

Original Research Article

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

Abstract

Nitrogen is one of the limiting element 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 in designated research stations. The Experiments were laid out in Randomized Complete Block Design (RCBD) in a factorial arrangement with three replicates giving a total of 36 plots each measuring 3m by 4m. Two nitrogen forms including Urea and Ammonium sulphate were applied in three levels (0kg ha^{-1} , 25kg ha^{-1} and 50kg ha^{-1} respectively). Data on yield was collected at harvest, while Zinc, Iron and protein concentrations were done in the laboratory using standard protocols. 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 mgkg^{-1} and 195.5 mgkg^{-1} in Ahero and Mwea respectively. A similar trend was observed in both crude protein and Iron accumulation in the rice grain. The Unfertilized plot 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 hidden hunger, hence enhancing food insecurity and reducing malnutrition through increased yield and quality of rice.

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

36 **1. Introduction**

37 Nitrogen nutrition in rice crop is a major requirement in growth and development. It is a
38 limiting factors in plant and development and has also been reported to affect uptake of other
39 micro-nutrients that are essential for human nutrition (Fageria *et al.*, 2008). In Kenya, rice is
40 the third most important cereal crop that contributes a greater percentage toward curbing the
41 issue of food insecurity in the country (Atera *et al.*, 2018). Of essence, the micronutrients
42 play great role in promoting growth and development of the rice plants. Zinc is one of the
43 micro-nutrients essential in growth and reproduction of rice and is useful in synthesis of
44 substrates and enzyme that enhance transcription. It has been reported to be one of the
45 element leading to the greatest disorders and limits growth and production in rice especially
46 in lowland countries (Cakmak, 2008). Zinc's major function is to also promote physiological
47 metabolisms of the rice plants. Its deficiency has also been viewed as one of the major
48 challenges limiting rice production and its contribution to the maximum nutritional quality
49 upon consumption. When substantial amount of the Zn is accumulated in the tissues, it is
50 later remobilized and stored in the grain. The amounts of micronutrients that accumulated in
51 the grain are affected by proper nitrogen nutrition (Yuan *et al.*, 2013).

52 Iron on the other hand, is an element essential for oxidation and transportation of the electro
53 elements. It also maintains the structure and function of the chloroplasts. Several reports have
54 denoted that there is low accumulation of Zinc and Fe in the grain of rice hence leading to
55 low quality of nutritional needs (Pearson and Rengel, 1994, Lee *et al.*, 2009). Low
56 concentration of zinc and iron in the grain of rice has been associated to be the leading factors
57 in increased rates of malnutrition the world (Hänsch and Mendel, 2009). Crude protein
58 provides another measure of quality in grain as it determines its nutritional content (Kim *et*
59 *al.*, 2013).

60 The protein content in the grain is also a representation of nitrogen harvest index which
61 depends on the ability of the plants to uptake the available and supplied Nitrogen into
62 potential yield (Gweyi-Onyango, 2018). Therefore, this implies that that there is great
63 problem that needs to be addressed on crude protein content , zinc and iron concentration in
64 the grain to ensure the required nutritional quality is achieved. Nitrogen fertilization has been
65 marked as one of the strategies that can enhance improved quality of the grain (Clarkson and
66 Hanson, 1980, Tester and Langridge, 2010). Therefore, the current study was carried out to
67 evaluate the effects of N fertilization under different levels and forms on yield, crude protein
68 content, zinc and Fe concentration in rice grain.

69 **2. MATERIALS AND METHODS**

70 **2.1 Description of the study site**

71 The study was carried out in two locations; Ahero in Kisumu county and National
72 Research Irrigation Board research station and in Mea Kirinyaga county at Kirogo farm
73 research station. Kisumu receives rainfall almost throughout the year and thus does not have
74 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
75 $59.99''$ W. The average temperature is 22.9°C and an annual rainfall of between 1200 mm
76 and 1300 mm in various locations. Mwea, Kirinyaga County on the hand is located in central
77 parts of country. It lies between 1158 M and 5380 M above the sea level and Latitude (05°
78 and 04° S) and Longitude (37° and 38° E) with temperatures ranging between 12°C and 26°C
79 (Njinju *et al.*, 2018). It has both the tropical climate and the equatorial rainfall patterns
80 annual rainfall ranges between 800-2200 mm and this influenced by its position along the
81 equator and on the windward side of Mt. Kenya. The experiment was carried out during the
82 min growing season of rice in Kenya from July to December 2017.

83

84 **2.2 Experimental Design and Data collection.**

85 The experiment was laid as Randomized Complete Block Design (RCBD) in a factorial
86 arrangement and replicated three times. The treatments included; two forms of nitrogenous
87 fertilizers which were: Sulphate of Ammonia (SA) and Urea at different levels (0kg ha^{-1} , 25kg
88 ha^{-1} and 50kg ha^{-1}). There were two methods of applications; full dose and two splits. The plot
89 sizes measured 3m by 4m. Three weeks old seedlings were transplanted at a spacing (25×15)
90 cm in the 36 plots. The fields were kept weed-free through manual weeding at different
91 stages. Watering was done regularly and any incidences of pests and diseases were managed.
92 Birds were managed through physical scaring while the fungal diseases were controlled using
93 fungicides.

94 At maturity, the rice grain was harvested, and dried up to 14 % moisture content. They were
95 ground and analysis of Zinc and Iron done through the use of the ash solution using an ICP
96 spectrophotometer in a procedure described by Jones and Case (1990). Nitrogen content was
97 determined following the Micro Kjeldahl method described in AOAC (2007). The grain crude
98 protein content was determined through multiplication of N content (this was determined by
99 Kjeldahl method according to (Bremner, 1996) with a standard value of 6.25.

100

101 **2.3 Data Analysis**

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

107

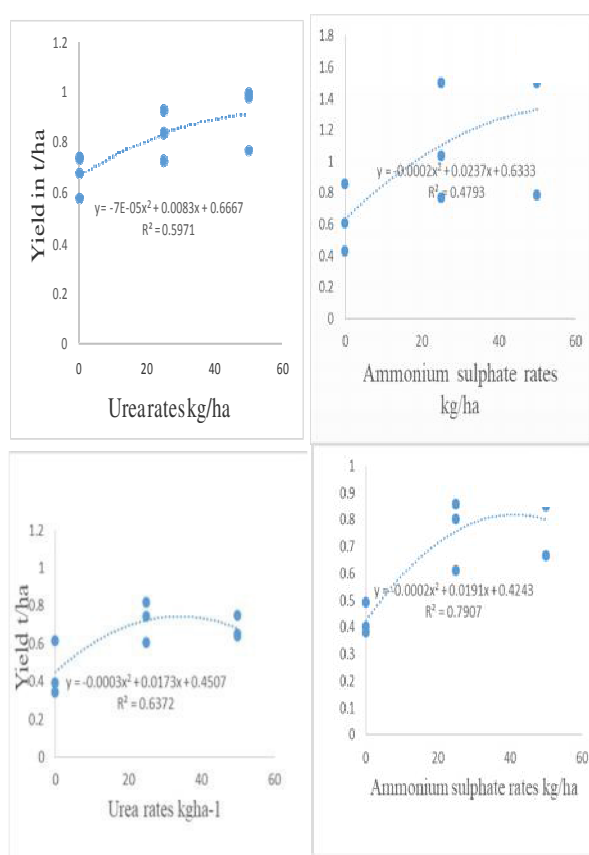
108

109

110 3 Results and Discussions

111 3.1 Influence of nitrogen forms and levels on grain yield

112 From the regression analyses shown in figure 2, it was revealed that yield increased with N
 113 rates and the response assumed polynomial function, particularly at Mwea site where the R^2
 114 values were 0.63 and 0.70 for urea and ammonium sulphate respectively. The trends were
 115 however very different at Ahero site with R^2 values being quite low and hence meant that
 116 yield increase could barely be explained by increasing N rates. For instance, nitrogen rates
 117 could only explain 47 percent of yield when N was applied as ammonium sulphate at Ahero
 118 site (Fig. 2 b) while the remaining 53 percent of yield were due to other factors other than N.
 119 It was also clear that rice yield still responded to additional N supply at Ahero (irrespective to
 120 N source). This was not reported in Mwea site which showed an optimal rate at about 45 kg
 121 $N\ ha^{-1}$.



The shapes are not clear. More clearly and separately a, b, c, d should indicate**Figure 2:**
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 increment in the addition would probably lead to more yield. This may be pointer that the doses used by the farmers are well below the plant's needs, which to partially agree with the high NUE in these sites (Gweyi-Onyango 2018). 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 Kirinyaga results in our current work (Fig. 2 c and d) there seemed to be 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 Njinju *et al.* (2018) 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.* (2015) in Ghana who reported an increment in grain yield in rain fed rice up to a certain level of 90kg ha^{-1} and declining beyond this level. The disagreement could be probably due to the different environment conditions since in rain fed 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 in agreement with those of Ahemed *et al.* (1993) 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 addition of N levels (Sabir *et al.*, 2002). The results are also in agreement with those of Zhu *et al.* (2017) who reported an increased 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*

147 *al.*, 2017) who reported a similar trend finger millet when supplied with different levels of
148 phosphorous.

149 3.2 Effects of nitrogen form and levels on grain Iron (Fe) Uptake

150 The iron uptake in rice grain was significantly ($P<0.05$) different due to levels and forms or
151 sources of N in both experimental sites. Ammonium sulphate applied at 50kg ha^{-1} elicited an
152 increased uptake of iron in the grain in both sites with 163.8mg kg^{-1} and 195mg kg^{-1} in ahero
153 and Mwea respectively, this was followed by urea 50kg ha^{-1} while the control(unfertilized
154 plots had the lowest concentration of 119.5mg kg^{-1} in Ahero and 139mg kg^{-1} in Mwea
155 (Table 1).

156 **Table 1: Crude protein and Iron concentration in rice grain as affected by nitrogen**
157 **forms and levels**

	Ahero		Mwea	
	Crude protein	Iron Fe	Crude protein	Iron
	Percent	mg kg^{-1}	Percent	mg kg^{-1}
N rates kg ha^{-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

158 **Means followed by the same letter within the same column are not significantly**
159 **different ($P\leq 0.05$).**

160

The increase of N concentration in the grain 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 (2002), who reported that there 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 as an effect of nitrogen fertilization is associated with high uptake of micronutrients like Iron due to a high pool of translocation to the grain (Khoshgoftarmanesh *et al.*, 2010). This argument is in concurrence with those of Barunawati *et al.* (2013). The results of this study thus suggests that nitrogen fertilization is promising strategy can be used in natural biofortification process (Bouis and Saltzman, 2017) hence proper management can help in eliminating the issue of malnutrition and hidden hunger among the world's population as result of Fe deficiency.

3.3 Grain crude protein as affected by nitrogen forms and levels

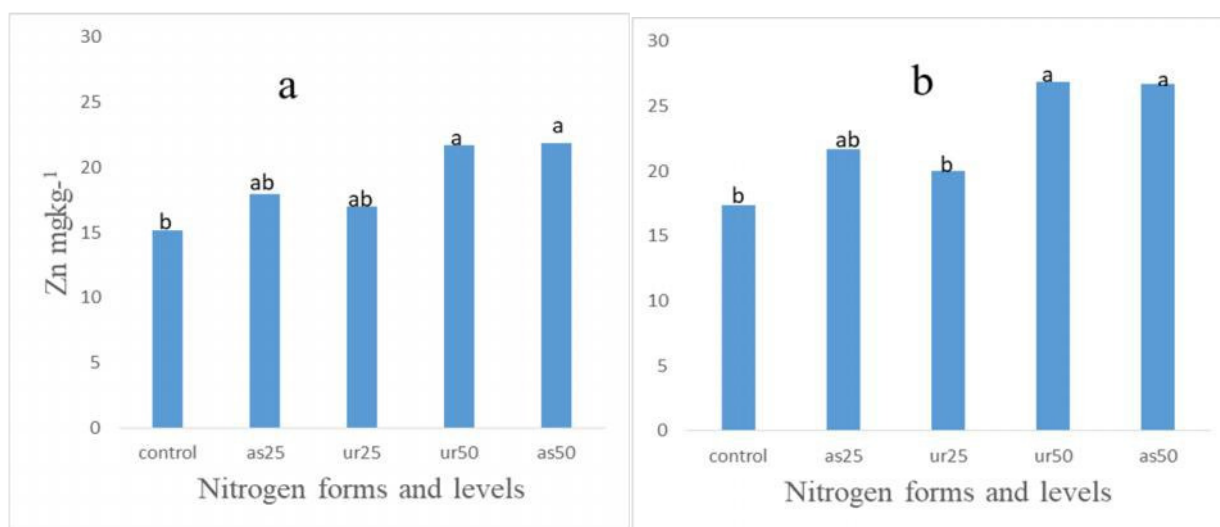
Significant differences ($p < 0.05$) on crude protein content were observed between nitrogen rates and sources in the two study sites. There was a relative increase in the protein content in the grain with the increase in nitrogen fertilization of different N forms/sources. Ammonium sulphate applied at the dosage of 50 kg ha⁻¹ had the highest crude protein content with values of 15.06% and 22.60 % in Ahero and Mwea respectively (Table 1). Just like in case of Fe uptake, the control for in case also had the lowest crude protein content of 10.25% in Ahero and 15.41% in Mwea sites. The low levels of crude protein content in the unfertilized plot is a clear indication that nitrogen fertilizer is key enhancing quality of the rice grain. According to the report given by Silva *et al.* (2005), nitrogen application at silking also increased kernel crude protein content of maize grain. This is due to increase uptake and

high demand for nitrogen by the plants specifically at reproductive stage. Moreover, this process is an added advantage at this stage since there are less nitrogen losses from the field hence reducing the production cost on the farmer's side. In another report done by Khalil *et al.* (1997~~It doesn't match the references. You must check.~~), it was found out that slow release fertilizers like Urea lead to a significant increase in grain protein content of cereals crops such as the wheat and rice. The slow release N fertilizers are effective in maintaining a balanced uptake of applied N that matches the needs of the crop at different growth stages. Therefore, due to increased nitrogen efficiency throughout the growing period implies a higher amount of crude protein in the grain, hence promoting the quality of the grain effective for human consumption. Similar findings were reported by Song *et al.* (2012) were reported, indicating that an increase in supply of nitrogen to rice plants 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 ($P < 0.05$) between nitrogen sources and rates in both Ahero and Mwea sites (figure 1). There was an increase in the concentration of Zn in the grains with an increased supply of N. Urea 50kg ha^{-1} was the most superior in both sites with 21.67mg kg^{-1} and 26.83mg kg^{-1} .

205



206

207 **Figure 1. Zinc concentration as affected by nitrogen levels and forms in Ahero (a) and**
 208 **Mwea study sites (b) respectively. –**

209 This was followed by Ammonium sulphate at 50kg ha⁻¹ in both study sites and the
 210 unfertilized had the lowest zinc concentration in the grain. These findings agree with those of
 211 Kutman *et al.* (2011) who reported an increase of Zn accumulation in wheat grain upon
 212 increase of N fertilization. Importantly, they argued that the uptake of Zn to the grain is
 213 facilitated by the metal chelating compounds. In addition, increase of N supply enhanced
 214 remobilization of Zn from pre-anthesis phase to the grain (Hao *et al.*, 2007). The improved
 215 uptake of Zn in the rice grain is an indication of improved remobilization and transportation
 216 of microelements from the roots to the shoot and then to the grain during grain filling stage
 217 (Chandel *et al.*, 2010). Similar reasoning had previously been advanced in an excellent
 218 review by Cakmak, (2008).

219 Conclusion

220 Nitrogen fertilization had a significant effect in yield and quality of rice grain. Increase in
 221 nitrogen levels regardless of the forms led to an increase in both yield and quality of the rice
 222 grain. The micronutrients including, Iron and Zinc have been were 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 [NWhat should be at the rate. Please explain](#) forms to be applied to enhance economic and health returns from their production systems.

REFERENCES

- Ahmed, I. A., Abo-Elenin, R. A., and Ahmed, W. E. (1993). Interactive effects of salinity and both forms and levels of nitrogen fertility on growth and yield of barley. In *Towards the rational use of high salinity tolerant plants* (pp. 121-128). Springer, Dordrecht.
- 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.
- Baranawatti N, Giehl RFH[It doesn't fit the rules. You must check.](#), Bauer Ba and Von Wirén N (2013). Influence of inorganic nitrogen fertilizer forms on micronutrient retranslocation in grains of winter wheat. *Frontiers in Plant Science*. 4(320): 1-11
- Bouis H E and Saltzman A (2017). Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security*. 12: 49–58
- Bremner, J. M (1996). Nitrogen-Total. In *Methods of Soil Analysis*. Part 3, Chemical Methods; Sparks, D. L., Ed.; Soil Science Society of America, American Society of Agronomy: Madison, WI, pp 1085–1121.
- Cakmak, I. (2008). Enrichment of cereal grains with zinc: agronomic or genetic biofortification?. *Plant and soil*, 302(1-2), 1-17.
- Chandel, G., Banerjee, S., See, S., Meena, R., Sharma, D. J., and Verulkar, S. B. (2010). Effects of different nitrogen fertilizer levels and native soil properties on rice grain Fe, Zn and protein contents. *Rice Science*, 17(3), 213-227.

- 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 Duhan, B. S., & Singh, M. (2002). Effect of green manuring and nitrogen on yield and uptake
251 of micronutrient by rice. *Jouranl of the Indian Society of Soil Science (India)*.
- 252 Fageria, N. K., Baligar, V. C., and Li, Y. C. (2008). The role of nutrient efficient plants in
253 improving crop yields in the twenty first century. *Journal of plant nutrition*, 31(6),
254 1121-1157.
- 255 ~~Fitzgerald, M. A., McCouch, S. R., & Hall, R. D. (2009). Not just a grain of rice: the quest~~
256 ~~for quality. *Trends in plant science*, 14(3), 133-139.~~
- 257 Gweyi-Onyango, J P. (2018). Effects of Nitrogen Levels on Yield and Nitrogen Utilization
258 Efficiencies of Two Rice Varieties in Kirinyaga County, Kenya. *African Journal of*
259 *Horticultural Science*, 14(23), 23-34.
- 260 Hänsch, R., and Mendel, R. R. (2009). Physiological functions of mineral micronutrients (Cu,
261 Zn, Mn, Fe, Ni, Mo, B, Cl). *Current opinion in plant biology*, 12(3), 259-266.
- 262 Hao, H. L., Wei, Y. Z., Yang, X. E., Ying, F. E. N. G., and Wu, C. Y. (2007). Effects of
263 different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and
264 grain quality in rice (*Oryza sativa*). *Rice Science*, 14(4), 289-294.
- 265 Khalil, J. K., Sawaya, W. N., Hamdallah, G., and Prasad, J. D. (1987). Effect of nitrogen
266 fertilization on the nutritional quality of wheat variety, Yecora Rojo. *Plant Foods for*
267 *Human Nutrition*, 36(4), 279-293.
- 268 Khoshgoftarmanesh AH, Schulin R, Chaney R L, Daneshbakhsh B and Afyun M (2010).
269 Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable
270 agriculture. A review. *Agron. Sustain. Dev.* 30: 83–107 .

- 271 Kim, J. W., Kim, B. C., Lee, J., Lee, D. R., Rehman, S., and Yun, S. J. (2013). Protein
272 content and composition of waxy rice grains. *Pakistan Journal of Botany*, 45(1), 151-
273 156.
- 274 Kutman, U. B., Yildiz, B., and Cakmak, I. (2011). Effect of nitrogen on uptake,
275 remobilization and partitioning of zinc and iron throughout the development of durum
276 wheat. *Plant and Soil*, 342(1-2), 149-164
- 277 ~~Lakshmanan, R., Prasad, R., & Jain, M. C. (2005). Yield and uptake of micronutrients by rice~~
278 ~~as influenced by duration of variety and nitrogen fertilization. *Archives of Agronomy*~~
279 ~~and *Soil Science*, 51(1), 1-14.~~
- 280 Lee, S., Jeon, U. S., Lee, S. J., Kim, Y. K., Persson, D. P., Husted, S., . and An, G. (2009).
281 Iron fortification of rice seeds through activation of the nicotianamine synthase gene.
282 *Proceedings of the National Academy of Sciences*, 106(51), 22014-22019.
- 283 Moro, B. M., Nuhu, I. R., Ato, E., and Naathanial, B. (2015). Effect of nitrogen rates on the
284 growth and yield of three rice (*Oryza sativa* L.) varieties in rain-fed lowland in the
285 forest agro-ecological zone of Ghana. *International Journal of Agricultural Science*,
286 5(7), 878-885.
- 287 Njinju, S M, Samejima H, Katsura K, Kikuta M, Gweyi-Onyango J P, Kimani J M.,
288 Yamauchi A and Makihara D (2018). Grain yield responses of lowland rice varieties
289 to increased amount of nitrogen fertilizer under tropical highland conditions in central
290 Kenya. *Plant Production Science*. 21:2, 59-70
- 291 Pearson, J. N., and Rengel, Z. (1994). Distribution and remobilization of Zn and Mn during
292 grain development in wheat. *Journal of Experimental Botany*, 45(12), 1829-1835.
- 293 Sabir, S., Bakht, J., and Shafi, M. (2002). Effect of foliar vs. broadcast application of
294 different doses of nitrogen on wheat. *Asian Journal of Plant Sciences*

- 295 Silva, P. R. F. D., Strieder, M. L., Coser, R. P. D. S., Rambo, L., Sangoi, L., Argenta, G., and
296 Silva, A. A. D. (2005). Grain yield and kernel crude protein content increases of
297 maize hybrids with late nitrogen side-dressing. *Scientia Agricola*, 62(5), 487-492.
- 298 Song, Y. J., Choi, I. Y., Sharma, P. K., and Kang, C. H. (2012). Effect of different nitrogen
299 doses on the storage proteins and palatability of rice grains of primary and secondary
300 rachis branches. *Plant Production Science*, 15(4), 253-257.
- 301 Tester, M., and Langridge, P. (2010). Breeding technologies to increase crop production in a
302 changing world. *Science*, 327(5967), 818-822.
- 303 Welch, R. M. (2005). Biotechnology, biofortification, and global health. *Food and Nutrition*
304 *Bulletin*, 26(4_suppl3), S304-S306.
- 305 Wekha N. Wafula, Korir K. Nicholas, Ojulong F. Henry, Moses Siambi and Joseph P.
306 Gweyi-Onyango (2017). Finger millet (*Eleusine coracana* L.) grain yield and yield
307 components as influenced by phosphorus application and variety in Western Kenya.
308 Tropical Plant research: An Agricultural Journal. 3(3): 673–680, 2016 DOI:
309 10.22271/tpr.2016.v3.i3.08
- 310 Yuan, L., Wu, L., Yang, C., and Lv, Q. (2013). Effects of iron and zinc foliar applications on
311 rice plants and their grain accumulation and grain nutritional quality. *Journal of the*
312 *Science of Food and Agriculture*, 93(2), 254-261.
- 313 Zhu, D. W., Zhang, H. C., Guo, B. W., Ke, X., Dai, Q. G., Wei, H. Y., ... and Huo, Z. Y.
314 (2017). Effects of nitrogen level on yield and quality of japonica soft super rice.
315 *Journal of integrative agriculture*, 16(5), 1018-1027.