NITRATE ASSIMILATION

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Abstract

Although most legumes obtain nutrient nitrogen through symbiotic fixation of atmospheric nitrogen to ammonia, uptake and assimilation of nitrate from soil is also a significant process and often it is possible to modulate growth and productivity of nitrogen fixers as well as non-fixer legumes through the nitrate assimilation process. The process of nitrate reduction in legumes is well studied and the characterization of nitrate reductase, the rate limiting enzyme in the process, is almost similar to that in non-legumes. It is possible to manipulate process through the endogenous levels of carbohydrates and other photosynthates, nitrate supply, growth regulators and some of the environmental factors. The molecular genetics of nitrate assimilation process is now better understood than earlier. Transgenic tobacco (a non-legume) with altered levels of nitrate reductase activity have been produced, although it has not been possible to increase growth and productivity by increasing the expression of nitrate reductase gene in the transgenics. Clearly, the process is quite complex and many inter-linked metabolic aspects are also to be manipulated to achieve the desired effects.

1. Introduction

Legumes belonging to the family Leguminosae are important for their high nutritional value both for humans and cattle, primarily on account of high protein content in their seeds and also in the leaves. Morphologically the leguminous species range from small annual herbs such as pea and peanuts to large perennial trees such as tamarinds. The nitrogen requirement of many of the legumes is satisfied by the symbiotically associated microorganisms, which are present in specialised root nodules. However even in these nitrogen fixing species all the nitrogen requirement is not fulfilled by nitrogen fixation (Sinha, 1978) and they have to absorb and assimilate inorganic nitrogen from the soil to achieve optimum growth and productivity (Asthana and Srivastava, 1979). The soil nitrogen, which is usually in the form of nitrate is assimilated in to amino acids and other organic nitrogenous molecules through its reduction to nitrite and then to ammonia, by
the sequential action of the enzymes nitrate reductase and nitrite reductase. The ammonia is incorporated primarily to glutamic acid and glutamine. The enzyme nitrate reductase (NR, E.C.1.6.6.1-2) is considered to be the rate limiting enzyme in the pathway and its activity is often correlated with the protein and total organic nitrogen content of the tissue (Srivastava, 1980). A positive correlation between NR activity and growth had been observed in suspension cultures of soybean also (Bayley et al., 1972). Further, the activity of the enzyme is maximum during flowering and pod formation, when the requirement for nitrate assimilation for the synthesis of protein in the grains and over all pod development may be very high (Liu et al., 1997). Perhaps the nitrogen fixer legumes have developed nitrate assimilation potential as an evolutionary strategy to make maximum use of whatever nitrogen is available in the environment. When nitrate is available as a source of nitrogen the process of nodulation and the activity of the enzyme nitrogenase, which is responsible for N₂ fixation in symbiotically associated legumes, are inhibited (Streeter, 1988). This inhibition is apparently due to resistance in O₂ diffusion in short term exposure to nitrate and linked to nitrate metabolism in long term effects (Kaiser et al., 1997). Thus, nitrate reduction and assimilation is a significant process in the nitrogen nutrition of nitrogen fixer legumes also and it is possible to modulate growth and productivity of legumes through nitrate assimilation process.

2. Nitrate reduction in legumes

As mentioned earlier, both nodulating and non-nodulating legumes have the potential of absorbing and assimilating nitrate derived nitrogen. The nitrate absorbed from the soil is assimilated either in the roots or/and in shoots. Green leaves are undoubtedly the most active centres of active nitrate assimilation as they are for most other activities. However, the process depends upon the availability of nitrate in the leaves. For example, it has been demonstrated in soybean that nitrate reduction in the leaves is determined by the nitrate flux from the xylem. In other words, the export of nitrate from roots determines the nitrate reducing potential of the leaves (Gojon et al., 1991). However, for short term requirement the nitrate from the storage pool in the leaves is mobilized to the metabolic pool, where it is reduced and assimilated (Gojon et al., 1991). Stem also plays an important role in nitrate assimilation in herbaceous legumes of tropical origin (Andrews et al., 1984). The legumes of the temperate region have very little nitrate reduction in the stem and they reduce nitrate mostly in the leaves (Sprent, 1980). It is apparent from these accounts that the factors affecting the growth and metabolic status of the leaves will have a significant effect on nitrate assimilation process.

The distribution of nitrate reduction in roots or in leaves varies and is affected by the nitrate supply. At low nitrate concentrations, NR activity is localised primarily in the roots, and contribution by the leaf increases with the increasing level of nitrate supply to the plant (Glaab and Kaiser, 1993). Apparently, the supply of nitrate from roots to shoots increases with the increase in external nitrate concentration. As indicated in the earlier paragraph, the distribution of NR activity also varies according to the species. In legumes belonging to the Phaseolae (Glycine max, Phaseolus coccineus, P. vulgaris, Vigna radiata and V. unguiculata), the enzyme activity is substantially lower (15% of the total) in roots than