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Distant and intraspecific hybridization, induced mutagenesis in soft bread wheat

Abstract: Increase wheat yields by improving its genotype – one of the most urgent problems of the rural economy. At present, using traditional methods of selection and genetic studies, such as backcross selection, distant hybridization, experimental mutagenesis, increased efficiency of obtaining genetically modified and improved forms of wheat. The high content of mono phosphorus (H_3PO_4, 5%) leads to a weak mutagenic effect, and its 0.1% concentration promotes the development of plants biomass. Fertility rates of soft wheat hybrids crosses with wild species (T. timopheevii, T. dicoccum and T. kiharae) depend on the direction of crossing and genotypes of initial varieties. Wild species as the parent component positively affect the high percentage of grain fertility. Genome of Nadezhda variety is compatible with genomes of wild species [1, 2].

Key words: breeding, chemical mutagenesis, isogenic lines, replacement of chromosomes, distant hybridization, variety, wheat chromosomes.

Introduction

By chemical mutagenesis new forms are obtained qualitatively, such as, dwarf mutants in wheat and barley, ultra-fast mutants in wheat and barley resistant to fungal diseases of plants form, high-lysine and highly productive mutants [3]. These facts indicate that mutants obtained by using chemical mutagens can successfully serve as progenitors of new high-yielding varieties. However, obtaining mutants and their study – is only the first stage of selection work. It is possible to use hybridization in selection of mutations. More important is the use of mutants in hybridization to obtain positive transgressions [4-8]. Preparation of mutants and their use for hybridization requires the study of genetic nature of emerging changes, which is crucial for the selection of effective mutagens with specific action, and to broaden and deepen understanding of the nature of wheat evolution. Mutants having complex morphological, physiological and biochemical changes affecting economically valuable properties can be further used to locate genes that determine the trait followed Intervarietal replacement of chromosomes. Isogenic lines are convenient objects for many biological and agricultural experiments. Main advantage of these lines is high genetic similarity among themselves and with the control line, which allows estimating the contribution to the formation of crop marking characteristics and applying them as effective donor marker signs [1, 2]. One of the possibilities to create new varieties with economically valuable traits, and primarily in the direction of selection on productivity and disease resistance to wheat rust, and their improvement in economically valuable attributes is the method of hybridization. Interspecific hybridization in wheat breeding to leaf rust resistance requires use of *T. timopheevii* [3]. In order to overcome hybrids sterility methods facilitating the gene transfer from distant wheat species have been recently developed. Some of them are based on the methods of chromosome engineering, others on methods of genetic control of meiotic recombination, third on the methods of genetic engineering. The aim of the current research is the obtainment of mutants with agriculturally valuable traits, distant and interspecific wheat hybrids and their breeding analysis.

Materials and methods

Spring soft wheat Kazakhstanskaya 126 variety (*Triticum aestivum* L. *var. ferrugineum* Al.), a series of its monosomic lines and such varieties as Nadezhda, Kazakhstanskaya 4 and Shagala served the objects of the current research. Kazakhstanskaya 126 variety was developed at Kazakh SRI of Agriculture and crop production by crossing soft wheat Lutescence 47 with the local variety Kozhebiday and subsequent two-time selection. Isogenic lines of Avocet variety by *Yr* genes, *T. timopheevii* species. Wheat grains of Nadezhda and Kazakhstanskaya 126 varieties were processed by phosphoric acid (H_3PO_4) in 5-10% aqueous solutions. For that different concentrations of phosphoric acid: 0.01; 0.1 and 0.5% were tested. Wheat grains were then incubated in a solution of appropriate concentration.

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Results and their discussion

Comparative study of the effect of different concentrations of phosphoric acid has shown that 5% is set as the optimum concentration of the substance to study the ontogeny and cell division activity of root meristem of wheat germ. Effect of chemical compounds has been considered previously in studies of different directions. However, the genetic basis of variations in plant reaction to the action of these compounds has not been studied. Below is the data for the study of reactions of treated grains under laboratory conditions (Table 1).

Table 1 – Study of cell division and aberrations in anaphase of mitosis

Mutagen and its concentration,%	Total number of analyzed cells	Aberrations	The average percentage of affected cells
Kazakhstanskaya 126			
Control	750	5	0.66±0.01
H_3PO_4 0.1%	750	8	1.00±0.01
H_3PO_4 0.01%	750	11	1.40±0.01
H_3PO_4 0.5%	750	29	3.80±0.40
Nadezhda			
Control	750	3	0.40±0.01
H_3PO_4 0.1%	750	14	1.86±0.02
H_3PO_4 0.01%	750	17	2.26±0.01
H_3PO_4 0.5%	750	37	4.9±0.04

The treated grains were sown in test sites. Phenological observations showed that high level of mono phosphorus (5%) leads to a weak mutagenic effect, while its 0.1% concentration contributes to the development of the biomass. Weak mutagenic effect of 5% concentration is apparently linked to a strong acidification of pH. This is proven by some aberrations of chromosomes in mitosis and meiosis disturbances in plants treated with H_3PO_4 (Figure 1). Mitosis in mutant plants was accompanied by a massive sticking of chromosomes (pyknosis) and offset spindle of metaphase plate (Figure 2).

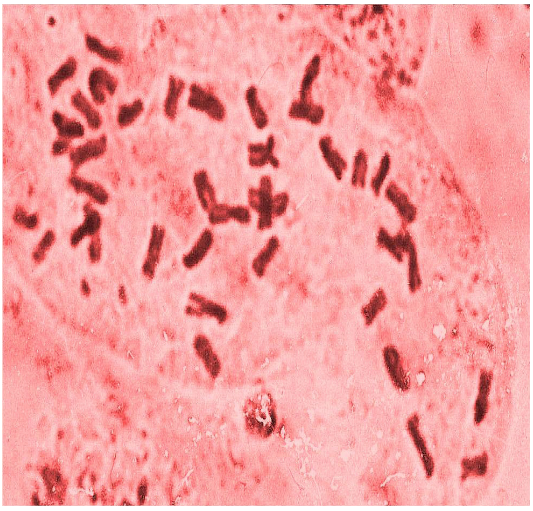

Figure 1 – Mitosis of mutant plants, ditelocentrics are indicated by arrow

Figure 2 – Sticking of chromosomes in plants, treated with phosphoric acid

Mutagenic effect and its importance in the breeding are determined by the results of the mitotic activity and the nature of the aberrations in cell division. They allow us to determine the degree of variability in plants obtained by the action of chemical and physical factors.

The inducing activity of a specific phosphoric acid concentration (5%) on grain germination, cell division and aberrations in mitosis of meristematic cells of test options compared with control obtained by the action of H_3PO_4 was studied. The action of the different concentrations of the chemical compound (H_3PO_4) was observed within the plant ontogeny. Thus, 0.1% H_3PO_4 concentration has a minor deviation (1.00 ± 0.01) on the normal course of mitosis compared to control (0.66 ± 0.01).

Structural analysis of elements of productivity isogenic lines revealed three lines – IL-Hg, IL-BgHg and IL-Pc, differing significantly by increase of productivity indicators of the spike and 1000 grain weight in comparison with control (Table 2). Isogenic line IL-Hg with hairy spike can be morphologically well-tested during the heading stage and has more saturated color of glume compared to the control. Indicators of spike productivity and weight of 1000 grains of the line IL-Hg was significantly higher than such in control (Table 2).

The length of the spike averaged 13.0 ± 0.2 cm. with the number of spikelets counting 20.0 ± 0.4. The number of grains in the main spike counts 63.2 ± 1.0 with a weight of 2.9 ± 0.1 g. Grain is medium size, oval with shallow groove. The average value of the weight of 1000 grains was 48.1 ± 1.4 g, in comparison with control – 44.7 ± 0.7 g.

Isogenic line IL-BgHg has a hairy, black ear. The median length of the spike in IL-BgHg line was 13.1 ± 0.1 cm. The number of spikelets on average counts 20.0 ± 0.1. the number of grains 65.4 ± 0.2, which was significantly higher than control. Grain size is medium, the groove is not deep. The weight of 1000 grains in line IL-BgHg significantly exceeds such in control counting 49.7 ± 0.3 g ($P<0.001$). Observed increase in spike productivity indices in lines IL-BgHg and IL-Hg can possibly be associated with the presence of a dominant allele of glume pubescence Hg in these lines. Isogenic line IL-Pc is characterized by the purple color of straw. The length of the spike in line IL-Pc in average is 12.5 ± 0.5 cm. Number of spikelets 19.6 ± 0.2, number of grains 63.2 ± 1.0. Weight of grain from the main spike in average is 2.7 ± 0.1. Major grain has articulate groove. The weight of 1000 grains in line IL-Pc is 48.4 ± 0.6 g, deviation from control is significant under at $P<0.001$. The increase in the average weight of grain from isogenic line IL-Pc is confirmed by the improved grain filling. This can possibly be associated with the increase in productivity of photosynthesis, due to the intensification of this process in anthocyanin containing plants.

Table 2 – Elements of productivity of spike of morphologically marked isogenic lines

Variety/line	Productivity of the main spike				
	Length of spike, cm	Number of spikelets, pc.	Number of grains, pc.	Weight of grain, g	Weight of 1000 grains, g
K. 126	12.2±0.1	19.0±0.3	51.7±1.6	2.4±0.1	44.7±0.7
IL-Hg	13.0±0.2***	20.0±0.4**	63.2±1.0***	2.9±0.1***	48.1±1.4**
IL-Pc	12.5±0.5	19.6±0.2	63.2±1.0***	2.7±0.1*	48.4±0.6***
IL-BgHg	13.1±0.1***	20.0±0.1***	65.4±0.2***	3.0±0.1***	49.7±0.3***
Deviation from control is significant under * $P<0.05$ ** $P<0.01$ *** $P<0.001$					

Earlier upon action with 0.01% aqueous solution of cadmium chloride as a mutagen on the variety Kazakhstanskaya 3 and Shagala mutant lines: L1, L2 and L3 were selected. Mutant forms have long spikes, elongated glumes, glassy large grain, anthocyanin coloration of the stem and leaf axils eyelets, as well as the high weight of 1000 grains. Several lines were higher and thicker culm, thickening and lengthening of the stem nodes, increased productive tillering. These selection and mutant forms of the important features, firmly inherited from generation to generation (M1-M6). In this regard, one of the objectives of this study is to localize genes responsible for economic-valuable signs of mutant forms. It is known that the elongation glumes spike was positively correlated with elongated grains. This fact is a direct proof of the high productivity of the ear mutant form A1. Under natural conditions, when intraspecific hybridization to obtain such form is rarely possible. Therefore, before using a mutant in order to hybridization it was necessary to genetically examine this property of wheat using the method of chromosome engineering. To carry out this work obtained F_1 hybrids seeds using a variety of monosomic lines Kazakhstanskaya 126 in the amount of 22 crossing combinations. Comparative monosomic analysis of the hybrids on the basis of extension glume will be held study of F_1 offspring based on harvest of 2013. Identification of mono- and disomics of Kazakhstanskaya 126 variety served as prerequisite for this work.

Interspecific hybridization. Tetraploid endemic species Triticum timopheevii Zhuk. (gentic formula AtAtGG) is characterized by a unique gene pool controlling resistance to many diseases of wheat. Creating and intensive involvement in the selection process of wheat donors with effective Lr-resistance genes transmitted from wild relatives could significantly expand its genetic basis for one or the other economically valuable traits. However, despite the difficulties (the sterility of hybrids and cytological instability), the literature contains information about the migration of a number of genes resistant to brown stem rust, powdery mildew by T. timopheevii to soft wheat. The published information about the compatibility of species T. aestivum and T. timopheevii is contradictory due to specific ecological zones [3, 4]. Using Twell method contributed to a sharp increase in the volume and improve the pollination quality, but in general – hybridization. High performance pollinating (up to 90 spikes per hour) by native pollen permitted analysis of the actual compatibility of the initial parental forms. Table 3 shows the results of cross-species hybridization.

Table 3 – Fertility of reciprocal hybrids of distant hybridization

№	Combination of breeding	Number of		Percentage of grain folding,%
		pollinated flowers	folded grains	
	Soft wheat x T.timopheevi			
1	F_0(*T.timopheevi* x Nadezhda)	190	119	62.63
2	F_0(Nadezhda x *T.timopheevi*)	72	11	15.28
3	F_0(*T.timopheevi* x к-2780)	150	61	40.67
4	F_0(к-2780 x *T.timopheevi*)	56	6	10
5	F_0(32 shortst. x *T.timopheevi*)	56	0	0
	Soft wheat x T.dicoccum			
1	F_0(*T. dicoccum* x Nadezhda)	282	181	64.18
2	F_0(Nadezhda x T.*dicoccum*)	156	41	26.28
3	F_0 (T.*dicoccum* x к-2780)	150	71	47.33
4	F_0(к-2780 x *t.dicoccum*)	130	14	10.77
5	F_0(*T.dicoccum* x 32 shortst.)	32	17	53.12
6	F_0(32 shortst. x *T.kiharae*)	33	0	0
	Soft wheat x T.kiharae			
1	F_0 (*T.kiharae* x Immune1498)	84	34	40.47
2	F_0 (Immune1498 x *T.kiharae*)	108	12	11.11
3	F_0 (*T.kiharae* x к-2780)	32	17	53.12
4	F_0(к-2780 x *T.kiharae*)	102	17	16.66
5	F_0(*T.kiharae* x 15/20977)	18	8	44.44
6	F_0(15/20977 x *T.kihara*)	118	14	11.86
7	F_0 (T.*kiharae* x Nadezhda)	50	29	58
8	F_0 (Nadezhda x *T.kiharae*)	52	10	19.23
9	F_0(USA18 x *T.kiharae*)	48	4	8.33
10	F_0 (*T.kiharae* x USA18)	22	12	54.54
11	F_0 (*T.kiharae* x USA19)	24	12	50
12	F_0 (USA19 x *T.kiharae*)	82	23	28.05

Hybrids with T. timopheevii. Experimental data shown in Table 3 suggests that the hybridization with different wild cultures of wheat species is successful. However, tying of grains in various combinations ranges from 0 to 64.18%. Apparently, the percentage depends on the genotype of variety from which samples were taken for cross-breeding, as well as from the crossing direction. Thus, the percentage of successful crosses of T. timopheevii with soft wheat is relatively high in the case, when the wild form is taken as the parent form. Depending on the number of successfully pollinated spikes the number of hybrid progeny grains varies. Compatibility level of T. timopheevii with soft wheat variety Nadezhda is relatively high, and the average is about 62.63%, k-2780 – 40.67%, and the percentage of backcrossing luck in hybrid progeny plummets 15.28% and 10%, respectively. F_1 hybrids (T. timopheevii to x-2780) of 150 – 61%, and 56 from the reciprocal mating pollinated flowers ensued only 10% of the grain.

Hybrids with T. dicoccum. F_1 hybrid offspring with wild species T. dicoccum with soft wheat was similar to the results of previous combinations produced with T. timopheevii. It is interesting to note that in this case the percentage of luck is much higher than, those combinations where grade Nadezhda served as a father. Thus, from 282 pollinated flowers luck percentage was 64.18%, and in the reciprocal crosses from 156 pollinated flowers tie a percentage of grains appeared 26.28%. Percentage of luck in direct (T. dicoccum x k-2780) crossed with the sample to 2780, amounted to 47.33% and -10.77% in reverse.

Hybrids with T. kiharae. Hybrids of wheat with T. kiharae, less productive than the hybrids with the previous combinations. However, in this case, there is a sharp drop in interest luck compared with those combinations where the parent form is taken T. kiharae. For example, the percentage of good luck in the forward mating ranged from 54.54% to 40.47% and 28.05% from reverse to 8.33%. This variety of indicators can be explained by the genotype – by environmental conditions for growing plants.

Thus, the study of reciprocal hybrids F_1, obtained by crossing wheat with wild species – T. timopheevi, T. dicoccum, revealed clear differences in the percentage of zavyazyvaemost grains. In plants, a hybrid combination with T. timopheevi, T. dicoccum and T. kiharae cytoplasm wheat, ie, under which the mother plants as soft wheat has been used, the percentage of luck somewhat lower compared to the hybrids, which served as the parent form of wild species. Hence, one can adopt clearly that the use of the wild-type form as maternal genomes increases compatibility than in the opposite mating. For hybrids derived from crosses with T. timopheevi soft wheat characteristic heteroplasmic condition: simultaneously present copies of the wild (the parent) and wheat (paternal) types.

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