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Optimized Conditions for Biolistic-mediated Transformation of *Lilium longiflorum* 'Nellie White'

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ABSTRACT

A variety of tissues were used for biolistic-mediated transformation of *Lilium longiflorum* 'Nellie White'. Transgenic plants were not recovered from five-month-old, "non-select" callus or suspension cells that had been bombarded with pDM327 that contains the *bar-uidA* fusion gene under control of the CaMV 35S promoter. In comparison, ten transgenic plants were recovered from "select" callus that had been selected for its embryogenic-like appearance. Transgenic plants were also obtained following direct bombardment of precultured bulb scales. Both preculture time and preculture medium had an impact on transformation frequencies. Bulb scales cultured overnight on Murashige and Skoog's medium containing 1.0 mg/L picloram before bombardment with 0.6 µm gold resulted in a transformation frequency of 0.9%. A one week or one month preculture time on Murashige and Skoog's medium containing 1.0 mg/L picloram yielded higher, 4.2% and 5%, transformation frequencies, respectively. When bulb scales were cultured on Murashige and Skoog's medium with either 2.0 mg/L dicamba or 0.5 mg/L picloram, lower frequencies of transformation, 1.2% and 0.8%, respectively, were observed. Direct bombardment of bulb scales eliminates the time needed for callus induction and multiplication when embryogenic callus is bombarded.

Keywords: flower bulb crops, flower transformation, gene gun
Abbreviations: BA, 6-benzyladenine; **GUS**, β-glucuronidase; **MS medium**, Murashige and Skoog medium; **NAA**, 1-naphthaleneacetic acid; **PCR**, polymerase chain reaction; **PPT**, phosphinothricin; **SDS**, sodium dodecyl sulfate; **SSC**, sodium chloride/sodium citrate

INTRODUCTION

Lilies are one of the most important floral crops as they are grown in gardens, sold as a pot plant, and popular as a cut flower. Currently lilies are the number one cut flower in the U.S. with an annual wholesale value of $6.1 million USD (National Agricultural Statistics Service Floriculture Crops 2011). *Lilium longiflorum* 'Nellie White' was used in this study because it is currently and has been the predominant Easter lily cultivar in the U.S. for many years.

Most reports on transformation of lilies have used the *uidA* reporter gene, which codes for β-glucuronidase, *Gus*. The transformation efficiency of lilies is generally very low, except for a few recent reports (Li *et al*. 2008; Azadi *et al*. 2010; Liu *et al.* 2011; Núñez de Cáceres *et al.* 2011). Both *Agrobacterium* (Mercuri *et al.* 2003; Hoshi *et al*. 2004, 2005; Ogaki *et al*. 2008; Krens *et al*. 2009; Azadi *et al*. 2010; Liu *et al*. 2011; Núñez de Cáceres *et al*. 2011; Wang *et al.* 2012) and the gene gun (Watad *et al*. 1998; Cohen *et al*. 2004; Ahn *et al*. 2004; Shi *et al*. 2012) have been used to transform lilies. Most studies with *L. longiflorum* used 'Snow Queen' (Watad *et al*. 1998; Mercuri *et al*. 2003; Cohen *et al.* 2004). To date, *Lilium* ×*formolongi* has been transformed with multiple genes of the carotenoid biosynthesis pathway (Azadi *et al*. 2010), *Lilium* 'Acapulco' with a defective cucumber mosaic replicase gene for virus resistance (Azadi *et al*. 2011), and *L. davidii* with L-galactono-1, 4-lactone dehydrogenase for stimulating vitamin C levels (Shi *et al*. 2012).

There is one report of low frequency transformation of *Lilium longiflorum* 'Nellie White' using biolistics in which the selective agents, shooting pressure, and distance for gene gun bombardment were compared (Kamo and Han 2008). The low transformation efficiency for lilies has hindered development of transgenic lilies with potentially useful genes. To further improve the transformation frequency, in this study I investigated several factors influencing biolistic-mediated transformation of 'Nellie White'. These included the type of explants (select and non-select callus, suspension cells, and bulb scales), preculture conditions on bulb scale explants (preculture time and preculture medium), and size of the gold particles used for bombardment (0.6 µm vs. 0.75 µm). Transgenic plants were obtained after direct bombardment of precultured bulb scales which significantly decreases the 3-12 months needed for callus induction and multiplication.

MATERIALS AND METHODS

All chemicals were purchased from the Sigma-Aldrich Chemical Co., St. Louis, MO unless stated otherwise.

Plant materials

L. longiflorum 'Nellie White' plants were grown *in vitro* on MS medium (Murashige and Skoog 1962) containing 3% sucrose, 2% Phytagel and the following in mg/L: 1.0 glycine, 1.0 thiamine, 0.5 nicotinic acid, 1.0 pyridoxine, and 100 *myo*-inositol (Kamo and Han 2008). Plants were grown under cool-white fluorescent lights (40-60 $\mu mol \cdot m^{-2}s^{-1}$) with a 12 h photoperiod. These plants were the explant source of bulb scales for direct bombardment and for callus induction. All bulb scales were used from a bulb, except for the innermost ones that were difficult to peel away from the inner core without damaging them. Bulb scales used directly for bombardment and callus induction were cultured in the dark at 25°C.

Preculture treatments for bulb scales prior to bombardment included: (1) One month culture on MS medium supplemented with either 2.0 mg/L 4-amino-3,5,6-trichloropicolinic acid (dicamba), 0.5 or 1.0 mg/L 3,6-dichloro-o-anisic (picloram); (2) One week preculture on MS medium containing 1.0 mg/L picloram; (3) 24 h preculture on MS medium containing 1.0 mg/L picloram; and (4) One week preculture on MS medium containing 3.0 mg/L BA

Received: 31 August, 2012. Accepted: 5 October, 2012.

Original Research Paper

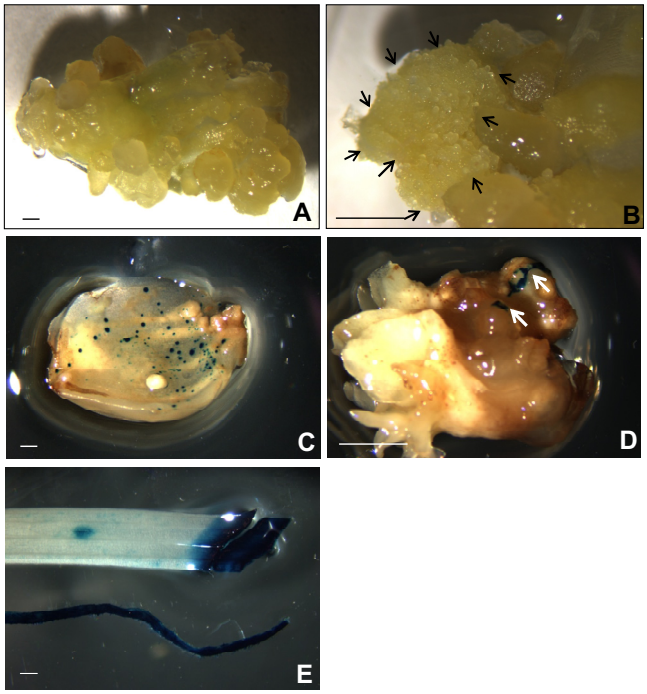

Fig. 1 Both (**A**) non-select and (**B**) select callus (indicated by arrows) of *L. longiflorum* 'Nellie White' were induced from bulb scales cultured 3 months on MS medium with 0.5 mg/L picloram. *Gus* staining one month (**C**) and three months (**D**) after bombardment of bulb scales that had been precultured for one month on MS medium containing 1.0 mg/L picloram before bombardment with pDM327. Arrows in (**D**) indicate region where *Gus* expressing cells have undergone cell division. (**E**) Transformed plant showing *Gus* positive staining in its leaf (top) and root (bottom). Magnification bar represents 1 mm.

and 0.1 mg/L NAA. Non-select callus was induced from bulb scales cultured on MS medium supplemented with either dicamba (2.0 mg/L) or picloram (0.5 or 1.0 mg/L) (**Fig. 1A**). Non-select callus was not used for selecting embryogenic-like callus so it was a mixture of both non-embryogenic and embryogenic callus. Approximately 3 months after callus induction, embryogenic-like callus ("select" callus) was excised and cultured separately for six months until it had multiplied (**Fig. 1B**). Both five-month-old non-select callus and select callus were used for bombardment. All callus was grown in the dark at 25°C and transferred monthly to fresh medium.

Suspension cells derived from non-select callus were cultured in a 125 ml flask containing 30 ml of liquid MS medium supplemented with 0.5 mg/L picloram. The cells were aerated on a gyratory shaker at 120 rpm in the dark at 25°C. Once a month the liquid suspension medium was completely replaced with fresh medium, and the cells were divided in half and placed in 30 ml of fresh medium.

Transformation

Plasmid DNA was pDM327 (received from David McElroy, Verdia, Redwood, CA) and consists of the *bar-uidA* fusion gene under control of the CaMV 35S promoter. Several experiments used co-bombardment with a Bluescript vector containing Oc1ΔD86 that codes for a modified cystatin gene under the CaMV 35S promoter (Howard Atkinson and Catherine Lilley, Leeds University, Leeds, UK) and pDM307 that contains the *bar* gene under the CaMV 35S promoter (D. McElroy). Alkaline lysis followed by purification on a cesium chloride gradient was used for plasmid DNA isolation (Dellaporta *et al.* 1983).

Target tissues were bombarded with either 0.6 or 0.75 µm diameter gold particles (Alameda Scientific Instruments Richmond, CA) that had been coated with plasmid DNA according to the method of Sanford *et al.* (1993). The PDS-He system (Bio-Rad, Richmond, CA) set with a 1 cm gap and a 1 cm flying membrane distance was used to shoot cells 1x/plate at 1100 psi and at a 12 cm target distance. All callus, suspension cells, and bulb scales, except for the bulb scales precultured overnight, were cultured four days on MS medium supplemented with the appropriate hormone and 0.125 M mannitol prior to bombardment. After bombardment, cells or bulb scales were transferred to the same medium lacking mannitol. In some experiments, non-select callus was cultured on MS medium containing 2.0 mg/L dicamba and an antioxidant (either 10 mg/L silver nitrate, 200 mg/L L-cysteine, or 500 mg/L citric acid) or 100 µM validamycin A for 5 days prior to bombardment. Callus maintained on MS medium containing either an antioxidant or validamycin A and 0.1 mg/L phosphinothricin (PPT, received from AgrEvo, Pikeville, NC) for one month after bombardment.

Selection

One week after bombardment, callus, suspension cells, and bulb scales were transferred to MS medium supplemented with the appropriate hormone and 0.1 mg/L PPT that was filter sterilized using a 0.45 µm Millex PF filter (Millipore, Bedford, MA) and added to autoclaved medium. Selection subcultures were performed monthly. One month later, shoots were excised and transferred to MS medium that lacked hormones but contained 1 mg/L PPT. Culture of shoots was under the same light conditions as for *in vitro*-grown plants. The PPT selection pressure was increased to 0.5 mg/L PPT for the first subculture and to 1 mg/L for the remaining subcultures until shoots formed and the callus or bulb scales were dead. Bulb scales precultured on MS medium containing 3.0 mg/L BA and 0.1 mg/L NAA were transferred to MS medium with 3.0 mg/L BA, 0.1 mg/L NAA, and 1 mg/L PPT rather than 0.5 mg/L PPT after the second subculture because numerous shoots regenerated vigorously from these bulb scales.

Gus staining

Histochemical staining was performed to visualize *Gus* expression one and three months following bombardment. Three plates of target cells were stained for each time point and for each replicate, except for the experiments using select callus because of an insufficient amount of callus available. The tissues were incubated for 16 h at 37°C in staining solution (Jefferson *et al*. 1987) modified with the addition of 20% methanol, 0.1% Triton X-100, 0.5 mM potassium ferricyanide, and 0.5 mM potassium ferrocyanide, pH 7.0. Tissue was destained in 70% ethanol until no visible chlorophyll remained.

PCR analysis

Genomic DNA was isolated from leaves of in vitro-grown plants using the FASTPREP plant DNA isolation kit according to the manufacturer's directions (Qbiogene, Carlsbad, CA). Each 50 µl PCR reaction contained 200 ng DNA, 1.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer specific for either the *bar* gene (5′-CAT GCC AGT TCC CGT GCT TGA AG-3′ and 5′-GTC AAC TTC CGT ACC GAG CCG CAG-3′) or *uidA* gene (5′-TAA CCT TCA CCC GGT TGC CAG AGG-3′ and 5′-CTT TAA CTA TGC CGG AAT CCA TCG-3′) and 0.5 units of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). Amplification was performed using an MJ Research PTC-0200 Peltier Thermal Cycle (MJ Research, Waltham, MA) programmed for 94°C for 4 min, followed by 36 cycles (94°C for 20 s, 67°C for 30 s, 72°C for 2 min), and then 72°C for 10 min.

Southern blot hybridization

Genomic DNA was isolated from leaves of plants grown in vitro using the method of Dellaporta *et al.* (1983). DNA (30 µg) was digested with *Xba* I followed by electrophoresis in a 0.7% agarose gel with TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) and then capillary transfer of DNA from the agarose gel to a Nytran membrane (Schleicher-Schuell, Keene, NH). Southern blot hybridization was performed using a probe resulting from PCR using the *bar* gene primers. This PCR probe was gel-purified using a Prepagene kit (Bio-Rad) according to the manufacturer's directions followed by random prime labeling with α-[^{32}P]dCTP

Table 1 *Gus* staining and transformation results for select callus, non-select callus, and suspension cells grown on MS medium containing either dicamba (2.0 mg/L) or picloram (0.5 or 1.0 mg/L) and then bombarded with 0.6 µm gold particles.

Hormone (mg/L)	*Gus* staining (No. blue spots/plate)		Putative transformants	
	One month	Three months	No. plates	No. plants
Non-select callus				
2.0 dicamba	296 ± 80 a*	53 ± 22 a	11	0
0.5 picloram	171 ± 22 a	79 ± 16 a	7	0
1.0 picloram	108 ± 25 a	54 ± 10 a	11	0
Select callus bombarded with pDM327				
2.0 dicamba	ND	18 ± 6	11	0
0.5 picloram	91 ± 13	21 ± 14	9	0
1.0 picloram	ND	ND	10	1
Select callus co-bombarded with p35S cystatin and pDM307				
0.5 picloram (1-year-old)			10	1
0.5 picloram (10-months-old)			10	4
0.5 picloram (6-months-old, attached to bulb scale)			10	2
1.0 picloram (10-months-old)			13	0
1.0 picloram (2-4 months-old, attached to bulb scale)			9	2
Suspension cells				
0.5 picloram	27 ± 9	6 ± 2	23	0

*Values with different letters are significantly different at $P < 0.05$ according to Dunn's Method used to compare the number of blue spots resulting from *Gus* expression for each treatment. Because there was not enough callus for some treatments, the tissue was not stained as indicated by no data (ND).

using the DECAprime II kit (Ambion, Austin, TX). Prehybridization, Southern blot hybridization, and washing after hybridization were performed according to Maniatis *et al*. (1982). The membrane was hybridized with the probe for 16 h at 60°C. The blot was then washed in either 2X SSC, 0.2% SDS; 1X SSC, 0.2% SDS, and lastly 0.1X SSC, 0.2% SDS. Each wash was 15 min and 60°C. The membrane was exposed to X-ray film with an intensifying screen for 5 days at -70°C.

Statistical analysis

Each bombardment experiment consisted of 9 plates of plant material, except for the experiments using select callus which varied depending on the availability of the callus. Three plates were stained for *Gus* expression one month after bombardment, three plates were stained 3 months after bombardment, and the remaining three plates were cultured on selection medium for the regeneration of transformed plants. The number of blue spots indicating *Gus* expression was counted for each plate and the standard error calculated. Each treatment was repeated twice. An ANOVA followed by Dunn's Method was performed using Sigmastat (www.systat.com) to determine if the treatments were significantly different at $P \leq 0.05$.

Ethics

All experiments were done in a laboratory approved for recombinant DNA research by the Animal Plant Health Inspection Service of the U.S. Department of Agriculture and the U.S.D.A. Beltsville Area Research Biosafety Committee under recombinant DNA projects #61 and #64.

RESULTS AND DISCUSSION

Non-select callus, select callus, and suspension cells

There were no putatively transformed plants recovered from non-select callus (**Table 1**). *Gus* positive spots, 108-296/plate one month after selection and 53-79/plate two months after selection, were observed on the bombarded, non-select callus**.** More importantly, the size of the *Gus* positive spots did not show an obvious increase in size during the 1-3 months following bombardment, possibly indicating that either the initially bombarded cells were not destined for further cell division or possibly they had been damaged from the gold particles which consequently prevented them from undergoing further cell division. These results were not affected by the hormones dicamba or picloram used for callus induction and multiplication.

Application of three antioxidants (silver nitrate, L-cysteine, and citric acid) did not result in a significant increase in the number or size of *Gus* positive spots (data not shown) although it was anticipated that these antioxidants would help to reduce the necrosis and browning resulting from wounded lily callus cells following particle bombardment. These results were different from previous publications where antioxidants improved the regeneration and transformation efficiencies for other species (Dan 2008). Validamycin A inhibited trehalase accumulation in transgenic tobacco and transgenic potato tubers, and this resulted in the accumulation of high intracellular levels of the osmoprotectant, trehalose which promoted plant regeneration (Goddijn *et al*. 1997). Inclusion of validamycin A in the medium following Agro-infection increased the transformation efficiency for strawberry (Husaini 2010), but in this study it did not increase the number of *Gus* positive spots for bombarded lily callus (data not shown).

In the experiment using pDM327 to bombard select callus, 800 bulb scales were cultured on MS medium for each hormone concentration, and only 11 plates of select callus were obtained from bulb scales cultured on MS medium containing 2.0 mg/L dicamba, 9 plates using 0.5 mg/L picloram, and 10 plates using 1.0 mg/L picloram. Histochemical staining for Gus expression showed that the number of Gus-positive spots spots for select callus was 91 blue spots/plate and 18-21 spots/plate one and three months after bombardment, respectively. This is lower than the number observed on non-select callus (**Table 1**). Eventually, one putatively transformed plant was recovered after one year of selection on MS medium containing 1.0 mg/L picloram and 1 mg/L PPT from 10 plates of select callus bombarded with pDM327 (**Table 1**).

Nine putatively transformed plants were recovered from 52 plates of select callus that had been cultured on MS medium containing either 0.5 or 1.0 mg/L picloram and then co-bombarded with pDM307 and p35S-cystatin that contains the cystatin gene under control of the CaMV 35S promoter (**Table 1**). PCR analysis indicated that all nine plants contained the *bar* gene and 8 plants the cystatin gene (data not shown). Select callus that was 2-6-months-old and still attached to the bulb scale resulted in transformed plants as well as older, 10-12-month-old select callus that was no longer attached to the bulb scale. Apparently selection using PPT could successfully be applied to callus that was attached to the bulb scale. It is unclear as to why the transformation efficiency was higher when callus was co-bombarded with pDM307 and p35S-cystatin as compared to pDM327, but generally the more actively growing, select callus appears to be better for transformation.

Transgenic plants were not recovered from suspension cells grown in MS medium containing 0.5 mg/L picloram.

Table 2 *Gus* staining and transformation results for bulb scales (BS) cultured one month on MS medium containing either dicamba (2.0 mg/L), picloram (0.5 or 1.0 mg/L) before bombardment with gold particles that were either 0.6 or 0.75 µm.

Hormone (mg/L)	Gold size (µm)	*Gus* staining (No. blue spots/plate)		Putative transformants	
		One month	Three months	One month	Three months
2.0 dicamba	0.6	107 ± 84 a*	30 ± 16 a	80	1 (1.2%)
2.0 dicamba	0.75	152 ± 57 a	34 ± 9 a	90	1 (1.1%)
0.5 picloram	0.6	106 ± 23 a	17 ± 3 a	110	0 (0%)
0.5 picloram	0.75	109 ± 33 a	12 ± 5 a	120	1 (0.8%)
1.0 picloram	0.6	136 ± 59 a	110 ± 29 b	100	5 (5%)
1.0 picloram	0.75	92 ± 38 a	26 ± 6 a	120	1 (0.8%)

*Values with different letters are significantly different at P<0.05 according to Dunn's Method used to compare the number of blue spots indicating *Gus* expression for each treatment.

Bombarded suspension cells showed fewer *Gus* positive spots than both non-select and select callus, and the size of the spots did not increase during the three months after bombardment (**Table 1**). This result was different from previous reports where Cohen *et al.* (2004) reported that the transformation frequency of suspension cells of *Lilium longiflorum* 'Snow Queen' was significantly higher than that of callus. Tribulato *et al*. (1997) described suspension cells of 'Snow Queen' as rapidly-growing and consisting of fine cell clusters that were regenerable. Our 'Nellie White' suspension cells were relatively slow-growing and consisted of large cell clusters, and this difference in quality of the suspension cells between the cultivars probably contributed to our inability to transform 'Nellie White' suspensions.

One month precultured bulb scales

The hormone and its concentration in the MS medium had an impact on transformation of bulb scales (**Table 2**). MS medium containing 1.0 mg/L picloram yielded the highest number of *Gus* positive spots/plate 3 months after bombardment, when dark blue spots or areas indicated cell division of the primary bombarded cell (**Fig. 1C, 1D**). In addition, 5% of the bombarded bulb scales produced putative transformants after 18-24 months of selection. A significant effect of gold size on transformation was only observed on MS medium containing 1.0 mg/L picloram; gold 0.6 µm in diameter resulted in a higher transformation frequency than gold 0.75 µm in diameter (**Table 2**).

Gus staining was observed in both leaves and roots of five transgenic plant lines (**Fig. 1E**). Two lines (20 and 31) did not show blue staining, and two lines (32 and 35) showed blue staining in some of their leaves but not roots indicating that lines 32 and 35 could be chimeric. PCR amplification of the *uidA* and *bar* genes indicated that lines 20 and 31, that did not express *Gus* in leaves and roots, contained both genes (**Fig. 2**). The result indicated that silencing or weak expression of the *uidA* gene could be responsible for the *Gus* negative result in lines 20 and 31. Thus, 5 out of 9 transgenic lines obtained from bulb scales in this study appeared to be non-chimeric transformants. This ratio is higher than that of our previous report where 3 out of 8 transgenic lines derived from callus explants were apparently non-chimeric transformants (Kamo 2011).

Bulb scales cultured overnight to one week

A relatively low transformation frequency, 0.9%, was observed for bulb scales precultured overnight (**Table 3**). These bulb scales showed only 18 *Gus* positive spots/plate one month after bombardment. Bulb scales precultured one week on MS medium containing 1.0 mg/L picloram produced 5 PPT-resistant plants from 120 bulb scale explants after 10-12 months of selection. This 4.2% transformation frequency was comparable to the 5% frequency for the bulb scales precultured one month on MS medium with 1.0 mg/L picloram.

The one transgenic plant (line 21) that was isolated following overnight culture of the bulb scale was *Gus* negative in both its leaves and roots, and PCR analysis confirmed that the *uidA* gene was absent, but the *bar* gene was

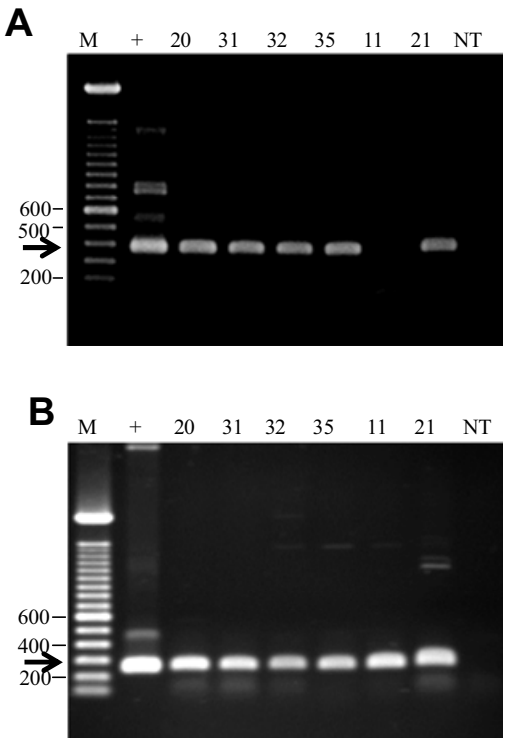

Fig. 2 PCR amplification of putatively transformed plants that had *Gus* negative leaves and roots following *Gus* staining showed that (**A**) a fragment of the *uidA* gene was present in five of the six independently transformed lines, and (**B**) a fragment of the *bar* gene was present in all six lines (lower gel). Numbers above each lane indicate the line number of each plant. A 100-bp ladder (M) was used as the molecular marker. The positive control (+) was pDM327, and non-transformed (NT) plants were negative.

present (**Fig. 2**). The 5 transgenic lines resulting from bulb scales cultured one week before bombardment showed variable *Gus* staining. Two transgenic lines were *Gus* positive for both leaf and root tissues, one line was *Gus* positive in root but not leaf tissues, and two lines were *Gus* negative. PCR analysis showed that line 11, *Gus* negative in its leaves and roots, lacked the *uidA* gene but contained the *bar* gene (**Fig. 2**).

'Nellie White' plants can be regenerated directly from bulb scales with minimal callus formation when the bulb scales are cultured on MS medium containing 3.0 mg/L BA and 0.1 mg/L NAA. Bulb scales were cultured on this medium for one week prior to bombardment with pDM327. Although many phenotypically normal shoots formed less than one month after bombardment, none of these shoots grew when transferred to MS medium lacking hormones and containing 1 mg/L PPT. Recently, Shi *et al*. (2012) recovered transformed plants following bombardment of bulb scales from *L. davidii* var. unicolor, the edible lily. Bulb scales of the edible lily were taken from plants grown *in vitro* on MS medium containing 1.0 mg/L BA and 0.3 mg/L NAA. Following bombardment the bulb scales of edible lily

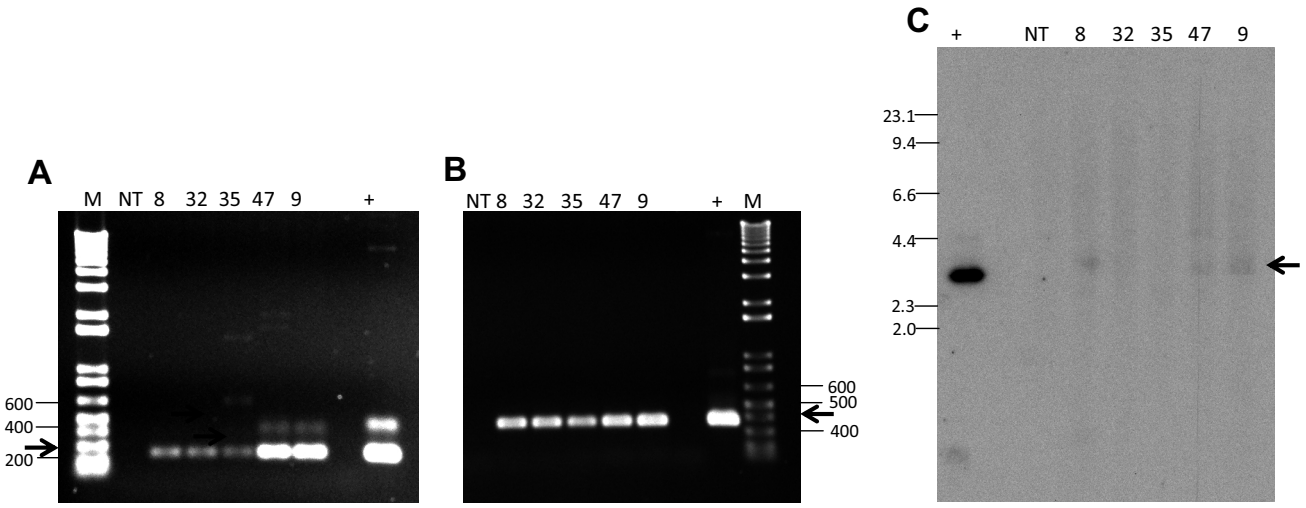

Fig. 3 Southern blot hybridization and PCR amplification of *Gus* positive 'Nellie White' plants. PCR amplification indicated that leaves from the five plant lines contained (**A**) the *uidA* gene as indicated by the 253 bp PCR product and (**B**) the *bar* gene as indicated by the 379 bp PCR product. Plant line numbers and a non-transformed plant (NT) are indicated above each lane. PCR gels show a 100-bp ladder as the molecular markers (M). The positive control (+) is pDM327. (**C**) DNA blot of genomic DNA digested with *Xba* I (30 µg/lane) followed by Southern hybridization with a PCR generated probe of the *bar* gene labeled with [^{32}P]-dCTP. The positive control (+) is 50 pg of the 3 kb fragment following digestion of pDM327 with *Xba* I.

were cultured on MS medium for 9 days and then transferred to MS medium containing 1.0 mg/L BA and 0.3 mg/L NAA, and transgenic plants were recovered at a 1.3% transformation frequency.

Southern hybridization

Five transgenic lines that showed positive *Gus* staining were subjected to Southern blot hybridization. Genomic DNA was digested using *Xba* I that digests pDM327 at the beginning of the *bar* gene and at the end of the *nos* terminator resulting in a 3 kb fragment. A faint, approximately 3 kb band was detected in lines 47 and 9, and no bands were observed in non-transformed plants and lines 8, 32, and 35 (**Fig. 3C**). The large lily genome might be responsible for the faint hybridizing bands for lines 47 and 9. The absence of hybridizing bands for lines 8, 32, and 35 suggested that these three lines could be chimeric. PCR amplification showed that lines 8, 32, and 35 contained both the *uidA* and *bar* genes, but because the PCR bands were faint for the *uidA* gene fragment and relatively weak for the *bar* gene fragment, these plant lines were probably chimeric (**Fig. 3A, 3B**).

CONCLUSION

Obtaining transgenic plants from select callus of 'Nellie White' is very time-consuming and laborious. Transgenic plants were not recovered using non-select callus and suspension cells.

Bulb scales cultured either one week or one month on MS medium containing 1.0 mg/L picloram prior to bombardment with 0.6 µm gold particles resulted in the highest frequencies (4.2-5%) of transgenic plants. This is the highest transformation frequency reported to date using gene gun bombardment of lily.

ACKNOWLEDGEMENTS

Dr. Keerti Rathore (Texas A&M, College Station, TX) is thanked for his helpful suggestions in this study. Anne O'Connor prepared the DNA blot for Southern hybridization. Mention of a trademark, proprietary product or vendor does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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