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E.M. KISHCHENKO, I.K. KOMARNITSKII, N.V. KUCHUK
Institute of Cell Biology & Genetic Engeneering
Natl. Acad. Sci. of Ukraine, Kyiv
E-mail: iicb@iicb.kiev.ua

TRANSGENIC SUGAR BEET TOLERANT TO IMIDAZOLINONE OBTAINED BY *AGROBACTERIUM*-MEDIATED TRANSFORMATION

Sugar beet is highly sensitive to imidazolinone herbicides thus rotational restrictions exist. In order to develop imidazolinone tolerant sugar beets als gene from Arabidopsis thaliana encoding acetolactate synthase with S653N mutation was used for genetic transformation. Transgenic sugar beet plants were obtained by Agrobacterium-mediated transformation of aseptic seedlings using vacuum-infiltration. The efficiency of genetic transformation was 5.8 %. RT-PCR analysis of obtained plants revealed accumulation of specific als transcript. The resistance to imidazolinone was proved for developed transgenic sugar beet plants in vitro and in greenhouse conditions after spraying with imazethapyr (Pursuit®, BASF).

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Introduction. The imidazolinone group of herbicides provides effective and prolonged control of a broad spectrum of weeds. However these herbicides applied at recommended rates are very toxic to conventional sugar beet varieties. Moreover the carryover effect was determined on sugar beet in more than 12–24 months after application of imidazolinone herbicides in other resistant rotational crops [1, 2]. Thus suggested interval before sowing of sugar beet is 26 months at least (Pursuit®, BASF). Production of sugar beet tolerant to imidazolinone herbicides could reduce the risk of herbicide carryover injury, dispose of rotation restriction for sugar beet and provide efficient chemical weed control.

Imidazolinone herbicides inhibit acetolactate synthase (ALS, EC 4.1.3.18, also known as acetohydroxyacid synthase, AHAS), the first enzyme unique to the biosynthesis of the branched-chain amino acids (valine, leucine, and isoleucine) in plastids, that results in accumulation of toxic levels of alpha-ketoglutarate and decrease in protein synthesis [3, 4]. Besides imidazolinones ALS is the target of several classes of herbicides like sulfonylureas [5, 6], triazolopyrimidines [7], pyrimidinylthiobenzoate [8] and sulfonylamino-carbonyl-triazolinone [9]. Some point mutations within *als* gene give rise to herbicide resistance by reducing the herbicide binding capacity of ALS inhibitors with enzyme. Such mutated *als* gene has been identified in bacteria, fungi and plants [10–13]. At least 12 amino acid residues in ALS (G121, A122, M124, P197, A205, K256, M350, H351, D375, M570, W574 and S653) have been identified where mutations confer tolerance to imidazolinone herbicides [17–22]. Some of these amino acid substitutions result in cross-resistance to herbicides of the other abovementioned classes.

In general imidazolinone-resistant plants have been obtained by different strategies: 1) selection for naturally occurring mutations; 2) chemically induced seed or pollen mutagenesis; 3) selection of somaclonal variants from embryo or suspension cultures; 4) selection of somaclonal variants from microspore cultures following chemical mutagenesis; 5) genetic transformation [23–26]. Several of such developed crops as sunflower, wheat, rice, canola (BASF Inc.), maize (Syngenta Seeds, Inc., Pioneer Hi-Bred International Inc.) and soybean (Pioneer Hi-Bred International Inc.) are commercially available (see AGBIOS DATABASE for details, http://www.agbios.com/dbase.php).

In this paper we describe development of transgenic sugar beet tolerant to imidazolinone via *Agrobacterium*-mediated transformation using *A. thaliana als* gene with S653N mutation.

Materials and methods. *Agrobacterium strain and plasmid***.** *Agrobacterium tumefaciens* nopaline strain GV3101 harboring the binary vector pCB004 was used. T-DNA region of pCB004 contains neomycin phosphotransferase II gene (*nptII*) with nopaline synthase promoter and terminator and *als* gene from imidazolinone tolerant line of *A. thaliana* linked to its native promoter and terminator. Such variant of *als* gene differs from the wild type *A. thaliana* gene by the single point mutation. The resulting ALS enzyme contains serine by asparagine amino acid substitution at position 653. *Agrobacterium* culture was grown in LB medium containing 50 mg/l rifampicin and 50 mg/l kanamycin for 48 hours at 28 °C on rotary shaker (200 rpm) and then used for genetic transformation.

*Plant material***.** Seeds of diploid sugar beet line (*Beta vulgaris* L.) SC 023–2 were kindly provided by the Institute of Sugar Beet Research (Kyiv, Ukraine). Sugar beet seeds were incubated at +4 °C for a week then soaked in water overnight at room temperature prior to surface sterilization. Seeds were sterilized in 40 % (v/v) formalin for 2 min, transferred to 70 % ethanol for 30 s, treated with 30 % (v/v) bleach (1.5 % sodium hypochlorite) for 20 min and washed in autoclaved distilled water 3 times for 10 min. Seeds were germinated on MS medium [27] containing 15 g/l sucrose and 2 mg/l BAP at 22 °C in the dark.

*Agrobacterium-mediated transformation***.** Genetic transformation of sugar beet was carried out according to the previously reported protocol [28]. The *Agrobacterium* culture was sedimented, resuspended with equal volume of solution containing MS macrosalts (440 mg/l $CaCl_2 \cdot 2H_2O$, 1650 mg/l NH_4NO_3, 170 mg/l KH_2PO_4, 1900 mg/l KNO_3, 370 mg/l $MgSO_4 \cdot 7H_2O$), 20 g/l sucrose, 2 g/l glucose, 2 mg/l BAP, 0.2 mM acetosyringone and then incubated on rotary shaker (200 rpm) at 27 °C for 1 hour. Etiolated derooted seedlings three centimeters long were used as start material for *Agrobacterium*-mediated transformation. After vacuum-infiltration the seedlings were transferred to sterile filter paper and incubated in the dark at 22–24 °C for 3 days. Then they were cut into 7–10 mm pieces, incised and placed on MS medium containing 15 g/l sucrose and 2 mg/l BAP, 500 mg/l cefotaxime and 100 mg/l kanamycin at 28 °C in the dark. Within 6–8 weeks friable callus arose from the explants. Kanamycin-resistant callus was isolated and then cultured on the regeneration medium at 24 °C in scattered light and 16-h photoperiod with 3 weeks subcultivation period. The regeneration medium was composed of MS basal salts, Morel vitamins [29], 30 g/l sucrose, 29 mM silver thiosulfate, 0.5 g/l polyvinil pirrolidone, 1 mg/l BAP, 0.3 mg/l indoleacetic acid and 0.4 mg/l gibberellic acid supplemented with 300 mg/l cefotaxime and 100 mg/l kanamycin. Shoot regeneration occurred within 4–10 weeks. The selected shoots were transferred to MS medium supplemented with 100 mg/l cefotaxime and 100 mg/l kanamycin for root formation.

*PCR analysis***.** Genomic plant DNA was extracted from leaves with by CTAB-method [30]. To amplify the coding sequence of the transgenes the PCR primers were chosen: 5'-AAGGTTCTGATAATCACCGG-3' and 5'-CCGAGCTCACACATTTCTCG-3' for 300 bp portion of the *als* sequence; 5'-GAGGCTATTCGGCTATGACTG-3' and 5'-AATCTCGTGATGGCAGG-3' for 800 bp fragment of *nptII* gene. Amplification was performed on Mastercycler® personal (Eppendorf) with initial denaturation step at 94 °C for 3 min, followed by 33 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C (for *als* gene) or 60 °C (for *nptII* gene) 30 s, elongation at 72 °C for 30 for *als* or 45 s for *nptII*. The final extension reaction was performed at 72 °C for 3 min, after that the reaction was held on at 16 °C. Amplification products were fractionated by electroforesis in 1 % agarose gel in TBE buffer.

RNA isolation and RT-PCR. For reverse transcription polymerase chain reaction (RT-PCR) 1 μg of total RNA isolated from young leaf tissues of greenhouse-grown plants was used. The RNA extraction protocol proposed by Logemann et al. [31] was followed. To eliminate DNA contamination RNA samples were treated with DNase I (RNase-free). Then the first strand cDNA was synthesized from 1 μg total RNA by M-MLV reverse transcriptase and random hexamer primers (Fermentas) at 37 °C for 1 h. After that 2 μl of the reaction mixture were used as a template for PCR with primers for *als* transgene.

Herbicide application. Micropropagated transgenic sugar beet plants were transferred into soil in

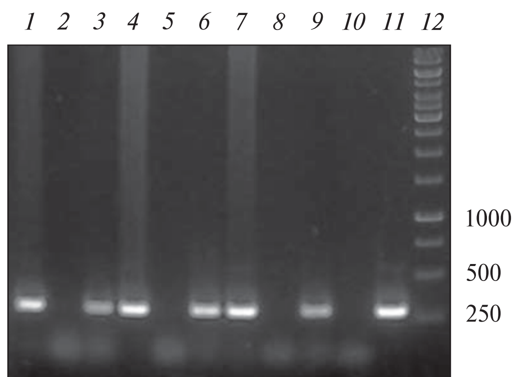

Fig. 1. PCR and RT-PCR analysis of transformed sugar beet plants using primers for mutated *als* transgene: *1*, *4*, *7* – amplificated DNA from transformed plants (clones № 4, 5, 6); *2*, *5*, *8* – amplificated RNA from transformed plants (clones № 4, 5, 6), *3*, *6*, *9* – amplificated cDNA samples of transformed plants (clones № 4, 5, 6); *10* – negative control, master mix; *11* – positive control, plasmid DNA pCB004; *12* – DNA marker GeneRuler™ 1 kb Ladder (Fermentas)

Fig. 2. Untransformed (*a*) and transgenic (*b*) plants 10 days after greenhouse treatment with Pursuit® (BASF) (scale bar = 2 cm)

greenhouse at 25 °C and under 18 h light/6 h dark photoperiod. Then they were sprayed with aqueous solution of Pursuit® (BASF) with working concentration 250 mg/l imazethapyr to evaluate the resistance to imidazolinone herbicide.

Results and discussion. For long time sugar beet was considered as recalcitrant crop for genetic transformation. Whereas transformation frequency depends on efficient and reliable tissue culture regeneration system the early experiments on genetic transformation of sugar beet were mainly focused on the search for the genotypes with high regenerative potential and the explants whose cells were the most competent for regeneration. Such high-efficiency regeneration systems for sugar beet are (1) direct regeneration from petioles [32–34], cotyledonary node explants [35, 36], shoot bases [37, 38], bud clumps from immature inflorescence and (2) indirect regeneration via callus from cotyledons and hypocotyls [39, 40]. Sugar beet transformation frequency of different explants was reported mainly within 1–5 % [28, 35, 39–42] and arrived at 19.2 % in case of shoot base transformation [37, 38]. Vacuum infiltration of plant tissues or application of Silwet L-77 increased the frequency of genetic transformation as such treatments improved the delivery of agrobacteria to deeply buried cells competent for regeneration [43]. Direct regeneration from transformed sugar beet explants resulted in formation of chimeric shoots with the different percentage of transgenic cells and non-transgenic shoots, the so-called escapes, among transgenic shoots [33–35, 37, 38, 41, 43, 44]. Transgenic cells had not any unambiguous selective advantages over the non-transgenic ones in direct regeneration from the meristem zones of explants. Selection on kanamycin resulted in production of escapes amounted to 50–95 % among the regenerated kanamycin-tolerant shoots [33, 34, 38, 41, 44, 45]. Similar results were obtained when 15 mg/l phosphinothricin was used for selection after transformation of embryogenic callus of cotyledonary origin [44] or 10 mg/l hygromycin after transformation of bud clumps from immature inflorescence [43]. Among selected plants only 30 % and 29.8–38.7 % were transgenic, respectively.

When indirect system was used for transgenic sugar beet regeneration the vital difference was observed since far fewer escapes were formed. The selection efficiencies were 100 % and 85.7–90.9 % on media with kanamycin and phosphinothricin respectively. However the frequency of subsequent regeneration from transgenic callus clones averaged out 10–56 % and strongly depended on the genotype [28, 39, 40]. Though the indirect regeneration had such disadvantage, we have used this system for our study because it was accompanied by effective selection. Hence we used sugar beet

seedlings which cotyledons and hypocotyls regenerated plants *via* callus as start material for genetic transformation.

Genetic transformation of sugar beet line SC 023–2 was carried out with nopaline *A. tumefaciens* strain GV3101 harboring binary vector pCB004 by vacuum infiltration. The frequency of friable callus formation from cotyledon and hypocotyl explants on selection medium with 100 mg/l kanamycin was about 9 %. Nine of fourteen transgenic callus clones were mophogenic and were able to regenerate shoots on selective medium. The number of individual transgenic plants depended on morphogenic potential of each callus clone. Since the binary vector pCB004 harboring *als* gene with point mutation provided imidazolinone tolerance we transferred callus clones and regenerated plants on the media supplemented with 20 μg/l imazethapyr (Pursuit®, BASF). The selected clones and plants were resistant to herbicide.

The presence of *als* and *nptII* transgenes in regenerated plants was confirmed by PCR-analysis (Fig. 1, lines *1*, *4*, *7*). RT-PCR of total RNA was performed to confirm *als* expression in transgenic plants. Accumulation of specific *als* transcript (the predicted 300-bp fragment) was revealed for each transgenic plant (Fig. 1, lines *3*, *6*, *9*). Samples of total RNA were also used for PCR to prove the absence of contamination by DNA that could gives false-positive result (Fig. 1, lines *2*, *5*, *8*).

The micropropagated transgenic sugar beet plants were transplanted into soil, adapted to the greenhouse conditions and sprayed with aqueous solution of imazethapyr (250 mg/l) to test obtained plants for imidazolinone tolerance *ex vitro*. Resistance of the obtained plants to imidazolinone was evaluated after herbicide application in transgenic sugar beet. The control untransformed plants perished with herbicide (Fig. 2). Thus transgenic sugar beet plants with imidazolinone resistance have been obtained in our study.

Current weed control necessarily includes herbicide use since weeds cause considerable losses in sugar beet yield and taproots quality. Cultivation of herbicide-resistant crops has certain financial and environmental benefits. To date transgenic sugar beet plants resistant to different herbicides such as glufosinate ammonium [28, 44, 46], glyphosate [47] and sulfonylurea [44] have been developed by *Agrobacterium*-mediated and protoplast-based techniques. Using another strategy based upon suspension cell selection imidazolinone-resistant sugar beets had been produced by Wright and Penner [48]. In the present work transgenic sugar beet plants tolerant to imidazolinone were obtained by *Agrobacterium*-mediated transformation. The created transgenic sugar beet as other herbicide-resistant plants would improve weed control practices in this crop and reduce in the number of herbicide applications. Our results supplement the list of transgenic sugar beet tolerant to nonselected broad spectrum and environmentally preferable herbicides.

Е.М. Кищенко, И.К. Комарницкий, Н.В. Кучук

ПОЛУЧЕНИЕ ТРАНСГЕННОЙ САХАРНОЙ СВЕКЛЫ, УСТОЙЧИВОЙ К ИМИДОЗАЛИНОНАМ, С ПОМОЩЬЮ АГРОБАКТЕРИАЛЬНОЙ ТРАНСФОРМАЦИИ

Сахарная свекла очень восприимчива к имидозалинонам, поэтому существуют определенные ограничения в ее высеве после культур, обрабатываемых этими гербицидами. Создание трансгенной сахарной свеклы, устойчивой к имидозалинонам, путем переноса мутантного гена *als* из *Arabidopsis thaliana*, кодирующего ацетолактатсинтазу с S653N-заменой, позволяет обойти такое препятствие в севообороте. Трансгенные растения сахарной свеклы, устойчивые к имидозалинонам, были получены путем агробактериальной трансформации с помощью вакуум-инфильтрации. Частота генетической трансформации составила 5,8 %. Полимеразная цепная реакция, сопряженная с обратной транскрипцией, выявила накопление специфического *als* транскрипта у полученных растений. Устойчивость к имидозалинонам созданных растений была доказана как в тестах *in vitro*, так и в условиях теплицы после опрыскивания их имазетапиром (Pursuit®, BASF).

О.М. Кіщенко, І.К. Комарницький, М.В. Кучук

ОТРИМАННЯ ТРАНСГЕННОГО ЦУКРОВОГО БУРЯКУ, СТІЙКОГО ДО ІМІДОЗАЛІНОНІВ, ЗА ДОПОМОГОЮ АГРОБАКТЕРІАЛЬНОЇ ТРАНСФОРМАЦІЇ

Цукровий буряк дуже чутливий до імідозалінонів, тому існують певні обмеження його висіву після культур, що оброблялися цими гербіцидами. Створення трансгенного цукрового буряку, стійкого до імідозалінонів, шляхом переносу мутантного гена *als* з *Arabidopsis thaliana*, що кодує ацетолактатсинтазу з S653N-заміною, дозволяє обійти таку перешкоду в сівозміні. Трансгенні рослини цукрового буряку зі стійкістю до імідозалінонів було отримано шляхом агро-

бактеріальної трансформації за допомогою вакуум-інфільтрації. Частота генетичної трансформації становила 5,8 %. Полімеразна ланцюгова реакція, поєднана зі зворотною транскрипцією, виявила накопичення специфічного *als* транскрипта у отриманих рослин. Стійкість до імідозалінонів створених рослин була доведена як у тестах *in vitro*, так і в умовах теплиці після обприскування їх імазетапіром (Pursuit®, BASF).

ABBREVIATIONS

ALS – acetolactate synthase,
BAP – 6-benzylaminopurine,
MS – Murashige and Skoog,
RT-PCR – reverse transcription-PCR

REFERENCES

1. *Renner K.A., Powell G.E.* Response of sugarbeet (*Beta vulgaris*) to herbicides residues in soil // Weed Technol. – 1991. – **5**. – P. 622–627.
2. *Alister C., Kogan M.* Efficacy of imidazolinone herbicides applied to imidazolinone resistant maize and their carryover effect on rotational crops // Crop Prot. – 2005. – **24**. – P. 375–379.
3. *Shaner D.L., Anderson P.C., Stidham M.A.* Imidazolinones: potentinhibitors of acetohydroxyacid synthase // Plant Physiol. – 1984. – **76**. – P. 545–546.
4. *Singh B.K., Stidham M.A., Shaner D.L.* Assay of acetohydroxyacid synthase // Anal. Biochem. – 1988. – **171**. – P. 173–179.
5. *Challeff R.S., Mauvais C.J.* Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants // Science. – 1984. – **224**. – P. 1443–1445.
6. *Ray T.B.* Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants // Plant Physiol. – 1984. – **75**. – P. 827–831.
7. *Gerwick B.C., Subramanian M.V., Loney-Gallant V., Chander D.P.* Mechanism of action of the 1,2,4-triazolo[1,5-a]pyrimidine // Pestic Sci. – 1990. – **29**. – P. 357–364.
8. *Sprague C.L., Frasier A.L., Penner D.* Identifying acetolactate synthase inhibitors for potential control of quackgrass (*Elytrigia repens*) and canada thistle (*Cirsium arvense*) in corn (*Zea mays*) // Weed Technol. – 1999. – **13**. – P. 54–58.
9. *Geier P.W., Stahlman P.W., Hargett J.G.* Dose responses of weeds and winter wheat to MKH 6561 // Weed Sci. – 2001. – **49**. – P. 788–791.
10. *Winder T., Spalding M.* Imazaquin and chlorsulfuron resistance and cross resistance in mutants of *Chlamydomonas reinhardtii* // Mol. Gen. Genet. – 1988. – **213**. – P. 394–399.
11. *Saxena P., King J.* Herbicide resistance in *Datura innoxia*: cross-resistance of sulfonyl urea resistance lines to imidazolinones // Plant Physiol. – 1988. – **86**. – P. 863–867.
12. *Haughn G.W., Somerville C.R.* A mutation causing imidazolinone resistance maps to *csrl* locus in *Arabidopsis thaliana* var Columbia // Plant Physiol. – 1990. – **92**. – P. 1081–1085.
13. *Trucco F., Hager A.G., Tranel P.J.* Acetolactate synthase mutation conferring imidazolinone-specific herbicide resistance in *Amaranthus hybridus* // J. Plant Physiol. – 2006. – **163**. – P. 475–479.
14. *Ott K.H., Kwagh J.-G., Stockton G.W., Sidorov V., Kakefuda G.* Rational molecular design and genetic engineering of herbicide resistant crops by structure modeling and site-directed mutagenesis of acetohydroxyacid synthase // J. Mol. Biol. – 1996. – **263**. – P. 359–368.
15. *Chang A.K., Duggleby R.G.* Herbicide-resistant forms of *Arabidopsis thaliana* acetohydroxyacid synthase: characterization of the catalytic properties and sensitivity to inhibitors of four defined mutants // Biochem. J. – 1998. – **333**. – P. 765–777.
16. *Lee Y.-T., Chang A.K., Duggleby R.G.* Effect of mutagenesis at serine 653 of *Arabidopsis thaliana* acetohydroxyacid synthase on the sensitivity to imidazolinone and sulfonylurea herbicides // FEBS Lett. – 1999. – **452**. – P. 341–345.
17. *Oh K.J., Park E.J., Yoon M.Y., Han T.R., Choi J.D.* Roles of histidine residues in tobacco acetolactate synthase // Biochem. Biophys. Res. Communs. – 2001. – **282**. – P. 1237–1243.
18. *Duggleby R.G., Pang S.S., Yu H., Guddat L.W.* Systematic characterization of mutations in yeast acetohydroxyacid synthase: interpretation of herbicide-resistance data // Eur. J. Biochem. – 2003. – **270**. – P. 2895–2904.
19. *Le D.T., Yoon M.Y., Kim Y.T., Choi J.D.* Roles of conserved methionine residues in tobacco acetolactate synthase // Biochem. Biophys. Res Communs. – 2003. – **306**. – P. 1075–1082.
20. *Le D.T., Yoon M.Y., Kim Y.T., Choi J.D.* Two consecutive aspartic acid residues conferring herbicide resistance in tobacco acetohydroxy acid synthase // Biochim. Biophys. acta. – Proteins Proteomics. – 2005. – **1749**. – P. 103–112.
21. *Kolkman J.M., Slabaugh M.B., Bruniard J.M., Berry S., Bushman B.S., Olungu C., Maes N., Abratti G., Zambelli A., Miller J.F., Leon A., Knapp S.J.* Acetohydroxyacid synthase mutations conferring resistance to imidazolinone or sulfonylurea herbicides in sunflower // Theor. Appl. Genet. – 2004. – **109**. – P. 1147–1159.
22. *Preston C., Stone L.M., Rieger M.A., Baker J.* Multiple effects of a naturally occurring proline to threonine substitution within acetolactate synthase in two herbicide-resistant populations of *Lactuca serriola* // Pestic Biochem. Physiol. – 2006. – **84**. – P. 227–235.
23. *Newhouse K.E., Singh B.K., Shaner D.L., Stidham M.A.* Mutations in corn (*Zea mays* L.) conferring resistance to imidazolinone herbicides // Theor. Appl. Genet. – 1991. – **83**. – P. 65–70.

24. *Newhouse K.E., Smith W.A., Starrett M.A., Schaefer T.J., Singh B.K.* Tolerance to imidazolinone herbicides in wheat // Plant Physiol. – 1992. – **100**. – P. 882–886.
25. *Wright T.R., Penner D.* Cell selection and inheritance of imidazolinone in sugarbeet (*Beta vulgaris*) // Theor. Appl. Genet. – 1998. – **96**. – P. 612–620.
26. *Andersson M., Trifonova A., Andersson A.-B., Johansson M., Bulow L., Hofvander P.* A novel selection system for potato transformation using a mutated AHAS gene // Plant Cell Rep. – 2003. – **22**. – P. 261–267.
27. *Murashige T., Skoog F.* A revised medium for rapid growth and bioassays with tobacco tissue culture // Physiol. Plant. – 1962. – **15**. – P. 473–497.
28. *Kishchenko E.M., Komarnitskii I.K., Kuchuk N.V.* Production of transgenic sugarbeet (*Beta vulgaris* L.) plants resistant to phosphinothricin // Cell Biol Int. – 2005. – **29**. – P. 15–19.
29. *Morel G., Wetmore R.H.* Fern callus tissue culture // Amer. J. Bot. – 1951. – **38**. – P. 141–143.
30. *Doyle J.J., Doyle J.L.* Isolation of plant DNA from fresh tissue // Focus. – 1990. – **12**. – P. 13–15.
31. *Logemann J., Schell J., Willmitzer L.* Improved method fort he isolation of RNA from plant tissues // Ann. Biochem. – 1987. – **163**. – P. 16–20.
32. *Tertivanidis K., Goudoula C., Vasilikiotis C., Hassiotou E., Perl-Treves R., Tsaftaris A.* Superoxide dismutase transgenes in sugarbeets confer resistance to oxidative agents and the fungus *C. beticola* // Transgenic Res. – 2004. – **13**. – P. 225–233.
33. *Norouzi P., Malboobi M.A., Zamani K., Yazdi-Samadi B.* Using a competent tissue for efficient transformation of sugarbeet (*Beta vulgaris* L) // In Vitro Cell. Dev. Biol. – Plant. – 2005. – **41**. – P. 11–16.
34. *Jafari M., Norouzi P., Malboobi M.A., Ghareyazie B., Valizadeh M., Mohammadi S.A., Mousavi M.* Enhanced resistance to a lepidopteran pest in transgenic sugar beet plants expressing synthetic *cry1Ab* gene // Euphytica. – 2009. – **165**. – P. 333–344.
35. *Krens F.A., Trifonova A., Keizer L.C.P., Hall R.D.* The effect of exogenously-applied phytohormones on gene transfer efficiency in sugarbeet (*Beta vulgaris* L.) // Plant Sci. – 1996. – **116**. – P. 97–106.
36. *Мишуткина Я.В., Камионская А.М., Скрябин К.Г.* Создание трансгенных растений сахарной свеклы, экспрессирующих ген *bar* // Приклад. биохимия и микробиология. – 2010. – **46**, № 1. – С. 89–95.
37. *Hisano H., Kimoto Y., Hayakawa H. et al.* High frequency *Agrobacterium*-mediated transformation and plant regeneration via direct shoot formation from leaf explants in *Beta vulgaris* and *Beta maritima* // Plant Cell. Rep. – 2004. – **22**. – P. 910–918.
38. *Lindsey K., Gallois P.* Transformation of sugarbeet (*Beta vulgaris*) by *Agrobacterim tumefaciens* // J. Exp. Bot. – 1990. – **41**. – P. 529–536.
39. *Snyder G.W., Ingersoll J.C., Smigocki A.C., Owens L.D.* Introduction of pathogen defence genes and a cytokinin biosynthesis gene into sugarbeet (*Beta vulgaris* L.) by *Agrobacterium* or particle bombardment // Plant Cell Rep. – 1999. – **18**. – P. 829–834.
40. *Zhang C.-L., Chen D.-F., McCormac A.C., Scott N.W., Elliot M.C., Slater A.* Use of the GFP reporter gene as a vital marker for *Agrobacterium*-mediated transformation of sugar beet (*Beta vulgaris* L.) // Mol. Biotechnol. – 2001. – **17**. – P. 109–117.
41. *Joersbo M., Donaldson I., Kreiberg J., Petersen S.G., Brunstedt J., Okkels F.T.* Analysis of mannose selection used for transformation of sugar beet // Mol. Breed. – 1998. – **4.** – P. 111–117.
42. *Kuykendall L.D., Stockett T.M., Saunders J.W.* *Rhizobium radiobacter* conjugation and callus-independent shoot regeneration used to introduce the cercosporin export gene *cfp* from *Cercospora* into sugar beet (*Beta vulgaris* L.) // Biotechnol. Lett. – 2003. – **25**. – P. 739–744.
43. *Yang A.F., Duan X.G., Gu X.F., Gao F., Zhang J.R.* Efficient transformation of beet (*Beta vulgaris*) and production of plants with improved salt-tolerance // Plant Cell Tissue Organ Cult. – 2005. – **83**. – P. 259–270.
44. *D'alluin K., Bossut M., Bonne E., Mazur B., Leemans J., Botterman J.* Transformation of sugarbeet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants // Bio\Technology. – 1992. – **10**. – P. 309–314.
45. *Ivic-Haymes S.D., Smigocki A.C.* Biolistic transformation of highly regenerative sugar beet (*Beta vulgaris* L.) leaves // Plant Cell Rep. – 2005. – **23**. – P. 699–704.
46. *Hall R.D., Riksen-Bruinsma T., Weyens G.J. et al.* A high efficiency technique for the generation of transgenic sugar beets from stomatal guard cells // Nat. Biotechnol. – 1996. – **14**. – P. 1133–1138.
47. *Mannerlöf M., Tuvesson S., Steen P., Tenning P.* Transgenic sugar beet tolerant to glyphosate // Euphytica. – 1997. – **94**. – P. 83–91.
48. *Winder T., Spalding M.* Imazaquin and chlorsulfuron resistance and cross resistance in mutants of *Chlamydomonas reinhardtii* // Mol. Gen. Genet. – 1988. – **213**. – P. 394–399.

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