Transfer of gene conferring herbicide bialaphos resistance into buckwheat plants

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Bar gene conferring resistance to herbicide bialaphos (phosphinothricin) was cloned from Streptomyces hygroscopicus. Bar gene under 35S promoter of cauliflower mosaic virus was introduced in binary pBin19 vector and constructed plasmid was transferred into Agrobacterium tumefaciens strain. Conditions of genetic transformation of cultivated buckwheat and interspecific hybrid Fagopyrum esculentum × F. tataricum were worked out. Buckwheat explants were inoculated by the strain with a plasmid carrying bar gene nearby NPTII gene. Molecular analysis of 8 regenerated plants that were selected for kanamycin resistance was performed. 5 plants gave positive signal by dot- and Southern hybridization using as a probe DNA fragment with bar gene that is an evidence of integration of bar gene into plant genome. Transformed plants grow and rooted at Basta concentrations in the medium that totally inhibited nontransformed plants.

Introduction. The implementation of herbicides in modern agriculture is inevitable [1]. They control the weed growth and thus increase harvest [2]. Use of herbicides of a new generation that are very effective and as a rule inhibit single definite enzyme at very low concentration is very attractive. These herbicides are not toxic for human and animals. The ecological safety is connected to their quick degradation in soil and absence of target enzymes in vertebrates [1, 3].

Bialaphos is an example of a herbicide of this generation. It is a tripeptide and its acting substance is phosphinothricin (PPT) a toxic analog of glutamic acid [4]. But bialaphos as many other herbicides of this generation is not selective, inhibiting the growth of any plant and this character diminished the use of these herbicides in agriculture. Using gene engineering approach by introducing into plant genome of gene(s) conferring resistance to herbicides of this type it is possible to produce transgenic plants resistant to these herbicides [5].

The source of these genes is soil and other microorganisms. Bialaphos resistance gene (bar) was found in the genome of bialaphos producing streptomycetes, conferring their resistance to produced tripeptide [4].

Production of transgenic tobacco, potato, tomato, aspen plants resistant to herbicide PPT is reported [1, 3, 6, 7]. Earlier we described the introduction of modified aroA gene conferring resistance to herbicide glyphosate (Roundup) into potato, sugarbeet and soybean plants [8, 9]. In this paper we report about transfer of bar gene and its expression in buckwheat plants.


Alcali plasmid isolation, restriction, DNA ligation and electrophoresis, DNA elution and its cleaning, transformation of bacteria were done by the described methods [12, 13]. The binary vectors were transferred by tri-parental matings to the disarmed Agrobacterium tumefaciens strains 3850 [12] and LBA 4404.

All the enzymes used in this work were obtained from NPK «Biotekh» (Russia).

In all experiments we used mutant form of buckwheat Homostilnaya with high regeneration potential and hybrid of this form with tartari buckwheat (k-17). This hybrid was obtained via in vitro culture of immature interspecific embryos [14].

Internodes, petioles and leaves of these hybrids and Homostilnaya were inoculated by 3850 strain of
A. tumefaciens by two hours co-cultivation in overnight culture of agrobacteria in the presence of acetosyringone. Then explants were transferred onto Murashige-Skoog basal media with the addition of 1 mg/l BA and 0.5 mg/l NA for plant regeneration. To suppress agrobacteria regrowth and for kanamycin selection (NPTII gene is located on plasmids alongside bar gene) antibiotics kanamycin, carbenicillin and claforan were also added to the culture medium. Explants were cultivated first in the dark and after appearance of regenerants under 16:8-hr photoperiod with constant temperature of 26 °C. Regenerants that came through kanamycin selection were rooted and microclonally propagated. For evaluation of their herbicide resistance these regenerants were transferred onto MS medium with 3—30 μl/l concentrations of PPT (Basta).

Results and Discussion. It was shown that recombinant pBA-1 plasmid conferring bialaphos resistance has additional 1.7 kb pair PstI fragment. To improve bar gene expression GTG translation initiation codon was changed for eukaryotic ATG codon.

35S promoter of cauliflower mosaic virus and 3′ region from nopaline synthase gene were introduced into bar gene expression cassette. Recombinant pBA-3 plasmid was constructed for transfer of bar gene into buckwheat plants using agrobacteria transformation system. For this purpose HindIII fragment containing 35S promoter, bar gene and poly A region of nopaline synthase gene was cloned into corresponding site of pBin19 binary vector.

Resulting A. tumefaciens strains 3850/pBA-3 and LBA 4404/pBA-3 appeared to be resistant to 10 mg/l of bialaphos in nutrient medium.

Plants regenerated on kanamycin containing medium that was changed every 12—14 days were analyzed by dot- and Southern blot-hybridization. Out of 8 kanamycin resistant regenerants positive autoradiographic response gave 5 plants: 4 plants of hybrid Homostilnaya x k-17 and one plant of Homostilnaya that is a proof of bar gene integration into plant's genome (Fig. 1, 2).

These 5 plants were microclonally propagated and transferred into MS medium containing 3, 10, 15, 20 and 30 μl/l of herbicide Basta. On medium added with 3 and 10 μl/l of Basta all these plants formed roots and their growth did not differentiate from the growth of plants on MS medium without herbicide.

At 15 and 20 μl/l of Basta only 2 plants rooted and their development was normal. At 30 μl/l Basta concentration only one plant survived. The growth and root formation of nontransformed plants of Homostilnaya and Homostilnaya x k-17 hybrid were inhibited already at 3 μl/l of Basta.

That is a difference among transformed plants by the level of transferred bar gene expression was clearly seen.
мации растений. Идеальная рекомбинационная плазмида пере­
несена в штамм 3850 Agrobacterium tumefaciens. Подобные условия трансформации культурной гречихи и межвидового гибрида Fagopyrum esculentum × F. taiaricum. Экспланты гре­
хи инокулировали агробактериальным штаммом, в котором bar-ген находился на T
плазмиде рядом с геном NPTII. Проведен молекулярно-биологический анализ восьми регенеран­
тов, прошедших канамициновый отбор. Пять растений дали
положительный радиоавтографический ответ при дот- и Сау­
зерн-гибридизации с меченным зондом, несущим bar-ген, что
свидетельствует об интеграции этого гена в геном растений.
Трансформированные растения росли и образовывали корни
при концентрации гербицида басты в среде, полностью инги­
биррующей не трансформированные растения.

REFERENCES
6. Devillard C. Transformation in vitro du tremble (Populus tremula × Populus alba) par Agrobacterium rhizogenes et regen­
8. Левенко В. А., Смехин И. Н., Билека Г. М. и др. Введение гена устойчивости к глифосату в растения картофеля и сахарной свеклы // Физиология и биохимия культур. растений.—1993.—25.—С. 197—200.
14. Рубцова М. А., Левенко В. А., Гараничук Л. К., Шаповал А. И. Получение межвидовых гибридов между гречихой обыкновенной и гречихой татарской с помощью эмбриокультур // Физиология и биохимия культур. растений.—1994.—26, № 6.—С. 563—566.

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