Article

The use of low-density SNP array for identification of interspecific introgressions of genetic material from the bread wheat into homologous chromosomes of the durum wheat

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Abstract: The hexaploid bread wheat *Triticum aestivum* L. (2n = *6x* = 42) and the tetraploid durum wheat *Triticum durum* (2n = *4x* = 28) are two commercially the most important wheat species worldwide. These two species are components of the same gene pool, which evolved under different conditions, which made it possible to acquire different adaptations to a wider range of environmental conditions. Identification and characterization of introgressed fragments of one species in the genome of another can help in the search for new loci responsible for economically valuable traits. SNP microarrays provide information on the genotypes of thousands of positions throughout the genome. This information can be used to obtain the distribution of alleles of one species in the genome of another and to determine the location of introgressed fragments. In our work, we used genotyping data from the SNP microarray to identify durum wheat chromosome fragments containing bread wheat alleles in the offspring from crossing bread and durum wheat. We have studied the distribution of the genetic material of bread wheat in 15 combinations from crosses of bread and durum wheat. Introgressed fragments of bread wheat into the durum wheat genome were identified on chromosomes 1A, 1B, 4A, 5B, 6B, and 7A. The introgressed fragments showed polymorphism in different breeding lines. A functional enrichment analysis of genes in the introgression fragments was performed, which showed the presence of statistically significant enrichment of genes responsible for various molecular functions, biological processes and cellular components. The obtained data can be used to identify new QTL of economically valuable traits for obtaining durum wheat varieties with improved agronomic traits.

Keywords: bread wheat; durum wheat; introgression; homologous chromosomes; SNP microarray; functional enrichment

1. Introduction

The two main wheat species, the hexaploid bread wheat *Triticum aestivum* L. (2n = *6x* = 42) and the tetraploid durum wheat *Triticum durum* (2n = *4x* = 28), are commercially the most important wheat species worldwide. Almost 95% of cultivated wheat is hexaploid bread wheat, while only 5% is tetraploid durum wheat [1]. Hexaploid wheat has three diploid sets of seven chromosomes belonging to the A-, B- and D-genomes (AABBDD), while tetraploid wheat has only two diploid sets of seven chromosomes belonging to the A- and B-genomes (AABB). Such a combination in one organism of genomes of different species that have evolved in different conditions and adapted to them gives these species the potential for adaptation to a wider range of environmental conditions. A constant flow of genes between tetraploid and hexaploid wheat species occurred during evolution [2].

T. aestivum and *T. durum* are closely related species with potentially different adaptive abilities and only a few different technological properties, but they represent two components of the same gene pool. Breeders can take advantage of interspecific crossbreeding to create wheat varieties that combine pasta or bread quality traits with adaptability, using not only tetraploid, hexaploid, and synthetic hexaploid lines, but also using lines with introgressions from *T. aestivum* to *T. durum* or *vice versa* [1]. Despite the fact that the main

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difference between bread and durum wheat is the presence of the D genome, and many works are aimed at transferring genes from the D genome of bread wheat to the A and B genomes of durum wheat [3–5], many other genes were transferred from the A and B genomes of hexaploid to tetraploid wheat. The introgression of alleles from the Vernalization locus from hexaploid wheat made it possible to make tetraploid wheat more resistant to cold conditions [6]. Several Fusarium head blight resistance loci have been transferred from bread to durum wheat, making durum wheat more resistant to these diseases [7]. The resistance of durum wheat to preharvest sprouting was increased by the transfer of a large QTL from chromosome 3B of bread wheat [8]. Durum wheat with improved tolerance to elevated concentrations of Al^{3+} ions in acidic soils was obtained by introgression of two genes from bread wheat [9].

The development of SNP microarrays makes it possible to simultaneously genotype thousands of variants in genomes. Today, SNP microarrays of various densities have been developed for many plant species [10–15] and have become a powerful tool in genetic research [16]. The first SNP microarray for wheat was developed using Illumina iSelect technology and contained 9,000 SNPs [17]. The same technology was used a year later to create an array that included 90,000 SNPs [18], which was subsequently used to generate a selection-oriented SNP microarray containing 15,000 variants [19]. The Affymetrix Axiom 820K SNP is the largest SNP microarray for bread wheat to date [10]. Subsequently, it was also used to obtain the 35K Axiom^{TM} Wheat Breeder's Genotyping Array SNP microarray for use on elite bread wheat lines [20]. SNP microarrays for bread wheat can be used for durum wheat genotyping [21]. Although more than 8,000 years of independent D-genome evolution separate the A and B genomes of *T. aestivum* and *T. durum*, overall sequence similarity remains a common feature [1]. For example, the SNPs contained in the 90K iSelect Infinium microarray for bread wheat [18] were fully informative when tested in a large panel of elite durum wheat varieties [22]. Nearly 90% of the SNPs located on chromosome 3B of the durum wheat consensus map [23] had significant agreement and collinearity with the bread wheat pseudomolecule 3B sequence [24].

In this work, we analyzed a number of breeding lines derived from a cross between *T. aestivum* × *T. durum* and (*T. aestivum* × *T. durum*) × *T. durum* using a low-density SNP microarray developed for the bread wheat. The amount of genetic material in the homologous chromosomes of the offspring originating from *T. aestivum* and *T. durum* in the offspring was estimated. Fragments of introgression between homologous chromosomes of *T. aestivum* in *T. durum* were revealed. The chromosomal distribution of SNPs and from parental lines in the offspring was analyzed and a functional enrichment analysis of genes in the regions of introgressed fragments enriched with bread wheat alleles was carried out.

2. Results

2.1. The total proportion of the genome of the offspring with the genetic material of the parents

Analysis of homozygous SNPs shows that for most SNPs (about 80%) it is impossible to determine the origin in their offspring due to the identity of their allelic state in the parents (Table 1). For only about 20% of homozygous SNPs the parent of origin (bread wheat or durum wheat) can be clearly defined by their allelic state. The studied offspring lines showed a different degree of saturation with the genetic material of bread wheat. Breeding lines 4528h1 and 4529h25 contained the minimum proportion of SNPs from bread wheat (2.41%), while breeding line 4686h35 contained almost three times higher (6.34%) proportion of SNPs from bread wheat parent. The same breeding line 4686h35 had the fewest portion of SNPs that originate from durum wheat parent (13.42%), and the proportion of variants with undefined origin was almost the maximum among all the analyzed triplets (80.23%).

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Table 1. Summary information about SNPs of different origin in combination analyzed in this work.

Parent 1 (TA[1])	Parent 2 (TD[2])	Offspring (TD)	Total SNPs in analysis	SNPs from bread wheat in offspring	SNPs from durum wheat in offspring	SNPs of undefined origin in offspring
Zhong Pin 1583	Alyona	4135h7	13148	687 (5.23%)	2188 (16.64%)	10273 (78.13%)
Zhong Pin 1583	Agat donskoy	4135h7	13069	648 (4.96%)	1990 (15.23%)	10431 (79.81%)
Zhiva	Cristella	4306h15	12189	732 (6.01%)	2060 (16.90%)	9397 (77.09%)
Alekseich	Cristella	4320h38	9452	558 (5.90%)	1530 (16.19%)	7364 (77.91%)
Sila	Amazonka	3903h9-17-3	12664	469 (3.70%)	2255 (17.81%)	9940 (78.49%)
Vostorg	Amazonka	3902h3-18-31	12643	647 (5.12%)	2224 (17.59%)	9772 (77.29%)
Markiz	Laska	4521h13	11917	574 (4.82%)	1973 (16.56%)	9370 (78.63%)
Rigi	Lazurit	4686h35	10594	672 (6.34%)	1422 (13.42%)	8500 (80.23%)
Rigi	Crucha	4686h35	10542	602 (5.71%)	1453 (13.78%)	8487 (80.51%)
Rigi	Krupinka	4528h1	11096	267 (2.41%)	2076 (18.71%)	8753 (78.88%)
Rigi	Lazurit	4529h25	10764	259 (2.41%)	1940 (18.02%)	8565 (79.57%)
Grom	Kordon	4646h1	10285	461 (4.48%)	1638 (15.93%)	8186 (79.59%)
Bezostaya 100	Kurant	4675h11	11626	623 (5.36%)	1911 (16.44%)	9092 (78.20%)
Ayvina	Kurant	4518h20	12768	495 (3.88%)	2406 (18.84%)	9867 (77.28%)
Vostorg	Amazonka	3902h3-18-3	12633	483 (3.82%)	2380 (18.84%)	9770 (77.34%)

1 - *Trticum aestivum*

2 - *Trticum durum*

2.2. Distribution of SNPs from bread wheat across chromosomes of offspring

We studied the distribution of alleles of different origin for each chromosome in triplets (Supplementary Tables 1-15). For all chromosomes, the same trend is observed - variants whose allele origin cannot be accurately identified are always several times higher than variants that are polymorphic in parents (Figure 1). The proportion of the shares of unidentified variants in the analyzed combinations are in the range from 70 to 90%. Similar numbers were obtained earlier in works about the correspondence between chromosomes of the A- and B-genomes of bread and durum wheat [22,24]. Among all the studied combinations, chromosome 7A has the largest range of variation of the proportion of wheat alleles and the smallest median value of the proportion of alleles whose origin in the offspring cannot be identified. Chromosome 2A, in contrast, has the highest median proportion of alleles of undefined origin. Chromosome 1B has the largest range of variation in the proportion of durum wheat alleles and alleles of unidentified origin compared to all other chromosomes, however, the range of variation in the proportion of bread wheat alleles is alomost the same as other chromosomes. This may indicate that 1B chromosomes are more polymorphic in the analyzed combinations and often contain different numbers of heterozygous and miscalled variants.

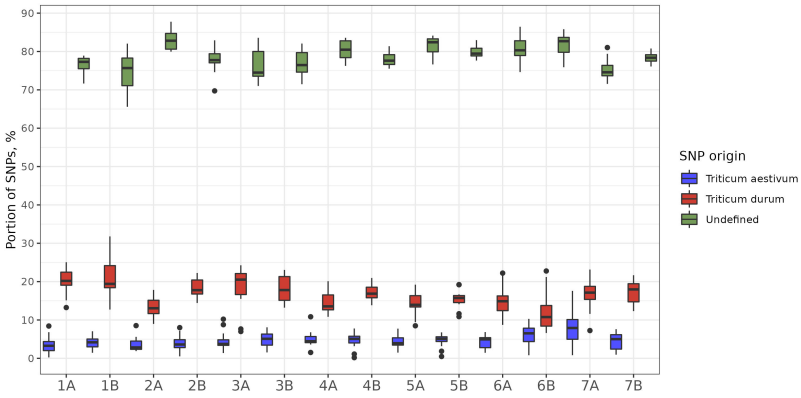

Figure 1. Distribution of the proportion of alleles of various origins on each chromosome in all studied combinations.

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2.3. Distribution of the genetic material of bread wheat and durum wheat in the offspring across the chromosomes

We have analyzed the distribution of species-specific alleles in the chromosomes of offspring lines. Only species-specific polymorphic markers, that is, those whose source (bread wheat or durum wheat) in the offspring could be clearly identified, was used for analysis. Increasing of proportion of species-specific polymorphic markers towards the edges of the chromosomes was observed (Supplementary Figures 1-15). Axiom^{TM} 35K Wheat Breeder's Genotyping Array is the least dense commercially available not custom SNP microarray for bread wheat. The distribution of variants along the chromosome in this SNP microarray is not uniform but shifted to the edges of the chromosomes, where most genes are located in bread wheat [14,24]. In our work, we observed a shift in the proportion of informative variants to the edges of chromosomes. Together with the low density of the SNP microarray, this imposes limitations of the possibility of identification for short introgression fragments, however, it makes it possible to identify rather long fragments containing an increased number of bread wheat alleles. Next, we analyzed chromosome fragments of offspring lines that we identified as introgression from bread wheat in some breeding lines at least. Distribution patterns of species-specific alleles for each chromosome across all combinations analyzed in the work are presented in Supplementary Data 1.

2.3.1. Chromosome 1A

Chromosome 1A has two regions containing introgressions (Figure 2). In the first region, combinations 1, 2, 6, 8, 9, and 15 show a local increase in the number of bread wheat alleles compared to the number of durum wheat alleles. Other combinations in this region almost or completely lack windows containing bread wheat alleles. The size of the introgressions varies approximately from 10 to 30 Mbp in different combinations. According to the annotation, the region includes 471 genes (Supplementary Table 16). In the second region, combinations 1, 2, 6, and 15 show a local increase in the number of bread wheat alleles compared to the number of durum wheat alleles. Other combinations in this region almost or completely lack windows containing bread wheat alleles. The size of the introgressions varies from 40 to 60 Mbp in different combinations. According to the annotation, the region includes 963 genes (Supplementary Table 16).

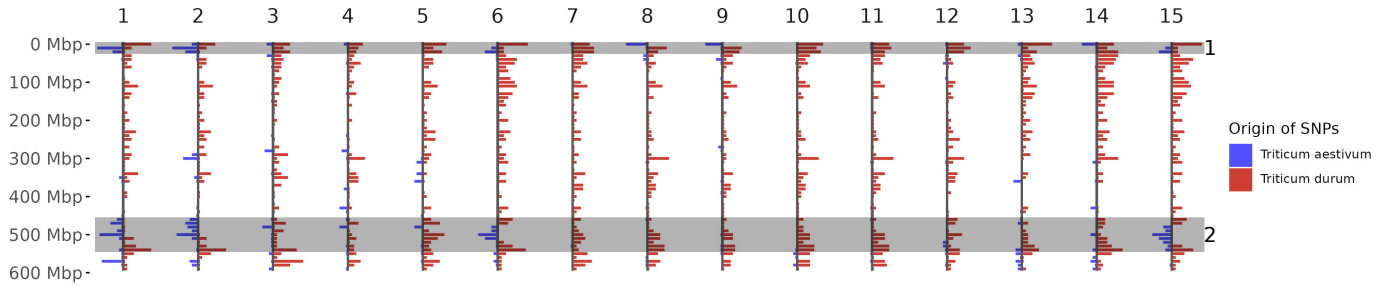

Figure 2. Distribution of species-specific SNPs along chromosome 1A of all analyzed offspring lines. The upper numbers are the index of the combination according to Table 2. The fragments containing introgression are marked with gray rectangles. One column corresponds to a 10 Mbp chromosome fragment. Length of column is a number of SNPs in window.

Functional enrichment analysis showed that in the first region of introgression there is a statistically significant enrichment with wound response genes (GO:0009611), enzymes involved in phosphorylation (GO:0006468), proteins involved in pollen recognition (GO:0048544). In molecular functions terms, genes encoding proteins involved in the binding of ATP (GO:0005524), ADP (GO:0043531) and polysaccharides (GO:0030247), serine/threonine kinases (GO:0004674) and endopeptidases (GO:0004867), as well as storage proteins (GO:0045735) are significantly enriched (Figure 3A, Supplementary Table 22). Wound response genes are often expressed in response to pathogen exposure [25]. Nine wound response genes were found on chromosome 1A between 12 Mbp and 13 Mbp on

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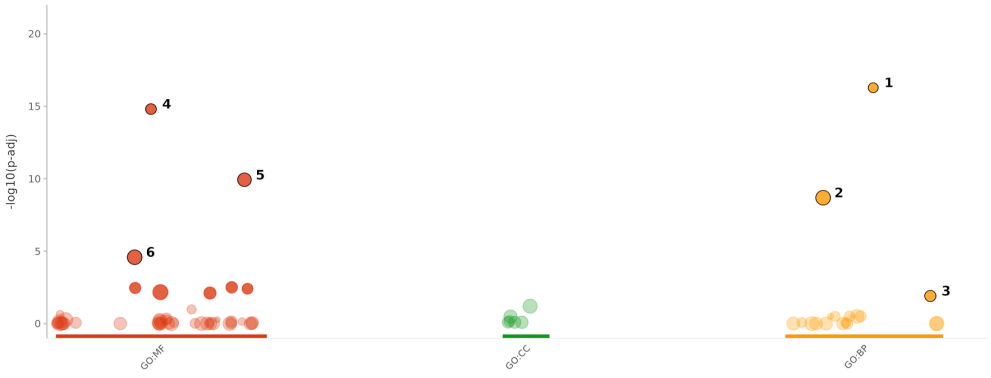
pseudochromosomes of bread wheat assembly [26]. The introgression region identified in this work includes these coordinates. In the region of the second introgression, there is enrichment with proteins involved in the response to oxidative stress (GO:0006979, GO:0004601), as well as proteins involved in the assembly of nucleosomes (GO:0006334) (Figure 3B, Supplementary Table 22). Since durum wheat is more often cultivated in dry conditions, which can lead to the accumulation of ROS (Reactive Oxygen Species), which in turn will lead to oxidative stress, the use of loci that increase the resistance of durum wheat to oxidative stress can increase yield. At the moment, the literature does not mention loci of bread wheat or durum wheat located on chromosome 1A that affect resistance to oxidative stress.



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0009611	response to wounding	209	5.2e-17
2	GO:BP	GO:0006468	protein phosphorylation	5355	2.0e-09
3	GO:BP	GO:0048544	recognition of pollen	433	1.2e-02
4	GO:MF	GO:0004867	serine-type endopeptidase inhibitor activity	323	1.5e-15
5	GO:MF	GO:0043531	ADP binding	2230	1.2e-10
6	GO:MF	GO:0004672	protein kinase activity	5350	2.6e-05

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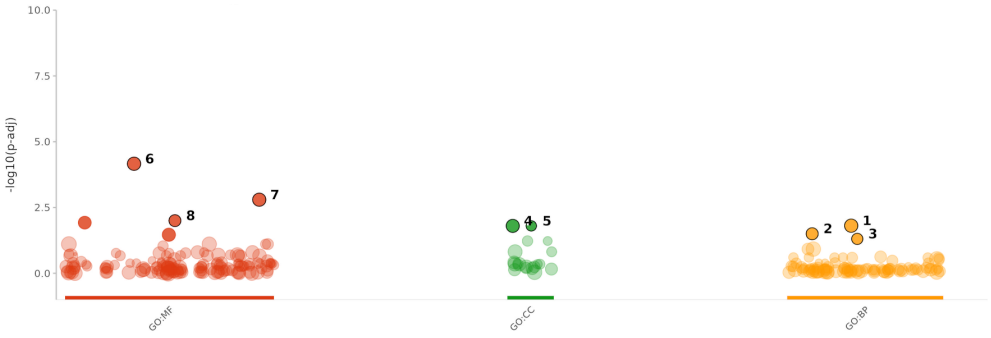
A



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0006979	response to oxidative stress	728	1.6e-02
2	GO:BP	GO:0006334	nucleosome assembly	205	3.1e-02
3	GO:BP	GO:0007264	small GTPase mediated signal transduction	91	4.9e-02
4	GO:CC	GO:0000786	nucleosome	603	1.6e-02
5	GO:CC	GO:0009507	chloroplast	47	1.6e-02
6	GO:MF	GO:0004601	peroxidase activity	741	6.9e-05
7	GO:MF	GO:0046982	protein heterodimerization activity	676	1.6e-03
8	GO:MF	GO:0008171	O-methyltransferase activity	188	1.0e-02

g:Profiler (biit.cs.ut.ee/gprofiler)

B

Figure 3. Results of functional enrichment analysis of genes located in the first (A) and the second region (B) of introgression on chromosome 1A. On the Manhattan plots the size of the dots is the number of genes of a certain GO term in the introgression zone. On the X-axis - functional GO term, colored by category. The height of the points along the Y-axis is $-log_{10}$ FDR-adjusted p-values. The table below each graph provides detailed information about GO terms with the top three $-log_{10}$ FDR-adjusted p-values in each functional group. See Supplementary Table 22 for complete functional group information. MF: Molecular Function; BP: Biological process; CC: Cellular component.

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2.3.2. Chromosome 1B

The region containing introgression on chromosome 1B revealed in combinations 6, 7, 10 and 15 (Figure 4) and contains fragments enriched with alleles of bread wheat from 30 to 130 Mbp long in different combinations. Other combinations in this region almost or completely lack windows containing bread wheat alleles. According to the annotation, the region includes 1341 genes (Supplementary Table 17).

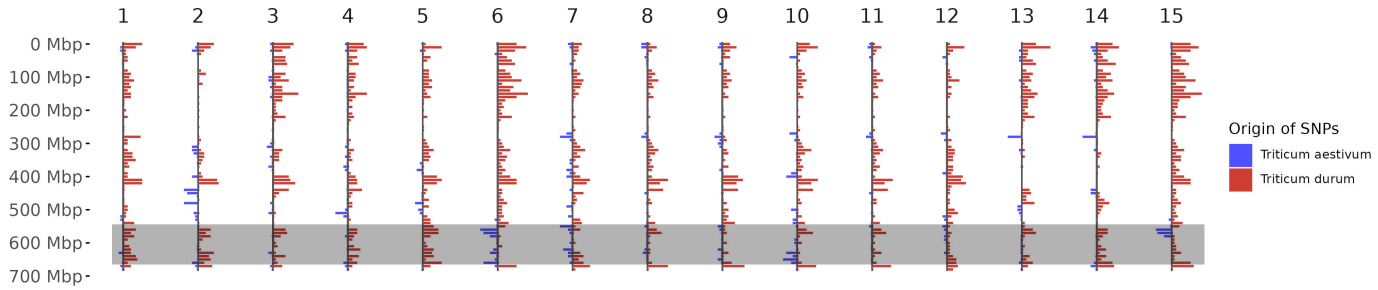
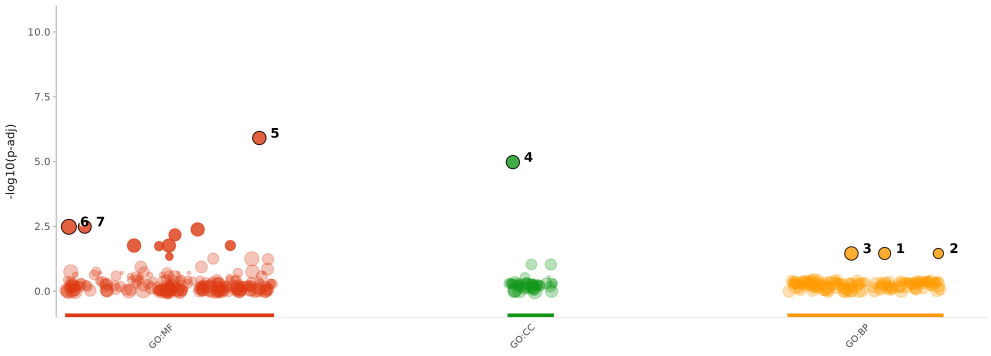


Figure 4. Distribution of species-specific SNPs along chromosome 1B of all analyzed offspring lines. The upper numbers are the index of the combination according to Table 2. The fragments containing the introgression are marked with gray rectangles. One column corresponds to a 10 Mbp chromosome fragment. Length of column is a number of SNPs in window.

Functional enrichment analysis showed that in the region of introgression there is saturation with genes encoding proteins associated with nucleosomes (GO:0000786), involved in the response to oxidative stress (GO:0006979), in folate binding (GO:0005542), and in the inhibition of endopeptidase activity (GO:0004866), as well as in methyltransferase activity (GO:0008171). There is also gene saturation of DNA-binding proteins (GO:0003677), in particular, transcription factors (GO:0017025) (Figure 5, Supplementary Table 23).



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0015986	proton motive force-driven ATP synthesis	174	3.5e-02
2	GO:BP	GO:0070897	transcription preinitiation complex assembly	31	3.5e-02
3	GO:BP	GO:0006979	response to oxidative stress	728	3.5e-02
4	GO:CC	GO:0000786	nucleosome	603	1.1e-05
5	GO:MF	GO:0046982	protein heterodimerization activity	676	1.2e-06
6	GO:MF	GO:0003677	DNA binding	4857	3.3e-03
7	GO:MF	GO:0003924	GTPase activity	430	3.3e-03

g:Profiler (biit.cs.ut.ee/gprofiler)

Figure 5. Results of functional enrichment analysis of genes located in the region of introgression on chromosome 1B. On the Manhattan plot the size of the dots is the number of genes of a certain GO term in the introgression fragment. On the X-axis - functional GO term, colored by category. The height of the points along the Y-axis is $-log_{10}$ FDR-adjusted p-values. The table below Manhattan plot provides detailed information on the top three $-log_{10}$ FDR-adjusted p-values in each functional group. See Supplementary Table 23 for complete functional group information. MF: Molecular Function; BP: Biological process; CC: Cellular component.

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2.3.3. Chromosome 4A

The region containing introgression on chromosome 1B revealed in combinations 1, 2, 7, 8, and 9 (Figure 6) and contains fragments enriched with alleles of bread wheat from 30 to 170 Mbp long in different combinations. According to the annotation, the region includes 2258 genes (Supplementary Table 18).

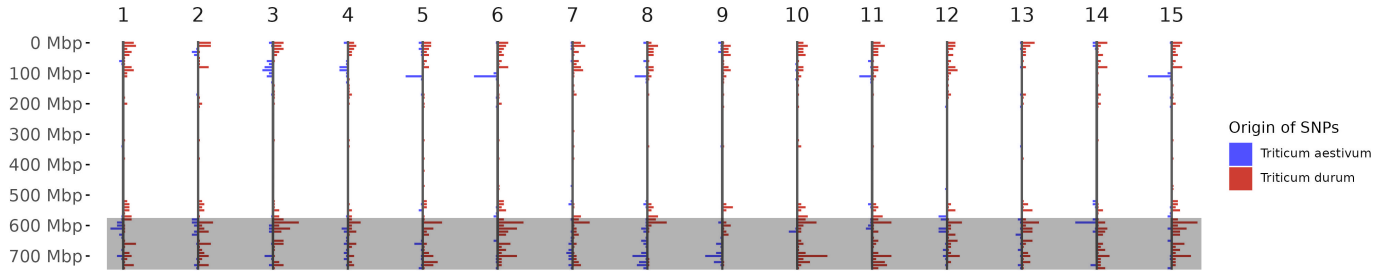
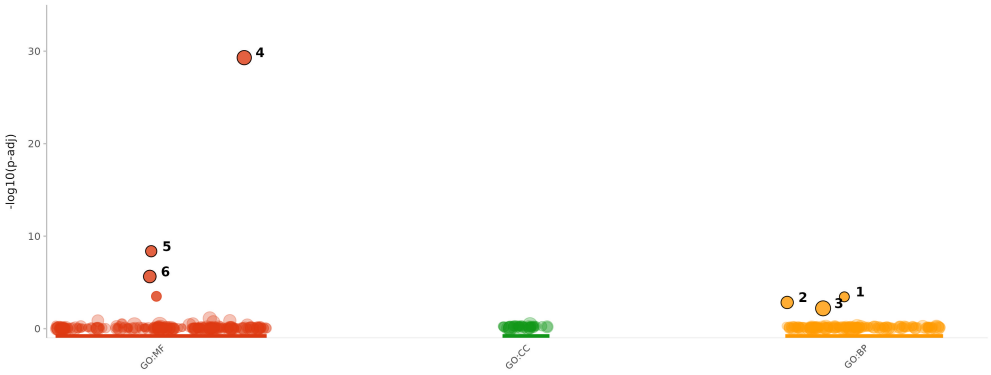


Figure 6. Distribution of species-specific SNPs along chromosome 4A of all analyzed offspring lines. The upper numbers are the index of the combination according to Table 2. The fragments containing introgression are marked with gray rectangles. One column corresponds to a 10 Mbp chromosome fragment. Length of column is a number of SNPs in window.

Functional enrichment analysis of genes in the fragment of introgression on chromosome 4A showed enrichment with genes involved in phosphate metabolism - protein phosphorylation (GO:0006468), phosphate transport through the membrane (GO:0005315) and phosphate signaling (GO:0000160) (Figure 7, Supplementary Table 24). These results support the results of a recent work, in which a QTL was found on chromosome 4A, which, according to the coordinates on the assembly of the bread wheat genome, is located inside the introgression zone and is associated with the efficiency of phosphorus uptake [27].



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0006817	phosphate ion transport	33	3.6e-04
2	GO:BP	GO:0000160	phosphorelay signal transduction system	271	1.5e-03
3	GO:BP	GO:0006468	protein phosphorylation	5355	6.4e-03
4	GO:MF	GO:0043531	ADP binding	2230	5.0e-30
5	GO:MF	GO:0004871	obsolete signal transducer activity	97	4.3e-09
6	GO:MF	GO:0004857	enzyme inhibitor activity	356	2.3e-06

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Figure 7. Results of functional enrichment analysis of genes located in the region of introgression on chromosome 4A. On the Manhattan plot the size of the dots is the number of genes of a certain GO term in the introgression fragment. On the X-axis - functional GO term, colored by category. The height of the points along the Y axis is $-log_{10}$ FDR-adjusted p-values. The table below Manhattan plot provides detailed information on the top three $-log_{10}$ FDR-adjusted p-values in each functional group. See Supplementary Table 24 for complete functional group information. MF: Molecular Function; BP: Biological process; CC: Cellular component.

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2.3.4. Chromosome 5B

The fragment of introgression on chromosome 5B revealed in all analyzed combinations except for 10, 11 and 14 (Figure 8) and contains fragments enriched with alleles of bread wheat from 30 to 90 Mbp long in different combinations. According to the annotation, the region of putative introgression includes 1076 genes (Supplementary Table 19).

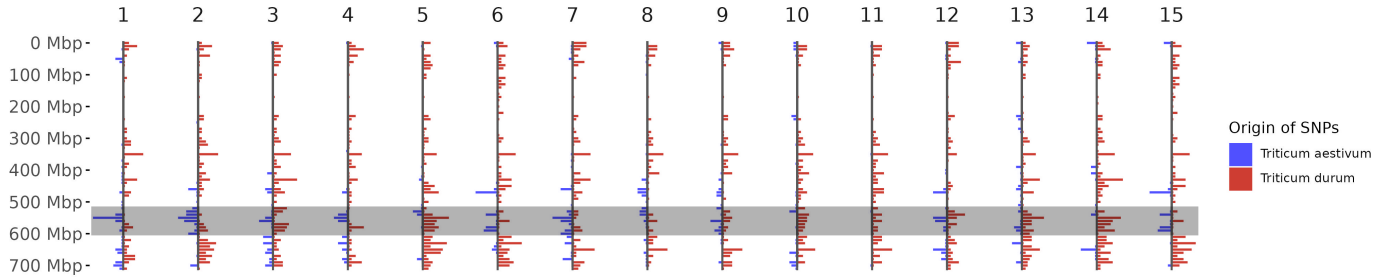
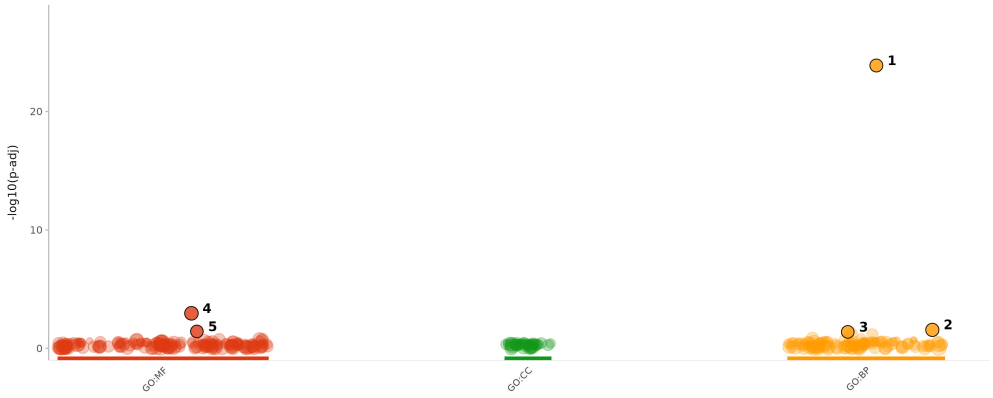


Figure 8. Distribution of species-specific SNPs along chromosome 5B of all analyzed offspring lines. The upper numbers are the index of the combination according to Table 2. The fragments containing introgression are marked with gray rectangles. One column corresponds to a 10 Mbp chromosome fragment. Length of column is a number of SNPs in window.

Functional enrichment analysis shows that the fragment of introgression is enriched with genes encoding proteins involved in the response to auxin (GO:0009733), as well as transmembrane transport proteins for xenobiotics (GO:0006855) (Figure 9, Supplementary Table 25) . Auxin is critically required for various biological processes during plant growth and development, such as organogenesis, apical dominance, tropisms, elongation, division, and extension at the cellular level [28]. At the moment, 69 auxin response genes have been identified in bread wheat on all chromosomes, but its functions are not completely clear for each of them, however, more and more data are emerging on their participation in responses to various abiotic stresses [29].



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0009733	response to auxin	308	1.3e-24
2	GO:BP	GO:0048544	recognition of pollen	433	2.7e-02
3	GO:BP	GO:0006855	xenobiotic transmembrane transport	223	4.0e-02
4	GO:MF	GO:0009055	electron transfer activity	679	1.1e-03
5	GO:MF	GO:0015297	antiporter activity	223	3.7e-02

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Figure 9. Results of functional enrichment analysis of genes located in the zone of introgression on chromosome 5B. On the Manhattan plot the size of the dots is the number of genes of a certain GO term in the introgression fragment. On the X-axis - functional GO term, colored by category. The height of the points along the Y axis is $-log_{10}$ FDR-adjusted p-values. The table below Manhattan plot provides detailed information on the top three $-log_{10}$ FDR-adjusted p-values in each functional group. See Supplementary Table 25 for complete functional group information. MF: Molecular Function; BP: Biological process; CC: Cellular component.

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2.3.5. Chromosome 6B

The fragment of introgression on chromosome 6B revealed in all analyzed combinations except for 5, 9, 10, and 11 (Figure 10) and contains fragments enriched with alleles of bread wheat from 20 to 100 Mbp long in different combinations. According to the annotation, the region of putative introgression includes 579 genes (Supplementary Table 20).

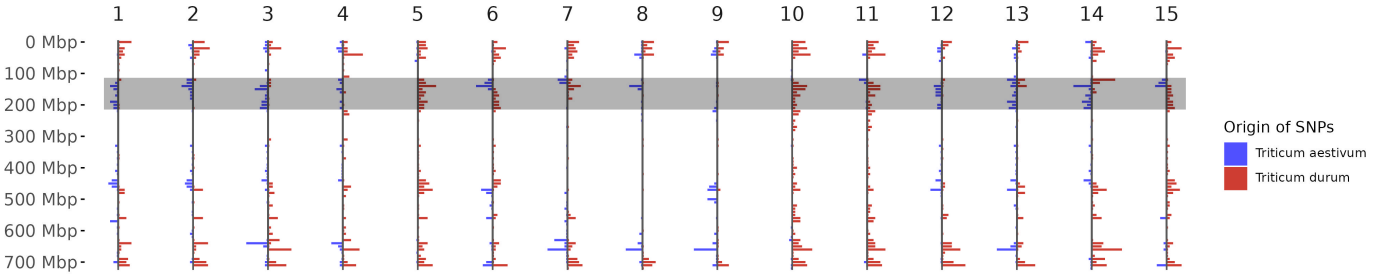
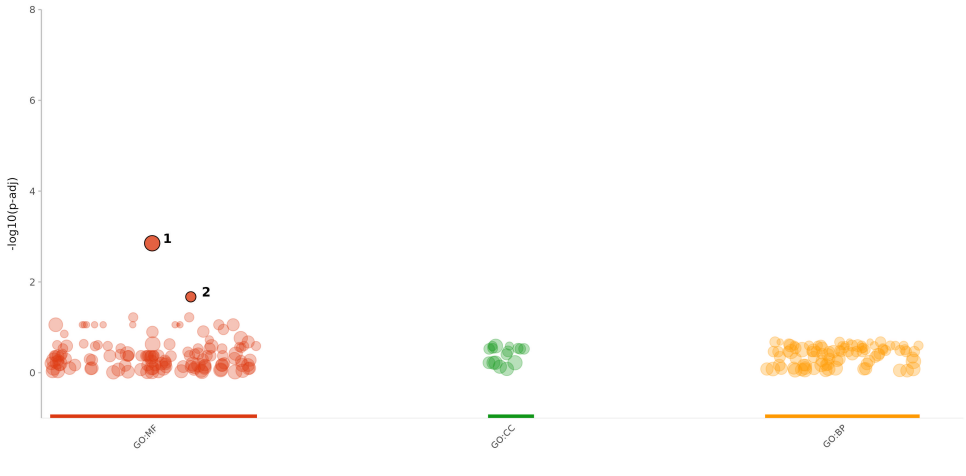


Figure 10. Distribution of species-specific SNPs along chromosome 6B of all analyzed offspring lines. The upper numbers are the index of the combination according to Table 2. The fragments containing introgression are marked with gray rectangles. One column corresponds to a 10 Mbp chromosome fragment. Length of column is a number of SNPs in window.

Functional enrichment analysis shows that the zone of introgression on chromosome 6B is saturated with genes encoding proteins with lipase (GO:0016298) and protein-binding activity (GO:0005515) (Figure 11, Supplementary Table 26). Although lipids are a small part of wheat flour, they important for the bread making [30]. To date, there is a single work about the identification of the genetic basis of lipid content in bread wheat [31]. Identification the loci responsible for increasing lipid levels can help make wheat more nutritious [30]. It has also been shown that reduction of grain hardness is associated with increased lipid content [32].



id	source	term_id	term_name	term_size	p_value
1	GO:MF	GO:0005515	protein binding	15411	1.4e-03
2	GO:MF	GO:0016298	lipase activity	25	2.1e-02

g:Profiler (biit.cs.ut.ee/gprofiler)

Figure 11. Results of functional enrichment analysis of genes located in the zone of introgression on chromosome 6B. On the Manhattan plot the size of the dots is the number of genes of a certain GO term in the introgression fragment. On the X-axis - functional GO term, colored by category. The height of the points along the Y axis is $-log_{10}$ FDR-adjusted p-values. The table below Manhattan plot provides detailed information on the top three $-log_{10}$ FDR-adjusted p-values in each functional group. See Supplementary Table 26 for complete functional group information. MF: Molecular Function; BP: Biological process; CC: Cellular component.

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2.3.6. Chromosome 7A

Chromosome 7A has two regions containing introgressions (Figure 12). In the first region, combinations 6, 8, 9, and 15 show a local increase in the number of bread wheat alleles compared to the number of durum wheat alleles. Other combinations in this region almost or completely lack windows containing bread wheat alleles. The size of the introgressions is about 40 Mbp in different combinations. According to the annotation, the region includes 715 genes (Supplementary Table 21). In the second region, combinations 3, 4, 5, 6, and 12 show a local increase in the number of bread wheat alleles compared to the number of durum wheat alleles. Other combinations in this region almost or completely lack windows containing bread wheat alleles. The size of the introgressions varies from 90 to 110 Mbp in different combinations. According to the annotation, the region includes 872 genes (Supplementary Table 21). Chromosome 7A in the sixth combination contains 17.59% of wheat alleles out of all homozygous alleles on this chromosome, which is significantly higher than in all other chromosomes in this combination (Supplementary Table 6).

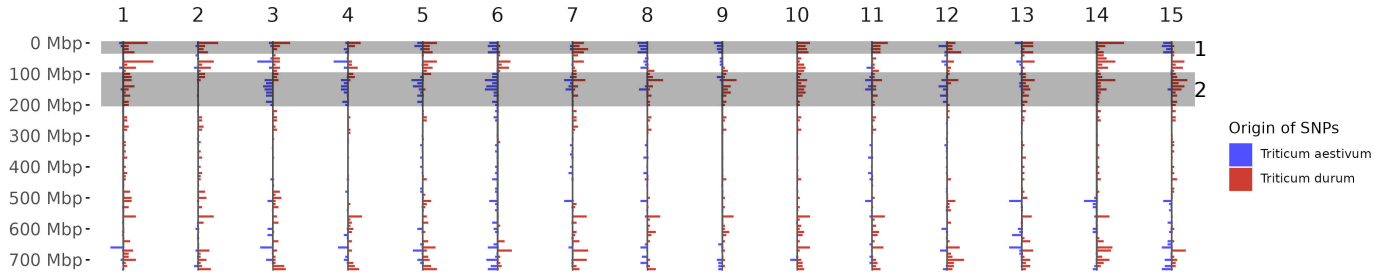

Figure 12. Distribution of species-specific SNPs along chromosome 7A of all analyzed offspring lines. The upper numbers are the index of the combination according to Table 2. The fragments containing introgression are marked with gray rectangles. One column corresponds to a 10 Mbp chromosome fragment. Length of column is a number of SNPs in window.

Functional enrichment analysis showed that in the first fragment of introgression on chromosome 7A there is enriched with genes involved in the development and dormancy of seeds (GO:0009793) - DNA damage checkpoint proteins during cell division (GO:0000077), inhibitors of enzymatic activity (GO: 0004857), G proteins (GO:0007186, GO:0031683), and carbohydrate converting enzymes (GO:0004564, GO:0004575) (Figure 13A, Supplementary Table 27). The fragment of the second introgression is saturated with genes for glutamate receptor membrane proteins (GO:0004970), as well as enzymes of carbohydrates conversion (GO:0008107, GO:0016758) (Figure 13B, Supplementary Table 27). Seed dormancy is controlled by the timing of fertilization and is an important adaptive strategy for weathering long periods of unstable environmental conditions. For agronomy, regulation of seed dormancy is important to avoid pre-harvest sprouting and to ensure uniform pollination in fields [33]. Many QTLs controlling pre-harvest germination have been found in cereals [34]. The second fragment of introgression is also enriched with genes involves in glutamate signaling (Figure 13B). In plants, glutamate involves in various physiological processes such as seed maturation [35], root system architecture [36,37], pollen maturation and pollen tube growth [38,39], wound and pathogene response [40–44], response and adaptation to abiotic stresses [45–49]. Glutamate also acts as a signal transmitter in the plant over long distances through cells, tissues, organs, and even entire plants by activating other signal pathways such as Ca_{2+} ions, ROS, and electrical signals [41–43].

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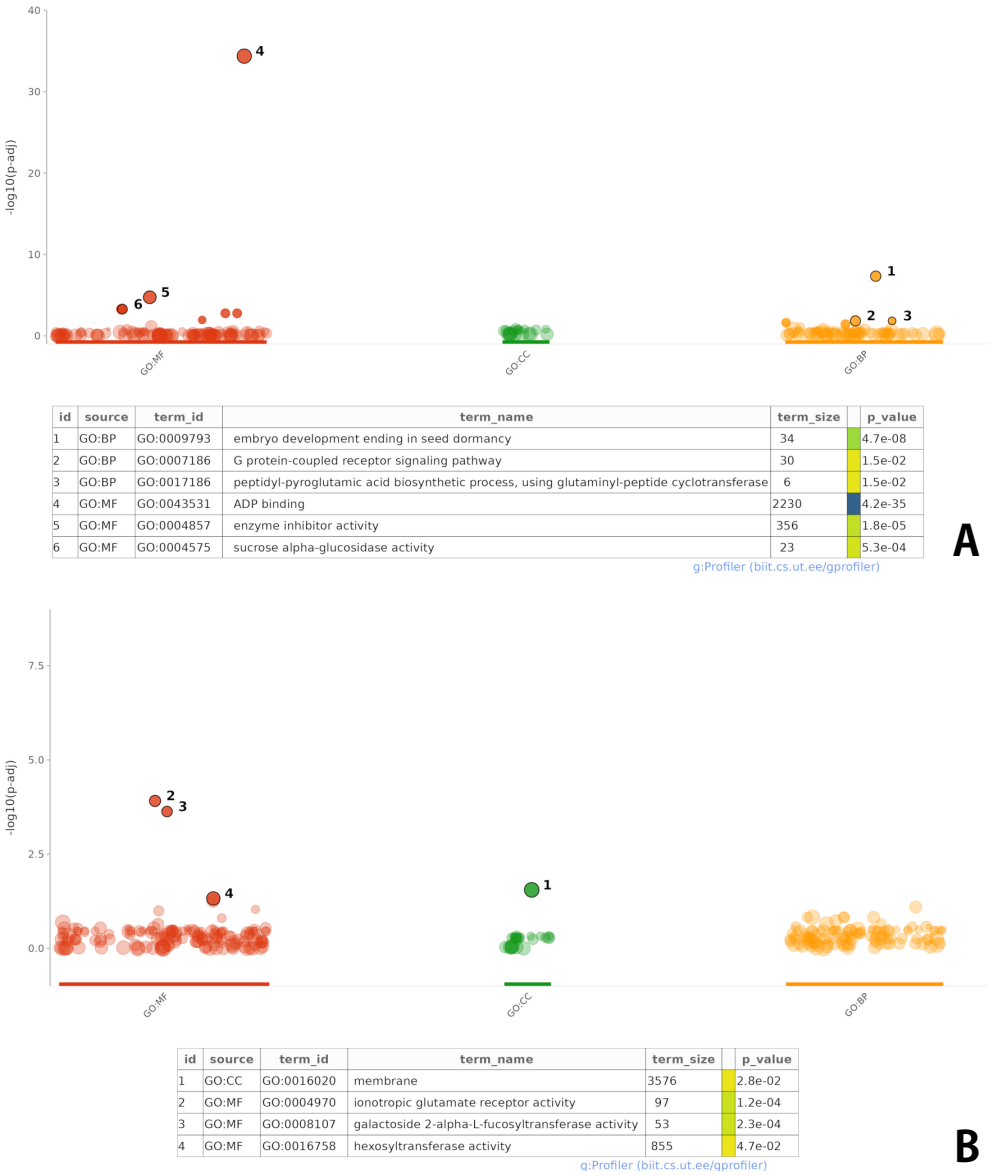

id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0009793	embryo development ending in seed dormancy	34	4.7e-08
2	GO:BP	GO:0007186	G protein-coupled receptor signaling pathway	30	1.5e-02
3	GO:BP	GO:0017186	peptidyl-pyroglutamic acid biosynthetic process, using glutaminyl-peptide cyclotransferase	6	1.5e-02
4	GO:MF	GO:0043531	ADP binding	2230	4.2e-35
5	GO:MF	GO:0004857	enzyme inhibitor activity	356	1.8e-05
6	GO:MF	GO:0004575	sucrose alpha-glucosidase activity	23	5.3e-04

g:Profiler (biit.cs.ut.ee/gprofiler)

A

id	source	term_id	term_name	term_size	p_value
1	GO:CC	GO:0016020	membrane	3576	2.8e-02
2	GO:MF	GO:0004970	ionotropic glutamate receptor activity	97	1.2e-04
3	GO:MF	GO:0008107	galactoside 2-alpha-L-fucosyltransferase activity	53	2.3e-04
4	GO:MF	GO:0016758	hexosyltransferase activity	855	4.7e-02

g:Profiler (biit.cs.ut.ee/gprofiler)

B

Figure 13. Results of functional enrichment analysis of genes located in the zone of introgression on chromosome 7A. On the Manhattan plot the size of the dots is the number of genes of a certain GO term in the introgression fragment. On the x-axis - functional GO term, colored by category. The height of the points along the Y axis is $-log_{10}$ FDR-adjusted p-values. The table below Manhattan plot provides detailed information on the top three $-log_{10}$ FDR-adjusted p-values in each functional group. See Supplementary Table 27 for complete functional group information. MF: Molecular Function; BP: Biological process; CC: Cellular component.

3. Discussion

In this work, we have demonstrated the possibility of using data obtained from the low-density Axiom^{TM} 35K Wheat Breeder's Genotyping Array SNP microarray to identify the events of introgression of the bread wheat genetic material into the durum wheat genome. The analyzed breeding lines contained vary amounts of genetic material of the bread wheat parent, however, the proportion of identified homozygous alleles specific for bread wheat does not exceed 6.5% (Table 1). In our work, we did not taking into account heterozygous alleles to identify introgressions because, when using SNP microarrays, heterozygote means rather a difference or similarity when compared with the reference which was used during an SNP microarray development, and not when comparing two samples with each other. For the majority of homozygous variants (about 80%), it was

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impossible to determine their origin in the offspring line due to their identical allelic state in the parents of bread and durum wheat.

Chromosome 1B among all the analyzed combinations showed the widest range of variation of the SNPs of undefined origin (Figure 1). This may indicate a greater polymorphism of this chromosome in the studied combinations, which may be the result of the presence of the 1BL/1RS translocation in some breeding lines [50]. Chromosome 7A shows the widest range of variation of the proportion of the bread wheat alleles. This is most likely the result of the presence of the 3902h3-18-31 breeding line, which has the vast proportion of species-specific alleles of bread wheat across chromosome 7A in comparison with all other breeding lines (17.59% of alleles of bread wheat, Supplementary Table 6). Chromosome 2A in all analyzed offspring lines has the largest median alleles of undefined origin, and one of the lowest median values of the species-specific alleles from durum wheat. This may indicate a higher conservation of chromosome 2A in the analyzed offspring lines (Figure 1).

The distribution of bread wheat alleles, despite their small number, is not random. Bread wheat's alleles often creates regions where these variants are significantly increased relative to the rest of the chromosome. In our work, we were able to identify no more than two fragments containing introgressions per chromosome. The revealed introgression framgments often show a different distribution pattern of species-specific alleles in different lines. The points where there is a decrease in the frequency of alleles of one species and an increase in the frequency of alleles of another species are most likely evidence of the recombination event in this region, which led to the exchange of fragment of homologous chromosomes in meiosis and the creation of a recombinant fragment of the chromosome (for example, Figure 8, lines 3, 4, 5 and 8). A small number of positions on the SNP microarray limits the resolution of introgression detection. The use of SNP microarrays with a large number of variants will allow much shorter introgressions to be identified, as well as to more accurately determine their boundaries.

The functional enrichment analysis of genes in the introgression regions showed the presence of significantly enriched GO terms. The discovered regions of introgressions enriched with genes for the response to wounding (1A), oxidative stress (1A, 1B), involved in phosphate transport (4A), auxin response (5B), lipase activity (6B), seed dormancy, and glutamate signaling (7A).

Thus, the fragments of introgression discovered in this work may carry new loci of valuable traits. In different breeding lines studied in this work, in the introgression fragments, a different pattern of species-specific alleles was found, which can lead to a different effect of the loci. Information about bread wheat introgressions in the durum wheat genome and the genes included in these fragments can be used in the breeding process to find associations between specific distribution patterns of introgressed fragments and phenotype.

4. Materials and Methods

4.1. Plant material

The offspring from crossing breeding lines and varieties of Russian and Chinese breeding were analyzed (Table 2). Winter durum wheat lines (offspring) were obtained by crossing winter bread wheats with winter durum wheats according to the schemes: *T. aestivum* × *T. durum* and (*T. aestivum* × *T. durum*) × *T. durum*. After the crosses were completed, the F_1 hybrids were self-pollinated and individual selection of ears was carried out in the F_2 generation, if necessary, additional individual selection of ears was carried out in the next generation. The seeds from the selected ears was sown in a breeding nursery, in which the selection of the best family was carried out. The selected family was studied in the control nursery, and then in the nurseries of competitive variety testing for 3 years. The study of the line in competitive variety testing was carried out in several repetitions. The presented samples are stable, homogeneous lines of durum wheat, prepared for state variety testing and subsequent commercial use.

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Table 2. Breeding lines and varieties used for analysis in this work.

Combination number	Parent 1 (TA[1])	Parent 2 (TD[2])	Offspring (TD)
1	Zhong Pin 1583	Alyona	4135h7
2	Zhong Pin 1583	Agat donskoy	4135h7
3	Zhiva	Cristella	4306h15
4	Alekseich	Cristella	4320h38
5	Sila	Amazonka	3903h9-17-3
6	Vostorg	Amazonka	3902h3-18-31
7	Markiz	Laska	4521h13
8	Rigi	Lazurit	4686h35
9	Rigi	Crucha	4686h35
10	Rigi	Krupinka	4528h1
11	Rigi	Lazurit	4529h25
12	Grom	Kordon	4646h1
13	Bezostaya 100	Kurant	4675h11
14	Ayvina	Kurant	4518h20
15	Vostorg	Amazonka	3902h3-18-3

1 - *Trticum aestivum*

2 - *Trticum durum*

4.2. Extraction of genomic DNA

Seeds were placed in Petri dishes onto two layers of filter paper soaked with ddH_2O and were grown in 24 °C. Total genomic DNA was extracted from 4-days old seedlings using CTAB-method with minor modifications [51]. Homogenization of samples were performed in 1% CTAB buffer using plastic pounder.

4.3. Genotyping of parental lines and offspring

Parental lines (bread wheat and durum wheat) and their offspring (durum wheat) were genotyped using Axiom^{TM} 35K Wheat Breeder's Genotyping Array (Genomed, Moscow). SNP-calling was performed using the Analysis Suite v5.1.1.1 software with standard settings for a diploid organism.

4.4. Identification of introgression chromosome fragments of T. aestivum *in* T. durum

Fragments of introgression were identified in triplets - two closest parents (durum wheat and bread wheat) and offspring (durum wheat) (Table 2). Only SNPs of the A and B genomes were selected for subsequent analysis. To identify introgression fragments, only homozygous SNPs were selected, the origin of which could be clearly identified in the offspring. For each SNP that met the selection criteria, the species of origin in the progeny was determined (durum wheat or bread wheat). The number of SNPs of each origin and SNPs of undefined origin were calculated for each chromosome in non-overlapping 10 Mbp windows using custom R-script. Chromosome distribution of SNPs was visualized using the same custom R-script (www.github.com/alermol/Introgression2022/blob/main/SNP_distribution/SNP_abundance_per_chr_per_window.R). Genotyping data for each combination is available (www.github.com/alermol/Introgression2022/tree/main/SNP_distribution/source_data/triples_geno_data).

4.5. Functional enrichment analysis of genes in introgression fragments

Functional enrichment analysis of genes in introgression zones was performed using the gprofiler2 v0.2.1 R package [52]. The GMT (Gene Matrix Transposed) file for the assembly of bread wheat IWGSC RefSeq v1.0 was created based on the annotation for this assembly version using a custom bash script (www.github.com/alermol/Introgression2022/blob/main/functional_enrichment/create_gmt.sh). The analysis was carried out in three categories of GO terms - cellular component, molecular function and biological process.

Filtration of statistically significant GO terms was carried out using the False Discovery Rate (FDR) < 0.05 criteria.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/1010000/s1

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Abbreviations

The following abbreviations are used in this manuscript:

QTL	Quantitative Trait Loci
GO	Gene Ontology
SNP	Single Nucleotide Polymorphism
TA	*Triticum aestivum*
TD	*Triticum durum*
ROS	Reactive Oxygene Species
FDR	False Discovery Rate

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