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ORIGINAL PAPER

Phosphomannose-isomerase as a selectable marker to recover transgenic orchid plants (*Oncidium* Gower Ramsey)

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Abstract A transformation method using the phosphomannose-isomerase (*pmi*) gene as a selectable marker was developed for orchid *Oncidium* Gower Ramsey. The *pmi*-gene, which converts mannose-6-phosphate to fructose-6-phosphate allowing for selection of transgenic plants on mannose selective medium. Genetically transformed plants of *Oncidium* were regenerated after cocultivating protocorm-like bodies with *Agrobacterium tumefaciens* strain GV3101 containing the vectors pEPYON-42P and pEPYON-42H with 35S::*PMI* and 35S::*HPTII* genes respectively. We observed that 35S::*PMI* (pEPYON-42P) produced high rate (27 plants) of mannose resistant transgenic plants compared to 35S::*HPTII* (pEPYON-42H) in which only fourteen hygromycin resistant transgenic plants were obtained. Mannose resistant transgenic plants were confirmed by PCR and Southern blot. The *pmi* gene expression in 35S::*PMI* (pEPYON-42P) transgenic plants was confirmed by RT-PCR. Furthermore, the duration of regeneration time of transgenic plants was significantly shorter in mannose selected system (4 months) than in hygromycin selected system (8 months). The *pmi*/mannose selection system is shown to be highly efficient for producing transgenic *O.* Gower Ramsey without using antibiotics or herbicides. For the first time, the *pmi*/mannose-based "positive" selection system has been used to obtain genetically engineered *O.* Gower Ramsey.

Keywords *Agrobacaterium tumefaciens* · Mannose · *Oncidium* · Positive selection · Protocorm-like bodies

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Abbreviations

AS	Acetosyringone
PLB	Protocorm-like body
MS	Murashige and Skoog
Man	Mannose
PMI	Phosphomannose-isomerase
Suc	Sucrose

Introduction

Orchids are one of the most important ornamental plants that occupy top position among all flowering plants which has been valued for cut flower production and potted plants. The genus *Oncidium* (*Orchidaceae*) comprises a number of commercially important species that have produced a wide range of attractive varieties, cultivars or hybrids as a result of intensive breeding. *Oncidium* Gower Ramsey is a major and popular ornamental plant for the worldwide market and high value cash crop plant. The demand of this plant for cut flowers and potted plants has been increasing worldwide. Its many characteristics have been improved through conventional breeding programs. Compared with conventional breeding, the molecular transformation technique is an alternative approach to introduce specific characteristics into plants and is advantageous for crop improvement, especially for the modification of ornamental characteristics such as flowering time, shelf life, flower colour and architecture. The currently available methods for selecting transgenic *Oncidium* are based on antibiotic selection using hygromycin (Liau et al. 2003; Li et al. 2005) and kanamycin (Thiruvengadam and Yang 2010). However, the presence of antibiotic marker genes, seen as an unpredictable hazard to the ecosystem and human health, can be solved by removing

the selectable antibiotic marker gene. The avoidance of antibiotic or herbicide resistance markers in genetic engineering plants has been encouraged. Several positive selection systems have been developed in recent years. These include systems based on nonmetabolizable agents such as xylulose (Haldrup et al. 1998), mannose (Joersbo et al. 1998; Negrotto et al. 2000; Reed et al. 2001), 2-deoxyglucose (Kunze et al. 2001), benzyladenine-N-3-glucuronide (Joersbo and Okkels 1996) or based on the promotion of plant regeneration without the use of a selective agent, such as isopentenyl transferase (Endo et al. 2001; Zuo et al. 2002). In previous reports of *Oncidium* transgenic plant selection utilizing the sweet pepper ferredoxin-like protein (*pflp*) gene and *Erwinia carotovora* as the selection agent (You et al. 2003).

The PMI enzyme is common in nature and found across kingdoms, but is less common in the plant kingdom (Goldsworthy and Street 1965). The enzyme is present in soybeans and other legumes, but absent in many other plants (Lee and Matheson 1984). The *manA* gene codes for the enzyme PMI (E.C. 5.3.1.8) isolated from *Escherichia coli* (Miles and Guest 1984). In the presence of mannose in transformed cells, the PMI converts mannose-6-phosphate into fructose-6-phosphate that can be immediately incorporated in the plant metabolic pathway (Privalle et al. 1999). Thus, the mannose can be used as sole source of carbohydrate for the transformed cells. Mannose cannot be usually metabolized by non-transformed cells and is converted into mannose-6-phosphate by endogenous hexokinase. Therefore, when mannose is added to the culture medium, it could minimize the plant growth due to mannose-6-phosphate accumulation. This PMI selection system is extremely efficient (Joersbo et al. 1998). In addition, the selection gene product is harmless; because of the safety assessment for PMI has revealed that purified PMI protein has no unfavorable effects in a mouse toxicity test and does not change glycoprotein profiles in PMI transformed plants (Reed et al. 2001).

The pmi/Man system has been successfully used in several plant systems such as sugar beet (Joersbo et al. 1998), cassava (Zhang and Puonti-Kaerlas 2000), maize (Negrotto et al. 2000; Wright et al. 2001), Arabidopsis (Todd and Tague 2001), wheat (Wright et al. 2001), tobacco and potato (Kunze et al. 2001), pepper (Kim et al. 2002), sweet orange (Boscariol et al. 2003), pearl millet (O'Kennedy et al. 2004), tomato (Sigareva et al. 2004), papaya (Zhu et al. 2005), bentgrass (Fu et al. 2005), apple (Degenhardt et al. 2006), onion (Aswath et al. 2006), almond (Ramesh et al. 2006), cucumber (He et al. 2006), cabbage (Min et al. 2007), sugarcane (Jain et al. 2007), flax (Lamblin et al. 2007), citrus (Ballester et al. 2008), sorghum (Gurel et al. 2009) and chickpea (Patil et al. 2009). However, the pmi/Man system has not been tested previously in *Oncidium* orchid. In the present study, we report the development of an efficient system with mannose as a selectable agent for the *Agrobacterium*-mediated transformation of *Oncidium* orchid.

Materials and methods

Bacterial strains and plasmids

Genomic DNA from *Escherichia coli* strains TOP10 was extracted by TRIzol reagent (Invitrogen). The PMI coding sequence was amplified by PCR using the forward primer EcPMI-For 5′-GCTCGAG**CATG**CAAAAACTCATTAACT CAG-3′ and reverse primer EcPMI-Rev 5′-GCTCGAG **TTA**CAGCTTGTTGTAAAC-3′. The specific forward and reverse primers for PMI contained the generated XhoI recognition site (5′-CTCGAG-3′, underlined) to facilitate the cloning of the DNA. The plant transformation vector pEpyon-42H has the *hygromycin phosphotransferase* (*hptII*) gene driven by duplicated (2×) *CaMV35S* promoter and contains GFP marker gene fused with promoter and first intron of monocot maize ubiquitin 1 (Ubi-1) gene (data not shown). The vector pEpyon-42P was constructed by removing *hygromycin phosphotransferase* (*hptII*) gene from pEpyon-42H by *XhoI* digestion and replaced with the *pmi* gene (Fig. 1, CHY Lab, Taichung, Taiwan, unpublished). The plasmid pEpyon was introduced into *Agrobacterium tumefaciens* strains GV3101 by Freeze Thaw method (Höfgen and Willmitzer 1988). A single colony of *Agrobacterium* was incubated overnight in 5 ml liquid LB medium with 100 mg l^{-1} kanamycin and 15 μg/ml gentamycin on a shaker (180 rpm) at 28°C. An aliquot of the bacterial suspension (25 ml) was transferred to 50 ml fresh medium and cultured for 12–18 h to an OD600 0.7–1.0.

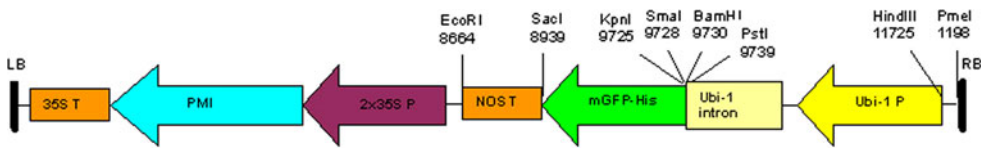

Fig. 1 **a** schematic representation of the plant transformation vector pEpyon-42P (12,057 bp). The pEpyon-42P contained the *phosphomannose-isomerase* (*pmi*) gene driven by duplicated (2×) *CaMV35S* promoter and a GFP marker gene fused with promoter and first intron of monocot Maize Ubiquitin 1 (Ubi-1) gene

Bacterial cells were harvested by centrifugation at 3,000 rpm for 10 min in a 50-ml sterile centrifuge tube (Corning, NY, USA) and then resuspended in 30 ml of in liquid MS medium supplemented with 100 μM acetosyringone (Sigma, Saint Louis, MO, USA).

Plant material and culture conditions

PLBs of the commercial variety of *Oncidium* Gower Ramsey were used as explants. They were maintained by subculturing chopped PLBs onto G10 (Liau et al. 2003) medium modified with MS salts, 20 g l^{-1} sucrose, 3.0 g l^{-1} phytagel, 1.0 g l^{-1} tryptone (Sigma), 1.0 g l^{-1} charcoal, 30 g l^{-1} potato tubers and 30 g l^{-1} banana at pH 5.6 and incubated with a 16-h photoperiod at 25°C. Light was provided by cool white fluorescent lamps with an intensity of 120 μmol m^{-2} s^{-1}. Newly formed PLBs of 45 day-old cultures were chopped and cultured in G10 medium, and then incubated at 25°C in the dark for 3 days.

Determination of mannose and hygromycin concentration for selection

To determine the optimum concentration of mannose and hygromycin inhibiting shoot formation of PLBs, explants were placed on regeneration medium containing various concentrations of mannose (0–30 g l^{-1}) in combination with different concentration of sucrose (0–20 g l^{-1}) (Fig. 2) and hygromycin (1.0–7.0 mg l^{-1}) (data not shown). After 5 weeks, explants were morphologically evaluated by determination of percentage of necrotic explants, and the number of produced new PLBs was recorded.

Genetic transformation of *Oncidium*

The 3-days precultured PLBs were transferred into *Agrobacterium* suspension with 100 μM acetosyringone and soaked for 30 min, with occasional shaking, in a sterile flask, then blotted dry on sterile filter paper. The PLBs were plated on cocultivation medium, which was MS (Murashige and Skoog 1962) medium supplemented with 200 μM acetosyringone, 30 g l^{-1} sucrose, and 2.0 g l^{-1} Gelrite at pH 5.8 and cultured in the dark for 3 days. The infected PLBs were washed with MS medium containing 300 mg l^{-1} timentin to prevent *Agrobacterium* over growth and then transferred to the selection medium containing G10 supplemented with MS salts, 25 g l^{-1} mannose (partially in place of sucrose, different concentrations of mannose and sucrose listed in Fig. 2 were used), 5.0 g l^{-1} sucrose, 1.0 g l^{-1} tryptone, 1.0 g l^{-1} charcoal, 30 g l^{-1} potato tubers, 30 g l^{-1} banana, 300 mg l^{-1} timentin and 3.0 g l^{-1} phytagel at pH 5.6, and cultured under a 16/8-h (light/dark) photoperiod at 26 ± 1°C and subcultured every week for 1 month. Transformed PLBs were transferred to G10 medium supplemented with 25 g l^{-1} mannose 5.0 g l^{-1} sucrose and 300 mg l^{-1} timentin for selecting putative transgenic plantlets within 3 months. Regenerated plants about 3–4 cm in height with five to six leaves and three to four roots were eventually transferred to pots containing sphagnum moss and acclimatized under greenhouse conditions. The same transformation protocol followed in 35S::*HPTII* (pEPYON-42H) and selection medium containing 5.0 mg l^{-1} hygromycin and 300 mg l^{-1} timentin was used.

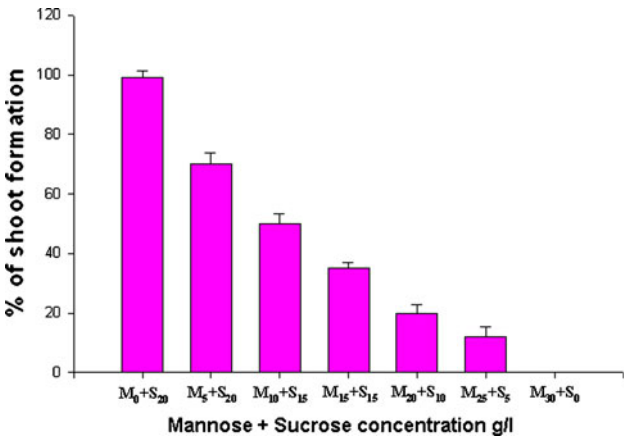

Fig. 2 Effect of various combinations of mannose and sucrose on regeneration of *Oncidium* Gower Ramsey. *M* Mannose, *S* Sucrose

PCR assay

Genomic DNA was isolated from the fresh leaves (100 mg) of putative transgenic and non transgenic plants using DNeasy Plant Mini Kit (Qiagen, Valencia, CA). PCR analysis was carried out with the genomic DNA to identify the presence of transgene. Primers used for the PCR to detect *pmi* gene insertion were EcPMI-For and EcPMI-Rev. The total volume of reaction mixtures was 20 μl, including 20 ng genomic DNA, 0.5 μl of each primer (20 μM), 1.5 μl of dNTP mix (2.5 mM), 2.0 μl buffer (10×) with $MgCl_2$ (15 mM) and 0.5 μl Taq DNA polymerase. Cycling parameters began with an initial hot start at 95°C for 5 min, then 35 cycles of denaturation (95°C; 1 min), annealing (55°C; 1 min), and extension (72°C; 45 s), followed by a final extension of 20 min at 72°C. The expected PCR products were 700 bp. PCR amplification products were analyzed by electrophoresis in 1% agarose gels.

Southern hybridization

Genomic DNA (15 μg) isolated from fresh leaves of PCR-positive transgenic plants, as well as nontransgenic control plants, was treated with *BamHI*, which digested at a unique site within the plasmid DNA, and separated on 1% agarose gels. Following gel electrophoresis, DNA was transferred

to Hybond N+ (Amersham, Buckinghamshire, UK) nylon membrane as described by Sambrook et al. (1989). A PCR-generated *pmi* gene fragment (700 bp) was used as a probe. The probe was radiolabelled with αP^{32} dCTP according to the manufacturer's instructions (DECAprime™ II, random primed DNA labeling kit, Ambion) and used for hybridization. Prehybridization, hybridization and washing were performed according to standard methods (Sambrook et al. 1989). The membranes were washed at 60°C twice with 2× SSC and 0.5% SDS (20 min each) and twice with 1× SSC and 0.1% SDS for 20 min. The washed blots were exposed to X-ray film (Kodak X-omat) with intensifying screens for signal detection at −80°C.

Total RNA isolation and RT-PCR

Total RNA was isolated from leaves of PCR positive transgenic plants and non transformed plants using the Trizol method according to instructions of the manufacturer. For cDNA synthesis, total RNA (1 µg) was reverse-transcribed in a 20 µl reaction mixture using the BcaBEST™ RNA PCR system (TaKaRa Shuzo Co., Shiga, Japan). A 5.0 µl of cDNA sample from RT reaction was used for PCR. Primers used for the RT-PCR to detect *pmi* gene expression were EcPMI-For and EcPMI-Rev. The fragment was amplified under the following conditions: one cycle of 95°C for 5 min; 35 cycle of 95°C for 1 min, 55°C for 1 min, and 72°C for 45 s; and finally elongated at 72°C for 20 min. The expected PCR products were 700 bp. The RT-PCR products were separated on a 1% agarose gel by electrophoresis.

Experimental design and data analysis

All the experiments were conducted with a minimum of 3 replicates per treatment. The experiments were repeated three times. The data were analyzed statistically using SPSS ver 14 (SPSS Inc., Chicago, USA). The significance of differences among means was confirmed using Duncan's multiple range test at $P = 0.5$. The results are expressed as a means ± SE of three experiments.

Results

Selection of mannose-resistant PLBs

As the pEpyon-42P contains the *pmi* gene which confers mannose resistance, we used the mannose as selection agent in our transformation experiments. In order to determine the sensitivity of mannose, *Oncidium* PLBs were cultured in various concentrations and combinations of mannose and sucrose (Fig. 2). The shoot growth decreased when mannose concentration increased (Fig. 2). In addition, with mannose in the medium, the shoot did not grow if the sucrose is not present. Sucrose is clearly the energy source for shoot regeneration. On medium lacking mannose, 100% of control PLBs (untransformed explants) produced shoots (Fig. 2). The inhibitory effects of mannose were observed in 1 month culture of wild type PLBs (Fig. 3a). However, with mannose concentrations over 25 g l^{-1} without sucrose, the new PLBs did not develop (Fig. 2). We had chosen as the most appropriate concentration of mannose 25 g l^{-1} and sucrose 5 g l^{-1} for the selection of transformants in subsequent experiments.

Agrobacterium-mediated transformation and selection of putative transgenic plants

The pEpyon-42P containing *pmi* gene was transformed into *Oncidium* orchid PLBs via *A. tumefaciens*-mediated transformation. After the proliferation stage, mannose was used to select the putative transformants. After subculture, the transformed PLBs were green and rapidly grew whereas the untransformed PLBs turned brown and starved in the G10 medium with 25 g l^{-1} mannose and 5 g l^{-1} sucrose (Fig. 3b). After the surviving PLBs were sub-cultured, shoots developed rapidly, and roots were formed

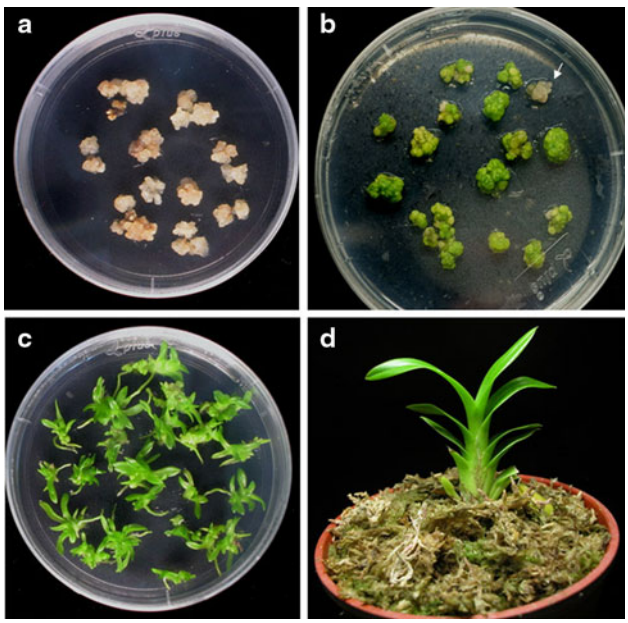

Fig. 3 Regeneration of PLB explants of *Oncidium* in mannose selection medium. **a** Non transgenic PLBs cultured on G10 medium with 25 g l^{-1} mannose and 5 g l^{-1} sucrose. **b** Transgenic PLBs (pEPYON-42P) cultured on selection medium (G10 + 25 g l^{-1} mannose and 5 g l^{-1} sucrose + 300 mg l^{-1} timentin). (*Arrow* indicated of untransformed PLBs). **c** Shoots and roots were well developed from pEPYON-42P transformants cultured on G10 medium containing 25 g l^{-1} mannose with 5 g l^{-1} sucrose and 300 mg l^{-1} timentin. **d** A transgenic plant of 35S::*PMI* (pEPYON-42P) grown on sphagnum moss

spontaneously after 1 month (Fig. 3c). From one hundred transformed PLBs used for transformation experiment, 69 PLBs survived mannose selection and 27 putative transgenic plants were regenerated from culture (Tables 1, 2). All 27 putative transgenic plants were produced from PLBs within 4 months. These transgenic plants exhibited a normal phenotype (Fig. 3d). We randomly selected four independent mannose resistant putative transgenic orchid plants for molecular analyses.

Molecular analyses of transgenic plants

To confirm that these plants were transformants, total DNA and RNA were isolated and analyzed from the leaves of both non transformed plants and four pEpyon-42P transgenic plants. All tested transgenic lines showed the specific fragments of *pmi* gene (700 bp) detected by PCR, while no fragment was amplified in non transformed wild type plants (Fig. 4a). Foreign gene integration into the transgenic plant was confirmed by Southern blot analysis. Southern blot analysis was performed on genomic DNA from four independent PCR positive transgenic *Oncidium* plants (Fig. 4b, lanes 1–4). Results show that all four transgenic lines have a single copy of the transgene integrated into their genome, while no signal was detected in the untransformed control (Fig. 4b, lane 5). The presence of DNA for *pmi* gene in pEpyon-42P transgenic leaves testified truth of the transgenic plants. Furthermore, RT-PCR was performed and the results confirmed that *pmi* was strongly expressed in four pEpyon-42P transgenic plants (Fig. 4c). According to the results of PCR, Southern blot and RT-PCR, we believed that the *pmi* was introduced into these transformed plants.

Comparison of transformation efficiencies in mannose and hygromycin resistant plants

Parallel experiments were performed to compare the transformation efficiencies and culture duration for regeneration in mannose (pEpyon-42P) and hygromycin (pEpyon-42H) selection systems (Fig. 5a, b). A total 69/100 for pEpyon-42P and 42/100 for pEpyon-42H were survived in mannose and hygromycin selection respectively. Twenty-seven putative pEpyon-42P mannose resistant plants and fourteen pEpyon-42H hygromycin resistant plants were successfully regenerated from culture (Table 2). PCR screening revealed that the *pmi* gene was detected in 21.0% of the plants regenerated after mannose selection (Table 1, Fig. 5a) and the hygromycin selection system was 9% (Fig. 5a). In addition, we compared the duration of

Table 1 Transformation efficiency of pEPYON-42P transformed *Oncidium* PLBs using various concentrations of mannose and sucrose

Selection marker		No. of PLBs explants infected	No. of rooted plantlets	PCR positive plants	Transformation frequency (%)
Mannose	Sucrose				
0	20	100	12cd	0	0
5	20	100	10c	0	0
10	15	100	6e	0	0
15	15	100	10c	1c	1c
20	10	100	16b	3b	3b
25	5	100	27a	21a	21a
30	0	100	0	0	0

Each value represents the mean ± SE of three replicates per treatment. The data were statistically analyzed using Duncan's Multiple Range Test (DMRT). In the same *column*, significant differences according to the least significant difference (LSD) at the $P = 0.5$ level are indicated by different *letters*

Table 2 Comparison of the efficiency to generate the putative transgenic plants (mannose and hygromycin resistant) using different genes (PMI and HPTII) in *Oncidium*

Name of the constructs and selectable marker genes	No. of PLBs explants infected	No. of PLB's surviving on Mannose/hygromycin medium	No. of regenerated shoots	No of rooted plantlets (Mannose/hygromycin resistant)
pEPYON-42P (35S::*PMI*)	100	69a	31a	27a
pEPYON-42H (35S::*HPTII*)	100	42b	22b	14b

Each value represents the mean ± SE of three replicates per treatment. The data were statistically analyzed using Duncan's Multiple Range Test (DMRT). In the same *column*, significant differences according to the least significant difference (LSD) at the $P = 0.5$ level are indicated by different *letters*

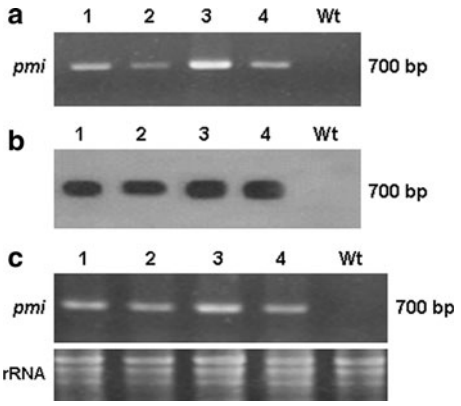

Fig. 4 PCR, Southern hybridization and RT-PCR assay for pEPYON-42P transgenic *Oncidium* plants. **a** PCR assay of *pmi* gene by using primers EcPMI-For and EcPMI-Rev. A 700 bp DNA fragment was amplified. Total genomic DNA was isolated from four pEPYON-42P transgenic T_0 *Oncidium* plants (*lane 1–4*) and one non transgenic wt plant (*lane 5*). **b** Southern blot analysis of transgenic plants. *Lanes 1–4* transgenic plants, *5* nontransgenic plant. Genomic DNAs were digested with *BamHI* and hybridized to a 700 bp *pmi*-probe. **c** RT-PCR assay of *pmi* expression by using primers EcPMI-For and EcPMI-Rev. A 700 bp cDNA fragment was amplified. Total RNA was isolated from four pEPYON-42P transgenic T_0 *Oncidium* plants in (**a**) (*lane 1–4*) and one non transgenic wt plant (*lane 5*)

regeneration time in hygromycin and mannose resistant plants of *Oncidium* (Fig. 5b). Mannose resistant plants were obtained from PLBs within 4 months. In contrast, hygromycin resistant plants required the period of 8 months (Fig. 5b).

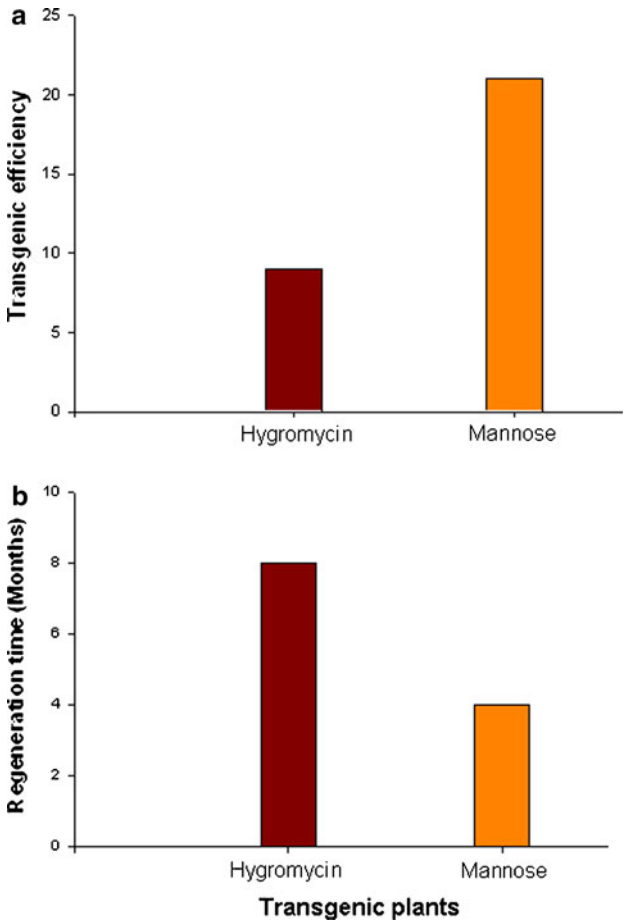

Fig. 5 Transformation efficiency and regeneration of transgenic *Oncidium* with hygromycin or mannose selection system. **a** Transgenic efficiency of pEPYON-42H and pEPYON-42P transgenic *Oncidium* plants. **b** Culture duration for regeneration of pEPYON-42H and pEPYON-42P transgenic *Oncidium* plants

Discussion

The system based on *pmi* as the selectable marker and mannose as the selective agent was successfully used to recover transgenic *Oncidium* plants. Genetic transformation using the PMI/mannose system has been carried out in many plant species using mannose (Boscariol et al. 2003; Wang et al. 2000; Todd and Tague 2001; Wright et al. 2001). The addition of sucrose to the selection medium can modulate the inhibitory effect of mannose on shoot formation and can make the recovery of transgenic shoots better in many plants (Joersbo et al. 1998; Negrotto et al. 2000; Zhang and Puonti-Kaerlas 2000; He et al. 2006). In our study, we used 25 g l^{-1} mannose and 5 g l^{-1} sucrose. The addition of sucrose and higher concentration of mannose was required for better repression of non transformed shoot formation. He et al. (2004) used 15–20 g l^{-1} mannose in combination with 5 g l^{-1} sucrose to obtain the highest transformation frequency (6.0%) for rice plants. In order to eliminate the non-transgenic escapes, the mannose concentration was increased in the course of selection (Table 1; Fig. 2).

In our transformation study, chopped PLBs pre-treated with 3 days darkness produced more mannose-resistant PLBs. In contrast, 7 days of darkness were needed for *Oncidium* transformation (Liau et al. 2003). *Agrobacterium*-mediated transformation, phenolic compounds such as AS serve as a potent inducer for the expression of the virulence (*vir*) genes located on the Ti plasmid of *A. tumefaciens* (Ashby et al. 1987). Since orchids are not natural hosts of *A. tumefaciens*, previous studies have shown that addition of AS to infection and coculture medium is effective in producing transgenic plants in several orchid species belonging to *Dendrobium* (Men et al. 2003), *Phalaenopsis* (Belarmino and Mii 2000) and *Oncidium* (Liau et al. 2003). Our present results also indicate that the presence of AS during infection and cocultivation period improved the transformation efficiency.

The most common selective protocols for plant transformation are the use of kanamycin, hygromycin, and

phosphinothricin. We demonstrated that kanamycin was ineffective up to 200 mg l^{-1} (our unpublished data). Orchids have some level of endogenous resistance to kanamycin (Chai and Yu 2007). The selection of hygromycin inhibits the root formation in transgenic plants of *Oncidium* (You et al. 2003). Kanamycin and hygromycin are very expensive, it is not recommended because of its high toxicity to humans (Altmann et al. 1992).

The selection strategy based on the use of a PMI gene as selectable gene and mannose as selective agent has proven to be highly efficient. Thus, we obtained transformation frequencies that were significantly higher than hygromycin selection system (Table 2, Fig. 5a). This observation agrees well with the earlier studies in sugar beet (Joersbo et al. 1998), sweet orange (Boscariol et al. 2003), rice (Lucca et al. 2001), maize (Wright et al. 2001) and almond (Ramesh et al. 2006). However, *Oncidium* orchid is one of the difficult crops to transform using kanamycin selection with a transformation rate of less than 5% (our unpublished data) and hygromycin system produced transformation efficiency of 9% (Fig. 5b). You et al. (2003) reported that 12% of *E. carotovora* resistant plants (*pflp*) were obtained. In our present investigation the transformation efficiency was observed higher (21%), compared to previous *Oncidium* transformation. In sugar beet, the mannose selection system using *Agrobacterium* was reported to result in tenfold higher transformation frequencies as compared to kanamycin selection (Joersbo et al. 1998). However, in cassava, the efficiency of hygromycin selection was about twofold higher than that of mannose selection-using PEG mediated particle bombardment (Zhang and Puonti-Kaerlas 2000). This indicated that transformation frequency on mannose selection varies with each crop. In this study, mannose resistant transgenic plants were obtained less than 4 months (Fig. 5b). In contrast, hygromycin resistant plants were obtained within 8 months (Fig. 5b). This indicated that mannose selected system is not only increased the transformation efficiency but also shorten the duration of regeneration time for *Oncidium* orchids.

In conclusion, we demonstrated for the first time, that "positive" selection based on mannose is compatible with popular ornamental plants of *Oncidium* orchid transformation. This mannose system provides an efficient way for transforming the selectable genes of potential commercial value such as genes that regulate flower color, shape, scent and longevity in orchid improvement in the future.

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