

Stable genetic transformation of lily scales without tissue culture

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Article

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Abstract

Lilium brownii is a perennial monocot with significant edible, medicinal, and ornamental value, but its genetic transformation system requires sterile tissue culturing to induce callus formation. However, the existing genetic transformation system for this plant is underdeveloped, resulting in limited research on the genetic improvement of lily and the functional annotation of its genes. Using *L. brownii* scales, this study established a tissue culture-free genetic transformation system involving *Agrobacterium tumefaciens*. Additionally, the effects of different *A. tumefaciens* strains, infection conditions and durations, and the addition of paclobutrazol (PBZ) on infection and callus induction efficiency were systematically investigated. The addition of PBZ increased the scale induction rate, thereby promoting differentiation and survival. Moreover, it increased the bulblet rooting rate and enhanced root growth. According to the study data, the optimal infection system for lily scales comprised the following: *A. tumefaciens* strain EHA105 (resuspension concentration of OD600 = 1.0) supplemented with PBZ for a shaking culture for 30 min. This system successfully integrated a target gene into the lily genome with a transformation rate of 11.76%. This study optimized key parameters of a tissue culture-free genetic transformation system for lily scales, providing a reference for establishing efficient and stable genetic transformation protocols, with implications for gene editing and molecular breeding of lilies.

1. Introduction

Lilium brownii, a perennial monocot, is a valuable edible, medicinal, and ornamental lily species. Considering the lack of a highly efficient and reliable genetic transformation system for this plant, gene function research and validation typically depends on tissue culture for callus induction. However, such research requires tissue culture expertise and sterile conditions. Furthermore, the lily callus induction system has not been fully optimized, with issues during induction often resulting in yields that are insufficient for genetic transformation. This suboptimal genetic transformation of lilies has restricted molecular breeding and gene-editing research. Therefore, this study was conducted to establish a stable, tissue culture-free genetic transformation system for lilies, thereby providing researchers with a novel method for the functional validation of genes and molecular mechanisms.

To date, genetic transformation of lilies has primarily relied on transient transformation methods, including *Agrobacterium tumefaciens*-mediated transformation and pollen magnetization-mediated transformation (Zhang et al., 2023; Li et al., 2025b). As a novel transient transformation approach, pollen magnetization-mediated transformation involves the use of nanomagnetic beads complexed with DNA plasmids to introduce foreign genes into lily pollen via magnetic force, which is followed by the insertion of the transformed pollen into lilies via vacuum infiltration. In a previous study of *Lilium regale*, optimizing the medium composition and magnetic bead ratios during pollen magnetization-mediated transformation resulted in a transformation efficiency that was as high as 88.32% (Zhang et al., 2023). However, *A. tumefaciens*-mediated transformation is the most widely used method for the genetic transformation of lilies. Advantages of this method include its relative simplicity, low cost, and high transformation efficiency (Krensek et al., 2015), but its efficiency is influenced by various factors (e.g.,

explant type, *A. tumefaciens* strain, and culture conditions) (Yan et al., 2019; Fan and Sun, 2024). Earlier research showed that the regenerative capacity of lily calli decreases with age and varies significantly depending on the callus source. Furthermore, during the transient transformation of lily, the regeneration frequency remains low and the growth performance of regenerated plants is often unstable (Wang et al., 2012). Although transient transformation technology enables rapid gene expression in plants, its low integration efficiency leads to inconsistent expression patterns and unstable inheritance by subsequent generations. These limitations have significantly restricted the utility of gene-editing technologies for the genetic improvement and breeding of lily varieties.

Methods for the stable genetic manipulation of lilies often use plant tissue culture techniques for callus induction. Notably, callus induction varies among lily varieties, explant types, developmental stages, medium formulations, and culture conditions. A previous study that used filaments, pedicels, styles, ovaries, and anthers as explants for callus induction revealed that the filaments of the Oriental hybrid lily ‘Sorbonne’ generated the most embryogenic calli, with the callus induction rate inversely related to the developmental stage (Du et al., 2014). In a recent study, the callus induction rate for an *in vitro* anther culture of *Lilium longiflorum* peaked when 0.75–1.0 mg/L 2,4-D and 4.0 mg/L kinetin were used (Li et al., 2025a). For *Lilium cernuum*, the optimal medium for scale-derived callus induction reportedly comprises Murashige and Skoog medium (MS), 2.0 mg/L 6-BA, and 1.0 mg/L NAA (Hwa and Soo, 2013). Furthermore, cultivation conditions influence callus formation. For example, a low-temperature pretreatment (4°C) and subsequent culturing in darkness can enhance callus induction from *L. longiflorum* anthers, although a prolonged low-temperature pretreatment may decrease the callus induction rate (Li et al., 2025b). To date, there has been some progress in lily callus-related research, but several challenges remain. Numerous studies indicate that lily calli are prone to browning, which adversely affects regeneration (Martínez and Whitaker, 1995; Chun et al., 2017). Additionally, relatively inefficient regeneration is a concern because callus differentiation rates are suboptimal in certain lily varieties, necessitating the optimization of hormone ratios, culture conditions, and morphogenetic processes (Li et al., 2017; Lestari et al., 2019). Some studies have established genetic transformation systems using calli as recipient materials for *A. tumefaciens*-mediated transformation, but several issues remain unresolved, including strong genotype effects, unstable transformation efficiency, and difficulties in regenerating transformed plants (Fan and Sun, 2024).

A stable lily genetic transformation method involving callus induction relies on tissue culture techniques and sterile environments, but is associated with low differentiation and regeneration efficiencies, ultimately limiting the stable inheritance of foreign genes and hindering functional gene research and the genetic improvement of lilies via breeding. In this study, a stable, tissue culture-free genetic transformation system was established based on *A. tumefaciens*-mediated transformation, thereby providing the technical basis for the functional annotation of lily genes and the breeding of new lily cultivars.

2. Materials and Methods

2.1. Experimental Materials

Healthy *L. brownii* bulbs with a similar growth status were purchased from a local grower in Wanzai County, Yichun City, Jiangxi Province, China. No specific permit was required for these commercially obtained materials.

2.2. Preparation of LhERF4-Overexpressing Strains

The pCAMBIA3301-*LhERF4* (Cao et al., 2024) overexpression vector was inserted into *A. tumefaciens* GV3101 and EHA105 strain cells, which were then added to LB solid medium supplemented with 100 mg/L kanamycin and 50 mg/L rifampicin in plates. All plates were inverted and incubated at 28°C for 48 h. The cell growth status was examined during the culture period. Individual colonies were analyzed by PCR, with confirmed transformants (i.e., those that yielded a PCR product of the expected size) used for the inoculation of LB liquid medium containing 50 mg/L kanamycin and 50 mg/L rifampicin. Cells were cultured at 28°C with shaking (200 rpm) for 24 h.

2.3. *Agrobacterium tumefaciens* Infection

Healthy middle-layer *L. brownii* scales with no detectable disease symptoms (e.g., spots) were immersed in MMA resuspension medium (4.43 g/L MS without vitamins, 20 g/L sucrose, 1.95 g/L MES, 10 mM MgCl₂, and 100 µM acetosyringone) with or without 10 µM PBZ (pH adjusted to 5.6). Bacterial cultures were diluted to OD₆₀₀ = 1.0 or 1.5. For each treatment, 50 scales were infected and incubated at 28°C with shaking (200 rpm) for 15 min or 30 min. Scales were then collected and embedded in trays. Details regarding scale treatment groups are provided in Table 1.

Table 1
Treatment of scales with different *Agrobacterium tumefaciens* cell cultures.

Treatment	OD ₆₀₀	Time	PBZ addition or not
GV	1.0	15 min	-
GV	1.0	30 min	-
GV	1.5	15 min	-
GV	1.5	30 min	-
GV	1.0	15 min	+
GV	1.0	30 min	+
GV	1.5	15 min	+
GV	1.5	30 min	+
EH	1.0	15 min	-
EH	1.0	30 min	-
EH	1.5	15 min	-
EH	1.5	30 min	-
EH	1.0	15 min	+
EH	1.0	30 min	+
EH	1.5	15 min	+
EH	1.5	30 min	+

2.4. Lily Scale Induction and Differentiation

The infected bulb scales were subjected to vernalization at 10°C. Cold-induced lily scales were inserted into substrate at a 45° angle (1/3 depth, with the tip facing upward) and then incubated in a climate-controlled chamber maintained at 20–25°C with appropriate humidity (about 70%) to induce differentiation and rooting.

After small bulbs differentiated from scale bases, they were transferred to plug trays and cultured separately at approximately 20°C. The number of differentiated small bulbs and their differentiation rates were recorded. Some small bulbs were randomly selected for a phenotypic analysis and root growth comparison. Samples were mixed and collected, with 10 bulbs as one replicate per treatment, and then stored at -80°C for future use.

2.5. Extraction and Analysis of DNA From Mixed Root Samples

To minimize the risk of residual *Agrobacterium* contamination on the tissue surface, all samples collected from differentiated bulblets (roots and leaves) were subjected to a rigorous surface sterilization protocol prior to DNA extraction. Tissues were washed thoroughly with running tap water, immersed in 75% ethanol for 30 s, followed by 2% sodium hypochlorite for 10 min, and finally rinsed five times with sterile distilled water. Genomic DNA was then extracted using an EasyPure Universal Plant Genomic DNA Kit (Beijing TransGen Biotech Co., Ltd.).

2.6. PCR Amplification and Analysis of Small Bulb Leaf Samples

For the treatment group in which positive bands were detected for mixed root samples, small bulb leaves were collected for a PCR analysis, which was completed using a HyperMB Extraction-Free Plant Genomic PCR Direct Amplification Kit (BBI).

3. Results

3.1. Analyses of the Lily Scale Differentiation Rate and Number of Small Bulbs

Treatment groups with optimal lily scale induction and differentiation were identified following infections with different *A. tumefaciens* strains, concentrations, durations, and systems. Experiments involving treatments without paclobutrazol (PBZ) revealed the following. For the *A. tumefaciens* GV3101 treatment group, a 15 min treatment was conducive to differentiation and survival, with $OD_{600} = 1.0$ resulting in a relatively high scale induction rate. For the *A. tumefaciens* EHA105 treatment group, a 30 min treatment was conducive to differentiation and survival, with $OD_{600} = 1.5$ resulting in a relatively high induction rate. When PBZ was included, a 30 min treatment was conducive to differentiation and survival for the *A. tumefaciens* GV3101 treatment group. By contrast, for the *A. tumefaciens* EHA105 treatment group, a 15 min treatment was conducive to differentiation and survival. For the *A. tumefaciens* GV3101 treatment group, a 30 min treatment that included PBZ, with $OD_{600} = 1.5$, resulted in a high scale induction rate, which was appropriate for differentiation and survival (Table 2). For the *A. tumefaciens* EHA105 treatment group, a 15 min treatment that included PBZ, with $OD_{600} = 1.5$, resulted in a high scale induction rate, which was appropriate for differentiation and survival (Table 3).

Table 2
Statistical analysis of lily scales and differentiated small bulbs following a treatment with *Agrobacterium tumefaciens* GV3101.

Treatment	Scale-induced survival quantity	Scale-induced differentiation rate	Bulb quantity
GV	35	70%	87
GV	9	18%	14
GV	14	28%	38
GV	10	20%	18
GV	11	22%	20
GV	10	20%	29
GV	11	22%	16
GV	28	56%	77

Table 3
Statistical analysis of lily scales and differentiated small bulbs following a treatment with *Agrobacterium tumefaciens* EHA105.

Treatment	Scale-induced survival quantity	Scale-induced differentiation rate	Bulb quantity
EH	13	26%	18
EH	25	50%	66
EH	9	18%	25
EH	27	54%	84
EH	22	44%	83
EH	9	18%	17
EH	32	64%	62
EH	17	34%	30

3.2. Phenotypic Examination of Lily Scales and Comparative Root Analysis

To analyze the effects of different treatments on lily bulb root growth, differentiated small bulbs were photographed and compared. For the PBZ-treated lily plantlets, when the PBZ concentration was 0.3–1.0 mg/L, the rooting rate and average number of roots increased significantly, indicating that a low PBZ concentration promotes rooting (Li et al., 2008). In addition, PBZ-treated samples exhibited better root development (i.e., length and quantity) than non-PBZ-treated groups. Thus, PBZ increased the rooting rate of lily bulbs and enhanced root growth. In the *A. tumefaciens* GV3101 treatment group, a 15 min

treatment resulted in better root growth than a 30 min treatment, suggesting that a relatively short treatment duration is suitable for this group (Fig. 1). However, in the *A. tumefaciens* EHA105 treatment group, a 30 min treatment resulted in better root growth than a 15 min treatment, implying that a relatively long treatment duration is appropriate for this group (Fig. 2).

3.3. Identification of Transgenic Plants

After the small bulbs that differentiated from infected scales had rooted, samples were collected for PCR analysis. Importantly, to assess whether the transgene was present in newly developed tissues rather than merely persisting as surface contamination on the original infected scales, we performed PCR on genomic DNA extracted specifically from the roots and leaves of the regenerated bulblets—tissues that developed after the infection event and are spatially separated from the initial inoculation site (the scale base). As shown in Fig. 3, target bands were detected in the leaf samples of treatments EH-7, EH-1, and EH-3. The detection of the target gene in these newly differentiated organs suggests that the transgene has been transmitted through cell division during organogenesis and is likely integrated into the plant genome. According to these results, *A. tumefaciens* EHA105 with OD₆₀₀ = 1.0 and infecting for 15–30 min was ideal for infecting lily plants. The target gene was detected in regenerated T0 plantlets, with a transformation efficiency of 1.15% for treatment group EH and 11.76% for treatment group EH.

We acknowledge that definitive proof of stable germline transmission would require analysis of the T1 generation. However, due to the extended life cycle (2–3 years to flowering) and self-incompatibility characteristics of *Lilium brownii*, obtaining T1 progeny was not feasible within the timeframe of this study due to these biological constraints. Therefore, the current results represent successful detection of the transgene in T0 regenerants, with evidence supporting integration based on multi-tissue analysis.

4. Discussion

This study used *A. tumefaciens* to establish a convenient, rapid, and tissue culture-free stable genetic transformation system for lilies (Fig. 4). Conventional lily genetic transformation methods rely on callus induction, which requires a sterile culture environment and is constrained by inefficient callus induction, differentiation, and regeneration. These limitations severely inhibit the stable inheritance of exogenous genes in lilies, thereby restricting research on gene functions as well as the genetic improvement of lily varieties. The transformation system developed in this study can overcome these technical bottlenecks, providing researchers with an efficient and reliable platform for gene functional analyses and molecular breeding of lilies.

Previous studies on lilies showed that genetic transformation efficiency can be enhanced by modulating key factors during the genetic transformation process. For example, during an earlier optimization of embryogenic callus induction and *A. tumefaciens*-mediated genetic transformation, a comparative analysis of different *A. tumefaciens* strains (EHA105, AGL1, LBA4404, and GV3101) demonstrated that EHA105 has the highest infection capability (Qi et al., 2014; Chen et al., 2023). The injection of *A.*

tumefaciens cells carrying *DsRed2* into *L. regale* bulbs can significantly promote gene expression, with a transformation efficiency of 86.58% (Deng et al., 2024). In a separate study on *A. tumefaciens*-mediated genetic transformation of lilies with the *ACO* antisense gene, the infection of a bulb-derived callus from the Oriental hybrid lily 'Sorbonne' using strain EHA105 resulted in a final transformation efficiency of 7.14% (Zhang et al., 2008). In the current study, a method for the stable genetic transformation of lilies under non-sterile conditions was preliminarily established. Notably, *A. tumefaciens* EHA105 was the most suitable strain for infecting lily plants. However, the faint bands observed for the mixed root samples may be attributed to suboptimal extraction of DNA from lily root tissues and/or inefficient transformation. Additionally, the inclusion of PBZ in the resuspension medium significantly influenced lily bulb root development, likely via the regulation of the endogenous hormone balance. Earlier research on the effects of PBZ on lily plantlet growth under artificial conditions showed that an appropriate PBZ concentration can enhance both rooting and adventitious bud differentiation, resulting in dwarfism, increased leaf number, and well-developed root systems. However, treatments with high PBZ concentrations reportedly induce leaf curling and even chlorosis (Hua and Li, 2015). Adding PBZ to the transient transformation system for Oriental lilies can significantly increase the floral tepal infection efficiency (Fatimah et al., 2019). In the present study, lily scales were infected using resuspension media with and without PBZ. Consistent with the results of previous research, treatments that included PBZ were conducive to the integration of foreign genes into the lily genome.

The lily genome is large, highly heterozygous, and complex, making genetic transformations difficult. Transient transformation technology is inappropriate for stably integrating genes into the genome for the subsequent inheritance by progeny. Moreover, the stable transformation of lilies relies on sterile tissue culture. Furthermore, the lily callus induction system remains underdeveloped, which has significantly constrained research on lily gene functions. Therefore, there is an urgent need for more efficient stable transformation methods. This study established a simple, rapid, and stable transformation system involving *A. tumefaciens* under non-sterile conditions, providing technical support for molecular breeding and the functional characterization of lily genes.

While this study establishes a convenient and rapid *Agrobacterium*-mediated transformation system for lily scales without tissue culture, we acknowledge certain limitations inherent to working with perennial species. The confirmation of stable transformation currently relies on molecular analysis of T0 regenerants, with evidence for integration supported by detection of the transgene in newly differentiated organs (roots and leaves) that are spatially and developmentally distinct from the original infection site. Definitive proof of germline transmission, which would require analysis of T1 progeny, is hindered by the biological constraints of *Lilium brownii*, including its prolonged vegetative growth phase (2–3 years to reach reproductive maturity) and self-incompatibility. Consequently, inheritance studies are beyond the scope of the present work. Nevertheless, this system provides a valuable platform for functional genomics studies in lily, enabling rapid in vivo validation of candidate genes without the need for time-consuming tissue culture procedures. Future research will focus on accelerating the flowering cycle or employing cross-pollination strategies to obtain T1 generations and confirm the heritability of the transgenes.

5. Conclusions

In summary, this study has preliminarily established a genetic transformation system for *L. brownii* mediated by *Agrobacterium tumefaciens* without relying on tissue culture techniques. According to the study findings, treatment with *A. tumefaciens* EHA105 (OD₆₀₀=1.0) and PBZ for 30 min is suitable for infecting lily plants to introduce exogenous genes, with a transformation rate of 11.76%. This system represents a viable alternative to other stable genetic transformation systems requiring sterile tissue culture for callus induction. Moreover, it may be useful for future research on lily gene functions as well as the genetic improvement of lilies.

Declarations

CRedit authorship contribution statement

Conceptualization, Y.C.; methodology, Y.C.; validation, R.Z.; formal analysis, J.Y.; investigation, Y.M.; resources, Y.C.; data curation, Z.Z. and J.C.; writing—original draft preparation, R.Z.; writing—review and editing, Y.C.; visualization, R.Z.; supervision, Y.C.; project administration, Y.C.; funding acquisition, Y.C. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

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Data Availability

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

References

1. Cao, Y.W., Song, M., Bi, M.M., Yang, P.P., He, G.R., Wang, J., Ming, J., 2024. Lily (*Lilium* spp.) LhERF4 negatively affects anthocyanin biosynthesis by suppressing *LhMYBSPLATTER* transcription. *Plant Sci.* 342, 112026.
2. Chen, Y., Hou, X., Zheng, Y., Lyu, Y., 2023. The establishment of a genetic transformation system and the acquisition of transgenic plants of Oriental hybrid lily (*Lilium* L.). *Int. J. Mol. Sci.* 24, 782.

3. Chun, L., Xiao, Y., Min, H., Rong, L., Li, C., Xiu, L., 2017. Techniques of reducing tissue culture browning of *Lilium lancifolium*. *Subtrop. Plant Sci.* 46, 122-125.
4. Deng, J., Li, W., Li, X., Liu, D., Liu, G., 2024. A fast, efficient, and tissue-culture-independent genetic transformation method for *Panax notoginseng* and *Lilium regale*. *Plants* 13, 2509.
5. Du, Q., Liu, T., Zhou, D., Wang, H., 2014. Embryogenic cultures of lily (*Lilium* spp.): optimising callus initiation, maintenance, and plantlet regeneration. *J. Hortic. Sci. Biotechnol.* 89, 159-166.
6. Fan, X., Sun, H., 2024. Correction: Exploring *Agrobacterium*-mediated genetic transformation methods and its applications in *Lilium*. *Plant Methods* 20, 138.
7. Fatihah, H.N.N., Moñino López, D., Van Arkel, G., Schaart, J.G., Visser, R.G.F., Krens, F.A., 2019. The ROSEA1 and DELILA transcription factors control anthocyanin biosynthesis in *Nicotiana benthamiana* and *Lilium* flowers. *Sci. Hortic.* 243, 327-337.
8. Hua, Z.R., Li, X.L., 2015. Effect of paclobutrazol and chloride on the growth of lily plantlets. *Chin. Agric. Sci. Bull.* 31, 144-149.
9. Hwa, K.B., Soo, E.Y., 2013. Plant regeneration through the callus culture induced from bulb scales of an endangered species *Lilium cernuum* Komarvo. *J. Plant Biotechnol.* 40, 65-71.
10. Krenek, P., Samajova, O., Luptovciak, I., Daskocilova, A., Komis, G., Samaj, J., 2015. Transient plant transformation mediated by *Agrobacterium tumefaciens*: Principles, methods and applications. *Biotechnol. Adv.* 33, 1024-1042.
11. Lestari, K.D., Deswiniyanti, N.W., Astarini, I.A., Arpwi, L.M., 2019. Callus and shoot induction of leaf culture *Lilium longiflorum* with NAA and BAP. *Nusantara Biosci.* 11, 133-138.
12. Li, H., Zhang, J., Yang, Y., Jia, N., Wang, C., Sun, H., 2017. miR171 and its target gene SCL6 contribute to embryogenic callus induction and torpedo-shaped embryo formation during somatic embryogenesis in two lily species. *Plant Cell Tissue Organ Cult.* 130, 591-600.
13. Li, M., Bi, H., Zhu, Y., Zhou, Q., Yong, Y., Xing, W., 2025a. *In vitro* regeneration and *Agrobacterium*-mediated genetic transformation of *Lilium lancifolium*. *Plant Cell Tiss. Organ Cult.* 162, 60.
14. Li, X.L., Hua, Z.R., 2008. Effects of PP333 on cultivating strong seedlings and rooting of test-tube lily seedlings. *J. Anhui Agric. Sci.* 36, 5307-5308.
15. Li, Y.Y., Li, Y.F., Dong, X.K., Cai, Y.F., Chen, J.R., Liu, R., Zhu, F., 2025b. Cultivating callus from anthers and regenerating haploid plants in *Lilium longiflorum*. *Horticultrae* 11, 349.
16. Martínez, M.V., Whitaker, J.R., 1995. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* 6, 195-200.
17. Qi, Y., Du, L., Quan, Y., Tian, F., Liu, Y., Wang, Y., 2014. *Agrobacterium*-mediated transformation of embryogenic cell suspension cultures and plant regeneration in *Lilium tenuifolium* Oriental × Trumpet 'Robina'. *Acta Physiol. Plant.* 36, 2047-2057.
18. Wang, Y., Kronenburg, B., Menzel, T., Maliepaard, C., Shen, X.H., Krens, F., 2012. Regeneration and *Agrobacterium*-mediated transformation of multiple lily cultivars. *Plant Cell Tiss. Organ Cult.* 111, 113-122.

19. Yan, R., Wang, Z.P., Ren, Y.M., Li, H.Y., Liu, N., Sun, H.M., 2019. Establishment of efficient genetic transformation systems and application of CRISPR/Cas9 genome editing technology in *Lilium pumilum* DC. Fisch. and *Lilium longiflorum* White Heaven. *Int. J. Mol. Sci.* 20, 2920.
20. Zhang, J.X., Liu, Y.L., Meng, R., Wang, Y.J., 2008. Research on genetic transformation of ACO antisense gene into lily mediated by *Agrobacterium tumefaciens*. *Acta Agric. Boreali-Occident. Sin.* 17, 158-163.
21. Zhang, M.F., Ma, X., Jin, G., Han, D.Y., Xue, J., Du, Y.P., Chen, X.Q., Yang, F.P., Zhao, C.L., Zhang, X.H., 2023. A modified method for transient transformation via pollen magnetofection in *Lilium* germplasm. *Int. J. Mol. Sci.* 24, 15304.

Figures

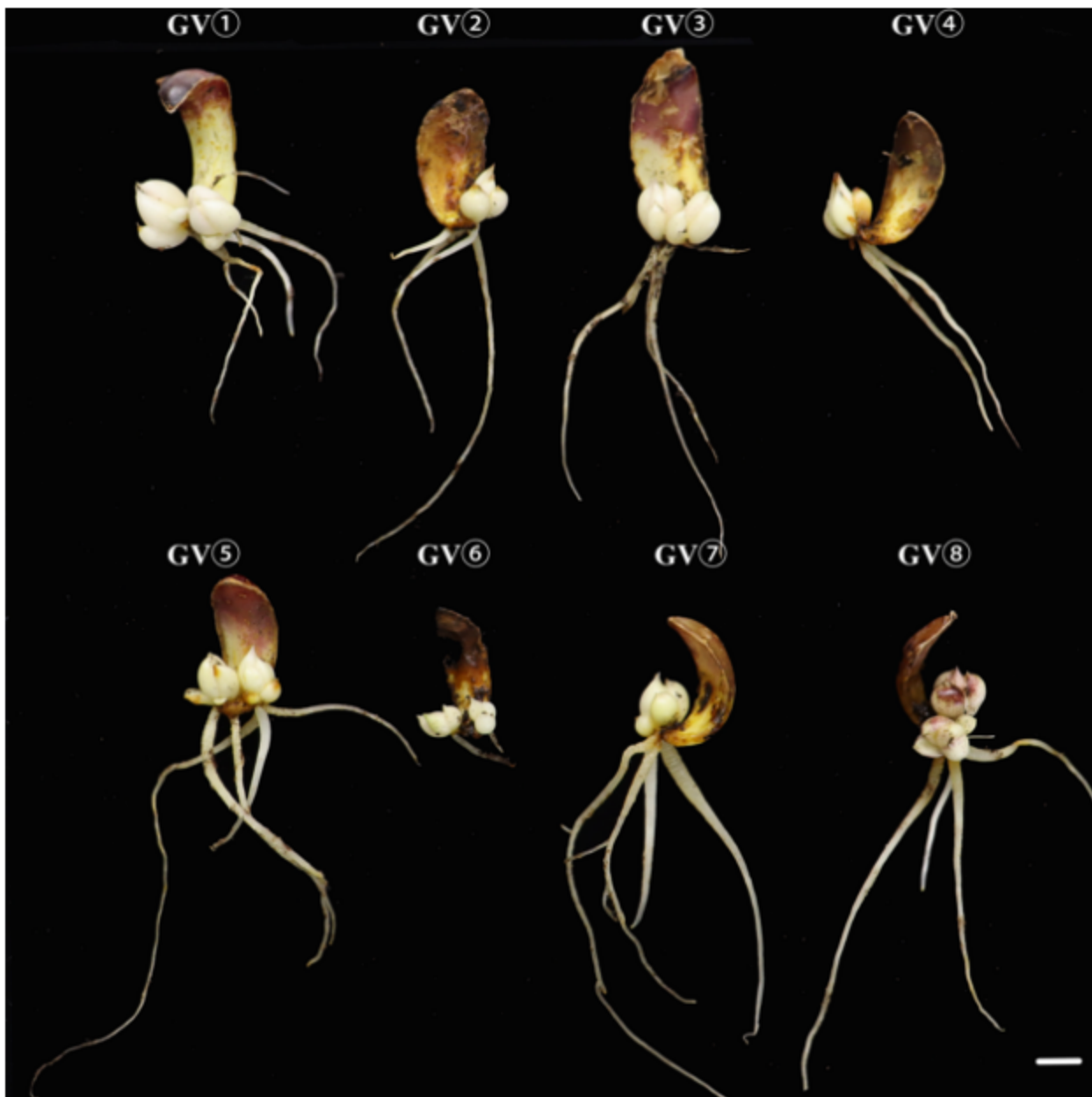


Figure 1

Lily bulb differentiation after a treatment with GV3101. The scale bar represents 1 cm.

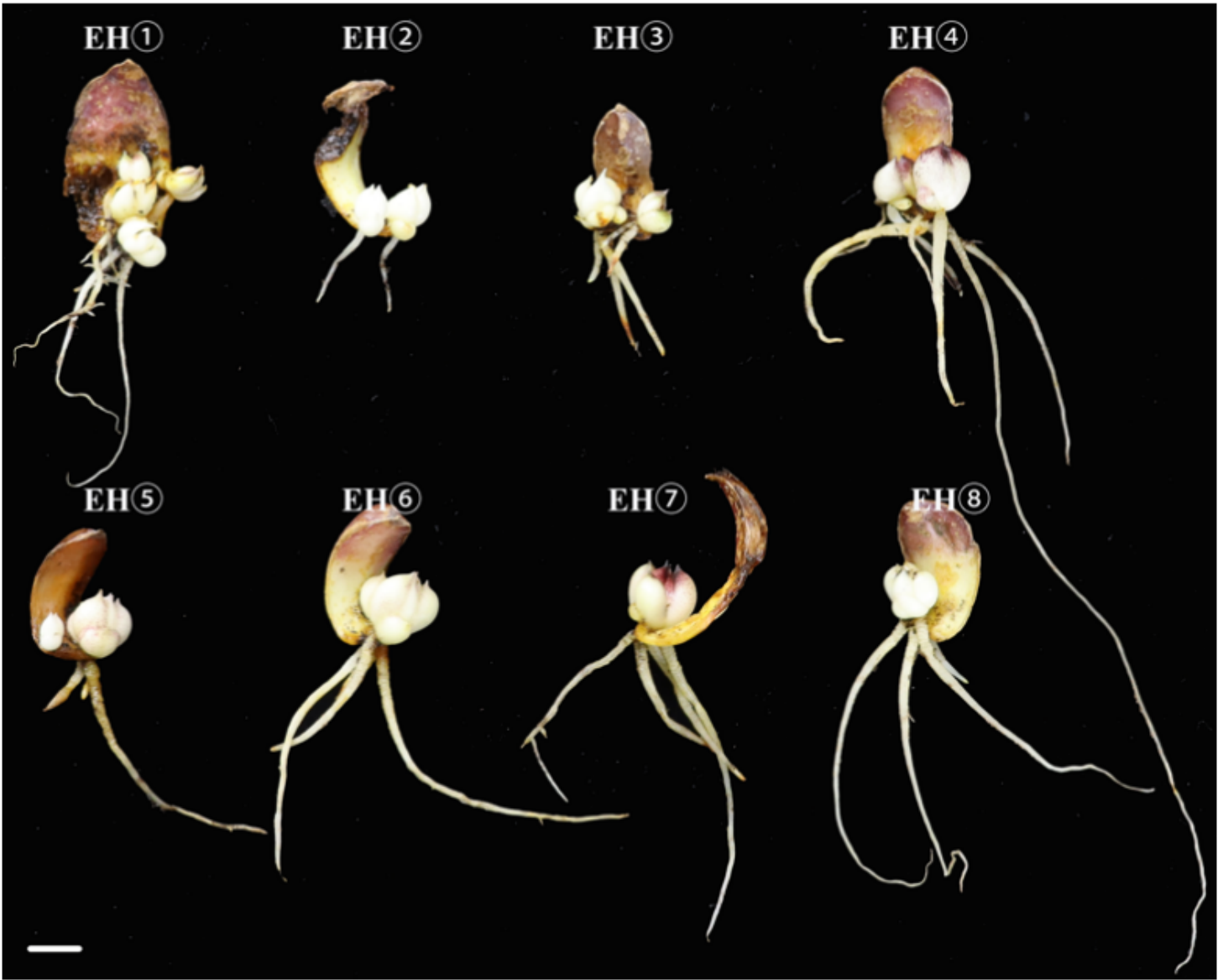


Figure 2

Lily bulb differentiation after a treatment with EHA105. The scale bar represents 1 cm.

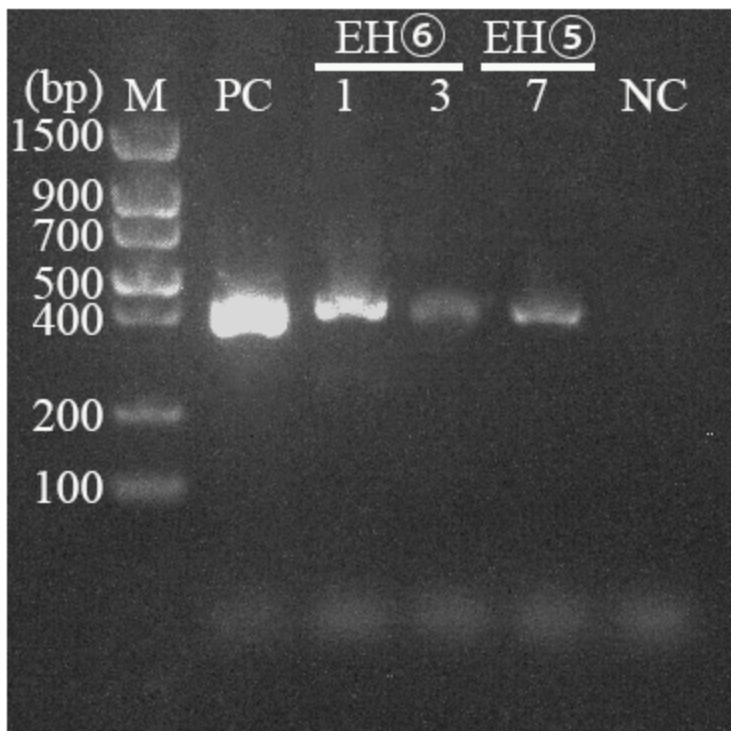


Figure 3

PCR-based detection of differentiated small bulbs after infections.

M: DNA Marker; PC: Positive control plasmid; NC: Negative control

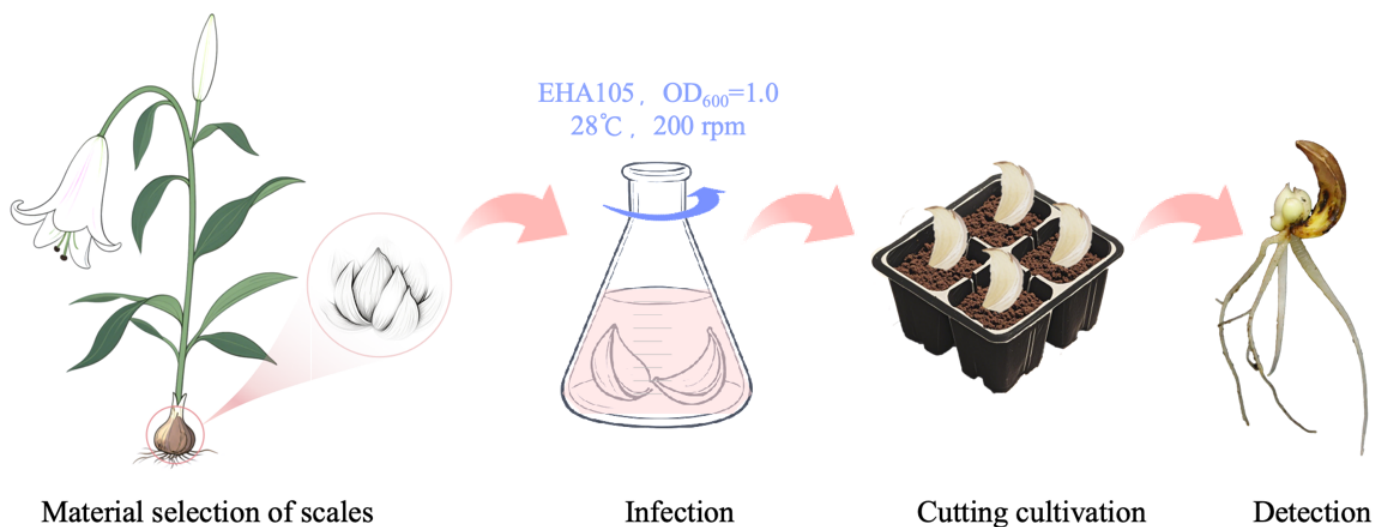


Figure 4

Stable genetic transformation of lily.