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# Multiple Shoot and Callus Induction of Herbaceous Peony (*Paeonia lactiflora* Pall.)

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## ABSTRACT

Underground buds of herbaceous peony (*Paeonia lactiflora* Pall.) 'Zhong Sheng Fen' were used as explants for axillary shoot induction while stems, petioles and leaves of 'Da Fu Gui' and 'Tao Hua Fei Xue' were used as explants for callus induction. The effects of different basal media and concentrations of plant growth regulators (PGRs) on induction were investigated to establish an aseptic regeneration system. The best medium to induce and proliferate shoots was modified half-strength Murashige and Skoog (MS) medium with double the concentration of $Ca^{2+}$ supplemented with 1 mg $l^{-1}$ gibberellic acid ($GA_3$) plus 1 mg $l^{-1}$ 6-benzyladenine (BA). Two successive steps were adopted for rooting shoots. Shoots were first cultured on Woody Plant Medium (WPM) plus 0.5 mg $l^{-1}$ 1-naphthyleneacetic acid (NAA) for 10-15 days in the dark, then shoots were transferred to PGR-free WPM medium containing 0.2% activated charcoal; in this case, rooting could reach 50%. The best explants for callus induction were young stems, and the best basal medium for callus induction was WPM for 'Da Fu Gui' and 'Tao Hua Fei Xue'.

**Keywords:** axillary shoot, callus, explant, media, plant growth regulators
**Abbreviations: BA**, 6-benzyladenine; **2,4-D**, 2,4-dichlorophenoxyacetic acid; **$GA_3$**, gibberellic acid; **IBA**, indole-3-butyric acid; **MS**, Murashige and Skoog medium; **NAA**, 1-naphthyleneacetic acid; **TDZ**, thidiazuron; **WPM**, Woody Plant Medium

## INTRODUCTION

Peony is a herbaceous perennial in the genus *Paeonia*, family *Paenoiaceae*. It has a long cultivation history in China. Propagation of herbaceous peony can be conducted by division, cutting, grafting and layering. Division is the main method for propagation in which a grower may double stock every 3 years by dividing tuberous root clumps containing 3-5 dormant buds (Shannon and Kamp 1959). However, conventional propagation is time-consuming – 3 years is an excessively long turn-over period – and cannot meet the increasing needs of the Chinese and international markets. Tissue culture and rapid propagation of herbaceous peony can raise the propagation rate and shorten the breeding period and this has important significance for production. Although many advances have been made in the micropropagation of peony (Gabryszewska 2010; Wang *et al*. 2010), several serious problems still remain unsolved, such as disinfection, contamination, browning, hyperhydricity, indirect shoot induction via callus and rooting (Gabryszewska 1998; Wang *et al*. 2010; Tian *et al*. 2010; Wu *et al*. 2011). Many kinds of explants from herbaceous peony have been known to generate callus, such as p*etal*s, vegetative buds, stems (with node), petioles, leaves, hypocotyls, among others (Yamada and Sinoto 1966; Orlikowska *et al*. 1998; Guo 2001; Tian *et al*. 2010; Wang 2010; **Table 1**), among which buds are optimal to obtain a high frequency of callus induction. Regeneration via adventitious buds had been very difficult for herbaceous peony (Zhang *et al*. 2007), and according to a recent study, only callus from hypocotyls succeeded in producing adventitious buds, but at a low regeneration rate (7.95%) (Wang *et al*. 2010). Another study, however, indicated that manipulation of the light spectral quality can be used to increase shoot production under photoautotrophic conditions (Ding *et al*. 2010). In general, for herbaceous peony, excessive browning of the tissues and development of callus prevents further bud development. Cotyledons, anthers and seed can be used to induce somatic embryos (Lee *et al*. 1992; Brukhin and Batygina 1994; Kim and Lee 1996; Kim *et al*. 2006). Bud and nodal stem sections are always used for direct shoot induction (Hosoki *et al*. 1989; Hu *et al*. 2003), In addition, direct shoot regeneration was observed only on leaf segments and the base of petioles and p*etal*s within 2 months, but the rate of recovery was always extremely low, never exceeding 10% (Orlikowska *et al*. 1998). Rooting and final transplanting are also big obstacles for successful herbaceous peony micropropagation (Guo 2001; Zhang *et al*. 2006) and in fact, in a recent paper that showed the successful shoot regeneration from multiple explants, this issue was completely ignored, or avoided (Tian *et al*. 2010).

In this experiment, we chose three herbaceous cultivars (which have high ornamental value and vigorous growth *in vitro*) as experimental materials, 'Zhong Sheng Fen', 'Da Fu Gui' and 'Tao Hua Fei Xue', to induce shoots and callus and explore the optimum level and combination of plant growth regulators (PGRs) for rooting.

## MATERIALS AND METHODS

### Materials

Four types of explants were investigated in this study: underground buds of 'Zhong Sheng Fen', and stems, petioles and leaves of 'Da Fu Gui' and 'Tao Hua Fei Xue'. Underground buds of 'Zhong Sheng Fen' were thicker than other cultivars, so they were better for shoot induction, and were thus selected for our study. Buds were collected in winter while other explants were collected in spring. All material was obtained from the Jiu Feng Herbaceous Peony Experimental Base of Beijing Forestry University.

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Original Research Paper

**Table 1** Adventitious shoot regeneration and callus formation studies in herbaceous peony (*Paeonia lactiflora* Pall.).

| Explant used | Basal medium | Cytokinin (mg $l^{-1}$) | Auxin (mg $l^{-1}$) | Other additives or growth regulators (mg $l^{-1}$) | Species or cultivar | No. shoots/explant OR % Explants forming callus | Reference |
|---|---|---|---|---|---|---|---|
| **Adventitious shoot formation** | | | | | | | |
| Leaf | 1/2 MS | 1 BA | – | 5 $AgNO_3$ | *Paeonia mloksewitschii* Lom. and *P. tenuifolia* L. | Not exceeding 10% | Orlikowska *et al*. 1998 |
| Petiole | 1/2 MS | 1 BA | – | 5 $AgNO_3$ | *Paeonia mloksewitschii* Lom. and *P. tenuifolia* L. | Not exceeding 10% | Orlikowska *et al*. 1998 |
| Fork petiole | MS | Pretreated with 2, 15, or 60 min of 20 mg $l^{-1}$ TDZ | – | – | Xi Shi Fen | 100% | Daike *et al*. 2010 |
| Node stem | 1/2 MS | 0.1–3 TDZ | – | – | Yang Fei Chu Yu | 100% | Daike *et al*. 2010 |
| | MS | 3.5 BA | – | – | *Paeonia lactiflora* | 0.9 shoots/callus section | Hu *et al*. 2003 |
| Petal | 1/2 MS | 1 BA | – | 5 $AgNO_3$ | *Paeonia mloksewitschii* Lom. and *P. tenuifolia* L. | Not exceeding 10% | Orlikowska *et al*. 1998 |
| Hypocotyl | 1/2 MS | 0.1-1.0 TDZ | 0.1 NAA | – | *Paeonia lactiflora* | 9.1% | Wang *et al*. 2010 |
| Shoot tip | 1/2 MS | 0.5 BA | – | 1 GA | *Paeonia lactiflora* | 2.4 axillary buds | Hosoki *et al*. 1989 |
| Vegetative bud | 1/2 MS | 2 BA | – | – | Peony cv. Jadwiga | 2.4 axillary shoots | Gabryszewska *et al*. 1998 |
| Underground bud | 1/2 MS | 0.5 BA | – | 1 GA | Qi Hua Lu Shuang | 85% | Guo 2001 |
| | 1/2 MS | 1 6-BA | – | 1 $GA_3$ | *Paeonia lactiflora* | 75.46% | Zhang *et al*. 2007 |
| | 1/2 MS (double-strength $CaCl_2$) | 1 6-BA | 0.1 NAA | 1 $GA_3$ | Zhong Sheng Fen | 81.25% | This study |
| **Callus formation** | | | | | | | |
| Petal | – | – | – | – | *Paeonia japonica* | – | Yamada and Sinoto 1966 |
| Mature seed embryoids | MS (double decreased nitrogen ($KNO_3$ and $NH_4NO_3$)) | 1 or 0.5 BA | 1 NAA | – | *Paeonia anomala* | 100% | Brukhin and Batygina 1994 |
| Hypocotyl | 1/2 MS | 0.2 TDZ | 0.1 NAA | – | Fen Yu Nu; Lian Tai | 100% | Wang *et al*. 2010 |
| Leaf | 1/2 MS | 1 BA | 0.1 NAA | – | Zhong Sheng Fen | 42.5% | Guo 2001 |
| | Modified MS | 0.5 BA | 0.5 2,4-D | – | *Paeonia lactiflora* | 74.29% | Zhang *et al*. 2007 |
| | WPM | 2 BA | 0.2 NAA, 0.2 2,4-D | – | Tao Hua Fei Xue | 69.74% | This study |
| Petiole | 1/2 MS | 1 BA | 0.1 NAA | – | Zhong Sheng Fen | 67.2% | Guo 2001 |
| | 1/2 MS | 1.0 BA | 2.0 NAA | – | *Paeonia lactiflora* | 85% | Wang *et al*. 2010 |
| | WPM | 2 BA | 0.2 NAA, 0.2 2,4-D | – | Da Fu Gui | 94.74% | This study |
| Stem | 1/2 MS | 2 ZT | 0.4 IAA | 100 VC | Da Fu Gui | 66% | Huang *et al*. 2009 |
| | WPM | 2 BA | 0.2 NAA, 0.2 2,4-D | – | Da Fu Gui | 100% | This study |
| Underground buds | 1/2 MS | 1 BA | 0.1 NAA | – | Zhong Sheng Fen | 84.9% | Guo 2001 |

Media: MS, Murashige and Skoog (1962); WPM, Woody Plant Medium (Lloyd and McCown, 1980); $PGR_S$ and other chemicals: $AgNO_3$, silver nitrate; BA, 6-benzyladenine; $CaCl_2$, calcium chloride; GA or $GA_3$, gibberellin or gibberellic acid; IAA, indole-3-acetic acid; NAA, 1-naphthyleneacetic acid; $NH_4NO_3$, ammonium nitrate; TDZ, thidiazuron; Vc, vitamin C; ZT, zeatin; 2,4-D, 2,4-dichlorophenoxyacetic acid

All the explants were cultured on agar (0.7%)-solidified medium. Two basal media supplemented with 30 g $L^{-1}$ sugar were used: 1) half-strength MS medium (Murashige and Skoog 1962) with double-strength calcium chloride (880 mg $l^{-1}$ $Ca^{2+}$); 2) Woody Plant Medium (WPM) (Lloyd and McCown 1980) with full vitamins. Different combinations of PGRs, including $GA_3$, BA, NAA, TDZ, IBA and 2,4-D (Sigma-Aldrich), were added.

## Surface sterilization and culture conditions

Since a high contamination rate of underground buds was observed in previous studies, a three-step method was used in this study to sterilize them. Before inoculating, buds were washed in tap water for 30 min. Outer scales were peeled off and buds were soaked in 75% ethanol for 30 s followed by 15 min of sterilization

**Table 2** Effects of different media on induction of underground buds of herbaceous peony cv. 'Zhong Sheng Fen' (n = 10).

| **Treatment [a] (mg $l^{-1}$)** | **Sprouting (%) (mean ± SD)** | **Axillary bud differentiation (%) (mean ± SD)** | **Callus induction (%) (mean ± SD)** |
|---|---|---|---|
| 1 $GA_3$ + 1 BA | 100 | 56.25 ± 7.22 | 12.50 ± 5.10 |
| 1 $GA_3$ + 1 BA + 0.1 NAA | 93.75 ± 6.25 | 81.25 ± 10.83 | 100 |

[a] Treatment: underground buds were inoculated on half-strength MS medium (double-strength $Ca^{2+}$) supplemented with different PGRs. BA, 6-benzyladenine; $GA_3$, gibberellic acid; NAA, 1-naphthyleneacetic acid.

with a diluted solution of sodium hypochlorite (2% active chlorine). Plant material was rinsed three times for 5 min in autoclaved distilled water (ADW). The remaining scales were peeled off, and buds were soaked in a diluted solution of sodium hypochlorite (1% active chlorine) for 12 min. The material was rinsed in ADW four times then cultured in 100-ml Erlenmeyer flasks containing 30 ml agar solidified medium with one or two buds per flask.

Other explants (i.e., stems, petioles and leaves) were sterilized more simply. The explants were washed in tap water for 30 min and cut into 2-4 cm long sections. Sections were soaked in 75% ethanol for 30 s followed by 15 min sterilization with a diluted solution of sodium hypochlorite (0.5% active chlorine). Plant material was rinsed three times, 5 min each, in ADW. After sterilization, explants were cut into shorter (1-2 mm) sections and cultured in Petri dishes (90 mm diameter) containing 30 ml of solidified agar (0.7%) medium.

The pH of all media was adjusted to 5.8 prior to autoclaving at 118°C for 18 min. Culture vessels were placed at 25 ± 2°C in a 14-h photoperiod with 50 μmol $m^{-2} s^{-1}$ PPFD using cool white fluorescent tubes. For callus induction, cultures were placed in complete darkness. Data were collected after 30 days of culture.

## Treatments

To initiate culture, shoot tips of underground buds of 'Zhong Sheng Fen' were cultured in half-strength MS medium (double-strength $Ca^{2+}$) supplemented with 1 mg $l^{-1}$ $GA_3$ + 1 mg $l^{-1}$ BA or 1 mg $l^{-1}$ $GA_3$ + 1 mg $l^{-1}$ BA + 0.1 mg $l^{-1}$ NAA. In shoot proliferation culture, vigorous tissue-cultured plants were separated at the base into 2-3 plantlets, and then placed on half-strength MS supplemented with 1 mg $l^{-1}$ $GA_3$ + 1 mg $l^{-1}$ BA, 1 mg $l^{-1}$ $GA_3$ + 0.1 mg $l^{-1}$ TDZ, 1 mg $l^{-1}$ BA or 0.2 mg $l^{-1}$ BA. At the rooting stage, shoots were placed on different rooting medium for 10-15 days, and then transferred to PGR-free WPM medium containing 0.2% activated charcoal (AC; Sigma). The rooting media were WPM (PGR-free), WPM + 0.5 mg $l^{-1}$ IBA, WPM + 1 mg $l^{-1}$ NAA, or WPM + 0.5 mg $l^{-1}$ NAA. For callus induction, stems and petioles were cut into 1-2 mm segments. The edges of leaf laminae were trimmed and the remaining leaf tissue was divided into 5 $mm^2$ squares and placed abaxial side down on the surface of media. Explants were cultured on half-strength MS (double-strength $Ca^{2+}$) or WPM medium supplemented with different PGRs as follows: (1) half-strength MS (double-strength $Ca^{2+}$) + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA + 0.2 mg $l^{-1}$ 2,4-D; (2) half-strength MS (double-strength $Ca^{2+}$) + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA; (3) WPM + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA + 0.2 mg $l^{-1}$ 2,4-D; (4) WPM + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA. The growth regulators, concentrations and combinations were selected on the basis of the domestic and foreign researches in tissue culture of peony. The same growth of shoots was used in every research.

## Experimental design and statistical analyses

Data was sampled in a completely randomized design. Means were separated by one-way analysis of variance and significant differences were assessed using Duncan's multiple range test at $P = 0.05$ using SPSS software version 13.0. Each experiment contained at least three replications with at least 10 explants per replications.

# RESULTS

## Shoot induction

After inoculation, yellowish-white buds turned green in the light. The shoots elongated and leaves expanded with the axillary bud sprouting. Contaminated buds could in fact sprout and differentiate, but died as the contamination aggravated. Some callus appeared at the base of buds. Although NAA, when added to the medium, benefited lateral bud differentiation, it also induced callus in 100% of explants (**Table 2, Fig. 1B**) and shoots grew poorly. $GA_3$ (1 mg $l^{-1}$) + BA (1 mg $l^{-1}$) stimulated the growth of shoots whose stems were sturdy and leaves were large (**Fig. 1A**).

## Shoot proliferation

The highest number of axillary shoots (3.9) was obtained on medium with 1 mg $l^{-1}$ $GA_3$ + 0.1 mg $l^{-1}$ TDZ (**Table 3**); however, TDZ had a negative effect on the growth of shoots, which gradually became abnormal. Leaves were twisted and the plants tended to become hyperhydric (**Fig. 1D**). Most of the axillary shoots induced by TDZ could not be used in subsequent culture and proliferation percentage was lowest. TDZ was better than BA for shoot proliferation (**Table 3**). The highest proliferation rate (2.3) and good quality (i.e., vigorous growth, strong stems, dark-green leaves) shoots were obtained on medium with 1 mg $l^{-1}$ $GA_3$ + 1 mg $l^{-1}$ BA (**Fig. 1C**); 1 mg $l^{-1}$ of BA was more effective than 0.2 mg $l^{-1}$ in terms of shoot proliferation. $GA_3$ had no significant effect on shoot multiplication.

## Rooting of shoots

The highest rooting percentage (50%) was obtained on medium with 0.5 mg $l^{-1}$ NAA (**Fig. 1E; Table 4**) in which the average root number was 2.3. Even though NAA induced callusing at the base of shoots, roots were not connected to the callus, only to the stems, i.e. adventitious roots. Other media induced lower rooting percentages and fewer roots. Roots could also be induced in auxin-free medium but, among all media, parameters were lowest in this case.

## Callus induction

Different explant types reacted differently on callus induction medium, although the result was similar for 'Da Fu Gui' (**Table 5**) and for 'Tao Hua Fei Xue' (**Table 6**): Stems were significantly the best explants to induce callus (**Fig.**

**Table 3** Effects of different media on proliferation of multiple shoots of herbaceous peony cv. 'Zhong Sheng Fen' (n = 10).

| **Treatment [a] (mg $l^{-1}$)** | **Number of axillary shoots/axillary bud** | **Proliferation ratio** |
|---|---|---|
| 1 $GA_3$ + 1 BA | 3.2 ab | 2.3 b |
| 1 $GA_3$ + 0.1 TDZ | 3.9 b | 1.3 a |
| 1 BA | 3.1 ab | 2.1 b |
| 0.2 BA | 2.8 a | 2.0 b |

[a] Treatment: plantlets with one axillary bud were inoculated on half-strength MS medium (double-strength $Ca^{2+}$) supplemented with different PGRs. BA, 6-benzyladenine; $GA_3$, gibberellic acid; NAA, 1-naphthyleneacetic acid; TDZ, thidiazuron. Proliferation ratio = number of normal axillary shoots which can be used for subculture / number of axillary shoots when cultured

Different letters within a column indicate significant differences according to Duncan's multiple range test ($P = 0.05$).

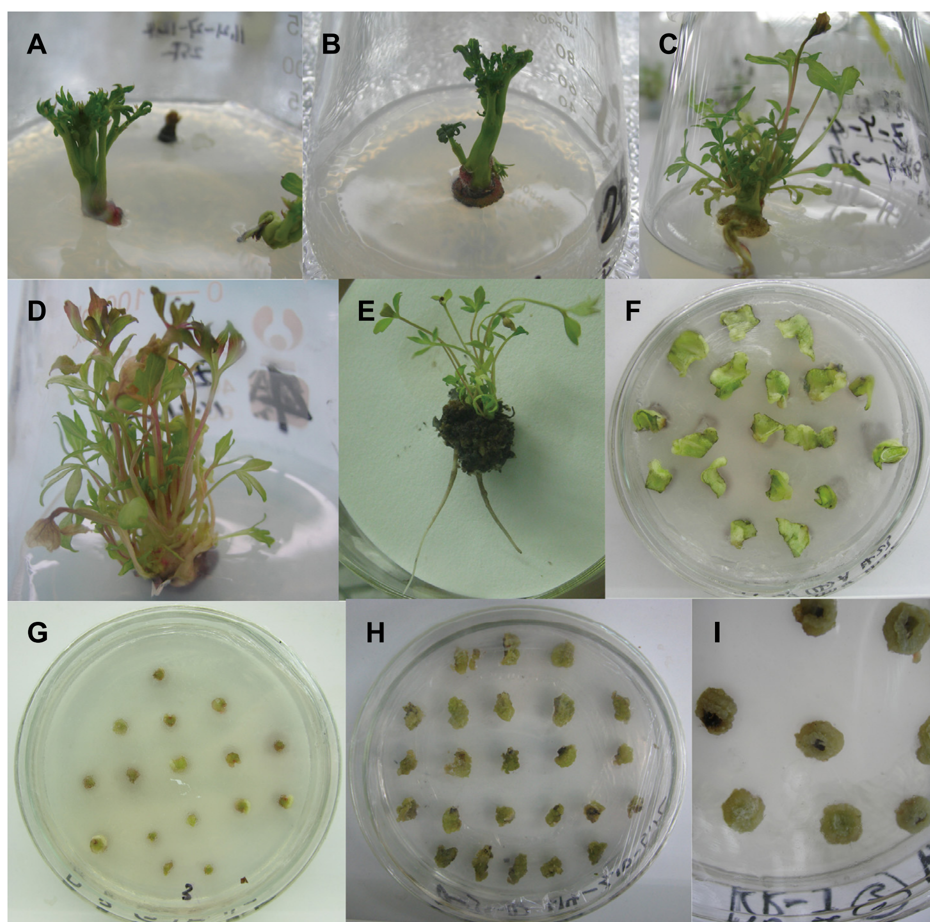


**Fig. 1 Two ways to rapidly propagate herbaceous peony.** (**A**) Underground buds cultured on 1/2 MS medium supplemented with 1 mg $l^{-1}$ $GA_3$ + 1 mg $l^{-1}$ BA; (**B**) Underground buds cultured on 1/2 MS medium supplemented with 1 mg $l^{-1}$ $GA_3$ + 1 mg $l^{-1}$ BA + 0.1 mg $l^{-1}$ NAA ('Zhong Sheng Fen'); (**C**) Shoots cultured on 1/2 MS medium supplemented with 1 mg $l^{-1}$ $GA_3$ + 1 mg $l^{-1}$ BA; (**D**) Shoots cultured on 1/2 MS medium supplemented with 1 mg $l^{-1}$ $GA_3$ + 0.1 mg $l^{-1}$TDZ ('Zhong Sheng Fen'); (**E**) A rooted plantlet of 'Zhong Sheng Fen' by two successive steps for rooting. The root induction medium was WPM medium supplemented with 0.5 mg $l^{-1}$ NAA; (**F**) Callus generated on leaves; (**G**) Callus generated on petioles. (**H, I**) Callus generated on stems.

**Table 4** Effects of different media on rooting of multiple shoots in herbaceous peony cv. 'Zhong Sheng Fen' (n = 10).

| **Treatment** [a] **(mg $l^{-1}$)** | **Number per treatment** | **Rooting frequency (%)** | **Average root number** | **Average root length (cm)** |
|---|---|---|---|---|
| Auxin-free medium | 15 | 13.3 a | 1.5 a | 4.5 a |
| 0.5 IBA | 14 | 28.6 ab | 1.3 a | 1.5 b |
| 1 NAA | 20 | 35.0 b | 2.0 a | 1.7 b |
| 0.5 NAA | 14 | 50.0 b | 2.3 a | 1.6 b |

a Treatment: shoots were inoculated on WPM medium supplemented with different PGRs for 10-15 days. They were then transferred to PGR-free WPM medium containing 0.2% activated charcoal. IBA, indole-3-butyric acid; NAA, 1-naphthyleneacetic acid; WPM medium, Woody Plant Medium.
Different letters within a column indicate significant differences according to Duncan's multiple range test ($P$ = 0.05).

**1H, 1I**), despite serious browning, followed by petioles (**Fig. 1G**) then leaves (**Fig. 1F**). The latter, however, showed less browning than stems and petioles. In the dark, callus was white or yellowish-white and friable. Stem segments generated callus within 1 or 2 days of culture. For all explant types, WPM medium was better than 1/2 MS medium, resulting in greater callus production and less browning while the addition of 2,4-D to medium improved callus growth for 'Da Fu Gui' (**Table 5**) and for 'Tao Hua Fei Xue' (**Table 6**).

## DISCUSSION

This paper studied two ways to rapidly propagate herbaceous peony: One was direct shoot induction; the other was indirect shoot induction via callus. It was more efficient to induce axillary shoots by direct shoot induction (Gabryszewska 2010; Yu *et al*. 2011), although parent plants can be injured when underground buds are sampled. In addition, underground buds are in close contact with soil bacteria thus making disinfection difficult. In the induction of axillary shoots, $GA_3$ played an important part in breaking dormancy, inducing and proliferating underground buds of herbaceous peony (Hosoki *et al*. 1989; Guo 2001). TDZ induces shoots more efficiently than BA (**Table 1**), although shoots induced on medium with TDZ are too frail for successive culture, making BA a better choice for auxiliary bud proliferation. Rooting and transplanting are the main obstacles for rapid propagation of herbaceous peony (Guo 2001; Jin *et al*. 2005; Wu *et al*. 2011). IBA and NAA were often used for rooting, the former being more effective than the latter (Hosoki *et al*. 1989; Guo 2001). However, the study indicated NAA was more effective than IBA. In addition, root was induced on auxin-free medium with the lowest rooting frequency but the longest root length (**Table 4**).

Stem explants were optimum to induce callus in both cultivars while lea explants performed poorly, although, in terms of browning, leaf explants showed the least browning among all explants (**Tables 5** and **6**, respectively). This response was cultivar-independent. 1/2 MS medium has been the most commonly used basal medium in the micropropa-

**Table 5** Effects of different explants and medium on callus induction of herbaceous peony cv. 'Da Fu Gui' (n = 10).

| Explants | Treatment [a] | Sum total | Contamination (%) | Browning (%) | Callus induction (%) | Survival (%) |
|---|---|---|---|---|---|---|
| Stem | (1) | 78 | 8.97 b | 100 d | 88.46 c | 89.74 b |
| | (2) | 80 | 0 a | 100 d | 97.50 c | 97.50 b |
| | (3) | 77 | 0 a | 100 d | 100 c | 100 b |
| | (4) | 89 | 0 a | 100 d | 100 c | 100 b |
| Petiole | (1) | 76 | 0 a | 69.74 bc | 82.89 c | 93.42 b |
| | (2) | 80 | 0 a | 81.01 cd | 60.76 b | 67.09 a |
| | (3) | 77 | 0 a | 61.84 bc | 94.74 c | 96.05 b |
| | (4) | 89 | 0 a | 48.51 b | 88.12 c | 95.05 b |
| Leaf | (1) | 77 | 0 a | 25.97 a | 35.06 a | 94.81 b |
| | (2) | 76 | 0 a | 22.37 a | 18.42 a | 100 b |
| | (3) | 81 | 0 a | 20.99 a | 23.46 a | 100 b |
| | (4) | 78 | 0 a | 14.10 a | 20.51 a | 100 b |

[a] Treatment: explants were inoculated on medium containing (1) half-strength MS (double-strength $Ca^{2+}$) + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA + 0.2 mg $l^{-1}$ 2,4-D; (2) half-strength MS (double-strength $Ca^{2+}$) + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA; (3) WPM + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA + 0.2 mg $l^{-1}$ 2,4-D; (4) WPM + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA. NAA, 1-naphthyleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; BA, 6-benzyladenine; WPM medium, Woody Plant Medium.
Different letters within a column indicate significant differences according to Duncan's multiple range test ($P$ = 0.05).

**Table 6** Effects of different explants and medium on callus induction of herbaceous peony cv. 'Tao Hua Fei Xue' (n = 10).

| Explants | Treatment | Sum total | Contamination (%) | Browning (%) | Callus induction (%) | Survival (%) |
|---|---|---|---|---|---|---|
| Stem | (1) | 77 | 0 a | 100 c | 92.21 d | 96.10 c |
| | (2) | 75 | 5.33 ab | 100 c | 72.00 cd | 90.67 bc |
| | (3) | 75 | 10.67 b | 100 c | 86.67 d | 86.67 b |
| | (4) | 76 | 3.95 a | 100 c | 89.74 d | 100 c |
| Petiole | (1) | 77 | 1.30 a | 98.70 c | 83.12 d | 97.40 c |
| | (2) | 75 | 0 a | 100 c | 49.33 abc | 70.67 a |
| | (3) | 75 | 0 a | 100 c | 81.33 d | 100 c |
| | (4) | 78 | 0 a | 96.15 c | 85.90 d | 96.15 c |
| Leaf | (1) | 75 | 0 a | 18.67 a | 40.00 ab | 100 c |
| | (2) | 76 | 3.95 a | 15.79 a | 27.63 a | 100 c |
| | (3) | 76 | 0 a | 28.95 b | 69.74 cd | 100 c |
| | (4) | 76 | 0 a | 14.47 a | 53.95 bc | 100 c |

[a] Treatment: explants were inoculated on medium containing (1) half-strength MS (double-strength $Ca^{2+}$) + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA + 0.2 mg $l^{-1}$ 2,4-D; (2) half-strength MS (double-strength $Ca^{2+}$) + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA; (3) WPM + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA + 0.2 mg $l^{-1}$ 2,4-D; (4) WPM + 2 mg $l^{-1}$ BA + 0.2 mg $l^{-1}$ NAA. NAA, 1-naphthyleneacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; BA, 6-benzyladenine; WPM medium, Woody Plant Medium.
Different letters within a column indicate significant differences according to Duncan's multiple range test ($P$ = 0.05).

gation of herbaceous peony (Tian *et al*. 2010; Wang *et al*. 2010). In this study, WPM medium was superior to 1/2 MS medium because of its higher callus induction percentage and lower levels of browning. This response was also cultivar-independent although 'Da Fu Gui' and 'Tao Hua Fei Xue' showed different callus induction abilities, morphology and color of callus (**Tables 5** and **6**, respectively). Regeneration via adventitious buds has been shown to be very difficult for herbaceous peony (Orlikowska *et al*. 1998; Guo 2001; Zhang *et al*. 2007; see a comparison of studies in **Table 1**). According to a recent study, only callus from hypocotyls succeeded in producing adventitious buds, although with a subsequently low regeneration rate (7.95%) (Wang *et al*. 2010).

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