

Original Research Article

Combined Effect of Nitrogen and Phosphorus 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 varieties were chosen and planted as test crop using split plot design and replicated 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/hac) 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 NO₃ 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 shown the rise in nitrate reductase activity in response to more nitrogen and the increase vegetative reduced nitrogen. A substantial positive connection was discovered between nitrate reductase activity (expressed as moles N/hac each season) and grain nitrogen (kg N/hac) 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/hac.). 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: Flag leaf, Lodging, Wheat, Phosphorus, Nitrate reductase activity (NRA)

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 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 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 converted into the 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 and boosting soil fertility.

41 Proteins, Rubisco, nucleic acids, and chlorophyll all contain nitrogen as a structural
42 component. N fertilisation plays an important agronomic management strategy for increasing crop
43 productivity (Astanehet *al.*,[2]). The supply of N in plants has a significant impact on the functional
44 activity of the photosynthetic apparatus in leaves. Furthermore, it has been observed that effective N
45 feeding has the capacity to mitigate drought stress effects by maintaining metabolic processes even at
46 low tissue water potential (Abidet *al.*, [1]). Excess nitrogen allows many plants to grow aggressively with
47 dark green coloured lush growth, but it also causes developmental alterations and may alter the
48 biology of plants such as a longer vegetative phase, delayed maturity, a longer plant life cycle, and
49 enhanced succulence (Dietz, *et al.*,[5]).

50
51 The majority of nitrogen in the biosphere is in the form of N₂ in the atmosphere, which is
52 useless to most of the plants species until it is "fixed" biologically or abiotically (by lightning or aurorae,
53 or industrially). It is usually either absorbed and converted into biological N or nitrified into NO₃ once it
54 is fixed into NH₃. Ammonification is a process that converts organic nitrogen back into NH₃. Nitrate can
55 be transformed to N₂O and N₂ through nitrification and denitrification, respectively. As a result of this
56 N₂O and N₂ production, ecosystems lose nitrogen while the atmospheric nitrogen stored in gains
57 nitrogen.

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

64
65 Phosphorus (P) is an important nutrient for plant growth and productivity. It's content in plants
66 ranges from 0.05 to 0.5 percent of the total dry weight of the plant. Despite the fact that the
67 concentration of P in soil is 2000 times higher than in plants, its fixation in the form of aluminium/iron or
68 calcium/magnesium phosphates prevents plants from absorbing it (Malhotra *et al.* [11]). Phosphorus is
69 involved in a variety of biological functions, including membrane structure maintenance, biomolecule
70 synthesis and the production of high-energy molecules. Nitrogen is the fourth most prevalent element
71 in living and it is utilized to essential biological components including amino acids and nucleic acids
72 (Luo *et al.*,[10]). All plants require the element phosphorus (P). Low amounts of phosphorus in the soil
73 will cause plants to grow slowly, but for agricultural production reasons, the soil should have an
74 adequate level of phosphorus to promote healthy plant growth (Watson and Mullen [16]).

75
76 Phosphorus (P) is a structural component of major bio molecules such as nucleic acids, sugar
77 phosphates, adenosine triphosphates, and phospholipids, and a substitutable essential element for
78 plant growth. Phosphorus (P) is one of the most important nutrients for plant growth and survival. It is
79 essential for cellular bioenergetics and metabolic pathways within the plant body. The primary function
80 of mineral fertilizers is to increase crop yield but the biggest impediment to realize known crop potential
81 is the low or none use of fertilizers, notably P and N (Irfan *et al.*,[9]).

82
83 In agricultural systems, the application of P to the soil is required to ensure plant productivity
84 (Simpson *et al.*,[15]). Seed P reserves are rapidly mobilized and translocated to emerging root and
85 shoot tissues after germination, as it is the sole P source available to sustain seedling growth. This P
86 supply is then replenished by P uptake by the root system as it develops (Julia *et al.*,[7]). When root P
87 acquisition is insufficient to meet the P requirement for new growth, biochemical, physiological, and

88 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 RNAs and excess sucrose from the shoot to the root by 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.

94 Phosphorus is the second most commonly limiting macronutrient for plant growth, behind N. It 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. It is also involved in the regulation of metabolic pathways and the control of important enzyme processes (Zhan *et al.*, [20]).

99 Kaure *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 were 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 Nitrogen use efficiency (NUE) and nitrogen content, suggesting that these enzymes may be the rate limiting enzymes in nitrogen metabolism. Crops grown with standard nitrogen fertiliser had higher nitrogen and biomass contents, and the accompanying soils had more nitrification and denitrification genes in abundance (Robinson *et al.*, [18]).

108 **1. Material and methods**

109 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.

116 The reduction of nitrate to nitrite was assayed *in vitro* by incubating the enzyme extract with KNO₃ in the presence of an electron donor (NADH). Nitrite was diazotized with sulphanilamide and then reacted with N-(1-naphthyl) ethylene diamine dihydrochloride (NEDD) to produce an azo dye which was measured spectrophotometrically at 540nm (Nicholas and Nason [12]). The 0.2 gm leaf sample was taken and put into 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 in boiling water at 100 °C. Aliquot (0.5 ml) was taken to which 1 ml of 1N sulphanilamide and 1ml of 0.02% NEDD was added and 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 and make up the volume in each to 2 ml with water. The enzyme activity was expressed as $\mu\text{mol KNO}_2/\text{h/g}$ fresh weight. The sampling for NRA was done at 50, 65 and 85 DAS (Days after sowing) on the morning hours.

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132 **Table.1 Details of experiment**

| | | | |
|-----|---------------------------|---|-----|
| 1. | Design of experiment | Split plot design | 133 |
| 2. | No. of replication | 3 | |
| 3. | No. of treatments (plots) | 36 | 134 |
| | Main plot | 4 | |
| | Sub plot | 9 | |
| 4. | Net plot size | 2m x 2m | 135 |
| 5. | Gross plot size | 2.5m x 2.5 m | |
| 6. | Row spacing | 22.5 cm | 136 |
| 7. | No. of rows per plot | 9 | |
| 8. | Crop | Wheat | |
| 9. | Plant population per plot | 190 | 137 |
| 10. | Seed rate | 100 kg ha ⁻¹ | |
| 11. | Fertilizer dose (RDF) | 120-60-40 kg ha ⁻¹ of N, P and K | 138 |
| 12. | Date of sowing | 25 November 2020 | |

139 **Nine varieties of wheat**

140 V₁ to V₉=HI8663, HI8737, HI8713, HI1563, HI1544, HI1531, GW366, LOK1, NARMADA14

141 Recommended dose of fertilizer (RDF) @120:60:40 kg/ha of N, P₂O₅ and K₂O were supplied through
 142 Urea, SSP and MOP, respectively. 50% of N, 100% of P₂O₅ and 100% of K₂O of the respective
 143 treatments were applied as basal and rest 50% of the N dose was applied in two top dressings at 22
 144 DAS and 45 DAS. The remaining N was top dressed in equal splits at 25 and 45 DAS, whereas 50% of
 145 the applied N was delivered as basal.

146 **3. RESULTS AND DISCUSSIONS**

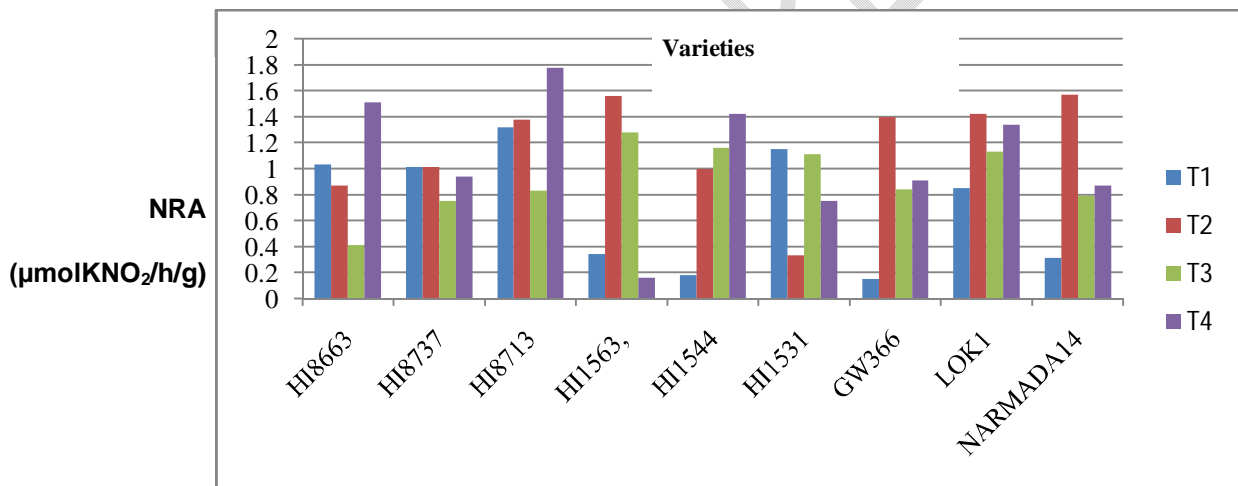
147 **Nitrate reductase activity (NRA)**

148 At 50 DAS, there were non-significant differences in NRA observed between varieties of wheat
 149 and fertilizer treatments. The range of NRA was found in between 0.22(Naramada14) to 2.43
 150 μmoleKNO₂/h / gfrwt(GW366) among all the treatments (Table 2& Fig.1). The mean NRA was higher
 151 in Normal dose treatment followed by reduced phosphatic fertilizer dose treatment, reduced nitrogen
 152 dose fertilizer treatment and lower in control plots. Among all the treatments, the highest magnitude of
 153 NRA was observed in GW366 (2.43 μmole KNO₂/h/g) followed by HI8663 (2.17 μmoleKNO₂/h/g) in
 154 normal Dose fertilizer treatment and lower NRA was observed in NARMADA14 (0.22
 155 μmoleKNO₂/h/g)(Table2& Fig.1).

156 At 65 DAS,the range of NRA was found in between 0.41(LOK1) to 1.73 μmoleKNO₂/h/g
 157 (HI1544) among all the treatments. The mean NRA was higher in Normal dose treatment followed by
 158 reduced phosphatic fertilizer dose treatment and reduced nitrogen dose fertilizer treatment and lower in
 159 control plots(Table2& Fig.2). Among the varieties grown in full dose of N & P treatment, the
 160 highestNRA was found in HI1544 (1.41 μmoleKNO₂/h/g) and the lowest NRA was found in HI8713
 161 (0.43 μmoleKNO₂/h/g).Among the varieties grown in half dose of N fertilizer treatment the highestNRA
 162 was observed in HI1544 (1.54 μmoleKNO₂/h/g) followed by HI1531 (1.44 μmoleKNO₂/h/g) and among
 163 the varieties grown in half dose of P, the highestNRA was found in HI1544 (1.73 μmoleKNO₂/h/g)

164 followed by HI8713(1.28 $\mu\text{moleKNO}_2/\text{h/g}$). Among all the treatments, the highestNRA was observed in
 165 HI1544 and lowest NRA was observed in LOK1 (0.41 $\mu\text{moleKNO}_2/\text{h/g}$) in reduced phosphorus dose
 166 fertilizer(Table2& Fig. 2).

167 At 85 DAS, there were significant differences in NRA observed between fertilizer
 168 treatment and varieties. The range of NRA was found in between 0.15(GW366) to 1.78 $\mu\text{moleKNO}_2/\text{h/g}$
 169 (HI8713) among all the treatments(Table2& Fig. 3). The mean NRA was higher in Normal dose
 170 treatment followed by reduced phosphatic fertilizer dose treatment, reduced nitrogen dose fertilizer
 171 treatment and lower in control plots. Among the varieties grown in full dose of N & P treatment, the
 172 highest NRA was found in Narmada14 (1.57 $\mu\text{moleKNO}_2/\text{h/g}$) and the lowest NRA was found in
 173 HI1531 (0.33 $\mu\text{mole KNO}_2/\text{h/g}$).Among the varieties grown in half dose of N fertilizer treatment the
 174 highest NRA was observed in HI1563 (1.28 $\mu\text{moleKNO}_2/\text{h/g}$) followed by HI1544 (1.16
 175 $\mu\text{moleKNO}_2/\text{h/g}$) and among the varieties grown in half dose of P, the highest NRA was found in
 176 HI8713 (1.78 $\mu\text{moleKNO}_2/\text{h/g}$) followed by HI1544(1.42 $\mu\text{moleKNO}_2/\text{h/g}$). Among all the treatments, the
 177 highest NRA was observed in HI8713 (1.78 $\mu\text{moleKNO}_2/\text{h/g}$) grown in reduced P dose and lower NRA
 178 was observed in GW366 (0.15 $\mu\text{mole KNO}_2/\text{h/g}$) grown in control plots. Across all nutrient treatment
 179 leaf area in selected wheat varieties followed the following trends:-
 180 HI8713 > LOK1 > HI8663 > HI1544 > HI8737> NARMADA 14> HI1563 > HI1531 >GW366 (Table2&
 181 Fig.3)



183 **Fig.1Effect of N & P on NRA activity in leaf of wheat genotypes at 50 DAS**

184 The enzyme NR nitrate reduces nitrogen for protein metabolism in plant system. Nitrate is the principal
 185 source of nitrogen for wheat plant, wherein NRA is the rate limiting, and hence protein synthesis is
 186 mostly dependent on NR activity. At all growth phases, NR activity was found to have a strong and
 187 positive relationship with grain protein. As a result, a favourable relationship between these two
 188 traitswere inevitable (Adaviet al.,[14])

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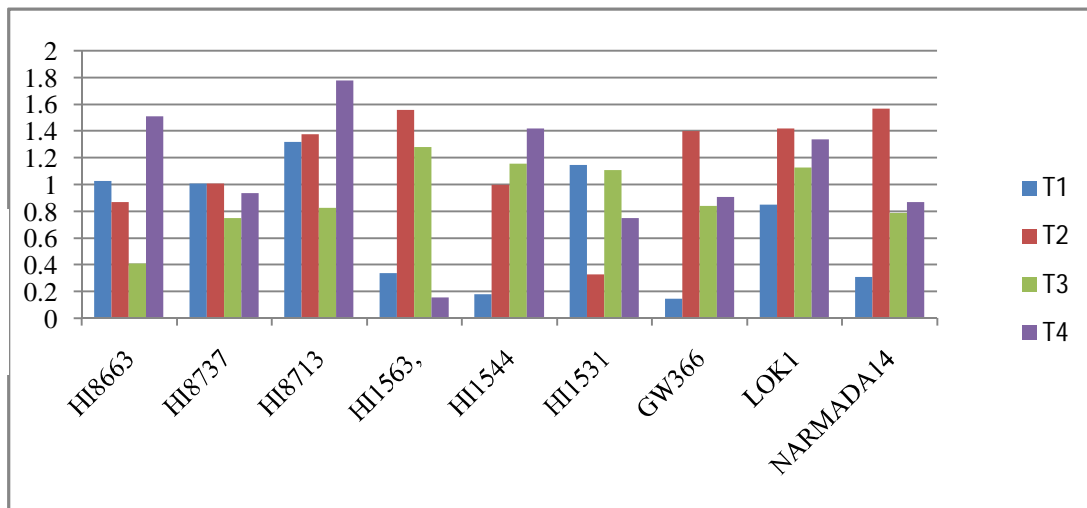
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NRA
($\mu\text{molKNO}_2/\text{h/g}$)



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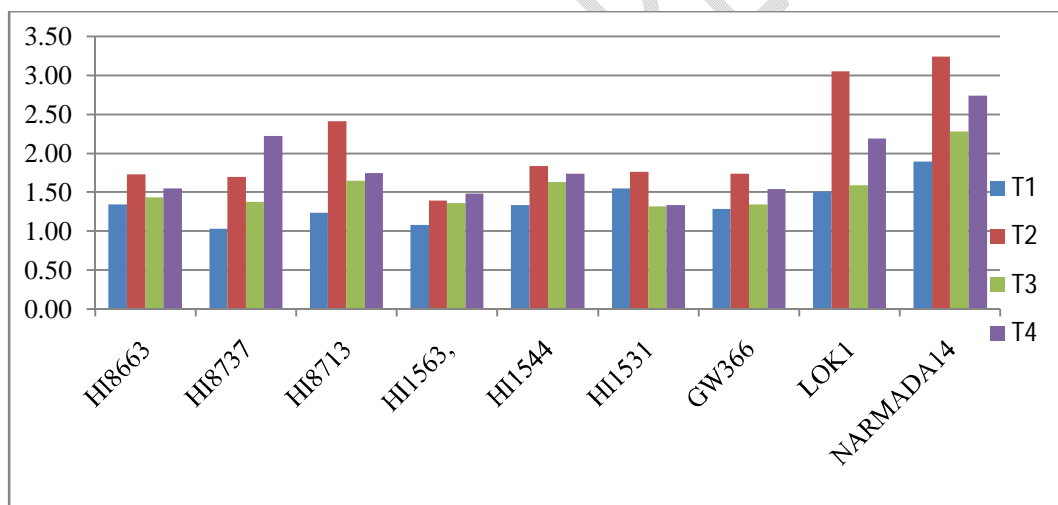
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Fig. 2 Effect of N & P on NRA activity in leaf of wheat genotypes at 65 DAS

NRA
($\mu\text{molKNO}_2/\text{h/g}$)



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

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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.*, [6], 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.

Table2 Effect of N & P on nitrate reductase enzyme activity ($\mu\text{molKNO}_2/\text{h/g}$) 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 |

T₁=Control T₂=100% (N+P+K) T₃= 50% N+ 100% (P+K) T₄=50%P+100%N+K

4. SUMMARY AND CONCLUSIONS

At 50 DAS, GW366 had highest NRA content (2.43 $\mu\text{moleKNO}_2/\text{h/g}$) followed by HI8663 (2.17 $\mu\text{moleKNO}_2/\text{h/g}$) and at 65 and 85 DAS HI 1544 variety had highest average NRA content.

All of the investigated wheat varieties showed different levels of NRA activity, with some showing the highest concentrations at the early phases of flowering, others at the post-flowering stage, and still others at the vegetative stage.

The main findings are that (a) The rate of enzyme activity was significantly affected by the amount of nitrate present in the tissue. (b) Although not statistically, nitrate reductase activity was correlated with leaf protein content. (c) On a field scale, split dose of nitrogen proved successful in producing nitrate reductase. (d) Increases in protein (%) were associated with higher enzyme activity, which is achieved by dividing the nitrogen dose.

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