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Micro-propagation and Appraisal of Hereditary Constancy through RAPD Analysis of *Eclipta alba*: A Significant Curative Plant Species

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Authors' contributions

This work was carried out in collaboration among all authors. Author PP designed the protocols and wrote the first draft of the manuscript. Author SSS designed the study, wrote the protocols, analyzed the study and guided the research investigated and edited the manuscript. Authors PP and SSS managed the literature searches. Authors PP, SSS and SS performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To set up a prudent and standard protocol to frame *in vitro* recovery of *Eclipta alba*, a therapeutically significant plant, from nodal district by shoot culture initiation.
Study Design: Our examination was designed arbitrarily and treatment had threefold samples of 30 explants each. All components including number of shoots per explants, shoot length, root length and so forth were determined from the complete number of explants from which cultures

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were set up. The contaminated cultures were not regarded. Data were analyzed by using the "Statistical Analysis Software (SAS)" and all the information were directed to one-way classified analysis of variance (ANOVA) and means of treatments were compared based on Turkey's honestly significant different test (HSD) at "0.01 probability level" using SAS.

Place and Duration of Study: Department of Botany, College of Basic Science and Humanities, and Department of Agricultural Biotechnology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar, between July 2019 and December 2020.

Methodology:

- Surface sterilization and preparation of explants
- Preparation of Culture medium and culture conditions for *in vitro* plant regeneration
- Acclimatization of regenerated plantlets
- Assessment of genetic fidelity through PCR based RAPD investigation

Results: BAP is the most effective Plant Growth Regulator for regeneration in *E. alba* and MS medium supplemented with BAP and Kin the foremost successful combination for multiple shoot proliferation. Soil: vermicompost (1:1) mixture has shown the best promising result of hardening. All the plantlets developed through *in vitro* culture have attended genetic stability with the mother plant. There is no genetic variation shown through RAPD analysis.

Conclusion: It is a successful attempt to generate a quick, economic and reproducible protocol for the micropropagation of *E. alba* without any genetic alternation and maintaining homologous clonal propagation.

Keywords: In vitro micro-propagation; Eclipta alba; genetic fidelity; RAPD; BAP; NAA.

1. INTRODUCTION

Biotechnological approaches nearby plant tissue culture offers an enormous apparatus for improving quality and measures of yields [1]. It can also provide steady planting material for the generation of new variety. An expanded enthusiasm for in-vitro culture strategies opens up scopes for mass increase and germplasm protection of uncommon, imperiled, and compromised therapeutic plants [2]. The technique further comes up with opportunities for genetic improvement by enhanced drug yield [3] higher disease resistance against virus, fungi and bacteria and most importantly a higher aptitude of abiotic tolerance (Jain *et al.,* 2001). All this has been achieved by the accustomed techniques of tissue culture such as organogenesis and somatic embryogenesis. The recent emanation of cell suspension culture for enormous scope creation of auxiliary metabolites has become another incredible asset of in vitro culture [4]. The same technique has been reported in *Eclipta alba* [5] as well. Among all, micropropagation through organogenesis has end up being the most dependable method of recovery in the region of tissue culture. Anyway, hardly any reports have been recorded on commercial large-scale engendering of *Eclipta alba* utilizing nodal explants [6,7] and Defeat et al., 2006) till date.

Genetic instability is a major problem in the course of commercial micro propagation (Lázaro-Castellanos *et al.,* 2018). A suitable conservation program requires genetic characterization of plants and their assessment for genetic stability. However, plant tissue culture creates large spectrum of fluctuation in recovered plants [8]. Induction of such variations is employed by manipulation of growth media, explants variants, regeneration mode, culture conditions and plant growth regulations through mutagenesis or somatic variation [9-11]. All such variations put a question mark on the hereditary analogy of the regenerated plants.

At the same time, many reports also have archived the lower chances of genetic changes and variability, if an organized tissue like meristem is chosen for direct plant regeneration (Salvi et al., 2002; [10,11]. Further genetic stability can be inspected by a comparison of fidelity among mother plant to micropropagated plants. Bairu et al., [12] explains about all such conformations by several methods ranging from morphological, physiological, biochemicals to molecular level. Among every such strategy molecular markers are routinely being utilized for investigation of hereditary relations, populace hereditary qualities and hereditary portrayal among taxa of different ranks. [13,14,9,10]. RAPD (Random Amplified Polymorphic DNA) markers are always being popular among the scientific Diasporas for its enormous use in appraisal of all such studies related to diversity. It has been extensively utilized for evaluation of

genetic diversity in a few plant types for example- Triticum (Cao et al., 1999), Glycine max (Hotmans et al., 2004), Geranium [15] and furthermore in in vitro recovered propagules in various plants as detailed by Goel et al., [14] Kishor et al., [9] Rai et al., [16] Das et al., [11] and Sharma et al., [17] Artichoke [18] Bamboo [19] Jackfruit [20]. Due to its comparative easy management, cost effectiveness together with high reproducibility abilities, it is preferred over other molecular markers like ISSR, SSR, RFLP, AFLP and so forth [21,22].

Eclipta alba (L.) is a little, spreading, yearly restorative herb with white blossom head in the family Asteraceae and is regularly known as false daisy (in English) and Bhringraj (in Hindi). It is nearby to the tropical and subtropical locale of the world. In India it is especially found as a weed mostly in countryside. The ethno-information portrays the utilization of the plant in the treatment of liver maladies, chest contamination, short-sidedness, skin issue, untimely turning grey of hair and furthermore exceptionally useful for memory improvement. [23] Ray and Bhattacharya, 2010[24]. The leaves of *E. alba* contain many bioactive compounds like stigmasterol, a-terthiemymethanol, wedelolactone, demeathylwedelolactone and olemethylweedelolactone-7 glucoside etc. [25]. The medicinal properties of the plant manifest a wide range of attribute from anti-inflammatory to bronchodilator [26] hepato-protective to anti-hyperglycemic [27] anti-viral, anti- bacterial, spasmogenic, hypo-tensive, analgesic to anti-oxidant [17] Karthikumar et al., 2007 [28] and Yadav et al., [29] cardio protective [30] to anti-cancerous [29] and anti-diabetic [31] to anti-anaphylactic [32] impacts. Hence its mass clonal propagation is a prerequisite step for large scale phytochemicals extraction by pharmaceutical industries to fulfil the global market demand. Thus, it is imperative to build up an effective micropropagation technique for E. alba for quick dispersal of recognized predominant clones. So, the current investigation intends to develop a standardized convention for in-vitro mass clonal proliferation of E. alba, its acclimatization and evaluation of hereditary loyalty of recovered plants through RAPD.

2. MATERIALS AND METHODS

2.1 Explant Source, Surface Sterilization and Preparation of Explants

Nodal segments of the *E. alba* excised from the field grown plants from farm of Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India were used as a source of explant. Elongated shoots (each having one node) were thoroughly washed with sterile double distilled water to clear away the sand and soil particles and deliquated with 2% (v/v) cetrimide (a blend of various quaternary ammonium salts counting cetrimonium bromide) and three to four drops of Polysorbate-20 (MP Biomedicals, US) trailed by again washing with clean twofold refined water for the removal of all the detergents and disinfectants. A short time later, surface disinfection was completed under aseptic conditions in laminar wind current in 0.05% mercuric chloride prepared afresh as long as 3-5 minutes. Then it was washed thoroughly with sterile distilled water for four to multiple times. The nodal region of the stem section was cut transversely into pieces of 1-3mm thickness. These bits of nodes were utilized as explants for plant regeneration.

2.2 Culture Medium and Culture Conditions for *In vitro* Plant Regeneration

In vitro micro propagation was finished with the nodal explants by refined on MS (Murashige and Skoog's, 1962) basal medium enhanced with 3% (w/v) sucrose and various concentrations of development controllers like BAP, Kin (Kinetin), NAA (Sigma Chemical Co., St. Louis, MO, USA). The pH was changed in accordance with 5.8± 0.02 with 0.1 N NaOH or 0.01 N HCl preceding autoclaving at 121 ◦C for 15 min. Agar 0.8% (w/v) was added to solidify the media (Merck India Ltd., Mumbai, India). The explants were inoculated in the MS medium after sterilization. All cultures were maintained at a temperature of 26 ± 20C, under a photoperiod of 16-h and light intensity of 40 µmol m−2 s−1(executed by cool white, fluorescent cylinders, Crompton, India). The inoculants were sub-cultured in fresh MS medium every 3-4 weeks. The culture societies were subjected to different convergence of hormonal combinations in order to ascertain the accelerated recovery from the in vitro engaged explants.

2.3 Acclimatization of Regenerated Plantlets

In vitro developed plantlets from nodal explants having very much evolved shoots and roots were moved to 2.5 cm earthen pots containing sterilised soil. Plantlets were secured with polyethylene packs to keep up formidable

moistness and sprinkled with water at time intervals. Three blends were taken e.g., only soil, soil: sand (1:1) and soil: vermicompost (1:1) for hardening. Pots containing the plantlets were managed under nearly similar reputation of temperature at 24 ± 10 C, artificial light intensity of 50 µmol m−2 s−1 around fourteen days. Short time later those were moved to sunshine for a short period of time (2–2.5 h/day) at first. The time was further extended, a tiny bit at a time till approaching the normal photoperiod. It took 2.5–3 months for establishment of the plantlets from pots to soil under field condition.

2.4 Assessment of Genetic Fidelity

To survey the hereditary devotion between the mother plant and in vitro recovered plants, 22 recovered plants were haphazardly chosen. Genomic DNA was detached from lively and fresh leaves by C-TAB convention of Doyle and Doyle (1987). A short time later the quality and convergence of DNA were inspected by agarose gel electrophoresis (0.8%) and UV-visible spectrophotometer respectively. For PCR based RAPD investigation arbitrary decamer Operon Primers (Operon Tech., Alameda, USA) broke up in twofold disinfectedT10E1 buffer, pH8.0. 30 chose preliminaries from OPA, OPD, OPC, OPN and OPAF arrangement (Operon Tech., Alameda, CA.) were utilized. Every reaction mixture has 25µl volume which contains 2.5µl of 10X PCR buffer, 2.5µl MgCl2 (1.5mM), 1µl of dNTPs 25mM each (dATP, dCTP, dGTP and dTTP), 1µl of irregular preliminary (10pM), 0.5µl of Taq DNA polymerase (3 units; Bangalore Genei Private Ltd. Bangalore India) and 1µl of 50ng of template DNA. The rest volume was adjusted by molecular grade water (MP Biomedicals, US). The amplification response was conveyed in Gene Amp PCR system 9700 (Applied Biosystems, Germany). The strengthening was acted in three phases PCR. Beginning with, denaturation of layout DNA was done at 94^0C for 5 minutes in a solitary cycle. The subsequent step was completed in 40 cycles and each cycle comprises of three thermal reading steps i.e., one minute at 94^0C for final denaturation of template, one minute at 37^0C for annealing trailed by two minutes at 72^0C for chain extension. The last expansion step comprised of just one cycle i.e., 10 minutes at 72^0C. Samples were stored at 4^0C until examination was completed. The PCR items for investigation were finished by 1.5% agarose (Seakem, FMC, USA) gels, by utilizing 1X Tris-acetic acid–ethylene diamine tetra-acetic acid (TAE) buffer and recoloured with the recolouring operator ethidium bromide solution. After electrophoresis, the gel was archived in Gel Doc 2000 (Bio-Rad, USA) for scoring the bands. The whole procedure was rehashed in any event threefold to affirm the reproducibility.

2.5 Design of Experiment and Statistical Analysis

Our examination was planned in a totally randomized way and treatment had threefold imitates of 30 explants each. All components including number of shoots per explants, shoot length, root length and so forth were determined from the complete number of explants from which cultures were set up. The contaminated cultures were not contemplated. Data were analyzed by using the “Statistical Analysis Software (SAS)” as per Swain et al., 2018. All the information were exposed to one-way classified analysis of variance (ANOVA) and means of treatments were compared based on Turkey’s honestly significant different test (HSD) at “0.01 probability level” using SAS.

3. RESULTS AND DISCUSSION

In an attempt to establish an efficient and suitable methodology for rapid micropropagation the present study also aims for large scale clonal progenies within a short period of time. So, selection of suitable explants is crucial for development and success of such methodology. As the source of explants and the regeneration mode also control the hereditary strength of the *in vitro* recovered plants [33,34] the ongoing study took on the choice of nodal segments of *E. alba* as reliable explants.

Adventitious shoot buds are induced within 1.5 weeks of culture of explants in MS medium with and without growth regulators. Again, BAP was observed effective over other growth regulators for the quantity of shoots per explants, average length of shoot and response of shoot proliferation percentage (Table 1). The adventitious buds on shoot were marked initially leaning on peripheral region of the explants (Fig. 1A and 1B), followed by the basal part of the explants (Fig. 1B).

Finally, the adventitious buds grew into clumps. A similar perception was accounted for by Ghnaya et al., [35] and Singh et al., [36] in Spilanthes acmella and Brassica napus

respectively. The response for number of shoot proliferation was significantly affected by BAP at 1% of Tukey's HSD for various convergences of BAP inspected. Greatest percentage of acclamation for shoot multiplication was found in 13.2µM, 15.4µM and 11µM which are statistically in a same group followed by 8.8µM, 4.4µM and 17.6µM (Table 1).

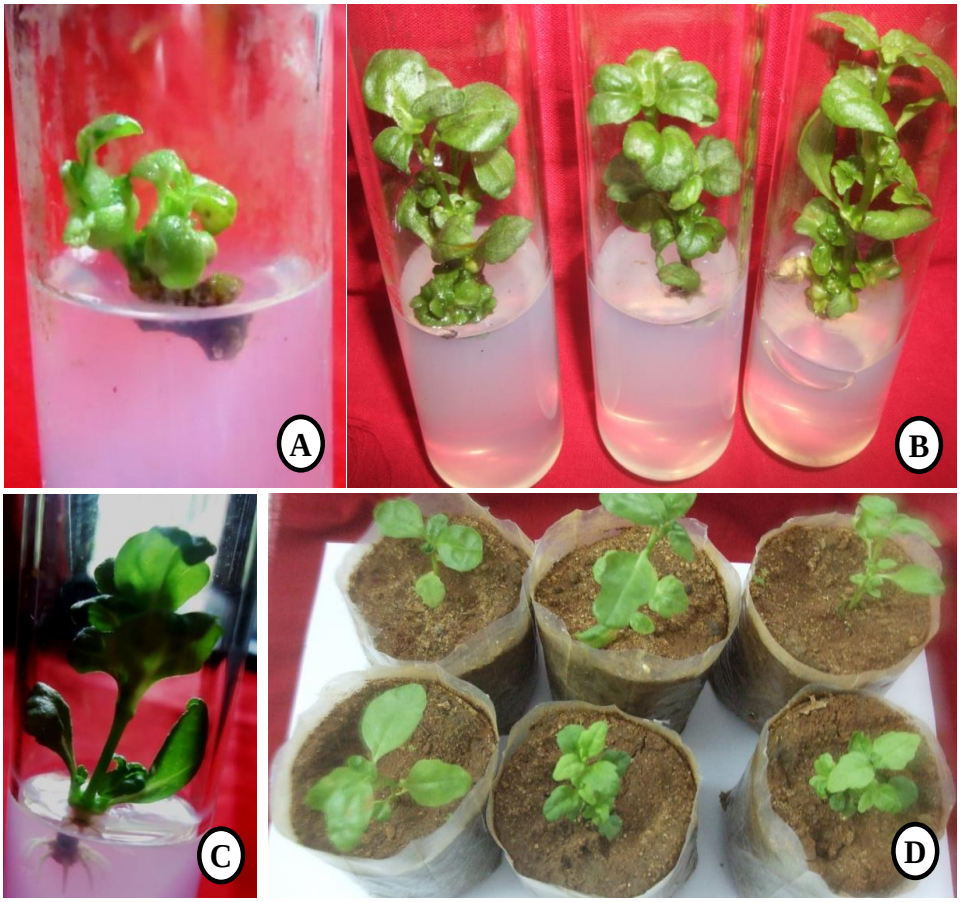

Fig. 1. *In vitro* plant regeneration of *E. alba* through nodal explants: (A) Shoot bud induction from nodal explant; (B) Induction of multiple shoot buds from basal region of explant; (C) Rooting in *in vitro* regenerated shoot on growth regulator free MS medium; (D) Acclimatised and well hardened plants of *E. alba*

Table 1. Effect of BAP on shoot multiplication from nodal explant

MS medium supplemented with BAP (µM)	Response of explant (%)	Shoots / explant	Average shoot length (cm)
-	71.00^{d}	1.90^{e}	4.50^{a}
2.2	75.83^{c}	4.33^{e}	4.10^{a}
4.4	89.33^{b}	9.00^{d}	3.80^{ab}
8.8	90.33^{b}	15.33^{c}	3.27^{bc}
11	97.33^{a}	25.00^{b}	3.00^{cd}
13.2	99.67^{a}	29.00^{a}	2.40^{de}
15.4	98.67^{a}	26.67^{ab}	2.10^{ef}
17.6	86.67^{b}	18.00^{c}	1.33^{f}
p-Value	<.0001	<.0001	<.0001
CV (%)	1.25	5.18	7.23
Tukey HSD at 1%	4.0132	3.0206	0.7997

** Means with same letter are not significantly different at p≤0.01*

Interestingly exact similar type of observation was found in case of number of shoots per explant. Result of the present investigation strongly support that shoot regeneration capacity of the explant was positively affected by BAP. A consolidated impact of streamlined concentration(13.2µM) of BAP was additionally assessed with Kin and NAA on shoot proliferation. In the thick of entire treatment; the medium of MS accommodating 13.2µM, BAP with 4.6µM and 2.3µM Kin instituted as the best effectual assembly for shoot proliferation from the explant (Table2). Besides this 6.9µM Kin may be recommended as an effective concentration for shoot multiplication as per the statistical records. Upon 4.6µM and 2.3µM Kin with 13.2µM of BAP in MS medium, response of shoot proliferation (%) shoot multiplication was 100% and 99.67% with average shoots of 36 and 32 per explant respectively [37-40]. When the mother explants were cultured on the same medium after removal of elongated shoots, average shoot number as well as shoot length increased. Moreover, application of combined BAP and NAA did not have any promising effect over the BAP to Kin combinations on the parameters studied.

Shoots recovered from nodal explants were established on the MS medium (without growth regulator) following 7-10 days of subculture from multiplication media. The average percentage of rooting moved towards 100 % around. 13-15 roots for every shoot were prompted after 3.5-4 weeks of further culture (Fig. 1C). Ray and Bhattacharya [39-42] had communicated similar reports propounding the development of roots on shoots in medium, free of growth regulators. The plantlets having root were acclimatized and established in the soil successfully (Fig. 1D). The percentage of survival was observed to be highest (i.e. 93%) in soil: vermicompost (1:1) mixture as compared to soil and soil sand mixture (Fig. 2). The regenerated plantlets then were tested for homogeneity with the mother plant.

30 different RAPD primers were tried out under the standardized PCR conditions. Out of these only 10 primers delivered resolvable, reproducible and scorable bands. Intensification consequence of all the 10 primers were monomorphic in nature over all the recovered plantlets and the mother plant. The extent of intensified groups created by the primers reached out from 225 to 2100 bp and the amount of expound results noticed from 2 to 6. Fig. 3 shows RAPD enhancement designs procured with primers OPC-20 (Fig. 3A) and OPN-11 (Fig. 3B). This sort of banding configuration approves the hereditary constancy of all the recovered plants with the original plant taken for regeneration as well as themselves [43-46]. *In vitro* recovered plants are presented to various

Table 2. Combined effect of optimized concentration of BAP (13.2µM) with KIN or NAA on shoot multiplication from nodal explant

Optimum concentration of BAP with plant growth regulators (µM)		Response of explant (%)	Shoots/ explant	Average shoot length (cm)
KIN	**NAA**			
-	-	99.67^{a}	26.00^{cd}	2.43^{a}
2.3	-	99.67^{a}	32.00^{b}	2.33^{ab}
4.6	-	100.00^{a}	36.00^{a}	2.13^{bc}
6.9	-	96.00^{ab}	31.00^{b}	1.93^{c}
9.2	-	93.00^{bc}	29.00^{bc}	1.60^{d}
11.5	-	90.00^{cd}	26.00^{cd}	1.63^{d}
13.8	-	86.33^{d}	24.00^{d}	1.53^{d}
-	2.6	78.00^{e}	19.00^{e}	0.87^{e-g}
-	5.3	71.67^{f}	17.67^{e}	1.10^{e}
-	7.9	69.67^{f}	17.67^{e}	1.00^{ef}
-	10.6	63.33^{g}	16.00^{ef}	0.87^{e-g}
-	13.3	61.00^{g}	14.00^{fg}	0.73^{fg}
-	15.9	51.33^{h}	12.00^{g}	0.67^{g}
p-Value		<.0001	<.0001	<.0001
CV (%)		1.41	4.26	5.15
Tukey HSD at 1%		4.0942	3.5177	0.2667

** Means with same letter are not significantly different at p≤0.01*

inherited and epigenetic changes by factors like types of explants, strategy for recovery, culture reputation, etc. So, the choice of a sorted-out tissue like nodal meristem in the present study has demonstrated to avoid genetic changes and variability and did not manifest any polymorphic bands in the RAPD analysis.

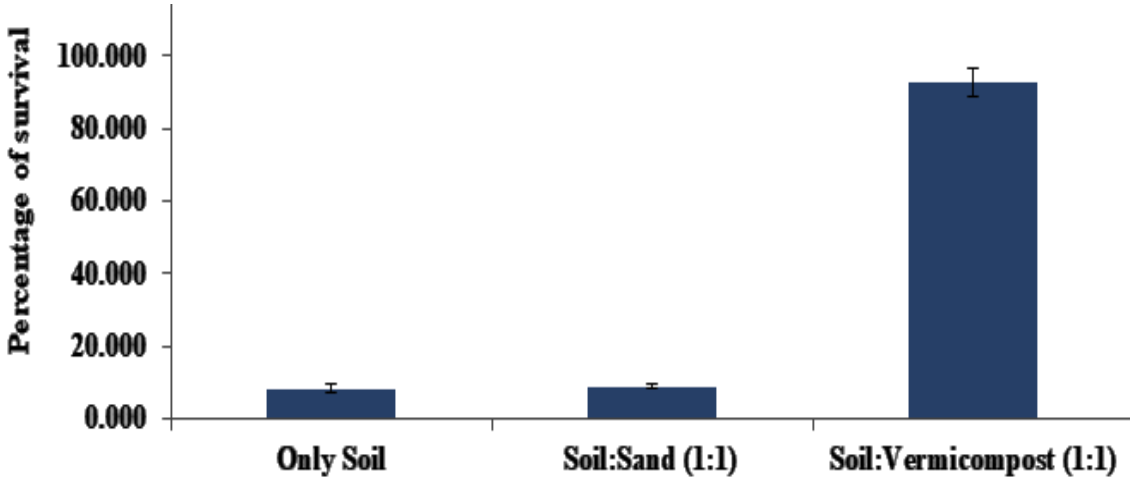

Fig. 2. Survival percentage of in vitro regenerated plants in different potting substrate

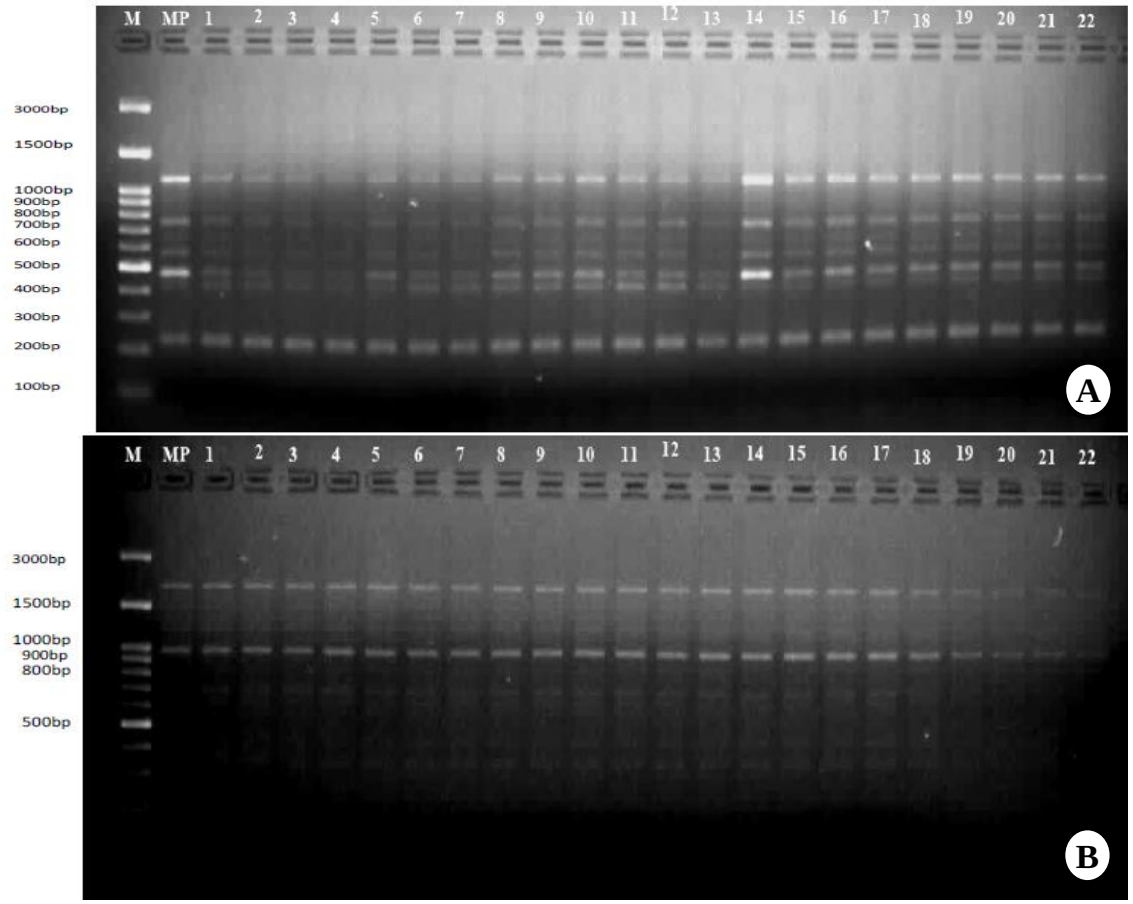

Fig. 3. DNA amplification obtained with RAPD primers: (A) OPC 20 and (B) OPN 11; M: DNA marker; MP: Mother plant; Lane 1 - 22: Micropropagated plants

A large number of the literature has announced the utility of transverse thin cell layer (tTLC) culture procedure in the in-vitro recovery together with hereditary changes investigations of different plant groups including E. alba [47-53]. They have described the use of tTLC as homogeneous explants which avoid genetic variations. Again, various reports demonstrated that the straight plant recovery over sorted out tissues of meristematic nature maintain a strategic distance from hereditary revisions and show lesser probability of inherited variability [37,10] (Salvi et al., 2002). However, the greater part of the writing is quiet on the use of nodal segments in E. alba as explants which offer similar homogenous property [54-56]. These explants have a bit of leeway due to the meristematic homogenous cell nature. Reiteratively the simple accessibility of development substances to such tissues elucidates the success of outstanding plant recovery. Our result of using nodal explants in *Eclipta alba* were in conformation with similar studies on tissue culture by Franca *et al*., [6,7,45,23,37]. Finally, RAPD has comprehensively checked hereditary loyalty or inconstancy of in vitro recovered plantlets in various plant groups [14,9,11,10,17].

4. CONCLUSION

From the current study, it is concluded that BAP is the most effective Plant Growth Regulator for regeneration in *E. alba* and MS medium supplemented with BAP and Kin the foremost successful combination for multiple shoot proliferation. Soil: vermicompost (1:1) mixture has shown the best promising result of hardening. All the plantlets developed through *in vitro* culture have attended genetic stability with the mother plant. There is no genetic variation shown through RAPD analysis. This study aims to develop a standard protocol to initiate shoot culture at a standardized media and hormonal concentration of *E. alba* that will be beneficial for large scale *in vitro* production. Hence it is a successful attempt to generate a quick, economic and reproducible protocol for the micropropagation of *E. alba* without any genetic alternation and maintaining homologous clonal propagation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chandran H, Meena M, Barupal T, Sharma K. Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnology Reports. 2020;26:e00450.
2. Delgado-Paredes GE, Vásquez-Díaz C, Esquerre-Ibañez B, Bazán-Sernaqué P, Rojas-Idrogo C. In vitro tissue culture in plants propagation and germplasm conservation of economically important species in Peru. Scientia Agropecuaria. 2021;12(3):337-49.
3. Hesami M, Baiton A, Alizadeh M, Pepe M, Torkamaneh D, Jones AM. Advances and perspectives in tissue culture and genetic engineering of cannabis. International Journal of Molecular Sciences. 2021;22 (11):5671.
4. Kaur K, Dolker D, Behera S, Pati PK. Critical factors influencing in vitro propagation and modulation of important secondary metabolites in *Withania somnifera (L.)* dunal. Plant Cell, Tissue and Organ Culture (PCTOC). 2022;149 (1):41-60.
5. Salma U, Kundu S, Ali MN, Mandal N. Elicitor mediated enhancement of wedelolactone in cell suspension culture of *Eclipta alba (L.)* Hassk. Plant Cell Tiss Org Cult 2018;134(3):409-21.
6. Franca SC, Bertoni BW, Pereira AMS. Antihepatotoxic agent in micropropagated

plantlets of Eclipta alba. Plant Cell Tiss Org Cult 1995;40:297-299
7. Borthakur M, Dutta K, Nath SC, Singh RS. Micropropagation of Eclipta alba and Eupatorium adenophorum using a single-step nodal cutting technique. Plant Cell Tiss Org Cult 2000;62:239-242.
8. Bednarek PT, Orłowska R. Plant tissue culture environment as a switch-key of (epi) genetic changes. Plant Cell, Tissue and Organ Culture (PCTOC). 2020;140(2):245-57.
9. Kishor R, Devi HS. Induction of multiple shoots in a monopodial orchid hybrid (Aerides vandarum Reichb.f ×Vanda stangeana Reichb.f) using thidiazuron and analysis of their genetic stability. Plant Cell Tiss Org Cult. 2009;97:121-129.
10. Rai SK, Sharma M, Jain M, Awasthi A, Purshottam DK, Nair NK, Sharma AK. Rapid *In vitro* production of cloned plants of Uraria picta (Jacq.) DC - a rare medicinal herb in long-term culture. Appl Biochem Biotechnol. 2010;162:1929-1937.
11. Das A, Kesari V, Rangan L. Plant regeneration in Curcuma species and assessment of genetic stability of regenerated plants. Biol Plant. 2010;54:423-429.
12. Bairu MW, Aremu AO, VanStaden J. Somaclonal variation in plants: Cause sand detection methods. Plant Growth Regul. 2011;63:147-173.
13. Undal VS, Ahir MR. The methods of molecular markers in genome analysis: A brief biotechnological approach. Weser Books. 2023;149.
14. Goel MK, Kukreja AK, Bisht NS. *In vitro* manipulations in St. John's wort (*Hypericum perforatum L.*) for incessant and scale up micropropagation using adventitious roots in liquid medium and assessment of clonal fidelity using RAPD analysis. Plant Cell Tiss Org Cult. 2009;96:1-9.
15. Yin J, Khayatnezhad M, Shakoor A. Evaluation of genetic diversity in Geranium (Geraniaceae) using rapd marker. Genetika. 2021;53(1):363-78.
16. Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan AK. Developing stress tolerant plants through in vitro selection - an overview of the recent progress. Environ Exp Bot. 2011;71:89-98.
17. Sharma S, Sudheer Pamidimarri DVN, Vijay Anand KG, Reddy MP. Assessment of genetic stability in micropropagules of Jatropha curcas genotypes by RAPD and AFLP analysis. Ind Crop Prod. 2011;34:1003-1009.
18. Abdalla N, Ragab M, El-Miniawy S, Arafa N, Taha H. A new aspect for in vitro propagation of Jerusalem artichoke and molecular assessment using RAPD, ISSR and SCoT marker techniques. Egyptian Journal of Botany. 2021;61(1):203-18.
19. Suwal MM, Lamichhane J, Gauchan DP. Assessment of genetic stability of micropropagated Bambusa balcooa Roxb. Using RAPD marker. Plant Tissue Cult. Biotechnol. 2021;31:81-95.
20. Kader A, Sinha SN, Ghosh P. Clonal fidelity investigation of micropropagated hardened plants of jackfruit tree (*Artocarpus heterophyllus L.)* with RAPD markers. Journal of Genetic Engineering and Biotechnology. 2022;20(1):145.
21. Ceasar SA, Maxwell SL, Prasad KB, Karthigan M, Ignacimuthu S. Highly efficient shoot regeneration of *Bacopa monnieri L.* using a two-stage culture procedure and assessment of genetic integrity of micropropagated plants by RAPD. Acta Physiol Plant. 2010;32:443-452.
22. Asthana P, Jaiswal VS, Jaiswal U. Micropropagation of Sapindus trifoliatus L. and assessment of genetic fidelity of micropropagated plants using RAPD analysis. Acta Physiol Plant 2011;33:1821-1829.
23. Dhaka N, Kothari SL. Micropropagation of *Eclipta alba (L.)* Hassk - an important medicinal plant. *In vitro* Cell Dev Biol Plant 2005;41:658-661.
24. Singh SK, Rai MK, Asthana P, Sahoo L. Alginate-encapsulation of nodal segments for propagation, short-term conservation and germplasm exchange and distribution of *Eclipta alba (L.).* Acta Physiol Plant. 2010;32:607-610.
25. Thakur VD, Mengi SA. Neuropharmacological profile of *Eclipta alba* (Linn.) Hassk. J Ethnopharmacol. 2005;102:23-31.
26. Leal LK, Ferreira AA, Bezerr GA, Matos FJ, Viana GS. Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing Coumarin: A comparative study. J Ethnopharmacol. 2000;70:151-159.
27. Ananthi J, Prakasam A, Pugalendi KV. Antihyperglycemic activity of Eclipta alba

leaf on alloxan-induced diabetic rats. Yale J Biol Med. 2003;76:97-102.

28. Veeru P, Kishor MP, Meenakshi M. Screening of medicinal plant extracts for antioxidant activity. J Med Plant Res. 2009;3(8):608-612.
29. Yadav N K, Arya RK, Dev K, Sharma C, Hossain Z, Meena S, Arya KR, Gayen JR, Datta D, Singh RK. Alcoholic extract of *Eclipta alba* shows in vitro antioxidant and anticancer activity without exhibiting toxicological effects. Oxidative Med Cellular Longevity. 2017;9094641.
30. Baliga MS, Jagetia GC, Venkatesh P, Reddy R, Ulloor JN. Radio protective effect of abana, a polyherbal drug following total body irradiation. Br J Radiol. 2004;77:1027-1035.
31. Gupta A, Kumar A, Kumar D, Nandan S, Shankar K, Varshney S, Rajan S, Srivastava A, Gupta S, Kanojiya S, Narender T. Ethyl acetate fraction of Eclipta alba: A potential phytopharmaceutical targeting adipocyte differentiation. Biomed Pharmacother. 2017;1(96):572-83.
32. Patel MB, Panchal SJ, Patel JA. Antianaphylactic activity of alcoholic extract of *Eclipta alba*. J Young Pharma. 2010;1:244-250.
33. Ebrahimzadegan R, Maroufi A. *In vitro* regeneration and Agrobacterium-mediated genetic transformation of Dragon's Head plant (*Lallemantia iberica*). Scientific Reports. 2022;12(1):1784.
34. Halder M, Majumder A, Ray S, Jha S. Medicinal plant research at crossroads: Biotechnological approaches for conservation, production and stability in tissue cultures and regenerated plants. Medicinal Plants: Domestication, Biotechnology and Regional Importance. 2021;459-544.
35. Ghnaya AB, Charles G, Branchard M. Rapid shoot regeneration from thin cell layer explants excised from petioles and hypocotyls in four cultivars of *Brassica napus L*. Plant Cell Tiss Org Cult. 2008;92:25-30.
36. Singh SK, Rai MK, Asthana P, Sahoo L. An improved micropropagation of *Spilanthes acmella L*. through transverse thin cell layer culture. Acta Physiol Plant. 2009;31:693-698.
37. Rout GR, Mohapatra A, Jain SM. Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnol Adv. 2006;24:531-560.
38. Swain H, Adak T, Mukherjee AK, Mukherjee PK, Bhattacharya P, Behera S, Bagchi T, Patro R, Shasmita, Khandual AK, Bag MK, Danger TK, Lenka S, Jena M. Novel Trichodermastrains isolated from tree barks as potential biocontrol agents and biofertilizers for direct seeded rice. Microbiol Res. 2018;214:83-90.
39. Ray A, Bhattacharya S. An improved micropropagation of *Eclipta alba* by *In vitro* priming with chlorocholine chloride. Plant Cell Tiss Org Cult. 2008;92:315-319.
40. Kartiki kumar S, Vigneswari K, Jegatheesan K. Screening of antibacterial and antioxidant activities of leaves of *Eclipta alba (L*.). Sci Res Eassay. 2007;2(4):101-104.
41. Rai MK, Rathour R, Behera S, Kaushik, Naik SK. Encapsulation technology: an assessment of its role in *In vitro* conservation of medicinal and threatened plant species. In: Agricultural biotechnology: Latest research and trends (eds Srivastava DK, et al.), Springer; 2021. DOI: doi.org/10.1007/978-981-16-2339-4_5
42. Bui VL, Hang Phuong NT, Anh Hong LT, Tran Thanh Van K. High frequency shoot regeneration from Rhynchostylis gigantea (Orchidaceae) using thin cell layers. Plant Growth Regul. 1999;28:179-185.
43. Lázaro-Castellanos JO, Mata-Rosas M, González D. *In vitro* propagation of endangered Mammillaria genus (Cactaceae) species and genetic stability assessment using SSR markers. In Vitro Cell Dev Biol-Plant. 2018;54:518-529.
44. Mohanty P, Behera S, Swain SS, Barik DP, Naik SK. Micropropagationof Hedychium coronarium J. Koenig through rhizome bud. Physiol Mol Biol Plant. 2013;19(4):605-610.
45. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with Tabacco tissue cultures. Physiol Plant. 1962;15:473-497.
46. Pandey MK, Singh GN, Sharma RK, Lata S. Antibacterial activity of *Ecliptaalba (L.)* Hassk. J Appl Pharm Sci. 2011;1:104-107.
47. Bui VL, Thao DMN, Gendy C, Vidal J, Tran Thanh Van K. Somatic embryogenesis on thin cell layer of a C4 species, *Digitaria sanguinalis (L.)* Scop. Plant Cell Tiss Org Cult. 1997;49:201-208.

48. Carimi F, De-Pasquale F, Crescimanno FG. Somatic embryogenesis and plant regeneration from pistil thin cell layers of Citrus. Plant Cell Rep. 1999;18:935-940.
49. Mulin M, Bellio-Spataru A. Organogenesis from hypocotyls thin cell layers of Lupinus mutabilis and Lupinus albus. Plant Growth Regul. 2000;30:177-183.
50. Nhut DT, Bui VL, Teixeira da Silva JA, Aswath CR. Thin cell layer culture system in Lilium: Regeneration and transformation perspectives. *In vitro* Cell Dev Biol Plant. 2001;37:516-523.
51. Chattopadhyaya B, Banerjee J, Basu A, Sen SK, Maiti MK. Shoot induction and regeneration using internodal transverse thin cell layer culture in *Sesamum indicum L*. Plant Biotechnol Rep. 2010;4:173-178.
52. Scherwinski-Pereira JE, da Guedes RS, Fermino Jr PCP, Silva TL, Costa FHS. Somatic embryogenesis and plant regeneration in oil palm using the thin cell layer technique. *In vitro* Cell Dev Biol Plant. 2010;46:378-385.
53. Gawde AJ, Paratkar GT. Micropropagation of *Eclipta alba* Hassk: An approach to shorten the protocol. Indian J Biotechnol. 2004;3:128-132.
54. Behera S, Barik DP, Naik SK. Micropropagation of Symplocosracemosa Roxb., a threatened medicinal tree of India. Curr Sci. 2017;113(4):555-558.
55. 9. Behera S, Kamila PK, Rout KK, Barik DP, Panda PC, Naik SK. An efficient plant regeneration protocol of an industrially important plant, *Hedychium coronarium J*. Koenig and establishment of genetic and biochemical fidelity of the regenerants. Ind Crops Prod. 2018;126:58-68.
56. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 1987;19:11-15.

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