

APPROBATION OF AGROBACTERIAL TRANSFORMATION OF SUNFLOWER BY IN PLANTA METHOD

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The results of approbation of agrobacterial transformation of sunflower by in planta method are given. The evaluation of resistance of derived forms of sunflower to herbicide with phosphinothricin as active ingredient is made. Plants that inherited resistance to the herbicide were selected.

Sunflower is the main oil crop in Ukraine. Seeds of its recognized varieties and hybrids contain 50-52 % of oil and selection varieties contain up to 60%. Compared with other oil crops sunflower provides the highest yield per unit area (750 kg/ha nation average).

However, high weediness of crops and weed infestation of soil create intense competition to sunflower plants, lead to significant losses of nutrients and moisture, their shading and suppression, and finally to reduction of yields of sunflower hybrids and varieties [2]. Usage of non-selective herbicides is highly efficient method of combating dicotyledonous, monocotyledonous, annual and perennial weeds [3].

To create plants resistant to herbicides more and more attention is paid to methods of transformation, which allow preventing long-term manipulations with recipients plants. Today it is possible to obtain transgenic plants without any procedures *in vitro* using natural transformation of Ti-plasmid plants (Eng. *Tumor inducing*), soil agrobacteria (*Agrobacterium tumefaciens*), through which you can introduce the foreign genes and relatively large gene constructs in the genome of dicotyledonous and some monocotyledonous plants [1]. To do this the method called *in planta* offered by Bechtold and co-auth. in 1993 is used [5].

The aim of the research was the approbation of agrobacterial transformation of sunflower by *in planta* method.

Methods of the research

The research was conducted at the Department of Genetics, Plant Selection and Biotechnology of Uman National University of Horticulture during 2011-2013, in two phases; laboratory and field.

As the recipient sterile parent forms of sunflower hybrid Ukrainskyi F1, Ukrainskyi skorostyglyi, Ukrainske sonechko, registered in the State Register of Ukraine, were taken. We took sterile forms because it gives the possibility to control the process of pollination and fertilization by isolating these forms and further pollination. That's why for agrobacterial transformation of plants with bisexual flowers sterile forms were used, which makes it possible to synchronize the process of fertilization and incorporation of T-DNA into the genome of plants.

For transformation soil bacteria (*Agrobacterium tumefaciens*) strain LBA4404 were used, which carry the binary vector construction with non-onkogenous plot of T-DNA,

which has the target gene *bar* - resistance to the herbicide Basta and selective antibiotic resistant genes.

In the laboratory the agrobacteria were grown on a nutrient medium LB (Luria-Bertani) 10 g tryptone, 5g NaCl, 5 g yeast extract, antibiotics were added 50 mg/l kanamycin, 50 mg/l rifampicin, 25 mg/l gentamicin. Cultivation of bacteria was carried out on a shaker (150-200 rpm/min) in the dark at a temperature of 28 °C within 24 hours. After the cultivation of bacteria sucrose and surfactant *Silwet L- 77* were added [4].

In the field environment (*in planta*) baskets were isolated cotton sheeting insulators till flower opening. When 3-4 rows of flowers opened sunflower was artificially pollinated, removed with clean cloth pollen from fertile plants and transferred to sterile ones. Sterile plants, located close to fertile ones for getting them pollen were pressed with the baskets to each other. After 1.5 hours (time necessary for germination of pollen tube of sunflower) by immersion of baskets into the suspension with agrobacteria inoculation was performed during 1 minute. Baskets were left for 24 hours in high humidity and then covered with cotton sheeting insulators for ripening of seeds.

Results of the research

In general 25 plants of sterile form of hybrid Ukrainskyi F1, 20 plants of sterile form of hybrid Ukrainske sonechko and 23 plants of sterile form of hybrid Ukrainskyi skorostyglyi were treated with agrobacterial suspension. The obtained seeds were sown in test plots in accordance with the terms of sowing this culture and in the phase of 4-6 true leaves the plants were sprinkled with the herbicide Basta 7 ml/l (Table 1).

1. Frequency of transformation of T₀ sunflower obtained by *in planta* method (2012)

Sunflower hybrids	Total number of seedlings	Number of white plants	Number of green plants	Transformation frequency
	units	units	units	%
Ukrainskyi F ₁	956	947	9	0,4*±0,02**
Ukrainske sonechko	523	518	5	1,1*±0,14**
Ukrainskyi skorostyglyi	754	750	4	0,6*±0,3**

Note * – accurate by usage the Student's coefficient ($p = 0,05$), ** – ± standard deviation.

The number of plants before chemical cultivation was: form of the hybrid Ukrainskyi F₁- 956 plants, hybrid Ukrainske sonechko - 523 plants, hybrid Ukrainskyi skorostyglyi - 754 plants.

After usage of herbicide as a spray on the fourth day most of the plants became "white" and died. In general 947 plants of sterile form of hybrid Ukrainskyi F1, 518 plants of sterile form of hybrid Ukrainske sonechko and 750 plants of sterile form of hybrid Ukrainskyi skorostyglyi died, which are not herbicide resistant. Plants of sunflower T₀, survived after herbicide impact had a green color and continued to form

vegetative organs according to the phases of ontogeny. Totally survived: Ukrainskyi F₁- nine plants, Ukrainske sonechko - five plants and Ukrainskyi skorostyglyi: four plants. The transformation frequency was the following: sterile hybrid form of Ukrainskyi F₁- 0,9, sterile hybrid form of Ukrainske sonechko - 1.1, sterile hybrid form of Ukrainskyi skorostyglyi - 0.6 respectively.

To confirm the transfer of gene *bar*, which determines resistance to the herbicide with the active ingredient phosphinothricin, sterile resistant forms were pollinated with the sterility binder not resistant to the herbicide impact (Tab.2).

2. Inheritance of phosphinothricin -resistance T₁ of sunflower, (2013)

Sunflower hybrids	Plants total						H*o	χ^2
	Before chemical cultivation		died		resistant			
	unit	%	unit	%	unit	%		
Ukrainskyi F ₁	141	100	62	44,0	79	56,0	1:1	2,0496
Ukrainske sonechko	122	100	54	44,3	68	55,7	1:1	1,6066
Ukrainskyi skorostyglyi	138	100	74	53,6	64	46,4	1:1	0,7246

Note:

1. Ho* - theoretically expected ratio nonresistant plants and resistant plants;
2. Maximum permitted value $\chi^2_{05} = 3,84$; $\chi^2_{01} = 6,63$.

The young crops of sunflower after chemical cultivation were obtained in the amounts: Ukrainskyi F₁ -141 units, which is 100 %, Ukrainske sonechko - 122 units, which is 100 %, Ukrainskyi skorostyglyi - 138 units, which is 100 % respectively. Selection of plants of sunflower according to phenotype was performed by spraying with herbicide. As a result of herbicide impact 62 units died, what makes 44.3 % of plants of sterile parent form of hybrid Ukrainskyi F₁, 54 units, what makes 44.0% of plants of sterile parent form of hybrid Ukrainske sonechko and 74 units, what makes 53.6% of plants of sterile parent form of hybrid Ukrainskyi skorostyglyi. Obtained resistant plants: Ukrainskyi F₁- 79 units, which is 55.7 %, Ukrainske sonechko – 68 units, which is 56.0 % and Ukrainskyi skorostyglyi - 64 units, which is 46.4 % respectively. Thus, herbicide resistant sterile forms T₁ of sunflower were obtained.

By individuals a relatively equal ratio of quantity of herbicide resistant and nonresistant offsprings were revealed. This shows the expression gene *bar* and heterozygosity of initial transgenic materials with dominant transgene. According to the genetic patterns by sterile forms T₁ between plants that died and stable ratio is 1:1.

Plants of sunflower with transferred resistance genes did not differ phenotypically from normal plants (non-transgenic). We can assume that the introduced into plant

genome *bar* construction has no effect on the expression of functional and structural genes of plants.

Conclusions. After cultivation with agrobacterial suspension of sunflower by *in planta* method nine herbicide-resistant plants of sterile parent form of hybrid Ukrainskyi F₁, five plants of sterile parent form of hybrid Ukrainske sonechko and four plants of sterile parent form of hybrid Ukrainskyi skorostyglyi were received.

The transformation frequency was the following: Ukrainskyi F₁- 0.4 %, sterile form of hybrid Ukrainske sonechko - 1.1 %, sterile form of hybrid Ukrainskyi skorostyglyi - 0.6 % respectively.

After pollination of derived forms in generation of sunflower T1 1:1 splitting between resistant and nonresistant plants took place which shows the expression of the resistance gene and heterozygosity of starting materials for the dominant transgene.

LIST OF THE USED SOURCES

1. Vyktorək-Smagur A. Sravnenye dvuh metodov transformacyy Arabidopsis thaliana: pogruzhennye cvetochnyh rostek y vakuumnaja ynfyl'tratsiya / A. Vyktorək-Smagur, K. Hnatushko-Konka, A. K. Kononovych // Fyzyologiya rastenyj. – 2009. – T. 56. – № 4. – S. 619-628.
2. Bur'jany v zemlerobstvi Ukrai'ny : prykladna gerbologija / [I.D. Prymak, Ju.P. Man'ko, S.P. Tanchyk ta in.]. – Bila Cerkva, 2005. – 664 s.
3. Klajv Dzhejms. Svitovyj stan komercializovanyh biotekhnologichnyh genetychno modyfikovanyh kul'tur: 2000–2010 rik: [Elektronnyj resurs]. – Rezhym dostupu: <http://www.isaaa.org> // Mizhnarodna sluzhba z vprovadzhennja agrobiotekhnologichnyh rozrobok (ISAAA).
4. Chumakov M. Y. Agrobakteryal'naja transformacyja nepovrezhdennyh rastenyj / M. Y. Chumakov, Y. V. Kurbanova, G. K. Solovova // Fyzyologiya rastenyj. – 2002. – T. 49. – №6. – S. 898-903.
5. Bechtold D. In Planta Agrobacterium Mediated Gene Transfer by Infiltration of Adult Arabidopsis thaliana Plants / D. Bechtold, J. Ellis, G. Pelletier // R. Acad. Sci., Life Sci. – 1993. – V. 316. – P. 1194-1199.