Androgenic Plant Families in Breeding

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One of the limitations in obtaining the genetic diversity of doubled haploid (DH) lines via anther culture is the development of families of regenerants, and each family represents a clone. This work examines the results of studying this phenomenon in anther culture of alloplasmic (*H. vulgare*)–*T. aestivum* and euplasmic lines with 1RS.1BL and 7DL-7Ai translocations and hybrids between them. Parameters of androgenesis such as the number of embryo-like structures, the total number of regenerants, and the number of green regenerants per 100 anthers varied depending on the genotype. In all genotypes from embryo-like structures, predominant development of families of plantlets rather than single plantlets was found. The source of family plantlets was polyembryos. About 75% of families consisted of regenerants at the same fertility level. On average, 37.74%4% of the R0 plants were fertile. The sister DH lines of three hybrid combinations were formed from seeds of R1 plants ($2n = 42$) with high fertility and in the presence of wheat–alien translocations. After four years of breeding trials, the sister DH lines of three families with fungal disease resistance increased yield, and some parameters of grain quality exceeding the controls were identified as promising for breeding.

anther culture   families of regenerants   (H. vulgare)–T. aestivum lines   breeding

1. Introduction

One of the goals of increasing the genetic diversity of bread wheat is to create new genotypes that are resistant to fungal pathogens that affect plants, leading to decreased grain yield and quality [1]. The main approach to obtaining new bread wheat genotypes is introgressive hybridization aimed at transferring valuable genes from cultivated and wild relatives to their genome [2][3]. Alloplasmic lines combining the nuclear genome of one species with the cytoplasm of another species are considered to be an additional source of biodiversity for cultivated plants [4]. The new nuclear–cytoplasmic interactions in alloplasmic genotypes can cause epigenetic modifications of nuclear genes [5], leading to changes at the level of transcription and metabolism [5][6]. Given that variability in the chloroplast and mitochondrial genomes contributes significantly to the adaptation of plants to biotic and abiotic environmental factors [7], alloplasmic lines with restored fertility can be valuable genotypes for creating new varieties of bread wheat [8], including those with introgression of alien genes [9].

Earlier, we reported the production of recombinant alloplasmic lines of bread wheat derived from backcross progenies of barley–wheat hybrid *H. vulgare* × *T. aestivum* and restoration of their fertility [10][11]. Alloplasmic (*H. vulgare*)–*T. aestivum* lines characterized by different fertility levels proved to be valuable models for studying the variability of both nuclear and organellar (mitochondrial and chloroplast) genomes in the process of nuclear–cytoplasmic co-adaptation as a result of backcrossing wide hybrids with paternal genotypes [12][13][14]. Some of the

recombinant alloplasmic (*H. vulgare*)–*T. aestivum* lines with full fertility restoration and their doubled haploid (DH) lines were effectively used to obtain new introgression genotypes [14][15] and their involvement in breeding [16]. As a result of selections in different regions, alloplasmic lines L-311(4), L-311(5), and L-311(6), carrying translocation 1RS.1BL with gene complex *Lr26*/*Sr31*/*Yr9*/*Pm8* controlling resistance to fungal pathogens, became commercially valuable varieties of spring bread wheat (Uralosibirskaya 2, Sigma, and Ishimskaya 11, respectively) [15][16]. Varieties with the 1RS.1BL translocation are widespread [17]. Mass cultivation of such varieties has resulted in the appearance of a highly aggressive race of stem rust, Ug99, virulent to the gene *Sr31* [18]. Currently, the gene *Sr31* in Russia remains effective in protecting against stem rust [19], but the genes *Yr9* [20] and *Pm8* [21] linked to *Sr31* have lost their effectiveness, and the gene *Lr26* is most effective in combination with other leaf rust resistance genes [22]. In order to ensure the protection of wheat varieties from the possible spread of the race Ug99 as well as other fungal pathogens, it is necessary to conduct pyramiding of disease resistance genes in the genetic background of highly productive genotypes. Alloplasmic (*H. vulgare*)–*T. aestivum* lines of the L-311 group were used as such genotypes in our research. Resistance gene donors were lines derived from wheat varieties Omskaya 37 and Omskaya 38 with translocations 1RS.1BL and 7DL-7Ai (carrier of genes *Lr19*/*Sr25*) and showed resistance to stem rust when studied in an international nursery in Kenya [23], as well as lines with introgression of genes from different species of *Triticum*.

Developing a new variety is a lengthy and costly process; therefore, this process is accelerated by using homozygous DH lines in many breeding programs [24][25]. DH lines are created within one year and can be quickly analyzed in repeated trials, accelerating the selection of genotypes with the desired traits in the process of variety development [26], especially in combination with marker-assisted selection (MAS) [27]. When using traditional methods to stabilize desired traits, for example, in bread wheat, it is necessary to obtain six to eight self-pollinated generations [24]. DH lines are successfully used in hybridization to create new breeding material [28] since their use makes it possible to fix in one genotype a combination of a series of target genes (gene pyramiding) introgressed from different parents. This can provide long-term resistance to biotic stress [29], obtain genotypes with resistance to abiotic factors [30], or lead to fixation of heterosis [31]. Obtaining DH lines is an important and widely used method for the selection of mutations [32], for the detailed study of many traits in plants, including quantitative ones [33][34], transformation [35]; and genomic editing [36].

Several technologies are used to produce DH wheat lines. One includes the crossing of wheat with haploproducers (e.g., *Zea mays* and *Imperata cylindrica*) [37]. In such hybrid combinations, haploproducer chromosomes are eliminated at the early stages of embryo development, which leads to the development of haploid wheat embryos. Using embryo rescue techniques makes it possible to grow haploid plants, which, after doubling the number of chromosomes, become sources of DH lines. Another approach for producing DH lines is based on the induction of androgenesis in anther [38][39] and isolated microspore culture [39]. Stressful conditions are created that cause the reprogramming of microspores from the gametophyte to the sporophyte pathway during in vitro cultivation [40]. This leads to the formation of embryo-like structures (androgenic structures) from microspores on the induction culture medium. Embryo-like structures develop into seedlings during cultivation on regeneration medium. The efficiency of anther and microspore culture methods is measured by the frequency of obtaining viable green plants in order to form DH lines that are necessary for further work [41].

Potentially, under culture conditions, each microspore can become a source of one androgenic plant, and one anther, for example, of bread wheat, contains more than 1500 microspores [42]. However, many factors limit the production of androgenic plants in anther culture. The success of androgenesis induction in anther culture is influenced by the conditions of the growth of donor plants; the methods of anther pretreatment; the stage of development of microspores; the composition of culture medium, in which there is an important role for growth regulators; and the culture conditions of anthers and androgenic structures [26]. Despite the possibility of optimizing the whole complex of methods, the reaction of anthers to the culture conditions is determined by the influence of the plant genotype [43][44]. This is due to the fact that each main stage of androgenesis (embryo regeneration, embryoid induction to seedling regeneration, development of green seedlings and albinos) is under independent genetic control by the nuclear genome [45] and cytoplasm [46]. Restrictions in the production of DH lines by anther and microspore cultures are associated with gametoclonal variation, particularly for plants that have regenerated from gametic cells under in vitro conditions. Typical manifestations of gametoclonal variation associated with deletions in the chloroplast genome are the development of albinism [47] and changes in the number of chromosomes and their structure [41][48]. However, spontaneous doubling of the number of chromosomes in regenerants leads to the restoration of fertility, which excludes their treatment with colchicine [48]. Cytogenetic variation in androgenic plants is especially pronounced when hybrid genotypes characterized by cytogenetic instability in meiosis are used as donors for anther and microspore cultures [41][48][49]. Another serious limitation of the use of DH lines in breeding and genetic research is reduced genetic diversity through the formation of genetically identical plants, i.e., clones, among androgenic regenerants [50]. These authors emphasize that this problem is practically ignored in research papers.

2. Data and Influences

Androgenesis and the formation of families of regenerants in anther culture in alloplasmic (*H. vulgare*)–*T. aestivum* and euplasmic genotypes and their hybrids were studied. It was shown that parameters of androgenesis such as the number of embryo-like structures and the total number of regenerants and green regenerants per 100 anthers varied depending on the genotype. Among the studied genotypes, the lowest values of these parameters were in the euplasmic lines Om37 and Om38 and the alloplasmic recombinant line L-17(3). The highest values of the three studied indicators of androgenesis were in the alloplasmic line L-311(4), carrier of translocation 1RS.1BL. These results were consistent with our previously obtained data in a study of androgenesis of other lines isolated from varieties Omskaya 37 and Omskaya 38 and the hybrid population L-311/00-22 in anther culture [48][51]. The high androgenesis rates in the L-311(4) line can be explained by the presence of the 1RS.1BL translocation. It is known that in anther culture of bread wheat, depending on the genotype, the 1RS.1BL translocation can have a positive effect on the formation of embryoids and the development of seedlings [52][53], including green seedlings [53].

The Om37 and Om38 lines, with low values of androgenesis, carried wheat–rye 1RS.1BL and wheat–wheatgrass 7DL-7Ai translocations. In some bread wheat genotypes with 7DL-7Ai translocations, the formation of androgenic embryoids and the regeneration of green seedlings are suppressed [54]. In other genotypes of wheat with this

translocation, only increased green plant regeneration has been observed [44]. Thus, in the presence of the wheat–rye 1RS.1BL translocation, the negative effect of the 7DL-7Ai translocation on androgenesis dominated in the Om37 and Om38 lines. However, in the genetic background of hybrids L-311(4) × Om37, L-311(4) × L-134, and L-311(4) × Om38, the negative effect of the 7DL-7Ai translocation on androgenic ability in anther culture did not occur in the presence of the wheat–rye 1RS.1BL translocation. In these hybrids, the values of the studied indicators of androgenesis are significantly higher than in the Om37 and Om38 lines. In the hybrid combination L-311(4) × 2870, in addition to the 1RS.1BL translocation, there is genetic material from the 2870 line, the pedigree of which includes *T. dicoccoides* [55]. The number of green plantlets/100 anthers in the hybrid combination L-311(4) × 2870 was significantly lower than that of hybrids L-311(4) × Om37 and Om37 × L-311(4). These results show that the genotype significantly affects the efficiency of anther culture, which is consistent with the data of other works [43][44][54]. Moreover, as discussed in [44][56], in vitro androgenic response could be transferred from a variety with the ability to regenerate in its F1 hybrids.

It was noted that in the L-17(3) line and hybrids L-311(4) × Om37 and L-311(4) × 2870, the number of all regenerants per 100 anthers was higher than the number of ELS/100 anthers. This is because most often, one ELS did not produce single plantlets, but clusters (families) of regenerants that developed from polyembryos. This concerned both albino and green plantlets. With regard to the analysis of green plantlet development, it has been observed that regardless of the genotype, families of regenerants are formed rather than single plantlets. The formation of androgenic polyembryos was noted in anther culture of bread wheat [57] and triticale [50]. Inducers of polyembryoid formation include 2,4-D present in the induction medium [57].

It is also possible that the development of families of multiple regenerants depends on the genotype. The present work noted a tendency for the number of green plantlets to increase in families of alloplasmic genotype L-311(4), and some of its alloplasmic hybrids in comparison with euplasmatic lines Pyr28 and Om37. It can be assumed that in alloplasmic genotype (*H. vulgare*)–*T. aestivum* carrying the 1RS.1BL translocation, increased induction of polyembryos was the result of the mutual influence of barley cytoplasm and rye chromosome 1RS. This is consistent with the presence of polyembryony and the development of twins from the seeds of F1 hybrids obtained from pollination of (*H. vulgare*)–*T. aestivum* lines with pollen of wheat–rye substitution lines 1R(1A) and 1R(1D) [58]. The development of twins in the alloplasmic lines of bread wheat variety Salmon carrying the cytoplasm of *Aegilops kotschyi* Boiss. and rye chromosome 1RS has been described previously [59].

In our work, the level of fertility of regenerants within families was studied. Approximately half of the green regenerant families (46.82%) were found to have all sterile sister plants. The frequency of families in which all sister plants were partially fertile was 9.09%, all plants were fertile in 20% of families, and 24.09% of families consisted of regenerant clusters at different fertility levels. The presence of such families can be explained by the participation of several polyembryoids in their formation. Thus, in 75.91% of families, regenerants with identical fertility were found. We assumed that fertility is not an accurate measure of kinship between sister lines within the same family. However, for a quick selection of required families, this method can be convenient. In this work, the frequency of families with green R0 regenerants identical in fertility was not significantly distinguishable from the

value revealed in the study of androgenic families of triticale, which showed that in about 80% of cases, all members of a family were genetically identical using DNA markers [50].

In our work, the average percentage of R0 regenerants that restored fertility varied depending on genotype and was 37.74%. Among the families of R1 sister lines grown from the seeds of individual fertile R0 spikes of the alloplasmic line L-311(4) and its three hybrid combinations (L-311(4) × Om37, L-311(4) × L-134, and L-311(4) × 2870), three groups were identified that differed in the level of plant fertility. On average, only 50% of families had sister lines, all of whose plants were fertile. In two other groups of families with different frequency, the sister lines segregated for fertile, partially fertile, and sterile plants. The frequency of families with plant fertility segregation was higher for hybrid combinations compared to their maternal line L-311(4). Cytogenetic analysis showed that the majority of plants with reduced fertility were aneuploids, which is consistent with our previous data [48][49] and data of other authors [41][44]. In this regard, for field trials, sister DH lines of families of hybrid combinations L-311(4) × Om37, L-311(4) × L-134, and L-311(4) × 2870 were formed only from seeds of the 42-chromosome plants of individual R1 sister lines with full fertility (more than 30 seeds in the main spike) carrying target genes localized in wheat–alien translocations.

Fourteen alloplasmic sister DH lines from three families of hybrid combination L-311(4) × Om37, nine DH lines from three families of hybrid combination L-311(4) × L-134, and eleven DH lines from three families of hybrid combination L-311(4) × 2870 were selected for field trials. By the fourth year of testing, three families of sister DH lines of three hybrid combinations were selected and studied. It was established that in terms of resistance to fungal pathogens, alloplasmic sister DH lines within families did not differ, showing the same level of resistance as in parental genotypes, or higher. The increased resistance to leaf rust and powdery mildew in sister DH lines compared to the maternal alloplasmic line L-311(4) indicates that new resistance genes from donor lines used as paternal genotypes during hybridization were introgressed into the genetic background of this line. It can be assumed that the resistance to powdery mildew in sister lines R28-DH1 and R28-DH2 of the hybrid combination L-311(4) × L-134 is due to the influence of the *Pm4b* gene, the carrier of which is the variety Reno [59], which is part of the L-134 pedigree. High resistance (immunity) to leaf rust and powdery mildew in sister lines R51-DH1-R51-DH4 of the hybrid combination L-311(4) × 2870 were transmitted from their paternal line 2870, which carries these genes introgressed from *T. dicoccoides* [53]. High resistance to leaf rust in sister lines R17-DH1 – R17-DH4 can be explained by the mutual influence of genes *Lr 26* and *Lr19*, and high resistance to stem rust by mutual influence of genes *Sr31* and *Sr25* due to the presence of wheat–rye 1RS.1BL and wheat–wheatgrass 7DL-7Ai translocations, respectively [60].

The high susceptibility of the Omskaya 33 variety to leaf rust and stem rust, and its susceptibility to powdery mildew, shows that in the year of testing there was a strong spread of fungal pathogens. Thus, the infectious background was strongly pronounced, which makes it possible to objectively judge the resistance of DH lines to leaf rust, stem rust, and powdery mildew. According to agronomic characteristics, sister DH lines were either at the level of parental genotypes or exceeded them. The studied DH lines are promising for further breeding work.

3. Conclusions

It was shown that the values of parameters of androgenesis such as number of embryo-like structures and number of all regenerants and green regenerants/100 anthers varied depending on genotype. Despite the different responses of genotypes to cultivation conditions, all of them are characterized by a common feature, the predominant development of families of regenerants originating from polyembryoids rather than single regenerants. Families can be ranked by the level of fertility of regenerants, or family members. However, families that differ in fertility and ploidy can develop as single mixed clusters, which is associated with the participation of several embryo-like structures in their formation. In order for DH lines not to be populations of different genotypes and not to include aneuploids, they must be formed from the most productive 42-chromosomal plants R1, which are grown from seeds set in a separate fertile spike of regenerants R0. Pre-breeding should include cytogenetic and molecular analysis to prove the presence of introgressed genes. This work shows the possibility of obtaining novel introgression DH lines with complex resistance to fungal pathogens with the involvement of alloplasmic genotypes (*H. vulgare*)–*T. aestivum* with fertility restoration and fixed nuclear–cytoplasmic compatibility.

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