Advances in Studies on Effects of Nitrogen Metabolism Pathways on Seed Germination and Seedling Growth of Plants

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Abstract Nitrogen is an important component of plant nucleic acids, proteins and chlorophyll. It is an essential nutrient element required for the plant growth and development. It is also one of the important factors limiting the growth of plants. This review introduces several important aspects of nitrogen metabolism including arginine metabolism, urea metabolism pathway and glutamine synthetase assimilation process, and also induces and summarizes the response mechanisms of nitrogen metabolism on the seed germination and seedling growth. In addition, it also importantly summarizes the research advances in the effects of the nitrogen metabolism on seed germination and seedling growth of plants. At the same time, this review briefly summarizes the application values of nitrogen in crop production, environmental pollution and ecological conditions and so on, and also discusses the limitations and deficiencies of nitrogen metabolism research, providing the certain theoretical foundations for the research of the effects of nitrogen metabolism pathway on the seed germination and seedling growth of plants.

Keywords Nitrogen metabolism; Germination; Seedling stage

Background Nitrogen metabolism is an indispensable physiological response in the entire metabolism of plants. Nitrate nitrogen (NO₃⁻-N) and ammonium nitrogen (NH₄⁺-N) are the main inorganic nitrogen forms absorbed by plants. Among them, nitrate nitrogen is assimilated as NO₃⁻ in stems, leaves and roots (Crawford et al., 1998; Andrews et al., 2010), while the principle of ammonium nitrogen absorption by plants is inconclusive. A series of toxic ammonia ions and nitrites are produced after the nitrogen assimilation. The GS/GOGAT (glutamine synthetase/glutamic acid synthetase) pathway is an ammonia assimilation pathway that plants have evolved (Chen et al., 2004), and GS can catalyze the synthesis of glutamine with ammonia ions and glutamic acid in the presence of ATP in order to reduce the toxic effect of ammonia on plants. It has been known that GS is a key enzyme involved in nitrogen metabolism of plants, and its activity reflects the nutritional status and the level of nitrogen assimilation in plants to a certain degree. Seed germination and seedling growth are key periods in the plant growth, and a series of complex physiological processes occur in these two periods. Recent studies have found that stresses such as salt, drought, high temperature, and nutritional elements seriously affect the plant growth and development. Nitrogen is called the life element and it is also the essential nutritional element for the survival of plant, and its effect on plant growth and development has attracted more and more attention from researchers (Peter et al., 1991). This review summarizes the main research advances and response mechanisms of several key factors in plant nitrogen metabolism, focusing on the exploration for the effects on the seed germination and seedling stages of plants.

1 The effect of arginine metabolism pathway on seed germination and seedling growth

During the seed germination, the storage proteins in plants are largely degraded into various amino acids. Among them, arginine is the amino acid with highest carbon-to-nitrogen ratio and account for 40%-50% of total nitrogen. It is the main transportation and storage form of plant organic nitrogen and the important signaling molecule in the metabolic process. It is also involved in the regulation process of plant stress responses (Cvikrová et al., 2013). The
arginine content of plants also increases significantly at the seedling stage (Takahashi et al., 2003; King et al., 2013). Zhang Xiaoxu found that during the seed germination period of Arabidopsis, both the arginase genes AtARGH1 and AtARGH2 were expressed in large amounts in situ hybridization. During the period of the radicle breakthrough, its main expression site was the root tip, while in the late stage of germination, the main expression site was the radicle elongation zone. Shi et al. (2013) that in Arabidopsis mutants argah1, argah2, and argah1argah2, the resistant abilities to drought, salt and low temperature were improved due to the higher content of NO and Pas (Flores et al., 2008). Therefore, understanding the effects of arginine metabolism on seed germination and seedlings is beneficial to improve plant productivity and resistant ability by employing some technologies such as breeding and genetic engineering.

There are mainly three ways of arginine decomposition in the organism: 1. The nitric oxide synthase produces NO from arginine, which is the main way of NO production in the organism. Wang et al. (2018) reported that the addition of NO could effectively increase the arginine content of tomato seedlings under the metal stress, and Liu et al. also reported that the exogenous addition of NO could promote the arginine content of roots and leaves in oats under the lanthanum stress. However, the physiological and biochemical mechanism of its signaling regulation was not clear; On the other hand, Zhang et al. (2003) reported that the nitric oxide donor sodium nitroprusside could significantly promote the seed germination and the elongation of hypocotyls and embryo buds in wheat under the osmotic stress. It could also enhance the activity of various enzymes during the germination and accelerate the degradation of storage proteins; 2. Spermine is produced from arginine by arginine decarboxylase, and then consequently, putrescine is produced by spermase; 3. Under the activity of arginase, arginine produces ornithine and urea, and participates in the urea cycle (Wang et al., 2016). Arginase is widely present in plants and animals, and it is the only enzyme of producing urea in plants as divalent metal ions as an activator. The content of arginase is increased ten-fold from the period of seed germination to seedling stage of Arabidopsis thaliana, indicating arginase is actively expressed in plants during the seed germination period. In organisms, both NOS and ARG uses arginine as a substrate. When the activity of NOS in the organism decreases, the activity of ARG rises. Researches showed that the NO content of arginine mutants increased significantly compared with wild-type Arabidopsis thaliana. The number of lateral and adventitious roots also increased considerably (Flores et al., 2008); The transgenic cotton mutant that overexpressed the rice arginase also inhibited the development of lateral roots and growth (Zhi et al., 2015); Meng et al. (2019) reported that the addition of arginase inhibitors reduced the ROS activity and aging level in agaricus bisporus; During the germination period of soybean seeds, the arginase transcription level also rapidly increased (Goldrai et al., 1999). These results show that arginase has an important biological significance in the seed germination and seedling growth.

2 The effects of urea metabolism pathway on seed germination and growth

The urea cycle, also known as ornithine cycle, is the first cyclic metabolic pathway in the organism (Holmes, 2007). This pathway is as follows. With the carbamoyl phosphate and ornithine as substrates, ornithine carbamoyl transferase (OCT), argininosuccinate synthase (ASS) and the argininosuccinate-ate lyase (ASL) catalyzes the formation of arginine (arginine, Arg) via citrulline (Cit), and then continues to be decomposed into the urea and ornithine (Orn) under the action of arginase (ARG), thus forming a urea cycle.

Urea is the main source of nitrogen nutrition for crops and also an important intermediate product of nitrogen metabolism. Part of the urea used in plants come from the soil (Hoshida et al., 2000), and some parts comes from synthesis by plant itself. There are two main ways of urea synthesis in plants: such as arginine degradation pathway and ureide and purine degradation pathway. In the arginine degradation pathway, arginine is decomposed into ornithine and urea under the catalysis of arginase, and urea is hydrolyzed by urease into ammonia and CO2. After that, the ammonia is assimilated in the GS/GOGAT cycle and used for the plant growth and development (Yang, 2010). Therefore, urease is a key enzyme in urea metabolism (Mobley et al., 1989; Wang et al., 2008), and it depends on nickel ions to break down urea in plants (Murphy et al., 2011). Kou (2016) reported that during the germination period of Arabidopsis seeds, as the seeds absorbed water and swelled, the stored proteins in the organism were largely degraded, which might be one of the reasons for the gradual increase in urea content, and
the hydrolysis of urea might produce a large amount of NH$_4^+$. Because the most of plants are relatively sensitive to NH$_4^+$, high concentrations of NH$_4^+$ will inhibit plant growth and development (Gerendás et al., 1997; Dev et al., 2002). Youngdah et al. (1968) also reported that NH$_4^+$ could affect the nitrogen uptake by rice seedlings. At the same time, as the accumulated ammonia derivatives change the alkalinity of the soil, it will also affect seed germination and seedling growth, which will bring serious problems to the economy and the environment. Therefore, understanding the effects of urea metabolism on seed germination and growth is beneficial for improving plant productivity and reducing environmental pollution.

3 The effects of nitrogen assimilation on seed germination and seedling growth

Glutamine synthetase (GS) is a key enzyme in the process of nitrogen metabolism. It can convert ammonia in inorganic salt form into the organic form of nitrogen and participate in different kinds of regulation on the nitrogen assimilation. GS exists in almost all tissues or organs in the form of isoenzymes (Oliver et al., 1998; Wang et al., 2002). However, the gene expression of GS has tissue and organ specificity, and it is also affected by some factors such as nitrogen source, temperature, light, moisture and NaCl (Lam et al., 1996). Both ammonium nitrogen and nitrate nitrogen can induce the expression of GS gene. The types of GS in different plants are also different. GS can be mainly divided into two types GS1 and GS2 based on the subcellular localization:

GS1 is mainly located in the cytoplasm and vacuoles. When NH$_4^+$ was used as the sole nitrogen source, the enzymatic activities of root glutamine synthetase (GSr) and leaf cell glutamine synthetase (GS1) in wheat seedlings were higher than that in case of NO$_3^-$ as the sole nitrogen source, indicating NH$_4^+$ could promote the GS mRNA synthesis at the root part. Tabuchi et al. Found Arabidopsis that the developmental rate of rice gsr1 mutant slowed down during the growth process and the grain yield decreased significantly. Guan et al. (2015) also showed that the fruit pods, seed setting rate and the size of seeds in the GS1 functional mutant gln1.2 were significantly smaller than those of wild type, indicating that GS play a regulatory effect on the development of Arabidopsis seeds. At the same time, a lot of other researches also proved that GS1 gene was expressed in large amounts in germinated seeds and senescent leaves of Arabidopsis thaliana. Therefore, it can be said that GS1 probably play a function as the nitrogen source transport in the seed germination of Arabidopsis thaliana. GS2 is mainly present in chloroplasts and plastids and can assimilate the ammonia released from photorespiration (Ramón et al., 2003). It also participated in the reassimilation of photorespiration ammonia, reduced ammonia (primary nitrogen) and circulating ammonia. Zhang et al. observed that the exogenous ammonia induced the production of kind of GS isoenzyme (GSrb) in the root of rice and also promoted the total activity of GS and the activity of GS2 in rice leaves (Zhang et al., 1992). With the elongation of Arabidopsis leaves, the gene expression of GS2 gradually increased to the maximum. However, in the leaves in senescence, the expression of GS1 increased significantly while GS2 expression decreased considerably (Li et al., 2001). Moreover, under the normal photorespiration, the gs2 mutants showed the abnormal growth phenomena such as yellowing of leaves, slow growth trend and increased ammonia content and so on (Wang et al., 2004). These results indicate that GS2 has a great effect on the growth of plant seedlings. In summary, it can be said that GS play a huge influence on plant seed germination and seedling growth.

4 The application of nitrogen in agricultural production

Nitrogen participates in all the stages of plant growth and development and plays an important regulatory role in plant life activities (Gao et al., 1999), affecting cell growth and differentiation (Tian et al., 2011). Therefore, Nitrogen is also widely used in life. For example, it has important application values in the economic, environmental and other aspects of life.

Economically, nitrogen can increase crop yield. Studies have found that the levels of exogenous nitrogen and different fertilization can affect the plant growth potential, cotyledon morphology, stem and leaf weight and yield (Chai et al., 2004; Li et al., 2011). Qi et al. (2019) reported that the nitrogen fertilizer could promote the wheat growth, enhance the wheat photosynthesis and accelerate the accumulation and transformation of nutrients. If nitrogen is deficient, it will produce short plants, which will eventually reduce the wheat yield. Sun et al reported
that the different forms of nitrogen source ratios had significant promotive effects on citrus orange seedlings. In summary, nitrogen can increase crop yield and economic benefits, but excessive nitrogen fertilizer may cause the environmental pollution. Zhang et al. (1995) reported that the nitrate content in groundwater had a certain relationship with the large-scale fertilization in farmland. Therefore, even if nitrogen is an essential element for crop growth and development and also can increase economic benefits, the environmental pollution caused by excessive nitrogen fertilizer cannot be ignored, which may cause groundwater nitrate pollution and eutrophication of rivers and lakes. At present, there has been some results about the effects of nitrogen on the plant response to stresses at the molecular level, but the research mechanism in different forms of nitrogen is not deep yet. It mainly stays in the initial stage of the physiological analysis of plants, and thus, the in-depth molecular mechanism of signaling pathways and transport is not clear.

There are differences in the mechanism of nitrogen metabolism at different growth stages of plants. Therefore, in order to understand the effect of nitrogen on plant, the comprehensive studies have to be conducted on the full growth cycle of plants including germination, seedling, senescence and so on. Seed germination and seedling are the critical periods of plant growth and development. During this period, a large amount of nitrogen-containing substances are degraded, which provide the nitrogen nutrition for the plant organism, and it is easily affected by external environmental factors. In addition, the effect of nitrogen on seed germination and growth is extremely complicated, which involves the participation of multiple pathways and enzymes, but the content and methods of a lot of researches mainly stay on the surface phenomena. How to improve the utilization efficiency of nitrogen is the most important issue at present. Therefore, it is necessary to further screen the key genes taking part in the nitrogen metabolism and to deeply explore the regulative and signal transduction mechanism of nitrogen in controlling the transcription factors related with the seed germination by using the molecular biological methods. It is also important to improve the utilization of nitrogen and enhance the resistant ability to stress. I personally think that the following aspects should be considered: (1) Explore the regulation mechanisms of key genes in nitrogen metabolism in-depth; (2) Explore the maximum utilization of nitrogen; (3) Analyze the mechanism of nitrogen transport at the protein level in-depth (4) Improve the crop varieties and screen the varieties with high nitrogen utilization.

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References
Andrews M., 2010, The partitioning of nitrate assimilation between root and shoot of higher plants, 9(7): 511-519
https://doi.org/10.1111/j.1365-3040.ep1616228

Crawford N.M., Glass A.D.M., 1998, Molecular and physiological aspects of nitrate uptake in plants, Trends in Plant Science, 3(10): 390-395
https://doi.org/10.1016/S1360-1385(98)01311-9

Chen Y., Zhu B.G., Zhang J, and Liang Z.S., 2004, Effects of different nitrogen sources on the activity and protein content of nitrate reductase and glutamine synthetase in soybean, Soy Science (02): 64-67

Cvikrová M., Geperlová L., Martinová O., and Vanková R., 2013, Effect of drought and combined drought and heat stress on proline-overs-producing tobacco plants, Plant Physiology and Biochemistry, 73: 7-15
https://doi.org/10.1016/j.plaphy.2013.08.005
PMId:24029075

Chai W.D., Liu Y.P., Li H.Q., Liu Z., Li H.M., 2004, Preliminary Report on the Effects of Nitrogen Application on Hollow Multistem Plant Type Short-Stage Cotton, 12(3): 92-94

Dev T.B., Herbert J.K., 2002, NH$_4^+$ toxicity in higher plants: a critical review, Journal of Plant Physiology, 159(6): 567-584
https://doi.org/10.1078/0176-1617-0774

Flores T., Todd C.D., Tovar-Mendez A. et al., 2008, Arginase-negative mutants of Arabidopsis exhibit increased nitric oxide signaling in root development, Plant Physiologvy, 147(4): 1936-1946
https://doi.org/10.1104/pp.108.121459
PMid:18567826 PMCid:PMC2492630
Flors T., Todd C.D., Tovar-Mendez A., et al., 2008, Arginine-negative mutant of Arabidopsis exhibit in decreased nitric oxide signaling in root development. Plant 
https://doi.org/10.1104/pp.108.121459
PMid:18567826 PMCid:PMC2492630

Gerendis J., Zhu Z., Bendixen R., Ratcliffe R.G., Sattelmacher B., 1997, Physiological and biochemical processes related to ammonium toxicity in higher plants, Zeitschrift für Pflanzenzüchtung und Bodenkunde, 160(2): 239-251
https://doi.org/10.1002/zp.19971600218

Guan M., Möller L.S., Schjoerring J.K., 2015, Two cytosolic glutamine synthetase isoforms play specific roles for seed germination and seed yield structure in Arabidopsis, Journal of Experimental Botany, 66(1): 203-212
https://doi.org/10.1093/jxb/eru411
PMid:25316665 PMCid:PMC4265158

Gao J.F., Wang Z., 1999, Mineral and nitrogen nutrition of plants, Plant Physiology, Beijing: China Agricultural Press, 80-110

Holmes F.L., 2007, Hans krebs and the discovery of the ornithine cycle, Federation proceedings, 39(2): 216

Li C.J., Lin Q.H., Zhang C.F., 2001, Advances in research on glutamine synthetase in higher plants, Journal of Biology (04): 2

Kim J.E., Gifford D.J., 2013, Amino acid utilization in seeds of loblolly pine during germination and early seedling growth: i. arginine and arginase activity, Plant Physiology, 113(4): 1125-1135
https://doi.org/10.1104/pp.113.4.1125
PMid:12223664 PMCid:PMC158235

Lam H.M., Coschigano K.T., Oliveira I.C., Melo A.J., 2003, The role of arginase in regulating postharvest storage quality of Agaricus bisporus fruiting bodies, China Food Science, 24(5): 209-212

Li G.Q., Tang L., Zhang W.Y. et al., 2011, Effects of nitrogen application rate on leaf type vertical distribution characteristics of different plant type wheat varieties, Crop Journal, 3(1): 127-137
https://doi.org/10.13724/SPJ1006.2011.00127

Li C.J., Lin Q.H., Zhang C.F., 2001, Advances in research on glutamine synthetase in higher plants, Journal of Biology (04): 2-4

Meng D.M., Zhang Y.X., Yang R. et al., 2019, The role of arginase in regulating postharvest storage quality of Agaricus bisporus fruiting bodies, China Food News, (07): 201-209

Murphy T.F., Brauer A.L., 2011, Expression of urease by haemophilus influenzae during human respiratory tract infection and role in survival in an acid environment, BMC Microbiology, 11(1): 183
https://doi.org/10.1186/1471-2180-11-183
PMid:21843372 PMCid:PMC3166929

Mobley H.L.T., Hausinger R.P., 1989, Microbial ureases: significance, regulation, and molecular characterization. Microbiological reviews, 53(1): 85-108
https://doi.org/10.1128/MMBR.53.1.85-108.1989
PMid:2651866 PMCid:PMC372718

Oliver M.J., Quisenberry J.E., Trolinder N.L.G., Keim D.L., 1998, Control of plant gene expression, US

Peter M.V., Robert W.H., 1991, Nitrogen limitation on land and in the sea: how can it occur, Biogeochemistry, 13(2): 87-115
https://doi.org/10.1007/BF000902772

Qi B.Y., 2019, Effects of nitrogen metabolism on wheat yield and quality, Seed Industry Guide (4), 8-10

Ramón S., Judith M., Shishkova S., Georgina H., 2003, Overexpression of alfalfa cytosolic glutamine synthetase in nodules and flowers of transgenic lotus japonicus plants, Physiologia Plantarum, 117(3): 326-336
https://doi.org/10.1043/1399-3054.2003.00053.x
PMid:12634032

Shi H., Ye T., Chen F. et al., 2013, Manipulation of arginase ex-pression modulates abiotic stress tolerance in Arabidop-sis: effect on arginine metabolism and ROS accumulation, Journal of Experimental Botany, 64(5): 1367-1379
https://doi.org/10.1093/jxb/ers400
PMid:23378380 PMCid:PMC3598423

Sun M.H., Lu X.P., Cao X.J., Li J., Xiong J., Xie S.X., 2017, Effects of Different Nitrogen Forms on Nitrate Reductase Activity and Related Gene Expression in Loquat Orange Seedlings, Journal of Fruit Science, (04): 20-27
Takahashi M., Uematsu Y., Kashiwaba K., Yagasaki K., Hajika M., and Matsunaga R. et al., 2003, Accumulation of high levels of free amino acids in soybean seeds through integration of mutations conferring seed protein deficiency, Planta, 217(4): 577-586
https://doi.org/10.1007/s00425-003-1026-3
PMid:1268487

Tian J., Guo S.R., Sun J., Wang L.P., Yang Y.J., Li B., 2011, Effects of exogenous spermidine on nitrogen metabolism of cucumber seedlings under high temperature stress, Journal of Ecology (10): 85-90

Wang H.F., Feng X., Zhang Y.Y., Feng L.F., Zhang Y., and Chen G. et al., 2016, Cloning and expression analysis of cotton arginase gene gharg1 cdna, North China Agricultural Journal, 33(04): 21-28

Wang J., Yu S.X., Wang Y.H., Dou Q.H., and Cui X.M., 2014, Effects of exogenous no on the chelate peptide and arginine metabolism of tomato plants under copper stress, Journal of Soil and Water Conservation, 28(4): 317-323

Wang Y.F., Yu Z.W., Li S.X., Yu S.L., 2002, Effects of nitrogen nutrition level on key enzyme activities of nitrogen metabolism and grain protein content in winter wheat, Acta Crop Sinica (06): 24-29

Wang J.C., Liu F.L., Li D.F., Jiang J.C., 2004, Antioxidant effect of free glutamine in the body, Chinese Journal of Biophysics, 20(6): 429-433

Wang W.H., Khler B., Cao F.Q., and Liu L.H., 2008, Molecular and physiological aspects of urea transport in higher plants, Plant Science (Oxford), 175(4): 470-477

Yang Y.S., 2010, Fertilizer characteristics and taboos of urea, Shandong Vegetables, (2):33-34
https://doi.org/10.1016/j.plantsci.2008.05.018

Zhang H., Shen W.D., and Xu L.L., 2003, Effects of Nitric Oxide on Wheat Seed Germination and Active Oxygen Metabolism under Osmotic Stress (English), Acta Botanica Sinica, 45(8): 901-905

Zhi G.M., Zhao H.M., Rui Z., Cheng Z.L., Jian M.W., and Yan L.W. et al., 2015, Expression of the rice arginase gene osarg in cotton influences the morphology and nitrogen transition of seedlings, PLOS ONE, 10(11): e0141530
https://doi.org/10.1371/journal.pone.0141530
PMid:26528551 PMCid:PMC4631492

Zhang L., Tian Z.X., Zhang N., and Li X.Q., 1995, Investigation of groundwater nitrate pollution caused by agricultural nitrogen fertilizer in northern China, Journal of Plant Nutrition and Fertilizer, (02): 84-91

Zhang C.F., Peng S.B., Bennett J. et al., 1992, Glutamine synthetase ferredoxin dependent glutanate synthase in maize, Plant Cell Physiology, 33: 1193-1198

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