The influence of combinations of alien translocations on *in vitro* androgenesis in lines of spring common wheat

Timonova E.M.*, Adonina I.G., Salina E.A.

Kurchatov Genomic Center, Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

*e-mail: eegorova@bionet.nsc.ru

Androgenesis *in vitro* is potentially the most effective technique for doubled haploid production of wheat. However, it is not widely used in breeding programs due to its main limitation: the genotype dependence. The basic approach to the production of new genotypes of wheat - introgressive hybridization and enrichment of the *Triticum aestivum* L. gene pool by genes from different species of goat grass, wheatgrass, rye and other relatives are continued. The method of *in vitro* anther culture for doubled haploid production could facilitate this process. Due to genetic differences between wheat and related species, it was assumed that alien genetic material are different in their capacity to conduct androgenesis and the influence of foreign translocations on androgenesis *in vitro* has been shown [1-4].

The aim of this study was to develop a set of doubled haploid (DH) wheat lines containing wheat-alien translocation in genome and to study the influence of foreign translocations on the ability to androgenesis in anther culture of those lines.

Methods and results

Plant Material. The material used in this study included: spring wheat cultivar Novosibirskaya 16; line 991 carrying wheat-alien translocations 1RS.1BL from rye and 5BS.5BL-5SL from *Aegilops speltoides* and four hybrid F_3 generation lines (10-7, 14-8, 15-8, 15-12) from their crossing and differing in the content of foreign translocations.

Anther Culture. The spikes were cut at the late one-nuclear stage. Anthers after isolation were incubated at 32°C for 72 h in the dark and kept for 7 days at 4 °C. The embryo-like structures (ELS) were induced on the N6 medium [5] supplemented by 60 g of sucrose, 30 g maltose and 1 mg/l of 2,4-D. The ELS were isolated and transferred onto the medium for regeneration B5 [6] containing 0.5 mg/l of NAA and 0.5 mg/l of kinetin. The green haploid regenerants were treated with a 0.2% colchicine solution to induce their diploidization.

Efficiency of anther culture *in vitro* was analyzed according to the following parameters: number of productive anthers per 100 anthers, number of ELS per 100 anthers and amount of albino and green plants (Table 1).

Table 1. Efficiency of anther culture in four F_3 lines 10-7, 14-8, 15-8, 15-12 and their parents Novosibirskaya 16 and line 991.

Genotype	Number of cultivated anthers	Productive anthers (PA)		Embryo-like structures (ELS)		Albino plantlets (AP)		Green plantlets (GP)	
		Number of PA	Number of PA/100 Anthers	Number of ELS	Number of ELS/100 Anthers	Number of AP	Number of AP/100 Anthers	Number of GP	Number of GP/100 Anthers
N16	972	78	8	160	**16,4**	70	**7,2**	2	0,2
line 991 (1R,5S)	462	34	7,4	60	**13**	26	**5,6**	17	**3,7**
line 15-12 (5S)	674	22	3,3	29	4,3	9	1,3	8	1,2
line 10-7 (1R)	904	189	21	348	**38,5**	151	**16,7**	38	**4,2**
line 15-8 (no transl.)	780	28	3,6	36	4,6	20	2,5	4	0,5
line 14-8 (1R, 5S)	979	188	19	488	**50**	211	**21,5**	111	**11,3**

Methods and results

Ploidy level of total 151 R_0 plants were determined by chromosome counts of root tips cells stained by acetocarmine or DAPI (Fig. 1a) and by measurement the size of stomatal guard cells (Fig. 1b). The regenerated plants are separated into three groups of ploidy (haploids, spontaneous doubled haploids and aneuploids). Spontaneous chromosome doubling rates among from wheat genotypes are 41%. Data summarized in Table 2.

Table 2. Ploidy level of green plantlets regenerated from lines 10-7, 14-8, 15-8, 15-12 and their parents Novosibirskaya 16 and line 991

Genotype	Number of analyzed green plantlets	Number of haploids (1n)	Number of doubled haploids (2n)	Frequency (%) of spontaneous doubled haploids	Number of aneuploids
N16	2	1	1	50	0
line 991 (1R,5S)	15	7	5	33,3	3
line 15-12 (5S)	7	5	2	28,6	0
line 10-7 (1R)	29	15	10	34,5	4
line 15-8 (no transl.)	3	0	2	66,6	1
line 14-8 (1R, 5S)	96	48	42	43,7	6
Total	151	76	62	**41**	14

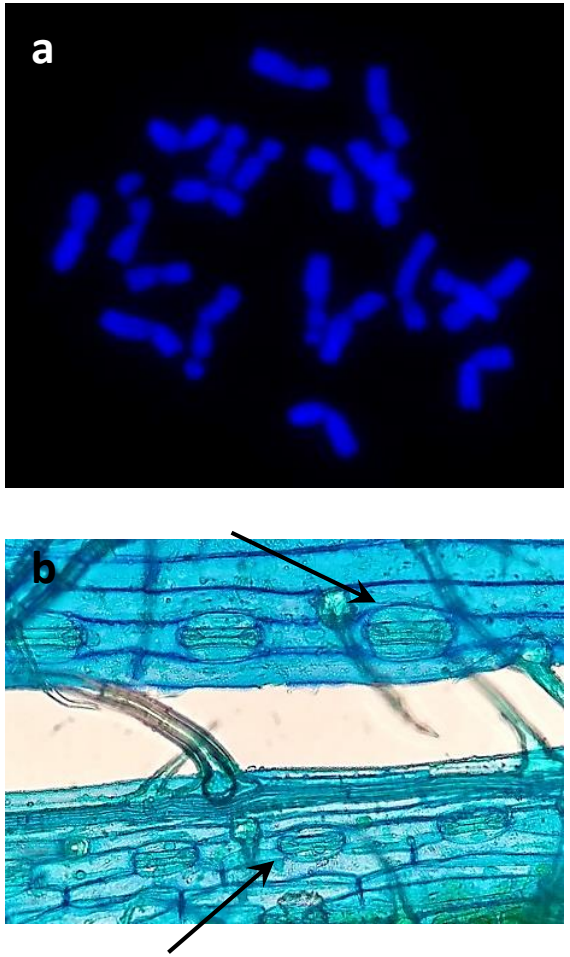
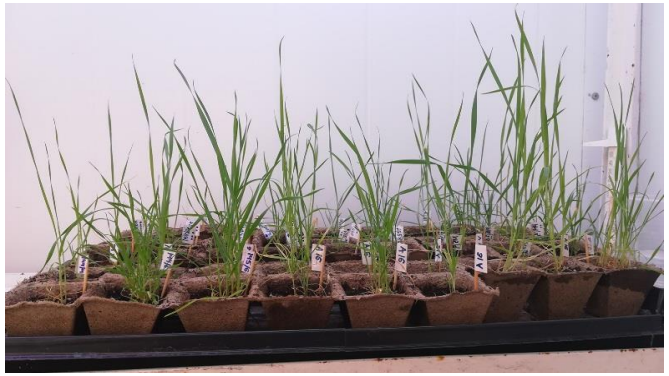


Figure 2. Metaphase chromosomes of a haploid regenerant plant (n = 21) (a); stomatal guard cell of double haploid (above) and haploid plants (under) (b)

Figure 4. Wheat doubled haploid plants growing in the greenhouse



Conclusion

Thus, double haploid common wheat lines with different combinations of alien translocations in the genome were produced and it was shown that parameters of androgenesis varied depending on line. According the results, the best-responding lines 991, 10-7 and 14-8 are characterized by the presence of 1RS.1BL wheat-rye translocation chromosome in genome. So, number of ELS/100 anthers of those lines was recorded to be 13; 38,5 and 50 and frequency of green plants – 4%, 4,2% and 11,3% respectively. The values of the parameters for lines 15-12 (carrying 5BS.5BL-5SL translocation from *Aegilops speltoides)* and 15-8 (without translocations in the genome) did not differ significantly. Therefore, it can be concluded that the presence of the introgressive fragment of chromosome 5S did not affect the efficiency of androgenesis and short shoulder of the chromosome 1R carries genes that stimulated androgenesis in anther culture. The seeds of 48 DH plants obtained from hybrid lines were replicated to be used in breeding programs

References

1) Pershina L., Trubacheeva N., Badaeva E., Belan I., Rosseeva L. Study of Androgenic Plant Families of Alloplasmic Introgression Lines (*H. vulgare*) –*T. aestivum* and the Use of Sister DH Lines in Breeding. *Plants*. 2020; 9(6):764. https://doi.org/10.3390/plants9060764
2) Сибикеева Ю.Е., Сибикеев С.Н. Влияние комбинаций чужеродных транслокаций на андрогенез *in vitro* у почти изогенных линий яровой мягкой пшеницы Генетика. 2014. т.50. № 7. С. 831 – 839. Weigt D., Kiel A., Nawracała J., et al. Obtaining doubled haploid lines of the Lr19 gene using anther cultures of winter wheat genotypes. *BioTechnologia*. 2016, 97(4):285-293. doi:10.5114/bta.2016.64545
3) Osadchaya, T.S., Pershina, L.A., Trubacheeva, N.V., Belan, I.A., Rosseeva, L.P., Devyatkina, E.P. Androgenetic ability in euplasmic lines of common wheat and alloplasmic recombinant lines (*H. vulgare*)–*T. aestivum* carrying 1RS.1BL and 7DL_7Ai translocations and development of double haploid lines. *Russ. J. Genet. Appl. Res.* 2015, 5, 174–181.
4) Chu C.C., The N6 Medium and its Application to Anther Culture of Cereal Crops. *Science Press*, 1978, Beijing, China, pp: 43-50.
5) Gamborg O.L., Miller R.A., Ojima K. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 1968, 50, 151–158

This work was done within the framework of State Assignment Kurchatov Genomic Center of ICG SB RAS (075-15-2019-1662).