DISCUSSION
The current rate at which massive fragmentation of habitat and extinction of many of the species is taking place and the extent of devastation it has caused in a short time frame, has resulted in resetting the future evolution of the planet's biota (Novacek and Cleland, 2001).

The dramatic loss of biodiversity is seeing severe repercussions all over the world in recent times particularly in phyto sphere. Thousands of species are being lost forever for which there are no records.

In the past few decades Complementary Alternative Medicinal Systems (CAMs) have received global acceptance and Botanical Dietary Supplements (BDS) have surfaced as a major segment of CAMs (Brevoort, 1994; Krochmal, 2004). Also, indigenous medicinal systems that were so far undocumented are now moving towards global significance along with other CAMs like Chinese, Siddha & Unani, etc. The keen interest of the Pharma Industries in these and the increasing attention being given to these systems at the international level are evidences of the same (Berliner & Salmon, 1980). India with its huge area and various climatic conditions has not only been blessed with a variety of forests, but also associated folklores and several of these CAMs.

Because of developmental pressures and related deforestation, the phyto flora is already under threat. To add to these problems, due to the increasing usage of herb based therapies, there has been an uncontrolled uprooting of medicinal plants from the wild which is not accounted for. The forest dwellers, many a times, not knowing the importance of ecoequilibrium, for some petty cash, uproot these plants in bulk, which are then utilized by pharmaceutical companies to incur huge profits. In the process the forests suffer. Hence, there are newer approaches required to protect these plants in the wild.

Plant tissue culture makes one very important component of such an integral activity. Because of these reasons, there is a growing interest in plant tissue culture of various medicinal plants, both for conservation purposes as well as for their developmental studies and for their generation to meet the growing deficit between the demand and supply.
There also needs to be a research based validation for these plant species so as to ensure unscrupulous material from entering markets and being consumed by people who generally self treat using these formulations. With the advent of the current sciences, it is high time we verify the claims and efficacy of these drugs/formulations. The concept is being promoted by Government bodies like ICMR (ICMR Reports, 2006).

Thus, there is a growing need for a holistic analysis of these plants from the point of view of their regenerative potentials so as to generate a continuous supply of raw bulk as well as conserving these plants in their natural habitats and ensuring undisturbed ecology and prevention of extinction of the species.

Keeping this in mind, three important medicinal plants were chosen for the current work. These plants have been mentioned in catalogued indigenous systems and are also being used extensively by the tribal communities. The tribals use it for many such diseases which are not reported and can give a novel insight from the application point of view. These plants were chosen on the basis of a range of their significance. *Argyreia boseana* Santapau & Patel is an unexplored species as it hasn’t been reported in the books on the flora of Gujarat. The plant was reported by our laboratory in a doctoral study (D’Cruz, 2002) for the first time in Gujarat. *Curculigo orchioides* Gaertn. was chosen because of its endangered status and on the other hand its high medicinal values. The plant serves as a herbal Viagra and is one of the main ingredients for the formulations made by chiro practitioners (*Ayurvaidyas*) to cure sexual impotency. However, the plant has many other medicinal advantages, which have not got their due recognition and require in depth research based analysis to support the claims made in various CAMs. *Hemidesmus indicus* R. Br. was chosen as it is an important plant, for which the demands are always high in comparison to the supply. Since the roots is a part useful for herbal formulations, the method of collection of plant results in destruction of the plants. Thus, there has to be a regular supply of plant material in such a way that a significant number of plants are conserved *in situ*.

**Establishing plants ex situ:**

As a first step, we brought the plants from the forests of the South Gujarat region and these were then established in the Ecological Niche of St. Xavier’s College, Ahmedabad. At this stage itself because of the difference in the climatic conditions, various problems were encountered. Problem like lower survival rate and apical dominance were seen predominantly in *Argyreia boseana*.
Santapau & Patel and *Hemidesmus indicus* roxb. *Curculigo orchioides* survived well. However, the proliferation was seen to be less in the first year. Once the plants got acclimatized to the *ex situ* environment, the work was initiated.

**Plant Tissue Culture of three species:**

Plant Tissue Culture work for all three species was carried out. To the best of our knowledge, there are no reports on the plant *Argyreia boseana*, specially on the Gujarat based mother plants as it has been missed out in all the books cataloguing the flora of Gujarat. We could successfully establish calli cultures from the plants using leaf, petiole and nodal explants. The callus in 2,4 D: BAP was seen to be morphogenic and when the histology was studied, a well differentiated shoot structure was seen under the microscope. However, all the attempts to sprout those shoots were unsuccessful and we had to look for alternative protocols to generate plantlets. On the other hand, callus induction and proliferation was achieved *in vitro* and this callus was later used for phytochemical analysis. The shoots were generated using BAP 0.5 mg/l and the roots were generated to these shoots using IAA 0.2 mg/l. The plantlets were hardened at the lab scale as they were to be used for further analyses.

Entire plantlets of *Curculigo orchioides* Gaertn. were generated using MS Basal medium. Similar results have been reported by many other groups of scientists like D'Souza *et al*., 1997. However, there are no reports on optimization of culture conditions for higher rate of plantlet regeneration. Initial work on the effect of various parameters has been reported by Vaidya *et al*., 2005. This has been carried forward to the extent that complete statistical analysis was possible. On the basis of the results generated, the conditions were modified and optimization of the protocol was achieved. This optimization increased the plantlet production by five to seven folds, as compared to earlier reports by D’Souza *et al*. when the medium was fortified with BAP.

The effect of BAP was studied on leaf explants and it was found that lower concentrations of BAP did not allow any dedifferentiation. It, on the other hand produced results closer to that in case of MS basal medium. Only when BAP 3.0 mg/l, 4.0 mg/l and 5.0 mg/l were used for supplementation of MS medium, callus was generated. The callus was blackish brown and was highly granular. It was found to be very hard. On transferring the calli on basal medium, it sprouted into shoots. Francis *et al* have reported a protocol for *in vitro* clonal propagation of *Curculigo*
Curculigo orchioides Gaertn. through apical meristem culture using MS medium supplemented with 1.5 mg l⁻¹ 6-benzyladenine (BA), 100 mg l⁻¹ adenine sulfate (Ads) and 3% sucrose. The group’s findings are very close to our results showing the potentials of BAP to differentiate the leaf explants into plantlets. (Francis et al., 2007). Nagesh has reported use of BAP to germinate the shoot buds and shoot tips into plantlets. However, our studies were limited only to the leaf explants as in case of root discs or rhizomes, very high rate of contaminations was seen, which were difficult to curb (Nagesh, 2008).

Many other groups of scientists have also worked on the plant tissue culture of Curculigo orchioides Gaertn. having either identical or closely similar results with the ones mentioned above (Suri et al., 2002; Dhenuka & Balakrishnar, 1999; Suri et al., 1999; Suri et al., 1998).

However, to the best of our knowledge, this work is the first to report direct rhizogenesis from the leaf explants. Using 2, 4-D 5 mg/l, the leaf explants produced root clusters. These root clusters further developed into tubers.

Since Curculigo orchioides is an endangered plant (IUCN) and grows only for three months of the monsoon, the plant is being over exploited as it holds an important position in the ethnic folklore and CAMs. While through tissue culture the plant can be regenerated into multiple plants and the depleting numbers can be restored, success of direct rhizogenesis raises real possibilities of production for commercialization through a novel route. The rhizomes can be grown in vitro and can be sold both as planting material as well as for extraction of important phytochemicals, thus saving the plants from being lost from the forests. However, large scale standardization needs to be carried out before it can be adopted for commercial benefits.

The third plant, Hemidesmus indicus showed results identical to the reported ones. The callus was generated mainly to study the pattern of accumulation of compounds in undifferentiated mass of cells to establish preliminary results for fermentation and bulk production of these compounds. Direct organogenesis was achieved using BAP 2.0 mg/l, which were then multiplied on medium containing BAP 2.0 mg/l, IBA 0.1 mg/l fortified with ADS 30 mg/l. Results reported by Saha et al. show similar medium composition. In their work BAP 2.0 was fortified with NAA 0.5 for production of axillary shoots. Misra et al. report adventitious shoot induction using BAP 1.0 mg/l with NAA 0.1 mg/l fortified with 15 mg/l of ADS.
Though there are little differences found in the combinations chosen, essentially effect of higher cytokinin to auxin ration is evident in case of Hemidesmus indicus R. Br. Which is an established principle for obtaining multiple shoots. In case of plant tissue culture work, several factors play important roles viz. the age of explant, the climatic conditions in which the mother plant grows, season as well as the quality of chemicals used for the work. That makes the process highly susceptible to lab to lab variations. Thus, standardization of protocols are exclusively done on a lab to lab basis. However, the variations observed in case of this particular species are not so drastic.

In all three cases, the plantlets were hardened mainly in the in vitro conditions. As they were required for other analytical purposes whose results and implications are discussed further below.

**Need for Validation:**
Concerns have been expressed over the quality of plant material generated in vitro from the point of view of their pharmaceutical potentials. Since in in vitro condition, there are no stresses- neither biotic nor abiotic, the secondary metabolism machinery might function differently and may either completely not express the phytomolecules or will express them in concentrations which would make the process impractical from the market-economy point of view. Since in case of ornamental plant species, the metabolites are not that important, there is a ready market for that. But, when it comes to medicinal plants, there have been conflicting theories and that has affected the commercialization of plant tissue culture of medicinal plants, and further exacerbating the cost effectiveness issue.

Another major issue related to tissue culture regenerants is their genetic deviations or instability. Since there are both dedifferentiation and redifferentiation processes of tissues involved, the genetic variations are expected to occur and reported to occur (Larkin & Scowcroft, 1981). The details related to the factors of these variations have been covered in the review of literature in depth. Thus, if the product plantlet is not genetically identical, the term ‘clonal propagation’ cannot be employed. More than the term, there are other associated concerns as variations in genetic make up might lead to synthesis of compounds that are toxic or not as beneficial as the compounds expressed by the original plant material.
Since in direct organogenesis, the tissue doesn’t go through dedifferentiation, it is believed that only through direct organogenesis genetically identical plants can be produced. However, direct organogenesis is not always redifferentiation only. At an internal- sub epidermal level, the tissue has to undergo mild to severe dedifferentiation before redifferentiating. Thus, there are equal chances for the tissues to deviate from their parental genetic make up.

In the present work, we have demonstrated in our plant tissue culture experiments, that the products produced using the protocols developed by us, do not show any significant variation when compared with the source plant materials in terms of the presence of phytochemicals and even their genetic banding patterns as well as their protein profiling.

**Phytochemical Analysis:**

As the first step in this direction, phytochemical profiling was carried out using simple qualitative analysis tests. Comparative phytochemical analysis was carried out for 6 different groups of phytochemical viz. Alkaloids, Flavonoids, Tannins, Saponins, Proanthocyanidins & steroids. The results were found to be identical for both *in vitro* as well as *in vivo* plants. However, since these tests are specific for a group of phytochemicals and not for a specific phytochemical, this could not be confirmed for a specific compound level. However, since for both *in vitro* and *in vivo*, the results obtained were identical, it does confirm the presence of all important phytochemical groups.

Further comparative analysis was carried out using High Performance Thin Layer Chromatography (HPTLC), on which phytochemical fingerprints of methanolic extract of natural plant parts as well as *in vitro* regenerants were created. Three major classes of phytochemicals- Alkaloids, Flavonoids and Steroids were isolated and purified from natural plant part as well as *in vitro* regenerants and resolving solvent systems were established for them by trying various solvent systems. Callus was collected after 10, 20, 30, 40, 50 & 60 days, dried and run along the *in vitro* regenerants in case of *Argyreia boseana* Santapau & Patel.

As it is evident from the results, with advancing age of callus, the accumulation of these compounds was seen to be increasing and in *in vitro* regenerants, it was seen to be reaching very close to levels of the natural plants. This indicated that the plants were able to produce these phytochemicals in comparable amounts.
In case of *Curculigo orchioides*, however, it was observed that the plants once hardened in the field, showed a mild decrease in the compounds. This can be attributed to the absence of stress factors or decrease in them. Since the plant had germinated and had enough humidity and nutrition available, it did not face any threat to produce higher contents of compounds. It has been well established that the phenolics are synthesized in a plant especially when there is an immediate threat of infection or extinction. On the other hand, when the same hardened plants were used as the source plants for the tissue culture work, they produced phytochemicals in comparable amounts.

In certain cases, in comparative fingerprints, some new bands were found present at some specific stage of development in callus. Unless confirmed structurally, they cannot be presumed to be new compounds synthesized by the plants. They might be intermediates in the process of chemical conversion to ultimately result into the compounds present the mature plant *in vivo*. As these ‘new’ compounds were found present in very low concentrations and they were generally seen too closely placed to one of the isolates, the likelihood of them being intermediates is higher than the possibility of being a novel chemical entity *in toto*. However, the possibility of it being a new compound cannot be ruled out completely either.

**Phytochemical marker studies:** 7 different phytochemical markers were run along with these plant extracts on HPTLC and their presence and concentrations were checked. As it can be gathered from the data presented in the chapter of results, it was seen that both *in vivo* as well as *in vitro* plantlets showed presence of these marker compounds. And in case of callus analyses they were seen to be increasing with advancing age of callus.

However, the presence of these markers cannot be concluded solely on the basis of densitograms generated using HPTLC as Rf value may change with variations in solvent system. Also, structurally similar compounds may show very close Rfs which then creates confusion. To overcome this limitation, spectra of each band was taken at the specific absorbance maxima (Emax) of that marker. With this the identities of compounds was confirmed. As can be seen in the photoplates coinciding peaks of spectra indicate presence of compounds in all the cultures.

**HPLC Profiling:** HPLC profiling was also carried out to support the data generated through HPTLC. Since the marker compounds were a gift and were limited in quantities, only those
which were available were analyzed for HPLC. The HPLC profile showed presence of all the compounds in the extracts both \textit{in vivo} as well as \textit{in vitro}. However, in case of \textit{Hemidesmus indicus} R. Br., a slight shift in the spectra of samples was seen in comparison to standard, which can be because of the presence of interfering substances in the methanolic extracts.

**Confirmatory analyses:** Further confirmatory analysis was carried out by subjecting the isolates to IR, GC MS and NMR spectra. As can be seen from the results, the identities were confirmed using these methods.

**IR Spectra of some of the isolated compounds:** IR of three compounds, viz. Vanillin, Stigmasterol and Caffeic Acid were taken along with the reference standards. The purity spectra were taken which showed high purity. In Vanillin, a broad -OH stretch was seen at 3300 cm\(^{-1}\) while the carbonyl band was found present around 1740-1720 cm\(^{-1}\). In Ferulic Acid, -COOH stretch was tracked by a sharp peak at 1724-1700 cm\(^{-1}\) which overlaps with the C-H stretch, and a narrow stretch near 33 cm\(^{-1}\) indicating the presence of O-H were found present. Similarly -OH stretch was observed in stigma sterol isolated from \textit{Argyreia boseana}.

**GC- MS of Methanolic extracts of plants:** GC- MS showed presence of fatty acids and sterols in these extracts. In case of \textit{Hemidesmus indicus} R. Br., two important compounds viz. Lupeol Acetate and 4- Methoxysalicylaldehyde were found to be present both \textit{in vivo} as well as \textit{in vitro}.

**NMR Spectra of 9 different isolates:** The NMR spectra were taken for 9 different isolated compounds. In that, the NMR spectra was generated which was then compared with the online databases available and the structures were thus confirmed to be Ferulic Acid, Vanillin, Caffeic Acid, Rutin, Catechin, Gallic Acid and Ellagic Acid. The analysis of NMR spectra could not be done completely till the time the thesis was submitted and so the spectra were confirmed with the online databases. Further structural elucidation is in the process to verify the results.

Thus, through a series of analytical techniques, our work confirmed the presence of Stigmasterol, Caffeic Acid, Ferulic Acid and Rutin in \textit{Argyreia boseana} Santapau & Patel. Out of these Caffeic Acid and Ferulic Acid are known to have hepatoprotective properties along with Rutin. Stigma sterol has been reported to have protective roles in cancer. Thus, the claims made by the Vasava tribals about the plant species having liver protecting and liver damage curing properties
might be true. However, further animal model studies as well as clinical feasibility of the same needs to be verified.

As mentioned earlier, presence of Rutin and Stigmasterol have been reported in Argyreia nervosa (See Review of Literature). But no reports are found on the presence of Caffeic acid and Ferulic Acid in the genus. Though Ferulic Acid is a common secondary metabolite, it is generally found only in seeds, while in this case it is found in abundance in leaves as well as roots.

These experiments can be extended to high yield throughput of ferulic acid via cell suspension cultures or fermentor technology. Ferulic acid, which is a hepatoprotective compound, can be biotransformed using simple microbes into compounds like Vanillin which also holds very high commercial value.

In Curculigo orchioides Gaertn. B-sitosterol, Rutin, Catechin, Gallic Acid, Ellagic Acid were found to be present. HPLC profile also indicated the presence of Caffeic Acid. No literature reports presence of Catechin and Ellagic acid.

Catechin has also been reported to be astringent, antiviral and immunostimulant compound (Duke et al., 2002).

Curculigo orchioides Gaertn. is a highly medicinal plant and has diverse curing properties (). While the high contents of phenolics like Rutin, Catechin, Gallic Acid, Ellagic Acid make the plant highly anti oxidative in nature, which has been confirmed through our anti oxidant experiments, this property makes the plant a useful remedy for curing cancer. The anti oxidant property leads to anti cancer therapies too and many flavonoids and phenolics have been suggested as a cure for cancer (Malaveille, 1996).

In Hemidesmus indicus R. Br., as reported in literature Vanillin and Rutin were confirmed to be present by HPTLC. Further analysis with GC MS confirmed the presence of 4-Methoxy salicyl aldehyde as well as Lupeol Acetate.

While Vanillin holds commercial value, it also has many medicinal properties. It is a known anti oxidant and anti microbial compound. The compound has also been reported to be a very good
hypotriglyceridemic (Srinivasan et al., 2008). Vanillin has also been proposed as a potent compound for curing sickle cell anaemia (Abraham et al., 1991).

Lupeol and its derivatives are reported to possess anti-asthmatic activity that include strong antioxidant, antimutagenic, anti-inflammatory and anti-arthritic properties (Saleem et al., 2004).

Presence of these compounds are important from the point of view of verification of ethnomedicinal claims made about these plant species. As mentioned earlier, the Vasava tribals of South Gujarat Forests have been using these plants to cure various disorders.

Arqyreia boseana Santapau & Patel has been used by these tribals to cure a disease, locally known as ‘Nagot’, the symptoms of which are very similar to liver cirrhosis (D’Cruz, 2002). Presence of compounds like Ferulic Acid and Rutin confirm the hepatoprotective and hepatocuring properties of the plant as these compounds have long been known to possess liver protecting potentials. Caffeic acid, on the other hand has been reported in curing hepatocarcinoma and preventing DNA methylation (Chung, 2004). Ferulic Acid, Caffeic Acid and Rutin are also reported to possess antioxidant activity.

All the compounds found present in Curculigo orchioides Gaertn. are phenolics and are reported to possess antioxidant activities. Gallic Acid is a polyhydroxyphenolic compound which can be found in various natural products, such as green tea, grapes, strawberries, bananas and many other fruits (Inoue, 1994). GA was reported as a free radical scavenger and as an inducer of differentiation and apoptosis in leukemia, lung cancer, and colon adenocarcinoma cell lines, as well as in normal lymphocyte cells (Kawada et al., 2001; Salucci, 2002; Sohi et al., 2003). It has been postulated that GA plays an important role in the prevention of malignant transformation and cancer development (Taraphdar, 2003).

**Histo cytological experiments to study accumulation of various phytochemicals:** The results were further confirmed by carrying out microscopy based experiments, which showed less lignin deposition in in vitro tissues as compared to natural plants. However, amount of flavonoids as seen from the results also was found to be very high in case of callus, especially in Hemidesmus indicus. The higher accumulation of these compounds indicate active metabolism which might be due to the rearrangements and differentiation of cells or because of some stress condition being faced by the cultures.
Biochemical parameters: Certain biochemical parameters were compared for *in vivo* as well as *in vitro* plants. These were proteins, sugars—total sugar and reducing sugars, phenolics and tannins. It was seen that of the three species *Hemidesmus callus* showed very high rate of accumulation of compounds. The concentrations were seen to be very similar in case of both *in vivo* and *in vitro* plant materials.

Plant enzyme analysis: In all five enzymes tested for the difference in activities, it was observed that their activities were similar when *in vivo* and *in vitro* plantlets were considered, but drastic variations were observed in case of calli. Polyphenol oxidase and catalase were seen to be increasing in case of calli as compared to natural material. This can be correlated to the higher concentrations of phenolics present in the cells. It can be believed that higher concentrations of phenolic compounds trigger the polyphenol oxidase activity. Similarly increase in invertase indicates more release of sugar, which could be required to provide energy to the growing cells to cope with the metabolic rates.

All three plant species were analyzed for two medicinal properties viz. anti microbial and antioxidant.

Antimicrobial properties: Anti microbial activity was carried out against ten bacterial species and three fungal species. It was carried out by both streak plate as well as bioautography methods. Moderate to strong inhibition was observed in these plant species. However, except *Hemidesmus* leaf extract which showed maximum inhibition at 750 µg/ml, all the others showed highest inhibition at 1000 µg/ml.

Out of the microorganisms tried for this experiment, five gram +ve and gram –ve species, though identical results were obtained as far as concentrations were concerned, the susceptibility of the microorganisms was found to be different.

Five species viz. *Staphylococcus epidermidis, Bacillus pumilus, Bacillus cereus, Streptococcus faecalis* and *Bordetella bronchiseptica* were found to be less susceptible to phyto compounds as compared to the rest as they produced zones of inhibition only at 2-3 bands, while the other five showed more number of bands, showing higher susceptibility. However, the synergistic effect of the inhibitory compounds ultimately create identical effects as all the
organisms were optimally inhibited at 1000 ig/ml. In general, the plant antibiotic substances appear to be more inhibitory to Gram-positive organisms than to the Gram-negative type. It may be remembered that penicillin and some of the other prominent antibiotic agents of fungal origin are also rather selective in their inhibitory action, most of them being inhibitory to Gram-positive organisms. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (Burn, 1988). However, here we have got contradictory results. The Gram positives are less susceptible as compared to Gram–ve. This is indicative of presence of such specific compounds which specifically targets the lipopolysaccharide layers of gram–ve and thus making them vulnerable to inhibition.

Infections caused by Pseudomonas aeruginosa are among the most difficult to treat with conventional antibiotics (Levison and Jawetz, 1992). The growth of Pseudomonas aeruginosa was inhibited by all five extracts. However, Hemidesmus root extracts showed very high rate of inhibition as compared to the others.

While most Bacillus species are regarded as having little pathogenic potential, both Bacillus cereus and Bacillus subtilis have been known to act as primary invaders or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning (Turnbull and Kramer, 1991). Since these plants are used for edible purposes by the tribals, it is essential to check whether they are susceptible to such infectious agents. It was seen that though both bacilli species got inhibited by less number of compounds, Bacillus cereus was inhibited more by these extracts as compared to bacillus subtilis.

When the activity was verified by bioautography method, it was observed that the all three root extracts showed lesser number of inhibitory compounds to be present as compared to leaf extracts. However, the inhibition in all except Hemidesmus leaf extract was seen at 1000 ig/ml. This indicates that the compounds that are present in root extracts are more inhibitory as compared to those present in leaves. Further work on isolation and identification of these compounds could lead to develop newer antibiotic molecules.

In case of anti fungal experiments, out of the three species, maximum inhibition was observed against Aspergillus flavus. Rhizopus and Penicillin showed resistance. However, at 1000 ig/ml, all the three species showed maximum inhibition, thus showing similar behaviours as of microbes.
**Anti oxidant activity:** In the three anti oxidant activities carried out, it was observed that root extracts showed more inhibitory power as compared to the leaf extracts. Out of these extracts, *Curculigo* root extracts showed highest inhibition as compared to *Argyreia* and *Hemidesmus* root samples. The data can be correlated as in case of *Curculigo orchioides*, if we check the markers, more phenolics are ofund to be present. Also in the biochemical analysis the plant has showed maximum amount of phenolics as compared to others. Phenolics are responsible for anti oxidative activity and thus the results further corroborate the findings through biochemical estimations.

**Molecular analysis:** The presence of variability within species (among populations, and also between individuals within populations) is essential for their ability to survive and to successfully respond to environmental changes (Ryman et al., 1995). Unfortunately the process of extinction as well as complexity of gene erosion is ever-increasing (Hammer et al., 2003). Reports of considerable genetic degradation in the economically important species of India prompted us to comparatively analyse the extent of polymorphism amongst these plants and their in vitro products.

The molecular make up was checked by protein as well as RAPD profiling of both *in vitro* as well as *in vivo* plant materials.

**RAPD Analysis:** RAPD was carried out for *Hemidesmus indicus* and *Curculigo orchioides* Gaertn. In both cases, the tissue cultured regenerants were found to be identical to their sources. This indicates that the plants are identical in their genetic make up with the mother plant. Making this tissue culture technique a reliable clonal propagation technique for these plant species.

*Curculigo orchioides* Gaertn. has been reported to be an endangered species. The RAPD results obtained in a way support the environmental status of the plant on the basis of genetic theory of extinction (See Review of Literature). However, from the point of tissue culture work, the generation of true to type plantlets indicate that the protocol is safe to employ for mass propagation for *in situ* and *ex situ* cultivation for restoring the depleting numbers of the species.

Similar results were obtained in case of RAPD of *Hemidesmus indicus* too. However, when different mother plants were used, the band pattern changed. Thus, the tissue culture protocol allows the production of true to type plants, but between two different mother plants, the variations
were found to be present. Thus making it less susceptible to environmental erosion in comparison to *Curculigo*.

**Protein Profiling:** Protein profiling was mainly carried out to further support the true to type nature of the plant material, identical band patterns were obtained in the gel for in vitro and in vivo specimens.

**Barcoding of *Curculigo orchoides Gaertn.*:** Looking at the endangered status of the plant species, the barcoding work of the plant was initiated. The plant was bar coded using a forward and reverse primer each of ten mer which generated a gene segment of 550 bases in length at the Leucine tRNA region of the genome. The segment was then submitted to NCBI, which was accepted and the accession no. **GQ903691** was granted. We are the first ones to report genes of *Curculigo orchoides* on NCBI. This is further proof that plant hasn’t been explored to its maximum potential. When the BLAST was carried out for the sequence generated by us, it was seen that it showed 96% similarity to the plant *Vincetoxicum stocksii* which is a variety of wild capsicums and a member of Asclepiadiaceae family, which happens to be a dicot. Thus, very high conservation is seen across the monocotyledonous and dicotyledonous taxa. This needs further investigation to establish molecular basis for classification.