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Attempts to produce transgenic *Beta vulgaris* L. plants via combined gene transfer methods

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ABSTRACT We have started to elaborate a general transformation and plant regeneration system for *Beta vulgaris* L. using combined gene transfer procedure. Three methods were tested: particle bombardment with pure wolfram micro-carriers prior to *Agrobacterium* treatment, vortexing and infiltration of explants in *Agrobacterium* suspension, halving of explants. The histochemical analysis of transient and stabile *gus*-gene expression showed the beneficial effect of explant halving and infiltration in *Agrobacterium* suspension.

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KEY WORDS

combined gene transfer
Beta vulgaris L.
Agrobacterium tumefaciens
gus-gene expression

Recent research activities in the field of traditional and molecular genetics, plant physiology, *in vitro* plant cell cultures and plant breeding make it possible to improve the characteristics of sugar and fodder beets (*Beta vulgaris* L.) with a complex procedure. To produce an "ideal" genotype of sugar and fodder beets with classical breeding methods nearly impossible or a real time consuming work. This genotype should be resistant against different diseases, should have a high, at least 17.5-19.0 %, sugar content and biomass. Synchronized flowering and monogermity, as well as high germination rate (at least 90-95 %) are also demands from crop producers. The characteristics of today's breeding methods used in beets, such as low-rate *in vitro* propagation, limited application of species and genus crosses, unsuccessful induced mutagenesis, shortage of *B.vulgaris* gene resources did not lead to any proper result. The above mentioned conditions have justified the attempts on plant biotechnology (*in vitro* mutagenesis and genetic transformation to widen the genetic resources) in sugar and fodder beet breeding.

The aim of our work was to start the elaboration of a general transformation and plant regeneration system for *Beta vulgaris* L. using combined gene transfer procedure. The rapid and effective transformation with valuable gene constructs of these species via this system will serve as a tool for the improvement of some characteristics which are important in crop production and processing industry.

Materials and Methods

Plant material: cotyledon and hypocotil explants from *in vitro* grown seedlings of two beet varieties ("Aranymono" and "Vöröshenger") derived from BETA Research Ltd. (Sopronhorpács) were used.

Culture conditions: the explants were cultured on the surface of solidified MS (Murashige and Skoog 1962) medium, supplemented with 5 mg/l BAP. Gene transfer took place after three days of cultivation.

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Plasmid DNA: binary plasmids pRGG *hpt* and pRGG *neo* derived from Agricultural Biotechnology Center (ABC) at Gödöllő were introduced into the target tissues. The constitutive expression of *gus*-gene served as a sign of successful transformation.

Gene transfer: three parallel transformation technique were used:

1) direct gene transfer via particle bombardment using the GENEBOOSTER™, produced by ELAK Ltd. Co. (Budapest) (Jenes et al. 1996);

2) combined gene transfer with *Agrobacterium tumefaciens* and biolistics;

3) genetic transformation via *Agrobacterium tumefaciens* bombardment.

The virulence of *Agrobacterium tumefaciens* C58C1 strain was enhanced by acetosyringon, aldose type carbon source, low pH value and macroelement concentration of culture media.

Results and Discussion

The penetration efficiency of bacteria into the target tissues were tested with different methods: particle bombardment with pure wolfram micro-carriers prior to *Agrobacterium* treatment, vortexing and infiltration of explants in *Agrobacterium* suspension, halving of explants. The effect of this procedures on gene transfer efficiency was evaluated by histochemical analysis of transient and stabile *gus*-gene expression, and the determination of hygromicin resistant calli (Table 1). According to our results the volume of stabile transgene integration and the bacterial penetration were not increased by vortexing. In contrary, short time (5 min) infiltration of explants in bacteria suspension resulted nearly two-fold increase of hygromicin resistant calli (Table 1). Transient GUS-expression was more enhanced by longer infiltration (10-20 min), but short time treatment has more significant effect on genetic transformation of these beet varieties. Bombardment of target tissue with pure wolfram particles alone and with *Agrobacterium* suspension did not

Table 1. The effect of different transformation and explant-wounding methods on penetration of *Agrobacterium* suspension and transgene integration from bacteria into the target beet tissues.

Explant preparation	No. of transformed explants	Explants with callus development on selective medium	Resistant callus tissues (%)
Halved explants	339	25	7.37
Vortexed explants	42	3	7.14
Halved and vortexed explants	273	21	7,7
Halved and infiltered explants (5 min)	14	2	14.3
Halved and infiltered explants (10 min)	929	83	8.9
Halved and infiltered explants (20 min)	26	3	11.5

enhanced neither transient nor stabile *gus*-gene expression comparing to the conventional *Agrobacterium* transformation system.

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