**In vitro** propagation via organogenesis and formation of globular bodies of *Salvia plebeia*: a valuable medicinal plant

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**Abstract**

As a valuable medicinal plant, *Salvia plebeia* R. Brown (*S. plebeia*) belongs to the Lamiaceae family that has been subjected to over-exploitation in its natural habitat for phytochemical and pharmacological studies. The present study focuses on the development of a micropropagation protocol for sustainable propagation and conservation of *S. plebeia*. Direct organogenesis (from shoot tips and cotyledonal nodes explants) and globular bodies (GBs) induction (from hypocotyl explants) systems have been established in *S. plebeia*. Alternative collection methods need to be developed for the large-scale propagation of *S. plebeia*. In addition, roots as explants can also induce adventitious shoots via callus. The highest and number of regenerated shoots (7.0 ± 0.8) per shoot tips was obtained on Murashige and Skoog (MS) medium supplemented with a combination of 0.1 mg L\(^{-1}\) indole-3-acetic acid (IAA) and 1.0 mg L\(^{-1}\) 6-benzyladenine (6-BA), the proliferation of shoots and shoots rooted were carried out on the same medium treatments almost synchronously. Similarly, MS medium supplemented with 0.1 mg L\(^{-1}\) IAA and 1.0 mg L\(^{-1}\) thidiazuron (TDZ) yielded the maximum number of shoots (37.5 ± 1.3) with 100% shoot sprouting frequency. Simultaneously, a protocol was developed for GB induction from hypocotyl explants, and it produced 17.4 GBs per explant with 82.7% response on MS medium supplemented with TDZ (1.0 mg L\(^{-1}\)) and IAA (0.1 mg L\(^{-1}\)), and produced GBs were characterized by globular, heart-shaped, and cotyledonary stages and successfully germinated on hormone-free MS medium, the developmental process of which was similar to that embryo. The acclimatized plantlets with well-developed root systems were successfully shifted to the natural soils with a 100% survival rate. Taken together, it is the foremost report on **in vitro** regeneration of *S. plebeia*; this work will facilitate in germplasm preservation and genetic transformation and provides necessary plant materials for various biotechnological and pharmaceutical applications.

**Keywords** *Salvia plebeia* R. Brown · Organogenesis · Globular bodies · Propagation · Thidiazuron

**Introduction**

*Salvia plebeia* R. Brown (*S. plebeia*) belongs to the family Lamiaceae and genus *Salvia*. Many *Salvia* species have been used as herbal medicines all around the world since ancient times (Ulubelen et al. 2002). *S. plebeia* is an annual or biennial herb famous for its medicinal properties, and it is distributed in China, South Korea, Japan, India, Iran, and Australia. This plant is extensively used in traditional systems of medicine in many parts of the world (Bang et al. 2016; Seo et al. 2019). In China, *S. plebeia* is popularly known as “Ha-Ma-cao,” and it is distributed mainly in Anhui, Zhejiang, and Jiangsu provinces. As a traditional Chinese herb, *S. plebeia* has a huge potential to be used in traditional prescriptions and home health remedies (Bang et al. 2016). It was first recorded 490 years ago in the *Compendium of Materia Medica*, which was written in the Ming dynasty by Shizhen Li, a famous Chinese medicine master. There are 21 traditional prescriptions that contain *S. plebeia*. In recent years, *S. plebeia* has attracted considerable scientific attention due to its immense medicinal properties. The whole plant can be used as a medicine and has been extensively explored in traditional as well as modern...
therapeutic practices for the treatment of numerous inflammatory diseases, such as nephritis, cough, hepatitis, diarrhea, hemorrhoids, rheumatoid arthritis, and even tumors (Jin et al., 2008, 2015; Jung et al., 2009; Liang et al. 2020). Thus far, phytochemical studies have revealed the presence of diterpenoids, flavonoids, lignans, and sesquiterpenoids in this herb, which exhibits various biological activities, such as antioxidant, antiproliferative (Jung et al. 2009; Ma et al. 2017), antiviral (Bang et al. 2016), anti-inflammatory (Jung et al. 2009; Akram et al. 2015; Zou et al. 2018), and antihyperlipidemic properties (Rai et al. 2015). To the best of our knowledge, micropropagation studies on S. plebeia have not been previously performed. The present investigation intended to develop an efficient system for in vitro plant regeneration via direct organogenesis and GBs formation (not via callus stage) of S. plebeia, using different concentrations and combinations of plant growth regulators (PGRs). In addition, morphologic observations were conducted to confirm the bipolar structure, developmental stages, and viability of the GB of S. plebeia.

Materials and methods

S. plebeia seeds were collected from mature plants, no permission is required to collect these seeds. The seeds were authenticated by Professor Minghua Luo of Mianyang Normal University. The voucher specimen of this material has been deposited in a publicly available herbarium. The seeds were surface-sterilized using 70% (v/v) ethanol for 45 s, followed by 0.1% (w/v) mercuric chloride (HgCl₂) (Guizhou Wanshan Special Zone Jinxin Mercury Industry Co., LTD, Tongren, China) for 4 min, supplemented by few drops of Tween 20, and then they were washed in sterile distilled water and set to germinate on Murashige and Skoog (MS) medium (Murashige and Skoog 1962, PhytoTechnology Laboratories™, Lenexa, KS) fortified with 1.0 mg L⁻¹ gibberellic acid (GA₃). All plant growth regulators were purchased from Sigma-Aldrich (St. Louis, MO). MS basal medium was gelled with 3% (w/v) sucrose, and the pH was set to 5.8 before adding 0.6% (w/v) agar and autoclaving at 121 °C for 15 min. The dimension of Petri dishes is 9 cm in diameter. The shape of the culture bottle is cylindrical, with a diameter of 7.0 cm and a height of 8.0 cm. The cultures were maintained at a temperature of 25 ± 2 °C and light intensity of 40 µmol m⁻² s⁻¹, which was provided by cool white fluorescent light (Philips, Shenzhen, China) under a 16-h photoperiod in all experiments. Shoot tips, cotyledonal nodes, and hypocotyls were excised from 16-d-old in vitro germinated seedlings are used as explants for experimentation.

In vitro plant regeneration via organogenesis or callus

Shoot tips and cotyledonal nodes (with two cotyledons) were grown on Petri dishes within a vertical orientation and cultured on MS basal media supplemented with 0.5, 1.0, and 2.0 mg L⁻¹ 6-benzyladenine (BA) or thidiazuron (TDZ) in combination with a lower concentration (0.1 mg L⁻¹) of indole-3-acetic acid (IAA) or 1-naphthaleneacetic acid (NAA). Adventitious roots were placed on 0.2 mg L⁻¹ IAA and 2.0 mg L⁻¹ TDZ medium. The plant growth regulators were purchased from Sigma-Aldrich, and 12 to 14 explants were cultured per Petri dish. The number of explants initiating shoot buds and the average number of shoot buds per explant were recorded after 6 wk. In each treatment, 50 explants were used, and the experiment was repeated three times.

Globular body induction from hypocotyl explants

For GB induction, in vitro hypocotyl explants (1.0 to 1.5 cm in length, with radicle) used as explants were flatted onto a petri dish and cultured on MS medium containing 1.0 mg L⁻¹ TDZ or 6-BA in combination with 0.1 mg L⁻¹ IAA. After a total of 5 wk of culture, the GB induction process was recorded. After another 2 wk of culture, plant recovery from GB was investigated. In each treatment, 50 explants were used, and the experiment was repeated three times.

Shoot proliferation, elongation, root formation, and transplanting

Adventitious bud clusters were cultured on MS medium containing 0.2 mg L⁻¹ NAA, 1.0 mg L⁻¹ 6-BA, and 0.5 mg L⁻¹ GA₃ for proliferation and elongation. The regenerated shoot buds were transferred to the half-strength MS medium containing 0.5, 1.0, and 1.5 mg L⁻¹ IBA (3-Indolebutyric acid) or NAA and were cultured for 15 d to induce the growth of shoots and roots. After 2 wk of culture, the percentage of rooted shoots was evaluated. The in vitro rooted shoots were harvested and were moved to plastic pots containing a 3:1 (v/v) mixture of peat and perlite and placed
in the greenhouse (25 ± 1 °C) under natural photoperiod conditions. Irrigated every alternative day with quarter-strength MS basal nutrient solution, and were maintained in a greenhouse use (25 ± 1) under natural photoperiod conditions and 75 to 85% relative humidity. Plantlet survival was recorded after 4 wk.

**Statistical analysis** In each treatment, 50 explants were used, and all experiments were repeated three times. The data were separated by one-way analysis of variance (ANOVA), and the treatment means were considered to be significantly different from the controls by the least significant difference (LSD) test at $P \leq 0.05$ using SPSS v. 18.0″ (IBM, New York, NY).

**Results**

At the beginning of the experiment, *S. plebeia* seeds were germinated on MS medium fortified with 1.0 mg L$^{-1}$ GA$_3$ at the rate of 100% (Fig. 1A). Since no previous information on the regeneration protocols of *S. plebeia* has been reported, the shoot tips, cotyledonal nodes (with two cotyledons), and hypocotyl (with radicle) of in vitro-grown seedlings were initially investigated after 16-d. All three explant types displayed a regeneration response under the conditions; therefore, they all would be used for subsequent further studies.

**Screening of hormones** Cytokinins (6-BA and TDZ) used at the same concentration (1.0 mg L$^{-1}$) were used to induce shoot regeneration from shoot tips. By the 36 d, adventitious shoots were produced directly from the cut surface of shoot tips. TDZ had a significant positive effect on adventitious shoot formation and produced relatively shorter shoots than the 6-BA (Fig. 1B; Table S1); however, the impact of 6-BA on the elongation and growth of shoot tips was superior to that of TDZ (Fig. 1C; Table S1). These usually developed roots, and no callus was visible. Previous studies have shown that TDZ has a better proliferation effect on plant regeneration induced by nodal segments explant (Khanam and Anis, 2018). Therefore, 6-BA was used for shoot tips regeneration and TDZ was used for cotyledonal nodes organogenesis in subsequent experiments.

The effect of the combination of concentration of 6-BA (0.5, 1.0, 1.5 mg L$^{-1}$) alone or with different auxin IAAs or NAAs was also tested. For 36 d, the inclusion of IAA at 0.1 mg L$^{-1}$ to the MS medium containing 1.0 mg L$^{-1}$ 6-BA remarkably increased the mean number of shoots and shoot elongation per explant and was accompanied by the emergence of mass adventitious roots (Fig. 2A, B). The mean number of shoots and roots significantly reduced with 0.1 mg L$^{-1}$ NAA and 1.0 mg L$^{-1}$ 6-BA (Fig. 2A, B). Therefore, IAA was used in subsequent experiments.

**Effect of PGRs on adventitious shoot regeneration from shoot tips and cotyledonal node explants of *S. plebeia***

Adventitious shoot regeneration and morphogenesis from shoot tips Adventitious shoot regeneration and morphogenesis *in vitro* were achieved from shoot tips explants. Initially, the shoot tips grew straight up, few pale yellow calluses were observed at the basal end of the explant (Fig. 3B), a few buds appeared from the base of the shoot tips cut within 36 d (Fig. 3C). When the culture period was prolonged, adventitious shoots grew normally and developed multiple shoots, abundant buds with broad leave were detected and accompanied by mass adventitious roots (Fig. 3D, E).

Data on multiple shoot induction from the shoot tip explants cultured on medium with different concentrations of 6-BA alone or combined with 0.1 mg L$^{-1}$ IAA are presented in Table 1. The inclusion of IAA is essential for shoot tips organogenesis (Fig. 2C). In this study, combinations of IAA and 6-BA were more effective for shoot regeneration, which yielded significantly higher shoot numbers and lengths (Table 1; Fig. 3D, E). These cultures induced broad leaves (approximately, 0.6 × 2.0 cm). In addition, 1.0 mg L$^{-1}$ 6-BA and IAA 0.1 mg L$^{-1}$ produced the mean maximum number of shoots, and a higher 6-BA concentration (2.0 mg L$^{-1}$) decreased both the frequency of shoot induction and mean the number of shoots per explant (Table 1). In the present study, the growth and proliferation of shoots and

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**Figure 1** (A) *Salvia plebeia* R. Brown seedlings germinated after 16-d of culture. The development of direct adventitious shoot regeneration on Murashige and Skoog medium containing 1.0 mg L$^{-1}$ TDZ (thidiazuron, B) and 1.0 mg L$^{-1}$ 6-BA (6-benzyladenine, C) from the shoot tips after 36-d of culture. *Bar*= 2 cm
rooting were conducted by the same medium treatments. The rooting and root length were strongly affected by the medium containing IAA (0.1 mg L$^{-1}$) (Table 1, Fig. 2C). The rooted shoots were acclimatized ex vitro (100%), and these roots were thick and white in color (Fig. 3D, E). However, the highest number (7.0 ± 0.82) of shoots was achieved for direct adventitious shoots of S. plebeia in 0.1 mg L$^{-1}$ IAA and 1.0 mg L$^{-1}$ 6-BA with 95.4% frequency (Table 1). The present investigation showed that the concentration and combination of PGRs played a vital role in enhancing of healthy shoots.

Figure 2 (A) Direct adventitious bud induction from Salvia plebeia R. Brown shoot tips on Murashige and Skoog (MS) medium containing 0.1 mg L$^{-1}$ NAA (1-Naphthylacetic acid) or IAA (indole-3-acetic acid) combination with1.0 mg L$^{-1}$ 6-BA (6-benzyladenine) and roots generated from the bottom of the shoot tips after 36-d. (B) Reverse side of A. (C) Shoot regeneration in MS medium with 1.0 mg L$^{-1}$ 6-BA alone or with 0.1 mg L$^{-1}$ IAA after 36 d. Bar = 2 cm.

Figure 3 Direct adventitious shoot regeneration and plant formation from shoot tips (A–E) and cotyledonary node (F–J) explants of Salvia plebeia R. Brown. (A) Shoot tip explants. (B) Shoot tips after 16 d of culture. (C) Proliferation of shoot tips for 24 d. (D) and (E) Development of adventitious shoots on MS medium with 0.1 mg L$^{-1}$ IAA (indole-3-acetic acid) and 1.0 mg L$^{-1}$ 6-BA (6-benzyladenine) accompanied by abundant adventitious roots generation from the bottom of the shoot tips after 36 d and 43 d, respectively. (e) is a local magnification of (E), showing mass shoots induced at the base. (F) Cotyledonary node explants. (G) Cotyledonary nodes after 16 d. (H) Proliferation of cotyledonary nodes for 24 d. (I) and (J) Development of adventitious shoots on MS medium with 0.1 mg L$^{-1}$ IAA and 1.0 mg L$^{-1}$ TDZ (thidiazuron) after 36 d and 43 d of culture, respectively. Bar = 2 cm.
Adventitious shoot regeneration and morphogenesis from cotyledonary nodes. In this study, a highly efficient micropropagation system, capable of sustainable multiplications of shoots, has been developed for *S. plebeia* using cotyledonary nodes (with two cotyledons) explants obtained from *in vitro* seedlings (Fig. 3F). Initially, two axillary shoots were developed from cotyledonary nodes (Fig. 3G). Auxins were found to be essential for the development of multiple shoots. On the medium with TDZ (0.5, 1.0, 1.5 mg L⁻¹) alone or combination of 0.1 mg L⁻¹ IAA, the cotyledonary nodes developed axillary shoots (without callus formation) (Table 2, Fig. 3H). Then, a great number of adventitious buds emerged from the basal of the axillary bud of cotyledonary nodes (Fig. 3J), clusters of adventitious buds were obtained by proliferation culture on the same medium (Fig. 3J) eventually. Based on the results presented in Table 2, the best multiplication results were recorded when the cotyledonary nodes explants were cultured on a medium containing 0.1 mg L⁻¹ IAA and 1.0 mg L⁻¹ TDZ, and the mean shoot number was approximately 37.5 ± 1.34 per explant with 100% shoot sprouting frequency.

**Table 1**: Effect of plant growth regulators on adventitious shoot regeneration from shoot tips explants of *Salvia plebeia* R. Brown

| Plant growth regulators (mg L⁻¹) | Shoot sprouting frequency (%) | Shoot explants⁻¹ | Shoot length (cm) | Roots explants⁻¹ | Root length (cm) |
|---------------------------------|-------------------------------|------------------|------------------|-----------------|-----------------|
| IAA 6-BA                        |                               |                  |                  |                 |                 |
| 0.5                             | 62.4 ± 1.36d                  | 2.4 ± 0.17c      | 0.6 ± 0.05c      | 1.7 ± 0.24d     | 0.8 ± 0.07c     |
| 1.0                             | 77.5 ± 2.21c                  | 3.2 ± 1.07bc     | 1.0 ± 0.13b      | 4.1 ± 0.21c     | 1.3 ± 0.14bc    |
| 2.0                             | 72.4 ± 3.51 cd                | 3.5 ± 0.58b      | 1.1 ± 0.11ab     | 4.4 ± 0.35c     | 1.2 ± 0.31bc    |
| 0.1 0.5                         | 87.5 ± 1.72b                  | 3.7 ± 0.36b      | 1.2 ± 0.04a      | 8.7 ± 0.43b     | 2.4 ± 0.58ab    |
| 0.1 1.0                         | 95.4 ± 1.21a                  | 7.0 ± 0.82a      | 1.6 ± 0.08a      | 12.4 ± 0.59a    | 3.5 ± 0.32a     |
| 0.1 2.0                         | 92.7 ± 2.42ab                 | 5.1 ± 0.32ab     | 1.3 ± 0.07a      | 10.0 ± 0.31ab   | 3.3 ± 0.17a     |

IAA indole-3-acetic acid; 6-BA 6-benzyladenine. Values within a column followed by different letters indicate significant differences according to the LSD test at *P* < 0.05. All treatments consisted of 50 explants and the experiments were repeated three times.

Effect of PGRs on globular bodies from hypocotyl explants

Hypocotyl explants cultured on medium in the presence of 1.0 mg L⁻¹ 6-BA alone did not regenerate callus and morphogenic structures, the addition of auxin IAA was recorded to be effective for induction (Table 3). After 24 d of incubation, globular bodies (GBs) (embryo-like structure) have occurred on the upper cut surface of the hypocotyl. The performance of TDZ was clearly superior to that of BA when in combination with IAA at 0.1 mg L⁻¹, and which produced a mean of 17.4 light greenish-yellow GBs explant⁻¹ with a 92.7% frequency (Table 3; Fig. 4C). To obtain plantlets, GBs were transferred to a PGR-free medium. Plantlet formation was observed from the second week of culture in the same treatments (Fig. 4D, E). The frequency of GB development into plantlets was above 70% for all treatments after culturing for 7 wk (Table 3).

The morphogenesis analysis revealed various developmental stages of the GBs from the hypocotyl. Initially, the transverse surface of the hypocotyl (not near the radicle) swelled and turned green (Fig. 4B). Green protuberances originated from the epidermis at the upper end of the hypocotyl and then extended to the GB structure after 24 d (Fig. 4C). Stereomicroscope observations showed an enhanced number of GBs (Fig. 4G and H); additionally, the GBs with a bipolar structure developed without any intermediate callus development. During bipolar development, the shoot pole (Fig. 4I black arrow) produced the shoot tip, heart-shaped embryos (Fig. 4J) and cotyledon-shaped embryos were also observed (Fig. 4K). However, the root pole remained embedded without any root formation on protuberances (Fig. 4J black arrow). GBs successfully germinated and converted into plantlets on a hormone-free MS medium (Fig. 4E).

Seedling hypocotyls are preferred for organogenesis and embryogenesis for kinds of species (Gerszberg et al. 2015; Banjac et al. 2019; García-Forlea et al. 2020; Kara-\_kas 2020). The present study describes an efficient protocol.
for *S. plebeia* GB (embryo-like structure) formation, derived from the hypocotyl.

**Shoot regeneration from root explants** Roots have been used as explants in many species, and they exhibited low regeneration compared with other explants (Zou *et al.* 2017; Banjac *et al.* 2019; Zeng *et al.* 2019). This experiment indicated that adventitious roots (Fig. 5A) from regenerated plants (Fig. 5A) were placed medium containing 0.2 mg L⁻¹ IAA and 2.0 mg L⁻¹ TDZ, leading to prolific regeneration shoot. No morphogenesis was observed under PGR free medium. Especially, the shoots regenerate in an interesting way: initially, the roots (in some parts) started to swell and then produced compact green callus that formed small balls arranged regularly on the root (Fig. 6B); thereafter, a strange phenomenon occurred in which some root apices formed light green callus and maintained the absorptive capacity. The proliferation of prolific shoots arose from the green nodal callus after 24 d (Fig. 5C, D), and the compact green callus is continuously proliferated and differentiated (Fig. 5C black arrow), abundant shoots covered the root explants finally (Fig. 5F), and some apices still possessed a distinct root hair zone with absorptive capacity (Fig. 5F). Previous reports have shown that the variety of regeneration abilities among different explants is attributed to variations in the physiological conditions, such as different levels of proteins (Tian *et al.* 2003) and endogenous hormones (Wang *et al.* 2015; Zeng *et al.* 2019). In this study, we found that root explants exhibited regeneration capacity (Fig. 5); however, when we induced GB from hypocotyls (with radicles) as explants, we found that the radicles did not respond (Fig. 4A–C), which may be related to endogenous hormones.

**Table 3** Effect of plant growth regulators on the formation of GBs from hypocotyl explants of *Salvia plebeia* R. Brown

| Plant growth regulators (mg L⁻¹) | Callus formation frequency (%) | Number of nodal embryogenic explant⁻¹ | Average plants recovered from embryos |
|---------------------------------|-------------------------------|---------------------------------------|-------------------------------------|
| IAA 6-BA TDZ                    |                               |                                       |                                     |
| 1.0 0d 0d                       | 64.5 ± 1.9b                   | 10.5 ± 1.32b                          | 7.0 ± 0.30b                         |
| 0.1 1.0 1.0                     | 11.4 ± 0.7c                   | 3.2 ± 0.72c                           | 2.0 ± 0.14c                         |
| 0.1 1.0 1.0                     | 82.7 ± 2.8a                   | 17.4 ± 2.16a                          | 12.0 ± 0.58a                        |

IAA indole-3-acetic acid; 6-BA 6-benzyladenine; TDZ thidiazuron; GBs globular bodies. Values within a column followed by different letters indicate significant differences according to the LSD test at *P* < 0.05. All treatments consisted of 50 explants and the experiments were repeated three times.

**Figure 4** *In vitro* propagation through the formation of GBs (globular bodies) and morphological observations of GBs in the multiplication and differentiation stage of *Salvia plebeia* R. Brown. (A–E) *In vitro* propagation through the formation of GBs. (A) Hypocotyl explant (with radicle). (B) Hypocotyl that swelled and became green. (C) Light greenish yellow GB formation from the hypocotyl after 24 d of culture. (D) GB development into a cluster of plantlets after 36 d of culture. (E) Germination of a cluster of GBs after 49 d of culture. Bar = 10 cm. (G–H) Morphological observation of *Salvia plebeia* GBs in the multiplication differentiation stage. (G) and (H) Magnification of F from different angles. (G, H) Multiplication of GBs. The red arrow indicates the globular stage of GBs, and the black arrow indicates the cotyledonary stage of GBs. (I) Globular stage (red arrow indicates shoot pole, black arrow indicates root pole) and (J) heart stage of GBs. (K) GB development into a cluster of plantlets. Bar = 2 cm (A, B), 1 cm (C, D), 1 cm (E), 1.5 cm (F), 5 mm (G, H, K).
Shoot proliferation and elongation and root formation and transplanting For further development of adventitious shoots, these (Fig. 6A) were transferred to a medium containing 0.5 mg L⁻¹ GA₃ in combination with 0.2 mg L⁻¹ NAA and 1.0 mg L⁻¹ 6-BA to elongate growth. Moreover, as the subculture was extended to 2 wk, the number of well-developed shoots increased significantly (data not shown, Fig. 6B–E). The well-developed shoots (3–5 cm in length) were excised and transferred to half-MS medium supplemented with NAA (0.1, 0.5, 1.0 mg L⁻¹) or IBA (0.5, 1.0,
1.5 mg L\(^{-1}\)), and these shoots rejuvenated and rooted spontaneously (Fig. 6F). Root length and morphogenesis in vitro were observed after 2 wk of culture according to the media used. The maximum root number (22.4 ± 0.42) and length (4.6 ± 0.32 cm) per shoot were obtained with a half-strength MS medium containing 0.5 mg L\(^{-1}\) NAA (Table 4; Fig. 6F, G). Rooted plantlets were transferred into soil and successfully acclimatized under the greenhouse conditions with a 100% survival rate (Fig. 6H).

### Discussion

*Lamiaceae* species are some of the most popular ornamental and medicinal plants with high economic values (Widoretno and Wahyu 2016). To date, there are no peer-reviewed publications on *Salvia plebeian* in vitro regeneration. The establishment of an efficient micropropagation system not only better meets market demand via a large-scale multiplication but also forms the basis for genetic transformations. In this experiment, we detected the effect of plant growth regulators and explants type on the regeneration capacity as well as Globular body (GB) formation.

#### Effect of PGR

TDZ is one of the most effective plant growth regulators and a derivative of phenyl urea (Kumari et al. 2018), and it is believed to be the best synthetic cytokinin present for the regeneration of numerous plant systems, including *Salvia* (Sivanesan et al. 2011b). Our results demonstrated that TDZ played a distinctive role in shoot regeneration and Globular bodies (GBs) (embryo-like structure) formation from cotyledonary nodes and hypocotyl explants respectively, which are consistent with the results of various species, including *Cibotium barometz* (Yu et al. 2017), *Portulaca pilosa* L. (Chen et al. 2020), *Metrosideros ovalifolia* W. T. Wang (Ouyang et al. 2016), and *Solanum nigrum* (Xu et al. 2014).

On increasing the concentration of TDZ above the optimum level, the shoot regeneration capacity decreased. The continuous presence of TDZ has a deleterious effect on the growth and multiplication, and this result has been reported in numerous plants species, like *Rauwolfia tetraphylla* (Faisal et al. 2005), *Allamanda cathartica* (Khanam and Anis 2018), and *Withania somnifera* (Fatima and Anis 2011). In our study, when contiguously exposed to TDZ, the differentiated adventitious bud from cotyledonary nodes failed to elongate, even after 2 wk of incubation (Fig. 3J). This finding could be due to the lack of degradation of TDZ by cytokinin oxidase enzymes present in plant tissues (Zattoukal et al. 2008; Podwysznyska et al. 2014). Our results also indicated that without TDZ media (NAA at 0.2 mg L\(^{-1}\), 6-BA at 1.0 mg L\(^{-1}\) and GA\(_3\) at 0.5 mg L\(^{-1}\)) was effective for shoot elongation (Fig. 5B–E).

Additionally, in our study, shoot regeneration capacity from shoot tips and rooting cultured without TDZ (only 6-BA alone or in combination with IAA) far surpassed those with TDZ, the combination of BA and IAA was reported to be effective for shoot regeneration in *Albizia lebbeck* (Pereven and Anis 2015), *Populus* (Zeng et al. 2019), and *Elliottia racemosa* (Woo and Wetzstein 2008).

#### Effect of explant type

Different explant types create diverse regeneration capacity in many species like *Cotoneaster wilsonii* (Sivanesan et al. 2011a), *Populus* (Zeng et al. 2019), and *Caryopteris terniflora* (Wu et al. 2020). Therefore, the selection of explants plays a decisive role in different effectiveness. In this study, shoot tips, cotyledonary nodes and hypocotyls gave rise to high regeneration frequency, more importantly, diverse inductive phenomena were observed. Similar phenomenon was reported by Wu et al. (2020) in *Caryopteris terniflora*, in *Elliottia racemosa* Woo and Wetzstein (2008). Our results also have shown that root explants exhibited high regeneration capacity, consistent with the results observed in *Gymnocladus dioicus* (Genew 2005).

#### Globular body formation

Seedling hypocotyls are preferred for in vitro regeneration, somatic embryogenesis and genetic transformation in various species, such as in *Brassica oleracea* (Banjac et al. 2019), in *Albizia odoratissima* (Rajeswari and Paliwal 2008), and in *Lycium barbarum* (Karacas et al. 2020). In our work, we observed that GBs (embryo-like structure) were formed from hypocotyls explants. The novel structure of developmental process was similar to that of somatic embryos, which has been reported in other species, including *Cibotium barometz* (Yu et al. 2017), *Cyclamen* (Da et al. 2016), and *Portulaca pilosa* (Chen et al. 2020). Such observation notes that the regeneration pathway might

### Table 4

| Plant growth regulators (mg L\(^{-1}\)) | Rooting (%) | Mean number of roots | Root length (cm) |
|--------------------------------------|-------------|---------------------|------------------|
| NAA | IBA |
| 0.5 | 72.4 ± 1.37c | 6.3 ± 2.11c | 1.4 ± 0.25bc |
| 1.0 | 87.5 ± 1.25b | 8.7 ± 0.32c | 2.3 ± 0.43b |
| 1.5 | 80.7 ± 2.15b | 6.8 ± 1.19c | 2.1 ± 0.38b |
| 0.1 | 91.0 ± 2.34a | 11.4 ± 1.62b | 2.4 ± 0.15b |
| 0.5 | 100.0 ± 0.00a | 17.5 ± 0.43a | 4.6 ± 0.32a |
| 1.0 | 100.0 ± 0.00a | 14.3 ± 2.31b | 2.7 ± 0.38b |

NAA 1-naphthalacetic acid; IBA 3-indolebutyric acid. Values within a column followed by different letters indicate significant differences according to the LSD test at P < 0.05. All treatments consisted of 50 explants and the experiments were repeated three times.
vary with explant tissue, which could be dependent on the endogenous hormone content.

Conclusions

*S. plebeia* is a highly valued traditional plant. In the present investigation, a highly efficient system was developed for the micropropagation of *S. plebeia* via direct organogenesis and globular body (embryo-like structure) formation. Specific concentrations and combinations of plant growth regulators influenced shoot organogenesis of *S. plebeia*. Overall, we found TDZ played a distinctive role in shoot regeneration and GBs induction. The present work revealed that shoot tips and cotyledonal node explants were more suitable explants than root explants for shoot regeneration. Meanwhile, hypocotyl explants were used for GBs induction. The study showed that the process of GB development was similar to that of the somatic embryo. This protocol developed in the present study will help can be facilitated in generating uniform propagules, germplasm conservation, and genetic engineering processes in *S. plebeia*.

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Author contribution LJZ designed and guided the experiments. QGW and CZ performed the experiments, analyzed the data and wrote the article. HLY, YXS, and JYH made substantial contributions to the data analysis and the manuscript revision. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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