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In vitro Propagation and the conservation procedure followed is suitable and relevant 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 diverse climatic, altitudinal differences and ecological environments. There have been acquisitive rates of intimidations of running down to these biological assets due to enormous biotic and abiotic stress. Unselective collection of plants for their medicinal, ornamental, perfumery practices, the prompt loss of genetic diversity and territory come predominantly from resource-poor areas of the world and from comprehensive biodiversity hotspots and degration are possible causes of threats. This article defines the efforts towards savings plants from destruction. Conservation of genetic materials of many vulnerable plants, which contains vanishing 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.

1. Introduction

Medicinal and aromatic plants are wildlife's aptitude and have been used for alleviating disease and educating the quality of life for ages. Plant-based medicines are used in all societies and cultures and have been one of the necessary lines of defense in sustaining health and fighting 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 assessed that plant based 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 assessed the current ultimatum for medicinal plants is approximately US $14 billion per year and the response is likely to increase more than US $5 trillion in 2050. In India, medicinal plant-related trade is assessed to be around US $1 billion per

year (Kala *et al.*, 2006; Kala and Sajwan, 2007). Over the years herbal medicines have increased rising trend for ingesting exclusively with the advance and normalisation of herbal medicines.

The industrial ingesting, substantial amounts of medicinal plant resources are expended in the country under its traditional health precaution practices at the domestic level, by traditional naturopaths and by physicians. An idea about the lushness and diversity of these health care performs in India can be had from the diversity of plant species used in these systems. It is assessed 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 considering 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 *Aedesaegypti* 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 (Rai*et 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 numerous pharmacological accomplishments, e.g., anti-cancer, antimicrobial, anticancer, wound healing, anti-inflammatory and altered T-cell proliferative activities and antifertility actions. (Kini*et al.,* 1997, Aparanji*et al.,* 2005, Chen *et al*., 2009).

The genetic biodiversity of some significant medicinal plants may be a threatening disorder due to inhabitants growth, urbanization, the unobstructed collection of medicinal plants from the natural lifestyles. To cope up with this startling position, it is essential to theimmediate conservation of that essential medicinal plant species through micropropagation practice (Hassan, 2012).

Karyomorphological investigation is a valuable method for characterizing plant chromosomes. The cytological stability of micropropagated plants necessities 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* derivative secondary metabolite synthesis have many advantages over the other bases 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- upsurge in the propagation rate of plants; the rapid multiplication of plants which in a specific climate do not give seeds or whose seeds have a low germination aptitude; obtainability of plants throughout the year, *i.e*., in all the seasons; uniform plants of a selected genotype; conservation of genetic assets of species and threatened plants.

In edict to continue true to type high yielding elite clones of above point out five species, plant regeneration via direct organogenesis is the ideal revenue. Since our goal is to improve a unfailing source of necessary pharmacologically active chemotypes of five species through *in vitro* propagation, it necessitates the establishment of a tissue culture system that should consist of negligible or no callus development in order to reduce the probability of induction and recapture 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 issues (light- intensity, quality of light, photoperiod, temperature, humidity etc. issues are of paramount importance to successfully acquire viable tissue culture aspects are of paramount importance to successfully obtain viable tissue culture.

In vitro practises deliver valuable resources 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 advantage of producing uniform planting material for balancing the heaviness 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, reproduciblemethod 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.

2. 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.) MiersHook. 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 trial 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; *Tinosporacordifolia* (Willd.) Miers ex Hook.f.& Thomson- CNH/2014/Tech.II/78/200 and *Withaniasomnifera* (L.)Dunal- CNH/ 37h /2014 /Tech.II / 137.

- **Methods:**

Morphological Study:

The key analytic morphological features, viz. plant habit, plant height, leaf characteristics, flowers number, fruiting calyx, berry colour, seed set/fruit, and seed weight, were noted from the living plants in the field. For each character an average of 20 readings from as many plants within an accession/cutivar 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, due to its high native and export demands, five species in the wild populations are overexploited at an unrestrained 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 acme cultivation of its high-yielding genotypes for profitable 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 developedusing HPLC methods.

For Colchicine from *G. superba*15 tubers per district and 5 per population. The tubers were systematically washed, dried, powered and extracted with methanol for 6 h at 40^0C. The extract was evaporated, and the residue redissolved in distilled water, separated against n-hexane and finally the aqueous phasecontaining colchicine was extracted with chloroform. The chloroform was evaporated and the residue dissolved inhigh-pressure liquid chromatography (HPLC)-grade methanol. A quantitative analysis was performed on Waters liquid chromatograph (Waters 2489 UV/Visible Detector) employing aXBridge C18 HPLC Column with acetonitrile: methanol: water(35:5:60). The flow rate was 1 ml/min and all chromatographswere plotted at 243nm, 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 additional 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 40^0C. The extract was moved 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 (25–30^0C) 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 45^0C. 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 defined 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	*T. cordifolia*	Stem	Acetonitrile: Water (60:40, v/v)	265
2	Colchicine	*G. superba*	Tuber	Acetonitrile: Methanol: Water (35:5:60)	243
3	Plumbagin	*P. zeylanica*	Root	Methanol: Water (70:30, v/v)	270
4	Withanolide A &withaferin A	*W. somnifera*	Root	Acetonitrile: Water (60:40, v/v)	227

3. Micropropagation method

***Culture media and incubation condition*:**

Murashige and Skoog (MS), basal and improved media (consisting of varying amount of salts, vitamins and several 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 numerous concentrations to MS medium before the pH of the medium was accustomed 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 separately or with low level of auxin (s) were verified and were documented periodically. For shoot elongation multiple shoots induced were excised separately and sub-cultured on to MS medium augmented with BAP (1.0 mg/l) and variable concentrations of gibberellic acid (GA_3) (0.2, 0.4, 0.6, 0.8, and 1.0 mg/l) or MS medium augmented 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±2°C, 60% RH and a 16-h photoperiod (using white fluorescent tubes) under a photosynthetic photon flux density of 30 $\mu molm^{-2\ s-1}$.

***Surface Sterilization, Initiation and Subsequent Stages of Culture*:**

Various types of explants (mainly shoot tips and rhizomatous tuber in case of *G. superba*) were collected from elite selected healthy plants growing inside the shade-net house. Then, the explants were treated with 2.0 % non-systematic fungicide (thiram) for 10- 15 min followed by 3.0 % liquid detergent (Tween-20) for 1 min, then washed systematically in running tap water, and lastly 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 augmented with altered concentrations and mixture of plant growth regulators (PGRs) and different additives as experimental necessities. 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 edited and implanted on both MS and ½ MS basal media, which facilitated 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 ½ MS media along with 1- 3% sucrose.

4. *In vitro* Conservation:

Slow growth is usually reached by reducing the culture temperature, by amending culture media (reducedmedia strength) with additions of osmotic agents (mannitol and sorbitol 1.0 –3.0 %, w/v), and removing all pant growth regulators.

5. 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 poly-bags to continue 90–99 % relative humidity. Ultimately, the acclimatized plants were moved 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).

6. 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-orcine staining method. Root tips were pretreated in saturated solution of *p*- dichlorobenzene (*p*DB) for 3 to 6 hrs at 12- 16°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-orcein: 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.

7. Results

Selected elite chemotype:

For selection of elite genotype or accessionthe 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) 0.0291% (root)	Withanolide A Withaferin A	Bankura, W.B., India (wild)
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 inselected district (s) synthesize more metabolite rather than some available cultivars (*W. somnifera*).So furtherall experimental works were carried out in selected accession only.

8. Micropropagation

Micropropagtion 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 shoots 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 number 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 KNO_3 and 200 mg/l myo-inositol within 30 days of culture. For shoot elongation phenomena GA_3 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 GA_3 depending onspecies. 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.*

9. *In vitro* conservation

This method has the probable of extending 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%)
G. superba	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)
A. vulgaris	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)
W. sominifera	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)
T. cordifolia	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)
P. zeylanica	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)

10. 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. In periods of hardening plantlets grow about 2.0- 3.5 cm with two or four new leaves formation. The hardened plants were then moved 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 full sunlight and nearly100 % survival recorded after 4 months of field transfer.

Table: 3:

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

11. Evaluation of morphological characters in field growing regenerated plants

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

12. 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 changes were noted in regenerated plant chromosome in associate with karyotype analysis of mother plant. Thus, the somatic chromosome supplements of *in vitro* generated plants continued stable even after passing through three cycles of multiplication. It also authorises that no chromosomal inconsistency was induced from the short term conserved plants among the regenerates. It shows that reduced growth conditions useful in our studies along with the inherent genotypic potentiality and stability of the species

aided to maintain germplasm over such a short period of time in maintained and chromosomal uniformity among the regenerates. This present outcome is supported with some other revisions where regenerants are originate cytological stable.

13. 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) 0.031% (root)	Withanolide A Withaferin A	Bankura (wild)
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

4. Tinospora cordifolia, 5. Plambo zeylanica

14. Conclusion

In this research work, an effort has been made to provide the solicitation 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 practise monitored is proper and relevant for the clonal mass propagation of elite five medicinally important plant species.

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