International Journal of Environment, Agriculture and Biotechnology
Vol-10, Issue-4; Jul-Aug, 2025
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Impact of foliar Nano-DAP on nitrogen metabolism in wheat seedlings *in vitro*

Vikash Kumar[1]*, Vinod Saharan[1], Deepak Rajpurohit[1], Abhay Dashora[2], N.L. Meena[3], Kinjal Mondal[1], Pooja[1], Poonam Kumari[1], Vinod Kumar Sen[1], Laxmi[1]

[1]Department of Molecular Biology and Biotechnology, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India
[2]Department of Genetics and plant breeding, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India
[3]Department of Plant Pathology, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India
*Corresponding author

Received: 12 Jun 2025; Received in revised form: 10 Jul 2025; Accepted: 14 Jul 2025; Available online: 20 Jul 2025

Abstract— *Nano-DAP are known to enhance plant defence and metabolism. In this study, a foliar application of Nano-DAP was applied in varied concentrations (2-6 ml/L), to 14-day-old wheat seedlings in vitro. To evaluate the responses of Nano-DAP on nitrogen metabolism in wheat, key enzymes were targeted to be checked at 8 hours after foliar application. Results showed significant increases in nitrate reductase (NR) and glutamine synthetase (GS) activities, along with varied free amino acid (FAA) levels and chlorophyll content, compared to conventional DAP (2%, w/v) as well as control. These findings suggest that Nano-DAP enhances nitrogen assimilation and photosynthetic efficiency, demonstrating its potential as a plant metabolic enhancer.*

Keywords— ***Nano-DAP, Wheat, Foliar application.***

I. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a cereal crop belonging to the family Poaceae, preferably grown in temperate climates and requires specific conditions for optimal growth, including cool temperatures during germination and warmer temperatures during grain filling (Porter *et al.,*1999). During the 2022-23 *Rabi*, India produced over 110 million metric tons (MMT) of wheat from 31.4 million hectares, accounting for approximately 37% of the nation's total food grain production with a record average productivity of 3520.7 kg/ha (FAO, 2023). Nitrogen (N) is a fundamental nutrient required for plant growth, particularly in cereal crops like wheat, which has a high nitrogen demand for optimal development and yield. As an essential component of amino acids, proteins, nucleic acids, and chlorophyll, nitrogen is involved in key physiological and biochemical processes including cell division, enzyme activation, and photosynthesis (Lea & Azevedo, 2006; Fageria & Baligar, 2005). In wheat, adequate nitrogen availability enhances vegetative growth, tillering, leaf area index, and promotes grain filling, ultimately leading to increased productivity and improved grain protein content (Good *et al.,* 2004). N acquisition efficiency of cereals is less than 50% of the N supply (Raun and Johnson, 1999), this value is lower than the N acquisition rate (60%) which is required for maximizing plant growth and yield (Sylvester-Bradley *et al.,* 2009). Currently, improving plant N absorption ability in soil may be a critical means to improve plant N efficiency and plant growth (Garnett *et al.,* 2013). Despite the widespread use of nitrogen fertilizers in wheat cultivation, nitrogen use efficiency (NUE) remains relatively low. It is estimated that only 30–50% of applied nitrogen is taken up by the plant, with the rest lost through volatilization, leaching, surface runoff, or denitrification (Raun & Johnson, 1999). Such losses not only reduce fertilizer efficiency and raise production costs but also lead to significant environmental issues, such as nitrate pollution of water bodies and emissions of greenhouse gases (Cowan *et al.,* 2019). Therefore, enhancing the efficiency of nitrogen use has become a critical goal in sustainable wheat production systems. In recent years, foliar application of nitrogen has

been explored as an effective strategy to supplement traditional soil fertilization methods. Foliar feeding allows nutrients to be absorbed directly through leaf stomata and cuticular pathways, enabling rapid nutrient uptake and utilization, especially during peak demand periods or when root function is compromised (Fernández & Eichert, 2009). This method is particularly advantageous during late growth stages, such as grain filling, when root uptake becomes less efficient and the plant requires an immediate nitrogen source to sustain photosynthesis and protein synthesis (Tahir *et al.,* 2024). Given the importance of nitrogen in wheat physiology and the potential advantages of foliar feeding, this study aims to evaluate the role of foliar-applied nitrogen sources in enhancing the growth, physiological traits, and yield performance of wheat plants. The findings may contribute to developing more efficient and sustainable nutrient management practices in wheat production. Since the Green Revolution, nitrogen fertilizers have significantly increased wheat yields; however, their overuse has caused environmental concerns such as soil acidification and nitrous oxide emissions (Tilman *et al.,* 2002; Harty *et al.,* 2016). This necessitates a shift toward sustainable and eco-friendly nutrient management practices. Di-Ammonium Phosphate (DAP) is one of the most widely used phosphorus-based fertilizers, known for its high nutrient content and rapid solubility, making it essential for early plant development. Di-ammonium phosphate (DAP) serves as a valuable fertilizer by supplying both N (16%) and P (48%), thereby supporting various metabolic processes in wheat (Zheng *et al.,* 2023). But, excessive use of DAP possesses several significant drawbacks, both to agricultural productivity and the environment. One of the primary issues is the alteration of soil pH; when DAP is applied in large amounts, the resulting ammonium ions can acidify the soil, affecting nutrient availability and microbial activity. Overuse also leads to nutrient imbalances, as continuous application of phosphorus-rich fertilizers can suppress the uptake of other essential nutrients like zinc and iron, thereby impairing plant growth (Zhao *et al.,* 2022). Environmentally, the surplus P from DAP often leaches into nearby water bodies, contributing to eutrophication — a process that triggers excessive algae growth, which depletes oxygen levels in the water, disrupts aquatic ecosystems, and can lead to the death of fish and other marine life (Ngatia and Taylor, 2018).

II. MATERIALS AND METHODOLOGY

2.1 Synthesis and characterization

In this study, Nano-DAP was procured from IFFCO and subjected for characterization. Dynamic light scattering (DLS) has followed to understand the hydrodynamic diameter of particles, poly-dispersity index (PDI) and zeta potential (mV). In addition, viscosity of the formulation was measured using a rotational viscometer (ViscoQC 100-L, Anton-Paar, Austria, Europe).

2.2 *In vitro* seedling bioassay to measure real time impact on nitrogen metabolism

Wheat seeds were grown in a mini-glass house. Afterwards, seeds were sown in different seedling trays (10 seeds in each pot hole) filled with cocopeat and vermicompost mixture (1:1) in controlled light condition. Seedling trays (having 35 pot holes) were kept at mini-glass house set up. Regular watering was done to keep the germination area satisfactorily moisten. Foliar application of various forms of Nano-DAP, were performed with various concentration 2ml/l, 4ml/l,6ml/l, concentration on 14-days old wheat seedling. Leaf samples were collected from mini glass house at 8h after foliar application to measure nitrate reductase (NR; EC 1.6.6.1), glutamine synthetase (GS; EC 6.3.1.2) using available protocols (Hageman and Hucklesby, 1971; Lillo, 1984). Likewise, leaf samples were also be used for the estimation of free amino acid and chlorophyll content (a and b) (Lichtenthaler, 1987) 8 h after foliar application.

2.2.1 Nitrate reductase activity

The activity of nitrate reductase (NR; EC 1.6.6.1) in leaves of 14-days old wheat seedlings grown in a mini-glass house was measured as described by Hageman and Hucklesby, 1971; along with in-house modifications. First, 1 g of fresh leaves were ground into powder using liquid N and 100 mM Tris HCl buffer. After centrifugation at 10,000 rpm for 10 min, the supernatant was transferred into a reaction mixture containing a solution of nicotinamide adenine dinucleotide (1 mM NADH), Tris HCl buffer (25mM) and KNO_3 (100 mM). The mixture was incubated at 30°C for 30 min in the dark. After incubation, the NO_2^- produced pink colour, which was measured at 540 nm after addition of 1% Sulfanilamide in 3M HCl and 0.2% NED (N-1-naphthylethylenediamine dihydrochloride) were added to terminate the reaction.

2.2.2 Glutamine synthetase activity

Following Lillo, 1984; 1 g fresh samples taken from mini glass house grown plant leaves were ground into powder to determine the activities of glutamine synthetase (GS) (EC 6.3.1.2). After centrifugation at 10,000 rpm for 10 min at 4°C, the supernatant was retained for reaction. The reaction mixture for GS was a solution containing 0.1M imidazole, 0.08M $MgSO_4$ $7H_2O$, 0.02M Glutamic acid and 58mM Hydroxylamine hydrochloride at pH 7. Then, 10mM ATP (pH >7) was added to the reaction mixture, after incubation at 30°C for 15 min, terminate the reaction by adding 1ml of $FeCl_3$ reagent. The absorbance of the

supernatant was measured at 540 nm for the calculation of GS activity.

2.2.3 Free amino acid content

Free amino acids were extracted from 14-days old wheat seedlings grown in a mini-glass house was measured according to (Rosen, 1957) 1g of fresh leaves were ground into powder using liquid N and 80% ethanol. After centrifugation at 10,000 rpm for 10 min, the supernatant was transferred into 0.8% ninhydrin reagent and kept in water bath at 100℃ for 20 min; afterwards, the solution is allowed to cool at room temperature and the absorbance of the supernatant was measured at 570 nm and the enzyme activity was calculated using double-beam UV-VIS spectrometry.

2.2.4 Chlorophyll content

To extract chlorophyll from 100 mg of finely cut leaf fragments, a solution of 10 ml dimethyl sulfoxide (DMSO) was employed, and the mixture was subjected to a constant temperature of 65°C for a period of 3h. Occasional gentle agitation was performed one or two times during this interval to ensure complete chlorophyll extraction. Subsequently, the supernatant was meticulously collected, and its optical absorbance was measured at two specific wavelengths, namely 663 nm and 645 nm, employing a UV-VIS spectrophotometer. This analytical procedure adhered to the methodology detailed by Hiscox and Israelstam (1979).

Chlorophyll a, chlorophyll b, and total chlorophyll concentrations (expressed in mg/g) were then calculated employing the following precise formulas:

Chlorophyll a (Chl. a) = (12.7 × A663 - 2.63 × A645) × V / (1000 × W)

Chlorophyll b (Chl. b) = (22.9 × A645 - 4.48 × A663) × V / (1000 × W)

Total chlorophyll = (20.2 × A645 + 8.02 × A663) × V / (1000 × W)

Where,

V = represents the volume of the sample (10 ml)

W = corresponds to the weight of the leaf sample (100 mg)

2.3 Statistical analysis

The statistical analysis was used as post hoc test to determine the significance difference between the treatments at P=0.05 level. The analysis will be performed with JMP software version 12 (SAS, 2010) using Turkey Kramer HSD test.

2.4 Location of Research experiment

Mini-glass house experiment was conducted at the Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Udaipur.

III. RESULTS AND DISCUSSION

3.1 Physicochemical characterization of Nano-DAP

The commercially available IFFCO-based Nano-DAP is reported to contain 16% P, 8% N. In this study, Nano-DAP has procured from IFFCO and subjected for characterization. Dynamic light scattering (DLS) has followed to understand the hydrodynamic diameter of particles, Z Avg. is 271.2, poly-dispersity index (PDI) is 1.0 and zeta potential (mV) is -2.75, count per rate(kcps) is 265. In addition, viscosity of the formulation has measured using a rotational viscometer (ViscoQC 100-L, Anton-Paar, Austria, Europe). Stability has studied by measuring the size, zeta and PDI values for extended storage period. Viscosity of Nano-DAP is 7 centipoises.

3.2 Bio-efficacy evaluation of Nano-dap on nitrogen metabolism in wheat

Foliar application of various forms of Nano-DAP was performed with 2ml/L, 4ml/L, 6ml/L, concentration on 14-days old wheat seedling. Leaf samples were collected from mini glass house at 8h after foliar application to measure various enzyme activity. After 8 h NR activity increased in Nano-DAP (6ml/L), (5.1 µg/min/g) as compared to control (2.01µg/min/g). After 8 h GS activity increased in Nano-DAP (6ml/L), as compared to control (0.27mg/min/g) and control (0.11 mg/min/g). After 8 h FAA content increased in Nano-DAP (6ml/L) (1.1 mg/g) and in control (1.053 mg/g). Chlorophyll content found higher in treatment Nano-DAP (4ml/L). After 8 h chlorophyll a increased in Nano-DAP (1.79mg/g) as compared to control (1.21mg/g) and chlorophyll b increased in (1.11 mg/g) as compared to control (0.096 mg/g) and total chlorophyll content increased in Nano-DAP (2.88 mg/g) as compared to control (1.34 mg/g). All the results have graphically been explained in Figure 1.

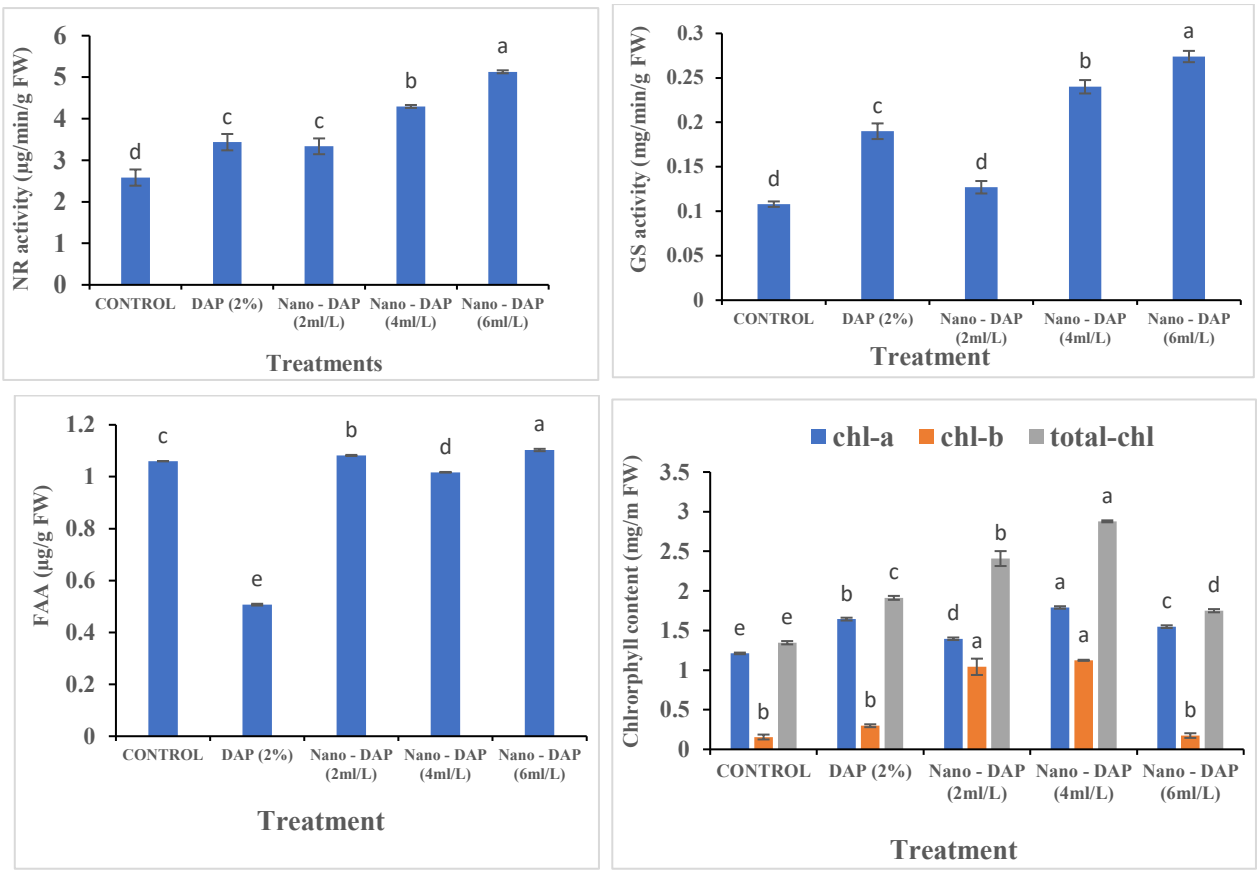

Fig.1 Biochemical responses of wheat seedlings towards foliar Nano-DAP. Each value is mean of triplicates. Graph is with same latter is not significantly different at p=0.05 as determined by Tukey-Karmer HSD.

IV. CONCLUSION

The findings of this study clearly demonstrate that foliar application of Nano-DAP at concentration 6 ml/L concentration significantly enhances nitrogen metabolism and photosynthetic efficiency in wheat seedlings under mini glass house conditions. The marked increases in NR and GS activities, free amino acid content, and chlorophyll levels high in 4ml/L concentration within just 8 hours of treatment indicate a rapid physiological response. These enhancements suggest improved nitrogen assimilation and utilization, likely mediated by Nano-DAP induced activation of key metabolic pathways. Overall, Nano-DAP show promising potential as eco-friendly plant growth regulator to improve crop nutrient efficiency and reduce reliance on synthetic nitrogen fertilizers, contributing to sustainable agricultural practices.

REFERENCES

[1] Cowan, N., Levy, P., Moring, A., Simmons, I., Bache, C., Stephens, A., & Skiba, U. (2019). Nitrogen use efficiency and N_2O and NH_3 losses attributed to three fertiliser types applied to an intensively managed silage crop. *Biogeosciences*, *16*(23), 4731-4745.

[2] Fageria, N. K., & Baligar, V. C. (2005). Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy*, *88*, 97-185.

[3] FAOSTAT, 2023. https://www.fao.org/faostat/en/#data/QCL

[4] Fernández, V., & Eichert, T. (2009). Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. *Critical Reviews in Plant Sciences*, *28*(1-2), 36-68.

[5] Garnett, T., Conn, V., Plett, D., Conn, S., Zanghellini, J., Mackenzie, N.,& Kaiser, B. N. (2013). The response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. *New Phytologist*, *198*(1), 82-94.

[6] Good, A. G., Shrawat, A. K., & Muench, D. G. (2004). Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production. *Trends in Plant Science*, *9*(12), 597-605.

[7] Hageman, R. H., & Hucklesby, D. P. (1971). Nitrate reductase from higher plants. In *Methods in Enzymology* (Vol. 23, pp. 491-503). Academic Press.

[8] Harty, M. A., Forrestal, P. J., Watson, C. J., McGeough, K. L., Carolan, R., Elliot, C., & Lanigan, G. J. (2016).

Reducing nitrous oxide emissions by changing N fertiliser use from calcium ammonium nitrate (CAN) to urea-based formulations. *Science of the Total Environment*, *563*, 576-586.

[9] Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, *57*(12), 1332-1334.

[10] Jmp, A., & Proust, M. (2010). Design of experiments guide. *Cary, NC: SAS Institute Inc*.

[11] Lea, P. J., & Azevedo, R. A. D. (2006). Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. *Annals of Applied Biology*, *149*(3), 243-247.

[12] Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In *Methods in Enzymology* (Vol. 148, pp. 350-382). Academic Press.

[13] Lillo, C. (1984). Diurnal variations of nitrite reductase, glutamine synthetase, glutamate synthase, alanine aminotransferase and aspartate aminotransferase in barley leaves. *Physiologia Plantarum*, *61*(2), 214-218.

[14] Ngatia, L., & Taylor,R. (2018). Phosphorus eutrophication and mitigation strategies. *Phosphorus-recovery and recycling*, 45-61.

[15] Porter, J. R., & Gawith, M. (1999). Temperatures and the growth and development of wheat: a review. *European Journal of Agronomy*, *10*(1), 23-36.

[16] Raun, W. R., & Johnson, G. V. (1999). Improving nitrogen use efficiency for cereal production. *Agronomy journal*, *91*(3), 357-363.

[17] Rosen, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. *Archives of Biochemistry and Biophysics*, *67*(1), 10-15.

[18] Sylvester-Bradley, R., & Kindred, D. R. (2009). Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *Journal of Experimental Botany*, *60*(7), 1939-1951.

[19] Tahir, M., Arshad, M. A., Akbar, B. A., Bibi, A., Ain, Q. U., Bilal, A., Arqam, S., Asif, M., Ishtiaq, H., Rasheed H.U., & Pervaiz, R. (2024). Integrated nitrogen and irrigation management strategies for sustainable wheat production: Enhancing yield and environmental efficiency. *J. Pharmacogn. Phytochem*, *13*, 209-222.

[20] Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature*, *418*(6898), 671–677.

[21] Zhao, Y., Li, R., Huang, Y., Sun, X., Qin, W., Wei, F., & Ye, Y. (2022). Effects of various phosphorus fertilizers on maize yield and phosphorus uptake in soils with different pH values. *Archives of Agronomy and Soil Science*, *68*, 1746-1754.

[22] Zheng, L., Wang, R., Zhou, P., Pan, Y., Shen, R., & Lan, P. (2023). Comparative physiological and proteomic response to phosphate deficiency between two wheat genotypes differing in phosphorus utilization efficiency. *Journal of Proteomics*, *280*, 104894.