Effects of Copper (Cu) on Yield Components and Associated Traits in Segregating Populations of Lowland Rice (O. sativa L.)

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Abstract: Trace elements are very critical for rice growth of which Cu is one of the essential trace elements for rice and excess of copper becomes toxic to rice growth. The aim of this study was to determine the productivity increase in rice crop and genotype reactions to application of Copper under the tropical rainforest condition. Three experiments were established concurrently in randomized complete block design in three replications in pots. Treatment comprised of 6 breeding lines each from two rice populations of F2 and F3 generations and two popular checks. Experiment one is the control without CuSO₄ treatment, while experiment two and three is the F2 and F3 populations, respectively treated with CuSO₄ solution. Three concentration levels of CuSO₄ solution (15mg Cu/kg of soil, 30mg Cu/kg of soil and 60mg Cu/kg of soil) were applied into each pots a week before transplanting in the treated experiments. This study observed that at 30mg of Cu/kg of soil is the optimum level for rice performance based on these experiments beyond, reduction in rice performance. Reduction of 24.92% and 22.12% of total grain yield of F2 and F3 populations at 60mg of Cu/kg of soil as compared to the control were recorded, stable and high yielding genotypes across the copper concentration levels were identified for copper breeding programme.

Keywords: Genotypes, Populations, Micronutrients, GGE Biplot, Rice

1. Introduction

Rice is one of the cereal crops globally consumed and is now become a staple crop mostly in Asian countries and part of the developing counties in the world. The demand for rice is far outstrip its production globally and there is need to address some of the limiting factors of rice production at least closing the gap between demand and production of rice. The soil fertility level is depleting annually due to human activities as well, the scenario of global warming thus affecting rice production. Soil micronutrients as the name implied but very important elements in rice production, often, they are one of the most depleted by the aforementioned factors, thus, there is need to mitigate the effects on rice production. Copper being a transition metal is considered as a trace element with a low concentration in biological tissues but essential for life [1]. Copper was first identified as a plant nutrient and its solubility in soil is greatly dependent on soil pH and dissolved organic matter content [2] and becomes readily available at a pH below 6 [3, 4]. The requirement of Cu for healthy plant growth and development varies with plant species and cultivars [5]. Excess Cu in plants can also be genotoxic, means is capable of generating genetic mutations. In rice roots, excess Cu specifically altered levels of genes involved in fatty acid metabolism and cellular component biogenesis. Toxic effects of Cu in plants can be observed by reduction of yield, poor seed germination, stunted leaf and root growth [6, 7]. In plants, Cu deficiency altered root and leaf construction, as well as significant
reduction in chlorophyll pigments and photosynthesis [8].

The requirement of Cu for plant growth particularly rice varies with species and genotypes [5]. Phytotoxicity of Cu depends mostly on its solubility and availability in the soil. However, the deficiency and excess of Cu affect plant growth and this could alter important biochemical processes. There are reports on the threshold for Cu deficiency in plants, however, it depends on crop species and other environmental factors [5].

The plant height of rice was reduced as a result of Copper toxicity [10]. Plant height could be used for assessing crop performance [9]. A report showed that tillering ability is delayed with increasing levels of soil Copper and excess Cu concentration led to slow recovery from transplanting, delayed tillering and reduction of maximum tiller numbers [10]. Soil Cu treatments had a large impact on number of spikelets per panicle, which decreased with the increase levels of soil Cu [10]. The toxic effect of Copper on rice yield significantly increased with increasing level of Cu concentration. Micronutrient fertilization also referred to agronomic fortification improves crop yield for human consumption, and could also address crop nutritional quality and micronutrient dietary of humans’ health [11]. The aim of this study was to determine the productivity increase in rice crop and genotype reactions to the application of Copper under the tropical rainforest condition.

### 2. Materials and Methods

The study was conducted in the greenhouse using soil collected from the experimental farm of the International Institute of Tropical Agriculture (IITA) Onne, (longitude 7°95′28″E and latitude 4°43′78″N) in the Humid forest ecological zone of Nigeria. Mean annual rainfall in the zone is 2310.9 mm and it falls mainly within the months of February to November with peak rainfall received in September. This is a pot experiment and Soil was collected from the research station field at 0 – 15 cm depth, sterilized and filled into 4kg pot to minimize uneven distribution of CuSO₄ in the pots [12].

Three experiments were established concurrently in randomized complete block design in three replications in pots. Treatment comprised of 6 breeding lines each from two rice populations of F2 and F3 generations and two popular checks (Table 1.). Experiment one is the control without CuSO₄ treatment, while experiment two and three are F2 and F3 populations, respectively treated with CuSO₄ solution. Three concentration levels of CuSO₄ solution (15mg Cu /kg of soil, 30mg Cu /kg of soil and 60mg Cu /kg of soil) were applied into each pots a week before transplanting in the treated experiments. The rice seeds were raised in the normal seedling nursery beds with untreated soil. The seedlings were transplanted at 21 days after sowing into treated pots with CuSO₄ [13] two seedlings per pot.

#### Table 1. Genetic material used for the experiment.

| S/N | Genetic materials | Pedigree | Source          |
|-----|-------------------|----------|-----------------|
| 1   | UPN 59            | 325845/FARO 44 | Uniport Germplasm Uniport |
| 2   | UPN 82            | 325861/UPIA 3 | Uniport Germplasm Uniport |
| 3   | UPN 86            | 325865/UPIA 2 | Uniport Germplasm Uniport |
| 4   | UPN 95            | 325876/FARO 52 | Uniport Germplasm Uniport |
| 5   | UPN 103           | 325879/FARO 44 | Uniport Germplasm Uniport |
| 6   | UPN 107           | 325892/FARO 57 | Uniport Germplasm Uniport |
| 7   | Checks            |           | Uniport Germplasm Uniport |
| 8   | FARO 44           |           | Uniport Germplasm Uniport |
| 9   | UPIA 2            |           | Uniport Germplasm Uniport |

#### 2.1. Data Collection

Data was collected at appropriate stage of the crop development. The agronomic characters were measured at weekly intervals. The ‘Standard Evaluation System (SES) for Rice’ reference manual [14] was used for all trait measurements except where stated otherwise.

#### 2.2. Statistical Analysis

Analysis of variance (ANOVA) was performed separately on the individual experiments using the PROC GLM of SAS [15]. Simple linear correlation analysis was performed using the PROC CORR program of SAS. Biplot analysis was employed to investigate the cultivar-by-environment interaction (site regression model) [16]. Biplot construction was based on the first two principal components (PC1 and PC2). The PC1 and PC2 are referred to as primary and secondary effects, respectively, and were derived from singular-value decomposition (SVD) of the environment-centred data [16]. The environment-centred data were subjected to SVD for the construction of the biplots. This resulted in three component matrices: singular value (SV) matrix, the cultivar eigenvector matrix, and the environment eigenvector matrix. Thus, the biplot was constructed based on the following model,[17]:

\[
Y_{ij} - G - E_j = \sum \lambda_n \epsilon_i \eta_j + e_{ij},
\]

where \(Y_{ij}\) = the measured mean trait of cultivar i in environment j; \(G\) = the grand mean; \(E_j\) = the mean effect of environment j; \((G + E_j)\) being the mean trait in environment j; \(\lambda_n\) = the SVD of nth principal component (PC), the square of which is the sum of square explained by PCn; \(\epsilon_i\) = the eigenvector of cultivar i for PCn; \(\eta_j\) = the eigenvector of environment j for PCn; and \(e_{ij}\) = the residual variation associated with genotype i in environment j.
3. Results

3.1. Agronomic Performance of the Tested Genotypes

Plant height showed highly significant difference among the tested genotypes (Table 2). Plant height increased with increasing concentration of copper in the soil up to 30mg Cu/kg of soil concentration and declined when above this level. It was observed that genotypes from F2 populations were taller than those from F3 population. These genotypes (UPN 103 and UPN 107), performed better than the overall mean in all concentration levels. The number of tillers increases with increasing copper concentration up till 30mg Cu/kg of soil and beyond, which the maximum tillering declined. Generally, the F3 population produced more tillers than the F2 population in all concentration levels. Genotype UPN 59 tiller more at 60mg Cu/kg of soil while UPN 86 and UPN 95 had more tillers at 30mg Cu/kg of soil level. (Table 3)

Table 2. Effects of copper concentrations on plant height (cm) of genotypes within F2 and F3 populations.

| Genotype | Control | 15mg of Cu | 30mg of Cu | 60mg of Cu |
|----------|---------|------------|------------|------------|
|          | F2      | F3         | F2         | F3         | F2         | F3         | F2         | F3         |
| UPN 59   | 72.50c  | 69.75de    | 77.75c     | 75.50c     | 88.50c     | 81.25e     | 68.25d     | 63.75d     |
| UPN 82   | 70.25c  | 66.00f     | 82.75c     | 80.00c     | 95.25b     | 90.50c     | 65.75f     | 62.25f     |
| UPN 86   | 73.75c  | 71.25d     | 81.75c     | 79.75c     | 87.50c     | 85.25d     | 74.00c     | 71.25c     |
| UPN 95   | 72.25c  | 68.25ef    | 79.00c     | 75.25c     | 87.75c     | 82.00de    | 66.50ef    | 63.25d     |
| UPN 103  | 87.25b  | 77.25c     | 106.50a    | 95.75a     | 123.25a    | 111.00a    | 80.25b     | 76.75b     |
| UPN 107  | 96.25a  | 94.00a     | 101.00ab   | 90.75b     | 119.75a    | 107.50a    | 91.00a     | 87.50a     |
| UPNIA 2  | 82.50b  | 85.25b     | 89.75bc    | 89.75b     | 98.25b     | 97.25b     | 74.50c     | 73.25c     |
| FARO 44  | 74.75c  | 76.50c     | 78.75c     | 80.00c     | 83.75c     | 84.50de    | 70.25d     | 71.75c     |
| Mean     | 78.69   | 76.03      | 87.16      | 83.34      | 98         | 92.41      | 73.81      | 71.22      |
| Coefficient of variation | 3.58 | 1.49 | 5.72 | 2.27 | 1.94 | 1.67 | 1.33 | 1.33 |
| Level of Significance | ** | ** | ** | ** | ** | ** | ** | ** |

*=significant at the 1%.

Table 3. Effect of copper concentration on Maximum number of tillers of genotypes within F2 and F3 population.

| Genotype | Control | 15mg of Cu | 30mg of Cu | 60mg of Cu |
|----------|---------|------------|------------|------------|
|          | F2      | F3         | F2         | F3         | F2         | F3         | F2         | F3         |
| UPN 59   | 9.00abc | 9.75cde    | 11.25ab    | 12.50bc    | 14.00abcd  | 15.00cd    | 10.00a     | 9.25a      |
| UPN 82   | 8.25bc  | 9.25e      | 10.00b     | 11.00de    | 12.75cd    | 15.30de    | 8.50b      | 8.75ab     |
| UPN 86   | 9.5ab   | 10.00bcd   | 12.00a     | 13.00ab    | 16.25a     | 17.50a     | 7.75b      | 8.75ab     |
| UPN 95   | 9.50ab  | 10.25abc   | 12.25a     | 14.00a     | 14.75abc   | 17.00ab    | 8.25b      | 9.00a      |
| UPN 103  | 8.00c   | 9.50cde    | 10.25b     | 11.75cd    | 13.75bcd   | 14.00def   | 6.25c      | 8.50b      |
| UPN 107  | 6.25d   | 7.75f      | 8.25c      | 10.00e     | 12.00d     | 13.00f     | 5.00d      | 5.75c      |
| UPNIA 2  | 10.25a  | 10.50ab    | 12.75a     | 13.00ab    | 15.25ab    | 16.00bc    | 8.00b      | 8.75ab     |
| FARO 44  | 10.25a  | 10.75a     | 11.50ab    | 11.50cd    | 13.25bcd   | 13.50ef    | 8.50b      | 8.75ab     |
| Mean     | 8.88    | 9.72       | 11.03      | 12.09      | 14         | 15.06      | 7.78       | 8.38       |
| CV       | 6.39    | 3.04       | 5.67       | 3.96       | 6.47       | 3.32       | 5.66       | 4.51       |
| LOS      | **      | **         | **         | **         | *          | **         | **         | **         |

*= significant at the 5%, **=significant at the 1%.

3.2. Performance of Post-harvest Traits of the Tested Genotypes

Effective tiller is the tiller that produce economic panicle at the time of harvest, which is very important in the determination of total grain yield of genotype. There was significant difference among all the genotypes both in F2 and F3 populations in all the Cu concentration levels except F3 population at 60mg Cu/kg of soil (Table 4). The 30mg Cu/kg of soil of copper concentration had the highest effective tillers for all the genotypes and UPN 95 and UPN 86 were highest in F3 population. At 60mg Cu/kg of soil, all genotype had reduction in effective tiller number, while FARO 44 had the highest number of effective tillers (Table 4).
For panicle length, there was significant difference among all the genotypes both in F2 and F3 populations in all the Cu concentration levels except at F3 population at 30mg of Cu and 60mg Cu/kg of soil (Table 6). It was observed that the two checks (UPIA 2 and FARO 44) had longer panicles than the test genotypes and F3 populations had longer panicle length than F2 in all the experiments. (Table 6).

The grain yield (GY) per hectare increased with increasing copper concentration in the soil but decreased at 60mg Cu/kg of soil. There is no significant difference among the genotypes in both F2 and F3 populations at 60mg Cu/kg of soil (Table 7). The F3 populations perform better than the F2 population in all Cu concentration levels based on grain yield. The UPN 59, UPN 82 and UPN 86 were among the best yielded tested genotypes in all Cu concentration and population levels. (Table 7)
populations (Figure 2). Three mega environments were identified for the two populations, environment one and two respectively, while FARO 44 yield highest in environment three for F2 and UPN 59 for F3 population. The highest yielding genotype were UPN 86 and UPN 59 for F2 and F3 populations, respectively (Figure 1 and Figure 2). The environment two and three were opposite of both side of the perpendicular double-headed arrows indicated the mean grain yield of the experiments. Environment one for both populations is very closed to the appendicular line, which indicates to be close to an ideal environment for the experiment. The genotypes at the vertices of the pentagon had highest GY at that environment, UPN 86 and UPN 95 had the highest GY in environment one and two respectively, while FARO 44 yield highest in environment three for F2 and UPN 59 for F3 population (Figure 1 and Figure 2).

Performance of genotypes were ranked in the direction indicated by the single-headed arrow (average tester coordinate) in ascending order of the mean grain yield of the experiments. Therefore, Stability of genotypes ranked on the basis of their projection from the average tester coordinate (axis) on the average environment main effect. The greater the length of the projection of a genotype, the more unstable that genotype was (Figure 3 and Figure 4).

Table 8. Linear correlation coefficient of growth and yield parameters for F2 and F3 population (Copper environment).

| TRAITS      | PHT_C2 | NTI_C2 | ET_C2 | PAL_C2 | NPPP_C2 | 1000GWT_C2 | YLD_C2 |
|-------------|--------|--------|-------|--------|---------|------------|--------|
| PHT-C3      | 0.39ns |        |       |        |         |            |        |
| NTI_C3      | 0.40*  | 0.95***|       |        |         |            |        |
| ET_C3       | 0.66** | 0.50** | 0.57**|        |         |            |        |
| PAL_C3      | 0.36*  | 0.90***| 0.90***| 0.34*  | 0.56**  | 0.56**     | 0.58** |
| NPPP_C3     | 0.20ns | 0.51*  | 0.56**| 0.51*  | 0.65**  | 0.65**     |        |
| 1000GWT_C3  | 0.31** | 0.64** | 0.65**| 0.11ns | 0.65**  |            |        |
| YLD_C3      |        |        |       |        |         |            |        |

C2 and C3 at the end of variables represent F2 and F3 populations, respectively. ns= not significant, *= significant at 5%, **=significant at 1%, ***=significant at 01%, * = significant at the 5%, ** =significant at the 1%.
Figure 1. F2 Cu which wins where.

Figure 2. F3 Cu which wins where.
Figure 3. F2 Cu GGE biplot analysis of Yield.

Figure 4. F3 Cu GGE biplot analysis of yield.
4. Discussion

4.1. Agronomic Performance of the Tested Genotypes

Micronutrient elements are very important for rice growth of which Cu is one of the essential trace elements for rice. As a trace element, copper is also an enzyme cofactor and played an important role in the inhibition of plant uptake of toxic trace elements. Plant height is a parameter for assessing crop performance [9]. This present finding revealed that plant height increased with increasing in concentration CuSO₄ solution up to 30mg of Cu/kg of soil level and decreased when soil at 60mg of Cu/kg of soil. In rice, plant height was reduced as a result of Copper toxicity [10], although UPN 103 and UPN 107 were the tallest even at high copper concentration, however, smaller plants do not necessarily affect yield, especially in the absence of water stress [18].

In most abiotic stressed environments, rice crop performed very well to some concentration levels, beyond, which experienced decline in performance. In this study, tiller number increased up to (30mg of Cu/kg of soil) and decreased at (60mg of Cu/kg of soil), this corroborate the report on Cu in wheat [6]. Similar report on salinity and its effects on tillering ability on rice [19, 20]. The increase in tiller number even at high level 60mg of Cu/kg of soil may be attributed to the nitrogen fixation an attribute of copper as well as the role of copper in biochemical processes like photosynthesis and respiration [21]. It was observed that F3 populations had more tiller numbers than the F2, this could be due to biased selections made at early stages of the crops.

4.2. Performance of Post-harvest Traits of the Tested Genotypes

The effective tillers which, is the number of tillers harvestable per plant, is very important in the determination of total grain yield of genotype. This study showed that the optimal level of copper concentration is at 30mg Cu/kg of soil, where all the genotypes had the highest numbers of effective tiller both in F2 and F3 populations. Reports also showed that there was 10% reduction on the rice performance at 100 mg Cu/kg and beyond, reduction by 50% in rice performance [22, 23]. The genotypes UPN 95 and UPN 86 had more effective tiller number even at 60mg of Cu/kg of soil, which could be used for population improvement in copper breeding programme.

The 1000 grain weight and panicle length are major yield components that determine the ultimate grain yield of rice. The panicle length determines the number of grains to be accommodated and higher grain yield for varieties with longer panicle length have been reported [24]. Most of the genotypes tested had high values of 1000 grain weight and long panicle length even at 60mg of Cu/kg of soil, which could be deployed to copper stressed environments.

The genotype UPN 59 performed better based on grain yield across in all copper concentration levels. Therefore, this genotype could have potential variety in copper stressed environment. Reduction of 24.92% and 22.12% of total grain yield of F2 and F3 populations at 60mg of Cu/kg of soil as compared to the control were recorded, the performance of F3 populations could be biased selection at the early generation of the crop. The decrease in grain yield as compared to the control could be a combination from the effects of yield components in Cu treated experiments as stated earlier.

4.3. Phenotypic Correlation Among Traits in the Populations

The grain yield had significant correlation at probability of 0.01 with plant height, effective tiller, 1000 grain weight. These results corroborate earlier reports [25, 19]. The existence of correlation may be attributed to the presence of linkage or pleiotropic effect of genes or environmental effect or combination of all [26]. The significant and positive correlation values observed could be used as secondary traits for yield selection especially, in early generation of the varietal development in rice.

4.4. GGEbiplot Analyses

The GGE refers to the genotype main effect (G) and the genotype x environment interaction (GE), which are the two most important sources of variation for cultivar evaluation in a multi environment trials [27]. GGE has been recognized as a useful tool to analyze and visualize the pattern of genotype x environment interaction of cultivar in multi environment and evaluation of different crops including cereals [27, 20]. Three mega environments were identified and could assist the breeders in prioritizing screening environments and genotypes for each environment as UPN 86 and UPN 95 had the highest GY in environment one and two, respectively. Screening for copper resistant genotypes at the early generation of the breeding cycle will assist breeders to reduce the number of genotypes to be carried into the next breeding cycles of the programme. The most stable genotype for F2 were UPN 59 and UPIA 2, while UPN 82 and UPN 86 for F3 population. The highest yielding genotype were UPN 86 and UPN 59 for F2 and F3 populations, respectively, these promising genotypes could accelerate breeding for copper resistance and immediate deployment to copper stressed environments.

5. Conclusion

Trace elements are very critical for rice growth of which Cu is one of the essential trace elements for rice. Selecting and breeding staple food crops which are more efficient in the uptake of micronutrients from the soil are beneficial for agricultural productivity. This study observed that 30mg of Cu/kg of soil is an optimum level for rice performance based on these experiments beyond, which reduction in rice performance. Reduction of 24.92% and 22.12% of total grain yield of F2 and F3 populations at 60mg of Cu/kg of soil as compared to the control were recorded, stable and high
yielding genotypes across the copper concentration levels were identified for copper breeding programme.

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