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# Phosphomannose isomerase gene for selection in lettuce (*Lactuca sativa* L.) transformation

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**A positive selection system using phosphomannose isomerase was employed for *Agrobacterium tumefaciens* mediated transformation of lettuce (*Lactuca sativa* L. var. 'Achát'). It was shown that the mannose-based selection system works very well with the lettuce genotype used, reaching up to 25% transformation efficiency on the medium with 20 g/L mannose and 20 g/L sucrose. The best transformation efficacy with the commonly-used kanamycin at 100 mg/L as a selection agent was 21%. Southern blot analyses of thirteen chosen mannose-resistant regenerants revealed that some of them have clonal origin, about one-half harbour a single T-DNA copy and one plant contains an incomplete T-DNA segment with only the left part of T-DNA with the *pmi* gene present in the genomic DNA. The following Northern analysis showed transcriptional activity of the introduced *pmi* gene in all plants analysed with very high differences in the level of *pmi* specific mRNA. The results demonstrate that both mannose and kanamycin provide comparable transformation efficiencies in our lettuce genotype. An alternative selection method with mannose as a selection agent is now available for lettuce transgenosis.**

**Keywords**: phosphomannose isomerase, *pmi*, *npt II*, *Lactuca sativa*, kanamycin, transformation efficiency

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## INTRODUCTION

When Fraley and coworkers published in 1983 the first successful transformation of a plant genome with foreign genes, a new key tool for basic plant research as well as applied research was created. It was shown that genes for antibiotic resistance or herbicide tolerance could be used as reliable markers for transgenic tissue selection. Such traditional and frequently used plant selectable marker genes are, for example, the *nptII* gene granting resistance to aminoglycoside antibiotics like kanamycin, neomycin, paromomycin and G-418, the *hph* gene conferring hygromycin B resistance, or the bialaphos resistance genes *bar* and *pat* providing resistance to herbicides with phosphinothricin active compound. Unfortunately, antibiotic resistance markers are not appropriate for monocots (Wilmink & Dons, 1993) and other plant species, and they are not accepted by the public. The same is true for the markers based on herbicide resistance. In addition, the traditional selective agents often adversely affect the transformed plant cells, bringing about a decrease in the regeneration of transformed cells by the accumulation of toxic compounds from killed, non-transformed cells (Hansen & Wright, 1999). Also, for the introduction of several genes into a single transgenic plant, the development of further types of selectable markers is desirable.

Up to now, a number of marker genes have been employed for the development of alternative selection systems that avoid the use of either antibiotics or herbicides (Sundar & Sakthivel, 2008). Such promising alternative systems were developed by Joersbo and Okkels (1996) with the selectable agent benzyladenine *N*-3-glucuronide, Haldrup *et al.* (1998) with D-xylose, Kunze *et al.* (2001) with 2-deoxyglucose, Erikson *et al.* (2004) with D-amino acids, Erikson *et al.* (2005) with D-serine, Yamada *et al.* (2005) and Hsiao *et al.* (2007) with 5-methyltrytophan. Also, You *et al.* (2003) used a ferredoxin-like protein gene and Ebmeier *et al.* (2004) the *Escherichia coli* threonine deaminase gene as selectable markers. The mannose-based selection system with phosphomannose isomerase (*pmi*) gene as a selectable marker was first reported by Joersbo *et al.* (1998) for the transformation of sugar beet. In the following years, PMI was shown to be a useful marker in the transformation of a number of plant species such as cassava (Zhang *et al.*, 2000), maize (Negrotto *et al.*, 2000; Reed *et al.*, 2001; Wright *et al.*, 2001), *Arabidopsis* (Todd & Tague, 2001), wheat (Reed *et al.*, 2001; Wright *et al.*, 2001), barley and watermelon (Reed *et al.*, 2001), durum wheat (Gadaleta *et al.*, 2006), rice (Lucca *et al.,* 2001), sweet orange (Boscariol *et al.*, 2003), hemp (Feeney & Punja, 2003), pearl millet (O'Kennedy *et al.*, 2004), tomato (Sigareva *et al.*, 2004), bentgrass (Fu *et al.*, 2005), papaya (Zhu *et al.*, 2005), sorghum (Gao *et al.*, 2005), almond (Ramesh *et al.*, 2006), onion (Aswath *et al.*, 2006), cucumber (He *et al.*, 2006), Chinese cabbage (Ku *et al.*, 2006; Min *et al.*, 2007), *Torenia* hybrids (Seitz *et al.*, 2007), flax (Lamblin *et al.*, 2007), sugarcane (Jain *et al.*, 2007), apple (Degenhardt *et al.*, 2007), plum (Mikhailov *et al.*, 2007), potato (Bříza *et al.*, 2008), and citrus (Ballester *et al.*, 2008).

Cells of the majority of plant species take up mannose and convert it by endogenous hexokinase into mannose-6-phosphate (M6P). This inhibits glycolysis, depletes the cells of inorganic phosphate and induces endonucleases to degrade DNA (Stein & Hansen, 1999). The non-transformed plant cells starve and cease growing. In PMI-harbouring cells, however, the enzyme catalyses

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**Abbreviations:** BAP, 6-benzylaminopurine; MS, Murashige and Skoog medium; NAA, α-naphthalene acetic acid; NPT II, neomycin phosphotransferase II; PMI, phosphomannose isomerase.

the isomerisation of M6P to fructose-6-phosphate that is immediately metabolised through gluconeogenesis (Privalle *et al.*, 2000) and positively influences the growth of transformed cells.

The PMI enzyme occurs in bacteria, yeast and mammals (including humans) but there are only a few plant species like soybeans and several other legumes where the enzyme has been reported to be present (Lee & Matheson, 1984). Therefore, the PMI-based selection system employing the *E. coli manA* gene (Miles & Guest, 1984) is highly versatile in plants. In addition, the selection gene product is harmless; the safety assessment for PMI (Reed *et al.*, 2001) has revealed that purified PMI protein has no unfavourable effects in a mouse toxicity test and does not change glycoprotein profiles in PMI-transformed plants.

This work reports the establishment of an efficient mannose-based selection system for the production of transgenic lettuce (*Lactuca sativa* L.) plants of a Czech cultivar 'Achát'.

## MATERIALS AND METHODS

**Plasmid construction.** The pNOV2819 plant vector from Syngenta Seeds AG (Basel, Switzerland) was used as the *pmi* gene source. *nptII* gene from plasmid pGA472 (An *et al.*, 1986) was inserted into the polylinker sequence near the right border of pNOV2819, creating the binary vector pCB3160 with two selection systems (Bříza *et al.*, 2008). The *pmi* gene was driven by a short version of the cestrum yellow leaf curling virus promoter (CMPS), the *nptII* gene by the nopaline synthase promoter, and both genes had nopaline synthase terminator (tNOS). pCB3160 was transfected into *Agrobacterium tumefaciens* strain LBA4404 using the freeze-thaw method of Holsters *et al.* (1978) and the spectinomycin resistance gene of pNOV2819 was used for bacterial selection.

**Effect of mannose on shoot formation of explants.** To estimate the optimal combination of mannose/sucrose concentration in selection media, the mannose inhibition of shoot formation of explants was evaluated. Non-transformed lettuce cotyledon leaves were plated for one day on 0.75% agar complete MS medium (Murashige & Skoog, 1962) with 3% sucrose and pH 5.7 and thereafter transferred on R media comprising MS macro-, micronutrients and vitamins, 0.93 mg/L NAA, 0.56 mg/L BAP, and 16 different mannose/sucrose concentrations (Table 1). Three Petri dishes, each with eight explants, were used for every mannose/sucrose combination. The dishes were cultured under a 7 h photoperiod of 90 μmol/m² per s and 23 °C for 6 weeks with medium change after 3 weeks and the number of explants with shoots, total number of shoots and shoot size was determined.

**Plants and transformation.** For transformation of lettuce (*Lactuca sativa* L. cv. 'Achát') the modified cotyledon leaves method (Michelmore *et al.*, 1987) was used. Briefly, seeds were surface-sterilised in 70% (v/v) ethanol for 2 min then immersed in diluted commercial bleach Domestos solution for 30 min. After being washed four times (5 min each) in sterile distilled water the seeds were planted on 0.75% high gel-strength agar (Serva Electrophoresis Ltd., Heidelberg, Germany) complete MS medium supplemented with 1% sucrose and with pH adjusted to 5.7 before sterilisation by autoclaving. The culture conditions were as follows: 7 h photoperiod, irradiance of 90 μmol/m² per s and 23 °C. Ten days later, the cotyledons were scarfed to get large wounded area and incubated with suspension of *A. tumefaciens* (adjusted to an optical density of $OD_{600}$ 0.9) for 20 min with occasional gentle shaking. After the excess liquid of the bacterial suspension had been dried off on a sterile filter paper, the cotyledon explants were placed upside up for one day onto Petri dishes with agar (0.75%) complete MS medium with 3% sucrose, pH 5.7. Thereafter, the explants were transferred to 0.75% agar selection SA medium consisting of MS macro-, micronutrients and vitamins, 0.93 mg/L NAA, 0.56 mg/L BAP, 400 mg/L Timentin and with 100 mg/L kanamycin and 30 g/L sucrose or different concentrations of mannose and sucrose (sucrose 20 g/L + mannose 20 g/L = S20+M20, S25+M20, S30+M20, S20+M30, and S30 + M30). The dishes were placed in a growth chamber SGC097.CFX.F (Sanyo Biomedical Europe BV, Nijverheidsweg, Netherlands) with a 10 h photoperiod (90 μmol/m² per s) and 15/21 °C night/day temperature. When the calli and small shoots appeared after about 4 weeks, the explants were transferred onto regeneration agar SE medium with the same composition as SA medium except for plant hormones (BAP 1 mg/L, NAA was omitted). Regenerated shoots were separated from calli and cultured on rooting SC medium (complete MS medium, 0.1% 4-morpholineethanesulfonic acid, 0.2% gerlite, 400 mg/L Timentin and 100 mg/L kanamycin or combination of sucrose with mannose as in the corresponding SA medium).

**Molecular analysis of transgenic plants.** PCR assays were performed according to Klimyuk *et al.* (1993) using leaves from mannose or kanamycin resistant regenerated plants. NPT-1 (5'-ACGCAGGTTCTCCGGCCGCTTG-3') and NPT-2 (5'-GAAGCGGTCAGCCCATTCGCCG-3') primers resulted in an *nptII* gene fragment of 699 bp, and the presence of the *pmi* gene was verified using primers PMI-1 (5'-ACAGCCACTCTCCATTCA-3') and PMI-2 (5'-GTTTGCCATCACTTCCAG-3') designed by Syngenta, resulting in the amplification of a 514 bp fragment. Both PCR for *nptII* and for *pmi* were performed as duplex reactions with primers LSM-1 (5'-GAG ATT GCA AGG GTA AAT GCC ACC-3') and LSM-2 (5'-AAA ACC GGG AGA GGT TTG CAG GGG-3') amplifying a 263 bp fragment of lettuce gene coding for endo-β-1,4-mannanase and with PCR enhancer MasterAmp™ (Epicentre, Madison, WI, USA). Amplification of the 263 bp fragment in duplex reactions served as a positive control of PCR implementation in the tube. The PCR consisted of 35 cycles of 45 s denaturation at 94 °C, 30 s annealing at 55 °C for *nptII* gene or 60 °C for *pmi* and 3 min extension at 72 °C.

Genomic DNA (15 μg) extracted from leaves according to Tai and Tanksley (1991) was used for Southern blot analysis. DNA was digested with *Bam*HI restriction enzyme, electrophoresed for 16 h in 1% agarose gel with TBE (Tris/borate/EDTA) buffer (Sambrook *et al.*, 1989) and transferred onto nylon Hybond-N⁺ membrane. Hybridisations were performed according to Church and Gilbert (1984) and the membranes were probed either with the 514 bp fragment of the *pmi* gene or the 699 bp of the *nptII* gene probes labelled with [α-$^{32}$P]dCTP ($1.11 \times 10^8$ MBq/mmol) using a random priming kit, Rediprime™ II (Amersham Biosciences, Little Chalfont, UK). Hybridisations were detected by autoradiography using a phosphoimager Typhoon system (Amersham Biosciences, Little Chalfont, UK) after 5 h exposure.

For Northern blot hybridisation, total RNA was extracted from 100 mg of leaf tissue of selected transgen-

otes using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), 25 μg of RNA was fractionated in a 1% agarose gel in Mops running buffer with 1.2 M formaldehyde, transferred onto nylon Hybond-$N^+$ membrane and probed with 514 bp long *pmi* gene DNA probe labelled with [α-$^{32}$P] dCTP. The hybridisation buffer and temperature as well as conditions during membrane washing and detection were the same as for the Southern hybridisation.

## RESULTS AND DISCUSSION

### Effect of mannose on shoot formation of explants

Sixteen different combinations of mannose/sucrose in R media were used to evaluate the effect of mannose on the organogenesis of cotyledon explants (Table 1). After 6 weeks' cultivation we found that no explants produced shoots on media without sucrose or on medium with low sucrose (10 g/L) but high mannose (20 g/L) concentration. Overall it is obvious that sucrose demonstrated a protective effect against mannose — caused inhibition of the organogenesis of lettuce cotyledon leaves — the higher protection was observed for intermediate sucrose level (20 g/L) while the total number of regenerated shoots in the media with 30 g/L sucrose was a little lower. Similar results were described by Sigareva *et al.* (2004) in tomato — the addition of sucrose to the selection medium modulated the inhibitory effect of mannose on shoot formation. Analogous outcomes were also reported by Joersbo *et al.* (1999) testing the phytotoxic effect of mannose in the presence of four different non-toxic saccharides on the dry mass of sugar beet cotyledonary explants after 3 weeks of growth, by Wright *et al.* (2001) for wheat or by Negrotto *et al.* (2000) for maize. In 2008, we published (Bříza *et al.*, 2008) sucrose concentration dependency of callogenesis inhibition in tomato and potato.

### Plant transformation

On the basis of a previous evaluation of the inhibitory effects of mannose on callo- and organogenesis, we decided to use in transformation experiments five selection media differing in mannose/sucrose concentrations (see Materials and Methods). The total survey of results from all transformation experiments is shown in Table 2. Altogether, we obtained 68 putative transgenic plants (from 700 explants) forming roots on SC medium after selection on mannose media and 48 rooting plants (from 200 explants) after kanamycin selection. All these plants were assayed by PCR for both transgenes (Fig. 1). Some of the plants did not show the presence of transgenes, i.e. the regenerants presented escapes, or they revealed clonal origin (see below) despite our struggle to avoid collecting regenerated shoots harbouring the same transformation events by careful marking of shoot positions on the cotyledon explants. Basing on these results the transformation efficiency (expressed as the ratio of transgenic plants harbouring independent transgenic event to total number of planted explants) reached 25% in one experiment after mannose/sucrose 20/20 g/L (M20 + S20) selection and 21% using 100 mg/L kanamycin as a selection agent (Table 2). Southern hybridisations using *nptII* gene-derived probe (Fig. 2A) or *pmi* gene-derived probe (Fig. 2B) of the thirteen assumed transgenic plants originating from a few explants after M20 + S20 selection confirmed that some of them were of clonal character — three plants in lanes 4, 5 and 12, two plants in lanes 6 and 13, and two plants in lanes 10 and 11. Out of the nine independent transposition events in Fig. 2, a complete single copy of T-DNA is present in six cases (lanes 1, 4+5, 7, 9, 10+11, 12) whilst two or three T-DNA copies are found in three cases (lanes 2, 3, 6+13). A comparison of blot hybridisations with both probes for the plant in lane 2 showed that the plant contained one complete T-DNA and one partial sequence consisting of the right part of the T-DNA

**Table 1. Effects of mannose/sucrose on callo- and organogenesis of lettuce cotyledon explants after 6 weeks of cultivation**

| Medium | Mannose (g/L) | Sucrose (g/L) | No of explants | Feature of explants | Feature and size of calli | Explants with shoots (%) | Size of shoots (mm) | Number of shoots |
|---|---|---|---|---|---|---|---|---|
| R11 | 0 | 0 | 24 | green | none | 0 | 0 | 0 |
| R12 | 5 | 0 | 23 | greenish yellow | pale green, small | 0 | 0 | 0 |
| R13 | 10 | 0 | 24 | yellowish green | pale green, small | 0 | 0 | 0 |
| R14 | 20 | 0 | 24 | green | none | 0 | 0 | 0 |
| R21 | 0 | 10 | 24 | green | green, large | 8 | 1–2 | 2 |
| R22 | 5 | 10 | 24 | green | green, large | 50 | 3–5 | 12 |
| R23 | 10 | 10 | 24 | greenish yellow | pale green, medium | 17 | 4–7 | 4 |
| R24 | 20 | 10 | 23 | yellowy | brownish, small | 0 | 0 | 0 |
| R31 | 0 | 20 | 16 | green | green, very large | 43 | 3–6 | 7 |
| R32 | 5 | 20 | 23 | yellowish green | brownish, large | 91 | 6–20 | 21 |
| R33 | 10 | 20 | 24 | green | yellowy, large | 75 | 6–15 | 18 |
| R34 | 20 | 20 | 24 | yellow | brownish, medium | 37 | 5–8 | 9 |
| R41 | 0 | 30 | 24 | yellowish green | pale green, large | 58 | 5–7 | 14 |
| R42 | 5 | 30 | 16 | brownish yellow | brownish, large | 56 | 4–9 | 9 |
| R43 | 10 | 30 | 23 | yellowish brown | brown, medium | 56 | 5–15 | 13 |
| R44 | 20 | 30 | 24 | yellowy | brown, medium | 33 | 5–15 | 8 |

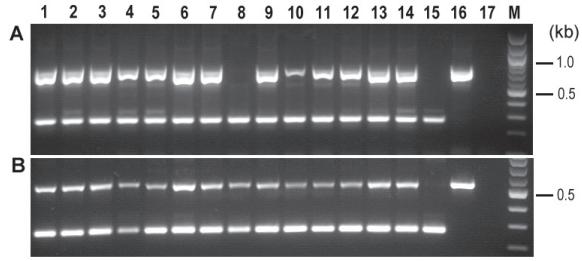


**Figure 1. PCR analyses of rooted lettuce regenerants**
Duplex reactions were performed by amplifying a 699 bp fragment of the *nptII* gene (**A**) or a 514 bp fragment of the *pmi* gene (**B**) and a 263 bp fragment of lettuce gene coding for endo-β-1,4-mannanase as a positive control of PCR implementation. Lanes 1–13, randomly chosen rooting shoots from experiments using SA34 medium; lane 14, regenerated shoot after kanamycin selection; lane 15, non-transformed lettuce plant; lane 16, *A. tumefaciens* harbouring binary vector pCB3160; lane 17, negative control; lane M, DNA marker (100 bp ladder, NEB).

with the *nptII* gene. In addition, the plant in lane 8 harboured only the left part of the T-DNA with the *pmi* gene but the right part was missing. PCR assays of this plant proved the presence of the *pmi* transgene and absence of *nptII* one. The ratio of single-copy/multiple-copy bearing transgenic plants is definitely different from the findings of Michelmore *et al.* (1987), who reported that only two out of the sixteen plants analysed appeared to have a single T-DNA insertion and other plants showed multiple independent insertions, tandem insertions or rearrangements of the T-DNA. Conversely, Lamblin *et al.* (2007) reported findings similar to ours when out of eight randomly chosen primary transformants five harboured a single T-DNA copy with *pmi* chimeric gene and three plants bore two to three copies. Control cotyledon explants (i.e., without *Agrobacterium* cocultivation) showed limited level of callogenesis and a very low level of organogenesis on the mannose media and none on kanamycin. If any shoots rooted (very rarely) on mannose they represented escapes without any PCR products, of course.

The highest transformation efficiency for mannose medium M20 + S20 reached 25%, the average was 15%. This represents a relatively high efficiency of transformation, when compared with literature data for other plant species. For example, Negrotto *et al.* (2000) reported in maize a 30% transformation efficiency, Wright *et al.* (2001) for wheat 25%, Feeney and Punja (2003) for hemp 31%, Lucca *et al.* (2001) for rice 41%, He *et al.* (2006) for cucumber 23%, Aswath *et al.* (2006) for onion 23% or 27%, Degenhardt *et al.* (2007) for apple up to 24%, Ballester *et al.* (2008) for citrange 30%, and Bříza *et al.* (2008) for potato up to 53%. On the other hand, there are plant species where mannose-based selection of transgenic shoots does not work with such a high efficiency — 0.72% transformation efficacy was described for pearl millet (O'Kennedy *et al.*, 2004), 0.94% for sugar beet (Joersbo *et al.*, 1998), 1.14% for durum wheat (Gadaleta *et al.*, 2006), and 1.4–3% for Chinese cabbage (Min *et al.*, 2007). In addition, there are reports in which transformation efficiency depends on plant genotype, type of the plasmid construct or explant type (Boscariol *et al.*, 2003; Sigareva *et al.*, 2004). Finally, some dicotyledonous plant species like carrot, tobacco, sweet potato or leguminous crops demonstrate a substantial resistance to mannose (Sundar & Sakthivel, 2008) and therefore a mannose-based selection system can not be employed for their successful transformation.

Northern hybridisation analysis of the same plant set as in Southern analysis with the *pmi* gene probe revealed the presence of *pmi*-specific mRNA of expected size in all transgenic plants assayed (Fig. 3). A markedly weak signal was observed in four out of thirteen mannose-selected plants because lanes 3 and 6 contained samples from the multiple-copy T-DNA plants, and lanes 2 and 8 from plants harbouring truncated T-DNA (see above).

The plasmid construct used allowed either mannose- or kanamycin-based selection. From Table 2 it is apparent that kanamycin gave a slightly lower transformation efficacy compared with mannose selection in single experiments (21% against 25%), but the average transformation efficiency was slightly better for kanamycin — 17.5% against 15% for experiments with M20 + S20 selection medium. A similar situation was reported by Lamblin *et al.* (2007) for flax when transformation efficacy for a mannose selection system was comparable with the kanamycin one — 3.6% *vs.* 3.3%. Our values for kanamycin are probably higher than in the work of Torres *et al.* (1993) where the authors described that only 6% of lettuce cotyledons gave rise to GUS-positive shoots for cv. South Bay after kanamycin selection. Curtis *et al.* (1994) reported genotype-independent *Agrobacterium*-mediated transformation of thirteen lettuce cultivars but it is not possible to derive transformation efficacy from their findings. They concluded, however, that al-

**Table 2. Transformation efficiency of lettuce cotyledon explants using mannose or kanamycin (Kn, 100 mg/L) selection**

| Medium | Mannose (g/L) | Sucrose (g/L) | No of explants | Explants with calli (%) | Number of rooting shoots | Number of independent transformants | Transformation efficiency (%) |
|---|---|---|---|---|---|---|---|
| SA34 | 20 | 20 | 100 | 72 | 37 | 25 | 25 |
| SA34 | 20 | 20 | 100 | 29 | 12 | 5 | 5 |
| SA51 | 20 | 25 | 100 | 34 | 3 | 0 | 0 |
| SA44 | 20 | 30 | 100 | 76 | 7 | 3 | 3 |
| SA44 | 20 | 30 | 100 | 13 | 4 | 0 | 0 |
| SA35 | 30 | 20 | 100 | 58 | 5 | 1 | 1 |
| SA45 | 30 | 30 | 100 | 49 | 0 | 0 | 0 |
| SA/Kn | – | 30 | 100 | 59 | 30 | 21 | 21 |
| SA/Kn | – | 30 | 100 | 40 | 18 | 14 | 14 |

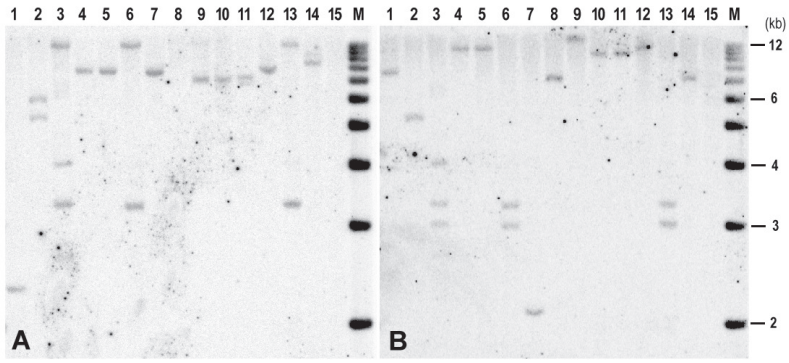


**Figure 2. Southern hybridisation analyses of transformed lettuce plants bearing pCB3160 T-DNA**
DNA digested with *Bam*HI was hybridised in successive steps with a 699 bp *nptII* probe (**A**) and a 514 bp *pmi* probe (**B**) with stripping between hybridisations. The minimal expected band size was about 2.25 kb for the *nptII* probe and 1.6 kb for the *pmi* probe. Lanes 1–15 see Fig. 1; lane M, DNA size marker (1 kb ladder, Gibco-BRL).

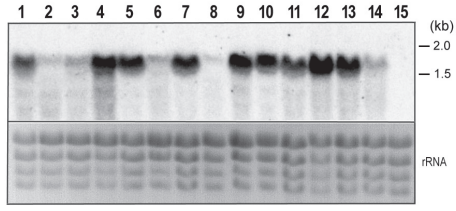


**Figure 3. Northern hybridisation analysis of transformed lettuce plants with pCB3160 T-DNA**
About 25 µg of total RNA was probed with 514 bp *pmi* gene derived probe. Lanes 1–15 see Fig. 1.

though not all cultivars could be successfully transformed at the same efficiency using a single procedure, a reliable protocol with kanamycin had been developed for routine production of transgenic lettuce plants. After that the transgenesis of lettuce using kanamycin as a selectable agent has become a standard method for different studies like introduction of a gene conferring freezing resistance (Pileggi *et al.*, 2001), systematic silencing of tobacco nitrate reductase transgene (Dubois *et al.*, 2005), expression of an oxalate decarboxylase gene from *Flammulina* sp. (Dias *et al.*, 2006), synthesis and assembly of *E. coli* heat-labile enterotoxin B subunit (Kim *et al.*, 2007) or for production of the *Mirafiori lettuce virus* resistant lettuce plants by introduction of inverted repeats of the viral coat protein gene (Kawazu *et al.*, 2009). Another selectable transgene for use in lettuce transgenesis was, however, missing.

In conclusion, we demonstrate here for the first time that a selection system based on mannose is applicable for lettuce transformation, in particular for the cultivar 'Achát', with the transformation efficiency comparable to that obtained with the classical selectable marker gene *nptII*. This opens the door for advanced plant biotechnology requiring introduction of several genes into a single transgenic lettuce plant.

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