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Micropropagation of Dwarf Tree Peony from Lateral Buds

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Abstract: Tree peony is known as china's national flower which is very beautiful, graceful and looked as the best of all the flowers. Tree peony Var. *spontanea Rehd* was micropropagated by using lateral buds as explants, the effect of pruned and cut method on developing adventitious shoots was investigated, a regeneration system which is the first report was developed for dwarf peony from a lateral bud. A good disinfection protocol for lateral buds was as follows: Disinfecting with 75% alcohol for 30 sec and then soaking with 0.1% $HgCl_2$ for 7 min adventitious shoots regenerated from lateral buds with different pruned and cut treatments and the control, the highest mean number of shoots (5.0) was obtained on pruned and cut wound. A high-frequency multiplication rate was achieved on woody plant medium supplemented with BA 3.0 mg L^{-1} and IAA 0.2 mg L^{-1} multiple shoots were formed within 5 weeks; adventious shoots were efficiently rooted (75.62%) on half-strength woody plant medium with NAA 1.0 and IBA 4.0 mg L^{-1} and rooted plantlets survived after acclimatization to the greenhouse.

Key words: Micropropagation, pruned and cut, adventitious shoots, multiplication, rooting

INTRODUCTION

Dwarf tree peony (*Paeonia suffruticosa*, Andr. Var. *spontanea Rehd*) is a endangered plant and an important germplasm resource. Dwarf tree peony belong to the family Paeoniaceae which are world-famous flowers (Wang, 1997). Paeoniaceous plants have been used as an important source of crude drugs in traditional Chinese medicine and have been prescribed for women's diseases, The peony with its extraordinary beauty is known as the queen of all flowers which is always held in esteem by people. There is an increased demand for high quality peony and increased intrest in establishing plantations with improved peony genotypes. Clonal propagation or genetically improved genotypes will help to increase the establishment of high-quality plantations. The traditional propagation of peonies was mainly by grafting, this causes many problems such as irregularity, low propagation rates and so on. The micropropagation obstacles for peonies are as follows: Tissue browning (Barzilay *et al*., 2002; Tan and Dai, 1997; Beruto *et al*., 2004; Jia *et al*., 2006) and lower induction rates of adventitious shoots (Jia and Liu, 2009). Although, successes have been reported in clonal propagation of tree peony through axillary bud culture and embryo culture (Beruto *et al*., 2004; Jia *et al*., 2006; Liu *et al*., 2009; Brukhin and Batygina, 1994), these *in vitro* techniques have not been used in actual production. The objective of this study was to establish a regeneration protocol for dwarf peony from lateral buds through the pruned and cut method. We also evaluated the effect of BA, NAA, IBA and IAA on micropropagation of dwarf tree peony. A highly efficient and reproducible plant regeneration procedure for dwarf tree peonies was developed.

MATERIALS AND METHODS

Explant sources: *Paeonia suffruticosa* Andr. Var. *spontanea Rehd* was used in our experiments. The lateral buds were obtained in spring from the 6 year old stock plants which grew in TaiHang mountain in the north china.

Effects of the pruned and cut method on adventitious shoots: The explants were surface disinfested in 75% ethanol for 30 sec, then in $HgCl_2$ solution (0.2% w/v) for 5, 7 and 9 min, respectively to obtain the suitable sterilization time, followed by 6 rinses with sterile double distilled water, the explants were placed in glass jars containing 40 mL of WPM medium. The contamination rate of inoculated shoots were investigated after 15 days culture.

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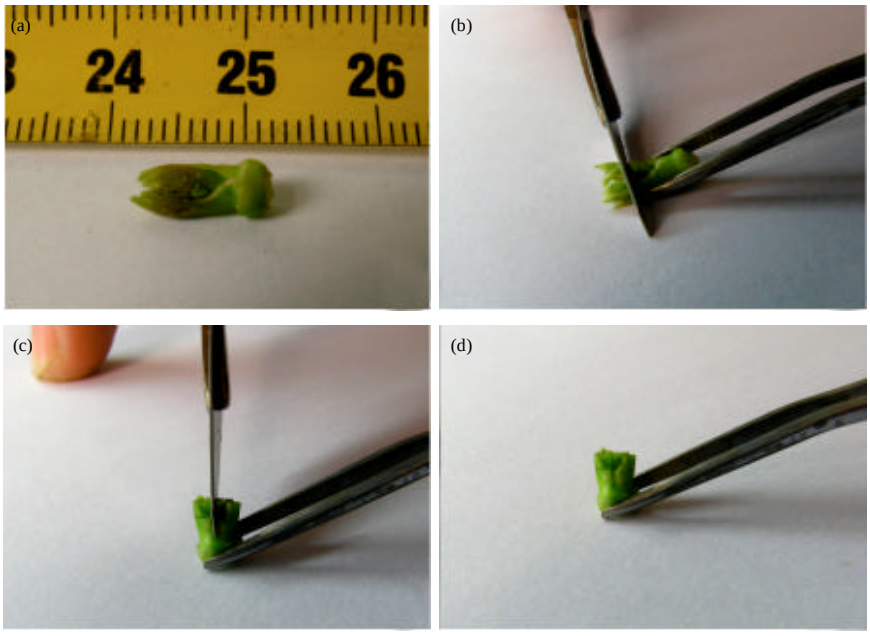

Fig. 1(a-d): Pruning and cutting of adventitious shoots (a) Lateral buds, (b) Pruned (c) Cut in the twig axil and (d) Wounded edges

Table 1: Effects of plant growth regulators on the adventitious root induction

Growth regulators			
IBA	NAA	Mean No. of roots	Rooting rate (%)
2.0	1.0	3.40±0.50^{c}	60.25±0.50^{c}
3.0	1.0	2.90±0.25^{d}	65.30±0.50^{b}
4.0	1.0	4.65±0.50^{a}	75.62±0.25^{a}
5.0	1.0	3.40±0.75^{c}	62.49±0.50^{c}
2.0	2.0	2.40±0.25^{e}	51.15±0.25^{d}
2.0	3.0	3.65±0.50^{b}	52.94±0.50^{d}

Duncan's test ($p = 0.010$), different letters indicate significant differences between means

Once subjected to the best disinfection treatment, the effect of five explants on adventitious shoot induction were evaluated: (I1): Pruned 2/3 of buds, cut in the twig axil; (I2) Pruned 1/2 of buds from the base of buds, cut in the twig axil; (I3) Pruned 1/3 of buds, cut in the twig axil; (I4) Cut in the twig axil; (I5) Control: lateral bud (Fig. 1). These explants were respectively inoculated in WPM (Woody Plant Medium of Lloyd and McCown, 1980; Razdan, 2006) supplemented with BA 2.5 mg L^{-1} and IAA 0.2 mg L^{-1}, the experiment was conducted three times (total of 100 explants per treatment. The adventitious-shoot induction rate and the number of adventitious shoots (size ≥ 0.5 cm) were recorded after 35 days of culture.

Effects of plant growth regulators on multiplication: The elongating shoots were cut into nodal sections and cultured on WPM supplemented with different growth regulators (Table 1). Culture room conditions were: Photoperiod, 14 h day^{-1}; light intensity, 2500 lux; room temperature, 20±1°C. Multiplication rate and multiplication number among the sixteen treatments was recorded after culture of 35 days.

Effects of plant growth regulators on the adventitious root induction: The shoots of 1.5-3 cm were cultured on half-strength WPM supplemented with different growth regulators (Fig. 2-4). The experiment was conducted three replicates. Half of the bottle was covered by black paper. Culture room conditions were: Photoperiod, 14 h day^{-1} light intensity 2500 Lux; room temperature, 25±1°C during the day and 16±1°C in the night. Number of available roots (length≥0.2 cm) per shoot and rooting rates were recorded after 45 days of culture.

The rooted plantlets were transplanted to the pots filled with peat and vermiculite (1:1, v/v) after being maintained in the closed bottles under increasing light intensity (1000-15000 lux) for 20 days and later on in opened bottles for 10 days. The acclimatization period after transplanting is 30 days and during this period excessive humidity accumulation in the vessel should be avoided by removing the polyethylene cover and progressively increasing the gas exchange.

Statistical analysis: The results of the observations were evaluated by analysis of variance. The means that differed significantly were identified using Duncan's multiple test at the significance level of $p \leq 0.01$.

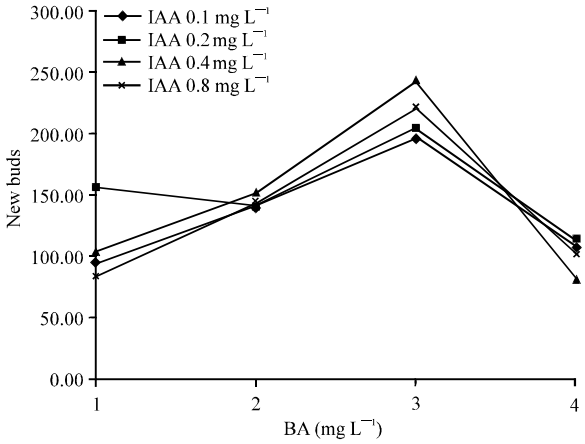

Fig. 2: Effects of plant growth regulators on adventitious shoots

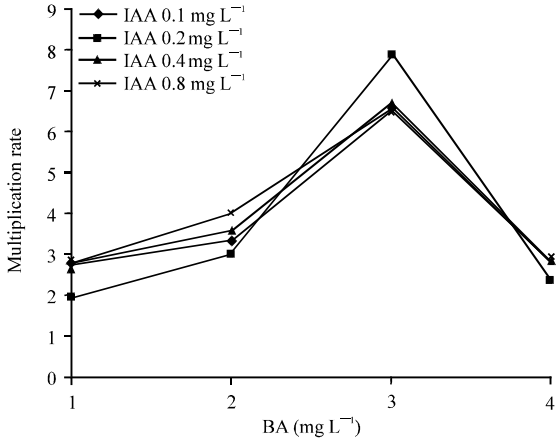

Fig. 3: Effects of plant growth regulators on multiplication rate

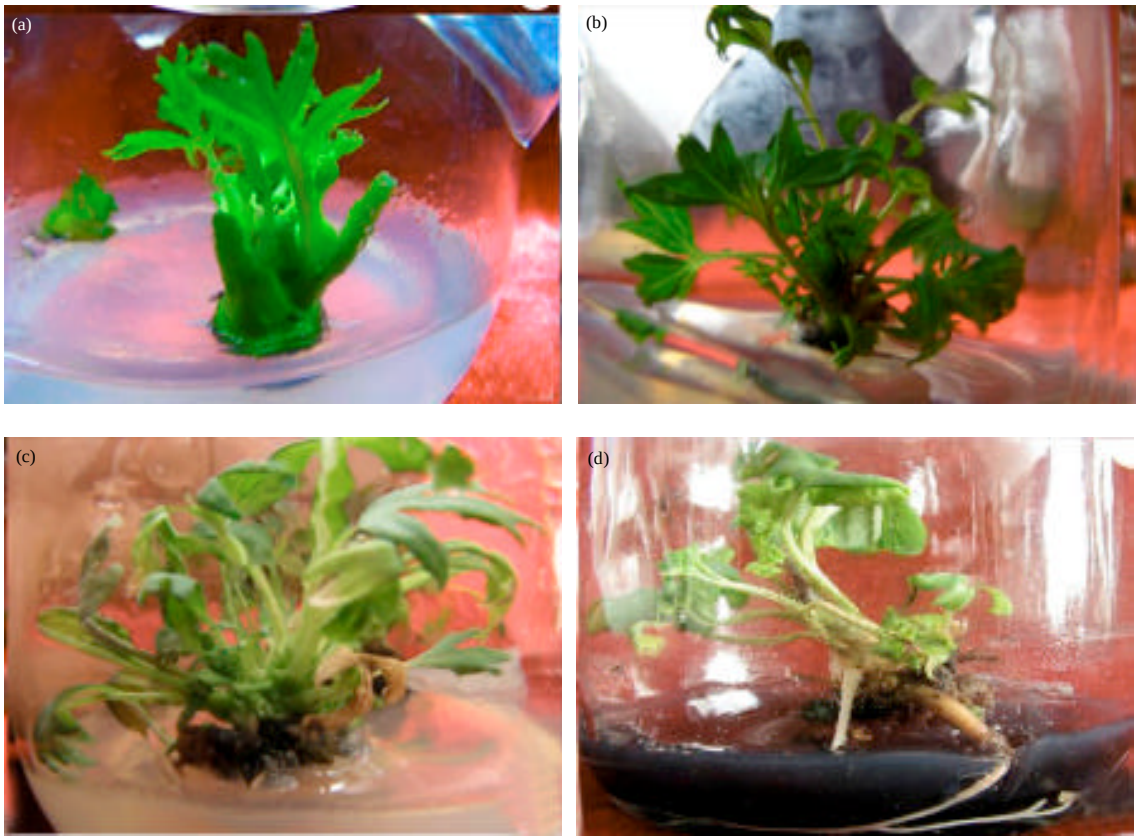

Fig. 4(a-d): Adventitious shoots (a) From I1 treatment, (b-c) Multiplication and (d) Rooting of adventitious shoots

RESULTS

Disinfection treatment: The contamination rates were significantly lower by soaking with 0.1% $HgCl_2$ for 7 min than for 5 min, 26% explants turned brown and died when the soaking time increased to 9 min, Therefore, a disinfection with 75% alcohol for 30 Sec and then a soak in a $HgCl_2$ solution (0.1%) for 7 min was an effective disinfection protocol.

Effects of different pruned and cut methods on adventitious shoots: The lateral buds began to grow after 5 days culture and changed from brown green to yellow green in color. Adventitious shoots and callus appeared mainly on the wounded after 25 days culture.

There were notable differences of induction number and number of shoots per explant between five treatments (Table 2). Adventitious shoots began to develop after 15 days culture, shoots developed much faster from the wounded than from other parts.

The induction rate of adventitious shoots was only 20% on the control (I5) and the mean number of shoots per explant was only 3.05. The highest induction rate of shoots (13.50) and the highest regeneration number (13.50) occurred on the I1 treatment which is 4.7-4.8 times higher than on the control. Adventitious shoots developed mainly on the wounded edges, this indicated the pruned and cut method treatment can significantly increased induction rate and induction number of shoots per explant. The pruned and cut method was the most important factor for adventitious shoot regeneration, as fewer adventitious shoot on the control without the pruned and cut treatment, Regeneration efficiency increased when lateral buds were cut and pruned.

This experiment indicated that adventitious shoots mainly appeared from wounded edges of basal segment of branches (43.25%) (Fig. 4a).

Effects of different medium on multiplication of adventitious shoots: Plant growth regulators in medium and have been recognized as important factors controlling the *in vitro* multiplication of tree peony. The experiments showed that adventitious shoots formed from two ways: (1) From calli and (2) Mainly from wounded edges.

Table 2: Effects of different pruned and cut treatments on adventitious shoots

Treatments	Mean No. of adventitious shoots per explant	Induction rate
I1	13.50±0.21^a	94.50^a
I2	10.50±1.01^b	80.00^b
I3	9.50±0.69^b	78.00^b
I4	4.24±0.54^c	52.00^c
I5 (Control)	3.05±0.38^d	40.00^c

Duncan's test ($p = 0.010$), different letters indicate significant differences between means

The experiments showed both BA and IAA had a significant effect on adventitious shoots. BA at low concentration (1 mg L^{-1}) promoted shoot elongation but was ineffective in promoting shoot regeneration. The influence of BA on regeneration rate also showed a quadratic regression, this can be explained as in a range of BA concentration (<3 mg L^{-1}), regeneration rate increased as BA increased. Higher concentration of BA were tested for one replication, however, adventitious shoots formed as a cluster and it took a much longer time for the cluster to develop into individual shoots (data not shown). IAA also had a significant effect on regeneration rate; the optimal concentration of IAA for shoot regeneration was between 0.1 and 0.4 mg L^{-1} (Fig. 2-3). A satisfactory multiplication was achieved when BA 3 mg L^{-1} was in combination with IAA 0.2 mg L^{-1} (Fig. 4b-c), there was interaction between BA and IAA for regeneration rate.

Effects of plant growth regulators on the adventitious root induction: Roots appeared as early as 15 days after being cultured in the media, most roots developed 15-30 days after being cultured in the media, IBA concentration had a significant effect on rooting efficiency for the shoots, Root percentage, mean root number and mean root length decreased with increasing IBA (<4.0 mg L^{-1}), root induction was not enhanced as the IBA concentration (>4.0 mg L^{-1}) increased. The shoots responded best with the rooting percentages of 75.62% and 4.65 roots per shoots at NAA 1.0 mg L^{-1} and IBA 4.0 mg L^{-1} (Fig. 4d and Table 2).

Rooted plantlets were transplanted into plastic pots containing autoclaved peat and vermiculite (1/1, v/v) for further aseptic growth in the green house, after 60 days, the surviving plants (about 90%) were planted into the field.

DISCUSSION

The number of adventitious shoots induced by the pruned and cut method is higher than by not-treated buds (Jia and Liu, 2009). In our experiments, similar results were obtained; the pruned and cut method was more efficient in promoting shoots regeneration form lateral buds. The shoot regeneration frequency was about 94.50 when the pruned and cut method was used, as compared to 40% for the control. Furthermore, the pruned and cut method initiated the formation of compact and nodular calli on the cutting edge and the surface of the lateral buds.

Plant growth regulators are important factors which can selectively influence the trigger differentiation of cells in culture (Bouza *et al*., 1994; Albers and Kunneman, 1992). BA and IAA are efficient growth regulators for shoot regeneration from calli, stem and embryo of peony.

The number of adventitious shoots induced by BA combined with IAA is more than by BA alone (Gildow and Mitchell, 1977; Linsmaier and Skoog, 1965). In this experiment, similar results were obtained, a IAA:BA ratio of 0.2:3.0 was effective to shoot multiplication; this results agree with the previously reported by Bouza *et al*. (1994) and by Wang and Van Staden (2001).

Many woody plants developed adventitious roots readily (Alvarez *et al*., 1989) and tree peony (*Paeonia suffruticosa* Andr.) is one of them. This experiment has achieved good results, the rooting percentages of shoots was about 80% on half-strength WPM medium supplemented with NAA 1.0 mg L^{-1} and IBA 4.0 mg L^{-1}.

CONCLUSION

The results from the current study have confirmed that the pruned and cut treatment is effective to develop adventitious shoots from the lateral buds, the pruned and cut treatment was found to be superior to the control for developing adventitious shoots. In addition, the result showed BA is important to multiplication of adventitious shoots .The present study, conducted over two years, indicated that dwarf tree peony is not yet routinely micro propagated. However, the information given here will be valuable for future study.

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