Chapter 6
Summary
and Conclusions
SUMMARY AND CONCLUSIONS

India has probably the oldest, richest and most diverse cultural traditions in the use of medicinal plants. The dependency of human beings for utilizing plants for basic preventive and curative health care can be related from the ancient time. India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. Millions of Indians use herbal drugs regularly, like spices, home-remedies, health foods as well as over-the-counter as self-medication or also as drugs prescribed in the non-allopathic systems (Gautam et al. 2003). Presently, medicinal plants play a very important role in the modern economy. However, it is evaluated that more than 15,000 species of medicinal plants are globally threatened due to several causes like habitat loss, destructive harvesting which jeopardized the population of medicinal plants. Hence the conservation of medicinal plants is therefore, an important objective for the health and human welfare. In vitro technology has a huge potential and is emerging as an alternative tool for ex-situ conservation. The present study deals with the development of in vitro protocols for mass propagation and conservation of two endangered medicinal plant species, Decalepis arayalpathra and D. salicifolia coupled with various physiological, molecular and chemical profiling of regenerated plantlets. However, fig. 59 is showing overall experiments carried out for the selected plants and obtained results were summarized as follows.

1.4.1 Decalepis arayalpathra (J. Joseph & V. Chandras.) Venter

In vivo explants (NS, ST and leaf) and axenic explants (NS and ST) were examined for the propagation of D. arayalpathra via the different mode of organogenesis. In vivo explants turned better than axenic explants and similarly, NS was more responsive than ST explants.

In the case of in vivo derive NS, out of all the three cytokinins (BA, Kn and 2iP) examined, 5.0 µM BA induced a maximum of 4.0 ± 0.4 shoots/explant with an average shoot length of 4.8 ± 0.4 cm, in 85.8 ± 1.8% cultures. The nutrient medium comprising of MS + 5.0 µM Kn produced 3.3 ± 0.1 shoots/explant with an average shoot length of 4.4 ± 0.3 cm in 75.0 ± 1.8% cultures after 6 weeks of the incubation
period. However, 2iP was least responsive wherein, only 3.0 ± 0.2 shoots/explant with an average shoot length of 4.6 ± 0.1 cm was formed in 64.2 ± 1.4% culture on MS + 5.0 µM 2iP. Therefore, BA resulted to be the best cytokinin followed by Kn and 2iP. Although the use of single cytokinin resulted in axillary bud induction but some growth abnormalities such as basal callusing, the formation of stunted microshoots, lesser expansion in the lamina and early drop of leaf were observed in the microshoots.

An improved regeneration response along with enhanced shoot number and shoot length was achieved when different concentration of auxins (NAA, IAA and IBA) was supplied with optimized cytokinin containing media. A treatment of MS + 0.5 µM BA + 0.5 µM NAA was found optimal for the maximum production of 6.2 ± 0.2 shoots/explant with an average shoot length of 5.8 ± 0.3 cm in 93.61 ± 0.3% culture through *in vivo* derived NS after 6 weeks of an incubation period. However, the growth abnormalities remained the same as the duration of the inoculation advanced.

Application of growth additives (Ads, Glu and PG) at various concentrations in combination with the optimized cytokinin and auxin exhibited a significant improvement in all the parameter studied. Moreover, the addition of growth additives was also helpful to eradicate the associated abnormalities of the culture. Among all growth additives used, Ads was most suitable and a treatment consisting of MS + 5.0 µM BA + 0.5 µM NAA + 20 µM Ads resulted in the maximum production of 11.8 ± 0.1 shoots/explant with an average shoot length of 9.2 ± 0.1 cm in 93.61 ± 1.3% culture through *in vivo* NS after 6 weeks of incubation period. Thus, the use of growth additives with optimized cytokinin and auxin concentration proved effective to get prominent results than the single cytokinin and auxin-cytokinin combination media.

For axenic NS, MS medium augmented with 5.0 µM BA was found optimal with the production of only 2.96 ± 0.08 shoots/explant with an average shoot length of 2.53 ± 0.17cm in 85.00 ± 1.15% response, after 6 weeks of the incubation period. To improve the mean number of shoots/explant, the optimized concentration of cytokinin was used with various concentrations of auxins. A treatment consisting of MS + 0.5 µM BA + 0.5 µM NAA was able to produce only 3.90 ± 0.4 shoots/explant with an average shoot length of 4.50 ± 0.17 cm in 94.66 ± 1.7% cultures. However, the cultures were witnessed with the abnormalities either on single cytokinin or in
**Decalepis arayalpathra**

- **Axenic**
- **In vivo**

**Decalepis salicifolia**

- **Axenic**
- **In vivo**

**Direct organogenesis**

**Axenic**

**In vivo**

**Rooting**

**Acclimatization**

**Physiological**

**Biochemical**

**Genetic Fidelity**

**SEM**

**2H4MB content**

**GC-MS analysis**

**Elicitation**

**2H4MB evaluation**

**Fig. 60**: A schematic diagram of overall experiments.
combination treatments. The MS medium supplemented with optimized cytokinin and auxin concentrations was found most effective for the maximum shoot regeneration of 7.10 ± 0.20 shoots/explant with an average shoot length of 8.00 ± 0.11 cm in 92.0 ± 1.21% culture after 6 weeks incubation period on a treatment consisting of 5.0 µM BA + 0.5 µM NAA + 20 µM Ads. The treatment was also effective in the elimination of associated abnormalities in the cultures.

Similar to the in vivo derived NS, in vivo derived ST was found superior to axenic ST explants. Among all the cytokinins evaluated singly, 5.0 µM BA was found optimal with the production of a maximum number of 3.0 ± 0.1 shoots/explant with an average shoot length of 2.9 ± 0.2 cm in 60.0 ± 2.9% cultures, through in vivo derived ST explants. While, axenic ST explants gave only 2.30 ± 0.11 shoots/explant with an average shoot length of 2.00 ± 0.1cm in 80.00 ± 1.50% cultures. When cytokinin-auxin combination treatments was used, in vivo derived ST explants exhibited improved shoot regeneration as an average number of 4.0 ± 0.1 shoots/explant with an average shoot length of 2.3 ± 0.1cm in 72.8 ± 2.0% cultures were produced on MS + 5.0 µM BA + 0.5 µM NAA, in comparison to 3.50 ± 0.17 shoots/explant with an average shoot length of 4.00 ± 0.12 cm in 92.66 ± 1.76% cultures through axenic ST explants on same treatment and incubation period. On the optimal medium comprised of MS + 5.0 µM BA + 0.5 µM NAA + 20 µM Ads, the in vivo derived ST explants resulted in the maximum production of 5.5 ± 0.2 shoots/explant with an average shoot length of 4.8 ± 0.1cm, in 90.2 ± 2.0% cultures while, axenic ST explants produced a maximum of 5.06 ± 0.12 shoots/explant with an average shoot length of 4.76 ± 0.14 cm in 93.00 ± 1.70% cultures on the same treatment and incubation period.

For the indirect organogenesis, non-meristematic explants viz. in vivo leaf segments of D. arayalpathra was inoculated on MS medium supplemented with different PGRs either singly or in combination treatments. Different kinds of callus were formed according to the PGRs combination and concentrations. However, there was no differentiation of shoot buds from the callii on any of the treatment and even the different subculture passages.

In the present study, different factors like pH of the medium and sucrose concentration were evaluated, which directly affects the shoot morphogenesis. During
our observation, it was reported that among the different value of pH, 5.8 was consistently found to be optimal and responsible for the production of the maximum number of shoots/explant. Beyond the optimal pH value, a decrease in shoots/explant was recorded. Similarly, among the different concentration of sucrose tested, 3% sucrose was found quintessential for the maximum regeneration, differentiation and proliferation of shoots irrespective of the various explants. Moreover, sucrose concentration beyond the optimal level responsible for the reduction in all the growth parameters.

For long-term maintenance and multiplication of shoots, the regenerating tissue was subcultured after every 4 weeks on to the optimal regenerating medium (MS + 5.0 µM BA + 0.5 µM NAA + 20 µM Ads). An optimum production of shoots/explant was consistently observed after every 4 weeks of subculture passages.

The obtained microshoots of *D. arayalpathra* were further exploited for rhizogenesis. *In vitro* rooting was best obtained on agar gelled half-strength MS medium fortified with 2.5 µM NAA, wherein a maximum of 5.1 ± 0.1 roots/microshoot with an average root length of 4.9 ± 0.1cm in 91.6 ± 0.5% cultures. While in the case of phytogel gelled half-strength MS medium containing same treatment induced only 4.0 ± 0.2 roots/microshoot with an average root length of 2.4 ± 0.1cm in 90.6 ± 0.5% cultures, after 4 weeks of incubation period. The complete plantlets accompanied with a healthy shoot and root was acclimatized in different potting substrates (soilrite, vermi-compost and garden soil). However, soilrite proved helpful in promoting the root thickness. The acclimatized plantlets were hardened accordingly and transfer to the natural field conditions wherein, a survival rate of 92.3 % was recorded.

For the production of synthetic seeds, *in vivo* NS were encapsulated in encapsulation matrix, where 3.0% sodium alginate with 100 mM CaCl$_2$·2H$_2$O was found ideal for perfect bead formation when subjected to a complexing time period of 30 min. Application of PGRs proved favorable for the shoots re-growth and overall development and a concentration of 2.5 µM BA had a profound effect on % re-growth frequency. Furthermore, PGR augmented nutrient encapsulation was executed to corroborate the effect of nutrient augmentation (artificial endosperm) along with exogenous nutrient supplementation in the planting media, wherein 2.5 µM BA + 0.5 µM NAA + 20 µM Ads proved more advantageous when supplied in the
encapsulation matrix and resulted in re-growth frequency of 71.26 ± 1.12% culture after 6 weeks of incubation period.

To evaluate the germination and short-term preservation of encapsulated and non-encapsulated NS at various storage periods, was also carried out at 4 °C. The optimum regeneration frequency was achieved by encapsulated NS when matrix composition was supplemented with MS basal medium + PGR wherein, a maximum regeneration frequency of 78.13 ± 1.27% was recorded at 0 weeks of storage and a minimum of 44.10 ± 1.24% re-growth was obtained after 8 weeks of storage. Thus, it is distinct from the above results that nutrient encapsulation of NS was advantageous with the maintenance of viability of alginate beads for longer duration that too with optimum regeneration frequency.

Considering the importance of acclimatization periods, the physiological and biochemical parameters were also evaluated for in vitro raised plantlets of D. arayalpathra. The net photosynthetic rate and chlorophyll content showed an initial fall followed by gradual increased in their values as the day advanced. The value reached to their maximum value after 28 days of acclimatization. Similarly, transpiration rate and stomatal conductance was also evaluated and the result clearly depicts the initial fall in their value followed by linear increased as the acclimatization days advanced. The obtained results of physiological parameter clearly illustrate the sustainability of in vitro raised plantlets of D. arayalpathra in natural environment. In the present investigation, the higher concentration of antioxidant enzyme was recorded which clearly exhibits the adaptation of in vitro raised plantlets to the natural environment. After initial increased in SOD level, a decreased SOD concentration was observed on 28 days of acclimatization. However, a gradual increased was observed in CAT, APX and GR during the acclimatization period which depicts the adaptability of in vitro raised planets in natural environment.

The evaluation and quantification of 2-hydroxy-4-methoxybenzaldehyde (2H4MB) present in in vitro raised plants of D. arayalpathra was also carried out by using HPLC analysis. The quantification of 2H4MB was carried out at different periods of acclimatization (0, 2, 4, 6 and 8 weeks). The results clearly depicts the correlation between the 2H4MB content and biomass content. However, the synthesis of 2H4MB
started after 2 weeks of acclimatization and increased linearly as the weeks of acclimatization increased.

Histological observations at various stages of differentiation obtained through NS clearly exhibited the direct mode of origin of organogenesis of the regenerating tissue.

Randomly selected in vitro raised plantlets were chosen along with the mother plant to assess the genetic homogeneity using two molecular markers viz. RAPD and ISSR. The obtained monomorphic banding pattern did not exhibit any variation in morphological or growth characteristics when compared with the mother plant, thus, confirming the genetic stability of regenerants.

The investigation of the leaf surface during acclimatization period was carried out by using SEM technique to examine the fabric and texture of leaves of micropropagated plants of D. arayalpathra. The lower surface of stomata witnessed the presence of dens stomata with waxy deposition. The SEM analysis clearly revealed that in vitro raised plantlets of D. arayalpathra when subjected to external environment produced no morphological variation in leaves.

GC-MS analysis of root tuber extract of both in vitro raised plant and mother plant has also been carried out to investigate the minor and major metabolites present in the root tuber. As the root tuber is the most important plant part and methanol was found a suitable solvent. The GC-MS analysis also confirms the chemical homogeneity of the in vitro raised plant and mother plant. Similar kinds of compound have been reported in both in vitro raised and mother plant.

6.2 Decalepis salicifolia (Bedd. Ex Hook.f.) Venter

In vivo explants (NS, ST and leaf) and axenic explants (NS and ST) were examined for propagation of D. salicifolia via different mode of organogenesis. In vivo explants turned better than axenic explants and similarly, NS was more responsive than ST explants.

In case of in vivo derived NS, out of all the three cytokinins (BA, Kn and 2iP) evaluated, a treatment of MS + 5.0 µM BA produced an average of 2.06 ± 0.09 shoots/explant with an average shoot length of 2.84 ± 0.09cm in 88.6 ± 1.07% cultures. The treatment of 2iP was least while Kn was moderately effective.
Therefore, application of BA was found superior to Kn and 2iP. Although, the treatment of single cytokinin induced axillary shoot buds but with certain growth abnormalities viz. basal callusing, stunned microshoot growth, lesser expansion in lamina and premature leaf drop were observed in the regenerating cultures as the time advanced.

The application of different range of auxins (IAA, NAA and IBA) in combination with optimized concentration of cytokinin improved the mean shoots number and shoot length in comparison to single cytokinin treatment. Among the different auxins tested, best response was observed on a treatment consisting of MS + 5.0 µM BA + 0.5 µM NAA, wherein a maximum of 4.04 ± 0.05 shoots/explant were produced with an average shoot length of 4.99 ± 0.05 cm in 90.2 ± 1.28% cultures. However, the associated growth abnormalities were same as observed in single cytokinin treatment, irrespective of the type of explants.

Application of growth additives (Ads, Glu and PG) at various concentrations in combination with optimized auxin and cytokinin concentration not only revealed a remarkable increased in the shoot production but also eliminated the growth abnormalities that observed during single cytokinin and combination treatment. A treatment consisting of MS + 5.0 µM BA + 0.5 µM NAA + 30 µM Ads was found effective with the production of 9.97 ± 0.01 shoots/explants with an average shoot length of 6.46 ± 0.1 cm in 91.2 ± 1.16% cultures, after 6 weeks of incubation periods. Thus, the treatment of growth additives with optimized cytokinin and auxin concentration proved much effective to get remarkable results than single cytokinin and cytokinin-auxin combination media.

For axenic NS, MS medium augmented with 5.0 µM BA proved better than other cytokinins with the production of only 1.50 ± 0.17 shoots/explant with an average shoot length of 2.50 ± 0.1 cm in 86.56 ± 1.69% cultures, after 6 weeks of incubation period. Similar to the in vivo derived NS, axenic NS exhibited poorer response on the single cytokinin treatment. A treatment consisting of MS + 5.0 µM BA + 0.5 µM NAA exhibited significant improvement in number of shoot regeneration and produced a mean of 1.96 ± 0.14 shoots/explant with an average shoot length of 3.80 ± 0.11 cm in 91.03 ± 1.83% cultures. However, the cultures were witnessed with various growth abnormalities viz. basal callusing, early leaf drop and lesser expansion in
lamina. The application of growth additives did not exhibit significant improvement in mean shoot number but increased length was observed and also the associated abnormalities in cultures were eliminated. A treatment consisting of MS + 5.0 μM BA + 0.5 μM NAA + 30 Ads produced 2.56 ± 0.20 shoots/explant with an average shoot length of 5.23 ± 0.15 cm in 92.66 ± 1.76% cultures, after 6 weeks of inoculation. The obtained results clearly depicts that, axenic NS was lesser responsive than in vivo derived NS.

Similar to the NS, in vivo derived ST explants were found more responsive in comparison to the axenic ST explants. Among all cytokinins tested singly, a treatment consisting of MS + 5.0 μM BA was found optimal with the production of maximum number of 2.00 ± 0.11 shoots/explant with an average shoot length of 3.00 ± 0.11cm in 88.00 ± 1.15% cultures. While, only 1.30 ± 0.17 shoots/explant with an average shoot length of 2.0 ± 0.1cm were obtained through axenic ST explants in 76.56 ± 1.68% cultures on same treatment. When auxin-cytokinin combination treatment was used, the in vivo derived ST produced 3.0 ± 0.11 shoots/explant with an average shoot length of 4.00 ± 0.11 cm on MS + 5.0 µM BA + 0.5 µM NAA in 90.6 ± 0.66% cultures, whereas only 1.46 ± 0.14 shoots/explant with an average shoot length of 2.90 ± 0.11cm were obtained through axenic ST explants on similar treatment and incubation period with 90.03 ± 1.82% response The media supplied with cytokinin-auxin-growth additives combination exhibited better response in comparison to the single cytokine and cytokinin-auxin combination treatment. A treatment consisting of MS + 5.0 μM BA + 0.5 μM NAA + 30 Ads produced 5.00 ± 0.11 shoots/explant with a mean shoot length of 6.00 ± 0.11 cm in 96.76 ± 1.00% cultures through in vivo derived ST explants. On the other hand, same treatment induced only 2.20 ± 0.21 shoots/explant with an average shoot length of 4.33 ± 0.14 cm in 91.62 ± 1.75% cultures, through axenic ST explants in similar incubation period.

For the indirect organogenesis, nonmeristematic explants viz. in vivo leaf segments of D. salicifolia was inoculated on MS medium augmented with various PGRs either singly or in combination treatments. Different kinds of callus were formed according to the PGR combination and concentrations. However, no differentiation of shoot buds from the callii was observed onto any growth medium even after different subculture passages.
Similar to *D. arayalpathrta*, pH value of 5.8 and 3% sucrose concentration was found optimum and exhibited maximum regeneration potential irrespective of explants type and mode of organogenesis. Moreover, for long-term maintenance and multiplication of shoots obtained through best-optimized medium, the regenerating explants were subcultured on to the fresh medium and maintained up to the 6\textsuperscript{th} passages, wherein the regeneration efficiency was maintained up to 4\textsuperscript{th} subculture passages. Thereafter, a reduction in regeneration frequency and the number of shoots differentiation was observed.

*In vitro* rooting in the microshoots of *D. salicifolia* was best obtained on agar gelled half-strength MS medium supplemented with 2.5 µM IBA, wherein a maximum of 6.10 ± 0.07 roots/microshoot with an average root length of 2.30 ± 0.06 cm was observed with 91.0 ± 1.00% response in 4 weeks of incubation period. While on phytigel gelled medium, a mean of 4.3 ± 0.07 roots/microshoot with an average root length of 4.8 ± 0.06 cm was observed in 90.0 ± 1.00% cultures, on same treatment and incubation period.

The micropropagated plantlets accompanied with healthy shoots and roots were acclimatized using different potting substrate viz. soilrite, vermin-compost and garden soil. The plantlets were acclimatized in soilrite wherein healthy growth was observed. Acclimatized plantlets were hardened off accordingly and thereafter, transplanted to natural environment wherein 89.3% survival rate was recorded.

Synthetic seeds were produced by encapsulating in vivo NS explants in a matrix of 3.0% sodium alginate and 100 mM CaCl\textsubscript{2}·2H\textsubscript{2}O. PGR treatment of 2.5 µM BA + 0.5 µM NAA + 30 µM Ads when supplied in the encapsulation matrix, resulted in the maximum re-growth frequency of 71.30 ± 1.22% after an incubation period of 6 weeks when inoculated on MS + 5.0 µM BA. Storage of encapsulated and non-encapsulated NS explants was also carried out at 4\textdegree C, where optimum regeneration frequency was achieved by encapsulated NS explants when the matrix composition was fortified with MS + PGRs. A maximum regeneration frequency of 77.13 ± 1.22% was recorded at 0 weeks of storage while 43.10 ± 1.11% of regeneration frequency was observed after 8 weeks of storage on the medium comprised of MS + 5.0 µM BA + 0.5 NAA µM + 30 µM Ads.
Similar to the *D. arayalpathra*, physiological parameters (net-photosynthetic rate, chlorophyll a, b, carotenoids, transpiration rate and stomatal conductance) evaluated for *in vitro* raised plantlet of *D. salicifolia* during their acclimatization period. Similarly, biochemical parameters (SOD, CAT, APX, GR, TBARS content and electrolyte leakage) were also been evaluated. The obtained results clearly specify that the plantlets have sustained the different environmental stress and adapted for the growth in natural environment.

The evaluation of 2-hydroxy-4-methoxybenzaldehyde (2H4MB) was also carried out for root tuber of *in vitro* raised plants of *D. arayalpathra* through HPLC analysis. The quantification of 2H4MB was carried out at different periods of acclimatization (0, 2, 4, 6, 8 and 10 weeks). The results clearly depicts the correlation between the 2H4MB and biomass content. However, the synthesis of 2H4MB started after 2 weeks of acclimatization and increased linearly as the weeks of acclimatization increased.

Histological examination of the regenerating tissue of in vivo derived NS explants clearly exhibited the direct mode of origin of organogenesis.

Genetic fidelity of *in vitro* raised plantlets and mother plant of *D. salicifolia* was also performed via using molecular markers viz. RAPD and ISSR. The obtained amplification products were monomorphic and the plantlets did not show any detectable variation in morphological or growth characteristics when compared with the source plant, thus confirming the genetic homogeneity of regenerants.

SEM analysis was carried out to examine the fabric and texture of leaves of *in vitro* raised plants of *D. salicifolia* revealed the definite characteristics as that of stomata and unique appearance were observed on both the surface of leaves. However, stomatal density was higher at lower surface. The observation for SEM analysis revealed that no significant morphological differences in the leaves were observed with that of mother plant when exposed to the external environment.

GC-MS analysis of the both mother and *in vitro* raised plants of *D. salicifolia* was performed and results clearly revealed the chemical homogeneity. The GC-MS analysis also explores the unexplored minor and major metabolites present in the root tuber of *D. salicifolia*. 
The suspension culture of root tuber derived callus of *D. salicifolia* were used for the study of impacts of elicitation on biomass production and 2H4MB content. A treatment consisting of MS + 1.0 µM TDZ + 1.0 µM NAA was found optimal for maximum (84.8%) callus induction from the root tuber. Further, two important biotic elicitors, chitosan (CH) and yeast extract (YE) at various concentrations (50, 100, 200 and 300 µM) were used with different contact period (24, 48, 72 and 96). The obtained results clearly exhibits the supremacy of CH over YE, wherein a maximum biomass of 9.7 DW g/l was achieved at optimum contact period of 72 h when suspension culture was treated with 200 µM CH. Similarly, a maximum content of 2H4MB (14.8 µg/g) was recorded on similar chitosan concentration and contact period.

**Conclusions**

The following conclusions have been drawn from the present study:

- Direct organogenesis was obtained on premeristem containing explants of both the species, wherein, maximum regeneration was obtained on optimum cytokinin-auxin-growth additives combination medium (MS + 5.0 µM BA + 0.5 µM NAA + 20 µM Ads for *D. arayalpathra* and MS + 5.0 µM BA + 0.5 µM NAA + 30 µM Ads for *D. salicifolia*).
- Successful in vitro rooting in the microshoot was best obtained on agar gelled half-strength MS medium supplemented with 2.5µM NAA for *D. arayalpathra* and 2.5 µM IBA for *D. salicifolia*.
- For the maximum re-growth of synthetic seeds, PGR supplemented nutrient encapsulation proved to be more advantageous in both the plants and planting medium composed of MS + 5.0 µM + 0.5 NAA µM + 20 µM Ads and MS + 5.0 µM + 0.5 NAA µM + 30 µM Ads was more suitable for *D. arayalpathra* and *D. salicifolia*, respectively.
- Amongst various planting substrates, soilrite was found to be the best for the successful acclimatization of plantlets with the maximum survival rate of above 90% in both *D. salicifolia* and *D. arayalpathra*.
- Enhanced level of photosynthetic pigments after transfer of plantlets from in vitro to ex vitro condition correspond to the stability of the plant against light stress.
Significant change in antioxidant enzymes during acclimatization proved the preventive role of the plant against various environmental stresses.

Quantitative analysis of 2H4MB in the roots tubers of \textit{in vitro} raised plantlets revealed the correlation between 2H4MB and biomass content.

The Histological section clearly revealed the direct mode of regeneration.

Genetic stability through RAPD and ISSR markers clearly exhibited monomorphic banding pattern of the randomly selected regenerants of both the species and thus proving the clonal propagation.

SEM analysis of leaf surface revealed that \textit{in vitro} raised plantlets of both \textit{D. arayalpathra} and \textit{D. salicifolia} exhibit similar morphological development to the mother plant when subjected to external environment.

Metabolic profiling of root tuber through GC-MS analysis resulted in the identification of several minor and major metabolites confirming the chemical homogeneity of \textit{in vitro} raised plants and mother plant of both the species.

Chitosan was found to be more suitable elicitor over Yeast extract and results showed enhanced accumulation of 2H4MB in suspension culture.