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Ectopic Expression of *PgF3′5′H* in Commercial *Gypsophila paniculata* Cultivar through Optimized Agrobacterium-Mediated Transformation

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Abstract: *Gypsophila paniculata* is one of the most popular cut flowers in the world whose major cultivars are blooming white. As is well known, blue flowers could be generated via the overexpression of the gene encoding flavonoid 3′5′-hydroxylase (F3′5′H) in species that naturally lack it. In this study, we established the regeneration and a genetic transformation system for the commercial cultivar 'YX4' of *G. paniculata* and introduced the *F3′5′H* of *Platycodon grandiflorus* (*PgF3′5′H*) successfully into 'YX4' using the established protocol. A total of 281 hygromycin (Hyg)-resistant plantlets were obtained, and 38 of them were polymerase chain reaction (PCR) positive, indicating a 13.5% transformation efficiency. Shoot apex without meristem was more suitable for explant due to its high regeneration capacity, and the supplement of thidiazuron (TDZ) provided the most efficient promotion of adventitious bud induction, whereas the supplement of 6-Benzyladenine (6-BA) and 1-naphthaleneacetic acid (NAA) did not affect much. Additionally, the combination of 1 day (d) pre-culture, 5 d co-culture, 10 min infection, 30 mg·L^{-1} additional acetosyringone (AS) supplement, and 10 mg·L^{-1} Hyg selection formed the optimized system for 'YX4' transformation. This reliable and efficient agrobacterium-mediated transformation of the valuable commercial cultivar 'YX4' will contribute not only to the creation and improvement of *G. paniculata* cultivars, but also to the function research of genes associated with important ornamental traits.

Keywords: *Gypsophila paniculata*; regeneration; genetic transformation; commercial cultivar; *PgF3′5′H*

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1. Introduction

Gypsophila paniculata, also known as baby's breath, is a perennial herbaceous flower of the Caryophyllacae family [1]. Since the clouds of tiny white or pink flowers cover the bunches of the branching stems after blooming, *G. paniculata* is commonly used as fresh or dried filler in flower arrangements and bouquets [2]. As the only species in the genus *Gypsophila* used as a cut flower, *G. paniculata* is one of the top ten best-selling cut flowers in the world [3]. To meet the large demand for seedlings in production, tissue culture, which can rapidly and massively provide standard and uniform seedlings in plant factories, is widely used in the propagation of *G. paniculata* [4].

In contrast to the systematic and mature propagation industry, the cultivar innovation of *G. paniculata* remains slow. There were only 78 *G. paniculata* cultivars registered in the EU until 2017, while hundreds of commercial cultivars of other ornamental crops have been released every year [5]. Difficulties in crosses and low seed formation rates due to the double flower phenotype might explain the slowness of the *G. paniculata* cultivar's release. To overcome this slowness, researchers have tried to establish a transgenic system for *G. paniculata* since 1990s. Regenerated shoots were obtained from leaf or segmented stem explants for several cultivars [6,7]. Later, an *Agrobacterium tumefaciens* (*A. tumefaciens*)-mediated transformation system assisted by gibberellic acid was established for three

cultivars [8]. However, neither a gene function study nor the traits modified using genetic transformation have been reported in *G. paniculata* since then.

Flower colour is one of the most critical ornamental traits and is the primary breeding target of many floricultural species, such as rose, carnation, lisianthus, etc. Most of the natural *G. paniculata* cultivars bloom white except 'Flamengo', with pale-pink flowers [9]. Nevertheless, there is a huge market demand for the colourful *G. paniculata*. Thus, dyeing technology has been developed to change the flower colour into carmine, tartrazine, blue, and so on. However, the artificial variegation fails to stain the petals evenly, therefore the dyed flowers usually display white spots, and some of them even wither. It is also environmentally risky due to the potential pollution to the river system.

Over decades, the genetic and molecular networks regulating the formation of flower colour have been studied in various ornamental species [10–14]. The water-soluble anthocyanins belonging to flavonoids are responsible for the flower colour, which ranges from orange/red to violet/blue [15]. Moreover, three well-known flavonoids, pelargonidin, cyanidin, and delphinidin, contribute to the development of the red, purple, and blue colours of the flowers, respectively [16]. An anthocyanin biosynthetic pathway is conserved in most plant species, which involves various enzymes. 4-coumaroyl-CoA and 3-malonyl-CoA are the first substrates in the synthetic pathway, which are then catalyzed by chalcone synthase (CHS) and form naringenin chalcone. This is then converted to naringenin flavanone under the action of chalcone isomerase (CHI). Flavanone 3-hydroxylase (F3H) turns naringenin into dihydrokaempferol. To display blue, a precursor chemical, dihydromyricetin, is necessary, and flavonoid 3′,5′-hydroxylase, encoded by *F3′5′H*, is considered a critical enzyme to introduce hydroxylation of the flavonoid B ring of dihydrokaempferol. The dihydromyricetin is then catalyzed by dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and flavonoid 3-O-glucosyltransferase (UFGT) step by step and is finally converted into delphinidin 3-glucoside [17]. Lacking F3′5′H, the key enzyme that promotes delphinidin synthesis, in species such as rose, chrysanthemum, and carnation, are unable to bloom blue through traditional hybridization or mutation [7,18], which might be fixed by introducing exogenous *F3′5′H* into the non-blue cultivars [11,19–21]. The previous RNA-seq data [22] of 'YX4' from three independent stages (flower bud stage, flower semi-open stage, and flower fully open stage) revealed that *G. paniculata* harbouring *F3′5′H* did not express somehow (Figure 1), resulting in the formation of white flowers.

It was reported that *F3′5′H* of different species exhibit diversity in catalytic efficiency, and that the *Kal* from *Campanula medium* displayed the highest catalytic efficiency with a unique sequence structure of nine amino acids (SKLDSSASA) [23,24]. The *F3′5′H* of *Platycodon grandifloras* (*PgF3′5′H*) was the second gene reported to possess this special sequence, whereas its catalytic function required further exploration [25]. In this study, we established the regeneration and genetic transformation system for the commercial cultivar 'YX4' of *G. paniculata* and introduced *PgF3′5′H* into this cultivar, providing a basal tool for further gene function analyses and gene editing.

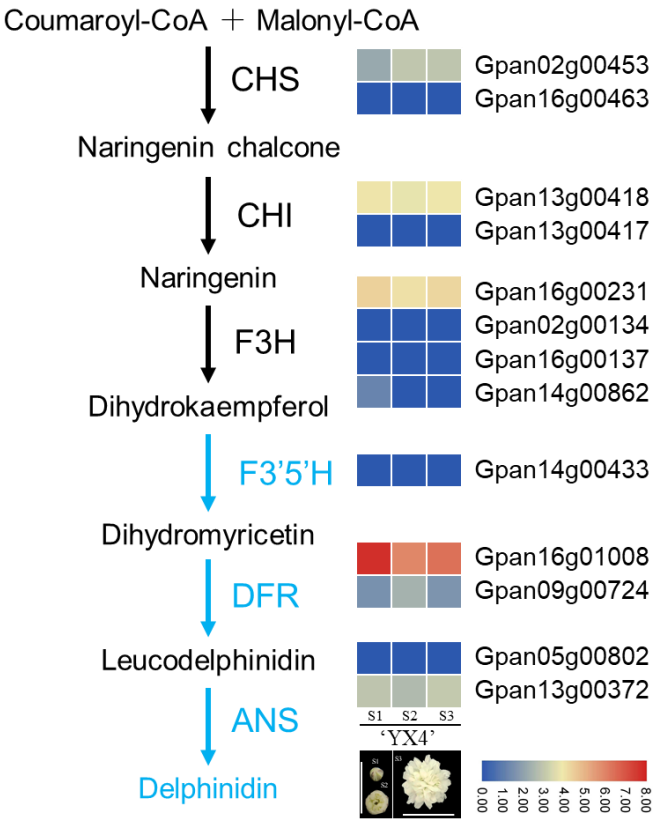

Figure 1. The delphinidin biosynthesis pathway in *G. paniculata*. Heat map of the expression of delphinidin biosynthesis pathway genes was constructed using TBtools. The S1, S2, and S3 are flower bud stage (S1), semi-open stage (S2), and fully open stage (S3), respectively. The scale bar is 1 cm. Abbreviations include CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavanone 3-hydroxylase; F3′5′H: flavonoid 3′,5′-hydroxylase; DFR: dihydroflavonol 4-reductase; ANS: anthocyanidin synthase.

2. Materials and Methods

2.1. Plant Materials and Culture Conditions

Aseptic plantlets of *G. paniculata* cultivar 'YX4' were obtained from Yuxi Yunxing Biological Technology Co., Ltd. (Yuxi, Yunnan Province, China). The aseptic plantlets were propagated every month with Murashige–Skoog (MS, Duchefa, Haarlem, The Netherlands) [26] medium supplemented with 1 mg·L^{-1} 6-Benzyladenine (6-BA, Sigma–Aldrich, St. Louis, MO, USA), 0.1 mg·L^{-1} 1-naphthaleneacetic acid (NAA, Sigma–Aldrich), 30 g·L^{-1} sucrose, and 8 g·L^{-1} agar. The pH of the propagation medium was adjusted to 5.95. The plantlets were cultured at 23 ± 2 °C with 1500 lx light intensity (16 h light/8 h dark, GreenPower LED with red and blue light in 4500 K, Philips, Amsterdam, The Netherlands).

2.2. Establishment of Regeneration System of G. paniculata

One-month-old robust plantlets were used in this study. First, the shoots were cut, and all the leaves were removed. Then, the stem apexes were cut and sliced up into 2–3 pieces. The segments were inoculated on MS-based adventitious bud induction medium supplemented with different concentrations of cytokinin (6-BA or thidiazuron, TDZ, Sigma–Aldrich) combined with 0.1 mg·L^{-1} NAA (Table 1). MS1 medium containing only 0.1 mg·L^{-1} NAA was taken as control. All the medium contained 30 g·L^{-1} sucrose and 8 g·L^{-1} agar and were adjusted to pH = 5.95. Thirty explants were included in each treatment, which was repeated three times. The explants were cultured in a growth room with the conditions mentioned above and relative data were collected one month later.

Table 1. Composition of mediums used to establish regeneration system.

NO.	PGRs' Combination	NO.	PGRs' Combination
T1	0.5 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA	B1	0.5 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA
T2	1.0 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA	B2	1.0 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA
T3	1.5 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA	B3	1.5 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA
T4	2.0 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA	B4	2.0 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA
T5	2.5 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA	B5	2.5 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA
T6	3.0 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA	B6	3.0 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA

PGRs, plant growth regulators, TDZ, thidiazuron; NAA, 1-naphthaleneacetic acid; 6-BA, 6-Benzyladenine.

2.3. Determination of Hygromycin (Hyg) Selection Pressure

Twenty explants were cultured with MS medium containing Hyg at different concentrations (0, 5, 10, 15, 20 mg·L^{-1}), respectively. The explants were cultured in the dark for 5 d and then transferred to a regular growth room. The number of surviving adventitious buds was counted after one month.

2.4. A. tumefaciens-Based Transformation

The *A. tumefaciens* strain EHA 105 containing pCAMBIA1301 with 35S-*GUS* and 35S-*PgF3′5′H* cassettes (pCAMBIA1301-*GUS-PgF3′5′H*, provided by Dr. Lulin Ma) was activated on the solid Luria–Bertani (LB) medium containing 50 mg·L^{-1} kanamycin (Kan) and rifampicin (Rif), followed by incubation at 28 °C for 16 h. The positive single colony was then inoculated into the liquid LB medium containing 50 mg·L^{-1} Kan and shaken cultured overnight (28 °C, 180 rpm) until the OD_{600} value reached 0.5–0.6. Stem apexes pre-cultured for 1 d were soaked in the *A. tumefaciens* solution and shaken cultured at 200 rpm for 10 min. The explants were blotted on sterile filter paper and grown on the M3 (for co-culture) medium in the dark for 5 d. They were then transferred to the M4 (for first selection) medium and cultured for two weeks. The adventitious buds were cut and grown on the M5 (for second selection) medium when they reached 1.5 cm. Two weeks later, the selected adventitious buds were transferred to the M6 medium and recovered for one month. Regeneration plantlets with 6–7 pairs of leaves were rooting on the M7 medium. The compositions of the medium mentioned above were listed in Table 2.

Table 2. Mediums used for *G. paniculata* genetic transformation.

Medium Number	Composition
Pre-culture: M2	MS + 2.5 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA
Co-culture: M3	MS + 2.5 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA + 10 mg·L^{-1} AS
First selection: M4	MS + 2.5 mg·L^{-1} TDZ + 0.1 mg·L^{-1} NAA + 200 mg·L^{-1} Cef + 10 mg·L^{-1} Hyg
Second selection: M5	MS + 1.0 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA + 200 mg·L^{-1} Cef + 10 mg·L^{-1} Hyg
Recover: M6	MS + 1.0 mg·L^{-1} 6-BA + 0.1 mg·L^{-1} NAA + 200 mg·L^{-1} Cef
Rooting: M7	MS + 0.2 mg·L^{-1} IAA + 0.3 mg·L^{-1} NAA

AS, acetosyringone; Cef, cefotaxime sodium; Hyg, hygromycin; IAA, Indole-3-Acetic Acid.

2.5. Effects of Different Factors on Transformation

The effects of the pre-culture period (1, 2, and 3 d), co-culture period (3, 4, and 5 d), infection period (10, 20, and 30 min) and AS concentration (10, 20 and 30 mg·L^{-1}) on the *A. tumefaciens* transformation efficiency were tested using an orthogonal experiment. All the treatments had three replicates, and each replicate was conducted using 50 explants. The number of adventitious buds was counted one month later.

2.6. β-glucuronidase Test

β-glucuronidase (GUS, Sigma–Aldrich) staining was performed according to [27]. Callus induced from explants co-cultured with *A. tumefaciens* containing pCAMBIA1301-*GUS-PgF3′5′H* were used for the histochemical analysis of GUS expression. The calli were soaked in x-gluc staining solution (Solarbio, Beijing, China) at 37 °C for 12 h and decolourised with 70% (*v*/*v*) ethanol for 2 h to remove chlorophyll.

2.7. Verification of Transgenic Plantlets Using PCR

The total DNA of plantlets which were resistant to Hyg and non-transformed plants (used as negative control) was extracted using the hexadecyltrimethylammonium bromi (CTAB) method [28]. The presence of the *Hyg* and *PgF3′5′H* genes was detected by using polymerase chain reaction (PCR) with pCAMBIA1301-*GUS-PgF3′5′H* as the positive control. The primers used were Hyg-F: GTTTCCACTATCGGCGAGTA, Hyg-R: GAGCCTGACC-TATTGCATCTC; *PgF3′5′H*-F: TTCCTCCTCATCGTCCTC, *PgF3′5′H*-R: TGGCTAGGCAGT-GTAAGC. The PCR was performed with the following reaction system: 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 58 °C for 30 s, 72 °C for 1 min; 72 °C for 5 min. The amplified products were separated via electrophoresis using 1% agarose gel.

2.8. Statistical Analasis

Images of callus in the GUS staining experiment were taken using a stereomicroscope. To determine the effects of plant growth regulators on adventitious bud regeneration, one-way analysis of variance (ANOVA) was used, and means were compared using a Tukey's Honest Significant Difference (HSD) test ($p > 0.05$). The statistic calculation was performed using the Data Processing System (v15.10, Hangzhou Ruifeng Information Technology Co., Ltd., Hangzhou, China).

3. Results and Discussion

3.1. Establishing the Regeneration System of G. paniculata Cultivar 'YX4'

Thirteen mediums with different combinations of plant growth regulators were tested for 'YX4' regeneration using segments of shoot apex without meristem as explants. All the mediums showed 100% callus induction capacity while the adventitious bud induction rates and induction co-efficiency varied. The induction rate of adventitious bud was up to 90% in our test, and it was 67% when using leaves as explants [7]. The supplement of TDZ increased the number of adventitious buds induced significantly whereas 6-BA did not contribute to the induction of adventitious shoot much, correlating with the former report [7]. It was also observed that the higher concentration (2.5 mg·L^{-1}) of TDZ reduced the number of explants that formed the adventitious shoot but increased the induction coefficient to 4.14 ± 0.22 (Table 3). Thus, we consider MS + 2.5 mg·L^{-1} TDZ + 0.01 mg·L^{-1} NAA as the optimized regeneration medium for further study.

3.2. Determination of Hyg Screening Pressure

To screen the Hyg-resistant concentration of *G. paniculata* shoot apical segments, we cultured the explants on M2 mediums containing 0, 5, 10, 15, or 20 mg·L^{-1} Hyg. The results showed that the survival ratio of the regenerated buds dropped as the concentration of Hyg increased. The survival ratio dropped to 25% when supplemented with 10 mg·L^{-1} Hyg, and no bud survived when the concentration was 20 mg·L^{-1} (Table 4). We also noticed that, when adding 10 mg·L^{-1} Hyg to the medium, the meristem of the regenerated bud was bleached although the leaves remained green, indicating the death of buds. Thus, 10 mg·L^{-1} was chosen as the selection concentration for 'YX4' transformation.

Table 3. The effects of plant growth regulators on adventitious bud regeneration.

Medium Number	Number of Explants	Number of Explants Forming Callus [1]	Number of Explants with Adventitious Buds	Number of Adventitious Buds [2]	Bud Induction Rates [3]	Induction Coefficient [4]
MS1	30	30	27.33 ± 0.58 ab	37.33 ± 1.53 f	0.91 ± 0.02 ab	1.37 ± 0.07 fg
T1	30	30	27.00 ± 2.00 ab	80.67 ± 4.51 b	0.90 ± 0.07 ab	3.00 ± 0.33 b
T2	30	30	27.33 ± 1.15 ab	66.00 ± 3.61 cd	0.91 ± 0.04 ab	2.42 ± 0.22 cd
T3	30	30	26.33 ± 1.53 ab	55.33 ± 2.52 de	0.88 ± 0.05 ab	2.10 ± 0.03 de
T4	30	30	25.00 ± 1.73 abc	69.00 ± 18.68 bc	0.83 ± 0.06 abc	2.74 ± 0.56 bc
T5	30	30	23.00 ± 3.46 c	95.00 ± 13.57 a	0.77 ± 0.12 c	4.14 ± 0.22 a
T6	30	30	27.67 ± 1.53 a	80.00 ± 3.00 b	0.92 ± 0.05 a	2.89 ± 0.12 b
B1	30	30	25.33 ± 2.08 abc	44.33 ± 8.14 ef	0.84 ± 0.07 abc	1.74 ± 0.19 ef
B2	30	30	24.33 ± 1.53 bc	43.00 ± 5.57 ef	0.81 ± 0.05 bc	1.76 ± 0.12 ef
B3	30	30	26.67 ± 2.52 ab	45.67 ± 7.37 ef	0.89 ± 0.08 ab	1.72 ± 0.26 efg
B4	30	30	27.33 ± 0.58 ab	35.67 ± 8.02 f	0.91 ± 0.02 ab	1.31 ± 0.31 g
B5	30	30	26.33 ± 2.08 ab	43.33 ± 1.53 ef	0.88 ± 0.07 ab	1.65 ± 0.09 fg
B6	30	30	26.67 ± 0.58 ab	46.67 ± 1.53 ef	0.89 ± 0.02 ab	1.75 ± 0.07 ef

Statistical analysis was performed using Tukey's HSD analysis ($p > 0.05$), different lowercase letters (a–g) indicate significant differences. [1] explants display visible callus; [2] bud regenerated from calli with visible leaves; [3] number of explants with adventitious buds/number of explants forming callus; [4] number of adventitious buds/number of explants with adventitious buds.

Table 4. The screen of Hyg selection pressure for commercial cultivar 'YX4'.

Concentration of Hyg ($mg \cdot L^{-1}$)	Number of Explants	Number of Explants Forming Callus	Number of Explants Forming Survived Adventitious Buds	Survival Ratio (%)
0	20	20	20	100
5	20	20	13	65.0
10	20	20	5	25.0
15	20	20	2	10.0
20	20	20	0	0

3.3. Optimization of Transformation Conditions and Exogenous Gene Transformation

The only reported *G. paniculata*- and *A. tumefaciens*-based transformation was performed using stem segments as explants, and the inoculation in *A. tumefaciens* was conducted directly without pre-culture [8]. To obtain the best procedure for the genetic transformation of 'YX4' using shoot apexes without meristem, we conducted the orthogonal experiment, including four factors, pre-culture period, co-culture period, infection period, and AS concentration, with three levels. *GUS* promoted by 35S promoter was introduced into the explants in this experiment. As shown in Figure 2, *GUS* expression was detected in the transgenic calli but exhibited blue pigmentation with varying degrees compared with the non-transgenic calli, indicating the successful transformation of pCAMBIA1301-*GUS*-*PgF3′5′H*. The observation of regenerated buds indicated that the infection time was the most influential factor for 'YX4' transformation, and the co-culture period ranked second, followed by the pre-culture period and AS concentration (Tables 5 and 6). The shorter pre-culture period and longer co-culture duration provided a higher differential ratio while the elongation of the infection duration affected the regeneration capacity obviously. To summarize, the combination of 1d pre-culture, 5d co-culture, 10 min infection, and 30 $mg \cdot L^{-1}$ additional AS forms the optimized system for 'YX4' transformation.

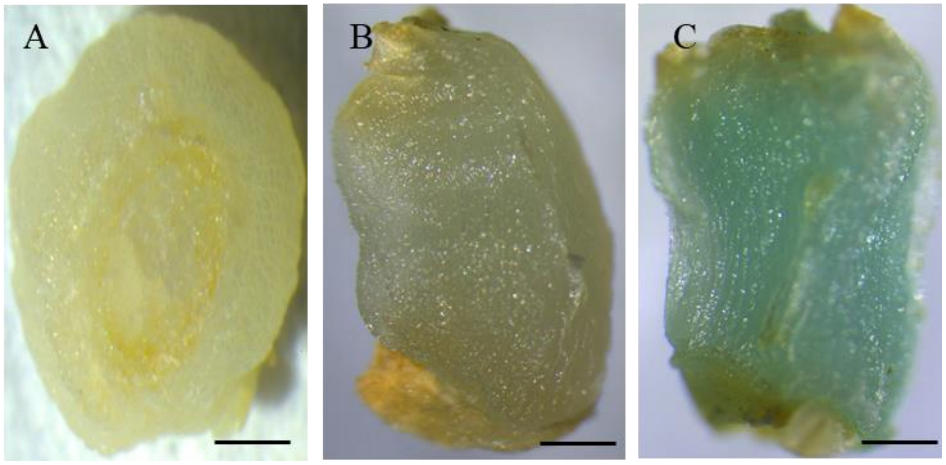

Figure 2. Histochemical staining of GUS transgenic calli. (**A**), wild type callus (**B**), transgenic calli. (**C**), Scale bar = 500 μm.

Table 5. The differentiation ratio of each orthogonal test group.

Test Number	Number of Explants	Pre-culture Period (d)	Co-culture Period (d)	Infection Period (min)	AS Concentration ($mg \cdot L^{-1}$)	Number of Explants Forming Buds	Differentiation Ratio
1	60	1	3	10	10	50	0.83
2	60	1	4	20	20	41	0.68
3	60	1	5	30	30	47	0.78
4	60	2	3	20	30	30	0.50
5	60	2	4	30	10	35	0.58
6	60	2	5	10	20	53	0.88
7	60	3	3	30	20	33	0.55
8	60	3	4	10	30	55	0.92
9	60	3	5	20	10	36	0.60

Table 6. Statistics analysis of the intersubjective effect.

T Value	Pre-culture Period	Co-culture Period	Infection Period	AS Concentration
T1	138	113	158	121
T2	118	131	107	127
T3	124	136	115	132
t1	46.00	37.67	52.67	40.33
t2	39.33	43.67	35.67	42.33
t3	41.33	45.33	38.33	44.00
R	6.67	7.66	14.34	3.67

Note: T1, T2, and T3 represent the sum of the number of the differentiation explants under different levels of the same factor, respectively; t1, t2, and t3 represent the average number of differentiation explants under different levels of the same factor; R is the range value.

3.4. Construction of Transgenic Blue G. paniculata

To test whether exogenous *F3′5′H* would promote the synthesis of delphinidin and facilitate the creation of blue *G. paniculata*, we introduced *PgF3′5′H* into 'YX4' through the optimized *A. tumefaciens*-mediated genetic transformation. The explants were cultured on the M2 medium for 1 d, followed by a 10 min infection of *A. tumefaciens* and a subsequent 5 d co-culture on the M3 medium. The induction of transgenic adventitious buds was then controlled by two rounds of Hyg selection (Figure 3). The regenerated Hyg-resistant

plantlets were then verified using PCR. To increase the reliability of PCR verification, we detected both *Hyg* and *PgF3′5′H* in the Hyg-resistant plants. A total of 38 out of 281 resistant plantlets which possessed both *Hyg* and *PgF3′5′H* in the genomic DNA were obtained, indicating that the transformation efficiency of 'YX4' reached 13.5%. The PCR-positive plantlets were then rooted and transferred to the soil for growth. Unfortunately, we did not observe blue flowers in any of them, which indicated that the function of exogenous *F3′5′H* was also silenced in 'YX4'. This could be caused by chimeric adventitious bud formation, defects in exogenous gene transcription, or translation and silence in gene function. However, the underlying mechanisms remain to be further explored.

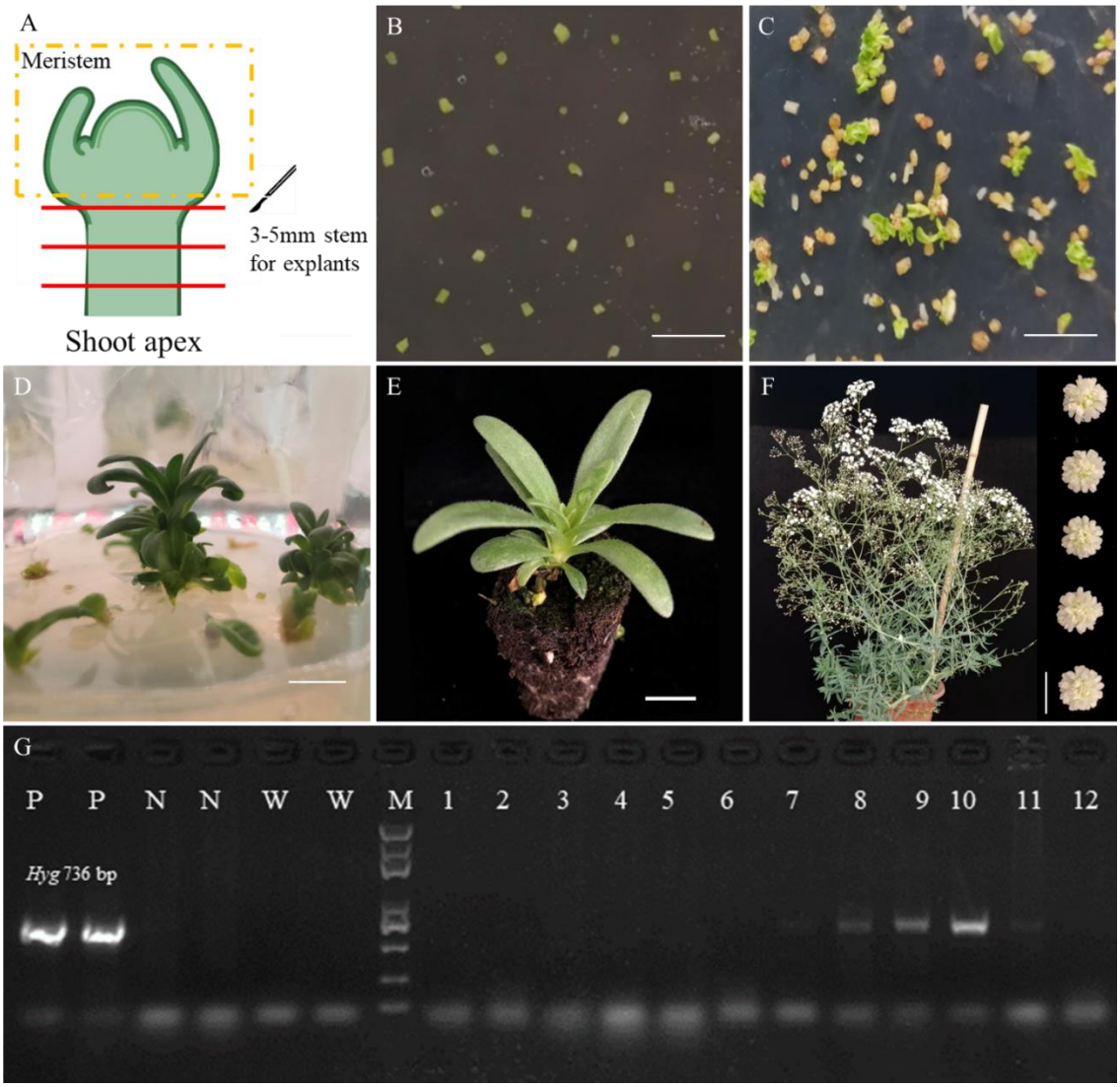

Figure 3. Construction of *PgF3′5′H* transgenic *G. paniculata*. (**A**), the schematic diagram of explants' acquisition; (**B**), the freshly cut explants; (**C**), regenerated shoots under first selection; (**D**), surviving regenerated shoots after second selection; (**E**), rooted seedlings on soil; (**F**), grown-up transgenic 'YX4' plant and flowers; (**G**), represented gel of PCR verification (*p*, plasmid; N, Non-transgenic plant; W, water; M, DNA ladder; 1–12, Hyg-resistant plantlets), Scale bar = 1 cm.

4. Conclusions

G. paniculata is the only species in the genus *Gypsophila* that has been used as cut flowers, and there is massive demand for new cultivars in the floricultural market. In contrast to its rising status, the breeding of *G. paniculata* has stagnated, and new cultivars with novel or improved traits are badly in need. Molecular genetics has become an important

tool in floricultural breeding, in which novel flower cultivars have been created through genetic transformation, such as the blue rose. In this study, we established the regeneration and genetic transformation system for the commercial cultivar 'YX4' of *G. paniculata* and introduced the *PgF3′5′H* successfully into 'YX4' using a shoot apex without meristem as an explant. This reliable and efficient agrobacterium-mediated transformation of the valuable commercial cultivar 'YX4' will facilitate genetic improvement, as well as the application of gene-editing technology in this species. Nevertheless, one may notice that the expected phenotype was not obtained in this study, as well as in many other attempts when the traits of ornamental crops were modified. In addition to the efficient transformation system, genetic study of the gene regulatory network controlling key traits also plays a critical role in the breeding procedure, which requires more attention and should be involved.

Author Contributions: C.J. and D.S. conceived and designed the research; L.M. provided the *PgF3′5′H* plasmid and cultivated the transgenic plants. C.J., D.S. and F.L. wrote the manuscript; F.L., X.M. and C.Y. supervised the project. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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