

The influence of the toxic effect of zinc and mineral starvation on the growth and development of buckwheat regenerants in vitro culture

Borovaya S.A., Klykov A.G.

Federal Scientific Center of Agrobiotechnology in the Far East named after A.K. Chaika, Timiryazevsky stl., Ussuriysk, Russia



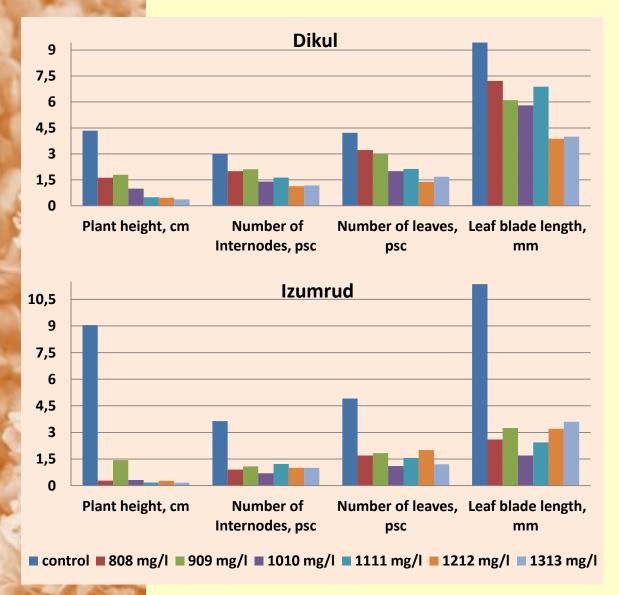
Purpose of the work: studying morphological characteristics and general non-specific adaptive response of common buckwheat plant regenerants *in vitro* culture, resistant to the toxic effect of zinc ions and mineral starvation.

Materials and Methods

Initial plant material: varieties of *Fagopyrum esculentum* Dikul and Izumrud. Primary explants are mature seeds sterilized according to V.A. Tilba (1971). They were placed in test tubes with cotton gauze plugs on Murashige-Skoog medium (hereinafter MS) and cultured for 30 days for microcloning and obtaining regenerants (5 passages).

The experiment and composition of selective media. We used zinc salts at a concentration of 808-1313 mg/l $ZnSO_4 \times 7H_2O$, the control variant - MS. MS was prepared without macrosalts to simulate mineral starvation. Aseptic single-node cuttings 0.7-1.5 mm long with an axillary bud obtained by dividing the stem of the resulting regenerants were cultivated for 33 days. on control and selective media with Zn^{2+} (20 tubes per each variant). Surviving plants were microcloned on nutrient media without macrosalts, incubation duration was 40 days. Subsequently, 2 passages were made, lasting 33 days on the MS.

Morphological parameters of buckwheat varieties Dikul and Izumrud *in vitro* culture on selective media with Zn²⁺ on the 33rd day of cultivation



Cultivation of buckwheat varieties on selective media with zinc for 33 days led to inhibition of the growth and development of microplants in all variants. Izumrud turned out to be more sensitive to the toxicant.

In the first three variants (808-1010 mg/l of zinc salt) of Izumrud variety, 25-30% of microplants were culled, in the next three, with an increase in the concentration of zinc salt (1111-1313 mg/l) - 50-67%, while in Dikul variants 9-18% and 18-45% were culled, respectively.



Influence of mineral starvation on the survival of buckwheat plants obtained after cultivation on selective media with zinc (808-1313 mg/l ZnSO₄ x 7H₂O)

Variety	The number of microplants in the variants of the experiment, pcs.							The total number of
	Control (without zinc)	808 mg/l (l)	909 mg/l (II)	1010 mg/l (III)	1111 mg/l (IV)	1212 mg/l (V)	1313 mg/l (VI)	survived micro- plants, pcs.
Izumrud	50/11	14/4	18/4	14/10	12/2	8/2	8/2	35
Dikul	50/34*	20/10*	18/0	14/6	16/6	16/6	12/4	50

Note: above the line - the number of microclones at the beginning of cultivation; below the line - the number of surviving microplants at the end of cultivation;

Cultivation of microclones obtained on control variants and selective media with zinc salt under conditions of complete mineral starvation in vitro on MS medium without macrosalts for 40 days turned out to be the strongest stress for them: most plants died, and the rest looked like underdeveloped leaf rosettes or small microplants 1.5-2 cm long with 1-2 small (2-3 mm) leaf blades and without roots. Dikul showed an increased adaptive response to a complex stressor.

^{* -} presence of roots in microplants.



Microplants of Dikul and Izumrud varieties tolerant to the complex stressor





Dikul Izumrud

The result of the study made it possible to identify regenerants of common buckwheat sowing varieties Izumrud and Dikul, the most resistant to complex stress caused by high doses of zinc and mineral starvation *in vitro* culture. A higher level of stress resistance and regenerative capacity of the Dikul variety was revealed in comparison with the Izumrud variety. The test tube regenerant buckwheat plants obtained under selective conditions are a promising material for the purposes of further breeding, as well as for studying the possibility of their use as phytoremediators.



We would like to express our gratitude to E.N. Barsukova, the Head of the Laboratory of Agricultural Biotechnology, for the methodological guidance in conducting experiments.

Thank you for your attention!