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Review Article (Open Access)

GENETICS OF NITROGEN METABOLISM IN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

N fertilization significantly increased photosynthetic parameters and N metabolite enzymes at all growth stages. Nitrogen harvest index, partial productivity factor, agronomic recovery efficiency, and nitrogen agronomic efficiency were decreased with increased N.

N-metabolism genes such as NRTs, AMTs, NRs and NiRs are up-regulated rapidly after N treatment and functioned in the uptake and transport of nitrate and ammonium or in the reduction of nitrate and nitrite. Also, many differentially expressed genes are involved in the metabolism of amino acids such as glutamate, alanine and aspartate. Under low nitrogen conditions, the activities of nitrogen metabolism-related enzymes (GS, NR, GDH), antioxidant enzymes (SOD, POD, CAT) and soluble protein contents are higher in high NUE varieties. A major QTL was observed on the short arm of chromosome 6B controlling grain protein content in wheat accounting for 66% of the phenotypic variation where the cloning of functional gene named Gpc-B1is elucidated. Under N limitation, there was a genetic negative correlation between grain yield and grain protein concentration. Deviation from this negative relationship (grain protein deviation or GPD) was related to genotypic differences in post-anthesis N uptake. 3949 (2414 down-regulated, 1535 up-regulated) differentially expressed genes (DEGs) were identified in the shoot, and 3911 (2675 down-regulated, 1536 up-regulated) DEGs were identified in the root of the wheat plants grown under NDC.

Keywords: *Wheat (Triticum aestivum L.), Nitrogen utilization efficiency, Low N tolerance, Nitrogen metabolism, Gene expression*

INTRODUCTION

Rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.) are the important cereals in terms of human nutrition, accounting for 90% of global grain output. Since the Green Revolution, the importance of cereal crops in world agriculture has expanded dramatically. Among the factors found to influence the quality and quantity of cereal crops produced; nitrogen availability is one of them.

The International Food Policy Research Institute projected that the world demand for wheat will rise from 552 million tons in 1993 to 775 million tons by 2020, and by a total of 60% by 2050 (Singh *et al*., 2016). Benzian and Lane (1979) analyzed the relationship between nitrogen supply, grain yield, and grain protein for wheat. It was found that a greater nitrogen supply increased grain protein concentration linearly while grain yield response to added nitrogen had a diminishing return relationship. They also found that when nitrogen was very limiting, small nitrogen additions resulted in greater grain yield with decreased protein concentration caused by dilution of the plant nitrogen. However, at higher levels of nitrogen, which are far more common, grain and protein yields usually increased but, threonine, methionine, and cystine concentrations are lower than those recommended by the World Health Organization (WHO) (Simmonds, 1978). Kies and Fox (1974) determined that lysine was the most limiting amino acid for human nutrition in wheat protein. Maximum accumulation of proteins occurred at 30 DAA and changed almost a little afterwards. Nitrate reductase and protease activity also increased up to 30 DAA and declined sharply later during development of wheat grains.

Soil nitrogen abundance, as well as nitrogen use efficiency (NUE), significantly affect the crop yield and grain protein content (GPC). Depending on the genotype, a negative correlation between the yield and GPC can occur (Fig.1).

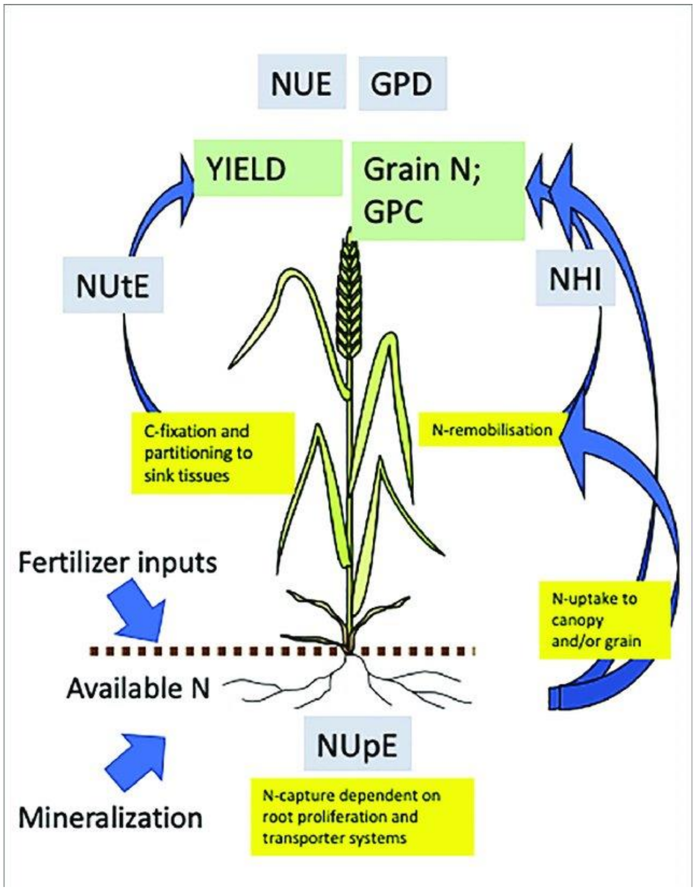


Fig.1.Pathway for NUE and GPD in wheat crop.

NITROGEN UTILIZATION EFFICIENCY

Nitrogen management requires special attention in its use so that the large losses can be minimized and the efficiency maximized. Site-specific nutrient management (SSNM) has been found especially useful to achieve the goals of improved productivity and higher N use efficiency (NUE).

Leaf colour charts and chlorophyll meters assist in the prediction of crop N needs for rice and wheat, leading to greater N-fertilizer efficiency at various yield levels (Fig.2). Remote sensing tools are also used to predict crop N demands precisely. At the same time, traditional techniques like balanced fertilization, integrated N management (INM), split application and nutrient budgeting, among others, are also used to supplement recent N management techniques to attain higher productivity and NUE, and reduce environmental pollution through the leakage of fertilizer.

Plant growth and development are largely determined by nutrient availability; therefore, to ensure better productivity of crop plants, it becomes essential to understand the dynamics of nutrients uptake, transport, assimilation, and their biological interactions (Wawrzyńska and Sirko, 2014). Protein-coding genes involved in the uptake, mobilization, storage, and assimilation of macro/micro-elements have been characterized; and regulatory networks affecting their expression in response to the changing nutritional status are being elucidated (Briat *et al.*, 2015; Chaiwong *et al.*, 2020).

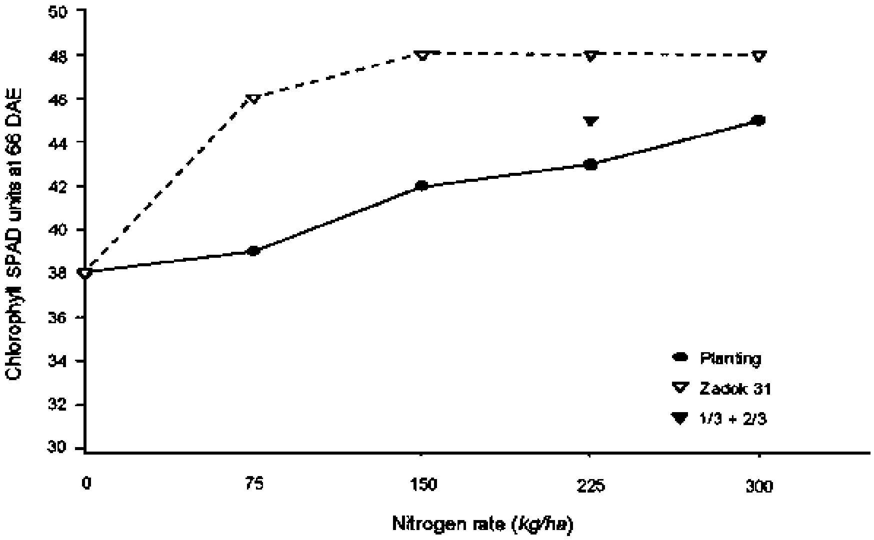


Fig.2. Changes of Chlorophyll content in leaves with nitrogen rate in wheat.

In two durum wheat cultivars, “Aureo” and “Vespucci”, after fertilization, the nitrogen content and values of some of the agronomic parameters and yield-related traits increased in both cultivars; nevertheless, a simultaneous rise in both the yield and GPC occurred only in Aureo. The biochemical parameters, analyzed at tillering, confirm the genotypic specificity of nitrogen use. In Vespucci’s roots, the nitrogen supply did not affect the nitrate reductase (NR), but greatly increased the amino acids and proteins, suggesting that ammonium is preferentially assimilated. In Aureo, nitrate is in part assimilated by the roots, as suggested by the ammonium increase and NR enhancement. In the leaves of both cultivars, organic nitrogen significantly increased after fertilization; however, the rise in amino acids, as well as in NR activity, was higher in Aureo than in Vespucci. These indicate that the different nitrogen use, and in particular the diverse NR behaviour, at tillering, are in part responsible for the cultivar differences in grain yield and GPC.

In durum wheat, researchers found the involvement of *GS* and *GOGAT* candidate genes in the control of GPC (Nigro *et al.*,2017). Interestingly, the plastidic *GS2* and the cytosolic *GS1.3* have been associated with QTL for GPC in homoeologous regions to those reported in bread wheat on the 2B and 4A chromosomes, respectively (Gadaleta *et al.*,2011,2014). The development of two distinct sets of heterogeneous inbred family (HIF)-based NILs segregating the *GS2* and *Fd-GOGAT* genes obtained from heterozygous lines at those loci confirm the previously identified GPC QTLs on the 2A and 2B chromosomes, and the involvement of these genes in GPC control (Nigro *et al.*, 2020). Due to the high number of QTLs related to NUE, GPC, and yield mapped to the chromosomal regions containing *GS2* in both bread and durum wheat, it is plausible that these regions may be useful in breeding to obtain wheat varieties with improved agronomic performance and NUEs (Habash *et al.*,2007; Laperche *et al.*, 2007).

The domestication of crops is associated with the genome-wide loss of nucleotide diversity caused by selection and genetic drift. 32 genotypes representing key stages in the domestication of tetraploid wheat, i.e. wild emmer, emmer and durum wheat, their RNA-Seq analysis was combined with estimates of rate of evolution, heritability and QST to characterize the plasticity of gene expression and its adaptive role in primary and secondary domestication under different nitrogen growth conditions. It was noted that nitrogen starvation induced the expression of a larger number of genes in durum wheat compared to emmer and wild emmer. QST distributions and QST–FST comparisons revealed distinct selection signatures at each domestication stage. While primary domestication affected the expression of genes involved in biotic interactions, secondary domestication was associated with changes in expression of genes involved in metabolism of amino acids, particularly lysine (Pieri *et al.*,2023) (Fig.3).

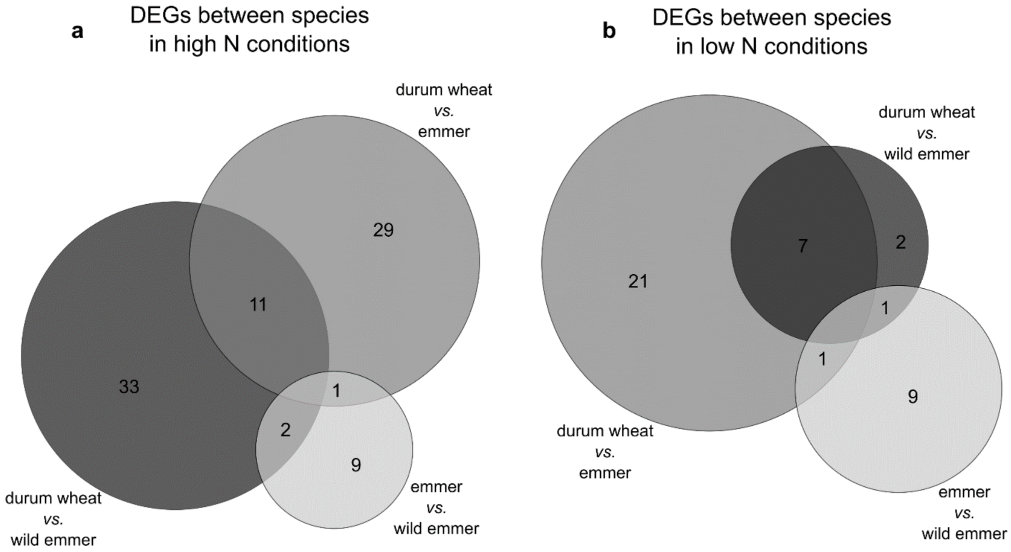


Fig.3.Venn diagrams show the numbers of DEGs between each subspecies in pair-wise comparisons under a high nitrogen conditions and b low nitrogen conditions.

Several kinds of plasma membrane-associated transporter proteins have been identified as being engaged in active transport and have been classed as high- and low-affinity transporters (Loque and Von Wiren,2004; Dechorgnat *et al.*,2010). In higher plants, three types of transport systems are active based on affinity and NO_3^- content in the rhizosphere—inducible high-affinity transport system (iHATS), constitutively expressed high-affinity transport system (cHATS), and non-saturable low-affinity transport system (LATS). iHATS is activated at low NO_3^- concentrations (1–200 µM), and its activity varies depending on plant type and environmental conditions (Feng *et al.*,2011). Both cHATS and iHATS have the trait of becoming saturated once the external NO_3^- concentration reaches a particular threshold. The third, LATS, has low-affinity transporters and is activated when there is a high concentration of NO_3^- in the soil (250 µM). *NRT1* and *NRT2* are two important gene families involved in NO_3^- in higher plants. NRT1/PTR stands for nitrate transporters, the peptide transporter family (NPF), and the main facilitator super family (MFS) of the *NRT2* family (Leran *et al.*,2014). In the absence of NO_3^-, the plant growth hormone abscisic acid activates the high-affinity transport system in wheat, which is controlled by five genes (*TaNRT 2.1*, *TaNRT* 2.2, *TaNRT 2.3*, *TaNAR 2.1*, and *TaNAR2.2*) (Cai *et al.*,2007).

It is critical for spring wheat (*Triticum aestivum* L.) production in the semi-arid Loess Plateau to understand the impact of nitrogen (N) fertilizer on changes in N metabolism, photosynthetic parameters, and their relationship with grain yield and quality. The photosynthetic capacity of flag leaves, dry matter accumulation, and N metabolite enzyme activities from anthesis to maturity were studied on a long-term trial under different N rates [0 kg ha^{-1}(N1), 52.5 kg ha^{-1} (N2), 105 kg ha^{-1} (N3), 157.5 kg ha^{-1} (N4), and 210 kg ha^{-1} (N5)]. It was observed that N3 produced optimum total dry matter (5407 kg ha^{-1}), 1000 grain weight (39.7 g), grain yield (2.64 t ha^{-1}), and protein content (13.97%). Results showed that N fertilization significantly increased photosynthetic parameters and N metabolite enzymes at all growth stages. Nitrogen harvest index, partial productivity factor, agronomic recovery efficiency, and nitrogen agronomic efficiency were decreased with increased N. Higher N rates (N3–N5) maintained higher photosynthetic capacity and dry matter accumulation and lower intercellular CO_2 content. The N supply influenced NUE by improving photosynthetic properties. The N3 treatment produced highest chlorophyll content, photosynthetic rate, stomatal conductance and transpiration rate, grain yield, grain protein, dry matter, grains weight, and N metabolite enzyme activities compared to the other rates (N1, N2, N4, and N5) (Fig.4) (Effah *et al.*, 2022).

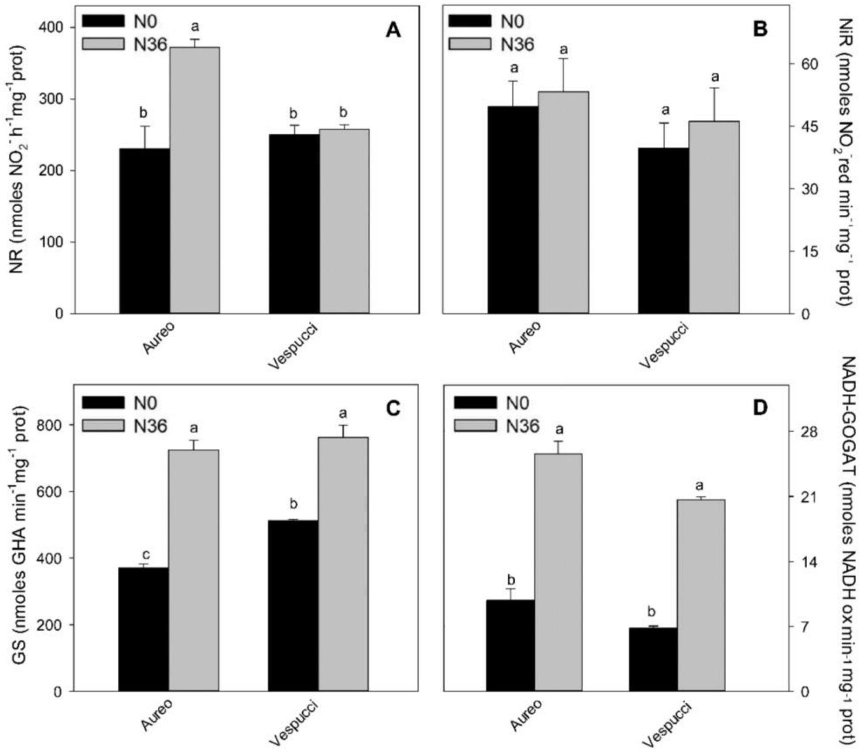


Fig.4. Influences of nitrogen on NR,NiR,GS and GOGAT in different wheat varieties under varying nitrogen levels.

The synthesis of glutamate is the starting point for the synthesis of most amino acids (Konishi *et al.*, 2014; Krapp, 2015).Although each amino acid has its unique catabolic pathway, all enzymes and metabolites involved in amino acid catabolism have the common characteristics across organisms. These metabolites of amino acid include ammonia, CO_2, glucose, long-chain and short-chain fatty acids, H_2S, ketone bodies, nitric oxide (NO), urea, uric acid, polyamines and other nitrogenous substances, which were generated under the catalyzing of enzymes. N-metabolism genes such as NRTs, AMTs, NRs and NiRs are up-regulated rapidly after N treatment. The expression level of *CHL1* (classified as an NRT transporter) is rapidly up-regulated in 30 min and reaches a peak within 2 h. NRTs, AMTs, NRs and NiRs functioned in the uptake and transport of nitrate and ammonium or in the reduction of nitrate and nitrite. Also, many differentially expressed genes are involved in the metabolism of amino acids such as glutamate, alanine and aspartate.

Accelerated senescence along with enhanced breakdown of protein and starch in flag leaves was correlated with higher grain yield, NUE for grain yield, and NUE for grain protein under N limitation. The more dramatic reductions in flag leaf N compounds and carbohydrate reserves in VA08MAS-369 were linked with significantly elevated expression of genes and enzymes associated with these metabolic pathways in this high NUE genotype. Consistent with the gene expression data, nitrate reductase, glutamine synthetase, and NAD-dependent glutamate dehydrogenase activities were highly induced under limited N in VA08MAS-369. A combination of elevated NAD-dependent GDH and GS activities may be a key mechanism underlying efficient N remobilization from senescing leaves to grains under low N availability. These processes enable continuous ATP production even under C starvation at the latter senescence stage, supporting energetically expensive N transport and assimilation during grain filling (Alpuerto *et al.*,2021).

TaPAP, *TaUPS* and *TaNMR* were all up-regulated across the treatments with different N chemotypes when compared to the control. However, their expression patterns and intensities varied across N chemotypes. *TaUPS* and *TaNMR* showed an 8.6-fold intensity of *TaPAP* at 1 d under NH_4NO_3 treatment, and both *TaPAP* and *TaUPS* showed significantly higher expression intensities at 1 d under NH_4NO_3 treatment than their corresponding intensities at 1 d under NH_4^+ and NO_3^- treatments, and up-regulations of *TaNMR* were not significant among the three N chemotypes. These suggested that *TaPAP* and *TaUPS* may be subjected to the interaction between NH_4+ and NO_3-chemotypes and *TaNMR* is not, and it is also possible that these three genes may be involved in distinct pathways of N-metabolism (Fig.5) (Li *et al.*,2019).

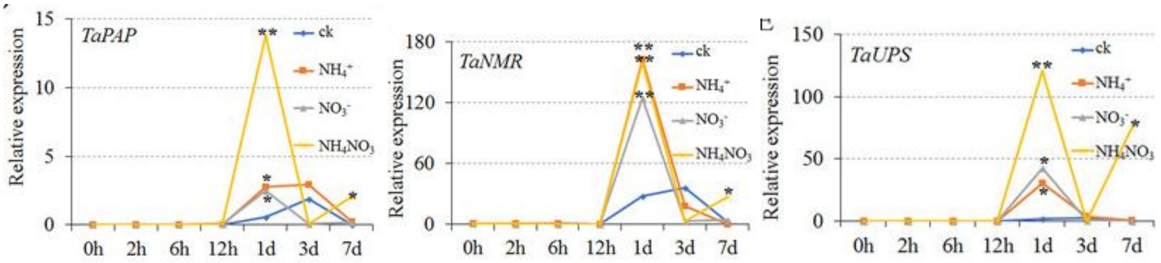


Fig.5. Verification and the expression patterns of conserved nitrogen metabolism-related genes. N0, N2 and N4 represent the treatment with 0 g, 2 g or 4 g of carbamide per pot, respectively.

Under low nitrogen, the activities of nitrogen metabolism-related enzymes (GS, NR, GDH), antioxidant enzymes (SOD, POD, CAT) and soluble protein contents of ZM366 (high NUE cultivar) were higher than those of JD8 (low NUE cultivar). The hybrid cultivar of ZM366 and JD8 showed mid-parent or over-parent heterosis. Transcriptome analysis revealed that ‘alanine, aspartate and glutamate metabolism’, ‘terpenoid backbone biosynthesis’ and ‘vitamin B6 metabolism’ pathways play key roles in nitrogen use efficiency in wheat. The significant enhancement of the ‘Calvin cycle’ and ‘photorespiration’ in ZM366 contributed to its higher level of carbon metabolism under low nitrogen stress. In addition, the activation of ABA signal transduction and biosynthesis pathways also helps to maintain NUE under low- nitrogen conditions. Moreover, bHLH transcription factors were also found to play a positive role in wheat NUE (Wang *et al.,* 2022).

QUANTITIVE TRAIT LOCI

Significant QTLs were detected in the wheat RIL population (TN18 × LM6) for grain yield; root NUE and shoot dry weight (Zhang *et al.*,2019). A major QTL was observed on the short arm of chromosome 6B controlling grain protein content in wheat accounting for 66% of the phenotypic variation where the cloning of functional gene named Gpc-B1 was carried out (Uany *et al.,* 2006). Various novel NUE-related traits and alleles in adapted breeding materials (Fontaine *et al.*,2009), landraces (Pozzo *et al.*,2018; Van Deynze *et al*.,2018), and wheat wild relatives (Hu *et al.*,2015) were identified. One such report is on winter wheat where the QTL associated with NUE on chr 1D, 6A, 7A, and 7D with LOD scores ranging from 2.63 to 8.33 and phenotypic variation up to 18.1% were instigated (Brasier *et al.*,2020) (Table 1).

Table 1. Genes associated with nitrogen use efficiency in wheat (Raigar *et al.*, 2022).

Category	Gene	Chr	Location	IWGSC Gene ID
Nitrogen transporters	*TaNPF6.2*	1A	373,766,258– 373,768,702	*TraesCS1A02G210900*
	TaNPF6.5	1A	14,519,757– 14,525,659	*TraesCS1A02G031300*
	TaNPF7.7	1A	355,624,056– 355,628,073	*TraesCS1A02G197600*
	TaNPF7.7	1B	385,644,930– 385,648,470	*TraesCS1B02G212200*
	TaNPF7.7	1D	284,040,636– 284,044,349	*TraesCS1D02G201100*
	TaNPF2.3	2A	17,869,278– 17,871,731	*TraesCS2A02G045500*
	TaNPF1.1	3A	540,654,271– 540,656,804	*TraesCS3A02G304400*
	TaNPF2.4	3A	660,436,466– 660,444,074	*TraesCS3A02G418700*
	TaNRT2.3	3B	457,633,984– 457,635,782	*TraesCS3B02G285900*
	TaNRT2.3	3D	356,623,041– 356,624,585	*TraesCS3D02G254900*
	TaNAR2.1	4A	640,232,228– 640,233,158	*TraesCS4A02G367300*
	TaNRT1	4B	483,508,916– 483,514,108	*TraesCS4B02G231500*
	TaNPF2.1	5A	3,085,412– 3,088,853	*TraesCS5A02G004400*
	TaNPF2.2	5A	34,980,804– 34,986,700	*TraesCS5A02G037900*
	TaNPF6.6	5A	599,204,895– 599,208,619	*TraesCS5A02G409600*
	TaNPF6.1	6A	486,547,388– 486,550,355	*TraesCS6A02G263500*
	TaNPF7.1	6AL/BL/DL	486,547,388– 486,550,355	*TraesCS6A02G263500*
	TaNAR2.2	6B	415,788,848– 415,790,024	*TraesCS6B02G238700*
	TaAMT1.2/1.3	6B	458,486,050– 458,487,918	*TraesCS6B02G254800*
	TaNAR2.2	6D	267,236,634– 267,237,837	*TraesCS6D02G193100*
	TaAMT1.2/1.3	6D	293,801,873–	*TraesCS6D02G208200*

			293,803,683	
	NRT1 PTR	7A	169,020,411–169,025,550	*TraesCS7A02G206400*
	TaLHT1	7A	109,262,804–109,265,004	*TraesCS7A02G156600*
	TaNRT2.4	7B	583,923,053–583,926,829	*TraesCS7B02G328700*
N assimilation	*TaNiR1*	6B	636,392,631–636,397,024	*TraesCS6B02G364600*
	TaNiR1	6D	422,078,484–422,081,985	*TraesCS6D02G313100*
Amino acid biosynthesis (glutamine synthase)	*TaAlaAT10–1/TaAlaAT-4*	1A	71,689,760–71,695,155	*TraesCS1A02G085600*
	TaASN2	1A	553,535,726–553,542,082	*TraesCS1A02G382800*
	TaASP6	1A	287,681,550–287,684,692	*TraesCS1A02G160200*
	TaAlaAT10–1/TaAlaAT-4	1B	112,748,629–112,753,960	*TraesCS1B02G102700*
	TaASN2	1B	635,920,024–635,926,285	*TraesCS1B02G408200*
	TaASP6	1B	317,791,804–317,795,107	*TraesCS1B02G176400*
	TaASP6	1D	221,915,283–221,918,343	*TraesCS1D02G157400*
	TaGOX4	2D	301,816,850–301,819,891	*TraesCS2D02G251800*
	TaASP4	3A	541,257,235–541,261,301	*TraesCS3A02G305400*
	TaASP4	3B	536,074,881–536,079,450	*TraesCS3B02G331100*
	TaGOGAT1/3	3B	481,595,302–481,606,660	*TraesCS3B02G299800*
	TaGOGAT1/3	3D	369,790,549–369,802,074	*TraesCS3D02G266400*
	TaASN1	4B	417,737,785–417,741,607	*TraesCS4B02G194400*
	TaGS1	4B	499,898,695–499,901,767	*TraesCS4B02G240900*
	TaGGT2	4B	573,273,107–573,276,702	*TraesCS4B02G288100*
	TaGGT3	4B	363,644,060–	*TraesCS4B02G167100*

			363,647,074	
	TaAlaAT10–2	5B	74,659,823–74,670,378	*TraesCS5B02G066600*
	TaAS	5B	107,190,378–107,196,256	*TraesCS5B02G084600*
	TaGDH1	5D	494,216,160–494,219,691	*TraesCS5D02G442000*
	TaASP1	6B	668,432,728–668,437,537	*TraesCS6B02G393600*
	TaGS1	6B	577,183,711–577,187,787	*TraesCS6B02G327500*
	TaGS1	6AL/BL/DL	531,394,366–531,398,363	*DQ124209;DQ124210; DQ124211*
Transcription factors	*TaNF-YB2.1*	1A	572,334,701–572,336,969	*TraesCS1A02G411700*
	TaNF-YB2.1	1B	662,783,949–662,786,278	*TraesCS1B02G442000*
	TaNF-YB2.2	3B	605,665,548–605,668,470	*TraesCS3B02G385600*
	TaNF-YB2.2	3D	458,624,044–458,626,934	*TraesCS3D02G347000*
	TaHLHm1	4B	639,452,139–639,453,299	*TraesCS4B02G345800*
	TaFBX94	5B	133,417,326–133,419,111	*TraesCS5B02G100300*
	TaHLHm4	5B	13,081,769–13,086,120	*TraesCS5B02G013000*
	TaHLHm4	5D	13,313,304–13,318,505	*TraesCS5D02G020600*
	TaNAC9/NAM	6B/1B	51,579,298–51,580,659	*TraesCS6B02G075200*
Other genes (kinases)	*TaSAPK6*	1A	381,819,326–381,822,599	*TraesCS1A02G215900*
	TaSAPK6	1B	411,987,863–411,990,884	*TraesCS1B02G229400*
	TaSAPK6	1D	304,838,300–304,841,343	*TraesCS1D02G218200*
(Rubisco)	*Rbcs*	2A	171,076,784–171,079,172	*TraesCS2A02G198700*

REMOBILIZATION

To quantify the genetic variability in N partitioning and N remobilization in Indian spring wheat cultivars and identify traits for improved grain yield and grain protein content for application in breeding N efficient cultivars. Growth analysis was conducted at anthesis and harvest to assess above-ground dry matter (DM) and dry matter and N partitioning. Flag-leaf photosynthesis rate (Amax), flag-leaf senescence rate and canopy normalized difference vegetation index (NDVI) were also assessed. Significant N × Genotype interaction was observed for grain yield and N-use efficiency. A positive linear association between post-anthesis flag-leaf Amax and grain yield amongst the 30 genotypes was observed under high N (HN) conditions. Flag-leaf Amax was positively associated with N uptake at anthesis (AGNA). Under both HN and low N (LN) conditions, higher N uptake at anthesis was associated with delayed onset of flag-leaf senescence and higher grain yield. Under N limitation, there was a genetic negative correlation between grain yield and grain protein concentration. Deviation from this negative relationship (grain protein deviation or GPD) was related to genotypic differences in post-anthesis N uptake (Table 2) (Nehe *et al.*,2022).

Table 2. Genetic ranges in N-remobilization efficiency for the flag-leaf lamina, stem and remaining leaf lamina and whole crop in 30 wheat cultivars under HN and LN conditions. Values represent means across years 2014 and 2016.

	Crop NRE		Flag-leaf NRE		Stem+Rem Lam NRE	
	HN	LN	HN	LN	HN	LN
Mean	0.79	0.84	0.82	0.85	0.78	0.82
Min	0.74	0.78	0.71	0.78	0.71	0.75
Max	0.84	0.87	0.87	0.87	0.84	0.86

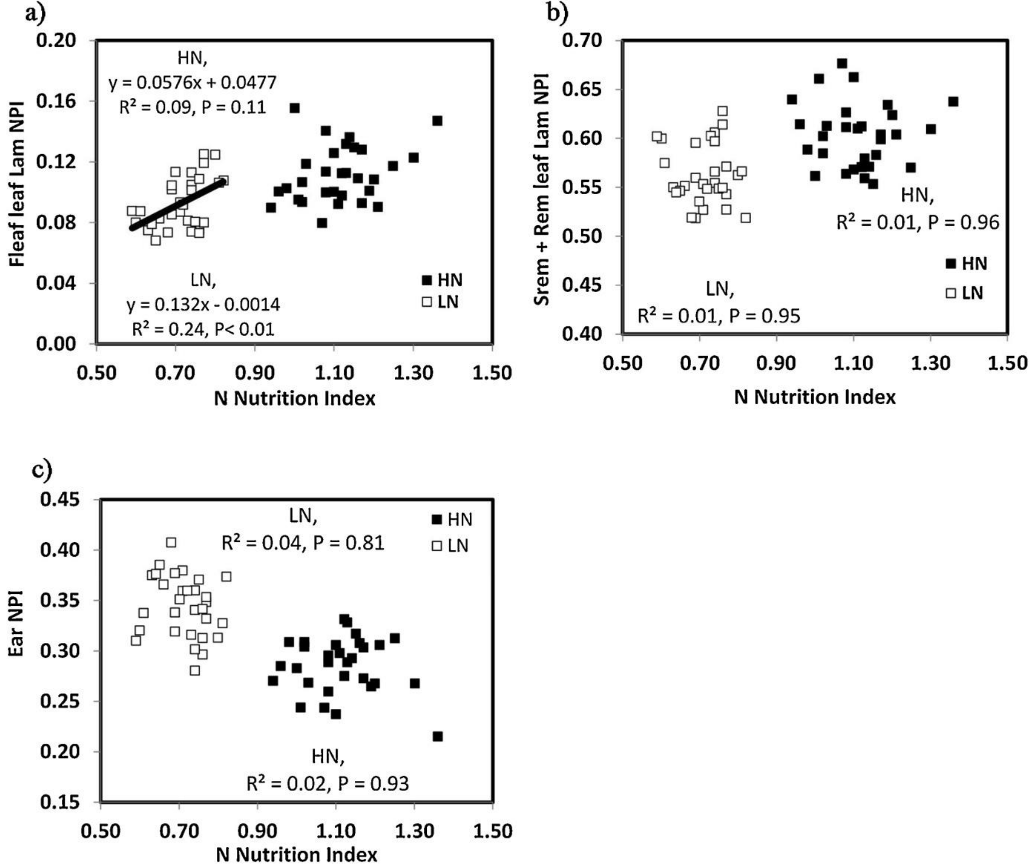


Fig.6. NPI and NNI relationships in flag leaf, stem+leaf and ear of wheat under high and low nitrogen.

The effect of the environment on the relationship between nitrogen uptake at flowering and nitrogen remobilization depended on nitrogen uptake during grain-filling period and disease pressure and was also affected by genotype. Assessment of genetic variation for accumulation, partitioning, and remobilization of N towards grain, and its association with grain protein concentration (GPC) and yield in a nested association mapping (NAM) RILs (195 lines). A genotype dependent positive association of grain yield with pre- and post-anthesis biomass accumulation was observed. The N-remobilization efficiency (NRE) was correlated with aboveground N accumulation at anthesis (AGNA) and grain yield but negatively associated with AGN at post-anthesis (AGNPA) suggesting higher N uptake to anthesis would favour higher N remobilization towards grain filling.

Hierarchical cluster analysis based on NRE and grain yield revealed four clusters of RILs defined as efficient (31), moderately efficient (59), moderately inefficient (58), and inefficient (47). In the N-efficient lines, AGNA contributed to 77% of total N accumulated in grains, whilst in the N inefficient lines, it was 63%. Among the four clusters, 16 lines of P×Cim, 10 from P×Wat, and 5 from P×Baj were categorized as N efficient while most of the P×Wya lines were inefficient or moderately inefficient. Several N efficient lines exhibited positive grain protein deviation (GPD), combining high grain yield and GPC. These positive GPD lines could be deployed as varieties or serve as genetic resource for improving grain yield with high GPC under limited N condition (Figs.6&7) (Sharma *et al.*,2022).

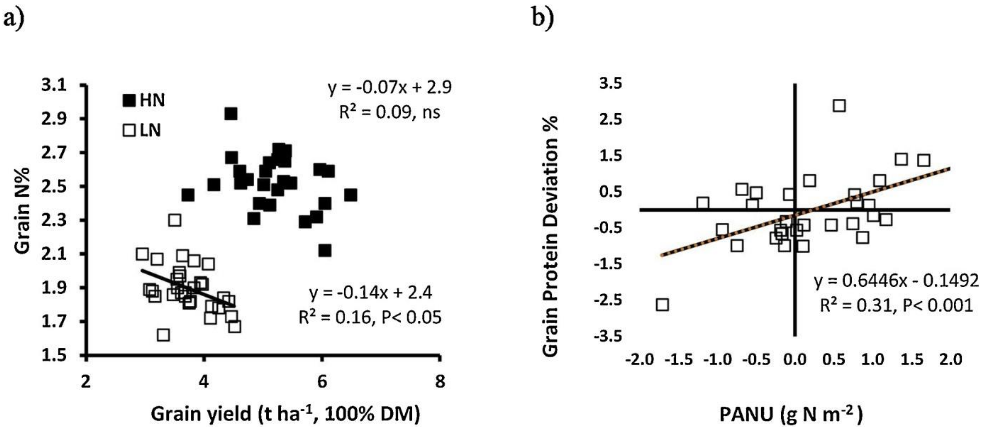


Fig.7. (a) Grain N percent and Grain yield relationships under high and low nitrogen (b) GPD and PANU relation in wheat.

QTLs related to N remobilization from senescing leaves for grain filling have been found in barley and durum wheat (Joppa *et al.* 1997; Mickelson *et al.* 2003) mapped a QTL on chromosome 6 for seed protein content in durum wheat. For grain protein in wheat, two QTLs were mapped on chromosomes 2 and 6. One of the QTL was cloned through positional cloning and fine mapping (Uauy *et al.*, 2006). The locus encodes a NAC transcription factor, NAM-B1, which accelerates leaf senescence and increases nutrient remobilization from leaves to developing grains. The role of NAM-B1 was confirmed by reducing the RNA levels of multiple NAM homologs by RNA interference, this resulted in delayed senescence and reduced wheat grain protein, zinc and iron content. At the same time as the identification of the NAM-B1 gene at the QTL locus in durum wheat, functional analysis of an AtNAP homolog in *Arabidopsis* was reported (Guo and Gan, 2006).

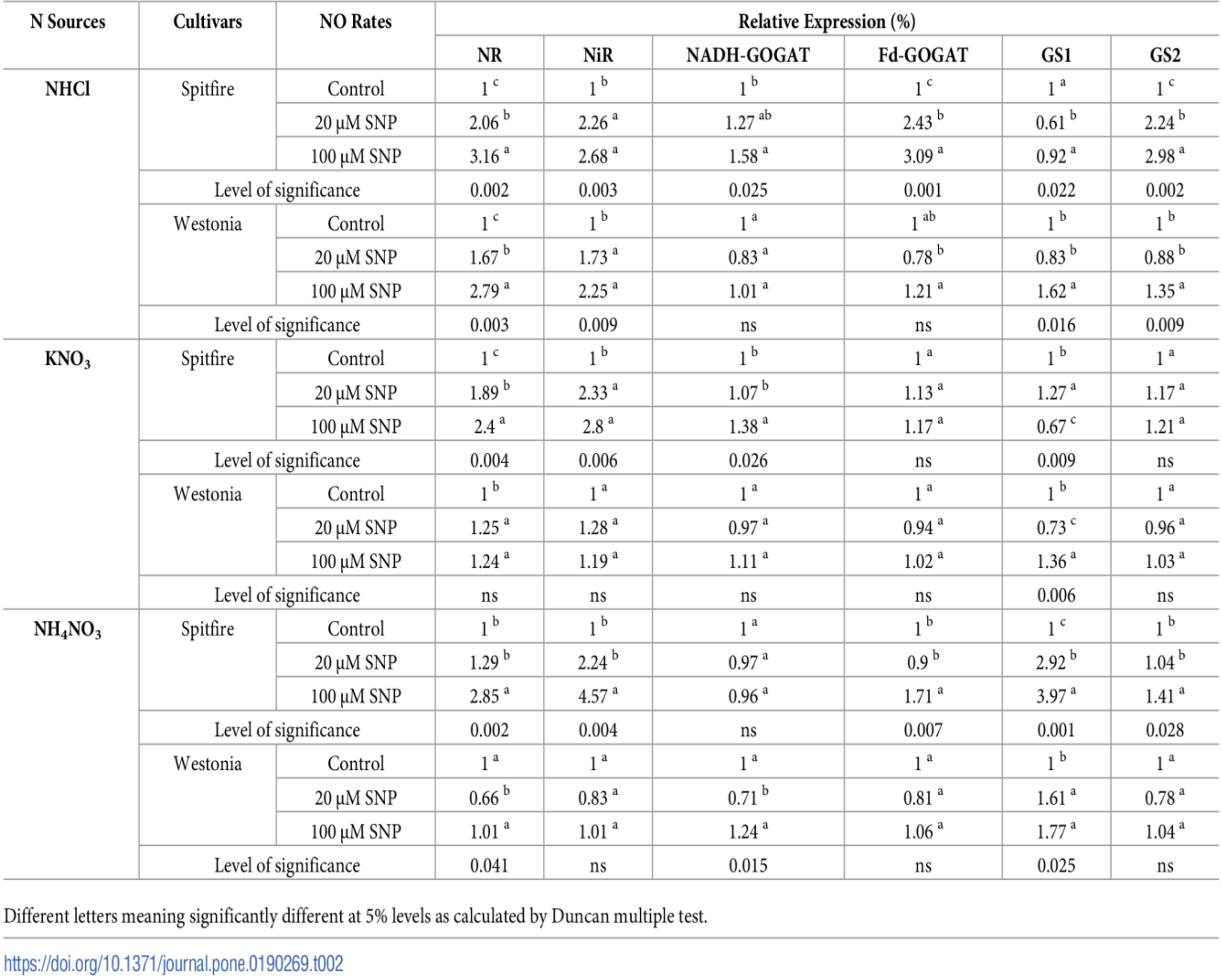
SULPHUR

Wheat (*Triticum aestivum*) quality is mainly determined by grain storage protein composition. Sulphur availability is essential for the biosynthesis of the main wheat storage proteins. The impact of different sulphur fertilizer regimes manifested important traits and associated gene networks was studied Results revealed that sulphur supplementation had a significant effect on grain yield, harvest index, and storage protein compositions. Consequently, two comparative sulphur fertilizer treatments (0 and 30 kg ha^{-1}

sulphur, with 50 kg ha^{-1} nitrogen) at seven days of post-anthesis, transcriptomics analysis to screen for differentially expressed genes (DEGs) involved in the regulation of sulphur metabolic pathways. Higher sulphur supply led to one up-regulated DEG and sixty-three down-regulated DEGs. Gene ontology enrichment showed that four down-regulated DEGs were significantly enriched in nitrogen metabolic pathway related annotation, three of which were annotated as glutamine synthetase. The Kyoto Encyclopedia of Genes and Genomes pathway enrichment identified three significantly enriched pathways involved in nitrogen and amino acid metabolism (Yu *et al*., 2018).

Tissue-specific transporters are activated during the reproductive stage which is important for nitrogen remobilization. NRT1.7 is an important nitrogen transporter and its gene is reported to be controlled by nitrogen limitation adaptation regulators which are further under the control of miRNA827 (Liu *et al*.,2016). This double-level control over tissue-specific nitrogen transporters suggests that the remobilization of nitrogen is tightly regulated. The remobilization process is under multiple regulatory controls along with transporters. The enzymes, such as GOGAT, are reported to be involved in ammonia recycling during remobilization (Zeng *et al.*,2016). Along with its certain transcription factors, such as NAM-B1, efficiently increase nitrogen remobilization toward grains from mature leaves in wheat (Uauy *et al.*, 2006).

Table 3. N assimilation gene expression and enzyme activity under exogenous NO and N.



N Sources	Cultivars	NO Rates	Relative Expression (%)					
			NR	NiR	NADH-GOGAT	Fd-GOGAT	GS1	GS2
NHCl	Spitfire	Control	1^{c}	1^{b}	1^{b}	1^{c}	1^{a}	1^{c}
		20 µM SNP	2.06^{b}	2.26^{a}	1.27^{ab}	2.43^{b}	0.61^{b}	2.24^{b}
		100 µM SNP	3.16^{a}	2.68^{a}	1.58^{a}	3.09^{a}	0.92^{a}	2.98^{a}
	Level of significance		0.002	0.003	0.025	0.001	0.022	0.002
	Westonia	Control	1^{c}	1^{b}	1^{a}	1^{ab}	1^{b}	1^{b}
		20 µM SNP	1.67^{b}	1.73^{a}	0.83^{a}	0.78^{b}	0.83^{b}	0.88^{b}
		100 µM SNP	2.79^{a}	2.25^{a}	1.01^{a}	1.21^{a}	1.62^{a}	1.35^{a}
	Level of significance		0.003	0.009	ns	ns	0.016	0.009
KNO_3	Spitfire	Control	1^{c}	1^{b}	1^{b}	1^{a}	1^{b}	1^{a}
		20 µM SNP	1.89^{b}	2.33^{a}	1.07^{b}	1.13^{a}	1.27^{a}	1.17^{a}
		100 µM SNP	2.4^{a}	2.8^{a}	1.38^{a}	1.17^{a}	0.67^{c}	1.21^{a}
	Level of significance		0.004	0.006	0.026	ns	0.009	ns
	Westonia	Control	1^{b}	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}
		20 µM SNP	1.25^{a}	1.28^{a}	0.97^{a}	0.94^{a}	0.73^{c}	0.96^{a}
		100 µM SNP	1.24^{a}	1.19^{a}	1.11^{a}	1.02^{a}	1.36^{a}	1.03^{a}
	Level of significance		ns	ns	ns	ns	0.006	ns
NH_4NO_3	Spitfire	Control	1^{b}	1^{b}	1^{a}	1^{b}	1^{c}	1^{b}
		20 µM SNP	1.29^{b}	2.24^{b}	0.97^{a}	0.9^{b}	2.92^{b}	1.04^{b}
		100 µM SNP	2.85^{a}	4.57^{a}	0.96^{a}	1.71^{a}	3.97^{a}	1.41^{a}
	Level of significance		0.002	0.004	ns	0.007	0.001	0.028
	Westonia	Control	1^{a}	1^{a}	1^{a}	1^{a}	1^{b}	1^{a}
		20 µM SNP	0.66^{b}	0.83^{a}	0.71^{b}	0.81^{a}	1.61^{a}	0.78^{a}
		100 µM SNP	1.01^{a}	1.01^{a}	1.24^{a}	1.06^{a}	1.77^{a}	1.04^{a}
	Level of significance		0.041	ns	0.015	ns	0.025	ns

Different letters meaning significantly different at 5% levels as calculated by Duncan multiple test.

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NITRIC OXIDE

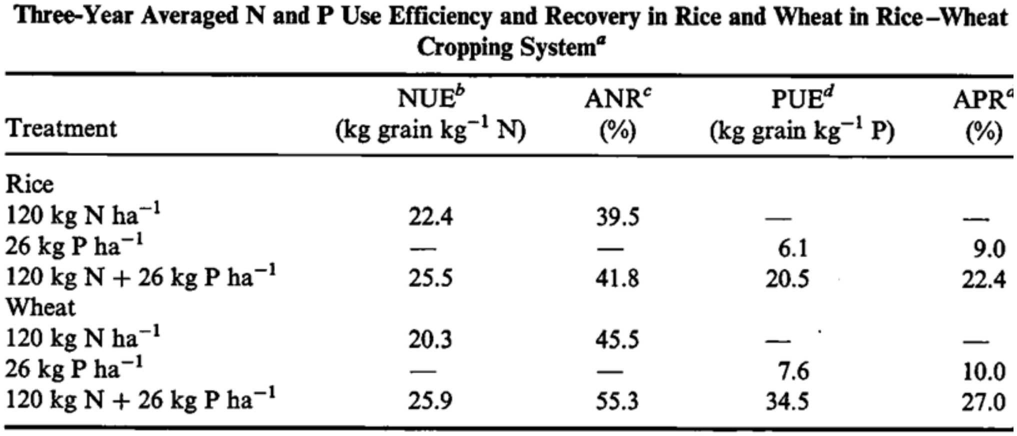
Nitric oxide (NO) is a signaling plant growth regulator involved in nitrogen assimilation. Influence of exogenous NO on nitrogen metabolism at the gene expression and enzyme activity levels under different sources of nitrogen is vitally important. Two Australian wheat cultivars, a popular high NUE cv. Spitfire and a normal NUE cv. Westonia, under different combinations of nitrogen and sodium nitroprusside (SNP) as the NO donor revealed that application of NO increased the gene expressions and activities of nitrogen assimilation pathway enzymes in both cultivars at low levels of nitrogen. At high nitrogen supplies, the expressions and activities of N assimilation genes increased in response to exogenous NO only in cv. Spitfire but not in cv. Westonia. Exogenous NO caused an increase in leaf NO content at low N supplies in both cultivars, while under high nitrogen treatments, cv. Spitfire showed an increase under ammonium nitrate (NH_4NO_3) treatment but cv. Westonia was not affected. N assimilation gene expression and enzyme activity showed a clear relationship between exogenous NO, N concentration and N forms in primary plant nitrogen assimilation (Baloff *et al.*,2018) (Table 3).

NITROGEN X PHOSPHORUS INTERACTION

Nitrogen (N) and phosphorus (P), are essential nutrients required in large amounts by plants to ensure growth and development, grain yield and quality. N is involved in various cellular processes including biosynthesis of chlorophyll, co-enzymes, phytohormones, secondary metabolites, nucleic acid, and proteins (Nazir *et al.*, 2016; Ganie *et al.*, 2017). Plants grown with low N, exhibit less biomass accumulation and reduced shoot-to-root ratio in comparison to plants grown with sufficient N. Field screening for nutrient efficiency is often limited due to environmental and economic constraints. However, repeating the experiments in the field followed by an in-depth study of selected genotypes in the controlled condition delineates a better understanding of the mechanisms governing the efficiency (Soumya *et al.*, 2021a). The selection of contrasting genotypes thus provides a high level of confidence.

On the other hand, excessive use of N and P fertilizers has caused nutrient imbalances, particularly disequilibrium of the N:P ratio (Penuelas *et al.*,2023). Besides, use of high doses of fertilizer has a negative impact on environmental health as the average N use efficiency (NUE) and P use efficiency (PUE) at agricultural fields is of the order of 33% and10–30%, respectively (Raun and Johnson, 1999; Manske *et al.*, 2000). Excess use of N and P fertilizer also leads to multi-nutrient deficiencies with a negative impact on crop growth as well as human health (Bindraban *et al.*, 2020). This can be achieved by identifying nutrient use efficient crops or crops tolerant to nutrient limited conditions, showing both internal and external efficiency (Vinod and Heuer, 2012). Research endeavours made so far are concerned with improving the use efficiency of individual nutrients, including evaluation of wheat genotypes for NUE (Gaju *et al.*, 2011; Ranjan *et al.*,2019; Mahmoud *et al.*, 2020; Ranjan and Yadav, 2021) (Table 3& Fig.8).

Table 3.



Three-Year Averaged N and P Use Efficiency and Recovery in Rice and Wheat in Rice–Wheat Cropping System^{a}

Treatment	NUE^{b} (kg grain kg^{-1} N)	ANR^{c} (%)	PUE^{d} (kg grain kg^{-1} P)	APR^{a} (%)
Rice				
120 kg N ha^{-1}	22.4	39.5	—	—
26 kg P ha^{-1}	—	—	6.1	9.0
120 kg N + 26 kg P ha^{-1}	25.5	41.8	20.5	22.4
Wheat				
120 kg N ha^{-1}	20.3	45.5	—	—
26 kg P ha^{-1}	—	—	7.6	10.0
120 kg N + 26 kg P ha^{-1}	25.9	55.3	34.5	27.0

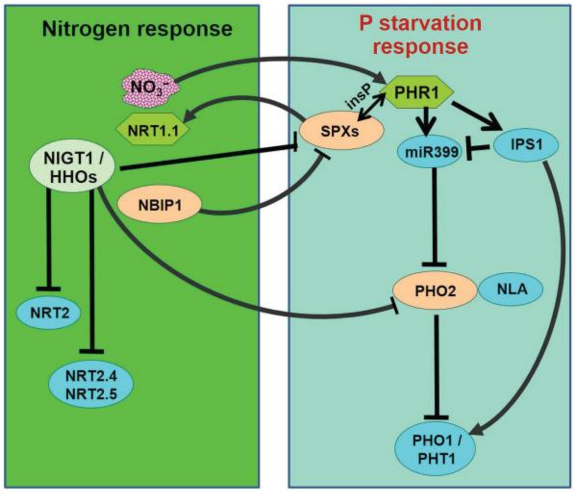


Fig.8. Schematic representation of nitrogen-starvation response and phosphate-starvation responses explaining N/P interactions.

NITROGEN STRESS

In the N efficient group, the percentage reduction in TBm, SBh and grain yield was 16.8, 14.3, and 21.7% respectively, while N inefficient group exhibited 22.0, 16.7, and 31.5% reduction respectively under low N as compared to sufficient N. Similarly, total N uptake and grain N uptake differed significantly between the N efficient and N inefficient groups.

The transcript accumulation of N assimilation genes, TaNia, TaAlaAT, TaGS1, and TaGDH showed significant reduction under all treatments that was more prominent in root rather than shoot tissue. However, in shoot of HD2781, there was meagre increase in the expression of these genes under low N treatment, whereas it was down regulated in C306.

Similarly, for low N stress tolerance in rice, Singh *et al.* (1998) proposed grain yield, N harvest index, N uptake, as well as physiological N use efficiency. Moreover, recent work on N remobilization efficiency in 195 wheat recombinant inbred lines grown in low N soil revealed that the aboveground biomass, grain yield, N harvest index, and grain protein concentration can be used as physiological markers (Sharma *et al.*,2023b).

The wheat plants grown under the nitrogen-deficient conditions (NDC) showed reduced crop height, leaf area, root volume, photosynthetic rate, and crop weight and increased root length, root surface area, and root/shoot ratio as compared to control. 3949 (2414 down-regulated, 1535 up-regulated) differentially expressed genes (DEGs) were identified in the shoot, and 3911 (2675 down-regulated, 1536 up-regulated) DEGs were identified in the root of the wheat plants grown under NDC.GO pathway and KEGG pathway enrichment analysis of these DEGs were also conducted. 24 expansin genes (such as treasCS5B02G528400) and 9 Nrt genes (such as TreasCS6A02G031100) were correlated to increased N absorption. Besides, 3 Pet genes (such as TreasCS7B02G226200) and 8 Psb genes (such as TreasCS3D02G523300) were correlated to the inhibition of the photosynthetic pathway; also, 3 Nar genes (such as TreasCS6A02G326200) and 1 Nir gene (TreasCS6D02G333900) were correlated to the inhibition of nitrogen metabolism pathway in wheat plants grown under NDC (Liu *et al.*,2020).

Wheat homologues of N-responsive miRNAs mediate NUE-related responses through downstream signalling pathways. miRNAseq analysis revealed the expression details of miRNAs involved in signal transduction in high NUE Indian dwarf wheat (*Triticum sphaerococcum* Perc.) genotypes identified under low N conditions. Findings infer that the effective and coordinated signal transduction network involving the miRNAs targeting and reprogramming N metabolism, secondary metabolism, N signal perception and signalling helps to achieve high NUE. The miRNAs targeting key genes could serve as a potential biotechnology target for enhancing nitrogen efficiency in wheat and other crops (Das *et al*.,2023).

This indicates that the deficiency of N in the media suppresses the secretion of acid phosphatase enzyme (extracellular) from root, while the gene TaPAP was significantly expressed in the root tissue (intracellular), particularly in HD2781.

The activity of N assimilation enzymes was reduced under low N stress (Masclaux-Daubresse *et al.*, 2010; Wen *et al.*, 2019). The genotypes HD2781 and HD2824 exhibited relatively higher activities of NR, GS, and GOGAT under low N, whereas under low P, the activity was higher for GS, GOGAT, and GDH as compared to Ajantha and C306. Studies revealed in rice (Sevanthi *et al.*, 2021) and wheat cultivars (Kocheva *et al.*, 2020) with higher specific activity of N assimilating enzymes under N starvation. The nitrate reduction process is regulated by availability of nitrate at the site of the NR, whereas enzymes nitrite reductase and GOGAT requires reducing powers (NADH or ferredoxin-reduced), while GS and asparagine synthetase needs ATP (Masclaux-Daubresse *et al.*, 2010). So, nitrate assimilation being an energy demanding process, the decrease in activity of enzymes not only under low N but also under low P or low NP is justified.

WATER STRESS

The study of morpho-physiological and cytological responses, as well as elements of the anatomical structure of primary roots of three wheat species, *Triticum monococcum L., Triticum dicoccum Shuebl., and Triticum aestivum L.*, to osmotic stress. It was shown that the degree of plasticity of root morphology in water deficit affected the growth and development of aboveground organs. It was found that in conditions of osmotic stress, the anatomical root modulations were species-specific. In control condition the increase in absolute values of root diameter was reduced with the increase in the ploidy of wheat species. Species-specific cytological responses to water deficit of apical meristem cells were also shown. The development of plasmolysis, interpreted as a symptom of reduced viability apical meristem cells, was revealed (Table 4)(Terletskaya *et al.*, 2020).

Table 4. Influence of water deficit on the anatomical parameters of the roots of different wheat species (Terletskaya *et al.*,2020).

Water	Thickness of epiblem,µm	Thickness of exoderm,µm	Thickness of cortical parenchyma ,µm	Thickness of endoderm ,µm	Dia.of central cylinder ,µm	Root Dia.µm
			T.monococcum			
Control	14.8±0.7	13.9±0.4	74.9±1.7	18.6±0.6	191.4±2.2	435.8±7.1
Sucrose,17.6%	14.1±0.7	27.7±0.2*	85.1±1.9*	18.6±0.5	187.6±1.4	478±2.2*
%to cont.	95	199	113	100	98	110
			T.dicoccum			
Control	19.4±0.2	16.7±0.8	67.3±0.4	12.8±0.6	217.5±3.5	449.9±5.2
Sucrose17.6%	13.7±0.1*	30.4±0.6*	80.0±0.7*	26.4±0.8*	192.9±1.1*	493.9±0.6*
%to cont.	71	182	119	206	89	110
			T.aestivum			
Control	16.6±0.8	28.5±0.9	70.1±1.2	19.7±0.5	205.8±3.6	475.5±9.2
Sucrose17.6%	18.5±0.1*	25.6±0.8*	75.9±0.5*	24.5±0.3*	234.0±8.9	523.0±9.8*
%to cont.	112	90	108	124	114	110

*Note: * indicate significant differences at $p \leq 0.05$,* A significant increase in enzymatic activity of superoxide dismutase under osmotic stress was found to be one of the mechanisms that could facilitate root elongation in adverse conditions. The tetraploid species *T. dicoccum Shuebl*. were confirmed as a source of traits of drought tolerant primary root system for crosses with wheat cultivars.

The comparison of varieties, seed size classes and water stress levels showed that in terms of seedling growth variety UP-2338 was more resistant to water stress as compared to other varieties, whereas in terms of germination variety PBW-154 was more resistant. Variety UP-2425 showed maximum reduction in seed (Shahi *et al*., 2015).

When measured the changes in various parameters related to nitrogen (N) metabolism in flag leaf and grain of wheat (*Triticum aestivum* L.) plants (cv. Jinan 17 and Lumai 21), which were subjected to five irrigation regimes until physiological maturity. Severely deficient or excessive irrigation during grain filling decreased the photosynthetic rate (*A*), the concentrations of N, free amino acid, and soluble protein, as well as the activities of nitrate reductase (NR) and glutamine synthetase (GS) and increased malondialdehyde (MDA) accumulation and endopeptidase (EP) activity, though grain protein concentration might mainly depend on genotype. The activities of NR and GS were significantly positively correlated with *A*, but those of EP were significantly negatively correlated with *A*. The results indicate that while severe water stress aggravates the adverse effect on nitrogen metabolism, excessive soil moisture is also not useful during the grain-filling stage, resulting in lower grain yield and quality. Results suggest that applying an optimal irrigation regime in wheat fields still plays an important role in the improvement of grain yield and quality (Xu andYu, 2006).

Wheat (*Triticum aestivum* L.) root growth in the subsoil is usually constrained by soil strength, although roots can use macropores to elongate to deeper layers. The quantitative relationship between the elongation of wheat roots and the soil pore system, however, is still to be determined. When studied the depth distribution of roots of six wheat varieties and explored their relationship with soil macroporosity from samples with the field structure preserved. Undisturbed soil cores (to a depth of 100 cm) were collected from the field and then non-destructively imaged using X-ray computed tomography (at a spatial resolution of 90 **μ**m) to quantify soil macropore structure and root number density (the number of roots cm^{-2} within a horizontal cross-section of a soil core). Soil macroporosity changed significantly with depth but not between the different wheat lines. There was no significant difference in root number density between wheat varieties (Zhou *et al.*, 2021).

Drought	**+ temperature**
* Inhibition of primary root growth *Reduction of lateral root growth and angle * Hydra-patterning response: shallow root system and branching	*Increased primary root inhibition * Reduction of root partitioning of carbon assimilates * Decreased root respiration * Cellular damage by oxidative stress
Salinity	**+ temperature**
*Inhibition of primary root growth * Cytotoxicity and oxidative stress damage * RSA alteration	*Enhanced inhibition of primary root *Increased oxidative stress damage * Alteration of root ions update
Nutrition starvation	**+ temperature**
*Nutrient-dependent alteration of RSA: low nitrogen enhancement of root elongation, low phosphate induced lateral root density	*Increased root growth inhibition * Impaired heat response * Reduction in nitrogen efficiency

Plants have a greater water demand in warmer environments due to increased water loss by evapo-transpiration and decreased water uptake by the root, causing an overall water deficit situation

(Heckathorn *et al.,* 2013). Water uptake takes place in the root either through aquaporins, membrane channels that facilitates water transport inside the cells, or by diffusion through plasmatic membrane (Maurel *et al.,* 2016). Studies with several crops have shown different response of aquaporins and plasmatic membrane fluidity to higher temperatures in roots. Thus, in pepper and wheat, water uptake in warmer soil seems to positively correlate with aquaporin activity (Carvajal *et al*., 1996; Cabañero *et al.*, 2004), whereas in broccoli (*Brassica oleracea* var. italica) and maize, warmer temperatures decrease aquaporin quantity and activity but increase membrane fluidity. When temperature is extreme, the membrane starts to rigidify heavily decreasing even more water uptake (Iglesias-Acosta *et al.*, 2010; Ionenko *et al.*, 2010). Nutrient balance is also altered by changes in temperature. Similarly to water, temperature effect on nutrient uptake varies depending on the crop. ROOT SYSTEM ARCHITECTURE

Acquisition of water and nutrients is a major determinant of yield and adaptation in wheat. The distribution of roots in the soil influences the pattern of water and nutrient uptake and modelling studies have indicated variation in root architecture can alter grain yields (Dreccer *et al*., 2002; Manschadi *et al.*, 2006). One aspect of root architecture that can influence root distribution is the angle of the seminal roots. Significant variation in root angle occurs in wheat and it has been suggested that this is related to the geographical adaptation of varieties and in particular their adaptation to the patterns of soil moisture (Oyangi, 1994; Manschadi *et al*., 2006, 2008). Plants with narrow seminal roots angles tend to have deeper roots (Oyangi ,1994), which has may be advantageous to the use of moisture from the subsoil.

Large phenotypic variability in root system morphology in wheat genotypes was observed at the tillering stage using established semi-hydroponic phenotyping techniques. Phenotypic differences in and trait correlations among some interesting root traits may be considered for breeding wheat cultivars with efficient water acquisition and better adaptation to abiotic stress (Fig.9.)(Chen *et al.*,2020).

Fig.9. a **Wheat plants grew in a semi-hydroponic system in a glasshouse,** b **a close shot of plants in the bin, and** (c) **example plants of four wheat genotypes with contrasting shoot and root system morphology grown in the semi-hydroponic phenotyping platform in a temperature-controlled glasshouse 35 days after transplanting.**

The response of plant root development to nutrient deficiencies is critical for crop production. Auxin, nitric oxide (NO), and strigolactones (SLs) are important regulators of root growth under low-nitrogen and -phosphate (LN and LP) conditions. Polar auxin transport in plants, which is mainly dependent on auxin efflux protein PINs, creates local auxin maxima to form the basis for root initiation and elongation; however, the *PIN* genes that play an important role in LN- and LP-modulated root growth remain unclear. qRT-PCR analysis of *OsPIN* family genes showed that the expression of *OsPIN1b* is most abundant in root tip and is significantly down regulated by LN, LP, sodium nitroprusside (SNP, NO donor), and GR24 (analogue of SLs) treatments. Seminal roots in *ospin1b* mutants were shorter than those of the wild type; and the seminal root, [3H]IAA transport, and IAA concentration responses to LN, LP, SNP, and GR24 application were attenuated in *ospin1b-1* mutants. *pCYCB1;1::GUS* expression was upregulated by LN, LP, SNP, and GR24 treatments in wild type, but not in the *ospin1b-1* mutant, suggesting that *OsPIN1b* is involved in auxin transport and acts as a downstream mediator of NO and SLs to induce meristem activity in root tip in rice under LN and LP**.**

It was demonstrated that TaSRL1 functions as a direct regulator of *TaPIN2* in the auxin-dependent pathway, and integrates auxin and jasmonate signaling by interacting with TaTIFY9 to repress root growth. Further, the molecular marker of *TaSRL1-4A* is valuable for the improvement of the root system, plant architecture, and yield in the wheat breeding process. Nitric oxide (NO) and auxin are important regulators of root growth and development under NO_3^- supply (Sun *et al.*,2014).

SALINITY

Variation in response to salinity was measured by subjecting seedlings of six wheat to one control treatment (-0.1 bars) and two stress treatments (-3.5 and -10.4 bars) from NaCl, $MgSO_4$, and $MgCl_2$ in hydroponic solutions. Salinity stress retarded growth of wheat significantly at -3.5 bars but not at -10.4 bars. Stress decreased root and shoot nitrate N and total N contents. Studies with one wheat cultivar showed that salinity affected decreased activity of nitrate reductase and stimulated accumulation of proline. Salinity more adversely affected at vegetative stages than reproductive stages of plants grown to maturity. It revealed that salinity affected wheat by both osmotic effects and antagonism of nitrate metabolism from chloride. Absolute growth and relative growth at different stress levels were superior to correlate in nitrogen metabolism as selection criteria for salinity tolerance (Abdul-Kadir and Paulsen,1982).

Studies on the effect of salinity on amino acid, proline and glycine betaine accumulation in leaves of different stages of development in durum wheat under high and low nitrogen supply suggest that protective compounds against salt stress are accumulated in all leaves. The major metabolites are glycine betaine, which preferentially accumulates in younger tissues, and proline, which is found predominantly in older tissues. Proline tended to accumulate early, at the onset of the stress, while glycine betaine accumulation was observed during prolonged stress. Nitrate reductase (NR) and glutamate synthase (GOGAT) are positively correlated with these compatible solutes: proline is associated with NR in the oldest leaves of high-nitrate plants and glycine betaine is associated with GOGAT in the youngest leaves of both low- and high-nitrate plants. In high-nitrate conditions proline accounts for more than 39% of the osmotic adjustment in the cytoplasmic compartments of old leaves. Its nitrogen-dependent accumulation may offer an important advantage in that it can be metabolised to allow reallocation of energy, carbon and nitrogen from the older leaves to the younger tissues. The contribution of glycine betaine is higher in young leaves and is independent of nitrogen nutrition (Carillo *et al.*,2008).

Salinity more adversely affected at vegetative stages than reproductive stages of plants grown to maturity. It revealed that salinity affected wheat by both osmotic effects and antagonism of nitrate metabolism from chloride. Absolute growth and relative growth at different stress levels were superior to correlate in nitrogen metabolism as selection criteria for salinity tolerance.

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