INTRODUCTION

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Of the numerous formulations synthesized employing modern technology in organic chemistry, still today, medicinal plants supply the maximum raw materials required for the pharmaceutical industries (Rhodes *et al.*, 1986). This raw material contained several compounds known as secondary metabolites which are so complex that their synthesized substitutes are not as effective as the natural prouducts (Mantell *et al.*, 1985). Besides that, it has been reported that a major constraint in using the natural flora as the raw material for drug manufacturing would be their chemovariability (Heble, 1993). Hence, efforts by various agencies to develop uniform plantation of medicinal plants of superior 'elite' quality, giving high yields of desired products are needed.

Withania somnifera (L.) Dunal (Family: Solanaceae) is a perennial shrub commonly known as 'Ashwagandha'. It is well known for its medicinal applications against nervous disorders, rheumatism, inflammatory conditions, cardiac diseases, and as a potential antitumor agent as well as a general tonic (Suffness and Douros, 1982; Heble, 1993). The extract of roots of this plant has been used as a drug against various rheumatic diseases. The plant has high potential value as stress reducing agent (Bhattacharaya *et al.*, 1987).

Earlier, (1911) Power and Salway conducted chemical investigations of this plant, which were later undertaken by Majumdar and Guha (1933). They isolated three amorphous bases. Majumdar (1952, 1955)

continued the investigations further and revealed the presence of several nitrogenous bases and characterised these as somniferine, somniferinine, somnine, withanine, withananinine, withananine and pseudowithanine. Out of these the first six were the alkaloids, while the last one was a disintegrated product of withanine. A major breakthrough was made when eight alkaloids were isolated from the roots and further characterised as tropine, pseudotropine, 3α-tigloxyloxytropane (3-tropyl tigloate), choline, cusohygrine, dl-isopelletierine, anaferine and anahygrine (Schwarting *et al.*, 1963).

Thus, it was evident from these reports that this plant possessed the capacity for synthesis / accumulation of numerous tropane alkaloids. Recent research work has revealed presence of new alkaloids and steroids of medicinal value.

NATURAL PLANT PRODUCTS :

Plants naturally possess the power of using solar energy for synthesizing chemical compounds classified as primary and secondary metabolites (Balandrin and Klocke, 1988). These secondary metabolites are produced by plants to ward off potential predators, attract pollinators or combat infectious diseases (Harborne 1972, 1978, 1982; Wallace and Mansell, 1976; Timmermann *et al.*, 1984). Numerous reports of their occurrence in the vacuoles of the cells has been known. For their extraction the cell biomass has to be harvested and cells are to be ruptured for the release of the secondary metabolites (Fowler, 1986a). The plant owes its medicinal / pharmaceutical importance to secondary compounds such as alkaloids, steroids, terpenoids,

flavonoids, tannins, anthocyanins, anthraquinones, napthaquinones and coumarins. A few of these are listed in Chart I.

CHART - I

MEDICINALLY IMPORTANT PLANTS

Sr.No.	PLANT	PLANT PRODUCT	PHARMACEUTICAL USE
1.	*Catharanthus roseus*	Vincristine (alkaloid)	Antileukaemic
2.	*Cinchona ledgeriana*	Quinine (alkaloid)	Antimalarial
3.	*Datura stramonium*	Scopolamine (alkaloid)	Antihypertensive
4.	*Digitalis lanata*	Digoxin (cardiac glycoside)	Cardiotonic
5.	*Dioscorea deltoidea*	Diosgenin (steroid)	Anti-fertility agent
6.	*Papaver somniferum*	Codeine (alkaloid)	Analgesic

Since the plant under investigation is *W.somnifera* here, the reports of medicinal value of alkaloids in various plants has been surveyed.

MEDICINAL IMPORTANCE OF ALKALOIDS

Literature survey on alkaloids indicated that they are one of the oldest drugs and even with the dramatic progress of organic chemistry which resulted in enormous production of synthetic drugs, some of the most powerful remedies are still of plant origin. Plants during their life span manufacture several compounds with apparent ease (Waller and Nowacki, 1978).

An exact definition of alkaloid still remains to be done as these secondary compounds are with complex structure, have significant pharmacological activity and are known for their medicinal value. Today, 5000 alkaloids of all the types have been known to occur in 15% of the land flora which comprises of hundreds of families. Plants belonging to solanaceae family in particular include plants mostly having tropane type of alkaloids which possess excellent medicinal applications as seen in Chart II.

CHART - II

ALKALOIDS FROM SOLANACEOUS PLANTS WITH THEIR MEDICINAL APPLICATIONS

Sr.No.	PLANT	NAME OF THE ALKALOID	USEFUL AGAINST
1.	*Atropa belladonna*	Atropine	Anticholionergics
2.	*Datura stramonium*	Scopolamine	Hypertension
3.	*Hyoscyamus niger*	Hyoscyamine	Cholinergics
4.	*Scopolia stramanifolia*	Hyoscyamine	Cholinergics
5.	*Duboisia myoporoides*	Scopolamine	Hypertension
6.	*Nicotiana tabacum*	Nicotine	Insecticide

Tropane alkaloids are mostly used today for chemical derivatization. For instance 30% of all spasmolytic sold in Japan in 1983 was about 19 million $, which were derivates of tropane alkaloids (Anonymous, 1985). In Brazil, plantation of *Datura* sp. were established so as to guarantee

the supply of scopolamine alkaloid for the production of derivates. New production centres were also set up in India, Iran and South Africa for various pharmaceutical industries (Strauss, 1989) .

Alkaloids are routinely extracted from the plant material which involves the destruction of individual organs/whole plants. Thus, in recent years man has disturbed the natural flora which has led to a decreased population of medicinal plants. Besides,there are several difficulties in their cultivation (Tabata, 1977) due to the use of toxic agricultural chemicals, seasonal influences and diseases caused by inscets/microbes. As an alternative, plant tissue culture technology has gained momentum for the production of medicinal compounds.

Mitra and Jain (1994) have cited that plant tissue culture technology has opened new vistas in the rapid propagation of uniform population of medicinal plants.

REVIEW OF PLANT TISSUE CULTURE

Plant tissue culture is a technique in which plant cells, tissues and organs are grown in aseptic conditions, in a nutrient medium, keeping the environmental conditions precisely controlled. Plant tissue culture dates back nearly to a century when a German botanist Haberlandt in 1902 for the first time cultured isolated, fully differentiated cells derived from palisade tissue of *Lamium purpureum*. His observations revealed that the cells could grow but failed to divide, the reason being that the cells were already in a differentiated state. Further in 1922, working independently Robbins and Kotte reported

that *in vitro* culture could be achieved using meristematic cells such as those that operate in root tips or in buds. Kotte (1922) succeeded in cultivating root tips of pea in a variety of nutrient solutions, whereas Robbins (1922) working with maize roots failed to achieve success in establishing *in vitro* excised root cultures. However, White (1934) could for the first time successfully grow continuous cultures of tomato root tips. In 1943, after studying the nutritional requirements of these cultured excised roots he formulated a medium suitable for their culture which is known after him. Dormer and Street (1949) studied the carbohydrate requirement of cultured roots and reported the superiority of sucrose over the other carbohydrates.

Skoog and Miller in 1957 put forth the concept of hormonal control of organ formation which was regulated by the auxin - cytokinin ratio in the medium. A significant contribution to a formulation of a defined growth medium suitable for a wide range of applications was made by Murashige and Skoog in 1962. Immediately after this significant contribution to plant tissue culture, Street and Jones (1963) stated that the excised organs of a plant when cultured under uniform conditions provided opportunities to study the individual factors *viz.*, nutritional/ hormonal requirements for their optimal growth and development during culture period.

In the same decade Muir (1953) succeeded in developing a technique for the culture of single isolated cells. Later, in 1954 Muir, Hildebrandt and Riker reported the growth of single cells and cell clumps of *Nicotiana tabacum* and *Tagetes erecta* in liquid medium. Another technical advancement in the field of tissue culture was brought about by Bergmann

(1960), whereby he plated cells of fine suspension which gave rise to single cell clones.

An important development during the 1960's was the isolation of protoplasts (Cocking, 1960) which opened new 'vistas' with its far reaching implications in tackling fundamental and pragmatic problems in plant biochemistry.

In 1964 Guha and Maheshwari at Delhi University observed the development of embryos and plantlets from the cultured anthers of *Datura innoxia*. Later on, they traced the origin of embryos to pollen grains and found the plants to be haploids. Bourgin and Nitsch (1967) working on the same aspect reported the formation of complete haploid plants of *Nicotiana tabacum* from cultured anthers. Further, Nitsch (1977) demonstrated that androgenesis could be achieved in isolated pollens (microspores).

By the late 1970s, it had become evident that plant tissue culture technology was begining to make significant contributions to agriculture and industry (Murashige, 1978; Zenk, 1978). In agriculture, the major areas were haploid breeding, clonal propagation, mutant cultures, pathogen-free plants, production of secondary products and genetic engineering.

ADVANTAGES OF PLANT TISSUE CULTURE

Tissue culture technology finds a number of applications which are as follows :-

(i) Rapid clonal propagation

The application of plant tissue culture technology as an

alternative means of plant vegetative propagation is well established by now. Morel (1960) was the pioneer in this field. He applied tissue culture techniques for the rapid multiplication and propagation of orchids. Several industries have standardised the protocols for multiplication of ornamentals like gerberas, carnations, roses and plantation crops like banana and cardamom (Pierik, 1991). No serious attempt has been made to clone medicinal plants on industrial scale. Heble (1993) has reported that by standardising protocols for clonal propagation of 'elite plants' an eight-to ten-fold increase in the products per unit area is achieved. Clonally propagated plants exhibited uniformity in their chemical constitutions which otherwise vary a lot from plant to plant. Moreover, these plants showed improved vigour and quality as compared to seed derived plants (Bhagyalakshmi, 1991).

Broadly, three approaches have been followed to achieve *in vitro* shoot multiplication. These are; (a) through callusing, (b) adventious bud formation, and (c) enhanced axillary branching. Only adventious bud formation is discussed below.

A number of plant species produce adventious bud *in vitro* which has leads to the concept of 'totipotency' of plant cells and tissues (Krikorian and Berquam, 1969). *In vitro* method for mass propagation of *Begonia* by adventious buds have proved to be successful (Takayma and Misawa, 1982). Another merit of this technique is that a small explant which would otherwise fail to survive in nature is able to produce adventious buds in cultures (Hussey, 1978). Bapat and Rao in 1977 reported adventitious shoot bud induction in *Physalis minima*. It has proved to be helpful in case of *Physalis ixocarpa* which exhibits gametophytic self-incompatibility (Malagon and Alejo, 1991).

(ii) Root cultures

Excised root cultures have been continuously tapped for their potential to produce medicinally important products. These cultures are an alternative route to natural product synthesis as they are not affected by various environmental factors, geographical and seasonal constraints. Root clones have a rapid growth rate and there are no difficulties in multiplying the clone in order to yield any desired quantity of products (Butcher and Street, 1964).

As early as in 1957 West and Mika reported that the excised root cultures of *Atropa belladonna* could synthesize/ accumulate atropine alkaloid, and *Nicotiana* root cultures could synthesize anabasine and nicotine (Solt *et al.*, 1960). The nicotine synthesis in these tobacco root cultures was confined to the root apices and it was related to the rate of their growth (Roddick and Butcher, 1972). Anabasine production increased with the incubation period (Solt, 1957 and Solt *et al.*, 1960). Whereas in cultured roots of *Duboisia myoporoides*, *D. leichhardtii* and *D. hopwoodii* hyoscyamin production decreased in the early stage of incubation, but began to increase when roots reached the exponential phase of growth (Endo and Yamada, 1985).

Cultured roots of *Hyoscyamus niger* synthesized/ accumulated higher quantities of scopolamine than the intact mature roots (Hashimoto and Yamada, 1983). Similarly, roots of *Datura stramonium* produced tropane alkaloids at higher levels than the cell cultures and plant roots (Maldonado - *et al.*, 1992). One of the possible reasons may be the presence of large number of enzymes in cultured roots, which are related to the synthesis of tropane alkaloids (Hashimoto *et al.*, 1990; Drager, 1990).

Nutritional and hormonal requirements are known to vary with different types of excised roots in culture and these simultaneously affect the synthesis/accumulation of alkaloids. Endo and Yamada in 1985 reported that high sucrose concentration in the medium stimulated nicotine production in *Duboisia leichhardtii*.

Auxins have also shown to affect the growth and biosynthesis of Hyoscyamine in *Hyoscyamus* root cultures (Hashimoto *et al.*, 1986). Shrivastav in 1995 has reported that excised roots of *Boerhaavia diffusa* cultured in IBA-containing medium synthesized / accumulated highest amount of punarnavine.

Thus, it became evident that plant species under study need to be investigated for the standardisation of its nutritional/hormonal requirements for the optimal growth of excised roots.

Incorporating biosynthetic precursors of the desired compound in the culture medium, enhances the yield of the product (Hay *et al.*, 1986). In *Nicotiana rustica* feeding nicotinic acid to the cultured roots enhanced the product yield (Robins *et al.*, 1987). It has been established that the change in alkaloid composition in terms of total amounts and the ratio of different alkaloid, of *Nicotiana alata* root cultures changed with the concentrations of the precursor (Friesen *et al.*, 1992). Feeding L-tryptophan to the excised roots of *Boerhaavia diffusa* increased punarnavine alkaloid in it (Shrivastava, 1995).

In the last decade, transformed root cultures have gained importance because of their ability to synthesize more amount of secondary

metabolites. These 'hairy roots' are obtained by genetic transformation of plant by *Agrobacterium rhizogenes* which are fast growing and show a biosynthetic capacity close to that of the roots of intact plants (Parr and Hamill, 1987). *Datura stramonium* plantlets were infected with different strains of *A. rizogenes* out of which monopine strain TR-105 was the most effective and the hairy root cultures developed a great stability in alkaloid production for more than five year (Maldonado *et al.*, 1993).

As the mature roots of *W. somnifera* are medicinally important, modern research work needs to be carried out on its excised root culture which would serve as an ideal source of alkaloid production on an industrial scale.

(iii) Callus Cultures

Routier and Nickell, as early as in 1956, reported that plant cells and callus cultures had the potential to produce secondary metabolites of medicinal value. Thus, in the progressive years, plant cell cultures have been exploited as an alternative means for production of commercially valuable secondary compounds (Fowler, 1983). There are certain advantages in producing secondary plant products using plant cell cultures which are as follows :

(a) Cultures grow under uniform controlled conditions,

(b) Cultures are free of seasonal variation,

(c) Callus in culture exhibits short growth cycles,

(d) Cultured cells are simple in organisation and minimize the problems of translocation, permeability, segregation of metabolic pools and,

(e) Incorporation and turnover of precursor can be studied over a short period.

Culturing of plant cells for the production of secondary metabolites needs some insight into the fundamental knowledge about factors influencing their synthesis (Kreis and Reinhard, 1989). These secondary metabolites which accumulate at any stage in the culture is the reflection of the dynamic balance between the rates of their biosynthesis and biodegradation.

One of the major factors influencing the secondary metabolites is the explant used for initiation of callus cultures which plays a key role in the production of high yields of desired compounds. As the explant consists of a cell population which has heterogeneous cell types, they exhibit different growth rates, ploidy levels, including their physiological and biosynthetic activities (Holden *et al.*, 1988). It has been reported that high yielding plants alone produce callus cultures having the ability to synthesize and produce high yields of secondary compounds, because the production of secondary compounds is a genetically determined character (Zenk *et al.*, 1977; Kinnersley and Dougall, 1981).

In 1975 Nagel and Reinhard found that the shoot-derived callus cultures produced shoot-specific essential oils whereas those derived from the roots produced root-specific oils in *Ruta graveolens*. In an extensive study of alkaloid production by plant and cell cultures in *Catharanthus roseus* and in general, it was found that cultures established from high yielding parent plants accumulated / synthesized high alkaloid content as compared with the low yielding plants (Zenk *et al.*, 1977). In *Crataeva nurvala* and *Azadirachta indica* the stem callus could not synthesize / accumulate kaempferol, like their

stem bark which too showed extremely low levels of kaempferol accumulation. Whereas the petal callus synthesized / accumulated maximum kaempferol, like the flowers which too showed maximum amounts of kaempferol (Ramesh, 1992).

Hence, in the present study high yielding plants of *W. somnifera* need to be identified and cultivated.

The composition of the culture media due to the presence of different levels of macro, microelements, and vitamins affect the biomass production of callus cultures and thereby indirectly affect the secondary metabolite synthesis. One of the fundamental factor and of major importance for biomass production is the nature and the quantity of the supplied carbohydrate. The ability of various carbohydrates to support callus and suspension cultures of plant cells was reviewed (Maretzki *et al.*, 1974). From their studies, it was concluded that sucrose or its component monosaccharides, like glucose or fructose were the carbon sources resulting in the optimal growth of most plant cell cultures. Yasuda and his coworkers (1972) showed that growth of *N. tabacum* var, *xanthi* cultures varied with different concentrations of sucrose. Similarly in *Achyranthes aspera* and *Boerhaavia diffusa* out of the various levels of sucrose tried for biomass production, 3% was found to be optimal level required for the growth of stem and leaf callus tissues (Shrivastava, 1995).

Previously it was reported that there was a substantial increase in alkaloid yield in *Catharanthus roseus* cell cultures with corresponding increase in the levels of sucrose (Zenk *et al.*, 1977).

The other supplements added to the culture medium are the phytohormones *viz.*, cytokinins and auxins, which are needed for the growth of the cultured tissues. These growth regulators have shown to be effective in stimulating and inhibiting secondary product synthesis in cell cultures, of which auxins have the greatest influence on the biosynthetic activity (Dougall, 1980). It has been shown that 2,4-D in the medium inhibited nicotine production in tobacco cells (Tabata and Hirakoa, 1976), whereas in the presence of IAA the cell could synthesize nicotine, anatabine as well as anabasine (Furuya *et al.*, 1971). Petri and Bajaj in 1989 studied various factors affecting the alkaloid production in callus cultures of *Datura innoxia*. Here the root derived callus cultured in a medium containing kinetin and 2,4-D, incubated in the dark showed highest cell biomass and alkaloids.

(iv) Organogenesis

The classical findings of Skoog and Miller (1957) have continued to be the guiding principles on *in vitro* organogenesis. Under a particular set of experimental conditions, specific type of organogenesis occurs with the exclusion of other types implying that the cultured cells vary in their competence for morphogenesis and responsiveness to inducers (Yeoman, 1987). Organogenesis from callus tissue is an important step leading to plant regeneration. In addition, regenerated organs such as shoot / root / plantlet become excellent experimental material for studying their properties and functions without the influence of other parts of the plant in isolation (Kitamura, 1988).

In many medicinally important plants, plant regeneration through callus cultures have been carried out so as to compare the alkaloid profile of the regenerated plants with that of the parent plant. Complete plants have been regenerated from callus cultures of *Atropa belladonna* (Raj Bhandary *et al.*, 1969). In *Datura innoxia*, the plants regenerated from callus showed tropane alkaloids (Hiraoka and Tabata, 1974). Another solanaceous plant in which plant regeneration has been reported is *Hyoscyamus niger* (Yamada and Hashimoto, 1982).

Thus, with this review of literature, the organogenetic studies of callus cultures of *W. somnifera* need to be analysed.

(v) Cell suspension cultures

The use of callus cultures in many studies is hampered by the lack of direct cell contact with the medium since the cells are piled upon each other. Suspension cultures have some advantages over callus cultures which are as follows :

(a) Cultures show a faster growth rate

(b) The liquid medium is in direct contact with almost every cell

(c) The medium is easily altered by addition or rinsed away and fresh medium added and,

(d) The cells can be plated directly

Over the years the use of cell suspension cultures as productive cell culture systems capable of synthesizing specific products to levels

equivalent to or higher than the plant from which they were derived has been well established. Chart IV comprises the product level of cultured cells achieved to date for the most common and essential drugs manufactured from higher plants.

CHART IV

PLANTS SHOWING THE PRODUCT LEVELS OF CULTURED CELLS

Sr.No.	PLANT	COMPOUND	CULTURE TYPE	SPECIFIC YIELD (% dry mass)	TOTAL YIELD (mg/l)
1.	*Dioscorea deltoidea*	Diosgenin	Suspension	7.8	150
2.	*Papaver somniferum*	Codeine	Suspension	0.1	4
3.	*Papaver somniferum*	Morphine	Suspension	0.1	5
4.	*Rauwolfia serpentina*	Reserpine	Suspension	0.5	6

Some examples of secondary products which are accumulated in cell cultures in higher amounts than in the differentiated plants are lucidine (Zenk *et al.*, 1975), serpentine (Zenk *et al.*, 1977), shikonin (Fujita *et al.*, 1982) and rosmarinic acid (Ulbrich *et al.*, 1985).

Plant cell suspension cultures often do not synthesize the same products as that of the differentiated plant. On the other hand, *in vitro* cultured cells can release a genetic potential which is not expressed in the differentiated plant. The examples of such secondary compounds detected only in plant cell cultures are paniculide C from *Andrographis paniculata* (Butcher and Conolly,

1971) and pericine from *Picralima nitida* (Arens *et al.*, 1982).

Hence, in the present study the cell suspension cultures of *W. somnifera* needs to be established for enhancement of the secondary product.

Feeding the culture with a known precursor and/or intermediates has indicated increase in the potential of plant cell cultures to produce particular metabolites (Hay *et al.*, 1986). Nicotine levels increased in the cultures when fed with ornithine (Ohta *et al.*, 1978). Boulanger and others (1973) observed a large increase in quinoline alkaloids in *Ruta graveolens* cultures with the addition of 4 hydroxy-2-quinoline. In *Dioscorea deltoidea* cultures, a 100% increase in diosgenin contents was recorded when the cell cultures were fed with cholestrol (Chowdhary and Chaturvedi, 1979). The codeine producing suspension culture of cells was capable of converting the precursor codeinone to codeine (Tam *et al.*, 1980, 1982).

There are various methods employed for the isolation of high-yielding cell-lines of the desired compound. Fujita and his coworkers (1985) selected high producing cell-lines of shikonin from protoplast cultures of *Lithospermum erythrorhizon*. Many cells which accumulate high levels of various coloured compounds have been selected by visual selection. Selection of high producing strains is also accomplished by cell plating techniques (Bergmann, 1960). The colonies thus formed are analysed for their alkaloid / steroid / flavonoid contents. The high producing colonies are cultured, replated and retested after which stable high-yielding strains are isolated. In the present study on *W. somnifera*, attempts for establishing high yielding cell-lines ~~need to be~~ were carried out.

It has been pointed out that the plant cells exhibiting a certain degree of differentiation when cultured under suitable conditions have the potential to produce, either by *de novo* synthesis or by bio-transformation, a range of secondary plant compounds (Alfermann *et al.*, 1985). The bio-transformation involves sterospecifc reactions such as hydroxylation, glycosylation, esterfication, epoxidation, isomerisation, and converts one molecule into another providing a possibility of developing new derivatives of known compounds. Immobilization of cells provides conditions that are conducive to differentiation thereby encouraging production of high yields of secondary compounds (Lindsey and Yeoman, 1983). *Catharanthus roseus* cells immobilized in agarose or alginate were most efficient for synthesizing ajmalicine from precursors than the freely suspended cells of suspension cultures (Brodelius and Nilsson, 1980).

(vi) Haploids

Natural haploidy has been known for a long time, but it was the studies on *in vitro* induction of androgenesis in *Datura innoxia* (Guha and Maheshwari, 1964) which emphasised the potentialities of a number of other angiosperm pollens for cell division, differentiation and in the production of a plantlet. The totipotent nature of pollen grains has been exploited to advantage in many plants of economic and ornamental value. The significance of production of haploids in studies of induction of mutations and as a tool in various biological disciplines has been stressed by Vasil and Nitsch (1975) and Nitzche and Wenzel (1977).

Literature pertaining to anther culture to date shows that this technique has been especially successful in species belonging to the family Solanaceae and Graminae. Most solanaceous plants have proved versatile in development of embryoids directly from the pollens, unlike the monocotyledons where there is an intervention of a callus before organogenesis sets in. Table Chart V gives the list of solanaceous plants in which haploid embryoids/calli/ plantlets have been developed.

Table 4
CHART - V

SOLANACEOUS PLANTS IN WHICH SUCCESSFUL PRODUCTION OF ANDROGENIC EMBRYOID/CALLI/PLANTLETS HAVE BEEN ACHIEVED BY ANTHER/POLLEN CULTURE

Sr.No.	PLANT	NATURE OF REGENERATION	REFERENCES
1.	*Atropa belladonna*	embryoids and plantlets	Zenkteler (1971), Narayanaswamy and George (1972); Rashid and Street (1973)
	Datura deltoidea	embryoids and callus	Gair & Kohlenbach (1973)
2.	*Datura innoxia*	"	Guha and Maheshwari (1964, 1967), Sunderland *et al.*, (1974)
3.	*Hyoscyamus niger*	"	Corduan (1975), Raghavan (1975)
4.	*Lycopersicum esculentum*	callus, embryoids and plantlets	Debergh and Nitsch (1973)
5.	*Nicotiana tabacum*	embryoids and plantlets	Bourgin and Nitsch (1967)
6.	*Physalis minima*	"	George and Rao (1979)
7.	*Solanum dulcamara*	"	Zenkteler (1973)
Add → 8.	*Datura metel* L.	"	Iyer and Raina (1972)
Add → 9.	*Solanum melongena* L.	callus, "	Raina and Iyer (1973)
Add → 10.	*Petunia* sp. triploids		Engvild (1973)

Success in the production of embryoids depends to a large extent upon the constituents of the media used for culturing the anthers. Since the anthers contain both somatic and gametophytic tissues, it is essential to induce division in the microspore alone, which has been achieved by the judicious applications of auxins and cytokinins (Narayanswamy and George, 1982). Other growth adjuvants used to augment cell division and growth are coconut milk (Guha and Maheshwari, 1964), plumjuice (Guha and Maheshwari, 1967), and casein hydrolysate (Zenkteler and Misiura, 1974). Nitsch (1969) demonstrated that for tobacco, only sucrose is essential for the initiation of cell division in pollen. For most species 2-3% of sucrose in the medium has been shown to be most favourable although in special cases higher levels have been used (Clapham, 1971; Thomas and Wenzel, 1975). Thus, the nutritional requirements vary from species to species and the same is true for phytohormones. For instance, it has been demonstrated that in case of *Datura* (Guha and Maheshwari, 1967) and *Petunia* (Engvild, 1973) presence of only kinetin in the medium triggered off division in the pollen, whereas for *Atropa* (Zenkteler, 1971) and *Solanum dulcamara* (Zenkteler, 1973), an auxin and a cytokinin in certain specific concentrations are essential for pollen division. In case of *Datura*, coconut milk in the medium initiates pollen division (Guha and Maheshwari, 1967; Geier and Kohlenbach, 1973).

The correlation between the developmental stage of pollen and embryoid formation has been critically examined in *Nicotiana tabacum* (Sunderland and Wicks, 1971) and *Datura innoxia* (Engvild *et al.*, 1972). The yield of these embryoids could be significantly increased by culturing the

anthers containing uninucleate microspores (Nitsch and Nitsch, 1969). But young anthers with meiotic stages of pollen mother cells and with starch filled grains failed to produce embryoids.

Since haploids contain only one set of chromosomes, all the genes present in them, even the recessive ones ~~were~~ get expressed in the phenophyte (Narayanaswamy and George, 1982). Homozygous diploids were produced in large number in *Nicotiana tabacum* by treating the haploid plants with colchicine (Sunderland, 1970). Doubled haploids that were homozygous and fertile were readily obtained, which enabled the selection of desirable gene-combination. So also in production of new mutant forms, haploids provide excellent material for experimentation.

In vivo and *in vitro* studies on *W. somnifera* were carried out with the following objectives :

1. Screening of *W. somnifera* (L.) Dunal plants for the presence of alkaloid/s.
2. Selection of 'elite' plant.
3. Screening of individual organs of the 'elite' plant and locating the site of synthesis/accumulation of the alkaloid/s.
4. Establishment of excised root cultures and standardisation of its nutritional / hormonal levels,
5. Ascertaining the biosynthetic ability of these excised root cultures for accumulation of alkaloid/s ,
6. Enhancement in the synthesis/accumulation of alkaloid by precursor feeding .

7. Establishment of callus cultures of individual organs of 'elite' plant, and standardising their nutritional/hormonal levels.

8. Establishment of cell suspension cultures and selection of high yielding cell-line for alkaloid production.

9. Regenerating plantlet from callus cultures.

10. Raising of haploids.

11. Production of homozygous diploid plants.