

Exogenous Elicitors Driven optimization: a boon for in vitro propagation and acclimatization of Euphorbia milii plant

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Abstract

Background

Euphorbia milii is a flowering ornamental plant that plays an important role in folk medicine. It has a variety of therapeutic effects due to its high content of many active compounds. This research presents a simple protocol for micropropagation of *Euphorbia milii* and studies the effect of certain elicitors on shoot multiplication. Root induction and the adaptation stage in vitro were studied.

Results

Nodal stem segments from regenerated shoots were cultured on MS medium containing different levels of benzyladenine (BA) (0, 0.5, 1, 1.5 mg·L⁻¹). Regenerated microshoots were cultured on different rooting media including MS-salt-free, ¼ MS salts, ½ MS salts, and full-strength MS salts. Biotic elicitors (malt extract, yeast extract and peptone) and abiotic elicitors (salicylic acid, abscisic acid and gibberellic acid) at different concentrations were added to MS medium fortified with 1 mg·L⁻¹ BA. The results showed that 1 mg·L⁻¹ BA was the optimal treatment for shoot multiplication. Full-strength MS medium produced the highest number of roots. In the case of biotic elicitors' effect on shoot multiplication, malt extract (400 mg·L⁻¹) was produced the highest number of shoots per explant and shoot length followed by yeast extract at 3 g·L⁻¹ concentration. In the case of abiotic elicitors' effect, abscisic acid (0.5 mg·L⁻¹) gave both the highest number of shoots per explant and shoot length, followed by salicylic acid at 2 mg·L⁻¹. The plantlets were successfully acclimatized by transferring them into a potting mixture of peat moss: sand in equal proportion with 100 percent survival was observed.

Conclusions

Stem nodal explants were the optimal source for proliferation and rapid in vitro propagation of *Euphorbia milii* on a large scale. Biotic and abiotic elicitors had a significant effect on plant growth; especially, malt extract and abscisic acid which are the preferable elicitor for shoot multiplication. Plants derived from the treatment of 1.0 mg·L⁻¹ BA + 400 mg·L⁻¹ ME showed good growth and flowering performance in nursery pots.

Background

Euphorbia milii Des Moul. (Crown of thorns) is a flowering plant which belongs to family Euphorbiaceae and is native mainly to Madagascar. Euphorbiaceae family consists of 2000 species, including a large number of medicinal plants. *Euphorbia milii* is a succulent shrub with spiny stems that produces flowers all year round (Riina et al. 2013). Phytochemical studies of *E. milli* leaves revealed rich content of various bioactive compounds such as glycosides, alkaloids, flavonoids, phenols, amyirin acetate, tannins, lupeol

acetate, triterpenes, β -sitosterol and lectin (Anju et al. 2022; Amtaghri et al. 2022). Regarding pharmacological value, *E. milii* was used for the treatment of several systemic diseases, including cancer and tumors as reported by Mahendiran et al. 2023. *E. milii* is propagated by cuttings but limited-branching and slow growth are main problems for its propagation. So, it is preferable to investigate efficient micropropagation protocols for it (Kapadiya et al. 2017). Successful in vitro propagation for *Euphorbia* species was mentioned by Almzori et al. 2023, Xiyan et al. 2024, Farid et al. 2025.

It has been found that the use of different abiotic elicitors (temperature, light, salinity, drought, ozone, chemical elements, and phytohormones) or biotic elicitors (bacteria, fungi, polysaccharides, peptone and organic additives) promote shoot multiplication and induce the formation of secondary metabolites of different plants (Rasouli et al. 2021, James-Forest et al. 2025, Razzaq et al. 2025). Among abiotic elicitors, some phytohormones can be used as elicitors such as salicylic acid (SA), jasmonic acid (JA), gibberellin (GA3), abscisic acid (ABA), cytokinin and Ethylene. GA3 at $2 \text{ mg}\cdot\text{L}^{-1}$ gave the highest level of multiple shoots of *Stevia rebaudiana* (Ghazal et al., 2024). On the other hand, biotic elicitors such as organic additives including) malt extract (ME), yeast extract (YE), casein hydrolysate (CH), coconut water (CW), alginate (ALG), and chitosan (CHI) have positive effects on plant growth and bioactive compounds production. For instance, application of $1 \text{ mg}\cdot\text{L}^{-1}$ malt extract gave the highest biomass production of *Centella asiatica* (Kundu et al., 2016), while yeast extract at $100 \text{ mg}\cdot\text{L}^{-1}$ induced shoot multiplication and biomass yield in *Arnica montana* L. cultures (Petrova et al. 2025). Also, the addition of $2 \text{ g}\cdot\text{L}^{-1}$ peptone induced multiple shoot formation of orchid cultures as reported by Utami and Hariyanto 2020. The current study aimed to investigate an efficient and successful protocol of *Euphorbia milii* in vitro plantlet mass production and their acclimatization using medium supplemented with different types of biotic and abiotic elicitors.

Methods

Plant material preparation and aseptic culture initiation

New shoot flushes of *E. milii* plants were separated and thoroughly washed under running water for 15 minutes, then submerged in 70% ethyl alcohol for 10 s, washed with sterilized water, followed by rinsing for 10 min in 25% sodium hypochlorite. Finally, explants were washed with sterilized water. Shoot tips were cultured on MS medium salts (Murashige and Skoog, 1962) plus sucrose ($30 \text{ g}\cdot\text{L}^{-1}$), agar ($7 \text{ g}\cdot\text{L}^{-1}$) and $0.5 \text{ mg}\cdot\text{L}^{-1}$ benzyladenine (BA) for initiation of aseptic shoot cultures.

Shoot multiplication stage

Nodal stem segments from regenerated shoots were cultured on MS medium plus $30 \text{ g}\cdot\text{L}^{-1}$ sucrose, $7 \text{ g}\cdot\text{L}^{-1}$ agar and BA at different levels (0, 0.5, 1, $1.5 \text{ mg}\cdot\text{L}^{-1}$), with pH 5.8. Three explants were used per culture jar and kept at $25 \pm 1^\circ\text{C}$ under 16/8 h light photoperiods and 2000–3000 lux of light intensity. Subculturing was done every four weeks.

Effect of different elicitors on shoot cultures

In the first experiment, stem segments were cultured on MS medium containing $1 \text{ mg}\cdot\text{L}^{-1}$ BA along with three different biotic elicitors as follows: malt extract (ME) at 400, 800 and $1200 \text{ mg}\cdot\text{L}^{-1}$; yeast extract (YE) at 1, 3 and $5 \text{ g}\cdot\text{L}^{-1}$ and peptone at 2, 4 and $6 \text{ g}\cdot\text{L}^{-1}$. In the second experiment, stem segments were cultured on MS medium containing $1 \text{ mg}\cdot\text{L}^{-1}$ BA along with three different abiotic elicitors including salicylic acid (SA) at 1, 2 and $3 \text{ mg}\cdot\text{L}^{-1}$; abscisic acid (ABA) at 0.5, 1.0 and $1.5 \text{ mg}\cdot\text{L}^{-1}$ and gibberellic acid (GA3) at 2, 4 and $6 \text{ mg}\cdot\text{L}^{-1}$.

Root induction

Regenerated shoots (0.5 cm) were transferred to different root induction treatments: free MS salts, $\frac{1}{4}$ MS salts, $\frac{1}{2}$ MS salts and full-strength MS salts. All treatments were also contained $30 \text{ g}\cdot\text{L}^{-1}$ sucrose and solidified with $7 \text{ g}\cdot\text{L}^{-1}$ agar.

Acclimatization stage

The obtained rooted shoots were removed gently from the culture medium and were washed with water, then they were transplanted into 6 cm plastic pots contained a mixture of peat moss to sand (1:1), supplemented with MS salts at transplant time under a $25 \pm 2^\circ\text{C}$ air temperature and covered with polyethylene bags. After 7–10 days, these bags were removed gradually to adapt plants to greenhouse environmental conditions.

Statistical analysis

The experiments were designed as a complete randomized design. One-way analysis of variance (ANOVA) using MATATC software and the least significant difference (LSD) test with probability level ($p < 0.05$) was applied. The test identifies the significant differences between the means of the treatments (Waller and Duncan 1969).

Results and discussions

Shoot tips were cultured on MS medium plus $0.5 \text{ mg}\cdot\text{L}^{-1}$ BA for initiation of aseptic shoot cultures. Nodal stem segments from regenerated shoots were excised and used as explants for shoot multiplication experiment.

Shoot multiplication stage

The nodal stem explants under direct organogenesis on MS medium contained various levels of BA were studied (Table 1). All stem segments on the different treatments started to regenerate after two weeks

of culture with a frequency of 100%. The explants cultured on 1.0 mg·L⁻¹BA gave the highest average number of shoots per explant (3.5 shoots) as shown in Fig. 1 that was significantly higher than the other treatments. Whereas, the free basal MS medium gave the lowest mean number of shoots (1 shoot per explant). The highest shoot length (1.7 cm) and maximum number of leaves (5 leaves per shoot) were obtained on free-BA medium, followed by those cultured on 1.0 mg·L⁻¹BA without significant difference. The same results were obtained with the number of leaves per shoot. It could be concluded from data in Table (1) that 1 mg·L⁻¹BA was the optimal treatment for shoot multiplication.

Effect of different biotic elicitors (malt extract, yeast extract, peptone) on *Euphorbia milii* shoot cultures

Stem nodal segments were grown on the optimal treatment for multiple shoots induction (MS-medium + 1.0 mg·L⁻¹BA) fortified with malt, yeast and peptone extracts. Table 2 shows that the highest shoot number per explant was produced with applying lower concentrations of ME (400, 800 mg·L⁻¹) followed by YE (3 g·L⁻¹) without significant differences among them, whilst peptone treatments gave the lowest shoot number. The shoot length and the number of leaves were significantly higher (0.8 cm, 5.3 leaves/shoot) with a concentration of each ME (400 mg·L⁻¹), YE (3 g·L⁻¹) and peptone (2 g·L⁻¹) as shown in Fig. 2.

Table 1
Effect of BA with different concentrations on shoot development of *Euphorbia milii* from nodal stem explants after 4 weeks in culture.

Concentration of BA (mg·L ⁻¹)	Percentage of explants producing shoots	average number of shoots per segment	average shoot length cm	Average number of leaves per shoot
0	100	1 ± 0.0577 ^c	1.7 ± 0.2962 ^a	5 ± 0.5773 ^{ab}
0.5	100	1.5 ± 0.1154 ^c	1 ± 0.1732 ^b	3 ± 0.2886 ^b
1.0	100	3.5 ± 0.2886 ^a	1.2 ± 0.1154 ^{ab}	5 ± 0.3527 ^a
1.5	100	2.5 ± 0.2886 ^b	1 ± 0.2886 ^b	3 ± 0.5773 ^b

Averages ± SE introduced with different letters are significantly different at (P < 0.05).

1 mg·L⁻¹BA after 4 weeks of culture

Effect of different abiotic elicitors (salicylic acid, abscisic acid and gibbrellic acid) on *Euphorbia milii* shoot cultures

Stem nodal segments were grown on the selected treatment for multiple shoots induction (MS-medium + 1.0 mg·L⁻¹ BA) fortified with salicylic acid, abscisic acid and gibberellic acid, as shown in Table 3. The results shown that the optimal level of SA was 2 mg·L⁻¹ as it produced 2.5 average shoots per explant. In case of using ABA, the highest significant average number of shoots per explant (2.7 shoots) was obtained with 0.5 mg·L⁻¹ ABA. Whereas, GA3 gave the least number of shoots with all its concentrations, as shown in Fig. 3. For the average shoot length and also the number of leaves, ABA at 1.5 mg·L⁻¹ produced the best shoot growth t, as shown in Fig. 2

Table 2

Shoot development of *Euphorbia milii* stem segments on 1.0 mg·L⁻¹ BA plus different biotic elicitors after 8 weeks of culture

Treatments	Average no. of shoots/exp.	Average shoot length	Average no of leaves/shoot
ME 400 mg·L ⁻¹	2.8 ± 0.1145 ^a	0.8 ± 0.1155 ^a	5.0 ± 0.5773 ^a
ME 800 mg·L ⁻¹	2.5 ± 0.2886 ^a	0.7 ± 0.0577 ^{ab}	4.5 ± 0.2887 ^{ab}
ME 1200 mg·L ⁻¹	1.5 ± 0.2886 ^{bc}	0.6 ± 0.0577 ^{ab}	4.6 ± 0.1155 ^{ab}
YE 1 g·L ⁻¹	1.0 ± 0.2886 ^c	0.5 ± 0.0577 ^b	4.0 ± 0.5773 ^b
YE 3 g·L ⁻¹	2.3 ± 0.1732 ^a	0.8 ± 0.0577 ^a	5.3 ± 0.1155 ^a
YE 5 g·L ⁻¹	1.6 ± 0.0577 ^b	0.6 ± 0.1155 ^{ab}	4.6 ± 0.1155 ^{ab}
Peptone 2 g·L ⁻¹	1.3 ± 0.1732 ^{bc}	0.8 ± 0.0577 ^a	5.3 ± 0.1155 ^a
Peptone 4 g·L ⁻¹	1.0 ± 0.2886 ^c	0.5 ± 1155 ^b	4 ± 0.5773 ^b
Peptone 6 g·L ⁻¹	1.0 ± 0.2886 ^c	0.5 ± 1155 ^b	4 ± 0.5773 ^b

Averages ± SE introduced with different letters are significantly different at (P < 0.05).

Table 3

Shoots development of *Euphorbia milii* stem segments on 1.0 mg/l BA plus different abiotic elicitors after 8 weeks of culture

Treatments	Average no. of shoots/exp.	Average shoot length	Average no of leaves/shoot
SA 1 mg·L ⁻¹	1.0 ± 0.2887 ^b	0.5 ± 0.0577 ^b	4 ± 0.5773 ^b
SA 2 mg·L ⁻¹	2.5 ± 0.2887 ^a	0.5 ± 0.0577 ^b	4 ± 0.5773 ^b
SA 3 mg·L ⁻¹	1.0 ± 0.2887 ^b	0.5 ± 0.0577 ^b	4 ± 0.5773 ^b
ABA 0.5 mg·L ⁻¹	2.7 ± 0.1155 ^a	0.5 ± 0.0577 ^b	4 ± 0.5773 ^b
ABA 1.0 mg·L ⁻¹	1.7 ± 0.1155 ^b	0.5 ± 0.0577 ^b	4 ± 0.5773 ^b
ABA 1.5 mg·L ⁻¹	2.0 ± 0.5773 ^{ab}	1.0 ± 0.2887 ^a	6 ± 0.5773 ^a
GA3 2 mg·L ⁻¹	1.3 ± 0.0577 ^b	0.6 ± 0.0577 ^b	4 ± 0.5773 ^b
GA3 4 mg·L ⁻¹	1.0 ± 0.2887 ^b	0.5 ± 0.0577 ^b	4 ± 0.5773 ^b
GA3 6 mg·L ⁻¹	1.0 ± 0.2887 ^b	0.5 ± 0.0577 ^b	4 ± 0.5773 ^b

Averages ± SE introduced with different letters are significantly different at (P < 0.05).

acid (f).

Rooting stage

The shoots were transferred to root induction media with different strengths of MS salt free -PGR. The rooting responses are shown in Table 4. During 3–4 weeks of culture on rooting media, 100% of shoots formed roots on all treatments, but the number of roots was different among the different strengths of MS as full MS gave the highest mean number of roots per shoot (7.5 roots), as shown in Fig. 3. Whereas, shoots cultured on free MS salts medium gave the lowest root number (3 roots). On the other hand, the maximum average root length (4.6 cm) was found on a medium with ½ MS. Whereas, roots on full MS medium were 3 cm on average. The plantlet growth (length and number of leaves) on rooting media was better on full MS medium (8 leaves/ plantlet) than the others, as it decreased with decreasing of MS strength. Generally, the results showed that rooting and plantlet growth were better on full-strength MS basal medium followed by ½ MS medium.

Acclimatization stage of *Euphorbia milii* in vitro plantlets

Plantlets with good roots were successfully transplanted into pots contained a mixture of peat to sand (1:1). Normal growth with developed new leaves was observed, and after 4 months of adaptation, the plantlet began to flower, as shown in Fig. 3. Plants derived from treatment (1.0 mg·L⁻¹ BA + 400 mg·L⁻¹ ME appeared to be faster and more vigorous in growth and flowering than those derived from treatment 1.0 mg·L⁻¹ BA alone, as shown in Fig. 3.

Table 4
Effect of MS strength on root induction and development of *Euphorbia milii* microshoots

treatments	Frequency (%) of explants producing roots	average number of roots per shoot	average root length Cm
Free-MS	100	3.0 ± 0.5773 ^c	4 ± 0.2886 ^a
¼ MS	100	4.5 ± 0.2886 ^b	4.5 ± 0.2886 ^a
½ MS	100	5.5 ± 0.2886 ^b	4.6 ± 0.2886 ^a
Full MS	100	7.5 ± 0.4041 ^a	3 ± 0.1154 ^b

Averages ± SE introduced with different letters are significantly different at (P < 0.05).

Discussion

Nodal segments were the preferred explant type used in many researches for shoot culture initiation of different plants with high genetic stability (Farooq et al. 2021, El-Wakeel et al. 2025, **Pandit et al. 2025**). For shoot culture development, using plant growth regulators, especially BA alone or combined with auxins, has the main effect on cell division, differentiation, and axillary bud activation (Beura et al., 2020; Mekonen et al., 2021; Alsoufi et al., 2021). Our results showed that frequency of shoot induction was 100% at all treatments, while the number and lengths of shoots varied according to the concentration of BA, as the highest shoot number and leaves was produced on cultures treated with 1 mg·L⁻¹ BA compared with other concentrations. These results are close to those found by Kodad et al., 2021 who reported that the use of 1 mg·L⁻¹ of BA was favorable for increasing the proliferation rate and mean shoot number of of“Beldi”almond. Lakho et al. 2023 also found that 2 mg·L⁻¹ of BA produced the highest number of f healthy shoots of pineapple cv. ‘Smooth Cayenne’. On the other hand, our results showed that the longest shoot (1.7 cm) had been obtained on medium free of BA, indicating that medium without any growth regulators was the best treatment for shoot elongation. The current results were in line with data observed by Qarachoboogh et al 2025 who mentioned that the highest shoot elongation of *uniperus excelsa* L. was obtained at MS medium without any growth regulators. However, Almozori et al., (2023) observed that MS medium containing 1.0 mg. L⁻¹ BA promoted shoot length for *Euphorbia pulcherrima* Willd.

In general, root induction is commonly achieved by a high level of auxin to cytokinin; therefore, adding an auxin to the growth medium plays an important role in root formation, such as IAA, IBA and NAA (Ahmad and Dumanoğlu, 2022, Meyad et al. 2023, Song et al. 2024). However, a medium-free plant growth regulator can be effective for rooting, as it often results in stronger roots compared to some PGR-supplemented media. However, its success for rooting depends on some factors such as plant species, basal culture medium, mineral composition and sucrose concentration. In some cases, absence of PGRs leads to successful rooting and can even improve acclimatization, but in other reports, it resulted in low rooting percentages. In the present study, different MS salts strengths without PGR were applied for root induction of *Euphorbia milii* shoots. The results indicated that the percentage of rooting was 100% in all treatments and proved that this species can be successfully rooted without the addition of any PGR. Full-strength MS medium was the best treatment for producing high root number per shoot, whereas the highest length of root was achieved on both $\frac{1}{2}$ MS and $\frac{1}{4}$ MS strengths and even on free MS treatment. These results are in harmony with the observations of Mohammadi et al 2026 and **Asmono et al.,2021** who reported that reducing the salt strength to a half or a third was optimal for root growth in many medicinal plants, leguminous species and fruit trees. On the other hand, **Mabotja et al.,2025** recorded a maximum root number with full-strength MS medium without PGR. Different biotic and abiotic elicitors were widely applied for enhancing in vitro shoot multiplication in many species Rasouli et al. 2021, Razzaq et al. 2025). Malt extract is a valuable supplement as a biotic elicitor that promotes shoot multiplication due to its rich and complex nutritional content as a natural source of carbohydrates and growth stimulants like auxins and gibberellins. The results of our study indicated that malt extract was the best among the biotic elicitors used, as the highest number of shoots was produced with applying lower concentrations of ME ($400, 800 \text{ mg}\cdot\text{L}^{-1}$). These results were in harmony with Kundu et al. 2016 who found that MS medium containing $1 \text{ mg}\cdot\text{L}^{-1}$ of malt extract and $1.5 \text{ mg}\cdot\text{L}^{-1}$ of BAP gave the highest biomass production of *Centella asiatica* among all elicitors. Also, yeast extract at 100 mg/L promotes shoot multiplication and biomass yield of *Arnica montana* L. shoot cultures (**Petrova et al.2025**). This may be due to its high content of amino acids, inositol and thiamine. These results are close to the results of Rasouli et al. 2021 who found that $50 \text{ mg}\cdot\text{L}^{-1}$ of yeast extract induced the shoot multiplication of *Stevia rebaudiana*. Although ABA is known as a plant growth inhibitor, it is commonly used for this purpose in plant tissue culture (Lorrai et al. 2018), but its function can be stimulatory or inhibitory depending on different factors such as concentrations, media ingredients, timing, and tissues light intensity, or plant species (**Humplik, et al. 2017**). Our results showed that $0.5 \text{ mg}\cdot\text{L}^{-1}$ and $1.5 \text{ mg}\cdot\text{L}^{-1}$ ABA produced the highest shoot number and shoot length, respectively. These findings were in good agreement with Humplik et al. 2017 who noted that ABA could also exhibit a stimulating effect in shoot and root growth via inhibiting the ethylene production. Also, salicylic acid (SA) at $2 \text{ mg}\cdot\text{L}^{-1}$ gave a high number of shoots per explant. Similar results were reported by Dobhal et al. 2023 who found that culture medium supplemented with $2 \text{ mmol}\cdot\text{L}^{-1}$ SA showed significantly higher buds' number of date palm Ovidi. Petrova et al. 2025 reported that SA at $100 \text{ }\mu\text{M}$ was effective in the induction of vigorous shoot growth and biomass accumulation of *Arnica montana* L.

Rooted plantlets were successfully acclimatized in a soil mixture (1 peat: 1 sand) with a high survival rate (100%). This result was similar to those obtained by Farid et al. 2025 who reported that *Euphorbia milli* plantlets were acclimatized successfully (100%) in peat:sand (1:1). Also, Xiyan et al, 2024 reported that the survival rate of adapted *Euphorbia milli* plantlets reached 96%.

Conclusions

In summary, the authors recommended employing the stem nodal explants as the permanent optimal source for proliferation and rapid in vitro propagation of *Euphorbia milii* on a large scale. Biotic and abiotic elicitors had a significant effect on plant growth; among them, malt extract and abscisic acid are the preferable elicitors for shoot multiplication. Plants derived from treatment $1.0 \text{ mg}\cdot\text{L}^{-1}$ BA + $400 \text{ mg}\cdot\text{L}^{-1}$ ME performed growth and flowering significantly in nursery pots containing soil mixture. In the future, the in vitro plantlets and the adapted *Euphorbia milii* provided baseline cultures for further detailed investigations of important bioactive compounds for various biological activities and healthy food supplements.

Abbreviations

MS Murashige and Skooge

BAP Benzylamino purin

ME malt extract

YE yeast extract

SA salicylic acid

ABA Absciscic acid

GA3 Gibbrellic acid

Declarations

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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Author Contribution

N.D.G.: Methodology, Interpretation of data for the work, Writing – original draft and Analysis. A.E.M: Design of the work, Supervision, Review & editing. N.M.A.: Project administration, Writing, Review & editing.

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Data Availability

All data generated or analyzed during this study included in this published article

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Figures



Figure 1

Multiplicated shoots on MS medium plus

$1 \text{ mg} \cdot \text{L}^{-1}$ BA after 4 weeks of culture

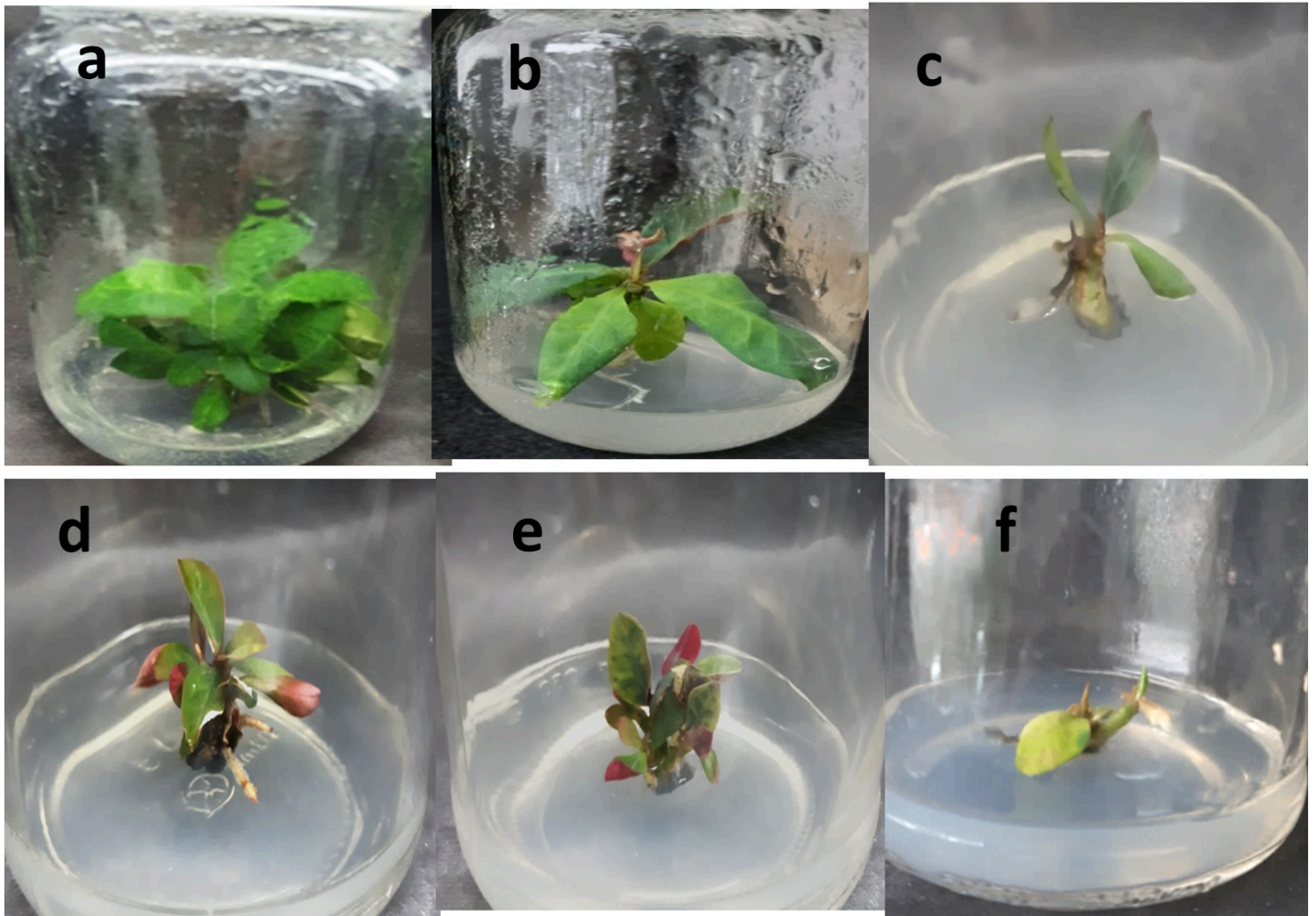


Figure 2

Shoot proliferation on medium supplemented with biotic and abiotic elicitors after 8 weeks: $400 \text{ mg} \cdot \text{L}^{-1}$ malt extract (a), $3 \text{ g} \cdot \text{L}^{-1}$ yeast extract (b), $2 \text{ g} \cdot \text{L}^{-1}$ peptone (c), $2 \text{ mg} \cdot \text{L}^{-1}$ salicylic acid (d), $0.5 \text{ mg} \cdot \text{L}^{-1}$ abscisic acid (e) and $2 \text{ mg} \cdot \text{L}^{-1}$ gibberellic acid (f).



Figure 3

Rooting of shoots on full-strength basal MS medium after 4 weeks (left), acclimatization after 4 months (right); 1: Flowering plant derived from treatment with a biotic elicitor ($1.0 \text{ mg}\cdot\text{L}^{-1}$ BA+ $400 \text{ mg}\cdot\text{L}^{-1}$ ME), 2: Flowering plant derived from treatment without elicitors ($1.0 \text{ mg}\cdot\text{L}^{-1}$ BA).