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# Synergistic use of nitrogen and zinc to bio-fortify zinc in wheat grains

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## Abstract

Our daily diet is largely contributed by cereals, which have low genetic abilities to amass higher concentrations of micronutrients in their grains. Hence, wide spread deficiencies iron, zinc and other essential nutrients have prevailed. Present study focuses the bio-fortification of Zn in wheat grains, taking advantage of nutrient-nutrient synergy between Zn and N. Three wheat genotypes (NIA-Amber, BWQ-4 and SD-998) were tested in a field experiment following randomized complete block factorial design with three replicates. Urea fertilizer was applied at the rates of 120 (recommended), 150 and 180 kg N ha$^{-1}$ in combination with three levels of Zn (0, 5 & 10 kg ha$^{-1}$). Outcomes of the experiment revealed that NIA-Amber had the highest grain yield of 6.03 tons/ha against 150 kg N ha$^{-1}$ and 10 kg Zn ha$^{-1}$. Maximum Zn contents of 447.86, 429.56 and 395.56 g ha$^{-1}$ were observed in BWQ-4, SD-998 and NIA-Amber at 180 kg N ha$^{-1}$ in combination with 10 kg Zn ha$^{-1}$. Maximum enhancement in protein contents was observed in BWQ-4 (743 kg ha$^{-1}$) at 180 kg N ha$^{-1}$and combined with 5 kg Zn ha$^{-1}$. For NIA-Amber, 180 kg N ha$^{-1}$ in combination of 10 kg Zn ha$^{-1}$ proved the most suitable in terms of Zn concentration and other quality attributes. Nitrogen @ 180 kg N ha$^{-1}$ with 5 kg Zn ha$^{-1}$ depicted appreciable zinc and protein contents in grains of BWQ-4 and SD-998.

**Keywords**: Bio-fortification, human nutrition, nutrient management.



## Introduction

Bio-fortification is the production of crops with increased concentration of essentials minerals and protein to be provided to consumers through agricultural research and food trades (Allen, 2000). Such crops can serve as sustainable solution to the prevailing malnutrition among half of the world population (Riezzo et al., 2005).

Industrial fortification by supplementation and food processing; contrasts with bio-fortification (Vincent and Menefee, 2007; Mayer et al., 2008). Bio-fortification depends upon plant physiological efficiency of crops to accumulate higher concentration of desired mineral/nutrient (UNO, 2004; FAO, 2013). Breeding fortified crops, by using genetic variations and genetic modification can be a fruitful approach (Welch and Graham, 2004; Vijayaraghavan, 2002; Stein, 2006; Stein et al., 2007). Yet, fortification through nutritional management is of greater adaptability at farm level and less laborious (Bouis and Welch, 2010).

Cereals fulfill almost 61% of the total daily protein needs in human diet. Nitrogen has prime role in protein synthesis and crop yield especially in the country, where cereals are growing in almost 100% N deficient soils (Warraich et al., 2002). Crops respond to applied N due to its vital role in metabolism and growth (Abedi et al., 2010; Marino et al., 2011). Particularly, nitrogen in plants has role in proteins assimilation, protoplasm formation, nutrient regulation (Rodrigues et al., 2000), enzymatic functions and cell division (Oscarson, 2000; Warraich et al., 2002).

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Zinc is an important plant nutrient needed in small concentration. Its acute deficiency can lead up to 40% reduction in crop yield (Salvagotti and Miralles, 2007; Kutman et al., 2010; Kutman et al., 2011). Chlorophyll and auxin synthesis necessitate adequate Zn contents in plant tissues (Nadim et al., 2012; Jiang et al., 2013). Zinc also performs as cofactor to various enzymes hence, controls their activities. Wheat in comparison with other field crops has relatively better tolerance to Zn deficiency (Chauhan et al., 2014). However, its continuous cultivation in Zn deficient soils has resulted in wide spread Zn deficiency in grains; hampering crop production and causing low Zn supply to human (Chauhan et al., 2014).

Nitrogen and Zinc supplementations in wheat have prominent role in sustaining wheat production. The synergy between both can be used to augment Zn concentration in the edible portion and improving quality (Kutman et al., 2010; Shi et al., 2010; Cakmak, 2010; Cakmak et al., 2010a,b). Zinc uptake from soil to roots, mobilization in plant body and accumulation to sink is supported by nitrogenous proteins. (Wang et al., 2011). N is involved in the production of natural chelating agents (Kutman et al., 2010) like nicotianamine (NA) (Takahashi et al., 2003) and deoxymugeinic acid (DMA), involved in translocation of metals from flag leaves to grain (Barunawati et al., 2013). Therefore, the present study was executed to assess to N-Zn interaction to improve plant growth, nutrient contents, quality and Zn concentration in wheat grains.

## Material and Methods

An field experiment was conducted at Nuclear Institute of Agriculture (NIA), Tando Jam, Sindh-Pakistan from 25$^{th}$ November, 2015 to 18$^{th}$ April, 2016. The experimental site was 29 m above sea level at latitude of 20$^{0}$24'49.4" north and longitude of 68$^{0}$31'03.7" east. The climate of the area is arid with an average high and low temperatures of 41$^{0}$C and 11$^{0}$C, respectively and annual precipitation less than 10.8mm (NAMC, 2016). Eighty one experimental units each of 4m × 4 m were allotted to three wheat genotypes (NIA-Amber, BWQ-4 and SD-998). Nine treatment combinations of three N levels (120, 150 and 180 kg ha$^{-1}$) and three levels of Zn (0, 5 and 10 kg ha$^{-1}$) were applied to each genotype with three replications. Phosphorus in the form of di-ammonium phosphate (90 kg $P_2O_5$ ha$^{-1}$) and potassium in the form of sulfate of potash (60 kg $K_2O$ ha$^{-1}$) were applied at the time of sowing. Urea as nitrogen source was applied in three equal splits viz., at the time of sowing, tillering and panicle initiation. Zinc sulfate was applied along with second split of nitrogen to maintain three levels of zinc in the respective treatments. Experiment was irrigated with canal water at critical growth stages. Manual and chemical eradication of weeds and other cultural practices were performed as and when required. At harvest, yield attributes were recorded. From each replication, grain and straw samples were randomly collected and dried in oven at 70 $^{0}$C for three days. A uniform portion of dried plant samples (straw and grain) was digested in di-acid ($HNO_3$ and $HClO_4$) mixture for the determination of phosphorus and Zn (Chapman and Pratt, 1961). Vanadomolybdate yellow color method was used for the determination of phosphorus (Ryan, 2000). Zn concentrations were determined as described by Rashid (1986) by using atomic absorpotion spectrophotomerty (Analytical jena AAS-NOVA-400, Germany) in digested samples. Grain N was determined through Kjeldal method (Jones, 1991). Subsequently, protein contents were calculated by multiplying grain N with 6.25 (FAO, 2003). Statistical analysis were performed using Statistix 8.1 at 5% probability level.

## Results

Data recorded for the yield attributes (Table 2) of wheat depicted that higher doses of N appreciably increased grain yield in comparison to 120 kg/ha N (recommended dose). The grain yield increased by 29, 24 & 32 % over 120 kg N ha$^{-1}$ when NIA-Amber, BWQ-4 and SD-998 were supplemented with 150 kg N ha$^{-1}$. On application of 180 kg N ha$^{-1}$ recorded grain yields of NIA-Amber, BWQ-4 and SD-998 were 2, 1 & 4.6 % higher than that of recommended N level. Similarly, straw yields were also enhanced up to 16, 1 & 24 % by increasing N dose for 120 kg ha$^{-1}$ to 150 kg ha$^{-1}$. Highest straw yields were recorded when N was supplemented @ 180 kg ha$^{-1}$. Crop yield was affected positively by the application of Zn in combination with all levels of N input. Maximum grain yield of 6.03 t/ha was recorded in NIA-Amber with 5 kg Zn + 150 kg N ha$^{-1}$ which was statistically similar to grain yield (6.01 t/ha) of genotype SD-998 achieved by the application of 10 kg Zn +180 kg N ha$^{-1}$. However, grain yield remained almost stagnant with the increase of input rates from higher to highest. Straw yield was also improved by the interactive use of N and Zn 10.45 t/ha was recorded as highest among all genotypes shown by SD-998 at 10 kg Zn + 180 kg N ha$^{-1}$.

Increasing the N dose in wheat from 120 to 180 kg N ha$^{-1}$ escalated N contents (Table 3) in grain from 53.64 to 88.64 kg N ha$^{-1}$ in NIA-Amber, from 78.45 to 104.77 kg N ha$^{-1}$ in BWQ-4 and from 64.68 to 102.32 kg N ha$^{-1}$ in SD-998. Upon application of Zn doses (5 and 10 kg ha$^{-1}$) further increase in N contents was recorded.

Grain contents of 135. 64 kg N $ha^{-1}$ by BWQ-4 in response to interactive use of 10 kg Zn+180 kg N $ha^{-1}$ was highest among all the genotypes in comparison to all Zn + N combinations. Increase in phosphorus uptake (Table 3) was recorded with increasing the N in all the genotypes however with the application of Zn, P uptake tended to decrease slightly. In NIA-Amber, BWQ-4 and SD-998 P uptake decreased from 13.78 to 11.58 kg P $ha^{-1}$, from 13.53 to 9.88 kg P $ha^{-1}$and from 15.99 to 15.10 kg P $ha^{-1}$ with increasing Zn from 5 to 10 kg $ha^{-1}$ in combination with 120 kg N $ha^{-1}$. While average P uptake of the genotype was reduced from 22.44 to 18.74 kg P $ha^{-1}$ when Zn was applied with 150 kg N $ha^{-1}$. Similar, results were recorded in case of 180 kg N $ha^{-1}$ applied to wheat along with 5 and 10 kg Zn $ha^{-1}$ and mean reduction of 1.8 kg P $ha^{-1}$ was observed. Zn contents (Table 4) in edible portion of wheat were enhanced by the interactive use of N and Zn. Significant increments from 55.78 g Zn $ha^{-1}$ to 171.79 g Zn $ha^{-1}$, 135.59 to 320.65 g Zn $ha^{-1}$and 173.33 to 424.33 g Zn $ha^{-1}$ were recorded by supplementing Zn in combination with 120, 150 and 180 kg N $ha^{-1}$, respectively. Highest uptake was recorded in SD-998 when supplied with 10 kg Zn + 180 kg N $ha^{-1}$.

Momentous and steady increase in wheat grain protein (Table 4) contents were recorded with increasing levels of N and maximum were recorded with 180 kg N $ha^{-1}$. Increasing protein contents are the direct indication to improved quality of produce. Zn application also influenced protein contents significantly as 10 kg Zn + 180 kg N $ha^{-1}$ showed maximum protein contents (847.75 kg $ha^{-1}$) in grains of BWQ-4 followed by SD-998 (740.22 kg $ha^{-1}$) and NIA-Amber (613.25 kg $ha^{-1}$).

## Discussion

Increased input levels of N imparted higher photosynthetic activity (Rodrigues et al., 2000), cell division (Warraich et al., 2002), increased number of leaf, vigorous vegetative growth (Protic et al., 2007) and better assimilation and metabolic rates (Oscarson, 2000) which resulted in better crop productivity in wheat (Goswami, 2007). Similar responses of various levels of N application have been reported by Yadav et al. (2012) and Singh et al. (2013). Increase in grain and straw yield (Table 1) is due to role of Zn in stimulation and catalysis of various metabolic processes. As co-factor of enzymes Zn is involved in growth and development of plants eventually leading to higher yields of wheat (Imran et al., 2015). However, lower rate of Zn was more favorable than higher rate in terms of grain yield. Grain yield was improved with increasing rates of N at each level of Zn. Combination of 5 kg Zn and 150 kg N $ha^{-1}$ has more pronounced effect because these levels might have maintained optimal balance between the nutrients as artificially modified soil fertility through the addition of fertilizer in proportion to the crop needs can promote growth and increase or sustain yield. This is comparable with the results recorded by Sahay et al. (2009) and Protic et al. (2007) while studying the effect of N and Zn on wheat in deficient soils. Significant positive correlations (r = 0.896) between mean nitrogen contents of grains and mean grain yields of tested genotypes illustrate the role of nutrients contents in better crop yield (Figure 1). The correlation (r = 0.755) between grain zinc contents and grain yield (Figure 2) supports the role of zinc in yield enhancement.

Table 1. Physico-chemical characteristics of the soil

| Characteristics | Value |
|---|---|
| Textural class | Silty clay loam |
| Organic matter | 0.73 % |
| Electrical conductivity | 1.20 dS $m^{-1}$ |
| pH | 7.56 |
| Kjeldhal nitrogen | 0.035% |
| Extractable Phosphorus | 6.8 μg $g^{-1}$ |
| Extractable potassium | 145 μg $g^{-1}$ |
| Extractable Zinc | 0.36 μg $g^{-1}$ |

Improved nutrient contents can be attributed to enhanced nutrient demands of plants due to escalated grain and straw yield (Brown et al., 2005). Application of N improved Zn uptake majorly due to the improved enzymes activities and more efficient metabolic processes. (Imran et al., 2015).

Phosphorus uptake (Table 3) was improved by the higher doses of N explains the role of N in enhanced P uptake as ammonium ions temporarily lowers the pH in the micro-sites of the plant roots which facilitates more P uptake in plants (Chaudhary et al., 1997; Singh et al., 2007) . This increased uptake of P in grains can also be physiological prompted rather than root system ramification. On the contrary, P uptake declined with the application of Zn might be due to antagonism between the two nutrients (Singh et al., 2010).

Table 2. Effect of synergistic use of nitrogen and zinc on grain and straw yields of wheat genotypes

| Treatment | NIA-Amber | BWQ-4 | SD-998 | Mean | NIA-Amber | BWQ-4 | SD-998 | Mean |
|---|---|---|---|---|---|---|---|---|
| | Grain yield (ton/ha) | | | | Straw yield (ton/ha) | | | |
| 120 kg N/ha | 4.48 b | 4.39 b | 4.25 b | 4.37 B | 4.54 ij | 5.20 g-j | 5.69 f-j | 5.14 B |
| 120 kg N/ha + 5 kg Zn/ha | 4.54 b | 4.50 b | 4.60 b | 4.55 B | 4.61 ij | 5.81 e-j | 7.48 b-h | 5.97 B |
| 120 kg N/ha + 10 kg Zn/ha | 4.75 b | 4.64 b | 4.67 b | 4.69 B | 4.39 j | 6.33 d-j | 7.83 b-g | 6.18 B |
| 150 kg N/ha | 5.78 a | 5.44 a | 5.65 a | 5.62 A | 5.31 g-j | 5.29 g-h | 7.11 b-i | 5.90 B |
| 150 kg N/ha + 5 kg Zn/ha | 5.80 a | 5.83 a | 5.96 a | 5.86 A | 5.15 h-j | 6.46 d-j | 7.48 b-h | 6.36 B |
| 150 kg N/ha + 10 kg Zn/ha | 6.03 a | 5.91 a | 5.96 a | 5.97 A | 5.55 f-j | 6.59 c-j | 8.31 a-e | 6.82 B |
| 180 kg N/ha | 5.87 a | 5.49 a | 5.91 a | 5.76 A | 7.88 a-f | 7.95 a-f | 9.15 a-c | 8.33 A |
| 180 kg N/ha + 5 kg Zn/ha | 5.92 a | 5.75 a | 5.98 a | 5.88 A | 8.04 a-f | 8.63 a-c | 9.43 ab | 8.70 A |
| 180 kg N/ha + 10 kg Zn/ha | 5.99 a | 5.82 a | 6.01 a | 5.94 A | 8.39 a-e | 9.39 ab | 10.45 a | 9.41 A |
| Mean | 5.46 A | 5.31 B | 5.44 A | 5.4 | 5.98 C | 6.85 B | 8.1 A | 6.97 |

Means sharing similar letters are statistically similar to each other at $p < 0.05$

Table 3. Effect of synergistic use of nitrogen and zinc on grain nitrogen and phosphorus contents of wheat genotypes

| Treatment | NIA-Amber | BWQ-4 | SD-998 | Mean | NIA-Amber | BWQ-4 | SD-998 | Mean |
|---|---|---|---|---|---|---|---|---|
| | Nitrogen contents in grains (kg/ha) | | | | Phosphorus contents in grains (kg/ha) | | | |
| 120 kg N/ha | 53.64 k | 78.45 f-j | 64.69 i-k | 65.59 F | 13.79 d-b | 13.53 d-h | 15.99 b-f | 14.44 C |
| 120 kg N/ha + 5 kg Zn/ha | 59.60 jk | 91.52 e-h | 73.97 h-k | 75.03 F | 12.04 f-h | 12.79 e-h | 15.65 c-g | 13.49 C |
| 120 kg N/ha + 10 kg Zn/ha | 76.47 g-k | 95.87 b-h | 82.59 e-h | 84.98 E | 11.58 f-h | 9.88 gh | 15.10 c-g | 12.18 C |
| 150 kg N/ha | 81.78 e-j | 100.56 b-f | 90.89 d-h | 91.08 DE | 22.32 a-c | 21.9 a-c | 23.09 a | 22.44 A |
| 150 kg N/ha + 5 kg Zn/ha | 83.92 e-i | 103.55 b-e | 93.61 b-h | 93.69 C-E | 20.70 a-c | 19.49 a-e | 22.08 a-c | 20.76 AB |
| 150 kg N/ha + 10 kg Zn/ha | 92.63 e-h | 117.05 a-d | 95.01 b-e | 101.56 BC | 18.85 a-e | 16.88 a-f | 20.51 a-d | 18.75 B |
| 180 kg N/ha | 88.64 e-h | 104.77 b-e | 102.32 b-e | 98.58 B-D | 23.21 a | 22.73 ab | 23.06 a | 23.00 A |
| 180 kg N/ha + 5 kg Zn/ha | 90.24 e-h | 118.97 ab | 104.69 b-e | 104.64 B | 22.01 a-c | 21.48 a-c | 22.92 a | 22.14 A |
| 180 kg N/ha + 10 kg Zn/ha | 98.12 b-g | 135.64 a | 118.43 a-c | 117.40 A | 21.82 a-c | 20.33 a-d | 21.44 a-c | 21.20 AB |
| Mean | 80.56 C | 105.16 A | 91.8 B | 92.51 | 18.48 B | 17.67 B | 19.98 A | 18.71 |

Means sharing similar letters are statistically similar to each other at $p < 0.05$

Table 4. Effect of synergistic use of nitrogen and zinc on grain zinc and protein contents of wheat genotypes

| Treatment | NIA-Amber | BWQ-4 | SD-998 | Mean | NIA-Amber | BWQ-4 | SD-998 | Mean |
|---|---|---|---|---|---|---|---|---|
| | Zinc contents in grain (g/ha) | | | | Protein contents in grain (kg/ha) | | | |
| 120 kg N/ha | 56.68 m | 58.52 m | 52.13 m | 55.78 E | 335.25 k | 490.34 f-j | 404.29 i-k | 409.96 F |
| 120 kg N/ha + 5 kg Zn/ha | 84.86 l | 145.99 jk | 179.67 hi | 136.84 D | 372.48 jk | 572.02 e-h | 462.33 h-k | 468.94 F |
| 120 kg N/ha + 10 kg Zn/ha | 135.56 jk | 181.42 g-i | 198.38 f-h | 171.79 D | 477.95 g-k | 599.19 b-h | 516.21 e-j | 531.12 E |
| 150 kg N/ha | 123.30 k | 128.59 jk | 154.87 ij | 135.59 D | 511.14 e-j | 628.62 b-f | 568.07 e-h | 569.28 DE |
| 150 kg N/ha + 5 kg Zn/ha | 188.53 f-h | 268.49 de | 289.99 d | 249.01 C | 524.47 e-i | 647.16 b-e | 585.07 d-h | 585.57 C-E |
| 150 kg N/ha + 10 kg Zn/ha | 209.55 fg | 375.02 c | 377.38 c | 320.65 B | 578.91 e-h | 731.54 a-d | 593.83 c-h | 634.76 BC |
| 180 kg N/ha | 154.23 ij | 147.96 jk | 217.79 ab | 173.33 D | 554.00 e-h | 654.83 b-e | 639.51 b-e | 616.11 B-D |
| 180 kg N/ha + 5 kg Zn/ha | 255.51 e | 424.77 ab | 418.91 a | 366.40 B | 564.02 e-h | 743.58 ab | 654.31 b-e | 653.97 B |
| 180 kg N/ha + 10 kg Zn/ha | 395.56 bc | 447.87 a | 429.57 a | 424.33 A | 613.25 b-g | 847.75 a | 740.22 a-c | 733.74 A |
| Mean | 178.2 B | 242.07 A | 257.63 A | 225.96 | 503.5 C | 657.23 A | 573.76 B | 578.16 |

Means sharing similar letters are statistically similar to each other at $p < 0.05$

Increased Zn uptake in wheat grains can be attributed to the role of N dependent proteins in Zn uptake and translocation in plant (Cakmak et al., 2010b). This phenomenon can excellently be understood by taking in account of pre-anthesis accumulation of Zn in vegetative parts of wheat and role of N in extending Zn supply to grains during anthesis and grain filling (Kutman et al., 2012; Barunawati et al., 2013; Sperotto et al., 2013). Very strong correlation was found (r = 0.904) between the mean N contents in grains and mean Zn contents in grains (Figure 3), explains the positive influence of nitrogen on zinc uptake in crop.

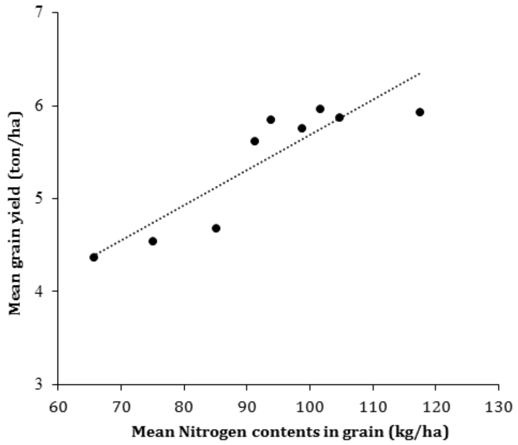


Figure 1. Correlation (r=0.896) between mean nitrogen contents in grains to mean grain yield of wheat genotypes

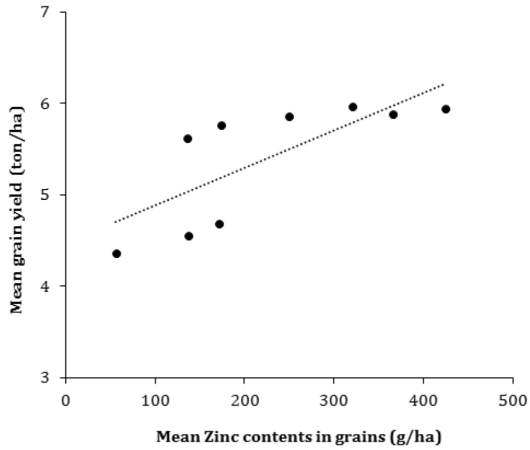


Figure 2. Correlation (r = 0.755) between mean grain zinc contents to grain yield of tested wheat genotypes

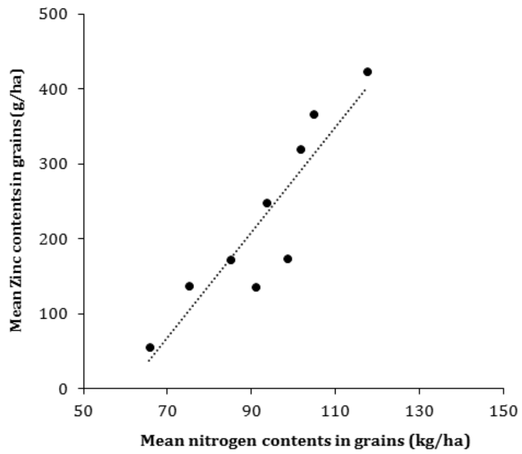


Figure 3. Correlation (r = 0.904) between grain nitrogen contents to grain zinc contents of tested wheat genotyptes

Plant accumulated larger concentration of N with the increasing N input levels hence depicted more grain protein contents. N plays pivotal role in amino acids and protein synthesis (Brown et al., 2005; Abedi et al., 2011). Therefore, its higher doses can be attributed to increased protein contents. Levels of Zn also played important role in stimulating protein contents in grains (Nadim et al., 2012; Jiang et al., 2013) Zn is indispensable nutrient for N metabolism due to its catalytic influence on numerous enzyme systems, biochemical activities responsible for nitrate reduction and protein synthesis (Khare and Dixit, 2011).

## Conclusion

On the basis of experimental results it is concluded that N and Zn fertilization is crucial not only for the yield maximization in wheat but also to improve quality of produce. 150 kg N $ha^{-1}$ in combination with 5 kg Zn $ha^{-1}$ can be recommended as optimal dose to achieve better growth, productivity and quality of wheat.

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