

Impact of nitrogen forms and levels on yield and quality of rice in Kirinyaga and Kisumu counties Kenya

Abstract

Nitrogen is one of the most limiting elements in crop production. The lack of nitrogen is even more pronounced in Sub-Saharan African as farmers are poor and cannot afford required amount for crop production. Depending on form, nitrogen is known to affect the uptake of other nutrient elements. It is therefore important to understand strategies that can enhance the availability of N as well as other element. The N content is also a putative precursor for protein, an important component of rice quality.

An experiment was therefore carried out to evaluate the effects of nitrogen forms and levels on yield and quality of rice grain in Ahero Kisumu county and Mwea Kirinyaga county.

The trial took place during the main growing seasons of July to December. The Experiments were laid out in Randomized Complete Block Design (RCBD) in a factorial arrangement with three replicates giving a total of 36 plots, with each plot measuring 3 m by 4 m. Two nitrogen forms, including urea and ammonium sulphate were applied in three levels (0, 25 and 50kg ha^{-1} respectively).

The results showed a positive relationship between levels of nitrogen and the measured parameters. There were observed increase in yield, grain crude protein, as well as more zinc and iron accumulations. Ammonium sulphate supplied at 50kg ha^{-1} recorded an increase in grain zinc concentration of 163.8 mg kg^{-1} and 195.5 mg kg^{-1} in Ahero and Mwea respectively. A similar trend was observed in both crude protein and iron accumulation in the rice grain. The unfertilized plots had the lowest accumulation and uptake of the two elements and lower quality of tested aspects. Proper application of nitrogen with the recommended rates has the potential of eliminating the challenges of hidden hunger, hence enhancing food insecurity and reducing malnutrition through increased yield and quality of rice.

Key words: cation competition, hidden hunger, iron, protein content, protein, Zinc

35 **1. Introduction**

36 Nitrogen nutrition in rice crop is a major requirement in plant growth and development. It is a
37 limiting factors in plant and development and has also been reported to affect uptake of other
38 micro-nutrients that are essential for human nutrition (1). In Kenya, rice is the third most
39 important cereal crop that contributes a greater percentage toward curbing the issue of food
40 insecurity in the country (2). Of essence, the micronutrients play great role in promoting
41 growth and development of the rice plants. Incidentally, zinc is one of the micro-nutrients
42 essential in growth and reproduction of rice and is useful in synthesis of substrates and
43 enzyme that enhance transcription. It has been documented to be one of the elements leading
44 to the greatest plant growth and developmental disorders and limits growth and production in
45 rice especially in lowland countries (3). Zinc's major function is to also promote
46 physiological metabolisms of the rice plants. Its deficiency has also been viewed as one of the
47 major challenges limiting rice production and its contribution to the maximum nutritional
48 quality upon consumption. When substantial amount of the Zn is accumulated in the tissues,
49 it is later remobilized and stored in the grain. The amounts of micronutrients that
50 accumulated in the grain are affected by proper nitrogen nutrition (4).

51 Iron on the other hand, is an element essential for oxidation and transportation of the electro
52 elements. It also maintains the structure and function of the chloroplasts. Several reports have
53 denoted that there is low accumulation of Zinc and Fe in the grain of rice hence leading to
54 low quality of nutritional needs (5, 6,). Low concentration of zinc and iron in the grain of
55 rice has been associated to be the leading factors in increased rates of malnutrition the in
56 world (7). Crude protein provides another measure of quality in grain as it determines its
57 nutritional content (8). The protein content in the grain is also a representation of nitrogen
58 harvest index which depends on the ability of the plants to uptake the available and supplied
59 Nitrogen into potential yield (9). Therefore, this implies that there is a gap that needs to be

addressed to give more insights to crude protein content, zinc and iron concentration in the grain to ensure that the required nutritional quality is achieved. Nitrogen fertilization has been marked as one of the strategies that can enhance improved quality of the grain (10, 11). Therefore, the current study was carried out to evaluate the effects of N fertilization under different levels and forms on yield, crude protein content, zinc and Fe concentration in rice grain.

2. MATERIALS AND METHODS

2.1 Description of the study site

The study was carried out in two locations; Ahero in Kisumu county and National Research Irrigation Board research station and in Mea Kirinyaga county at Kirogo farm research station. Kisumu county receives rainfall almost throughout the year and thus does not have a true dry season. It lies between latitude of $0^{\circ} 10'$ to $0^{\circ} 60''$ N and a longitude of $34^{\circ} 54'$ E and $59.99''$ W. The average temperature is 22.9°C and an annual rainfall of between 1200 mm and 1300 mm in various locations. Mwea, Kirinyaga county on the hand is located in central parts of country. It lies between 1158 m and 5380 m above the sea level and Latitude (05° and 04° S) and Longitude (37° and 38° E) with temperatures ranging between 12°C and 26°C (12). It has both tropical climate and the equatorial rainfall patterns, annual rainfall ranges between 800-2200 mm and this is influenced by its position along the equator and on the windward side of Mt. Kenya. The experiment was carried out during the main growing season of rice in Kenya from July to December 2017.

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2.2 Experimental Design and Data collection.

The experiment was laid out as Randomized Complete Block Design (RCBD) in a factorial arrangement and replicated three times. The treatments included; two forms of nitrogenous fertilizers which were: Sulphate of Ammonia (SA) and Urea at different levels (0, 25 and

50kg ha^{-1}). There were two methods of applications; full dose and two splits. The plot sizes measured 3 m by 4 m. The seeds were sourced from the National Irrigation Board. Three weeks' old seedlings were transplanted at a spacing of 25 x 15 cm in the 36 plots. The fields were kept weed-free through manual weeding at different stages. Watering was done regularly and any incidences of pests and diseases were managed. Birds were managed through physical scaring while the fungal diseases were controlled using fungicides. At maturity, the rice grains were harvested, and dried up to 14 % moisture content. They were ground and analysis of zinc and iron done through the use of the ash solution using an ICP spectrophotometer in a procedure described by Jones and Case (13). Nitrogen content was determined following the Micro Kjeldahl method described in AOAC of 2007 (14). The grain crude protein content was determined through multiplication of N content (this was determined by Kjeldahl method according to (14, 15) with a standard value of 6.25.

2.3 Data Analysis

The collected data on yield, Fe, Zn and crude proteins were managed in the excel spreadsheet and subjected for analysis (ANOVA) using GenStat statistical software version 15.1. Separation of means was done at 5% level of significance using Fischer's Protected Least Significance Difference. Associations between the collected variables were determined by regression analyses

3 Results and Discussions

3.1 Influence of nitrogen forms and levels on grain yield

From the regression analyses shown in figure 1, it was revealed that yield increased with N rates and the response assumed polynomial function, particularly at Mwea site where the R^2 values were 0.63 and 0.70 for urea and ammonium sulphate respectively. The trends were however very different at Ahero site with R^2 values being quite low and hence meant that

yield increase could barely be explained by increasing N rates. For instance, nitrogen rates could only explain 47 percent of yield when N was applied as ammonium sulphate at Ahero site (Fig. 2 b) while the remaining 53 percent of yield were due to other factors other than N. It was also clear that rice yield still responded to additional N supply at Ahero (irrespective of N source). This was not reported in Mwea site which showed an optimal rate at about 45 kg N ha⁻¹.

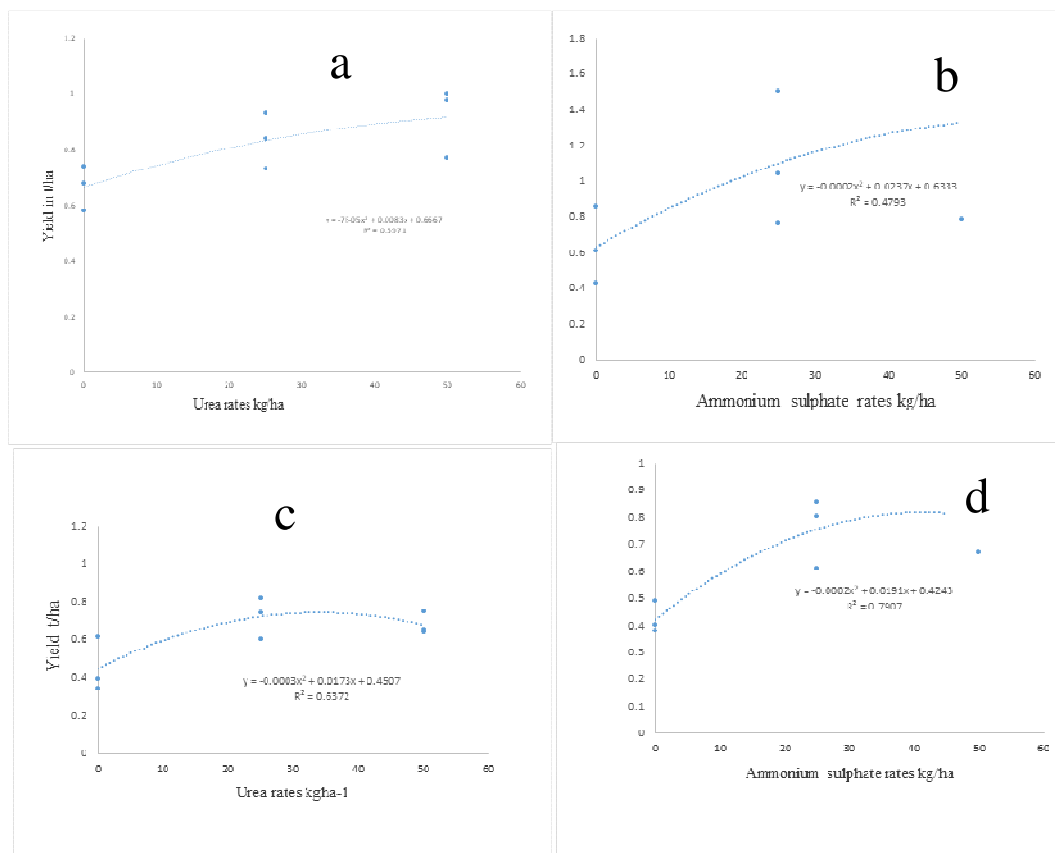


Figure 1: Regression analysis between nitrogen and grain yields based on different nitrogen sources in Ahero study site (a and b) and Mwea study site (c and d)

In the two sites, there was a corresponding increase in the grain yield with increase in nitrogen rates irrespective of the source (urea or ammonium) in the two sites. These may imply that the plants were responding to the increase of the nitrogen nutrition and an additional amount would probably lead to more yield. This may be a pointer that the doses

used by the farmers are well below the plant's needs, which partially agree with the high NUE in these sites (9). The low R^2 value in regression between yield and N rates had earlier reported by the above author in Kirinyaga county with Nerica 4 and Nerica 10. But just like in Mwea site in Kirinyaga county results in our current work (Fig. 1 c and d), there were no further yield response to N with Nerica 4, an indication that other factors other than N led to poor response. Similar arguments had been advanced by Njiru *et al.* (12) who ascribed the low yield to unfilled grains when more N was supplied.

The results of this study are in disagreement with those of Moro *et al.* (16) in Ghana who reported an increment in grain yield in rain fed rice up to 90 kg ha⁻¹ and thereafter declined beyond this level. The disagreement could probably be due to the different environment conditions since in rain fed conditions the rate of emission and leaching are minimal compared to low land rice hence leading to high efficiency that maximize yield. The findings of this study are however in agreement with those of Ahmed *et al.* (17) who reported an increase in the yield of barley in response to an increase in nitrogen levels. In another study done in India, it was also reported that the wheat grain yield increased with an increase of N levels (18). The results are also in agreement with other reports (19) that showed an increase in yield of japonica soft super rice with an increment with nitrogen levels in Nainjing in China. A similar trend was also observed in a study done by Wekha *et al.*, (20) who reported a similar trend finger millet when supplied with different levels of phosphorous.

3.2 Effects of nitrogen form and levels on grain Iron (Fe) Uptake

The iron uptake in rice grain was significantly ($p \leq 0.05$) different in respect to levels and forms /sources of N in both experimental sites. Ammonium sulphate applied at 50 kg ha⁻¹ elicited an increased uptake of iron in the grain in both sites with 163.8 mg kg⁻¹ and 195 mg kg⁻¹ in Ahero and Mwea respectively, this was followed by urea 50 kg ha⁻¹ while the

control (unfertilized plots had the lowest concentration of 119.5 mgkg⁻¹ in Ahero and 139 mgkg⁻¹ in Mwea (Table 1).

Table 1. Crude protein and iron concentration in rice grain as affected by nitrogen forms and levels in Ahero and Mwea sites.

	Ahero		Mwea	
	Crude protein	iron Fe	Crude protein	iron
	Percent	mg kg ⁻¹	Percent	mgkg ⁻¹
N rates kgha ⁻¹				
Control	10.25 ^c	119.5 ^b	15.41 ^c	139.2 ^c
As50	15.06 ^a	163.8 ^a	22.60 ^a	195.5 ^a
As25	12.51 ^b	144.8 ^a	19.02 ^b	172.0 ^{ab}
Ur50	14.72 ^a	155.3 ^a	22.15 ^a	188.0 ^a
Ur25	12.27 ^b	147.8 ^a	18.34 ^b	177.8 ^a
LSD	0.70	14.05	1.13	16.97

Means followed by the same letter within the same column are not significantly different (p≤0.05).

The increase of N concentration in the grain in response to the increase in the levels of nitrogen could be due to synergistic relationship between nitrogen sources and iron translocated to the grain. The findings of this study are similar to those of Duhan and Singh (21), who reported a notable increase in iron concentration in the grain when nitrogenous fertilizers were applied in rice. Moreover, nitrogen is associated with potential to increase availability of micronutrient such as iron hence the high uptake in the grain following adequate fertilization. The increase in biomass production of the rice grain due to nitrogen fertilization is associated with high uptake of micronutrients like iron due to a high pool of translocation to the grain (22). This argument is in concurrence with those of Barunawati *et*

163 *al.* (23). The results of this study thus suggests that nitrogen fertilization is promising strategy
164 can be used in natural biofortification process (24) hence proper management can help in
165 eliminating the issue of malnutrition and hidden hunger among the world's population as
166 result of Fe deficiency.

168 **3.3 Grain crude protein as affected by nitrogen forms and levels**

169 Significant differences ($p<0.05$) on crude protein content were observed between nitrogen
170 rates and sources in the two study sites. There was a relative increase in the protein content
171 in the grain with the increase in nitrogen fertilization of different N forms/sources.
172 Ammonium sulphate applied at the dosage of 50 kg ha^{-1} led to the highest crude protein
173 content, with values of 15.06% and 22.60 % in Ahero and Mwea respectively (Table 1). Just
174 like in case of Fe uptake, the control also had the lowest crude protein content of 10.25% in
175 Ahero and 15.41% in Mwea sites respectively. The low levels of crude protein contents in the
176 unfertilized plots is a clear indication that nitrogen fertilizer is key in enchasing quality of the
177 rice grain. According to the report given by Silva *et al.* (25), nitrogen application at silking
178 also increased kernel crude protein content of maize grain. This is could be due to increased
179 uptake and high demand for nitrogen by the plants, specifically at reproductive stage.
180 Moreover, this process is an added advantage at this stage since there are less nitrogen losses
181 from the field hence reducing the farmers' production costs. In another research (26), it was
182 found out that slow release fertilizers like urea led to a significant increase in grain protein
183 content of cereals crops such as the wheat and rice. The slow release N fertilizers are
184 effective in maintaining a balanced uptake of applied N that matches the needs of the crop at
185 different growth stages. Therefore, due to increased nitrogen efficiency throughout the
186 growing period, it implies a higher amount of crude protein in the grain, hence promoting the
187 quality of the grain effective for human consumption. Similar findings (27) were reported,

indicating that an increase in supply of nitrogen to rice plants had a corresponding increase in crude protein. This was associated with plant mechanisms to translocate absorbed nitrogen in high supply to essential storage organs such as the grain hence boosting the quality of the rice grains.

3.4 Grain Zinc uptake as affected by nitrogen forms and levels

Zinc accumulation in the rice grain revealed significant differences ($p \leq 0.05$) between nitrogen sources and rates in both Ahero and Mwea sites (Figure 2). There was an increase in the concentration of Zn in the grains with an increased supply of N. Urea at 50 kg ha^{-1} was the most superior in both sites with 21.67 mg kg^{-1} and 26.83 mg kg^{-1} in Ahero and Mwea sites respectively.

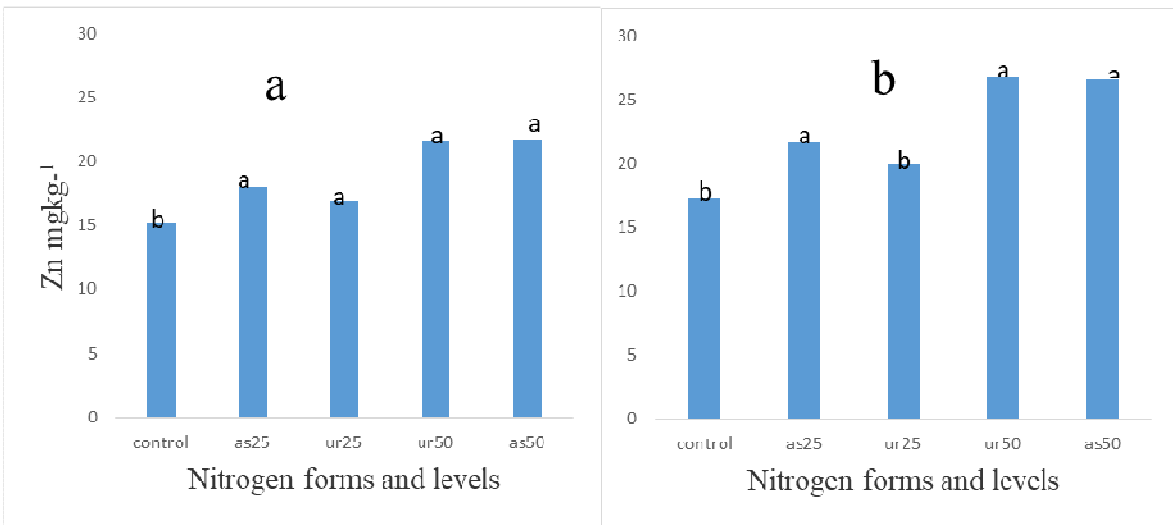


Figure 2. Zinc concentration as affected by nitrogen levels and forms in Ahero (a) and Mwea study sites (b) respectively.

The 50 kg ha^{-1} urea rate was followed by ammonium sulphate at 50 kg ha^{-1} in both study sites and the unfertilized treatments had the lowest zinc concentration in the grain. These findings agree with those of Kutman *et al.* (28) who reported an increase of Zn accumulation in wheat

grain upon increase of N fertilization. Importantly, they argued that the uptake of Zn to the grain is facilitated by the metal chelating compounds. In addition, increase of N supply enhanced remobilization of Zn from pre-anthesis phase to the grain (29). The improved uptake of Zn in the rice grain is an indication of improved remobilization and transportation of microelements from the roots to the shoot and then to the grain during grain filling stage (30). Similar reasoning had previously been advanced in an excellent review by Cakmak, (3).

Conclusion

Nitrogen fertilization had a significant effect in yield and quality of rice grain. Increase in nitrogen levels regardless of the forms led to an increase in both yield and quality of the rice grain. Therefore, farmers can use both urea and ammonium sulphate at higher rates (50 kg ha⁻¹) for improved yield. The concentrations of micronutrients including, iron and zinc increased in rice grains in response to nitrogen levels in both study sites. Therefore, there is clear indication that supply of adequate nitrogen fertilizers to rice crop is promising strategy to curb the issue of food insecurity as well malnutrition. Rice growers need to be advised on the optimal rates and N forms to be applied to enhance economic and health returns from their production systems.

REFERENCES

- Fageria, N. K., Baligar, V. C., and Li, Y. C. (2008). The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of plant nutrition*, 31(6), 1121-1157.
- Atera, E. A., Onyancha, F. N., and Majiwa, E. B. (2018). Production and marketing of rice in Kenya: Challenges and opportunities. *Journal of Development and Agricultural Economics*, 10(3), 64-70.

230 Cakmak, I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic
231 biofortification?. *Plant and soil*, 302(1-2), 1-17.

232 Yuan, L., Wu, L., Yang, C., and Lv, Q. (2013). Effects of iron and zinc foliar applications on
233 rice plants and their grain accumulation and grain nutritional quality. *Journal of the*
234 *Science of Food and Agriculture*, 93(2), 254-261.

235 Pearson, J. N., and Rengel, Z. (1994). Distribution and remobilization of Zn and Mn during
236 grain development in wheat. *Journal of Experimental Botany*, 45(12), 1829-1835.

237 Lee, S., Jeon, U. S., Lee, S. J., Kim, Y. K., Persson, D. P., Husted, S., . and An, G. (2009).
238 Iron fortification of rice seeds through activation of the nicotianamine synthase gene.
239 *Proceedings of the National Academy of Sciences*, 106(51), 22014-22019.

240 Hänsch, R., and Mendel, R. R. (2009). Physiological functions of mineral micronutrients (Cu,
241 Zn, Mn, Fe, Ni, Mo, B, Cl). *Current opinion in plant biology*, 12(3), 259-266.

242 Kim, J. W., Kim, B. C., Lee, J., Lee, D. R., Rehman, S., and Yun, S. J. (2013). Protein
243 content and composition of waxy rice grains. *Pakistan Journal of Botany*, 45(1), 151-
244 156.

245 Gweyi-Onyango, J P. (2018). Effects of Nitrogen Levels on Yield and Nitrogen Utilization
246 Efficiencies of Two Rice Varieties in Kirinyaga County, Kenya. *African Journal of*
247 *Horticultural Science*, 14(23), 23-34.

248 Clarkson, D. T., and Hanson, J. B. (1980). The mineral nutrition of higher plants. *Annual*
249 *review of plant physiology*, 31(1), 239-298.

250 Tester, M., and Langridge, P. (2010). Breeding technologies to increase crop production in a
251 changing world. *Science*, 327(5967), 818-822.

252 Njinju, S M, Samejima H, Katsura K, Kikuta M, Gweyi-Onyango J P, Kimani J M.,
253 Yamauchi A and Makihara D (2018). Grain yield responses of lowland rice varieties

254 to increased amount of nitrogen fertilizer under tropical highland conditions in central
 255 Kenya. *Plant Production Science*. 21:2, 59-70

256 Jones, J. B. Jr and Case, V W. (1990). Sampling, handling and analyzing plant tissue
 257 samples. In: R. L. Westerman (ed), Soil testing and plant analysis. 3rd ed. *Soil Sci.*
 258 *Soc. Am*, Inc. Madison, WI. p. 389-447

259 AOAC. (2007). *Official Methods of Analysis*. (17th edition, Revision 2). AOAC
 260 International. Gaithersburg, Maryland 20877-2417, USA.

261 Bremner, J. M (1996). Nitrogen-Total. In *Methods of Soil Analysis*. Part 3, Chemical
 262 Methods; Sparks, D. L., Ed.; Soil Science Society of America, American Society of
 263 Agronomy: Madison, WI, pp 1085–1121

264 Moro, B. M., Nuhu, I. R., Ato, E., and Naathanial, B. (2015). Effect of nitrogen rates on the
 265 growth and yield of three rice (*Oryza sativa* L.) varieties in rain-fed lowland in the
 266 forest agro-ecological zone of Ghana. *International Journal of Agricultural Science*,
 267 5(7), 878-885.

268 Ahmed, I. A., Abo-Elenin, R. A., and Ahmed, W. E. (1993). Interactive effects of salinity and
 269 both forms and levels of nitrogen fertility on growth and yield of barley. In *Towards*
 270 *the rational use of high salinity tolerant plants* (pp. 121-128). Springer, Dordrecht.

271 Sabir, S., Bakht, J., and Shafi, M. (2002). Effect of foliar vs. broadcast application of
 272 different doses of nitrogen on wheat. *Asian Journal of Plant Sciences*

273 Zhu, D. W., Zhang, H. C., Guo, B. W., Ke, X., Dai, Q. G., Wei, H. Y., ... and Huo, Z. Y.
 274 (2017). Effects of nitrogen level on yield and quality of japonica soft super rice.
 275 *Journal of integrative agriculture*, 16(5), 1018-1027.

276 Wekha N. Wafula, Korir K. Nicholas, Ojulong F. Henry, Moses Siambi and Joseph P.
 277 Gweyi-Onyango (2017). Finger millet (*Eleusine coracana* L.) grain yield and yield
 278 components as influenced by phosphorus application and variety in Western Kenya.

279 Tropical Plant research: An Agricultural Journal. 3(3): 673–680, 2016 DOI:
 280 10.22271/tpr.2016.v3.i3.08

281 Duhan, B. S., & Singh, M. (2002). Effect of green manuring and nitrogen on yield and uptake
 282 of micronutrient by rice. *Jouranl of the Indian Society of Soil Science (India)*.

283 Khoshgoftarmanesh AH, Schulin R, Chaney R L, Daneshbakhsh B and Afyun M (2010).
 284 Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable
 285 agriculture. A review. *Agron. Sustain. Dev.* 30: 83–107 .

286 Baranawatti N, Giehl RFH, Bauer Ba and Von Wirén N (2013). Influence of inorganic
 287 nitrogen fertilizer forms on micronutrient retranslocation in grains of winter wheat.
 288 *Frontiers in Plant Science*. 4(320): 1-11

289 Bouis H E and Saltzman A (2017). Improving nutrition through biofortification: A review of
 290 evidence from HarvestPlus, 2003 through 2016. *Global Food Security*. 12: 49–58

291 Silva, P. R. F. D., Strieder, M. L., Coser, R. P. D. S., Rambo, L., Sangoi, L., Argenta, G., and
 292 Silva, A. A. D. (2005). Grain yield and kernel crude protein content increases of
 293 maize hybrids with late nitrogen side-dressing. *Scientia Agricola*, 62(5), 487-492.

294 Khalil, J. K., Sawaya, W. N., Hamdallah, G., and Prasad, J. D. (1987). Effect of nitrogen
 295 fertilization on the nutritional quality of wheat variety, Yecora Rojo. *Plant Foods for*
 296 *Human Nutrition*, 36(4), 279-293.

297 Song, Y. J., Choi, I. Y., Sharma, P. K., and Kang, C. H. (2012). Effect of different nitrogen
 298 doses on the storage proteins and palatability of rice grains of primary and secondary
 299 rachis branches. *Plant Production Science*, 15(4), 253-257

300 Kutman, U. B., Yildiz, B., and Cakmak, I. (2011). Effect of nitrogen on uptake,
 301 remobilization and partitioning of zinc and iron throughout the development of durum
 302 wheat. *Plant and Soil*, 342(1-2), 149-164

303 Hao, H. L., Wei, Y. Z., Yang, X. E., Ying, F. E. N. G., and Wu, C. Y. (2007). Effects of
304 different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and
305 grain quality in rice (*Oryza sativa*). *Rice Science*, 14(4), 289-294.

306 Chandel, G., Banerjee, S., See, S., Meena, R., Sharma, D. J., and Verulkar, S. B. (2010).
307 Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe,
308 Zn and protein contents. *Rice Science*, 17(3), 213-227.

309