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Using isogenic analysis to study genotype effect in in vitro cell and tissue culture of wheat.

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Isogenic analysis using NILs that differ in alleles for only one gene is an exact tool, allowing the establishment of straight and pleiotropic effects of concrete genes for various characters. Using this method it is possible to search genes with a strong positive effect for interesting characters.

Isogenic analysis is seldom used to study morphogenetic processes in cell and tissue culture *in vitro*. Practical use of in vitro isogenic analysis is especially useful when the genes are known to have a precise phenotypic effect and economic value, such as genes for short stalk.

For a number of years, we have screened a set of NILs of soft and hard wheat that differ for the short stalk character in *in vitro* anther and somatic tissue culture.

We found that the *Rht*, *s1*, and *Q* genes in the soft wheat Saratovskaja 29 influence morphogenic anther and haploid formation and regeneration in *in vitro* anther culture, the formation of meristematic tissue in somatic calli, and regeneration ability during long-term callus cultivation.

We also compared the influence of the *Rht-B1b* gene on stages of *in vitro* anther and somatic tissues cultivation in three varieties of hard wheat. For all investigated genes, the greatest positive effect was on haploids in anther culture and morphogenesis in somatic calli in a line with the *Rht-B1c* gene. The *Q* gene increases the frequency of haploid formation and plant regenerants in anther culture.

A serious restriction in using isogenic analysis is the creation of the NILs. Nevertheless, studying the effects of genes will help answer the question of the genetic processes proceeding in vitro cell and tissue culture.

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Wheat plant growth in the presence of aluminum ions is an indication of tolerance to aluminum toxicity.

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Aluminum toxicity is one of the most important issues for poor soils. Wheat makes poor growth and productivity if Al ions are present in the soil. We have established that some wheats have a positive reaction Al ions. When the spring wheat cultivar Lada was grown in an aluminum solution (Ca (1 x 10^{-4} M) + Al (1 mg/l)), leaf length was greater than that of the control plants (Ca (1 x 10^{-4} M)) (Fig. 1). This result was unexpected. We later determined that the increase in yield in same variant in vegetation tests. However, not all wheat plants are capable of increased growth and yield in the presence Al ions.

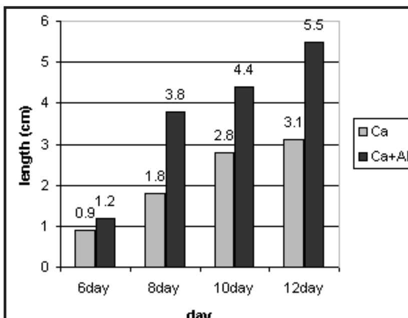

Fig. 1. Leaf length of the spring wheat cultivar Lada grown in an aluminum solution (Ca (1 x 10^{-4} M) + Al (1 mg/l)) and the control plants (Ca (1 x 10^{-4} M))

Materials and Methods. For vegetation tests, the spring wheat cultivars Voronegskaya, Yugo-vostochnaya 2, Kerba, and Omskaya 24 were grown in cells filled with sod-podzol soil with medium macroelement availability, pH 5.2–5.6 (in different test years). The cultivars were grown in 6l plots. The procedure included a control (without aluminum), Al1 (6 mg/kg soil), Al2 (13 mg/kg soil), Al1+K (100 mg K/kg soil), and Al2 + K (100 mg K/kg soil).

Results and Discussion. We determined that productivity gains in Al1 and Al2, in the presence of aluminum ions, exceeded the control and the variants with potassium. These data do not confirm nor disprove the hypothesis that potassium ions in a soil solution would diminish aluminum toxicity; aluminum is toxic as it is. However, detailed analysis of the situation helped unveil the reason of such results. Initially, the test in distilled water was conducted to exclude irrelevant effects. Wheat seedlings were grown in distilled water with using a control, 1 mg/l Al, 3 mg/l Al, 12 mg/l Al, and 40 mg/l Al. The results did not show a strict dependence in root length in the test variants, but we explicitly established activate growth in the above-ground parts of the plant at very low concentrations of aluminum in the medium (1 and 3 mg/l). These plants were called aluminum sensitive (Table 1). Less sensitive cultivars would have above-ground growth at higher aluminum concentrations.

Table 1. Length of wheat lseedlings at the 10 day (cm) in solution containing aluminum ions.

Aluminum concentration	Cultivar				
	Voronegskaya	Irgina	Priokskaya	Omskaya	Kerba
Control	9.5	13.5	12.1	12.1	14.3
1 mg/l Al	11.0	14.8	12.1	8.7	14.2
3 mg/l Al	10.7	13.2	13.9	10.5	14.0
12 mg/l Al	10.5	13.5	12.7	12.5	10.7
40 mg/l Al	10.6	13.3	11.3	9.2	13.4

Exactly which cultivars are capable of active growth in the presence of aluminum at the lowest possible concentrations have yield ability exceeding that of the control (Fig. 2). These results show that those cultivars capable of steady growth on soils containing aluminum ions are also highly sensitivity to aluminum ions at the lowest possible concentrations at the earliest stages of development. The capability for sensing aluminum ions enabled plants to activate the mechanism to adapt and gain steady growth.

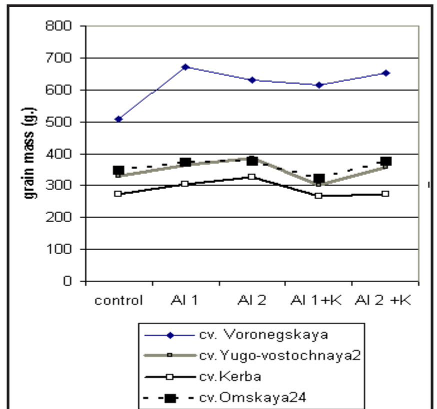

Fig 2. Grain yield (g/plant) for the cultivar Lada in the control (Ca) and in a solution of Ca + Al.

We know that potassium ions diminish the effects of aluminum toxicity and increase the general competition of ions in solution. However, in vegetative tests, the variants with added potassium ions showed low fertility, below that of control plants. Based on the activation of adaptation to aluminum ions by the reduction in the effects of aluminum toxicity by potassium ions, we believe that potassium ions are the reason for a plants inability to develop full tolerance against aluminum. The early growth phases, i.e., the period of adaptation against edaphic stress, on basis of aluminum ion sensitivity is vitally important for wheat cultivars capable of steady growth and adaptation to aluminum ions.

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The oxidation of saturated free fatty acids by winter wheat mitochondria.

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The most important catabolic pathway of free fatty acids (FFA) is β-oxidation with acyl-CoA formation, which is further fully oxidized in the Krebs cycle to CO_2 and H_2O. Animal β-oxidation is known to take place in mitochondria and peroxisomes (Schulz H 1991). Questions about the localization of plant mitochondrial β-oxidation was under discussion