Establishment of a Genetic Transformation System for Microtuber Immersion in *Polygonatum cyrtonema* Hua

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Abstract [**Objectives**] This study was conducted to establish an efficient in vitro regeneration system for *Polygonatum cyrtonema* Hua. [**Methods**] *P. cyrtonema* was selected as the experimental material to investigate the effects of different *Agrobacterium OD* values and infection durations on the transformation efficiency of microtubers. The transgenic plants were also identified. [**Results**] ① After treatment with 0.1% mercuric chloride, the apical buds of *P. cyrtonema* exhibited significant elongation, strong germination vigor, and a low contamination rate, and an efficient in vitro regeneration system with a proliferation and differentiation coefficient above 4 was established. ② An *Agrobacterium OD* value of 0.8 and an infection duration of 20 min yielded the highest transformation efficiency; ③ PCR identification results and the red coloration indicative of the RUBY reporter gene preliminarily confirmed successful genetic transformation. [**Conclusions**] In this study, an efficient in vitro regeneration system and a microtubers-based genetic transformation system via immersion infection were successfully established for *P. cyrtonema*, a medicinal plant that is difficult to transform. This study provides a foundation for subsequent functional gene studies.

Key words *Polygonatum cyrtonema* Hua; Microtubers; Genetic transformation; *In-vitro* regeneration; Immersion infection

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Polygonatum cyrtonema Hua is a perennial herbaceous plant of the genus *Polygonatum* in the family Liliaceae[1]. It is a traditional medicinal plant in China, and its rhizomes are rich in active constituents such as polysaccharides, saponins, and flavonoids, possessing pharmacological effects including immunomodulation, antioxidant activity, and hypoglycemic activity. It has been widely used in traditional Chinese medicine and the development of health products[2-5]. With the growth of market demand, wild *Polygonatum* resources are at risk of overharvesting. Traditional breeding methods for *P. cyrtonema* are time-consuming and inefficient, whereas genetic transformation technology can rapidly introduce target traits and greatly shorten the time required for breeding new varieties, providing a shortcut for the cultivation of superior varieties[6-7].

Genetic transformation is an important means for verifying gene function. Establishing a relatively complete and efficient genetic transformation system for *Polygonatum* will provide technical support for gene function verification and germplasm improvement of plants in the genus *Polygonatum*. Many medicinal plants have been successfully genetically transformed to improve their traits or produce valuable secondary metabolites[8-10]. The common plant genetic transformation methods are biolistic bombardment and *Agrobacterium*-mediated transformation, which are currently widely applied in various plant species[11-14]. However, these methods also have several limitations, such as low transformation efficiency, high cost, strong species dependence, damage to plant tissues, and long time requirements. In 2022, Zhu Jiankang's research team developed the cutdipbudding (CDB) delivery system, in which *Agrobacterium* rhizogenes is used to infect the cut root-stem junction, and transformed roots are produced from the upper stem and subsequently give rise to transformed plants[15]. The CDB system has been successfully applied to achieve genetic transformation in plant species from multiple plant families. Moreover, this method enables efficient transformation or gene editing in these plants under nonsterile conditions, without the need for tissue culture, using a very simple explant soaking procedure. However, this technology has not yet been applied to genetic transformation in the genus *Polygonatum* at present.

As an important medicinal plant, *Polygonatum* has made certain progress in genetic transformation and application research in recent years. Among these efforts, the research team led by Jin Qing at Anhui Agricultural University[16] developed an efficient genetic transformation system mediated by hairy roots, stably obtaining transgenic hairy roots of *P. cyrtonema* and applying them to gene function verification in this species. The research results on genetic transformation and application of *Polygonatum* can provide a reference for the rapid propagation of medicinal rhizomes, rhizome growth, and stress resistance of *P. cyrtonema*. They also lay a foundation for further indepth studies on the biological functions of related genes in regulating the growth and development of rhizome organs in plants, as well as for the genetic improvement of *P. cyrtonema*[17-19]. Efficient in vitro regeneration from explants is the foundation of genetic transformation research and the guarantee for breaking through the bottlenecks constraining the development of genetic transformation technology. Therefore, establishing an efficient and stable genetic transformation system for

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P. cyrtonema is of profound significance for scientific research, industrial development, and ecological conservation. In this study, *P. cyrtonema* was selected as the experimental material to establish an efficient in vitro regeneration system based on microtuber formation, and simultaneously to establish a microtuber-based genetic transformation workflow for *P. cyrtonema* through *Agrobacterium*-mediated transformation and the CDB system. This study provides a technical reference for the genetic improvement of germplasm resources in the genus *Polygonatum*.

Materials and Methods

Plant materials

The rhizomes of *P. cyrtonema* were kindly provided by the Boda Flower and Tree Professional Cooperative in Donggang District, Rizhao City. Rhizomes with apical buds were selected to establish the sterile *in vitro* regeneration system. Microtubers (3 – 5 mm in diameter, cultured for 30 d) induced from aseptically obtained tissue cultured plantlets of *P. cyrtonema* were used as materials for genetic transformation.

Agrobacterium strains and vectors

The strains tested were *Agrobacterium rhizogenes* strain K599 and *Agrobacterium tumefaciens* strain GV3101. Five recombinant vectors were used: UBQ (ubiquitin promoter): RUBY, DR5 (tissuespecific promoter): RUBY, 35S (cauliflower mosaic virus strong promoter): RUBY, 121GFP (green fluorescent protein), and PTPGFP (green fluorescent protein).

Experimental methods

Establishment of an efficient in vitro regeneration system for *P. cyrtonema* Rhizomes of *P. cyrtonema* with apical buds were selected and rinsed thoroughly under running water. The microtubers with apical buds were immersed in 75% ethanol for 50 s and then rinsed 4 – 5 times with sterile water. Subsequently, they were treated with 0.1% mercuric chloride for 10 – 15 min and rinsed 4 – 5 times with sterile water. After being blotted dry on sterile filter paper, the explants were transferred to prepared MS medium (MS + 4 mg/L 6-BA + 0.5 mg/L NAA) for primary induction culture of adventitious buds at 23 – 25 ℃. After 40 d, the sprouting rate and contamination rate were calculated. After 40 d of culture, the induced adventitious buds were transferred to prepared MS medium (MS + 2 mg/L 6-BA + 0.2 mg/L NAA + 2 mg/L adenine) for subculture and proliferation of adventitious buds, so as to establish an efficient in vitro regeneration system for *P. cyrtonema*. The explant contamination rate, explant sprouting rate and proliferation and differentiation coefficient were calculated according to following formulas: Explant contamination rate (%) = Total number of contaminated explants/Number of inoculated explants × 100%, Explant sprouting rate (%) = Total number of sprouted explants/Number of inoculated explants × 100%, and Proliferation and differentiation coefficient = Total number of newly proliferated explants/Total number of inoculated explants. Data were recorded using Excel, and the experimental data were processed and statistically analyzed using SPSS 26.0. Graphs were generated using GraphPad Prism 9.

Preparation of materials for genetic transformation After 30 d of subculture of the *P. cyrtonema* rhizomes cultured according to the procedure in "Establishment of an efficient in vitro regeneration system for *P. cyrtonema*". The formed microrhizomes (each containing one apical bud) were selected and cut into 0.5 cm × 0.5 cm squares, which were used as explants for *Agrobacterium*-mediated transformation. The explants were precultured for 2 d before use.

Transformation and identification of recombinant vectors Two types of vectors were used for genetic transformation. One was the RUBY reporter system, which allows direct visual observation of red betalain accumulation in plant tissues[15,21]. Three vectors containing this reporter gene (UBQ: RUBY, DR5: RUBY, and 35S: RUBY) were transformed into competent cells by the freeze-ethaw method, and after restriction enzyme digestion and identification, they were used to transform *Polygonatum* microtubers. The other type contained the GFP reporter gene, for which two vectors (pBI121-GFP and PTP-GFP) were constructed. After transformation into *Agrobacterium* and identification by restriction enzyme digestion, they were stored for later use.

Activation of *Agrobacterium* and preparation of infection solution After routine activation of the two types of *Agrobacterium*[22], the *Agrobacterium* culture with an OD_{600} of 0.8 was collected and centrifuged at 4 ℃ and 5 000 rpm for 10 min, and the supernatant was discarded. The bacterial pellet was resuspended in an equal volume of infection buffer (MS medium + hygromycin + acetosyringone) and gently pipetted to homogeneity. The OD_{600} was finally adjusted to 0.6 – 0.8 for later use.

Effects of different genetic transformation factors on the efficiency of *Agrobacterium*-mediated genetic transformation of *P. cyrtonema* Different concentrations of *Agrobacterium* suspension (OD_{600} of 0.6, 0.7, and 0.8) and different infection times (10, 20, and 30 min) were tested to investigate the effects of different genetic transformation factors on the efficiency of *Agrobacterium*-mediated genetic transformation of *P. cyrtonema*. The microtuber transformation rate was calculated according to following formula: Microtuber transformation rate (%) = Number of microtubers showing fluorescence or red coloration/Total number of inoculated microtubers × 100%.

Establishment of the CDB delivery system The method described in the literature[15,21] was adopted with some modifications. Healthy *Polygonatum* microtubers were selected and precultured for 2 d. After activation of the *Agrobacterium* harboring the RUBY reporter gene vector, the bacterial pellet was resuspended in an equal volume of infection buffer (as described above), and the cut microtubers were immersed in the suspension for infection. After coculture of the infected microtubers for 2 d, they were transferred to the corresponding selection medium (hygromycin 50 mg/L) for culture and observation.

Identification of transgenic plants Genetically transformed plants can first be subjected to phenotypic analysis, including

RUBY color development (red) or GFP fluorescence observation. Since betalain exhibits a very bright red color, the RUBY gene can serve as a reporter gene for tracking gene expression or visualizing transformation events, and the primary genetic transformants of *Polygonatum* microtubers can be easily identified by their red coloration. In addition, after 40 – 50 d of culture, when the resistant shoots grew to 2 – 3 cm or color changes appeared in the tubers, the shoot tips or tubers were excised, and their genomic DNA was extracted. Universal primers of the vectors were used for molecular detection of positive transgenic plants by PCR.

Results and Analysis

Establishment of an efficient *in vitro* regeneration system for *P. cyrtonema*

Explant disinfection is the first step in establishing an aseptic culture system for plant tissue culture. When *Polygonatum* rhizome explants are collected from the field, their surfaces carry soil with large numbers of attached bacteria and viruses. The type and concentration of disinfectant, as well as the sterilization time, directly affect the sterilization efficacy of the explants. The experimental results (Fig. 1 and Fig. 2) show that a disinfection time of 15 min was optimal for both disinfectants, yielding the lowest contamination rate. With prolonged disinfection time, the explant contamination rate showed a trend of first increasing and then remaining stable. Among the treatments, the disinfection effect of 0.2% sodium hypochlorite was significantly better than that of 0.1% mercuric chloride. Its contamination rate in the 5th week reached 43%, lower than the 50% observed for 0.1% mercuric chloride, but the difference between the two treatments was not significant. Overall, the contamination rate during primary culture of *P. cyrtonema* explants remained relatively high. Reducing the contamination rate at the primary culture stage is critical for subsequent in vitro regeneration.

Different types of disinfectants had significant effects on the sprouting rate of *P. cyrtonema* rhizome explants. Analysis of the sprouting rates showed that after disinfection with 0.1% mercuric chloride and 2% sodium hypochlorite solution, the sprouting rate at week 6 reached 50% in both treatments (Fig. 3). However, after treatment with 0.1% mercuric chloride, the apical buds of *P. cyrtonema* elongated markedly and were green with strong sprouting vigor (Fig. 4A – Fig. 4C), which was clearly superior to the results obtained with 2% sodium hypochlorite solution. By inoculating primary tissue-cultured plantlets onto subculture proliferation and differentiation medium, it was found that the combination of 6BA and NAA significantly affected the proliferation efficiency of *Polygonatum* tissue-cultured plantlets. After 5 weeks of culture, the average proliferation coefficient reached 4.2 ± 0.27 (Fig. 4D). Under the medium formulation used in this experiment, the adventitious buds grew vigorously, showing expanded dark green leaves and markedly elongated stem segments, and the average plant height was (3.47 ± 0.25) cm (Fig. 4E). A batch of subcultured differentiated tissue-cultured plantlets was successfully obtained, providing materials for subsequent genetic transformation (Fig. 4F).

Transformation and identification of genetic transformation vectors

The three vectors containing the RUBY reporter gene were separately transformed into competent *Agrobacterium* cells. On LB plates containing dual antibiotics (spectinomycin + rifampicin + kanamycin), unevenly distributed single colonies, white or pale yellow in color, were observed, with colony diameters of approximately 1 – 2 mm. No bacterial lawns or contamination were observed, indicating successful transformation (Fig. 5). Subsequent colony PCR identification also confirmed the amplification of the target fragment (Fig. 6), further verifying the correct insertion of the target gene, which could be used for subsequent genetic transformation experiments of *Polygonatum* microtubers. The vectors harboring the GFP reporter gene were not successfully transformed.

Effects of different genetic transformation factors on the efficiency of *Agrobacterium*-mediated genetic transformation of *P. cyrtonema*

A large number of studies have demonstrated that plant genetic transformation efficiency is usually influenced by the concentration of the infection bacterial suspension and the infection time[22]. Based on preliminary experiments, the results of genetic transformation experiments on *P. cyrtonema* microtubers revealed that the infection time had a significant effect on the genetic transformation rate of *P. cyrtonema* microtubers. When *Agrobacterium* cultures harboring the three vectors 35S:RUBY, DR5:RUBY, and UBQ:RUBY were used for infection at a bacterial suspension OD of 0.8, the highest microtuber transformation rates were observed with a 20min infection, reaching 98.9%, 80%, and 60%, respectively (Fig. 7). The above conditions were adopted for the subsequent infection and transformation experiments (Fig. 8).

Identification of genetically transformed plants

Genetically transformed plants of *P. cyrtonema* were randomly selected, and their total DNA was extracted as templates for PCR amplification to detect the transformed plants. The PCR amplification results showed that target bands were amplified from all positive *P. cyrtonema* plants (Fig. 9), indicating that genetic transformation was preliminarily successful.

The leaves of both positive shoots and positive plants transformed with the UBQ:RUBY reporter gene exhibited a pale red color (Fig. 10, indicated by arrows), allowing transgenic and nontransgenic plants to be distinguished by the naked eye, which significantly improved the screening efficiency of transgenic plants. In this study, it was observed that the RUBY reporter gene exhibited pale red instead of red. This may be because the expression of this gene needs to accumulate to a certain level before red coloration becomes visible. Meanwhile, the inherent color of *Polygonatum* tubers may mask weak red signals, resulting in the observation of only a weak red signal. Therefore, further research is needed to investigate this phenomenon.

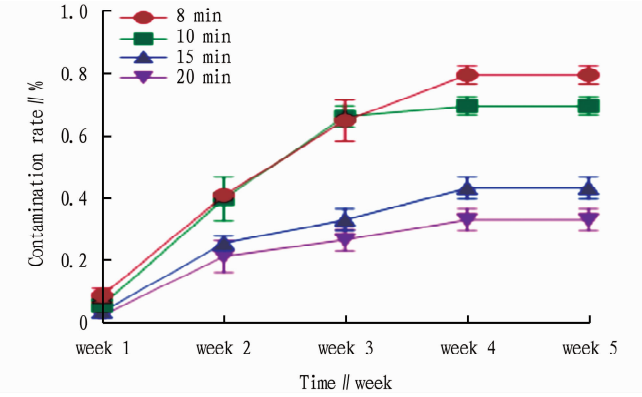


Fig. 1 Disinfection effect of 0.1% mercuric chloride

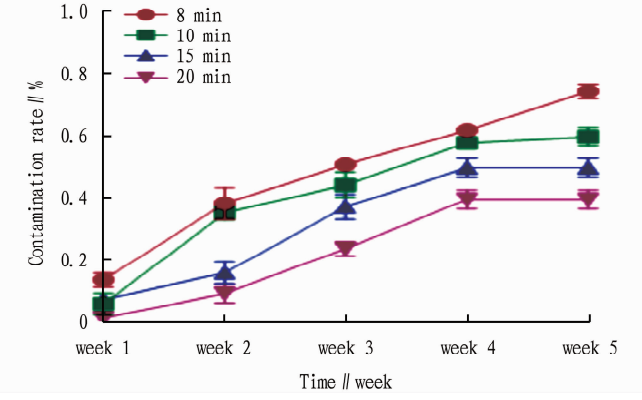


Fig. 2 Disinfection effect of 2% sodium hypochlorite

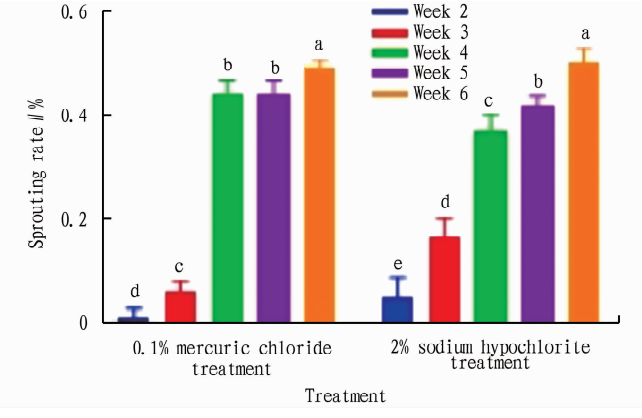
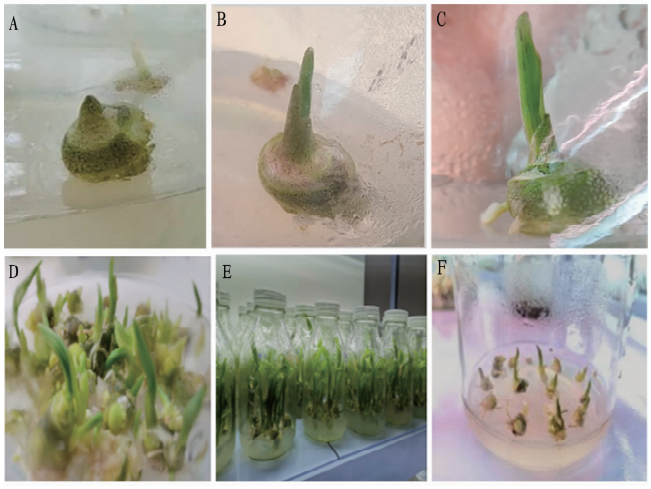


Fig. 3 Comparison of sprouting rates between the two disinfectants



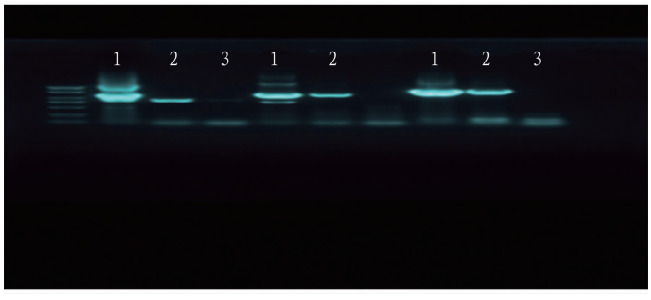
(A – C) Sprouting of *P. cyrtonema* treated with 0.1% mercuric chloride at weeks 1, 3, and 6 after inoculation; (D – E) Proliferation and culture of *P. cyrtonema* tissue-cultured plantlets after 2 and 5 weeks of subculture; (F) Microtubers obtained from in vitro culture.

Fig. 4 Efficient in vitro regeneration system of *P. cyrtonema*



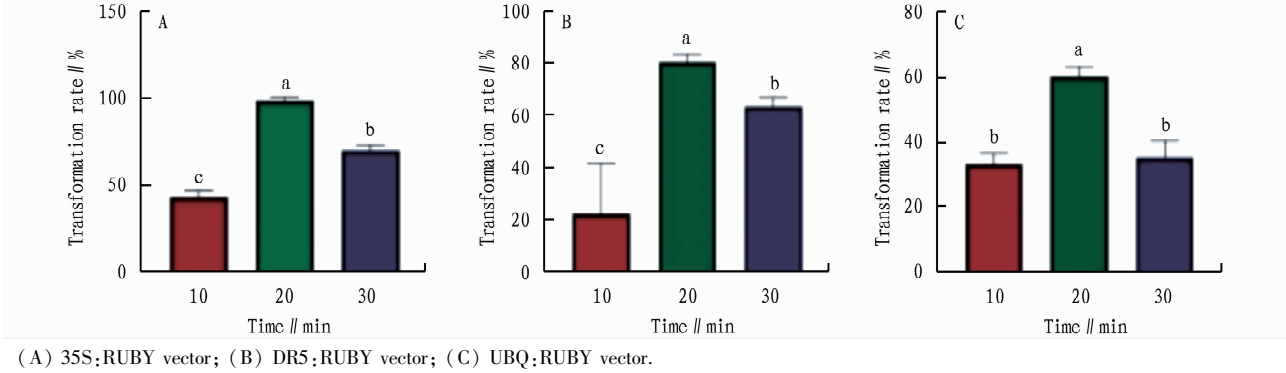
From left to right: DR5:RUBY, 35S:RUBY and UBQ:RUBY.

Fig. 5 Colony growth of *Agrobacterium* transformed with partial vectors



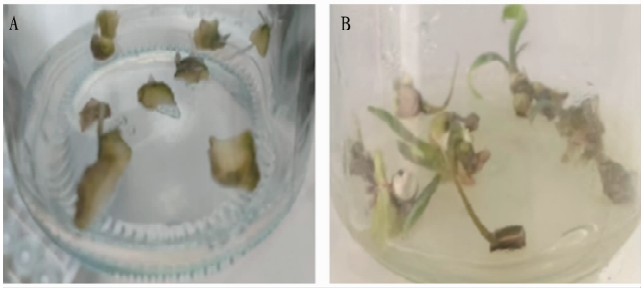
1. Plasmid; 2. Colony; 3. Blank control.

Fig. 6 Identification by colony PCR



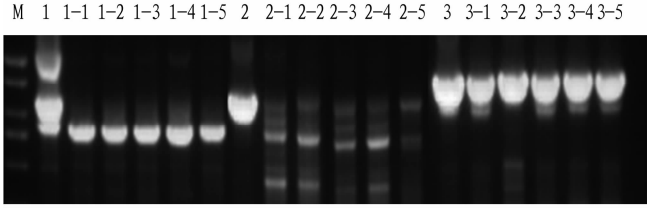
(A) 35S:RUBY vector; (B) DR5:RUBY vector; (C) UBQ:RUBY vector.

Fig. 7 Effects of different infection durations on transformation efficiency



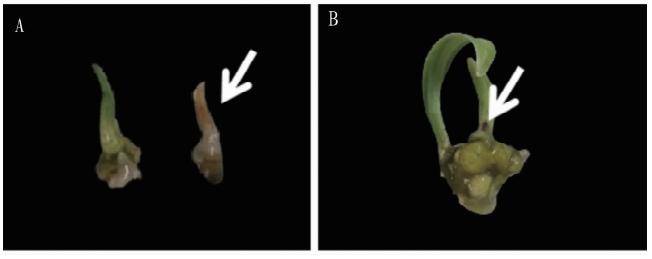
(A) Co-cultivation; (B) The 6th week after infection.

Fig. 8 Infection of *P. cyrtonema* microtubers by *Agrobacterium* GV3101



M: DL2000; 1: 35S:RUBY vector; 1 - 1 to 1 - 5: Transgenic plants with 35S:RUBY; 2: DR5:RUBY vector; 2 - 1 to 2 - 5: Transgenic plants with DR5:RUBY; 3: UBQ:RUBY vector; 3 - 1 to 3 - 5: Transgenic plants with UBQ:RUBY.

Fig. 9 PCR identification of transgenic plants



(A) Control on the left, positive shoots harboring the UBQ: RUBY reporter gene on the right; (B) Leaves of positive plants harboring the UBQ:RUBY reporter gene.

Fig. 10 Screening of positive plants based on the RUBY reporter gene

Conclusion and discussion

The choice of explant is a key factor affecting the success of genetic transformation. Leaves are widely used in genetic transformation due to their high frequency of successful transformation and regeneration, and have been broadly applied in many plant species[23-24]. The genetic transformation material used in this study was the medicinal plant *P. cyrtonema*. For this plant, establishing a leaf regeneration system is extremely difficult, and most scholars have established rhizome regeneration systems and callus regeneration systems[17,25-26]. This study successfully established an efficient in vitro regeneration system for *P. cyrtonema*, which serves as an "enabling platform" for genetic transformation. It not only addresses the low efficiency and long cycle of conventional transformation methods, but also provides scalable technical support for gene editing and metabolic engineering.

In this study, an *Agrobacterium*-mediated infection transformation system for *P. cyrtonema* was successfully established, and for the first time, a CDB-mediated genetic transformation system for *P. cyrtonema* microtubers was established. It was found that the introduction of the RUBY reporter gene significantly simplified the screening process for positive transformants. It eliminated the need for resistance markers or sophisticated equipment, as well as the need for strain culture and co-cultivation steps, thereby reducing the risk of contamination. These findings are consistent with previous reports[27-28]. In this study, it was observed that the CDB system exhibited pigment interference, for example, the inherent color of *Polygonatum* tubers might mask weak red signals, resulting in the observation of only a weak red signal. This is similar to the findings reported by other researchers[21,29]. Therefore, further optimization of the system is still needed. The results of this study indicate that the transformation efficiency of the CDB system is better than that of the *Agrobacterium*-mediated method. Furthermore, the RUBY system enables high-efficiency screening of the style of "what you see is what you get" for genetic transformation of *P. cyrtonema*. This strategy is especially suitable for medicinal plants lacking stable transformation systems. It provides an *Agrobacterium*-free transformation protocol for medicinal plants which are difficult to transform, such as *P. cyrtonema*, and accelerates functional gene research.

References

[1] YIN SY, QIAN LH. Research progress in diversity of origin plants of Chinese medicinal Polygonati Rhizoma and its utilization[J]. Chinese Wild Plant Resources, 2022, 41(2): 4957. (in Chinese).

[2] SI JP, ZHU YX. Polygonati Rhizoma: A new high-quality crop with great potential and not occupying farmland[J]. Scientia Sinica (Vitae), 2021, 51(11): 14771484. (in Chinese).

[3] TAO MT, LI LX, WANG HY, *et al.* Comparison of polysaccharide components and antiinflammatory and antioxidant effects of *Polygonatum sibiricum*, *Polygonatum cyrtonema*, and *Polygonatum kingianum*[J]. Chinese Traditional Patent Medicine, 2023, 45(4): 13671372. (in Chinese)

[4] WANG JJ, SHI JD, LI CX, *et al.* Nutritional components and antioxidant enzyme activity of *Polygonatum cyrtonema* and *Polygonatum kingianum* seeds[J]. Guizhou Agricultural Sciences, 2022, 50(1): 9095. (in Chinese).

[5] PENG T, WU XX, LI Y, *et al.* Herbal textual research on the names and origins of *Polygonatum*[J]. Chinese Journal of Modern Applied Pharmacy, 2023, 40(1): 7581. (in Chinese).

[6] ZHENG LY, MA XL, SUN YQ. Resource distribution and development and application status of *Polygonatum* as a medicinal and edible plant[J]. Zhejiang Agricultural Sciences, 2022, 63(8): 16791684. (in Chinese).

[7] LIU L, QU J, WANG C, *et al.* An efficient genetic transformation system mediated by *Rhizobium rhizogenes* in fruit trees based on the transgenic hairy root to shoot conversion[J]. Plant Biotechnol J., 2024, 22(8): 2093-2103.

[8] ZENG WD, YAN HB, WU ZD, *et al.* Agrobacterium rhizogenes-mediated transformation system of *Pueraria lobata* hairy roots[J]. Chinese Bulletin of Botany, 2025, 60(3): 425434. (in Chinese).

(Continued on page 18)

species and its ITS molecular identification[J]. Journal of Zhejiang Agricultural Sciences, 2022, 63(6): 1257 - 1261. (in Chinese).
[68] FENG LT, XIE W, LUO YF, *et al.* Inhibitory effects of two *Chaetomium* strains on the pathogen of potato dry rot[J]. China Plant Protection, 2021, 41(12): 5 - 8, 50. (in Chinese).
[69] LIU ZW, SHI XJ, ZHAO JH, *et al.* The control efficiency of three fungus on the rhizome rots of ginger caused by *Pythium myriotylum*[J]. Northern Horticulture, 2010(10): 189 - 191. (in Chinese).
[70] GE XH, YA ML, NING XY, *et al.* The potential of *Trichoderma brevicompactum* for controlling root rot on *Atractylodes macrocephala*[J]. Canadian Journal of Plant Pathology, 2021, 43(6): 794 - 802.
[71] GAO F, REN XX, WANG ML, *et al.* Research progress in root rot diseases of Chinese herbal medicine and control strategy by antagonistic microorganisms[J]. China Journal of Chinese Materia Medica, 2015, 40(21): 4122 - 4126. (in Chinese).
[72] ZHANG MX, PENG N, WANG YY, *et al.* Study on the biocontrol effect of antagonistic bacteria XT-25 against *Panax quinquefolium* root rot [J]. Journal of Nuclear Agricultural Sciences, 2024, 38(7): 1249 - 1257. (in Chinese).
[73] LI WJ. Screening and identification of antagonistic actinomycetes against fungal diseases of potato[D]. Lanzhou: Northwest Normal University, 2017. (in Chinese).
[74] SUN XC, PENG JF, ZHANG N, *et al.* Inhibitory effect and identification of an antagonistic actinomyces strain JY-22 against *Fusarium solani* causing potato dry rot[J]. Journal of Plant Protection, 2013, 40(1): 38 - 44. (in Chinese).
[75] NIU SQ, LI WJ, LI HY, *et al.* Screening of actinomyces on antagonism to *Fusariun* spp. isolated from saline-alkali soils in Hexi corridor[J]. Journal of Northwest Normal University: Natural Science, 2017, 53(2): 94 - 98. (in Chinese).
[76] CHEN J, TANG L, GUO TW, *et al.* Resistances of actinomycetes against potato soil-borne pathogenic fungi and their promoting effects [J]. Journal of Northwest A&F University: Natural Science Edition, 2014, 42(1): 111 - 119. (in Chinese).
[77] KHUCHAROENPHAISAN K, RODBANGPONG K, SAENGPAEN P, *et al.* Exploration on soil actinomycetes against *Phytophthora* sp. causing root rot of cassava and plant growth promoting activities[J]. Journal of Plant Sciences, 2016, 11(1): 38 - 44.

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(Continued from page 10)

[9] SUN SJ, XIA XY, ZHAO YQ, *et al.* Study on a rapid shoot regeneration and genetic transformation system from leaves of *Salvia miltiorrhiza*[J/OL]. Molecular Plant Breeding, 2025, 19. (in Chinese).
[10] HE XX, YU YH, ZHANG Y. Genetic transformation of *PSY* gene in ginseng[J]. Chinese Bulletin of Botany, 2008(5): 579584. (in Chinese).
[11] QIAO LY, LI R, HAO YQ, *et al.* Construction of a genetic transformation system mediated by biolistic particle for winter wheat Linfen 5064 [J]. Journal of Shanxi Agricultural Sciences, 2023, 51(12): 13471352. (in Chinese).
[12] SHIVANI I, HARI SM, SUSAN E. Genetic transformation of chickpea (*Cicer arietinum* L.) with insecticidal crystal protein gene using particle gun bombardment[J]. Plant cell reports, 2007, 26(6): 755 - 63.
[13] XIA QY, JIANG YM, LIU YN, *et al.* An efficient genetic transformation method for broomcorn millet mediated by *Agrobacterium tumefaciens* [J]. Chinese Journal of Tropical Crops, 2025, 46(1): 4450. (in Chinese).
[14] YANG S, SONG XQ, ZHAO ST, *et al.* Establishment of regeneration tissue culture and genetic transformation system by roots of *P. deltoides* cl. 'Danhong' × P. nigra cl. '44'[J]. Forest Research, 2024, 37(5): 7484. (in Chinese).
[15] CAO XS, XIE HT, SONG ML, *et al.* Cut-dip-budding delivery system enables genetic modifications in plants without tissue culture[J]. The innovation, 2023, 4(1): 100345.
[16] JIN Q, PENG FL, ZHANG WW, *et al.* A genetic transformation method for *Polygonatum cyrtonema* and its application [P]. Anhui Province: CN202211592302.3, 20230303. (in Chinese).
[17] BAN JJ. Microrhizome induction of *Polygonatum cyrtonema* Hua and functional study of PcDREB10[D]. Fuzhou: Fujian Agriculture and Forestry University, 2023. (in Chinese).
[18] ZHU TY. Industrialized production of *Polygonatum cyrtonema* test-tube plantlets and identification and expression analysis of the PLT family [D]. Fuzhou: Fujian Agriculture and Forestry University, 2023. (in Chinese).
[19] ZHANG H. Effects of light on tissue culture and medicinal components of *Polygonatum cyrtonema* and identification and expression of the ACY1 family[D]. Fuzhou: Fujian Agriculture and Forestry University, 2023. (in Chinese).
[20] WAN K, YANG FJ, NAN YL, *et al.* Transcription factor PcERF114 is involved in the regulation of flavonoid accumulation of *Polygonatum cyrtonema* Hua[J]. Industrial Crops and Products, 2025, 230: 121082.
[21] YANG H, ZHANG Y, FU Q, *et al.* The DR5 and E8 reporters are suitable systems for studying the application of the Ruby reporter gene in tomato[J]. Vegetable Research, 2023, 3: 12.
[22] MAHESHWARI P, AND KOVALCHUK I. Agrobacterium-mediated stable genetic transformation of *Populus angustifolia* and *Populus balsamifera*[J]. Front. Plant Sci., 2016, 7: 296.
[23] FENG MQ, WANG RY, SIMA L, *et al.* Genetic transformation system of plants with non-tissue culture and its application[J]. Journal of Huazhong Agricultural University, 2025, 44(2): 228242. (in Chinese).
[24] ZHANG DH. Study on an efficient regeneration system suitable for genetic transformation of oriental lily 'Sorbonne'[D]. Nanjing: Nanjing Agricultural University, 2019. (in Chinese).
[25] ZHAO Q, WU CF, WANG WG, *et al.* In vitro plantlet regeneration system from rhizomes and mannose-binding lectin analysis of *Polygonatum cyrtonema* Hua[J]. Plant Cell Tiss Organ Cult, 2009, 99: 269 - 275.
[26] ZHOU JJ, LIN YL, WANG JD, *et al.* Technical regulations for tissue culture and rapid propagation of *Polygonatum cyrtonema*[J]. Fujian Agricultural Science and Technology, 2024, 55(7): 5761. (in Chinese).
[27] PRUSTY MR, SHATIL-COHEN A, KUMAR R, *et al.* Pigments to precision: RUBY aiding genetic transformation and genome editing in wheat and barley[J]. Physiol Mol Biol Plants, 2025, 31: 545 - 554.
[28] LIN K, LU LX, PAN BZ, *et al.* *Agrobacterium rhizogenes*-mediated hairy root genetic transformation using *Agrobacterium* gel inoculation and RUBY reporter enables efficient gene function analysis in Sacha Inchi (*Plukenetia volubilis*)[J]. Int. J. Mol. Sci., 2025, 26: 2496.
[29] HE YB, ZHANG T, SUN H, *et al.* A reporter for noninvasively monitoring gene expression and plant transformation [J]. Horticulture Research, 2020, 7: 152.

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