Vol.65: e22210264, 2022
https://doi.org/10.1590/1678-4324-2022210264
ISSN 1678-4324 Online Edition

BABT
BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY

Article - Human and Animal Health

Efficient Tissue Culture and Regeneration Systems for the Medicinal Plant *Tanacetum parthenium*

Leila Abdi Saray[1]
https://orcid.org/0000-0002-6071-9186

Mohammad Ahmadabadi[*1]
https://orcid.org/0000-0002-0466-7051

Rana Valizadeh Kamran[1]
https://orcid.org/0000-0001-5288-4547

[1]Azarbaijan Shahid Madani University, Faculty of Agriculture, Department of Biotechnology, Tabriz, Iran.

Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Jane Manfron Budel

Received: 23-Apr-2021; Accepted: 11-Sep-2021.

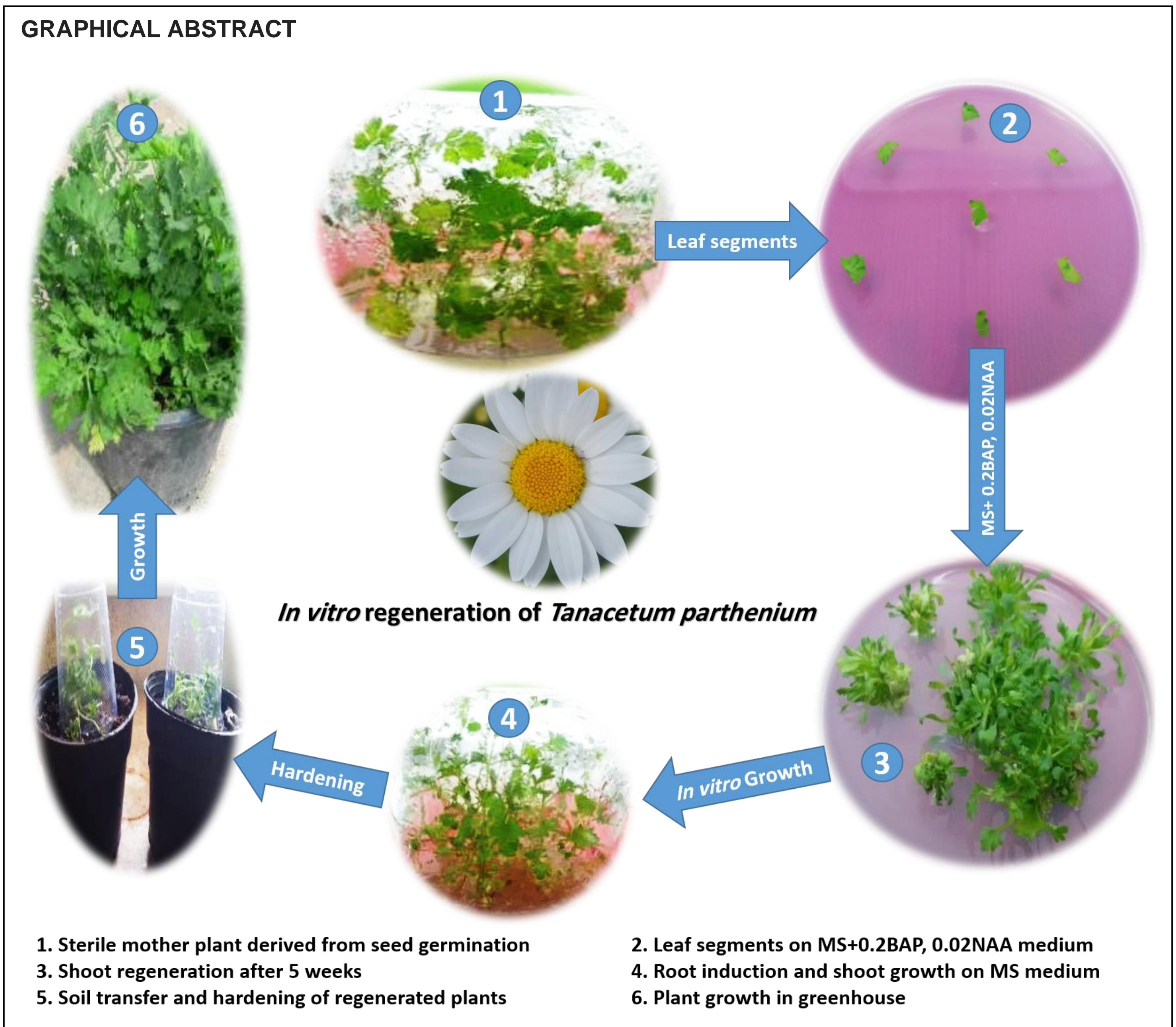
*Correspondence: ahmadabadiir@yahoo.com; Tel.: +98-41-34327572 (M.A.)

HIGHLIGHTS

- Highly efficient regeneration protocols are reported for *Tanacetum parthenium.*
- The highest regeneration was obtained in leaf explants on 0.2 BAP and 0.02 NAA (mg L^{-1}).
- The protocols are fast and reliable, suitable for rapid propagation of Feverfew.
- *GUS* reporter gene was transiently transformed to leaf tissue using biolistic method.

Abstract: *Tanacetum parthenium* L. Schultz-Bip. is an important medicinal plant with valuable secondary metabolites such as parthenolide. Due to the low amount of active parthenolide in plant tissues, the establishment of cell and plant lines with larger amounts of this metabolite through genetic engineering is essential for cost-effective production at the commercial level. Having a reliable tissue culture and regeneration protocol is critical to the success of plant genetic engineering. In this study, we report reliable *in vitro* shoot regeneration protocols for *T. parthenium* from leaf and internode explants. Using MS as the basal medium, among the 16 hormonal combinations tested, the highest regeneration rate from leaf fragments (more than 98%) was obtained on medium containing 0.2 mg L^{-1} BAP cytokinin and 0.02 mg L^{-1} NAA auxin. Also, internodes showed the highest percentage of regeneration (85%) in auxin-free medium containing 0.2 mg L^{-1} BAP. For both explants, the highest number of regenerated shootlets per explant was observed in the same media as above. Regenerated plants efficiently performed root formation and acclimatization to greenhouse condition. Transient expression of GUS gene in leaf explants via gene gun method proved their suitability for use in gene transfer experiments for molecular breeding programs.

Keywords: *Tanacetum parthenium*; Feverfew; Tissue culture; Shoot regeneration; Micropropagation.



INTRODUCTION

Tanacetum parthenium L. Schultz-Bip. (Feverfew) is a perennial, diploid herbaceous plant (2n = 2x = 18), belonging to the Asteraceae family. This plant which is native to Asia and has a wide distribution in Europe and the Mediterranean (1), is a valuable medicinal plant and is used in traditional medicine as a fever reducer, and in the treatment of certain diseases such as skin disorders and urinary and genital problems. In addition, research has shown that Feverfew essential oil has antimicrobial (2) and anti-cancer (3) properties. At present, the plant is mostly used to prevent migraines, which is attributed to its main active ingredient, parthenolide (4, 5). This compound accumulates specifically in relatively small amounts in the superficial bilobed glands of the plant (6).

The parthenolide level has been reported to vary largely in different tissues, ranging from nearly 5 mg g^{-1}FW in flowers to zero in root tissue (7). This amount has been estimated less than 1 mg g^{-1}FW in leaf and stem tissues (7). Establishing an efficient tissue culture and regeneration protocol for *T. parthenium*, in addition to providing the molecular modification requirements of this plant through genetic engineering, may provide a good opportunity to produce more parthenolide through micropropagation under controlled culture conditions. Despite the widespread use of Feverfew as a traditional medicine, little attention has been paid to tissue culture, micropropagation and genetic engineering in this plant. There are only a few previous reports on Feverfew tissue culture which are mainly focused to produce parthenolide (8-10). Although an increase in parthenolide production has been reported with the use of some elicitors in Feverfew cell suspension cultures (9, 10), these amounts are still a long way from the commercial and low-cost production of this important metabolite. One of the most important ways to increase the production of secondary

metabolites is to identify key enzymes in their synthetic pathway and regulate their expression through genetic engineering (11). A germacrene A synthase (TpGAS) and an (E)-b-caryophyllene synthase (TpCarS) have been reported as key enzymes in parthenolide biosynthesis pathway (7), expression of which has been shown to be correlated with parthenolide levels in different tissues of Feverfew (12).

An efficient tissue culture system is essential for successful plant molecular breeding through genetic engineering (13). In addition, tissue culture systems can be used in basic research to understand plant evolution (14), study gene function (15), commercial propagation (16), and germplasm maintenance (17). The latter case has become very important in recent years, due to the widespread use of herbal medicines and the increased risk of important medicinal species extinction. In this study, by intensive evaluation of different ratios of selected auxin and cytokinin hormones, the shoot regeneration response of *T. parthenium* from leaf and internode explants was investigated. Also, since the gene transfer system for this plant has not been reported so far, transient transformation and expression of *GUS* reporter gene in leaf tissue was evaluated.

MATERIAL AND METHODS

Seed disinfection and establishment of sterile culture

Seeds of *Tanacetum parthenium* L. (provided by Pakanbazr Seed Company, Isfahan-Iran) were sterilized using 70% ethanol for 1 min, and then 6% sodium hypochlorite solution for 3 min. After 3 to 5 times washing with sterile distilled water, the seeds were cultured in glass jar vessels containing MS basal medium. Three-week-old plants were used for regeneration experiments (Figure 1).

Explant preparation and hormonal combinations of the culture media

Leaf and internode explants prepared from 3-week-old plants were used as starting materials to evaluate tissue culture and regeneration response (Figure 1a). Leaf explants were prepared in the size of about 3 mm^2 (Figure 1b), and internodes were cut in about 3 mm fragments using a sharp scalpel (Figure 1d). Seven pieces of each explant type were cultured at equal intervals in a 10 cm in diameter petri dishes containing about 35 ml of MS medium containing different concentrations of hormones (Figure 1b,d). In this study, 16 hormonal combinations of NAA auxin and BAP cytokinin at 4 levels (0, 0.02, 0.2, and 2 mg L^{-1}), were examined as a factorial experiment based on a completely randomized design with 4 replicas. The pH was adjusted to 5.8 and media were solidified using 8 g L^{-1} agar (Merck KGaA, Darmstadt, Germany). The cultures were placed in a growth chamber with a diurnal cycle of 16 hours of light (50 µE) and 8 hours of darkness at 25 °C. After 4-5 weeks of culture, shoot regeneration was evaluated in explants.

Root induction and acclimatization of regenerated plants

Root induction from regenerated plants was performed in hormone-free MS basal medium. To transfer the plants to the greenhouse environment (16/8 hour light/dark cycle, with a light intensity of 120-150 µE and 22-26 °C), the roots were thoroughly washed with tap water and cultured in a mixture of soil and perlite (1:1 ratio) followed by transferring to the greenhouse for adaptation and growth.

Transient transformation of *GUS* gene to leaf explants

The pFF19G vector (18), which contains the *GUS* gene under the control of the promoter and terminator of the 35S gene of cauliflower mosaic virus (CaMV), was used to evaluate the efficacy of gene transfer and expression to leaf explants by gene gun method. For gene transfer, young leaves of 3-week-old sterile plants were placed on the Adaxial side in the center of a petri dish containing MS medium (19). Plasmid-coated gold particles (1 µm in diameter) were bombarded on the leaves using a Bio-Rad Helium-driven PDS-1000/He particle gun (Bio-Rad, USA).

Histochemical GUS staining

For histochemical GUS staining, bombarded leaves with pFF19G-coated particles, after 24 h incubation at 28 °C, were incubated overnight in GUS staining solution [1 mM EDTA (pH=8), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 200 mM potassium phosphate (pH=7.0), 1% Triton-X-100, 1 mg mL^{-1} X-Gluc], at 37 °C. To remove leaf pigments, stained leaves were incubated for one hour in 50% ethanol followed by incubation in pure ethanol for several hours at room temperature.

Statistical analysis of data

Statistical analysis of data was performed based on a factorial experiment using MSTAT-C software, and mean comparison was performed with Duncan's multiple range test.

RESULTS

Seed germination and establishment of sterile cultures

Germination of Feverfew seeds and their growth to produce sterile mother plants was performed on MS medium after about 2 to 3 weeks with very high efficiency (nearly 100%) (Figure 1a). These results show that in addition to high seed vigor, disinfection method and culture medium used in this study, is suitable for this plant and its use is recommended for future experiments.

The effect of hormonal combinations in culture medium on Feverfew shoot regeneration

After 5 weeks of *in vitro* culture of leaf and internode explants on MS medium containing different concentrations of NAA and BAP, the regeneration response of explants was evaluated. The results showed that there are significant differences in shoot regeneration potential of both explants among different media (Table 1). The highest regeneration rate for internode explant (85%) was obtained on the MS medium containing 0.2 mg L^{-1} BAP and without auxin (Table 1; Figure 1e). For leaf explant, the highest regeneration rate (more than 98%) was observed on MS medium containing 0.2 mg L^{-1} BAP and 0.02 mg L^{-1} NAA (Table 1; Figure 1c).

Table 1. Mean comparison for the shoot regeneration of *Tanacetum parthenium* L. in *in vitro* culture.

Hormonal combinations	Regeneration rate (%)		Number of regenerated plants	
	Internode	Leaf	Internode	Leaf
0 BAP + 0 NAA	8.125^{I-K}	5^{L-N}	3.25^{c}	2^{c}
0 BAP + 0.02 NAA	5^{L-N}	2.5^{N}	2^{c}	1^{c}
0 BAP + 0.2 NAA	5.625^{K-M}	6.25^{J-M}	2.25^{c}	2.5^{c}
0 BAP + 2 NAA	3.75^{MN}	11.875^{H}	1.5^{c}	4.75^{c}
0.02 BAP + 0 NAA	8.75^{IJ}	6.25^{J-M}	3.5^{c}	2.5^{c}
0.02 BAP + 0.02 NAA	10.625^{HI}	10.625^{HI}	4.25^{c}	4.25^{c}
0.02 BAP + 0.2 NAA	30.625^{D}	15.625^{FG}	12.25^{b}	6.25^{bc}
0.02 BAP + 2 NAA	3.75^{MN}	8.125^{I-K}	$1/5^{c}$	3.25^{c}
0.2 BAP + 0 NAA	85^{B}	39.375^{C}	34^{a}	15.75^{b}
0.2 BAP + 0.02 NAA	28.75^{D}	98.125^{A}	11.5^{b}	39.25^{a}
0.2 BAP + 0.2 NAA	16.25^{FG}	10.625^{HI}	6.5^{bc}	4.25^{c}
0.2 BAP + 2 NAA	17.5^{EF}	14.375^{G}	7^{bc}	5.75^{c}
2 BAP + 0 NAA	16.25^{FG}	8.125^{I-K}	6^{bc}	3.25^{c}
2 BAP + 0.02 NAA	18.75^{E}	10^{HI}	7^{bc}	4^{c}
2 BAP + 0.2 NAA	17.5^{EF}	11.875^{H}	7^{bc}	4.75^{c}
2 BAP + 2 NAA	5.625^{K-M}	6.875^{J-L}	2^{c}	2.75^{c}

Different letters in superscript show significant difference based on Duncan's multiple range test in corresponding feature.

It should be noted that the highest number of shoots per explant was obtained on the media with the highest regeneration rate (Table 1). For leaf explants, the maximum number of regenerated shoots in each explant was 39.25 on average, and for internode explants, 34 shoots. Regenerated plants easily produced roots on hormone-free MS medium, followed by successful adaptation and growth in greenhouse environment (Figure 1f). Adaptation rate to greenhouse conditions for rooted seedlings was about 90%.

Transient transformation and expression of GUS gene in leaf explants

As the leaf material showed better response to shoot regeneration *in vitro*, we then evaluated the potential of leaf tissue for gene transfer using biolistic method. Several blue spots could be observed in leaf tissue (Figure 1g), demonstrating successful transformation and expression of GUS transgene in leaf tissue.

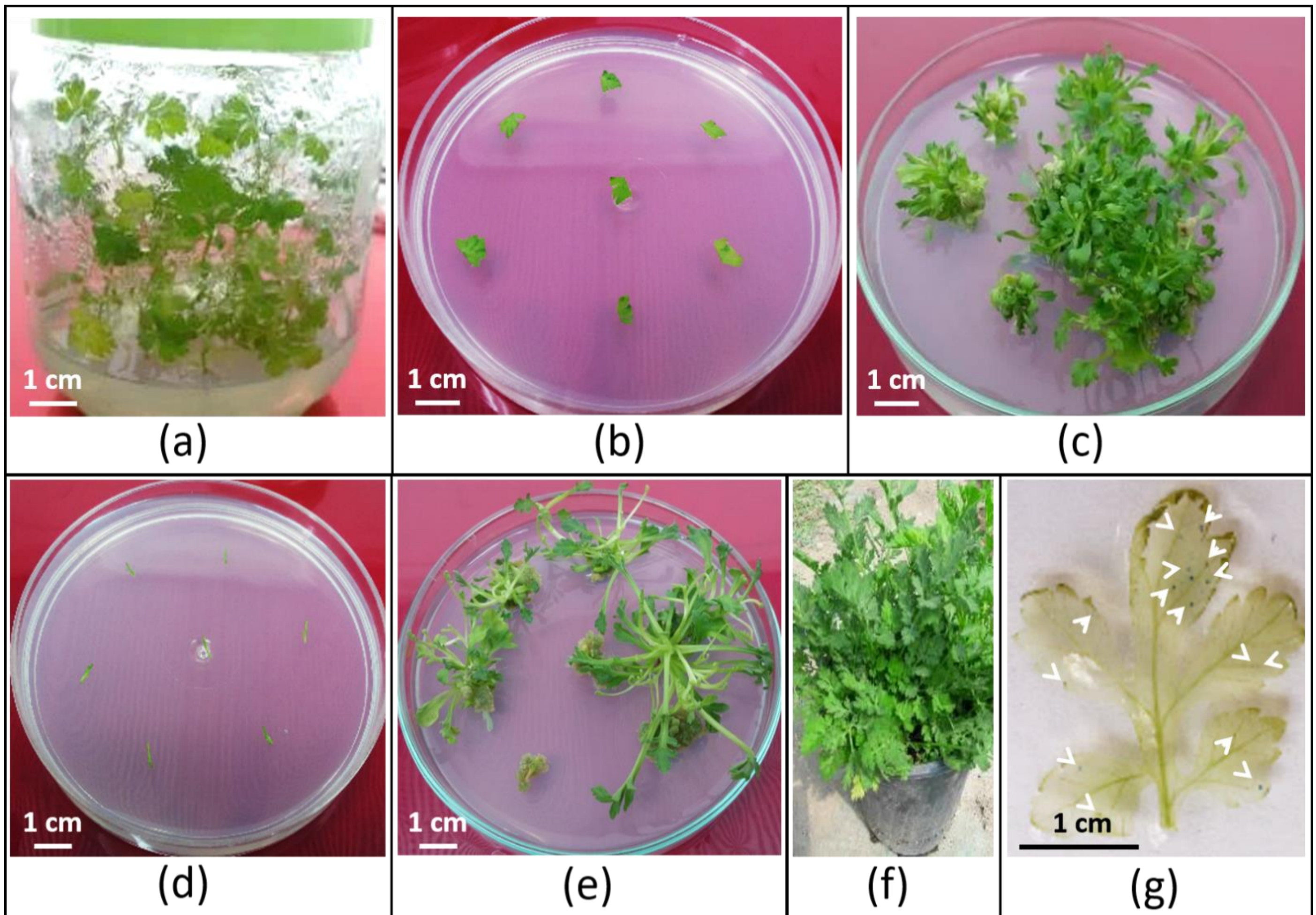


Figure 1. (a) Sterile cultures resulting from seed germination and growth in in vitro conditions after 3 weeks. (b) Leaf segments placed on regeneration medium (MS medium containing 0.2 mg L^{-1} BAP and 0.02 mg L^{-1} NAA). (c) Shoot regeneration from leaf explants after a 5 week culture on regeneration medium. (d) Internode explants on regeneration medium. (e) Shoot regeneration from internode segments after a 5 week culture on regeneration medium. (f) Regenerated plants adapted to greenhouse conditions. (g) An example of the results obtained from the transient transformation and expression of the *GUS* gene in leaf tissue using biolistic transformation system. Blue spots indicating GUS expression are shown in the figure by arrow-heads.

DISCUSSION

The members of the Asteraceae family include several medicinal and endangered species with valuable pharmaceutical properties against a variety of diseases (20). *Tanacetum parthenium* is an important perennial medicinal herb with a wide distribution worldwide (1). Despite its great implications at traditional medicine, efficient tissue culture and regeneration protocols for *T. parthenium* have not been reported so far. Reliable tissue culture systems can provide important tool for rapid *in vitro* propagation as well for basic research. Due to the fact that leaf and internode explants can be easily derived from sterile plants, therefore, the use of these explants as starting material in most plant tissue culture protocols is preferred. So far, regeneration protocols from leaf explant has been developed for a number of taxa in this family, including *Senecio candicans* DC. (21), *Plectranthus barbatus* Andrews (22), *Leuzea carthamoides* (Willd.) Iljin (23), *Sphaeranthus indicus* L. (24), *Echinacea pallida* Nutt. (25), *Tagetes erecta* L. (26), *Matricaria chamomilla* L. (27) and *Achillea wilhemsii* C. Koch (28). In Feverfew, a few reports have been published on tissue culture (9, 29). However, in most of these reports, the main focus is on parthenolide production and no intensive study has been carried out on optimization of shoot regeneration. In this study, the basal MS medium and different concentrations of two hormones, NAA (0, 0.02, 0.2 and 2 mg L^{-1}l) and BAP (0, 0.02, 0.2 and 2 mg L^{-1}), were used to establish efficient protocols for Feverfew *in vitro* regeneration that is suitable for use in genetic engineering and vegetative propagation.

After 5 weeks of culture, the results showed that there is a significant difference in the response of explants to different hormonal combinations (Table 1). The highest regeneration rate for internode explant

(85%) was obtained on the medium containing 0.2 mg L^{-1} BAP and without auxin (Table 1; Figure 1e), while for the leaf explant, the highest regeneration rate (more than 98%) was observed on medium containing 0.2 mg L^{-1} BAP and 0.02 mg L^{-1} NAA (Table 1; Figure 1c). These results may be related to differences in internal auxin content between internode and leaf tissue. Most of the long-distance transmission of auxin occurs through the phloem vessels (30), and this factor can lead to an increase in the auxin content of stem internodes. Whereas, auxin transport in leaf tissue is carried out polarly using molecular carriers (30). In Feverfew, the auxin content of the leaves seems to be lower than that of the stem tissue, and therefore, the presence of exogenous auxin at low levels is required to establish hormonal balance in this explant to stimulate shoot formation. An increase of auxin by 0.2 mg L^{-1} severely affected the regeneration of leaf tissue, so that the regeneration rate decreased by about 90%. The regeneration rate in the internode explant, however, decreased with a lower slope by increasing auxin concentration, compared to the leaves (Table 1). This may also be related to the faster translocation of hormones in the internode tissue (via vessels) than in leaf tissue (via polar transmission through carriers), that may lead to relatively rapid hormonal balance partly due to the faster exchange of internal and external hormones in internode explants.

In most of the regeneration protocols developed for different members of Asteraceae family, BAP is used as the exogenous cytokinin in the range of 0.2 to 2 mg L^{-1}, albeit in combination with other plant growth regulators (22-28). In *Senecio candicans*, for example, the best results were obtained using MS medium supplemented with 2 mg L^{-1} BAP in combination with 2 mg L^{-1} IAA and 3 mg L^{-1} NAA (21). Regeneration in *S. indicus* was the most efficient when two different cytokinins including BAP and kinetin were used together (24). Overall, due to the large differences among Asteraceae family members, in vitro regeneration and micropropagation protocols should be standardized for important members of this family (31).

The workflow for complete procedure (about 13-16 weeks) of our optimized regeneration protocols can be summarized as follows:

- Seed sterilization by 70% EtOH for 1 min, followed by 6% NaOCl for 3 min
- Seed wash with sterile distilled water for 3 to 5 times, followed by culture on hormone-free MS basal medium for germination (2 weeks)
- Plant growth in *in vitro* condition to provide sterile mother plants (3 weeks)
- Explant preparation: 3 mm^2 pieces for leaf samples, and 3 mm long segments for internodes
- Culture 7 explants with equal distances in Petri dishes (10 cm in diameter) containing 35 ml MS medium complemented with (i) 0.2 mg/l BAP and 0.02 mg/l NAA for leaf explants, and (ii) only 0.2 mg/l BAP for internodes
- Incubate cultures in growth chambers with diurnal cycle of 16 h light (50 µE) and 8 h darkness, constant temperature of 25 °C, and cabin humidity of 70% (5-6 weeks)
- Transfer regenerated shoots on shoot elongation and root induction medium (hormone-free MS basal medium) (2-3 weeks)
- Wash roots with tap water and transfer plants on soil (1:1 ratio mixture of pot soil and perlite) for acclimatization (1-2 weeks)

Note that if the time for establishment of sterile mother plants is excluded, the time for performing whole shoot regeneration procedure is reduced to around 8-11 weeks, which is very fast and cost-effective.

As the leaf tissue showed better performance to shoot induction *in vitro*, we then evaluated its potential to receive foreign genes using particle bombardment method. To this end, pFF19G vector (18) containing *GUS* reporter gene was used. On average, about 50 blue spots were observed on each shot leaf (about 2 cm^2 in size) with 20× magnification under a stereo microscope, examples of which can be seen in Figure 1g. It should be noted that the displayed image has been taken without magnification, in which case usually only parts of the sample where several transgenic cells are gathered together and have a high staining intensity, are visible. In this study, 1 micrometer in diameter gold particles, 1100 psi launch pressure, 8 cm sample distance from the stopping screen, -0.8 bar vacuum level, and mono adapter were used. Successful expression of GUS in an acceptable number of leaf tissue cells showed that these parameters are suitable for the transfer and expression of foreign genes to leaf tissue by biolistic transformation system.

CONCLUSION

In this study, we developed efficient protocols for *T. parthenium* regeneration from leaf and internode explants. The efficiency of these protocols was very high and shoots were formed in a short time and with a

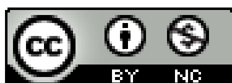
high frequency through direct organogenesis and sometimes through callus intermediates (~20%). In addition to the genetic engineering experiments, these protocols can be used in the vegetative micropropagation of this plant to produce parthenolides. Production of acceptable parthenolide levels in tissue culture obtained shoots has been previously reported (9, 29). In addition, in this study, we demonstrated the potential of leaf explants for transformation and expression of foreign genes using transient expression of the *GUS* reporter gene.

Funding: This research was funded by the Azarbaijan Shahid Madani University.
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

1. Polatoglu K, Demirci F, Demirci B, Gören N, Baser KHC. Antibacterial activity and the variation of *Tanacetum parthenium* (L.) Schultz Bip. essential oils from Turkey. J Oleo Sci. 2010;59:177-84.
2. Izadi Z, Esna-Ashari M, Piri K, Davoodi P. Chemical composition and antimicrobial activity of Feverfew (*Tanacetum parthenium*) essential oil. Int J Agric Biol. 2010;12(5): 759-63.
3. Lesiak K, Koprowska K, Zalesna I, Nejc D, Duchler M, Czyz M. Parthenolide, a sesquiterpene lactone from the medical herb feverfew, shows anticancer activity against human melanoma cells in vitro. Melanoma Res. 2010;20(1):21-34.
4. Pittler MH, Vogler BK, Ernst E. Feverfew for preventing migraine. Cochrane Database Syst Rev. 2000(3):CD002286.
5. Vogler BK, Pittler MH, Ernst E. Feverfew as a preventive treatment for migraine: a systematic review. Cephalalgia. 1998;18(10):704-8.
6. Banthorpe DV, Brown GD, Janes JF, Marr IM. Parthenolide and other volatiles in the flowerheads of *Tanacetum parthenium* (L.) Schultz Bip. Flavour Fragr J. 1990;5(3):183-5.
7. Majdi M, Liu Q, Karimzadeh G, Malboobi MA, Beekwilder J, Cankar K, et al. Biosynthesis and localization of parthenolide in glandular trichomes of feverfew (*Tanacetum parthenium* L. Schulz Bip.). Phytochemistry. 2011;72(14-15):1739-50.
8. Banthorpe DV, Brown GD. *Tanacetum parthenium* (L.) Schultz Bip. (Feverfew): In vitro culture and prospects for the production of parthenolide. In: Bajaj YPS, editor. Medicinal and Aromatic Plants V. Berlin, Heidelberg: Springer Berlin Heidelberg; 1993. p. 361-72.
9. Brown AMG, Lowe KC, Davey MR, Power JB. Feverfew (*Tanacetum parthenium* L.): tissue culture and parthenolide synthesis. Plant Sci. 1996;116(2):223-32.
10. Pourianezhad F, Rahnama H, Mousavi A, Khosrowshahli M, Mafakheri S. Parthenolide production in cell suspension culture of feverfew. Bioresour Bioprocess. 2019;6(1):23.
11. Dasgupta A, Chowdhury N, De RK. Metabolic pathway engineering: Perspectives and applications. Comput Methods Programs Biomed. 2020;192:105436.
12. Majdi M, Abdollahi M, Maroufi A. Parthenolide accumulation and expression of genes related to parthenolide biosynthesis affected by exogenous application of methyl jasmonate and salicylic acid in *Tanacetum parthenium*. Plant Cell Rep. 2015;34.
13. Loyola-Vargas VM, Ochoa-Alejo N. An introduction to plant tissue culture: advances and perspectives. Methods Mol Biol. 2018;1815:3-13.
14. Firn RD, Sharma N, Digby J. Physiology, growth and development of plants and cells in culture—the way ahead. Lumsden PJ, Nicholas JR, Davies WJ, editors. Dordrecht: Springer; 1994. 430 p.
15. Deng Y, Johnson DR, Guan X, Ang CY, Ai J, Perkins EJ. In vitro gene regulatory networks predict in vivo function of liver. BMC Syst Biol. 2010;4:153.
16. Kumar N, Redd MP. In vitro plant propagation: a review. J For Sci. 2011;27:61–72.
17. Fay MF. Conservation of rare and endangered plants using in vitro methods. In Vitro Cell Dev Biol - Plant. 1992;28(1):1-4.
18. Timmermans MCP, Malig P, Viera J, Messing J. The pFF plasmids: Cassettes utilising CaMV sequences for expression of foreign genes in plants. J Biotechnol. 1990;14:333-44.
19. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15:473-97.
20. Heywood V, Brummitt, Culham A, Seberg O. Flowering plant families of the world. Richmond Hill (Canada): Firefly Books; 2007. 424 p.
21. Hariprasath L, Jegadeesh R, Arjun P, Raaman N. In vitro propagation of *Senecio candicans* DC and comparative antioxidant properties of aqueous extracts of the in vivo plant and in vitro-derived callus. S Afri J Bot. 2015;98.
22. Thangavel P, Britto S, Senthilkumar S. Adventitious shoot regeneration from leaf explants of the valuable medicinal herb *Plectranthus barbatus* Andrews. Afr J Biotechnol. 2011;10:8562-9.
23. Akhtar Z, Babaei A, Reza O, Elham D. Study on callus induction and plant regeneration of *Leuzea carthamoides* via tissue culture system. J Med Plant Res. 2014;8:260-8.

24. Rajesh Y, Aileni M, Anil Kumar V, Kokkirala VR, Umate P, Abbagani S. Direct shoot regeneration from mature leaf explants of *Sphaeranthus indicus* L., a multipurpose medicinal plant. J Phytol. 2010;2:5-11.
25. Wang H-M, Jeng S-T, To K-Y. In vitro regeneration, Agrobacterium-mediated transformation, and genetic assay of chalcone synthase in the medicinal plant *Echinacea pallida*. Plant Cell, Tissue Organ Cult. 2017;130:117-30.
26. Misra P, Datta SKD. Direct differentiation of shoot buds in leaf segments of white marigold (*Tagetes erecta* L.). In Vitro Cell Dev Biol - Plant. 2001;37:466-70.
27. Sayadi V, Mehrabi A, Saidi M, Nourollahi K. In vitro culture and callus induction of chamomile (*Matricaria chamomilla* L.) explants under different concentrations of plant growth regulators. Int J Biosci. 2014;4:206-2011.
28. Pashmforoosh N, Ahmadabadi M. Efficient in vitro tissue culture and regeneration systems for yarrow (*A. wilhelmsii* C. Koch). Zeitschrift fur Arznei- & Gewurzpflanzen. 2019;23 (3):134-7.
29. Stojakowska A, Kisiel W. Production of parthenolide in organ cultures of feverfew. Plant Cell, Tissue Organ Cult. 1997;47(2):159-62.
30. Morris DA, Friml J, Zažímalová E. The transport of auxins. In: Davies PJ, editor. Plant hormones: Biosynthesis, signal transduction, action! Dordrecht: Springer Netherlands; 2010. p. 451-84.
31. Abraham J, Thomas D. Recent advances in Asteraceae tissue culture. In: Anis M, Ahmad N, editors. Plant tissue culture: Propagation, conservation and crop improvement. Singapore: Springer; 2016. p. 161-95.



© 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY NC) license (https://creativecommons.org/licenses/by-nc/4.0/).