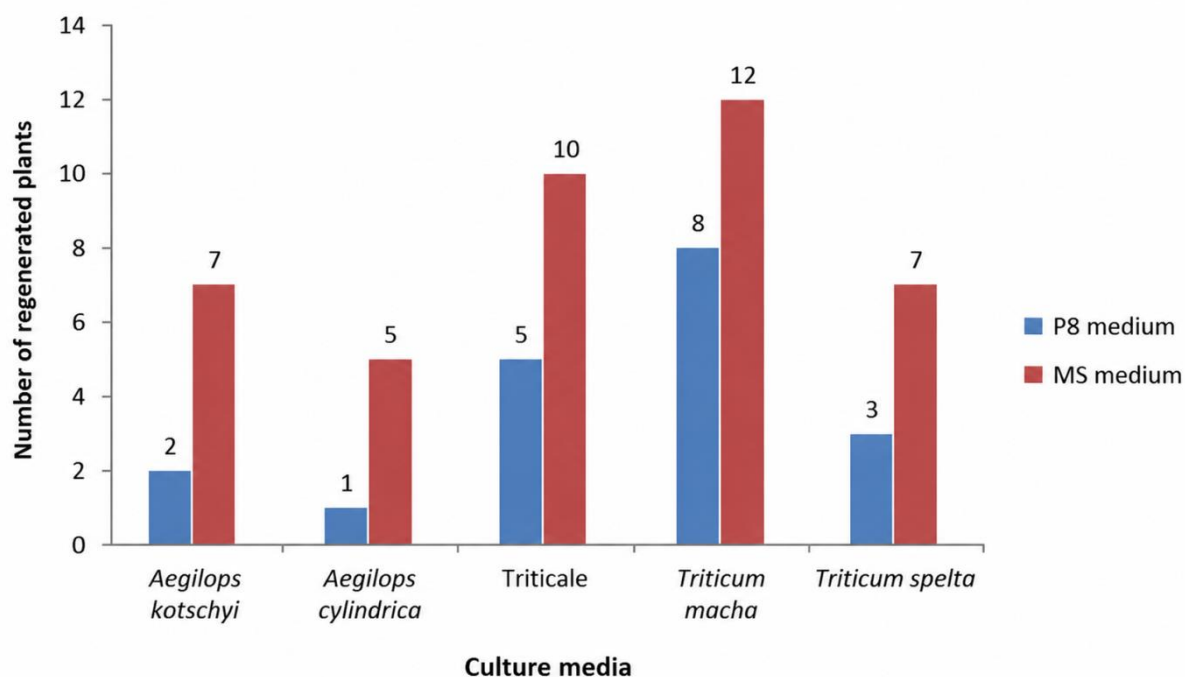


Figure 2. Effect of P8 and MS culture media on plant regeneration.

Table 5. Composition of the modified MS and P8 media for *in vitro* rescue of hybrid embryos.

Culture medium components	MS (mg/l)	P8 (mg/l)
Macronutrients		
KNO ₃	2500	2500
NH ₄ NO ₃	1650	-
(NH ₄) ₂ SO ₄	-	134
CaCl ₂ ·2H ₂ O	150	-
MgSO ₄ ·7H ₂ O	250	250
NaH ₂ PO ₄ ·H ₂ O	150	150
Micronutrients		
H ₃ BO ₃	6,2	3
MnSO ₄ ·H ₂ O	-	10
MnSO ₄ ·4H ₂ O	22,3	-
ZnSO ₄ ·7H ₂ O	8,6	2
Na ₂ MoO ₄ ·2H ₂ O	0,25	0,25
CuSO ₄ ·5H ₂ O	0,025	0,025
CoCl ₂ ·6H ₂ O	0,025	0,025
KI	0,83	0,75
Fe-Na-(EDTA)	43	40
Vitamins		
Nicotinic acid	0,5	2
Thiamine hydrochloride	2,5	10
Pyridoxine hydrochloride	0,5	2
Myo-inositol	100	100
Vitamins according to Staba		
Folate	-	0,5
Riboflavin	-	0,5
Biotin	-	1
Choline Chloride	-	1
Calcium pantothenate	-	1
Cyanocobalamin	-	0,0015
Amino acids		
L-Asparagine	200	-
Glycine	1	-
Plant growth regulators		
Kinetin	0,1	0,1
Absciscic acid	0,1	-
Indole-3-Acetic Acid	0,1	0,1
Other components		
Casein hydrolysate	200	200
Sucrose	30000	30000
Agar	7000	8000
pH	5,5	5,7

P8 medium (Новолоцкий, 1985), Staba Modified Vitamins

MS modified medium (Murashige and Skoog, 1962), Vitamins (Chu, Hill, and Brule-Babel, 1990), Macro elements (Gamborg, Miller, and Ojima, 1968)

The results obtained show that the modified MS medium containing abscisic acid provides a higher regeneration potential for immature embryos from distant crosses compared with the P8 medium. The better result is probably due to the optimal composition of the mineral salts in the MS medium, including the higher concentrations of ammonium and nitrate ions, as well as the inhibitory effect of ABA on the precocious germination of undifferentiated embryos. This creates conditions for their better development and regeneration under *in vitro* conditions.

3.2.2. Application of the embryo rescue method in crosses of *Triticum durum* with species of the genera *Triticum* and *Aegilops* and the synthetic species *Triticale*

The study investigated the possibility of rescuing embryos from distant crosses with durum wheat using our modified MS culture medium. The crosses were carried out with species of the genera *Aegilops* and *Triticum* and the synthetic species *Triticale*. Of a total of 414 isolated embryos, 126 germinated and developed into whole plants (30.4 % of the embryos), of which 30 F₁ plants from twelve hybrid combinations were successfully acclimatized after transfer to soil (Tables 6 and 7, Photo 1). The number of isolated embryos and the degree of endosperm development varied depending on the specific cross, as shown in Tables 6 and 7.

Substantial differences in endosperm development were observed in crosses of durum wheat with species from the different genera. The endosperm was well developed in crosses with species of the genus *Triticum* when homologous genomes were present in durum wheat and the wild species. In the absence of homologous genomes, as in crosses with species of the genus *Aegilops*, the endosperm was reduced or poorly developed. In crosses with *Triticale*, the endosperm was watery or absent in all hybrid combinations, despite the presence of two homologous genomes. Caryopses consisting only of a seed coat, without embryos or endosperm, were observed, probably having aborted at an early stage of development due to rapid endosperm degeneration or its absence.



Photo 1. Stages of embryonic development, regeneration and acclimatization of plants obtained through distant hybridization.

Table 6. Embryo rescue and stages of regeneration and acclimatization in distant hybridization with species of the genus *Aegilops*.

Hybrid combination			Number of embryos	Endosperm	Regenerated plants		Acclimatized plants	
7351	×	<i>Aegilops tauschii</i> (D)	2	reduced	-	-	-	-
F ₁ (Gergana × Vazhod)			4	poorly developed	1	25 %	1	100 %
о́бщо			6		1	16,7 %	1	100 %
Д-7383	×	<i>Aegilops umbellulata</i> (U)	2	reduced	2	100 %	2	100 %
Beloslava			11	poorly developed	4	36,4 %	2	50 %
Victoria			30	reduced	7	23,3 %	-	-
о́бщо			43		13	30,2 %	4	30,8 %
7121	×	<i>Aegilops cylindrica</i> (CD)	5	reduced	-	-	-	-
Victoria			2	reduced	2	100 %	2	100 %
Vazhod			27	reduced	-	-	-	-
о́бщо			34		2	5,9 %	2	100 %
7538	×	<i>Aegilops crassa</i> (DM)	2	reduced	-	-	-	-
Victoria			5	reduced	-	-	-	-
Progress			1	reduced	-	-	-	-
о́бщо			8		-	-	-	-
Victoria	×	<i>Aegilops ventricosa</i> (DU)	3	reduced	1	33,3 %	-	-
7110			21	reduced	4	19,1 %	-	-
о́бщо			24		5	20,8 %	-	-
7346	×	<i>Aegilops kotschyi</i> (SU)	14	reduced	7	50 %	-	-
Vazhod			2	reduced	-	-	-	-
Victoria			22	reduced	9	40,9 %	2	22,0 %
о́бщо			38		16	42,1 %	2	12,5 %
7208	×	<i>Aegilops juvenalis</i> (DMU)	2	reduced	-	-	-	-
7416			5	reduced	1	20 %	-	-
Vazhod			14	reduced	4	28,6 %	1	25,0 %
Beloslava			3	reduced	-	-	-	-
о́бщо			24		5	20,8 %	1	20,0 %

Table 7. Embryo rescue and stages of regeneration and acclimatization in distant hybridization with species of the genus *Triticum* and *Triticale*.

Hybrid combination			Number of embryos	Endosperm	Regenerated plants		Acclimatized plants	
7552	×	<i>Triticum dicoccoides</i> (AB)	29	normal	18	62,1 %	6	33,3 %
7346			12	normal	5	41,7 %	1	20,0 %
7307			17	normal	3	17,6 %	-	-
Vazhod			21	normal	9	42,9 %	2	22,2 %
о́бщо			79		35	44,3 %	9	25,7 %
<i>Triticum dicoccum</i>	×	<i>Triticum durum</i>	14	normal	8	57,1 %	3	37,5 %
о́бщо			14		8	57,1 %	3	37,5 %
7383	×	<i>Triticum carthlicum</i> (AB)	10	normal	4	40,0 %	1	25,0 %
о́бщо			10		4	40,0 %	1	25,0 %
F ₁ (Vazhod × Victoria)	×	<i>Triticum timopheevii</i> (AG)	6	normal	6	100 %	2	33,3 %
о́бщо			6		6	100 %	2	33,3 %
Vazhod	×	<i>Triticum macha</i> (ABD)	12	normal	4	33,3 %	1	25,0 %
о́бщо			12		4	33,3 %	1	25,0 %
7346	×	<i>Triticum spelta</i> (ABD)	9	normal	1	11,1 %	-	-
7505			5	normal	-	-	-	-
F ₁ (Progress × Beloslava)			4	normal	4	100 %	3	75,0 %
о́бщо			18		5	27,8 %	3	60,0 %
7192	×	TCL (ABR)	10	watery	3	30 %	1	33,0 %
6948			34	reduced	7	20,6 %	-	-
Vazhod			50	watery	12	24 %	-	-
Progress			4	watery	-	-	-	-
о́бщо			98		22	22,4 %	1	4,5 %

The percentage of regenerated plants in the individual hybrid combinations used in the present experiment varied widely. In crosses with species of the genus *Aegilops*, further development of the hybrid embryos and plant regeneration were observed at the following rates: 5.9 % for *Aegilops cylindrica* and 16.7 %, 20.8 % and 20.8 % for *Aegilops tauschii*, *Aegilops ventricosa* and *Aegilops juvenalis*, respectively. Higher percentages of regenerated plants were recorded in crosses with *Aegilops kotschy* – 42.1 % and *Aegilops umbellulata* – 30.2 % (Table 6). In the hybridization of durum wheat with species of the genus *Triticum*, the observed regeneration ranged from 44.3 % for *Triticum dicoccoides* to 100 % for *Triticum timopheevii* (Table 7). Lower regeneration was recorded in hybrid combinations with hexaploid species of the genus *Triticum*. The regeneration potential was 33.3 % for *Triticum macha* and 27.8 % for *Triticum spelta*. Regeneration in *Triticale* was 22.4 %.

Variation in this trait was also observed within hybrid combinations involving the same species when different durum wheat genotypes were used as female parents.

The study revealed a comparatively low acclimatization potential of the regenerated plants.

Regeneration and acclimatization potential varied depending on the hybrid combination and the durum wheat genotype used, without a direct relationship being established between the degree of crossability and the ability to regenerate. This confirms the need for careful selection of parental forms and optimization of embryo rescue conditions.

The present study investigated the optimal age for embryo isolation and culture on a nutrient medium. It was established that early embryo isolation, on the 13th or 15th day after pollination, was unfavorable for their subsequent *in vitro* development. The embryos were small and poorly differentiated, and no growth was observed when they were cultured on a nutrient medium. The stage of embryo development from the 16th to the 20th day after pollination was the most suitable for successful transfer to an artificial medium and their subsequent *in vitro* development into whole plants.

3.3. Studies on the development of F₁ hybrid plants and harvesting of subsequent backcross generations

The hybrid nature of the acclimatized plants underlies the occurrence of processes that hinder their normal development. Reduced viability, leading to the death of hybrid plants, and the sterility observed in the F₁ progenies are abnormalities that limit their reproduction.

Despite their ability to acclimatize, some of the plants obtained from crosses with *Aegilops kotschy* and *Aegilops juvenalis* showed deterioration in viability and died at a later stage of development. The remaining acclimatized plants obtained from crosses with *Aegilops umbellulata*, *Aegilops tauschii*, *Aegilops cylindrica*, *Triticum dicoccoides*, *Triticum dicoccum*, *Triticum carthlicum*, *Triticum timopheevii*, *Triticum macha*, *Triticum spelta* and *Triticale* were grown to maturity.

Normal heading was observed in the hybrid plants, which exhibited an intermediate phenotype resulting from the combination of traits of the two parental genotypes. Spike morphology differed depending on the hybrid combination, and the morphological and cytological characteristics of the hybrid plants from crosses with *Aegilops cylindrica* and *Aegilops umbellulata* are presented in detail below.

The number of seeds per spike in F₁ hybrids obtained from crosses with species of the genus *Triticum* was comparatively low, but sufficient to obtain hybrid plants in the next generation. Some of the spikes of the F₁ hybrids were emasculated and pollinated with durum wheat, and backcross progenies (BC₁) were obtained. During each year of the study, first, second and third backcrosses were carried out using F₁, BC₁ and BC₂ plants from different hybrid combinations.

3.3.1. Characterization of F₁ hybrids obtained from crosses with *Aegilops cylindrica* and *Aegilops umbellulata* and their inclusion in backcrossing schemes

The acclimatized plants from crosses with *Aegilops cylindrica* and *Aegilops umbellulata* were grown to full maturity and successfully harvested. Some of the spikes of the F₁ hybrids were left to self-pollinate, while the remaining spikes were used for backcrossing, with durum wheat (*Triticum durum*) used as the recurrent parent.

Complete sterility, both male and female, was observed in the F₁ hybrids obtained from the cross with *Aegilops cylindrica*. As a result, no F₂ or BC₁ generations were obtained.

In plants from the cross with *Aegilops umbellulata*, the self-pollinated F₁ hybrids exhibited partial fertility. The seeds obtained from them were sown, but no germination was recorded.

Following backcrossing of the F₁ hybrids from the combination (Beloslava × *Aegilops umbellulata*) with the recurrent parent, durum wheat cultivar Beloslava, BC₁ seeds were obtained. Crossability did not exceed that recorded in the F₁ hybrids (Tables 7 and 8).

Table 8. Crossability in the BC₁ and BC₂ generations from the cross Beloslava × *Aegilops umbellulata*

Backcross generations	Number of pollinated florets	Number of seeds	Crossability, %
(Beloslava × <i>Aegilops umbellulata</i>) BC ₁	397	7	1,8
(Beloslava × <i>Aegilops umbellulata</i>) BC ₂	164	43	26,2

The use of backcrossing restores hybrid fertility and allows the introgression of desirable traits from *Aegilops umbellulata* into durum wheat.

3.3.1.1. Morphological evaluation of F₁ hybrids obtained from crosses of *Triticum durum* with *Aegilops cylindrica* and *Aegilops umbellulata*

Morphologically, all F₁ hybrids obtained from the cross of cultivar Victoria with *Aegilops cylindrica* were completely uniform and exhibited traits characteristic of both parental forms. The plant form, hybrid spike shape, leaf pubescence and tendency towards increased tillering were typical of the wild species. Leaf length and width and plant height were intermediate between the two parental forms. Spike length in the hybrid plants was greater than that of both the wild and cultivated parents. The spikes were straight and cylindrical, resembling those of the wild species, but were wider (Photo 2). The awns were located in the apical part of the spike.



A



B

Photo 2. A: Spikes of the F₁ hybrid and the durum wheat parent, cultivar Victoria; B: Spikes of the wild species *Aegilops cylindrica* used as the male parent.

The mean values of various morphological and agronomic traits of the F₁ hybrids from the cross Beloslava × *Aegilops umbellulata* and their parents are presented in Table 9. All F₁ hybrid plants obtained were uniform, showed good tillering ability and exhibited traits of both parents.

Table 9. Morphological comparison between the F₁ hybrids (Beloslava × *Aegilops umbellulata*) and the parental forms.

Morphological traits (cm)	<i>Triticum durum</i> cultivar Beloslava	<i>Aegilops umbellulata</i>	F ₁ hybrid
plant height	90,3	52,3	50,0
number of tillers	15,0	29,0	21,0
spike length	8,5	4,0	5,5
leaf length	40,0	11,5	20,2
leaf width	2,2	1,2	0,6
leaf hairiness	weak	strong	weak
plants shape	erect	rosette	erect
angled stem	no	yes	no



Photo 3. F₁ hybrid plants

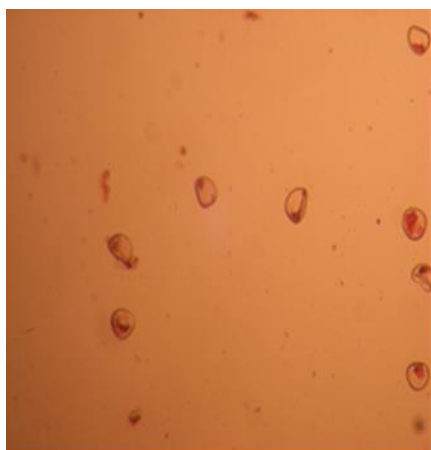


Photo 4. Spikes of the parental forms and the F₁ hybrid plant

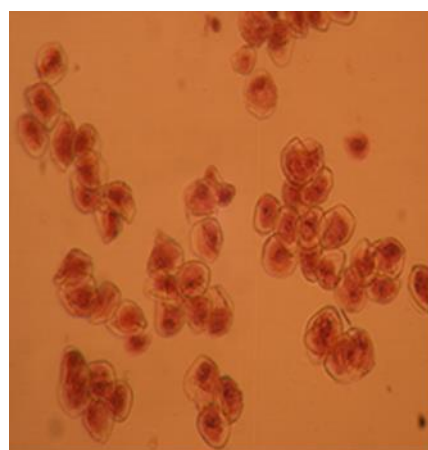
The form of the plants and spikes was similar to that of durum wheat, with an erect plant habit. The F₁ hybrid spikelets and seeds had wheat-like morphology (Photos 3 and 4). Leaf length, leaf width and number of tillers were intermediate between the two parents. In other traits, such as plant height, spike length and strong leaf pubescence, the hybrid plants resembled the wild parent, *Aegilops*.

3.3.1.2. Cytological observations in F₁ hybrids obtained from crosses of *Triticum durum* with *Aegilops cylindrica* and *Aegilops umbellulata*

Cytological examination of microspores from F₁ hybrids obtained from the cross between cultivar Victoria and *Aegilops cylindrica* revealed abnormalities in microspore formation. Microspores with irregular shape and smaller than normal size were observed (Photo 5A). Examination of mature pollen also revealed numerous abnormalities. The number of pollen grains was greatly reduced, and very small, irregularly shaped pollen grains with transparent protoplasm and poorly differentiated nuclei were also observed (Photo 5B).



A



B

Photo 5. Abnormalities in microspore formation in the distant cross (Victoria × *Aegilops cylindrica*): A – microspores of small size; B – irregularly shaped microspores.

Cytological investigations of hybrids in the F₁ and BC₁ generations from the cross Beloslava × *Aegilops umbellulata*, during anaphase II and the uninucleate microspore stage, revealed irregular meiotic progression. During late anaphase II, numerous bridges and fragments (Figure 3a), as well as irregular cytokinesis and lagging chromosomes (Figure 3b), were detected at a low frequency. Abnormal tetrads were observed, manifested by the frequent formation of dyads, triads and polyads (Figure 3d–g), as well as irregular positioning of microspores within the tetrads (Figure 3h). The formation of micronuclei and microcytes was also detected (Figure 3c–d).

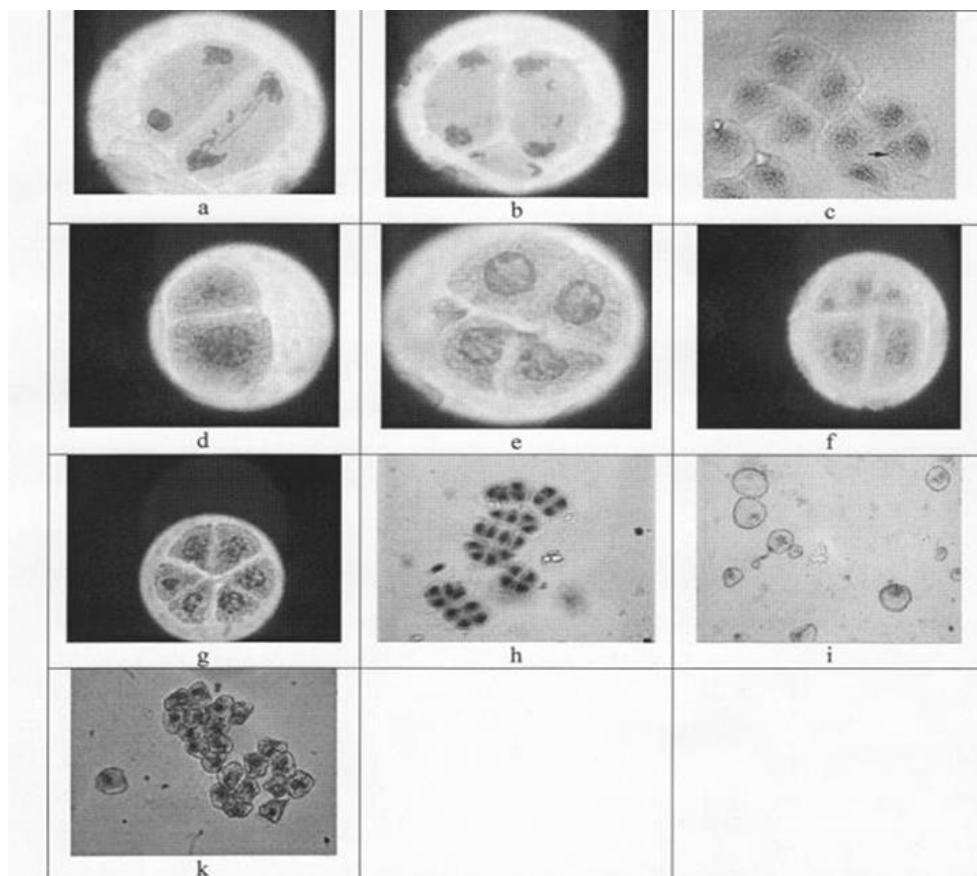


Figure 3. Some aspects of meiotic behavior in a hybrid between durum wheat and *Aegilops umbellulata*: a – late anaphase II with bridges and fragments; b – late anaphase II with irregular cytokinesis and lagging chromosomes; c – tetrads with microspores containing micronuclei; d – dyad with microspores of different size and micronuclei; e – triad with a microcyte; f – triad with one trinucleate microspore; g – hexad; h – irregular arrangement of microspores in the tetrads; i – microspores with irregular shape and size; k – degenerating microspores.

The meiotic abnormalities observed in the hybrids with *Aegilops umbellulata* – the presence of dyads and triads, as well as chromosome bridges and fragments during anaphase II – provide grounds for suggesting the possible formation of unreduced ($2n$) gametes and potential recombination between the homoeologous chromosomes of the parents. In accordance with published data, these cytological features could partially explain the fertility retained during subsequent backcrossing with the cultivated parent.

3.4. Identification of the transfer of genetic information in advanced backcross lines derived from distant hybridization of durum wheat with species of the genera *Triticum* and *Aegilops* and *Triticale*

The study included ten advanced backcross lines obtained as a result of our long-term work on distant hybridization of six Bulgarian durum wheat cultivars (*Triticum durum* Desf.) ($2n = 28$, AABB) with nine distantly related species of the family Gramineae and the synthetic species *Triticale*, using a backcrossing strategy with durum wheat as the recurrent parent (Table 10).

Table 10. Lines obtained through distant hybridization and a subsequent backcrossing strategy.

Cross combinations	Year of F ₁ generation development	Molecular analysis 2009
(Gergana × Vazhod)F ₁ × <i>Aegilops tauschii</i> F ₁ × <i>Tr. durum</i> BC ₁ × Victoria	2006	BC ₂ F ₂
(Beloslava × <i>Aegilops umbellulata</i>) F ₁ × Beloslava) BC ₁ × Victoria)	2007	BC ₂ F ₂
(Vazhod × <i>Triticum dicoccoides</i>)F ₁ × Vazhod	2007	BC ₁ F ₁
(<i>Triticum dicoccum</i> × <i>Triticum durum</i>)F ₁ × Progress	2006	BC ₁ F ₂
(<i>Triticum polonicum</i> × <i>Triticum durum</i>)F ₂ × <i>Triticum durum</i>)BC ₁ × Progress	2005	BC ₂ F ₁
(7383 × <i>Triticum carthlicum</i>)F ₂ × Victoria	2007	BC ₁ F ₁
(Vazhod × Victoria)F ₁ × <i>Triticum timopheevii</i>)F ₁ × Vazhod	2006	BC ₁ F ₃
(<i>Triticum durum</i> × <i>Triticum macha</i>)F ₁ × Progress	2005	BC ₁ F ₄
(Progress × Beloslava)F ₁ × <i>Triticum spelta</i>)F ₁ × Vazhod	2005	BC ₁ F ₄
(Д7192 × <i>Triticosecale</i>)F ₁ × Vazhod)BC ₁ F ₁ × <i>Triticum durum</i>	2006	BC ₂ F ₄

The backcrossing method is a breeding strategy commonly used to introduce a single or a small number of valuable genes, most often from a wild relative (an exotic, unadapted species), into an elite cultivar or germplasm. The method involves crossing a recurrent parent with a donor parent. This approach makes it possible to reduce the proportion of the donor parent genome after each successive backcross and to avoid the masking effect of deleterious wild recessive alleles.

Despite the methods developed for the introgression of useful genes from distantly related species or unadapted germplasm (BIL, F₂, F₃, BC₁, etc.), one of the main challenges in distant hybridization remains the selection of recombinant plants. This is due to the limited frequency of recombination events between homoeologous chromosomes, which makes it difficult to isolate plants carrying the desired segments of the donor genome. The identification of introgressed genomic segments inherited from wild species would facilitate the selection of lines with specific traits and support breeding programs. The detection of introgressions from distantly related species in the durum wheat genome can be further assisted by molecular marker-based techniques.

To identify chromosomal regions introgressed from the distantly related parents, a total of 29 microsatellite markers with known chromosomal locations were selected on the basis of published genetic maps (Gupta et al., 2002; Röder et al., 1998; Somers et al., 2004), amplifying 33 SSR loci in the durum wheat genome. Thirteen microsatellite loci were mapped to the A genome, 10 to the B genome and 10 loci to the D genome. They were used to analyze and identify polymorphic fragments for each of the fourteen chromosomes belonging to the A and B genomes, as well as for the additional D and G genomes of the two parental lines.

Overall, the level of polymorphism between the distantly related species and the durum wheat cultivars was much higher than that observed among the durum wheat cultivars involved in the hybridization (25 % to 35 %), owing to the genetic distance between the distantly related species and durum wheat (Table 11). The polymorphic markers identified between the distantly related species and the cultivated durum wheat cultivars were

subsequently used to demonstrate introgressions from the wild species into the genome of the backcross lines.

Table 11. Polymorphic loci amplified in distantly related species of the genera *Triticum* and *Aegilops*, *Triticale*, and durum wheat cultivars using microsatellite markers.

Hybrid combination	Polymorphic loci, %	Hybrid combination	Polymorphic loci, %
<i>Tr. dicoccoides</i> (AABB) Vazhod	60,6	<i>Tr. spelta</i> (AABBDD) Vazhod	90,1
<i>Tr. carthlicum</i> (AABB) Victoria	78,8	<i>Tr. macha</i> (AABBDD) Progress	90,9
<i>Tr. dicoccum</i> (AABB) Progress	69,7	<i>Ae. tauschii</i> (DD) Victoria	78,8
<i>Tr. polonicum</i> (AABB) Progress	57,6	<i>Ae. umbellulata</i> (UU) Beloslava	75,75
<i>Tr. timopheevii</i> (AAGG) Vazhod	66,7	<i>Triticale</i> (AABBRR) Vazhod	72,72

3.4.1. Detection of introgressions resulting from crosses of *Triticum durum* with distantly related tetraploid species possessing AABB and AAGG genomes (*Triticum carthlicum*, *Triticum dicoccoides*, *Triticum dicoccum*, *Triticum polonicum* and *Triticum timopheevii*)

In our study, 23 SSR markers mapped to the A and B genomes of durum wheat, covering all 14 chromosomes except 2A and 6B, and 10 SSR markers mapped to the D genome were used for genotyping four tetraploid species of the genus *Triticum* possessing AABB genomes (*Triticum carthlicum*, *Triticum dicoccoides*, *Triticum dicoccum* and *Triticum polonicum*) and three durum wheat cultivars (Victoria, Vazhod and Progress).

SSR primers amplifying loci in the D genome were included not only to detect the transfer of D-genome chromatin from the distantly related species *Aegilops tauschii* (DD, $2n = 14$) and the hexaploid species *Triticum macha* (AABBDD, $2n = 42$) and *Triticum spelta* L. (AABBDD, $2n = 42$), but also to amplify loci in diploid, tetraploid and hexaploid distantly related species possessing A and B genomes. The reason for this is the increasing evidence in recent years that some microsatellite primers specific to the A and B genomes can also amplify loci in the diploid species *Aegilops tauschii*, which possesses only the D genome, and, conversely, that primers specific to the D genome can also amplify loci in the A and B genomes (Zhang et al., 2004; Zhang et al., 2007).

Analysis of the backcross lines derived from crosses of durum wheat with *Triticum dicoccum* and *Triticum polonicum*, using the selected polymorphic *Xgwm* and *Xwmc* SSR markers, led to the identification of introgressed chromatin segments from *T. dicoccum* and *T. polonicum* in the A and B genomes of the backcross lines. Introgressed chromatin segments from *Triticum dicoccum* were detected using the markers *Xgwm136* (12 cM) and *Xwmc24* (48 cM), mapped to chromosome arm **1AS**. Introgressed chromatin segments from *Triticum polonicum* were identified using markers on chromosome **5B** (*Xgwm234-5BS* [38 cM] and *Xgwm408-5BL* [117 cM]), as well as the markers *Xgwm46* (56 cM) and *Xgwm302* (86 cM) on chromosome arms **7BS** and **7BL**, respectively (Table 12).

Table 12. Introgressions of chromosomal segments from distantly related tetraploid (AABB), diploid (DD) and hexaploid (AABBDD) species into the durum wheat genome (cultivars Victoria, Vazhod and Progress) and newly appearing alleles in their backcross lines.

Crosses	Introgressions	New alleles	Crosses	Introgressions	New alleles
<i>Tr. carthlicum</i> (AABB) Victoria	<i>Xwmc327-5AL</i> <i>Xgwm639-5DL</i> <i>Xwmc9-7AL</i>	<i>Xgwm268-1BL</i> <i>Xgwm285-3BS</i> <i>Xgwm165-4BL</i> <i>Xgwm234-5BS</i> <i>Xgwm282-7AL</i>	<i>Tr. timopheevii</i> (AAGG) Vazhod	NONE	<i>Xgwm136-1AS</i> <i>Xgwm285-3BS</i> <i>Xgwm165-4BL</i> <i>Xgwm644-6BL</i> <i>Xgwm233-7AS</i> <i>Xwmc 9-7AL</i> <i>Xgwm302-7BL</i>
Total	3	5	Total	0	7
%	11,5	19,2	%	0	31,8
<i>Tr. dicoccoides</i> (AABB) Vazhod	<i>Xgwm312-2AL</i> <i>Xgwm165-4AS</i> <i>Xgwm639-5DL</i>	<i>Xwmc 24-1AS</i> <i>Xgwm285-3BS</i> <i>Xwmc327-5AL</i> <i>Xgwm639-5BL</i> <i>Xgwm644-6BL</i> <i>Xgwm 46-7BS</i> <i>Xgwm233-7AS</i>	<i>Ae.</i> <i>tauschii</i> (DD) Victoria	<i>Xgwm639-5DL</i>	<i>Xgwm268-1BL</i> <i>Xgwm644-6BL</i> <i>Xwmc 83-7AS</i>
Total	3	7	Total	1	3
%	15,0	35,0	%	3,8	11,5
<i>Tr. dicoccum</i> (AABB) Progress	<i>Xgwm136-1AS</i> <i>Xwmc 24-1AS</i> <i>Xgwm 5-3AS</i> <i>Xgwm165-4AS</i> <i>Xgwm234-5BS</i> <i>Xgwm639-5DL</i> <i>Xgwm644-6BL</i> <i>Xwmc 83-7AS</i>	<i>Xgwm136-1AS</i> <i>Xgwm 99-1AL</i> <i>Xgwm268-1BL</i> <i>Xgwm312-2AL</i> <i>Xgwm285-3BS</i> <i>Xgwm639-5BL</i> <i>Xgwm233-7AS</i> <i>Xwmc 9-7AL</i> <i>Xgwm 46-7BS</i> <i>Xgwm302-7BL</i>	<i>Tr. macha</i> (AABBDD) Progress	<i>Xgwm136-1AS</i> <i>Xgwm 99-1AL</i> <i>Xgwm285-3BS</i> <i>Xgwm165-4AS</i> <i>Xgwm233-7AS</i> <i>Xwmc 83-7AS</i> <i>Xwmc 9-7AL</i> <i>Xgwm282-7AL</i>	<i>Xwmc 24-1AS</i> <i>Xgwm268-1BL</i> <i>Xgwm234-5BS</i> <i>Xgwm233-7AS</i> <i>Xgwm 46-7BS</i> <i>Xgwm302-7BL</i>
Total	8	10	Total	8	6
%	34,8	43,5	%	26,7	20,0
<i>Tr. polonicum</i> (AABB) Pogress	<i>Xgwm165-4AS</i> <i>Xwmc327-5AL</i> <i>Xgwm234-5BS</i> <i>Xgwm408-5BL</i> <i>Xwmc 9-7AL</i> <i>Xgwm 46-7BS</i> <i>Xgwm302-7BL</i>	<i>Xgwm136-1AS</i> <i>Xgwm268-1BL</i> <i>Xgwm285-3BS</i> <i>Xgwm234-5BS</i> <i>Xgwm408-5BL</i> <i>Xgwm644-6BL</i>	<i>Tr. spelta</i> (AABBDD) Vazhod	<i>Xwmc 24-1AS</i> <i>Xgwm408-5BL</i> <i>Xgwm469-6DS</i> <i>Xgwm302-7BL</i>	<i>Xgwm136-1AS</i>
Total	7	6	Total	4	1
%	36,8	31,6	%	13,3	3,3

No introgression of A- or G-genome chromosomal segments from *Triticum timopheevii* (AAGG) was detected in the genome of cultivar Vazhod using the set of 33 SSR markers mapped to the A/B and D genomes of bread wheat (Table 12). The results obtained are consistent with earlier studies (Schneider et al., 2010), which demonstrated the limited effectiveness of SSR markers in characterizing the G, U and M genomes, owing to the greater nucleotide sequence divergence between these genomes and those of bread wheat (Molnár et al., 2011).

3.4.2. Detection of introgressions resulting from crosses of *Triticum durum* with hexaploid species possessing the AABBDD genome (*Triticum macha* and *Triticum spelta*)

Most of the introgressions from the genome of *Triticum macha* were located on chromosomes 7A and 1A (Table 12, Figure 4A). According to the published map of Somers et al. (2004), the largest introgressed chromatin segment from *Triticum macha* was identified on chromosome 7A using the markers *Xgwm233* (4 cM), *Xwmc83* (55 cM), *Xwmc9* (72 cM)

and *Xgwm282* (100 cM). This provides clear evidence of introgression/replacement of the entire or nearly entire chromosome 7A in the genotype of the recipient cultivar Progress by the homologous chromosome of *Triticum macha*. Introgression events were also observed on chromosome 1A using the markers *Xgwm136* (12 cM) and *Xgwm99* (126 cM), but the large distance between the two markers is more indicative of separate introgression events than of the introgression of a single large chromosomal segment.

Compared with the interspecific cross *Triticum macha* × Progress, introgressions from *Triticum spelta* (AABBDD), although fewer in number, were detected at loci mapped to the A, B and D genomes of the backcross line using the markers *Xwmc24-1AS*, *Xgwm408-5BL*, *Xgwm469-6DS* and *Xgwm302-7BL* (Table 12, Figure 4B). Only one marker located in the D genome indicated introgression of a DNA fragment from *Triticum spelta*.

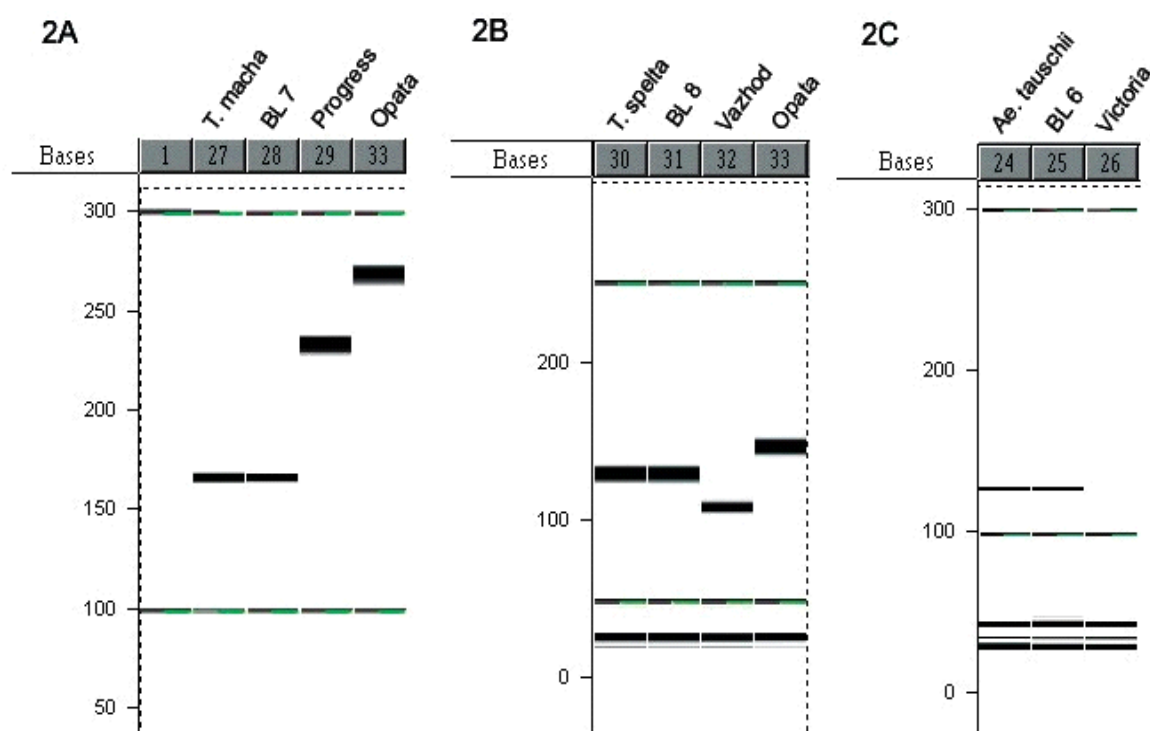


Figure 4. Introgressions of chromosomal segments from distantly related hexaploid species with the AABBDD genome and diploid species with the DD genome into durum wheat: **(A)** from *Triticum macha* into the genome of cultivar Progress, detected with marker *Xgwm282-7AL*; **(B)** from *Triticum spelta* into the genome of cultivar Vazhod, detected with marker *Xwmc24-1AS*; **(C)** from *Aegilops tauschii* into the genome of cultivar Victoria, detected with marker *Xgwm639-5DL*.

3.4.3. Detection of introgressions resulting from crosses of *Triticum durum* with distantly related diploid species possessing DD and UU genomes

Introgression of D-genome segments from the donor parent *Aegilops tauschii* (DD) was detected in the backcross line developed in our study only with the SSR marker *Xgwm639-5DL* (Table 12, Figure 4C). Analysis of the line involving *Aegilops umbellulata* revealed introgression of only one chromatin segment from the wild relative into the backcross line, detected with the primer pair *Xwmc24-1AS* (Figure 5A).

The set of polymorphic SSR markers used in the study of the distantly related diploid species was insufficient to detect a larger number of introgressions from the donor genomes of *Aegilops tauschii* and *Aegilops umbellulata*. Therefore, a larger number of SSR markers is

necessary for a more comprehensive analysis of the genomes of the parental lines involved in the interspecific and intergeneric crosses and for a more accurate assessment of genomic introgressions in the progenies.

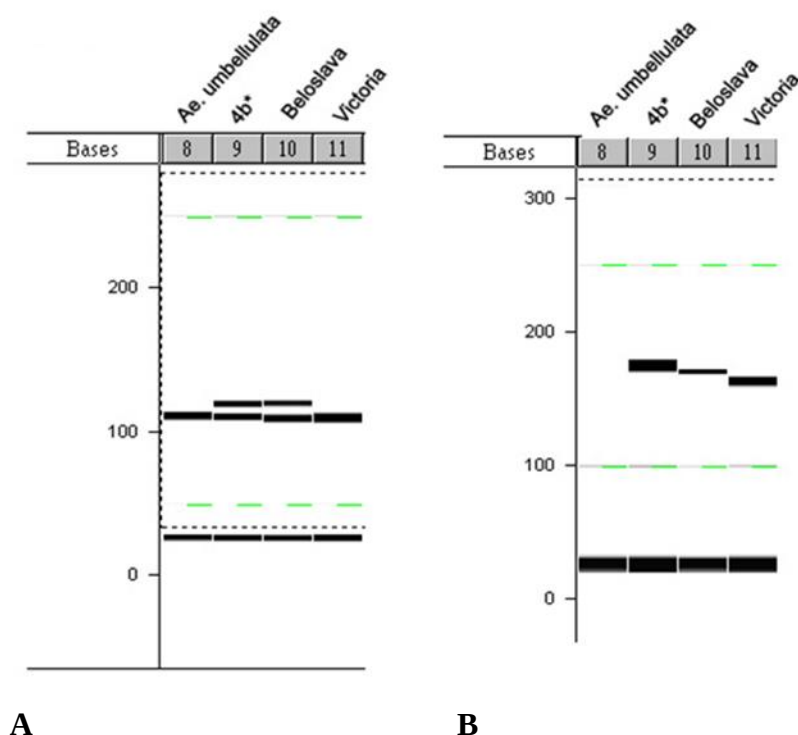


Figure 5A. Introgression of genomic DNA from *Aegilops umbellulata* into cultivars *Beloslava* and *Victoria* at the microsatellite locus **Xwmc24-1AS**. The sizes of the DNA fragments transferred from the wild species into the hybrid were as follows: *Aegilops umbellulata* – 112 bp; **4b*** – 112/120 bp; *Beloslava* – 110/120 bp; *Victoria* – 110 bp.

Figure 5B. Presence of a new allele in hybrid **4b*** from the cross involving *Aegilops umbellulata* and cultivars *Beloslava* and *Victoria* at locus **Xgwm46-7BS**.

3.4.4. Novel alleles in backcross lines

In addition to the detected introgressions, novel alleles were identified in the backcross lines that generally did not correspond to the alleles of either parent. The highest percentages of novel alleles were recorded in the backcross lines from the following hybrid combinations: *Triticum dicoccum* × Progress (43.5 %), *Triticum dicoccoides* × Vazhod (35 %), *Triticum timopheevii* × Vazhod (31.8 %) and *Triticum polonicum* × Progress (31.6 %). The presence of novel alleles is probably an indication of active recombination between the genomes of the distantly related species and the durum wheat cultivars, rather than residual heterozygosity in the parental genotypes.

3.4.5. Detection of introgressions using ISSR markers

Three ISSR markers, (GA)9C, (AC)8G and (CT)9G, were selected on the basis of their widespread use in previous studies for distinguishing wheat cultivars and distantly related species (Zhu et al., 2011). Of the three ISSR primers, two, (GA)9C and (AC)8G, amplified polymorphic fragments in three of the eight distantly related species that were not detected in the durum wheat genome.

The application of ISSR markers in addition to SSR markers enabled the detection of introgressed fragments from several distantly related species. Such introgressed fragments

from *Triticum polonicum* and *Triticum macha* were detected in the genome of cultivar Vazhod. The several introgressed genomic segments from *Triticum timopheevii* (AAGG) observed in the backcross line using the ISSR primer (AC)8G (Figure 6) provide evidence of the usefulness of employing a large set of different markers for comprehensive analysis of the genomes of the parental forms involved in distant hybridization and their subsequent application for molecular characterization of the progenies obtained through backcrossing.

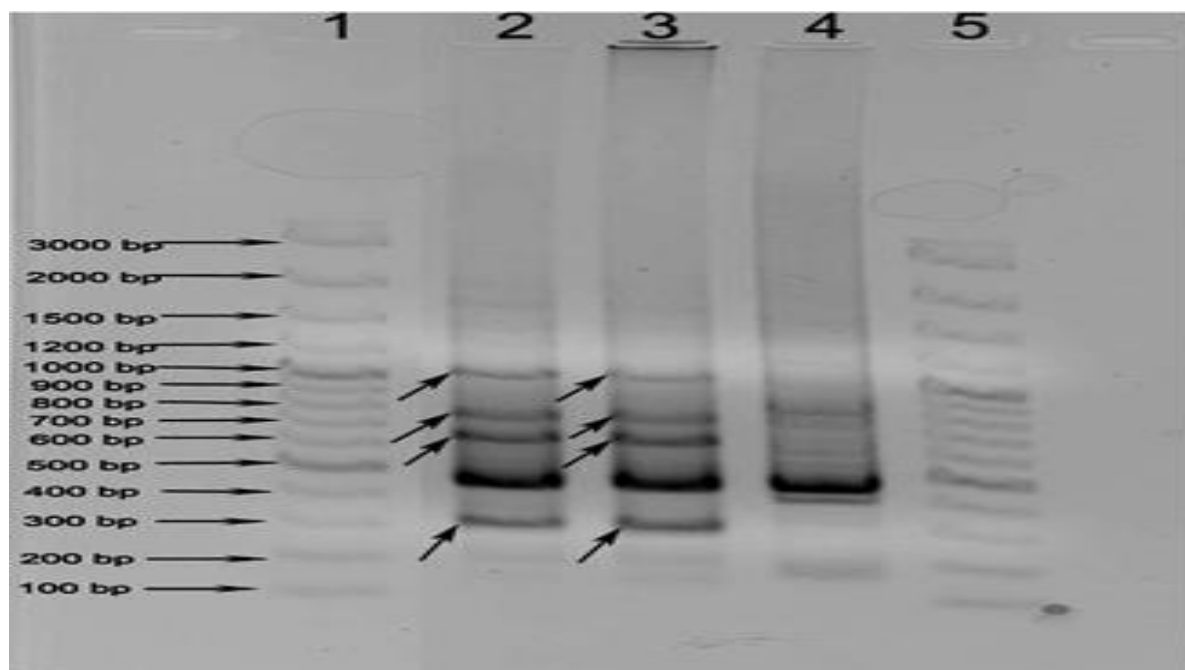


Figure 6. Introgression of four chromosomal segments (300 bp, 600 bp, 750 bp and 1000 bp) from the distantly related tetraploid species *Triticum timopheevii* with the AAGG genome into the genome of cultivar Vazhod, identified using the ISSR marker (AC)8G.

Lane 1: 100 bp ladder; **Lane 2:** *Triticum timopheevii*; **Lane 3:** Backcross line; **Lane 4:** cultivar Vazhod; **Lane 5:** 100 bp ladder.

3.4.6. Detection of introgressions resulting from crosses of *Triticum durum* with the synthetic species *Triticale* possessing the AABBRR genome

The 33 SSR markers used amplified 24 loci in the genome of triticales (*Triticosecale* Wittmack) ($2n = 42$, AABBRR) and the same number of loci in the genome of durum wheat (cultivar Vazhod), used as the recurrent parent in backcrossing of the resulting hybrid (Table 13).

In the backcross line studied, introgression of 10 chromosomal segments derived from the triticales parent was detected. In addition to the identified introgressions, novel alleles that did not correspond to the alleles of either parent were also detected in the backcross line studied. These results confirm the presence of triticales chromatin introgressions in the homologous A and B genomes of durum wheat, as well as recombination between them in the backcross line studied.

Table 13. Amplification of microsatellite loci in the parental forms and introgressions and recombinations of genomic DNA from triticale in the backcross line.

Backcross line Parents	Monomorphic locus	Loci with no PCR amplification in the parent (null loci)	Polymorphic loci	Introgressions	Recombinations
Triticale (AABBRR)	<i>Xgwm 642-1D</i>	<i>Xgwm 484-2D</i>	<i>xw24-1A</i> , <i>xg136-1A</i> , <i>xg99-1A</i> ,	<i>Xwmc 24-1A</i>	<i>Xwmc 327-5A</i>
Backcross line		<i>Xgwm 261-2D</i>	<i>xg268-1B</i> , <i>xg95-2A</i> , <i>xg312-2A</i> ,	<i>Xgwm 136-1A</i>	<i>Xgwm 644 6B</i>
Durum wheat cultivar Vazhod (AABB)		<i>Xgwm 456-3D</i>	<i>xg5-3A</i> , <i>xg285-3B</i> , <i>xg165-4A</i> ,	<i>Xgwm 268-1B</i>	<i>Xgwm 282-7A</i>
		<i>Xgwm 165-4D</i>	<i>xg165-4B</i> , <i>xw327-5A</i> , <i>xg639-5A</i> ,	<i>Xgwm 95-2A</i>	<i>Xgwm 46- 7B</i>
		<i>Xgwm 190-5D</i>	<i>xg639-5B</i> , <i>xg639-5D</i> , <i>xg408-5B</i> ,	<i>Xgwm 5-3A</i>	<i>Xwmc 83-7A</i>
		<i>Xgwm 469-6D</i>	<i>xg234-5B</i> , <i>xg644-6B</i> , <i>xw9-7A</i>	<i>Xgwm 165-4A</i>	<i>Xgwm 312-2A</i>
		<i>Xgwm 325-6D</i>	<i>xw83-7A</i> , <i>xg282-7A</i> , <i>xg233-7A</i> ,	<i>Xgwm 165-4B</i>	<i>Xgwm 400-7B</i>
		<i>Xgwm 437-7D</i>	<i>xg400-7B</i> , <i>xg302-7B</i> , <i>xg46-7B</i>	<i>Xwmc 9-7A</i>	<i>Xwmc 327-5A</i>
		<i>Xgwm 484-2D</i>	<i>xw24-1A</i> , <i>xg136-1A</i> , <i>xg99-1A</i> ,	<i>Xwmc 83-7A</i>	<i>Xgwm 644- 6B</i>
		<i>Xgwm 261-2D</i>	<i>xg268-1B</i> , <i>xg95-2A</i> , <i>xg312-2A</i> ,	<i>Xgwm 400-7B</i>	<i>Xgwm 282-7A</i>
Total	1	8	24	10	7
%			72,72	41,66	29,16

3.5. Phenotypic evaluation of advanced backcross lines obtained from crosses of durum wheat with distantly related species of the genera *Aegilops* and *Triticum*

Eight advanced backcross lines developed through distant hybridization were evaluated for phenological, morphological and agronomic traits together with the parental durum wheat genotypes. All lines studied had confirmed introgressions of genomic segments from distantly related diploid (*Aegilops tauschii*), tetraploid (*Triticum dicoccoides*, *Triticum*

dicoccum, *Triticum polonicum*, *Triticum carthlicum* and *Triticum timopheevii*) and hexaploid (*Triticum macha* and *Triticum spelta*) species and were at different stages of the breeding process (Table 10).

The field experiment conducted in 2011 showed significant variation in the agronomic and grain quality traits of the backcross lines studied. Statistically significant differences from the respective parental durum wheat genotypes were established in both positive and negative directions.

With regard to phenological development, three of the backcross lines headed 3 to 8 days later than the parental cultivars, whereas the line derived from a cross involving *Triticum carthlicum* was characterized by earlier heading than cultivar Victoria. In most lines, plant height varied within a narrower range, and the plants were shorter than the parental cultivars, with the exception of the combination involving *Triticum dicoccoides*, in which the plants exceeded cultivar Vazhod in height (Table 14).

Statistically significant variation was also established in the morphological characteristics of the flag leaf. The backcross lines exhibited a wider range of variation in flag leaf length than the parental cultivars. In some lines, greater leaf length, width and area were recorded, indicating the possibility of positive changes in traits associated with photosynthetic potential (Table 14).

Table 14. Phenotypic evaluation of eight backcross lines with confirmed genomic introgression from distantly related species and their durum wheat parents for traits related to flowering date, plant height and flag leaf parameters.

Backcrosses	Heading date (days after May 1)	Plant height (cm)	Flag leaf parameters (cm)		
			Length	Width	Leaf area
(Gergana × Vazhod)F ₁ × <i>Aegilops tauschii</i> F ₁ × <i>Tr. durum</i> BC ₁ × Victoria	20 ^{c*}	98,6 ^{de}	22,7 ^{bc}	1,78 ^b	33,6
(Vazhod × <i>Triticum dicoccoides</i>)F ₁ × Vazhod	20 ^c	91 ^{cde}	23,2 ^{bcd}	2,11 ^d	35,8
(<i>Triticum dicoccum</i> × <i>Triticum durum</i>)F ₁ × Progress	23 ^e	75 ^{6a}	21,3 ^{ab}	1,98 ^{cd}	32,0
<i>Triticum polonicum</i> × <i>Triticum durum</i> F ₂ × <i>Triticum durum</i> BC ₁ × Progress	20 ^c	94,4 ^{cde}	20,7 ^a	1,94 ^{cd}	30,1
(7383 × <i>Triticum carthlicum</i>)F ₂ × Victoria	19 ^b	80,2 ^{ab}	22,7 ^{ab}	1,92 ^{cd}	32,7
(Vazhod × Victoria)F ₁ × <i>Triticum timopheevii</i> F ₁ × Vazhod	21 ^d	79,2 ^{ab}	22,7 ^{ab}	2,1 ^d	35,1
(<i>Triticum durum</i> × <i>Triticum macha</i>)F ₁ × Progress	26 ^g	86,8 ^{abc}	27,4 ^f	1,98 ^{cd}	40,68
(Iporpec × Beloslava)F ₁ × <i>Triticum spelta</i> F ₁ × Vazhod	24 ^e	80,6 ^{ab}	25,9 ^{ef}	1,63 ^a	31,83
Progress	18 ^a	97,4 ^{de}	23,8 ^{cd}	1,84 ^{bc}	32,96
Vazhod	21 ^d	88,2 ^{bcd}	23,3 ^{bcd}	1,95 ^{cd}	34,14
Victoria	19 ^b	102,6 ^e	19,6 ^a	1,92 ^{cd}	28,33

Note: Within each column, mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Traits related to the spike are of considerable importance for grain yield formation. The backcross lines studied were characterized by shorter spikes than their respective backcross parents, with the exception of the lines involving *Triticum polonicum* and *Triticum macha*. In terms of the number of grains per spike, the introgression lines showed values close to those of the parental durum wheat cultivars, with the lines involving *Triticum polonicum*, *Triticum macha* and *Triticum dicoccum* exceeding cultivar Progress (Table 15).

Grain weight per spike varied within a comparatively narrow range, with the highest value recorded in the line derived from the cross with *Triticum macha*. All lines studied were characterized by lower thousand-kernel weight than the parental durum wheat cultivars (Table 16).

Table 15. Phenotypic evaluation of eight backcross lines with confirmed genomic introgression from distantly related species and their durum wheat parents for traits related to spike parameters.

Backcrosses	Spike parameters			
	Length (cm)	Number of spikelets	Number of grains	Grain weight(g)
(Gergana × Vazhod) F ₁ × <i>Aegilops tauschii</i> F ₁ × <i>Triticum durum</i> BC ₁ × Victoria	7,3 ^{bcd}	19 ^a	27 ^a	1,1 ^a
(Vazhod × <i>Triticum dicoccoides</i>) F ₁ × Vazhod	7,4 ^{bcd}	21,2 ^{ab}	43,6 ^{bc}	1,7 ^{ab}
(<i>Triticum dicoccum</i> × <i>Triticum durum</i>) F ₁ × Progress	6,5 ^d	20,8 ^{ab}	51,4 ^c	2,2 ^{cd}
(<i>Triticum polonicum</i> × <i>Triticum durum</i>) F ₂ × <i>Triticum durum</i> BC ₁ × Progress	8,7 ^{ab}	22 ^{bc}	50,2 ^{bc}	2,0 ^{bcd}
(7383 × <i>Triticum carthlicum</i>) F ₂ × Victoria	7,7 ^{bcd}	19,2 ^a	46,8 ^{bc}	1,96 ^{bcd}
(Vazhod × Victoria) F ₁ × <i>Triticum timopheevii</i> F ₁ × Vazhod	7,8 ^{bcd}	20,8 ^{ab}	43,6 ^{bc}	1,98 ^{bcd}
(<i>Triticum durum</i> × <i>Triticum macha</i>) F ₁ × Progress	8,8 ^{ab}	22 ^{bc}	51 ^c	2,5 ^d
(Progress × Beloslava) F ₁ × <i>Triticum spelta</i> F ₁ × Vazhod	6,8 ^d	18,8 ^a	37,6 ^b	1,7 ^{ab}
Progress	9,5 ^a	20,9 ^{ab}	46,4 ^{bc}	2,34 ^{cd}
Vazhod	8,3 ^{abc}	21,1 ^{ab}	42 ^{bc}	1,98 ^{bcd}
Victoria	7,5 ^{bcd}	21 ^{ab}	40 ^{bc}	1,78 ^{bc}

Note: Within each column, mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Grain quality in durum wheat is determined primarily by protein and gluten content, as well as gluten strength, which are important breeding traits in *Triticum durum*. The backcross lines studied were characterized by higher protein content than the parental durum wheat genotypes. The values ranged from 16.9 % in a line involving *Triticum timopheevii* to

22.2 % in a line involving *Triticum carthlicum*, whereas protein content in the parental cultivars ranged from 14.9 % to 16.5 % (Table 16).

Higher wet and dry gluten content was also established in the backcross lines compared with the durum wheat cultivars used in backcrossing. The lines involving *Triticum dicoccoides*, *Triticum macha* and *Triticum spelta* were characterized by the highest gluten content (Table 16).

Table 16. Phenotypic evaluation of eight backcross lines with confirmed genomic introgression from distantly related species and their durum wheat parents for traits related to thousand-kernel weight and protein and gluten content.

Backcrosses	1000 grain weight	Protein content (%)	Gluten content (%)	
			Wet	Dry
(Gergana × Vazhod)F ₁ × <i>Aegilops tauschii</i> F ₁ × <i>Triticum durum</i> BC ₁ × Victoria	40,7 ^a	16,86 ^d	40,4	13,8
(Vazhod × <i>Triticum dicoccoides</i>)F ₁ × Vazhod	39 ^a	19,91 ^g	44	15,2
(<i>Triticum dicoccum</i> × <i>Triticum durum</i>)F ₁ × Progress	42,8 ^{ab}	19,43 ^f	42,5	14,4
<i>Triticum polonicum</i> × <i>Triticum durum</i> F ₂ × <i>Triticum durum</i> BC ₁ × Progress	40 ^a	18,14 ^e	39,5	14
(7383 × <i>Triticum carthlicum</i>)F ₂ × Victoria	42,7 ^{ab}	22,16 ^g	41,4	14,1
(Vazhod × Victoria)F ₁ × <i>Triticum timopheevii</i> F ₁ × ВЪЗХОД	45,9 ^{abc}	16,86 ^d	34,8	13,2
(<i>Triticum durum</i> × <i>Triticum macha</i>)F ₁ × Progress	49,01 ^{cd}	19,27 ^f	43,1	15
(Progress × Beloslava)F ₁ × <i>Triticum spelta</i> F ₁ × Vazhod	45,3 ^{abc}	20,07 ^g	46,9	16,3
Progress	51,9 ^{cd}	15,9 ^b	35,6	13,3
Vazhod	45,5 ^{abc}	16,54 ^c	34	12,2
Victoria	47,3 ^{bcd}	14,93 ^a	31,2	11,1

Note: Within each column, mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

The lines derived from crosses of *Triticum dicoccum*, *Triticum polonicum* and *Triticum macha* with the recurrent parent, cultivar Progress, were characterized by the best combination of traits related to spike productivity and grain quality. It should be noted that these lines also showed the highest level of introgressions from the donor genomes of the above-mentioned distantly related species, as established by molecular analyses.

The backcross lines did not differ substantially in a negative direction from the durum wheat cultivars, owing to the repeated negative phenotypic selection against unfavorable agronomic traits originating from the alien parents.

3.6. Evaluation of yield and quality in advanced durum wheat breeding lines obtained through distant hybridization with species of the genera *Triticum* and *Aegilops*

To determine the breeding value of advanced durum wheat lines obtained through distant hybridization with species of the genera *Triticum* and *Aegilops*, a comparative field trial was conducted.

Six breeding lines were included in a comparative variety trial over three consecutive years, 2020–2022 (Table 17). The breeding material was developed through hybridization of *Triticum durum* with the distantly related species *Triticum polonicum*, *Triticum dicoccum*, *Aegilops tauschii* and *Aegilops kotschy*. The lines were evaluated for traits related to grain yield and quality, using the durum wheat cultivar Predel as the standard.

Table 17. Origin and breeding stage of advanced durum wheat lines obtained through interspecific and intergeneric hybridization.

Line No.	Crosses	Year of development	Breeding stage (2020/2021/2022)
DV-8357	<i>Triticum polonicum</i> × <i>Triticum durum</i>	2010	F ₁₁₋₁₃
DV-8358	<i>Triticum polonicum</i> × <i>Triticum durum</i>	2011	F ₁₀₋₁₂
D-8572	<i>Triticum polonicum</i> × <i>Triticum durum</i>	2011	F ₁₀₋₁₂
DV-8360	<i>Triticum durum</i> (7208) × <i>Aegilops kotschy</i>	2011	F ₁₀₋₁₂
DV-8417	(<i>Triticum durum</i> × <i>Aegilops tauschii</i>)F ₂ × <i>Triticum durum</i> BC ₁ F ₁) × <i>Tr.durum</i>	2013	BC ₂ F ₃₋₅
D-232	(Victoria × <i>Triticum dicoccum</i>)F ₃ × Victoria)BC ₁ F ₁ × Predel	2013	BC ₂ F ₂₋₄

The analysis of variance showed a significant effect of genotype, growing year and the interaction between the two factors on the variation of almost all traits studied (Table 18). The variation in SDS sedimentation value (97.34 %), vitreousness (85.55 %), yellow index b* (77.85 %), grain protein content (50.08 %), grain yield (44.24 %) and thousand-kernel weight (36.50 %) was attributable to the greatest extent to genotype.

The growing year had a significant effect on the variation in plant height (59.74 %) and thousand-kernel weight (36.31 %). Despite the greater contribution of genotype to yield variation, the environment (39.61 %) and genotype × environment interaction (15.17 %) also contributed to it. A number of authors have associated the considerable variation in this trait with the influence of environmental factors (Chairi et al., 2020; Evlice et al., 2023). A significantly greater effect of genotype on the variation in SDS sedimentation value and yellow pigment content has been reported by many researchers (Martini et al., 2015; Evlice et al., 2023). The genotype × environment interaction contributed to the greatest extent to the variation in wet gluten content (52.94 %) and test weight (63.93 %).

Table 19 presents the results for grain yield and plant height by year and as mean values. Averaged over the three years of the study, two of the analyzed lines exceeded the yield of the standard cultivar Predel (636 kg/da). Line DV-8417 originated from distant hybridization with *Aegilops tauschii*, and line DV-8360 from hybridization with *Aegilops kotschy*. The good yield potential of line DV-8417 may be associated with the transfer of genomic segments from the wild parent, which has been reported in the literature as a source of genes for increased biomass, grain yield and resistance to abiotic stresses. The results reported in Section 3.4 provide evidence of introgressions and the occurrence of novel alleles in hybridization between durum wheat and *Aegilops tauschii*, which may play an important role in the breeding improvement of durum wheat.

Averaged over the three years of the study, line DV-8417 was characterized by the greatest plant height (105 cm), while line D-8572 had the lowest (88.7 cm). Three of the lines, D-8572, DV-8360 (89.3 cm) and D-232 (90.3 cm), were shorter than the standard cultivar Predel (94 cm).

Table 18. Analysis of variance of selected traits in advanced durum wheat lines obtained through interspecific and intergeneric hybridization.

Traits	Source of variation, % of total variation								
	Genotype			Year			Interaction		
	SS	MS	η^2 %	SS	MS	η^2 %	SS	MS	η^2 %
Grain yield	166945	27824 ***	44,2	149473	74736 ***	39,61	57234	4769 ***	15,1
Plant height	1774,8	295,8 ***	28,4	3732	1866 ***	59,74	695,5	58 ***	11,1
1000 grain weight	219,6	36,6 ***	36,5	218,5	109,3 ***	36,31	163,2	13,6 ***	27,1
Protein content	23,9	3,98 ***	50,0	7,65	3,82 ***	16,03	15,63	1,3 ***	32,7
Wet gluten content	107,04	17,84 ***	35,1	36,01	18,00 ***	11,81	161,5	13,45 ***	52,9
SDS sedimentation value	5498,9	916,48 ***	97,3	56	28,00 ***	0,99	52	4,33 ***	0,9
Yellow index b*	32,75	5,46 ***	77,8	5,82	2,91 ***	13,83	3,5	0,29 ***	8,3
Vitreousness	532,9	88,8 ***	85,5	43,7	21,9 ***	7,02	45,9	3,8 ***	7,3
Test weight	26,5	4,4 ***	32,5	0,2	0,1ns	0,25	52,1	4,3 ***	63,9

*** Significant at $p < 0.001$, ** Significant at $p < 0.01$, * Significant at $p < 0.05$ ns – not significant

Table 19. Mean values of grain yield and plant height in advanced durum wheat breeding lines obtained through interspecific and intergeneric hybridization.

Genotype	Grain yield, kg/da				Plant height, cm			
	2020	2021	2022	Mean	2020	2021	2022	Mean
DV-8357	538,1	524,2	455,3	505,9	93	102	92	95,7
DV-8358	587,74	648,1	514,6	583,48	89	108	90	95,7
D-8572	668,5	651,8	566,1	628,8	85	102	79	88,7
DV-8360	654,3	729,4	634,2	672,6	92	99	77	89,3
DV-8417	666,28	666,28	605,7	646,1	107	115	94	105,3
D-232	653,4	742,1	470,8	622,1	85	102	84	90,3
Predel	660,7	679,8	567,5	636	99	101	82	94,0

The results for traits related to the milling quality of the breeding materials studied are presented in Table 20. Test weight is a major indicator of milling quality in durum wheat. Desirable values for grain test weight are above 77–78 kg/hl. Averaged over the three years of the study, the analyzed lines were characterized by good values for this trait. The lowest test weight was recorded for line D-232 (76.1 kg/hl), and the highest for line D-8572 (78.2 kg/hl), which slightly exceeded the standard cultivar Predel (78.0 kg/hl).

All lines studied were characterized by medium-sized grains. Averaged over the three years, line DV-8358 had the largest grains (46.5 g) and exceeded the standard cultivar Predel (45.9 g) for this trait. Line D-8572 (45.7 g) was also characterized by large grains over the study period. Both lines originated from *Triticum polonicum* × *Triticum durum*. Vitreousness is a major indicator of the structural and mechanical properties of durum wheat grain. High-quality grain is highly vitreous, with vitreousness above 75–80 %. All lines studied were characterized by high vitreousness. Two of the analyzed lines exceeded the standard cultivar Predel (90.7 %): D-8572 (96.6 %) and D-232 (92.2 %).

Table 20. Mean values of test weight, 1000 grain weight and vitreousness in advanced durum wheat lines obtained through interspecific and intergeneric hybridization.

Geno type	Test weight				1000 grain weight				Vitreousness			
	2020	2021	2022	Mean	2020	2021	2022	Mean	2020	2021	2022	Mean
DV-8357	77,9	77,5	78,0	77,8	41,4	44,8	42,1	42,8	89,9	87,8	88,2	88,6
DV-8358	77,8	77,6	78,0	77,8	44,6	48,4	46,5	46,5	90,2	88,1	89,3	89,2
D-8572	79,1	77,4	78,0	78,2	45,2	47,2	44,6	45,7	96,3	94,8	98,8	96,6
DV-8360	75,9	78,2	78,5	77,5	39,4	46,6	45,2	43,7	88,2	87,3	86,4	87,3
DV-8417	76,4	77,4	78,5	77,4	38,4	42,6	41,6	40,9	89,6	88,2	88,4	88,7
D-232	77,2	77,4	73,7	76,1	43,4	48,0	38,4	43,3	92,4	90,1	94,1	92,2
Pre del	78,2	77,8	78,0	78,0	41,8	48,2	47,6	45,9	92,6	89,2	90,3	90,7

The results for grain protein and wet gluten content in the durum wheat lines studied, by year and as mean values, are presented in Table 21. According to studies by many authors, durum wheat possesses better agronomic characteristics and protein quality than other tetraploid wheats, but has a lower protein content (Ruan et al., 2012; Cabas-Lühmann et al., 2023). All lines analyzed in this study were characterized by good grain protein and wet gluten content. Three of the lines studied showed higher mean values for these traits over the three years of the study compared with the standard cultivar Predel (14.9 % protein and 30.9 % wet gluten, on a dry matter basis). These were lines D-232 (15.7 % protein and 31.9 % wet gluten, respectively), DV-8360 (15.1 % and 31.3 %, respectively) and DV-8417 (14.8 % and 31.3 %, respectively).

The main traits related to durum wheat grain quality are yellow pigment content and gluten strength. The results for yellow pigment content and SDS sedimentation value are presented in Table 22. Line D-232 was characterized by a higher yellow index b* and, correspondingly, higher yellow pigment content than the standard cultivar Predel (17.29 b*), averaged over the three years of the study (18.09 b*). However, the grain of *Triticum*

dicoccum has been found to have lower β -carotene content than traditional wheat cultivars (Kuznetsova et al., 2019). Two of the lines, D-8572 and DV-8357, showed a yellow index b^* close to that of the standard cultivar.

Table 21. Mean values of grain protein and wet gluten content in advanced durum wheat lines obtained through interspecific and intergeneric hybridization.

Genotype	Protein content, %				Wet gluten content, %			
	2020	2021	2022	Mean	2020	2021	2022	Mean
DV-8357	13,8	14,0	13,9	13,9	27,4	28,2	28,0	27,9
DV-8358	14,1	14,4	14,2	14,2	28,6	34,1	28,8	30,5
D-8572	12,3	14,1	14,8	13,7	24,8	31,5	31,0	29,1
DV-8360	15,1	14,5	15,1	14,9	31,4	31,9	30,6	31,3
DV-8417	15,1	14,1	15,1	14,8	31,2	30,8	32,0	31,3
D-232	15,6	14,8	16,6	15,7	31,4	29,6	34,6	31,9
Predel	14,9	13,9	15,8	14,9	30,5	30,2	32,1	30,9

Genotypes with good gluten quality are characterized by SDS sedimentation values above 30–40 cm³, while durum wheat genotypes with values above 40 cm³ are characterized by excellent quality and strong gluten (Wang et al., 2017). High SDS sedimentation values and, consequently, strong gluten were recorded, averaged over the three years of this study, for the standard cultivar Predel (43 cm³) and line D-232 (35.3 cm³). High SDS sedimentation values in *Triticum dicoccum* were reported by Petrenko et al. (2018).

Table 22. Mean values of grain yellow pigment content and SDS sedimentation value in advanced durum wheat lines obtained through interspecific and intergeneric hybridization.

Genotype	Yellow index b^*				SDS, cm ³			
	2020	2021	2022	Mean	2020	2021 a	2022	Mean
DV-8357	17,31	16,62	17,12	17,02	18	20	18	18,7
DV-8358	16,72	16,37	16,34	16,48	17	18	20	18,3
D-8572	18,10	16,32	17,21	17,21	16	18	18	17,3
DV-8360	16,40	15,84	16,12	16,12	17	20	18	18,3
DV-8417	15,94	15,68	15,81	15,81	16	18	18	17,3
D-232	18,44	17,32	18,52	18,09	36	35	35	35,3
Predel	17,53	17,11	17,23	17,29	42	45	42	43

To evaluate the γ -gliadin composition, A-PAGE electrophoresis was performed on the lines with the highest yield, DV-8360 and DV-8417, as well as on line D-232, characterized by the highest SDS sedimentation value. Two γ -gliadin fractions encoded by the *Gli-B1* locus were identified in the genotypes studied.

In line D-232, the γ -45 gliadin fraction, favorable for gluten quality and associated with the LMW-2 type, was identified (Figure 7), whereas in lines DV-8360 and DV-8417, the γ -42 fraction, associated with lower gluten quality and the LMW-1 type, was detected. The presence of γ -45 in line D-232 probably originates from cultivar Predel or from the wild parent *Triticum dicoccum*, in which this fraction has been identified in previous studies.

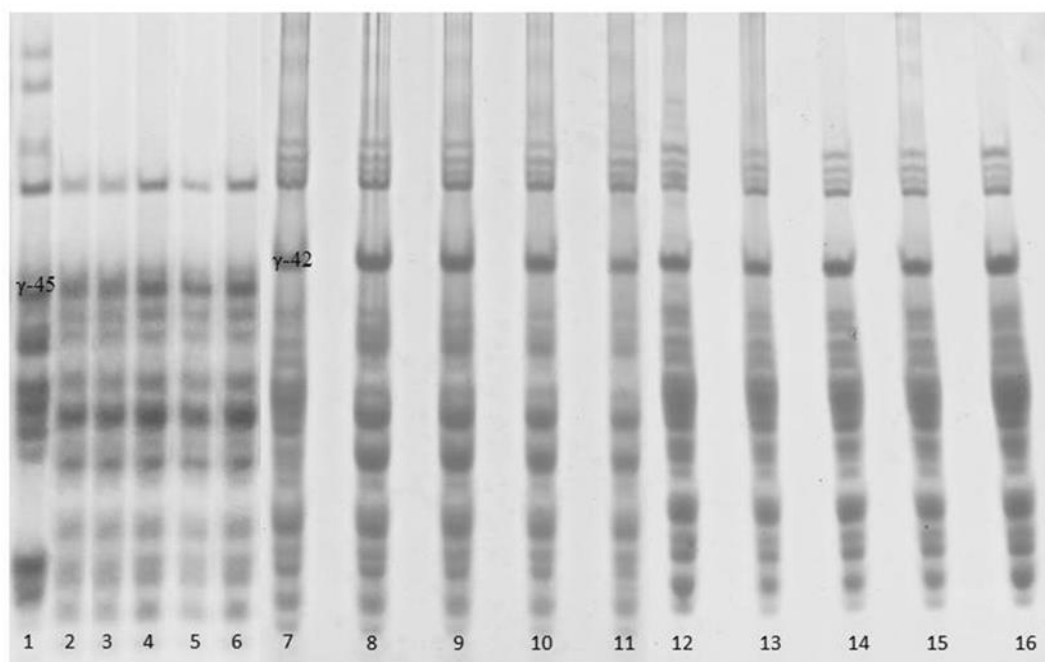


Figure 7. A-PAGE of gliadins: 1. Agridur (standard); 2–6. D-232; 7–11. DV-8360; 12–16. DV-8417.

4. CONCLUSIONS

1. Durum wheat exhibits low crossability with genetically distant species, with higher crossability observed in species possessing homologous genomes. The degree of crossability depends on the ploidy level and the number of homologous genomes, regardless of the presence of a common pivotal genome.
2. The existence of evolutionary genetic relatedness between non-homologous chromosomes accounts for the occurrence of crossability, albeit at a minimal level, in hybridization of durum wheat with species of the genus *Aegilops*.
3. Genotypic differences in crossability attributable to the female parent, *Triticum durum*, were established in the hybrid combinations studied.
4. The modified MS medium containing abscisic acid provides a higher regeneration potential for immature embryos. The better result is most likely associated with the combined effect of the mineral and organic composition of the medium and the inhibitory effect of ABA on the precocious germination of undifferentiated embryos.
5. In interspecific and intergeneric hybrids of durum wheat with species of the genera *Triticum* and *Aegilops* and the synthetic species *Triticale*, abnormalities in endosperm development were observed depending on the genetic distance between the species used.
6. The optimal period for embryo isolation for *in vitro* rescue is between the 16th and 20th day after pollination, when the embryo is at the differentiation or differentiated embryo stage.
7. The percentage of regenerated plants varies considerably both among individual hybrid combinations and within the same combination when different durum wheat

genotypes are used. No direct relationship was established between regeneration potential and the degree of crossability of the parental forms.

8. In distant crosses, hybrid fertility varies from complete sterility to full fertility. The initially low fertility of F₁ hybrids can be partially overcome through backcrossing.
9. Morphological analyses of F₁ interspecific hybrids show that hybridization between durum wheat and species of the genus *Aegilops* results in a combination of traits from both parents, with most morphological traits showing intermediate values.
10. Complete male and female sterility was established in F₁ plants from hybrids with *Aegilops cylindrica*. Male sterility was associated with the observed abnormalities in microgametogenesis, and female sterility with the absence of seed formation after backcrossing with durum wheat.
11. The cytological abnormalities observed in our study, suggesting the formation of unreduced gametes and possible chromosomal recombination in the cross with *Aegilops umbellulata*, together with the partial restoration of fertility after backcrossing, indicate a certain degree of genetic compatibility between the cultivated and wild species and the possibility of introgression of genetic material from the wild species into the durum wheat genome.
12. SSR markers are suitable for identifying polymorphism arising as a result of introgressions during hybridization of modern durum wheat cultivars with related species of the family Gramineae.
13. The highest level of introgressions was established in cultivar Progress following crosses with *Triticum polonicum* and *Triticum dicoccum*, as well as in cultivars Vazhod and Progress following crosses with *Triticale* and *Triticum macha*. The identified chromosomal introgressions and replacements, including the nearly complete replacement of chromosome 7A in hybridization with *Triticum macha*, indicate that genomic interactions have occurred in the hybrid lines.
14. The use of ISSR markers enables the detection of additional introgressed fragments from distantly related species in the durum wheat genome that could not be identified with the SSR markers used.
15. The backcross lines studied are characterized by improved agronomic and quality traits, including better grain set per spike and higher grain protein and gluten content.
16. The backcross lines obtained from crosses of *Triticum dicoccum*, *Triticum polonicum* and *Triticum macha* with cultivar Progress possess the best combination of agronomic traits related to spike productivity and grain quality.
17. The backcrossing strategy applied is effective for transferring donor alleles from alien species into elite durum wheat cultivars without substantially reducing their valuable agronomic characteristics.
18. Durum wheat breeding lines developed through distant hybridization and a backcrossing strategy and included in comparative variety trials outperform the standard cultivar Predel in agronomic traits.
19. The highest yields were recorded in lines involving *Aegilops kotschyi* and *Aegilops tauschii*, whereas lines derived from crosses with *Triticum polonicum* showed greater grain weight. The line obtained through hybridization with *Triticum dicoccum* was characterized by large grains, high protein content and strong gluten.

5. CONTRIBUTIONS

Scientific and theoretical contributions

1. Information was obtained on the crossability of durum wheat with a large number of distantly related and related species of the genera *Aegilops* and *Triticum* and *Triticosecale*, and on the possibility of obtaining F₁ hybrid plants through the application of *in vitro* embryo rescue.
2. The main stages of the *in vitro* embryo rescue procedure were optimized to overcome embryo incompatibility in distant hybridization of durum wheat.
3. Serious abnormalities in microsporogenesis were established, which account for the partial or complete sterility of hybrid plants obtained from crosses of durum wheat with *Aegilops umbellulata* and *Aegilops cylindrica*.
4. The applicability and effectiveness of SSR and ISSR markers in distant hybridization were demonstrated for detecting polymorphism and analyzing introgressions, thereby confirming the hybrid nature of the developed backcross lines.
5. The backcrossing strategy applied was shown to be an effective tool for introducing novel alleles from distantly related species without deterioration of the main agronomic characteristics of durum wheat.

Scientific and applied contributions

1. Protocols for *in vitro* embryo rescue in durum wheat were developed.
2. Durum wheat breeding lines were developed through distant hybridization and a backcrossing strategy and are currently undergoing comparative variety trials, thereby enriching genetic diversity for important agronomic traits.
3. Backcross lines derived from crosses with *Triticum dicoccum*, *Triticum polonicum* and *Triticum macha* were identified as possessing the best combination of traits related to spike productivity and grain quality, with potential for release as new cultivars.

Scientific publications related to the dissertation

1. Hadzhiivanova B., Bozhanova V., 2010. Obtaining interspecific hybrids from crosses of durum wheat with the wild species *Aegilops cylindrica* (Host.). Field Crops Studies, vol. VI (3): 355–360. (In Bulgarian).
2. Bozhanova V., Todorovska E., Hadzhiivanova B., Dechev D., 2014. Obtaining of interspecific hybrids between durum wheat (2n = 28) and triticale (2n = 42) and molecular evidence of alien introgressions in advanced backcross line. Turkish J Agric Nat Sci., 1: 868–874 (Special Issue).
3. Hadzhiivanova B., Taneva K., Bozhanova V., Dragov R., 2025. Evaluation of yield and quality of advanced durum wheat lines obtained by the distant hybridization method. Bulgarian Journal of Crop Science, 62(1): 3–16 (In Bulgarian).

Studies on the use of distant hybridization in durum wheat breeding (*Triticum turgidum* L. subsp. *durum* (Desf.))

Summary of PhD Thesis Boryana Hadzhiivanova

The main objective of the study is the use of distant hybridization as an approach for expanding the genetic diversity of durum wheat (*Triticum durum* Desf.) through the incorporation of genetic material from species of the genera *Triticum*, *Aegilops* and the synthetic species *Triticale*. The crossability and compatibility between different varieties and breeding lines of durum wheat and distant species were investigated, as well as the possibilities for overcoming embryo incompatibility through in vitro embryo rescue, the development of F₁ hybrids and subsequent backcross generations, and the presence of introgressions in advanced breeding lines.

It was established that the degree of crossability depends on the genetic relatedness between the parental forms, the ploidy level, the number of homologous genomes and the genotype of durum wheat used as the female parent. The highest crossability was observed in hybridization with species of the genus *Triticum*, followed by the synthetic species *Triticale*, while the lowest crossability was recorded with species of the genus *Aegilops*. Nevertheless, successful formation of ovaries and hybrid plants prove the possibility of using these distant species as a source of valuable genetic variability.

The embryo rescue method was optimized, with the modified MS nutrient medium supplemented with abscisic acid showing a higher regeneration potential compared to the P8 medium. The morphological and cytological analyses of the F₁ hybrids revealed an intermediate phenotype and different degrees of fertility depending on the hybrid combination. Complete sterility was established in the hybrids with *Aegilops cylindrica*, whereas in *Aegilops umbellulata* partial fertility and subsequent backcrossing allowed the development of further generations. The observed meiotic abnormalities, the formation of micronuclei and possible unreduced gametes indicate the complex nature of genome interactions in distant hybridization.

Using SSR and ISSR markers, introgressions from the distant species into the durum wheat genome were detected. Introgressed chromosomal segments from *Triticum dicoccum*, *Triticum polonicum*, *Triticum macha*, *Triticum spelta*, *Aegilops tauschii*, *Aegilops umbellulata* and *Triticale* were identified. Of particular importance is the established almost complete substitution of chromosome 7A in a line involving *Triticum macha*, as well as the presence of newly emerged alleles, which indicate active genome interactions and recombination processes.

The evaluation of the advanced breeding lines shows that distant hybridization, combined with a backcross strategy and phenotypic selection, is an effective approach for developing durum wheat lines with improved agronomic and quality traits. Some of the studied lines exceeded the standard variety Predel in grain yield, protein content, wet gluten content or other traits related to grain quality. The obtained results demonstrate the scientific and applied value of distant hybridization as a method for enriching the genetic base and developing promising breeding material in durum wheat.