CHAPTER I

INTRODUCTION

INTRODUCTION

The family leguminosae comprises approximately 600 genera with around 13000 species, out of which only a few - about 20 are of economic importance as food and are consumed by humans either in form of fully ripened, dried seeds or as fresh immature seeds. They constitute one of the richest and cheapest sources of dietary proteins for large segments of the world's population, particularly in those countries in which the consumption of animal protein is limited by non-availability or its self imposed ban because of cultural or religious habits. (Liener 1982). Thus legumes are a major source of protein food in the world with an annual production in excess of 100 million metric tonnes (FAO report, 1989).

Many of the cereals and millets are poor source of proteins because of their low protein value ranging between 4.5 to 9%. Besides, their proteins are deficient in some of the essential amino acids required in human diet. For example, wheat and maize are deficient in lysine and jowar and maize are deficient in tryphophan (Kuppaswamy et al., 1958). On the other hand legume grains contain twice as much protein as that of cereals. They are rich source of lysine, although relatively low in total sulfur containing amino acids (Elias and Bressani, 1974). Due to the high lysine content of most of the legumes, except for groundnut, they are a good supplement to cereals (Aykroyd et al, 1982). Besides lysine they also form a valuable source of nutrients like thiamine, niacin and iron (Hallab et al, 1974). Thus in third world countries, legumes act as a valuable supply of protein,

calcium, iron, thiamine and riboflavin. In the western culture, meat and fish are the chief source of proteins, but they are normally low in dietary fibre and complex carbohydrates. Supplementation with legumes would bring these diets to recommended dietary goals.

India has the largest area in the world under pulse crops, growing chiefly Cicer arietinum, Cajanus cajan and Phaseolus mungo, all of which are dehusked and decorticated. The whole legume seeds are referred as 'pulses' and the split cotyledons after removal of seed coat as 'dhals'. Lathyrus sativus or 'Kesari' is a minor pulse crop forming a staple diet of a major section of rural population in the states of Madhya Pradesh and part of Uttar Pradesh, Bihar and Gujarat. This pulse stands apart when comparison is made in terms of nutritive value with the above mentioned pulses. The content of protein on seed dry weight basis is (28 to 35% w/w) and stands next only to Glycine max which has 43% protein . The seeds are also rich in minerals, fibre, carbohydrates coupled with pigments like carotene and vitamins like riboflavin, thiamine and niacin. The seeds have got calorific value (345 K.Cal/100g) which is higher than that of Cajanus (Table -I.1), the most widely used 'dhal' in India.

Further the protein quality in terms of its amino acid composition (Table I.2) is superior comprising of good amounts of essential amino acids than other pulses. The unique character of L. sativus is that it is an extremely hardy crop and can be grown in areas having acute scarcity of water under extreme conditions of

drought, this is the only crop that remains available to the poor because of its hardy character (Misra et al., 1981). It requires no agronomical managerial practices and it is virtually immune to insects and pests attack.

Besides the 20 amino acids that are necessary for the formation of proteins in plants, there are many unusual amino acids found in free state, viz γ-aminobutyric acid (GABA), homoserine, citrulline, ornithine etc (Table I.3). Some of the free amino acids have deleterious effects on animals by feeding. A number of free but unusual amino acids have been recognised (Fowden, 1962) and many legumes are known to contain such unusual, harmful substances (Liener, 1975). They act in a competitive way in the biological system as antimetabolites, showing sometimes growth retardation and other abnormalities.

Non-protein amino acids :

The term non protein-amino acid, generally include those naturally occurring amino acids, amino acid amides and imino acids which are not usually found as protein constituents, excepting a few non protein amino acids such as homosorine, citrulline and ornithine, which are involved in primary metabolic pathways of plants. Great majority of these compounds can be fairly regarded as secondary products. There are more than 220 non-protein amino acids now detected in plants (Fowden,1990). Most of the non protein amino acids of higher plants occur in free state or as condensation compounds of low molecular weight such as oxalyl,gamma-glutamyl and acetyl derivatives which can be readily extracted in 80%

ethanol, buffer or water. Generally, the non-protein amino acids seem to be restricted to groups of closely related species and, indeed, some are still known only as constituents of the single species from which they were isolated and characterized.

The great majority of non-protein amino acids are simple molecules, most of which contain fewer than twelve carbon atoms. Inspite of this limitation of molecular size, the group includes compounds showing a wide range of structural types. It is probaly easiest to remember the structural types if we can relate them to the more familiar protein amino acids.

L. sativus - Non-protein amino acids

Two basic amino acids normally found in protein are lysine and arginine. 2,3-α,β-diaminopropionic acid (DAPA) a basic amino acid was first found as naturally occurring amino acid by Gmelin et al., (1959) in the Mimosaceae and it is the lowest member of the homologous series containing lysine. In the seeds of Lathyrus species, DAPA exist in oxalyl form as N-2-oxalyl diamino propionic acid (ODAP)(Bell et al., 1964).

Accumulation of Non-protein Amino acids

It is not possible to generalise about the accumulation of non protein amino acids in plants. High concentrations of azetidine-2-carboxylic acid are stored in the seeds of the legume Bussea massainensis (Watson and Fowden, 1973) while the same compound is absent in the seeds of another legume Delonix regia, yet is present in the seedlings (Sung and Fowden, 1968). While in

Lathyrus sativus oxalyl-diamino propionic acid is present in greater amounts, whereas Lathyrus sylvestris contains another non-protein amono acid 2,4-Diamino butyric acid (DABA).

Perhaps the most remarkable feature of non-protein amino acid accumulation is the way in which single compound may represent a major part of the plant soluble amino acid pool or stored nitrogen reserve. L-3-4-Dihydroxynylalanine amounts to 8% of the dry weight of Mucuna mutisiana (Bell and Janzen; 1971) While 5-hydroxy-L-tryptophan may account for as much as 14% of the seed weight of Griffonia simpliciolia (Bell et al ., 1976).

In general, secondary plant products are distinguished from primary plant products by definition as being important for interactions with environmental factors but not being necessary for general metabolism. This assumption often leads to the misinterpretation that the synecological function is the only function of secondary plant products. Neverthless, it must be considered that many secondary products, particularly nitrogen containing compounds are remetabolized by the plants and are utilized to build up new substances. It is interesting to note that many of these compounds are present in drought resistant species of legumes (Fowden,1962).

In case of many nitrogen containing secondary plant products it could be shown that they have dual functions as repellent factors to pests and insects as well as nitrogen store compounds eg. pyrrolizidine alakaloids, (Toppel et al., 1988), Oxalyl diaminopropionic acid and diamino butyric acid (Rosenthal, 1982).

etc; proving that the significance of these secondary products is not simply mono-but multifunctional. For example, the storage functions of β —Cyanoalanine a non protein amino acid is easy to demonstrate in the cyanogenic containing Unguadia speceiosa (Sapindaceae). During seedling development of Unguadia the total amount of cyanogenic compounds (400 μ mol/seed) is consumed totally and transferred into a non cyanogenic compound. At the same time other cyanogenic glycosides are produced, which might reveal the important synecological functions. Suffice to state here that many of the non-protein amino acids from plants are known to be toxic to vertebrates, invertebrate animals, higher plants and to microorganisms. They can also act as feeding deterents to insects (Bell, 1977).

Excessive oral intake seed of Lathyrus sativus,(chickling vetch) causes human and animals to develop a type of spastic paralysis or a crippling disease known as 'neurolathyrism' (Rao et al., 1969). Disease is characterised by slow or more rapid onset of spastic paralysis associated with corticospinal pathways which results in paralysis of leg muscles and in extreme cases death. (Sachdev et al., 1969; Streifler et al., 1977) (Table I.4 and Fig I.1) Neurolathyrism generally appears when the one third of the diet consists L. sativus seeds at a rate of about 200g or more per day for 3-6 months (Dwiwedi and Prasad, 1964)

Another significant observation is that the disease symptoms do not appear gradually, but very abruptly when the adequate level of toxin is built up in the body (Sarma and Padmanabhan, 1969). A recent outbreak of the lathyrism disease in Madhya Pradesh, India

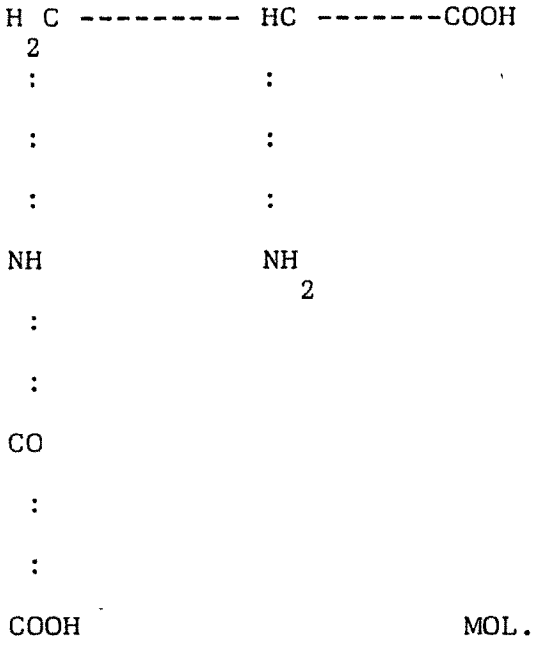
showed that the onset can occur much erlier, around 20 days (cf.Rutter and Percy, 1984.). Ganapathi and Dwiwedi on the basis of their epidemological survey in 1961 concluded that symptoms of the adverse effects of neurolathyrism do no manifest themselves in the same manner in both males and females suggesting that the estrogen hormone might play an important role in the females for resistance to the disease. This conclusion is supported by Das et al., (1974) who found the ratio of patients is 16.9 males to 3.3 females.

The cause of the disease is due to the presence of free ninhydrin-reacting toxic compounds in the species of Lathyrus latifolius, L. sylvestris, L. sativus, and Vicia sativa and V. angustifolia (Bell, 1979; Roy, 1981).

Table I-3 summarizes some of the free, toxic amino acids found in legumes. In L. sativus, the most widely consumed species, there are 3 toxic non-protein amino acids and their derivatives, which are (1) β-N-Oxalylamino-L-alanine (BOAA) (Nagarajan et al, 1965) or also called B-N-Oxalyl L, B-diaminopropionic acid (Ox-dapro or ODAP), (2)N-dioxalyl – diaminopropionic acid (Do-DAPA) and (3) N-D-glucopyronosyl-N-Diamino propionitrile.

Various claims have been made regarding the factors responsible for the disease neorolathyrism. Studies by Bell and Trimanna (1965) indicated β-N-Oxalyl-L α,β- diamono propionic acid (ODAP) is the principal causative factor for the disease.

The chemical structure of ODAP is as follows :



Three research groups in India (Murti et al., 1964; Roy et al.,1963 and Rao et al., 1964) have reported the isolation of neurotoxin (ODAP) from L. sativus almost at the same time. Bell and O'Donovan (1966) observed that the naturally occurring neurotoxin (ODAP) exists in two isomeric forms alpha and beta isomers ; the beta isomer ranges from 92% to 96% of the total ODAP content.

As described earlier the Lathyrus species are generally cultivated for preparation of food, fodder, green manure and ornamental purpose. In India,L. sativus occupies 4% of the total area under pulse crops cultivation and constitutes 3% of the total pulse production. Recent studies carried out by ICMR team headed by Gopalan and Dwiwedi (1986) showed that in Madhya Pradesh (India) 10-20% of the total grain production is occupied by L. sativus. Because of its low unit price and ease of cultivation it is used

as an adultrant of other pulses such as Cicer and Lentils (Sastri 1962). Due to the intermittent outbreak of neurolathyrism, Government of India had imposed a ban on its cultivation and consumption (Ganpathi and Dwivedi, 1961). However, an effective ban on the cultivation of L. sativus was not possible in veiw of the lack of suitable alternative crop acceptable to the poor farmers (Padmanabhan, 1980). However, recently in 1990, a resolution was passed in Maharashtra state to lift the ban on sale of Kesari dal and its products in India to solve the rising price of pulses. The principal arguments in favour of this resolution were the need for cheap, drought resistant, nutritious crop and the absence in the last 10 years of cases of lathyrism in population where daily consumption is rather low i.e., 35-70 grams per day. The latter argument has called for much debate among the policy makers and scientists at global level, since the 'no-effect' level of the neurotoxin has not been established with any degree of medical and scientific certainty.

An alternative approach is to find a method by which seeds can be processed suitably so as to free them from toxin and render it fit for human consumption keeping in mind its other nutritional merits.

A different approach was followed by Somayajulu et al., (1975) for the removal of ODAP from seeds, viz. development of purelines from low ODAP containing plants by conventional breeding methods. They were able to identify four lines of L. sativus containing a low ODAP content of which Pusa-24 was recommended as being relatively safe for human consumption. Results of bioassay studies conducted

on chicks indicated that 24 g of P-24 line produced symptoms similar to those produced by 5-6 g of seeds of local varieties.But a recent study by Nerkar (1990) indicated that extensive screening of the germplasm did not result in the identification of any variety free of the neurotoxin (ODAP). A mutation breeding programme was initiated in 1966 to induce neurotoxin-free mutants in Lathyrus. (Swaminathan, 1969) Seeds of four widely cultivated varieties were subjected to mutagenic treatments involving gamma rays and the chemical mutagen ethyl methanesulonate (EMS). In the M2 generation plants with about 80-90% reduction in ODAP content were isolated. The seggregation in the M2 generation indicated that the level of ODAP in seeds is governed by a single partially dominant gene. But the heritability of the low toxin lines was estimated to be low.

Post harvest processing is the second approach in which attempts have been made to process the L. sativus seeds to free them of the toxin (Table I.5). Mohan et al., (1966) suggested that removal of ODAP from seeds can be done by (a) steeping the dehusked seeds in hot water for several hours or (b) boiling the seed in water and discarding the water. The removal of neurotoxin from seeds by these methods was achieved by about 70% to 80%. Such methods of detoxification, however, would not only remove several of the useful nutrients, such as soluble proteins, amino acids and vitamins, along with ODAP, but also involve large-scale technological processing of the materials which is cumbersome. Rao et al., (1969) recommended that the roasting of seeds for about 15-20 min at 85 ^{o}C could make the seeds free of neurotoxin.

However, the Chapati-making properties of the legume may be effected adversely by continuous heat treatment. The simple methods suggested for detoxification of kesari,(Naved et. al.,1990) include (a) overnight soaking and then steeping for 1hr with saturated lime water : detoxification = 88.9% (b) overnight soaking and then steeping for 1 hr with 1.5% baking soda ($NaHCO_3$ solution : detoxification = 82.6 % and (c) direct baking of kesari paste after 15 min soaking with $NaHCO_3$ solution (1.86 g/100 seed,) detoxification = 50.9%. Considering the time, cost of heating and nutrient loss involved in the steeping procedures, direct baking is favoured, which retains the taste and flavour of Kesari.

The recently organised 'International Workshop on 'Non-protein amino acids, with special reference to L. sativus (Gent Symposia, 1990) has thrown much light on methods to overcome the neurotoxin problem. This includes a report on prospects of low ODAP Lathyrus from indigenous germplasm by Agrawal et al.,(1990). Nearly 2200 germplasm lines were collected of which 900 small seeded types were analysed for ODAP.A considerable variation in ODAP content was observed. Cultivars with as low as (0.3%) ODAP content were recorded. Further, genetical studies (Brigg & Campbell, 1990) to understand segregation patterns for ODAP contents in selected F2 progeny of L. sativus indicated that character for low ODAP content is recessive in nature. A further interesting observation has been the identification of high ODAP accessions which when crossed with medium or low ODAP lines produced F2 segregants with high levels of ODAP, upto double those of the high parent values.

The so called low neurotoxin containing cultivars like P-24, LD1, LD2, LD3 etc. failed to retain the low ODAP content under different agro-climatic conditions. Hence this achievement proved to be a futile exercise as the same cultivar revealed high ODAP content upto 0.8% or even more under different agro-climatic conditions at various Indian locations (Narsinghani, 1983).

Similar results of the poor stability of the trait of low neurotoxin contents in varieties P-24, LD1, LD2, LD3, LD4, LD-5 and LD-6 was also reported earlier by Ramanujan et al (1980). Thus breeding for low neurotoxin is of no use till we fully understand the physiology and biochemistry of neurotoxin synthesis in L. sativus.

The alternative solution could be to replace the hardy Lathyrus crop with other suitable legumes or cereals such as some of the promising pea lines or produce a variant with low or zero toxin using more sophisticated techniques of site specific mutagenesis (Narasinghani, 1983).

Physiological approaches made to detoxify Lathyrus seeds include foliar spray of micronutrients like cobalt and molybdenum in low concentrations at the reproductive stage of the plant. (Misra and Barat 1981). They observed reduction in toxin level upto 50% with cobalt and 30% by molybdenum, only in cultivar like P-24 which are low in ODAP responded to foliar , but the high ODAP containing line did not respond.

Biosynthetic pathway of ODAP :

There have been several attempts to establish the biosynthetic pathway of ODAP in plants. Based on the structural relationship of ODAP and its occurence in high concentrations in L. sativus, serine had been suggested to be the precursor of the diaminopropionic acid moiety in the biosynthesis of ODAP (Murti and Seshadri 1964). On the contrary Roy (1969) observed that when L. sativus seeds were germinated in the presence of DjL-serine-3-^{14}C no radio activity was associated in the ODAP.

It is interesting to note that the naturally occurring osteolathyrogen alpha-(glutamyl) aminopropionitrile and the neurotoxic amino acids including ODAP are biosynthetically inter-related.(Nigam and Ressler, 1966). With regards to biosynthesis of oxalyl moeity ODAP, that ^{14}C oxalic acid is incorporated as an unit into ODAP in the germinating seedlings of L. sativus (Malathi et al, 1968).It has been demonstrated that ^{14}C - labelled ODAP is produced when the diabasic acid, L,B -diaminopropionic acid is incubated with ^{14}C - labelled oxalic acid in presence of oxalyl activating enzyme system requiring Co-A, ATP and Mg^{+2} (Jhonston and Llyod, 1967). However, other investigators (Bell and O'Donovan 1966; Roy and Narsinga Rao, 1968; Wu et al., 1976) doubted the findings of Malathi et al., (1968) and indicated it could only be an isomeric rearrangement and was not enzymic.

No further work was done on biosynthetic pathway of neurotoxin ODAP until recently Lambein et al (1990) reported an heterocyclic unstable amino acid, namely Beta-(isoxozol-5-on-2-yl)-L-alanine

(BIA) as the precursor of ODAP. This was confirmed when ^{14}C labelled BIA incorporated into ODAP in young seedlings and 7-14% of the radioactivity in the amino acid fraction was found in ODAP. Also, when ODAP was further purified and hydrolysed, labelled DAPA was found. BIA is present in seedlings of the sweet pea (Lathyrus odoratus), the lentil (Lens culinaris) and grass pea (Lathyrus sativus).

In an attempt to elucidate ODAP synthesis Kuo and Lambein (1990) obtained ^{14}C-BIA purified from Pisum sativum seedlings after feeding ^{14}C-serine. Freshly cut callus tissue of L. sativus was supplemented with this ^{14}C-BIA alone or together with unlabelled DAPA or Oxalate in liquid media and incubated for six days. When callus tissue was supplemented with ^{14}C - BIA and DAPA, less incorporation of radioactivity was found into ODAP, than when ^{14}C -BIA alone was used.Further the inhibition by DAPA was concentration dependent. When oxalate was added together with ^{14}C - BIA, the incorporation into ODAP was increased. These results confirm that BIA is the in vivo precursor of the neurotoxin ODAP and that DAPA may be a short lived intermediate for this biosynthetic step.

Based on the above findings these workers proposed biosynthetic pathway of ODAP (Fig.III.11). Here the two compounds, Isoxozone - 5 - one and DAPA are not detected so far in the free form.

Non-protein amino acids in cell cultures.

The plant cell cultures have proved valuable in the description of pathways of secondary metabolism that are only expressed very low levels for limited periods in whole plants. Frequently this has been achieved by exploiting natural or induced variation in cultures to produce lines of high metabolic flux through such pathways. In a few cases specific inducers of these pathways have been identified, allowing studies of their regulation. For e.g,in Phaseolus vulgaris treatment of cells in culture with an elicitor prepared by heat released from cell walls of the fungus Lindemuthianum leads initially to the formation of isoflavone kievetone and later to the accumulation of pterocarpan phaseoltin (Robins et al., 1985). Further, the availability of cultures of plant cells active in secondary product biosynthesis independent of seasonal or climatic limitations has led to rapid advances in our knowledge of secondary metabolism, of their regulation and of the interface between these pathways and primary metabolism.

Much of the work on metabolic studies of amino acids in cell cultures have dealt with amino acids which involve primary metabolism protein synthesis . Very meagre information is available regarding metabolic studies of non-protein amino acids in cultured tissues. In flat pea, Lathyrus sylvestris L. the nuerotoxin 2,4-Diaminobutyric acid (DABA) was determined in the subcellular fractions from mesophyll protoplasts. (Foster et al., 1987). Chloroplasts contained about 15% of the cellular DABA. Atleast 75% of the DABA was vacuolar, based on the assumption that each protoplast contained a single vacuole. Coulombe et al.,

(1990) with an objective to optimise media requirement for whole plant regeneration and to characterise the variability of DABA among regenerated plants of Flatpea (L. sylvestris) found that callus tissue contained 20.2 mg/g dr.wt whereas regenerated roots and leaves contained 16.1 and 33.8 mg/g.dr.wt. of DABA, respectively. This indicated that regenerated plants did not show substantial reduction in DABA concentration.

Somatic Cell Genetic Approach :

The ability to culture plant somatic cells on chemically defined media and to regenerate plants from these cells either via somatic embryogensis or adventitious shoot bud formation offers great possibilities for genetic experimentation. Direct selection for defined mutant types, an experimental advantage previously restricted to microbes, can now be accomplished in vitro using somatic cells. This development places us on the threshold of what must prove to be a new realm of scientific discovery. The potentials for applying selection technique to obtain mutant lines for improvement of higher plants as well as to elucidate basic problem has been discussed in numerous reviews (Street, 1973; Chaleff and Carlson, 1974; Parke and Carlson, 1979; Flick, 1983; Widholm, 1984; Kucherenko, 1985; and Wershun, 1989).

The entire gamut of science in which either the selection of trait or hybridization or genetic transformation is carried out using somatic cell, has now been called as somatic cells genetics. By far the greatest experimental advantage of somatic cell for the geneticist is that it makes large number of cells available for

experimentation . Besides large quantity, the trememdous reduction in size of the experimental unit allows a scientist to handle numerous experimental units in a very small space, an advantage offered previously only by organism like yeast and bacteria.

Further, there is a considerable reduction of time than those of conventional genetics, e.g., it takes several years in case of tobacco to obtain a purely homozygous plants by repetitive selfing in the conventional genetic approach. Besides, in self-incompatible species these conventional methods cannot produce homozyogous plants at all. Both of these drawbacks of conventional genetics could easily be overcome by the technique of anther culture. Homozygous plants even from self incompatible species can be produced in one year period in Petunia and Brassica. Similarly tobacco plants resistant to fungal toxin (Selvapandiyan et al., 1988) or herbicides (Chaleff and Roy., 1984) can be produced in a period of one or two years using single cell plating technique of somatic cell genetics which requires 5 to 7 years in the conventional approach. Here selection can be done in highly controlled condition in the single cell plating technique of giving reproducibility of experiment. This reproducibility is difficult to achieve in the conventional approach as the selection done in the open environment which is variable.

Regeneration in Legumes :

The improvement of legumes is essential for their continued exploitation as source of human nutrition and other products. Their ability to fix gaseous nitrogen in symbiosis with Rhizobium speciess, will become on ever-increasing source of organic nitrogen fertilizer as inogranic fertilizer prices continued to rise. While some improvements will be brought about by traditional breeding, plant tissue culture facilitating genetic manipulations at the cellular level will play an increasing role in plant improvement programs. Fundemental to the exploitation of tissue culture is requirement to regenerate plants routinely and in sufficient numbers from cultured cells.

It is only in the last decade that significant advances have been made in regenerating plants from callus tissues of legumes (Hammatt et al., 1985). Most progress has been made in forage crops in which regeneration from callus cultures occurs through somatic embyogenesis and caulogenesis. Regeneration has also been demonstrated in leguminous trees. However, the large-seeded grain legumes (pulses) used mainly for human consumption, have shown in most cases, only very limited regeneration.

Because of such numerous advantages offered by the somatic cell genetic approach we decided to explore the possibility for the improvement of L. sativus. Regeneration of whole plant from somatic cells is the foremost requirement for the success of this approach.

Table I.6 describes progress made in regeneration of some important legumes in vitro, in the last decade. It is apparent that large number of leguminous plants have been regenerated from in vitro cultures and therefore there is possibility to achieve whole plant regeneration from our experimental material, i.e. cell cultures of L. sativus. We therefore in subsequent pages will describe how we have selected a cell line with low neurotoxin and then how they have been regenerated into whole plant.

TABLE I.1 : Proximate principles, minerals and vitamins of Lathyrus sativus compared to common legumes

Pulses and legumes	Moisture	Protein	Fat	Minerals	Fibre	Carbohydrates	Energy KCal 100g	Calcium	Phosphorus	Iron	β-Carotene	Thiamine	Riboflavin	Niacin
	gm%							mg%			(ug%)		(mg %)	
1. Kesari dhal (Lathyrus sativus)	10.0	28.2	0.6	2.3	2.3	56.6	345	90	317	6.3	120	0.39	0.17	2.9
2. Bengal gram (Cicer arietinum)	9.8	17.1	5.3	3.0	3.9	60.9	360	202	312	10.2	189	0.3	0.15	2.9
3. Black gram dhal (Phaseolus radiatus)	10.9	24.0	1.4	3.2	0.9	59.6	347	154	385	9.1	38	0.42	0.37	2.0
4. Green gram (Phaseolus aureus)	10.4	24.0	1.3	3.5	4.1	56.7	334	124	326	7.3	94	0.47	0.39	2.1
5. Pigeon pea (Cajanus cajan)	13.4	22.3	1.7	3.5	1.5	57.6	335	73	304	5.8	132	0.45	0.19	2.9
6. Feild beans Dolichos lablab)	9.6	24.9	0.8	3.2	1.4	60.0	347	60	433	2.7	-	0.52	0.16	1.8
7. Soybean (Glycine max)	8.1	43.2	19.5	4.6	3.7	20.9	432	240	690	11.5	426	0.73	0.39	3.2
8. Cow peas (Vigna catjang)	13.4	24.1	1.0	3.2	3.8	54.5	323	77	414	5.9	12	0.51	0.20	1.3

ICMR Food Table (1978)

TABLE : I.2 Amino acid composition of L.sativus

Amino acid	content (% of sample)	
	Seed meal	Protein concentrate
Lysine	1.85	5.99
Histidine	1.10	2.18
Arginine	1.41	5.69
Asparagine	1.80	7.41
Serine	1.20	4.02
Threonine	0.85	2.78
Glutamic acid	2.25	8.79
Proline	1.46	4.22
Glycine	0.72	2.90
Alanine	0.80	2.92
Cystine	Traces	Traces
Valine	0.81	2.76
Methionine	0.35	0.99
Isoleucine	1.01	3.32
Leucine	1.45	5.30
Tyrosine	0.62	2.54
Phenyl alanine	1.03	3.29

Source : Sarma and Padmanabhan (1969)

TABLE I.3 : Naturally Occuring Toxic Amino acids and Derivatives in Leguminosae

Toxic amino acids and derivatives	Source	Toxicity	Reference
1. L- γ-diamino butyric acid or L-2,4-diaminobutyric acid and γ-N-oxalyl derivative	Lathyrus latifolius L.sylvestris (Flat pea)	Neutotoxic	Ressler et al.,(1961,69
2. β-Cyanolanine and γ-glutamyl-β-Cyanoalanine	Vicia sativa (Common vetch) V.angustifolia	Neurotoxic	Bell & Tirimann (1965) Ressler(196
3. β-N-γ-Glutamyl)-amino-propionitrile	L.pusillus (Singletary pea) L. odaratus (Sweetpea)	Osteo-toxic	Duppy and Lea (1954)
4. β-N-Oxalyl-L-diamino	L. sativus (Chickling vetch)	Neurotoxic	Murti et al., (1964) Rao et al (1964) Bell (1973)
a. propionic acid (ODAP) or β-N-Oxalyamino L-alanine (BOAA)			
b. Dioxalyl diaminopropionic acid			
c. N-D-glucopyronosyl-L-diaminopropionitrile			
d. Phenolic compounds.			
5. Canavanline	Canavalia ensiformis (Jackbean)	Inhibits growth of Neurospora and other micro-organism.	Bell and Trimann (1965)
6. β-Nitropropionic acid	Indigofera eudecaphylla (Creeping indigo)	Hepato-toxic and Neurotoxic	Britten et al., (1959)

TABLE I.4 The Four Stages of Neurolathyrism
(Patwardhan, 1961)

Stage	Symptoms
I	Weakness of lower limbs with spasticity of several muscles, pain in ankles, knees, swing around the foot while walking
II	Flexion of knee: Inversion with tendency to walk with a stick.
III	Two stick stage prevails. Other symptoms become chronic
IV	Knees completely flexed. Atrophy of thigh and leg muscles. Neuromotor disturbance in upper extremeties. No sensory disturbance in affected limbs.

Fig.I.1. The Four stages of Lathyrism.

I

II

III

IV

TABLE I.5 : Effect of different food processing on neurotoxin (ODAP) contents :in L.Sativus

Reference	Method of processing	Degradation of ODAP	Remarks
ICMR (1968) report	Soaking the seeds overnight followed by steaming for 30 min or sun-drying the grains	80%	No loss of thaimin, but substantial loss of riboflavin and nicotinic acid.
Mohan et al., (1966)	Cooking the pulse in excess of water and then draining off the excess water	70%	Loss of water soluble 'B' Vitamins
Rao et al., (1969)	Roasting the seeds at 85 °C for 20 min.	85%	- Not economical for the poor - Binding capacity of the flour is lost.
Shiv Prakash et al., (1977)	Germination	Significant reduction	--
(Cf.Rutter and Percy, 1984)	Parboiling the dahl	80-90%	--
Naveed et al., (1990)	Overnight soaking and then steeping in soda solution.	50%	--

TABLE : I.6 Tissue Culture Work in Legumes (1980-91)

Species	Mode of regeneration						Reference
	Meristem	De novo (direct)	Callus/suspension Organogenesis	Callus/suspension Embryogenesis	Protoplasts	Plantlets	
Acacia senegal	+					+	Dave et al., 1980.
Albizia lebbeck			+			+	Upadhyay and Chandra,1983., Tomar and Gupta, 1988.
"		+					Gharyal and Maheshwari, 1981.
Alhagi camelorum			+			+	Bharal and Rashid,1981.
Arachis hypogaea	+		+				Bajaj et al.,1981b.
"			+				Mroginiski et al., 1981.
"		+				+	Hisajima, 1982c, Bhatia et al., 1985, Mhatre et al. 1985.
"				+		+	Hazra et al., 1989.
"		+					Atreya et al., 1984.
"			+				
"			+			+	Mckently et al., 1990.
Arachis villosa			+ (anther)				Bajaj et al., 1980b.
Cajanus cajan		+				+	Usha Mehta and Mohan Ram, 1980.

TABLE : I.6 (contd.)

Species	Mode of regeneration						Reference
	Meristem	De novo (direct)	Callus/suspension Orgao-genesis	Callus/suspension Embryo-genesis	Protoplasts	Plantlets	
Cajanus cajan			+			+	Kumar et al., 1983.
"				+			Bajaj et al., 1980b.
Ceratonia siliqua	+					+	Thomas, 1982.
Cicer arietinum	+					+	Kartha et al., 1981.
			+				Singh et al., 1982b.
Clianthus formosus					+	+	Binding et al., 1983.
Coronilla varia			+				Mariotti and Arcioni, 198
Crotalaria juncea			+		+	+	Rao et al., 1982,1985,198
Dalbergia sissoo		+	+			+	Mukhopadhyay and Mohan Ram 1981. Sharma & Chandr
"	+					+	1987., Datta and Datta, 1983.
Glycine canescens		+				+	Kameya and Widholm, 1981. Widholm and Rick, 1983.
"					+	+	Newell and Luu, 1985.
"					+		Hammatt, 1987.
Glycine max.		+					Cheng et al., 1980.
"	+					+	Kartha et al., 1981.
"			+			+	Lipmann and Lipmann, 198
"			+			+	Li et al., 1985.

TABLE : I.6 (contd.)

Species	Mode of regeneration						
	Meri-stem	De novo (direct)	Callus/suspension Organo-genesis	Embryo-genesis	Proto-plasts	Plant-lets	Reference
Glycine max			+	+		+	Barwale et al.,; 1986.
"					+	+	Chowdhary and Windholm; 1985.
"				+		+	Finer; 1988.
"				+		+	Lazzeri; 1985, 1987.
"				+		+	Ranch et al.; 1985.
"					+	+	Wei and xu, 1988.
"				+		+	et al.
"				+		+	Parrot et al.; 1989. Bhatt, 19
Glyucine soja				+	+	+	Gamborg et al., 1983b.
Glycirrhiza glabra	+					+	Shah and Dalal, 1980.
Indigofera patoninii			+			+	Howell et al.; 1987.
Lathyrus sativus			+			+	Gharyal and Maheshwari, 1980
Leucaena leuco cephala	+					+	Ravishankar et al., 1983.
"	+					+	Goyal et al., 1985.
"	+					+	
Linum usitatissimum				+			Hughen and Swartz, 1984.
Lotus corniculatus			+			+	Swanson and Tomes, 1980.
"							
"				+		+	Arcioni and Mariotti, 1982.
Medicago coerula					+	+	Arcioni et al., 1982.
Medicago sativa			+			+	Staverek et al., 1980.

TABLE : I.6 (contd.)

Species	Mode of regeneration						Reference
	Meristem	De novo (direct)	Callus/suspension Organogenesis	Callus/suspension Embryogenesis	Protoplasts	Plantlets	
Medicago sativa					+	+	Dos Santos et al.,1980;
"				+			Santos et al. 1983.
"				+	+		Brown and Atanassov;1985. Nagrajan et al, 1986
Medicago Sps.				+		+	Bingham et al, 1988
Medicago sativa				+		+	"
Medicago truncatula				+		+	Nolan et al; 1989.
Melilotus-officinalis			+			+	Oelek and Schiener, 1983.
Melilotus-parviflora			+			+	Bajaj and Gosal, 1981.
Mimosa pudica			+				Gharyal and Maheshwari, 1982.
Onobrychis-viciaefolia			+			+	Arcioni and Mariotti,1982.
"					+	+	Hamill et al., 1986.
Phaseolus vulgaris	+					+	Kartha et al., 1981.
"			+				Zagorska et al., 1982.
"				+ (early stages)			Martins and Sondahl, 1984.
"			+		+		Crepy et al. 1986
Pisum sativum	+					+	Hisajima, 1982.

TABLE : I.6 (contd.)

Species	Mode of regeneration						Reference
	Meristem	De novo (direct)	Callus/suspension Organogenesis	Callus/suspension Embryogenesis	Protoplasts	Plantlets	
Pisum sativum	+					+	Hisajima, 1982; Hussey and Gunn, 1984.
"			+			+	Mroginski and Kartha,1981; Rubluo et al., 1982.
"			+		+		Johanna and Erikson; 1988.
"				+			Jacobson and Kysely; 1984.
Prosopis cineraria	+					+	Goyal and Arya, 1984.
Psophocarpus-tetragonolobus			+			+	Gregory et al., 1980
"		+	+			+	Mehta and Mohan Ram,1981;
"					+	+	Wilson et al., 1985.
Sesbania grandiflora		+	+			+	Khattar and Mohan Ram,1982.
Sesbania sesban		+	+			+	Khattar and Mohan Ram,1982.
Stylosanthes-guianensis			+			+	Mejjer and Broughton,1981. Kapoor and Gupta, 1986.
"					+	+	Meijer and Steinbiss, 1983;
Stylosanthes humilis			+			+	Meijer 1982.
Stylosanthes scabra			+			+	Godwin et al. 1987.
Tamarindus indica	+					+	Mascarenhas et al.,1982.
Trifolium alpestre	+					+	Parrott and Collins,1982.

TABLE : I.6 (contd.)

Species	Mode of regeneration						Reference
	Meri-stem	De novo (direct)	Callus/suspension Organo-genesis	Embryo-genesis	Proto-plasts	Plant-lets	
Trifolium-corniculatus				+	+		Lu et al., 1982.
Trifolium glutinosa					+	+	Arcioni et al., 1982.
Trifolium-incarnatum	+		+			+	Parrott and Collins,1982.
" medium				+		+	Choo; 1988
" pratense				+		+	Phillips and Collins,1980.
" "	+					+	Parrott and Collins, 1983.
"repens		+		+		+	Greshoff and Mohapatra,1981.
" "				+		+	Maheswaran and Williams1984, 1985,1986.
"					+	+	Greshoff,1980; and White, 1983.
Trifolium-				+		+	Mcgee et al., 1989.
" "			+			+	
"rubens	+			+		+	Parrott and Collins, 1982.
"subterraneum					+	+	Grosser and Collins, 1984.
" "	+			+		+	Parrott and Collins, 1982.
" "						+	Webb et al; 1987.
Trigonella corniculata				+	+	+	Lu et al., 1982.
"foenum-graecum			+		+		Xu et al., 1982.

TABLE : I.6 (contd.)

Species	Mode of regeneration						Reference
	Meristem	De novo (direct)	Callus/suspension Organogenesis	Callus/suspension Embryogenesis	Protoplasts	Plantlets	
Vicia faba	+					+	Galzy and Hamoui, 1981.
Vicia narbonensis				+		+	Albrecht and Kohlenbach, 1989.
Vigna aconitifolia			+			+	Kumar et al. 1988.
" "			+	+	+	+	Shekhawet and Galston, 1983; Krishnamurthy et al. 1984; Gill and Eapen,1986.
" "		+				+	Eapen and Gill, 1986.
" mungo			+				Sinha et al., 1982.
" radiata (var.aureus)	+					+	Singh et al., 1980.
Vigna radiata	+					+	Mathews and Rao, 1984.