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Nitrification Inhibitor and Plant Growth Regulators Improve Wheat Yield and Nitrogen Use Efficiency

Khadim Dawar[1] · Umar Rahman[1] · Syed Sartaj Alam[2] · Mohammad Tariq[1] · Aamir Khan[1] · Shah Fahad[3,4] · Rahul Datta[5] · Subhan Danish[6] · Shah Saud[7] · Muhammad Noor[8]

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Abstract
The field experiment was conducted to investigate the effects of applying urea with nitrification inhibitor (NI) (Nitrapyrine) alone or in combination with gibberellic acid (GA-K salt), on improving wheat yield and N-use efficiency at The University of Agriculture (34.1°′21″ N, 71°28′5′E), Peshawar-Pakistan. There were five treatments with four replications: control (no urea), urea (150 kg N ha^{-1}), urea + nitrapyrin (525 g ha^{-1}), urea + GA-K salt (60 g ha^{-1}), and urea + nitrapyrin + GA-K salt, respectively. Wheat plant biomass, grain yield and total N uptake were enhanced by 31, 37 and 44%, respectively, when urea was applied together with nitrapyrin and GA-K salt over control. In addition, 1000 grains weight, grain $spike^{-1}$, and spike length were also significantly increased when urea was applied with both nitrapyrin + GA-K salt. In conclusion, use of urea with 525 g ha^{-1} nitrapyrin or 60 g ha^{-1} GA-K salt has the potential to enhance N-use efficiency and yield components of wheat yield.

Keywords Urea · Nitrapyrin · GA_3-K · Wheat · Nitrogen use efficiency

Introduction

Nitrogen (N) is an essential nutrient and plays an important role for plant growth and health as it has a key role in the synthesis of protein and chlorophyll (Dawar et al. 2011), which are essential for plant development and crop yield (Zhu and Chen 2002). Farmers have led to high inputs of N fertilizer into cultivated and grass land (Qu et al. 2014). The low nitrogen use efficiency (NUE) 5–56% (Dawar et al. 2021) observed in many cropping systems is also largely the result of N losses associated with NO_3^--N leaching possesses (i.e., N losses from nitrification and denitrification) (Cui et al. 2011; Wu et al. 2017; He et al. 2018). Further increases in fertilization rates are not likely to be useful at increasing crop yields, as

Handling Editor: Mikihisa Umehara.

Khadim Dawar
khadimdawar@yahoo.com

Shah Fahad
shahfahad@uoswabi.edu.pk; shah_fahad80@yahoo.com

Rahul Datta
rahulmedcure@gmail.com

Subhan Danish
sd96850@gmail.com

1 Department of Soil and Environmental Science, The University of Agriculture Peshawar-Pakistan, Peshawar, Pakistan

2 Department of Plant Pathology, The University of Agriculture Peshawar-Pakistan, Peshawar, Pakistan

3 Hainan Key Laboratory for Sustainable Utilization of Tropical Bioresource, College of Tropical Crops, Hainan University, Haikou 570228, Hainan, China

4 Department of Agronomy, The University of Haripur, Khyber Pakhtunkhwa, Pakistan

5 Department of Geology and Pedology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska1, 61300 Brno, Czech Republic

6 Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan 60800, Punjab, Pakistan

7 Department of Horticulture, Northeast Agriculture University, Harbin, China

8 Department of Agriculture, Hazara University, Mansehra, Pakistan

the N-use efficiency of fertilizer sharply decreases at higher application rates, and attention related to N fertilizer application has shifted from its role in increasing crop yield to environmental problems. Nitrification, a key process in the global N cycle that provides NO_3^- through microbial activity may result in the conversion of relatively immobile NH_4^+ to highly mobile NO_3^-, making inorganic N susceptible to losses through leaching of NO_3^- to the ground water and gaseous N emissions (NH_3 and N_2O) to the atmosphere, potentially increasing environmental and health problems (Galloway et al. 2008; Schlesinger 2009; Megaritis et al. 2013).

Modern agricultural practices require a new concept of N fertilizer management in order to optimize N utilization and avoid N losses. There are a many new management practices and technologies that can enhance N-use efficiency and could reduce environmental pollution (Yao et al. 2009; Shoun et al. 2012; Blagodatskaya et al. 2014; Choudhary et al. 2018; Keuschnig et al. 2020). One of the mitigation option that can be highly effective in reducing N fertilizer losses and increasing N-use efficiency and yields in a few cropping systems is the application of nitrification inhibitors (NIs) (Majumdar et al. 2002; Cui et al. 2011; Moir et al. 2012; Wu et al. 2017; He et al. 2018; Dawar et al. 2021). Nitrification inhibitors are compounds that delay bacterial oxidation of the ammonium-ion (NH_4^+) by depressing over a certain period of time the activities of Nitrosomonas bacteria and also provide more opportunities for plant uptake and microbial immobilization of NH_4^+ within the soil profile. They are responsible for the transformation of ammonium into nitrite (NO_2^-) which is further changed into nitrate (NO_3^-) by *Nitrobacter* and *Nitrosolobus* bacteria. The inhibition of O_2 consumption by the nitrification process may also improve soil O_2 status and reduce N_2O loss through denitrification (Sun et al. 2015; Zhu et al. 2015).

Hundreds of NIs are tested, but only a few have gained commercial importance, such as dicyandiamide (DCD), which is one of the most commonly used NIs (De Klein et al. 2011; Ball et al. 2012). The application of DCD with N-based fertilizers has increased yield and reduced N losses (Majumdar et al. 2002; Cui et al. 2011; Moir et al. 2012). The commonly used NIs are nitrapyrin (NP) (2-chloro-6-(trichloromethyl) pyridine), commercialized with the name of N-Serve (He et al. 2018; Dawar et al. 2021; Borzouei et al. 2021). These inhibitors have been found to be effective, at low concentrations, in reducing N_2O loss and NO_3^- leaching in pasture or cropping systems and improved yield and N-use efficiency (Abalos et al., 2014; Sun et al. 2015; Wu et al. 2017; He et al. 2018; Dawar et al. 2021. Borzouei et al. 2021).

Plant growth regulators (PGRs), either produced naturally by the plant or synthetically by a chemist, are small organic molecules that act inside the plant cells and alter the growth and development of plants. Plant growth regulators can be broadly divided into two groups: plant growth promoters (auxins, gibberellins, and cytokinins) and bioinhibitors (ABA, methyljasmonate). Plant growth regulators are involved in cell division, cell enlargement, pattern formation, tropic growth, flowering, fruiting, and seed formation. Bioinhibitors play an important role in plant responses to wounds and stresses of biotic and abiotic origin, and they are also involved in various growth-inhibiting activities such as dormancy and abscission. The use of PGRs, as gibberellins, cytokinins, auxins, or their synthetic compounds, is becoming popular to ensure efficient Production. There are many reports which indicate that application of PGRs enhanced plant growth and crop yield (Ud-Deen 2009; Mostafa & Alhamd 2011). One frequently used gibberellic acid (GA3) increases stem length, the number of flower per plant and increasing yield (Kurepin et al. 2014).

However, the combined application of NIs and PGR has not previously been investigated. Therefore, we investigated the effects of NIs and PGR to compare with conventional N fertilization without NIs in intensive cropping systems in Pakistan. The objectives of this field study were therefore to investigate the effects of the use of NI and PGR on yield and NUE.

Materials and Methods

Field Management and Experimental Set-Up

A field experiment was conducted in the research farm of The University of Agriculture Peshawar, Pakistan (34.1°′21″ N, 71°28′5′ E) during Rabi 2015–0.2016. The soil was cultivated with the common cultivator (tine plough) up to a depth of 0.30 m followed by 2 cultivations across the field and planking was done in all plots to break the clods and smooth the field. Before sowing wheat, surface irrigation of 100 mm was applied and final seedbed was prepared when field moisture reached at 50% field capacity after six days of irrigation. Wheat variety, Atta Habib, was sown by mounted planter equipped with row cleaner wheels with seed rate of 100 kg ha^{-1} on November, 15, 2015. Eight irrigations were applied during the wheat-growing season; all were equivalent to 75 mm, except the first (pre-planting) which was 100 mm. Before sowing, a basal dose of phosphorus (P) at 90 kg P_2O_5 ha^{-1} in the form of single superphosphate (SSP) and of potassium (K) at 60 kg K_2O ha^{-1} in the form of potassium sulfate were applied and incorporated in to the soil. Wheat variety (Atta Habib) was sown with seed rate of 100 kg ha^{-1}. There were 5 treatments in the experiment and plot size was kept 5×3 m^2 containing 10 rows. The row-to-row distance was kept 30 cm apart. The experiment was laid out as a randomized complete block design (RCBD), having five treatments with four replications: control (no N), urea (150 kg N ha^{-1}), urea (150 kg N ha^{-1}) + nitrapyrin (525 g ha^{-1}), urea (150 kg N ha^{-1}) + GA_3-K (60 g ha^{-1}), and urea (150 kg N ha^{-1}) + nitrapyrin (525 g ha^{-1}) + GA_3-K (60 g ha^{-1}) treatments. All treatments were applied in 3 split applications, i.e., at sowing time (15th November), after first irrigation (December 05, 2015) and at stem elongation stage (50–60 days after sowing). Nitrapyrin and GA_3-K were applied at a rate of 0.35% and 0.03% of the applied N (w/w) and the mixtures were obtained by dissolving urea with nitrapyrin and GA_3-K in water. Nitrapyrin, GA_3-K, and urea were dissolved in water 30 min before application and surface applied by hand at 90 L solution per plot. Soil samples at (0–10 cm) depth were collected after 1, 3, 7, 14, 21, 28, and 36 days after fertilizer application to assess the trend of urea hydrolysis and nitrification (Soil NH_4^+ and NO_3^- concentrations).

All other cultural practices including hoeing, weeding, and insects control were carried out to all plots uniformly. Soil moisture and temperature probes were inserted at 0–10 cm soil depth to monitor moisture contents and temperatures. An on-site rain gauge enabled rainfall and irrigation to be monitored.

Determination of Mineral N in Soil

Total mineral N in soil was determined by the steam distillation method of Bremner and Mulvaney (1982). In this method, 20 g samples of moist soil were shaken with 100 ml of 1 M KCl for one hour and filtered. Twenty ml of the filtrate was distillated with either MgO to recover NH_4^+-N or with MgO + devarday's alloy to recover total mineral N. The distillate was collected in 5 ml boric acid mixed indicator solution and then titrated against 0.005 M HCl. The NO_3^--N was determined by subtracting the NH_4^+-N from the total mineral N.

Determination of Total N in Soil and Plant

Total N in soil and plant samples was determined by the Kjekdhal method (Bremner 1996). In this method, 0.2 g of finely ground samples of dry materials were digested with 3 ml of concentrated H_2SO_4 in the presence of 1.1 g digestion mixture containing $CuSO_4$, K_2SO_4, and Se on a heating mantle for about 1 h. The digest was transferred quantitatively to the distillation flask and distilled in the presence of 10 ml of 10 M NaOH solution. The distillate was collected in 5 ml boric acid mixed indicator solution and then titrated against 0.01 M HCl solution by adding 5 ml boric acid mix Indicator. Using the following formula, total N was calculated.

$$\text{Total nitrogen }\% = \frac{(\text{Sample} - \text{Blank}) \times 0.005 \times 0.014 \times 100}{\text{Weight of soil} \times \text{volume made}}$$

Crop Harvesting and Yield Measurement

Wheat crop from main plots was harvested at physiological maturity on and data were recorded on various agronomical traits (biomass, grain yield, and straw yield) and total N uptake in crop. Biomass yield was separated into grain and above-ground plant tissue (i.e., shoot and leaves) and their fresh bulk weight was recorded immediately. Five randomly chosen plant tissue subsamples (ca. 100 g fresh weight) from each subplot were transferred to sealable plastic bags and transferred to lab in container with ice to ensure no water losses occur from collected plant tissue. After transporting the plant tissue samples to the lab, fresh weight was immediately recorded. After recording the fresh weight, harvested material was placed in pre-weighed paper bags and dried at 65 °C for seven days. Dry weights of the plant tissue after seven days were recorded in order to calculate its moisture content or fraction. The grain yield was adjusted for moisture fraction, prior to obtaining its dry weight, using a moisture tester. For N uptake, the above-ground plant tissues (i.e., shoot and leaves) and the grains were taken, and then both these two tissues samples were ground separately to a fine powder (for determination of the total N by Kjeldahl method).

Grains yield was recorded after threshing of wheat plants taken from central four rows of each treatment and then converted into kg ha^{-1} by using the following formula:

$$\text{Grain yield (kg ha}^{-1}) = \frac{\text{Grain yield in four central rows}}{\text{Row} - \text{row distance} \times \text{Row length} \times \text{No. of rows}} \times 10{,}000.$$

Biological yield was recorded by harvesting 4 central rows in each plot, dried, and weighed, and then weight was converted into kg ha^{-1} using the following formula:

$$\text{Biological yield (kg ha}^{-1}) = \frac{\text{Biological yield in four central rows}}{\text{Row} - \text{row distance} \times \text{Row length} \times \text{No. of rows}} \times 10{,}000.$$

Total N uptake was calculated using formula below:

$$\text{Total N uptake} = \frac{\%\text{N in grains} \times \text{grain yield (kg ha}^{-1})}{100}$$

Nitrogen use efficiency was calculated using formula stated below:

$$\text{NUE} = \frac{(\text{Yield obtained from controlplot} - \text{yield obtained from fertilized plot})}{\text{Amount of fertilizer applied}}$$

Thousand (1000) grains weight was recorded by counting thousand (1000) grains randomly from each treatment and then weighing on electronic balance. This parameter was also recorded for each treatment separately. Plant height was recorded by selecting five plants which were randomly selected from each plot and tagged and their heights were recorded from the soil surface to the tip of each plant at physiological maturity. Then for precision, mean of five plants was taken for each treatment. For determination of spike length, five spikes were randomly selected from each treatment plot and measurements from the base of the rachis to the tip of the uppermost spikelet were taken. Five randomly selected spikes from each plot were thrashed individually to determine the number of grains per spike and then the mean of both spike length and grains spike^{-1} was taken for each treatment for statistical analysis and mean comparison.

Laboratory Soil and Plant Analyses

Before treatment application, four composite soil samples (0–10 cm depth) were taken. Each composite soil sample was comprised of 10 randomly collected soil cores which were collected from the experimental site and passed through a 2-mm sieve to remove visible plant litter and roots. Sieved soil samples were analyzed for key soil properties. Mineral N in soil samples was determined by the steam distillation method (Bremner and Mulvaney 1982). Soil pH and EC were determined in the saturated soil extract (1:5 suspensions). Soil organic matter was determined by the Walkley–Black procedure (Nelson and Sommers 1982). Soil texture was determined by hydrometer (Gee and Bauder 1986). Total N in both soil and plant samples was determined by Kjeldhal method (Bremner 1996).

Statistical Analysis

Analysis of variance (ANOVA) was calculated to compare fertilizer treatments with respect to various measured parameters. When significant differences at $P \le 0.05$ between treatments were found, adjusted LSD values of Turkey's test were calculated to make comparisons among the different fertilizer treatments. Minitab (Version 12, Minitab Inc., USA) was used to perform statistical analyses. The graphs were designed in sigma plot 12.

Results

Soil Physico-chemical Properties

The experimental site was silt clay loam in nature, alkaline in reaction with a pH 7.81 and EC 0.25 dSm^{-1}. The soil was highly calcareous in nature with lime content of 18.37%, organic matter (< 1%) and also was deficient in nitrogen and phosphorus (Table 1).

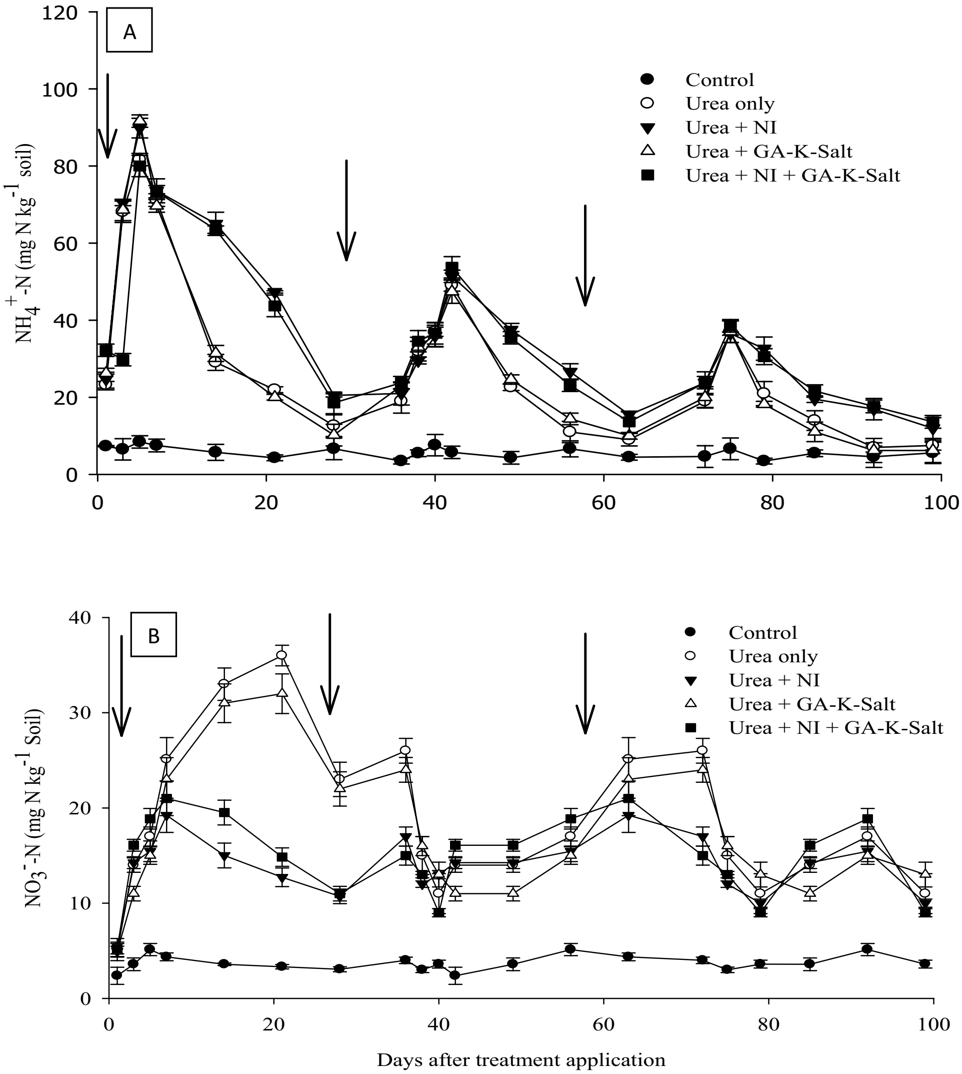
Effect of NI and PGR on Soil NH_4^+-N and NO_3^--N Concentrations

Urea applied alone or with nitrapyrine or with GA-K salt exhibited fast hydrolysis soon after its application, as evidenced by significantly ($P < 0.05$) higher concentrations of soil NH_4^+ in all treatments (Fig. 1a). While soil NO_3^- concentrations remained relatively low (Fig. 1b). After fertilizer application, soil NH_4^+ concentration in the urea-alone and urea with GA-K salt treatments reached its maximum in 5–7 days and sharply decreased afterward. Urea with nitrapyrin and mulch delayed nitrification rate, as evidenced by significantly ($P < 0.05$) higher concentrations of soil NH_4^+ up to 21 days compared with those of urea-alone or urea with GA-K salt treatments. Soil NO_3^--N concentration were significantly ($P < 0.05$) lower in nitrapyrin treatments compared with those of the urea-alone or urea with GA-K salt treatments during the experimental period (Fig. 1b).

Table 1 Physico-chemical properties of soil before wheat sowing

Soil physico-chemical properties	Units	Concentration
Silt	%	64.5
Sand	%	29.6
Clay	%	5.4
Textural Class	–	Silt loam
EC(e)	dS m^{-1}	0.25
pH	–	7.81
Organic matter content	%	0.76
Mineral nitrogen	mg kg^{-1}	8.76
AB-DTPA extractable P content	mg kg^{-1}	3.26

Fig. 1 Effects of urea with or without nitrification inhibitor (nitrapyrin) (NI) and GA-K salt on soil ammonium (**a**) and nitrate (**b**) (0–10 cm soil depth). Vertical bars indicate standard errors. The solid arrows indicate the time of N fertilization



Effect of NI and PGR on Yield and Yield Components of Wheat

Urea applied with nitrapyrine or with GA-K salt had a significant ($P<0.05$) influence on biological yield of wheat (Fig. 2). Pearson correlation showed that a significant positive correlation was existed between treatments and biological yield of wheat (Fig. 8). Principle component analyses showed that biological yield was more closely associated with treatment T3 (Fig. 9). Urea applied with nitrapyrine or with GA-K salt produced significantly higher biological yield (8622 kg ha^{-1} and 9502 kg ha^{-1}) compared to (7703 kg ha^{-1}) from the equivalent urea-alone treatment. Urea applied with nitrapyrine or with GA-K salt increased the biological yield by 12% and 23%, respectively, compared to the equivalent urea-only treatment. Wheat biological yield was increased further by 31%, when urea was applied together with nitrapyrin and GA-K salt.

Similarly, grain yield of wheat was significantly ($P<0.05$) higher when urea was applied with nitrapyrine or with GA-K salt than urea treatment (Fig. 2). Pearson correlation showed that a significant positive correlation was existed between treatments and grain yield of wheat (Fig. 8). Principle component analyses showed that grain yield was more closely associated with treatments T3 and T5 (Fig. 9). Urea applied with nitrapyrine or with GA-K salt enhanced the biological yield significantly (8622 kg ha^{-1} and 9502 kg ha^{-1}) compared to (7703 kg ha^{-1}) than the equivalent urea-alone treatment. Urea applied with nitrapyrine or with GA-K salt increased the biological yield by 12% and 23%, respectively, compared to the equivalent urea-only treatment (Fig. 3).

Urea applied with nitrapyrine or with GA-K salt had a significant effect on plant height (Fig. 4), 1000 grain weight (Fig. 5), grain per spike (Fig. 6), and spike length (Fig. 7) of wheat compared to the urea-alone treatment (Fig. 4). Pearson correlation showed that a significant positive correlation was existed between treatments, plant

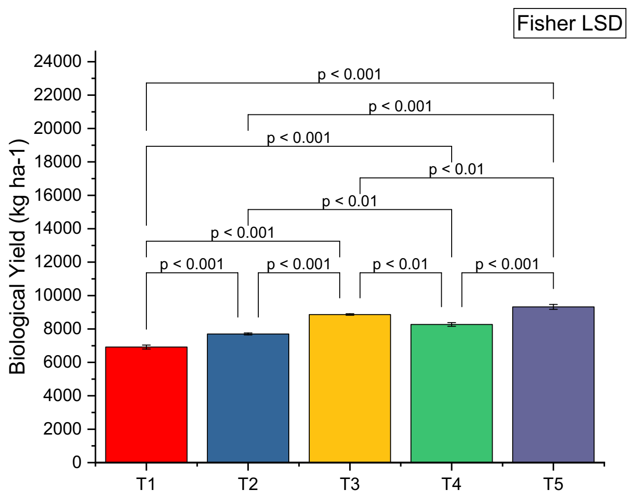


Fig. 2 Effect of urea applied with or without nitrapyrin and GA-K salt on biological (kg ha$^{-1)}$ of wheat. Values are means (n=4). T1=control (no N); T2=urea (150 kg N ha^{-1}); T3=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1}); T4=urea (150 kg N ha^{-1})+GA_3-K (60 g ha^{-1}); T5=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1})+GA_3-K (60 g ha^{-1})

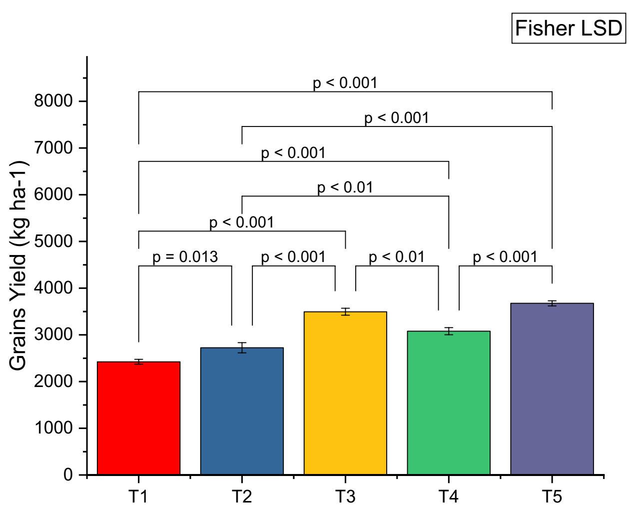


Fig. 3 Effect of urea applied with or without nitrapyrin and GA-K salt on grains yield (kg ha^{-1}) of wheat. Values are means (n=4). T1=control (no N); T2=urea (150 kg N ha^{-1}); T3=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1}); T4=urea (150 kg N ha^{-1})+GA_3-K (60 g ha^{-1}); T5=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1})+GA_3-K (60 g ha^{-1})

height, 1000 grains weight, grains per spike, and spike length of wheat (Fig. 8). Principle component analyses showed that plant height, 1000 grains weight, grains per spike, and spike length were more closely associated with treatments T3, T4, and T5 (Fig. 9). Plant height was significantly higher from treatments receiving urea with

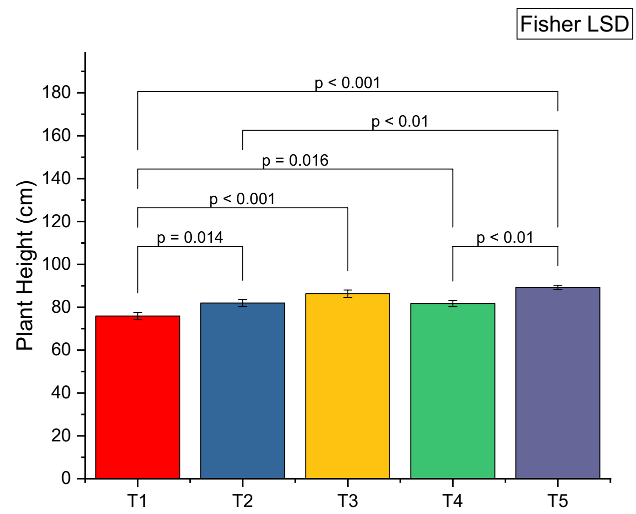


Fig. 4 Effect of urea applied with or without nitrapyrin and GA-K salt on plant height (cm) of wheat. Values are means (n=4). T1=control (no N); T2=urea (150 kg N ha^{-1}); T3=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1}); T4=urea (150 kg N ha^{-1})+GA_3-K (60 g ha^{-1}); T5=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1})+GA_3-K (60 g ha^{-1})

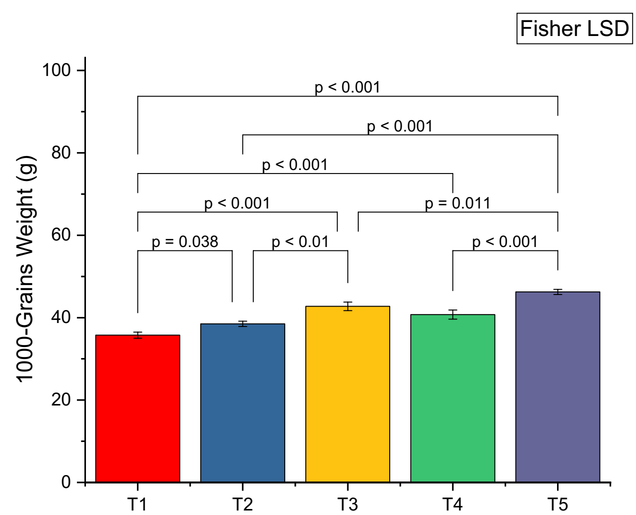


Fig. 5 Effect of urea applied with or without nitrapyrin and GA-K salt on 1000 grains weight (g) of wheat. Values are means (n=4). T1=control (no N); T2=urea (150 kg N ha^{-1}); T3=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1}); T4=urea (150 kg N ha^{-1})+GA_3-K (60 g ha^{-1}); T5=urea (150 kg N ha^{-1})+nitrapyrin (525 g ha^{-1})+GA_3-K (60 g ha^{-1})

nitrapyrine plus GA-K salt than in the urea-alone treatment. The highest plant height was recorded from treatments with nitrapyrine -treated urea plus GA-K 3Salt (90.3 cm), followed by urea + GA-K salt (87.5 cm) and urea + nitrapyrine (85.7 cm). This represents an increase in plant height by 10.2, 6.8, and 4.1%, respectively, than

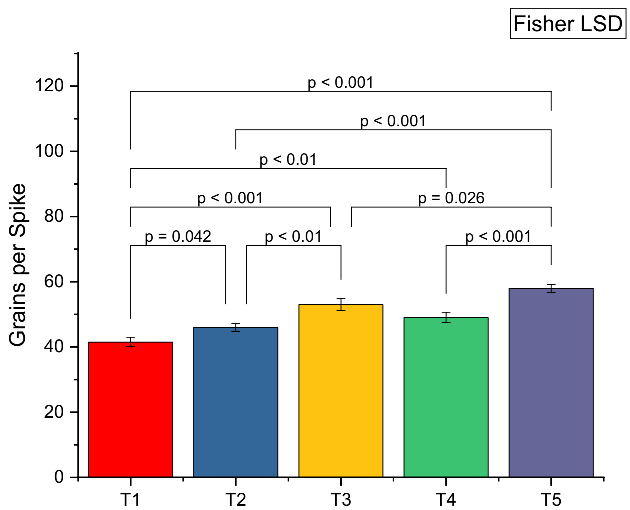


Fig. 6 Effect of urea applied with or without nitrapyrin and GA-K salt on grains per spike of wheat. Values are means ($n=4$). T1 = control (no N); T2 = urea (150 kg N ha^{-1}); T3 = urea (150 kg N ha^{-1}) + nitrapyrin (525 g ha^{-1}); T4 = urea (150 kg N ha^{-1}) + GA_3-K (60 g ha^{-1}); T5 = urea (150 kg N ha^{-1}) + nitrapyrin (525 g ha^{-1}) + GA_3-K (60 g ha^{-1})

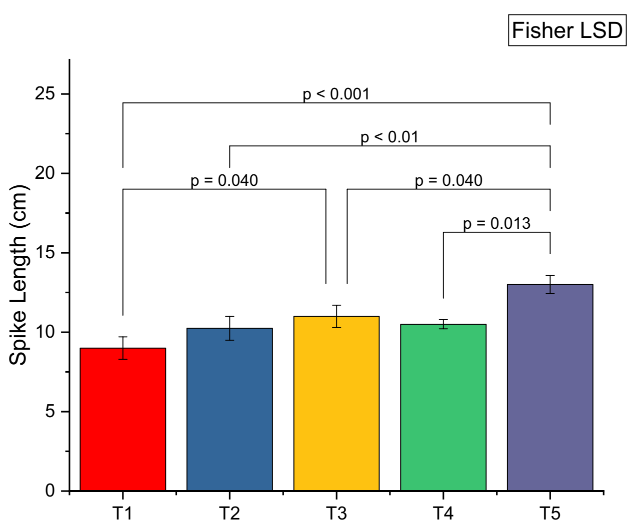


Fig. 7 Effect of urea applied with or without nitrapyrin and GA-K salt on spike length of wheat. Values are means ($n=4$). T1 = control (no N); T2 = urea (150 kg N ha^{-1}); T3 = urea (150 kg N ha^{-1}) + nitrapyrin (525 g ha^{-1}); T4 = urea (150 kg N ha^{-1}) + GA_3-K (60 g ha^{-1}); T5 = urea (150 kg N ha^{-1}) + nitrapyrin (525 g ha^{-1}) + GA_3-K (60 g ha^{-1})

in the urea-alone treatment. The maximum grain weight (g) was 46, 43, and 30 (g) for wheat receiving urea with nitrapyrine plus GA-K salt, urea with GA-K salt, and urea with nitrapyrine, respectively. This shows a 12.1, 4.8, and 2.4% increase, respectively, relative to the corresponding urea-alone treatment. Number of grains spike^{-1} in the urea with nitrapyrine and with GA-K salt were significantly higher (by 2.1 and 4.2%, respectively, over the urea-alone treatment (Fig. 6). Urea with nitrapyrine and with GA-K salt was significantly effective up to 8.2 and 17.5%, respectively, Compared to the urea-alone treatment. The combined application urea with nitrapyrine plus GA-K salt significantly increased the Spike length by 41.2% over the individual urea treatment. Spike length was significantly ($P<0.05$) affected by nitrapyrine with and without GA-K salt. Urea with nitrapyrine and with GA-K salt was significantly effective by 8.2 and 17.5%, respectively, Compared to the urea-alone treatment. However, the combined application of urea with nitrapyrine plus GA-K salt significantly increased the Number of grains spike−1 by 8.5% over the individuals urea treatment.

Effect of NI and PGR on Total N Uptake and N-Use Efficiency

The results obtained on total N uptake by wheat crops are presented in Table 2. All fertilizer treatments increased the total N uptake in the above-ground biomass compared with the control treatment. The maximum N uptake of 133 kg ha^{-1} was recorded in the urea with nitrapyrine plus GA-K salt followed by urea + GA-K salt 112 kg ha^{-1} and urea + nitrapyrine 112 kg ha^{-1} treatments, respectively. The results showed that total N uptake in the above-ground biomass was significantly ($P<0.05$) improved in the treatments where urea was applied with nitrapyrin and GA-K salt. It was observed that nitrapyrin and GA-K salt significantly ($P<0.05$) increased (15% and 32%) total N uptake by the wheat crop than urea-alone treatment (Table 2). Total N uptake was improved further (44%) when urea was applied with both nitrapyrin and mulch. Nitrogen use efficiency (kg DM kg^{-1} of N applied) also varied significantly ($P<0.05$) when urea was applied with nitrapyrin and with GA-K salt (Table 2). Nitrogen use efficiencies were 6, 12, 18, and 22 kg DM kg^{-1} N for urea-alone, urea with nitrapyrin, urea with mulch, and urea with nitrapyrin + mulch treatments, respectively.

Discussion

After application, urea was quickly hydrolyzed within 5 to 7 days (Dawar et al. 2011; Sanz-Cobena et al. 2011; He et al. 2018) by urease enzymes to NH_4^+, resulting the higher concentrations of soil NH_4^- from urea, with or without nitrapyrin, during the first 7 days after fertilizer application (Fig. 1Ba). After day 7, the soil that recived urea with nitrapyrin significantly increased NH_4^- compared to urea-alone treatment, and this trend of nitrapyrin on the nitrification inhibition continued till day 28 (Wang et al. 2015; Zhang et al. 2015; Borzouei et al. 2021; Dawar et al. 2021).

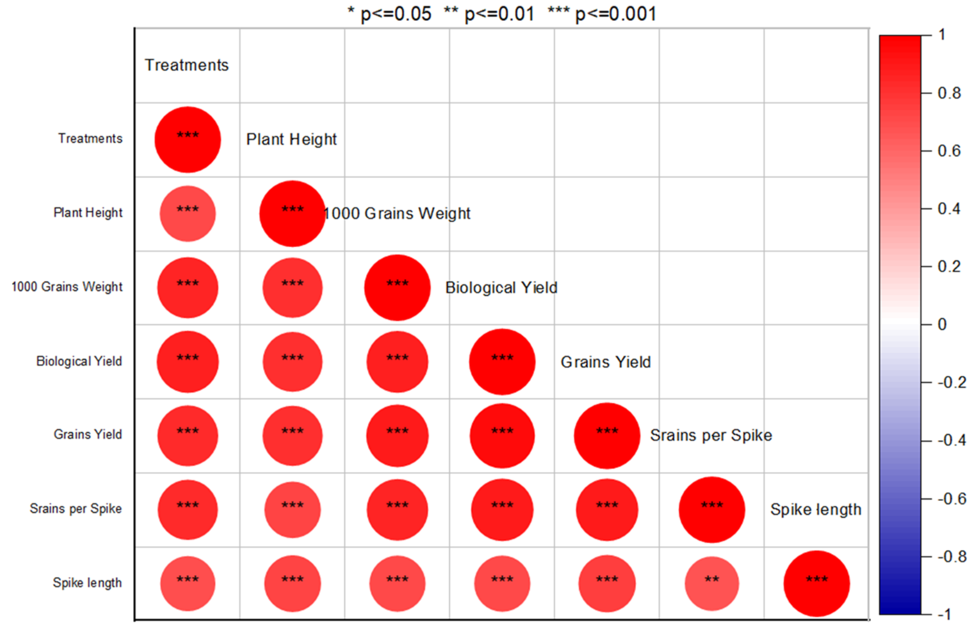


Fig. 8 Pearson correlation of treatments and wheat yield indices

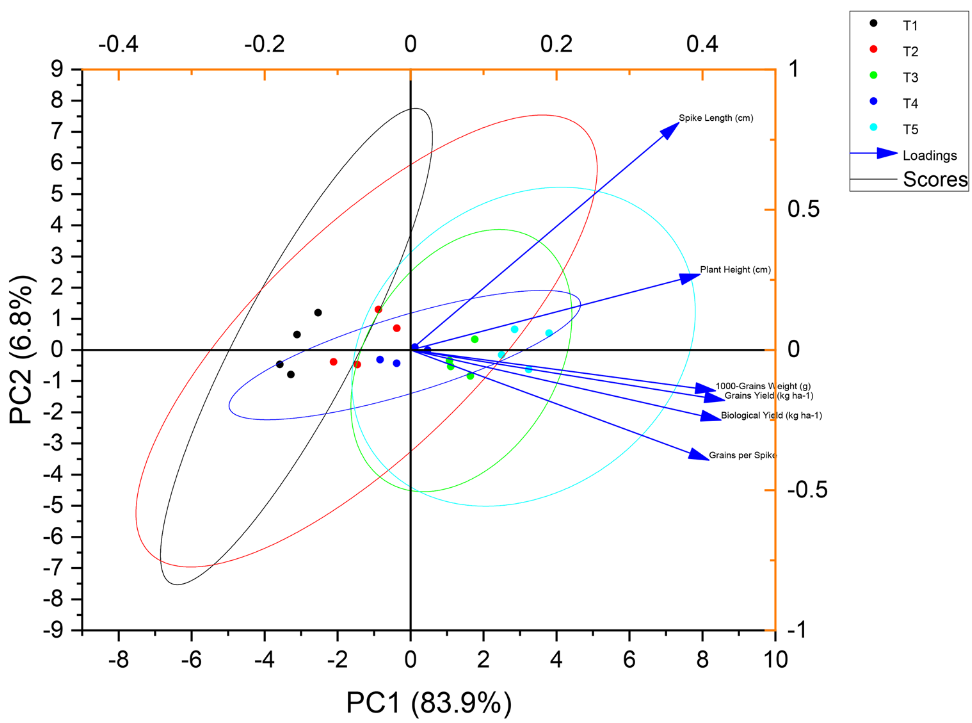


Fig. 9 Principle component analyses of growth and yield attributes of wheat under different application of treatments

Suppressing NH_4^+ oxidation by nitrification inhibitors for longer period of time minimized the risk of soil N being lost via NO_3^- leaching and N_2O emission and improved N uptake (Gioacchini et al. 2002; Belastegui Macadam et al. 2003; Abalos et al. 2012; Sun et al. 2015).

The application of urea with nitrapyrin or GA_3-K resulted in significant ($P < 0.05$) increases in yield, total N uptake, and N-response efficiency compared with the urea-alone treatment (Table 2 and Figs. 1, 2, 3, 4, 5, 6, 7). It had previously been reported by other researchers, that applying N fertilizers with nitrapyrin increases crop yield and fertilizer N efficiency by increased N uptake (Yuchun Ma et al. 2013a, b; Zhang et al. 2015; He et al. 2018; Borzouei et al. 2021; Dawar et al. 2021). These increases are highlights the fact that application of the nitrapyrin, which increases the proportion of mineral N in the NH_4^+ form for several days, had equal opportunity to take up applied N as NH_4^+ forms which may be incorporated into organic compounds

Table 2 Effect of urea applied with or without nitrapyrin and GA-K salt on biological and grain yields, total nitrogen uptake, and N-response efficiency

Treatment	Biomass (kg ha^{-1})		Grain yield (kg ha^{-1})		Total N uptake (kg ha^{-1})		Response efficiency (kg biomass yield kg^{-1} of applied N)
		% Change from urea treatment		% Change from urea treatment		% Change from urea treatment	
Control	5822 ± 211^{f}		2349 ± 48^{f}	–	54 ± 13^{f}	–	
Urea only	7703 ± 209^{d}		2712 ± 39^{d}	–	92 ± 11^{d}	–	6 ± 2^{d}
Urea + Nitrapyrin	8622 ± 202^{c}	12	3027 ± 56^{c}	12	106 ± 15^{c}	15	12 ± 5^{c}
Urea + GA-K salt	9502 ± 276^{b}	23	3408 ± 55^{b}	26	12 ± 19^{b}	32	18 ± 4^{b}
Urea + Nitrapyrin + GA-K salt	10,098 ± 236^{a}	31	3706 ± 61^{a}	37	133 ± 17^{a}	44	22 ± 3^{a}

Values are means (n = 4)

Means followed by same letter(s) within columns are statistically nonsignificant ($P < 0.05$)

and finally into plant protein at less energy cost compared to NO_3^-, suggesting that the plant may be left with extra energy to allocate to growth and crop yields (Aulakh et al. 2001; Dawar et al. 2011, 2021).

It is important to note that we found greater wheat yield and yield components, as well as N-use efficiency which could also be due in part to a lower energy requirement for assimilation of N into protein in plants when they take up NH_4^+ relative to when NO_3^- is the primary N source. Urea and NH_4^+ are both known to require less metabolic energy for conversion to plant protein (Middleton & Smith 1979; Dawar et al. 2021). Furthermore, NO_3^- N has to be reduced before assimilation, which requires additional energy (Raven 1985). Soil NO_3^- concentrations were lower than concentrations NH_4^+ and were significantly influenced by urea applied with nitrapyrin (Fig. 1b). This implies that nitrapyrin delayed nitrification process and maintained high soil NH_4^+ content for a longer period of time. Such reductions in NO_3^- content provide both environmental benefits by reducing N_2O emissions and the risks of NO_3^-N leaching (Wu et al. 2017; Dawar et al. 2021) and agronomic and economic benefits by increasing N-use efficiency especially in N-deficient soil, as evidenced by the increased total N uptake and crop yield in our study.

Similarly, N-response efficiency further improved when urea was applied with GA_3-K (Table 2). Plant growth regulators like gibberellins may thus play a role in plant tolerance to salinity and drought by enhancing plant growth. Gibberellins also affect N metabolism and N redistribution with increased nitrate reductase and carbonic anhydrase activities in plants and improve N-use efficiency through better utilization of soil-derived N. This suggests that plant growth hormones can increase plant N uptake when N fertilizer is applied, thus resulting in an increased yield (Giannakoula et al. 2012; Dawr et al. 2021).

Conclusions

Our field study provided important insights into the effects of urea applied with nitrapyrin and GA-K salt on N losses and improving wheat yield, N-use efficiency and N uptake under the semi-arid and hot climatic conditions of Pakistan. In conclusion, the co-application of urea with nitrapyrin and GA-K salt is likely to be significant steps toward improving N-response efficiency and both biomass and grain yields. Further long-term field research is, however, required under a wide range of soils and environmental conditions to evaluate the performance of different types of PGRs on crop biomass and to better understand the effect of nitrapyrin on N losses production under different crops and vegetable cultivation in Pakistan.

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Compliance with Ethical Standards

Conflict of interest We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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