INTRODUCTION

CHAPTER I

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In higher plants isoprenoid pathway is one of the most important pathways as it results into the formation of a wide range of physiologically important compounds including Carotenoids (Anderson and Porter, 1967), sterols, terpenoids and atleast 3 growth hormones viz; Gibberellic acids (Greabe et al.,1965, Sponsel,1987), the naturally occuring cytokinin isopentanyl adenine (Short and Torrey, 1972; Mc Gaw,1987) and abscisic acid (Milborrow,1974; Walton, 1987). In recent years, the interest in this pathway has been increasing. If the interest in this pathway of animals is due to the end product cholesterol, which is responsible for the hypercholesterolemia and related ailments in human beings (Goldstein and Brown,1990), the case with plants is due to physiologically important isoprenoid compounds (e.g. monoterpenes, Diterpenes, Sequiterpenes, Cholorophylls, Carotenoids) and commercially important products (e.g. Rubber, Essential oils, Steroidal alkaloids etc.) of this pathway (Gray, 1987). A summary of this pathway is as shown in Table 1 as recently described by Bach (1987).

There is basic similarity in this pathway among animals and plants as is evident from the literature on animals

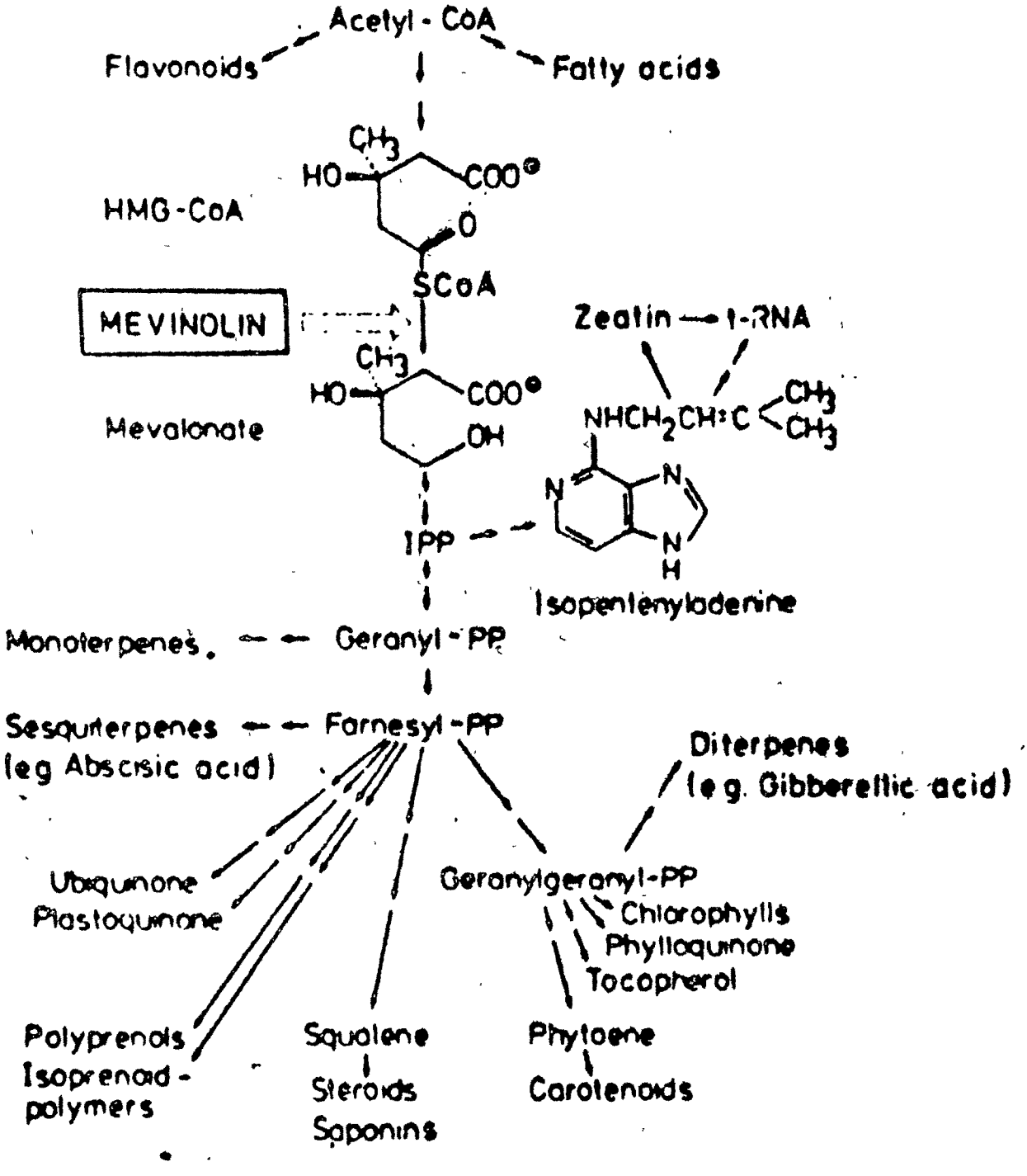


Fig. 2. Role of HMG-CoA reductase-directed synthesis of mevalonate and its flow into various classes of isoprenoid compounds in plant cells. The inhibition of HMG-CoA reductase by mevinolin is indicated.

reductase, and squalene synthetase. But, the metabolite mediated regulation of expression of these genes through LDL mediated control is not reported in plants except for the feed back inhibition of the enzyme HMG CoA reductase caused by exogenously supplied cholesterol and abscisic acid demonstrated in cellcultures of Solanum dulcamara (Bhatt & Bhatt, 1984) and Pea seedling respectively (Brooker and Russel, 1979). Stermer & Bostock (1987) has shown a positive modulation of the enzyme activity by elicitor arachidonic acid treatment to potato tissues. Similarly a phytochrome mediated control of this enzyme is shown by Broocker and Russel (1979) with pea seedling enzyme.

The history of regulation of isoprenoid pathway in higher plants begins with the proposal by Johnston (1954) who made observation that ^{14}C acetate can be incorporated in to HMG CoA by extracts of flax seedlings. Chemical degradation of in vivo synthesised ^{14}C carotene studied by Braithwaite and Goodwin (1960) supported Johnston's (1954) proposal. Hartmann and Lynen (1961) described the first step of formation of acetoacetate by the condensation of two molecules of acetate. It is a reversible reaction. Though Brodie et al (1963) suggested next step to be the condensation of acetoacetate with acetyl CoA to form HMG CoA with release of CO_2, Higgins and Kekwick (1973) based on

their studies on latex synthesis in rubber concluded that malonyl CoA is converted to acetyl CoA prior to its condensation with acetoacetate. Lately, the probable synthesis of R-S HMG-CoA from ^{14}C acetylCoA by extracts of sweet potato tissues is demonstrated to proceed optimally at pH 8.8 (Schulze-Siebert and Schulze,1985). The incorporation of labelled leucine into the HMG CoA through a degradative incorporation believed to be a method for direct formation HMG CoA (Johnston et al,1954) in flax seedlings is convincingly shown to be taking place by first getting broken down to acetate and then getting incorporated into HMG-CoA in Andrographis paniculata tissue cultures (Anastasis et al,1985).

However, the enzymes catalyzing the formation of HMG CoA from acetyl CoA have not been characterised from any plant source (Gray, 1987).

Next step in the isoprenoid pathway is the conversion of HMG-CoA to the mevalonate catalysed by the enzyme 3-Hydroxy 3-Methyl glutaryl CoA reductase E.C. 1.1.1. 34. This enzyme is studied in a few species of plants which is given below.

Distribution of HMGCOA in plants

Plant Material	Enzyme source	Reference
Hevea brasiliensis Higher plants latex	600 g bottom fraction 95% in lutoid fraction, 5% associated with Frey-Wyssling particles 49,000 g Pellet	Lynen, 1967; Hepper and Audley, 1969
Pea seedlings	Microsomes, mitochondria, Plastids	Booker and Ruseel, 1975a and b; 1979; Russel and Davidson, 1982; Wong *et al*, 1982.
Sweet potato roots	Microsome, mitochondria	Suzuki *et al*, 1975; 1976; Suzuki and Uritani, 1977; Yu-Ito- *et al*, 1982
Sweet potato cell cultures	Microsomes	Ito *et al*, 1979.
Tobacco seedlings	Microsomes	Douglas and Paleg, 1978.
Tobacco cell cultures	Microsomes	Chappel *et al*, 1989 Vogeli and Chappel, 1988.
Transformed cell cultures of tobacco	Microsomes	Bach, 1986
Radish seedlings	Microsomes, plastids	Bach, 1981.
Capsium annum	Chromoplasts	Camara *et al*, 1983.
Carrot cell cultures	10,000 and 105,000 g pellets	Nishi and Tsuritani, 1983.
Nepata cataria leaf and callus cultures	Microsomes, plastids	Arebalo and Mitchell 1984.

Spinach leaves	Microsomes	Kreuz and Kleinig, 1984.
Helianthus tuberosus tissue explants	15,000 and 105,000 g pellets	Ceccarelli and Lorenzi,1984.
Potato roots	Microsomes	Oba et al, 1985.
Parthenium argentatum leaves	Plastids, microsomes	Reddy & Ramdas, 1986.
Solanum tuberosum tubers infected with Phytophthora infestans	Microsomes	Stermer and Bostock, 1987
Lower plants		
Ochromonas malhamensis culture	Microsomes	Maurey and Goldbek 1985, Maurey et al, 1986.

Characteristics of plant HMG CoA reductase:

Only very little is known about the characteristics of HMG CoA reductase in plants. Some of the kinetic properties of HMG CoA reductase is given in the table below:

Source Enzyme	pH Optimum	Km RS-HMG CoA	Reference
Radish seedlings 16,000g membranes		$5.5x10^{-6}M$	Bach & Lichtenthaler (1984)
	7.5		
Purified enzyme		$3.0x10^{-6}M$	Bach *et al* (1986)
105,000g membranes		$4.75x10^{-6}M$	Bach & Lichtenthaler (1984)
Hevea brasiliensis latex 40,000g	6.6-6.9	$5.6x10^{-5}M$	Sipat (1982a)
Pea seedlings 3000 g membranes	7.9	$7.7x10^{-7}M$	Wong *et al*(1982)
50,000g membranes	6.9	$1x10^{-4}M$	Brooker and Russel (1975a)
Sweet potato roots, infected with *Phytophthora*	7.3-7.5	$1.3x10^{-6}M$	Suzuki *et al*(1975)
105,000g membranes			
Parthenium argentatum			
Cytosolic	7.0	$2.5x10^{-4}M$	Reddy and Ramdas (1986)
Plastidic			
Tobacco cell cultures	7.0	$1.8x10^{-5}M$	Vogeli & Chappel, 1988; Chappel, *et al* 1989

Determination of molecular weight by density gradient centri fugation and electrophoretic movement on polyacrilamide gel (Bach *et al*, 1986) and based on the calculations of Helenius and Simons (1975) that approximately 90,000 daltons would be contributed by the micelles when solubilized with triton

for molecular weight determination, this protein is believed to be a dimer of 45 kilo daltons each in the case of radish. HPLC analysis of the HMGR protein by Kondo, Oba (1986) indicated it to be a dimer of 55 k Da. Recently Learned and Fink (1989) sequenced the HMGR gene from Arabidopsis thaliana and has shown that it is different from HMGR genes of mammals and yeast at the COOH terminal (containing the active site of the enzyme), and the membrane bound amino terminus. The HMGR gene of Arabidopsis is of relatively smaller size (492 amino acids) compared to yeast (1050 amino acids) Chinese hamster (871 amino acids) or Drosophila (916 amino acids).

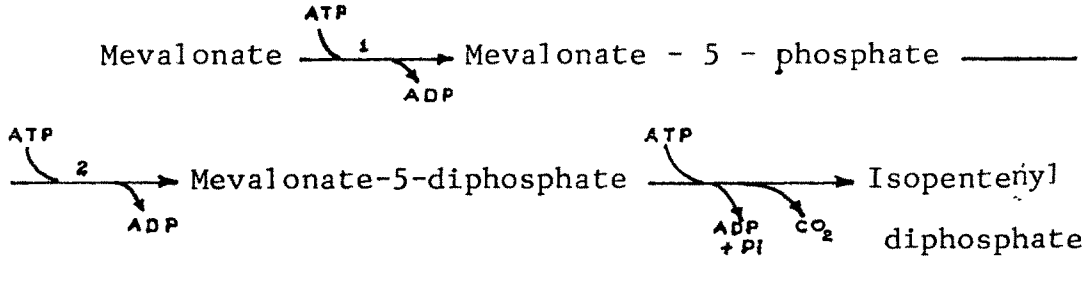
Control of the activity of an enzyme can be understood only if complete information on the enzyme is available. However, often there exists a lot of disparity with regard to different aspects of the properties of HMGR from different sources and even complete information on a single system is lacking. Based on the available information the control of plant HMGR activity is believed to be modulated by metabolites. By the studies on purified Radish enzyme it is shown that this enzyme activity is inhibited by coenzyme A and NADP (Bach and Lichtenthaler,1984; Bach et al, 1986) suggesting a possible regulation by the NADPH/NADP ratio. On the contrary studies on the effect of a number of isoprenoid products such as sterols, abscisic acid,

gibberellic acid and zeatin on pea microsomal enzyme did not show any feed back inhibition (Russel et al,1985). Bhatt and Bhatt (1984) have clearly showed that cholesterol mediated regulation of HMGR is taking place in Solanum Sp. using cell culture system. They used cell cultures of S.dulcamara and showed that the incorporation of ^{14}C acetate in to sterols was inhibited by exogenously supplied cholesterol while the incorporation of mevalonates was enhanced under this condition. Modulation by protein factors is proposed by many workers (Sipat,1982a; Isa and Sipat 1982; Russel,1985). Isa and Sipat (1982) have described the presence of a trypsin sensitive heat-stable activator of HMGR which doubles the HMGR activity in the in vitro studies with Hevea latex enzyme but the same is to be verified under physiological environment.

Some of the important findings related to the knowledge of animal HMGR regulation and properties have come out as a result of the studies on CHO cells using compactin as a molecular probe. The group lead by Goldstein could produce CHO cells resistant to compactin (upto 40 uM). This cell line designated as UT_1 showed amplification of the HMGR gene so that it produced HMGR protein 2% of total cell protein. At this state the anatomy of the cell changed and the endoplasmic reticulum became crystalloid (Chin et al, 1982a

and b; Luskey et al, 1983). This cell line (UT_1) permitted the easier isolation of this otherwise minor protein of the cell, and cloning of c-DNA to study the nucleotide sequence of HMGR gene (Goldstein and Brown, 1984). Studies of Goldstein's group could also establish that compactin resistant clones can be obtained by subjecting the cell cultures to the compactin stress and thus mutants with greater enzyme activity (500 fold) leading the over production of sterols as HMGR is the gate way enzyme of this pathway (Chin et al, 1982a and b).

The formation of isopentenyl diphosphate is the next step in the pathway and is well characterised (Beytia et al, 1969); Oba and Uritani, 1969; Potty and Bruemmer, 1970; Kekwick, 1973a and b; Peregrin et al, 1973; [illegible] Arebalo and Mitchel, 1984). Here three molecules of ATP are used sequentially to convert MVA into IPP.



Once IPP is formed it can get converted to natural cytokinin 2-isopentenyl adenine (Chen and Melitz, 1979; Hommes et al,1984). The formation of geranyl pyrophosphate takes place

by condensation of two molecules of IPP. It is the precursor of monoterpenes. The enzyme responsible for the formation of GPP is partially purified from different plant tissues (Nandi and Porter, 1964; Oruga et al,1972; Spurgeon et al 1984). The Geranyl pyrophosphate thus formed is in turn converted to Farnesyl pyrophosphate (FPP) by condensation with one mole of IPP which is a major branch point for the formation of different isoprenoid compounds viz; sesquiterpenes, side chains of uniquinone, plastoquinone, polyprenols and isoprenoid polymers.

In the next step FPP undergoes condensation with one IPP to form geranyl-geranyl pyrophosphate which act as the precursor of diterpenes, phytoene, carotenoids, side chains of chlorophylls, tocopherols and plastoquinones which are shown to have specific physiological functions,. The diterpene, gibberellic acid, is a well known plant growth regulator (Yabuta & Hayashi, 1935; Paleg, 1965; Hedden et al,1978), while chlorophyll and carotenoids are essential molecules required for biomass production through the biological carbon fixation process. (Gassman & Bogorad 1967; Akoyunoglou and Siegelman, 1968; Mathis and Sauer,1973; Goodwin,1980).

The commited step in the biosynthesis of triterpenes and sterols from isoprene units is the condensation of two molecules of FPP toform squalene in animal system (Faust et al, 1979). Evidences for this conversion in plant system come from the work of Graebe (1967) Green and Baisted (1971a,b). The flow of Squalene to the triterpenes is demonstrated by many workers (Suzuki et al, 1975, Stermer and Bostock, 1987, Stossl, et al, 1976; Tjamos and Kuc, 1982; Chappel and Nabel,1987; Chappel et al,1989).

With very few exceptions, sterols are present invariably in all eukaryotic organisms (Nes, 1984). In most fungi the major sterol both in quality and quantity, is 24 β-methyl cholestea-5, 7-22 E-trien-3 β-ol (Ergosterol) (Weete,1980), while most vascular plants possess predominantly 24α-ethyl-cholest5-en 3β-ol (Sitosterol), 24 α ethylcholesta-5, 22 E-dien-3 β-ol (Stigmasterol) and 24-methylcholest-5en-3-βol present either as 24 α (campesterol) or 24β -(dithyrobrassica sterol) epimer (Benveniste, 1986). Though cholesterol is predominantly a major animal sterol, is now known to occur widely in higher plants, usually in trace amounts (Heftmann, 1984), and in several taxonomic groups of fungi (Weete,1980). In spite of this generalization, many members of ascomycetes possess brassica sterol (Weete et al, 1985) and many members of

uridinales possess 24 ethylidene sterols (Weete, 1980). Some powdery mildews possess ergosta-5, 24(28)-dien 3-β-ol (Loeffler et al, 1984) and some members of ascomycetes possess cholesterol, 24 methyl cholesterol, desmosterol. Higher plants of the family Cucurbitaceae are exceptional in possessing Δ^7 sterols instead of usually found Δ^5 sterols. (Ito et al, 1986). A cactus, Lophocereus schottii is unique in having anomalous Δ^7 and $\Delta^{8,14}$ sterols (Campbell and Kircher, 1980). Various sterol derivatives are found in higher plants along with the free sterol, as long chain fatty acid esters, steryl-glycosides and acylated glycosides. In plants, any one of these forms predominate depending on the species, tissue, or developmental stage (Mudd 1980; Duperon et al, 1984; Bhatt and Bhatt, 1986; Wang and Faust, 1988). Fungi possess steryl esters in cytosolic lipid droplets (low et al, 1985). Among fungi, steryl esters may increase with age (Parks, 1978) or remain as free sterols (Evans and Gealt, 1985), but steryl glycosides and esterified steryl glycosides are not of wide spread occurrence in fungi (Ghannoum et al, 1986).

Inspite of the wide spread occurrence of a wide variety of sterols in plants, very little is known about their role in plant growth and development (Grunwald, 1978; Heftmann 1984). Earlier studies attributed two functions for sterols:

(1) sterols act directly as plant hormones or indirectly as the precursors of growth regulators (Geuns, 1978) and (2) sterols act as an integral component of the cell membranes (Grunwald, 1978). It is shown by Parks et al (1984) that about 15 ATP equivalent energy is used for a single methylation step in the biosynthesis of sterols with bulky side chains, which appears to be not suited to perform the functions attributed to the membrane sterols. However, studies with different experimental systems (Burden et al, 1989) higher plants and fungi have shown absolute necessity for different sterols in bulk amounts as well as in trace amounts, suggesting structural as well as hormonal roles respectively.

Quite how the structural features present in plant and fungal sterols and their derivatives relate to the role of these compounds in growth and development is intriguing but at present time, a largely unanswered question (Burden et al, 1989). However, from the evidences available at present for structure of the sterols in plants should be viewed with regard to their possible function either as a membrane component which keeps the integrity and fluidity of the cell membrane system or as cellular metabolite or hormone.

Early studies, to unravel the physiological functions of sterols carried out by Grunwald, (1968, 1970, 1975) Heftman

(1971) Kemp (1967, 1968) using different systems like red beet, tobacco and barley have lead to the establishment of the role of sterols as a membrane component. Results of Grunwald's (1971, 1974) work showed that cholesterol is the most effective type of sterol in regulating the membrane functions. However, plants possess multiplicity of sterols and sterol derivatives (Grunwald, 1978). Geuns (1978) triggered the idea of the hormonal role played by sterols. Recent reviews on this aspect by Adam (1986), Mandava (1988) and Roth (1989) have given evidences to this direction, but still much more is to be done to the understanding of the distribution and mode or action of these compounds.

One possible explanation for the presence of multiplicity of sterols in plants is to survive under a variety of changing environmental conditions, by allowing a more flexible alteration of membrane composition in response to stress than if cholesterol was the only sterol. This argument is based on the observations made by different workers under different environmental conditions such as light (Bae, 1970; Kasporzyk,1970; Bush and Grunwald, 1973; 1979), temperature and salinity stress (Erdei et al, 1981; Kuiper 1984; Unnikirshnan et al, 1991). However, this explanation is contradicted by Uemura & Yoshida (1984) Simons & Orcut 1988) and Brown & Dupont (1988) who show

that the turn over of sterols in the membrane is not high enough to account for the membrane flexibility. Thus this aspect remains inconclusive. Qualitative and quantitative changes are also observed by many workers during the different developmental stages (Duperon et al, 1983; Bhatt and Bhatt 1984; Bhatt et al, 1985; Heupel et al, 1986). However, the significance of these changes are not clear.

Different scientists have chosen alternate routes to go deep in to the functional aspects of the sterols. As detailed earlier, easy but less informative approach is the study of distribution of sterols (Kemp & Mercer, 1968b; Grunwald, 1970; Hartmann, 1975).Another approach is the use of chemicals that modify the sterols like nystatin (Chiu et al, 1982) or studying the factors viz., developmental, hormonal and environmental;influencing the qualitative and quantitative changes in plant sterols (Lancaster & Mann, 1974; Marshall & Staba, 1976; Bhatt and Bhatt,1984; Garg, 1987). Site specific sterol biosynthetic inhibitors (SBI) acting at various specific sites in the isoprenoid pathway could be powerful tool in unravelling the chemistry and physiology of plant sterols (Benevenite, 1986;Burden et al, 1987; 1989).

The ideal system to understand the functional aspects of plant sterols would be the use of biochemical mutants. Such

biochemical mutants have solved many of the physiological paradoxes in the plant world like dwarf mutants of maize in studying the role of gibberellins (Ingram et al, 1984; Phinney,1984) and biochemical mutants of corn in studying the role of abscisic acid.

Of all these methods described, we feel that the best would be utilizing biochemical mutants of the pathway to disclose the chemistry and physiology of sterols. It is shown in the case of fungi Ustilago which is deficient in sterol (Walsh and Sisler, 1982) and sterol auxotroph yeast GL 7 (Kawasaki et al,1985). Among the higher plants such mutants are not in existence except for the SKF resistant mutants of S.xanthacarpum and S.dulcarmara developed in our lab. by Subramani (1991). Variant cell lines of Solanum Spp. were selected by two different approaches. 1. Cells were plated on medium containing L.D. 50 - L.D.100 concentration of SKF-525A and resistant colonies were picked up and grown further on the SKF 525-A containing medium. Further, plants were regenerated from this resistant calli. ii. cell lines were first subjected to or x-ray irradiation to cause mutation and were then used as the plating units. Calli were developed from these colonies developed on medium containing diff. conc. of SKF 525-A (L.D. 50to L.D. 100) and plants were regenerated from these calli.

Analysis of these variant regenerants proved following facts -

i) In a leaf disc assay for the resistance to SKF these plants showed resistance upto L.D.50 conc. of SKF 525-A

ii) Analysis of sterols viz. free sterols, steryl esters and steryl glycosides showed a significantly higher amount of sterols in the variant clones.

iii) Analysis of the steroidal alkaloid (solasodine) content showed significantly more content of Solasodine.

So this study established a technique for the production of an over producer of solasodine by cell line selection and provided an experimental material for studying the steroid metabolism and function in higher plants. Therefore, it would be appropriate to generate such biochemical mutants through somatic cell genetic approach. Such defined biochemical mutants of amino acids and phenolic metabolism were generated in the past using somatic cell genetic approach (Widholm, 1972a,b, 1974, 1976; Bourgin,1978, berlin 1980; Jacobs, 1982; Dix 1984; Gonzales et al, 1984). Based on the results of Alberts et al (1980) a fungal metabolite mevinolin is used as a probe to study the physiological aspects of sterol.

The anticholesterolemic agent mevinolin is a fungal metabolite (Alberrts et al, 1980). It is a specific

inhibitor of HMG CoA reductase enzyme of the isoprenoid pathway. It has been used successfully in studying the physiological effects on 'animals, algae and plants (Alberts et al, 1980; Bach and Lichtenthaler, 1983, Maurey et al, 1986). However, this drug has never been used to generate a biochemical mutant of the isoprenoid pathway in plants.

The somatic cell genetic approach involves the selection of plant cells which can grow under inhibitory concentrations of specific analogues. Under such conditions resistance may be expressed because of many reasons as follows:

1. The enzyme targeted by the analogue/inhibitor may be structurally altered so that it is no more sensitive to them (Widholm, 1974; Mathews et al, 1980).
2. The enzyme in question may be over produced to nullify the effect exerted by the analogue or inhibitor (Berlin & Widholm, 1977, 1978; Berlin and Vollmer, 1979; Scott et al, 1979)
3. There may be new permeability barrier as a result of mutation which does not allow the entry of the inhibitor or the analogue (Berlin & Widholm, 1978)
4. Mutation leading to the detoxification of the analogue/ inhibitor (Berlin 1980).

A number of inhibitors of sterol biosynthesis have been used in animals, fungi, and algae, but not in higher plants to study the physiological role of sterols at cellular level. (Hosikawa, 1984). Some of the inhibitors used such as Triazole, Imidazole substituted pyrimidines and pyridines of the type, palcobutrazole and tripentenone are targetted at enzyme 14-methyl sterol demethylase (Langeake et al, 1984; Bach, 1985; Fletcher et al, 1986; Lenton, 1987; LurgSen, 1987 Scheinplug and Kuck, 1987). Plants possess a number of secondary products including alkaloids of different categories viz, alkaloids derived from amino acids (Fodor, 1980; Groger 1988), and alkaloids derived from isoprenoids (Roddick, 1980). Inspite of their wide spread occurrence a specific physiological function is not attributed to them (Roddick, 1980).

Steroidal alkaloids are shown to be toxic or inhibitory to a wide range of living organisms ranging from bacteria to higher animals. (Nishi et al,1974, Roddick, 1975b). Solamarine from Solanum dulcamara is shown to have tumour inhibiting properties (Kupchan, 1965). Few glycoalkaloids have shown to cause poisoning of human beings (Willimot,1933) and Cattles (Clarke, 1970). Embryo toxicity of α-Solanine (Bell et al, 1976) and teretogenicity of alkaloid rich blighted potato is reported (Willo, 1972).

Frank et al (1975) concluded that there is no direct correlation between the resistance to pathogen and alkaloid content as proposed earlier by (Sinder et al 1973). However, a recent review by Williams et al (1989) has affirmed that alkaloids among other secondary metabolites have a role as a natural defence system against other organisms.

Whatever may be the physiological roles played by these compounds a number of steroidal alkaloids are a central point of attraction to the pharmaceutical industries (Mann,1978; Ripperger & Schreiber 1981). This interest is because of their use as the starting material for the synthesis of cortico steroidal drugs (Mann, 1978).

Recently, technologies have evolved for the production of alkaloids among other secondary metabolites in cell cultures (Sakuta & Komamine, 1987) as well as microbial fermentores (Mahato et al, 1989). Plant cell cultures are also made use in the biotransformation which can be utilized for the production of alkaloids (Suga & Hirata, 1990). Immobilization of the cell cultures provide another area of research for the increased production of alkaloids (Subramani et al, 1989).

Inspite of all these available technologies developed, success of production ultimately depends on the cell strain

which itself should be a stable over producer of the desired natural product(s). Attempts towards achieving this goal have been made by different workers (Zenk et al, 1977; Tabata et al, 1978; Deus-Neumann & Zenk, 1982, 1984; Fujita et al, 1984) by using the analogues, cell line selection or by inducing mutations (Zank et al, 1977; Tabata et al, 1978, Berlin 1982a Sasse et al, 1983b; Deus-Neumann & Zenk, 1984; Maliga, 1984; [illegible] Subramani, 1991). However, in most of the cases the variants obtained were biochemically and genetically unstable as they were physiological variants rather than a genetic variant (Rhodes and Robins, 1987). There is evidence available regarding the successful genetic transformation with Agrobacterium to produce a hairy root cultures (Willnitzer et al, 1982b). However, information is not available in the literature regarding the production of stable mutant of the steroid pathway which is genetically characterised.

For quite some time, our laboratory was concentrating on the research on plant sterols and steroids (Bhatt & Bhatt, 1984; Bhatt et al, 1984 a and b; 1986, Chappel et al, 1989; Jacob et al, 1989; Subramani 1991). During this period the callus and cell suspension culture of solanum Spp. were well worked out (Bhatt, et al, 1979; 1981; 1986). Growth and development related sterol changes were studied in Solanum nigrum and it was shown that the quantitative composition of

the sterols changes with the change in the developmental stages of tissue in question. A qualitative change in the sterol composition ie.,the increase in the stigma sterol sitosterol ratio was established in Solanum nigram. Quantitatively, a three fold increase in the sterol content in the differentiation state was also shown. This study also demonstrated a change in the stigma sterol/sitosterol ratio as a result of hormonal changes in the medium (Bhatt & Bhatt, 1984).

Studies on the steroidal alkaloid (Solasodine) production by Solanum species showed a pronounced effect of hormones used (IAA and BAP) on the alkaloid production. Studies on the effect of sucrose and light showed the increase in the alkaloid production with increased supply of carbon source and darkness (Bhatt et al, 1983).

Effect of inorganic nutrients in the medium on steroidal biosynthesis was studied using Solanum dulcamara (Bhatt et al, 1986).

Studies on the regulation of sterol biosynthesis in two species viz., S. dulcamara and S. nigrum showed a negative correlation of the exogenous supply of sterol and incorporation of labelled $1\text{-}^{14}C$ acetate in to the

phytosterols without affecting the incorporation from ^{3}H mevalonate. It was also shown that the exogenous addition of cholesterol enhanced the incorporation of 1-^{14}C acetate in to fatty acids while reducing the incorporation of ^{14}C in to the isoprenoids suggesting a feedback control of sterol biosynthesis by cholesterol (Bhatt and Bhatt, 1984).

Other studies on the sterol biosynthesis was related to the biosynthesis of guggulsterone in the immobilised resin ducts of Commiphora wightii (Jacob et al, 1989).

Sterol and steroidal alkaloid levels in Solanum xanthocarpum and Solanum sysimbriifolium (Subramani et al, 1989).

As evidenced from the above description the interest of our lab. was revolving around higher plant steroidal chemistry and physiology. Techniques for the analysis of sterols and steroidal alkaloid (Solasodine) were well standardized in our laboratory. Cell line selection and regeneration procedure for Solanum xanthocarpum was standardized. We anticipated such a variant (resistant to mevinolin) of Solanum can be an over producer of sterols as well as steroidal alkaloid solasodine which has pharmaceutical potential. Thus we aim to convert this weed with wide geo-climatic adaptability to become a commercially

useful plant bearing Solasodine using the somatic cell genetical approach.

The only attempt that has so far been made is the SKF-A-525 (α-diethyl aminoethyl diphenyl propal acetic hydrochloride) resistant variant of Solanum xanthocarpum developed in our laboratory by Subramani (1991). The inhibitor SRF 525 A blocks the sterol synthesis in cell cultures by virtue of its inhibitory action on synthesis of 1 squalene from farnesyl pyrophosphate. My work aims to develop complimentary mutant of S. xanthocarpum to that ofSKF 525 A reistant variant developed by Subramani 1991. I selected mevinolin as the inhibitor because this inhibitor non competitively inhibits the enzyme 3-Hydroxy 3-methyl glutaryl CoA reductase (HMGR) that catalyses the first committed step in the isoprenoid pathway which produces the committed precursor mevalonic acid.