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Review Article

TRANSGENIC EFFORTS TO ENHANCE NITROGEN USE EFFICIENCY OF PLANTS

***Punjab Singh Malik**
**Department of Botany, DAV College, Muzaffarnagar-251001, India*
**Author for Correspondence*

ABSTRACT
The increasing food demands of a growing human population and the need for an environmental friendly strategy for sustainable agricultural development require significant attention when addressing the issue of enhancing crop productivity. Crop productivity is heavily dependent on the application of nitrogen (N) fertilizers. Increasing N fertilization levels, however, are subject to diminishing returns, quite apart from their deleterious impact on the environment. Improving N use efficiency (NUE) is therefore crucial for development of sustainable agriculture. Plant NUE is a complex trait determined by quantitative trait loci and influenced by environmental changes. The natural supply of soil N varies and is frequently limiting for plant growth and crop yield. Unraveling the molecular basis of how plants sense and respond to changes in N availability should enable the development of new strategies to increase NUE. This review discusses the latest advances in our understanding of the genetic manipulation of different genes that are responsible for nitrogen uptake and utilization or acquisition efficiency in plants.

Keywords: *Transgenic Plants, N Use Efficiency (NUE), Nitrate Transporter*

INTRODUCTION
In order for plants to grow, they must intercept light to enable photosynthesis to convert CO_2 into the basic molecules for metabolism such as sugars and amino acids. Water and nutrients are usually acquired from the soil and together with photosynthate, are used to create new plant tissues. At least 13 different nutrients are required by plants for normal growth. The mineral nutrient needed in greatest abundance by plants is nitrogen. N is quantitatively the most essential nutrient for growth and metabolism in the plant life cycle. In the biosphere nitrogen is available for plants in different forms, which include molecular nitrogen (N_2), volatile ammonia or nitrogen oxides (NH_3 or NOx), mineral nitrogen (NO_3 - and NH_{4+}) and organic N (amino acids, peptides etc.). Due to their high nitrogen demand plants can use almost all form of N with the exception that the use of molecular N_2 is restricted to plants species living in symbiosis with nitrogen fixing bacteria. However, the utilization of these N sources is determined strongly by environmental and particularly the soil conditions providing the different N form. Plant roots can absorb and assimilate N in many different forms, such as nitrate, ammonium, amino acids and other N-containing substances. Efficiency of nitrate uptake is largely determined by three interdependent factors that sense and respond to changes in nitrate availability: first, the functional properties of the root transporters in the roots; second, activities of high-affinity and low-affinity transporters at the plasma membrane of roots cells and third, the surface and architecture of the root system (Leran *et al.,* 2015). Most ammonium is taken up by the roots via ammonium transporters (AMTs) and assimilated within the organ by glutamine synthetase (GS); the product of glutamine (Gln) is further assimilated into glutamate via the GS/glutamine-2-oxoglutarate amino-transferase (GOGAT) cycle, or assimilated into asparagine by asparagine synthetase (Xu *et al.,* 2012). Nitrate is taken up by nitrate transporters (NRTs) in the roots, but the large part is translocated to the shoots (Leran *et al.,* 2013; Hsu *et al.,* 2013), where it is reduced to nitrite by nitrate reductase and further to ammonium by nitrite reductase (Crawford *et al.,* 2002). During the vegetative stage, the leaves are a sink for N that is later remobilized for use in the developing seeds, and metabolism of Gln in senescing organs is believed to play a crucial role in this dynamic N recycling (Kant *et al.,* 2011). In well aerated soil NO_3- is the most abundant form of nitrogen available for plants (von- Wiren *et al.,* 1997). The nitrogen use efficiency (NUE) includes N uptake, utilization or acquisition efficiency, expressed as a ratio of output (total plant N, grain N, biomass yield, total protein content, grain

yield) and input. The speed and precision of the transgenic approach enables one not only to test the candidate genes considered to be critical for NUE by over expressing them, but also to identify such genes by knock-out mutations. This review will focus on advances in the molecular understanding of the role of different genes that take part in enhancing the NUE in plants.

Genetic Manipulation of Nitrate Transporter Genes to Enhance Nitrogen Use Efficiency

Transgenic over expression of a *CHL1* cDNA (representing the constitutive HATS) driven by the cauliflower mosaic virus 35S promoter in a *chl1* mutant, recovered the phenotype for the constitutive phase but not for the induced phase (Liu *et al.*, 1999). Similarly, the NO contents in transgenic tobacco plants over-expressing the *NpNRT2.1* gene (encoding HATS), were remarkably similar to their wild-type levels, despite an increase in the NO_3 - influx (Vincent *et al.*, 1997). These findings indicate that genetic manipulation of nitrate uptake may not necessarily lead to concomitant improvement in nitrate retention, utilization or increasing NUE.

Genetic Manipulation of NR Gene to Enhance Nitrogen Use Efficiency

NR has long been considered to be the rate-limiting step in nitrate assimilation. Efforts to improve NUE by manipulating NR gene have yielded mixed results, with transformed *Nicotiana plumbaginifolia* plants constitutively expressing NR, showed a temporarily delayed drought-induced loss in NR activity, thereby allowing more rapid recovery of N assimilation following short term water-deficit (Ferrario-Mery *et al.*, 1998). The *NR* gene over expression by constitutive expression in transgenic plants caused a reduction in nitrate levels in tissues of tobacco (Quillere *et al.*, 1994), and potato (Djannane *et al.*, 2002). Nitrate reductase (NR) deficient mutant of *Nicotiana plumbaginifolia* totally impaired in the production of NR transcript and protein was restored for NR activity by transformation with a chimaeric NR gene fused with CaMV 35S promoter.

The transgenic plants were viable and fertile and expressed from one-fifth to three times the wild-type NR activity in their leaves. Although, there was similar leaf protein levels, total nitrogen, chlorophyll, starch and sugar were present (Vincentz and Caboche, 1991). While factors such as NO_3 – availability regulate flux through the pathway of N assimilation, the NR transformants were better equipped in terms of available NR protein, which rapidly restored N assimilation. However, no significant change on biomass accumulation could be attributed during the short term growth.

Genetic Manipulation of NiR Gene to Enhance Nitrogen Use Efficiency

Over expressing *NiR* genes in *Arabidopsis* and tobacco resulted in increased *NiR* transcript levels but decreased enzyme activity levels, due to the post translational modifications (Crete *et al.*, 1997, Takahashi *et al.*, 2001). The *NiR* enrichment improves assimilation of NO_2 in *arabidopsis thaliana* (Takahashi *et al.*, 2001). The *NiR* is regulated at post transcriptional level by nitrate or indirectly by NR activity. Over expressor of *NiR* with 35S promotor in *N. plumbaginifolia* shows an increase in NiR activity and protein level of transgenic plant grown in nitrate containing media but the same decreased in ammonia fed media (Crete *et al.*, 1997). Therefore, the utility of transgenic over-expression of NR/NiR for major improvements of NUE remains uncertain, though the possibility that different crops respond differently cannot be ruled out yet.

Genetic Manipulation of GS2 and Fd-GOGAT for Nitrogen Use Efficiency

Improvement in NUE via manipulation of plastidic GS2 and Fd-GOGAT genes has met with limited success. Transgenic tobacco plants with twofold over-expression of GS2 were shown to have an improved capacity for photorespiration and an increased tolerance to high light intensity. On the other hand, transgenics with reduced amount of GS2 had a diminished capacity for photorespiration and were photo inhibited more severely by high light intensity compared to control plants (Kozaki *et al.*, 1996). Over-expression of GS2 has also been reported in rice (Hoshida *et al.*, 2000), and tobacco (Ferrario-Mery *et al.*, 1998), with improved re-assimilation of ammonia in tobacco (Migge *et al.*, 2000). Studies on barley mutants with reduced Fd-GOGAT revealed changes in various nitrogenous metabolites, decreased leaf protein, Rubisco activity and nitrate content (Hausler *et al.*, 1994). While these studies hint at the potential of such transgenic attempts, most of them have been inconclusive regarding NUE so far, due to lack of physiological and agronomic data.

Genetic Manipulation of GS1 and NADH-GOGAT for Nitrogen Use Efficiency

Ectopic expression of cytosolic pea GS1 in tobacco leaves was suggested to provide an additional or an alternative route for the re-assimilation of photo respiratory ammonium, resulting in an increase in the efficiency of N assimilation and enhanced plant growth (Fuentes *et al.*, 2001). Efforts to raise more efficient GS1 transgenic lines have met with varying degrees of success (Vincent *et al.*, 1997; Chichkova *et al.*, 2001), with Man *et al.*, (2005) providing additional empirical evidence for enhanced nitrogen-assimilation efficiency in GS1 transgenic lines. Transgenic over expression and under expression studies to modulate the expression of NADH-GOGAT in alfalfa and rice plants (Schoenbeck *et al.*, 2000, Yamaya *et al.*, 2002), have implicated the involvement of GS1 in the export of N via phloem in senescing leaves. Over-expression of soyabean cytosolic glutamine synthetase (GS1) gene linked to organ specific promotors in pea plants grown in different concentration of nitrate showed that there was no significant increase in NUE of these over expressor plants although the activity of GS-1 was increased significantly (Fei *et al.*, 2003, 2006). Though these genes of secondary ammonia assimilation appear to be more viable candidates for improving NUE, the degree of success needs to be tested across crops and cropping conditions.

Genetic Manipulation of Glumate Dehydrogenase for Nitrogen Use Efficiency

Transgenic tobacco plants containing a *gdhA* gene encoding a NADP-GDH from *E.coli* under the control of the 35S promoter have also been produced (Ameziane *et al.*, 2000; Kisaka *et al.*, 2007). Increase in GDH activity has been observed during carbohydrates starvation, a process that could be reversed by the addition of soluble sugars (Robinson *et al.*, 1992; Athwal *et al.*, 1997; Dubois *et al.*, 2003). The function of enzyme in relation to carbohydrates metabolism has been observed by using 15N and 13C labeling experiments (Masclaux *et al.*, 2001). These studies clearly demonstrate that either in cells or in intact mitochondria, GDH was able to supply 2-oxoglutarate to tissues only when carbon was limited (Robinson *et al.*, 1992; Aubert *et al.*, 2001). A study performed on a maize null mutant deficient in the gene encoding gdh1 tended to indicate that roots were able to incorporate less 15N ammonium into total reduced nitrogen (Dubois *et al.*, 2003). Studies performed on transformed tobacco and corn plants over expressing bacterial GDH have shown increased tolerance to water stress accompanied by increase in biomass and yield (Ameziane *et al.*, 2000; Terce-Laforgue *et al.*, 2004). Development of quantitative genetics approaches have demonstrated the central role of NAD(P)H-GDH activity as a marker during the transition of sink leaves to source leaves (Hirel *et al.*, 2007).

Conclusion

Nitrogen sensing, signaling and responses are complicatedly regulated by various positive and negative components. It is very important to know how plants sense and respond to changes in nitrate availability to cope with diverse environmental conditions. Recent discoveries regarding the involvement of different proteins in regulation of nitrate responses and its crosstalk with other cellular signaling pathways have advanced our understanding of how plants sense and respond to changes in nitrate availability to cope with diverse environmental conditions.

Therefore, identification of new components of N signaling using systems-wide approaches and the understanding of their crosstalk with other nutrients and other cellular signaling pathways will provide new strategies to improve NUE in the major crops.

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