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Comparative analysis of urea and nano urea along with zinc fertilization on yield and nutrient uptake in wheat (*Triticum aestivum*)

KADAPA SREENIVASA REDDY[1], YASHBIR SINGH SHIVAY[1], DINESH KUMAR[1], VIJAY POONIYA[1], RADHA PRASANNA[1], SUNIL MANDI[2], SOMANATH NAYAK[3], KIRTTIRANJAN BARAL[1], GUNTURI ALEKHYA[1] and ROHIT BAPURAO BORATE[1]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

A study was carried out during winter (*rabi*) seasons of 2021–22 and 2022–23, at the ICAR-Indian Agricultural Research Institute, New Delhi to assess the effectiveness of different nitrogen sources combined with zinc fertilization on the yield and nutrient uptake by wheat (*Triticum aestivum* L.) in a split-plot design (SPD) with three replications. The experiment consisted of nitrogen (N) treatments in main plot (0 kg/ha, 130 kg/ha, 97.5 kg/ha + nano urea foliar sprays, 65 kg/ha + nano urea foliar sprays and 65 kg/ha + prill urea foliar sprays) and zinc (Zn) treatments in subplot (no Zn, 0.5% $ZnSO_4$ foliar spray, 0.1% nano-ZnO foliar spray and cyanobacterial (BF1)-*Anabaena* spp.). This study revealed that tillers/m^2, grain yield, straw yield, and N uptake increased by 19.8%, 27.7%, 28.3%, and 69.0%, respectively with nitrogen application of 130 kg/ha in comparison to control (0 kg/ha). However, nitrogen at 65 kg/ha + nano urea foliar spray reduced the grain yields by 10.1% and nitrogen uptake by 23.8% as compared to nitrogen application at 130 kg/ha over the two-year study period. Moreover, nitrogen application of 97.5 kg/ha + nano urea foliar spray had a statistically similar yield compared to 130 kg N/ha. Nano-zinc oxide applied at the rate of 0.1% foliar spray recorded significantly higher in wheat yields (4.1%) and Zn nutrient uptake (18%), compared to no Zn fertilization. Therefore, it may be recommended that nitrogen at 130 kg/ha combined with a foliar spray of 0.1% nano-zinc oxide at critical stages can be applied to enhance yields and nutrient uptake in wheat.

Keywords: Foliar spray, Nano urea, Nitrogen, Nutrient uptake, Yield, Zinc

Over recent decades, advancement in wheat production technologies has significantly enhanced crop yields. However, the depletion of soil nutrients is on the rise, attributed to the adoption of high-yielding crop varieties and increased cropping intensity. Wheat (*Triticum aestivum* L.) is the most extensively grown cereal globally and holds significant importance. In India, it is a crucial staple crop, second only to rice. Additionally, the escalating demand for food to sustain a growing population has led to an over reliance on chemical fertilizers, particularly nitrogen (N) (Prasad and Shivay 2019, Reddy *et al.* 2024). Despite balanced fertilization of N, P, and K, widespread micronutrient deficiencies have resulted in a decline in factor productivity. Zinc (Zn) emerges as one of the most deficient micronutrients in Indian conditions (Shivay *et al.* 2016). Zn deficiency has been observed to significantly affect crop productivity, confirming a positive response to zinc application as evidenced by Nayak *et al.* (2023). The deficiency of micronutrients in the soil not only diminishes crop yield but also compromises grain nutritional quality (Nayak *et al.* 2023). Therefore, biofortification under low zinc conditions can be accomplished through soil and foliar application (Prasad and Shivay 2020, Shahane and Shivay 2024).

In general, crop plants utilize only 30–40% of the applied nitrogen fertilizer efficiently. Increasing N fertilizer application may not result in a proportional enhancement in crop yields (Shivay *et al.* 2020). Nano-formulations release nutrients gradually, enhancing crop productivity and reducing environmental risks (Rathanayaka *et al.* 2018). Nano-urea (liquid) was developed by IFFCO as an alternative to commercial urea fertilizer, it contains 4% N (Kumar *et al.* 2021). Recently, nano-urea has gained importance in sustainable agriculture for improving nitrogen use efficiency, increasing crop production, enhancing nutrient use efficiency, and reducing the reliance on chemical fertilizers (Kumar *et al.* 2024, Reddy *et al.* 2024). Limited research exists on assessing the application of nitrogen particularly nano-urea and zinc in wheat plants, especially under field conditions. Consequently, the objectives of the experiment were designed to study the impact of different

[1]ICAR-Indian Agricultural Research Institute, New Delhi; [2]ICAR-Indian Agricultural Research Institute, Dhemaji, Assam; [3]Central Agricultural University, Imphal, Manipur. *Corresponding author email: ysshivay@hotmail.com

nitrogen sources (nano urea) and zinc fertilization on wheat crop growth, yield attributes, yield and nutrient uptake

MATERIALS AND METHODS

A study was carried out during winter (*rabi*) seasons of 2021–22 and 2022–23, at the ICAR-Indian Agricultural Research Institute (28º38'N, 77º10' E; and an altitude of 228.6 m amsl), New Delhi. During the wheat growing seasons, the total rainfall was 181.5 mm and 149.3 mm for the first and second-year seasons, respectively. The field was sandy clay loam in texture with the available nitrogen (N), phosphorus (P), and potassium (K) of 178.3 kg/ha, 23.6 kg/ha, and 228.1 kg/ha, respectively, analyzed following the procedures described by (Prasad *et al.* 2006). The wheat variety used in the experiment was WB–02, a biofortified variety. The recommended nutrients of phosphorous (P_2O_5), and potassium (K_2O) were uniformly applied to all treatments. The entire phosphorus and potassium, along with the nitrogen, was applied during sowing, while the remaining 50% of nitrogen was top-dressed at two stages (CRI and tillering). For nitrogen treatments, control (0 kg/ha), 130 kg N/ha (N_{130}), 97.5 kg N/ha + nano urea foliar sprays (FS) of 2.5 L/ha ($N_{97.5}$ + nano urea), 65 kg N/ha + nano urea FS of 2.5 L/ha (N_{65} + nano urea), 65 kg N/ha + 2% prill urea FS of solution at 500 l/ha (N_{65} + prill urea). The additional nitrogen supplied through nano urea was 100 g of N, equivalent to a 2.5 L nano urea foliar spray applied in treatment. For Zn fertilization, control (no Zn), three foliar sprays of 0.5% $ZnSO_4.7H_2O$ (Zn_1), 0.1% nano-ZnO (Zn_2) and Cyanobacterial formulation (BF1)–*Anabaena torulosa* (Zn_3) at maximum tillering stage, pre-flowering and grain filling stage was applied using a knapsack sprayer and the inoculation followed with methodology described by Prasanna *et al.* (2015). Leaf area was measured by leaf area meter and further, calculated the leaf area index (LAI). To determine dry matter, plant samples were dried in a hot air oven at 60 ± 2°C and expressed in g/m^2. Wheat was manually harvested from this area to estimate yields, and the grain yield and straw yield were calculated to determine the total biological yield, expressed in t/ha. Plant samples were first dried and then oven-dried at 60 ± 2 °C before being analyzed for nutrient content. After drying, the samples were finely ground and a 0.5 g portion was analyzed for nutrient content following the procedure (Prasad *et al.* 2006). Nutrient uptake of N, P, and K (kg/ha) was calculated by using the following expression:

$$\text{Nutrient uptake} = (\%\ \text{nutrient context} \times \text{yield}) \div 100$$

A pooled analysis was performed on data collected during experimental seasons, and the averages were reported. Data of all parameters was analyzed statistically by performing an analysis of variance (ANOVA). To assess the statistical difference between treatment groups, a 5% significance level was adopted and additionally, a post-hoc Tukey HSD test was performed. Pearson correlation analysis was used to examine the relationships between growth, yield parameters, and nutrient uptake by plants, utilizing R Studio version 2023.03.0 + 386 (R Core Team 2013).

Table 1 Growth, yield attributes and yields of wheat as influenced by nitrogen sources and zinc fertilization (pooled data of two years)

Treatment	Dry matter accumulation (g/m^2)		Leaf area index (LAI)		Effective tillers/ m^2	Grains/ spike (Nos.)	Grain yield (t/ha)	Straw yield (t/ha)	Biological yield (t/ha)
	60 DAS	90 DAS	60 DAS	90 DAS					
Year (Y)									
Year I	242.4^a	711.4^a	1.65^a	3.89^a	424.7^a	50.98^a	4.38^a	6.65^a	11.02^a
Year II	236.5^a	704.1^a	1.53^a	4.03^a	428.9^a	47.02^b	4.08^b	6.60^b	10.68^b
Nitrogen sources (N)									
N_0	192.4^d	613.4^c	1.45^b	3.65^c	386.4^c	42.29^d	3.64^c	5.78^d	9.41^d
N_{130}	280.6^a	768.2^a	1.75^a	4.23^a	463.1^a	55.39^a	4.65^a	7.29^a	11.94^a
$N_{97.5}$ + nano urea	260.6^b	743.0^{ab}	1.67^{ab}	4.12^{ab}	441.0^{ab}	51.16^b	4.41^{ab}	6.93^b	11.34^b
N_{65} + nano urea	230.3^c	703.8^b	1.54^{ab}	3.88^{bc}	417.6^b	46.38^c	4.18^b	6.46^c	10.64^c
N_{65} + prill urea	233.2^c	710.3^b	1.56^{ab}	3.93^b	425.8^b	48.80^{bc}	4.27^b	6.66^{bc}	10.91^{bc}
Zinc fertilization (Zn)									
Zn_0	232.7^b	693.6^b	1.56^b	3.89^b	419.9^b	46.89^b	4.13^b	6.50^b	10.63^b
Zn_1	241.5^{ab}	712.6^a	1.60^a	3.99^a	427.6^a	49.27^a	4.26^a	6.66^a	10.92^a
Zn_2	246.4^a	720.9^a	1.63^a	4.02^a	433.7^a	50.05^a	4.30^a	6.75^a	11.05^a
Zn_3	237.1^{ab}	704.0^a	1.58^a	3.94^a	425.9^a	48.80^{ab}	4.22^{ab}	6.58^{ab}	10.81^{ab}
N × Zn	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y × N	NS	NS	NS	NS	NS	S	S	S	S
Y × N × Zn	NS	NS	NS	NS	NS	NS	NS	NS	NS

S, Significant at p=0.05; NS, Non-significant at p=0.05.
Treatment details are given under Materials and Methods.

RESULTS AND DISCUSSION

Growth parameters, yield attributes, and yields of wheat: Dry matter production increased progressively towards crop maturity at 60 and 90 DAS. Significantly (p=0.05) higher dry matter accumulation was observed with the N application of 130 kg/ha, followed by nitrogen at 97.5 kg/ha + nano urea foliar sprays, resulting in a 45.8% and 35.4% increase in dry matter at 60 DAS, and a 25.2% and 21.2% increase at 90 DAS compared to the control, respectively (Table 1). Among zinc fertilization treatments, foliar spray of 0.1% nano-ZnO at three stages increased dry matter by 13.7 g/m^2 and 27.3 g/m^2 at 60 DAS and 90 DAS, respectively, compared to no Zn application. The effective number of tillers/m^2 (463), and LAI (4.23) at 90 DAS were recorded as the highest with nitrogen application of 130 kg/ha followed by N 97.5 kg/ha + nano urea foliar sprays. The highest grain yield (4.65 t/ha) and straw yield (7.29 t/ha) were recorded with the application of 130 kg N/ha, followed by $N_{97.5}$ + nano urea, N_{65} + prill urea, and N_{65} + nano urea, with overall yield reductions of 5.2%, 8.2%, and 10.1%, respectively, compared to N_{130}. The yield trend followed the order, N_{130} > $N_{97.5}$ + nano urea > N_{65} + prill urea > N_{65} + nano urea > N_0. However, with the application of 65 kg N/ha + nano urea, foliar sprays reduced the grain yield (10.1%) and straw yield (12.8%) as compared to nitrogen at 130 kg/ha. The reduction in yields was attributed due to a decrease in the dry matter accumulation, effective tillers/m^2, and grains/spike while reducing the nitrogen application. The Zn application resulted in a positive response, resulting in an increase of 4.1% grain yield and an increase of 4.8% straw yield with a 0.1% nano-ZnO foliar spray compared to no Zn application (Table 1). Grain yield revealed a significantly positive correlation with dry matter (R^2=0.96) and leaf area index (R^2 =0.92) (Fig. 1). The pooled data analysis for two years showed that the yields of wheat were positively influenced by N and Zn treatments. Achieving high total dry matter production is essential for higher yield, as the crop reaches maturity as corroborated (Gawdiya *et al.* 2023). This resulted in reduced dry matter accumulation and fewer effective tillers, ultimately leading to reduced yields. Poor nutrient uptake in nano-urea treatments further contributed to the decline in yields, consistent with findings from previous research studies (Sarkar *et al.* 2023, Kumari *et al.* 2024, Reddy *et al.* 2024). As nano urea, contains only 4% nitrogen and supplies approximately 50 g of nitrogen/ha for a single foliar spray, which may not adequately substitute for the nitrogen of 30 or 60 kg/ha required for wheat, in spite of the greater efficiency of nano-fertilizers (Sarkar *et al.* 2023). Zinc fertilization improved Zn availability in treated plots as compared to no Zn (control) plots resulting in higher biomass production and yields (Shivay *et al.* 2015, Ghasal *et al.* 2017). Manufacturers claim that 20 g of nitrogen from nano urea (NU) can replace a 45 kg bag of conventional urea (20.7 kg N) through foliar application, suggesting a 1000-fold increase in nitrogen use efficiency (NUE). However, these claims have been challenged due

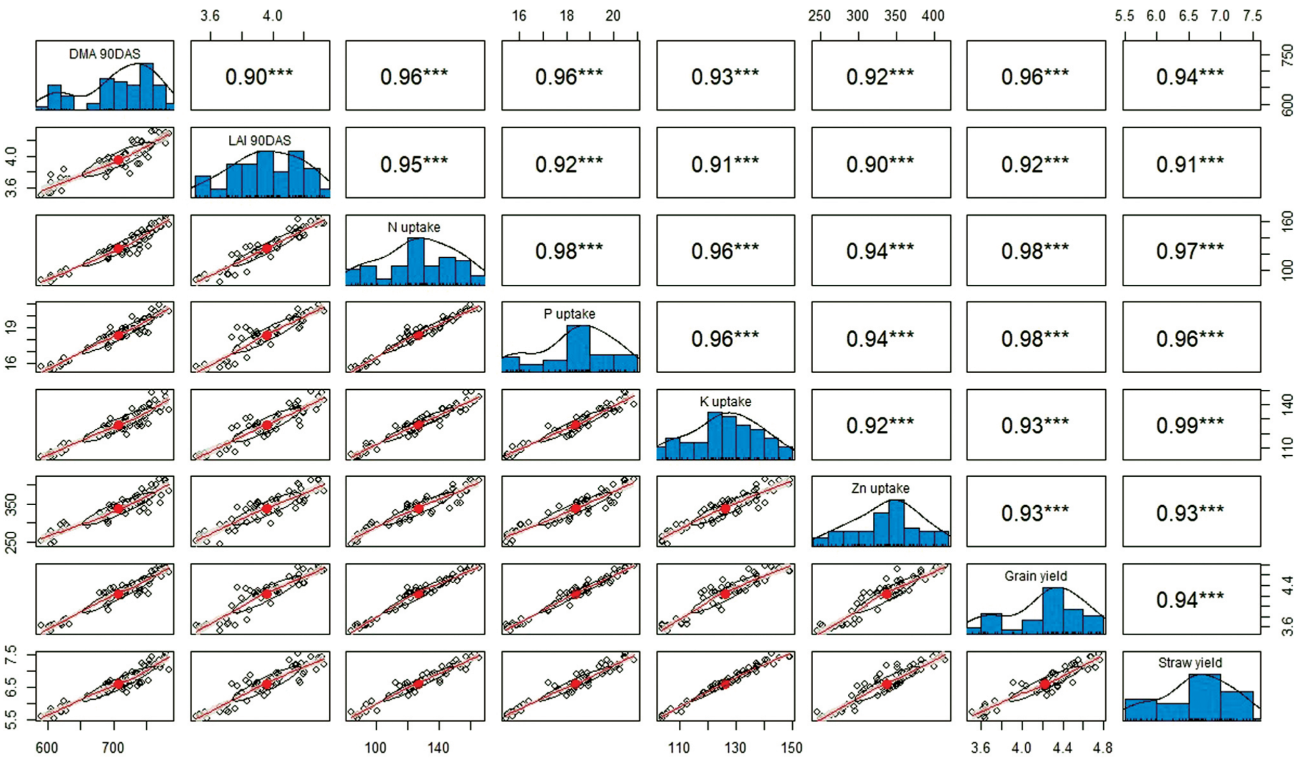

Fig. 1 Correlation matrix graph between different parameters of wheat (N=60).
DMA 90 DAS, Dry matter accumulation at 90 DAS; LAI 90 DAS, Leaf area index at 90 DAS; N uptake, Total nitrogen uptake; P uptake, Total phosphorus uptake; K uptake, Total potassium uptake; Zn uptake, Total zinc uptake; Significance level: *0.05, **0.01 and ***0.001.

Table 2 Total nutrient uptake of nitrogen, phosphorus, potassium and zinc of wheat as influenced by nitrogen sources and zinc fertilization (pooled data of two years)

Treatment	Nitrogen uptake (kg/ha)	Phosphorus uptake (kg/ha)	Potassium uptake (kg/ha)	Zinc uptake (g/ha)
Year				
Year I	133.9^{a}	18.8^{a}	127.7^{a}	344.8^{a}
Year II	119.8^{a}	17.9^{a}	124.2^{a}	329.9^{a}
Nitrogen sources (N)				
N_0	92.4^{d}	19.8^{c}	107.5^{c}	274.5^{d}
N_{130}	156.2^{a}	29.3^{a}	141.4^{a}	383.8^{a}
$N_{97.5}$ + nano urea	140.8^{b}	25.9^{b}	131.2^{b}	360.0^{b}
N_{65} + nano urea	119.1^{c}	21.5^{b}	126.2^{b}	329.2^{c}
N_{65} + prill urea	125.6^{c}	22.6^{b}	128.3^{b}	339.4^{c}
Zinc fertilization (Zn)				
Zn_0	120.8^{b}	21.9^{b}	122.8^{b}	306.2^{c}
Zn_1	128.7^{ab}	25.4^{a}	126.7^{a}	345.6^{ab}
Zn_2	132.3^{a}	26.1^{a}	129.0^{a}	361.3^{a}
Zn_3	125.6^{ab}	24.3^{a}	125.2^{a}	336.3^{ab}
N × Zn	NS	NS	NS	NS
Y × N	NS	NS	NS	NS
Y × N × Zn	NS	NS	NS	NS

S, Significant at 5% level of significance (p=0.05); NS, Non-significance at 5% level of significance (p=0.05).

Treatment details are given under Materials and Methods.

to insufficient scientific validation and inadequate product characterization (Frank and Husted 2024). No yield penalty was observed in N 75%, as optimal nitrogen levels supported crop establishment and efficient nano urea absorption. However, reducing nitrogen to N 75% with nano urea led to yield declines due to its low nitrogen concentration (4%), which was insufficient to enhance yield attributes and overall productivity (Kumar *et al.* 2024, Reddy *et al.* 2024).

Nutrient uptake by wheat: Nutrient uptake is influenced by both the concentration of nutrients in the plant and its dry matter yield. Different N sources showed a significant difference in nutrient uptake of wheat as compared to the control (without N application). N, P, K, and Zn uptake by wheat (156.2 kg/ha, 29.3 kg/ha, 141.4 kg/ha, and 383.3 g/ha, respectively) was recorded with the 130 kg N/ha compared to other nitrogen levels, followed by treatment with nitrogen at 97.5 kg/ha with two sprays of nano-urea (Table 2). Nitrogen at 130 kg N/ha increased the N, P, K, and Zn uptake by 69.0%, 47.9%, 31.5%, and 39.8%, respectively over control. Conversely, the treatment with 65 kg N/ha + nano urea foliar sprays resulted in a decrease in the uptake of N, P, K, and Zn by 31.1%, 36.2%, 12.1%, and 16.6%, respectively, in comparison to nitrogen at 130 kg/ha. Among Zn fertilization, the highest N, P, K, and Zn uptake was recorded with 0.1% nano-ZnO treatment and the contribution of zinc (Zn) to increasing nutrient uptake followed the order: $Zn_2 > Zn_1 > Zn_3 > Zn_0$. However, N applied at the rate of 65 kg/ha + nano urea foliar sprays recorded lower uptake of N (119.1 kg/ha), P (21.5 kg/ha), K (126.2 kg/ha) and Zn (329.2 g/ha) in the study conducted for two years. Grain yield resulted in a significantly positive correlation with nitrogen uptake (R^2=0.98) and zinc uptake (R^2=0.93) further supporting the increased nutrient uptake led to improved yields (Fig. 1). Our study found that N and Zn treatments significantly improved nutrient uptake in wheat, enhancing overall yield attributes and yield. Consequently, the higher nutrient concentration found in wheat grain and straw may be attributed to increased grain yield led to an increase in N uptake of wheat (Pappu *et al.* 2021). The nitrogen application led to an increase in leaf nitrogen concentration, which in turn improved plant's photosynthetic efficiency and enhanced nutrient uptake (Kumar *et al.* 2024). Reducing nitrogen application to 65 kg/ha along with nano urea reportedly decreased nitrogen uptake due to low nitrogen availability to the crop, consistent with findings of Sarkar *et al.* (2023) and Reddy *et al.* (2024). The study by Baral *et al.* (2023) highlighted the synergistic relationship between Zn and N, demonstrating how Zn nutrition enhances N uptake. In our study, zinc application was found to positively impact N content (Shahane *et al.* 2018), with higher zinc levels effective for Zn grain accumulation (Ghasal *et al.* 2018). Foliar spraying was also effective in increasing zinc grain concentration (Velu *et al.* 2014). Furthermore, microbial inoculation has been shown to enhance wheat yield and nutrient uptake (Prasanna *et al.* 2015). Zinc through soil and foliar application significantly enhanced N content, and subsequent accumulation in grain and straw, resulting in increased N and Zn uptake (Nayak *et al.* 2023).

Correlation studies between yield and nutrient uptake in wheat: Correlation analysis revealed a positive relationship between nutrient uptake and the yields of wheat grain and straw. Furthermore, this positive correlation extends to the relationship between growth parameters and yield. These results imply that the application of N and Zn has a beneficial impact on the yield of wheat (Fig. 1). The positive correlation between grain yield with LAI (R^2 = 0.67, R^2 = 0.66) and dry matter production (R^2= 0.77, R^2= 0.80) was observed during 2021–22 and 2022–23 years, respectively (Fig. 2 and 3). This validates the positive impact of nitrogen and zinc application as their application resulted in the experimental study. A strong positive correlation was observed between grain yield and total nitrogen uptake (R^2 = 0.89 in 2021–22 and R^2 = 0.92 in 2022–23) as well as total zinc uptake (R^2 = 0.83 in 2021–22 and R^2 = 0.82 in 2022–23) (Fig. 4 and Supplementary Fig. 1). Our results align with those of prior studies reported by Baral *et al.* (2023) and Nayak *et al.* (2023).

The experimental findings indicate that dry matter production, yield, and total nutrient uptake in wheat were optimized with a nitrogen application of 130 kg/ha. While the combination of 97.5 kg N/ha + nano urea foliar sprays produced comparable yields to 130 kg N/ha. Furthermore, a significant yield loss was observed with reduced nitrogen

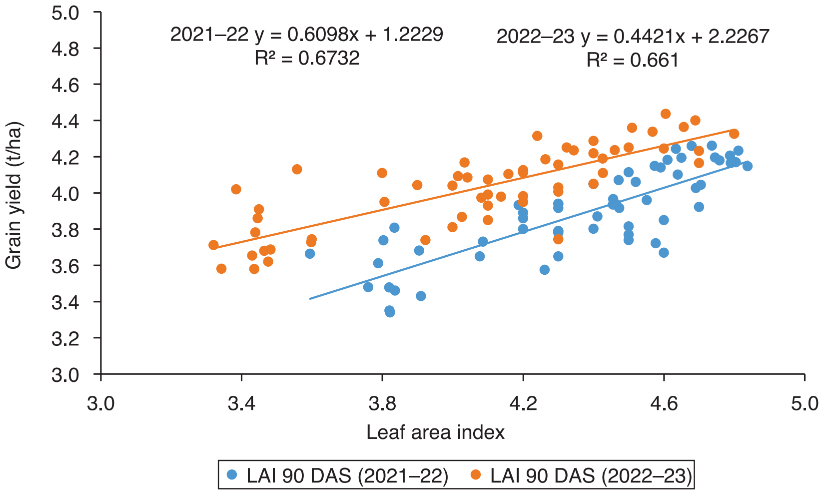

Fig. 2 Regression between grain yield and leaf area index (LAI) at 90 DAS of wheat as influenced by nitrogen sources and zinc fertilization.

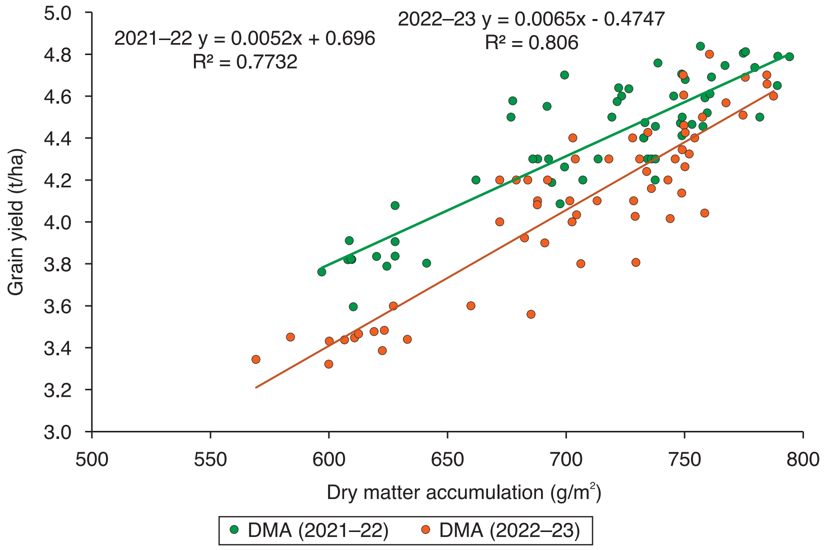

Fig. 3 Regression between grain yield and dry matter accumulation (DMA) at 90 DAS of wheat as influenced by nitrogen sources and zinc fertilization.

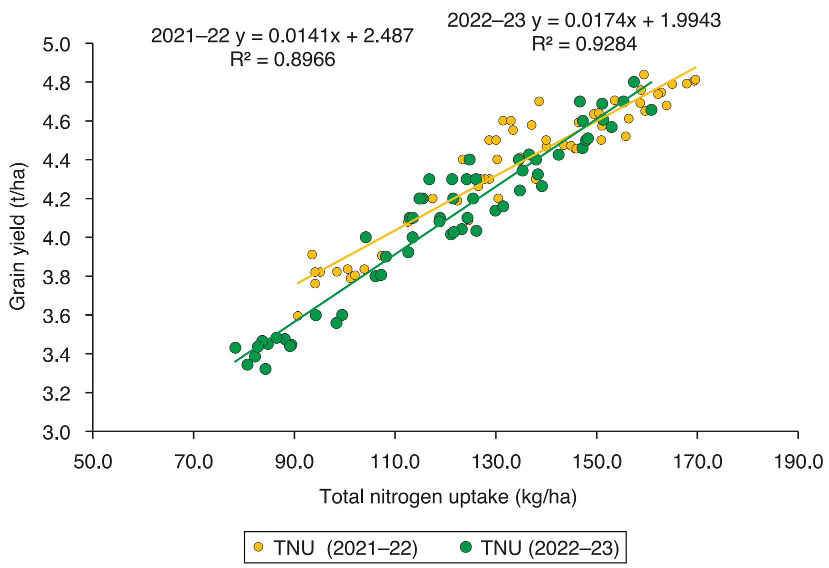

Fig. 4 Regression between grain yield and total nitrogen uptake (TNU) at harvest of wheat as influenced by nitrogen sources and zinc fertilization.

application (65 kg N/ha) combined with nano urea, indicating that lower nitrogen rates supplemented with nano urea did not enhance yield as expected. Among zinc fertilization treatments, 0.1% nano-ZnO showed significant yield and nutrient uptake compared to no zinc application. Our results suggested that applying 97.5 kg N/ha combined with 0.1% nano-ZnO foliar spray can achieve yields comparable to the application of 130 kg N/ha alone. Further research validation across different locations is needed to determine its effectiveness. However, the long-term effects of nano urea use across diverse regions in India, as well as its impact on soil nitrogen supply and nutrient depletion, need further evaluation.

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