

In Vitro Propagation and The Conservation Protocol Followed is Appropriate and Applicable for The Clonal Mass Propagation of Elite Five Medicinally Important Plant Species

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ABSTRACT

India has a rich biodiversity due to its varied climatic, altitudinal variations and ecological habitats. There have been increasing rates of threats of running down to these biological resources due to immense biotic and abiotic stress. Indiscriminate collection of plants for their medicinal, ornamental, perfumery uses, the rapid loss of genetic diversity and habitat come mainly from resource-poor areas of the world and from global biodiversity hotspots and degradation are potential causes of threats. This article describes the efforts towards saving plants from extinction. Conservation of genetic materials of many vulnerable plants, which includes endangered and vulnerable plant species. This review only describes the role of in vitro propagation techniques in medicinal plants.

Keywords: Genetic evaluation, medicinal plants, micropropagation, in vitro conservation

INTRODUCTION

Medicinal and aromatic plants are nature's gift and have been used for allaying disease and improving the quality of life for ages. Plant-based medicines are used in all civilizations and cultures and have been one of the indispensable lines of defense in maintaining health and combating diseases worldwide.

Numerous modern medicines can trace their origin to plants and plant extracts. To this date, most marginalized communities in developing countries rely on herbal remedies as the sole healthcare provision. The World Health Organization has estimated that herbal products constitute close to 25% of the total healthcare products market with a global sales volume at over US\$ 65 billion and an annual growth rate of 10-15%. The World Health Organization (WHO) has estimated the present demand for medicinal plants is approximately US \$14 billion per year and the demand is likely to increase more than US \$5 trillion in 2050. In India, medicinal plant-related trade is estimated to be approximately US \$1 billion per year (Kala et al., 2006; Kala and Sajwan, 2007). Over the years herbal medicines have gained upward trend for consumption especially with the development and standardization of herbal medicines.

The industrial consumption, significant quantities of medicinal plant resources are consumed in the country under its traditional health care practices at the household level, by traditional healers and by practitioners. An idea about the richness and diversity of these health care practices in India can be had from the diversity of plant species used in these systems. It is estimated that more than 6,000 higher plant species forming about 40% of the higher plant diversity of the country are used in its codified and folk healthcare traditions (NMPB Report, 2008).

All five species are medicinally and economically important and mention here in brief form: (i) *Gloriosa superba*, IUCN Red listed medicinal plant, contain an important high valued alkaloid i.e. colchicine due to its uses for treating arthritis, Mediterranean fever, gout, rheumatism, cancer etc. (Gupta, 2005; Jana and Shekhawat, 2011); (ii) *Artemisia vulgaris*, essential oil extracted from aerial parts is a potential larvicide against *Aedes aegypti* that can spread dengue fever, chikungunya, Zika fever, Mayaro and yellow fever viruses, red flour beetle and other

diseases (Silva, 2004, Govindaraj and Kumari, 2013); different parts can also be used as antibacterial, anti-inflammatory, antiseptic etc. purposes; (iii) *Withaniasomnifera*, contain steroidal lactones: withanolides, withaferins etc. effectively used as anti-stress (Kaur et al., 2001), anti-tumour, anti-cancer (Ralet al., 2016), immunomodulatory activity (Davis and Kuttan, 2000), also used against anxiety and depression (Bhattacharya et al., 2000) etc.; (iv) *Tinosporacordifolia*, contain berberine and other compounds used as anti-diabetic (Patel and Mishra, 2011), anti-cancer (Mishra and Kaur 2013), immunomodulatory activity (Sharma et al., 2012) etc. and (v) *Plumbagozeylanica*, contain plumbagin, which has been identified as significant bioactive component related to several pharmacological activities, e.g., anti-cancer, antimicrobial, anticancer, wound healing, anti-inflammatory and altered T-cell proliferative activities and antifertility actions. (Kiniet al., 1997, Aparanjiet al., 2005, Chen et al., 2009).

The genetic biodiversity of some important medicinal plants may be a threatening condition due to population growth, urbanization, the unrestricted collection of medicinal plants from the natural habits. To cope up with this alarming situation, it is essential to the immediate conservation of that important medicinal plant species through micropropagation methodology (Hassan, 2012).

Karyomorphological analysis is a useful method for characterizing plant chromosomes. The cytological stability of micropropagated plants needs to be checked before using this protocol at the commercial level (Ghosh and Sen, 1992 and 1997; Landey et al., 2015; Regalado et al., 2015; Tomiczak et al., 2015).

The contents of secondary metabolites may vary significantly due to different agro-climatic zone or soil characteristics and thus affect the therapeutic effects of the various species when they are used. So selected elite clone should be propagated for mass propagation in industrial level production. The in vitro derived secondary metabolite synthesis have many advantages over the other sources of production viz. seed-derived plants, synthetic sources etc.

Ex-situ conservation not only the essential for engendered medicinal plant species but also needed to conserve the elite genotype otherwise the wild medicinal plant genetic resources never be utilized or exploited for any improvement program in future.

Tissue culture provides efficient techniques for rapid and large scale propagation of medicinal plants and they're in vitro conservation of germplasms. The application of micropropagation methodology for medicinal plants gives alternative of seed propagation cultivation method have many benefits to the industry- increase in the propagation rate of plants; the rapid multiplication of plants which in a particular climate do not give seeds or whose seeds have a low germination capacity; availability of plants throughout the year, i.e., in all the seasons; uniform plants of a selected genotype; conservation of genetic resources of species and threatened plants.

In order to maintain true to type high yielding elite clones of above mentioned five species, plant regeneration via direct organogenesis is the preferred means. Since our goal is to develop a reliable source of desirable pharmacologically active chemotypes of five species through in vitro propagation, it necessitates the establishment of a tissue culture system that should involve minimal or no callus development in order to reduce the likelihood of induction and recovery of variants.

Several factors are critical to influencing the success of in-vitro propagation, mainly clonal micropropagation of different medicinal plants. The effects of chemical factors (plant growth regulators, vitamins, macro- and micro-nutrients etc.) biological factors (physiological status of source plant, explant type, etc.) and physical factors (light- intensity, quality of light, photoperiod, temperature, humidity etc. factors are of paramount importance to successfully obtain viable tissue culture factors are of paramount importance to successfully obtain viable tissue culture.

In vitro techniques provide valuable means for conservation and regeneration of valuable plant genetic resources. Although subculture at certain intervals can be used for the conservation of in vitro cultures, frequent subcultures of many clones is costly and labor-intensive. Conservation of plant germplasm by in vitro technology can be accomplished by using slow growth procedures or cryopreservation and has been practiced in many medicinal

plant species including *Ocimum* species, *Adhatoda vasica*, *Phyllanthus amarus*, *Withania somnifera*, *Dioscorea bulbifera* (Ahuja and Ramawat, 2014).

All above-mentioned plant species needed to develop simple efficient repeatable micropropagation method of a particular elite genotype by virtue of producing uniform planting material for offsetting the pressure on the natural populations especially for medicinal plants.

Therefore, the main objectives are –

1. Screening and selection of high yielding genotypes/accession based on their chemical profile from natural population and commercial cultivars.
2. To develop a simple, reproducible method for in vitro mass clonal propagation of all above mentioned species, comparison of micropropagated plants with the mother plants for consistency of phytochemical, chromosomal and morphological profiles and in vitro conservation of elite clones for future use.

MATERIALS AND METHODS

Materials:

Following medicinal plant materials were selected for all experimental purposes:

- a. *Artemisia vulgaris* L.
- b. *Gloriosa superba* L.
- c. *Plumbago zeylanica* L.
- d. *Tinospora cordifolia* (Willd.) Miers Hook. F. & Thoms.
- e. *Withania somnifera* (L.) Dunal

All plant species, *A. vulgaris*, *G. superba*, *P. zeylanica*, *T. cordifolia* and *W. somnifera* were collected from natural habitats of seven district of West Bengal, India and were grown in the experimental garden of the RKMVC College, Rahara, Kolkata, W.B., India. Collected specimens were confirmed by the authority of Botanical Survey of India. The accession no. of plant materials are- *Artemisia vulgaris* L. - CNH/63/2012/Tech.II/886; *Gloriosa superba* L.- CNH/2014/Tech.II/78/200.; *Plumbago zeylanica* L. CNH/37i/2014/Tech.II/137; *Tinospora cordifolia* (Willd.) Miers ex Hook.f.& Thomson- CNH/2014/Tech.II/78/200 and *Withania somnifera* (L.) Dunal- CNH/ 37h /2014 /Tech.II / 137.

METHODS

Morphological Study:

The main diagnostic morphological features, viz. plant habit, plant height, leaf characteristics, flowers number, fruiting calyx, berry colour, seed set/fruit, and seed weight, were recorded from the living plants in the field. For each character an average of 20 readings from as many plants within an accession/cultivar was taken.

Selection of elite genotype/ accession:

All five species *Gloriosa superba*, *Artemisia vulgaris*, *Withania somnifera*, *Tinospora cordifolia* and *Plumbago zeylanica* are important medicinal plants. In India, owing to its high domestic and export demands, five species in the wild populations are overexploited at an uncontrolled rate. To identify potential genotypes/chemotypes/ accession of above mentioned species from a different agro-climatic zone in seven districts namely, Darjeeling, North Dinajpur, Murshidabad, Bankura, West Midnapore, South 24- Parganas and Nadia, so as to emphasize cultivation of its high-yielding genotypes for commercial purposes. Five accessions collected from various

locations in each district and also collected materials were grown in a Field Gene Bank under identical environmental conditions in RKMVC college, Rahara, W.B., India campus. Field collected material of each accession were characterized through phytochemical profile. Considering the complexity of the chemistry of five medicinal plant species, like colchicine from *G. superba*, essential oil from *A. vulgaris*, withanolide A and withaferin A from *W. somnifera*, plumbagin from *P. zeylanica* and berberine from *T. cordifolia* the variation in its chemical content, simple and efficient method for each compound for qualitative and quantitative determination of the concentration of the above mentioned compound was developed using HPLC methods.

For Colchicine from *G. superba* 15 tubers per district and 5 per population. The tubers were thoroughly washed, dried, powdered and extracted with methanol for 6 h at 40°C. The extract was evaporated, and the residue redissolved in distilled water, partitioned against n-hexane and finally the aqueous phase containing colchicine was extracted with chloroform. The chloroform was evaporated and the residue dissolved in high-pressure liquid chromatography (HPLC)-grade methanol. A quantitative analysis was performed on Waters liquid chromatograph (Waters 2489 UV/Visible Detector) employing a XBridge C18 HPLC Column with acetonitrile: methanol: water (35:5:60). The flow rate was 1 ml/min and all chromatographs were plotted at 243 nm, using a UV detector (Ghosh et al., 2002).

For berberine from stem of *T. cordifolia*, dry powdered material (5 g) were defatted with 100 ml petroleum ether in a Soxhlet extractor for 12 h. The marc was air-dried and was further extracted with 50 ml methanol in a Soxhlet extractor for 12 h at 60°. The extract was filtered and concentrated to dryness under reduced pressure below 60° using a rotary evaporator. Different concentrations of the residue were prepared in methanol and used for HPLC analysis to quantify the berberine content.

For plumbagin from root of *P. zeylanica*, Powdered roots (0.5 g) were extracted using 10 ml methanol for 12 h and this was repeated thrice. The combined methanol extract was filtered and concentrated on a water bath at 40°C. The extract was transferred to volumetric flask and made up to 10 ml. This extract was used for quantification of plumbagin as per following table.

For withanolides from *W. somnifera*, the air dried (25–30°C) different plant parts (leaves and roots, (1.0 g) were extracted three times with 20 ml of methanol for 45 min in a sonicator. The combined extracts were concentrated to dryness under vacuum at 45°C. Dried extracts were re-dissolved in 1 ml of methanol. The examination of two withanolides (Withaferin A and Withanolide A) content in tissue was carried out by HPLC method (Nagella and Murthy, 2011).

Isolation of the essential oil from *A. vulgaris* by hydrodistillation: A 30–40 g portion of the powdered plant material was extracted by hydrodistillation for 3 h in a Clevenger-type collector apparatus. The resulting essential oil was isolated using the method described in the Indian Pharmacopoeia, 2014. The essential oil produced in this study was extracted from the entire aerial parts of the plant.

SL No.	Active Principle	Source plants	Plant Parts from which extracted	HPLC (UV-Vis detector, C18 Column)	
				Mobile phase	Wavelength (nm)
1	Berberine	<i>T. cordifolia</i>	Stem	Acetonitrile: Water (60:40, v/v)	265
2	Colchicine	<i>G. superba</i>	Tuber	Acetonitrile: Methanol: Water (35:5:60)	243

3	Plumbagin	<i>P. zeylanica</i>	Root	Methanol: Water(70:30, v/v)	270
4	Withanolide A & withaferin A	<i>W. somnifera</i>	Root	Acetonitrile: Water (60:40, v/v)	227

Micropropagation method

Culture media and incubation condition: Murashige and Skoog (MS), basal and modified media (consisting of varying amount of salts, vitamins and different percentage of sucrose) was used after solidifying with 0.7% (w/v) agar. Different plant growth regulators (PGRs) like α - naphthalene acetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA) N6-benzylamino purine (BAP), kinetin (KIN), thidiazuron (TDZ), 6-(γ,γ -dimethylallylamino) purine (2iP) were added at various concentrations to MS medium before the pH of the medium was adjusted to 5.7 (with 0.5 N NaOH).

For shoot multiplication MS medium with different concentrations and combinations of cytokinins (BAP, Kn, 2iP, BPA) either individually or with low level of auxin (s) were tested. and were recorded periodically. For shoot elongation multiple shoots induced were excised individually and sub- cultured on to MS medium supplemented with BAP (1.0 mg/l) and varying concentrations of gibberellic acid (GA_3) (0.2, 0.4, 0.6, 0.8, and 1.0 mg/l) or MS medium supplemented with GA_3 alone at different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/l) for elongation of shoots. A control group (basal medium without PGR) was also maintained.

Media were autoclaved at 1.06 kgcm^{-2} and 121°C for 15 min. Cultures at all growth stages were incubated under artificial conditions: $25 \pm 2^\circ\text{C}$, 60% RH and a 16-h photoperiod (using white fluorescent tubes) under a photosynthetic photon flux density of $30 \mu\text{molm}^{-2} \text{ s}^{-1}$.

Surface Sterilization, Initiation and Subsequent Stages of Culture: Different types of explants (mainly shoot tips and rhizomatous tuber in case of *G. superba*) were collected from elite designated healthy plants growing inside the shade-net house. Then, the explants were treated with 2.0 % nonsystematic fungicide (thiram) for 10-15 min followed by 3.0 % liquid detergent (Tween-20) for 1 min, then washed thoroughly in running tap water, and finally surface-sterilized with 0.1 % HgCl_2 for 5- 20 min (based on explant types) followed by washing three times with sterile distilled water. Then the explants were implanted on MS medium supplemented with different concentrations and combination of plant growth regulators (PGRs) and different additives as experimental requirements. The medium without any PGRs was used as a control. For rooting, in vitro grown micro shoots (2-3 cm) with 2-4 young light green leaves were excised and implanted on both MS and $\frac{1}{2}$ MS basal media, which served as a control. The individual micro shoots were subcultured with different concentrations of IBA, IAA and NAA (0.2, 0.5 and 1.0 mg/l) in full MS or $\frac{1}{2}$ MS media along with 1- 3% sucrose.

In vitro Conservation: Slow growth is usually achieved by reducing the culture temperature, by modifying culture media (reduced media strength) with supplements of osmotic agents (mannitol and sorbitol 1.0 –3.0 %, w/v), and removing all plant growth regulators.

Acclimatization and field performance of micropropagated plants: Regenerated plants were acclimatized for 15 -30 days (species dependent) by transferring to small earthen pots containing ‘Soilrite’ and covered with transparent polybags to maintain 90–99 % relative humidity. Ultimately, the acclimatized plants were transferred to earthen tubs containing a mixture of soil and vermicompost (3:1 ratio) and kept in the green shade-net house. Field performance like survival rate and growth rate of regenerated plants in the field conditions were evaluated for up to mature stage (flowering and fruiting).

Evaluation of cytogenetic status of micropropagated plants: Mitotic chromosome studies of mother plant as well as mature field growing regenerated plants were carried out from root tips using aceto-orceine staining method. Root tips were pretreated in saturated solution of p- dichlorobenzene (pDB) for 3 to 6 hrs at $12- 16^\circ\text{C}$ and fixed with dehydrated ethanol and glacial acetic acid (3:1, v/v) for 24 hours at 4°C . Fixed root tips were

stained with 2.0% aceto-orcin: 1(N) HCl (9:1, v/v) mixture followed by incubating for 2 h at room temperature. Metaphase plates were observed in Leica DM750 microscope and photographed with Leica DFC 295 camera.

RESULTS

Selected elite chemotype: For selection of elite genotype or accession the major secondary metabolite contents of medicinal interest isolated and estimated from the wild accessions as well as cultivars, here only mention the maximum content of natural product and their source plant material as below:

Plants	Maximum content	Compound	Population (District)
G.superba	0.391%	Colchicine	Darjeeling, W.B., India (wild)
A.vulgaris	0.9221%	Essential oil	Darjeeling W.B., India (wild)
W.sominifera	0.005% (leaf)	Withanolide A	Bankura, W.B., India (wild)
	0.0291% (root)	Withaferin A	
T.cordifolia	0.321%	Berberine	South 24-Parganas, W.B., India (wild)
P.zeylanica	0.603%	Plumbagin	Nadia. W.B., India (wild)

So it can be conclude that the plant growing in wild condition in selected district (s) synthesize more metabolite rather than some available cultivars (W. somnifera). So further all experimental works were carried out in selected accession only.

Micropropagation

Micropropagation of four different species through shoot tip culture and rhizomatous tuber (G. superba) trigger maximum number of axillary shoots in presence of cytokinin particularly BAP presence or absence of low level of NAA. But there are several factors like media components and physical condition(s) are also influenced for obtaining optimum number of micro-shoots. In case of G. superba maximum number of shoots (14-17) are produced in presence of 5.0 mg/l BAP along with SH vitamins and SH myo-inositol (1000 mg/l), whereas in MS vitamin and myo-inositol (100 mg/l) help to produce multiple shoots (5- 8) even in presence of same amount of cytokinin. In A. vulgaris optimum number of micro shoots (26- 30) obtained when culture in only 1.0 mg/l BAP within 8 weeks of culture but same number of shoot are produced within 6 weeks when 200 mg/l activated charcoal supplemented in culture media. The reduction of time for optimum multiplication may be due to the avoiding phenolic leaching problem which are generally inhibit normal growth. Maximum number of micro-shoots (22- 27) are triggered to multiply in presence of 2.0 mg/l BAP and 0.2 mg/l of NAA along with 3 times more MS micro elements in P. zeylanica. The additional amount of micro elements may help plant health particularly yellowing-leaf problem and also prevent premature leaf-falling problem also. In case of T. cordifolia maximum 17 micro shoots are produced in presence of more light intensity (4500 lux) in addition to cytokinin, BAP (5.0 mg/l). Maximum 26 number of micro shoots are induced in W. somnifera in presence of both cytokinins (BAP and Kn) in 1.0 mg/l level along with 2900 mg/l KNO₃ and 200 mg/l myo-inositol within 30 days of culture. For shoot elongation phenomena GA₃ play important role for elongation of micro shoots in all five species, the range of elongation from 18.0- 116.0 mm after 30 days of culture in presence of 0.2 – 0.8 mg/l GA₃ depending on species. The root induction in individual shoot in A. vulgaris occurred (5- 8 nos) and in G. superba (4 -6) in presence of 0.5 mg/l IAA after 30 days of culture. In P. zeylanica 7- 9 number of roots are induced within 40 days of culture when in MS media supplemented with 1.0 mg/l IBA whereas T. cordifolia induced maximum number of roots (9-12) in same PGR and same duration. Maximum 6- 8 numbers of roots are induced within 30 days of culture in presence of 0.5 mg/l IBA in W. somnifera.

In vitro conservation

This method has the potential of prolonging the subculture interval, thereby maintaining the elite germplasm and reducing the overall cost of labor. The result of subculture interval and survival potentiality is as below:

Table: 2, Maximum average survival rate (%) without subculture interval (days) in full viable condition with regeneration potentiality of multiple in vitro growing shoots.

Plants	MS with 3% Sucrose	½ MS with 1% sucrose	½ MS with 1% sucrose + mannitol (2%)	½ MS with 1% sucrose + sorbitol (2%)
<i>G. superba</i>	25C: 100 (30d) 10C: 68 (65d) 4C: 11 (48d)	25C: 78 (43d) 10C: 48 (61d) 4C: 5 (25d)	25C: 88 (102d) 10C: 68 (122d) 4C: 9 (56 d)	25C: 86 (100d) 10C: 65 (120d) 4C: 8 (40d)
<i>vulgaris</i>	25C: 100 (35d) 10C: 68 (65d) 4C: 9 (17d)	25C: 77 (40d) 10C: 40 (62d) 4C: nil (survive up to 10d)	25C: 90 (90d) 10C: 66 (110d) 4C: 4 (45 d)	25C: 93 (89d) 10C: 66 (119d) 4C: 3 (35 d)
<i>W. sominifera</i>	25C: 100 (28d) 10C: 53 (65d) 4C: 1 (30d)	25C: 70 (46d) 10C: 42 (58d) 4C: 2 (30d)	25C: 82 (106d) 10C: 42 (118d) 4C: 4 (40d)	25C: 82 (86d) 10C: 42 (100d) 4C: 4 (40d)
<i>T. cordifolia</i>	25C: 100 (30d) 10C: 68 (65d) 4C: 2 (22d)	25C: 78 (43d) 10C: 48 (61d) 4C: nil (survive up to 5d)	25C: 84 (96d) 10C: 52 (114d) 4C: nil (survive up to 15d)	25C: 81(100d) 10C: 50 (111d) 4C: nil (survive up to 11d)
<i>P. zeylanica</i>	25C: 100 (30d) 10C: 68 (65d) 4C: 5 (18d)	25C: 78 (43d) 10C: 48 (61d) 4C: nil (survive up to 5d)	25C: 88 (94d) 10C: 68 (108d) 4C: nil (survive up to 15d)	25C: 84 (91d) 10C: 72 (101d) 4C: nil (survive up to 10d)

Evaluation of field performance of micropropagated plants

A total of 125- 145 in vitro rooted plantlets were hardened for 14- 21 days with more than 90.5- 94.5% survival rate of five plant species. During these hardening periods plantlets grow about 2.0- 3.5 cm with two or four new leaf formation. The hardened plants were then transferred to earthen tubs filled with mixture of soil and

vermincompost at 3:1 ratio and acclimatized inside the poly-greenhouse for another 10 days with nearly 100 % survival rate. Ultimately the acclimatized plants were transfer to field condition under fullsunlight and nearly 100 % survival recorded after 4 months offield transfer.

Plant	For hardening transfer from in vitro to ex-vitro (No. of Plants transfer/ No. of plants survived)	Transfer to field (no. of potted acclimatized Plants / No. of plants survived in field)
G.superba	142/ 134	134/ 134
A.vulgaris	128/ 121	121/120
W.sominifera	132/ 123	123/120
T.cordifolia	138/129	129/126
P.zeylanica	125/ 119	119/ 116

Evaluation of morphological characters in field growing regenerated plants

Apparently no morphological variation are noticed in all field growing micropropagated five plant species.

Cytogenetical Evaluation of field growing regenerated plants

Chromosomal analysis of randomly selected root tips of one year old field growing in vitro regenerated plants (average 25 root tips and 5-10 metaphase/ root tip/ regenerated plant) showed predominantly diploid number of chromosomes (A. vulgaris, $2n=36$; G. superba, $2n=22$; P. zeylanica, $2n=24$; T. cordifolia $2n=24$ and W. somnifera, $2n=48$). All 15 regenerates so far analysed and same number of mitotic chromosome of mother plants and tissue culture raised plants were observed. No structural alternations alteration was observed in regenerated plant chromosome in compare with karyotype analysis of source plant. Thus the somatic chromosome complements of in vitro generated plants remained stable even after passing through three cycles of multiplication. It also confirms that no chromosomal variability was induced from the short term conserved plants among the regenerates. It indicates that reduced growth conditions applied in our studies along with the inherent genotypic potentiality and stability of the species helped to maintain germplasm over such a short period of time in maintained and chromosomal uniformity among the regenerates. This present finding is corroborated with some other studies where regenerants are found cytological stable.

Evaluation of secondary metabolites in field growing regenerated plants

After one year of in vitro regenerated field growing plants of five species evaluated their phytochemical profile and the results are as below (Table-4):

Table: 4: Evaluation of secondary metabolites average content of one year old field growing micropropagated plant species in poly-green house condition.

Plants	Maximum content	Compound	Population (District)
G.superba	0.421%	Colchicine	Darjeeling (wild)
A.vulgaris	1.22%	Essential oil	Darjeeling (wild)
W.sominifera	0.006% (leaf)	Withanolide A	Bankura (wild)

	0.031% (root)	Withaferin A	
T.cordifolia	0.291%	Berberine	South 24-Parganas (wild)
P.zeylanica	0.544%	Plumbagin	Nadia

The results showed more or less same amount of secondary metabolites are accumulated after one year in regenerated plants.



1. *Gloriosa superba*, 2. *Artemisia vulgaris*, 3. *Withania somnifera*



Tinospora cordifolia, 5. *Plambo zeylanica*

CONCLUSION

In this research work, an attempt has been made to provide the application of some plant biotechnological methods in medicinal plant production and present efforts to screen, propagate, and conserve elite clones of medicinally important five plant species for the production of respective medically important phyto-compounds.

Plants raised through in vitro propagation were compared with mother plants in terms of their gross morphology, secondary metabolite profile and chromosomal stability, therefore the micropropagation and the conservation protocol followed is appropriate and applicable for the clonal mass propagation of elite five medicinally important plant species.

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REFERENCES

1. Aparanji P, Kumar B V, Kumar S P, Sreedevi K, Rao D N and Rao R A. 2005. Induction of anti-inflammatory and altered T-cell proliferative responses by the ethanolic extract of *Plumbago zeylanica* in adjuvant-induced arthritic rats. *Pharm Biol.*, 43:784-789.
2. Ahuja MR and Ramawat KG. 2014. *Biotechnology and Biodiversity*. Springer International Publishing Switzerland.
3. Bennici A, Anzidei M and Vendramin G G. 2004. Genetic stability and uniformity of *Foeniculum vulgare* Mill. regenerated plants through organogenesis and somatic embryogenesis. *Plant Science*, 166: 221- 227.
4. Borthakur M, Singh R S. 2002. Direct plantlet regeneration from male inflorescences of medicinal yam (*Dioscorea floribunda* Mart. & Gal.). *In-vitro Cell Dev Biol-Plant*, 38: 183-185.
5. Chandra S, Lata H, Khan I A, ElSohly MA .2010. Propagation of elite *Cannabis sativa* for the production of D9-Tetrahydrocannabinol (THC) using biotechnological tools. In: Arora R (ed) *Medicinal plant biotechnology* (Chapter 7). CABI, Wallingford, pp 98–114.
6. Chen C A, Chang H H, Kao C Y, Tsai T H and Chen Y J. 2009. Plumbagin, isolated from *Plumbago zeylanica*, induces cell death through apoptosis in human pancreatic cancer cells. *Pancreatology*, 9:797-809.
7. Clarindo R. W. and Carvalho R. C. 2006. A high quality chromosome preparation from cell suspension aggregates of *Coffea canephora*. *Cytologia*, 71: 243- 249.
8. Davis L and Kuttan G. 2000. Immunomodulatory activity of *Withania somnifera*. *J Ethnopharmacol.*, 71:193-200.
9. Dodds J H and Roberts L. W. 1995 .*Experiments in plant tissue culture*. Cambridge University Press, New York.
10. Devi S P, Kumaria S, Rao S R and Tandon P. 2015. Genetic fidelity assessment in micropropagated plants using cytogenetical analysis and heterochromatin distribution: a case study with *Nepenthes khasiana* Hook f. *Protoplasma*, 252: 1305- 1312.
11. Fatima N and Anis M. 2011. Thidiazuron induced high frequency axillary shoot multiplication in *Withania somnifera* L. *Dunal. Journal of Medicinal Plants Research*. 5: 6681–6687.
12. Ghosh B and Sen S. 1992. Stable regeneration in *Asparagus cooperi* Baker. as controlled by different factors. *Plant Science*, 82: 119- 124.
13. Ghosh B and Sen S 1997. Suspension culture, somatic embryogenesis and stable regeneration in *Asparagus cooperi* Baker. *Cytobios*, 87: 189-200.
14. Ghosh S, Ghosh B and S. Jha. 2007. In vitrotuberisation of *Gloriosa superba* L. on basal medium. *Scientia Horticulturae* 114: 220–223.
15. Ghosh B, Mukherjee S, Jha T B and Jha S. 2002. Enhanced colchicine production in root cultures of *Gloriosa superba* by direct and indirect precursors of the biosynthetic pathway. *Biotechnology Letters*, 24: 231- 234.
16. Ghosh B and Sen S. 1991. Plant regeneration through somatic embryogenesis from spear callus of *Asparagus cooperi* Baker. *Plant Cell Reports*, 9: 667- 670.
17. Govindaraj S P and Kumari B D R. 2013. Composition and larvicidal activity of *Artemisia vulgaris* L. stem essential oil against *Aedes aegypti*. *Jordan J. Biol. Sci.*, 6: 11–16.
18. Gupta L. M., Rana, R. C., Raina R., and Gupta M. 2005. Colchicine contents in *Gloriosa superba* L. *SKUAST-J*, 4: 238-241.
19. Gururaj H B, Giridhar P and Ravishankar G A. 2007. Micropropagation of *Tinospora cordifolia* (Willd.) Miers ex Hook. & Thoms- a multipurpose medicinal plant. *Current Science*, 92: 23- 26.
20. Hassan R A B. 2012. *Medicinal Plants (Importance and Uses)*. *Pharmaceutica Anal Acta*, 3: 10.

21. Haque S M and Ghosh B. 2013. High frequency microcloning of Aloe vera and their true-to- type conformity by molecular cytogenetic assessment of two years old field growing regenerated plants. Botanical Studies, 54: 46-55.
22. Hemant L, Rita M. M and Bianca B. 2010. In vitro germplasm conservation of Podophyllumpeltatum L. under slow growth conditions In Vitro Cell. Dev. Biol. – Plant, 46: 22- 27.
23. Jaisi A and Panichayupakaranant P. 2016. Enhanced plumbagin production in Plumbago indica root cultures by L-alanine feeding and in situ adsorption. Plant Cell, Tissue and Organ Culture (PCTOC) doi:10.1007/s11240-016-1155-1156.
24. Jana S., and Shekhawat G.S. 2011. Critical review on medicinally potent plant species: Gloriosa superba. Fitoterapia. 82, 293-301.
25. Kala C P and Sajwan B S. 2007. Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through institutional networking and capacity building CURRENT SCIENCE, 93:797-806.
26. Kala C P, Dhyani P P and Sajwan B S. 2006. Developing the medicinal plants sector in northern India: challenges and opportunities. Journal of Ethnobiology and Ethnomedicine 2:32-46.
27. Kaur P, Mathur S, Sharma M, Tiwari M, Srivastava K K and Chandra R. 2001. A biologically active constituent of Withania somnifera (ashwagandha) with antistress activity. Indian J ClinBiochem., 16: 195–198.
28. Kini D P, Pandey S, Shenoy B D, Singh U V, Umadevi N, Kamath R, Nagarajkumari and Ramanarayan K. 1997. Antitumor and antifertility activities of plumbagin controlled release formulations. Indian J ExpBiol, 35: 374-379.
29. Landey R B, Cenci A, Guyot R., Bertrand B, Georget F, Dechamp E, Aribi JC, Lashermes P and Etienne H. 2015. Assessment of genetic and epigenetic changes during cell culture ageing and relations with somaclonal variation in Coffea arabica. Plant Cell Tissue Organ Cult, 122: 517- 531.
30. Lubaina A S , Nair G.M and Murugan K. 2011. Shoot multiplication and direct organogenesis of an important medicinal plant Plumbago zeylanica L. (Plumbaginaceae). Journal of Research in Biology, 6: 424-428.
31. Melo de F. N. 2002. Somatic Embryogenesis and Ploidy Stability in Cassava (Manihot esculenta Crantz) Cultivars Regenerated by in vitro Culture of Young Leaves. Cytologia. 67: 337- 341.
32. Maeda T. and Uchino A. 2004. Stability and variability of chromosome numbers in the Genus Schoenoplectus (Cyperaceae) , S. gemmifer, S. mucronatus var. S. mucronatus and S. triangulatus Cytologia, 69 : 75-83.
33. Mendoza M, Mills D E, Lata H, Chandra S, ElSohly M A and Almirall J. 2009). Genetic individualization of C. sativa by an STR multiplex. Anal Bioanal Chem, 393:719–726.
34. Mishra R and Kaur G. 2013. Aqueous ethanolic extract of Tinospora cordifolia as a potential candidate for differentiation based therapy of glioblastomas. PLoS ONE, 8: e78764. doi:10.1371/journal.pone.0078764.
35. Murch S J, Krishna Raj S, Saxena P K. 2000. Tryptophan is a precursor for melatonin and serotonin biosynthesis in in-vitro regenerated St. John's wort (Hypericum perforatum L. cv. Anthos) plants. Plant Cell Rep. 19: 698-704.
36. Nagella P and Murthy H N. 2011. Effects of macroelements and nitrogen source on biomass accumulation and withanolide-A production from cell suspension cultures of Withania somnifera (L.) Dunal. Plant Cell Tissue Organ Cult, 104:119–124.
37. Nayak S. and Sen S. 1995). Rapid and stable propagation of Ornithogalum umbellatum L. in long term culture. Plant Cell Rep. 15: 150D153.
38. Oliveira de Y, Pinto F, Silva da L L A, Guedes I, Biasi A L and Quoirin M. 2010. An efficient protocol for micropropagation of Melaleuca alternifolia (Cheel). In vitro 46: 192- 197.
39. Patel M B and Mishra S 2011. Hypoglycemic activity of alkaloidal fraction of Tinospora cordifolia. Phytomedicine, 18:1045–1052.
40. Pacheco G, Gagliardi R F I and Valls J F M. 2009. Micropropagation and in vitro conservation of wild Arachis species. Plant Cell Tissue Organ Cult. 99: 239- 249.
41. Perez-Bermudez P, Seitz H U and Gavidia I. 2002. A protocol for rapid micropropagation of Endangered Isoplexis. In-vitro Cell Dev Biol- Plant , 38: 178-82.

42. Raguhu A V, Geetha P S, Martin G, Balachandran I and Ravindran N P. 2006. In vitro Clonal propagation through mature nodes of *Tinospora cordifolia* (willd.) Hook.F. and Thoms.
43. Rai V R. 2002. Rapid clonal propagation of *Nothapody tesfoetida* (wight) sleumer– a threatened medicinal tree. *In-vitro Cell Dev Biol-Plant*, 38: 347-51.
44. Regalado J J, Martin E C, Castro P, Moreno R., Gil J and Encina C L. 2015. Study of the somaclonal variation produced by different methods of polyploidization in *Asparagus officinalis* L. *Plant Cell Tiss Organ Cult*, 128: 2019- 2035.
45. Sangwan R S, Chawrasiya N D, Misra L N, Lal P, Uniyal G C, Sharma R, Sangwan N S, Suri K A, Qazi G N and Tuli R. 2004. Phytochemical variability in commercial herbal products and preparation of *Withania somnifera* (Ashwagandha). *Current Sci*, 86:461–465.
46. Sen J. and Sharma A K. 1991. Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. *Plant Cell, Tissue and Organ Culture*, 26: 71-73.
47. Sharma U, Bala M, Kumar N and Bhalerao S. 2012. Immunomodulatory active compounds from *Tinospora cordifolia*. *Journal of Ethnopharmacology*, 141: 918-926.
48. Silva T D J A. 2004. Mining the essential oils of the Anthemideae. *Afr. J. Biotechnol.*, 3: 706–720.
49. Sivakumar G and Krishnamurthy K V. 2004. In vitro organogenetic responses of *Gloriosa superba*. *Russian Journal of Plant Physiology*, 51:790-798.
50. Silja P K, G P, Gisha G P and Satheeshkumar K. 2014. Enhanced plumbagin accumulation in embryogenic cell suspension cultures of *Plumbago rosea* L. following elicitation, 119: 469–477.
51. Sing A, Sah S K., Pradhan A, Rajbahak A and Maharajan N. 2009. In vitro study of *Tinospora cordifolia* (Willd.) Miers (Menispermaceae). *Botanica Orientalis- J Plant Sci*. 6: 103- 105.
52. Singh S K., Rai M K, Asthana P, Sahoo L. 2010. Alginate-encapsulation of nodal segments for propagation, short term conservation and germplasm exchange and distribution of *Eclipta alba* (L.). *Acta Physiologiae Plantarum*. 32: 607-610.
53. Sujatha G and Kumari R D B. 2007. High- frequency shoot multiplication in *Artemisia vulgaris* L. using thidiazuron. *Plant Biotechnology Rep*; 1:149-154.
54. Tan V P. 2016. Micropropagation of *Curcuma* sp., a threatened medicinal plant. *Advances in Bioscience and Biotechnology*, 7: 418- 427.
55. Tomiczak K, Mikula A, Sliwinska E and Rybezynski J. 2015. Autotetraploid plant regeneration by indirect somatic embryogenesis from leaf mesophyll protoplasts of diploid *Gentiana decumbens* L. F. In *In Vitro Cell Dev Biol Plant.*, 51: 350- 359.
56. Verpoorte R, Contin A and Memelink J. 2002. Biotechnology for the production of plant secondary metabolites. *Phytochem Rev.*, 1:13–25.
57. Wang J, Wang J, Liu K, Xiao X, Lu Y, Liu M, and Xu D. 2010. An efficient plant regeneration system with in vitro flavonoid accumulation for *Hylotelephium tatarinowii* (Maxim.) H. Ohba. 46: 445- 450.
58. Wink M. 1988. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theor Appl Genet*, 75: 225–233.
59. Wu C H, Murthy H N, Hahn E J and Paek K Y. 2007. Large-scale cultivation of adventitious roots of *Echinacea purpurea* in airlift bioreactors for the production of chichoric acid, chlorogenic acid and caftaric acid. *Biotechnol Lett.*, 29:1179–1182.