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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(2): 482-486

www.thepharmajournal.com
Received: 19-12-2019
Accepted: 23-01-2020

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Exploration of miRNA diversity for nutrient use efficiency

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Abstract
miRNAs are 20–24 nucleotides long bind to complementary transcripts, attenuating post-transcription or translational inhibition gene expression. In cell signalling pathways, small RNAs including micro RNAs (miRNA) play an indispensable role. Normally, miRNAs have emerged as the key regulator of various stress responses in plants, including low availability of nutrients. miRNAs have been documented to be critical for maintaining plant nutrient homeostasis by controlling the expression of transporters involved in nutrient uptake and mobilization. The present analysis emphasizes the function of different miRNAs in several plant deficiencies of macro-or micronutrients. Understanding regulation by miRNAs of various transporters may help to elucidate the underlying mechanisms of molecular signal transduction during nutritional stress. Recent findings on nutrient-related mRNAs and their gene-regulating machinery that delineate a new platform for future nutritional improvement of crop or crop biofortification programs.

Keywords: Plant, miRNA, micronutrient, homeostasis of nutrients

Introduction
Nutrient Use Efficiency (NUE) can be defined simply as yield per nutrient input unit. In agriculture input is generally related to fertilizers, whereas the NUE is often expressed in scientific terms as fresh weight or product yield per amount of available nutrient. NUE depends not only on the ability to take the nutrient from the soil effectively, but also on transportation, storage, mobilization, plant use, and even the climate. N, P, K, & S are the most essential nutrient that usually limits plant growth and its productive use. Efficiency of nutrient use can be demonstrated by following phenomena such as ratio of nutrient production, agronomic efficiency, physiological efficiency, agro-physiological efficiency, apparent recovery efficiency and quality of utilization. In a simple term, therefore, NUE is not only related to the absorption of nutrients, but also to their use within the plant system. Specific transporters are present in the plant system for nutrient uptake and their use which are directly or indirectly influenced by miRNAs.

Need for the enhancement of nutrient use efficiency
Plants do not make full use of the supplied nutrient. Most of the nutrient added is lost in ecosystems through immobilization, volatilization, denitrification, leaching etc. The consumption efficiency of N is typically 30-50%. P usage efficiency is 15-20%, low efficiency causes fastening in Al-P, Fe-P, Ca-P soils. K usage efficiency is 70-80%, low efficiency causes fastening in clay lattices. S use efficiency is 8-10%; micronutrients such as Zn, Cu, Fe, Mn, B use efficiency due to soil fixation are very low 1-2%. Excess application of fertilizer also causes certain health problems in Punjab and Haryana for example. The soil water sample of Haryana had nitrate N > 22mg / l. In India, phosphatic and potassic fertilizers are usually imported, increasing the cost factor. There is a shortfall of 2900 crore due to the loss of N fertilizer. In India, that's it. Rs. 10056 million will be saved according to an expected 1% increase in NUE in N and P.

Approaches for improving Nutrient use efficiency
NUE can be improved by following approaches such as

1) Agronomic Approaches
In this approach we can go for optimum phonological application based on crop nutrient

demand, soil-based nutrient status, plant nutrient uptake efficiency awareness, the use of controlled released fertilizers such as nitroform, nutralene, IB nitrogen, etc.

2) Physiological approach

NUE is influenced by the efficiency of the absorption of nutrients depending on the root characteristics, the partitioning of nutrients between the leaf and stem and the latency of the leaf Senescence. Higher nutrient output and canopy structure can be important parameter for improving NUE.

3) Genetic approach: It includes

I. Conventional approach: we usually go for the collection of those genotypes that have strong NUE in this approach and then we go for backcrossing. But it takes a very long time and not very effective approach.

II. Genomic approach: through QTL Mapping or Association Mapping, we try to identify the candidate gene in this approach and then go for assisted marker selection. It is an effective way of maximizing the NUE. But other factors known as miRNAs also control these candidate genes. So if we regulate these miRNAs we can manipulate our candidate genes easily and improve the NUE.

Role of miRNA in Nutrient use efficiency

The first miRNA was discovered in 1993 by Victor Ambros and Gary Ruvkun in the Lin-4 gene of C. elegans. miRNAs are generally 20-24 nucleotides long binding to specific complementary transcripts, attenuating post-transcription or translational inhibition gene expression. miRNAs play a very important role in preserving nutrient homeostasis in plants through regulating the expression of nutrient uptake and mobilization transporters. NUE-related miRNA diversity suggests various miRNA and target sequences involved in nutrient uptake, translocation, assimilation and use in plant systems.

Role of miRNA in Nitrogen use efficiency: Many miRNAs control certain transporters involved in the absorption, translocation and assimilation of nitrogen in plant systems. The gene of miRNA and its targets is as follows –

i. miR160 and auxin response factor 16 (ARF16)

ARF16 is the auxin response factor that is primarily responsible for controlling the formation of root cap. ARFs are binding DNA proteins that control auxin transcription and are only present in plants. N-deficiency induces miR160 expression, which increases degradation of ARF16 and therefore facilitates the formation of lateral root.

ii. miR393 and auxin signalling f-box protein 3 (AFB3)

Nitrate can transcriptionally induce AFB3 expression in roots and N metabolites produced after nitrate reduction and assimilation lead to a reduction in AFB3 levels due to induction of miR393. MIR393/ARF3 is the mechanism responsible for the suppression of primary root elongation and the induction of lateral root emergence under N.

iii. miR444 and MADS-Box transcription factor

The monocot specific miR444 regulates four MIKC-type MADS-box transcription factor genes in rice (*OsMADS23*, *OsMADS27a*, *OsMADS27b*, and *OsMADS57*. miR444 targets with Arabidopsis ANR1clade, a crucial NO3− signalling pathway in lateral root growth. Plants over-expressing this miRNA showed a decrease in the expression of the four MADS-box genes and a reduced nitrate induced lateral root growth.

iv. miR169 and nuclear factor Y subunit A (NFYA)

Also known as heme-activated protein (HAP) or CCAAT-box binding factor (CBF), NFYA is primarily associated with differentiation of nodule and tolerance to drought. Transgenic Arabidopsis plants showed a decrease in N accumulation over the expression miR169a. These plants displayed a higher N-limit sensitivity relative to the wild type, as NFYA regulates NRT1 and NRT2 nitrate transporters.

iv. miR156 and squamosa promoter binding protein like (SPL)

SPL proteins play critical roles throughout the plant life cycle in maintaining normal growth. Via their targets, members of the SPL transcription factors, these miRNAs are involved in phase change. MIR156 targets, SPL3, are down-regulated, indicating that the miR156/SPL3 module may work by reducing the availability of N by repressing vegetative phase changes. miR156 functions as a negative regulator of miR172 by regulating the expression of miR172 via SPL9 and SPL15 targets.

vi. miR171 and scarecrow-like 6 (SCL6)

Quantitative RT-PCR analyses showed that under N-deficient conditions the miR171c expression was three times higher than under N-deficient conditions. MIR171 induction stimulated by N-starvation, resulting in an inhibitory effect over its targets (SCL6-II, SCL6-III and SCL6-IV), and thereby suppressing primary root elongation during this stress state.

Role of miRNA in P use Efficiency: There is some miRNA that regulates the transporters involved in the take-up, transportation and assimilation of P within the plant system as follows –

i. miRNA 399 and PHO2

Two main proteins, phosphate trans-porter1 (PHT1) and phosphate 1 (PHO1), promote the uptake and assimilation of P in plants. PHT1 proteins can use energy for Pi and H+ co-transport and are therefore involved in the acquisition of Pi. PHO1 is involved in loading the purchased Pi into xylem, thereby facilitating the root-to-shoot transportation of this macronutrient in the field.

Pi Starvation Responses (PSR) is caused by plants under Pi stress. Since PHT1 and PHO1 proteins are essential to assimilating and allocating Pi in plants, PSR seeks to optimize their capacity word. The Phosphate Starvation Regulator 1 (PHR1) and Phosphate Starvation Regulator 1-like (PHR1-LIKE1) transcription factors of MYB contribute to over accumulation of PHT1 and PHO1. PHR1 and PHR1-LIKE1 induce the expression of Phosphate Transporter Traffic Facilitator 1 (PHF1), a protein that promotes the transportation of PHT1 to membranes, thus increasing the availability of Pi for assimilation.PHO2 mediates a post-translational inhibition of PHO1. Thus, by blocking PHO2, miR399 leads to both PHT1 and PHO1 accumulation during Pi starvation.

ii. miRNA 827 and NLA encoding gene

The Nitrogen Limitation Adaptation (NLA) protein, an E3 RING ubiquitin enzyme, may also cause PHT1 to suffer ubiquitin. NLA-mediated PHT1 ubiquitination results in endocytosis and protein degradation. Consequently, the NLA encoding gene's post-transcription regulation by miR827 helps to regulate PHT1 levels under stress conditions.

iii. miRNA, phosphorus, and mycorrhiza Symbiosis

The interaction with arbuscular mycorrhizal fungi is one of the most common adaptations to P deficiency. In this interaction, fungi promote a more efficient absorption of water and nutrients (including P) by roots and at the same time receive the carbohydrates needed for their metabolic process. In the model species M, microRNAs play a very important role in controlling this symbiosis. Truncatula, as participants in this physiological response, several miRNAs were expected. The miR171h operates by targeting the gene encoding Nodulation Signalling Pathway 2 (NSP2) required for the production of stimulating plant hormones in the spatial regulation of fungi root colonization.

The miR396, which exerts control over several root development-related transcription factors, undertakes a repressive action in mycorrhizal colonization in this species. Mutants expressing this miRNA were less colonized than control plants, while those inactivating the same miRNA were substantially more colonized by AM fungi.

Role of miRNA in K Homeostasis: Some miRNA controls the transporters involved in K uptake, their transportation and assimilation within the plant system as follows:

miRNA 444a and MADS Box gene

MADS-box genes encode a family of transcription factors and are associated with several developmental regulatory pathways, from root to flower and fruit production. The particular miRNA investigated for K+ signage is the common miR444a monocots. The ex-pression profile of this miRNA and its respective targets *(MADS-23, MADS-27a, MADS-27b, and MADS-57)* during K+ deprivation in rice roots. Compared with the control situation, the MADS-23 goal was strongly induced. The presence of NH4+ in medium-containing potassium facilitates the absorption of nitrogen by HAK5 protein.Suggested to be a K+ transporter with high affinity. Under this nutritional situation, even at low K+ concentrations, the protein AKT1 becomes the main K+ uptake protein. MADS-23 transcription factor, which is involved in potassium absorption, has been experimentally demonstrated as regulated in monocots by miR444.The transcripts of AKT (ankyrin potassium transporter), HAK, and HKT1 (high affinity potassium transporters) were predicted in rice andmiR168,miR842 and miR854 in Arabidopsis as putative targets of miR167 and miR443. The direct effect of miRNAs on the miRNA levels of potassium transporter members inhibited by NH4 + is, and remains to be confirmed, a hypothesis based on silico research.

Role of miRNA in S homeostasis: S takes up, transportation and assimilation within the plant system regulated by some miRNA, which are mentioned below

i. miR395 and its low affinity sulfate transporter SULTR2;1

Within Arabidopsis, sulfate is transported by cell-specific transporters such as sulphate transporters1; 1(SULTR1; 1), SULTR2; 1, and SULTR2; 2 by xylem or phloem. Sulfate limitation induces the expression of miR395 and its low affinity sulfate transporter SULTR2; 1, restriction of SULTR2; 1 expression of miR395 in xylem parenchyma promotes the translocation of sulfate ions from roots to shoots.

ii. miRNA 395 and ATP sulfury lase genes

In S assimilation, miR395 was also elucidated by suppressing the expression of ATP sulfurylase genes such as APS1, APS3, and APS4 that catalyze the first stage of S assimilation. S deficiency results in an elevated synthesis of SULFUR LIMITATION1 (SLIM1) protein in the roots, which in turn stimulates specific sulfate transporters to improve S absorption.

Role of miRNA in Copper homeostasis

There is some miRNA that controls the transporters involved in taking up Cu, their transportation and assimilation within the plant system as follows

i. miR398 and SOD1 (CCS1) gene

Cu plays an important role in combating oxidative stress responses by acting as a cofactor of Cop-per / Zinc superoxide dismutase (CSD). Superoxide dismutase SOD1 (CCS1) 1 is a protein containing chaperone.

ii. MIR397, miR408, miR857 and the genes of laccase and plastocyanine.

MiR397, miR408, and miR857 were found to be up-regulated during Cu starvation, which in turn suppresses laccase and plastocyanine gene expression. Three laccase genes (LAC), such as LAC3, LAC12, and LAC13, are reduced by miR408, and miR397 degrades the mRNA of LAC2, LAC4, and LAC17. MIR857 focuses primarily on LAC7 transcripts. Notably, miRNAs linked to Cu homeostasis promote biosynthesis of plastocyanine by reducing the biosynthesis of non-essential Cu enzymes, thus ensuring Cu homeostasis by altering the availability of Cu among different protein groups.

miRNA in other mineral homeostasis

Manganese (Mn), Fe, and Zinc (Zn) are essential plant growth and nutritional minerals. Several miRNAs have been found to be up-regulated during Mn hunger and are also associated with other mineral stresses. miR319, miR169, miR396, miR170 and miR167 are up-regulated during Mn toxicity and mitigate the expression of a wide gene group. Iron and Zn are important plant micronutrients as they are the main cofactors for several primary metabolic enzymes, including proteins from the Fe-S cluster and molecules of ferredoxin. Fe deficiency decreases the expression of miR397, miR398a, miR398b, miR398c, miR399, miR408 and miR2111, while Cu deficiency increases their expression and regulates the expression of CSD1 and CSD2 in turn.

Table 1: miRNA diversity related to Nutrient use efficiency

miRNA Family	Target gene or Protein	Description of function	Reference
156	Squamosa Promoter Binding Protein-Like (SPL) transcription factors	Shoot development Delayed vegetative phase change	Zhao *et al*,2012
160	Auxin response factors	Reduce auxin responsive activities, Vegetative growth, Lateral and adventitious root development and signal transduction	Liang *et al* ,2012
164	NAC transcription factors	Accelerate senescence N remobilization	Liang *et al* ,2012
169	HAP2 transcription factors, CAAT binding	Nitrogen homeostasis, stress response	Zhu *et al* ,2010
172	AP2 like transcription factors	Ethylene-responsive pathway, N remobilization, Flower development	Zhu *et al* ,2010
395	ATP sulfurylase; sulfate transporters	Sulfate homeostasis	Zeng *et al*,2010
397	Laccases	Laccases Reduce root growth,Copper homeostasis	Zhao *et al*,2013
444	MADS-box	Root development	Lundmark *et al*, 2010
171	SCARECROW-like transcription factors	Root development	Pant *et al*,2012

Table 2: miRNA expression under Nutrient Deficient condition

miRNA Family	Involvement under low N		Involvement under Low Pi		Reference
	Plant tissue	Plant sp.	Plant tissue	Plant sp.	
156	R (+)	maize	R(+)	Arabidopsis	Zhao *et al*,2012
160	R(+)	maize	R(+) L(-)	white lupin	Liang *et al* ,2012
164	L(+) R(-)	maize	R(+) L(-)	white lupin	Liang *et al* ,2012
169	R(-) S(-)	maize	R(-)	Arabidopsis	Zhu *et al* ,2010
172	L(+) S(+)	maize	L(-)	Tomato	Zhu *et al* ,2010
395	R(-)	Arabidopsis	R(-)	Arabidopsis	Zeng *et al*,2010
397	L(-) R(-)	maize	L(-)	White lupin	Zhao *et al*,2013
444	R(+)	rice	R(+)	Rice	Lundmark *et al*,2010
171	R(+)	Arabidopsis	L(-)	white lupin	Pant *et al*,2012

R; Root, S; Shoot, L; Leaf, (+) upregulated, (-) downregulated

Conclusion

Changes in soil nutrient levels can activate different signalling molecules that can serve as nutrient-responsive target repressors-miRNAs. Subsequently, the reduced accumulation of miRNAs stabilizes transporter expression. Many miRNAs involved in NPK deprivation are linked to mechanisms involved in adapting to stress conditions by affecting root architecture, controlling NO^3 or Pi transporters, controlling shoot production, affecting vegetative phase transition, and managing the leakage of these nutrients. miRNAs function in nitrate transporter control and metabolic enzymes such as aspartate amino transferase, glutamine synthase, glutamate dehydrogenase. Thus, miRNA plays a very important role in nutrient homeostasis in plants either by up-regulating or down-regulating transporters.

Future Prospects

- miRNA play key role in regulation of nutrients within the plant system. Future study should take following issues into consideration;
- Identify root-specific novel miRNAs under nutrient stress and investigate the function of miRNA-mediated activation or removal of transporter suppressors.
- Function of miRNAs in various groups of Fe and Zn root-to-seed transporters, miRNA promoter / genome editing.
- Investigation of novel miRNAs and their function in phytate biosynthesis, such as control of various inositol phosphate kinase genes, alteration of unique miRNA expression through over-expression or genome editing.

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