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Genetic engineering of russian wheat genotypes for abiotic stress resistance

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SUMMARY – The biolistic transformation protocol allowing generating a number of independent transgenic wheat plants has been worked out. A complete process from the introduction of foreign DNA into wheat cells to the field trials have been developed for several Russian wheat cultivars. Homozygous T2-T4 transgenic wheat progeny constitutively expressing herbicide resistance gene (*bar*) and soil salinity resistance gene (*hvnhx2*) were generated. Field tests of T3/T4 homozygous offspring demonstrated that agronomic traits and yields of transgenic lines treated with herbicide (1.0% Basta) and non-treated transgenic/non-transgenic plants are comparable or higher for transgenic lines. Transgenic wheat plants expressing gene of barley Na^+/H^+ vacuolar antiporter were able to grow, flower, and produce seeds in the presence of 200 mM sodium chloride. In the greenhouse the growth of the T1 transgenic wheat plants in saline conditions was about 80% of the same in non-saline conditions, while the non-transgenic progeny showed 50% delay in the growth.

Introduction

Genetic transformation of bread wheat (*Triticum aestivum* L.) offers numerous important opportunities for the improvement of existing varieties. Wheat is the major cereal crop in Russia. Transgenic wheat plants with constitutive expression of herbicide resistance gene (*bar*) and soil salinity resistance gene (*hvnhx2*) have been produced and investigated in order to satisfy the demand for Russian wheat varieties with resistance to diseases and other environmental stresses.

Generation of herbicide-resistant transgenic wheat plants

Currently the biolistic transformation approach is the most popular choice for generation of useful transgenic wheat germplasm in a genotype-independent manner. In our research a particle inflow gun was used to deliver DNA-coated tungsten particles into the immature embryos. A dual screening/selection approach based on the combination of *gfp* gene as vital reporter gene and *bar* gene as transgenes selection one was chosen for generation of transgenic wheat. 47 independent transgenic lines of Russian spring wheat variety 'Andros' were produced after transformation by vectors psGFP-BAR (Richards *et al*., 2001). GFP fluorescence-based segregation tests were performed at the expression level, and at the structural level by means of PCR to confirm the presence of *bar* gene. Most of transgenic lines belonged to T1 and T2 hetero- and homozygous progeny showed resistance to high concentration of Basta herbicide (up to 2.5 % while spraying. Following the segregation analysis fifteen T3 homozygous progenies from seven T0 transgenic lines were selected for evaluation of herbicide resistance in field tests.

In 2004 we performed field trials to assess the real effect of transformation on the agronomic performance of transgenic plants under field conditions. Field trials were designed as a randomized complete block and grown without irrigation. T3 homozygous plants resulted from T1 progenies of seven self-pollinated T0 plants of 'Andros' were sprayed with 1% Basta. Non-transgenic wheat plants showed no signs of resistance in a week after the treatment, while most of transgenic plants exposed high level of herbicide resistance (Fig. 1).

At the end of wheat cultivation, several traits such as plant height, weight of seeds at one spike and total yield were evaluated to determine the effect of herbicide treatment. In most cases the difference between transgenic lines treated with herbicide and non-treated transgenic/non-transgenic plants was not statistically confirmed, though certain losses and gains in yield were observed (Table 1). Two of 15 tested transgenic T1 lines had a genotypic difference against non-transgenic control. Line A-46/1 had a statistically valid lower plant height as compared to non-transgenic plants. Transgenic line A-18/47 showed the loss of yield (up to 30%) in sprayed and non-sprayed plots indicating that the observed difference is more related with somaclonal variation, rather than with herbicide treatment. The tendency to decrease yield compared to non- transgenic plants was observed for line A-6/12 also. For this line the post-treatment loss of yield seemed to be associated with insufficient expression of the *bar* genes, as the non-treated transgenic plants did not show such decrease. The result of this study showed that the agronomic traits and yields of transgenic and non-transgenic plants were comparable or even higher for most transgenic lines. Since the observed differences were unconfirmed statistically, we associated those variations with an environmental influence.

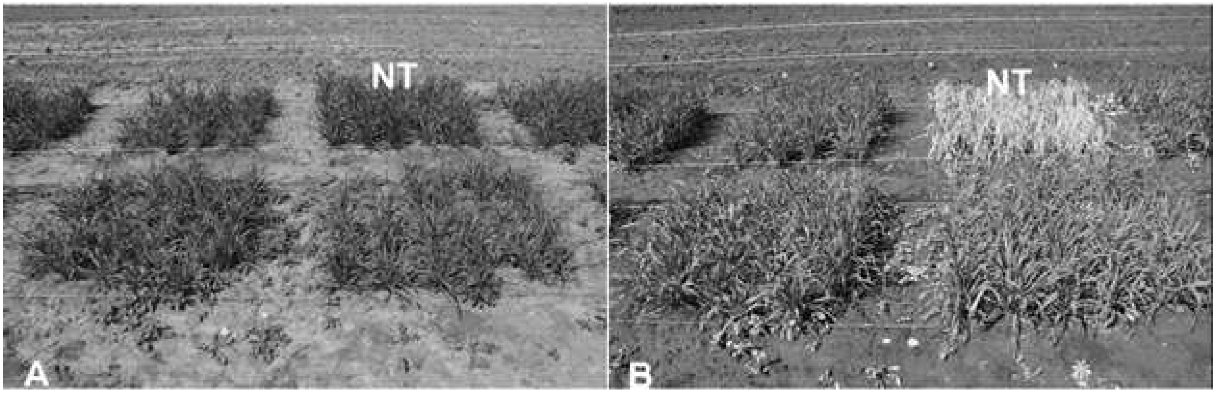

Fig. 1. Field trials for herbicide resistance of transgenic wheat lines (T3 progeny). A – plots before spraying with herbicide (1% Basta); B – resistance of plants after one week after spraying. NT – plots with non-transgenic plants.

Table 1. Evaluation of the field performance after herbicide (Basta 1.0%) spraying of transgenic wheat lines (T3 homozygous plants) transformed by *bar* gene

Transgenic line (T0→/T1)	Plant height, cm		Weight of seeds from one spike, g		Yield, kg/m^2	
	- Basta	+ Basta	- Basta	+ Basta	- Basta	+ Basta
Nontransgenic	1.13a	0.81c	1.32ab	0.83c	0.51ab	0.12c
A-03/4	1.07a	1.09a	1.56ab	1.48ab	0.48ab	0.63a
A-03/34	1.10a	1.12a	1.50ab	1.56ab	0.60ab	0.53ab
A-06/12	1.14a	1.15a	1.70a	1.40ab	0.56ab	0.44bc
A-18/47	1.04ab	1.12a	1.26ab	1.40ab	0.34bc	0.37bc
A-20/26	1.14a	1.14a	1.65ab	1.78a	0.47ab	0.50ab
A-20/28	1.10a	1.12a	1.63ab	1.46ab	0.65a	0.59ab
A-20/36	1.13a	1.07a	1.62ab	1.80a	0.59ab	0.59ab
A-20/38	1.14a	1.15a	1.78a	1.38ab	0.64ab	0.55ab
A-20/40	1.13a	1.12a	1.77a	1.60ab	0.47ab	0.55ab
A-34/9	1.09a	1.10a	1.68ab	1.66ab	0.61a	0.58ab
A-37/28	1.10a	1.08a	1.60ab	1.25ab	0.54ab	0.50ab
A-37/30	1.13a	1.15a	1.78a	1.71a	0.57ab	0.60ab
A-37/39	1.13a	1.12a	1.39b	1.47ab	0.51ab	0.52ab
A-37/45	1.14a	1.14a	1.55ab	1.37ab	0.61a	0.50ab
A-46/1	0.81c	0.77c	1.50ab	1.50ab	0.55ab	0.40bc

Production of salt-resistant wheat plants

At present almost 20% of the world’s cultivated lands and nearly a half of all irrigated lands are affected by salinity. In Russia soil salinity severely affects the productivity of cereals, including wheat. Recently genes encoding vacuole-type Na^+/H^+ antiporters have been shown to increase the salinity resistance of several transgenic species including rice (Ohta *et al*., 2002) and wheat (Xue *et al*., 2004). Na^+/H^+ antiporters exclude Na^+ from the cytosol and are localized in both plasma and vacuolar membranes. The barley *hvnhx2* antiporter gene was isolated by prof. A.V. Babakov's research group from All-Russia Research Institute of Agricultural Biotechnology. In this study the above-mentioned gene driven by the maize *Ubi1* promoter was introduced into the spring wheat genotype 'Andros'. The fourteen independent transgenic plants were generated via biolistic transformation. The expression of the *hvnhx2* gene in the transgenic plants was confirmed by mRNA detection. Three of nine analyzed transgenic lines showed the overexpression. Some of T1 offsprings obtained from self pollinated T0 transgenic wheat plants were grown in different saline conditions in order to examine whether the overexpression of the *hvnhx2* gene conferred the resistance to salt stress. Two-weeks-old plants were treated with 200 mM NaCl solution during 15 weeks. The appreciable differences in plant growth were observed between the *hvnhx2* transgenic and non-transgenic progeny of Hv-09 line (Fig. 2). The growth of the non-transgenic plants under the influence of NaCl was about 50% compared with the non-saline conditions. Transgenic progeny showed the delay of the growth as well; but it was about 20% only. Increased growth of the *hvnhx2* transgenic plants could be explained by the compartmentalization of Na^+ in vacuoles. These results demonstrate that transgenic wheat plants containing the *hvnhx2* gene are suitable for practical applications and are capable to produce crops even if they are exposed for a long period to a high concentration of saline.

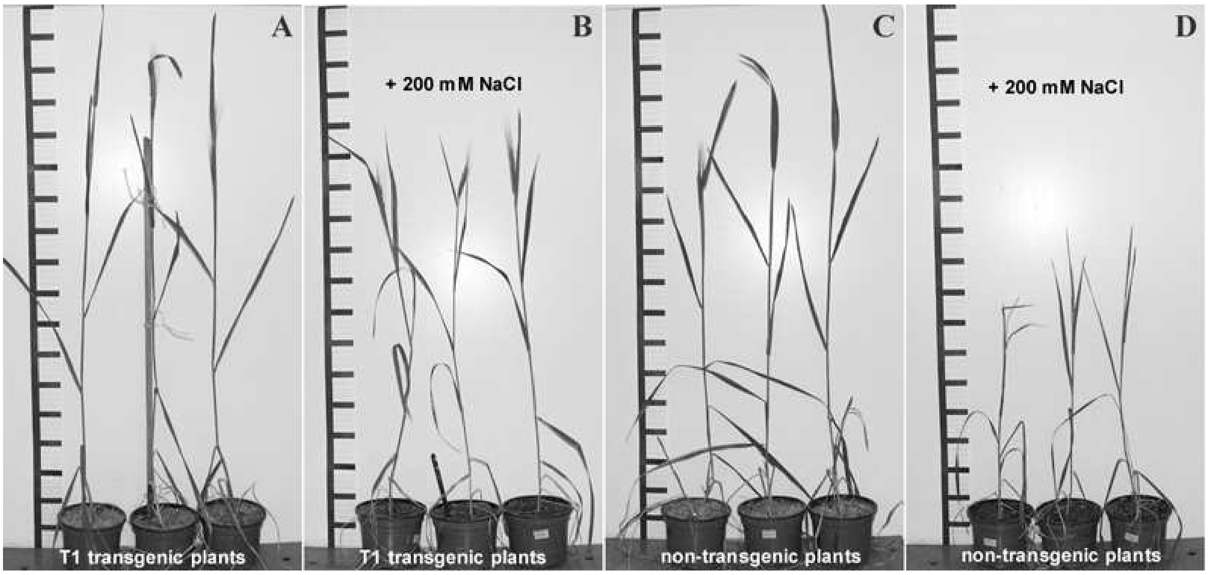

Fig. 2. Effect of salt on the growth of the T1 wheat progeny obtained by self-pollination of the primary T0 transgenic plant Hv-9. Plants were watered with a distilled water (A and C) or 200 mM NaCl (B and D). T1 transgenic plants (A and B) and non-transgenic plants (C and D) are shown after 15 weeks of salt treatment.

References

Ohta, M., Hayashi, Y., Nakashima, A., Hamada, A., Tanaka, A., Nakamura, T. and Hayakawa, T. (2002). *FEBS Letters*, 532: 279-282.

Richards, H.A., Rudas, V.A., McDanie, J.K., Tomaszewski, Z. and Conger, B.V. (2001). *Plant Cell Rep*., 20: 48-54.

Svitashev, S., Ananiev, E., Pawlowski, W.P. and Somers, D.A. (2000). *Theor. Appl. Genet*., 100: 872-880.

Xue, Z.-Y., Zhi, D.-Y., Xue, G.-P., Zhang, H., Zhao, Y.-X., Xia, G.-M. (2004). *Plant Sci.,* 167: 849-859.