Chapter I

* Review of Literature *

CHAPTER - I

(A) Historical Account :

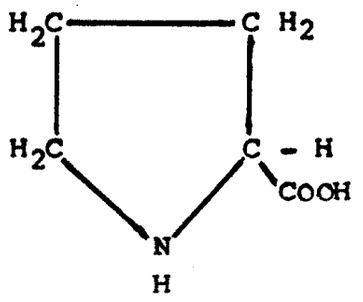
In 1901, Emil Fischer discovered L-Proline from Protein hydrolysate. He coined the name L-proline to the lengthy term α-pyrrololidin carbonsure. He also reported, L-proline from glutein, gliadin and zein. The occurrence of L-proline was first reported from lower organisms like bacteria (E. coli), fungi (Neurospora crassa) by Vogel (1953). In 1913 presence of L-proline in animals was repoted by Dakin and Abderhalden.

Later on after the long gap of fifty years, Indian workers Giri et al. (1950-52) reported occurrence of proline from plant leaves. Since that time L-proline is reported from various plant groups like algae, pteridophytes, gymnosperms and angiosperms by various workers. Further many chemical compounds, structurally related to L-proline have been reported in large number from living organisms. These are hydroxyproline, 4-Methylene DL-proline, 2,3-Cis-3,4 dihydroxy L-proline, L-azetidine - 2 - carboxylic acid, L-pipecolic acid and 4 - transhydroxy proline. L-proline is an amino acid and it serves as one of the building blocks in protein molecule. There is nitrogen atom$ in its ring, instead of carbon atom, hence it is termed as heterocyclic amino acid. Its biosynthesis was first discovered by Rose

and coworkers (1947-48), from glutamate. In plants L-proline exists in protein bound and free state. Under certain abnormal conditions, L-proline accumulates in free state in cell-cytoplasm in plants. For the first time its phenomenal accumulation under stress conditions was shown by Kemble and MacPherson (1954) in rye grass. Later on in 1957, Thompson and Morris confirmed it in turnip leaves. From this time, accumulation of L-proline in free state due to various environmental stress conditions was reported by many workers from all over the world. It is now very well established that, among the twenty amino acids, proline is perhaps the only amino acid which shows such heavy accumulation under stress conditions and hence considerable attention has been paid to the fate and role of this amino acid in plant species by plant physiologists in last two decades.

(B) Chemical Properties :

L-Proline is one of the protein amino acids. Since L-proline owes its origin from glutamate, it is included in glutamate family of amino acids. It is heterocyclic amino acid with following structure :



1) L-Proline is amorphous and yellow coloured with molecular weight 115.1 daltons.

2) It is sweet in taste.

3) It is soluble in ether, alcohol and highly soluble in water.

4) Its melting point ranges between 228°C. to 233°C.

5) It is optically active intermediate for organic synthesis.

6) α-amino carbon atom of L-Proline is asymmetric.

7) It gives yellow colour with ninhydrine on paper chromatograph. The other amino acids give violet or pink colour with ninhydrine. This unique property of L-proline is used for its detection and determination.

8) An amino group of L-Proline and hydroxyproline reacts with many acylating agents like Acetyl chloride (CH_3COCl), Benzoyl chloride (C_6H_5COCl), Benzene sulfonyl chloride ($C_6H_5SO_2Cl$) and Carbobenzoxy Chloride ($C_6H_5 - CH_2O COCl$). All these compounds react with L-proline in alkaline medium.

9) According to Palfi et al. (1975), L-proline is most stable amino acid to the oxidative acid hydrolysis.

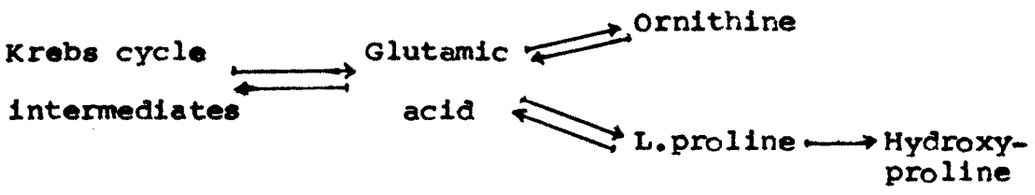
10) It does not react with nitrous acid to produce ammonia, but it produces nitrosoderivatives. This property of L-proline is also used for its detection.

11) L-proline gives rise to glutamic acid, organic acids

and CO_2 after its oxidation.

(C) Biosynthesis

The amino acids like L-Proline, glutamic acid, hydroxyproline and ornithine are structurally related to one another, and there exists metabolic interrelationship among them. Many research lines have given evidence regarding their interconversions and it can be very briefly represented as follows :



There are three major biosynthetic pathways leading to L-proline and these are -

(a) Glutamic acid ⟶ L - proline

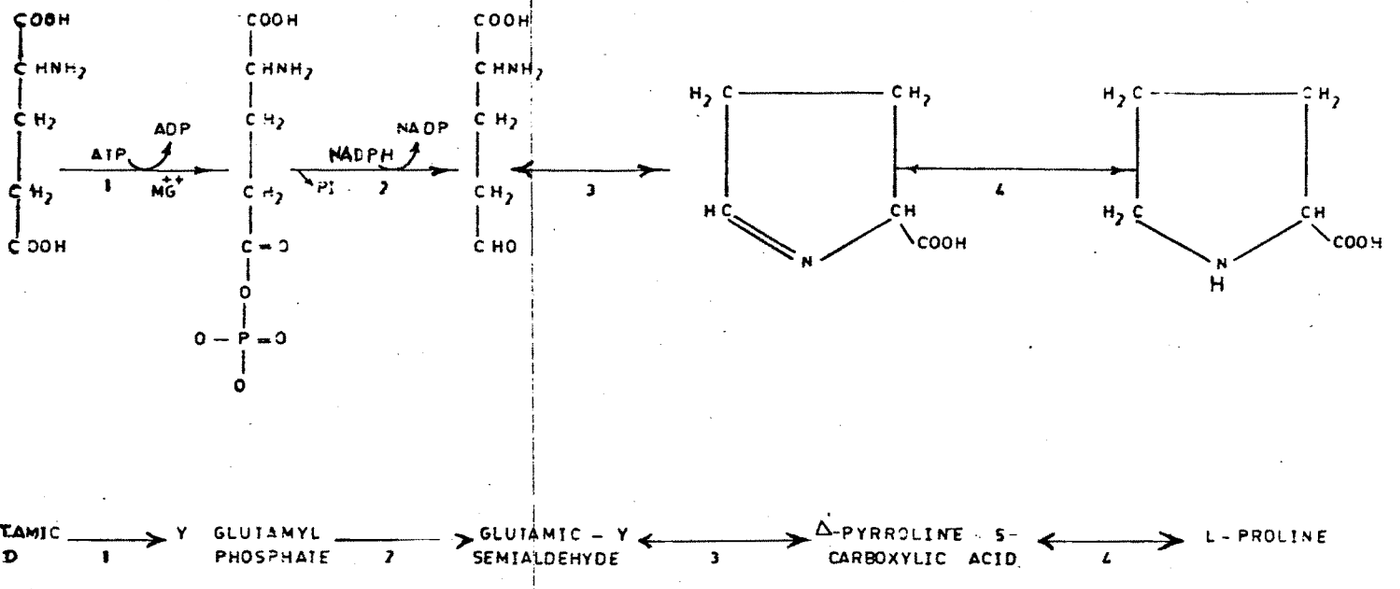
(b) Arginine ⟶ L - proline

(c) Ornithine ⟶ L - Proline

Besides above pathways, Hydroxyproline ⟶ L - proline, pathway is also reported to occur in few cases.

(a) Glutamic acid pathway :

The central role of glutamic acid in L-proline biosynthesis has been evident from the early times, when



GLUTAMIC ACID —1→ Y GLUTAMYL PHOSPHATE —2→ GLUTAMIC – Y SEMIALDEHYDE ⟷3 Δ-PYRROLINE 5-CARBOXYLIC ACID ⟷4 L-PROLINE

1 GLUTAMIC ACID KINASE 2 Y-GLUTAMYL PHOSPHATE REDUCTASE 3 SPONTANEOUS CYCLIZATION 4 PYRROLINE-5-CARBOXYLATE REDUCTASE

Fig. 2.

crude nutrition studies were performed and it is still valid in the days of radiotracer technique.

In 1912, Abderhalden extracted Casein hydrolysate in alcohol. This hydrolysate was free of L-proline but rich in glutamic acid when this was fed to dog, normal growth in it, took place. At the same time he found L-proline in the body of dog. The growth of dog was arrested due to the lack of L-proline, and it was made free by L-proline synthesized from the glumatic acid. This was the most possible suggestion made by Abderhalden.(1912)

Further Dakin 1913, pointed out that glutamic acid, L-proline and ornithine were all glucogenic in diabetic animals and he proposed that, these three amino acids might be metabolically related to one another. Later on in 1947-48, an indirect evidence was put forth by Rose and co-workers, regarding conversion of glutamic acid into L-proline and ornithine. In their experiment, they used radioactive glutamic acid and it was fed to rats. They found radioactivity in L-proline and ornithine. Further several other evidences were forwarded by many workers in support of the hypothesis that glutamic acid is a precursor of L-proline in bacteria and fungi (Bonner 1946, Ehrensvard and Reo 1949, Wiame 1951). Mainly the details regarding proline biosynthesis came from study with animals and lower organisms like bacteria and fungi.

Morris et al (1969) for the first time demonstrated L-proline biosynthesis from glutamate in higher plants. They found that in Swiss chard leaves L-proline biosynthetic path begins with glutamic acid and proceeds via Δ^1 - Pyrroline - 5 - Carboxylic acid in the manner described in Fig.1. Glutamic acid is converted into Y-glutamyl phosphate by taking phosphate bond from ATP. This reaction is catalysed by glutamic acid kinase with Mg^{++} as cofactor and imidazole. Such reaction was shown by Baich (1969) in E. coli. This enzyme has been isolated from crude extracts of Pseudomonas aeruginosa by Migula (Krishna and Loisinger et al. 1979). Smith and coworkers (1984) isolated and purified it from E. coli. In case of over production of free proline, glutamic acid kinase plays key role in case of E. coli, (Smith et al. and Thombras, 1986)

Y-glutamyl phosphate is further converted to y-glutamic semialdehyde by an enzyme y-glutamyl phosphate reductase. In this reaction NADPH or NADH supplies reducing power to substrate. Through this reaction Pi bond is given out. The enzyme y-glutamyl phosphate reductase was reported by Baich (1971) from E. coli. The cofactor NADPH or NADH requirement was investigated by Moses (1974).

Once the y-glutamic semialdehyde is produced, it can be converted to pyrroline-5-carboxylic acid through spontaneous cyclization.

The pyrroline-5-carboxylic acid is further converted to proline by an enzyme pyrroline-5-carboxylic acid reductase (Thompson and Morris et al., 1966). This enzyme requires NADPH or NADH as cofactor (Noguchi et al., 1966), and it works very well in pH range 7 to 7.6. This enzyme has been isolated and purified by Splittstoesser and Splittstoesser (1973) from Cucurbita maxima and C. moschata cotyledons, and Miler and Stewart (1976) from soybean leaves. This enzyme shows 4-5 fold greater activity in presence of NADH than NADPH in pH range 7 to 7.6. The activity of this enzyme was found to be inhibited by NH_2OH, NADP, ATP, GTP, phosphate and to lesser extent by proline. This suggests that, the activity of this enzyme may be regulated by end product inhibition.

In case of this pathway, Kueh et al. (1985) stated that, glutamate pathway is synthetic in case of proline biosynthesis and ornithine pathway is catabolic.

(b) Arginine Pathway :

Wrench et al. (1977) have demonstrated that, in osmotically stressed Artichoke tuber slices, arginine is quantitatively the more important precursor for proline synthesis than glutamate. Arginine is converted to ornithine by hydrolysis catalysed by orginase producing urea as the other product. It was first noted in mixed putrefactive organisms by Ellinger (1899). In 1940, Hills reported that

Streptococcus (gram +ve cocci) metabolized arginine to 2 NH_3 + CO_2 + ornithine. It was supported by Roloff and Rather (1940). An enzyme orginase is known to be present in cells of several plants (Bryan 1976).

(C) Orhithine Pathway :

The ornithine thus liberated, can also be converted to proline in plant tissue (Mazelis and Fowden 1969), Splittstoesser 1969, Stewart 1974). According to Muth and Castilow (1974) the conversion of ornithine to proline by ornithine cyclase involves the deamination of α-amino group prior to cyclization. This ornithine ⟶ Proline, conversion takes place by two possible routes. The difference between two routes is in group which is transaminated. If α-amino group of ornithine is transaminated, α-keto-δ-amino-valeric acid is produced. On the other hand, if δ-amino group of ornithine is transaminated, glutamic-y-semialdehyde is produced. This is represented in Fig.3.

The involvement of α-keto-δ-aminovaleric acid as a possible intermediate in this pathway was suggested by Krebs (1939).

But enzymes catalysing steps (4) and (6) in Fig.3 have not been isolated from plant tissues. But some early work indicated that they might be present in plants (Meister et al., 1957). The α-keto-δ-aminovaleric acid is cyclized to Δ'-pyrroline-2-carboxylic acid (step 5), which is then

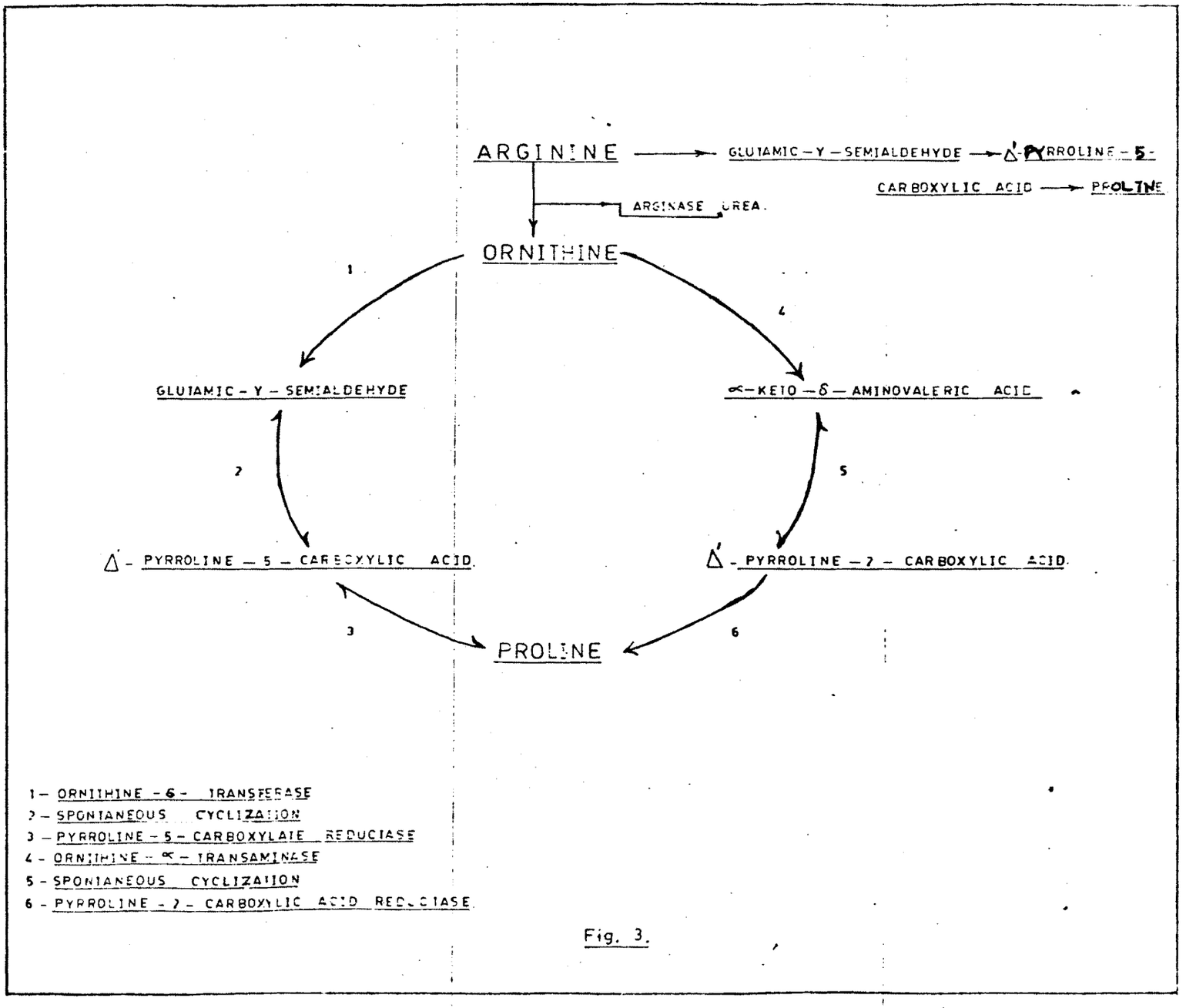

ARGININE
GLUTAMIC – Y – SEMIALDEHYDE
Δ' – PYRROLINE – 5 – CARBOXYLIC ACID
PROLINE
ARGINASE
UREA.
ORNITHINE
1
2
3
4
5
6
GLUTAMIC – Y – SEMIALDEHYDE
α – KETO – δ – AMINOVALERIC ACID
Δ' – PYRROLINE – 5 – CARBOXYLIC ACID.
Δ' – PYRROLINE – 2 – CARBOXYLIC ACID.
PROLINE
1 – ORNITHINE – δ – TRANSFERASE
2 – SPONTANEOUS CYCLIZATION
3 – PYRROLINE – 5 – CARBOXYLATE REDUCIASE
4 – ORNITHINE – α – TRANSAMINASE
5 – SPONTANEOUS CYCLIZATION
6 – PYRROLINE – 2 – CARBOXYLIC ACID REDUCIASE

Fig. 3.

reduced to proline (step 6) by an enzyme pyrroline-2-carboxylic acid reductase in presence of NADH or NADPH as an electron donor. This enzyme requires pH 7 for its optimum activity. Recently this pathway is demonstrated by Mestichelli et al. (1979) in plants.

Ornithine ⟶ Glutamic-y-semialdehyde ⟶
⟶ Δ' - Pyrroline-5-carboxylic acid ⟶ Proline.

In this route, δ-amino group of ornithine is transaminated. First ornithine is converted to glutamic-y-semialdehyde by an enzyme ornithine-δ-transaminase. This enzyme requires optimum pH and NADPH or NADH as an electron donor. Mazelis and Fowden (1969) have extracted and purified this enzyme from Peanut cotyledons. This enzyme requires α-ketoglutarate and prefers NADPH than NADH. This enzyme has been characterised from various plants by Lu and Mazelis (1975). Thus produced glutamic-y-semialdehyde spontaneously cyclizes to Δ' - Pyrroline-5-carboxylic acid. These two intermediates remain in equilibrium. The Δ'-Pyrroline-5-carboxylic acid is further converted to proline by an enzyme Δ'-Pyrroline-5-carboxylic acid reductase in presence of NADH or NADPH.

Of the above two proline biosynthetic routes from ornithine, second route is probably more dominant and this is supported by many workers with experimental proofs. Also recent radiotracer experiments go in favour of ornithine glutamic-y-semialdehyde ⟶ Δ' Pyrroline-5-carboxylic acid

Proline, pathway. The importance of ornithine α-keto-δ-aminovaleric acid → Δ'-Pyrroline-2-Carboxylic acid ⟶ Proline pathway is unknown but it may have importance in stress induced proline overproduction i.e. accumulation. According to Kueh et al. (1985) ornithine to proline pathway is catabolic.

(C) Hydroxyproline pathway :

The synthesis of proline from hydroxyproline has been demonstrated by Verner in carrot root slices. He stated that, the first intermediate in this pathway is 4,5-dihydroxy-L-proline. But this pathway is of limited significance since hydroxyproline is normally incorporated in protein (mainly of cell-wall).

The glutamate to proline and Orginine ⟶ ornithine ⟶ Proline pathways are of major significance so far as proline biosynthesis is concerned. At the same time it must be mentioned here that the glutamate → Proline pathway needs expenditure of one ATP in the first step of the process. It has been suggested by Kueh et al. (1984) that glutamate pathway is synthetic and the ornithine pathway is catabolic.

(D) Site of proline biosynthesis in plant cell :

According to Noguchi et al. (1966-68) stimulatory effect of light on proline biosynthesis from glutamate is due to presence of Δ'-Pyrroline-5-carboxylate reductase

enzyme in extract of washed chloroplasts. This observation suggests that there may be proline biosynthesis in chloroplasts. In addition, Noguchi et al. (1966) found the above reductase in the supernatant of chloroplast preparations. It indicates that there also may be a cytoplasmic enzyme. If NADPH and ATP are supplied, there is pronounced proline biosynthesis from glutamate in presence of light. Stewart and Lai (1974) found Δ^1-P5C reductase in the supernatant of mitochondrial preparations from etiolated pea seedlings. These findings suggest that, proline may be synthesized in chloroplasts or cytoplasm or both. Boggess et al. (1976 a) showed that, in turgid leaves of barley, glutamic acid giving rise to proline is separated from the glutamic acid derived from proline-oxidation. For their experiments, they used ^{14}C-glutamate and ^{14}C-proline. From above experiments, it appears that proline biosynthetic sites may be chloroplasts or Cytoplasm or both but not mitochondria where its oxidation occurs.

(E) Regulation of proline biosynthesis :

The proline biosynthesis is influenced by several factors like end products, proline analogues and light. End product inhibition - According to Vallee et al. (1973) pyrroline-5-carboxylate reductase can be inhibited by end product i.e. proline. Baich and Pierson (1965) showed that, first step in proline biosynthesis was sensitive to proline itself. Oaks et al. (1970) found that, proline inhibited the

incorporation of ^{14}C-acetate into proline in corn roots. In 1968 Noguchi et al., demonstrated that proline inhibited the incorporation of ^{14}C-glutamate into proline in illuminated tobacco leaves. Again in 1976, Boggess et al. found that, the incorporation of ^{14}C-glutamate into proline was inhibited by proline in turgid barley and tobacco leaves. The conversion of ^{14}C-glutamate into proline was also decreased by feeding unlabelled proline to the diatom Cyclotella cryptica (Liu and Hellebust 1976). Kueh et al. (1984), observed that, proline inhibited the biosynthesis of proline, from glutamate as well as ornithine in barley mutants.

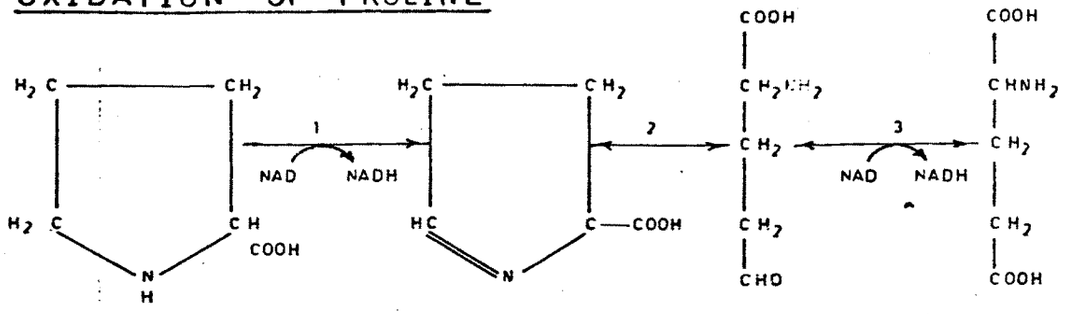
Proline analogues Oaks et al. (1970) have demonstrated that both hydroxyproline and azetidine inhibited the synthesis of proline, from glutamate in maize root tips. In contrast, 1% glucose enhanced incorporation of proline into protein and also reduced its oxidation and exaggerated its accumulation as soluble proline. Thomas and Stewart (1984) observed that, the proline analogue L-Thiozolidine-4-carboxylic acid inhibited the synthesis of proline, from glutamate in maize root tips. In contrast, 1% glucose enhanced incorporation of proline into protein and also reduced its oxidation and exaggerated its accumulation as soluble proline. Thomas and Stewart (1984) also observed that, the proline analogue L-Thiozolidine-4-carboxylic acid inhibited proline synthesis in wilted barley leaves.

(F) Fate of proline :

Since proline is a building block of protein, the major fate of proline is its incorporation into protein. Proline is a precursor of hydroxyproline which is found in a variety of plant proteins and is an important component in cell-wall biosynthesis, synthesis of this amino acid, differs significantly from the synthesis of the other protein amino acids in that proline is hydroxylated after incorporation into peptide linkage (Bryan 1976). This conversion is mediated by a prolyl hydroxylase which has been isolated from carrot phloem parenchyma cells and has been shown to require O_2, ferrous ion, ascorbate and an α-keto acid for activity (Sadava and Chrispeels 1971). This enzyme is located in Cytoplasm.

It is now clearly shown that plants have capability to oxidize proline (Wang 1968, Bernard and Oaks 1970, Rena and Splittstoesser 1974). The oxidation results in carbon being fed into Krebs cycle and eventually respired to CO_2, (Stewart 1972 c). Proline is oxidized to produce glutamic acid and this reaction can be represented in Fig.4. The conversion of ^{14}C-proline to glutamic acid, organic acids and CO_2 was shown by Wang (1968), Bernard and Oaks (1970) Stewart (1972a) and Rena and Splittstoesser (1974). The labelled carbon originating from ^{14}C-proline is also found in other amino acids, that are derived from glutamate i.e. y-aminobutyrate, glutamine and aspartate which would originate

OXIDATION OF PROLINE



PROLINE — Δ- PYRROLINE-5-CARBOXYLIC-ACID. — GLUTAMIC-γ-SEMIALDEHYDE. — GLUTAMIC ACID.

1) PROLINE DEHYDROGENASE OR PROLYL OXIDASE. 2) SPONTANEOUS CONVERTION. 3 GLUTAMIC-γ-SEMIALDEHYDE DEHYDROGENASE.

Fig. 4.

from Krebs cycle intermediates.

Regarding first step in proline oxidation, Mazelis and Fowden (1971) extracted a proline dehydrogenase from peanut seedlings. This enzyme was later found in Chlorella (McNamer and Stewart 1974), pumpkin (Rena and Splittstoesser 1974), wheat germ (Mazelis and Creveling 1974). The product of reaction is Δ'-Pyrroline-5-Carboxylic acid, (McNamer and Stewart 1974, Rena and Splittstoesser 1970) and this enzyme is apparently a cytoplasmic (Rena and Splittstoesser 1974 a).The recent findings that proline supported O_2 uptake in isolated mitochondria from barley, corn, mungbean, soybean and wheat, suggest that proline oxidation is a mitochondrial process (Boggess et al., 1978).

The second step in the proline oxidation pathway is spontaneous and identical to the same reaction in proline biosynthesis. In this step Δ'-Pyrroline-5-Carboxylic acid is spontaneously converted into glutamic-y-semialdehyde.

The last step is oxidative one, in which glutamic-y-semialdehyde is oxidized to glutamic acid. The enzyme glutamic-y-semialdehyde dehydrogenase has been isolated from mitochondrial preparations of pea, corn, castor bean and pumpkin seedlings (Stewart and Lai 1974) and barley (Boggess et al., 1975).

This enzyme requires pyridine nucleotide NAD as an

electron acceptor. The proline oxidase and Δ'-pyrroline-5-carboxylate dehydrogenase have been further characterised from mitochondrial location and electron transport by Elthon and Stewart (1981). Both enzymes are located on inner, mitochondrial membrane and are sensitive to rotenone, antimycin A and azide.

(G) Proline Accumulation :

L-proline has gained prominence in recent years due to its phenomenal accumulation in plants, subjected to various types of stress conditions. These stress conditions include following types of situations :

(a) Water deficit stress conditions, (b) Water logging stress conditions, (c) Salinity stress conditions, (d) Mineral deficiency conditions, (e) High and Low temperature stress conditions, (f) Chemical stress conditions, (g) Pathogenesis - attack of pathogens and pests, (h) Pollution stress conditions.

a) Water deficit stress conditions - An influence of water deficit stress, on free proline accumulation has been examined by several workers in large number of plant species. In majority of plant species, marked accumulation of free proline has been evident under the conditions of water deficit.

The very first report regarding free proline accumulation due to water deficit stress was given by Kemble and MacPherson (1954) in rye-grass. In 1957, Thompson and Morris

confirmed above report by experiment with turnip leaves. Later on in 1966, three laboratories reported the free proline accumulation due to water deficit stress (Barnette and Naylor, 1966; Routley 1966; Stewart et al., 1966; Thompson et al., 1966). They worked with the Cynodon dactylon. barley, bean and Brassica rapa L. plants respectively.

Since 1966, upto this date following plant species are reported from all over the world to accumulate free proline in response to water stress :

Table 1

Plant species	Investigators
Achyranthes aspera	Mohammed and Sen (1987)
Agropyron smithii	Bokhari and Trent (1985)
Ailanthus altissima	Dubroca and Bory (1983)
Alysicarpus vaginalis	Mohamed and Sen (1987)
Allium cepa	" "
Allium sativum	Palfi et al. (1974 c)
Amaranthes hybridus	Mohammed and Sen (1987)
Arachis hypogaea	Shashidar et al., (1981)
Artemisia absinthium	Pourrat and Hubac (1974)
Artemisia herba-alba	-,,-
Artemisia vulgaris	Palfi et al. (1974 c)
Avena sativa	-,,-
Beta vulgaris	Palfi et al. (1974 c)

Boerrhavia articularis	Mohammed and Sen (1987)
Boerrhavia diffusa	-,,-
Brassica napus	Palfi et al. (1974c)
Brassica oleracea	,, ,, (1974a)
Bromus arvensis	Palfi et al. (1974c)
Capsicum annum	Palfi et al. (1974c)
Carex pachystylis	Pourrat and Hubac (1974)
Carex setifolia	,, ,,
Carthamus tinctorius	Reddy and Sastry (1977)
Cassia mimosides	Mohammed and Sen (1987)
Chenopodium alloum	Palfi et al. (1974c)
Cicer arietinum	Singh and Rai (1981)
Citrus limon	Levy and Yoseph (1980)
Cleome viscosa	Mohammed and Sen (1987)
Colocyntheis citrullus	Mohammed, Palfi et al. (1974c)
Convolvulus microphyllus	Mohammed and Sen (1987)
Corchorus species	Roy Choudhury and Choudhuri (1986,87)
Crotalaria medicago	Mohammed and Sen (1987)
Cucumis melo	Palfi et al. (1974c)
Cucumis sativus	Palfi et al. (1974) and Mohammed and Sen (1987)
Cucurbita pepo	Palfi et al. (1974c)
Cyclotella cryptica	Schobert B. (1977)
Dactyloctenium aegypticum	Mohammed and Sen (1987)
Dicoma tomentosa	-,,-

Digera alternifolia	Mohammed and Sen (1987)
Dolichous biflorus	Nigavekar (1988)
Eleusine coracana	Chavan (1980)
Eleusine compressa	Mohammed and Sen (1987)
Eucalyptus melliodora	Clayton-Greene (1983)
Eucalypts spp	Pokhriyal et al. (1986)
Euphorbia granulata	Mohammed and Sen (1987)
Euphorbia hirta	,,
Fagonia cretica	-,,-
Festuca pratensis	Palfi et al. (1974 c)
Glycine max	Chang et al. (1985)
	Fukutoku et al. (1985)
Gossypium species	Chu et al. (1978), George (1978)
	Hubac and Camille (1980),
	Janagoudar et al. (1983-84),
	Stephen (1986)
Helianthus annuus	Wample and Bewley (1975)
Heliotropium marifolium	Mohammed and Sen (1987)
Heliotropium subulatum	,,
Hordeum vulgare	Singh et al. (1972), Stewart
	and Aspinall (1977), Tully and
	Hanson (1978), Stewart and Boggess
	(1978). Chu et al. (1978), Hanson
	and Tulley rs (1979), Stewart
	et al. (1987).
Hyoscyamus niger	Palfi et al. (1974 c)

Indigofera cordifolia	Mohammed and Sen (1987)
Justicia simplex	,, ,,
Lactuca sativa	Palfi et al. (1974)
Lens culinaris	,, ,,
Lolium aristatum	,, ,,
Lolium parenne	,, ,,
Lolium temulentum	Palfi et al. (1974 c)
Lycopersicon esculentum	Palfi et al. (1974 c)
	Singh et al. (1979)
	Aloni et al. (1984)
Matricaria chamamilla	Palfi et al. (1974 c)
Medicago sativa	,,
Papaver somniferum	Palfi et al. (1974 c)
Pheodactylum (alga)	Schobort B. (1978)
Phaseolus vulgaris	Palfi and Juhasz (1970)
	Palfi et al. (1974 c),
	Jager and Mayer (1977),
	Kapuya et al. (1986)
Pisum sativum	Palfi et al. (1974 c), Chu (1974)
Poa pratensis	,, ,, ,,
Raphanus sativus	Chu et al. (1974), Palfi et al.
	(1974 c).
Rubus caesius	Palfi et al. (1974 c)
Rumex scatatus	Palfi et al. (1974 c)
Saccharum species	HO.S.T., et al. (1985)
	Singh ~~et al~~. (1987)
	and Singh

Secale cereal	Palfi et al. (1974 c)
Setaria italica	Laxmi Narsimha Rao et al. (1985)
Sinapis alba	Palfi et al. (1974 c)
Solanum laciniatum	Palfi et al. (1974 c)
Solanum tuberosum	Palfi et al. (1975)
	Bansal and Nagarajan (1984,87)
Sorghum vulgare	Palfi et al. (1974 c)
	Bhaskaran et al. (1986)
Taraxacum officinalis	Palfi et al. (1974 c)
Tephrosia purpurea	Mohammed and Sen (1987)
Tephrosia portulacastrum	" " "
Trifolium rapens	Palfi et al. (1974 c)
Triticum aestivum	Tyankova (1967 b),
	Sinha and Rajagopal (1975),
	Sinha et al. (1977)
	Rajagopal et al. (1977).
	Rao and Nainawatee (1980),
	Pandey (1982), Singh and Singh (1983),
	Monneveux and Mahdi (1986)
Vigna sinensis	Prabha and Bhatri (1980),
	Mukherjee and Choudhuri (1981).
Vicia faba	Chu et al. (1978)
	Venekomp et al. (1987)
Zea mays	Pinter and Palfi (1979)
	Soldatini (1980),
	Caneller and Marta (1981),

Zea mays	Thakur and Rai (1981),
	Pahlich et al. (1984)
	Garcia et al. (1987)

b) Water logging stress conditions -

Besides water deficit, water logging of the soil is also a serious problem in many parts of the world. Singh and Singh (1981) reported that proline accumulated when maize plants were subjected to water logging conditions for 3 weeks. They screened two maize genotypes viz. Ganga 2, and D-747, for proline accumulation aspect. They found that the initial quantity of proline was same (0.1 μ mol/g. fr.wt.of leaves) in both genotypes before flooding. After 3 weeks of water logged conditions, they found more proline in Ganga-2 than D-747. Thus they concluded that Ganga-2 is more resistant to water logged conditions than D-747.

In 1983, Aloni and Rosenshtein performed similar type of experiment with tomato. They observed that this plant accumulates free proline due to flooding i.e. water logging conditions. According to them, proline accumulation is an indicator of sensitivity to dehydration and proline may play a role in the post-stress recovery response.

C) Salinity Stress Conditions :

Millions of hectares of soil, throughout the world are too saline to support normal plant growth and develop-

ment. Soil salinity is due to presence of excess of salts in soil solution. Salinity causes several metabolic disorders in the plants.

Free proline accumulation due to salinity was first reported by Goas et al. (1965) in Aster tripolium. Stewart et al. (1974) also observed this phenomenon in halophytes. Treichel (1975) found proline accumulation due to salinity stress in Salicornia fruticosa, Aster tripolium and Mesembryanthemum nodiflorum. Also he added that, young leaves contained more proline than mature leaves of A. tripolium.

Chu et al. (1976) studied proline accumulation, in barley due to salinity stress. There was increase in proline quantity due to salt-stress. Anthony and Anthony (1978) investigated, proline accumulation in eight species of marshy halophytes due to salinity stress. They found that, plants did not accumulate free proline, until a threshold salinity was reached. Wyn Jones and Storey (1978) investigated some plants of graminae for proline accumulation due to salt-stress. They found that, proline accumulates due to salt-stress and it happens so for osmotic adjustment. It is reported by many workers that besides osmotic effects, salinity also causes specific ion effects which are responsible for physiological disorders. Promotion of proline synthesis by K^+, Mg^{++} and Cl^- was evident in the experiments of Chu et al. (1976). Sheoran and Garg (1979) have studied

the effect of different types of salinities on proline accumulation in different organs of Phaseolus aureus seedlings. Accumulation of free proline was found to be more in the leaves than any organ. The chloride type of salinity resulted in greater proline accumulation in leaves and roots than sulphate and sodium salts and it was more effective than potassium. The authors concluded that, the effect of the salinity on free proline content varies with the type of the plant organ, growth state and kind of salinity used.

Based on work with crop species such as Triticum durum, T. aestivum, T. speltata, T. boeticum, Aegilops squarrosa, Hordeum vulgare, Secale cereal, Avena sativa and Zea mays, Drier (1983) recently tried to suggest a possible relationship between salt tolerance and proline accumulation. According to him there is a certain concentration of NaCl above which proline content of the plants strongly rises (critical point). In salt sensitive plants (wheat) the critical point lies below that of salt tolerant plants (barley). Drier further suggested that, the determination of the critical point by means of measurement of proline concentration may serve as a basis for the analysis of salt tolerance of crop plants. Panchal and co-workers (1980) reported proline accumulation in some tobacco cultivars due to salinity induced stress. They also suggested that, there may be decrease in protein synthesis due to free proline accumulation. Katz ~~et al~~ and Mocke (1980) reported that, in

Lycopersicon esculentum salinity induced more proline accumulation than its wild relative L. peruvianum. Free proline accumulation in plant species due to salt-stress is also reported by many workers from time to time. A list of these investigations is given as follows :

Table 2

Sr. No.	Name of the plant species	Year	Investigator
1.	Arachis hypogaea	1981	Shashidhar et al.
2.	Aster tripolium	1965	Goas, et al
2a.	Aegilops squarrosa	1975	Treichel
2b.	Avena sativa	1983	Drier
3.	Atriplex spongiosa	1979	Storey et al.
4.	Brassica napus	1984	Kumar, D.
		1987	Chadler ~~et al~~. and Thorpe
5.	Cicer arietinum callus	1986	Pandey and Ganapathy
6.	Cyamopsis tetragonoloba	1987	Garg et al.
7.	Cyclotella coyptica (alga)	1976	Liu and Hellebust
8.	Distichlis spicata	1979	Cavalieri and Huang
9.	Festuca rubra	1986	Torello et al.
10.	Glycine max	1987	Moftah and Michel
11.	Hordeum vulgare	1976	Chu et al.
		1983	Drier
12.	Hypochaeris radicata	1981	Siebren
13.	Lycopersicon esculentum	1980	Katz ~~et al~~. and Moshe
14.	,, peruvianum		

Sr. No.	Name of the plant species	Year	Investigator
15.	Mesembryanthemum nodiflorum	1975	Treichel
16.	Nicotiana var	1980	Panchal et al.
17.	Phaseolus aureus	1979	Sheoran and Garg.
		1985	Hug Imomul et al.
18.	Phaseolus mungo	1983	Kathiresan et al.
19.	Pisum sativum	1984	Klimashevskii
		1977	Poljakoff and Mayber
	Pina callus	1987	Newton et al.
20.	Pennisetum typhoides	1985	Reddy et al.
21.	Poa pratensis	1986	Torrello
22.	Puccinellia distans	1986	Torrello et al.
23.	Salicornia fruticosa	1975	Treichel
24.	Sesbania aculeata	1984	Karadage and Chavan
	Secale cereal	1983	Drier
25.	Tamarix tetragyna	1977	Poljakoff et al.
26.	Triticum durum	1983	Drier
27.	T. aestivum	,,	,,
28.	T. boeticum	,,	,,
29.	Vigna sinensis	1985	Imamul Hug and Larher
30.	Zeo mays	1983	Drier

Soil acidity : The acidic soils are known to create problems of aluminium toxicity in many agricultural crop plants. Klimashevskii (1984) reported proline accumulation due to soil acidity in pea, soybean, wheat, barley, maize and polygonum.

d) Mineral deficiency stress conditions :

Various mineral elements play a vital role in plant metabolism. The fact that mineral deficiency causes free proline accumulation in plants was noticed by Schobert (1977). In 1979, Geering and Thien observed, the free proline accumulation in the roots and shoots of maize seedlings subjected to deficiencies of nitrogen, phosphorus and potassium. In case of Zea mays, K^{++} deficiency was found to stimulate proline accumulation (Mukherjee and Ilabanta, 1980). Ebeid et al. (1984) investigated changes in proline and protein contents in seedlings of tree species Prosopis juliflora. They observed that, proline content increased in contrast to that of protein under K^{++} deficiency condition. Cincerova and Necasova (1979) observed that when exogenous L-proline was applied to germinating wheat seedlings, the exogenous application of proline reversed the manifestation of Ca^{++} deficiency symptoms in 10 days old seedlings. Growth was enhanced and pipecolic acid did not accumulate.

It has been noticed that, not only macroelements influence proline accumulation but microelements also exert influence on this process. Ghildiyal et al. (1987) studied the effect of Zn^{++} deficiency in Linseed vars. They reported that, this deficiency causes marked accumulation of free proline and decrease in protein nitrogen content.

e) 1) High temperature Stress conditions -

Free proline accumulation due to heat stress, has been reported in Avena sativa leaves and roots (Ozturk and Szawiawski 1981). Kathiresan et al. (1987) reported such phenomenon in blackgram seedlings. Heat stress induced proline accumulation is much more in roots than shoots (Ozturk and Szawiawski, 1981, Kathiresan et al., 1987). An accelerated incorporation of hydroxyproline into cell wall proteins has been observed in lessions induced by heat on hypocotyl sections of Phaseolus vulgare (Kliss et al.,1983).

According to Hong-Qi et al. (1984), if the pollen grains of Lilium longiflorum are exogenously supplied with proline, they show more resistance to heat. This is due to protection offered by proline in metabolic function and germination to pollen grains, from heat damage. The high proline concentration found in pollen of many species may confer resistance to germinating pollen grains at unfavourable temperatures thereby enhancing the chances of successful fertilization. Kuo et al. (1986) worked out effect of high temperature on proline content in tomato floral buds and leaves. High temperature reduced proline content in anthers regardless of the stages of floral bud development. However, high temperature increased the proline level in the leaves. The addition of proline to germination medium enhanced pollen germination rate and increased pollen resistance to heat.

These results suggest that, the low proline accumulation in anthers and pollen at high temperature may be the result of high accumulation in the leaves. Also high proline content in anthers may be necessary to confer heat resistance to pollen germination at high temperature.

Ozturk et al. (1987) studied responses of different plants to heat stress. For example, Helianthus annuus, Atriplex hortensis, (Plants of cool climate) and Cicer arietinum, Amaranthus retroflexus (Plants of warm climate) were subjected to high and low temperature stress. Especially their roots were exposed to 10, 18 and 28^{o}C temperature. At high root temperature, proline content of both roots and shoots tended to increase in cool climate plants and vice versa, to decrease in the case of warm climate species. On the other hand, when plant roots were subjected to temperature stress shifting from high to low or low to high and after the shifting they were kept at these temperatures for a short period such as 90 minutes, the content of free proline increased to both shoots and roots irrespective of temperature shift and ecophysiological difference of plants. In general, therefore, it seems that, the plants tend to accumulate free proline when they are subjected to temperature stress, at short time.

2) Low temperature stress conditions -

Some plants produce excess amount of proline if exposed to low temperature stress. This proline serves as biochemical to the plants for exhibiting resistance to the low temperature stress and this can be made clear by following observations.

Free proline accumulates in plants at cold temperature (LeSaint 1958; 1966; Heber 1958; LeSaint-Quervel 1966; Markowski et al., 1962; Ostoplyak 1967; Protsenko and Rubanyuk 1967; Zinganigirov 1968). It also decreases after removal of low temperature, or it increases further in hardier plants. Korimov et al. (1976) found increase in free proline in barley during winter season. They suggested that proline plays protective role against the low temperature of winter. Also they observed that, this proline disappeared in following March and April months. Suleimenova et al. (1978) found more and more free proline in cold-resistant vars of corn than control.

Yelenosky (1978) reported that due to cold hardening temperature treatment to Valencia orange tree, proline along with glutamic acid and valine were increased. LeSaint et al. (1978) also showed similar increase in free proline in savoy cabbage due to cold hardening temperature treatment. Chu et al. (1978) observed that, in case of Hordeum distichum

and Triticum aestivum cold hardening temperature treatment induces proline accumulation but it occurred in presence of light.

Kaldy and Fryman et al. (1984) in case of Triticum aestivum (winter wheat var) observed that proline and glutamic acid increased due to cold hardening temperature treatment.

Laliberty and Roger et al. (1985) studied effect of low temperature in Triticum aestivum. They observed that free proline increased in the leaves and stem of winter wheat, hardened at 1.0°C ± 0.5°C. Van Swaij et al. (1985) investigated effect of cold hardening and exogenous proline application in Solanum tuberosum regarding frost tolerance. They found, as free-proline amount increases, the frost tolerance also increases i.e. there is positive correlation between free proline accumulation and frost tolerance. An exogenous proline application to the young leaves also increases frost tolerance. Kushad and Yelenosky et al. (1987) reported that in case of citrus plants, free proline accumulation due to low temperature treatment was 3 to 6 fold higher in acclimated plants than non-acclimated. In 1987, Duncan et al. reported free proline accumulation in maize callus tissue. They also suggested that free proline accumulation increases the cold tolerance of maize callus.

f) Chemical Stress Conditions -

Free proline also accumulates due to treatments of chemicals applied to plants through soil, sprays, seed treatment and pollen treatment. These chemicals include salts like $CaCl_2$, NaCl, KCl and various plant growth regulators like abscisic acid, B_9 etc.

Shashidar et al. (1981) treated groundnut seeds with $CaCl_2$ and found increased proline quantity over control incase of groundnut seedlings. In case of sunflower (Kathiresan 1983) and black gram seedlings (Kathiresan et al.,1985), it was noticed that such overproduction of proline takes place due to $CaCl_2$ treatment to seeds. Proline accumulation has been found to be enhanced by exogenous application of KCl to black gram seedlings (Katheresan et al., 1985) and a positive correlation between K^+ content and proline accumulation has been noted by (Meenakshi, 1980; Shashidar et al., 1981; Katheresan et al., 1985). Chu et al. (1976) found that in barley Mg^{++} and Ca^{++} tended to promote proline accumulation.

ABA is one of the major naturally occurring growth inhibitors in plants and there are several reports which indicates that this compound induces accumulation of proline in response to stress conditions and causes growth inhibition. For the first time such phenomenon was reported by Rajagopal and Anderson (1978) in stressed leaves. Stewart et al. (1986)

also observed that exogenous ABA application induces proline accumulation in Hordeum vulgare leaves.

B_9 is one of the well known synthetic growth retardent, Siddique and Krishnamurthy (1987) noticed that, the presowing soaking treatment of gram seeds with B_9 causes accumulation of proline in cotyledons. The presowing soaking treatment with another growth retardent chlorocholine chloride (CCC) is also reported to cause accumulation of proline (Tretyakov and Gomer 1985). Beffegna et al. (1986) applied thioproline to the leaf sections of Hordeum vulgare and found that, there was a marked increase in proline quantity, with some decrease in glutamate quantity.

g) Pathogenesis - attack of pathogens and pests -

The pathogens (Viruses, bacteria, fungi) and insects and pests, many times become major limiting factor for crop production and silviculture in the world. Due to the attack of pathogens, insects and pests, on the plants, the free proline level increases in them. This free proline accumulation due to above causes is shown by many workers from time to time. This may be due to the breakdown of proteins and de novo synthesis of proline. Again proline utilization may also be stopped due to the abnormal conditions created by pathogens.

In case of tobacco stem the galls produced by infection

of Agrobacterium tumefaciens induced proline accumulation. This was reported by Seitz and Hochster in 1964. In tomato root and stem galls, free proline level was increased but, it was quite normal in uninfected roots and stem (Breyhan et al., 1974; Menon et al., 1978).

Meloidogyne is a nematode causing severe root-knot disease in many members of family solanaceae. In 1975, Levis et al. reported 2000 fold increase in free proline level due to the attack of this nematode. Mohanty et al. (1983) reported that Tungrovirus infection stimulates proline accumulation in leaves of rice (Oryza sativa L.) especially in sensitive cultivar Taichung native-1. They also reported that, the proline accumulation increases with the severity of the disease. Again in 1984, Mohanty et al. reported that, in Taichung native-1, Latisal and Ponkhari, free proline accumulates more due to the attack of leaf-hopper a vector for Tungro virus. Murray et al. (1987) reported proline overproduction and accumulation due to the powdery Mildew disease in barley seedlings.

In all above cases, free proline may serve in proliferation of damaged tissue for healing the wounds. It can be supported by the fact that meristematic cells contain high levels of free proline (Breyhan et al., 1954, Durzan and Steward 1963). Here they stated that proline accumulation may not help in adaptation on the part of plants, to the unhealthy conditions.

h) Pollution stress conditions -

Now-a-days most of the crop plants, fruit trees and vegetable crops face the problem of heavy air and water pollution stress conditions. These plants are directly or indirectly subjected to hazards of pollution conditions. The major air pollutants are SO_2, CO and O_3 in the industrial zone. Industrial wastes released in rivers, sewage city drainage, etc. cause tremendous water and air pollution. The uncontrolled and undirected industrial growth is catching more and more cultivable land. Air pollution reduces quality, quantity and taste of crops, fruits etc, and induces morphological and physiological modifications in them.

Among the various physiological modifications, some plants produce excess amount amino acids like proline to withstand air, water pollution stress conditions.

For the first time Jager and Pahlich (1972) reported proline accumulation due to SO_2 exposure in Pisum sativum. They exposed seedlings of above plant to 1.3 ppm SO_2 for 12 hrs. They observed the increased activity of Glutamic acid dehydrogenase in the direction of reductive amination. This led to excess proline synthesis and accumulation. Godzik and Linskens (1974) examined free proline accumulation in primary bean leaves after continuous and interrupted SO_2 fumigation. In their experiment they noticed that free

proline content increased as the duration of fumigation was increased from 6-24 h/day.

Similar results were obtained by Karolewski in Poland (1985) regarding proline accumulation in Weigela florida by SO_2 fumigation. He also added that bound proline and hydroxyproline decreased as free proline amount increased. Again in 1986, Karolewski, observed free proline accumulation in the leaves of Populus robusta.

Mumford et al. (1972) observed accumulation of amino acids like proline, glutamine and α-alanine on exposure to 3 ppm O_3 for 5.5 h/day for 60 days, in case of Zea mays L. var Harvard Hybrid-dwarf pollen grains. They further indicated that, O_3 promoted both the autolysis of structural glycoproteins and amino acids synthesis.

Besides air pollution, proline also accumulates in plants in response to water pollution. Soldatini et al.(1978) observed that the addition of sulfite to a medium supporting growth of Chlorella vulgaris resulted in increase in accumulation of free proline. Murumkar and Chavan (1985) noticed that, sugar factory effluents caused accumulation of free proline in chickpea seedlings.

H) Functions of Proline :

Several positive roles for proline accumulation in stress conditions have been suggested with greater or lesser conviction. All these roles are correlated with stress resistance of plant species, through different mechanisms. These mechanisms are briefly outlined as follows :

a) Osmoregulation : In case of higher plants accumulation of organic compounds due to stress has been linked primarily to maintain turgor pressure of cells (Kauss, 1977). Among these organic compounds, L-proline is prominent. Free proline has been involved in osmoregulation (Osmond, 1976). The idea of proline as an important cytoplasmic osmoticum is favoured strongly by the works of (Voetberg and Stewart 1983). They observed a positive correlation between Na^+ and K^+ concentration and proline in salt stressed barley leaves. This view is strongly supported by the fact that most of the halophytes contain large amount of proline Stewart and Lee 1974, Triechel 1975, Flowers et al., 1977 . In case of bacteria, Measures (1975) reported that, proline plays a role of osmotic adjustment in halophilic and non-halophilic types. Such phenomenon was also observed in marine algae Liu and Hellebust 1976 . In 1984, Handa et al. cleared that, proline helps in osmotic adjustment in culture plant cells adapted to water stress in Lycopersicon esculentum . At the same time, one cannot overlook the suggestion of

Schobert (1977) that, the accumulation of proline and possibly glycine-betaine in plant tissue should not be seen as a cytoplasmic osmoregulatory process correlated with retention of water by osmotic forces but as a non-osmotically lined protective phenomenon, maintaining hydration of macromolecules possibly by direct proline-protein association.

b) Enzyme stabilization : It is considered that this proline in solution, affect the solubility of various proteins and to protect bovine albumin from denaturation by $(NH_4)_2SO_4$ or ethanol (Schobert and Tschesche 1978). Here it is suggested that, proline interacts with hydrophobic surface of residues on the proteins and which increases the total hydrophilic area of the associated molecules and hence their stability. If such interaction occurs in cytoplasm, it will be significant in water deficit situation and will support Schobert's views on the importance of interactions between biopolymers and proline in stress responses.

Free proline at very high concentration does not inhibit growth and germination in plants as glutamine and aspargine does (Palfi et al.,1974). It suggests that, free proline does not interfere with plant metabolism at high concentration level during stress. This was confirmed by the in vitro experiment, that activity of several enzymes were unaffected in Triglochin maritima a proline accumulating species by 700 nM concentration of proline (Stewart and Lee

1974). These observations are supporting the view that, free proline accumulation during stress period has definite evolutionary advantage, in that it helps the cell in resistance. Yancey and Somero (1979), presented evidence that proline and other similar solutes can protect enzymes against the deleterious effects of biologically toxic compounds like urea. Since the enzymes play a key role in plant metabolism, the enzyme protector role of proline appears to be of great importance, especially during stress conditions.

c) Cellular metabolism : Bengston et al. (1978) stated that, accumulated proline serves as substrate for the synthesis of chlorophyll after stress relief. As we have already seen, proline is a precursor of hydroxy-proline which is compohent of proteins called extensions. The extensions are nothing but hydroxyproline rich glycoproteins most essential for cell-wall fabrication, in growth and plant morphogenesis Doshek and Erickson, 1981 . Stewart et al. (1977) observed that ^{14}C-proline can be easily converted to aminobutyric acid, alanine, aspartic acid, glutamine and glutamic acid in turgid tissues of Hordeum vulgare. Among all the amino acids, proline is most stable one, and less toxic to the plant. It is also more resistant for acid hydrolysis in water stressed plants (Palfi et al., 1974). Hence it is said to play a role of detoxification by the conversion of toxic amino acids into proline (Palfi and Juhaz 1970). Proline can also act as a

storage form for the otherwise injurious NH_3 released by proteolysis (Blum and Adelina 1976). In case of salt (NaCl) and water stressed halophytes, the conversion of ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene is promoted, but if proline is exogenously applied to the stressed tissue, the process of conversion is completely inhibited (Chrominski et al., 1989).

d) Conservation of energy, carbon and nitrogen : L-proline is translocated freely within the plant body through phloem during the water-stress and accumulates to the highest concentration in younger leaves and shoots (Singh et al., 1973). The concentration falls rapidly once the stress is relieved (Jager and Meyer 1977), and proline would appear to be a readily utilizable source of energy and amino groups. Proline is oxidized rapidly to glutamate in turgid leaves (Stewart et al., 1977), but there is no marked increase in glutamate or α-aminobutyrate concentration following stress relief. Blum and Ebercon (1976), stated that, proline is deaminated and its carbon skeleton is entering the Krebs cycle. This is supported by the fact that, $^{14}CO_2$ is rapidly given out following the re-watering of stress plants, previously loaded with ^{14}C-proline. Again in 1974, Dashek and Harwood have suggested that, in case of Lily pollengrains, as pollen tube increases in length, the concentration of proline decreases. Here, they have proposed that, proline serves as energy source to elongating tube. It is intellectually

attractive to consider the free proline accumulation in stress condition has evolved a mean of conserving both energy and nitrogen in a readily available form which has less physical and physiological harmful effects. Accumulated proline can also serve as a respiratory substrate in plant tissues (Britikov and Linskens 1970), and thus play a role of energy source.

e) Sink for soluble nitrogen : In case of water stressed plants, proteins are broken down to amino acids and ammonia. These ammonia ions are toxic to the plants. In such situation, proline acts as sink for soluble nitrogen (Savitskaya 1976). In stress period, protein synthesis is blocked but protein breakdown is not blocked, rather it is enhanced. Again the uptake of nitrogen from soil medium by plant is inhibited by stress and activity of nitrate reductase is also curtailed. All these will result in accumulation of soluble nitrogen containing compounds of low molecular weights. Such substances have an ability to inhibit cell-metabolism. Thus in addition to the roles cited above, evolution of proline accumulation confirred on at least some plants a means of coping with large amounts of soluble nitrogenous compounds which may themselves harm cellular metabolism.

Thus by forming a major component of soluble nitrogen pool, proline helps in protecting the metabolic machinary,

from toxic intermediates of nitrogen metabolism.

f) Growth : There are few reports which indicate that, proline is highly essential for growth process in higher plants. A genetic requirement for proline was demonstrated by Gavazzi et al. (1975) in maize mutant. Preil (1977), observed that, strong positive correlation between the proline content in leaves of 10 days old tomato plants and cumulative fruit yield of 175 and 192 days old plants. Armstrong and Green (1985), observed, involvement of L-proline in establishment and maintenance of friable embryogenic maize callus. They noticed that frequencies of friable-callus initiation and somatic embryoid formation increased linearly with addition to N_6 medium of up 25 mM L-proline.

g) Plant architecture : Melin (1977) observed that, in the twining shoots of Periploca gracea L., there was longitudinal distribution of free proline. It was maximum in the internode No.3 and were different in the concave and convex halves of stem. Recently Rajgopal and Madsen (1981) found that the proline level decreases during rolling of barley leaves and proline may play an important role in this process.

h) Reproduction : Britikov and Musatova (1964) have investigated, soluble proline content of pollen and pistils

of 200 plant species. They divided pollen into six groups by considering amounts of proline in them, from trace level to 1.5 % on dry weight basis. The amount of proline in pollen was greater than pistil. The significance of this observation was not made clear. Tupy (1963) has suggested that a proline to histidine index ratio may be a mean of differentiation between fertile and sterile apple varieties. Brooking (1976) observed that, the made sterility was induced in grain sorghum by exposure of plant to low night temperature. The sterile pollen at anthesis was devoid of starch and had low levels of free proline. Assays of anther proline levels proved to give a reliable index of pollen maturation. Dashek and Mills (1981) established that the proline of the pollen plays an important role in energy transformation and in the interaction of the pollen with the style. These authers also suggested that the proline content of the pollen and its fertility are corr lated (i.e. in Lilium, Petunia, Secale, Hordeum, Zea, Triticum, Oryza, Papaver and Solanum etc.). Palfi and Koves (1984) demonstrated that the vitality and fertility of pollen is proportional to proline content. The above observations suggest that, proline also plays a key role in plant reproductive processes.

i) Water relations : L-proline is highly soluble amino acid in water. Increase in bound water due to highly hygroscopic nature of proline has also been suggested by Palfi et al.

(1974). Since water retention is one of the important pre-requisite for drought tolerance, this property of proline is helpful for plants under stress conditions. Schobert and Tschesche (1978), suggested that, under stress conditions proline protects the proteins from denaturation by preserving the hydration level.

j) Cold tolerance : There are several reports which suggest that, proline contributes to cold hardening process in plants. In case of cold hardened plants, accumulated proline acts as chemical effector for transmission of hardening (LeSaint-Quervel 1960). This conclusion was based on the observation that hardening could be induced in cabbage shoots if they were allowed to absorb proline from solution at a non-hardening temperature. Heber et al. (1973) suggested that, proline acts as a membrane stabilizer. Again in 1979,Withers and King concluded that, proline is an effective cryoprotectant because, the storage of cultured Zea mays cells for 3-days in medium containing proline resulted in an increased freeze tolerance. The cryoprotected cells by proline, further showed reduced postthaw viability loss and greater tolerance to a range of post-thaw culture conditions. Withers and King further added that, proline may protect the cells against solution effects caused by dehydration during freezing.

k) Air pollution tolerance : In 1986, Karolewski suggested

that in case of polar plants, proline increased the tolerance of species to the air pollutant SO_2 gas. The pretreatment of proline increased resistance against injury changes in water content and chlorophyll loss in the leaves of plants exposed to the gas.