Front. Agric. China 2011, 5(3): 291–298
DOI 10.1007/s11703-011-1067-5

REVIEW

Molecular characterization, transcriptional regulation and function analysis of nitrate transporters in plants

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Abstract Nitrogen (N) is one of the most important inorganic nutrients in plants, and its less availability is an important limiting factor for plant growth in most agricultural systems. In this essay, the following aspects on nitrate transporters (NRT) in plants, such as the uptake features of nitrate (NO_3^-) mediated by roots, molecular characterization of NRTs, expression patterns and transcription regulation mechanisms of NRT genes, and functions of NRTs in plants, have been reviewed. Further studies of the molecular characterization, expression patterns, transcriptional regulation mechanisms, and functions of plant NRTs will provide a more detailed insight to understand the molecular mechanism of nitrate intake and nitrate transportation in plants. In the meantime, the researches on plant NRTs have potential roles for the improvement of nitrogen use efficiency (NUE) in crop production, as well as for the promotion of sustainable development in the agricultural ecosystem.

Keywords nitrate uptake, nitrate transporter, molecular characterization, transcriptional regulation, function

Introduction

Nitrogen (N) is one of the most important inorganic nutrients in plants, and its less availability is becoming an important limiting factor for plant growth in most agricultural systems. Around the world, approximately 85 to 90 million metric tons of nitrogen fertilizers is applied annually (Good et al., 2004). However, 50% to 70% of the applied nitrogen is lost from the plant soil system owing to its evaporation and leaching, resulting in air and water pollutions (Peoples et al., 1995). Therefore, improvement of nitrogen use efficiency (NUE) in crop production not only has a potential role on saving the production investment, but also has the additional values on protection of the agricultural ecosystem.

Nitrogen (N) can be absorbed by plant roots in several different forms, including nitrate (NO_3^-), ammonium (NH_4^+), and organic forms which are chiefly amino acids. Among them, NO_3^- is usually the most abundant source of N. Generally, NO_3^- is readily dissolved in soil water and is very mobile in the soil profiles.

The NO_3^- uptake by roots from the soil and the cellular transportation of NO_3^- in plants are mediated by nitrate transporters (NRTs) using the proton gradients between both sides of the membrane as driving power. It is recorded that the first eukaryotic NO_3^- transporter gene was isolated over 15 years ago from the fungus *Aspergillus nidulans* (Unkles et al., 1991). Subsequently, a gene encoding an NO_3^- transporter has also been isolated from *Arabidopsis*. Past several years, more and more nitrate transporter genes have been identified in plant species, owing to the rapid development in the scope of plant molecular biology. The molecular features, expression pattern and transcriptional regulation mechanism, and functions of the plant NRTs have been extensively studied.

Features of nitrate uptake mediated by plant roots

Early studies suggested that plants have developed four types of transport system to cope with the variations in nitrate concentrations in cultivated soils (Glass and Siddiqi, 1995), including a high-affinity transport system (HATS) generally taking up nitrate at low external concentrations (1 μmol/L – 1 mmol/L), a constitutive system (cHATS) functioning as

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Received September 19, 2010; accepted October 5, 2010
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taking up the external nitrate with an unchangeable pattern, an inducible system (iHATS) usually regulated by nitrate status in the external medium and a low-affinity transport system (LATS) displaying linear kinetics under high external nitrate supply condition (1 mmol/L) (Glass and Siddiqi, 1995).

Based on the transportation features mediated to NO_3^-, the nitrate transporters involved in the intake and distribution of NO_3^- in plants could be defined into NRT1 and NRT2 families. In *Arabidopsis*, totally 53 members of NRT1 and 7 members of NRT2 have been identified thus far. The coordinated actions of NRT1 and NRT2, the two nitrate transporter families, provide the molecular basis for the nitrate uptake and nitrate transportation in plants in the four patterns as mentioned previously.

Molecular characterization of plant nitrate transporters

Sequence similarity analysis shows that there are no sequence homologies between the NRT1 and NRT2 families (Trueman et al., 1996; Galván and Fernández, 2001). According to the classifications based on the secondary and tertiary structures of the proteins, the NRT1 transporters belong to the family of proton-dependent oligopeptide transporter (POT) (Paulsen et al., 1994). At the amino acid sequence level, the NRT1 proteins are predicted to have 12 transmembrane (TM) domains. Among them, the first six domains at the N-terminal are separated from the second six domains by a long hydrophilic loop.

The NRT2 transporters, also referring to nitrate/nitrite porter (NNP) family, belong to the major facilitator superfamily (MFS) (Pao et al., 1998). The secondary structure analysis reveals that the NRT2 proteins also contain 12 TM domains. Generally, a cytosolic loop is located at the position between the first six TM domains and the second six domains. In addition, a most striking feature in NRT2 proteins could be highlighted that they all share two conserved sequence motifs, including D/N-R-X-G-R-R/K between TM domains 2 and 3, and I-X2-R-X3-G-X3-G before and within TM domain 4.

Expression patterns and transcription regulation of plant nitrate transporters

In plants, the net NO_3^- uptake by roots is delicately regulated by demand which is determined by the concentrations of N metabolites, as well as the amount of NO_3^- in tissues (Vidmar et al., 2000). Feedback regulation of the nitrate transportation occurs from leaf to root, generally more specifically via the concentrations in the phloem. For that, the N metabolite, glutamine, plays a crucial role in the regulation process (Tillard et al., 1998). At the cellular level, the NO_3^- uptake mediated by the nitrate transporters can be fulfilled via various molecular mechanisms.

Gene expression

Generally, the expression of *NRT1* members is shown in a constitutive pattern. However, a recent study on *Arabidopsis AtNRT1.6* shows that the expression of this *NRT1* member is only detectable in reproductive tissues including the vascular tissues of the silique and funiculus. The expression of this gene can be increased immediately after pollination, suggesting that the expression patterns in the *NRT1* families are not always constitutive.

Among the seven *NRT* genes identified in *Arabidopsis* and other plant species, extensive studies have been conducted on the NRT2 members. We find that the members in *NRT2* group can be induced and delicately regulated by the depressed NO_3^- status.

Thus far, the regulation of *NRT2.1* expression has been thoroughly investigated at the mRNA level. In *Arabidopsis*, it is observed that the transcripts of *NRT2.1* are mainly located at epidermis and cortex of the mature root regions (Nazoa et al., 2003). Responding to the external NO_3^- conditions, the expression of *NRT2.1* is frequently induced by deprivation of NO_3^- and repressed by high N status through a negative feedback regulation which is delicately modulated by the reduced N metabolites such as NH_4^+ or amino acids (Nazoa et al., 2003). This could be observed clearly by detecting the transcripts of several *NRT2* genes in different plant species. In *Arabidopsis* and *N. plumbaginifolia*, the *NRT2* genes all prove to be induced by very low levels of NO_3^- (10–50 μmol/L) (Krapp et al., 1998; Filleur and Daniel-Vedele, 1999) and reach to the transient maximum in a short time. In barley, *NRT2* mRNAs accumulate to the highest level when the NO_3^- concentration maintains at 50 μmol/L under quasi steady-state conditions (Vidmar et al., 2000). In addition, the *NRT2.1* transcripts are also stimulated by light and sugars (Lejay et al., 2003).

The members of NAR2s act as the components of the MRT2/NAR2 system, also in a similar expression pattern to that of NRT2. It is observed that the expression pattern of *AtNAR2.1* almost exactly parallels to that of *AtNRT2.1* (Krouk et al., 2006). Furthermore, it is suggested that the two components *AtNRT2.1/NAR2.1* should be downregulated by NO_3^- itself via a mechanism specifically triggered by the dual-affinity transporter NRT1.1 and unrelated to the feedback repression loop exerted by the cellular N metabolites (Muños et al., 2004; Krouk et al., 2006).

Correlation analysis shows that the transcript level of *NRT2.1* is generally positively correlated with the HATS activity for NO_3^- uptake in plants, which provides the evidence that the transcriptional regulation of *NRT2.1* family members in plants plays a major role in controlling the root high-affinity NO_3^- uptake.

Transcriptional regulation of the nitrate transporter genes in plants

With the rapid progress of modern molecular biology, the regulatory mechanisms that the nitrate transporter genes are responsible for nitrogen responses in plants have been investigated (Leydecker et al., 2000; Zhang and Forde, 2000; Gutiérrez et al., 2005, 2007b). At the transcription level, several transcription factors putatively involved in regulating of transcription of the nitrate transporter genes have been identified, of which a MADS-box type transcription factor *ANR1* is induced by nitrogen deprivation and further controls lateral root branching and plant responses to external nitrate (Zhang and Forde, 1998; Gan et al., 2005). Under nitrogen-starvation condition, a Dof transcription factor was observed to play an important role in the improvement of plant nitrogen use efficiency (Yanagisawa et al., 2004).

Recently, it has been found that nitrate also acts as a signal in addition to serving as a nutrient. When plants are subjected to NO_3^- starvation condition, genes in the nitrate assimilation pathway (*NRT*, *NIA*, and *NiR*) are first rapidly induced (Wang et al., 2007). Other genes, required for reprogramming carbon metabolism and providing chemical energy for reduction and assimilation, are also induced subsequently (Wang et al., 2000, 2003, 2004; Stitt et al., 2002; Scheible et al., 2004; Fritz et al., 2006). Based on the transcriptome analyses, over 1500 genes are induced or repressed by nitrate within 20–180 min after treatment (Wang et al., 2003, 2004, 2007; Scheible et al., 2004; Gutiérrez et al., 2007a). Extension of the NO_3^- starvation period can result in the plant changes in root growth, development and architecture, in root-to-shoot ratios, and in germination rates (Forde, 2002; Alboresi et al., 2005; Filleur et al., 2005; Walch-Liu et al., 2005, 2006; Forde and Walch-Liu, 2009), owing to the delicate and complicate actions of the gene network responding to the nitrate.

The *NRT2* genes responding to the external NO_3^- are also regulated by distinct transcription factors that act as the key mediators in the signal transduction pathway. Therefore, further identification of the transcription factors involved in the regulation of NRT2 genes will be helpful to further elucidation of the transcription regulation mechanism in which NRT2 genes respond to the NO_3^- as well as to providing a more detailed new insight to understand the integrated nitrate signaling transduction mechanism.

Post-translational regulation of NO_3^- transporters

As the nitrate transporters are targeted onto the membranes at a cellular or subcellular level, the post-translational regulation of the NO_3^- transporters generally occurs before their involvement in NO_3^- intake and the transportation. For a long time, physiologic studies using chemical inhibitors have demonstrated that the NO_3^- uptake process is involved in the protein synthesis metabolism (Agüera et al., 1990).

In barley, the roots subjected to nitrate uptake inhibitors show that the iHATS has a relatively long half-life compared to the transportation system associated with the potassium uptake (Behl et al., 1988). In the yeast *Hansenula polymorpha*, the degradation of an NRT2-type protein (YNT1) in the vacuole is tightly associated with the removal of this transporter from the plasma membrane (Navarro et al., 2006). When the nitrogen metabolite, glutamine, is supplied to the cells, the YNT1 protein is feasible to be ubiquitinylated via the 26S/protesome pathway and is rapidly degraded by a specific proteinase A. The degradation of *Arabidopsis* AtNRT2.1 also shows a similar mechanism as the YNT1, providing a direct evidence for proving a fact that the posttranslational regulation of NRT2 in plants is dependent on the feedback of N metabolites.

As an important event in the signal transduction pathways, phosphorylation is also suggested to be involved in regulating the NRT2 activities. In barley, a number of conserved protein kinase C recognition motifs in the N- and C-terminal domains have been identified in HvNRT2.1 (Forde, 2000), being similar to that of AtNRT1.1 (Liu and Tsay, 2003). Based on the sequence similarity analysis, some potential 14-3-3 regulatory sites in the NRT2s have been identified. In tobacco, the NRT2.1 members share a perfect 14-3-3 binding consensus at the C terminus. In *Arabidopsis*, a perfect 14-3-3 binding motif with a consensus sequence RSXSXP has been identified in *AtNRT2.4*. These results suggest that the HTS system activities are mediated by 14-3-3, and the regulation effects are controlled via the phosphorylation in NRT2s. In addition, the 14-3-3 proteins can also regulate the activity of both NO_3^- reductase and glutamine synthetase. Thus, the uptake and assimilation of nitrate are performed via a delicate and coordinated mechanism involved in the posttranslational modifications of the NRTs.

Functions of nitrate transporter genes in plants

Functions of LATS type NRTs

Compared with HATS (generally NRT2 type), LATS (NRT1 type) is involved in the uptake of NO_3^- when the available concentration of NO_3^- in the soil is chiefly found in the mmol/L range. Currently, the molecular characterization of the LATS responding to the external NO_3^- is clear at the transcription level.

Some members of NRT1s in the *NRT1* (PTR) family are nitrate transporters (*NRT1*), while others are dipeptide transporters (PTR) (Tsay et al., 2007). In *Arabidopsis*, 53 *NRT1*(PTR) genes, including NRT1.2 (involved in nitrate uptake), NRT1.4 (involved in petiole nitrate storage), and NRT1.5 (involved in xylem loading), all prove to be the transporters with low-affinity features as we initially expected (Huang et al., 1999; Chiu et al., 2004; Tsay et al., 2007).

As an exception, *Arabidopsis* AtNRT1.1 (CHL1) was originally identified as a contributor to LATS (Tsay et al., 1993). The deletion mutants of AtNRT1.1 showed a deficiency in low affinity uptake when growing on a mixed

NO_3^- and NH_4^+ supply (Touraine and Glass, 1997). Afterwards, the gene transcription analysis and the LATS activity assays suggest that AtNRT1.1 is mainly involved in the uptake process at about 5 mmol/L external NO_3^- concentrations (Okamoto et al., 2003). However, the LATS is generally assumed to constitutively present in roots, and the expression of *AtNRT1.1* is NO_3^--inducible. In addition, with a phosphorylation switch between high and low affinity ranges of nitrate uptake, *AtNRT1.1* is presumed to actually act as dual affinity (Liu and Tsay, 2003). Gene deletion lines of *AtNRT1.1* show a 45% decrease in LATS (Huang et al., 1999), suggesting that *AtNRT1.1* plays a role to some extent in regulation of the NO_3^- uptake under the nitrogen-deficient condition.

Another number in LATs family with distinct expression pattern is *AtNRT1.2* which is not expressed constitutively in every location of the cells, tissues, and organs but only specifically expressed in root epidermal cells, with a K_m for NO_3^- around 6 mmol/L in oocytes (Huang et al., 1999). Antisense expression of *AtNRT1.2* results in a 50%–70% decrease in LATS activity (Huang et al., 1999). *AtNRT1.3* and *AtNRT1.4*, other two members of NRT1 type in *Arabidopsis*, also behave with variations on the expression patterns. *AtNRT1.3* is induced by NO_3^- in leaves and repressed in roots, suggesting that it is not a significant contributor to LATS (Okamoto et al., 2003). The *AtNRT1.4* has a distinct specific expression pattern in leaf petioles, where it plays a role in NO_3^- accumulation within the tissues (Chiu et al., 2004). Therefore, although the members in LATS type are expressed constitutively, more and more molecular information on the NRT1 members plays a key role in nitrate uptake in plants, which still remains to be explored.

Functions of HATS type genes

The functions of HATs in plants have been largely identified in the past several years. In *Arabidopsis*, it is observed that the mutants are defective in the HATS activity with impaired expression of *AtNRT2.1* and *ATNRT2.2*, which are two NRT2 members sharing in high sequence similarities with each other (Filleur et al., 2001; Orsel et al., 2004). In the meantime, the homolog of *AtNRT2.1* and *ATNRT2.2*, similar to that in *Arabidopsis*, is also detected in other plant species, for example, *Chlamydomonas* (Quesada et al., 1994) and rice (Araki and Hasegawa, 2006). Therefore, the NRT2 family members are suggested to exist with a pattern of parallel gene pairs in the genome of plants, indicating that the *NRT2* genes encoding major components of HATS may have evolved by gene-duplicating events in plants.

The observed significant correlations between the transcript abundance of *AtNRT2.1* and the high-affinity nitrate imply that *AtNRT2.1* exerts a major role in the high-affinity nitrate transport in *Arabidopsis* (Okamoto et al., 2003). This correlation is also evident when plants are subjected to different levels of nitrate and following exposure to various inhibitors of nitrate assimilation (Zhuo et al., 1999; Vidmar et al., 2000b).

Although the NRT members in plants generally contribute to the iHATs system for NO_3^- uptake, part of NRT2 members can be endowed with dual roles for plants. Recently, an *Arabidopsis* mutant uniquely disrupted in expression of *AtNRT2.1* has demonstrated that the NRT2 members contribute to both the nitrate-transportation systems of iHATS and cHATS systems (Li et al., 2006).

Functions of the two-component system of NRT2/NAR2.1

Generally, it is known that the NO_3^- uptake in plant roots is mainly mediated via the NRT2 members. However, the proteins except for NRT2 of NAR family are presently found to be involved in nitrate transportation process, via coordination with some NRT2 members. For example, the NO_3^- transport mediated by *Arabidopsis AtNRT2.1* requires a second protein as the coordinator (Orsel et al., 2006). The NO_3^- transportation process in *Xenopus* oocytes is mediated by *Arabidopsis* AtNRT2.1 and barley NRT2, which, as the homolog of AtNRT2, are both necessary to co-express a NAR2 protein (Zhou et al., 2000a; Tong et al., 2005; Orsel et al., 2006). In addition, mutants of NAR2 (*atnar2.1-1*) show a stronger phenotype with high deficiency in HATS than the *NRT2.1* mutants in *Arabidopsis* (Orsel et al., 2006).

Expression analysis identifies that the transcripts of *AtNAR2.1* are mainly modulated by the N status in plants. However, AtNAR2.1 does not exert roles on the regulation of *AtNRT2.1* expression. As being much smaller than AtNRT2.1 in the length of polypeptide, AtNAR2.1 is predicted to be sorted via endoplasmic reticulum (ER) by an N-terminal secretory pathway signal (Orsel et al., 2006). Further analysis by fusing of AtNRT2.1 with green-fluorescent protein (GFP) in the *atnar2.1-1* mutant background suggests that the transporter protein, AtNRT2.1, is not correctly targeted to the plasma membrane (Orsel et al., 2007), verifying that AtNAR2s are indispensable for targeting NRT2s to the plasma membrane (González et al., 2005). These results suggest that the NAR2 proteins are not a new type of NO_3^- transporters themselves (Okamoto et al., 2006), but rather they play a role in facilitating the targeting of some NRT2s to the plasma membrane.

Mutation analysis of distinct amino acid residue in the conserved domain in NAR2s demonstrates that one or several amino residues are critical to the regulation of the NAR2s function. For example, a mutation on an aspartate residue by replacement with asparagine in a highly conserved region of AtNAR2.1 reveals a great decrease in the NO_3^- uptake (Kawachi et al., 2006), resulting in a dwarf phenotype when growing on 5 mmol/L NO_3^-, the LATS concentration range of NO_3^- uptake (Okamoto et al., 2006; Orsel et al., 2006). Under nitrate-deficient condition, the dwarf plant phenotype is much more evident. However, there is no difference between the growth of wild-type and the *atnar2.1-1* mutant plants when supplied with enough N fertilizer (Orsel et al., 2006). The fact that the threshold NO_3^- concentration for the phenotype has been altered by a point mutation in *AtNAR2.1*, possibly owing to the modification of the affinity of AtNRT2.1 for NO_3^- after the mutation of a distinct amino acid residue in the conserved domain in NAR2s.

Various function of plant nitrate transporters

Roles in source-to-sink remobilization of nitrate

Recently, the NRT members have been demonstrated to exert various effects in plants, in addition to uptake and transportation of nitrate (Fan et al., 2009). For instance, *Arabidopsis* NRT1.7, one member of NRT1 family, was demonstrated to be responsible for the source-to-sink remobilization of nitrate. Based on the approaches of immunoblots, quantitative RT-PCR, β-glucuronidase reporter analysis, and immunolocalization, it is clearly confirmed that *NRT1.7* is expressed in the phloem of leaf minor veins, and the expression levels are increased coincidentally with the leaf source strength. In the loss-of-function mutants of *nrt1.7*, more nitrate is present in the older leaves. In the meantime, less $^{15}NO_3^-$ spotted on old leaves is remobilized into N-demanding tissues, and concurrently, less nitrate is detected in the phloem exudates of old leaves (Fan et al., 2009). These data indicate that NRT1.7 is responsible for phloem loading of nitrate in the source leaf to allow nitrate transport out of older leaves and into younger leaves. These results provide an evidence for the fact that nitrate itself, in addition to organic forms of nitrogen, is remobilized. Moreover, the remobilized nitrate mediated by phloem plays critical roles in sustaining vigorous plant growth under nitrogen deficiency conditions.

Involvement in nascent organ growth

The early identified NRT1 number in *Arabidopsis*, *AtNRT1.1* (*CHL1*), with dual affinities in nitrate transportation, is involved in controlling of the nascent organ development during vegetative and reproductive growth (Guo et al., 2001). Using fusions of *AtNRT1.1* and *GFP/GUS* marker genes, the *CHL1* expression is found to be concentrated in the tips of primary and lateral roots. A time-course study showed that *CHL1* is activated in the primary root tip early in seedling development and at the early stages of lateral root formation. In the meantime, a strong *CHL1* expression is also detected in shoots and concentrated in young leaves and developing flower buds. Further, the expression patterns confirmed by immunolocalization suggest that *CHL1* is functional specifically in the growth of developing organs. Mutants *chl1* showed a reduction in the growth of nascent roots, stems, leaves, and flower buds. Observation of the growth of nascent primary roots in the mutants found that the growth was inhibited even in the absence of added nitrate, whereas elongation of lateral root primordia was inhibited specifically at low nitrate. At the development stage, *chl1* mutants also displayed a late-flowering phenotype (Guo et al., 2001). These results clearly showed that *CHL1* is activated and functions in the growth of nascent organs in both shoots and roots during vegetative and reproductive growth.

Involvement in early embryo development and seed nitrate contents

With more and more studies on plant NRTs that have been conducted, a novel role of *Arabidopsis* NRT1.6 has been explored up to date (Almagro et al., 2008). RT-PCR, in situ hybridization, and β-glucuronidase reporter gene analysis show that the expression of *NRT1.6* is only detectable in reproductive tissues (the vascular tissue of the silique and funiculus) and that the expression increases immediately after pollination, suggesting that NRT1.6 is involved in delivering nitrate from maternal tissues to the developing embryo. The mutant analysis on *nrt1.6* indicates that the amount of nitrate accumulated in mature seeds can be reduced with an increase in the seed abortion rate. The abnormalities (i.e., excessive cell division and loss of turgidity) found in the mutants are mainly focused on the suspensor cells at the one- or two-cell stages of embryo development. However, the seed abortion rate of the mutant is reduced when grown under N-deficient conditions, suggesting that nitrate requirements in early embryo development can be modulated in response to external nitrogen changes mediated by AtNRT1.6 (Almagro et al., 2008). Our study indicates that nitrate is important for early embryo development. Several distinct NRT members play a crucial role in the coordinate regulation of the nitrate status during the embryo development.

Nitrate can also be accumulated in seeds and affect seed dormancy (Alboresi et al., 2005). A recent study shows that *Arabidopsis NRT2.7*, one member of the NRT2 family, is involved in nitrate accumulation in seeds (Chopin et al., 2007). NRT2.7 is located in the tonoplast and expressed in developing seeds, particularly at the end of seed maturation. The nitrate content of the *nrt2.7* mutant in mature seeds can be reduced, and fresh harvested seeds are more dormant than wild-type seeds (Chopin et al., 2007). Together, it is clear that ATNRT2.7 plays a specific role in nitrate accumulation in plant seeds.

Functions in the regulation of stomatal opening and drought susceptibility

The movement of guard cells in stomatal complexes can control water loss and CO_2 uptake in plants. Until now, the stomatal opening process is also regulated to some extent by the NRT actions. Based on the examination of the dual-affinity nitrate transporter gene *AtNRT1.1* (*CHL1*), the functions of this NRT1 member on the modulation of guard cells and stomatal aperture have been clarified (Guo et al., 2003). For that, the loss-of-function mutant *chl1* of plants grown under the nitrate conditions has been examined and can reduce the stomatal opening as well as the transpiration rates in the light or when deprived of CO_2 in the dark compared with wild-type plants. These effects result in enhanced drought tolerance in *chl1* mutants. In the meantime, the *chl1* mutants show the reduced nitrate accumulation in guard cells during stomatal opening without the nitrate-induced depolarization in the guard cells, in contrast to the wild-type guard cells, where the depolarization is induced by nitrate through threefold increasing of the nitrate concentrations during stomatal opening. Therefore, the NRT member AtNRT1.1 functions in stomatal opening regulation under nitrate conditions, in addition to its dual-affinity role in mediation of the NO_3^- intake and distribution in plants (Guo et al., 2003).

Remarks and conclusion

Physiologic measurements demonstrate that different transport systems of two types of affinity have different nitrate (NO_3^-) uptake by plant roots (high and low). In plant species, especially in the model plant *Arabidopsis*, more genes encoding both of these two uptake systems NRT1 and NRT2, respectively, have been identified during the past two decades. Most of the NRT1 members are constitutively expressed, playing key roles in NO_3^- intake when the available NO_3^- concentration in the soil is over 1 mmol/L, while the NRT2 members are chiefly contributed to HATS, which plays crucial roles in the intake of soil NO_3^- when the external NO_3^- is lower than 1 mmol/L or plants encounter a dramatic decline causing a nitrogen-starvation condition (μmol/L NO_3^-).

Several different regulatory mechanisms have been identified in response to the environmental NO_3^- status. Some of NRT2 members, such as *AtNRT2.1*, one of the membrane transporters, encode HATS and show a feedback regulation on the expression pattern, resulted from the signal transduction pathway initiated by the external NO_3^- variation. In the meantime, posttranslational regulations such as the phosphorylation are also involved in the regulation of functions of nitrate transporters.

From the range of concentrations in the soil NO_3^-, it is clarified that the NO_3^- inducible part of HATS could also act as a sensor for root NO_3^- availability. In addition to the intake and cellular distribution of NO_3^-, other various roles have been identified for the nitrate transporters in plants, including source-to-sink remobilization of nitrate, involvement of nascent organ growth, involvement of early embryo development and seed nitrate contents, and functions in the regulation of stomatal opening and drought susceptibility. With more and more nitrate transporters in plants identified, the molecular characterizations, expression patterns at the spatial and temporal level, delicate transcription regulation mechanisms, and their functions need further studies. Meanwhile, the signal components that result in the responses of *NRT2* genes at the levels of transcription or post-transcription, as well as translation and post-translation, also need to be further elucidated in the future. The integrated knowledge of the nitrate signaling and the transduction system covering the nitrate transporters will be helpful to shed light on the nitrogen use in plants with high efficiency to promote the development of the sustainable agriculture in China and worldwide.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No. 30971773), the Natural Science Foundation of Hebei (No. C2008000325) and the key Laboratory of Growth Regulation in Hebei.

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