***Original Research Article***

# Combined Effect of Nitrogen and Phosphatic fertilizer on the activity of the Nitrate reductase enzyme in different wheat cultivars

## ABSTRACT

Some characteristics of nitrogen metabolism were observed in the flag leaf on the main branch of wheat (*Triticum aestivum* L. and *Triticum durum* L.) In the current study, nine wheat varieties (T. aestivum and T. durum) were chosen and planted as a test crop using a split plot design that was reproduced three times, with nutrient dose as the main plot and varieties as the sub plot treatment cultivated at three different soil nitrogen and phosphorus levels (0, 60, and 120 kg ha-1) and (0, 30, and 60 kg/hac). These nitrogen and phosphorus levels were handled using four different treatments, where T1 served as the control, T2 as the nitrogen and phosphorus dose at its optimal level, and T3 as the half-nitrogen and full-phosphate fertilizer doses. Fertilizer dosages of T4 that were half nitrogen and half phosphorus were studied. Flag leaf blades were seen to be engaged in NO-3 assimilation. The flag leaf blade had the highest nitrate reductase activity, free amino acid content, and soluble protein content among all the leaf blades. The activity of nitrate reductase was markedly increased by the addition of nitrogen to the soil. The presence of substrate-dependent enzyme activity was demonstrated in flag leaf tissue. A coincidental association between enzyme activity and the buildup of reduced nitrogen in the plant can be shown between the rise in nitrate reductase activity in response to more nitrogen and the increase in vegetative reduced nitrogen. A substantial positive connection was discovered between nitrate reductase activity (expressed as moles N ha-1 each season) and grain nitrogen (kg N ha-1) at maturity because the transfer of vegetative nitrogen to the grain was homogeneous across treatments. Additionally, there was a strong and positive correlation between seasonal nitrate reductase and grain yields (kg ha-l). In cultivars resistant to lodging, maintaining nitrate reductase activity during the reproductive period might boost grain protein production and avoid the decrease of grain protein percentage that is usually seen when grain yields are high. In cultivars resistant to lodging, maintaining nitrate reductase activity during the reproductive period might boost grain protein production and avoid the decrease of grain protein percentage that is usually seen when grain yields are high.

**Key words:** Metabolism, Nutrient, Demonstrated, Vegetative, Optimum, Cultivars

## 1. INTRODUCTION

Nitrogen is the most critical nutrient that plants get from the soil in terms of quantity. (Lonhienne *et al.,[*17]) Plant roots have long been known to take up nitrogen molecules with a low molecular mass, such as ammonium, nitrate, and amino acids. In natural ecosystems, however, nitrogen is mostly found as proteins in the soil. This complex organic form of nitrogen is thought to be unavailable to plants directly. Although roughly 80% of the nitrogen in the atmosphere is di nitrogen (nitrogen gas, N2), most living organisms cannot use this form of the element and must convert it to a useful form, such as ammonia. (Bano and Iqbal [3]), opined that Leguminous legumes' ability to fix di nitrogen into useful reactive nitrogen molecules has traditionally been utilized by humans, boosting soil fertility. However, the amount of reactive nitrogen produced in this manner is currently dwarfed by that produced in the industrial sector. Nitrogen compounds released into the environment by human activities, along with nitrogen oxides, another kind of reactive nitrogen created as a by-product of combustion processes, are creating a web of unintended consequences.

The majority of nitrogen in the biosphere is in the form of $N_2$ in the atmosphere, which is useless to most species until it is "fixed" biologically or abiotically (by lightning or aurorae, or industrially). It is usually either absorbed and converted into biological N or nitrified into $NO_3$ once it is fixed into $NH_3$. Ammonification is a process that converts organic nitrogen back into $NH_3$. Nitrate can be transformed to $N_2O$ and $N_2$ through nitrification and denitrification, respectively. As a result of this $N_2O$ and $N_2$ production, ecosystems lose nitrogen while the atmospheric nitrogen store gains nitrogen.

Phosphorus (P), like nitrogen (N), is an important nutrient for plant growth and productivity. Its content in plants ranges from 0.05 to 0.5 percent of the total dry weight of the plant. Despite the fact that the concentration of P in soil is 2000 times higher than in plants, its fixation in the form of aluminium/iron or calcium/magnesium phosphates prevents plants from absorbing it (Malhotra *et al.* [11]). Phosphorus is involved in a variety of biological functions, including membrane structure maintenance, biomolecule synthesis, and the production of high-energy molecules. Nitrogen is the fourth most prevalent element in living creatures, and it is utilized to make essential biological components including amino acids and nucleic acids (Luo *et al.,* [10]).

Proteins, Rubisco, nucleic acids, and chlorophyll all contain nitrogen as a structural component. N fertilisation plays an important agronomic management strategy for increasing crop productivity (Astaneh *et al.,*[2]). The supply of N in plants has a significant impact on the functional activity of the photosynthetic apparatus in leaves. Furthermore, it has been observed that effective N feeding has the capacity to mitigate drought stress effects by maintaining metabolic processes even at low tissue water potential (Abid *et al.,* [1]).Excess nitrogen allows many plants to grow aggressively with dark green coloured lush growth, but it also causes developmental alterations and may alter the biology of plants such as a longer vegetative phase, delayed maturity, a longer plant life cycle, and enhanced succulence (Dietz, *et al.,95]*).

Phosphorus (P) is a structural component of major bio molecules such as nucleic acids, sugar phosphates, adenosine triphosphates, and phospholipids, and is an un substitutable, essential element for plant growth. Phosphorus (P) is one of the most important nutrients for plant growth and survival. It is essential for cellular bioenergetics and metabolic pathways within the plant body. The primary function of mineral fertilizers is to increase crop yields, but the biggest impediment to realize known crop potential is the low use of fertilizers, notably P and N. (Irfan *et al.,* [9]).

In agricultural systems, the application of P to the soil is required to ensure plant productivity (Simpson *et al.,* [15]).Seed P reserves are rapidly mobilized and translocated to emerging root and shoot tissues after germination, as it is the sole P source available to sustain seedling growth. This P supply is then replenished by P uptake by the root system as it develops (Julia *et al.,* [7]).When root P acquisition is insufficient to meet the P requirement for new growth, biochemical, physiological, and morphological responses occur to improve tissue P economy and increase soil P acquisition ( White *et al.*, [17]).Local and systemic signals involving gibberellins, auxin, cytokinins, ethylene, and strigalactones, as well as the translocation of regulatory mi RNAs and excess sucrose from the shoot to the root in the phloem, co-ordinate many of the responses of root tissues to P deprivation. Increased sucrose availability to the root, in particular, has been linked to increased P-uptake capability in P-deficient plants.

The amount of nitrogen in a plant's tissues determines its growth. According to the N productivity idea, plants that develop in N-rich environments have higher internal N concentrations and a higher relative growth rate. Limited N supply causes low shoot growth, a high root–shoot ratio, and decreased leaf growth in plants. As the leaves age, more nitrogen is assigned to the highest leaves, where there is a greater demand for photosynthetic enzymes and chlorophyll, and it is mobilized to seeds (Pilbeam [13]).

Phosphorus is the second most commonly limiting macronutrient for plant growth, behind N.P is a key plant macronutrient that accounts for around 0.2 percent of the dry weight of a plant. Phosphorus is a component of important compounds including nucleic acids, phospholipids, and ATP, plants cannot thrive without a consistent supply of this nutrient. The Pi is also involved in the regulation of metabolic pathways and the control of important enzyme processes (Zhang *et al.,* [20]).

Kaur *et al.,* [8]) reported that the activities of nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase all increased as the nitrogen rate increased, resulting in a rise in protein and amino acid content in all wheat genotypes. The amount of nitrogen and chlorophyll in the leaves decreased as the leaves grew. It was also shown that nitrogen assimilatory enzymes (nitrate reductase and glutamine synthetase) have a positive association with NUE and nitrogen content, suggesting that these enzymes may be the rate limiting enzymes in nitrogen metabolism.The nitrogen-efficient genotypes could be used to boost wheat crop output while using less nitrogen, saving the environment and money.

## 2. Material and methods

The field study was carried out at the ICAR-IISS research farm in Bhopal, Madhya Pradesh, during the rabi season of 2020–21. It is classified as semi-arid and subtropical and has scorching summers and frigid winters. The Vindhyan Plateau Agroclimatic Zone includes Bhopal. About 1100 mm of rainfall falls on average each year, with the majority falling between July and September during the monsoon season. The average maximum summer temperature is 35–40°C, while the average winter minimum is 2–9°C.There are 36 plots in a block (9 variety x 4 fertiliser N and P treatments). Each plot is 2 m x 2 m in size.

The reduction of nitrate to nitrite was assayed *in vitro* by incubating the enzyme extract with $KNO_3$ in the presence of an electron donor (NADH). Nitrite was diazotized with sulphanilamide and then reacted with N-(1- naphthyl) ethylene diaminedihydrochloride (NEDD) to produce an azo dye which was measured spectro photometrically at 540nm (Nicholas and Nason [12]). 0.2 g leaf sample was taken put it to 0.2 M phosphate buffer solution and 0.2M KNO3 (3 ML). The samples were kept in dark chamber for 1 hour for incubation of reaction. The reaction was terminated at 100 °C boiling water. Aliquot (0.5 ml) was taken to which 1 ml of 1N sulphanimide and 1ml of 0.02% NEDD was added the volume was made up to 6 ml. The absorbance of the resultant solution (Pink colour) was measured against the blank at 540 nm wavelength. For the formation of standard curve potassium nitrite solution (0.01M) was formed with series of test tubes. The standard curve was prepared with diluted KNO2 solution with series of test tubes, make up the volume in each to 2 ml with water and proceeds it. The enzyme activity was expressed as µmole $KNO_2$ /h / g fresh weight. The sampling for NRA was done at 50, 65and 85 DAS In the morning hours.

**Table .1 Details of experiment**

| | | |
|---|---|---|
| **1.** | Design of experiment | Split plot design |
| **2.** | No. of replication | 3 |
| **3.** | No. of treatments (plots) | 36 |
| | Main plot | Fertilizer nutrient levels (4) |

| | | |
|---|---|---|
| | Sub plot | Varieties (9) |
| **4.** | Net plot size | 2m x 2m |
| **5.** | Gross plot size: | 2.5m x 2.5 m |
| **6.** | Row spacing : | 22.5 cm |
| **7.** | No. of rows per plot | 9 |
| **8.** | Crop | Wheat |
| **9.** | Plant population per plot | 190 |
| **10.** | Seed rate | 100 kg $ha^{-1}$ |
| **11.** | Fertilizer dose (RDF) | 120-60-40 kg $ha^{-1}$ of N, P and K |
| **12.** | Date of sowing | 25 November 2020 |

### Nine varieties of wheat

$V_1$ to $V_9$=HI8663, HI8737, HI8713, HI1563, HI1544, HI1531, GW366, LOK1, NARMADA14

Recommended dose of fertilizer (RDF) @120:60:40 kg/ha of N, $P_2O_5$ and $K_2O$ were supplied through Urea, SSP and MOP, respectively. 50% of N, 100% of $P_2O_5$ and 100% of $K_2O$ of the respective treatments were applied as basal and rest 50% of the N dose was applied in two top dressings at 22 DAS and 45 DAS. The remaining N was top dressed in equal splits at 25 and 45 DAS, whereas 50% of the applied N is delivered as basal.

## 3. RESULTS AND DISCUSSIONS

### Nitrate reductase activity (NRA)

At 50 DAS, there were non-significant differences in NRA observed between varieties of wheat and fertilizer treatments. The range of NRA was found in between 0.22(Naramada14) to 2.43 µmole $KNO_2$ /h / gfrwt(GW366) among all the treatments (Table 1 & Fig. 1). The mean NRA was higher in Normal dose treatment followed by reduced phosphatic fertilizer dose treatment, reduced nitrogen dose fertilizer treatment and lower in control plots. Among all the treatments, the highest magnitude of NRA was observed in GW366 (2.43 µmole $KNO_2$ /h / g ) followed by HI8663(2.17 µmole $KNO_2$ /h / g ) in normal Dose fertilizer treatment and lower NRA was observed in NARMADA14 (0.22 µmole $KNO_2$ /h / g) (Table 1 & Fig.1).

At 65 DAS,the range of NRA was found in between 0.41(LOK1) to 1.73 µmole $KNO_2$ /h / g (HI1544) among all the treatments. The mean NRA was higher in Normal dose treatment followed by reduced phosphatic fertilizer dose treatment and reduced nitrogen dose fertilizer treatment and lower in control plots (Table 1 & Fig. 2).. Among the varieties grown in full dose of N & P treatment, the highest NRA was found in HI1544 (1.41 µmole $KNO_2$ /h / g) and the lowest NRA was found in HI8713 (0.43 µmole $KNO_2$/h / g).Among the varieties grown in half dose of N fertilizer treatment the highest NRA was observed in HI1544 (1.54 µmole $KNO_2$ /h / g) followed by HI1531 (1.44 µmole $KNO_2$ /h / g) and among the varieties grown in half dose of P, the highest NRA was found in HI1544 (1.73 µmole $KNO_2$ /h / g) followed by HI8713(1.28 µmole $KNO_2$/h / g). Among all the treatments, the highest NRA was observed in HI1544 and lowest NRA was observed in Lok1 (0.41 µmole $KNO_2$ /h / g) in reduced phosphorus dose fertilizer (Table 1 & Fig. 2).

At 85 DAS, there were significant differences in NRA observed between fertilizer treatment and varieties. The range of NRA was found in between 0.15(GW366) to 1.78 µmole $KNO_2$ /h / g (HI8713) among all the treatments (Table 1 & Fig. 3). The mean NRA was higher in Normal dose treatment followed by reduced phosphatic fertilizer dose treatment, reduced nitrogen dose fertilizer treatment and lower in control plots. Among the varieties grown in full dose of N & P treatment, the highest NRA was found in Narmada14 (1.57 µmole $KNO_2$/h / g) and the lowest NRA was found in HI1531 (0.33 µmole $KNO_2$ /h / g).Among the varieties grown in half dose of N fertilizer treatment the highest NRA was observed in HI1563 (1.28 µmole $KNO_2$/h / g) followed by HI1544 (1.16 µmole $KNO_2$ /h / g) and among the varieties grown in half dose of P, the highest NRA was found in HI8713 (1.78 µmole $KNO_2$ /h / g) followed by HI1544(1.42 µmole $KNO_2$ /h / g). Among all the treatments, the highest NRA was observed in HI8713 (1.78 µmole $KNO_2$ /h / g) grown in reduced P dose and lower NRA was observed in GW366 (0.15 µmole $KNO_2$ /h / g) grown in control plots. Across all nutrient treatment leaf area in selected wheat varieties followed the following trends:-
HI8713 > LOK1 > HI8663 > HI1544 > HI8737> NARMADA 14> HI1563 > HI1531 >GW366 (Table 1 & Fig 3

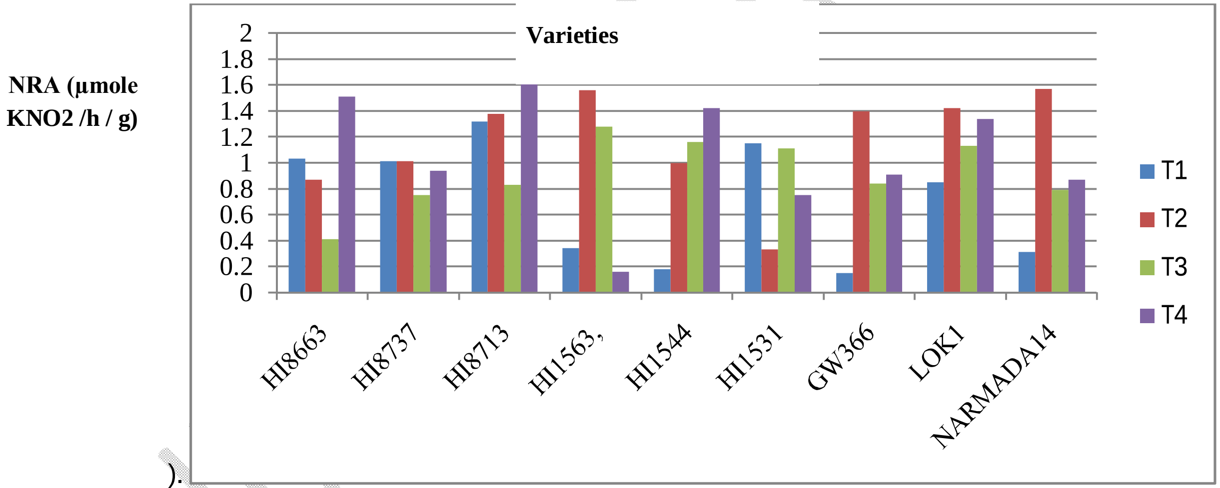

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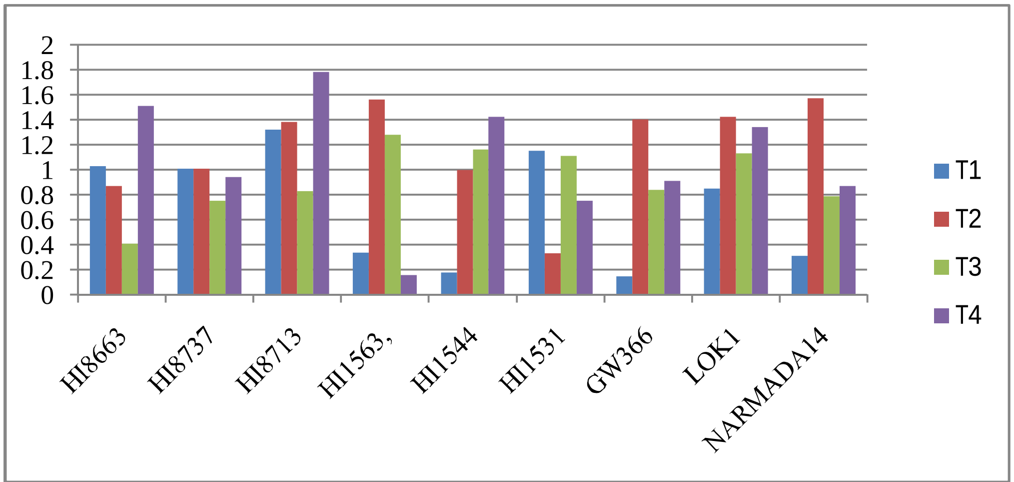
Figure : **1 Effect of N & P on NRA activity in leaf of wheat genotypes at 50 DAS**



**NRA (µmole KNO2 /h / g)**

Fig. 2 Effect of N & P on NRA activity in leaf of wheat genotypes at 65 DAS

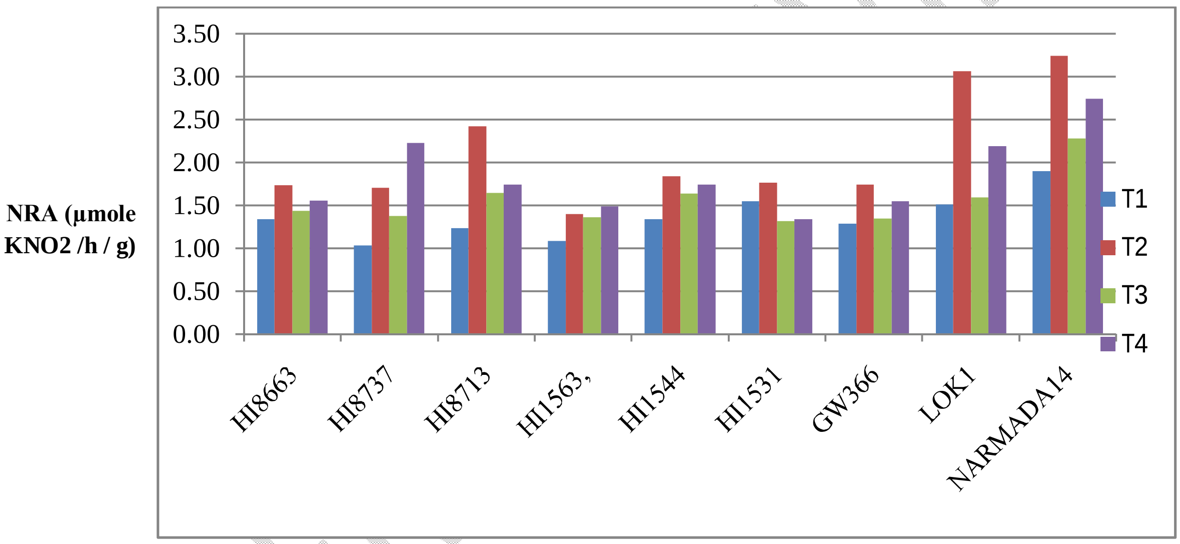


Fig. 3 Effect of N & P on NRA activity in leaf of wheat genotypes at 85 DAS

**Table 2 Effect of N & P on nitrate reductase enzyme activity of wheat genotype at 50, 65 and 85 DAS**

| | 50 DAS | | | | | 65 DAS | | | | | 85 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | Mean A | $T_1$ | $T_2$ | $T_3$ | $T_4$ | Mean A | $T_1$ | $T_2$ | $T_3$ | $T_4$ | Mean A |
| **HI8663** | 0.90 | 2.17 | 0.82 | 1.34 | 1.31 | 0.64 | 0.86 | 0.93 | 0.64 | 0.77 | 1.03 | 0.87 | 0.41 | 1.51 | 0.95 |
| **HI8737** | 1.51 | 0.67 | 0.44 | 1.13 | 0.93 | 0.49 | 1.05 | 0.67 | 0.59 | 0.70 | 1.01 | 1.01 | 0.75 | 0.94 | 0.93 |
| **HI8713** | 0.50 | 0.66 | 0.88 | 1.26 | 0.82 | 0.48 | 0.43 | 0.29 | 1.28 | 0.62 | 1.32 | 1.38 | 0.83 | 1.78 | 1.33 |
| **HI1563,** | 0.63 | 1.35 | 0.50 | 0.68 | 0.79 | 1.08 | 1.28 | 1.06 | 1.17 | 1.15 | 0.34 | 1.56 | 1.28 | 0.16 | 0.84 |
| **HI1544** | 0.93 | 0.81 | 0.84 | 0.76 | 0.84 | 1.57 | 1.41 | 1.54 | 1.73 | 1.56 | 0.18 | 1.00 | 1.16 | 1.42 | 0.94 |
| **HI1531** | 1.46 | 0.79 | 1.79 | 1.01 | 1.26 | 1.10 | 1.19 | 1.44 | 0.81 | 1.13 | 1.15 | 0.33 | 1.11 | 0.75 | 0.83 |
| **GW366** | 0.65 | 2.43 | 0.57 | 0.87 | 1.13 | 0.59 | 0.84 | 0.70 | 0.47 | 0.65 | 0.15 | 1.40 | 0.84 | 0.91 | 0.82 |
| **LOK1** | 0.55 | 1.26 | 1.22 | 0.84 | 0.97 | 0.41 | 0.59 | 0.74 | 0.70 | 0.61 | 0.85 | 1.42 | 1.13 | 1.34 | 1.18 |
| **NARMADA14** | 0.76 | 0.22 | 0.90 | 0.25 | 0.53 | 0.80 | 0.75 | 0.79 | 0.81 | 0.79 | 0.31 | 1.57 | 0.79 | 0.87 | 0.89 |
| **Mean B** | 0.87 | 1.15 | 0.89 | 0.90 | | 0.80 | 0.93 | 0.91 | 0.91 | | 0.70 | 1.17 | 0.92 | 1.07 | |
| **Factors** | **C.D.** | | **SE(d)** | | **SE(m)** | **C.D.** | | **SE(d)** | | **SE(m)** | **C.D.** | | **SE(d)** | | **SE(m)** |
| **Factor(A)** | NS | | 0.15 | | 0.10 | NS | | 0.08 | | 0.06 | 0.06 | | 0.02 | | 0.01 |
| **Factor(B)** | NS | | 0.41 | | 0.29 | 0.36 | | 0.18 | | 0.13 | 0.13 | | 0.07 | | 0.05 |
| **Factor(B)at same level of A** | NS | | 0.82 | | 0.31 | NS | | 0.35 | | 0.17 | 0.27 | | 0.13 | | 0.04 |
| **Factor(A)at same level of B** | NS | | 0.79 | | 0.56 | NS | | 0.34 | | 0.24 | 0.26 | | 0.12 | | 0.09 |

**$T_1$=Control, $T_2$=100% (N+P+K) $T_3$= 50% N+ 100% (P+K), $T_4$ =50%P+100%N+K**

The enzyme NR nitrate reduces nitrogen for protein metabolism in plant system. Nitrate is the principal source of nitrogen for wheat plant, wherein NRA is the rate limiting, and hence protein synthesis is mostly dependent on NR activity. At all growth phases, NR activity was found to have a strong and positive relationship with grain protein. As a result, a favourable relationship between these two traitsis inevitable (Adavi *et al.,*[14]). In this study, increased nitrate reductase enzyme activity was found in the HI1531 and HI8713 genotypes, and these genotypes also showed better grain production, indicating that the NRA and grain yield had a substantial relationship in the HI1531 and HI8713 genotypes. These findings are in congruence with Fortunato et al., 2019, wherein they found a highly substantial positive connection between NR activity and grain yield and grain protein. In wheat and triticale, (Zhan *et al.,* [4]) revealed a strong positive association between NRA and grain yield. In addition (Zhang et al., [19]) discovered a substantial relationship between NRA in the top leaf during the tillering stage and grain yield and grain protein. As a result, NRA can be utilised to select genotypes with high grain protein levels.

## 4. SUMMARY AND CONCLUSIONS

At 50 DAS, GW366 had highest NRA content (2.43 µmole KNO$_2$ /h / g) followed by HI8663 (2.17 µmole KNO$_2$ /h / g) and at 65 and 85 DAS HI 1544 variety had highest average NRA content.

Among all the wheat varieties tested, different varieties showed different stages of NRA activity, with maximum NRA activity at the early stage of flowering, some at the flowering stage, and some showing maximum NRA activity at the vegetative stage.

The main findings are that (a) the amount of nitrate in the tissue played a significant role in regulating the level of enzyme activity; (b) nitrate reductase activity was related to leaf protein content, though not numerically; (c) supplemental nitrogen was effective in inducing nitrate reductase on a field scale; and (d) increased enzyme activity from supplemental nitrogen treatments was linked to increases in gram-weight protein (% or total).

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