INTRODUCTION

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For centuries plants have been an important source of drugs. Medicinal plants always played the major role in the treatment of diseases. Inspite of the advances made in chemical technology, vegetable sources continue to provide new sources of drugs. Besides, the chemicals synthesized by any plant are so complex that the technologically synthesized substitutes are not as effective as those naturally occurring compounds (Mantell *et al.*, 1985). However, the major constraint in using the natural flora is the existence of chemovariability (Heble, 1993).

MEDICINAL IMPORTANCE OF *BOERHAAVIA DIFFUSA L.*

This plant is commonly known as Punarnava (Family:Nyctaginaceae). It is a perennial herb with stout woody root-stock and purple coloured stem swollen at nodes. Punarnava is one of the very few Indian drugs whose pharmacological activities are well established. Out of the 1650 herbal formulations served in Indian market, 52 contained punarnava (Chakraborty and Handa, 1989). This drug exhibited diuretic and anti-inflammatory activities (Bhalla *et al.*, 1971). A single drug useful for both these activities makes it a very useful drug for the treatment of inflammatory renal diseases where diuretic and anti-inflammatory effects are required simultaneously. It has been reported that leaf extract of punarnava plant cured ophthalmia (Wealth of India, 1978), and its root decoction improves digestion due to the enhancement in ß-amylase activity (Goswami and Sharma, 1993). Basu and Sharma (1947) identified a chemical from the *B.diffusa* plant extract and isolated punarnavine; an alkaloid in crystalline form ($C_{17}H_{22}N_2O$). Other compounds isolated were ß-sitosterol, myristic alcohol and myristic acid (Singh and

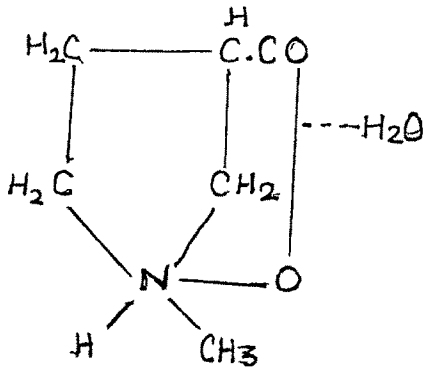
Udupa, 1972; Verma and Awasthi, 1979). The extract exhibited antiviral properties which might be due to the interference with synthesis of virus in the host cells (Verma and Awasthi, 1979). Seth *et al.* (1986) were successful in isolating punarnavoside and two more alkaloids which they named as punarnavine 1 and 2. Recently in 1991, Singh and his co-workers isolated potassium salts ketone and few carbohydrates from this plant extract. All these reports clearly indicate that *B.diffusa* possesses the medical potential which could and should be exploited using recent technology.

MEDICINAL IMPORTANCE OF *ACHYRANTHES ASPERA* L.

Achyranthes aspera L. the perennial herb (Family : Amaranthaceae) is extensively used as an Ayurvedic medicine. Decoction of whole plant is known for its diuretic action and as a cure in pneumonia. Its powder is consumed against gonorrhoea (Kirtikar and Basu, 1975). It is useful for lowering blood pressure due to its effect to dilate the blood vessels and increase the rate and amplitude of respiration (Neogi *et al.,* 1970). Butanol soluble fraction of it when consumed prevented pregnancy in mated rats when administered orally at 50 mg/1 kg of body weight (Wadhawa *et al.,* 1986). Recently, Shukla and his colleagues (1991) developed a medicated thread which they called as `Kshaarsootra' was coated with ash of this plant. This worked extremely well as a cure for anal fistula in patients suffering from it. Kshaarsootra is available in the market for sale.

An alkaloid achyranthine, a water soluble ($C_6H_{11}N_2O.H_2O$) compound was isolated from the alcoholic extract of *A. aspera* plant powder (Basu, 1957). Its

structure was as follows :



Kapoor and Singh (1967) were successful in isolating two more alkaloids from *A. aspera* plant extract. Ratra and Misra (1970) conducted detailed studies on the chemical constituents of this plant and reported that higher quantities of the alkaloid achyranthine was accumulated in the shoot portion rather than in the root portion. Gupta *et al.*(1972) isolated saponin from the seeds of *A. aspera* and according to them the diuretic effects of this plant lies in saponin. It appears that this plant possess excellent potential to be used as medicine which needs extensive research work.

PROBLEMS IN AVAILABILITY OF MEDICINAL PLANTS

Due to extensive industrialization, besides use of chemicals as pesticides, insecticides and several other factors, plants in their natural habitat manifest various symptoms of illness, the visible one lies in their stunted growth. Heble (1985) reported that only a few medicinal plant species have been cultivated against the numerous species growing naturally which are destroyed due to their indiscriminate cutting. Hence, rapid propagation of medicinal plants of superior selected species should be undertaken employing recent technologies.

A research study conducted in Botany Department of Aligarh University, reported in Indian Express (Baroda edition) dated 1st June, 1994 that plants of *A. aspera* growing in industrial areas not only produced low quantities of alkaloid, but the curative property of the alkaloid was decreased as well.

PLANT PRODUCTS OF MEDICINAL IMPORTANCE

Plants during their life-span synthesize several compounds which have been classified as primary and secondary metabolites. This distinction has been done on the basis that primary metabolites *viz.*, sugars, amino-acids, proteins, lipids are essential for plant growth and have a role in the physiological makeup of the plants. However, secondary metabolites *viz.*, steroids, alkaloids, terpenoids, flavonoids, anthocyanins and few others are complex chemical compounds produced by plants as a chemical interface between plants synthesizing them and their surrounding environment.

ALKALOIDS

Even with the dramatic progress of organic chemistry which has resulted in an enormous production of synthetic drugs some of the most powerful remedies are still by compounds of plant origin such as `the alkaloids' (Waller and Nowacki, 1978). Chart I, given below exhibits list of alkaloids isolated from medicinal plants which have been used against various diseases.

Chart I

List of plants and their alkaloids with their applications.

Plant	Name of the Alkaloid	Useful against
1. *Cinchona ledgeriana*	Quinine	Malaria
2. *Catharanthus roseus*	Vincristine	Leukaemia
3. *Datura stromonium*	Scoplamine	Hypertension
4. *Papver somniferum*	Codine	Analgesic
5. *Atropa belladonna*	Atropine	Cholinergic
6. *Rauwolfia serpentina*	Resperine	Hypertension
7. *Hyoscyamus niger*	Hyoscyamine	Cholinergic

Alkaloids are produced by plants as products of nitrogen reservoirs being useful to them as protective agents. Recently, Robins *et al.* (1991) reported that the major role of alkaloids is to protect the plants from being eaten by phytophagous, higher organisms or being invaded by pathogens.

Extraction of alkaloid from plants involves either their complete destruction or selectively harvesting of their specialised organs. An alternative source for the production of such important compounds could be developed by employing tissue culture technology. Recently Mitra and Jain (1994) have very rightly pointed out that tissue culture technology has opened up new vistas in the rapid propagation of uniform population of medicinal plants. Earlier, Heble (1993) pointed out that it was a major challenge for the plant biotechnologist to reduce a hundred year old tree to microscopic cells to make them produce the chemicals which the mature intact tree synthesizes during its life-span.

HISTORY OF PLANT TISSUE CULTURE

Haberlandt, as early as in 1902, imagined the concept of totipotency of cells. He cultured palisade parenchyma cells of *Lamium purpureum*, but failed in his attempt, as these cells being most differentiated ones failed to regain their meristematic competence. Kotte (1922) postulated that *in vitro* cultures could be made by using meristematic cells situated at root tips. Later, both the authors used root tips of pea and maize as culture material. White (1934) successfully developed excised root cultures of tomato which survived during successive cultures. In 1939 Gautheret, White and Nobecourt working independently established continuous cultures of tobacco and carrot, which laid the foundation for further work in the field of plant tissue culture.

Further work of White (1943) on excised root cultures for their nutritional requirements resulted in the formulation of culture medium which was named as `White's medium'. Dormer and Street (1949) studied carbohydrate requirement of cultured roots and reported superiority of sucrose over other carbohydrates due to its unique rate of absorption. Street and Jones (1963) stated that excised organ of a plant when cultured under aseptic conditions whereby the cultural conditions remained under control, provided opportunities to study individual factors *viz.*, nutritional/hormonal requirement for its optimal growth and development during culture period. The measurement of growth parameters of cultured roots such as fresh and dry weights, length of main axis, number of lateral roots initiated during the experimental period, provided data on the requirements of excised cultured roots of a particular species.

Callus tissues were initiated from an excised organ of a plant when cultured on a medium containing mineral salts, utilizable sugar and other growth factors (Gautheret, 1959). An organised tissue such as root/stem completely changed to a rapidly proliferating undifferentiated mass of cells when cultured under aseptic conditions on above said medium has been termed as callus. Miller and his associates (1955) isolated a compound from autoclaved yeast extract which they named as Kinetin (6-furfuryl aminopurine). Application of Kinetin was found necessary for the callus induction. However, Caplin and Steward (1948) were successful in enhancing the growth of callus tissue with the addition of coconut milk. They reported that certain growth promoting substances are present in the coconut milk. During this period a few other workers in this field added more information on the nutritional/hormonal requirements of callus cultures. Murashige and Skoog (1962) developed a culture medium which contained inorganic, organic salts, vitamins and supplemented with a utilizable source of carbohydrate and growth regulators. This medium was found suitable for variety of experiments and is popularly known as MS medium.

Narayanswamy (1977) reported that callus consisted of parenchymatous cells which could be grown continuously for unlimited period by subculturing it on fresh medium of the same composition at regular intervals. Callus cells are densely packed, but a soft friable callus is composed of undifferentiated and loosely attached cells. It is formed under conditions favouring rapid cell division. Callus cultures belonging to different species show variation in their friability, texture and colour. In *Citrus grandis*, whitish green coloured callus cultures have been reported (Mitra

and Chaturvedi, 1972).

Biomass production of callus tissue was enhanced by adjusting the level of carbohydrate as well as auxin in the culture medium. By manipulating the auxin concentrations of the culture medium, the growth rate of callus was increased in tobacco callus (Hiraoka and Tabata, 1974).

CYTODIFFERENTIATION

The cells of callus are mainly unspecialised and its biomass production when enhanced, results in the production of similar type of cells. Furthermore, the formation of differentiated cells appears to be correlated with organ development which results in response to the change in the quantities of growth regulators in the culture medium. The most commonly found type of differentiated cells in callus tissue were tracheids.

Callus tissues subjected to auxin-cytokinin variation seems to be equally dependent on the presence of sugar which plays a significant role in inducing xylem differentiation (Jacobs, 1952). Callus grown on lower levels of auxins turned nodular. Histological observation of each nodule revealed that few of its parenchymatous cells developed thickening and differentiated into tracheary elements without any change in its size or shape. This specialization of cells which is the basic event of development in eukaryotes is defined as cyto-differentiation (Fukuda and Komamine, 1980). In fact, the process of xylogenesis *in vitro* in callus tissue presents a model system for morphological and biochemical basis of cytodifferentiation (Bahadur *et al.*, 1991). Fadia and Mehta (1973) working with *Cucumis* callus reported that tracheary elements increased in the tissue in response

to the increase in the sucrose level of the culture medium thereby indicating sucrose was partially responsible for inducing xylogenesis.

APPLICATIONS OF TISSUE CULTURE

Following are the number of advantages offered by tissue culture technology in various fields :

(i) Rapid Multiplication and Propagation of Plants

Morel (1960) was the pioneer worker who applied tissue culture techniques for the rapid multiplication and propagation of orchids on mass scale. His achievements not only revolutionised the orchid industry but served as impetus for others who applied these techniques for the rapid multiplication and propagation of large number of other plant species.

Murashige (1974) was instrumental in further advancing research in this direction, he propagated number of the leafy ornamental and ferns. His techniques employed for angiosperms were through axillary bud sprouting which produced numerous propagules resulting in the rapid multiplication and propagation of genetically uniform population of plants. With the same procedure, large number of plants were propagated (Evans *et al.*, 1983; Sharma, 1991). Bud break was stimulated by culturing newly isolated axillary buds on medium supplemented with high levels of cytokinin (Van Arnold, 1988). However, micropropagated shoots exposed to high levels of cytokinins exhibited suppressed growth and often have a bushy appearance with small undeveloped leaves and hence it was discovered that cytokinin from culture medium should be omitted before shoots were to be

transferred for elongation. Second method of propagation of plants was through induction of adventitious meristem. Adventitious buds could be produced either directly or indirectly on the explants without intervening callus stage which was considered more reliable method for clonal propagation. Third method which produced plants on large scale was somatic embryogenesis (Ammirato, 1987). It was Steward (1958) who first reported that embryos could be produced in suspension cultures of carrot tissues. These were termed as `somatic embryos' because they developed directly from somatic cells without gametogenesis and syngamy. The availability of somatic embryos on large scale prompted investigators to find out the methods of their delivery (Ammirato, 1989). Somatic embryos were encapsulated and thereby developed `synthetic seeds', which could be stored for long time and were used as seeds for the propagation of the species. Moreover, plants developed from them were found genetically uniform. Such encapsulated somatic embryos were termed as `synthetic seeds'. A 100% conversion frequency of somatic embryos in synthetic seeds was achieved in *Santalum album* by Bapat and Rao, 1988.

(ii) Protoplast Culture

In plants, the plasma membrane is bounded by a rigid cellulosic wall and the adjacent cells are cemented together by a pectin rich matrix. Cocking (1960) isolated protoplast using various enzymes for the digestion of walls. His this achievement in particular, provided means to bring about genetic manipulations of the cells by insertion of DNA. Protoplast were cultured on agar media following the Bergmann's technique of cell plating. The first report of plant regeneration

from such cultured protoplast was in *Nicotiana tobaccum* (Takebe *et al.*, 1971). The characteristic feature of isolated protoplast was the ability of these naked cells to fuse with each other irrespective of their origin, which ultimately resulted in the process of somatic hybridization.

Schieder (1980) reported that in *Datura innoxia* and *D. Stramonium* somatic hybrids produced, exhibited vigorous growth as compared to the parental plant species. Besides these hybrids synthesized/accumulated higher quantities of alkaloid. Cultured cell protoplast could be useful for undergoing selective genetic changes as desired. For example, Carlson (1973) reported the uptake of chloroplasts by protoplast. Hence protoplast culture, and its genetic changes paved way for the progress of tissue culture technology.

(iii) Haploids

Haploid plant production using pollen/anther culture technique was an obvious benefit derived from tissue culture applications. Haploids are the plants having gametophytic number of chromosomes. The first successful raising of haploids through *in vitro* techniques was reported by Guha and Maheshwari (1964) in *Datura metal.* Haploids are of great importance in the studies on induction of mutations and also for the production of homozygous plants which are needed in large number. The presence of one set of chromosomes facilitate the isolation of mutants and isogenic diploids resulted by chromosome diplodization, could be useful for plant improvement programmes. Another use of haploid is in relation to self-incompatible plants. Amongst other users, homozygous plants obtained through anther culture have been observed in *Nicotiana tobaccum* producing high yields of

alkaloids (Collins and Sunderland, 1974).

(iv) Production of Secondary Metabolites

Plant tissue culture technology is potentially valuable for studying the biosynthesis of secondary metabolites as it eventually provides an efficient means of producing commercially important products (Butcher, 1977). Cultured callus tissue cells offer many advantages over intact plants for biosynthesis of medicinally valuable compounds as they are relatively easy to grow under strictly controlled nutritional and environmental conditions. Zenk *et al.* (1977) and Kinnersley and Dougall (1981) reported that plants yielding high quantities of secondary metabolite alone produce callus cultures with the ability to synthesize and produce high yields of secondary compounds, since the production of these compounds is of genetically determined character. In *Ruta graveolens*, Negal and Reinhard (1975) observed that shoot derived callus cultures produced shoot specific essential oil whereas those derived from the roots produced root specific oil inspite of the fact that the roots and shoots used for callus induction belonged to one and the same plant. This throws further light in indicating the importance of using specific organ from a plant for callus induction when high yielding callus cultures need to be initiated. It has been established that high yielding plants carry the required genetic make up which is passed onto their excised organs retaining the potential for secondary metabolite synthesis. Heble (1985) also reported that the alkaloid profile of various organs of *Rauwolfia serpentina* differed in their quantities which is shown in the chart II given below.

Chart II

Alkaloid profile of isolated organ cultures of *Rauwolfia serpentina*

Alkaloid name	Cultures of		
	Root	Stem	Leaves
Ajmaline	+	+	-
Ajmalidine	-	+	+
Reserpine	+	-	-
Ajmalicine	-	-	+
Serpentine	+	-	+
Total quantity	2.7%	0.7%	0.54%

It was evident from this results (Chart II) that highest quantity of alkaloid was synthesized/accumulated in root cultures of *Rauwolfia serpentina.*

(v) Root Culture

There are number of reports employing excised root cultures as a source for the production of medicinally important products. Tomatin the steroidal alkaloid, was synthesized in roots as well as the shoots of the plant but its site of accumulation was only roots (Sander, 1956). As early as 1957, West and Mika, reported that excised root cultures of *Atropa belladonna* synthesized/accumulated alkaloids. The qualitative and quantitative changes in the alkaloid contents of the excised root cultures might have been due to the absence of transportation of alkaloids to the aerial parts (Staba, 1982). A close and consistent association between the growth of the cultured tomato roots and the accumulation of tomatine suggested that the roots

might be the site of tomatin biosynthesis (Roddick and Butcher, 1972). Similarly, Nicotin synthesis was confined to root apices in tobacco root cultures which was related to the rate of their growth (Roddick and Butcher, 1972). Hence, Staba (1982) indicated the possibility of excised root cultures to serve as an alternative source for the production of desired medicinal compounds. This was proved true when transformed root cultures were initiated from plants selected for their high alkaloid spectrum relative to other plant of the same species. In excised root cultures, the favourable biosynthetic characteristic were introduced through *Agrobacterium rhizogenes* and higher quantities of alkaloid producing hairy root culture were established. Such hairy root cultures developed through genetic transformation by bacterium are usually fast growing and exhibited the identical biosynthetic capacity closely mirroring that of roots of intact plants (Parr and Hamill, 1987).

(vi) Shoot Culture

The inherent ability of a large number of medicinal plants to form multiple shoots in culture from axillary meristem has been exploited for the production of secondary metabolites from them. Shoot cultures of *Rauwolfia serpentina* produced excellent biomass while in culture and gave identical alkaloid profile of a ajmaline and ajmalidine (Roja *et al.*, 1987). In fact, Heble (1985) has successfully developed shoot cultures of *Atropa belladonna*, *Rauwolfia serpentina, Catharanthus roseus, Cinchona ledgeriana, Papaver bracteatum, Withania somnifera* and few others which resulted in the production of medicinal compounds with increased rate.

(vii) Callus Culture

Production of useful secondary metabolites of medicinal values were reported from plant cell and callus cultures as early as 1956 by Routier and Nickell. Over the last decade plant cell cultures have been exploited as an alternative means for production of commercially valuable secondary compounds (Fowler, 1983). There are some potential advantages in using tissue culture as a source for producing such compounds. They are as follows :

1. Independence from various environmental factors including climate, geographical and seasonal constraints,
2. Consistent production of superior quality of metabolites from selected cell lines,
3. Because of relative degree of control afforded by culturing cells under controlled nutritional/environmental conditions, production of these compounds could be geared up more accurately according to market demands,
4. Cultures of cells proved more suitable in cases where plants are difficult or expensive to grow in the fields due to their long life-cycles,
5. Cells and callus cultures could be easily subjected to various techniques whereby the desired product yield could be profitably enhanced such as bio-transformation, immobilization of cells etc. These are some of the advantages which acted as incentives for further research work in the application of compounds useful as drugs. Following chart (III) gives the list of alkaloids produced from tissue and cell cultures of medicinal plants.

Chart III

Plants used for raising tissue/cell cultures to produce medicinally important alkaloids :

Plant	Alkaloids
1. *Papaver somniferum*	Codeline
2. *Atropa belladonna*	Atropine
3. *Coffea arabica*	Caffeine
4. *Catharanthus roseus*	Ajamalicine
5. *Medicago sativa*	Choline
6. *Nicotiana tabacum*	Nicotine
7. *Rauwolfia serpentina*	Resperpine
8. *Lycopersicon esculentum*	Tomatine
9. *Datura innoxia*	Tropine
10. *Ruta graveolens*	Rutacridone

Besides alkaloids there are number of other secondary metabolites such as flavonoids-Kaempferol and quercetin from *Crotalaria juncea* (Jain and Khanna, 1974); Solasonine, a glycoside of solasodine from *Solanum xanthocarpum* (Heble *et al.,* 1971); anthraquinones by *Morinda citrifolia* (Zenk *et al.,* 1975) and nimbin from *Azadirachta indica* (Ramesh and Padhya, 1988) produced by employing this technology.

In case of alkaloid biosynthesis *in vitro*, callus cultures derived from explants of various parts of an alkaloid - producing plant were capable of synthesizing the

same spectrum of alkaloids which were found in the whole plant (Bohm, 1980). Traditionally the approach has been to initiate cultures from high yielding plants, since plant cells being totipotent, this characteristics gets transferred to the cultures. Callus cultures from parent plant of *Catharanthus roseus*, which produced high levels of serpentine proved that their cultures have retained this capacity (Zenk *et al.*, 1977). The cultural conditions which influenced the secondary metabolite accumulation *in vitro* needs to be investigated (Yeoman *et al.*, 1980).

Scanty information is available in the literature on temperature optima for the growth of cell cultures for secondary metabolite production since *in vitro* studies have been carried out as a matter of routine at temperature around 25°C. The medium most commonly used is Murashige and Skoog's (1962) medium for the tissue culture, but it is not the ideal one which could support the synthesis of alkaloids for the particular species under investigation. Sucrose which is incorporated in the culture medium as a energy source, its optimal level needs to be investigated for a particular callus tissue as the main effect of sucrose is to amplify the quantity of secondary metabolite production (Zenk *et al.*, 1975). For example, in callus cultures of *Catharanthus roseus*, sucrose at 3%, supported highest quantity of alkaloid production (Zenk *et al.*, 1977).

Other supplements added to the culture media are the phytohormones *viz.*, cytokinin and auxin which are required for the growth of cultured tissue *in vitro*. There are number of reports that the quality of auxin, NAA/IAA/2,4-D added to the medium affect the production of alkaloids. Furuya *et al.* (1971) reported that callus cultures of *Nicotiana tobaccum* in presence of 2,4-D failed to produce nicotine

while in presence of IAA in culture medium, detectable quantities of this alkaloid was extracted from the callus tissues. In general, 2,4-D was found less suitable for triggering secondary metabolism in plant cell cultures than either NAA or IAA (Mantell and Smith, 1983). However, with 2,4-D at higher levels supporting the production of carotenoids has been earlier reported in carrot cell cultures (Ikeda *et al.,* 1976). Effect of cytokinin alone on cultured tissues could not be assessed as they are usually supplemented in combination with auxin. In *Nicotiana tobaccum* callus cultures at higher levels of Kinetin suppressed nicotine production (Shiio and Ohta, 1973). Technique of selecting high yielding cell lines was developed by Widholm (1980) whereby from suspension cultures variant cell lines were isolated by cell plating. These cells when cultured, produced higher levels of desired compounds as has been seen in *Catharanthus roseus* (Widhlom, 1980), *Nicotina tobaccum* (Widholm, 1973).

By feeding precursors in particular, amino acids, to the cultured tissue in *Capsicum frutescens*, Lindsey and Yeoman (1983) reported higher levels of capsaicin production. Timing of precursor feeding played an important role. Tryptomine added during the second or third week of culture period stimulated cell growth and alkaloid metabolism (Alfermann and Reinhard, 1978). In *Dioscorea deltoidea* cultures, a 100% increased in diosgenin contents was recorded when the cell cultures were fed with cholesterol (Chowdhary and Chaturvedi, 1979).

Biotransformation is another means used to improve the biosynthetic ability of cultured cells which could be channeled to increase the yield of desired secondary metabolites. A number of biotransformation have been demonstrated, the most

advanced towards industrial applications being the hydroxylation of ß-methyldigitoxin to ß-methydigoxin (Reinhard and Alfermann, 1980). This conversion by means of biological systems is termed biotransformation and has been utilized on a massive industrial scale for the steroid industry (Mantell *et al.,* 1985). Various cell-lines of *Digitalis* have been isolated and identified from plants which are rich in digxin and could perform such conversions (Mantell *et al.,* 1985).

In recent years, a great progress in immobilisation of plant cells on a variety of supports *viz.,* agarouse, alginates or polyacrylamide have been developed. Immobilised cells of *Catharanthus roseus* exhibited enhanced quantities of the alkaloid (Fowler, 1983). The immobilisation of cells provided conditions which were conducive to cell differentiation thereby encouraging the production of high yields of secondary metabolites (Lindsey and Yeoman, 1983).

Inspite of all these advances made in tissue culture technology, one needs to standardize these techniques suitable for the species under investigation. Tissues/cell cultures produced secondary metabolites at much lower rate than the whole plant. The possible reason might be the lack of organ differentiation in them. In 1970, Thomas and Street reported that root differentiating callus of *Atropa belladonna* produced tropane alkaloids while non-differentiated callus could not do so. Reports of restoration of biosynthetic ability by regenerated Plantlets in digitalis was observed by Karting (1977). Similar reports of achieving biosynthetic ability were reported in *Atropa belladonna* L. (Epan *et al.,* 1978) and in *Tylophora indica* (Benjamin *et al.,*1979). In *Artemisia annua* callus differentiated into plantlets by manipulating the levels of phytohormons in the culture medium and it produced

higher yields of artemisinin (Fulzele *et al.,* 1991). All these reports clearly indicated that organised structures when produced resulted, in increase yield of secondary metabolites. Hence, the ability of various callus cultures for plantlet regeneration needs to be exploited.

(viii) Regenerative Potential of Excised Leaves

Regenerative potential of excised cultured leaves have shown that shoot buds have been produced in *Crateava nurvala* (Sharma and Padhya, 1991) and in *Azadirachta indica* (Ramesh and Padhya, 1991) while roots alone developed in *Boerhaavia diffusa* L. which showed the capacity for alkaloid production (Shrivastava and Padhya, 1995). Hence, it was necessary to evaluate the regenerative potential of excised organs of these two plants. From this review of literature, it became evident that there were hardly any tissue culture studies reported on *Achyranthes aspera* L. and *Boerhaavia diffusa* L. inspite of their immense medicinal use. This being the first attempt, basic studies in tissue culture field were conducted on both these plants with the following objectives :

1. Screening of *B. diffusa* L. and *A. aspera* L. plants for the presence of alkaloids for the selection of `elite' plants amongst them,

2. Screening of individual organs of the `elite' plant for locating the site of synthesis/accumulation of the alkaloids,

3. Establishment of excised root cultures of *B. diffusa* L. for their optimal growth by manipulating the cultural parameters, ascertaining their biosynthetic ability and attempts to improve their alkaloid production,

4. Establishment of callus cultures of individual organs of both the `elite' plants, standardization of nutritional/hormonal levels for their growth/alkaloid production,

5. Evaluation of organogenetic ability of these callus cultures with the objective to regenerate plantlets from selected callus tissue,

6. Lastly to investigate the regenerative ability of excised leaves of both the plants under study.

Results of the experimental work are presented in Chapter III. Part I deals with *B. diffusa* L. and part II with *A. aspera* L.