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ARTICLE

Genetic transformation of fodder beet (*Beta vulgaris* L.)

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ABSTRACT The signal metabolite fructose-2:6-bisphosphate (Fru-2:6-P2) interfering a key enzyme (cytosolic fructose-1:6-bisphosphatase (citFBPase)) of sucrose synthesis and allosterically activating the Pi-dependent fructose-6-phosphate kinase (PFP) in plant systems, which has important role in glycolysis. Theoretically, increasing levels of cytosolic Fru-2:6-P2 could lead to retard sucrose synthesis and to stimulate starch accumulation at the same time. Implicitly, the lower concentration of Fru-2:6-P2 could decrease the starch accumulation in chloroplasts and an increased volume of sucrose synthesis can be obtained in cytosol. The aim of our project was to produce fodder beet lines with enhanced sucrose content by lowering the endogenous Fru-2:6-P2 level using special gene transformation methods. These lines could beneficial as raw materials for the production of bioethanol
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KEY WORDS

genetic transformation
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Signal metabolites are commonly found in living organisms (plants, animals) not only to determine a simple gene expression level and enzyme activity but to control and synchronize complex metabolic pathways. Therefore changing of their endogenous concentrations will open more chance to alter a certain metabolic pathway in order to obtain plants which can be useful in theoretical (basic) and applied research works. Earlier sense and antisense techniques were used for the same purposes. The signal metabolite fructose-2:6-bisphosphate (Fru-2:6-P2) interfering a key enzyme (cytosolic fructose-1:6-bisphosphatase (citFBPase)) of sucrose synthesis and allosterically activating the Pi-dependent fructose-6-phosphate kinase (PFP) in plant systems, which has important role in glycolysis. Besides this it has stimulating effect on ADP-glucose pyrophosphorilase (AGPase) in chloroplasts, a regulator of starch synthesis. Theoretically, increasing levels of cytosolic Fru-2:6-P2 could lead to retard sucrose synthesis and to stimulate starch accumulation at the same time. Implicitly, the lower concentration of Fru-2:6-P2 could decrease the starch accumulation in chloroplasts and an increased volume of sucrose synthesis can be obtained in cytosol (Toldi et al. 2002).

The aim of our GVOP project was to produce fodder beet lines with enhanced sucrose content by lowering the endogenous Fru-2:6-P2 level using special gene transformation methods. These lines could beneficial as raw materials for the production of bioethanol.

Materials and Methods

Plant material: two fodder beet varieties (’Arany mono’ and ’Vöröshenger’) derived from BETA Research Ltd. (Sopronhorpács) were used. Seeds from field grown and flowered plants were surface sterilized and germinated in laboratory conditions. Axillary shoots and stem segments below them from *in vitro* grown seedlings were used as explants.

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Culture conditions: the explants were cultured on the surface of agar-agar solidified MS (Murashige and Skoog 1962) medium, supplemented with different concentrations and combinations of cytokinins and auxins: MSR - 4.5 mg L^{-1} BAP + 0.02 mg L^{-1} NAA; MSB - 2.0 mg L^{-1} BAP; TDZ1 - 0.5 mg L^{-1} TDZ; TDZ2 - 1 mg L^{-1} TDZ (abbreviations: BAP - 6-benzylaminopurine, NAA - α-naphtalene acetic acid, TDZ - thidiazuron).

Gene constructions: biphosphatase construction led by constituve promoter (pCaMV35S): pCaMV::Fru-2:6-P2ase, and kinase construction was labeled as pCaMV::6-PF-2-K.

Gene transfer: the production of transformed tissue cultures were done after three days of cultivation according to our previously elaborated method granted by an OM-KFHÁ project (Molnár et al. 2002). The penetration efficiency of *Agrobacterium tumefaciens* into the target fodder beet *in vitro* tissues were enhanced using particle bombardment (biolistics) with pure wolfram micro-carriers prior to *Agrobacterium* treatment (Jenes et al. 1996). Wounded surfaces of plant explants were produced by particle bombardment to the proper infection of *Agrobacteria*. The virulence of *Agrobacterium tumefaciens* C58C1 strain (exactly the activation of *vir*-region of *Ti*-plasmid) was enhanced by application of acetosyringon, aldose type carbon source, low pH value and decreased macroelement concentration of culture media. After particle bombardment the genetic transformation with *A.tumefaciens* was done according the commonly used agroinfection in dicots (Fig. 1). Hypothetically transgenic tissues and regenerated plantlets were analyzed with PCR technique. Expression

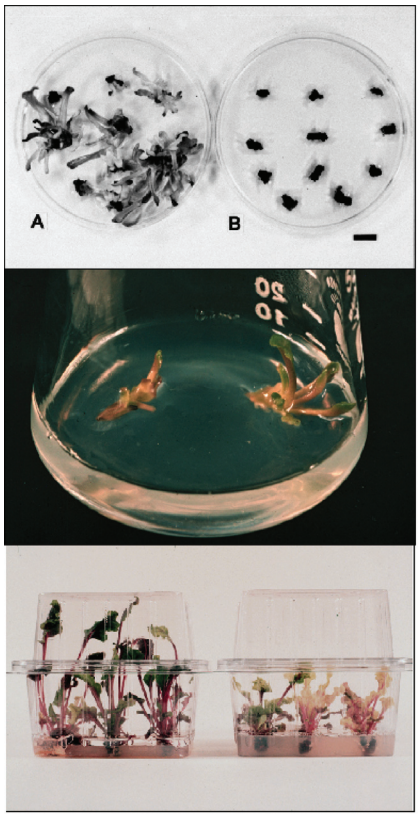

Figure 1. Transformation of shoot tip explants of fodder beet with *Agrobacterium tumefaciens*, selection and regeneration of hypotetic transgenic plantlets. *(A)* Shoot differentiation on transgenic explants in the presence of selection agent 100 mg L^{-1} kanamycin in culture medium, it was not occured in the case of non-transformed control ones *(B)*. Stabile transformation can be identified only when the plantlets have deep green leaves with normal morphology *(middle photo and left photo below)*. No transgene can be identified when the plantlets have pale green and yellow-white colours *(right photo below)*.

levels of useful genes were determined with RT-PCR. Also, altered carbohydrate profiles were determined with enzyme assays based on inter-conversion of NAD-NADH by spectrophotometry.

Results and Discussion

Lines bearing biphosphatase construction led by constituve promoter (pCaMV35S) were named pCaMV::Fru-2:6-P2ase. Two lines: one with a weak and one with a strong gene expression level were examined. With this schema: lines bearing kinase construction was labeled as pCaMV::6-PF-2-K, and also two lines, one with a weak and one with a strong gene expression level were examined. Transgenic plants were identified from transformed ones by PCR technique. Lines with high gene expression levels were selected with RT-PCR. To verify uniform sample delivery the constitutive expression of ubiquitin (UBQ) was used as internal control in RT-PCR analysis.

First, search for the explanation of a question was done: whether expression level differences detected by RT-PCR can be shown at the level of protein activity coded by transgenes? Therefore 6-PF-2-K and Fru-2:6-P2ase activity were measured, and steady-state Fru-2:6-P2 levels were determined in the photosynthetic leaves of fodder beet plant lines. The Fru-2:6-P2ase activity during the light period of the diurnal cycle on pCaMV::Fru-2:6-P2ase plants was increased from 140% to 390% compared to wild type plants. While 6-PF-2-K activity in pCaMV::6-PF-2-K plants was increased from 110% to 410% compared to wild type ones. On the one hand, fodder beet lines were produced with an enhanced 6-PF-2-K activity and Fru-2:6-P2 level (in the pCaMV::6-PF-2-K plants) in their photosynthetic leaves, and on the other hand increased Fru-2:6-P2ase activity and decreased Fru-2:6-P2 content (in pCaMV::Fru-2:6-P2ase plants) were detected. It has an effect on Fru-2:6-P2 content measured in pCaMV::Fru-2:6-P2ase plants: lowering the content with 10-15% compared to the wild type plants. In contrary Fru-2:6-P2 content were raised with 350-410% in pCaMV::6-PF-2-K plants. It was concluded that the experimental plant materials produced are convenient for starting physiological measurements.

Substantive change of endogenous Fru-2:6-P2 level led to the realignment of photosynthetic carbohydrate metabolism respecting not only their most abundant representatives, like sucrose and starch, but at the level of hexoses and phosphorilated intermediers. A significant starch accumulation was obtained in the leaves of pCaMV35S::6-PF-2-K plants: 200-230% of wild type plants. This phenomenon was connected to decreased sucrose content in them. While in the leaves of pCaMV::Fru-2:6-P2ase plants higher sucrose concentration (140-150% of the wild type ones) was measured at the expense of starch synthesis. We are planning to examine the tap root after measuring the above mentioned data in photosynthetic leaves, but our plantlets are not enough old now for this. Planned measurements will serve key factors to the production of bioethanol from fodder beet roots.

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