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Approaches to Improve Nitrogen and Phosphorous use Efficiency in Wheat

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ABSTRACT: Wheat productivity deliberately depends upon the large amount of N and P fertilizers. It becomes more challenging to meet the wheat N and P demand from soil with the growing population. But to meet that demand large amount of fertilizers uses affects the environment, water bodies and soil health and added cost to the crop production. To avoid the harmful effects of these chemical fertilizers we need to adapt the strategies for improving nitrogen and phosphorous use efficiency of the wheat. Strategies should be made in such a way that wheat genotypes must have higher uptake efficiency. Therefore, in this review, we present the importance of nitrogen and phosphorous to wheat, its uptake and utilization. We also mentioned the strategies to improve N/PUE, *i.e* via conventional breeding as well as molecular approaches such as marker assisted selection, Transgenic approaches, miRNAs in N and P stress and nanoparticles based approaches. These approaches will be helpful in future breeding programs and in improvement of N/PUE of wheat genotypes.

Keywords: NUE, PUE, miRNA, Marker assisted selection.

INTRODUCTION

Triticum aestivum L., commonly known as bread wheat, is hexaploid in nature (2n = 6× = 42, AABBDD genomes), originated from the combination of 3 interlinked diploid genomes having repetitive sequences accounting for approximately 80% of the total genome (~17 GB) (Venske *et al.*, 2019). Emmer wheat (*Triticum turgidum*=AABB) comes into existence by a combination of Triticum urartu (donor of A genome) and *Aegilops speltoides* (donor of B genome). Hexaploid wheat (*Triticum aestivum*) arose from the hybridization of *Triticum turgidum* and *Aegilops tauschii* (donor of D genome), which is cultivated today as bread wheat (Dubcovsky and Dvorak 2007; El *et al.*, 2017). India is the second wheat producer all over the world. To meets the world nutritional demand wheat production must be increased from the current level. Over the last two decades, India has produced 12.5% of the world's wheat after China who produced 17% of total worldwide wheat production. India keeps most of its wheat domestic because of significant food demand across the country. According to United States Department of Agriculture (USDA) estimation of wheat production will be 780.59 million metric tons that will represent the 0.16% enhancement in wheat production around the globe (http://www.worldagriculturalproduction.com/). Wheat contains the proteins near about 13%, vitamins, dietary fibres, carbohydrates and iron thus consumed in daily diet as a source of energy (Shewry and Hey 2015).

Grain yield and quality of wheat mainly depends upon considerable inputs of N. Thus to encounter the sustainable input of N in wheat, it is supplied with N fertilizer. Although wheat absorbed only 30-35% of applied fertilizer while remaining is lost through leaching and surface run off (Plett *et al.*, 2018; Correll, 1998). N loss results in depletion of nitrogen use efficiency (NUE). NUE is a measure of grain yield produced as a function of the N available to that plant. NUE is the product of two independent components- NUpE and NUtE (Moll *et al.*, 1982). Phosphorous is another macronutrients which plays role in photosynthesis, respiration, energy rich compounds like ATP, phospholipid and phosphoproteins. Like N, most of the P present in soil does not available to plants either its binding with soil particles or its conversion to organic P. A large supplementation of soil with these N and P fertilizers also leads to other multinutrient deficiencies that directly results in crop yield reduction. For sustainable agriculture, there must be an increase in understanding the mechanism of increased N/PUE. Therefore, this review presents the role of N and P in wheat growth and development, deficiency symptoms and approaches to improve N/P use efficiency in wheat.

Why N and P is important for wheat. Being a macronutrient, N is very essential for wheat growth and development. It is constituent of ADP and ATP which itself are important for energy production in plant during its various metabolic processes. N also contributes to plant heredity material as it is constituent of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). It is also a component of amino acids, alkaloids and chlorophyll which is main site of photosynthesis.

After N, P is the main limiting nutrient for plant growth and development and it contributes to 0.15-0.50% of wheat dry weight (Balyan *et al.,* 2016). It is constituent of nucleic acid, membrane proteins, phospholipids and ATP which is energy currency of cell. It is also involved in controlling the enzymatic reaction through reversible phosphorylation.

Consequences of N and P Deficiency. Chronic N starvation causes interference in the metabolic activities of plants. N deficiency can be diagnosed by visual symptoms such as stunted growth, reduction in tillers, spikes leaf area and, yellowing of leaves etc. N and P both are highly mobile and it moves from older to younger leaves therefore, its deficiency symptoms visible first in older leaves (Yadav *et al.,* 2017). Other traits influenced by its starvation are root system architecture, leaf development, yield and genes involved in nitrate transport, uptake, and assimilation and translocation pathway of plant (Bhardwaj *et al.,* 2009; Ghafoor *et al.*, 2021). Low NUE can cause some environmental consequences such as ground water pollution, eutrophication, emission of greenhouse gases etc. (Chen *et al.,* 2022).

Phosphorous nutrition is crucial at early growing stage of wheat. P starvation causes reduction in tillers and head formation to which plant cannot be recovered even after later supply of adequate P (Lin *et al*., 2020). P deficiency may results in stunted growth and purple leaf of plants.

Physiology of N uptake and utilization. Plants uptake N through the roots in the form of NO_3^- and NH_4^+ via combined action of low affinity transporters (LATS) and high affinity transporters (HATS). Nitrate is the most preferred source of N taken up by plants, which undergoes reduction from NO_3^- to nirite (NO_2^-) by Nitrate reductase (NR) in the cytoplasm and NO_2^- further moves to plastid or chloroplast which is further reduced to ammonium ion (NH_4^+) by Fd dependent nirite reducatse (NiR).

These NH_4^+ ions further assimilated to amino acids glutamate, glutamine, asparagine and aspartate and distributed in different plant parts (Liepman and Olsen 2003; Fontaine *et al*., 2005). These amino acids contribute to 95% of grain protein content. Nitrogen uptake to remobilization and assimilation into amino acids are censorious steps to improve NUE.

Physiology of P uptake and utilization. P can be taken up by the plants either in the form of inorganic P (Pi) or organic P (Po) while plants preferred the Pi form. Approximately 80% of the applied P does not available to plants due to its conversion into organic form by precipitation and adsorption (Smith 2002). Plants uptake Pi by its diffusion to plant roots. Soil pH, its ionic concentration and Pi concentration in the soil also affect the bioavailability of P. Absorbed Pi transported through roots to xylem and younger leaves. This absorbed P is further assimilated in the form of ATP, phospholipids, nucleic acid and glycoproteins (Balyan *et al.*, 2016).

Approaches to Improve Nitrogen and Phosphorous Use Efficiency

Conventional Breeding approaches. Nitrogen/Phosphorous use efficiency (N/PUE) is product of N/PUpE (amount of N/P absorbed out of the total N/P supply) and, N/PUtE (efficiency to utilize the absorbed N/P to produce grains) (Moll *et al.*, 1982). To enhance the N/PUE we need to understand the traits such as, increased leaf photosynthesis rate, root architecture to increase uptake and stay green traits associated with N/P remobilization that governs high N/PUE. Various studies showed the interaction between genotypes and N level (Cormier *et al.,* 2013; Wang *et al.,* 2021; Gorny *et al.*, 2011). Linina and Ruza (2018) reported the influence of N fertilizers on winter wheat. Winter wheat cultivars showed a significant effect of N fertilizer on grain yield ($p < 0.05$). N90 (6.29 t ha^{-1}) showed increased grain yield as compared to N60 (6.08 t ha^{-1}), while the further increase in N fertilizers dose does not affect the grain yield significantly. Nehe *et al*. (2018) reported the significant interaction between N×genotype for grain yield and NUE. An increment in NUpE is an attractive selection criterion for improving NUE. Genotypic variation with P level was reported in various studies (Batten *et al.*, 1986; Havlin *et al.*, 2016; Waraich *et al*. 2015). In case of P, PUE can be improved by an increased in uptake efficiency because plant available P is less as compared to total P present in soil. PUpE can be increased by enhancing the available P, increase in effective root area and, increasing root surface area, length and root hair development (Elanchezhian *et al.,* 2015). A schematic representation for improving N/PUE shown in Fig. 1.

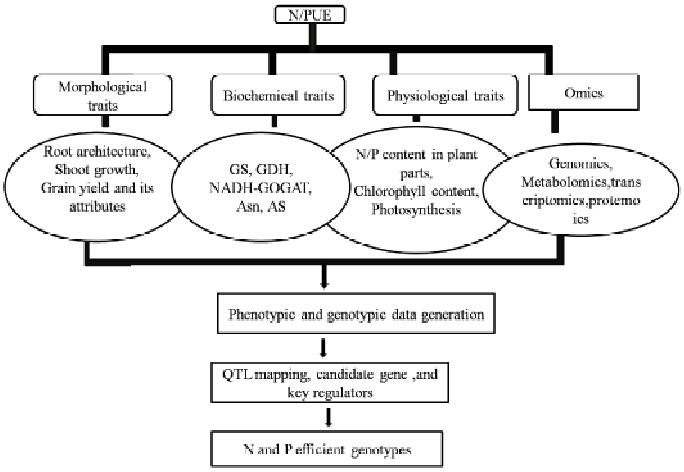

Fig. 1. A schematic representation for improving N/PUE**.**

B. Molecular approaches

Marker assisted selection. NUE is quantitative trait having two components -NUpE and NUtE which are controlled by a number of genes. Screening for N/PUE related traits becomes difficult to measure with classical plant breeding methods because these are time consuming. Thus, Marker assisted breeding helps in quantification of these N/PUE related traits in less time and at early stages of growth. Marker assisted selection becomes a selection method in improving the agronomic and quality traits related to wheat (Pandurangan *et al.*, 2021). Ranjan *et al.* (2021) carried out a study to dissect the trait through QTL mapping for NUE related traits under high N conditions in Indian wheat and detected the 28 QTL for 8 traits and 26 interacted QTL for three traits. Numerous studies concentrated on QTL analysis for NUE related agronomic traits and look into the enzyme namely GS,GDH etc. (Raigar *et al*., 2022; Ranjan *et al.*, 2021; Xu *et al.*, 2014; Guo *et al.*, 2012; Vitale *et al*., 2021). Grain protein content determines the important nutritional value of wheat flour which is used in baking and pasta making. Nigro *et al.* (2019) identified the candidate gene and QTL which migrates with N related genes. They found the four QTL related with grain protein content without affecting the final grain yield, hence, overcome the problems of negative correlation of grain yield with increasing grain protein content.

Numerous studies found the role of QTL in PUE, which may be potential target for marker assisted selection (Su *et al.*, 2006; Hitz *et al.*, 2017; Zhang *et al.*, 2015; Cui *et al.*, 2014; Yang *et al*., 2021; Ren *et al.*, 2017; Quarrie *et al*., 2005). To study the PUE, QTL mapping for 13 maturity and 11 seedling traits were studied in wheat in Recombinant Inred Lines. From this study, eleven high frequency QTL and four QTL clusters were detected with a total of 29 markers (Yuan *et al.*, 2017). Su *et al.* (2009) detected the QTLs associated with PupE and PUtE under low P and high P in wheat which would be helpful in improving the agronomic traits related with PUE. In another field study, it was reported that phosphorous acquisition efficiency (PAE) and PUE was enhanced in wheat cultivars due to higher expression of TaPHT1.2 in roots of KN92 (Deng *et al.*, 2018). They studied the PAE and PUE in two wheat cultivars under the 6 Phosphorous doses and conclude that PAE is under genotypic control and both the cultivars follow different root strategies for P uptake and utilization.

C. Micro-RNA and their targets in N/P equanimity

miRNAs role in N stress. Longer persistence of N starving conditions in plants can lead to senescence in leaves, reduction in photosynthetic activity and ultimately the growth and yield of crops (Fageria and Barbosa 2001). Several miRNAs have been characterized and show differential expression in response to N starvation conditions (Liang *et al.*, 2012; Sinha *et al.*, 2015). These miRNAs can be up and down-regulated under N starving conditions. Pant *et al.* (2009) detected that miR167 and miR398a were down regulated under N limiting conditions in Arabidopsis. Vidal *et al.* (2014) discovered the new miRNA-miR5640 and its target- AtPPC3 (phosphoenol pyruvate carboxylase) in roots of Arabidopsis through sequencing of small RNAs and mRNAs. Zhao et al. (2013) investigated the expression pattern of 38 miRNAs in wheat under optimum and low N conditions. Gao *et al.* (2016) studied and characterized the role of TaMIR444a in mediating plant tolerance to the N starvation stress. Under N limiting conditions, the expression of TaMIR444a was found to be upregulated in roots and leaves while its targets showed reversed expression pattern. Nitrate transporter and antioxidant enzymes were also found to be up-regulated in plants which show over expression of TaMIR444a. Liang *et al.* (2012) identified the N responsive miRNAs under N sufficient and deficient conditions. Under N starving conditions, miR160, miR167, and miR171 were found to be responsible for the development of root systems in

Arabidopsis. Sinha *et al.* (2015) studied the morphological response and assay of enzymes involve in the assimilation of N especially in the roots of wheat and check the expression of miRNAs (miR159a, miR159b, miR399, and miR408). All these studies confirmed the role of miRNAs in N uptake, transport, and assimilation. Sagwal *et al.* (2022), found miR171a and miR167a, miRNA-SSR markers which can differentiate the N and P efficient and deficient wheat genotypes, respectively.

miRNAs role in P stress. Phosphorous (P) is the second limiting element for the growth and development of plants followed by N. Plants uptake P in the form of inorganic phosphate (Pi) but it is less available to plants due to its precipitation. Therefore, plants solubilize, uptake, and transport this bounded P by various classes of nucleases, phosphatases and proteins (Poirier and Bucher, 2002). Phosphate transporter 1 (PHT1) plays a key role in the acquisition of Pi by using energy (Okumura *et al.*, 1998). Phosphate 1 (PHO1) helps in the transport of Pi from root to shoot by loading it into the xylem (Hamburger *et al.*, 2002). Several studies prove the involvement of miRNAs in the differential regulation of phosphate-related gene expression in various crops like wheat, rice, maize, soybean and model plant Arabidopsis (Fujii *et al.*, 2005; Lin *et al.*, 2010; Kuo and Chiou 2011). Valdes *et al.* (2008) revealed the role of miR399 in the signaling pathway of PvPHR1 (PHOSPHATE STARVATION RESPONSE 1) under P deficient conditionsin common bean roots. Liu *et al.* (2020) demonstrate the genome-wide identification of low P responsive miRNA in two soybean genotypes- Bogao and Nannong 94-156.

Target genes of N and P responsive miRNAs. MicroRNA plays a crucial role under N and P starvation conditions and a miRNAomics study revealed that how these miRNAs regulate the target genes. Most of the miRNAs suppress the transcript of the target; therefore, the expression of miRNAs is generally negatively correlated with that of their targets. Zuluga *et al.* (2018) studied the expression of miRNA and their target genes under N deprivation conditions. Novel miRNA106 expression was found 3-fold down-regulated in root tissues under N stress whereas its target MYB-A was found to be upregulated in the same root tissue. In the same study, expression of another miRNA miR399b significantly reduced after 24 h of stress treatment while its target gene PHO2 (PHOSPHATE 2) expression was increased after 24 h of imposing the stress. He *et al.* (2014) studied the role of two miRNA in the regulation of glucosinolate synthesis under N starvation conditions. Glucosinolates are N rich secondary metabolites and require the availability of N for their synthesis. AOP2 (ALKENYL HYDROXALKYL PRODUCING2) gene is accountable for the side-chain modification of glucosinolates. In Arabidopsis seedling, the AOP2 gene was found to be down-regulated under N deficient conditions in Arabidopsis. Expression of miR826 and miR5070 was found up-regulated in N deficient conditions. Upon target prediction, it was found that AOP2 is a common target of both the miR826 and miR5070. Kumar *et al.* (2018) reported the genome-wide identification of transcription factors of two sub-families-NLP (NIN-like proteins) and RKD (RWP-RK domain). These TF regulate the expression of genes involved in nitrogen use efficiency. The TaNLP7 gene showed significant upregulation in the roots and shoots of wheat genotypes with higher NUE during N-stress conditions. Target prediction depicts that these genes carried targets of 13 different miRNA. In an earlier study, it has been reported that miR171 and miR160 work in signaling pathways in root development in Arabidopsis. miR171 targets the SCL (SCARECROW-LIKE TF) and hence reduce the primary root elongation. Quantitative RT-PCR showed that miR171 up-regulated under N starvation condition and its expression was three-fold higher under N deficient condition as compared to N sufficient condition (Liang *et al.*, 2012). In one study, quantitative RT-PCR was performed to check the expression of miRNAs under P starvation conditions. miR159b, miR167, miR399, miR408, miR1122, miR1125, miR1135, and miR1136 were found to be up-regulated whereas miR408 was found to be down regulated. They also confirmed their results with RNA blot analysis. Expression analysis of these target genes was also performed. MYB3, Auxin response factor 8 (ARF8), basic blue protein/plantacyanin, annexin-like protein, NADH dehydrogenase subunit 6 target genes were down regulated by TamiR159b, TamiR167, TamiR1122, TamiR1125 and TamiR1135 respectively. Meanwhile, a target gene of TamiR408 was found to be up-regulated under P starvation conditions. PHOSPHATE STARVATION RESPONSE1 (PHR1) is a transcription factor of the MYB family that acts in the Pi starvation signaling pathway. It regulates a group of P starvation-induced genes. Kant *et al.* (2011) studied the nitrate dependent phosphate homeostasis under genetic regulation by NLA (NITROGEN LIMITATION ADAPTATION) and miR827. Their study revealed that NLA expression is regulated by miR827 which is itself induced by low Pi. In *Medicago truncatula*, miR171 targets the Nodulation Signaling Pathway 2 TF (NSP2) in response to cytokinin (Ariel *et al.*, 2012). A semi-quantitative and quantitative RT-PCR analysis disclosed that nine miRNAs including miR167a respond under P deficient conditions. miR167a is up-regulated under P starvation and targets the auxin response factor 8 (Zhao *et al.*, 2013). Zhang *et al.* (2021) performed a genome-wide analysis of long non-coding RNA in two soybean genotypes under P starvation conditions. RNA seq libraries were constructed from two genotypes of soybean. Out of the total 4,166 lncRNAs, 525 were found to be differentially expressed. Gene Ontology (GO) studies revealed the role of lncRNA in different biological processes and their targets were also predicted. Twenty-six TFs such as MYB, bHLH, NAC, and AP2 were reported to have a crucial role in P homeostasis.

Transgenic approaches for NUE/PUE. Roots are the major site for N and P uptake from the soil. Their volume, size and distribution affect the accession of N and P from the soil. Manipulation in roots traits have been proved to increase nutrient uptake from the soil and hence increase in productivity (Nguyen and Stangoulis, 2019; Fernando *et al.*, 2021). Stimulation of lateral root branching helps in steeper root growth and more acquisition of nutrients (Giehl and von Wiren 2014). Zhang *et al.* (2015) did a field study under different N environment in rice. Overexpression of Tolerance of Nitrogen Deficiency 1 (OsTOND1) in rice accelerates the primary root growth, N uptake, and N concentration in shoot as compared to control.

Transgenic manipulation in N transporter, ammonium transporter, N assimilated genes and Pi transporter and assimilated genes determines the improvement in N/UE in different crops (Ranathunge *et al.*, 2014; Fan *et al.*, 2016; Chen *et al.*, 2016; Ye *et al.*, 2015; Yan *et al.*, 2014). NAC transcription factor superfamily responses in number of stresses and plays role in nutrient availability, root development, leaf senescence and hormones responses regulation. Over expression of TaNAC 2-5A accelerates the N influx rate and nitrogen transport in aerial part of plant and hence, proves its importance in N signaling ultimately increase the grain yield in wheat. Another family of TF, TabZIP60 knockdown accelerates the activity of glutamate synthase (NADH-GOGAT) and increase the grain yield upto 25%, spikes number and N uptake (Yang *et al.*, 2019) (Table 1).

Nanoparticles to improve NUE/PUE. A loss of N and P from unwise use of fertilizers causes the main threat to environment and living beings. Therefore, we need to adopt some strategies to minimize the loss of these fertilizers in the environment. Nanoparticles especially nutrient coated urea can be used as slow release fertilizers (Mustafa *et al.*, 2022). Urea granules covered with thin layer of nutrients such as Zn and Cu, which helps in longer persistence of fertilizers in the soil and make the nutrients available to plant. Sulfur coated urea (SCU) with release time 120 days showed significant correlation among growth, yield and physiological parameters with N concentration in soil (Altaf *et al.*, 2021). In another study, sulfur coated urea and agrotain (a urease inhibitor) was applied in rice in a pot experiment. Grain yield, dry biomass and grain quality was observed higher as compared to application of granular urea (Khan *et al.*, 2015). Rundla *et al.* (2022) conducted a field study and observed the effect of salicylic acid foliar spray on the nutrient use efficiency (NPK) in mustard. As the chemical fertilizers are affecting the soil health as well as deteriorating the environment, so the use of nanoformulation and manures enhance the uptake efficiencies of nutrients as reported by Arvind *et al.* (2022).

Table 1: Transgenics reported in some crops with their altered phenotype.

Source	Target	Reference	Gene product	Phenotype Observed
TaGS2-2Ab	Wheat	Hu *et al.* (2018)	Glutamine synthetase (GS2)	Increase in root N acquisition, leaf chlorophyll content and grain yield
OsAnt1:HvAlaAT	Rice, wheat, barley	Tiong *et al.* (2021)	Alanine aminotransferase (AlaAT)	Higher nitrogen assimilation and increase in biomass
TaARE1	Wheat	Zhang *et al.* (2021)	ARE1 (abnormal cytokinin response1 repressor1)	Increase grain yield by delaying senescence
HvAlaAT	Sorghum and wheat	Pena *et al.* (2017)	AlaAT (alanine aminotransferase)	Increase in wheat yield ,no phenotypic outcomes for sorghum
ZMDof1	Sorghum and wheat	Pena *et al.* (2017)	Phosphoenol pyruvate carboxylase (PEPC)	Inc. in Photosynthesis rate, enhanced the yield and biomass of wheat and sorghum
AtASN1	Arabidopsis thaliana	Lam *et al.* (2003)	Asparagine synthetase	Increase seed protein and N starvation tolerance
TaNAC2-5A	Wheat	He *et al.* (2015)	Nitrate transporter and GS	Increase root NO3 – influx, N acquisition in aerial parts, grain yield
TaPHR1	Wheat	Wang *et al. (*2013)	PHR1(Phosphate starvation response gene)	Alleviated PUpE ,lateral root branching, increase in grain yield
OsMAD25	Rice	Yu *et al.* (2015)	ANR1 , Nitrate signaling genes	Increase in PR length, lateral root number and length, accelerate the NO3– expressions of transporter genes
OsTOL1	Rice	Gamuyao *et al.* (2015)	Phosphorous starvation tolerance	Increase in P uptake, root growth and grain yield
TaVP	Tobacco	Li et al. (2014)	vacuolar H+-pyrophosphatase	Enlargement and improvement in tobacco root system hence in plant growth
TabZIP60	Wheat	Yang *et al.* (2019)	NADH-GOGAT	Increases NADH-GS , lateral root growth, number , spikes number and yield of wheat

CONCLUSION

Wheat growth and productivity is largely affected by the dose of nitrogen and phosphorous fertilizers. Soil nutrient status is going to be deteriorating by the passing years due to higher use of chemical fertilizers to meet the N/P demand of crops. To enhance the N/P availability of plants we need to adopt the strategies which could be helpful in efficient uptake of N and P from the soil and directly to the growth and productivity of wheat.

FUTURE SCOPE

Conventional breeding approaches associated with marker assisted selection will be helpful in accelerating the release of new varieties. Therefore, we need to dig out more markers associated with N/P uptake, transport and utilization. There is need to work more on the use of slow release nanofertlizers instead of chemical fertilizers and they must be commercially available so that farmers can get benefit. A practical use of all approaches mentioned in review would be helpful in improving the soil health, its nutritional status and grain yield of wheat.

Conflicts of Interest. None.

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