SUMMARY

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Amongst the basic requirements of a green plant, nitrogen is undoubtedly of primary importance. It is one of the most prevalent elements in the living organism and is found in all essential compounds such as proteins, nucleic acids, some of the plant growth regulators and in many of the vitamins. The plants thus need a continuous supply of nitrogen. Though the earth's atmosphere consists of 80% of nitrogen most of the plants are unable to utilize nitrogen in this form. With the exception of those species capable of fixing molecular nitrogen, most plants absorb nitrogen in a fixed form from the soil. The forms of nitrogen available to the plant are nitrate, ammonia, organic nitrogen and molecular nitrogen. Very few species are capable of utilizing all four forms. Most the plants utilize nitrate as their nitrogen source, though some also grow well on ammonia. Nitrate is preferred probably because it can be absorbed and stored to a considerable quantity without causing deleterious effects to the plant while both nitrite and ammonia are known to cause various harmful effects, even at a slightly higher concentrations (above 2 mM). The stored nitrate can then be utilized under conditions of nitrogen starvation.

Nitrate taken up by the plant cannot be directly

utilized by the plant but is first reduced to ammonia before it is incorporated into nitrogenous compounds. The first step in nitrate reduction is its conversion to nitrite catalyzed by the enzyme nitrate reductase (NR). Nitrite is further reduced to ammonia by nitrite reductase (NiR). The ammonia so formed is incorporated into amino acids. The energy required for the reduction of nitrate is supplied by respiration.

Ammonia, whether produced from nitrate or absorbed from the soil may be assimilated via the glutamate dehydrogenase (GDH) pathway, whereby it is transaminated into other amino acids, or it may be first utilized to form glutamine by glutamine synthetase (GS). This enzyme in combination with glutamate synthase brings about a net synthesis of two molecules of glutamate. Recent investigations have shown that at physiological concentrations of ammonia it is the glutamine synthetase/glutamate synthase pathway which operates, while glutamate dehydrogenase is more functional at higher ammonia concentrations. The relative flux of ammonia between the two pathways is also decided by the energy status of the plant; glutamine synthetase operating at a high energy status and glutamate dehydrogenase operating at a relatively lower energy status.

Nitrate reductase, the first enzyme of the pathway is

believed to be the regulatory and inducible one. In addition to its substrate, other factors such as energy level and metabolites are known to affect the level of NR. The enzyme regulates the input of inorganic nitrogen into plant proteins and hence the nitrogen status and yield of a plant. It is often used as a marker for the growth of a plant. Light is known to influence nitrate reductase as well as other enzymes of the pathway and thus enhance the rate of assimilation.

Polyamines are aliphatic amines derived from arginine. They are now considered as universal cell constituents with a wide variety of functions. In plants they are known to promote macromolecular synthesis and mitosis, retard leaf senescence, stabilize cell membranes, specially under conditions of stress. They are known to modulate membrane-bound enzymes like ATPase and peroxidase. At physiological pH of the cell, they also act as cations and help to maintain homeostasis.

Much of the recent work on polyamines is centered on the possible role of these compounds in various growth processes. They have been implicated as regulators of various developmental processes. The polyamine content of young growing tissues is found to be high, while that of aging tissues is low. A possible link has been suggested between

the polyamine levels and physiological regulators like hormones and light, where polyamines are proposed to act as secondary messengers.

Guanidines, like polyamines, are also derived from arginine and are of widespread occurrence. They are known to possess surface active properties. In certain plant systems they exert an effect antagonistic to that of polyamines, such as inhibition of growth; while in other systems they may behave in a manner similar to that of polyamines.

Though much work has been done on polyamines during germination there are no reports of the effect of these compounds on nitrate assimilation or reserve mobilization. The present project was undertaken to study their effect on these processes in cotyledon in relation to growth of the seedling.

Nitrate assimilation was studied with respect to the levels of NR, NiR, GS and GDH. Protease and AAT levels were studied to measure reserve mobilization. The weight of embryonal axes was measured as an index of growth. Nitrate was found to increase growth of the seedling in light and dark. Ammonium however, inhibited growth in dark grown seeds. The level of NR, NiR and GS were maximal when seeds were grown in nitrate while maximum level of

GDH was obtained in the presence of ammonium. High levels of protease and AAT were obtained even in the absence of nitrogen source.

When seeds were grown in the presence of polyamines or guanidines, growth was enhanced by 40-50% in both light as well as dark grown seeds. This increase was observed throughout the period of germination irrespective of the nitrogen source supplied.

With regard to nitrate assimilation, both polyamines and guanidines did not have any effect on the level of enzymes when seeds were grown in the absence of a nitrogen source in light or dark. When seeds were grown in presence of nitrate, both the groups of compounds inhibited NR and GS in light grown seeds while NiR and GDH were not affected. In the dark grown seeds none of the enzymes were affected by the compounds. The inhibition of NR and GS activity in light was about 30-40%, lowering the level to the dark grown control group. The compounds thus inhibited only the light-mediated increase in enzyme activity. In the presence of ammonium, however, the compounds had no effect on the enzyme levels in light or dark grown seeds. Polyamines and guanidines increased protease activity in light as well as dark grown seedlings irrespective of the nitrogen source. The compounds enhanced AAT activity in

light grown seeds by about 40% while there was no effect in the dark grown ones.

Further studies were carried out to investigate the mechanism of inhibition or activation of the enzymes by the compounds. Since NR activity is known to be dependent upon the amount of nitrate available, nitrate content of the tissue in the absence and presence of the compounds was measured. However, there was no significant difference between the nitrate content of control group and the one treated with compounds. The catalytic activity of NR was not affected since the compounds had no effect when added to the assay *in vitro*. As NR activity is known to be induced in excised tissue, further studies were carried out during *in vitro* induction in excised cotyledons. When the metabolic pool of nitrate was determined (as assessed by *in vivo* NR activity) in the absence or presence of the compounds, the size of the metabolic pool was found to be decreased in the presence of the compounds. Thus, the compounds may affect the redistribution of nitrate. When the effect of polyamines was studied during various stages of induction, the inhibitory effect was maximum when the compounds were added at the start of induction. The effect decreased when added at later stages of induction and there was no effect when added after 2 hr of induction. Thus, the compounds probably affect the

synthesis of the enzyme. Polyamines have been earlier reported to inhibit macromolecular synthesis. A similar mechanism may operate in the present case also.

Studies on the stability of NR showed that the compounds did not affect the rate of decay when added to the tissue homogenate. However, when the cotyledons were treated with the compounds and enzyme was assayed at different intervals, NR activity was found to be lower in the treated tissue as compared to the control. Light increased the enzyme level in control tissue but not in the tissue treated with the compounds. However, the rate of decay of NR did not differ in both the groups from 2nd hr of storage. Thus, polyamines and guanidines did not affect the rate of breakdown but inhibited the synthesis of the enzyme. The lower level of NR is in agreement with the higher protease activity obtained in the presence of the compounds which may inactivate NR. The decay of NR may also be due to NR inactivating system detected in the cotyledons. The inhibitor of NR was a dialyzable, heat-stable molecule. The effect of the inhibitor was increased by pretreatment with NADH while pretreatment with $K_3Fe(CN)_6$ abolished the effect. Spermine however, did not cause an increase in the effect of the inhibitor.

Both polyamines and guanidines inhibited the light

mediated increase in GS activity and increased the activity of AAT in light grown seeds. Subcellular fractionation showed that the compounds affected only the chloroplastic GS and AAT activity while the cytosolic ones were not affected. The compounds did not affect the enzyme activity when added to the assay system *in vitro*. The increase in the activity of AAT by the compounds was abolished by cycloheximide suggesting it to be a *de novo* synthesis of the enzyme. The increase in protease activity by the compounds was, however, not affected by cycloheximide and polyamines and guanidines probably activated the preexisting enzyme. The compounds did not affect the activity of the protease when they were added to the assay system *in vitro*.

Polyamines and guanidines thus appear to increase the growth of radish seedling by enhancing the mobilization of reserve protein rather than by enhancing the nitrogen assimilation pathway.

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