Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 23 | No. 3 | pp. 1471-1482 | 2024

Original Research Paper

https://doi.org/10.46488/NEPT.2024.v23i03.017

Open Access Journal

Nitrogen Nutrition-Induced Changes in Macronutrient Content and Their Indirect Effect on N-Metabolism Via an Impact on Key N-Assimilating Enzymes in Bread Wheat (*Triticum aestivum* L.)

Vandna*, Vasundhara Sharma, Kalidindi Usha****, Dalveer Singh***, Ranjan Gupta*, V. K. Gupta* and Bhupinder Singh****†**

*Department of Biochemistry, Kurukshetra University, Kurukshetra, Haryana, India
**Department of Plant Physiology, ICAR-National Research Centre on Seed Spices (NRCSS), Ajmer, Rajasthan, India
***Division of Plant Physiology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India
****Division of Environment Science, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India
†Corresponding author: Bhupinder Singh; bhupindersinghiari@yahoo.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 22-12-2023
Revised: 02-02-2024
Accepted: 14-02-2024

Key Words:
Nitrogen deficiency
N-metabolism
N-Assimilating enzymes
Triticum aestivum L.

ABSTRACT

Judicious application of nitrogen (N) fertilizers in crop production is critical for reducing the nitrate pollution of groundwater and greenhouse gas emissions. It is, thus, important to improve the nitrogen use efficiency under the reduced application of nitrogen. A genotypic variation in N-uptake and N-use efficiency particularly under low N-input conditions exists across crops that can be deciphered and exploited for environmentally sustainable farming without any significant penalty of yield and quality. The present research conducted under the nutrient solution culture aimed to explore the inherent variability in the growth response of ten genetically diverse wheat varieties to low fertilizer N-application (N-, 10 μM N) in comparison to N sufficient control (N+, 8.5 mM N) viz., a viz., the activity of various key N-assimilating enzymes and to delineate the indirect effect of low N on uptake and partitioning of other major macronutrients viz., P, K, S, which may indirectly regulate the N-use efficiency. A notable increase in sulfur, potassium, and phosphorus content was observed under nitrogen-deficient conditions. Varieties such as Carnamah and HD 2824 exhibit a significant increase in shoot phosphorus content, emphasizing their potential to optimize phosphorus acquisition and utilization efficiency under nutrient-limited conditions. The findings highlight the complex interplay between nutrient availability and plant responses, showcasing varietal-specific adaptations to nitrogen limitations.

INTRODUCTION

Wheat, a cornerstone of global agriculture, is more than just a cereal crop; it's a primary source of sustenance, providing over 20% of the world's consumed calories (Giraldo et al. 2019). As we stand on the cusp of a global population surge, with projections soaring beyond 9 billion by 2050 (Dutia 2014), the demand for food is primed for a substantial upswing. To meet this burgeoning need, wheat production must surge by approximately 20% annually, an imperative recognized long ago, yet historical trends have shown a mere 0.9% annual increase (Ramadas et al. 2020). Nitrogen (N), a linchpin of plant physiology, is an elemental cornerstone found in an array of secondary metabolites within plants, including coenzymes, nucleic acids, amino acids, and proteins. Its multifaceted role extends beyond mere sustenance, as it orchestrates the very essence of growth and development in all plant species. Within the world of wheat, nitrogen takes center stage, profoundly influencing crop growth, yield, and nutritional composition. Effective nitrogen utilization is not only crucial for agricultural economics but also a matter of paramount importance, given that nitrogen ranks among the scarcest of plant nutrients in numerous agricultural regions worldwide. The dynamic nature of nitrogen, coupled with its propensity to escape through intricate plant-soil interactions, presents challenges in its effective regulation (Ladha et al. 2022). The insufficiency of essential nutrients in the soils of agricultural lands impedes optimal plant yield (Sandhu et al. 2021). Fertilizer application rates, notably nitrogen, directly impact crop production pace, yet excessive inputs may lead to poor nutrient use efficiency due to runoff water, where fertilizer inputs exceed crop requirements (Singh & Craswell 2021, Govindasamy et al. 2023). Paradoxically, certain agricultural fields grapple

with severe nutrient deficiencies, which detrimentally affect agricultural productivity (Hirel et al. 2011a).

In the Indian context, nitrogen assumes paramount importance as the most deficient macronutrient in soil, prompting farmers to apply elevated doses of external nitrogen to maximize crop productivity. This practice, while aiming to augment yield and returns, also precipitates environmental pollution, underscoring the need for judicious nitrogen fertilizer application (Paramesh et al. 2023). Historically, crop genetic improvement has yielded substantial increases in crop yields, contributing 50% to 60% of the overall growth. Simultaneously, the deployment of synthetic nitrogen (N), phosphorus (P), and potassium (K) fertilizers has substantially boosted yields. In crops like maize, high fertilizer usage has coincided with the selection of high-yielding genotypes tolerant of high seed density and receptive to increased N fertilizer inputs (Yin et al. 2018, Asibi et al. 2019, Gheith et al. 2022). However, the unbridled escalation of applied nitrogen may not necessarily result in enhanced yields. Instead, it raises concerns about environmental repercussions, including water pollution, greenhouse gas emissions, and soil degradation (Zhang et al. 2012, Nair 2021, Wang et al. 2021, Yadav et al. 2023). Consequently, the need to simultaneously elevate yields while moderating, or ideally decreasing, applied nitrogen becomes apparent, to optimize Nitrogen Use Efficiency (NUE) (Anas et al. 2020, Yan et al. 2020). The pivotal role of nitrogen in wheat goes beyond its direct influence on growth and yield; it is intricately woven into the biochemical processes that govern nutrient uptake and utilization. In wheat plants, nitrogen uptake typically occurs in the form of nitrate, subsequently reduced to nitrite by nitrate reductase (NR), further transformed into ammonium by nitrite reductase (NiR), and eventually converted into glutamine through the catalysis of glutamine synthetase/glutamate synthase (GS/GOGAT) and glutamate dehydrogenase (GDH). These biochemical reactions lay the foundation for amino acid production, an essential aspect of nitrogen metabolism. The activity of these enzymes is highly sensitive to nitrogen availability, with low nitrogen stress exerting a profound influence.

Within this intricate nitrogen assimilation network, glutamine synthetase (GS) emerges as a central player (Habash et al. 2001) and may be used as a marker for selecting genotypes with heightened NUE (Sharma et al. 2023). Similarly, glutamate synthase (GOGAT), with its two isoforms (NADH-GOGAT and Fd-GOGAT), assumes a vital role in photosynthetic tissues, recapturing ammonia released during photorespiration or senescence processes (Zhou et al. 2018, Sharma et al. 2023). The role of glutamate dehydrogenase (GDH), while still debated, remains pivotal in either ammonia assimilation or carbon recycling (Cooper 2012). It is worth mentioning here that nitrogen assimilation and yield often hinge not on the level and activity of NR/NiR enzymes, but rather on GS, which occupies the intersection of carbon and nitrogen metabolic pathways. Nutritional and biochemical factors that directly or indirectly induce or regulate the GS activity may, thus, hold promise for potentially enhancing the NUE by fostering efficient nutrient recycling (Foulkes et al. 2009, Cooper 2012). NUE as such cannot be increased by adding more N-fertilizer and the continued careless application of N fertilizer shall have ecological repercussions, including water pollution, greenhouse gas emission, and soil degradation. It is, thus, important to understand the N-nutrition-mediated regulation of mineral nutrient status and decipher their direct and indirect effect on the activity of key N-assimilating enzymes in order to determine the principle regulators of NUE under low N availability conditions of crop growth. The present research endeavor, thus, aimed to unravel the complex nexus between nitrogen, the growth attributes of wheat, and the realm of macro-nutrient management, seeking sustainable practices that reconcile the dual objectives of crop productivity and environmental stewardship. By scrutinizing the impact of nitrogen deficiency on wheat at multiple levels - from macronutrient dynamics to the underlying biochemical processes - we aim to contribute vital insights for a more resilient and sustainable future in wheat agriculture.

MATERIALS AND METHODS

Planting Material and Experimental Conditions

Bread wheat varieties viz., Ajantha, Atilla-12, C 306, Carnamah, HD 2824, KYPO 328, PBW 502, Stilleto, Turaco, and UP 2338 were procured from the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi and were raised under hydroponic condition, according to Sathee et al. (2018). For this, the wheat grains were rinsed twice with distilled water before being surface sterilized for 5 minutes with 0.1% mercuric chloride ($HgCl_2$). Grains were properly washed five to six times with double distilled water to eliminate any trace of $HgCl_2$. After 5-6 days of germination in petri plates lined with moist germination paper, uniform healthy wheat seedlings were selected and transplanted into plastic tanks containing 15 L Hoagland solution(Hoagland & Arnon 1950), at two levels of nitrogen (N) i.e., sufficient nitrogen control (SN, 8.5mM N) and N deficiency (LN, 10 μM N) under continuous aeration. To maintain a balanced nutrient availability during the growth period, the nutrient solution was replaced every 3 days up to 25 days after the transfer of seedlings to the nutrient solution culture, after which the experiment was terminated and observations

This publication is licensed under a Creative Commons Attribution 4.0 International License

were taken. For each treatment, three independent biological replications were maintained, and all observations were recorded in triplicate.

Variation in Growth Response of Wheat Varieties to Sufficient (SN) and Low N (LN) Input Condition

Shoot and root biomass: Shoot and root biomass (g dw plant^{-1}) was determined at 25 days after germination (DAG), after dissecting the respective N-treatment seedlings into the root and shoot tissues and drying them in hot air over for a few days until constant weights were obtained.

Variation in Macronutrient Acquisition in Wheat Varieties under Sufficient (SN) and Low N (LN) Input Condition

Shoot and root nitrogen (N): Total nitrogen (N) in the plant samples was determined using Kjeldahl's method (Jones et al. 1987). The method involved the digestion of pre-weighed shoot and root samples to convert organic and inorganic nitrogen compounds into ammonium ions which are subsequently quantified through titration.

Shoot and root phosphorus (P), potassium (K) and sulfur (S): A 100 mg dried shoot and root samples were digested in a 50 mL volumetric flask using 10 mL of di-acid mixture i.e., concentrated nitric acid and concentrated perchloric acid (10:3) pre-digested overnight. The pre-digested mixture was further digested on a hot plate, maintained at a temperature of 200-250°C for a minimum of 2 hours. The transition of the mixture from opaque to clear indicated the complete digestion of the organic matter. Subsequently, the mixture was allowed to cool to room temperature and was then adjusted to a final volume of 50 mL using double distilled water. The solution was then filtered using quantitative ashless filter paper (Whatman no. 42) and preserved for subsequent analysis. This resulting solution was suitable for a variety of analytical techniques. Phosphorus (P) levels in the samples were assessed using the ascorbic acid method (Murphy & Riley 1962) on a UV-Vis spectrophotometer (ECIL, India), while K was analyzed using a microcontroller based Flame photometer (Systronics India Ltd, India). The sulfur content was determined using the turbidity method described by Skwierawska et al. (2016).

Variation in Activity of Key Enzymes of N-Assimilating Pathway in Wheat Varieties under Sufficient (SN) and Low N (LN) Input Condition

Enzyme extraction: Extraction of enzymes and assays of GS, GOGAT, and GDH were done following the method of Mohanty & Fletcher (1980). Leaf samples were extracted in Tris-HCl buffer, which contained 100 mM Tris-HCl, 100 mM sucrose, 10 mM EDTA and 10 mM $MgCl_2$. Tissue samples were ground in a chilled pestle and mortar with an extraction buffer. Ground samples were centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant was collected and re-centrifuged at 12,000 rpm for 15 min at 40°C. The supernatant was collected in separate Eppendorf tubes and used for the estimation of GS and GOGAT. The pellet was dissolved in 1 mL of 50 mM phosphate buffer with 2.14 g.100 mL^{-1} sucrose with pH 7.5 and was used for estimation of GDH.

Leaf nitrate reductase (NR) activity: Estimation of *in vivo* nitrate reductase activity was done by estimating nitrite formed by the enzyme action, which was then diazotized using sulphanilamide in an acidic medium and NEDD using the method of Klepper et al. (1971) and modified by Nair & Abrol (1973). Nitrite was estimated by the method of Evans & Nason (1953), Xu et al. (2000) and Sun et al. (2003). The leaves and roots were cut into 2 mm pieces and after thorough mixing of the leaf samples, 0.3 g was weighed and added to ice cold incubation medium containing 3 mL each of phosphate buffer (0.2 M, pH 7.5) and potassium nitrate solution (0.4 M). To it, 0.2 mL of n-propanol was added. The leaf samples were infiltrated with the solution using a vacuum pump and then incubated in a water bath at 30°C for 30 minutes under dark conditions. At the end of the incubation period, tubes were placed in a water bath (70-80°C) for 3-4 minutes to stop the enzyme activity and for the complete leaching of the nitrite into the medium. The nitrite was then estimated by taking an adequate amount of aliquot in a test tube followed by the addition of 1 mL of sulphanilamide (1% in 1 N HCl). After mixing, 1 mL NEDD (0.02%) was added and again mixed well. The pink colour was formed immediately and after 20 minutes the total volume was made up to 3 mL with double distilled water. Absorbance was measured using a UV-visible spectrophotometer (model Specord Bio-200, Analytik Jena, Germany) at 540 nm. The calibration curve was prepared using a standard sodium nitrite solution. The enzyme activity was expressed as μmol nitrite formed g^{-1} DW h^{-1}.

Leaf glutamine synthetase (GS) activity: Estimation of GS activity was done by measuring the γ-glutamylhydroxamate formed. All the reagents viz., 0.35 mL of 200 mM Tris buffer, 0.25 mL of 200 mM $MgSO_4$, 0.1 mL of 50 mM cysteine, 0.25 mL of 500 mM α-glutamate, 0.1 mL of 50mM ATP, and 0.25 mL of 40 mM hydroxylamine were pipetted out along with 0.2 mL of aliquot and kept at 37°C for 30 min. To stop the reaction 0.5 mL $FeCl_3$ reagent was added and then centrifuged (sigma 3K30) at 1500-2000 rpm for 10 min. Absorbance was measured using a UV-visible spectrophotometer (Specord Bio-200, Analytik Jena, Germany). The

This publication is licensed under a Creative Commons Attribution 4.0 International License

enzyme activity was expressed as γ-glutamylhydroxamate formed g^{-1} protein h^{-1}.

Leaf glutamate synthase (GOGAT) activity: Estimation of GOGAT activity was done by measuring the NADH oxidization. All the reagents viz., 1 mL of 75 mM Tris-HCl, 0.2 mL of 50 mM α-ketoglutaric acid, and 0.2 mL of 200 mM L-glutamine were pipetted out with 0.1 mL of aliquot and volume made to 2.8 mL with double distilled water. To it, 0.2 mL of 1.5 mM of NADH was added into a cuvette with a reaction mixture just before taking absorbance reading mixed well and absorbance was measured using a UV-visible spectrophotometer (Specord Bio-200, AnalytikJena, Germany) at 340 nm for 60 second. The enzyme activity was expressed as μmol NADH oxidized g^{-1} protein h^{-1}.

Leaf glutamate dehydrogenase (GDH) activity: All the reagents viz., 1 mL of 75 mM phosphate buffer, 0.2 mL of 100 mM α-ketoglutaric acid, and 0.4 mL of 750 mM NH_4Cl were pipetted out with 0.2 mL of aliquot and volume made to 2.8 mL with double distilled water. To it, 0.1 mL of 1.5 mM of NADH was added into a cuvette with a reaction mixture just before taking absorbance reading mixed well and absorbance was measured using a UV-visible spectrophotometer (Specord Bio-200, AnalytikJena, Germany) at 340 nm for 60 second. The enzyme activity was expressed as μmol NADH oxidized g^{-1} protein h^{-1}.

Leaf alanine aminotransferase (AlaAT) activity: AlaAT activities were determined using the spectrophotometric method based on the procedure outlined by De Sousa & Sodek 2003. For AlaAT activity, the assay was conducted in the direction of alanine to pyruvate conversion, coupled with the oxidation of NADH by lactate dehydrogenase. The reaction mixture (1.5 mL) contained 0.1 M Tris–HCl buffer at pH 7.5, 0.5 M L-alanine, 15 mM 2-oxoglutaric acid, 0.18 mM NADH, 5 units of lactate dehydrogenase, and the enzyme extract. The reactions were carried out at 30°C, and the oxidation of NADH was continuously monitored at 340 nm. The activities of AlaAT were calculated using the absorption coefficient for NADH (e = 6.22 mM^{-1} cm^{-1}) and expressed in units, with each unit representing the amount of enzyme capable of catalyzing the formation of 1 nmol of product per minute.

Statistical analysis: Statistical analysis and graphical representation were carried out utilizing SPSS V26 and GraphPad Prism version 9.1 (La Jolla, California, USA) respectively. The statistical analysis involved the execution of a one-way analysis of variance (ANOVA) to compute adjusted P values, thereby establishing the significance level. To discern differences in means, Tukey's multiple comparisons test was applied.

RESULTS AND DISCUSSION

Growth Response of Wheat Varieties to Low Nitrogen Stress

Shoot and root biomass: Significantly higher shoot biomass was observed under sufficient nitrogen (SN) than low nitrogen (LN) conditions, while a reverse trend was observed for the root biomass which was distinctly higher under LN than SN treatment across all the ten experimental bread wheat varieties (Fig. 1, Table 1). The range of variation for the shoot, and root biomass under the SN and LN treatment was 0.18-0.29, 0.08-0.13, and 0.12-0.15, 0.18-0.28 $g.plant^{-1}$ respectively with an average value of 0.242, 0.123 and 0.15, 0.232 g $plant^{-1}$ under SN and LN treatments. The results reveal that LN is being perceived as a nutrient stress which gets reflected in the higher root growth across the varieties under N stress than N-sufficient control.

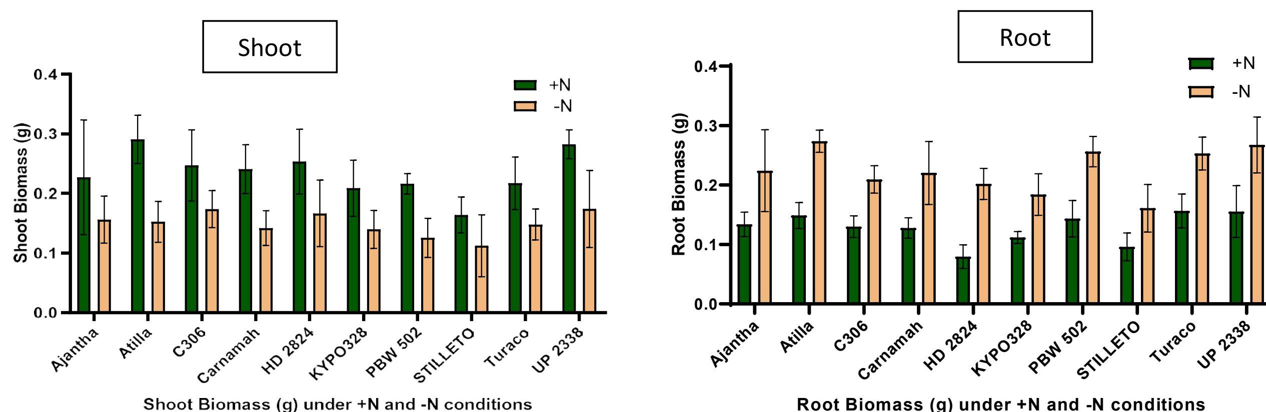

Fig. 1: Shoot and Root biomass of 25 days old seedlings of bread wheat varieties raised under sufficient (N+) and low N (N-) conditions in nutrient solution culture.

This publication is licensed under a Creative Commons Attribution 4.0 International License

Table 1: Shoot and Root biomass of 25 days old seedlings of bread wheat varieties raised under sufficient (N+) and low N (N-) conditions in nutrient solution culture (SN: Sufficient Nitrogen; LN: Low Nitrogen).

Varieties	Shoot Biomass		Root Biomass		Total Biomass	
	SN	LN	SN	LN	SN	LN
Ajantha	0.227	0.156	0.122	0.224	0.349	0.38
Atilla	0.290	0.153	0.149	0.273	0.439	0.426
C306	0.247	0.174	0.130	0.209	0.377	0.383
Carnamah	0.241	0.142	0.128	0.221	0.369	0.363
HD 2824	0.253	0.166	0.079	0.202	0.332	0.368
KYPO 328	0.209	0.139	0.112	0.184	0.321	0.323
PBW 502	0.216	0.125	0.143	0.246	0.359	0.371
Stilleto	0.164	0.112	0.096	0.161	0.26	0.273
Turaco	0.223	0.151	0.160	0.256	0.383	0.407
UP 2338	0.282	0.174	0.156	0.301	0.438	0.475

Macronutrient Profile of Bread Wheat Varieties under Low N Stress

Shoot and root nitrogen: In general, under nitrogen deficiency (LN, N-), wheat varieties exhibited a significant decline in the shoot nitrogen content, with a few exceptions (Fig. 2). Bread wheat variety Ajantha showed a 34.6% increase in leaf N under N- than N+ treatment. The inhibitory effect of low N availability on root growth was more pronounced than that observed for the shoot growth when compared with the sufficient N control (N+). Shoot N, in general, varied from 2.87 to 6.37%, while the root N content ranged from 1.55 to 4.46% under the N-sufficient (SN, N+) condition. On the other hand, under low nitrogen (LN, N-) conditions, both shoot and root nitrogen decreased significantly over N+ treatment, with a range of 2.63 to 4.42% for shoot N and 0.89 to 2.56%. The mean N content of shoot, and root under SN and LN treatments, averaged over the experimental bread wheat varieties was 4.28, 3.07, and 3.45, 1.64% respectively. In bread wheat var. Ajantha, in contrast to the observed low N mediated shoot growth induction, almost 70.5% decline in root growth was measured, when compared with N+ treatment. The results indicate the low N stress tolerance and an efficient N-uptake and N-translocation characteristic of var. Ajantha than the other experimental varieties.

Indeed, N deficiency typically triggers various adaptive mechanisms in plants, including enhanced uptake of other essential nutrients like P, K, and S, as observed in our study. This upregulation helps maintain cellular homeostasis and metabolic functions under stress (Amtmann & Hermans 2006, Hawkesford et al. 2012). However, the observation of higher leaf N content in Ajantha and UP 2338 under N-deficient conditions (Fig 2) can be due to the following reasons:

Enhanced N acquisition efficiency: These genotypes might possess a more efficient system for N uptake and utilization, even under N deficiency. This could involve improved root architecture, enhanced transporter activity, or better internal N mobilization mechanisms (Garnett et al. 2009, Sinclair & Gorissen 2009). For example, studies have shown that wheat genotypes with longer and denser root systems exhibit higher N uptake efficiency under limited N availability (Balemi et al. 2019).

Differential N allocation: While overall N uptake might be similar, these genotypes might prioritize allocation to leaves, maintaining higher N content for critical metabolic processes even under stress. This could involve altered signaling pathways or regulatory mechanisms governing N distribution within the plant (Xu et al. 2012, Hirel et al. 2011b). For instance, research suggests that certain wheat varieties preferentially allocate N to leaves under N deficiency to maintain photosynthetic capacity, potentially contributing to improved stress tolerance (Zheng et al. 2020).

Alternative N sources: Exploring potential contributions from atmospheric N fixation or N scavenging from organic matter in the soil could provide additional insights into the observed discrepancy. While not as common in wheat as in legumes, some genotypes exhibit inducible atmospheric N fixation capabilities under N stress (Kennedy & Roughley 2000). Additionally, efficient utilization of soil organic N through enhanced mineralization or root-microbial interactions could contribute to higher leaf N content (He et al. 2020).

Shoot and root phosphorus (P): The phosphorous content of shoot and root, under sufficient nitrogen (N+) conditions (Fig. 3), ranged from 1.22 to 3.53 ppm, and 1.71 to 4.66 ppm. However, under the low nitrogen (N-) condition, a significant increase in both shoot and root P was observed across the

This publication is licensed under a Creative Commons Attribution 4.0 International License

experimental wheat varieties. The range of variation in shoot and root P under N- treatment was 4.72 to 15.20 ppm and 6.52 ppm to 10.57 ppm. The mean P content of shoot, and root under SN and LN treatments, averaged over the experimental bread wheat varieties was 2.02, 2.99, and 9.86, 7.86 ppm respectively. A similar synergistic effect of P availability on N-metabolism of wheat including the expression of the N transport genes and enhanced activity of key N-assimilating enzymes (NR, GS, GDH, and GOGAT) has been recently reported by Li et al. (2023).

Shoot and root potassium (K): When subjected to nitrogen deficiency (LN, N-), a significant increase in the shoot K content of the wheat varieties was evidenced (Fig. 3). Under sufficient nitrogen (SN, N+) conditions, the shoot K ranged from 19.28 to 56.87 ppm, while the root K varied between 7.33 to 15.32 ppm. However, under low nitrogen (LN, N-) conditions, both shoot and root K increased significantly exhibiting a range variation of 79.99 to 115.88 ppm and 24.53 to 45.83 ppm respectively in the shoot and the root tissues of N-stressed (N-) plants. An increase in K application was reported to facilitate the uptake and transport of nitrate and also enhance the activity of N- assimilating enzymes in Spinach (Anjana & Iqbal 2009), while a reduced K was shown to impair the N- metabolism in sweet potato (Liu et al. 2022).

Shoot and root sulfur (S): In general, the shoot S content was significantly higher than root S under both N+ and N- conditions across the wheat varieties (Fig. 3). When compared between the N treatments, the tissue S content was found to be significantly higher under the N- than N+ condition of growth. The mean sulfur content across varieties varied significantly in a range from 18.78 to 33.85 ppm under sufficient nitrogen (SN, N+), while under N- treatment, the range of variation for shoot S was 26.46 to 49.65 ppm. In the case of root, the sulfur content under N+ and N- treatment varied between 4.93 to 14.93 ppm and 8.28 to 22.89 ppm respectively. The average shoot S content, across varieties, showed a significant increase from 27.34 under sufficient N to 38.65 ppm under low N stress. A similar pattern of increase in root S between N+ (10.25 ppm) and N- (15.66 ppm) was also recorded. Further, under nitrogen deficiency (N-), wheat varieties viz., Ajantha, Atilla, and Carnamah, demonstrated a heightened sulfur (S) uptake response in the shoot tissues. The above varieties, in general, also showed an increase in root S under low N (N-) than sufficient N (N+) treatment (Fig. 3). A lower content of S in the root than the shoot under N- over N+ treatment indicates a surge in root to shoot S-translocation, which may be a low N stress adaptation strategy. The findings highlight the nuanced changes in the sulfur content of wheat plants by nitrogen and thus, indicate an intricate interplay and influence of N and S metabolism on each other. Significant interaction between N and S on growth nutritional components and secondary metabolites (Jian et al. 2021) has been reported.

Activity of N-Assimilating Enzymes under Low N Stress

This study focused on evaluating the activity of key N-assimilating enzymes viz., glutamine synthetase (GS), glutamate synthase (GOGAT), nitrate reductase

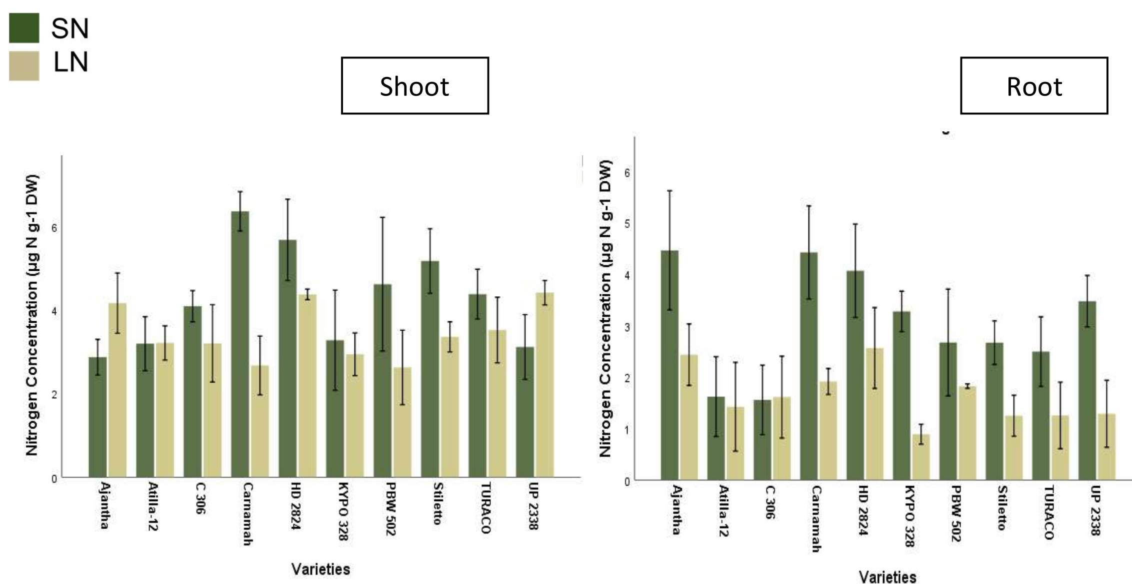

Fig. 2: Shoot and root nitrogen content of diverse wheat varieties under N-sufficient and N-deficient conditions of growth in nutrient solution culture.

This publication is licensed under a Creative Commons Attribution 4.0 International License

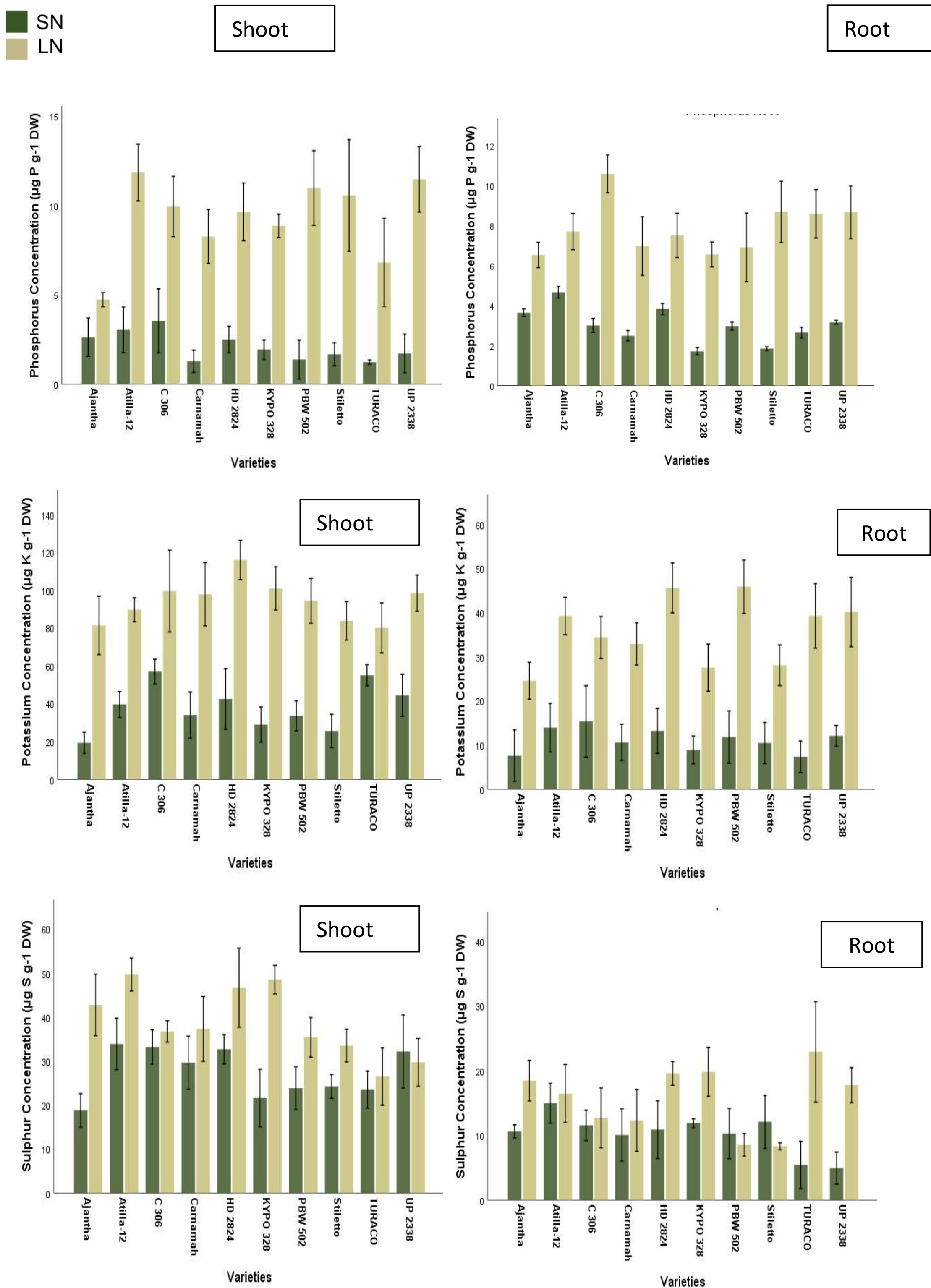

Fig. 3: Shoot and root phosphorus, potassium, and sulfur content of diverse wheat varieties under N-sufficient (SN) and N-deficient (LN) conditions of growth in nutrient solution culture.

This publication is licensed under a Creative Commons Attribution 4.0 International License

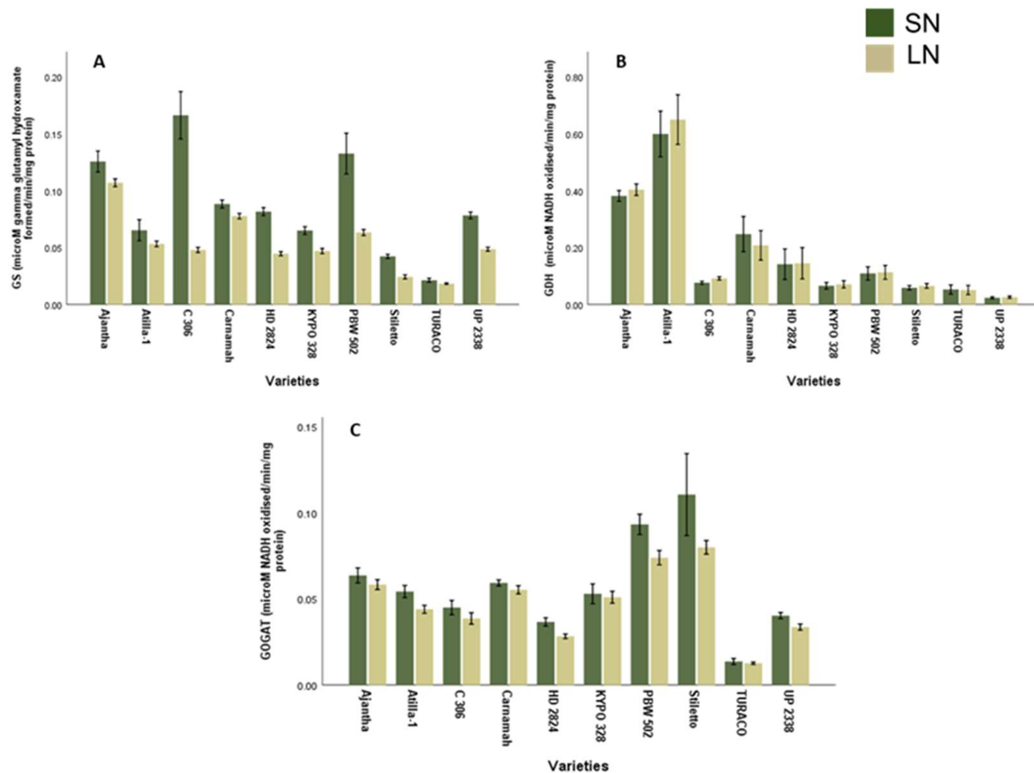

Fig. 4: Effect of N-stress on the activity of key N-assimilating enzymes viz., A) Glutamine synthetase (GS); B) Glutamate dehydrogenase (GDH); and C) Glutamate synthase (GOGAT) in wheat.

(NR), glutamate dehydrogenase (GDH), and alanine aminotransferase (AlaAT) as affected by nitrogen availability in bread wheat.

Glutamine synthetase (GS) activity: The glutamine synthetase (GS) activity varied between 0.021 to 0.166 and 0.018 to 0.107 μmol γ-glutamylhydroxamate formed per gram of protein per hour respectively under N+ and N- treatments. A decline in GS activity was recorded under N- over N+ condition (Fig. 4A). Mean GS activity averaged over the experimental wheat varieties, decreased significantly from 0.087 to 0.053 μmol γ-glutamylhydroxamate formed per gram of protein per hour under low nitrogen (N-) than N+ condition of growth. In general, three varieties i.e., Ajantha, Carnamah, and UP 2338 showed a relatively lesser decline in GS activity, also the bread wheat var. Turaco showed the least GS activity under the N- treatment.

Glutamate dehydrogenase (GDH) activity: In general, the GDH activity was not profusely affected by the N availability condition (Fig. 4B). In contrast to GOGAT, an increase in the GDH activity was recorded under the N-stress (0.183 μmol NADH oxidized per gram of protein per hour) when compared the N+ (0.176 μmol NADH oxidized per gram of protein per hour) treatment. The mean GDH activity, across the wheat varieties, varied from 0.024 to 0.600 and 0.026 to 0.650 μmol NADH oxidized per gram of protein per hour under the N+ and the N- treatment respectively. Bread wheat variety Atilla followed by Ajantha showed higher GDH activities, while the least activities across the N-treatments were observed for var., UP 2338.

Glutamate synthase (GOGAT) activity: A general decline in GOGAT activity under N- than N+ treatment was recorded (Fig. 4C). The GOGAT activity for the wheat varieties ranged between 0.014 to 0.111 and 0.013 to 0.080 μmol NADH oxidized per gram of protein per hour, respectively under the N+ and the N- treatment. For GOGAT, the average activity decreased from 0.057 to 0.048 μmol NADH oxidized per gram of protein per hour under N-stress. Another variety Turaco though showed the least GOGAT activity under the N-sufficient (N+) treatment, it did not decline under the low N (N-) input condition.

Nitrate reductase (NR) activity: A significant reduction in NR activity under the N- than compared with the N+ treatment was observed (Fig. 5A). The nitrate reductase (NR) activity, varied between 16.12 to 32.29 and 1.46 to 15.84 μmol nitrite formed per gram dw per hour respectively under the N+ and the N- condition of growth. A relatively

This publication is licensed under a Creative Commons Attribution 4.0 International License

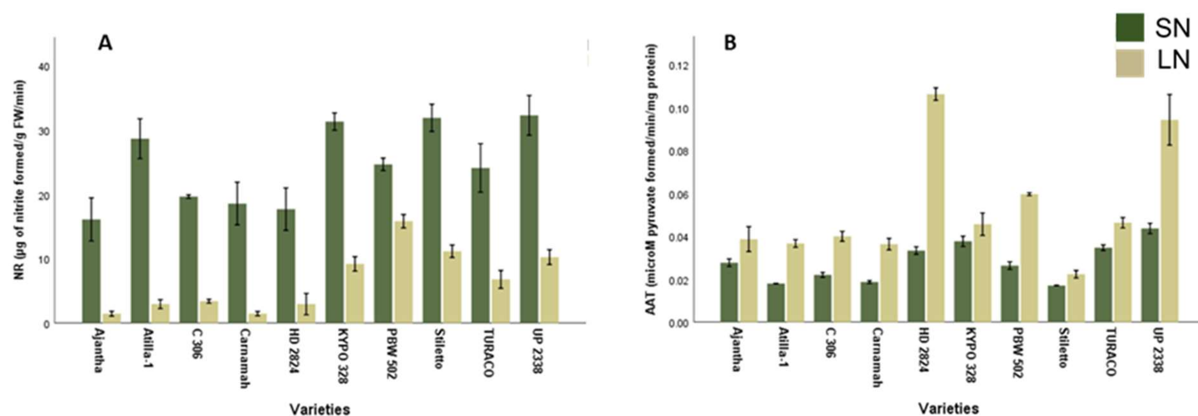

Fig. 5: Effect of N-stress on the activity of A) Nitrate reductase (NR) and B) Alanine aminotransferase (AlaAT) in wheat.

lower decline in the NR activity under the N- over the N+ treatment was recorded for var., PBW 502. Mean NR activity, averaged over the experimental varieties, under the N+ and the N- treatments was 24.51 and 6.55 µmol nitrite formed per gram dw per hour respectively.

Alanine aminotransferase (AlaAT) activity: Contrary to NR activity, the AlaAT activity across the bread wheat varieties was significantly induced under the N- than N+ treatment. Alanine Aminotransferase (AlaAT) activity, across varieties, varied from 0.017 to 0.044 and 0.022 to 0.106 µmol pyruvate formed per minute per mg protein under the N+ and the N- treatments respectively (Fig. 5B). Mean AlaAT activity, averaged over experimental varieties, increased from 0.028 (N+) to 0.053 µmol pyruvate formed per minute per mg protein under the N stress condition. A significantly higher N-stress-induced increase in AlaAT activity was recorded for var. HD 2824 and UP 2338 when compared to their respective enzyme activities under the N+ treatment.

Several studies have established the crucial role of macronutrients in influencing nitrogen (N) assimilation, particularly under N-deficient conditions. Anjana & Iqbal (2009) observed a significant decrease in glutamine synthetase (GS) activity, a key N-assimilating enzyme, in wheat experiencing combined S and N deficiency. Similarly, Jian et al. (2021) reported that co-limiting S and P in wheat significantly reduced both GS and glutamate dehydrogenase (GDH) activity, hindering N assimilation. Further emphasizing the interconnectedness of macronutrients, Liu et al. (2022) found that K deficiency under low N conditions further exacerbated the decrease in GS and GDH activity, highlighting the importance of balanced macronutrient availability for optimal N metabolism.

This current study builds upon this knowledge by demonstrating that supplementing S, P, and K under low N input (N-) can indirectly regulate N metabolism in wheat. Notably, this study observed an increase in the activity of N-assimilating enzymes in wheat plants receiving increased S, P, and K nutrition under N-deficient conditions. This enhanced enzyme activity is potentially linked to improved low N stress tolerance, as evidenced by Table 2.

Delving deeper into the mechanisms underlying this dependence holds immense potential for improving crop performance under N-limited conditions. Future research should focus on elucidating the specific roles of these macronutrients in enzyme synthesis, stability, and activity regulation. Additionally, investigating the species-specificity of these dependencies and exploring breeding strategies for enhanced N-assimilation efficiency and low N stress tolerance across various crops can significantly contribute to sustainable agriculture. Optimizing N use efficiency through balanced macronutrient management not only improves crop productivity but also minimizes environmental pollution associated with excessive N fertilizer application.

Table 2: Summary table showing an interactive effect of N-nutrition on macronutrients and activity of key N-assimilating enzymes in wheat. Upward and downward arrows indicate an increased and decreased activity for the observed parameter.

Nutrient	Shoot	Root
Nitrogen	Low ↓	Low ↓
Sulfur	High ↑	High ↑
Potassium	High ↑	High ↑
Phosphorus	High ↑	High ↑
N-assimilating enzymes	Shoot	
Glutamine synthetase	Low ↓	
Glutamate synthase	Low ↓	
Glutamate dehydrogenase	Low ↓	
Nitrate reductase	Low ↓	
Alanine aminotransferase	High ↑	

This publication is licensed under a Creative Commons Attribution 4.0 International License

Table 3: Relationship between the macronutrient content of wheat with the activities of key N-assimilating enzymes under the low N input condition (N-).

Parameter	GS	GOGAT	GDH	AlaAT	NR	Nitrogen	Phosphorus	Potassium	Sulfur
GS	1	0.331	0.483**	-0.083	-0.385*	0.320	-0.535**	-0.162	0.226
GOGAT		1	0.088	-0.460*	0.389*	-0.271	-0.184	-0.318	-0.296
GDH			1	-0.280	-0.514**	0.133	-0.166	-0.200	0.502**
AlaAT				1	0.101	0.550**	0.498**	0.767**	0.180
NR					1	-0.407*	0.149	0.060	-0.476**
Nitrogen						1	0.136	0.217	0.265
Phosphorus							1	0.673**	-0.058
Potassium								1	0.177
Sulfur									1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Further, the Pearson correlation analysis was performed to study the association between different macronutrients (N, P, K, and S) and nitrogen-related enzymes (GS, GOGAT, GDH, AlaAT and NR) under nitrogen deficit (N-) conditions. A significant positive correlation of AlaAT activity with nitrogen (r=0.550**) phosphorus (r=0.498**) and potassium (r=0.767**) nutrition of wheat was recorded (Table 3). Further, a significant positive correlation between the GS and the GDH activity (r=0.483**), but a negative correlation between the NR and the GDH activity (r=0.514**) and between S and GDH (r=0.502**) were observed. NR activity showed a significant negative correlation with sulfur (r=0.476*).

The observed variations in sulfur, phosphorus, potassium, and nitrogen content in both shoot and root tissues shed light on the complex interplay between nutrient availability and plant responses. The significant increase in sulfur content in the shoot tissues under nitrogen-deficient conditions aligns with previous studies emphasizing the role of sulfur in enhancing plant tolerance to abiotic stresses (Hasanuzzaman et al. 2018). A coordinated mediation between sulfur and nitrogen metabolism under salt stress has been reported (Jahan et al. 2021). The notable increase in shoot and root potassium content under nitrogen-deficient conditions is in agreement with previous research highlighting the role of potassium in enhancing plant osmoregulation and stress tolerance (Nieves-Cordones et al. 2016, Shabala 2017). The observed genotypic variation in macronutrients may be attributed to differences in nutrient uptake and utilization efficiency (Wang et al. 2015). The substantial increase in shoot phosphorus content in response to nitrogen deficiency, as exemplified by var. Carnamah and HD 2824, reflect the significance of phosphorus in enhancing plant metabolic processes and stress adaptation.

CONCLUSION

The observed variations in the activity of key enzymes such as glutamine synthetase (GS), nitrate reductase (NR), alanine aminotransferase (AlaAT), glutamate synthetase (GOGAT), and glutamate dehydrogenase (GDH) under nitrogen deficiency highlight the intricate regulation of nitrogen assimilation and amino acid metabolism in wheat. The ability of var. Turaco to maintain a similar level of GS activity under both the sufficient (N+) and the deficient N (N-) input/availability condition of growth, reflects upon its ability to tolerate low N stress. The trait can be introgressed into the high-yielding wheat varieties to improve their resilience against abiotic stresses. The findings further emphasize the importance of understanding the nuanced interplay between nitrogen availability, macronutrient uptake, and N-metabolism in wheat, and provide a foundation for targeted breeding and agronomic interventions aimed at enhancing the nutrient uptake efficiency and crop resilience in the face of changing environmental conditions. Understanding the nutrient interactions in controlled environments will further our knowledge and pave the way for developing more effective stress mitigation strategies for fortifying our quest for sustainable agriculture.

ABBREVIATIONS

GS:	Glutamine synthetase
GOGAT:	Glutamate synthase
GDH:	Glutamate dehydrogenase
AlaAT:	alanine aminotransferase
NR:	Nitrate reductase

REFERENCES

Anjana, S. U. and Iqbal, M. 2009. Effect of applied potassium in increasing the potential for N assimilation in spinach (*Spinacea oleracea L.*). Electron. Int. Fertil. Corresp., 20: 8-10.

Anas, M., Liao, F., Verma, K. K., Sarwar, M. A., Mahmood, A., Chen, Z.-L., Li, Q., Zeng, X.-P., Liu, Y. and Li, Y. R. 2020. Fate of nitrogen in agriculture and environment: Agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. Biol. Res., 53(1): 47. https://doi.org/10.1186/s40659-020-00312-4

This publication is licensed under a Creative Commons Attribution 4.0 International License

Amtmann, A. and Hermans, C. 2006. Coping with manganese toxicity in plants: physiological and molecular mechanisms. Annu. Rev. Plant Biol., 57(1): 361-385.

Asibi, A. E., Chai, Q. and Coulter, J. A. 2019. Mechanisms of nitrogen use in maize. Agronomy, 9(12): 775. https://doi.org/10.3390/agronomy9120775

Balemi, T. H., Ghafourizadeh, S. and Sohrabi, Y. 2019. Root traits contributing to nitrogen use efficiency in wheat genotypes under water stress. J. Plant Physiol., 233: 154-162.

Cooper, A. J. L. 2012. The role of glutamine synthetase and glutamate dehydrogenase in cerebral ammonia homeostasis. Neurochem. Res., 37(11): 2439-2455. https://doi.org/10.1007/s11064-012-0803-4

De Sousa, C. A. F. and Sodek, L. 2003. Alanine metabolism and alanine aminotransferase activity in soybean (Glycine max) during hypoxia of the root system and subsequent return to normoxia. Environ. Exp. Bot., 50(1): 1-8. https://doi.org/10.1016/S0098-8472(02)00108-9

Dutia, S. 2014. AgTech: Challenges and opportunities for sustainable growth. SSRN Electron. J. https://doi.org/10.2139/ssrn.2431316

Evans, H. J. and Nason, A. 1953. Pyridine nucleotide-nitrate reductase from extracts of higher plants. Plant Physiol., 28(2): 233. https://doi.org/10.1104%2Fpp.28.2.233

Foulkes, M. J., Hawkesford, M. J., Barraclough, P. B., Holdsworth, M. J., Kerr, S., Kightley, S. and Shewry, P. R. 2009. Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. Field Crops Res., 114(3): 329-342.

Garnett, T. P., Brundle, M. J. and Kniemeyer, A. 2009. Enhanced root shoot communication in nitrogen starved plants. Plant Cell Environ., 32(4): 382-395.

Gheith, E. M. S., El-Badry, O. Z., Lamlom, S. F., Ali, H. M., Siddiqui, M. H., Ghareeb, R. Y., El-Sheikh, M. H., Jebril, J., Abdelsalam, N. R. and Kandil, E. E. 2022. Maize (*Zea mays* L.) productivity and nitrogen use efficiency in response to nitrogen application levels and time. Front. Plant Sci., 13, 941343. https://doi.org/10.3389/fpls.2022.941343

Giraldo, P., Benavente, E., Manzano-Agugliaro, F. and Gimenez, E. 2019. Worldwide research trends on wheat and barley: A bibliometric comparative analysis. Agronomy, 9(7): 352. https://doi.org/10.3390/agronomy9070352

Govindasamy, P., Muthusamy, S. K., Bagavathiannan, M., Mowrer, J., Jagannadham, P. T. K., Maity, A., Halli, H. M., G. K., S., Vadivel, R., T. K., D., Raj, R., Pooniya, V., Babu, S., Rathore, S. S., L., M. and Tiwari, G. 2023. Nitrogen use efficiency-A key to enhance crop productivity under a changing climate. Front. Plant Sci., 14, 1121073. https://doi.org/10.3389/fpls.2023.1121073

Habash, D. Z., Massiah, A. J., Rong, H. L., Wallsgrove, R. M. and Leigh, R. A. 2001. The role of cytosolic glutamine synthetase in wheat. Ann. Appl. Biol., 138(1): 83-89. https://doi.org/10.1111/j.1744-7348.2001.tb00087.x

Hasanuzzaman, M., Bhuyan, M. H. M. B., Mahmud, J. A., Nahar, K., Mohsin, S. M., Parvin, K. and Fujita, M. 2018. Interaction of sulfur with phytohormones and signaling molecules in conferring abiotic stress tolerance to plants. Plant Signal. Behav., 13(5), e1477905. https://doi.org/10.1080/15592324.2018.1477905

He, J.Z., Wang, Z.L., Zhang, X.M., Li, X.L., Christie, P. and Li, F.S. 2020. Wheat genotypes differ in their rhizosphere priming effect and its regulation by nitrogen availability. Soil Biol. Biochem., 143, 107680.

Hirel, B., Piques, M. and Gouesnard, B. 2011a. Sustainability of crop production: how to reduce dependence on mineral nitrogen fertilization? Agric. Ecosyst. Environ., 144(1): 226-245.

Hirel, B., Tétu, T., Lea, P. J. and Dubois, F. 2011b. Improving nitrogen use efficiency in crops for sustainable agriculture. Sustainability, 3(9): 1452-1485. https://doi.org/10.3390/su3091452

Hoagland and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Circular. Calif. Agric. Exp. Station 1950 Vol.347 pp.32 pp. https://www.cabdirect.org/cabdirect/abstract/19500302257

Jahan, B., Rasheed, F., Sehar, Z., Fatma, M., Iqbal, N., Masood, A., Anjum, N. A. and Khan, N. A. 2021. Coordinated role of nitric oxide, ethylene, nitrogen, and sulfur in plant salt stress tolerance. Stresses, 1(3): 181-199. https://doi.org/10.3390/stresses1030014

Jian, S. F., Huang, X. J., Yang, X. N., Zhong, C. and Miao, J. H. 2021. Sulfur regulates the trade-off between growth and andrographolide accumulation via nitrogen metabolism in Andrographis paniculata. Front. Plant Sci., 12: 687954, https://doi.org/10.3389/fpls.2021.687954

Jones Jr, J. B. 1987. Nitrogen: Kjeldahl nitrogen determination what's in a name. J. Plant Nutr., 10(9-16): 1675-1682. https://doi.org/10.1080/01904168709363706

Kennedy, I.R. and Roughley, R.J. 2000. Nitrogen fixation in non-leguminous plants: an untapped resource for sustainable agriculture? Plant Soil, 228(2): 351-366.

Klepper, L., Flesher, D. and Hageman, R. H. 1971. Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. Plant Physiol., 48(5): 580-590. https://doi.org/10.1104/pp.48.5.580.

Ladha, J. K., Peoples, M. B., Reddy, P. M., Biswas, J. C., Bennett, A., Jat, M. L. and Krupnik, T. J. 2022. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. Field Crops Research, 283: 108541. https://doi.org/10.1016/j.fcr.2022.108541

Li, Y., Wang, Y., Chen, S., Gao, Y. and Shi, Y. 2023. Effects of different nitrogen and phosphorus synergistic fertilizer on enzymes and genes related to nitrogen metabolism in wheat. Phyton (0031-9457), 92(7).

Liu, J., Xia, H., Gao, Y., Pan, D., Sun, J., Liu, M. and Li, Z. 2022. Potassium deficiency causes more nitrate nitrogen to be stored in leaves for low-K sensitive sweet potato genotypes. Frontiers in Plant Science, 13: 1069181. https://doi.org/10.3389/fpls.2022.1069181

Murphy, J. A. M. E. S. and Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica chimica acta, 27: 31-36. https://doi.org/10.1016/S0003-2670(00)88444-5

Mohanty, B. and Fletcher, J. S. 1980. Ammonium influence on nitrogen assimilating enzymes and protein accumulation in suspension cultures of Paul's Scarlet rose. Physiologia Plantarum, 48(3): 453-459. https://doi.org/10.1111/j.1399-3054.1980.tb03285.x

Nair, K. P. 2021. Concluding comments and future imperatives. In K. P. Nair, Thermodynamics Of Soil Nutrient Bioavailability, (pp. 61-88). Springer International Publishing. https://doi.org/10.1007/978-3-030-76817-1_6

Nair, T. V. R. and Y. P. Abrol. 1973. Nitrate reductase activity in developing wheat ears. Experientia, 29.12: 1480-1481. https://doi.org/10.1007/BF01943867

Nieves-Cordones, M., Al Shiblawi, F. R. and Sentenac, H. 2016. Roles and transport of sodium and potassium in plants. The alkali metal ions: Their role for life, 291-324. https://doi.org/10.1007/978-3-319-21756-7_9

Paramesh, V., Mohan Kumar, R., Rajanna, G. A., Gowda, S., Nath, A. J., Madival, Y., Jinger, D., Bhat, S. and Toraskar, S. 2023. Integrated nutrient management for improving crop yields, soil properties, and reducing greenhouse gas emissions. Frontiers in Sustainable Food Systems, 7: 1173258. https://doi.org/10.3389/fsufs.2023.1173258

Ramadas, S., Kiran Kumar, T. M. and Pratap Singh, G. 2020. Wheat Production in India: Trends and Prospects. In F. Shah, Z. Khan, A. Iqbal, M. Turan, & M. Olgun (Eds.), Recent Advances in Grain Crops Research. IntechOpen. https://doi.org/10.5772/intechopen.86341

Sandhu, N., Sethi, M., Kumar, A., Dang, D., Singh, J. and Chhuneja, P. 2021. Biochemical and genetic approaches improving nitrogen use efficiency in cereal crops: A review. Frontiers in Plant Science, 12: 657629. https://doi.org/10.3389/fpls.2021.657629

Sathee, L., Adavi, S. B., Jain, V., Pandey, R., Khetarpal, S., Meena, H. S. and Kumar, A. 2018. influence of elevated CO_2 on kinetics and expression of high affinity nitrate transport systems in wheat. Indian Journal of Plant Physiology, 23(1): 111-117. https://doi.org/10.1007/s40502-018-0355-y

Shabala, S. (Ed.). 2017. Plant stress physiology. Cabi Publication, ISBN: 9781780647296

This publication is licensed under a Creative Commons Attribution 4.0 International License

Sharma, S., Kumar, T., Foulkes, M. J., Orford, S., Singh, A. M., Wingen, L. U., Karnam, V., Nair, L. S., Mandal, P. K., Griffiths, S., Hawkesford, M. J., Shewry, P. R., Bentley, A. R. and Pandey, R. 2023. Nitrogen uptake and remobilization from pre- and post-anthesis stages contribute towards grain yield and grain protein concentration in wheat grown in limited nitrogen conditions. CABI Agriculture and Bioscience, 4(1): 12. https://doi.org/10.1186/s43170-023-00153-7

Sinclair, T.R. and Gorissen, A. 2009. Wheat responses to agronomic nutrients and their efficiency in use. Soil Use and Management, 25(2): 196-209.

Singh, B. and Craswell, E. 2021. Fertilizers and nitrate pollution of surface and ground water: An increasingly pervasive global problem. SN Applied Sciences, 3(4): 518. https://doi.org/10.1007/s42452-021-04521-8

Skwierawska, M., Skwierawska, M., Skwierawski, A., Benedycka, Z. and Jankowski, K. 2016. Sulfur as a fertiliser component determining crop yield and quality. Journal of Elementology, 2/2016. https://doi.org/10.5601/jelem.2015.20.3.992

Sun, J., Zhang, X., Broderick, M. and Fein, H. 2003. Measurement of nitric oxide production in biological systems by using Griess reaction assay. Sensors, 3(8): 276-284. https://doi.org/10.3390/s30800276

Wang, Y. and Wu, W. H. 2015. Genetic approaches for improvement of the crop potassium acquisition and utilization efficiency. Current Opinion in Plant Biology, 25: 46-52.

Wang, X., Bai, J., Xie, T., Wang, W., Zhang, G., Yin, S. and Wang, D. 2021. Effects of biological nitrification inhibitors on nitrogen use efficiency and greenhouse gas emissions in agricultural soils: A review. Ecotoxicology and Environmental Safety, 220: 112338. https://doi.org/10.1016/j.ecoenv.2021.112338

Xu, J., Xu, X. and Verstraete, W. 2000. Adaptation of E. coli cell method for micro-scale nitrate measurement with the Griess reaction in culture media. Journal of Microbiological Methods, 41(1): 23-33. https://doi.org/10.1016/S0167-7012(00)00141-X

Xu, G., Fan, X. and He, H. 2012. Plant nitrogen assimilation and allocation under salt stress. Plant, Cell & Environment, 35(6): 1620-1635.

Yadav, M. R., Kumar, S., Lal, M. K., Kumar, D., Kumar, R., Yadav, R. K., Kumar, S., Nanda, G., Singh, J., Udawat, P., Meena, N. K., Jha, P. K., Minkina, T., Glinushkin, A. P., Kalinitchenko, V. P. and Rajput, V. D. 2023. Mechanistic understanding of leakage and consequences and recent technological advances in improving nitrogen use efficiency in cereals. Agronomy, 13(2): 527. https://doi.org/10.3390/agronomy13020527

Yan, S., Wu, Y., Fan, J., Zhang, F., Zheng, J., Qiang, S., Guo, J., Xiang, Y., Zou, H. and Wu, L. 2020. Dynamic change and accumulation of grain macronutrient (N, P and K) concentrations in winter wheat under different drip fertigation regimes. Field Crops Research, 250: 107767. https://doi.org/10.1016/j.fcr.2020.107767

Yin, Z., Guo, W., Xiao, H., Liang, J., Hao, X., Dong, N., Leng, T., Wang, Y., Wang, Q. and Yin, F. 2018. Nitrogen, phosphorus, and potassium fertilization to achieve expected yield and improve yield components of mung bean. PLOS ONE, 13(10): e0206285. https://doi.org/10.1371/journal.pone.0206285

Zhang, A., Liu, Y., Pan, G., Hussain, Q., Li, L., Zheng, J. and Zhang, X. 2012. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. Plant and soil, 351: 263-275. https://doi.org/10.1007/s11104-011-0957-x

Zheng, Z., Liu, M., Li, P., Li, J., Li, X. and Jing, Q. 2020. Wheat genotypes with different nitrogen use efficiency exhibit contrasting responses to nitrogen deficiency in terms of root architecture and root exudation. Journal of Agronomy and Crop Science, 206(2): 260-272.

Zhou, B., Serret, M. D., Pie, J. B., Shah, S. S. and Li, Z. 2018. Relative contribution of nitrogen absorption, remobilization, and partitioning to the ear during grain filling in Chinese winter wheat. Frontiers in Plant Science, 9: 1351. https://doi.org/10.3389/fpls.2018.01351

This publication is licensed under a Creative Commons Attribution 4.0 International License