INTRODUCTION

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Polyamines

Biogenic amines are low molecular weight, nonprotein nitrogenous bases that are widely distributed in nature. Amines are classified as mono-, di- or polyamines based upon the number of amino groups they contain. The polyamines, spermidine and spermine occur along with the diamines, putrescine and cadaverine. Polyamines are structurally related to putrescine, being derived from it by stepwise addition of propylamine residues to each amino group. The structures of the polyamines and the related diamines are shown below :

Putrescine	$H_2N-(CH_2)_4-NH_2$
Cadaverine	$H_2N-(CH_2)_5-NH_2$
Spermidine	$H_2N-(CH_2)_3-NH-(CH_2)_4-NH_2$
Spermine	$H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$

Though, spermine was discovered as early as 1678, polyamines remained relatively unfamiliar for a long time because the significance of their presence was not known. In recent years, however, there has been a renewed interest in these compounds due to the recognition of their possible role in various growth and developmental processes as well as other regulatory effects.

Several reviews concerning the occurrence, metabolism and functions of polyamines in animals, plants and micro-organisms have been published (Tabor and Tabor, 1964; Cohen, 1971; Smith, 1971; Bachrach, 1973; Jänne et al., 1978; Galston, 1983).

Occurrence in plants

Most of the earlier work involving plants has been centered on putrescine. Putrescine has been detected in several species of higher plants (Hiwatari, 1926; Smith, 1977 a). The polyamines spermidine and spermine also occur in leaves, roots and fruits of higher plants (Smith, 1971) and in especially high concentration in embryos of cereal grains. A number of plant seeds when analyzed for polyamine content showed that in general angiosperm seeds contain larger amounts of polyamines than the gymnosperm seeds (Bagni, 1968).

Cadaverine, a higher homologue of putrescine, was found in tobacco and tomato plants (Hohlt et al., 1970) and in Pisum, Lathyrus and Vicia seeds (Le Rudulier and Goas, 1971; Ramakrishna and Adiga, 1975). 1,3-diamino-propane, an oxidation product of polyamines, has also been detected in a variety of plants including barley (Smith, 1970 b) and cucumber (Flayeh et al., 1984). Some

uncommon polyamines and their derivatives also occur in plants. Homo-arginine and homo-spermidine have been reported in the leaves of Santalum album (Kuttan et al., 1971), Lathyrus (Ramakrishna and Adiga, 1974) and in root nodules of legumes (Smith, 1977 b).

Di- and polyamines have also been reported to occur as conjugates of alkaloids, steroids, glucosides and cinnamic acid in many plants (Smith, 1977 a; Smith et al., 1983). In the leaves of Nicotiana tabacum, p-coumaroyl-, caffeoyl and feruloylputrescine and caffeoylspermidine have been demonstrated on floral induction and these amides accumulate in considerable amounts in reproductive organs (Cabanne et al., 1977). On infection with TMV, these derivatives of polyamines were found to accumulate in hyper-sensitive lesions of Nicotiana tabacum (Cabanne et al., 1977) .

Knowledge about the intracellular distribution of polyamines is scanty. Being positively charged they bind to polyanions and are highly soluble in aqueous media which results into a redistribution of the amines on cell disruption and homogenization. In spite of these difficulties spermidine and spermine have been shown to be present in both particulate and soluble fractions of etiolated pea epicotyls (Bagni and Serafini-Fracassini, 1974).

Polyamine levels under different growth and physiological conditions

The level of polyamines in plants depends upon growth conditions as well as the type of nitrogenous compound supplied. Thus, polyamine concentrations have been reported to change during dormancy break, mitosis, germination, reproduction, growth of cultured cells, embryogenesis, stress, infection, mineral deficiency as well as under the influence of phytohormones and light.

Polyamine levels increased during the sprouting of Helianthus (Torrigiani and Serafini-Fracassini, 1979) and potato tubers (Kaur-Sawhney et al., 1982 b). During the first cell cycle after dormancy break, polyamine content increased during G and S phases and decreased as mitosis proceeded (Serafini-Fracassini et al., 1980). Germination increased the polyamine concentration in Phaseolus (Bagni, 1970), Hordeum (Smith and Best, 1977) and Oryza (Sen et al., 1981) seedlings. Highest polyamine content was found in the rapidly growing parts and lowest in the mature parts of the plant (Palavan and Galston, 1982). A correlation between the onset of mitotic activity and polyamine level was reported in the embryo of Triticum, Helianthus and Glycine max (Anguillesi et al., 1980).

Reproductive organs of the plant showed higher

polyamine levels compared to the vegetative ones (Cabanne et al., 1977) and the levels increased further after pollination (Heimer et al., 1982). Polyamines tend to have their own gradients where cell elongation is the main growth process (Dumortier et al., 1983).

Polyamine levels increased in the rapidly growing Paul's scarlet rose cultures (Smith et al., 1978). Putrescine and spermidine levels were higher in tumours induced by Agrobacterium tumefaciens compared to the normally growing tissues of Nicotiana (Bagni and Serafini-Fracassini, 1979). Polyamine levels also increased when actively growing Daucus carota cells were transferred from nonembryogenic to embryogenic medium (Montague et al., 1978, 1979).

Stress conditions such as salinity (Smith, 1977 a), SO_2 fumigation (Priebe et al., 1978), osmotic stress (Flores and Galston, 1982) and low pH (Young and Galston, 1983) increased putrescine levels. On infection with Cuscuta reflexa polyamine levels decreased in the host plants whereas part of the parasite in contact with the host showed an increase (Naik et al., 1976).

Putrescine was found to accumulate to high levels in plants grown in conditions of potassium deficiency

(Richards and Coleman, 1952; Coleman and Richards, 1956) and when grown in a medium high in NaCl (Stregonov et al., 1960). In Hordeum it increased under magnesium deficiency but decreased under phosphorous, sulfur and nitrogen deficiencies (Smith, 1977 a). The content of putrescine, cadaverine and spermidine of soybean seedlings was increased when they were grown in ammonium rather than in nitrate or urea as the nitrogen source (Le Rudulier and Goas, 1971). Similarly, putrescine level was high in Nicotiana grown in ammonium sulfate. Addition of KCl decreased putrescine level but increased cadaverine level (Hault et al., 1970).

Application of various growth hormones led to an increase in polyamine content. Application of indole acetic acid (IAA) (Cocucci and Bagni, 1968) and 2,4-dichlorophenoxy acetic acid (2,4-D) (Bagni et al., 1981 a) increased polyamine levels. Putrescine and spermidine levels in cucumber seedlings (Suresh et al., 1978) and that of polyamines in rice (Mukhopadhyay et al., 1983) increased on kinetin application. Application of abscisic acid decreased polyamine levels in rice (Mukhopadhyay et al., 1983).

External conditions such as light also change polyamine levels. Their levels increased in the bud and decreased in the subepicotyl segments of pea on exposure to red light and the red light induced changes were reversed by far-red light (Goren et al., 1982 a).

Metabolism

(a) Biosynthesis

Putrescine serves as the precursor of both the polyamines, spermidine and spermine. Putrescine is synthesized from the amino acid arginine. Successive addition of propylamine moiety supplied by methionine to putrescine leads to the formation of spermidine and spermine.

The biosynthesis of polyamines starting from arginine is shown in Fig. I. The pathway has been studied in bacteria (Gale, 1946), animals (Raina, 1963) as well as plants (Smith, 1970 a).

Two distinct pathways of putrescine biosynthesis have been described. The first route (A) is the decarboxylation of ornithine which is produced from arginine by the arginase reaction. The enzyme involved is ornithine decarboxylase (ODC). The other route (B) involves the decarboxylation of arginine to agmatine by the enzyme arginine decarboxylase (ADC). Agmatine is further converted to N-carbamyl putrescine, which in turn, is converted to putrescine. Mammalian tissues utilize the ODC pathway while bacteria and plants utilize one or both the routes.

The biosynthesis of putrescine in plants has been

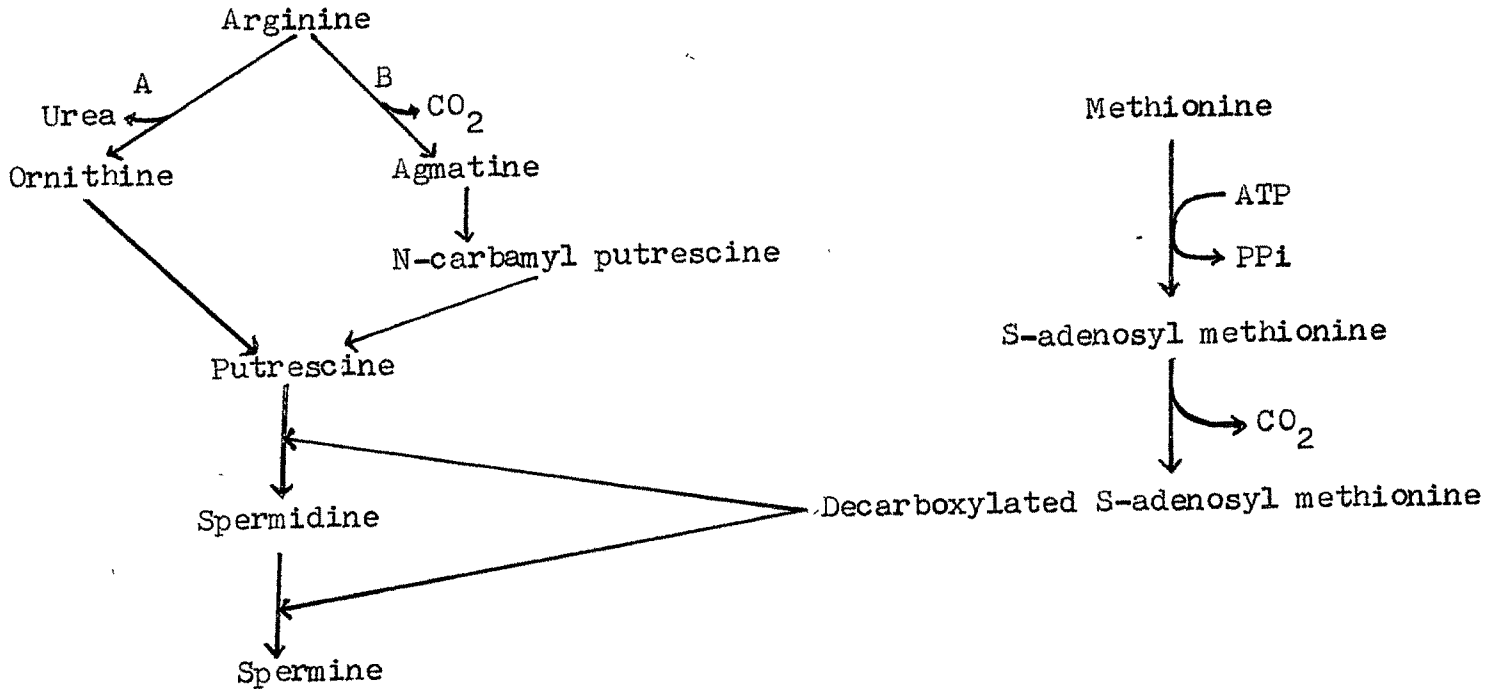


Fig. I : Polyamine biosynthetic pathways

reviewed extensively by Smith (1970 a, 1971, 1972). The major pathway of biosynthesis operating in higher plants appeared to be via ADC, while the enzyme ODC appeared to be of less importance (Smith, 1977 a). This was supported by the relatively high levels of ADC activity found in plant tissues in response to several growth stimuli. Thus, ADC activity was increased in embryonic cells of Daucus carota (Montague et al., 1979). However, other studies indicate that in certain species both ADC and ODC were active and may contribute to polyamine biosynthesis (Berlin, 1981). In tomato plants, fruit development was accompanied by a dramatic increase in ODC activity upto the 3rd day after pollination while ADC activity was very low (Heimer et al., 1979). Recent studies indicate that ADC and ODC activities may be similar with respect to some properties but may differ in other characteristics (Altman et al., 1983 a). Moreover, the inhibition of ODC pathway by ∝-difluoromethylornithine (DFMO) led to an increase in ADC activity, suggesting a compensatory mechanism to maintain the cellular level of putrescine (Altman et al., 1982).

Different ADC to ODC activity ratios were found in various tissues of a given plant and in various plant species (Berlin, 1981; Altman et al., 1982). In addition, various exogenous stimuli, which resulted in growth responses and stress related processes, induced differential

activation of ODC and ADC. Altman et al. (1983 a) have quoted the unpublished work of Friedman et al. that NaCl stress induced a 2-8 fold increase in ODC activity, while ADC activity was either inhibited or remained unaffected. These studies indicate that both ADC and ODC are active in plant tissues and their relative contribution to putrescine and polyamine biosynthesis is dependent upon the type of tissue.

The next two enzymes of the pathway, agmatine iminohydrolase and N-carbamyl putrescine amidohydrolase have been detected in sunflower seedlings and potassium deficient barley leaves (Smith, 1969).

The biosynthesis of spermidine and spermine in plants was studied by Smith (1970 a) who showed that ^{14}C-putrescine was readily incorporated into spermidine. Subsequently changes in polyamine content and their biosynthetic enzymes during cell proliferation were studied in rapidly growing embryonic axes (Ramakrishna and Adiga, 1975).

Spermidine and spermine are derived from putrescine by the stepwise addition of propylamine moiety derived from decarboxylated S-adenosyl methionine which is formed from methionine (Suresh and Adiga, 1977). Addition of propylamine moiety to putrescine for the synthesis of

spermidine and spermine is catalyzed by two separate synthases (Cohen et al., 1981).

In Lathyrus, spermidine may also be formed by an alternative route involving aspartic semialdehyde which spontaneously condenses with putrescine to form a Schiff's base. This is then reduced and decarboxylated to yield spermidine (Srivenugopal and Adiga, 1980). Spermine, however, is not formed by this mechanism.

(b) Degradation

Polyamines are degraded by oxidation which forms the major catabolic pathway. Several plants have been reported to oxidize both di- and polyamines. In most higher plants, especially in legumes, there occurs a diamine oxidase with a broad substrate specificity. Though this enzyme is particularly active with diamines as substrate it can also oxidize polyamines (Hasse and Schuhrer, 1962). Diamine oxidase has been reported to occur in pea (Hill and Mann, 1968) and young Glycine max plants (LeRudulier and Goas, 1977).

A polyamine oxidase has been reported in Gramineae including barley. The enzyme oxidized both spermine and spermidine, though spermine was oxidized faster than

spermidine (Smith, 1970 c). The enzyme in oat leaves was reported to occur in the cell wall and was particularly active in vascular tissue (Kaur-Sawhney et al., 1981).

Besides the major oxidative pathway there are other nonoxidative mechanisms of degradation which include transamination (Kim and Tchen, 1962), acetylation (Rosenthal and Dubin, 1962) and carbamylation (Kleczkowski and Wielgat, 1968).

Functions of polyamines

Most of the functions of polyamines may be attributed to their polycationic nature due to which they may bind and thus neutralize the negative charges of the molecules such as nucleic acids, proteins, neuraminic acid, phospholipids and other cellular anions.

a) Growth and development :

Recent interest in polyamines has been due to the fact that they were shown to be associated with a variety of growth processes in almost all systems studied. They have been shown to serve as growth factors for microorganisms (Herbst and Snell, 1948), mammalian cells (Ham, 1964) and higher plants (Bagni, 1966). In plants they are implicated with various growth regulatory functions and are being

considered as a new class of plant growth regulators (Galston and Kaur-Sawhney, 1982; Altman et al., 1983 b).

The effect of polyamines on plant growth was for the first time demonstrated in the dormant Helianthus tubers. Spermine promoted the growth of tubers in vitro (Bertossi et al., 1965; Bagni, 1966; 1967; Bagni, et al., 1971). Studies have indicated that growth factors for Helianthus tubers derived from coconut milk (Bagni, 1966) or from tomato (Bagni, 1967) contained polyamines. Low levels of polyamines as well as plant hormones present in the dormant tubers did not permit cell proliferation but when polyamines were supplied exogenously the tuber cells were able to divide and grow without any other growth substance (Serafini-Fracassini et al., 1980). The growth stimulation and vascular tissue arrangement was similar to that observed in treatment with IAA (Serafini-Fracassini and Alessandri, 1983). The release of dormancy by plant hormones may be mediated by polyamines and their biosynthetic enzymes (Coccuci and Bagni, 1968). Polyamines were also found to serve as the sole source of nitrogen for Helianthus explants (Bagni, 1979).

Polyamines have also been associated with other processes such as seed formation (Bagni et al., 1967) and germination (Bagni, 1970; Ramakrishna and Adiga, 1975;

Smith and Best, 1977). The content of polyamines during seedling growth may be related to their function as growth factors. Thus, during the growth of Phaseolus seedlings, spermidine and spermine decreased in cotyledons and increased in shoots with simultaneous and parallel changes in RNA and protein content (Bagni, 1970). Similar variations were also observed during later stages of growth (Palavan and Galston, 1982). Polyamine levels increased during the growth of embryonic axis of Lathyrus seedlings (Ramakrishna and Adiga, 1975).

The increase in plant growth was associated with a parallel enhancement of ADC and/or ODC, the two key enzymes of the biosynthetic pathway of polyamines. For example, ADC increased in carrot cell cultures on transfer to an embryogenic medium (Montague et al., 1979), in tobacco cells grown in culture and in developing tomato ovaries after pollination (Heimer et al., 1982) or after auxin spray (Cohen et al., 1982). Polyamine biosynthesizing enzymes increased in pea internodes on application of GA which causes increased internodal growth (Dai et al., 1982).

Polyamine concentration was also found to increase in response to external stimuli which brought about an increase in growth. Thus, application of red light to etiolated pea seedlings caused an increase in the growth of leaf and

terminal buds and a reduction in the rate of elongation. These changes in growth were paralleled by changes in the level of ADC activity. Red light increased ADC activity in light-stimulated tissue while there was inhibition in light-inhibited tissue. Both the effects were reversed by far-red light confirming phytochrome mediation of the light effect (Dai and Galston, 1981). The rise in ADC activity was the cause of bud growth (Goren et al., 1982 b) and not a consequence of the growth caused by red light.

Explant growth in Helianthus was inhibited by analogoues of arginine and ornithine such as canavanine and canaline as well as ∝-difluoromethylarginine (DFMA) and DFMO, respectively (Bagni et al., 1981 b, 1983). Similarly, auxin-induced growth in tomato ovaries was inhibited by DFMO which could be reversed by external application of putrescine (Cohen et al., 1982). Though polyamines could not induce root initiation, the rooting induced by indole butyric acid (IBA) was inhibited by canavanine and canaline indicating their involvement in rooting (Friedman et al., 1982).

Exogenous supply of polyamines to Helianthus explants was found to lead to a faster rate of RNA synthesis (Bagni, 1968) suggesting that the growth promoting effect of polyamines was probably due to their interaction with

nucleic acids, particularly, ribosomal RNA (Stevens, 1970). This is supported by the observation that inhibition of polyamine synthesis due to canavanine during the cell cycle of tubers after dormancy break paralleled the inhibition of RNA synthesis (Bagni et al., 1981 b). It was also found that after dormancy break polyamine biosynthesis begins within 15 minutes, whereas nucleic acid incorporation was detectable only later. The increase in polyamine biosynthesis is a general phenomenon of cycling cells (Serafini-Fracassini et al., 1980; Heby, 1981; Bagni et al., 1982) and there is a temporal correlation between polyamine synthesis and the start of chromosomal replication which suggests that polyamines are involved in the preparation of nuclear DNA synthesis.

b) Stabilization of cells and subcellular organelles :

Polyamines have been found to stabilize bacteriophages (Tabor, 1960), bacterial protoplasts (Bachrach and Cohen, 1961) and oat leaf protoplasts (Altman et al., 1977) from lysis. It was proposed that polyamines bind to the phospholipids of the membrane, thus stabilizing it and preventing the leakage of intracellular components (Harold, 1964). This effect was also observed in animal systems (Bernheim, 1970).

Subcellular particles like ribosomes and mitochondria are also stabilized in the presence of polyamines. Isolated mitochondria which tend to swell in hypotonic media were stabilized by various diamines (Tabor, 1960). Polyamines caused an aggregation of ribosomal subunits and microsomes (Cohen and Lichtenstein, 1960). Because of their stabilizing property polyamines stimulated RNA synthesis by intact liver nuclei (Macgregor and Mahler, 1967). Ribosomes isolated from bacterial and animal cells were found to contain polyamines (Bachrach, 1973).

c) Stabilization of membranes :

Polyamines are known to bind and stabilize cell membranes (Harold, 1964) thereby altering permeability of the membrane and preventing leakage of intracellular components. Freshly isolated oat leaf protoplasts were stabilized against lysis by either 1-10 mM arginine or lysine or by 0.1-1.0 mM polyamines (Altman et al., 1977). Spermine was more effective than spermidine, which in turn was more effective than putrescine or cadaverine. Spermine is also reported to interact with the thylakoid membrane of the chloroplasts resulting in the stabilization of the membrane during barley leaf senescence (Popovic et al., 1979). Naik and Srivastava (1978) and Naik et al. (1980) have shown that the efflux of betacyanin from beet root was

prevented by polyamines due to stabilization of the plasmalemma.

Polyamines have been found to stabilize several membrane bound enzymes like NADH-dehydrogenase (Hochstein and Dalton, 1968). They were found to affect the level of membrane bound ATPase as well as peroxidase (Srivastava and Rajbabu, 1983 a, b).

The stabilizing effect of polyamines on membranes and membrane-bound enzymes appears to be due to their association with the phospholipids (Gary-Bobo, 1970). Some studies, however, also indicate that polyamines may interact with -SH groups of the membrane proteins (Naik and Srivastava, 1981; Srivastava and Smith, 1982 a).

d) Macromolecules :

Polyamines have been reported to stabilize DNA and RNA in a variety of systems, prokaryotic as well as eukaryotic (Ames and Dubin, 1960). Due to their polycationic nature polyamines bind strongly to nucleic acids and stabilize and organize their structure (Zhurkin et al., 1980). Inhibition of nucleic acid synthesis by actinomycin-D was abolished by spermidine, suggesting that polyamines have a binding site on DNA which interfered with actinomycin-D binding (Bagni et al., 1971). Polyamines were found

in t-RNA isolated from pea epicotyls, spinach leaves (Cohen et al., 1969) and Helianthus tuber (Bagni et al., 1981 a), confirming their attachment with t-RNA. Polyamines are also known to be involved in the synthesis of nucleic acids. In oat leaf protoplasts, they promoted both DNA synthesis and inception of mitotic activity (Kaur-Sawhney et al., 1980). Spermine induced RNA synthesis, especially of ribosomal and t-RNA in dormant Helianthus tubers (Bagni et al., 1971). It also stimulated nucleotide incorporation and RNA polymerase activity in maize seedlings (Stout and Mans, 1967) and in soybean hypocotyls (Guilfoyle and Hanson, 1973).

Polyamines accelerate almost every step in protein biosynthesis in vitro. Polyamines have been shown to increase the fidelity of protein synthesis in wheat germ cell-free system (Igarashi et al., 1979) where the addition of spermidine reduced the misincorporation of leucine into proteins. Polyamines have been reported to stimulate the elongation of peptides by stimulating peptidyl transferase activity of ribosomes (Teraoka and Tanaka, 1973). A recent report (Igarashi et al., 1980) however, suggests that polyamines act at the initiation rather than at the elongation level of protein synthesis.

Much of the recent evidence suggests that polyamines

are also involved in several aspects of lipid metabolism such as suppression of lipolysis (Locwood and East, 1974) inhibition of phospholipases (Sechi et al., 1978) and regulation of glycerolipid biosynthesis (Jamdar, 1979). It is possible that the binding of polyamines to phospholipids could account for their binding to cells, protoplasts and membranes.

e) Senescence

Senescence of a plant or tissue is associated with various deteriorative processes such as disintegration of chloroplast structure leading to loss of chlorophyll and photochemical activities, increase in hydrolytic enzymes leading to breakdown of nucleic acids and proteins, increase in free radical formation resulting in membrane disintegration and ethylene production (Thimann, 1980).

Recent studies have shown that polyamines play a role in retarding senescence in both attached (Altman and Bachrach, 1981) and detached leaves (Cohen et al., 1979; Kaur-Sawhney et al., 1982 a; Altman et al., 1982). Further studies have shown that 1,3-diaminopropane, the oxidation product of polyamines, was responsible for delaying the senescence (Shih et al., 1982).

Leaves treated with polyamines were found to retain

chlorophyll effectively during senescence in dark (Kaur-Sawhney and Galston, 1979; Altman and Bachrach, 1981; Srivastava et al., 1983). Calcium was found to reverse the protective effect of polyamines, suggesting that the retardation of senescence by polyamines may be brought about by ionic mechanism (Kaur-Sawhney and Galston, 1979; Altman, 1982 a). In light however, polyamines promoted rather than retarded chlorophyll loss. This contradictory effect of the polyamines may be a result of the removal of magnesium from the chlorophyll which leads to its destruction either by direct photolysis or by an enzyme linked photodecomposition (Kaur-Sawhney and Galston, 1979).

Polyamines also delayed or prevented the rise in protease and RNAase activities along with the chlorophyll loss in dark incubated oat leaves (Kaur-Sawhney and Galston, 1979). Though the exact mechanism by which polyamines inhibit protease activity is not clear, it was postulated that the amines may either bind electrovalently to the enzyme protein, thus preventing the protease from acting on protein (Kaur-Sawhney and Galston, 1979) or by inhibiting the de novo synthesis of protease (Shih et al., 1982). Contrary to these reports, Srivastava et al. (1983) have shown that polyamines did not have any effect on amino nitrogen content which is an index of protease activity. The inhibition of RNAase by polyamines may be due to their

binding to RNA thus making it less available to the enzyme (Galston et al., 1978; Kaur-Sawhney et al., 1978; Altman, 1982 b).

Membrane deteriorating processes such as free radical induced lipid peroxidation are known to increase during senescence, while the activity of superoxide dismutase decreases (Dhindsa et al., 1981). Recent studies from our laboratory have shown that polyamines decreased lipid peroxidation and maintained the superoxide dismutase level (Srivastava et al., unpublished observations).

Most of the senescence retarding effects of polyamines observed oppose those brought about by ethylene, a hormone associated with senescence. Further, applied polyamines inhibited the production of ethylene (Apelbaum et al.,1981). Since ethylene and polyamines arise from a common precursor, S-adenosyl methionine, it has been suggested that the two pathways may be competitive and polyamines may retard senescence by inhibiting ethylene production (Fuhrer et al., 1982). In addition, polyamines inhibited 1-amino cyclopropane-1-carboxylic acid (ACC) synthase, an enzyme involved in ethylene biosynthesis (Yu et al., 1979) as well as the ACC converting system which is associated with membrane (Mattoo et al., 1981) thus inhibiting ethylene

biosynthesis. Polyamines were also found to retard senescence caused by exogeneous ethylene (Galston, 1983).

In addition to exogenous application of polyamines, their endogenous content was also studied in senescing tissues. Polyamine content was found to decline during senescence and this decline was proposed to be responsible for the senescence of the tissue (Altman and Bachrach, 1981). This view is supported by the observation that ADC activity and polyamine content declined progressively when excised leaves were kept in dark. Transfer of the leaves to light retarded senescence as seen by chlorophyll retention and increased ADC activity and polyamine content (Kaur-Sawhney et al., 1982 b). Contrary to these observations, Birecka et al. (1984) have shown that plants having low polyamine concentration have a weak senescence syndrome and the ones with high concentrations have a strong senescence syndrome.

f) Stress

Plants respond to various stress conditions by developing protective measures against them. Under stress conditions there is an activation of putrescine biosynthesis and a massive increase in diamine concentration. This increase occurs in case of both long term response such as in mineral deficiency or a short term response such as in osmotic stress and low pH. Di- and polyamines appear to

protect plants under stress conditions. Richards and Coleman (1952) found that potassium deficient barley plants accumulated high quantities of putrescine paralleled by an increase in ADC activity (Flores et al., 1984). Similarly, ammonium nutrition, the uptake and assimilation of which leads to H^+ production, causes an increase in ADC activity and putrescine content in pea, corn and wheat (Le Rudulier and Goas, 1979). SO_2 fumigation of plants leads to the formation of HSO_3^-, SO_3^- and SO_4^- ions (Priebe et al., 1978). In all these cases the increase in amine accumulation observed would function as a homeostatic mechanism to keep intracellular pH constant.

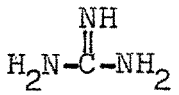
Certain stress conditions like K^+ or Mg^{2+} deficiency or pathogen infection led to an increase in free as well as bound polyamines such as mono- and dicaffeoyl-spermidine (Deletang, 1974; Martin-Tanguy et al., 1973).

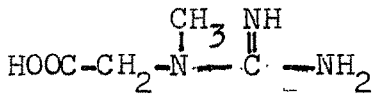
The increase in polyamine content may provide protection to the plant against these conditions since externally applied polyamines could protect the plants in some of these conditions (Hasnain et al., 1980). The protection provided by polyamines may be by protecting or reversing the membrane disruption caused by these conditions which may be brought about by their interaction with membrane lipids (Gary-Bobo, 1970) or with -SH groups of the membrane proteins (Naik and Srivastava, 1981).

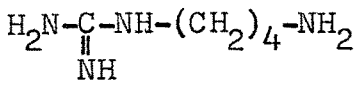
Guanidines

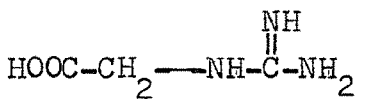
Guanidines are nitrogenous compounds of widespread occurrence. They have been detected in fungi (Boldt et al., 1971), animals (Pisano et al., 1963) as well as in plants (Reinbothe and Mothes, 1962).

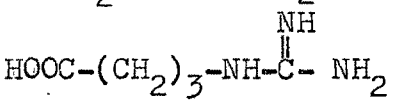
Guanidine was first synthesized from guanine in 1861 by Strecker. It was subsequently isolated from Vicia sativa seeds by Schulze (1892). The isolation of guanidine was followed by the discovery of other guanidino compounds in plants (cf. Guggenheim, 1958). The structure of some naturally occurring and synthetic guanidines is given below :

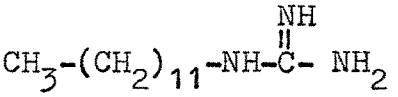
Guanidine 

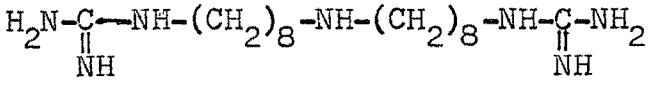
Creatine 

Agmatine 

Guanidinoacetic acid 

Guanidino butyric acid 

Dodine 

Guazatine 

All these compounds except dodine and guazatine have been found to occur naturally. Dodine and guazatine are synthetic guanidino fungicides (cf Srivastava and Smith, 1982 a,b).

Mono- and di-substituted guanidines have been detected though fewer disubstituted guanidines are known. N-phosphoryl guanidines have also been reported in plants. Disubstituted guanidines like arcaine were found in marine plants and seeds (Shibuya and Makizumi, 1953).

Crown gall tumour tissues also contain guanidines and compounds like octopine, not occurring in normal tissue (Goldmann et al., 1969; Morel, 1971). Several guanidino compounds have been detected in tobacco (Yoshida and Mitake, 1966), barley and spruce (Durzan and Steward, 1967) and Douglas fir (Ebell and McMullen, 1970). The natural occurrence of 3-guanidinoproline (Gallina et al., 1971) streptolidine (Bycroft and King, 1972) and guanidino amino acids has been reported (Wakamiya et al., 1973).

In general, guanidino compounds are derived from arginine. Many of the naturally occurring guanidines arise from arginine by transamidination. Transamidination reactions are also involved in the formation of biguanidines like arcaine in marine plants and other monosubstituted guanidines.

Besides transamidination, guanidines may also be synthesized by several other reactions. Agmatine is formed by the decarboxylation of arginine (Smith, 1963). In pine, γ-guanidinobutyric acid arises by oxidative pathways via α-keto-δ-guanidinovaleric acid (Barnes, 1962). This intermediate has also been found in spruce (Durzan, 1968 a). Barnes (1962) observed that radioactivity from ^{14}C-L-arginine was found in the γ-aminobutyric acid moiety of γ-guanidinobutyric acid, which is consistent with the oxidative pathway though the possibility of transamidination cannot be ruled out.

The ultimate source for monosubstituted guanidines in plants is inorganic nitrogen and guanidines were found to accumulate in response to ammonium-nitrogen (Durzan and Steward, 1967). However, Ebell and McMullen (1970) found that the level of guanidines in Douglas fir was higher when nitrate rather than ammonium was used as fertilizer.

Guanidines are degraded to yield amines as one of the products. Thus, agmatine gave putrescine (Smith, 1969) while canavanine formed the amine canaline and guanidinoacetic acid forms glycine (cf. Bidwell and Durzan, 1975).

The guanidine level was found to vary diurnally and with the plant part examined in spruce saplings (Durzan, 1968 b).

Functions of guanidines

Guanidines are known to inhibit mitochondrial respiration (Wilson and Bonner, 1970). They possess anti-lipolytic properties (Weitzel et al., 1972) and affect urate oxidase activity (Bentley and Truscoe, 1969).

Guanidines also affect metabolism in a number of ways and are used as fungicides for the prevention of apple scab (Woodcock, 1968). In white spruce callus, guanidines inhibited the assimilation of ^{14}C-urea (cf. Bidwell and Durzan, 1975).

Exposure of Valonia and Nitella to guanidines lowered the threshold of electrical excitability and produced a long series of quick action currents (Osterhout, 1942). The mitochondrial transport of sodium and potassium were also sensitive to guanidine (Gomez-Puyou et al., 1973).

Guanidine, above 0.1 ppm is toxic to plants (Kawakita, 1904-1905), probably because it acts as a very strong organic base. High levels of guanidines, like urea, break the hydrogen bonds of macromolecules (Katz and Miller, 1972).

Some guanidines, such as N-methyl-N'-nitro-N-nitrosoguanidine (Craddock, 1968) and nitrosoarginine (Endo and Takahashi, 1973) are reported to have mutagenic activity. They react with the nitrate in the food and form carcinogenic products (Archer et al., 1971).

Due to their fungicidal properties, synthetic guanidines like dodine and guazatine have been recommended for the control of a number of diseases of fruits, nuts and vegetable crops and on certain ornamental and shade trees (Byrde et al., 1962). Guazatine salts are effective as cereal dressing. They are effective in the control of brown spot, blast, alternaria leaf spot in the rice, apples and other fruits.

Some of the toxicity of guanidines may be associated with an increased permeability of plant membranes (Srivastava and Smith, 1982 b). They are found to increase permeability of the tonoplast surface by interacting with phospholipids of the membranes (Beatriz et al., 1979).

Guanidine salts also inhibit virus infection in plants such as the tobacco necrosis virus (Varma, 1968), as well as the infectivity of many animal viruses.

Recent studies have indicated that guanidines may have an action antagonistic to that of polyamines in many tissues. Thus, they inhibit the growth of cress, barley and oat seedlings (Srivastava and Smith, 1982 a). This toxicity of guanidines was reversed by spermine. Guanidines have been shown to destabilize cell membranes and this effect was again reversed with the application of polyamines (Srivastava and Smith, 1982 b). On the other hand, both

dodine and spermine were found to retain chlorophyll and retard the senescence of detached barley leaves (Srivastava et al., 1983). Similarly, both polyamines and guanidines increase membrane-bound ATPase activity in maize scutellum (Srivastava and Rajbabu, 1983 a) whereas peroxidase was activated by guanidines but inhibited by polyamines (Srivastava and Rajbabu, 1983 b). Moreover, guanidines reversed the effect of polyamines but polyamines could not reverse the effect of guanidines, indicating that guanidines probably had two separate binding sites on the membrane. The effects of guanidines thus appear to vary with the system studied.

Much of the current evidence thus suggests that polyamines and guanidines play an important role in the regulation of plant growth and at least polyamines have been suggested as a class of plant growth regulators by themselves. It has been proposed that polyamines, DNA and RNA must all reach a critical level before a cell can proliferate.

For the optimal growth and development of a plant, in addition to the intracellular hormones and metabolites, a number of major and trace elements obtained in inorganic form from the soil or atmosphere are required. One such element whose deficiency most frequently retards crop growth is nitrogen.

The growth and yield of a plant is often positively correlated with the amount of inorganic nitrogen input and assimilated (Hageman, 1979). Plants with a higher rate of nitrate assimilation were found to have a higher grain yield (Croy and Hageman, 1970). Nitrate reductase, the first enzyme of the nitrate assimilatory pathway is a substrate inducible and regulatory enzyme. The activity of nitrate reductase reflects the rate of supply of reduced nitrogen for plant growth. Elrich and Hageman (1973) have shown that nitrate reductase activity was well correlated with the accumulation of reduced nitrogen in a plant. In several studies nitrate reductase activity has been used as a criterion for the selection of cultivars having an ability to produce more grain protein. Dalling et al. (1975) have obtained high correlation between nitrate reductase and grain protein in several wheat varieties. Similar results were obtained with sorghum (Eck and Hageman, 1974) and soybean (Harper et al., 1972).

During the early period of germination of a seed, the growing embryo receives most of the nutrients from the cotyledonary reserves. The activity of proteases and aminotransferases which are involved in the mobilization and distribution of seed nitrogen increases during germination.

Though polyamines have been well correlated with growth processes, most of the work is centered on polyamine content and their biosynthetic enzyme levels during development, germination and growth. There are no studies on the effect of these compounds on various metabolic processes that contribute to growth like nitrate assimilation or reserve protein mobilization during germination of seeds.

Nitrate assimilation :

Green plants have comparatively simple nutrient requirements which comprise of a number of major and trace elements obtained from the atmosphere or from the soil. Nitrogen is one of these elements whose supply affects the development and growth of the plant. Though nitrogen is abundant in the earth's atmosphere, only a few symbiotic plants can utilize this ample store. A majority of the plants obtain their nitrogen in a fixed form from soil as nitrate or ammonium. Generally, nitrate is found to be the preferred source and plants grow well when supplied with nitrate. A few species however, may show a preference towards ammonium. Michael et al. (1970) have studied the comparative uptake of ^{15}N-labelled nitrate and ammonium from ammonium nitrate solutions. They suggest that the relative uptakes may be connected to the carbohydrate supply and age of the plant.

The other important factors governing the relative ammonium and nitrate uptakes are the stage of development of the plant, the nitrogen status, temperature and ambient pH (Tinker, 1979).

Nitrate may also be preferred due to the fact that it can be accumulated in substantial quantities without causing toxic effects to the plant. Some plants like spinach (Shaw, 1972) frequently contain nitrate upto 0.1-1.5% of their fresh weight, which may increase to 2% in conditions of molybdenum deficiency (Hewitt et al., 1979). Certain physiological conditions like high temperature and low light are also known to accumulate nitrate by lowering the activity of nitrate reductase (Nowakowski et al., 1965). Certain species have been found to accumulate more nitrate than others (Wright and Davidson, 1964). This probably represents a balance between the uptake and reduction process.

On the other hand, nitrite concentrations in fresh, undamaged plant tissues are very low, rarely exceeding 0.002% (Shaw, 1972) and probably under normal conditions the rate of reduction of nitrate to nitrite is matched by the rate of reduction of nitrite to ammonia. Similarly, ammonium concentration in plants is often between 0.001 to

0.01% of fresh weight and higher concentrations are toxic to the plant (Matsumoto et al., 1971).

Nitrate is taken up from the soil by roots of the plants. The uptake of nitrate is an energy dependent process, requiring the hydrolysis of ATP (Rao and Rains, 1976). Higher soil concentrations of nitrate may also lead to a higher uptake (Fowden, 1979). It is suggested that (Jackson et al., 1973) nitrate may induce the synthesis of a nitrate-specific permease. Antibiotics like actinomycin-D and puromycin were found to inhibit the induction of permease. The development of nitrate uptake system has also been studied in tobacco cells (Heimer and Filner, 1971) where the presence of either nitrate or nitrite was essential for the induction of the uptake system. Butz and Jackson (1977) proposed that the uptake and reduction of nitrate is catalyzed by the same protein, nitrate reductase, which spans the membrane and acts as a carrier of nitrate. An ATPase is associated with the enzyme protein supplying the required energy with the hydrolysis of ATP. This hypothesis was based on the observation that both nitrate reductase and nitrate transport activity appear to be obligatorily coupled (Goldsmith et al., 1973). The rate of induction of both the activities was increased by glucose or sucrose (Aslam and Oaks, 1975) and a coordinate regulation of both the processes has been suggested (Neyra

and Hageman, 1975), leading to the concept of a nitrate operon. However, studies on Phaeodactylum tricornutum (Cresswell and Syrett, 1979) showed that ammonium had differential effects on the two processes, while it inhibited nitrate uptake, nitrate reduction was unaffected. Similarly, tungsten selectively inhibited nitrate reduction but not the nitrate uptake when a diatom Skeletonema costatum was grown in the presence of tungstate (Serra et al., 1978), thus distinguishing between the process of nitrate uptake and subsequent reduction.

Nitrate entering the plant cell across the plasmalemma may undergo three possible fates. It may be reduced to ammonia and further to amino acids in the roots or may be transferred through the xylem to the shoots where it is assimilated or may be stored in the vacuole in times of excess external supply and be released later in times of nitrate deprivation (Oaks, 1979).

The relative proportions of nitrate reduced in the shoot and root depends upon the species studied as well as the soil nitrate concentration and the physiological status of the plant. Nitrate reductase may not be present in roots in all species of plants or the activity may be very low (Wallace and Pate, 1967). In these plants a major portion of the nitrate is transported to the shoots

and high concentration of nitrate in the soil also leads to increased translocation to the leaves (Beevers and Hageman, 1969). Higher reserves of photosynthates obtained from the phloem favour nitrate reduction in roots (Miflin, 1975).

Nitrate in the cell is probably stored in more than one pool (Ferrari et al., 1973) so that all is not available for reduction (Aslam et al., 1976). In fact, a greater part of nitrate may be stored in a pool of limited availability (Shaner and Boyer, 1976). Heimer and Filner (1971) hypothesized the presence of two pools, a smaller one, which is responsible for the induction of nitrate reductase activity and where nitrate is reduced and a larger one inaccessible to reduction. These were termed as metabolic and storage pools respectively (Aslam et al., 1976). Vacuoles in leaf cells have been shown to contain the stored nitrate of the cell, thus serving as storage pool (Granstedt and Huffaker, 1982). The vacuole was found to contain as much as 58% of the cellular nitrate. The proportion of nitrate found in the vacuole and the metabolic pool may be affected by several factors like the rate of uptake and the rate of reduction, the presence of light (Aslam et al., 1976), glucose (Aslam and Oaks, 1975) and certain toxins produced by pathogens (Yoder and Scheffer,

1973). The size of the metabolic pool in the cell may thus be a regulating factor in the assimilation of nitrate. It also serves as an important source for nitrate being transported to the xylem (Ashley et al., 1975). The first step in the assimilation of nitrate is its reduction to ammonia, the ultimate form of inorganic nitrogen. The ammonia so formed, or that absorbed from the soil, does not accumulate in the plant but is immediately incorporated into carbon skeletons to form amino acids; glutamine or aspargine being the primary products. Once assimilated into the amino group, nitrogen is subsequently distributed among a variety of amino acids and other metabolites.

The reduction of nitrate to ammonia is brought about by a two-step process, catalyzed by the enzymes, nitrate reductase (NR) and nitrite reductase (NiR) as shown in Fig. II. The ammonia formed may be assimilated by one of the two pathways catalyzed either by glutamate dehydrogenase (GDH) or glutamine synthetase-glutamate synthase (GS-GOGAT) (Fig. II).

For many years, GDH pathway was thought to be the most likely one for ammonia assimilation as it provided the only universally distributed, highly active mechanism for the production of amino nitrogen and kinetic studies

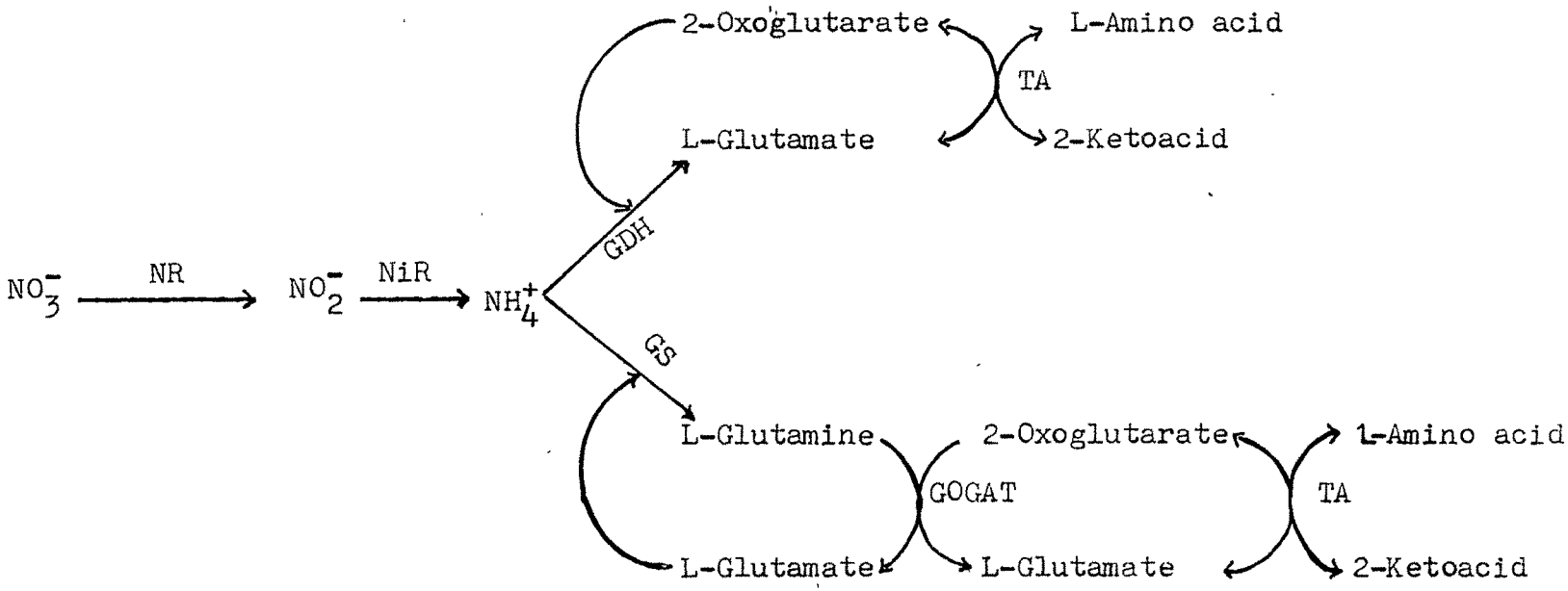


Fig. II : Nitrate assimilation pathway

had lent support for its operation (Sims and Folks, 1964). However, GDH has a very high Km for ammonia, in the range of 10-80 mM (Stewart and Rhodes, 1977). The high Km value of GDH for ammonia cast a doubt on its central role in ammonia assimilation since the tissue concentration of ammonia is low in the range of 0.1-1 mM (Stewart and Rhodes, 1977). Similarly, the chloroplastic GDH has a Km for ammonia of 5.2 mM while ammonia uncouples chloroplasts at concentrations of 2 mM (Miflin and Lea, 1976). Thus, GDH may function in higher plants chiefly under high ammonia concentration.

The other possible pathway for ammonia assimilation was through the reaction catalyzed by glutamine synthetase, forming glutamine from ammonia. But there was no known enzyme which could transfer the amide nitrogen to the α-amino position to form amino acids. However, Tempest et al. (1970) demonstrated the existence of a new enzyme in Aerobactor aerogenes, called glutamate synthase also acronymed as GOGAT (glutamine (amide) : 2-oxoglutarate aminotransferase). This enzyme in association with glutamine synthetase could provide an alternate pathway for the synthesis of glutamate. Subsequently, the enzyme was detected in leaves (Lea and Miflin, 1974), plant cell cultures, roots and cotyledons (Miflin and Lea, 1977). The

enzyme is located in chloroplasts in shoot tissue and uses ferredoxin as the electron donor. The activity of the enzymes of GS/GOGAT pathway were found to be high at low ammonia availability (Tyler, 1978) while that of GDH was high with a higher supply of ammonia. However, in most plant systems studied, GS/GOGAT appears to be the predominant pathway at physiological concentrations of ammonia.

The nitrogen of glutamate formed by either of the pathways is then distributed to form other amino acids by transaminases (TA).

Nitrate reductase (NR)

The enzyme catalyzes a 2 electron reduction of nitrate to nitrite according to the equation :

$$NO_3^- + 2e^- + 4H^+ \longrightarrow NO_2^- + 2H_2O$$

NR is widely distributed in nature occurring in all organisms capable of utilizing nitrate including bacteria, fungi and algae.

In higher plants, NR activity was first identified and characterized in soybean leaves (Evans and Nason, 1953). Since then the enzyme has been found to occur in a wide variety of plants and in almost all parts of a plant. It

has been detected in attached or excised root systems (Vaidyanathan and Street, 1959), germinating embryos of wheat and barley, aleurone cells, corn scutella and also cultured tobacco pith cells (Beevers and Hageman, 1969), as well as from leaves where it was found to be very active (Weissman, 1972). The proportion of NR activity in roots and shoots varies widely from species to species. In general, however, it appears that shortly after germination there is a measurable activity in both root and shoot preparations. But as the plant matures, the activity in the root disappears and the upper canopy apparently assimilates most of the nitrate (Oaks, 1979). NR activity was greatest in root tip regions of young seedlings (Oaks et al., 1972). It has been suggested that this decrease in stability and hence the level of NR is probably because of a NR-inactivating protein, found in mature regions of root, which is fairly specific for NR (Wallace, 1974, 1975).

In C_4 plants, NR was found to be localized chiefly in the mesophyll cells of the leaf (Neyra and Hageman, 1978). There has been much controversy regarding the intracellular location of NR. There are a few reports of NR being associated with a particulate fraction (Dalling et al., 1972 a; Butz and Jackson, 1977). However, most of the evidence is in favour of a cytoplasmic location

(Beevers and Hageman, 1969; Hewitt et al., 1976) but a possibility that NR may be loosely bound with a membrane fraction which may be broken during extraction procedure cannot be excluded. NR was purified first from soybean leaves by Evans and Nason (1953). Since then it has been characterized in several higher plants (Beevers et al., 1964).

The reduction of nitrate to nitrite requires the transfer of 2 electrons, which are donated by the physiological electron donors like NADPH or NADH. Generally, NADH is found to be the preferred donor (Beevers et al., 1964) except in the case of soybean leaves, where the enzyme was earlier shown to utilize NADH as well as NADPH (Evans and Nason, 1953). However, the lack of specificity was later attributed to the presence of a nucleotide phosphatase which probably converted NADPH to NADH (Wells and Hageman, 1974). A certain flexibility is found to occur occasionally and Shen et al. (1976) found two NRs in rice with differing preference for NADH or NADPH, depending on whether the enzymes were induced by nitrate or by chloramphenicol respectively.

In addition to nitrate reduction, NR also shows dehydrogenase activity, whereby it catalyzes the reduction

of a number of electron acceptors like cytochrome c, ferricyanide, dichlorophenol indophenol and various tetrazolium salts. It can also accept electrons from a variety of donors besides NADH which include reduced flavins and viologens like methyl viologen or benzyl viologen (Wray and Filner, 1970).

Several studies have been carried out to determine the source of NADH required in nitrate reduction. In non-photosynthetic tissue, nitrate assimilation was associated with enhanced respiratory metabolism indicating that respiration (Willis and Yemm, 1955) may provide NADH. In photosynthetic tissues light was thought to supply NADPH (Evans and Nason, 1953). However, a direct involvement of light was disputed later and it was suggested that following illumination, photosynthetic metabolites effluxed from the chloroplasts and during their glycolytic metabolism generated NADH for nitrate reduction (Beevers and Hageman, 1969). In contrast to the above observation, Sawhney *et al.* (1978) found that supply of TCA cycle acids stimulated *in vivo* nitrate reduction by soybean and wheat leaf sections. They thus suggested that TCA cycle rather than glycolysis supplies reduced power for nitrate reduction. The transfer of reductant from mitochondria to cytosol was proposed to occur via a malate oxaloacetate shuttle mechanism (Hageman *et al.*, 1980).

NR is susceptible to a number of factors, both internal as well as external. Being the first enzyme of the pathway it forms the logical point of control of nitrate assimilation. The amount of nitrate accumulating in plant tissues under various environmental conditions can be correlated well with the activity of NR. Thus, nitrate accumulates in plants grown in low light when NR activity is low (Hageman and Flesher, 1960). Similarly, studies on Aspergillus and Chlorella (Kessler, 1964) have shown that there is a correlation between the ability of an organism to use nitrate and the presence of NR in the organism.

NR is an inducible enzyme, nitrate serving as the inducer in most plants (Ferrari and Varner, 1969). The induction of NR is a protein synthesis dependent response (Afridi and Hewitt, 1965). Nitrate is also essential for the maintenance of the enzyme which declines on removal of nitrate from the medium (Schrader et al., 1968). The concentration of nitrate at which maximum NR is induced varies from species to species, ranging from 0.02-10.0 mM (Srivastava, 1980).

Nitrite, the product of the reaction was also found to induce NR activity in certain species (Lips et al., 1973). Ammonium has also been reported to induce NR

activity in plants like maize (Mehta and Srivastava, 1980). The effect of ammonium on the induction of NR by nitrate has also been studied and is found to vary from species to species. While ammonia had an inhibitory effect in some plants, it had no effect in others (Wallace, 1973). In the case of cultured Rosa cells (Mohanty and Fletcher, 1976), ammonium caused an increase in NR induction by nitrate.

Amino acids, rather than ammonia, have been found to inhibit NR activity in N. tabacum cells (Filner, 1966). Amino acids may inhibit NR by inhibiting any of the steps from the uptake of nitrate to the synthesis of NR. Thus, addition of amino acids to the medium resulted in a marked reduction in nitrate levels in roots and shoots of certain species and hence the level of NR (Heimer and Filner, 1971).

Many intermediates of carbohydrate metabolism also affect NR activity. Addition of glucose was found to maintain NR level (Sahulka et al., 1975), probably by serving as an energy source. However, it was also found to have a specific effect of increasing the metabolic pool of nitrate and hence the induction of NR (Aslam and Oaks, 1975) while the total nitrate content remained unaffected (Oaks, 1979).

The effect of various phytohormones has been studied in a variety of plants and was found to differ from species to species. In Trigonella foenum-graecium seedlings, NR was induced synergistically by nitrate and kinetin (Rijven and Prakash, 1971). Gibberellic acid (GA) along with cytokinins increased NR in leaves of N. tabacum (Lips and Roth-Bejerano, 1969) where they substituted the effect of light but gibberellins had no effect on NR in barley aleurone layer (Ferrari and Varner, 1970). Induction of NR by the hormones involves a de novo protein synthesis (Kende and Shen, 1972).

Abscisic acid showed no effect on the development of NR activity in Lemna (Stewart and Smith, 1972) while it markedly reduced the nitrate induced synthesis of NR in germinating cotton (Radin, 1974). The hormones may probably modify the NR activity by altering cellular metabolism and physiology of the plant.

External conditions like light and environmental stress greatly affect the level of NR. Light enhanced the induction of NR by nitrate (Travis and Key, 1971). NR may be induced in seedlings grown in dark conditions also, although in almost all cases studied, the level of NR in parts of light grown plants was higher than that in dark grown ones (Jordan and Huffaker, 1972). The duration as

well as the intensity of light was found to influence NR. NR activity was greater in plants grown at higher irradiance than at lower (Pellipe et al., 1975) and increasing the irradiance from 6 to 45 Klx resulted in an increase in NR activity in Glycine max (Nicholas et al., 1976). Regulation by white light has been demonstrated at various points in nitrate assimilation and nitrate uptake. NR level and the substrate turnover may each be affected by light (Beevers and Hageman, 1969). Various hypotheses have been advanced to explain the effects caused by light. Light has been proposed to increase the synthesis of mRNA (Williams and Novelli, 1968) or increase the polyribosome content of the tissue (Travis and Key, 1971) leading to an increased synthesis of NR. Light may bring about an increase in energy charge due to photosynthesis (Travis et al., 1970) as well as supply the reduced cofactors required to reduce nitrate to ammonia (Klepper et al., 1971). However, the presence of NR in dark grown tissues as well as nonchlorophyllous tissues (Srivastava, 1974) do not support this proposal. Besides these nonspecific effects of light, it has been proposed that light acts specifically on NR induction via phytochrome, since the inducing effect of light were red/far-red reversible (Jones and Sheard, 1975). Phytochrome may affect nitrate reduction by modulating the membrane properties, thus

possibly altering the compartmentation of nitrate in the cells (Wagner, 1975) resulting in the regulation of nitrate fluxes among intracellular nitrate compartments. Light is found to redistribute intracellular nitrate in etiolated peas (Jones and Sheard, 1973) releasing otherwise unavailable nitrate for induction. Thus, etiolated barley leaves, exposed to white light were found to show more metabolically active pool of nitrate as compared to the etiolated ones (Aslam et al., 1976). It has also been suggested that light acts on NR by modulating the supply of GA and kinetin and hence these hormones have been found to replace light requirement (Lips and Roth-Bejerano, 1969). GA and light have also been found to have similar effects (Lips, 1975). It is possible that one or more of these effects are responsible for the observed effect of light on NR activity.

Environmental stress like water stress may decrease NR activity by inhibiting either nitrate uptake or protein synthesis. It may also lead to a decline in polyribosome level (Hsaio, 1970) leading to a loss in NR activity. Water stress was found to decrease NR activity in Z. mays (Morilla et al., 1973).

Nitrite reductase (NiR)

Exogenous nitrite or that produced by the reduction of nitrate is further reduced to ammonia by the action of NiR. The reaction involves the transfer of six electrons as shown in the equation :

$$NO_2^- + 6e^- + 8H^+ \longrightarrow NH_4^+ + 2H_2O$$

Most studies on higher plants indicate that NiR in green leaves is associated with chloroplasts (Losada et al., 1963; Ritenour et al., 1967). These and additional studies (Magalhaes et al., 1974) showed that isolated illuminated chloroplasts could reduce nitrite to ammonia without supplemental cofactors and the rates were comparable with those in situ. In general, maximal levels of NiR are obtained in young leaves during the phase of leaf expansion (Beevers and Hageman, 1972).

NiR has also been found in nonchlorophyllous tissues like barley root (Bourne and Miflin, 1970). Dalling et al. (1972 b) found that 15% of the total NiR activity in wheat roots was associated with an organelle tentatively identified as the proplastid. This association has been also observed in tissue culture cells (Washitani and Sato, 1977).

Higher plant NiR requires an electron donor of a

very low potential for nitrite reduction (Cresswell et al., 1965). The physiological donor was found to be ferredoxin, an iron-sulfur protein, present in photosynthetic tissues. Support for the role of ferredoxin in nitrite reduction comes from the observation that light stimulates nitrite assimilation and also the fact that both NiR and ferredoxin are located in the chloroplast (Beevers and Hageman, 1972). Moreover, nitrite accumulation in green tissue in the presence of inhibitors or herbicides, like substituted urea, that prevent the reduction of ferredoxin by the photosynthetic electron transport system further supports the view that NiR is linked to ferredoxin as the electron donor (Neyra and Hageman, 1974).

In the nongreen tissue, however, the source of reductant has not been established. Butt and Beevers (1961) suggested that NiR in roots probably utilizes NADPH as the electron donor. Bourne and Miflin (1970) observed that a particulate preparation from barley roots converted nitrate to ammonia in the presence of pyruvate, ATP and various cofactors. Omission of ATP led to decreased ammonia production implying the possibility of an energized nitrite for the reduction to ammonia. NiR activity using reduced viologen dyes has been detected in root and scutella of maize (Hucklesby et al., 1972; Bourne and Miflin, 1973).

The enzyme showed properties very similar to that from leaves. However, the coupling of carbohydrate oxidation to ferredoxin via NADPH-ferredoxin dehydrogenase did not seem to be effective (Aslam et al., 1979).

NiR also appears to be an inducible enzyme. Nitrite is the common inducer but induction was also observed with nitrate (Ingle et al., 1966). In this case, however, it is difficult to identify the inducible ion which might be nitrate itself or nitrite generated as a consequence of the induction of NR. Kinetic studies of induction by nitrate in radish cotyledons (Ingle et al., 1966) appeared to indicate the sequential induction of the two enzymes. However, this may not necessarily be true for other plant systems.

Ammonia, the end product of the reaction, as well as amino acids were found to repress the synthesis of NiR in Lemna (Joy, 1969; Stewart, 1972) and in Chlorella (Losada et al., 1970). Cyanide also inhibits NiR, probably by competing with nitrite for the siroheme moiety of the enzyme (Vega and Kamin, 1977). Carbon monoxide, another inhibitor of NiR was found to inhibit it by reacting with the reduced form of the enzyme (Vega and Kamin, 1977). Light has an enhancing effect on NiR activity and the level of the enzyme was always higher in light

grown tissues as compared to dark grown ones (Canvin and Atkins, 1974). The chloroplastic location of the enzyme and reduced ferredoxin being the physiological donor explains the effect of light in enhancing NiR activity.

In normal growing conditions, NiR activity has a faster rate than NR (Goodman et al., 1974) since nitrite does not accumulate in plant tissue.

Glutamate dehydrogenase (GDH)

GDH mediates the combination of ammonia with 2-oxo-glutarate to yield glutamate and also catalyzes the reverse reaction leading to deamination of glutamate :

NH_3+2-Oxoglutarate + NAD(P)H $\rightleftharpoons$ L-Glutamate + $NAD(P)^+$

GDH has been studied in almost all organisms. In higher plants, almost all parts have been found to contain GDH (Barash et al., 1973; Nicklish et al., 1976).

Aminating GDH activity was found to increase during seedling growth with the supply of nitrogen source. This increase was well correlated with the organic nitrogen content and growth of the seedling (Singh and Srivastava, 1982). A similar correlation between GDH and organic nitrogen content was also observed in oat leaves (Barash et al., 1974).

GDH was found to be associated with the mitochondria (Thurman et al., 1965) and chloroplasts (Leech and Kirk, 1968) of the cell. A separate cytoplasmic GDH was, however, detected in pumpkin cotyledons by Chou and Splittstoesser (1972). The mitochondrial GDH generally utilizes NAD and the chloroplastic enzyme utilizes NADP as the coenzyme. Both the enzymes however, show dual coenzyme specificity to a certain extent. Mitochondrial GDH has been detected in seeds (Thurman et al., 1965), roots (Pahlich and Joy, 1971) and leaves (Barash et al., 1973). The chloroplastic enzyme has been studied from tissues including Vicia faba (Leech and Kirk, 1968) and lettuce (Lea and Thurman, 1972). The mitochondrial and chloroplastic GDH were found to differ in the Km value for substrates and electrophoretic mobility and were suggested to be due to separate isoenzymes. Isoenzymes of GDH have also been reported in various tissues including pea (Thurman et al., 1965) and oats (Barash et al., 1973). Pea seeds showed the existence of seven isoenzymes (Thurman et al., 1965) which decreased in number as the seed germinated and developed a new pattern in roots and shoots. The existence of such multiple enzyme forms could be associated with cellular differentiation, intracellular compartmentalization or physiological functions (Lea and Thurman, 1972).

Before the discovery of the GS/GOGAT pathway, GDH was considered to be the key enzyme of nitrogen assimilation and studies were carried out to determine the correlation between GDH activity and the formation of organic nitrogen during nitrogen supply. The supply of both nitrate and ammonia was found to increase the assimilatory GDH activity (Joy, 1969; Tsenova, 1975). Similar results have been obtained in cucumber (Buczek, 1979) and maize (Singh and Srivastava, 1982). The increase observed on the supply of ammonia was suggested to be due to the de novo synthesis of a specific isoenzyme of GDH (Barash et al., 1975). An alternate view is that the increase in GDH activity by ammonia was due to the activation rather than synthesis of the enzyme (Shepard and Thurman, 1973). This view is supported further by the observation that ammonia differentially affected the aminating and deaminating activities of GDH. Ammonia may change the physicochemical nature of the enzyme protein rather than its synthesis (Singh and Srivastava, 1982).

The increase in the activity of GDH obtained by the supply of ammonia indicates that the enzyme may play an important role in ammonia assimilation under conditions of high ammonia availability (Barash et al., 1975). Rhodes et al. (1976) suggested that it is glutamine rather than ammonia which regulates the level of GDH. Glutamate, the

product of the reaction has also been reported to inhibit the aminating GDH activity in many systems (Joy, 1969). The effect of other amino acids was found variable. They had no effect on the enzyme in Lemna (Stewart and Rhodes, 1977) while the enzyme in tea roots was stimulated by glutamine, threonine and alanine (Takeo, 1979). Cysteine inhibits the enzyme activity in maize leaves but not in the root. The inhibitory effect was also observed during the in vitro incubation and it is suggested that cysteine regulates GDH activity through allosteric modulation (Singh and Srivastava, 1983).

On the other hand, there are reports of high concentration of amino acids bringing about an increase in the activity of GDH (Bayley et al., 1972). It is possible that in higher plants, GDH could have a dissimilatory role of deamination. There may exist functionally distinct forms of GDH, one having an assimilatory role at high ammonia concentration and the other functioning in glutamate deamination.

Glutamine Synthetase (GS)

GS is found to occur throughout the plant and animal kingdom and has been studied from a wide variety of sources. It catalyzes the reaction

$$ATP + L\text{-Glutamate} + NH_3 \xrightarrow{Mg^{2+}} L\text{-Glutamine} + ADP + Pi$$

In addition to the biosynthetic reaction, GS can also utilize hydroxylamine as substrate, producing γ-glutamyl-hydroxamate (Varner, 1960) :

$$\text{ATP+L-Glutamate+ } NH_2OH \xrightarrow{Mn^{2+}} \gamma\text{-Glutamylhydroxamate + ADP + Pi}$$

Purified GS also catalyzes a γ-glutamyl transfer reaction with glutamine as its substrate, resulting in the formation of γ-glutamyl hydroxamate. The transferase activity appears to be several times higher than the synthetic one and is often used for measurement of GS (Shapiro and Stadtman, 1970).

The enzyme appears to be distributed in all parts of the plant but the activity differs markedly from one tissue to another. In general, specific activity of GS was higher in shoots than in roots, except in the case of species that assimilate a large quantity of nitrogen in the roots where GS activity was higher in roots (Lee and Stewart, 1978). The enzyme activity was found to be high in developing and germinating seedlings as well as young expanding leaves but decreased as the leaf senesced (Storey and Beevers, 1978).

Subcellular distribution of GS has been studied by several investigators (Wallsgrove et al., 1979; Mann et al., 1979) and the enzyme has been found to be present

in cytosol as well as the chloroplast of the cell. Studies on isolated chloroplasts have confirmed the presence of GS (O'Neal and Joy, 1973). The cytosolic and chloroplastic forms have been studied in barley (Mann et al., 1979) and found to have similar molecular weights. However, they differed in their pH optima and stability. Etiolated seedlings were found to contain only the cytosolic GS, designated as GS I, while green tissues contained both the cytosolic GS I and the chloroplastic GS, designated as GS II (Mann et al., 1980). Exposure of etiolated pumpkin seedlings to light resulted in the synthesis of the chloroplastic enzyme, which was absent in the etiolated tissue (Nishimura et al., 1982). It is proposed that the cytosolic GS is responsible for the assimilation of ammonia into the transport compound glutamine, while the chloroplastic GS is involved in the reassimilation of ammonia released during photorespiration (Nishimura et al., 1982).

GS studied from all sources has a very low Km for ammonia, in the range of 1-2 x 10^{-5} M (O'Neal and Joy, 1974) which supports the view that GS plays a major role in ammonia assimilation at the physiological concentration of ammonia.

GS forms a locus of control over the input of ammonia

into the organic form and the enzyme is found to be regulated by several factors. Studies of the ammonia assimilating enzymes in Lemna show a reciprocal pattern of control over GS and GDH (Rhodes et al., 1976). GS was active at a low ammonia and high energy level while GDH was active at high ammonia and comparatively lower energy state.

Consistent with this hypothesis is the observation that ADP and AMP inhibit GS in rice roots (Kanamori and Matsumoto, 1972), pea leaf (O'Neal and Joy, 1975) and in Lemna (Stewart and Rhodes, 1977). The inhibition was found to be competitive with respect to ATP.

The reaction catalyzed by GS is the regulatory step in a complex branched pathway which leads to the synthesis of several metabolites. In effect, GS has been found to be susceptible to a variety of metabolites, which modulate the enzyme activity.

Kapoor and Bray (1968) and Kapoor et al. (1968) observed that GS from N. crassa was inhibited by glycine and histidine as well as anthranilic acid which derive their amino group from glutamine. They suggested that a system of cumulative feedback inhibition operates in N. crassa. The effect of amino acids was, however, variable. Kanamori and Matsumoto (1972) found no inhibition of rice root

enzyme with any of the amino acids but Stewart and Rhodes (1977) reported that a mixture of amino acids inhibited GS in Lemna.

Various intermediates of metabolic pathways were also reported to affect GS activity. Carbamyl phosphate inhibits GS from many sources (O'Neal and Joy, 1975), while Lemna GS was not affected (Stewart and Rhodes, 1977). On the other hand, nitrate and 2-oxoglutarate activated the enzyme in pea seeds and rice roots while pyruvate and glyoxylate inhibited it (Kanamori and Matsumoto, 1972). These effects were also observed in Lemna leaf enzyme (Stewart et al., 1980).

Various cellular metabolites altering GS activity do so by either reversibly deactivating the enzyme or by dissociating it into less active monomers or by conformational modification of the enzyme. Reversible deactivation of the enzyme has been demonstrated in E. coli and other microorganisms (Stadtman et al., 1970) but not in higher plants. It involves adenylation of the enzyme catalyzed by an adenyl transferase. The modified enzyme had lower catalytic activity and was more susceptible to feedback inhibition (Stadtman et al., 1970). GS may exist in various states of adenylation depending upon the ATP present in the cell. Sims (1976) has extensively studied

GS activity in yeasts and found that GS was controlled by a reversible dissociation of an active octamer into tetramers. Different metabolites affected the degree of dissociation and association and hence the enzyme level.

GS has also been suggested to be subject to conformational modification. Rhodes et al. (1975, 1979) observed that GS in Lemna was reversibly inactivated when the plants were grown in ammonium or when ammonium was supplied to nitrate grown plants. Reactivation of the enzyme occurred on transfer of the plants to light or in vitro incubation with ATP, glutamate or reduced glutathione. They suggested that conformational changes in the enzyme in relation to thiol groups on the subunits lead to an active or inactive GS. Chloroplastic GS may also be regulated similarly, thus providing a means for the integration of assimilatory carbon and nitrogen metabolism.

Several studies indicate that the end product, glutamine controls the level of not only GS and GDH, but also NR and NiR (Stewart and Rhodes, 1977; Rhodes et al., 1979). It was suggested that a group of ammonia utilizing enzymes share a common regulatory metabolite, as in the case of fungi or bacteria.

Glutamate synthase (GOGAT) :

Glutamate synthase was first detected in Aerobacter aerogenes (Tempest et al., 1970). It catalyzes a glutamine dependent reductive amination of 2-oxoglutarate, resulting in the formation of two glutamate molecules.

Following its discovery in Aerobacter, the enzyme was detected in various higher plants (Wallsgrove et al., 1977; Stewart and Rhodes, 1978). The enzyme utilized ferredoxin as the physiological donor of electrons, though NADPH-glutamate synthase is also reported (Dougall and Bloch, 1976). Ferredoxin glutamate synthase is known to occur in roots (Stewart and Rhodes, 1978), shoots (Wallsgrove, et al., 1977) and developing seeds and legume root nodules (Miflin and Lea, 1976). In general, the activity of the enzyme was higher in shoots than in the roots. The enzyme activity was found to increase maximally during rapid growth and development in pea (Storey and Beeves, 1978).

In all the shoot tissues studied, the enzyme was found to be localized in chloroplasts (Rathnam and Edwards, 1976). This is consistent with the electron donor, ferredoxin, being restricted to the chloroplasts. The sub-cellular location of NADPH glutamate synthase is however, controversial. The enzyme in developing pea cotyledons was found not to be associated with any organelle (Beevers and

Storey, 1976), while the enzyme in roots (Emes and Fowler, 1978) was found to be almost exclusively in the plastids.

The enzyme is very specific for L-glutamine and 2-oxo-glutarate and NH_4Cl. L-Aspargine or oxaloacetate did not support the activity. However, it could utilize both NADH and NADPH as the electron donor (Dougall and Bloch, 1976).

Little is known at present about the regulation of this enzyme. In one strain of A. aerogenes, highest levels were found at low ammonia availability (Meers et al., 1970) while in another strain, high ammonia concentration resulted in high GOGAT levels. However, in L. minor, GOGAT was high in condition of low nitrogen availability (Rhodes et al., 1976).

Proteases

Proteolytic activity in plants has been studied from fruits, latex, leaves and particularly in germinating seeds, where it has been associated with the growth and development of the seedling.

Seeds contain considerable quantities of reserve proteins that are mobilized during germination by specific proteases. This process of mobilization is highly organized and the activity of proteases changes considerably during

this period according to the need of the plant (Mayer and Poljakoff-Mayber, 1963). Thus, protease activity either low or absent in ungerminated seed was found to increase rapidly in response to the initiation of germination (Jacobson and Varner, 1967) or it may be present at a high level at the onset of germination but may decline slowly as germination proceeds (Pusztai and Duncan, 1971).

Both exo- and endopeptidases were found to increase during germination (Yatsu and Jacks, 1968). Most plants appear to utilize sulfhydryl proteases, probably because of the high level of serine protease inhibitors present in plants (Richardson, 1977) which may mask the activity of these proteases. Endopeptidases have been detected in ungerminated as well as germinating seeds (Adams and Novelli, 1975), though the activity increased as germination proceeded (Jacobson and Varner, 1967; Beevers, 1968).

Most of the work done on localization of endopeptidases suggested that they are associated with protein bodies in the seed (Yatsu and Jacks, 1968). Protein bodies isolated from whole grains, aleurone layers and embryos were found to contain proteases (Adams and Novelli, 1975). However, Koroleva et al. (1973) found that, in addition to the enzymes present in the protein bodies, vetch seeds contained two cytosolic proteases, which could be differentiated from the former by pH optimas.

Besides endopeptidases, aminopeptidases (Moeller et al., 1970) and carboxypeptidases (Ihle and Dure, 1972) have also been found to increase during germination.

The increase in proteolytic activity and the resulting storage protein mobilization is a finely controlled phenomenon, occurring in an orderly manner. It is subject to control by various factors which include hormonal control of de novo synthesis, endogenous inhibitors and compartmentation. The increase in protease activity during germination was shown to be due to a protein synthesized de novo (Jacobson and Zwar, 1974). This synthesis occurs at a time when storage proteins are being degraded and appears to be dependent upon transcription and translation. On the other hand, the increase in carboxypeptidase in germinating cotton seeds (Ihle and Dure, 1972) was prevented by cycloheximide but not by actinomycin-D suggesting that the seeds may contain a preexisting mRNA, probably formed during seed development and maturation. The translation takes place only during germination when the mRNA undergoes post-transcriptional polyadenylation (Walbot et al., 1974).

The increase in protease activity was also found to be dependent upon the presence of axis tissue (Varner et al., 1963; Penner and Ashton, 1967). The removal of axis was found to reduce the increase in protease activity (Sze and Ashton, 1971). Addition of cycloheximide to the 3-day old

seedlings caused an immediate inhibition of the enzyme activity. It was suggested that at least a part of the increase in the protease activity was synthesized de novo and its synthesis was under the control of the embryonal axis. These effects of the embryonic axis were found to be mimicked by hormones like cytokinins (Penner and Ashton, 1966) or gibberellins (Tsay and Ashton, 1974) suggesting that the hormones probably originated in the embryonic axis and were translocated to the storage tissue during germination.

Another mode of regulation was suggested to occur through protease inhibitors present in the seeds. Shain and Mayer (1965) have reported the presence of an inhibitor in lettuce seeds which inhibited proteases. The inhibitor concentration decreased during germination with a corresponding increase in protease activity. Compartmentation of proteases from the storage protein body also appears to be a control mechanism (Ashton, 1976).

The level of released amino acids may also regulate the level of protease. Yomo and Varner (1973) found that if excised cotyledons were placed in a casein hydrolysate there was reduced level of protease, suggesting a control of protease level by amino acids. They also found that the level of protease increased in attached cotyledons, while

it did not increase in detached ones, probably because of the higher amounts of amino acids present in detached cotyledons.

Transaminases

Transaminases have been found widely in bacteria, animals as well as plants. They catalyze a reversible transfer of an amino group to the carbonyl group of an acceptor molecule. Most transaminations are freely reversible reactions.

The enzymatic transfer of amino group plays an important role in many metabolic processes where the interconversion of nitrogen containing molecules is involved, like synthesis of various amino acids from glutamate during nitrogen assimilation, porphyrin biosynthesis, synthesis of aliphatic amines from their aldehyde precursors and alkaloid biosynthesis. Transamination is also important in amino acid degradation.

Various transaminases have been studied in plants (Kretovitch, 1965). The best studied and characterized enzymes are aspartate aminotransferase (ASAT) and alanine aminotransferase (AAT).

The development of these enzymes has been studied

during the germination of various seeds. In early studies, a marked increase in both aspartate and alanine aminotransferases was observed in the embryo during the first few days of germination of H. vulgare and Z. mays (Albaum and Cohen, 1943). In many of the systems studied, they show a similar pattern of development (Forest and Wightman, 1971).

AAT has been studied during the germination of seeds (Smith and Williams, 1951), in leaves (Hatch and Mau, 1973) and fruits (Yu and Spencer, 1969) of various plants. In Cucurbita seedlings, grown in light or dark, the enzyme activity increased during the first eight days of germination but decreased rapidly after that (Splittstoesser et al., 1976). Forest and Wightman (1971) studied AAT level in the roots, shoots and cotyledons of Phaseolus seedlings. The enzyme activity in the cotyledons was high within 24 hr of the start of germination but decreased steadily thereafter. In the shoots, the activity increased steadily for first eight days and then decreased slowly. The decrease in activity obtained in roots is in agreement with the earlier reports where a similar decrease was observed in older roots of Lens culinaris (Pilet et al., 1972).

The level of AAT was found to correspond closely with protein synthesis in developing seeds of Lolium

(Hedley and Stoddart, 1972) suggesting that it exerts an important role during organ development.

The activity of AAT has also been studied in leaves of various species (Hedley and Stoddart, 1971). Light was found to stimulate AAT activity in dark grown first leaves of Lolium seedlings by more than 130% during the first 24 hr of light exposure. Red light treatments also produced an increase in AAT activity and return of plants to the darkness arrested the light stimulated increase in enzyme activity. In these investigations, a correlation between the effect of treatments on chlorophyll content and AAT activity was observed. In general, chlorophyll formation and AAT activity responded in a similar manner. By using light-grown leaves of various ages, a close similarity was found in chlorophyll content and AAT activity, suggesting that AAT may play a role in chlorophyll biosynthesis (Hedley and Stoddart, 1971).

In plants, most of the transaminase activity is found in the cytosol. Thus, in pumpkin cotyledons, 98% of AAT activity was present in the cytosol (Splitts-toesser et al., 1976). However, AAT has also been detected in particulate form and is suggested to be an isoenzyme (Thomas and Stoddart, 1974). AAT activity has been

detected in mitochondria (Yu and Spencer, 1969) and chloroplasts (Santarius and Stocking, 1969).

Considerable interest has been expressed in the activity and role of transaminases in chloroplasts. Activity of the enzyme has been found to increase during the greening of etiolated leaves on exposure to light (Hatch and Mau, 1973). Early studies have demonstrated the presence of AAT in isolated chloroplasts of Vicia and Nicotiana leaves (Santarius and Stocking, 1969). Kirk and Leech (1972) have suggested that transaminases play an important role in chloroplastic assimilatory pathways since glutamate, the primary product of photosynthesis, is transaminated to form alanine or aspartate from which other amino acids are synthesized. Aminotransferases thus provide an essential link between carbohydrate and amino acid metabolism in plant cells.

The literature reviewed above demonstrates that polyamines have now been assigned the role of a new class of plant growth regulators. Though, their levels and that of biosynthetic enzymes are closely associated with the growth of the plant, much of the evidence is still circumstantial and no systematic study is available on their role in various metabolic pathways. Since nitrogen

assimilation and mobilization of reserve play an important role in germination and growth of seeds, it was of interest to study the effect of polyamines and guanidines on these pathways in relation to growth. The results of these investigations in germinating radish seeds are incorporated in the thesis.

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