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REVIEW

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Exploring *Agrobacterium*-mediated genetic transformation methods and its applications in *Lilium*

Xinyue Fan[1] and Hongmei Sun[1,2*]

Abstract

As a typical bulb flower, lily is widely cultivated worldwide because of its high ornamental, medicinal and edible value. Although breeding efforts evolved over the last 10000 years, there are still many problems in the face of increasing consumer demand. The approach of biotechnological methods would help to solve this problem and incorporate traits impossible by conventional breeding. Target traits are dormancy, development, color, floral fragrance and resistances against various biotic and abiotic stresses, so as to improve the quality of bulbs and cut flowers in planting, cultivation, postharvest, plant protection and marketing. Genetic transformation technology is an important method for varietal improvement and has become the foundation and core of plant functional genomics research, greatly assisting various plant improvement programs. However, achieving stable and efficient genetic transformation of lily has been difficult worldwide. Many gene function verification studies depend on the use of model plants, which greatly limits the pace of directed breeding and germplasm improvement in lily. Although significant progress has been made in the development and optimization of genetic transformation systems, shortcomings remain. *Agrobacterium*-mediated genetic transformation has been widely used in lily. However, severe genotypic dependence is the main bottleneck limiting the genetic transformation of lily. This review will summarizes the research progress in the genetic transformation of lily over the past 30 years to generate the material including a section how genome engineering using stable genetic transformation system, and give an overview about recent and future applications of lily transformation. The information provided in this paper includes ideas for optimizing and improving the efficiency of existing genetic transformation methods and for innovation, provides technical support for mining and identifying regulatory genes for key traits, and lays a foundation for genetic improvement and innovative germplasm development in lily.

Keywords *Agrobacterium tumefaciens*, Genetically modified, Genetic transformation, Lily, Transgenic plants, CRISPR–Cas9

Background

Flowers are not only a globally important agricultural industry with great economic benefits but also necessary agents for mental health in people's daily lives. Many countries have given intensive attention to the development of the flower industry. At present, the world's flower cultivation area is 22.3 hm^2, and the international trade volume of flowers is expanding (AIPH, https://www.floraldaily.com). Lily is a typical perennial

*Correspondence:
Hongmei Sun
sunhm@syau.edu.cn; hmbh@sina.com
[1] Key Laboratory of Protected Horticulture of Education Ministry, College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China
[2] National and Local Joint Engineering Research Center of Northern Horticultural Facilities Design and Application Technology, Shenyang 110866, China

herbaceous bulb plant with more than 100 wild species and more than 9,000 varieties worldwide; additionally, this plant has high ornamental value, and the share of lily as a cut flower in the global flower market is increasing annually [1]. Some lily varieties are edible and have high medicinal value, and their extracts are rich in antioxidant and anti-inflammatory components, resulting in widespread use in medicine, functional food and cosmetics [2]. It is therefore unsurprising that lilies are the focus of much bulbous flower research. Improving the quality of seed balls and cut flowers has always been a key goal in the global lily industry [3, 4]. The target traits are dormancy, development, colour, floral fragrance and resistance to various biological and abiotic stresses to improve the quality of bulbs and cut flowers in planting, cultivation, postharvest, plant protection and marketing. Crossbreeding can quickly fuse good traits and easily produce heterosis. However, lily has a complex genetic background, high heterozygosity, and it is extremely difficult to carry out genome processing, because it is one of the plants with the largest genome, with nearly 30 Gb of genetic information. The long cycle of cross-breeding requires a lot of manpower and material resources. In many cases, sexual incompatibility is also an obstacle in the crossbreeding of lily. Likewise, physical and chemical mutagenesis methods are also highly uncertain. With the rapid development of molecular biology technology, genetic engineering has received increasing amounts of attention. Using molecular methods to improve germplasms can not only lead to the creation of new traits but also increase the efficiency and accuracy of breeding [5–7] (Fig. 1).

Genetic transformation is an important part of genetic engineering technology, and the main goals of flower genetic transformation are as follows: (1) genetic transformation for basic research on a single gene, gene family or gene regulatory network and (2) the application of basic research results as the theoretical basis for improving flower traits and creating new varieties. Plant genetic transformation includes target gene selection, delivery, integration into plant cells, and expression and, ultimately, the production of a complete plant after numerous processes [8]. Although genetically modified plants were obtained in 1983, the genetic transformation of bulb flowers such as lily has long been considered difficult or impossible [9]. With progress in the field of lily research, an increasing number of genes, including genes related to major factors involved in regulating various life activities, responding to various biological and abiotic stresses, and responding to various environmental signals, have been identified. However, many gene function studies still depend on the heterologous transformation of model

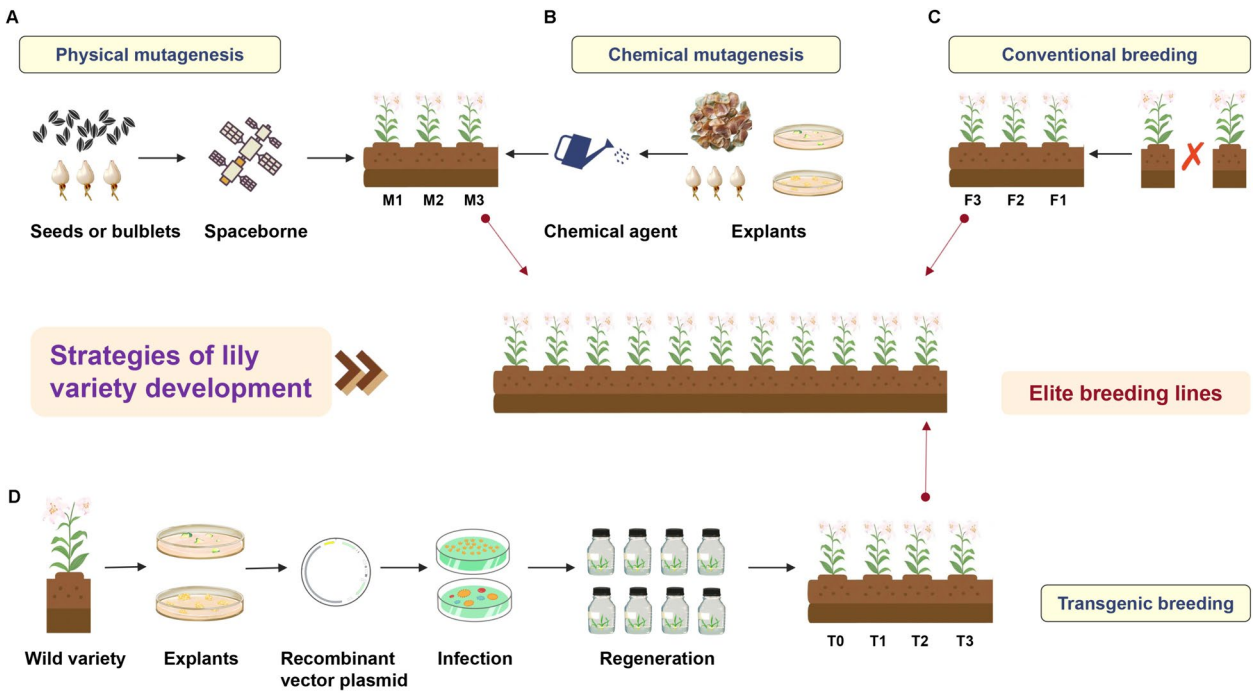

Fig. 1 Strategies for developing new varieties of lily using different plant breeding tools. **A** Physical mutagenesis (in which mutants are created by exposing seeds or bulblets to radiation). **B** Chemical mutagenesis (treatment of different explants with chemical agents to obtain mutants, such as EMS mutagenesis). **C** Traditional crossbreeding has led to the cultivation of new lily varieties. **D** Transgenic breeding

plants such as *Arabidopsis* and *Nicotiana benthamiana* (Fig. S1). In 1992, Cohen conducted the first genetic transformation experiment in lily through *Agrobacterium*-mediated transformation and detected foreign genes in the calli [10]. However, the low efficiency, difficulty of regeneration, and difficulty of integration into the lily genome remain obstacles to overcome. With increasing basic research on lily plants, instantaneous transformation based on virus induction has gradually become the first choice of many researchers because this method is simple and fast, and the research cycle is only a few hours or days. However, because these methods cannot enable integration into the genome and are sometimes limited to a single tissue, it is difficult to provide sufficient evidence for gene function, and the research results are uninformative and unfavourable for further application in breeding work. *Agrobacterium*-mediated transformation, particle bombardment, PEG and electric shock are common methods of plant genetic transformation at present (Fig. 2). Compared with other plant transgenic methods, *Agrobacterium*-mediated plant genetic transformation remains the most common and widespread lily transgenic strategy because of its advantages of high transformation efficiency, few transgenic copies, and stable transfer of integrated genes into offspring after continuous optimization and updating [11].

In the past 30 years, many researchers have attempted to improve and create technology by adjusting or changing various parameters and operating methods and have accumulated considerable valuable experience (Fig. 3). *Agrobacterium* is a gram-negative bacterial genus that is widely distributed in soil. At the beginning of the twentieth century, the principle that natural pathogens can infect plants through wounds was gradually elucidated. The main mechanism is the delivery of tumorigenic DNA molecules (transfer DNA or T-DNA) into plant cells through wounds in infected plants; these molecules are eventually integrated into the host genome and stably transmitted to the next generation of the plant through meiosis [12, 13]. The ability of *Agrobacterium* to integrate its own DNA into the host genome is primarily determined by the Ti plasmid [14], which can be modified by the insertion of target genes into the T-DNA region. With the help of the transferability of this region, genes

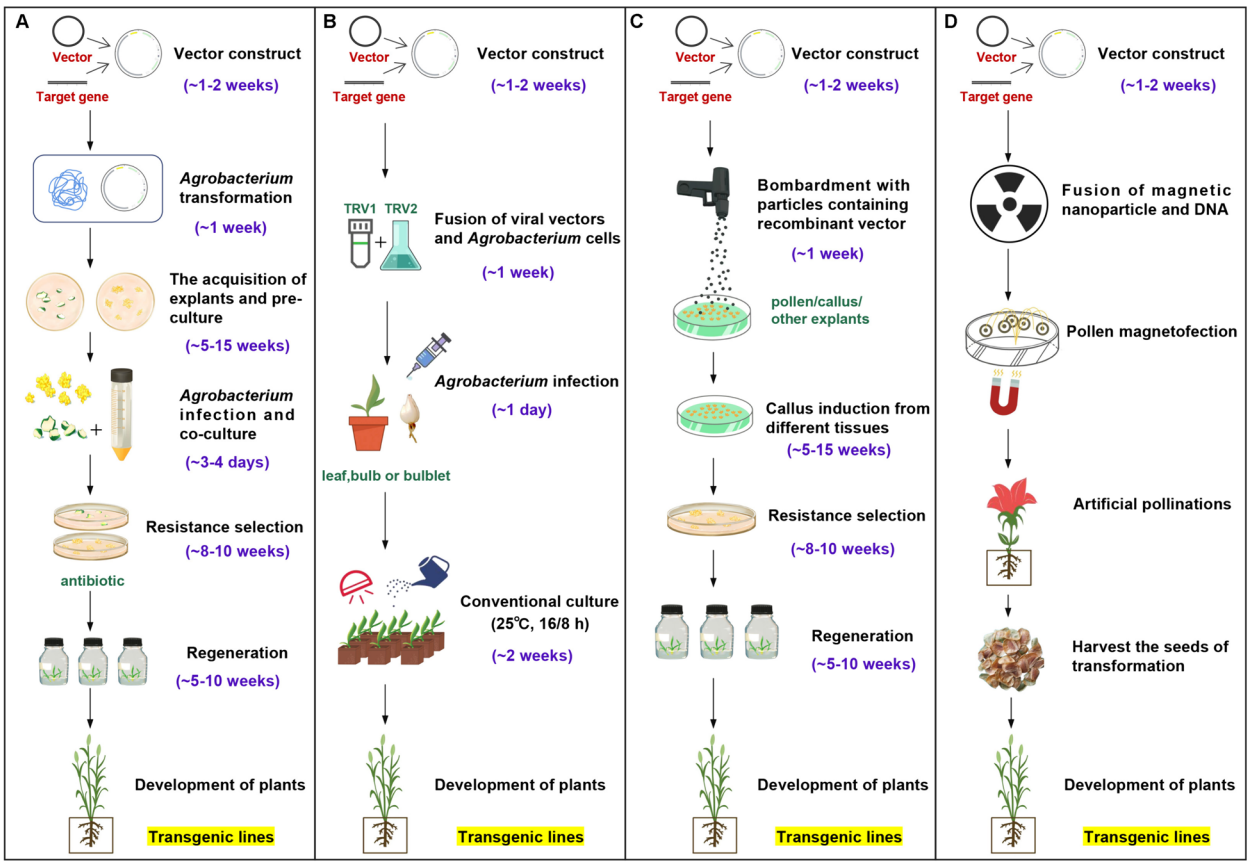

Fig. 2 Common methods for the genetic transformation of lily. **A** *Agrobacterium*-mediated stable genetic transformation **B** Virus-induced transient gene silencing (VIGS). **C** Particle bombardment. **D** Pollen magnetic effect method

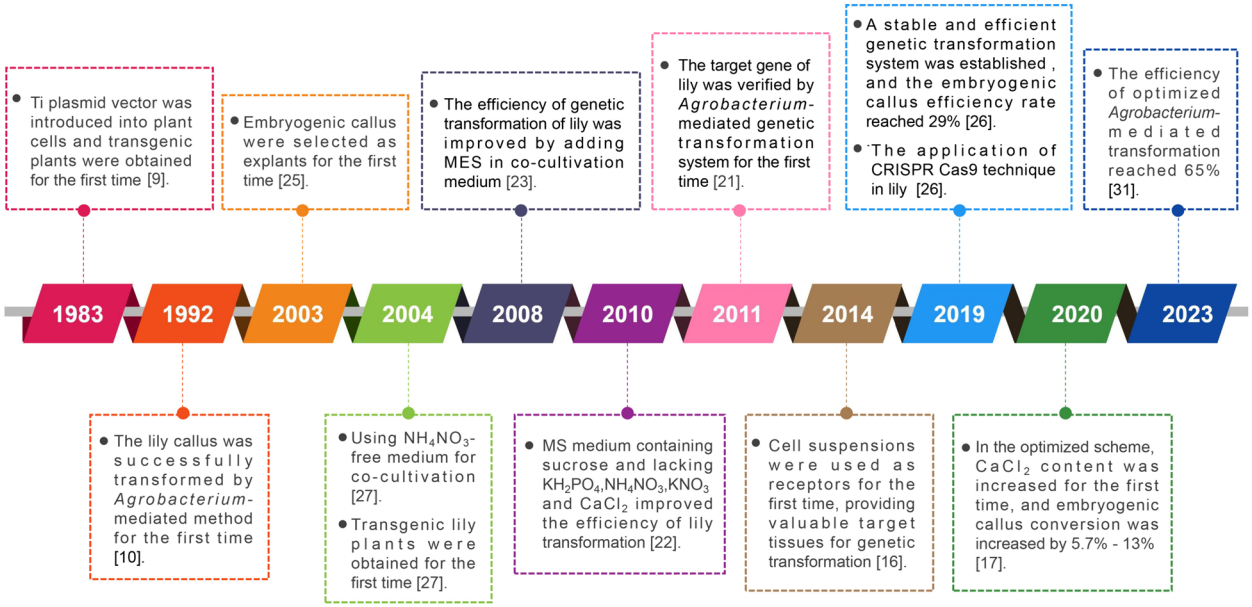

Fig. 3 Timeline of several major discoveries, applications and breakthroughs in the history of lily genetic transformation

can be introduced into plants by *Agrobacterium* infection and incorporated into plant genome, after which transgenic plants can be generated by cell and tissue culture technology [13]. Like most plant genetic transformation methods mediated by Agrobacterium, the genetic transformation procedures for lily mainly include vector construction, selection and culture of explants, preculture, *Agrobacterium* infection, coculture, resistance selection and transgenic plant regeneration (Fig. 2). At present, in addition to calli induced by roots, petals and leaves, scales and embryonic calli are common explants used for genetic transformation in lily [15–17]. With the continuous updating and optimization of genetic transformation technology for lily, functional verification of several genes by heterologous transformation of model plants has gradually increased, and many genes that regulate desirable traits in lily have been identified [3, 17–20]. Nevertheless, stable and efficient transformation of target genes has been achieved in only a few lily varieties, and the success rate of genetic transformation of some lily varieties is still low.

In this paper, the development of genetic transformation technology for lily plants over the past 30 years is reviewed, the factors and key technical points restricting the efficiency of genetic transformation in lily are described, the problems and limitations associated with the genetic transformation of lily are summarized, and the prospects for application and improvement are discussed. The purpose of this paper is to provide a technical reference for establishing a stable and efficient genetic transformation system for lily and to lay a foundation for directional breeding and genetic improvement of key characteristics.

Factors affecting the genetic transformation of lily

Genotype

Many factors affect lily regeneration and transformation. Genotyping is one of the key problems affecting the success of transformation [16]. Although genetic transformation systems have been established for different lily varieties, major differences exist between different genotypes [21]. Under the same conditions during the genetic transformation process, the genotype determines the difficulty of using *Agrobacterium* to successfully infect lily. At present, stable and efficient genetic transformation has been successfully achieved for few lily varieties (Table 1). Since the use of *Agrobacterium*-mediated genetic transformation of lily has been reported, several studies have aimed to optimize the transformation system or establish methods suitable for different lily species, including *Lilium formolongi* [22, 23], *Lilium longiflorum* [24–26], *Lilium pumilum* DC.Fisch. [26] and the *Oriental hybrid* Lily [15, 16, 27, 28]. Due to the strong genotypic dependence and difficult regeneration of explant materials after transformation, most related research results are restricted to certain genotypes [16, 25, 27, 28]. Yan et al. [26] established a stable and efficient transformation system through somatic embryogenesis and adventitious bud regeneration in *Lilium pumilum* DC. Fisch. and *Lilium longiflorum*. After method optimization,

Table 1 Summary of the main factors affecting the genetic transformation efficiency of lily

Genotype	Initial explant	Explant	Strain	Vector	Selection marker	Reporter gene	References
Lilium longiflorum 'Snow Queen'	Flower styles, pedicels	Embryogenic calli	LBA4404	pBin19	*nptII*	–	Mercuri et al. [25]
Lilium oriental 'Acapulco'	Filaments	Calli	EHA101	pIG121-Hm	*nptII, hpt*	*gus*	Hoshi et al. [27]
Lilium longiflorum 'Georgia'	Filaments	Calli	EHA101	pIG121-Hm	*nptII, hpt*	*gus*	Hoshi et al. [15]
Lilium formolongi	Seeds	Calli	EHA101	pIG121-Hm	*nptII, hpt*	*gus*	Ogaki et al. [23]
Lilium longiflorum × *L. formosanum*	–	Bulb scales	LBA4404	pBI121	–	–	Li et al. [29]
Lilium formolongi 'Akasu'	Seeds	Calli	EHA101	pIG121-Hm	*nptII, hpt*	*gus*	Azadi et al. [22]
Lilium oriental 'Acapulco'	Seeds	Calli	EHA101	pIG121-Hm	*nptII, hpt*	*gus*	Azadi et al. [22]
Lilium longiflorum	Bulb scales	Shoot segments	AGL1	pCAS04	*nptII*	*gus*	Liu et al. [24]
Lilium oriental × *trumpet* 'Robina'	Filaments, stem	Embryogenic cell suspension cultures	EHA105	pCAMBIA1301	*Hpt*	*gus*	Qi et al. [16]
Lilium oriental 'Sorbonne'	–	Bulb scales	EHA105	pJAM1890 pBAC9075	*nptII EPSP*	–	Wei et al. [28]
Lilium pumilum DC. *Fisch*	Bulb scales	Embryogenic calli	EHA105	pCAMBIA1301	*nptII*	–	Yan et al. [26]
Lilium longiflorum 'White Heaven'	–	Bulb scales	EHA105	pCAMBIA1301	*Hpt*	*gus*	Yan et al. [26]
Lilium oriental 'Manissa'	Bulb scales	Calli	EHA101	pCAMBIA 2301	*nptII, KPT*	*gus*	Abbasi et al. [30]
Lilium pumilum DC. *Fisch*	Bulb scales	Embryogenic calli	EHA105	pRI101-ON	*nptII*	–	Song et al. [17]
Lilium oriental 'Sorbonne'	–	Bulb scales	GV3101	pCAMBIA1300	*Hpt*	*gus*	Chen et al. [18]
Lilium oriental 'Siberia'	Stems, filaments	Embryogenic calli	GV3101	pCAMBIA1300	*Hpt*	*gus*	Chen et al. [18]
Lilium brownii var. *viridulum*	Bulb scales	Embryogenic calli	EHA105	pLGNe	*nptII*	*gus*	Fu et al. [31]

the transformation efficiency reached 29.17% and 4%, respectively. Although the transformation efficiency in 'White Heaven' is still low, it is relatively stable and can be regenerated within 1 month. Song et al. [17] improved the original transformation system by adjusting the pH and $CaCl_2$ concentration of the medium; the number of resistant plants increased by 2.7–6.4 times, the number of positive lines increased by 3–6 times transformation, and the genetic transformation efficiency increased by 5.7–13.0%. In the latest study, the genetic transformation system of *Oriental hybrid* lily was further optimized, and the efficiency was increased to 60% by screening for the lethal concentration of antibiotics, the concentration of the bacterial solution and the duration of infection.

Explants

Good explant material is the basis of plant genetic transformation. The success of transformation depends on the selection and totipotency of explants [32]. Researchers have tested different explants for genetic transformation of target genes based on the *Agrobacterium* system (Table 1). Calli generated from floral organs, scales, leaves, or seeds have been used for genetic transformation in most lily hybrids [27, 33, 34]. Related studies have shown that filamentous calli have a faster growth rate and may be more susceptible to *Agrobacterium* infection [35]. In the *Oriental hybrid* lily (*Lilium* cv. Acapulco), an *Agrobacterium*-mediated lily transformation system was successfully established by using filament-induced filiform calli as explants. Although transient expression of the *GUS* reporter gene could be detected by root–, leaf–, stalk–, ovary– and anther-induced callus infection, no positive transgenic tissues or plants were obtained [27]. In recent years, several other explants have been developed and offer additional possibilities for improving transformation efficiency. Liu et al. [24] discussed the effect of the direct regeneration pathway and the callus regeneration pathway on the transformation efficiency in *Agrobacterium*-based genetic transformation experiments using stem segments induced

by lily scales as explants. Notably, when stem segments were used as explants, adventitious buds obtained via the direct regeneration pathway after coculture significantly increased the regeneration rate of resistant plants and decreased the gene escape rate [24]. Another study showed that the direct use of scales as transformation explants did not significantly improve the transformation rate but did greatly shorten the genetic transformation cycle [26]. Different explant materials have their own advantages. Cohen and Meredith [10] used a particle bombardment approach to carry out lily genetic transformation and reported that the ability of embryonic calli to accept foreign genes was 50–70 times greater than that of ordinary calli. Embryogenic calli are composed of many embryogenic cells, and each cell has the potential to develop into adult somatic embryos. Therefore, using embryogenic calli as explant materials can result in a more stable transformation population with a lower chimaerism rate, which is very important for the research and development of plant genetic transformation [36–39]. Mercuri et al. [25] induced embryonic calli using the styles and peduncles of *Lilium longiflorum* 'Snow Queen', and they were found to be highly competent for transformation. Recently, two studies reported an efficient protocol with high transformation efficiency for *Lilium pumilum* DC.Fisch. using embryonic calli. Despite their long transformation cycle, embryogenic calli are the most common explant material for the genetic transformation of lily due to their high cell proliferation rate and genetic stability [17, 26].

Strains of agrobacterium

To date, many successful cases of stable genetic transformation of plants mediated by *Agrobacterium* have been reported [40–44]. The strain of *Agrobacterium* can also considerably influence the transformation frequency. With the continuous updating and optimization of plant genetic transformation technology, several researchers have attempted to improve the efficiency of plant transformation by changing various parameters, including *Agrobacterium* strains [45] (Table 1). Different plants have different preferences for the routinely used *Agrobacterium* strains EHA105, EHA101, LBA4404, GV3101, AGL1 and C58 [8, 27, 29, 46]. In alfalfa [47], tomato [48], grasspea [49], and pigeon pea [50], LB4404 and LBA4404 were found to be more virulent and highly effective, offering higher transformation efficiency. However, the LBA4404 strain has been less frequently reported in lilies. Mercuri et al. [25] reported that LBA4404 effectively promoted the infection of embryogenic calli from *Lilium longiflorum* 'Snow Queen'. According to another study of genetic transformation in lily, the use of the EHA105 strain to infuse embryonic calli seemed to be more beneficial for improving transformation efficiency [26]. In recent years, the strains EHA101 and EHA105 have been more widely used in lily transformation and are considered to result in greater transformation frequency [16, 18].

Selection of markers and reporter genes

The selection of marker genes also determines the efficiency of plant genetic transformation. They are usually delivered along with the target gene, conferring resistance to toxic compounds in plants and facilitating the growth of transformed cells in the presence of such unfavourable conditions. Normally, the marker gene and the target gene are connected to the same plasmid and delivered to the plant somatic cells via the *Agrobacterium*-mediated method. Suitable marker genes can help to quickly and efficiently screen many transformed materials and remove untransformed cells [8]. Conventional marker genes include the *hpt* gene, which encodes hygromycin phosphotransferase and confers resistance to hygromycin; the *npt-II* gene, which encodes neomycin phosphotransferase II and confers resistance to kanamycin, neomycin and geneticin; and the bar gene, which encodes phosphinothricin acetyltransferase and confers resistance to the herbicide phosphinothricin [23, 51, 52]. The type of resistance marker gene is determined in the vector, and *hpt* and *npt-II* are commonly used as marker genes for lily transformation [3, 17, 18, 24]. Linking the GUS gene to a transformation vector for double or even triple marker gene screening combined with GUS histochemical staining is also an effective strategy for reducing the false positive rate of resistant plants [16, 22, 26] (Table 1).

Key parameters in the genetic transformation program

pH of the medium

The expression of the *Agrobacterium* virulence gene *vir* is the basis for the transformation of plant cells and the key to ensuring infection efficiency, which is strongly dependent on the pH of the medium [17, 22, 53]. Previous studies have shown that an acidic pH is more conducive to the expression of *vir*, and as the pH of the preculture or coculture medium decreases, the expression of *vir* is significantly upregulated [54, 55]. Maintaining the pH at 5.2 effectively increased the genetic transformation efficiency of tomato cotyledons [56]. The *virA* and *virG* genes are switches that activate the expression of the *vir* gene [57]. *Agrobacterium* has a chemotactic system different from that of *E. coli* and is attracted to chemical inducers such as carbohydrates, amino acids and phenolic compounds [58]. High concentrations of chemical inducers bind to *virA* to induce the expression of the *vir* gene and trigger

T-DNA transfer [59]. Acetylsyringone (AS) is a common class of natural phenolic compounds that can promote the direct entry of microorganisms into plant cells through wounds and achieve T-DNA transfer by activating the expression of the vir gene [58, 60–62]. AS has been widely used in the genetic transformation of lily [23, 27]. Although pH 7.0 is the most suitable environment for the growth of *Agrobacterium*, *vir* is more easily expressed under acidic conditions after the addition of AS, while *vir* expression is hardly induced under neutral pH conditions [54, 63]. In a *Lilium pumilum* DC.Fisch. genetic transformation experiment, a stable pH of 5.8 in suspension and coculture media resulted in a somatic embryo transformation efficiency of 29.17% [26]. When the pH was adjusted to 5.0, the number of resistant calli increased significantly, and the transformation efficiency increased by 5.7–13% [17]. Ogaki et al. [23] reported that exogenous MES could effectively control the pH of the medium. This study further investigated the effect of adding different concentrations of MES (0, 10, 20, 50 and 100 mM) on the transformation efficiency of lily. The results showed that transient expression of the GUS gene could be observed only in coculture medium containing MES, and larger numbers of transgenic calli could be obtained by the addition of 10 mM MES buffer [30]. The above conclusions indicate that maintaining pH in the range of 5–6 values according to different varieties in the preculture and coculture stages is important for improving the efficiency of genetic transformation (Table 2).

Culture medium supplements

The composition of the medium is another rate-limiting factor affecting the genetic transformation efficiency of lily, and the process involves the preculture, coculture and regeneration of resistant plants. Many studies have shown that the addition or removal of certain compounds can significantly improve the efficiency of lily transformation (Table 2). MS medium, which contains 20.6 mM NH_4NO_3, is widely used in the tissue culture and genetic transformation of lily. However, the presence of NH_4NO_3 limits the efficiency of genetic transformation in lily [23, 26, 64]. Previous studies have shown that *virG* transcription can be activated by low concentrations of phosphate [53, 58]. When a low concentration of KH_2PO_4 was used as a salt source instead of NH_4NO_3, there was no significant change in the number of regenerated resistant calli. In contrast, the complete removal of KH_2PO_4 had a positive effect on lily transformation [22]. In a transformation study of the lily 'Sorbonne', it was found that the removal of KH_2PO_4, NH_4NO_3, KNO_3 or macroelements in the medium could significantly improve the transformation efficiency [28]. Montoro et al. [65] reported that Ga^{2+}-free media significantly increased GUS activity in Brazilian rubber trees. However, another study concluded that the effect of $GaCl_2$ on plant transformation efficiency appears to be strongly dependent on genotype. Ga^{2+} is considered one of the key factors involved in improving the efficiency of genetic transformation in improved lily genetic transformation systems. Increasing the $GaCl_2$ concentration from 0.44 g/L to 1.32 g/L significantly increased the germination coefficient of *Lilium*-resistant somatic embryos [17]. AS is an indispensable compound in the genetic transformation of lily, and its concentration is also a key factor; an AS concentration that is too high adversely affects T-DNA transfer [17]. Notably, researchers have found other compounds that can replace AS, and they can provide higher transformation efficiency. Chloroxynil (CX) is a class of phenolic compounds with a mode of action similar to that of AS that also improves the efficiency of treatment by activating the expression of *vir*. In the genetic transformation of lotus seeds, the transformation efficiency of explants treated with CX was 6 times greater than that of explants treated with AS [66]. Wei et al. [28] further confirmed the effect of CX in lily. When 4 μM CX was used, the transformation efficiency reached 11.1%, while 100 μM AS achieved only 6.6%, indicating that CX can replace AS in lily genetic transformation. Early studies showed that the promoting effect of carbohydrate substances other than glucose and xylose on *vir* gene activity was consistent with that of AS [67, 68]. Further studies by Azadi et al. [22] showed that MS media supplemented with monosaccharides significantly inhibited the expression of the GUS gene, and no hygromycin-resistant lily calli were obtained. In contrast, adding sucrose significantly improved the efficiency of genetic transformation. In summary, for most series lilies, removing NH_4NO_3 and adding an appropriate amount of AS has a positive effect on improving the genetic transformation efficiency. CX may be an excellent compound to replace AS, and it is worth further attempts in the future.

Bacterial concentration and infection time

The *Agrobacterium* concentration and infection time play pivotal roles in the transformation of lily. A low bacterial concentration and short infection duration will result in the failure of *Agrobacterium* to fully adhere to explant tissues, resulting in the inability to achieve effective transformation [69]. However, a high concentration of bacteria or long infection duration may also lead to rapid bacterial growth, which can cause severe damage to the recipient material [17]. Cell resistance varies greatly among different plant explants, and plant tolerance to different agrobacterium concentrations also differs. When the OD_{600} was 0.8, the highest GUS expression rate was detected in embryogenic calli, but the percentage of resistant calli

Table 2 Summary of parameters used in genetic transformation programs for lily

Genotypes	Medium (MS)	pH	Time			Selection of transformed plants(mg L^{-1})						OD_{600}	References
			Pre-cultivation	*Agrobacterium* infection	cocultivation	Kan	Hyg	G418	Carb	Cef	SM 7338 trihydrate		
Lilium longiflorum 'Snow Queen'	–	–	–	–	7 d	–	–	–	–	–	–	–	Mercuri et al. [25]
Lilium oriental 'Acapulco'	– NH_4NO_3, + AS	5.8	–	5 min	7 d	–	50	–	–	300	–	0.3	Hoshi et al. [27]
Lilium longiflorum 'Georgia'	– NH_4NO_3	5.8	–	–	7 d			–	–		–		Hoshi et al. [15]
Lilium formolongi	+ MES, + AS	5.8	–	10 min	3 d	–	25	–	–	–	12.5	0.6	Ogaki et al. [23]
Lilium longiflorum × *L. formosanum*	– NH_4NO_3, + AS	5.8	3 d	5 min	5 d	75	–	–	–	250	–	0.8	Li et al. [29]
Lilium formolongi 'Akasu'	– KH_2PO_4, – NH_4NO_3, – KNO_3, – $CaCl_2$, + AS, + MES	–	–	10 min	3 d	–	25	–	–	–	12.5	0.6	Azadi et al. [22]
Lilium oriental 'Acapulco'	– KH_2PO_4, – NH_4NO_3, – KNO_3, – $CaCl_2$, + AS, + MES	–	–	10 min	3 d	–	25	–	–	–	12.5	0.6	Azadi et al. [22]
Lilium longiflorum	+ AS	–	–	10 min	2 d	–	–	49.9	–	–	–	0.3–0.5	Liu et al. [24]
Lilium oriental × *trumpet* 'Robina'	+ AS, + MES	5.8	–	10 min	3 d	–	25	–	–	300	–	0.6	Qi et al. [16]
Lilium oriental 'Sorbonne'	– KH_2PO_4, – NH_4NO_3, – KNO_3, – Sucrose, + Maltose/Glucose	5.8	–	20 min	3 d	–	–	–	500	–	–	0.6	Wei et al. [28]
Lilium pumilum DC. *Fisch*	– NH_4NO_3, + AS	–	10 d	15 min	3 d	–	30	–	–	400	–	0.6	Yan et al. [26]
Lilium longiflorum 'White Heaven'	– NH_4NO_3, + AS	5.8	3 d	15 min	3 d	–	40	–	–	400	–	0.6	Yan et al. [26]
Lilium oriental 'Manissa'	– NH_4NO_3, – $CaCl_2$, + MES, + CX,	5.8	–	10 min	3 d	50	–	–	–	–	12.5	0.6	Abbasi et al. [30]
Lilium pumilum DC. *Fisch*	– NH_4NO_3, + AS, + $CaCl_2$	5.0	10 d	15 min	3 d	–	40	–	–	400	–	0.6	Song et al. [17]
Lilium oriental 'Sorbonne'	+ AS	–	3 d	20 min	3 d	–	50	–	–	300	–	0.6	Chen et al. [18]
Lilium oriental 'Siberia'	+ AS	–	3 d	15 min	3 d	–	35	–	–	300	–	0.4	Chen et al. [18]
Lilium brownii var. *viridulum*	– NH_4NO_3, + AS	–	–	10 min	3 d	–	75	–	–	300	–	0.4	Fu et al. [31]

significantly decreased compared with that when the OD_{600} was 0.6. For scales, the *GUS* expression rate and adventitious bud regeneration rate peaked when the OD_{600} was 0.6. Infection time is also the key to determining transformation efficiency, and research shows that for embryonic calli and scales of *Lilium pumilum,* DC. Fisch, 15 min is the optimal time for *Agrobacterium* infection [26]. However, for embryogenic calli of the *Oriental hybrid* lily 'Siberia', an OD_{600} of 0.4 was more beneficial for improving the transformation efficiency [18]. In addition, it has been reported that in the coculture process, the proliferation of *Agrobacterium* on the surface and around the callus increases with the removal of some elements, indicating that these elements have an inhibitory effect on *Agrobacterium*. Negative effects of bacterial overgrowth were observed when 10 mM MES was added to coculture media of sensitive varieties such as 'Red Ruby' and 'Casa Blanca'. Therefore, screening different varieties of MES can effectively reduce bacterial growth and improve the transformation efficiency of lily [22].

Antibiotic selection

In the process of plant genetic transformation, an appropriate concentration of antibiotics can effectively inhibit the growth of non-transformed Cefatothin, and this is also a crucial step in determining the success of genetic transformation. Kanamycin, hygromycin and glyphosate have been used extensively for lily transformation due to their high availability and low toxicity, despite the occurrence of false positives in the screening of resistant plants [70, 71] (Table 2). Lily explants of different genotypes have different antibiotic concentration requirements. Even within the same variety, different explant types have great differences in antibiotic tolerance [18]. Studies have shown that embryonic calli of *Lilium pumilum* DC. Fisch. The plants almost stopped growing and died after treatment with hygromycin supplemented at 40 $mg{\cdot}L^{-1}$, resulting in extremely low growth and induction rates. However, a few scales of 'White Heaven' still formed complete buds under these conditions. Adjusting the concentration of hygromycin to 30 $mg{\cdot}L^{-1}$ reduced the browning rate of embryogenic calli by approximately 20% and significantly increased the growth and transformation rate. Therefore, 30 $mg{\cdot}L^{-1}$ and 40 $mg{\cdot}L^{-1}$ were the best hygromycin concentrations suitable for embryogenic calli and scales of *Lilium pumilum* DC. Fisch., respectively [26]. Another necessary antibiotic is a bacteriostatic antibiotic, which is mainly used to prevent the transformation of material from dying or difficult regeneration due to excessive *Agrobacterium* contamination. Cef is a common bacteriostatic agent used in plant transformation that has extensive resistance and inhibits the growth of *Agrobacterium* [72, 73]. However, high concentrations of Cef can inhibit the growth of explant cells. Based on the results of studies on different lily varieties and explants, we believe that 300–400 $mg{\cdot}L^{-1}$ Cef may have a broad-spectrum effect [18, 26]. The concentration of *Agrobacterium* may be a prerequisite for screening Cef concentrations. Even 400 $mg{\cdot}L^{-1}$ Cef had no bacteriostatic effect when the concentration of the bacterial solution was too high. When the OD600 of the bacterial solution is maintained within 0.2–0.4, 300 $mg{\cdot}L^{-1}$ Cef can have good antibacterial efficacy [18]. Therefore, it is necessary to combine the concentration of the bacterial solution with the concentration of the bacteriostatic agent during screening.

Preculture, infection and coculture procedures

Preculture, infection and coculture are the key steps in determining the success of plant genetic transformation. Previous studies have generally been conducted under the belief that the preculture of explants before transformation can effectively promote cell division so that they can maintain the best life state during infection and integrate foreign genes more easily [74, 75]. The timing of preculture depends on the type and quality of the explants. Yan et al. [26] discussed the influence of preculture time on the transformation efficiency of *L. pumilum* and 'White Heaven'. The results showed that the expression rate of *GUS* was lower in uncultured calli or scale explants. Similarly, compared with those of the control group, the proliferation and survival rates of the explant-treated group were significantly lower. For embryogenic calli, *GUS* expression and the proliferation rate were the highest in resistant calli after 10 days of preculture (66.67% and 63.33%, respectively). After 4 days of preculture, *GUS* expression and bud resistance began to decrease for the traumatized scales. Although the percentage of resistant buds was the highest after 2 days of preculture, a higher *GUS* expression rate appeared after 3 days (Table 2).

In lily, wounded explant materials are often more conducive to the transfer and integration of T-DNA, which can greatly improve the efficiency of genetic transformation [27, 76]. Wei et al. [28] further confirmed this view. According to the results of *Agrobacterium*-mediated 'Sobone' genetic transformation, ultrasound treatment for 20 s can produce thousands of microwounds in explants, promote the penetration of *Agrobacterium* into the internal tissues of plants, and effectively improve the efficiency of transformation. The combination of heat shock and ultrasound had no significant effect. Coculture is an essential stage during which T-DNA is transferred into plant cells [77]. Therefore, coculture time is also an external factor that has been widely examined [75]. The time required for *Agrobacterium*-mediated gene transfer

and integration into the plant genome varies widely depending on the genotype and explant type, usually ranging from a few hours to a few days [78–81]. Wu et al. [82] compared the transformation efficiency of bulb sections of *Gladiolus* under coculture for 3 days and 12 days, and the results showed that the transformation rate of coculture for 12 days was more than twice that for 3 days, indicating that a longer coculture time may benefit *Agrobacterium* infection and transformation. However, in *Lilium pumilum* DC. After more than 5 days of coculture, Fisch, which is also a typical bulbous flower, will cause severe browning and death of embryogenic calli and scales [26]. However, for the calli of the other two kinds of lilies, coculture for 7 days still maintained a high transformation efficiency, indicating that the tolerance of lily to *Agrobacterium* may depend on the genotype [15, 27]. Drying plant tissue or cells before coculture can also promote T-DNA transfer [83]. The growth state and speed of calli in dry coculture were better than those in traditional media [84]. During subsequent resistance screening, only a few tissues were contaminated by the bacterial solution under dry conditions, and the regeneration rate of resistant calli increased significantly [34]. An appropriate low temperature during coculture also had a positive effect on T-DNA transfer [85, 86]. The transformation efficiency of *Boehmeria nivea* (L.) Gaud. was significantly improved by coculture at 20 °C compared with 15 °C, 25 °C and 28 °C [87]. In the genetic transformation system of *Gossypium hirsutum*, 19 °C can significantly increase the regeneration rate of resistant calli and completely inhibit the proliferation of *Agrobacterium*. However, the effect of temperature on the genetic transformation efficiency of lily has not been clearly reported. In future studies, we can try to optimize the genetic transformation system for lily by adjusting the ambient temperature at each link.

Application of genetic transformation technology

Generation of the CRISPR/Cas9 system

With the continuous development of gene function research technology, gene modification has been widely used in basic plant research and molecular breeding [88]. Since CRISPR/Cas9 gene editing technology was successfully applied in *Lotus japonicus*, because of its simple design and limited operation, this technique has been successfully used in the study of flower anatomy and morphology, flower colour, flowering time, fragrance and stress resistance in various ornamental plants [89–92]. Yan et al. [26] established a stable and efficient genetic transformation system for two lily genotypes using somatic embryos and scales as explants and generated completely albino, light yellow and albino green chimeric mutants via directional knockout of the PDS gene; these authors successfully applied CRISPR/Cas9 technology to lily for the first time. The CRISPR/Cas9 system also validated the feasibility and efficiency of the two genetic transformation systems.

Application for improvement of plant morphogenesis

Morphogenetic genes are key factors that control plant organogenesis and somatic embryogenesis and determine the location of target cells to produce different structures or whole plants [8]. The functions of many morphogenetic genes have been identified in model plants and important cash crops and have been applied in scientific practice to increase the efficiency of regeneration and genetic transformation [93, 94]. In lily, the somatic embryo has always been a good explant for genetic transformation. Plant somatic cells dedifferentiate into embryogenic stem cells under the action of external/internal genetic factors and then divide into somatic embryos. This process is the most critical stage for plant cells to become totipotent [95]. The most widely used method for somatic embryogenesis (SE) in various plants is the use of exogenous plant growth regulators, especially auxin [96]. Song et al. [17] reported that overexpression of *LpABCB21* in lily could shorten the time required for SE without changing the exogenous PIC (Picloram). In contrast, the *LpABCB21* mutant lines delayed somatic embryo generation by 1–3 days, but the induction rate of adventitious buds was significantly greater than that in the *LpABCB21*-overexpressing lines. The study also indicated that the PILS (PIN-LIKES) family member *LpPILS7* may participate in auxin regulation through the same mechanism as *LpABCB21*, and the somatic embryo induction efficiency of the *pils7* mutant was significantly reduced by approximately 10–60%. The importance of miRNAs in SE processes has also been validated in many dicot species and crops [82, 97]. In *Agrobacterium*-mediated *Lilium* embryo transformation experiments, silencing lpu-miR171a and lpu-miR171b promoted starch accumulation and the expression of key cell cycle genes in calli, significantly accelerated the SE process in *Lilium*, and resulted in the same phenotype as overexpressing *LpSCL6-II* and *LpSCL6-I*. WUSCHEL is a typical gene family involved in the regulation of plant morphogenesis, and its expression is upregulated in many plant SE processes [98, 99]. *LlWOX9* and *LlWOX11* reportedly play a positive regulatory role in the formation of bulbils by influencing cytokinin signalling [100]. However, the function of WUSCHEL members in *Lilium* embryogenesis remains to be further verified. It seems that changing the expression level of morphogenetic genes can be an effective means to improve the genetic transformation efficiency of lily, and this topic is worthy of further exploration in the future.

Genetic modification for agronomically important traits

At present, few studies have investigated genetic modification in lily, and most studies have validated the function of target genes only through transient gene transformation (Table 3). With the continuous improvement of the genetic transformation system for lily, a few key genes regulating important traits have been identified. In addition to influencing SE processes, many morphogenetic genes are involved in regulating plant organ formation or quality maintenance [101–103]. *LaKNOX1*, a member of the homeobox gene family involved in regulating plant organogenesis, was also further validated in 'Siberia' and 'Sorbonne' [18]. A recent study revealed that a key gene, *LdXERICO*, is involved in the regulation of dormancy in *Lilium davidii* var. *unicolour*, which indicated that the maintenance of dormancy depends on the ABA-related pathway and that the transcription of *LdXERICO* is inhibited by the temperature response factor LdICE1 during low-temperature storage, which eventually leads to lily sprouting [3]. Recently, the LoNFYA7-LoVIL1 module has also been shown to play a key role in orchestrating the phase transition from slow to fast growth in lily bulbs [104]. Biological and abiotic stresses have a great impact on plant growth and development, and these stresses usually disrupt cellular mechanisms by inducing changes at the physiological, biochemical, and molecular levels in plants [105]. The identification of key genes involved in the regulation of the stress response in lily was aimed at improving plant resistance to biotic and abiotic stresses. Low temperature, drought, salt stress and abscisic acid treatment can significantly upregulate the expression of *LlNAC2*, a member of the NAC transcription factor family. Overexpression of the *LlNAC2* gene in tobacco significantly enhances the tolerance of transgenic plants to various abiotic stresses [106]. Chen et al. [18] used the genetic transformation system of lily to transform *LlNAC2* and successfully generated a transgenic line, which provided favourable support for further clarifying the function of *LlNAC2* in coping with abiotic stress in lily species. Typical biological stresses, including bacteria, fungi, viruses, insects and other diseases and pests, seriously negatively affect the quality of ornamental plants [89]. Several researchers have attempted to increase the resistance of lily plants to pathogens or pests by increasing or decreasing the expression of certain genes. *Pratylenchus penetrans* (RLN) is one of the main pests and diseases encountered in lily production. The overexpression of the rice cystatin *(Oc-IΔD86*) gene in *Lilium longiflorum* cv. 'Nellie White' showed that the resistance of transgenic lily to RLN infection was significantly enhanced, and the total nematode population decreased by 75 ± 5%. Compared with wild-type plants, *OcIΔD86*-overexpressing plants also exhibited improved growth and development [107]. Plant resistance to viruses is usually established by transferring the coat protein-encoding gene of the virus into the plant [89]. Azadi et al. [22] introduced a cucumber mosaic virus (CMV) replicase defective gene (*CMV2-GDD*) into lily using an *Agrobacterium*-mediated genetic transformation system and identified two transgenic strains that showed stronger resistance to CMV. In *Lilium oriental* cv. 'Star Gazer', overexpression of the *rice chitinase 10* (*RCH10*) gene enhanced the resistance of lily to *Botrytis* elliptica [19]. Du et al. [108] identified a gene named *LhSorPR4-2*, which encodes a disease course-related protein involved in fighting *Botrytis elliptica* infection in lily, and the overexpression of *LhSorPR4-2* significantly enhanced the resistance of lily to *Botrytis*. This study also revealed that the function of *LhSorPR4-2* was closely related to its chitinase activity. Another study showed that the transcription level of the resistance gene *LrPR10-5* was significantly increased in transgenic 'Siberia' plants that overexpressed *LrWRKY1*, which subsequently promoted resistance to *F. oxysporum* [109].

Conclusions

Since the first successful transformation event in lily, remarkable progress has been made; a variety of lily genetic transformation systems have been gradually established, and many excellent new germplasms have been obtained. However, the genetic transformation of lily still faces great challenges due to its strong genotypic dependence. Most related studies have focused on optimizing existing systems, and applicable genetic transformation systems have not yet been established for most lily strains with high market value. For a long time, how to stably and efficiently deliver recombinant gene vectors into plant cells has been the focus of most scholars. At present, the most common delivery method is *Agrobacterium*-mediated transformation. In addition to the cumbersome tissue culture process, the transformation efficiency also depends greatly on the genotype. The choice of explants for DNA, strains and vectors; culture conditions; and effective selection markers are all major factors that play pivotal roles in successful transformation. At present, most transgenic work in lily is limited by laboratory-scale gene function verification, and even after successful transformation, it is not easy to obtain stable transgenic plants. In recent years, various types of *Rhizobium*, including *Ensifer adhaerens*, *Ochrobactrum haywardense* and *Sinorhizobium meliloti*, have shown great potential in the transformation of nonagricultural bacterial systems. Some studies have revealed an invisible mechanism for delivering DNA into plant cells, where *Sinorhizobium meliloti* can infect both monocotyledonous and

Table 3 Summary of genetic modifications in lily

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/RNAi(*Lilium*)	VIGS		
Lilium oriental 'Acapulco'										√		√					*Lilium* (35S)	Azadi et al. [22]
Lilium oriental 'Siberia'											√					√	*Lilium* (transient)	Tong et al. [110]
Lilium oriental 'Nellie White'										√		√					*Lilium* (35S)	Vieira et al. [107]
Lilium oriental 'Sorbonne'										√		√					*Lilium* (35S)	Wei et al. [28]
Lilium longiflorum 'White heaven', *Lilium oriental* 'Siberia'										√		√			√		*Arabidopsis* (35S) *Lilium* (transient)	Wu et al. [111]
Lilium longiflorum 'White heaven'										√		√					*Arabidopsis* (35S)	Wu et al. [112]
Lilium oriental 'Siberia'										√		√					*Nicotiana tabacum* (35S)	Zhang et al. [113]
Lilium longiflorum 'White heaven', *Lilium oriental* 'Siberia', *Lilium* Asiatic hybrid 'Jing He'										√		√					*Arabidopsis* (35S) *Nicotiana tabacum* (35S)	Wu et al. [114]
Lilium pumilum DC. *Fisch*										√				√			*Lilium*	Yan et al. [26]

Table 3 (continued)

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/RNAi(*Lilium*)	VIGS		
Lilium longiflorum 'White Heaven'										√				√			*Lilium*	Yan et al. [26]
Lilium leichtlinii 'Hakugin'											√					√	*Lilium* (transient)	Tasaki et al. [115]
Lilium pumilum DC. *Fisch*										√		√		√			*Lilium*	Song et al. [17]
Lilium regale wilson										√					√		*Lilium* (transient)	Fu et al. [116]
Lilium oriental 'Siberia'										√		√					*Nicotiana tabacum* (35S)	Zhang et al. [117]
Lilium tsingtauense Gilg											√					√	*Lilium* (transient)	Jiang et al. [118]
Lilium oriental 'Vivian'											√					√	*Lilium* (transient)	Yin et al. [119]
Lilium oriental 'Siberia'										√	√				√	√	*Arabidopsis* (35S) *Lilium* (transient)	Abbas et al. [120]
Lilium longiflorum 'White heaven'										√		√					*Arabidopsis* (35S)	Ding et al. [121]
Lilium oriental 'Siberia'										√					√		*Lilium* (transient)	Luo et al. [122]
Lilium langifolium											√					√	*Lilium* (transient)	He et al. [123]

Table 3 (continued)

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/RNAi(*Lilium*)	VIGS		
Lilium longiflorum 'White heaven', *Lilium orientalʻ* Siberia'										√	√	√				√	*Arabidopsis* (35S)	Liu et al. [124]
Lilium longiflorum 'White heaven'										√		√					*Arabidopsis* (35S)	Wu et al. [125]
Lilium orientalʻ Siberia'										√		√					*Arabidopsis* (35S)	Yuan et al. [126]
Lilium orientalʻ Siberia'										√		√					*Arabidopsis* (35S)	Li et al. [109]
Lilium langifolium										√	√				√	√	*Lilium* (transient)	He et al. [100]
Lilium longiflorum 'White heaven'											√					√	*Lilium* (transient)	Wang et al. [127]
Lilium orientalʻ Siberia'											√					√	*Lilium* (transient)	Fang et al. [128]
Lilium orientalʻ Siberia'											√					√	*Lilium* (transient)	Yang et al. [129]
Lilium langifolium 'White heaven'										√					√		*Lilium* (transient)	Wu et al. [130]
Lilium langifolium 'White Heaven'										√	√				√	√	*Lilium* (transient)	Wu et al. [131]

Table 3 (continued)

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/RNAi(*Lilium*)	VIGS		
Lilium langifolium 'White Heaven'										√		√					*Arabidopsis* (35S)	Li et al. [132]
Lilium langifolium 'White Heaven'										√		√					*Arabidopsis* (35S)	Li et al. [133]
Lilium pumilum DC. *Fisch*										√		√		√			*Lilium*	Yang et al. [129]
Lilium regale wilson											√					√	*Lilium* (transient)	Wang et al. [134]
Lilium longiflorum Thunb. cv. 'Snow Queen'										√					√		*Lilium* (transient)	Fu et al. [135]
Lilium langifolium 'White heaven'											√					√	*Lilium* (transient)	Zhou et al. [136]
Lilium oriental 'Siberia'										√					√		*Lilium* (transient)	Sun et al. [102, 103]
OT *Lilium* hybrid variety 'Robina'										√		√					*Arabidopsis* (35S)	Chen et al. [137]
Lilium oriental 'Sorbonne'										√		√					*Arabidopsis* (35S)	Du et al. [108]
Lilium longiflorum cv. 'Nellie White'										√		√					*Arabidopsis* (35S)	Westerdahl et al. [138]
Lilium oriental 'Siberia'										√	√				√	√	*Lilium* (transient)	Yue et al. [139]

Table 3 (continued)

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/RNAi(*Lilium*)	VIGS		
*Lilium orientalʻ*Siberia'											√					√	*Lilium* (transient)	Guo et al. [140]
*Lilium orientalʻ*Siberia'										√	√				√	√	*Lilium* (transient)	Feng et al. [141]
Lilium davidii var. *unicolour*										√	√	√				√	*Lilium*	Fan et al. [3]
*Lilium orientalʻ*Siberia'											√					√	*Lilium* (transient)	Fang et al. [142]
Lilium Brownii var. *Viridulum* 'Corvara'										√	√				√	√	*Lilium* (transient)	Liu et al. [143]
*Lilium orientalʻ*Siberia'										√	√	√				√	*Arabidopsis* (35S) *Lilium*	Liu et al. [144]
*Lilium orientalʻ*Siberia'											√					√	*Lilium* (transient)	Liu et al. [145]
*Lilium orientalʻ*Siberia'										√		√					*Lilium*	Chen et al. [18]
Lilium regale Wilson										√		√			√		*Nicotiana tabacum* (35S) *Lilium* (transient)	Deng et al. [146]
OT *Lilium* hybrid variety 'Robina'										√		√					*Lilium*	Wang et al. [147]
*Lilium orientalʻ*Siberia'										√	√	√				√	*Lilium*	Pan et al. [104]
Lilium pumilum DC. *Fisch*										√		√					*Arabidopsis* (35S)	Sun et al. [148]

Table 3 (continued)

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/RNAi(*Lilium*)	VIGS		
Lilium longiflorum 'White Heaven'										√	√	√			√	√	*Arabidopsis* (35S) *Lilium* (transient)	Wu et al. [149]
Lilium longiflorum 'White Heaven', *Lilium oriental* 'Sorbonne'										√	√	√			√	√	*Arabidopsis* (35S) *Lilium* (transient)	Wu et al. [150]
Lilium davidii, *Lilium davidii* var. *unicolour*										√					√		*Lilium* (transient)	Zhang et al. [151]
Lilium Asiatic hybrid 'Tiny Padhye'										√	√	√	√		√	√	'Orin' apple calli (35S) *Nicotiana tabacum* (35S) *Lilium* (transient)	Bi et al. [152]
OT *Lilium* hybrid variety 'Robina'										√		√					*Arabidopsis* (35S)	Feng et al. [153]
Lilium oriental 'Siberia'																		Yue et al. [139]
Lilium langifolium 'White heaven'										√	√					√	*Arabidopsis* (35S) *Lilium* (transient)	Ding et al. [121]

Table 3 (continued)

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/ RNAi(*Lilium*)	VIGS		
Lilium distichum Nakai										√		√					*Lilium*	Fu et al. [31]
Lilium pumilum										√		√					*Lilium*	Zhang et al. [154]
Lilium pumilum										√		√					*Nicotiana tabacum* (35S)	Jing et al. [155]
Lilium formolongi 'Raizan'										√	√	√				√	*Arabidopsis* (35S) *Lilium* (transient)	Wu et al. [156]
Lilium longiflorum 'White Heaven'										√	√	√			√	√	*Arabidopsis* (35S) *Nicotiana tabacum* (35S) *Lilium* (transient)	Wu et al. [157]
Lilium longiflorum 'White Heaven', *Lilium oriental* 'Sorbonne'										√	√	√			√	√	*Arabidopsis* (35S) *Lilium* (transient)	Wu et al. [158]
Lilium oriental 'Siberia'										√	√				√	√	*Lilium* (transient)	Tong et al. [159]
Lilium oriental 'Siberia'										√	√				√	√	*Lilium* (transient)	Liu et al. [161]
Lilium oriental 'Siberia'										√		√					*Arabidopsis (35S)*	Cao et al. [162]

Table 3 (continued)

Genotypes	GM traits									Transformation		Type					Remark	References
	Stress resistance			Plant attributes								Heritable			Transient			
	Pests	Fungi	Abiotic stress	Colour	Perfume	Dormancy	Longevity	Morphogenesis	Immune system	*Agrobacterium*	Virus	35S	RNAi	CRISPR–Cas9	35S/RNAi(*Lilium*)	VIGS		
Lilium Asiatic hybrid										√					√		*Lilium* (transient)	Cao et al. [163]
Lilium pumilum										√		√					*Nicotiana tabacum* (35S)	Liu et al. [160]

dicotyledonous plants [164]. Another way to improve the traditional transformation model is to coexpress developmental regulatory factors or morphogenetic genes during transformation. The overexpression of the developmental regulatory factors GROWTH-REGULATING factor (GRF) and Boby room (Bbm) in maize and sorghum, for which it is difficult to achieve genetic transformation, can significantly improve transformation efficiency [101, 165]. Pollen tube transformation is a transformation system that does not require tissue culture, but this method is suitable only for model plants such as *Arabidopsis* and a few closely related plants. The pollen magnetic transfection-mediated transformation method can be applied to lily, but it may be species- or varietal specific. Zhang et al. [166] optimized pollen culture conditions, established a new method for the transient transformation of pollen magnetic beads, and concluded that the transformation efficiency was positively correlated with the transverse diameter of pollen and negatively correlated with the ratio of longitudinal diameter to transverse diameter. This study also evaluated the transformation efficiency of *Lilium regale* L. 'Sweet Surrender' and *Lilium leucanthum*; L. 'Sweet Surrender' and *Lilium leucanthum* reached 85.80% and 54.47%, respectively, but successful transformation was not achieved in *Lilium davidii* var. *unicolour*. Particle bombardment and the electrical shock method are also common methods used in plant genetic transformation. At present, the main explants of the electroshock method are plant protoplasts, but because of their high cost, abundance of chimaeras after transformation and limited stable expression in offspring, these methods cannot be applied to large-scale lily plants. Therefore, exploring genetic transformation systems based on non-tissue culture methods is expected to alleviate the pressure of genetic transformation of lily in the future. Cao et al. [167] reported a cut-dip-bud (CDB) delivery system. Briefly, the CDB delivery system consists of cutting the junction of plant roots under nonsterile conditions, infecting the upper end with *Agrobacterium*, taking positive new roots after culture, cutting them into segments and culturing them again to obtain regenerated and transformed plants. Researchers have studied the effects on rubber grass (*Taraxacum koko-saghyz Rodin*, TKS), *Ipomoea batatas* [L.] Lam.), *Ailanthus altissima* (Mill) Swingle, and *Aralia elata* (Miq.) The CDB system has been tested in several difficult-to-transform plants, including three woody plants, one of which is *Clerodendrum chinense* Mabb. The results showed that the CDB delivery system has wide applicability in plant genetic transformation. Furthermore, there is a great need for the validation of promoters other than CaMV35S to achieve optimal expression of transforming genes [8].

Notably, various plant genetic transformation systems have been further applied to establish RNA interference (RNAi) and gene editing technology systems. To date, there have been few reports on the application of RNAi and CRISPR/Cas9-based gene editing techniques in lilies. In 2019, Yan established a stable and efficient genetic transformation system for somatic embryo regeneration for the first time and successfully conducted targeted gene editing based on CRISPR/Cas9 [20, 26]. As one of the sharpest tools in genetic technology, CRISPR/Cas9 "gene scissors" have set off a research boom in basic plant research and directed breeding work. It has great application potential for improving yield, quality, herbicide resistance, abiotic stress resistance and disease resistance. However, there are few successful cases of gene editing using CRISPR/Cas9 technology in lily, which may be related to its high heterozygosity. The stable genetic transformation system has not been widely used, which leads to many difficulties in the study of gene function. With the continuous improvement of the technical system of lily genetic transformation and the emergence of new delivery methods, the combination of multiple transformations may be the only way to develop functional lily genomics in the future, and major breakthroughs in genetic engineering applications in lily breeding are expected not to occur.

Abbreviations

SE	Embryogenesis
PIC	Picloram
Cef	Cefatothin
AS	Acetosyringone
Kan	Kanamycin
Rif	Rifampicin
PILS	PIN-LIKES
GRF	REGULATING factor
Bbm	Boby room
CDB	Cut-dip-bud
RNAi	RNA interference

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13007-024-01246-8.

Additional Files 1. Fig. S1: Literature keywords related to the field of lily research that appear together in the map. Keywords that appear more than 30 times are displayed, and different colours represent different cluster.

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Availability of data and materials
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request. No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study does not address all ethical issues and has been approved by all the authors. Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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