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ORIGINAL ARTICLE

Somaclonal variation in spring bread wheat

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We evaluated the quantitative and qualitative characteristics of somaclones of the first generation (SC_1) obtained in vitro from 11 spring wheat varieties in the culture of immature embryos. Phenotypic deviations from the parental forms were found in 28.7% of the regenerated plants. The frequency of variability ranged from 20 to 56.6%, depending on the donor of the explant. We found that the genotype, as well as the changes that occur at the cellular level during the cultivation, affect the variability of the traits.
Key words: spring bread wheat; callus; somaclonal variation; regenerant

The successful implementation of crop breeding programs is largely determined by the genetic diversity of the initial populations. Biotechnological approaches based on the possibilities of cultivating plant cells in vitro offer a new tool for increasing genetic variability, which allows combining classical and innovative methods in plant breeding (Rai et al., 2011). With the development of plant regeneration techniques from callus tissue, it became possible to obtain new forms that differ in various characteristics from the original plants. Such diversity among cell lines and regenerated plants is called "somaclones", and the phenomenon is called "somaclonal variation" (Larkin & Scowcroft, 1981). The genetic nature and mechanisms of somaclonal variability have been the subject of close attention over the past decades. Cytogenetic analysis of regenerants revealed the presence of such rearrangements of chromosomes as translocation, inversion, subchromatid exchange, partial loss of chromosomes. This proves that most phenotypic changes are due to genetic mechanisms. In addition to somaclonal variability associated with inherited rearrangements of the genome, phenotypic changes (“epigenetic”) are noted, which can be stably transmitted to daughter cells, but not manifested in regenerant plants or their sexual progeny. The emergence of somaclonal variations depends on the genetic heterogeneity of somatic cells of the explant and conditions of cell cultivation (Miguel et al., 2011), which primarily include nutrient media and plant growth regulators, as well as the genotype (Hussain et al., 2001).
To date, it has been proven that somaclonal variants can exist as genetically stable forms and inherit various traits (Larkin et al., 1984). The wide variability of regenerants is found in wheat (Akhtar et al., 2012), maize (Dolgikh, 2005), rice (Zhang et al., 2014), millet (Baer et al., 2007), soybeans, rapeseed and other crops. In particular, somaclonal variants of wheat with high yields, increased resistance to diseases, earlier maturity, drought, salinization and heavy metal tolerance (Arun et al., 2003; Akhtar et al., 2012; Mahmood et al., 2014) were created. Thus, somaclonal variability can be considered as an effective tool for creating genetic diversity of plants, which is an alternative to classical methods based on crossing and the using mutagens. The widespread involvement of somaclones in the breeding process of crops implies the availability of reliable information on the spectrum and frequency of such variations, beginning with the early generations of regenerants, as well as the stability of modified traits in the offspring.
The purpose of this study is to assess the quantitative and qualitative variability of spring bread wheat regenerants of the first generation (SC_1), obtained in vitro in the culture of immature embryos.

Materials and methods

We studied regenerants of 11 varieties of spring bread wheat of various ecological and geographical origin: Altajskaya 50, Skala, Zhnitsa, Tselinnaya 20, Tselinnaya 60, Zarnitsa, Tulunskaya 10, Botanicheskaya 2 (Bot. 2), Spektr, Vega, Leones. Donor plants were grown at the Altai Research Institute of Agriculture, The Altai Territory, Russia. For callus induction, we used immature embryos 1.3–1.5 mm in size. The explants were passaged on Linsmeier and Skoog nutrient medium (Linsmaier & Skoog, 1965) containing 0.8% agar, 3% sucrose, 2 mg / L 2.4-D. Cell cultures were grown in the dark at a temperature of 25 ± 1 °C, transplanting every 30-35 days to a differentiating medium containing 0.5 mg / L 2.4-D and 0.5 mg / L kinetin. The morphogenic zones were transferred to regeneration medium containing 0.2 mg / L indole-3-acetic acid (IAA). Seedlings, which reached 5–7 cm, were planted in vessels with soil and grown to maturity in a Memmert climate chamber at a temperature of 12 °C at night, 17 °C during the day with a 16-hour photoperiod.

We studied various morphological and economic traits of somaclones including productive tillering, the length of the main spike, the number of spikelets and grains of the main spike, the mass of grain in the main spike, the mass of grain in plant, the mass of 1000 grains, height of the plant. Quantitative variability was evaluated using the coefficient of variation. Statistical data processing was performed using Microsoft Excel 2010.

Results

We studied 24,800 spring bread wheat regenerants. About 37% of the plants did not form spikes or were sterile. Such serious deviations from normal development appear to be associated with large chromosomal rearrangements or aneuploidy. Seeds gave 40.7% (10,100 pcs.) of regenerants. The rest of the plants died due to poor survival when transplanted into the soil. Regenerants SC_1 showed slow growth in the early stages of development. A comparative analysis of the morphobiological features revealed their significant difference from the original parental forms. Deviations concerned both the spike phenotype and the stem. Figure 1 shows the spectrum and frequency of the release of plants of a certain phenotype, different from the parent variety, which was an explant donor.

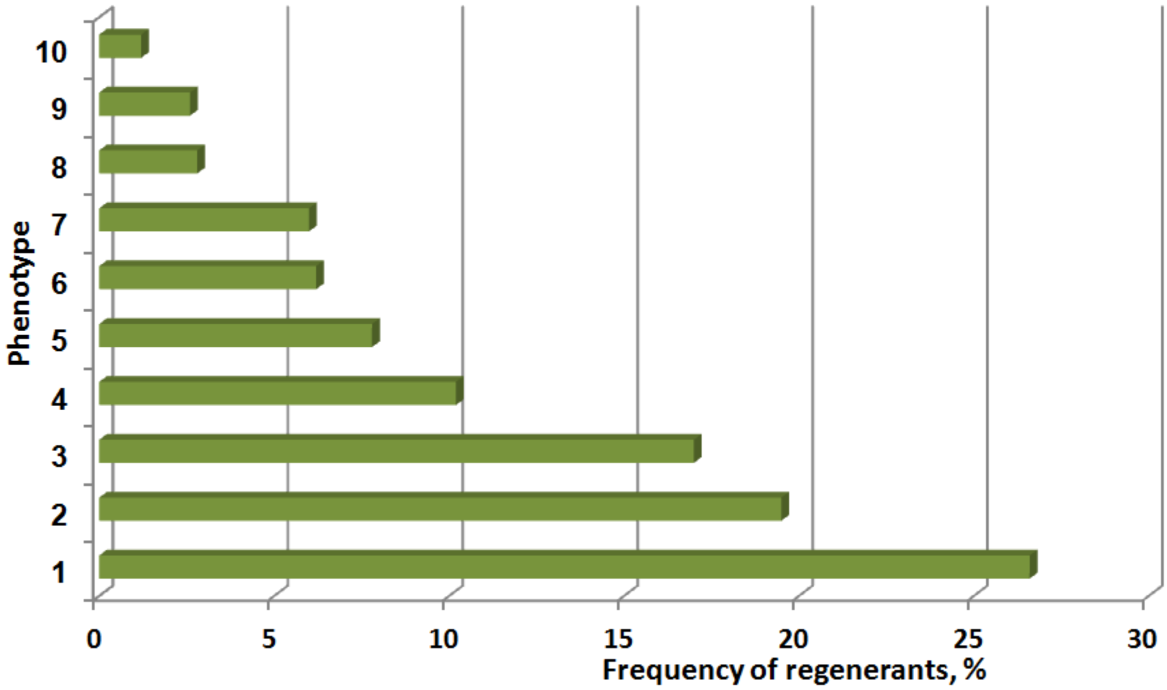

Fig. 1. Spectrum and frequency of modified traits in SC_1 regenerated plants of spring bread wheat, %:
1 – sterile spike; 2 – fine grain; 3 – intense tillering; 4 – modified spike color; 5 – shortened spike; 6 – dwarfism; 7 – awnless spike; 8 – curved stem; 9 – square-shaped spike; 10 – modified stem color

About a third part of regenerants (28.7%) had phenotypic deviations from the original varieties. Among them, more than 46% of the plants formed sterile spikes or very fine grains with low germination. Seventeen percent of somaclones were distinguished by intense tillering, which led to the formation of a large number of stems. A change in the color of the spike (red or white) was observed in 10.2% of the plants. Approximately in equal shares (6.0–7.8%) there were dwarfs, awnless forms and plants with a shortened spike with reduced spikelets. A small part of the plants had a curved stem, a modified stem color and a square-shaped spike. The latter phenotype had a club-shaped form with a compacted arrangement of spikelets in the apical part of the spike.

The spectrum and frequency of modified forms were specific for regenerants of each variety. The range varied from 20.0 (Tselinnaya 20) to 56.6% (Botanicheskaya 2), and the number of modified traits varied from 2 to 8, depending on the variety (Fig. 2). For example, only two modified traits were observed in Tselinnaya 60, eight traits were found in Spektr, and seven were revealed in Zhnitsa. It should be noted that some morphological features were found only in some genotypes. For example, a curved stem was found only in the regenerants of Spektr and Leones. Some somaclones of Zhnitsa and Vega had anthocyanin color of the stem and spike.

We studied economically valuable traits that affect yield, in 364 regenerants derived from 5 wheat varieties (Table 1). It was established that the frequency of modified traits differed significantly depending on the genotype of the explant donor. Somaclones derived from immature embryos of Botanicheskaya 2 and Zarnitsa were less variable in all tested traits, with the exception of productive tillering and the number of grains of the main spike. Therefore, it is possible that varieties with a wide range of variation, such as Skala, Spektr and Zhnitsa, will be of the greatest interest for obtaining modified forms. According to the coefficients of variation, most of the presented traits of regenerants obtained from these varieties belonged to the "highly variable" rank.

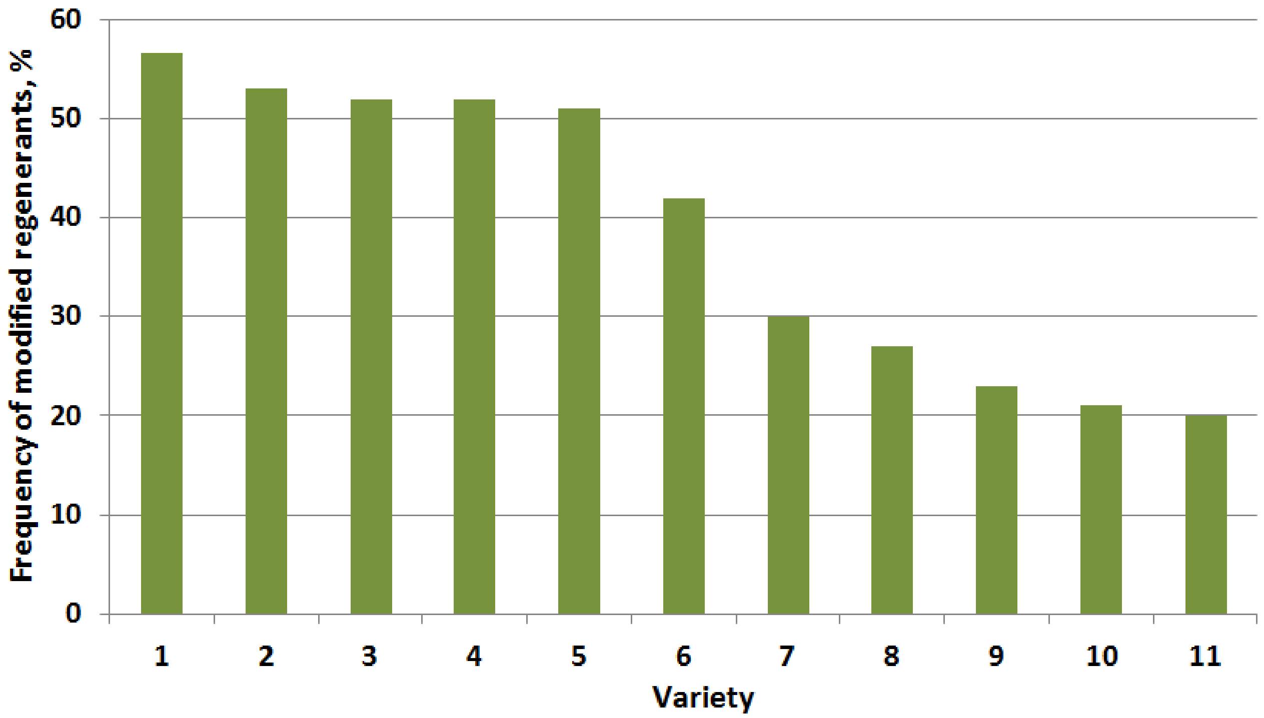

Figure 2. The frequency of plants with modified traits among regenerants of spring bread wheat, %:
1 – Botanicheskaya 2; 2 – Zhnitsa; 3 – Tselinnaya 60; 4 – Altajskaya 50; 5 – Tulunskaya 10; 6 – Vega; 7 – Skala; 8 – Zarnitsa; 9 – Spektr; 10 – Leones; 11 – Tselinnaya 20

Table 1. Characteristics of somaclones of the first generation (SC_1) of spring bread wheat

Variety, callus	Trait							
	Plant height, cm	Productive tillering, pcs.	Spike length, cm	Number of spikelets of the main spike, pcs.	The number of grains of the main spike, pcs.	Mass of 1000 grains, g	Mass of grain in the main spike, g	Mass of grain in plant, g
Skala, 36.6	49.9 29.7	1.9 57.9	4.6 18.0	6.3 25.6	12.9 30.1	34.0 16.9	0.6 52.7	0.5 67.3
Skala, 3.6	65.0 16.6	2.2 49.5	5.5 14.4	10.2 12.7	18.2 33.0	35.2 18.5	0.7 45.5	1.1 39.5
Skala, 2.4	66.8 34.2	4.0 22.3	6.1 35.0	8.3 35.3	21.0 38.8	33.1 7.1	0.9 54.2	2.2 77.8
Skala, 6.3	60.9 18.4	2.4 32.5	5.3 25.9	8.6 37.9	16.9 48.6	32.9 9.9	0.6 50.0	1.3 78.6
Skala, 40.4	76.4 7.2	3.4 64.4	7.2 16.0	12.6 16.4	24.2 13.8	41.7 7.7	1.0 22.5	2.7 88.8
Skala, 37.3	81.0 10.5	4.0 39.5	7.8 18.9	12.6 17.5	28.8 24.6	51.1 29.7	1.1 19.6	3.4 47.7
Skala	67.3 22.3	3.4 72.8	6.3 27.2	10.3 30.1	20.2 47.8	36.0 23.1	0.8 57.5	1.9 92.7
Spectr, 32.1	59.1 4.1	2.9 50.0	5.5 13.5	8.5 10.8	15.7 8.0	40.9 4.9	0.7 37.1	1.3 27.7
Spectr, 18.4	30.5 17.0	1.8 41.7	2.8 41.4	4.2 41.0	5.0 56.4	67.4 56.1	0.3 110.0	0.5 108.0
Spectr,	46.7 36.5	2.9 62.7	4.6 42.4	6.8 43.8	12.0 69.8	44.4 37.3	0.5 75.5	1.2 100.0
Bot. 2	66.9 13.4	3.1 40.0	6.8 15.7	11.6 16.6	21.8 57.2	41.9 14.8	1.0 56.9	2.2 58.2
Zarnitsa	68.1 17.5	3.0 59.0	7.5 21.2	10.3 30.7	19.4 51.6	42.2 25.0	0.9 39.5	4.0 66.8
Zhnitsa	72.8 42.3	3.5 68.0	8.5 35.3	11.5 45.7	23.0 62.2	57.2 46.0	1.2 70.0	3.7 58.2

Note: the numerator is the average value of the trait; denominator is coefficient of variation.

It should be noted that the manifestation of traits in plants is influenced not only by the donor's genotype, but also by changes that occur at the cellular level in vitro during the cultivation. This is evidenced by the differences among somaclones derived from the same callus. So, in the variety Skala, among 10 regenerants of one callus, there are plants with a height from 29 to 75 cm. That is, from one embryo one can get both long-stem and dwarf forms. In addition, the level of variability and the value of the trait varies depending on the calli obtained from the explants of the same variety. The coefficient of variation in plant height for regenerants of Skala ranged from 7.2 (weak variation) to 34.9% (strong variation). Productive tillering varied from 1.9 to 4.0 stems per plant, and the grain weight per plant reached 7-fold differences in regenerants originating from different explants.
We also studied a mass of 1000 grains. This feature is used in breeding for productivity, since it is closely related to it. In addition, it has low modification variability. Larkin et al. (1984), studying changes in yields, mass of 1000 grains, and other traits in three successive generations of regenerated plants, confirmed their genetic nature. Table 2 shows the data on the mass of 1000 grains in regenerants of 10 varieties.

Table 2. The mass of 1000 grains in the SC_1 plants of spring bread wheat

Variety, callus	The number of plants, pcs.	Mass of 1000 grains, g (variation)	The coefficient of variation, %
Spektr	501	40.1 ± 0.2 (7–24)	83.3
Spektr, 2.5	20	43.5 ± 0.8 (32–66)	27.0
Spektr, 1.2	28	45.0 ± 0.7 (30–60)	32.8
Spektr, 2.10	22	35.5 ± 0.8 (21–50)	40.3
Spektr, 1.12	20	35.3 ± 0.8 (23–47)	34.0
Spektr, 19.	22	41.0 ± 0.4 (10–54)	23.2
Spektr, 4.7	24	30.3 ± 0.6 (21–39)	29.5
Spektr, 3.2	29	37.9 ± 0.7 (25–51)	33.4
Spektr, 4.5	55	42.5 ± 0.9 (32–52)	23.3
Skala	465	30.1 ± 0.1 (20–40)	34.5
Skala, 6.5	21	25.8 ± 0.7 (14–17)	45.0
Skala, 6.2	17	34.2 ± 0.7 (26–43)	24.9
Skala, 16.6	24	33.0 ± 0.7 (21–45)	35.1
Skala, 19.1	24	32.2 ± 0.7 (20–35)	38.7
Skala, 13.3	37	29.7 ± 0.5 (21–40)	27.8
Skala, 16.1	20	30.0 ± 0.7 (19–41)	37.0
Skala, 1.7	23	35.5 ± 0.5 (31–41)	14.2
Skala, 4.6	16	37.4 ± 0.7 (32–42)	14.7
Zarnitsa	26	39.4 ± 0.6 (28–51)	28.5
Zhnitsa	25	34.0 ± 0.6 (24–44)	29.0
Tulunskaya 10	18	33.7 ± 0.7 (25–42)	25.5
Botanicheskaya 2	22	37.0 ± 0.7 (25–49)	32.3
Leones	33	30.3 ± 0.5 (23–39)	25.5
Tselinnaya 20	84	39.0 ± 0.6 (33–45)	14.4
Vega	37	30.4 ± 0.9 (19–42)	38.5
Altajskaya 50	7	42.5 ± 0.9 (36–49)	14.7

We found that the range of variation of the trait between varieties is less than within one variety. The average values of the trait depending on the parent genotype ranged from 30.1 (Skala) to 42.5 g (Altajskaya 50). Whereas in Spektr there are plants with a mass of 1000 grains from 7 to 66 g. In other varieties, the differences between the extreme values are less noticeable, as evidenced by the coefficients of variation, the values of which vary depending on the variety from 14.4 (Tselinnaya 20) to 38.5% (Vega). Analysis of the variability of the trait among regenerants of one callus showed its significant variation. The variability ranged from 23.2 to 40.3% in Spektr and from 14.2 to 45.0% in Skala.

Conclusion

Thus, a significant diversity among SC_1 regenerants of spring bread wheat, derived from the culture of immature embryos, indicates the possibility of obtaining forms with economic and biological modified traits, which allows them to be used as a starting material in the breeding program.

References

Akhtar, S., Niaz, M., Rahman, S., Asif, M. (2012). Study of somaclonal variation in wheat for the induction of salinity tolerance. Journal *of A*gricultural Research, 50(2), 165–176.
Arun, B., Joshi, A.K., Chand R., Singh B.D. (2003). Wheat somaclonal variants showing earliness, improved spot blotch resistance and higher yield. *Euphytica*, 132, 235–241.

Baer, G.Y., Emec, A.I., Stadnichuk, N.A., Rahmetov, D.B., Blyum, Y.B. (2007). Somaklonal'naya variabel'nost' kak istochnik dlya sozdaniya novyh sortov pal'chatogo prosa *Eleusine coracana* (L.) Gaertn. *Citologiya i genetika*, 4, 9–14. (In Russian)
Dolgih, Y.I. (2005). Somaklonal'naya izmenchivost' rastenij i vozmozhnosti ee prakticheskogo ispol'zovaniya (na primere kukuruzy): Theis of Doctoral Dissertation. Moscow. (In Russian)
Hussain, M., Khan, G.S., Shaheen, M.S., Ahmad, M. (2001). Somaclonal variation in regenerated plants of ten wheat genotypes. *Journal* of A*gricultural Research*, 39(1), 1–7.
Larkin, P.J., Ryan, S.A., Brettel, R.I. (1984). Heritable somaclonal variation in wheat. *Theoretical and Applied Genetics,* 67(2), 443–445.
Larkin, P.J., Scowcroft, W.R. (1981). A novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics*, 60(4), 97–214.
Linsmaier, E., Skoog, F. (1965). Organic growth factor reqularments of tobacco tissue culture. *Physiologia Plantarum*, 18, 1, 100–127.
Mahmood, I., Razzaq, A., Rasheed, M., Qayyum, A., Ahmad, M. (2014). Employment of immature embryo culture for in vitro selection of drought tolerant somaclones of wheat. *Bulgarian Journal* of *Agricultural Science*, 20, 155–161.
Miguel, C., Marum, L. (2011). An epigenetic view of plant cells cultured in vitro: somaclonal variation and beyond. *Journal of Experimental Botany*, 62(11), 3713–3725.
Rai, M.K., Kalia, R.K., Singh, R., Gangola, M.P, Dhawan, A.K. (2011). Developing stress tolerant plants through in vitro selection – An overview of the recent progress. *Environmental and Experimental Botany*, 71, 89–98.
Zhang, D., Wang, Z., Wang, N., Gao, Y., Liu, Y. et al. (2014). Tissue culture-induced heritable genomic variation in rice, and their phenotypic implications. *PLoS ONE*, 9(5), e96879. doi:10.1371/journal.pone.0096879.

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