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In vitro and *in vivo* Propagation of Green Perilla (*Perilla frutescens* L. Britton *var. crispa*) Plants: A Comparative Study

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ABSTRACT: An effective technique for rapid clonal micropropagation of Green perilla (*Perilla frutescens* L. Britton *var. crispa*, Lamaiaceae) was established. High percentage of seed stratification germination (95%) was recorded on Murashige and Skoog (MS) medium with 0.7% agar and 3.0% sucrose after one weekof culture. The shoots were cultured on MS medium with 6-Benzylaminopurine (BAP) and among the three auxin supplements: Indole-3-butyric acid (IBA); α-Naphthalene acetic acid (NAA) or Indole-3-acetic acid (IAA) at concentration 0.1 mg l^{-1} after four weeks of culture. The maximum number of shoots per explant (4.4) and shoot length (2.9 cm) gave MS medium with 1.0 mg l^{-1}BAP and 0.1 mg l^{-1} IAA. The best plant rooting was achieved on half-strength MS medium with 0.5 mg l^{-1} IBA after three weeks of culture. The multiple plants were successfully *ex vitro* adapted with 95% survival after two months of *ex vitro* conditions. Conventionally propagated plants by seeds was implemented, the persentage of seed germination without stratification was 75%, while that of stratified seeds was higher and reached 100% after one week of sowing.Some morphological characteristics as plant height, branches number per plant, leaves number perplant, leaf size, flowers number on main, flowers number per plant and seeds were measured after five months of field conditions. Different morphological features between *ex vitro* and *in vivo* propaged plants were not established. This is the first comparative study of micropropagated and *in vivo* seed derived perilla plants. The described protocol allows the establishment of numerous micropropagated of *P. frutescens* plants.

Keywords: *In vitro* and *in vivo* propagation; Morphometric analysis; Plant growth regulators

INTRODUCTION

Green perilla (*Perilla frutescens* L. Britton *var. crispa*, Lamaiaceae) is an annual medicinal herb adapted to warm and humid climates in China and Japan. This plant is an important oil seed crop and is widely cultivated in Asia (Pandey and Bhatt, 2008). It typically grows to 1.0 m in height, and contains small flowers and brown fruits. Seed color ranges from black to white (Brenner, 1993). The seeds of perilla have been used for food forbirds or humans and theleaves are used as a potherb for medicine or food coloring (Nitta *et al*., 2005; Hossain *et al*., 2010). Perilla seeds are rich in dietary fiber and dietary minerals such as calcium, iron, niacin, protein, and thiamine. Perilla leaves are also rich in dietary fiber and dietary minerals, such as calcium, iron, potassium and vitamins A, C and riboflavin. The amount of caffeic acid, ferulic acid, rosmarinic acid, quercetin and apigenin of perilla plants determined by high-performance liquid chromatography (HPLC), was 4.80, 5.10, 2.95, 6.46, and 3.93 mg/g DW, respectively (Ishikura, 1991). Perilla seeds contained 30-40% fixed oil, contained both saturated (palmitic acid 5-7%, stearic acid 1-3%), unsaturated or polyunsaturated (oleic acid 12-22%, linoleic acid 13-20%, γ-linolenic acid 0-1%, α-linolenic acid 52-64%, icosanoic acid 0-1%) fatty acids (Asif, 2011). It has been reported to have several positive health benefits effects, such as antitumor, antiinflammatory, hypotension and antiatherosclerosis activities (Okuyama *et al*., 2007; Jung *et al*., 2012; Sirilun *et al.,* 2016). The dried leaves of perilla contained more anticancer flavonoids than fresh leaves (Kagawa *et al.,* 2019). Yamazaki *et al.* (2003) used a LC-MS methodology to investigate metabolic differences in polar extracts of two red and green colored forms of *P. fructescens*. Striking difference in anthocyanin content was observed between the red and green forms. Anthocyanin was highly accumulated in the leaves of the red form but not in the green form (Yamazaki *et al*., 2003). In pigmented red form, leaves and stems are deep red-purple,for seed and oil production, but in

nonpigmented green form, leaves and stems are green, often used for traditional medicine (Yu *et al*., 1997; Nitta *et al*., 2005). The addition of silicone A to a culture in a stirred bioreactor produced a three-fold higher growth rate and a seven-fold increase in anthocyanin compared with surfactant-free cultures of *P. frutescens* (Zhong *et al*., 1992).

Plant micropropagation is one of the techniques to obtain large number of plants with constant biosubstances, irrespective of season. Organogenetic buds were induced from hypocotyl and cotyledon explants of oil crop *P. frutescens* in MS medium supplemented with 5.7 µM IAA and 8.9-3.3 µ MBA. Shoots were rooted on MS medium with 2.9 µM IAA and 1.4 µM gibberellic acid (GA_3) and the regenerated plants flowered and set seeds normally(Hou and Jia, 2005). A rapid plant regeneration system for *P. frutescens* was established from cotyledon and hypocotyl explants. A maximum of 91.06% cotyledon and 76.4% hypocotyl explants could directly produce shoots (3.09 shoots per explants) on MS medium with BA and IAA (Zhang *et al.,* 2005). Zhang (2007a) reported in the regenerated shoots, BAP with ammonium nitrate promoted such flowering and fruiting from cotyledon and hypocotyl explants of *P. frutescens* and offers a model system for studying the physiological mechanism of flowering of plants. Cryopreservation of *in vitro* grown shoot tips using the new V-Cryo-plate method has many advantages and may facilitate the cryo-storage of perilla (Matsumoto *et al.,* 2014). Some of *P. frutescens* species are at the verge of extinction in the Central Himalaya (Negi *et al*., 2011). Pandey *et al.,* 2009 reported that inoculated on MS medium supplemented with Kin (0.75mg/l) and NAA (0.5mg/l) which resulted in maximum proliferation of Lilium. These were subcultured for further multiplication on MS medium fortified with BAP(0.75mg/l) and NAA (0.5 mg/l) which resulted in maximum number of 7.2 bulblets per scale explant. The best rooting of Lilium 100% was obtained on MS (full-strength) medium fortified with NAA(1.0 mg/l). The significance of media × hormone interaction suggests that the effects of hormone concentration are influenced by media for all traits except number of node of *Stevia rebaudiana* Bertoni (Namdari *et al*., 2015). The soots formation, multiplication, and rooting under *in vitro* conditions can be improved if appropriate culture medium and precise levels of sucrose, 2ip, and NAA of *Phoenix dactylifera* were selected (Jazinizadeh *et al*., 2015). The addition of the ethylene inhibitor $AgNO_3$ to the best regeneration culture medium (MS + 4.40 µM BAP + 0.537 µM NAA) helped in good auxiliary shoot proliferation and elongation, as well as low callus formation of *Clitoria ternatea* (Mishra *et al.,* 2019). Maximum callus weight belongs to 0.25 mg/l Kinetin and 2 mg/l NAA treatment. Therefore, hormones amount which used can induced callus in *Taxus baccata* (Mahdinejad *et al*., 2015). Somaclonal variation for morphological traits was studied in both control and mutagen (chemical and physical) treated progenies of two varieties of *Solanum viarum.* The qualitative variations observed were less spine plants, dwarf plants, sterile plant types and complete pollen sterility (Kumar and Tejavathi, 2011). In its natural state, the yield of perilla leaves and seeds is not high. Biotechnological tools are important to conserve the critical genotypes of medicinal plants like perilla species. There are too few publications on the *in vitro* regeneration and micropropagation of *P. frutescens* (Hou and Jia 2005). The aim of the present study was focused on the development of efficient protocol for micropropagation of *P. frutescens* plants and the comparison of *ex vitro* and *in vivo* propagation.

MATERIALS AND METHODS

A. In vitro seed germination, shoot micropropagation, rooting and ex vitro acclimatization

The material, seeds of *P. frutescens* was obtained from Magic Garden Seeds, Germany and implemented by the Department of Plant-Soil Interactions, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences. The seeds were stratified at 4°C for two months. The mature seeds were washed under running water, surface sterilized with 70% (w/v) ethanol for one minute and agitated in 15% bleach solution (ACE Procter & Gamble Co., USA) for 15 min. Then they were washed three times each for 15 min in sterilized distilled water to remove bleach. The disinfected seeds were germinated on MS medium supplemented with 3.0% sucrose and 7.0% agar. The percentage of germination was determinated after one week of culture. Initial explants were taken from one week old seedlings for further *in vitro* cultivation. The micropropagation, growth and development of shoots were performed in full strength MS medium with vitamins, containing of the cytokinin: BAP at concentrations (0.5, 1.0, 1.5 and 2.0 mg l^{-1}) alone and combined with IBA, NAA or IAA at concentration 0.1 mg l^{-1} (Table 1). The explants were placed in the culture vessels (140 × 20 mm), one explant per10 ml medium. The percent of formed shoots, average number of shoots per explant and shoots length were measured after four weeks of culture. Each combination contained of two replicates with 10 seeds and was repeated three times.

For root induction, well developed shoots were cultivated on half strength MS medium with 2.0% sucrose, 7.0% agar and auxin IBA at concentrations 0; 0.1; 0.5 and 1.0 mg l^{-1}. As control ½ MS medium was used. The auxins were applied separately as described in Table 3. For each induction medium, shoots were cultured in tubes containing 10 ml medium. Data were recorded on percentage rooting, mean number of

roots per plant, and root length after four weeks of culture. Each treatment with three replicates. All the media were adjusted to pH 5.8 using NaOH or HCl and autoclaved at 121°C for 20 min at a pressure of 1.1 kg cm^{-2}. The chemicals used for preparing the media were purchased from Duchefa Biochemie B.V., the Netherlands. The *in vitro* cultures were maintained under growth room conditions at a temperature of 22±2ºC, relative humidity of 70% and a 16 hphotoperiod under 40 µmol m^{-2} s^{-1} illumination provided by Philips 36 W cool white fluorescent tubes.

The next phase was the adaptation of the plants from *in vitro* to *ex vitro* growth conditions. They were then transplanted under controlled conditions to plastic pots (6 cm diameter, 8 cm height) containing two combinations of soil mixtures: Mix 1 –soil and perlite (2:1, v/v) and Mix 2 – soil: sand: perlite (2:1:1, v/v/v). The potted plants were covered with a transparent polythene membrane to ensure high humidity (90%). The polythene membrane was opened after two weeks. The plants were grown in a culture room at a temperature of 24±1°C under 16 h illuminations (50 µmol $m^{-2}s^{-1}$ light intensity). Twenty plants were transplanted in each potting mixture. After two months, the percent of survived plants, plant height and number of leaves were measured. The fully acclimatized plants were transferred to the experimental field for further growth and development after two months of *ex vitro* adaptation.

B. Conventionally propagated plants by seeds

Perilla seeds were exposed to stratification temperature 5°C for two month in refrigerator. The *P. frutescens* seed was sown in small pots (7 cm diameter, 9 cm height) containing soil for germination (5 seeds per pot, 10 pots per treatment) at the beginning of February. Initially many seeds were planted and many seedlings sprouted. The seed germination percentage was determinated after one week of planting. The length of seedlings and leaf number were measured after three weeks. The pots were placed under 50 µmol m^{-2} s^{-1} illumination provided by 40-W cool-white fluorescent lamps in growth chamber at 24±1°C with a 16-h photoperiod. The pots were placed under the same *ex vitro* conditions. The seedlings were ready for transplantation, at the end of April. The plants were transplanted in the experimental field at a distance of 50 cm and plant spacing in the order of 30 cm. In total 10 plants from micropropagation and 10 plants from seedling propagation were measured. Both *ex vitro* and *in vivo* plants in the field, morphological characters like plant height, branches number per plant, leaves number perplant, leaf length and width, flowers number on main, flowers number per plant and seeds were measured after five months of field conditions. Collection of seeds was performed during the end of September from both*ex vitro* propagated and traditionally cultivated plants. The *ex vitro* and *in vivo* propagated plants were planted at the experiment field of the Institute of Plant Physiology and Genetics, Sofia, Bulgarian Academy of Sciences (42°50´N, 23°00´E, and altitude of 595 m). The soil type at Sofia, classified according tophysical characteristics was sandy loam (0-15 cm, top soil). Each trial was laid out in a randomized block design with two replications. The *ex vitro* and *in vivo* plants were planting byhand on 1.5 m × 1.2 m plots. In the temperate climate of Bulgaria, the planting were performed in early spring, the beginning of April.

C. Morphometric analysis

Ten selected plants from *ex vitro* and *in vivo* origin from each plot were tagged randomly for data recording. The *ex vitro* and *in vivo* derived plants of the same age from mid-August and the seeds from end of September were evaluated and comparedon the basis of selected morphometric parameters (Table 5).

D. Statistical analysis

All data were subjected to one-way ANOVA analysis of variance for comparison of meansand significant differences were calculated according to Fisher's least significance difference (LSD) test at the 5% significance level using a statistical software package (Statgraphics Plus, version 5.1 for Windows). Data were presented as means ± standard error.

RESULTS AND DISCUSSION

A. In vitro seed germination, shoot multiplication, rooting and ex vitro acclimatization

***In vitro* seed germination stage.** Preliminary our study has shown that *P. frutescens* plant successfully grown in Bulgaria. The maximum aseptic cultures (100%) were obtained when the seeds were immersed in 70% (w/v) ethanol for one minute and agitated in 15% bleach solution for 15 min. After sterilization, the dry cold stratification of the seeds germinated aseptically on MS medium without growth regulators. Due to the successful surface sterilization no bacterial and fungal contamination of the *in vitro* cultured seedlings of *P. frutescens* was observed. Seed germination was found effective on MS medium with germination rate from 95% after dry cold stratified seeds (Table 1, Fig. 1A). The seed germination with gibberellic acid of *P. frutescens* was improved (Vadivel *et al.,* 1981). The germination rates of perilla were fairly good (65–70%) for 5–8 years after harvest, stored at 4°C under dry conditions (Masumoto and Ito 2010). The effect of salt stress onseed germination of *P. frutescens*, seedling growth, root activity, contents of soluble sugar, proline, and malondialdehyde (MDA) and peroxidase (POD) enzyme activitywas investigated (Zhang *et al.,* 2012).

Table 1: Effect of plant growth regulators (PGRs) on shoot multiplication of *P. frutescens* shoots after four weeks of culture.

PGRs (mg l^{-1})	Shoot formation (%)	Number of shoots explant^{-1}	Shoot height (cm)
BAP			
0.5	80	2.7±0.23^{a}	1.2±0.10^{a}
1.0	90	3.5±0.26^{b}	2.3±0.17^{c}
1.5	85	2.4±0.21^{d}	1.5±0.12^{c}
2.0	80	1.8±0.15^{c}	1.6±0.14^{c}
BAP + IBA			
1.0 + 0.5	85	2.6±0.22^{e}	2.1±0.19^{d}
BAP + NAA			
1.0 + 0.5	80	3.1±0.24^{d}	1.8±0.16^{c}
BAP + IAA			
1.0 + 0.5	95	4.3±0.37^{c}	2.5±0.22^{d}

The data are presented as means of 10 plants per treatment ± standard error.Different letters indicate significant differences assessed by Fisher LSD test (5%) after performing ANOVA multifactor analysis.

Shoot multiplication stage. The efficacy of propagation depending on the explant and the type and concentration of used plant growth regulators and showed the highest shoot multiplication capacity. The data on shoot formation of tip explants isolated from seedling cultured on MS medium supplemented with different concentrations of BAP alone or BAP and auxin, are presented in Table 1. Various concentrations of BAP and auxins gave rise to adventitious shoots with different efficiencies. The number of induced shoots per explant varied depending onthe type and concentration of plant growth regulators. The maximum number of shoots per explant (4.3 with 2.5 cm average height) gave MS medium supplemented with 1.0 mg l^{-1} BAP and 0.1 mg l^{-1}IAA (Fig. 1B and 1C) followed by MS medium with 1.0 mg l^{-1}BAP (3.1 shoots per explant with 2.4 cm plant height) after four weeks of culture. The new shoots induced on MS medium, containing BAP/IAA in these concentrations were characterized by normal leaves and average shoot height. The cytokinin BAP and auxins positively influenced the plant growth and development and stimulated shoot formation.

In previous reports, plant regeneration of perilla was obtained from hypocotyl segment including cotyledon and hypocotyl (Zhang *et al*., 2005), apical bud (Hossain *et al.,* 2010), node culture (Matsumoto *et al*., 2014). Hossain *et al.* (2010) reported that higher concentration BA increased the shoots regeneration of *P. frutescens*. A cytokinin 0.5 mg/l BAP and auxin 1.0 mg/l IAA in MS medium on multiple shoot formation in *P. frutescens* has stimulatory effect (Zhang *et al*., 2005). The same stimulatory effect of BAP in MS medium on the shoot formation and was found to promote *in vitro* flowering of *P. frutescens* (Zhang, 2007b).

Rooting stage. The multiple shoots were transferred to half strength MS root induction medium with different concentrations of IBA (Table 2). Root formation from the basal end of the shoots was observed ten days after transfer to the rooting medium. The presence of an auxin (IBA) at a low concentration in half-strength MS medium was found to be more effective for rooting of *P. frutescens* plants. The best rhizogenesis was observed on ½ MS medium supplemented with 0.5 mg l^{-1} IBA, where 95% of plants produced high number of roots (average 7.1 roots per plant with 3.4 cm root length) after four weeks of culture (Fig. 1D). The formed roots were short and firmly fixed to the plant. The IBA at concentration 0.5 mg/l promote root formation and gave a greater number of roots. The shoots of perilla were rooted on MS medium with 2.9 M IAA and 1.4 M gibberellic acid (Houand Jia, 2005). Zhang *et al*. (2005) reported *in vitro* rooting on half-strength MS medium in the absence of external hormones of *P. frutescens* L. The presence of auxin for rizogenezis process is necessary in many types of medicinal plants. The concentration of IBA plays an important role in stimulating the root induction of medicinal plants (Zayova *et al*., 2013, 2016).

Table 2: Effect onauxins on the root induction of *P. frutescens* micropropagated plants after four weeks of culture.

Auxin (mg l^{-1})	Rooting (%)	Number of roots plant^{-1}	Root length (cm)
Control, ½ MS	15	0	0
½ MS+ 0.1 IBA	80	2.6±0.14^{a}	2.5±0.21^{b}
½ MS + 0.5 IBA	95	7.1±0.38^{c}	3.4±0.30^{a}
½ MS+ 1.0 IBA	70	3.2±0.22^{b}	2.7±0.25^{c}

The data are presented as means of 10 plants per treatment ± standard error. Different letters indicate significant differences assessed by Fisher LSD test (5%) after performing ANOVA multifactor analysis.

Acclimatization stage. The type of used potting substrate is important factor during acclimatization. In the present study, Mix 2 – soil: sand: perlite (2:1:1, v/v/v) was found to be most suitable planting substrate for hardening and its use ensured high frequency survival (95%) of propagated plants (Table 3, Fig. 1E). The plants were successfully transferred to the field where continue to grow and develop.The cloned perilla plants were identical in habit and stem height of development in field conditions (Fig. 1F). The micropropagated perilla plants flowered within 3-4 months after transplantation and produce seeds (Fig. 1G and 1H). Zhang *et al*. (2005) reported that the most of the regenerated plants (approximate 80%) could survive flower and bear seeds. Moreover, some morphological abnormalities were found among the regenerated plants.

Table 3: Effect of soil substrate on survival of *P. frutescens* plants after two months.

Soil mixture	Plant survival (%)	Plant height (cm)	Number of leaves plant^{-1}
Mix 1 - soil: perlite (2: 1, v/v)	65	2.3±0.18^{b}	4.0±0.27^{b}
Mix 2 - soil: perlite: sand (2: 1: 1, v/v/v)	95	3.6±0.25^{a}	6.2±0.38^{b}

The data are presented as means of 20 plants per treatment ± standard error. Different letters indicate significant differences assessed by Fisher LSD test (5%) after performing ANOVA multifactor analysis.

Fig. 1. Micropropagation of *P. frutescens*: A) *in vitro* seedling on MS medium; B) and C) *in vitro* propagated plants on MS medium with 1.0 mg l^{-1} BAP and 0.1 mg l^{-1} IAA; D) *in vitro* rooted plant on ½ MS medium with 0.5 mg l^{-1} IBA; E) *ex vitro* acclimatized plant; F) plants, grown under field conditions; G) plant with flowers and H) seeds from *in vitro* plants.

B. Conventionally propagated plants by seeds

The dry cold stratification of the seeds highly stimulated germination. Considerable differences in the germination rate between stratified and non stratified seeds cultured on the same nutrient substrate were observed (Table 4). Thus, the persentage of seed germination without stratification was 75%, while that of stratified seeds was higher and reached 100% after 5-6 days of sowing and the stratification process increases seed germination (Fig. 2A and 2B). The plants were ready to be transplanted in the field in the beginning of April. All plants were healthy and fully vigorous after planting in the soil (Fig. 2C). It should be noted that the plants produced aprolific amount of flowers (Fig. 2D and 2E). Much seeds were obtained until the end of culturing under the field conditions (Fig. 2F). One of the challenges is the problem of seed germination in perilla. It has been reported that the dormant period can be shorten if seeds kept in a low temperature (Bentsink and Koornneef, 2008).

Table 4: Seed germination of *P. frutescens* in non-sterile conditions.

Seed treatment	Seed germination (%)	Seedling height (cm)
No stratification	75	2.1±0.17^{a}
Dry cold stratification	100	2.5±0.22^{b}

C. Morphometric analysis

The presented results revealed some morphological variation between plants in the seed population, while all *ex vitro* propagated plants were morphologically similar and homogeneous. Using morphometric parameters, a comparative study was conducted between *ex vitro* and *in vivo* grown plants after five months of the experimental field (Table 5). Number of branches and leaves per plant and leaf size were not influenced by the propagation way, but the plant height and production of seeds were different. The *ex vitro* plants grown under field conditions were higher (average 92±0.52 cm) than those propagated *in vivo* (average 78±0.46 cm). On the other hands, there were morphometric differences among obtained *in vivo* plants; some generally had higher plant stature, whereas others had shorter ones. Each *ex vitro* and *in vivo* plant produced an average 14 branches number. Similar tendency was observed for the number of leaves. The *ex vitro* and *in vivo* derived plants showed very dark green leaves were broadly ovate with many deep wrinkles. Both *ex vitro* and *in vivo* perilla leaves looked similar with normal leaf shape, but differed in their size (Table 5). Moreover, the flowers number on main branch and per plant of both *ex vitro* and *in vivo* plants also showed similar morphology. Flower stalks were densely hairy. There were differences between seed characteristics of *ex vitro*-and seeds-derived *P. frutescens* plants. The seed production of *ex vitro* plants (81.5 g) was higher than *in vivo* plants (66.5 g) (Fig. 1H and 2F). Perilla seeds were globular in shape and white, grey and brownin colour. This means that micropropagation plants can be used as a seed source. Consequently, the type of plant propagation influenced only some morphological characteristics. Comparison between *ex vitro*- and *in vivo*- obtained plants revealed that all the features except plant heightand production of seeds, are almost the same to the propagation approach. The plants regenerated from cotyledon and hypocotyls of *P. frutescens* appeared no significant difference in morphology (Zhang *et al*., 2005).

Table 5: Morphometric characteristic of *ex vitro* and *in vivo* propagated *P. frutescens* plants grown in the experimental field

Morphological character	*Ex vitro* plants	*In vivo* plants
Plant height, cm	92.0±0.52^{d}	78.0±0.46^{c}
Branches number per plant	14.0±0.11^{a}	12.0±0.10^{a}
Leaves number per plant	76.0±0.65^{c}	74.0±0.62^{d}
Leaf length, cm	10.2±0.08^{b}	9.6±0.07^{b}
Leaf width, cm	8.4±0.06^{b}	7.5±0.05^{b}
Flowers number on main	8.0±0.06^{b}	6.0±0.04^{b}
Flowers number per plant	24.0±0.19^{ab}	20.0±0.17^{ab}
Seeds, g	81.5±0.64^{d}	66.5±0.53^{c}

The data are presented as means of 10 plants ± standard error

Fig. 2. *In vivo* propagation of *P. frutescens*: A) and B) *in vivo* seedlings from stratified seeds on the soil; C) *in vivo* plants, grown under field conditions; D) and E) plant with flowers and F) seeds from *in vivo* plants.

CONCLUSION

An effective protocol for micropropagation of *P. frutescens* using seedling culture in Bulgaria was successfully established. Data obtained in this study showed that the concentration of 1.0 mg/l of BAP alone or combination with 0.1 mg/l IAA can be used to induce multiple shoots. The best rooting of *P. frutescens* shoots was observed on MS medium supplemented with 0.5 mg/l IBA. Conventionally propagated plants by stratified seeds were accomplished. A micropropagation method may reduce the variation inherent in seed population. Comparison between both types of propagated plants revealed that some morphometric traits were influenced by the propagation way.The biotechnological tools may be applied for production of homogeneous plants with many seeds. Comparison between two types of plants revealed that all the traits except plant heightand production of seeds of *P. frutescens* were the same to the origin.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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