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Review article

Research advances on nitrate nitrogen reutilization by proton pump of tonoplast and its relation to nitrogen use efficiency

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Abstract

Large amount of $N0_3^-$-N are accumulated in vacuole, and cannot be timeously reducted, reutilized and transported into cytoplasm. It is the main reason for great $N0_3^-$-N accumulation in vacuole and nitrogen (N) use efficiency cannot be further improved. Transport mechanism of $N0_3^-$-N across tonoplast is explained in this paper, there are two proton pumps (H^+-ATPase and H^+-PPase) on tonoplast with absolutely different biology functions and physical characteristic. Mg·ATP and Mg·PPi are the specific substrates of H^+-ATPase and H^+-PPase respectively, hydrolysis H^+ is pumped into vacuole, and contribution to build electrochemical proton gradient between cytoplasm and vacuole. $N0_3^-$-N transport from vacuole to cytoplasm greatly depends on electrochemical proton gradient, $N0_3^-$-N transport from cytoplasm to vacuole is mainly achieved by vacuole $H^+/N0_3^-$ antiport system, while symport system (vacuole $N0_3^-$-N combined with anion) is of benefit for vacuole $N0_3^-$-N transporting into cytoplasm. $N0_3^-$-N transported by proton pump of tonoplast is influenced by NR activity in cytoplasm, $N0_3^-$-N can be continuing assimilation and reduction by NR in cytoplasm, and accelerating vacuole $N0_3^-$-N transported into cytoplasm. These results will supply references and research forecast for further study on efficiency and practicable methods of N utilization, and improving reuse efficiency of $N0_3^-$-N in plant tissues.

Keywords: Nitrate nitrogen; Proton pump of tonoplast; nitrogen use efficiency.
Abbreviations: N-nitrogen, V-ATPase-vacuole H^+-ATPase, V-PPase-vacuole H^+-pyrophosphatase, NR-nitrate reductase, DCCD-N,N′-dicyclohexylcarbodiimide, EDTA- ethylenediaminetetraacetic acid disodium salt, NRAact-activity nitrate reductase, NRAmax- maximum nitrate reductase.

Introduction

Nitrogen (N) fertilizer yearly consumption of China is 25 million tons (pure N) and leads the world. However, reward decline of N has become more and more serious, yield increasing of crop is stopped or even reduced with N fertilizer application level increased (Shen et al., 2003; Rahimizadeh et al., 2010). In addition, $N0_3^-$-N concentration in plant tissues were increased sharply with N fertilizer application level increased, it has become the mainly limiting factor for high quality of agricultural production (Liu et al., 2009). Therefore, improved N fertilizer use efficiency based on plant mechanisms, and exploitation of high N use efficiency potential of plants has become very important in plant nutrition research area in recent years (Rahman et al., 2009; Liu et al., 2006; Zhang et al., 2010). $N0_3^-$-N is main resources of N in plant tissues, and accumulated large amounts in plant tissues also (Miller et al., 2008). Over 90% volume of mature cell is occupied by plant vacuole, while $N0_3^-$-N concentrations in vacuole and cytoplasm are generally 30-50 $mol \cdot m^{-3}$ and 3-5 $mol \cdot m^{-3}$ respectively (Chen et al., 2005; Martinoia et al., 2000). Thus, vacuole is main tissues for $N0_3^-$-N accumulation, contribution of vacuole $N0_3^-$-N reutilization to N efficiency of plant cannot be ignored during N efficiency studying (Martinoia et al., 1981). Since NR is mainly located in cytoplasm, $N0_3^-$-N reduction is mainly processed in cytoplasm also (cytoplasm is referred to as $N0_3^-$-N metabolic pool). In addition, there is no NR in vacuole, which is known as $N0_3^-$-N storage pool. Accumulated $N0_3^-$-N in vacuole cannot be rapidly transported into cytoplasm, and transport speed depends on crop varieties and genotypes (Wang et al., 2008; Xu et al., 2007; Zhao et al., 2010). Zhang et al., (2007, 2009) reported that, regardless of N application levels, $N0_3^-$-N reuse efficiency of high N efficiency and high potential oilseed rape genotypes are higher than low N efficiency genotypes, it was suggested that N use efficiency can be obviously improved by $N0_3^-$-N reusing in plant tissues. Generally, assimilatory power of $N0_3^-$-N in cytoplasm of leaves is adequate. So, $N0_3^-$-N reuse efficiency of plant to a great extent depends on transport of $N0_3^-$-N from vacuole to cytoplasm (Cao et al., 2009). There is a definite relationship of

$N0_3^-$-N distribution and accumulation between vacuole and cytoplasm, realied by inspection of interaction mechanisms of $N0_3^-$-N metabolic and storage pools, whichare beneficial to discovery of physiological potential of N use efficiency in plant tissues, avoiding $N0_3^-$-N which is over accumulated in plant tissues (Jia et al., 2005). The above processes are all closely related to transport system of $N0_3^-$-N on tonoplast and investigation of this transport system can provide practicable technology for controlling concentration of $N0_3^-$-N in plant tissues and improving N efficiency (Martinoia et al., 2007). Many researches are involved in mechanisms of $N0_3^-$-N transport system on tonoplast, but the relationship between proton pump activity of tonoplast and $N0_3^-$-N transport and its influence on N efficiency are little reported (Zhao et al., 2010; Huang et al., 2006). In order to further study $N0_3^-$-N transport mechanisms of tonoplast and investigate effects of transport system on N efficiency, one should inspect the relative contribution of two proton pump on tonoplast to $N0_3^-$-N reuse and its dynamic resources. By studying responses of the two proton pump to different genotypes and N application levels can further define transport mechanisms of $N0_3^-$-N by proton pump of tonoplast and is providing a scientific basis for excavating plant potential of $N0_3^-$-N use efficiency and accelerating higher $N0_3^-$-N reuse of plant vacuole.

$N0_3^-$-N accumulation and utilization in plant tissues and their relation to N efficiency

$N0_3^-$-N accumulation and utilization in plant tissues are important contents of crop N use research and is closely related to $N0_3^-$-N concentration and N use efficiency in plant tissues. $N0_3^-$-N is the main N resource of plant and a large amount of $N0_3^-$-N can accumulate in plant tissues. Under extreme conditions, $N0_3^-$-N accumulation can account for morethan 2% of plant fresh weight, and 17% - 24% of plant dry weight (Huang et al., 2006). Plant $N0_3^-$-N is mainly distributed in vacuole, N use efficiency of plant is closely related to reuse ability of vacuole $N0_3^-$-N (Zhao et al., 2010). On account of $N0_3^-$-N is a reduction process in cytoplasm, and nitrite reduction occurs in chloroplast, in general, assimilatory ability of cytoplasm in leaves for $N0_3^-$-N reduction is adequate. Therefore, $N0_3^-$-N reuse in plant tissues to a great extent depends on transport of $N0_3^-$-N from vacuole to cytoplasm and its reverse transport (Shen et al., 2003; Lea et al., 2004). $N0_3^-$-N pool in plant tissues are separated into metabolic pool (cytoplasm $N0_3^-$-N) and storage pool (vacuole $N0_3^-$-N), the former quantity are few, but strongly influence on NR activity, the latter quantity are large, but bear little relation to NR activity (Granstedt et al., 1982; Glass et al., 2002). For some plants, vacuole $N0_3^-$-N can be transported into cytoplasm and assimilated rapidly, when N application deficient, and maintains low $N0_3^-$-N concentration level in vacuole. Vacuole $N0_3^-$-N concentration is increased significantly again and maintains high balance level until N application level recovery. These genotypes are generally tolerant under N deficiency or high N use efficiency conditions. However, vacuole $N0_3^-$-N concentration is still maintained at high levels in many plant tissues, when there is N application deficiency. Therefore, few $N0_3^-$-N are existing in cytoplasm and apparent N deficiency situations. These genotypes are generally sensitive under N deficiency condition or low N use efficiency (Huang et al., 2006). On the other hand, under the sufficient N application condition, how to maintain relative low vacuole $N0_3^-$-N concentration, and keep low $N0_3^-$-N concentration in plant tissues has become an impotant topic for these mechanisms can improve N use efficiency (Shen et al., 2003). All of the above cases are suggest a common question, that is the transport progress of $N0_3^-$-N from vacuole to cytoplasm by tonoplast system; definitely, this transport system can provide a scientific basis for controlling $N0_3^-$-N concentration in plant tissues and increasing N use efficiency.

Transport mechanism of $N0_3^-$-N across the tonoplast

Study on transport system of nutrient ions in cell membrane and tonoplast is one of the important aspects for definition of genotype differences of plant nutrition and is a basis for further study on molecular biological characteristics of nutrient ions transported on membranes (Leij et al., 1998). Generally, ions across membranes are achieved by membrane transport protein (Dschida et al., 1995). Currently, study on transport protein of $N0_3^-$-N in cytoplasm membrane is far deeper and extensive than transport protein of $N0_3^-$-N in tonoplast (Angeli et al., 2006). This is the same scenario with plant cytoplasm membrane, where there are large amount of nutrients transport proteins on tonoplast also, including activity ion pump, ion carrier, ion channels and receptor protein (Facanha et al., 1998). Research shows that there aretwo proton pumps (H^+-ATPase and H^+-PPase) on tonoplast with absolutely different biology functions and physical characteristics. Mg·ATP and Mg·PPi are the specific substrates for H^+-ATPase and H^+-PPase respectively, hydrolysis H^+ is pumped into vacuole, and contributes to building an electrochemical proton gradient between cytoplasm and vacuole. $N0_3^-$-N transport from vacuole to cytoplasm greatly depends on an electrochemical proton gradient, $N0_3^-$-N transport from cytoplasm to vacuole is mainly achieved by vacuole $H^+/N0_3^-$ antiport system, while symport system (vacuole $N0_3^-$-N combined with anion) is beneficial for vacuole $N0_3^-$-N transporting into cytoplasm (Angeni et al., 2006; Krebs et al., 2010). Transported speed of vacuole $N0_3^-$-N from vacuole to cytoplasm is much slower than speed of $N0_3^-$-N transported in metabolic pool. Using "Double-Barreled Nitrate Selective Microelectrodes" method to measure vacuole $N0_3^-$-N under N deficiency condition, results showed that reuse of vacuole $N0_3^-$-N is rather slow in cortex cells also (Leij et al., 1998). In addition, NR can not be induced by vacuole $N0_3^-$-N accumulation rapidly (Shen et al., 2003). It is suggested that $N0_3^-$-N transport between vacuole and cytoplasm is regulated by some physiological mechanisms. But these transport mechanisms are obscure currently and it is necessary to perform a large amount of experiments in order to inspect, investigate and analyze results.

Characteristics and regulation of proton pump on tonoplast

Proton pump is separated into three types according to composition, including F type which is located in inner mitochondrial membrane and thylakoid membranes of chloroplasts, P type is located in cytoplasm membrane, V type is located in tonoplast (Martinoia et al., 2007). H^+-ATPase is separated into two categories according to biological function: one category, ATPase is synthesized by using electrochemical proton gradient of transmembrane, F-H^+-ATPase belongs to this type, the other category, proton electrochemical gradient of transmembrane is built by using energy of ATP hydrolysis, P and V types H^+-ATPase belong to this type (Zheng et al., 2009). There are two types of proton pump (V-ATPase and V-PPase) on tonoplast of plant tissues, working together to build transmembrane ΔH^+ (Martinoia et al., 2000).

Characteristic and regulation of V-ATPase

V-ATPase is a primary transport protein and located on plant tonoplast (Brux et al., 2008), abundant accumulation on

tonoplast, and accounting for 6.5-35% of total vacuole membrane protein in different plant tissues respectively. V-ATPase as a "special enzyme" is crucial important for maintaining anionic equilibrium in cell, ion compartment distribution and cell metabolic (Chu et al., 2001). V-ATPase is using energy of ATP hydrolysis to build electrochemical proton gradient of transmembrane, and supply dynamic for solute (positive and negative ions, amino acid and carbohydrates) positive transport (Wang et al., 2000). V-ATPase showed a more crucially important status for coordination of function network of ion pump in cell and signal transport system (Xin et al., 2003). In recent years, more research results showed that content, activity and subunit composition changes of V-ATPase are closely related to different plant tissues, growth stages and environmental factors, especially flexible responses of V-ATPase to tolerant environment (salt, low temperature, water deficiency, etc.) (Ruan et al., 2004; He et al., 2006). Therefore, V-ATPase is one of the crucial positions for regulation of plant physiological function in cellular and physiological levels. ATPase activity adaption to pH range is relatively wider, activity peak appeared at pH 7.5-8.0, and high affinity with ATP (Blom-Zandstra et al., 1992). ADP and AMP are competition inhibitors of this enzyme. Catalysis activity of V-ATPase and proton pump can be activated by anion in varying degrees, and it is the common characteristic of V-ATPase. Activation V-ATPase molecular has binding site of anion and is involved in enzyme catalysis. Activity of vacuole H^+-ATPase is inhibited by high $N0_3^-$-N concentration in cytoplasm and the inhibiting function is dissolved along with exhausting of $N0_3^-$-N in cytoplasm, and H^+-ATPase activity is recovered; it is beneficial to building new proton degree and accelerating $N0_3^-/H^+$ symport transport, and promoting $N0_3^-$-N transport from vacuole to cytoplasm. Vacuole $N0_3^-$-N can be transported into cytoplasm by $N0_3^-/H^+$ symport transport mechanism under N deficiency conditions. Symport constituted by vacuole $N0_3^-$-N and other anions is beneficial to transport of $N0_3^-$-N from vacuole to cytoplasm (Garrido et al., 2008).

In addition, proton pump (H^+-ATPase) of plant tonoplast is a multi-subunit membrane protein, constituted of a solubility region of the outside membrane (V_1) and the membrane binding region (V_0), V_1 region has ATP hydrolysis and regulation functions, V_0 region has a built-in proton transmembrane pathway, the two regions combin tightly and activity disappears when the two regions are separated (Wang et al., 2000). Bafilomycin A_1 is an antibiotic with macrolide, combined with V_0 region of vacuole H^+-ATPase, blocking proton transmembrane pathway, and strongly inhibited activity of V-ATPase, as specificity inhibitor of H^+-ATPase on tonoplast, 64% enzyme activity can be inhibited by only 50 nm bafilomycin A_1 (Ma et al., 2003).

Characteristic and regulation of V-PPase

V-PPase is a distinguish H^+ transport enzyme from F-, P-, V-ATPase, universally located in plant and a few photosynthetic bacteria. V-PPase is also an abundant component of tonoplast, accounting for 1%-10% of membrane protein (Bao et al., 2006). Coupling free energy of PPi hydrolysis and H^+ transmembrane transport, formation proton impellent power ($\Delta\psi H^+$) provides dynamic secondary transport of ions and other solutions. Contribution of V-PPase building electrochemical proton gradient of transmembrane is almost the same with V-ATPase or even obtains a better effect, as well as mature vacuole volume which accounts for 40%-99% of total cell volume, thus V-PPase function cannot be neglected. V-PPase is mainly involved in biological energy accumulation, cytoplasm pH regulation, Pi-PPi exchange, and probably regulation of tissue pressure by transporting K^+ into vacuole (Zhu et al., 2001).

Current research considers that V-PPase function possessed reversibility. On the one hand, PPase as a hydrolysis enzyme which can hydrolyze PPi into Pi, and build electrochemical proton gradient of tonoplast. On the other hand, PPase as a synthesis enzyme can synthesize Pi into PPi using the electrochemical proton gradient of proton pump (V-ATPase and V-PPase) (Hsiao et al., 2002). Substrate of V-PPase is Mg/PPi complex, but the substrate has to be further studied (Zhu et al., 2001). The best pH condition for V-PPase activity is pH 7.5-8.5. N,N′-dicyclohexylcarbodiimide (DCCD) is a specific inhibitor of proton transport channel, strongly inhibited V-PPase activity, and slightly inhibited V-ATPase activity also (Maeshima et al., 1994; Yang et al., 1999). While the fourth transmembrane α-screw of subunit c in V-ATPase has a highly conservative Glu residue, this is the only function position between inhibitor (DCCD) of proton transport channel and V-ATPase, and combined position between DCCD and V-ATPase can be changed by sulfur, lead to DCCD cannot be combined with this special position, effectively resisting DCCD inhibiting V-ATPase hydrolysis, and does not relieve inhibition effectiveness of bafilomycin A_1 (Ma et al., 2003), therefore, DCCD+$NaSO_3$ treatment is a specificity inhibitor for V-ATPase activity. It can be concluded that V-ATPase and V-PPase are not only the key enzymes of proton pump in tonoplast, but also the decisive factor of vacuole $N0_3^-$-N reuse efficiency. But the mechanism of vacuole $N0_3^-$-N reused by proton pump of tonoplast and its relation to N efficiency are not clearly defined at this stage. Therefore, study on key enzyme activity of proton pump in tonoplast (as basic theory), to inspect mechanisms of vacuole $N0_3^-$-N reuse, has important meanings for improvement of crop N use efficiency.

Effect of NR, NRAact and NRAmax on proton pump activity of tonoplast

NR is an illumination and $N0_3^-$-N induced enzyme (Cao et al., 2007, 42009). NR activity is mainly regulated by NR phosphorylation (Garcia-Mata et al., 2003). Conception of NRAact and NRAmax is highlighted according to experiment results of NR activity degeneration (phosphorylation) in barley. Activity NR (NRAact) is measured by adding adequate EDTA, NR phosphorylation while extraction progress has been blocked by chelate action between EDTA and Mg^{2+}. Maximum NR (NRAmax) is measured by adding sufficient Mg^{2+}, NR phosphorylation while extraction progress has completed a reaction, and then maximum NR protein in plant tissues was measured by this method (Fan, 2005).Fan (2005) reported that there are no significant differences between NRAmax in leaves, between two rice cultivars (Yangdao 6 and Nongken 57) under 10 mmol·L^{-1} $N0_3^-$-N application level, but NRAact of Yangdao 6 is three times higher than Nongken 57. There is no effect of 24 hours N deficiency condition on NRAmax of Yangdao 6, but NRAmax of Nongken 57 has been reduced by 79.7%, and NRAact of the two rice cultivars has been decreased significantly, compared with 10 mmol·L^{-1} $N0_3^-$-N application level. Expression results of *OsNial* and *OsNia2* showed that a response of OsNial to N deficiency is faster than *OsNia2* (Fan et al., 2007). It is suggested that there are significant differences of NRAact and NRAmax between genotypes and N application levels. However, there are few researches studying effects of NRAact and NRAmax on proton pump of tonoplast. $N0_3^-$-N transported by proton pump of tonoplast is influenced by NR activity in cytoplasm, $N0_3^-$-N can be continuing assimilated and reduction by cytoplasm NR, and accelerating more vacuole $N0_3^-$-N transported into cytoplasm. NR activity

can be regulated by NR gene expression, NR amount and protein degradation (Dou et al., 2008). NR activity is also inhibited by additional chemical complex, Na_2WO_4 is a specificity inhibitor of NR. Chen et al., (2009) and Si et al., (2004) research results showed that NR activity and net photosynthesis rate of cabbage are decreased by Na_2WO_4 treatment significantly; it is specificity inhibitor of NR activity, and NR protein amount can not be influenced by this inhibitor. Therefore, effect of NR activity on proton pump activity on tonoplast can be further investigated by specificity regulation of NR activity.

Measurement method of $N0_3^-$-N concentration in cytoplasm and vacuole

I order to study transport progress of $N0_3^-$-N between cytoplasm and vacuole, it is necessary to measure electrochemical gradient of inside and outside the vacuole and measurement $N0_3^-$-N concentrations in cytoplasm and vacuole is a basis for studying transportation of $N0_3^-$-N between different cell compartments (Jia et al., 2006). This method can be divided into two types. Walker et al., (1995) suggest a "three-barreled nitrate selective microelectrodes" method and a "double-barreled nitrate selective microelectrodes" method. Although "double-barreled nitrate selective microelectrodes" method requires a high nitrate concentration ($\geq$ 5 mol·m^{-3}) in crop growth culture solution (Shen et al., 2003), its manufacturing process and measurement method are relatively simple, and this method has come into general application. However, the manufacturing process and measurement method of the "three-barreled nitrate selective microelectrodes" method is very difficult, and this method can not be generally used currently (Jia, 2006). Zhen et al., (1991) have used the "double-barreled nitrate selective microelectrodes" method to measure nitrate concentrations of inside and outside vacuole respectively, and measure nitrate concentration of the whole single cell using enzyme method, confirming availability of the "double-barreled nitrate selective microelectrodes" method for measurement of nitrate concentrations in inside and outside vacuole, it is a currently available method and technology for further studying on nitrate transport between cytoplasm and vacuole.

"Double-barreled nitrate selective microelectrodes" method has recently been generally used to measure nitrate concentration of plant tissues. More and more crop and plant have been used as plant materials for studying on changes of nitrate concentration between vacuole and other cell compartments, including barley, maize, bean, cabbage and rice, etc. (Wang et al., 2010; Jia et al., 2006; Jia et al., 2005), gradually formed a series of perfect and mature methods for measurement of nitrate concentration in cytoplasm and vacuole is a supplied available method for vacuole nitrate reusing studies.

Conclusion

It is noteworthy that there are some researches which focus on transport mechanisms of $N0_3^-$-N between cytoplasm and vacuole and how proton pump (V-ATPase and V-PPase) of tonoplast plays a key function during vacuole $N0_3^-$-N distribution (Martinoia et al., 2000). H^+-ATPase activity of tonoplast is regulated by $N0_3^-$-N concentration in cytoplasm, where H^+-ATPase activity is inhibited by high $N0_3^-$-N concentration in cytoplasm and recovered with low $N0_3^-$-N concentration; it providea a dynamic for secondary transport of ion and other solutions between cytoplasm and vacuole. V-PPase is another proton pump of tonoplast. On the one hand, PPase as a hydrolysis enzyme which can hydrolyze PPi into Pi, and build an electrochemical proton gradient of tonoplast and conversely, PPase as a synthesis enzyme can synthesize Pi into PPi (Hsiao et al., 2002). In addition, $N0_3^-$-N transported by proton pump of tonoplast is influenced by NR activity in cytoplasm, $N0_3^-$-N can be continuing assimilated and reduction by cytoplasm NR, and accelerating more vacuole $N0_3^-$-N transported into cytoplasm. But, transport mechanisms of tonoplast are not entirely clear yet and there is no still universally accepted transport mechanism, but a few researches are studying on proton pump activity of tonoplast combined with ion transport. For example, reusing mechanism of $N0_3^-$-N through proton pump of tonoplast and relation to N efficiency is not clear yet, where substrate of V-PPase is Mg/PPi complex, but the actual substrate used has to be further studied (Zhu et al., 2001). There are significant differences of NRAact and NRAmax between genotypes and N application levels, but few researches focus on effects of NRAact and NRAmax on proton pump activity of tonoplast. These must necessarily be further discussed and studied in the future during research progress of N use efficiency.

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References

Angeli AD, Monachello D, Ephritikhine G, Frachisse JM, Thomine S, Gambale F, Barbier-Brygoo H (2006) The nitrate/proton antiporter *AtCLC*a mediates nitrate accumulation in plant vacuoles. Nature 442:939-942.

Bao AK, Zhang JL, Guo ZG, Wang SM (2006) Tonoplast H^+-pyrophosphatase involved in plant salt tolerance. Plant Physiol Communi 42(4):777-783.

Blom-Zandstra M, Hattum JV, Koot HTM (1992) Relationship of nitrate and tonoplast ATPase activity in vacuoles and tonoplast vesicles from lettuce. Plant Sci 87(2):133-141.

Brux A, Liu TY, Krebs M, Stierhof YD, Lohmann JU, Miersch O, Wasternack C, Schumacher K (2008) Reduced V-ATPase activity in the trans-Golgi network causes oxylipin-dependent hypocotyl growth inhibition in Arabidopsis. The Plant Cell 20: 1088–1100.

Cao CL, Liu JZ, Yao C (2007) Effect of different respiratory inhibitors on the nitrate reductase activity. J Northwest A F Univer 35(8):185-188.

Cao YB, Gao ZQ, He JP, Wang M, Gao RF (2009) Effects of exogenous salicylic acid on nitrate accumulation and reduction and assimilation in the leaves of chinese chive. Acta Horticultur Sinica 36(3):415-420.

Chen LZ, Liang L, Xu H, Song B, Su XJ, Yuan XH (2009) Relationship of photosynthetic characters and nitrate reductase activity of pakchoi. Acta Botanica Boreali-Occidentalia Sinica 29(11):2256-2260.

Chen W, Luo JK, Yin XM, Jia JL, Zhang PW, Shen QR (2005) Distribution and remobilization of nitrate in two cultivars of pakchoi plant. Sci Agric Sinica 38(11):2277-2282.

Chu CL, Hsiao YY, Chen CH, Van RC, Lin WJ, Pan RL (2001) Inhibition of plant vacuolar H^+-ATPase by diethylpyrocarbonate. Bioch et Biophysi Acta 1506:12-22.

Dou ST (2008) Nitrate accumulation mechanisms and its agronomy regulation method in cabbage. PhD Thesi Zhejiang Univer.

Dschida WJA, Bowman BJ (1995) The vacuolar ATPase: Sulfite stabilization and the mechanism of nitrate inactivation. J Biol Chem 270(4):1557-1563.

Facanha AR, Meis LD (1998) Reversibility of H^+-ATPase and H^+-Pyrophosphatase in tonoplast vesicles from maize coleoptiles and seeds. Plant Physiol 116: 1487-1495.

Fan XR (2005) Physiological and molecular mechanisms of nitrate transmembrane transport in rice. PhD Thesi Nanjing Agric Univer: 1-59.

Fan XR, Jia LJ, Li YL, Smith SJ, Miller AJ, Shen QR (2007) Comparing nitrate storage and remobilization in two rice cultivars that differ in their nitrogen use efficiency. J Exp Bot 58(7):1729-1740.

Garcia-Mata C, Lamattina L (2003) Abscisic acid, nitric oxide and stomatal closure is nitrate reductase one of the missing links. Trends Plant Sci 8 (1): 20-26.

Garrido FDSRG, Garrido RG, Bucher CA, Souza SRD, Fernandes MS (2008) Rice varieties tonoplast and plasma membrane H^+-ATPases differences activities in responses to nitrate pulses. J Biol Sci 8(1):107-112.

Glass ADM, Britto DT, Kaiser BN, Kinghorn JR, Kronzucker HJ, Kumar A, Okamoto M, Rawat S, Siddiqi MY, Unkles SE, Vidmar JJ (2002) The regulation of nitrate and ammonium transport systems in plants. J Exp Bot 53:855-864.

Granstedt RC, Huffaker RC (1982) Identification of the leaf vacuole as a major nitrate storage pool. Plant Physiol 70:410-413.

He HY, He LF, Li XF, Gu MH (2006) Effects of sodium nitroprusside on mitochondrial function of rye and wheat root tip under aluminum stress. J Plant Physiol Mol Biol 32(2)：239-244.

Hsiao YY, Van RC, Hung HH, Pan RL (2002) Diethylpyrocarbonate inhibition of vacuolar H^+-Pyrophosphatase possibly involves a histidine residue. J Protein Chem 21(1): 51-58.

Huang CB, Wang ZH, Li SX (2006) Nutritional and physiological significance of nitrate accumulation in plant vacuolar. Soils 38(6):820-824.

Jia LJ, Fan XR, Yin XM, Cao Y, Shen QR (2005) Remobilization of nitrate in rice leaf vacuoles measured with double-barrelled nitrate-selective microelectrodes. Sci Agric Sinica 38(7):1379-1385.

Jia LJ, Fan XR, Yin XM, Cao Y, Shen QR (2005) Measurement of nitrate activity in leaf cells of chinese cabbage in vivo using double-barreled nitrate selective microelectrodes. Acta Pedol Sinica 42(3):447-452.

Jia LJ, Fan XR, Yin XM, Shen QR (2006) Effect of pH on nitrate uptake by rice seedlings. Plant Nutri Ferti Sci 12(5):649-655.

Jia LJ (2006) Study on the method of transmembrane transport electric potential and vacuole nitrate activity measurement by ionselective microelectrode. MS Thesi Nanjing Agric Univer.

Krebs M, Beyhl D, Gorlich E, Al-Rasheid KA, Marten I, Stierhof YD, Hedrich R, Schumacher K (2010) Arabidopsis V-ATPase activity at the tonoplast is required for efficient nutrient storage but not for sodium accumulation. Proce Nati Acad Sci USA 107:3251-3256.

Lea US, Hoopen FT, Kaiser FPWM, Meyer C, Lillo C (2004) Mutation of the regulatory phosphorylation site of tobacco nitrate reductase results in high nitrite excretion and NO emission from leaf and root tissue. Planta 219:59-65.

Leij MVD, Smith SJ, Miller AJ (1998) Remobilization of vacuolar stored nitrate in barley root cells. Planta 205: 64-72.

Liu Z, Wang ZH, Li SX (2006) A preliminary study on why it is difficult to reduce nitrate spinach petiole. Sci Agric Sinica 39(11):2294-2299.

Liu JX, Tian QY, Chen FJ, Mi GH (2009) Nitrate accumulation in maize and its role in adaptation to lasting low nitrogen environments. Plant Nutri Ferti Sci, 15(3):501-508.

Ma TJ, Xiang YY, Wang SS (2003) Effects of salt stress on the hydrolytic activity of H+ATPase from populus euphratica. J Xinjiang Agric Univer 26(2):43-48.

Maeshima M, Hara-Nishimura I, Takeuchi YK, Nishimura M (1994) Accumulation of vacuolar H^+-Pyrophosphatase and H^+-ATPase during reformation of the central vacuole in germinating pumpkin seeds. Plant Physiol 106: 61-69.

Martinoia E, Heck U, Wiemken A (1981) Vacuoles as storage compartments for nitrate in barley leaves. Nature 289: 292-294.

Martinoia E, Massonneau A, Frangne N (2000) Transport processes of solutes across the vacuolar membrane of higher plants. Plant Cell Physiol 41(11): 1175–1186.

Martinoia E, Maeshima M, Neuhaus HE (2007) Vacuolar transporters and their essential role in plant metabolism. J Exp Bot 58:83-102.

Miller AJ, Smith SJ (2008) Cytosolic nitrate ion homeostasis, could it have a role in sensing nitrogen status. Ann Bot 101:485-489.

Rahimizadeh M, Kashani A, Zare-Feizabadi A, Koocheki A R, Nassiri-Mahallati M (2010) Nitrogen use efficiency of wheat as affected by preceding crop, application rate of nitrogen and crop residues. Aus J Crop Sci 4(5):1835-2707.

Rahman MM, Amano T, Shiraiwa T (2009) Nitrogen use efficiency and recovery from N fertilizer under rice-based cropping systems. Aus J Crop Sci 3(6):336-351.

Ruan HH, Shen WB, Xu LL (2004) Nitric oxide modulates the activities of plasma membrane H^+-ATPase and PPase in wheat seedling roots and promotes the salt tolerance against salt stress. Acta Botanica Sinica 46 (4): 415-422.

Si JY, Wang XL, Chen P, Feng K (2004) Effect of NR inhibitor and NH_4^+ on NO3- absorpion of different rice genotypes. J Ynagzhou Univer 25(1):59-62.

Shen QR, Tang L, Xu YC (2003) A review on the behavior of nitrate in vacuoles of plants. Acta Pedolo Sinica 40(3):465-470.

Walker DJ, Smith SJ, Miller AJ (1995) Simultaneous measurement of intracellular pH and K^+ or NO_3^- in barley root cells using triple-barreled, lon-selective microelectrodes. Plant Physiol 108: 743-751.

Wang H, Wang TZ, Dong C H, Wang ZQ (2000) Purification and reconstitution of tonoplast H^+-ATPases from soybean. Chine J Biochem Mol Biol 16(1):110-115.

Wang B, Nai T, Jia JL, Shen QR (2008) Relationship between nitrate remobilization in root vacuoles and plant growth of two genotypes of lettuce. Acta Pedolo Sinica 45(3):555-560.

Wang XL, Sheng HJ, Liu Y, Tao HH, Feng K (2010) Effects of cadmium on membrane potential differences and membrane permeability of rice roots. J Agro-environ Sci 29(4):630-635.

Xin Y, Wang YQ, Zhang SQ, Huang CL, Wu ZY, Jia WS (2003) The effect of protein tyrosine phosphatases, ppases on ABA accumulation and involved in signal transportation of water tolerant in plant. Chine Sci Bullet 48(4):369-374.

Xu HR, Gu JT, Lu WJ, Deng RL, Cao YF, Xiao K (2007) Characterization and expression of nitrate transporter gene OsTNrt2.1 in rice (Oryza sativa L.). Acta Agron Sinica 33(5):723-730.

Yang SJ, Jiang SS, Kuo SY, Hung SH, Tam MF, Pan RL (1999) Localization of a carboxylic residue possibly involved in the inhibition of vacuolar H^+-pyrophosphatase by N,N'-dicyclohexylcarbodi-imide. Biochem J 342: 641-646.

Zhang ZH (2007) Studies on the relationship between the nitrogen distribution and the nitrogen physiological efficiency in oilseed rape. MS Thesi Hunan Agric Univer.

Zhang ZH, Song HX, Liu Q, Rong XM, Guan CY, Peng JW, Xie GX, Zhang YP (2009) Study on differences of nitrogen efficiency and nitrogen response in different oilseed rape (*Brassica napus* L.) varieties. Asia J Crop Sci 1(2):105-112.

Zhang ZH, Song HX, Liu Q, Rong XM, Xie GX, Peng JW, Zhang YP, Guan CY, Chen SY (2010) Absorption, distribution, and translocation of nitrogen at growth stages in oilseed rape plant. Acta Agron Sinica 36(2):321-326.

Zhao SP, Ye XZ, Zhang YZ, Zheng JC (2010) The contribution of *bnnrt*1 and *bnnrt*2 to nitrate accumulation varied according to genotypes in Chinese cabbage. Afric J Biotechnol 9(31):4910-4917.

Zhen RG, Koyro HW, Leigh RA, Tomos AD, Miller AJ (1991) Compartmental nitrate concentration in barley root cells measured with nitrate selective microelectrodes and by single cell sap sampling. Planta 185:356-361.

Zheng ZY (2009) Sugar uploading and transportation mechanisms of Ningxia Matrimony vine. MS Thesi Ningxia Univer :3-5.

Zhu ZJ, Qian YR, Pfeiffer W (2001) Effect of nitrogen form on the activity of tonoplast pyrophosphatase in tomato roots. Acta Botanica Sinica 43(11):1146-1149.