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Enhancing yield attributes in rainfed upland rice through incorporation of 6-benzylaminopurine in the cultivation program

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ABSTRACT

This experiment was carried out to evaluate the response of two upland rice varieties to incorporation of 6-benzylaminopurine (6-BAP) into the traditional fertilizer program. Treatments were laid out in a randomized complete block design (RCBD) in 2 x 5 x 2 factorial including 2 upland rice varieties (NERICA 3 (N3) and NERICA 8 (N8)), 5 treatments notably T0 = Controls (NPK 20:10:10 and Urea 46%), T1 = 6-BAP (25mg/l), T2 = 6-BAP (50mg/l), T3 = 6-BAP (75mg/l) and T4 = 6-BAP (100mg/l) all repeated twice. All the treatments received NPK (20:10:10) + Urea 46% while application of T1 to T4 started at the flag leaf stage and was done in 3 splits of foliar sprays. The results revealed that 6-BAP significantly influenced mean number of stay green tillers/m2 at 120 days after transplanting with corresponding scores of 16, 87.5, 111, 110 and 127 for T0, T1, T2, T3 and T4 in Nerica 3. Maximum paddy yield in N3 were 8.37 and 4.49 t/ha respectively for T3 and T0 while in N8, paddy yields were 7.36 and 4.6 t/ha correspondingly for T4 and T0. In N3, broken grains rate after milling was 34.45 and 26.30 % individually for the control and T3 while in N8 grain breaking rate of 28.81 and 25.16 % were respectively scored by T0 and T3. This study reveals that, foliar spray of 6-benzylaminopurine at 75 mg/l in three splits of 7 days during flag leaf stage optimizes upland NERICA yields and grain morphological characteristics after milling.

Contribution/Originality: To the best of our appreciation, studies on yield improvement in rice have been focused on exclusive use of synthetic fertilizers. In this study we incorporated plant growth regulator specifically cytokinin into the regular fertilizer program and the results showed significantly improve in paddy yield /grain physical quality after milling.

1. INTRODUCTION

Although rice is a staple food in sub–Saharan Africa, local production regrettably satisfies only 28.8% of its demand [1]. A typical example of low rice productivity is in Cameroon where average annual national production is only 105,000 tons while national demand stands at > 440,000 tons annually [2]. The causes of this low productivity are numerous ranging from policy through inadequate agronomic practices, sluggish acceptance/ implementation of new technologies and diminishing low lands. To solve the problem of diminishing lowlands by way of increase land suitable for rice cultivation, the Africa Rice Center developed and released 80 varieties called New Rice for Africa (NERICA) by interspecific crossing of *Oryza sativa, an* Asian rice variety known for its high yielding potential with *O. glaberrima*, an African rice known for its high adaptation to biophysical stresses [3].

Today 82 NERICA varieties exist; out of which 60 are lowland rainfed, 4 irrigated lowlands and 18 upland rainfed. The 18 interspecific varieties adapted for upland rainfed conditions have been named as NERICA 1 to 18 [4]. Amongst the upland varieties, NERICA-4 is the most widely adopted upland variety and it's been grown in more than 10 countries in sub-Saharan Africa (SSA) but in the Upper Noun valley of Cameroon, NERICA 3 and 8 are the most cultivated. Generally, upland NERICA varieties give good yields, are early-maturing (in 75-100 days) and are tolerant to major biophysical stresses in a wide range of rainfed upland environments [5]. Even with these benefits, acceptability of upland NERICA varieties is still very slow in many communities in SSA [6]. Though yield of upland rice is generally lower than lowland varieties [7] productivity in the Ndop plains of Cameroon are far lower compared to standard. In a field survey carried out in 2020 and 2021 farming seasons we noticed a leaf senescence rate of about 38 % per tiller when the crop were still at the R3 i.e. dough stage in about 81 % of upland rainfed rice farms visited. It was therefore hypothesized that this early senescence could be contributing significantly in maintaining yield lower than the genetic potential of the varieties under the farmers fertilization program notably Nitrogen 20% +Phosphorus 10% + Potassium 10% (NPK 20 10 10) complex fertilizer and urea applied in two splits of 50:50 at V4 and R1. A perusal of literature showed that Cytokinins delays leaf senescence [8, 9] and the longer the duration of stay green, the more efficient the photosynthetic apparatus in converting the solar energy to chemical energy and its eminent storage in the grain sinks. It was within scope that this study was initiated with the general objective to optimize grain yields in two upland Nerica rice field varieties by incorporating 6-BAP into the traditional fertilizer program.

2. MATERIALS AND METHODS

The field study was carried in the rice field of the Upper Noun Development valley authority (UNVDA) located in the Ndop plains of the North West Region of Cameroon while milling of paddy and assessment of quality traits were carried out at the JICA center in Nkolbisson, Yaounde, Cameroon. Ndop has geographical coordinates 5° 59'0" North, 10°15'0" East, with an altitude of 1558 m above sea level. This area has temperature ranges from 13-18 °C, characterized by annual rainfalls of about 2230 mm and average humidity of 70 % and 52% in the rainy season and dry season respectively. Two upland varieties were used namely NERICA 3 (N3) and NERICA 8 (N8). The choice of these two was based on their popularity among farmers in this region. The seeds were obtained from UNVDA and Seeds were pre -germinated and treated with a fungicide prior to sowing**.** The plant growth regulator 6BAP was imported from Sigma Aldrich.

2.1. Experimental Design and Field Layout

The experiment was made up of 5 treatments including a control plot for each variety. The treatments were arranged in 2 replicates and randomized in a complete block design. The replicates were arranged into 4 plots and each plot made up of 5 units given a total of 20 units. The replicates were separated from each other by a distance of 1m. Each unit measured 2m by 2m squares which covered a total surface area of $4m^2$ and separated with a distance of 0.5m apart. Each unit had 10 rows and each row is had 10 hills given a total of 100hills/unit. The slope of the experimental plots was on North to South direction hence the blocking was done on East to West orientation. The treatments were denoted as follows;

T0 = 200kg/ha NPK 20:10:10 + 100 kg/ha Urea applied in 2 splits.

T1 = T0 + 6-BAP (25mg/l).

T2 = T0 + 6-BAP (50mg/l).

T3 = T0 + 6-BAP (75mg/l).

T4 = T0 + 6-BAP (100mg/l).

2.2. Standard Method of Sowing/ Field Maintenance/ Application of Fertilizers

The seed rate was calculated at 11 kg/ha. Sowing of the seeds was by direct seeding and 4 pre germinated seeds were hand sown/hill. Missing hills were replaced by transplanting and this was done 15 days after sowing when the seedlings have developed 3rd leaves. Weeding and molding was carried out at interval of 30 days. Fertilizer (NPK 20:10:10) application was by placement with the first split of 50% i.e. 2.23g per hill applied 15 days after emergence (DAE) while the last split was applied 35 DAE. Urea was applied also in 2 splits but the first split was at 35 DAE while the second split was applied at flag leaf stage. Throughout the growth process, the fields were monitored for pests and diseases and were control by chemical spray depending on the symptoms observed.

2.3. Preparation of Hormonal Solution/ Application

Fours weights (25mg, 50mg, 75mg and 100mg) of 6-BAP corresponding to T1, T2, T3 and T4 were measured and placed each in a separate 1L containers. 5 ml of 70% ethanol was added in each of the concentrations and pipe borne water added to the 1L mark. Application of the various BAP solutions to their respective experimental units was by foliar spray and was done between 7 to 8 am. The first treatment was done when the crop was at the flag leaf stage and spraying was time spaced at 7 days interval for one month.

2.4. Harvesting/Threshing/Milling

Harvesting was carried out manually by cutting tillers using a sickle. Threshing was done immediately by beating the panicles with a stick over a tarpaulin. Paddy obtained from each treatment was sun dried for 5 days and winnowing carried out to get rid of unfilled grains and pieces of straws. The moisture content of the paddy samples was carried out using a moisture content tester from 10 seeds randomly collected. Each paddy sample was measured and converted to 14% standard moisture content using the international standard moisture content as follows

$$\frac{100 - x\ (\%)}{100 - 14\ (1\%)} xy\ (kg) \qquad where\ X = moisture\ content\ and\ Y = paddy\ weight$$

Any sampled that registered a moisture content >14, the paddy was further dried until its moisture content was 14 % before storage. Prior to milling, paddy from various treatments had a moisture content of 14%.

2.5. Paddy Dehusking, Brown Rice Polishing and Grading

Paddy was dehusked to brown rice using a small satake rice dehusker. The brown rice obtained was then polished in to white rice using a rice polisher. The white rice was later transferred in to a grain separator machine for grading. In this process the machine was programed to discharge only long grains considered as first grade while broken and unfilled grains considered as low grade were discharged into an inbuilt chamber.

2.6. Data Description and Collection

Parameters evaluated were percentage stay green of tillers, number of productive tillers, biometric parameters of grains and quality characteristics.

To calculate number of stay green tillers/ treatment at 128 DAE, each experimental unit was mapped in to 5 transects of 1 m^2. Three transects were randomly selected while taking into account the border effect and the mean number of tillers considered stayed green were counted. A tiller was considered to stay green if ≤ 90 % of its leaves were maintained green when the panicles are turned brown. Different sets of transects of 1 m^2 were also used to determine the mean number of productive tillers/m^2 for the various treatments expressed in percentage.

As concerns weight of 1000 paddy grains an electronic scale was used to take the mean weight of 3 samples of 1000 grains calculated at 14% standard moisture content. As for paddy length, a ruler was used to measure the length of 10 paddy grains randomly selected per treatment and the average recorded. Quality traits of milled rice like rate of broken grains were obtained as specified by Japan international cooperation agency (JICA) standards.

2.7. Data Analysis

Data collected were subjected to statistical analysis using analysis of Variance (ANOVA) to determine the significant effect on parameters measured. Data collected was analyzed using the statistical pack for social science (SPSS) version 17. Least Significant Difference at $P<0.05$ was used to carryout multiple comparison test aimed at detecting the significant differences between the means of the various treatments.

3. RESULTS

3.1. Number of Stay Green Tillers

Figure 1 illuminate the influence of foliar spray of different concentrations of 6-BAP on the mean number of stay green tillers per m^2 in two upland rice varieties. It was noticed that all trials under the four concentrations of 6-BAP showed highly significant differences compared to the control at $P<0.05$ in both rice varieties as far as number of stay green tillers as concerned. In both varieties, the control experiments recorded a mean of only 16 tillers /m2 stayed green tillers as oppose to 129 and 127.5 tillers/m^2 in experimental units treated with 100 mg/L 6-BAP respectively for NERICA 8 and NERICA 3 after the standard days to senescence of 120 for Nerica 3 and 110 days for Nerica 8. While significant differences were not recorded between 6-BAP 50 and 100 mg/l in both varieties, the later showed significant differences with BAP 25 mg/l (Figure 1).

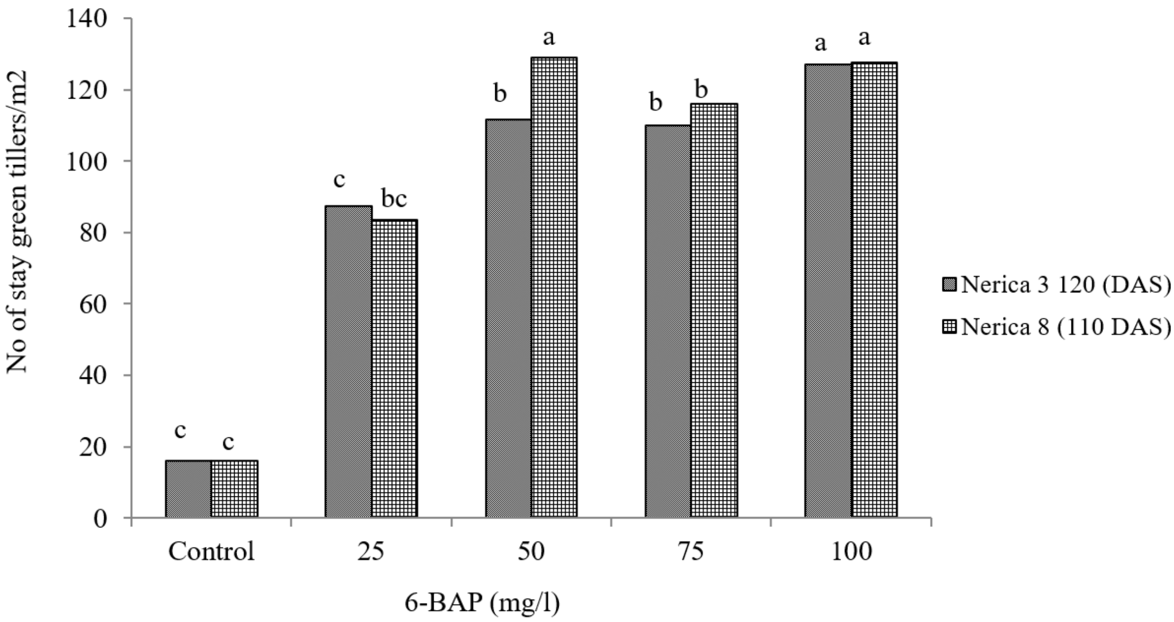

Figure 1. Influence of foliar spray of 6-BAP on number of stay green tillers in rice.

Note: Different letters on histograms represent significant differences within variety at $p < 0.05$.

3.2. Number of Productive Tillers

Figure 2 elucidates the influence of 6-BAP on rate of productive tillers/ m^2. Variations were observed between all treatments and the control and amongst the treatments. Statistically significant differences were obtained at $p < 0.05$ when the mean percentage of productive tillers/m^2 of the control trials was compared with the various concentrations of BAP. A steady increase in the number of productive tillers was noticed in both rice varieties under investigation with increase in 6-BAP concentration from 25 through 50 to 75 mg/l ranging from 87.1 % at dose of 25mg/L to 98.2 % at dosage of 100mg/L in N3 against 72.15% productive tillers in the control. In N8 maximum percentages of productive tillers recorded/m2 were 73.66 and 97.55 correspondingly for the control and dose of 100mg/L (Figure 2).

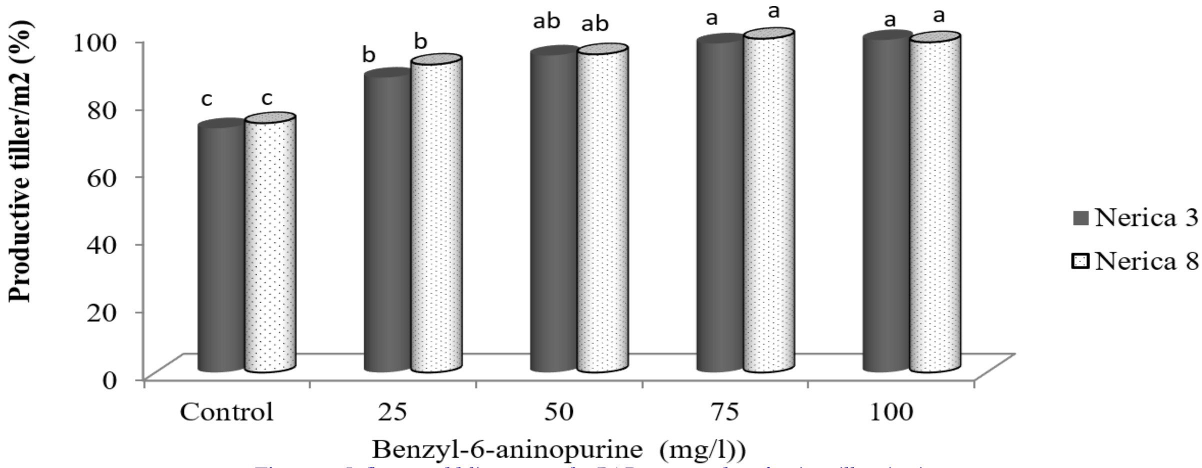

Figure 2. Influence of foliar spray of 6-BAP on rate of production tillers in rice.

Note: Different letters on histograms represent significant differences within variety at P<0.05.

3.3. Percentage Filled Grains

Figure 3 illuminates the influence of different concentrations of 6-BAP on percentage grain filled in two rice varieties studied. The results revealed a general increased in percentage grain filled in the trials under treatment with foliar spray of 6-BAP than in the control in the two varieties. Among the treatments, BAP 75 and 100 mg/l didn't show any significant differences but the later significantly increased grain filling against BAP at 25 and 50 mg/l. In N3, maximum mean percentage grain filled was 80.5 for the control and 97.7 % at concentration of 100mg/L (Figure 3).

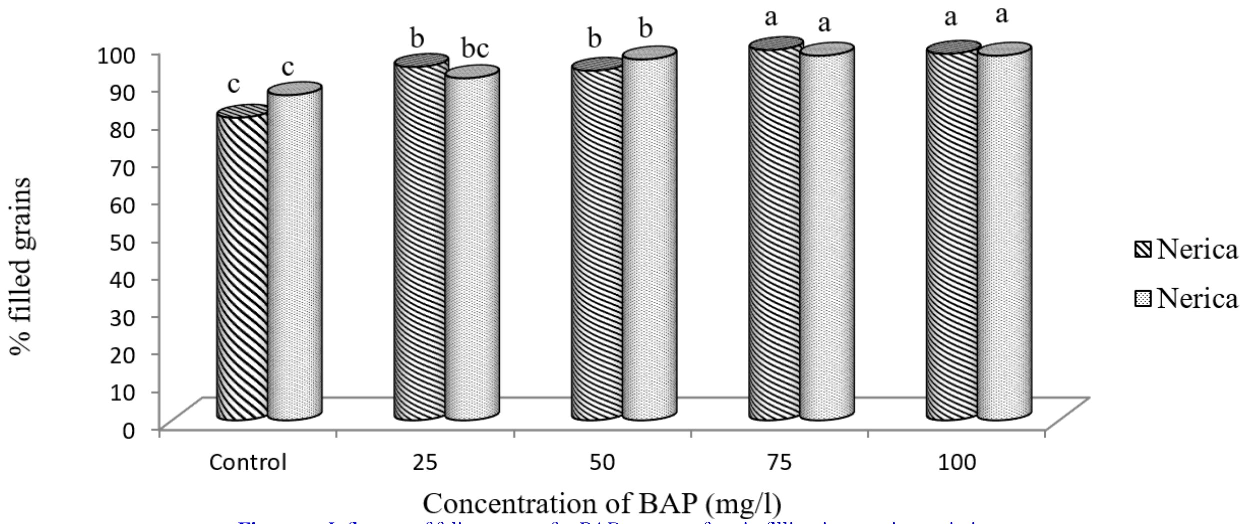

Figure 3. Influence of foliar spray of 6-BAP on rate of grain filling in two rice varieties.

Note: Different letters on histograms represent significant differences within variety at P<0.05.

3.4. Effect of BAP on Weight (g) of 1000 Paddy Grains at 14% M.C

Figure 4 illustrates the effect of foliar spray of different dosages of 6-BAP on mean weight of 1000 paddy grains in the two upland varieties under investigation. It was noticed that in N3, no significant differences were observed among all the treatments. However in N8 foliar spray of 6-BAP at 75 and 100 mg/L showed significant differences with respective scores of 29.5 and 30.5 g against 26 g for control, T1, T2 and T3 and 25 g for the UNVDA standard in terms of weight of 1000 paddy grains evaluated at a moisture content of 14 %.

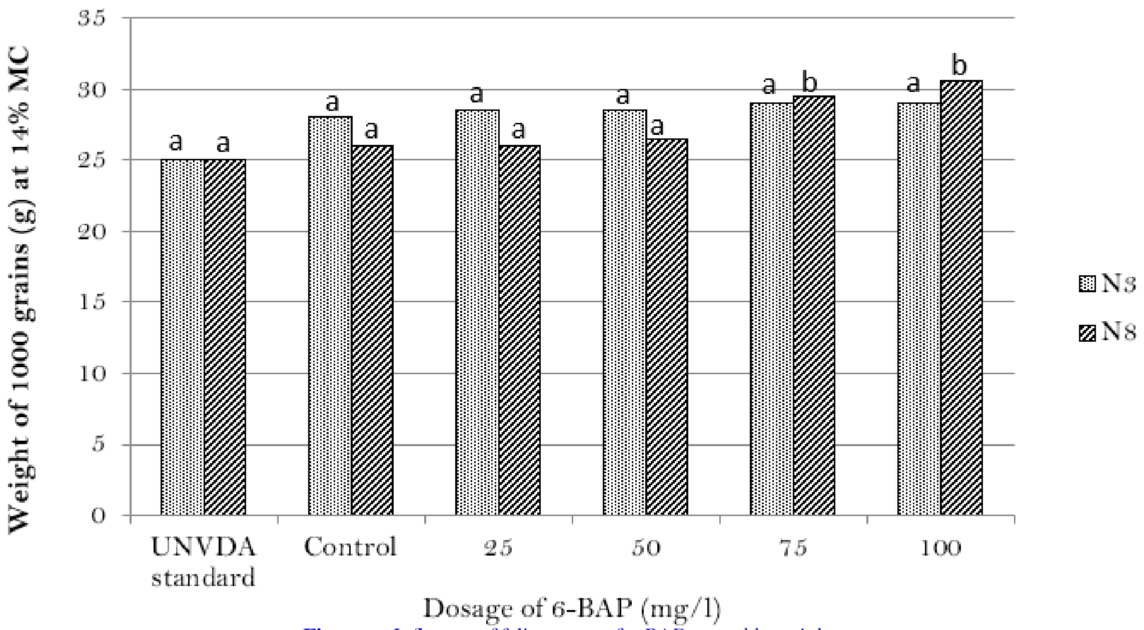

Figure 4. Influence of foliar spray of 6-BAP on paddy weight.

Note: Different letters on histograms represent significant differences at P<0.05 within variety.

3.5. Paddy Length

Figure 5 expatiates the effect of foliar spray of 6-BAP on mean paddy length at harvest. Results of this parameter were not consistent in both varieties. However, Nerica 3 recorded highest mean paddy grain length than Nerica 8 in 3 out of the 5 treatments. Comparison within Nerica 8 showed that foliar spray of 6-BAP generally had a significant effect on paddy grain length compared to the control. In Nerica 3, the control showed higher grain length (9.5 cm) against 8.8 cm and 9.1 cm individually for 6-BAP at 25 and 100 mg/l. Though BAP 50 mg/l recorded a higher mean paddy grain length (10cm) than the control, no significant difference was however noticed between the two treatments (Figure 5).

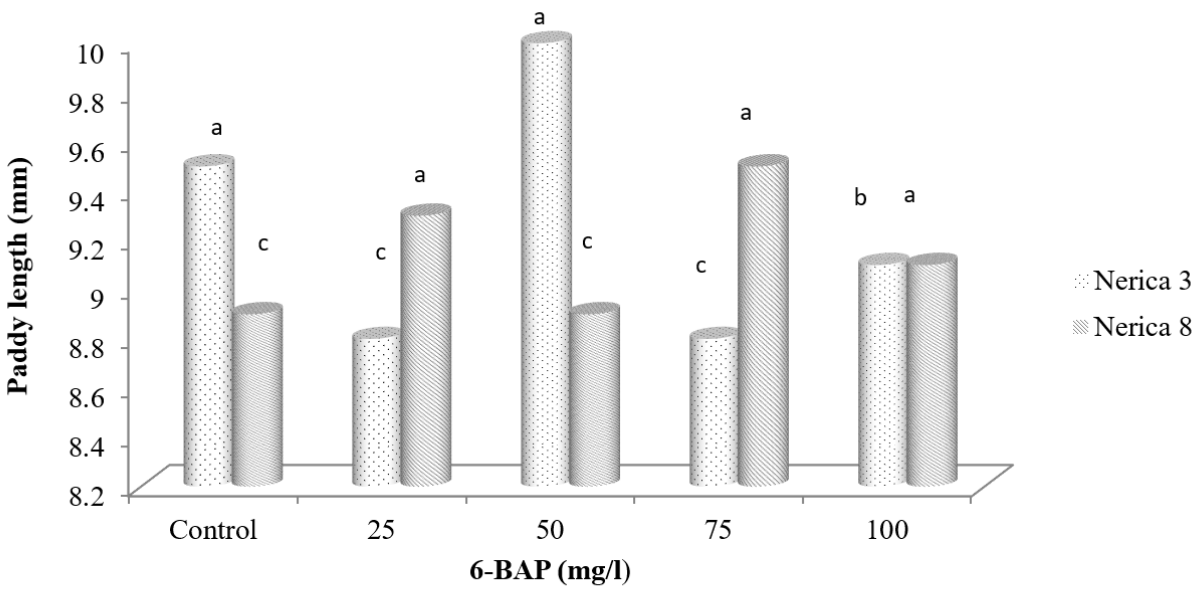

Figure 5. Influence of foliar spray of 6-BAP on paddy grain length.

Note: Different letters on histograms represent significant differences at P<0.05 within variety.

3.6. Paddy Yield (t/ha)

Figure 6 illustrate the influence of foliar spray of 6-BAP on paddy yield. Statistically no significant difference was noticed between the control and the check (UNVDA standard) in both rice varieties. Comparing the control to various dosages of BAP, significant differences were observed at all BAP application rates in both rice varieties. In the two varieties it was noticed that paddy yield was directly proportionate to 6-BAP dosage. Bap 100 mg/l increased paddy yield with a mean score of 8.52 t/ha, approximately two times the yield of control (4.8 t/ha) and check (4.6 t/ha) in Nerica 8. The same trend was observed in Nerica 3 where paddy yield was 8.37 t/ha in trials that were sprayed with 75 mg/l of BAP against 4.49 t/ha obtained in the control (Figure 6).

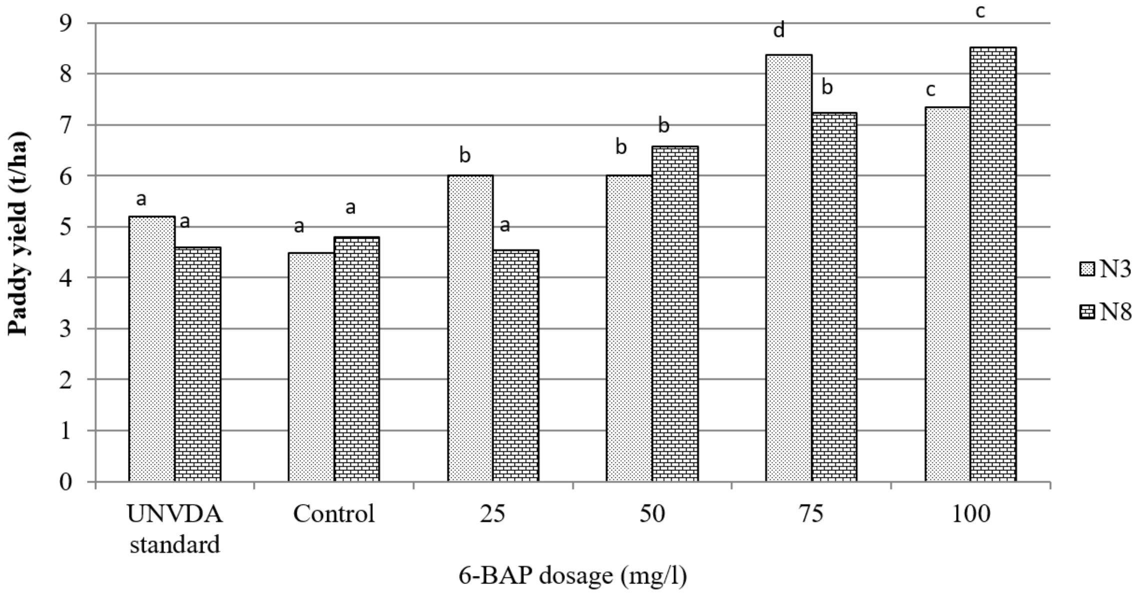

Figure 6. Influence of foliar spray of 6-BAP on paddy yield.

Note: Different letters on histograms represent significant differences obtained at $P<0.05$ within variety.

3.7. Grain Characteristics After Milling

3.7.1. Milling Rate

Figure 7 illustrates the influence of 6-BAP dosage on mean milling rate of paddy. As far as milling rate is concerned, the check or standard for upland rice according to UNVDA Ndop is 65-70 %. The result of the present study showed that in N3, no significant difference with the control though the control recorded a higher milling rate of 70.72%. However, significant differences were noticed between all various 6-BAP treatments and the control in N3. The milling rate increased with increase in dosage of 6-BAP, with 75 mg/l dosage scoring the highest milling rate of 74.32 % among the 6-BAP concentrations (Figure 7).

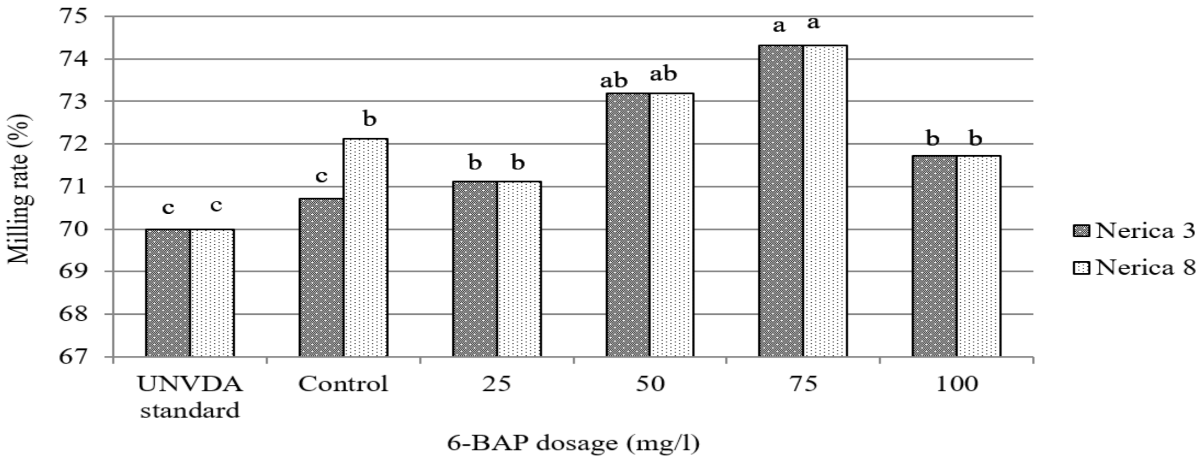

Figure 7. Influence of foliar spray of 6-BAP on milling rate of paddy.

Note: Different letters on histograms represent significant differences at $P<0.05$ within variety.

3.7.2. Broken Grains

Figure 8 presents the influence of 6-BAP on percentage broken grains during milling of paddy. Percentage broken grains were found to be lower in trials under BAP foliar spray compared to the control and the check from UNVDA. In each treatment, Nerica 8 generally showed a lower grain breaking rate than Nerica 3. The least broken grain percentage in Nerica 8 was 25.16 obtained in trials under 6-BAP at 50 mg/l. The same tendency was observed in Nerica 3 where BAP at 100 mg/l scored the least broken grain percentage of 26.45 against 40 and 34% respectively for the checker and the control (Figure 8).

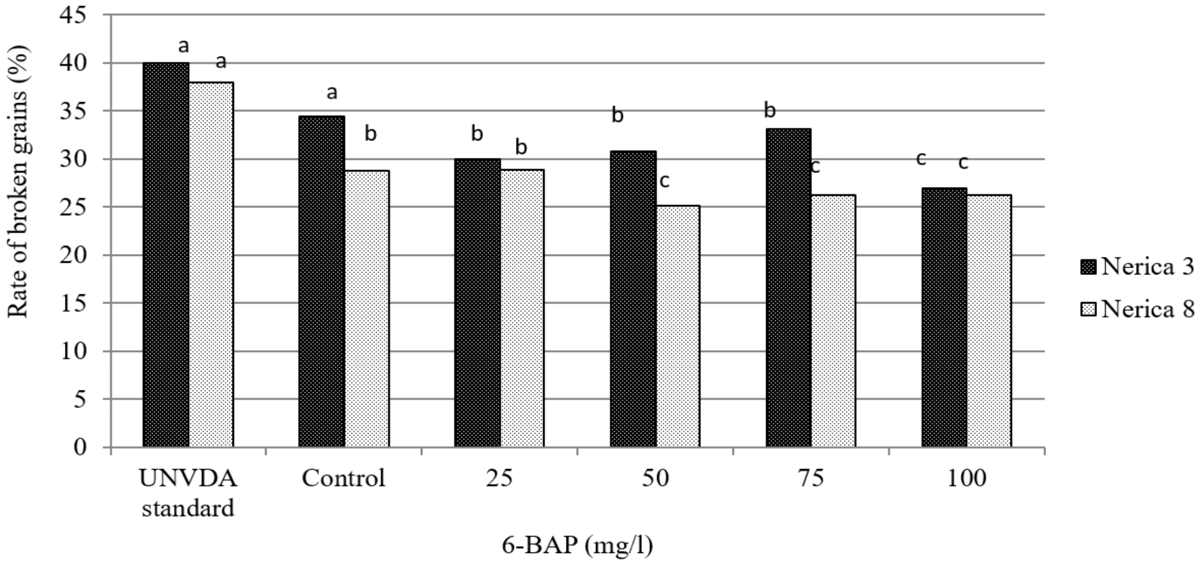

Figure 8. Influence of foliar spray of 6-BAP on rate of grain breakage during milling.

Note: Different letters on histograms represent significant differences at $P<0.05$ within variety.

4. DISCUSSION

In this study, it was observed that 6-BAP treatments showed highly significant differences with the control as concerns number of stay green tillers /m². Par results had been reported by Talla, et al. [10] using Benzylaminopurine (BA). Such results could be attributed to the fact that 6-BAP like other cytokinins, delays senescence as it plays a pivotal role in coordinating demand for and acquisition of nitrogen as well as nitrogen remobilization. Rapid leaf senescence have been reported after flowering in wet seeded rice and this is caused by a decline in cytokinin content in the flag leaf [11]. The former finding probably explains the need to supplement exogenous cytokinins (CK) during the reproductive phase of upland rice where intermediate drought periods could induce premature leaf senescence and decrease in yield as observed in the control experiment in the present study. In a related study, it was shown that delayed senescence induces high drought tolerance in angiosperms [12]. Supplementing Ck concentration by exogenous application at all concentrations could be the reason for significantly higher number of stay green tillers at all concentrations on the two upland rice varieties in the present study. In a similar study in wheat, it was reported that exogenous 6-BA significantly enhanced endogenous Zeatin riboxide (ZR) and decreased Abscisic acid (ABA). Delaying leaf senescence could maintain photosynthetic activity for a longer period and lead to a higher photosynthetic rate [13]. CKs modulate a number of important developmental processes, including the last phase of leaf development, known as senescence, which is associated with chlorophyll breakdown, photosynthetic apparatus disintegration and oxidative damage. There is ample evidence that CKs can slow down all these senescence-accompanying changes [14]. The general increase in grain yield in plants treated with 6-BAP over the control in this study could be attributed to increase assimilate synthesis during photosynthesis. Maximum grain yields obtained by spray of 6-BAP could be attributed to higher photosynthetic rate resulting from a maximal quantum yield of Photosystem II photochemistry [15]. The more the plants stayed green, the more the rate of photosynthesis and simultaneous translocation of assimilated to the grains.

In similar investigation with chlorophyceae algae it was reported that even under nitrogen deficiency photosynthesis and biomass productivity rates are enhanced by exogenous supplementation of cytokinin [16]. Several studies have reported the involvement of CKs in the functional as well as the structural aspects of photosynthesis at several levels. At the level of stomata, CKs acting as antagonists of abscisic acid can increase stomatal conductance [14] and thus, modulate leaf gas exchange and the availability of CO_2, the essential substrate for photosynthetically active tissue [17]. At the cellular level, CKs have a major effect on chloroplasts. CKs promote the differentiation of etioplasts, its transition to chloroplasts, chloroplast division and finally, increased the number of chloroplasts [18, 19]. At the level of the thylakoid membrane, Harvey, et al. [18] observed that CKs promote grana formation and increase the content of photosynthetically active pigments [20] and increase starch [21]. At the molecular level, CKs are reported to affect pigment-protein complexes involved in the light phase of photosynthesis, as well as enzymes of the dark phase. Of the genes most upregulated by CKs, the most widely documented genes are those coding for the light-harvesting chlorophyll *a/b* binding proteins of photosystem II (*CAB*) and small and large subunits of Ribulose biphosphate carboxylase oxygenase (RUBISCO) [19]. In the present study, paddy from trials treated with 6-BAP recorded significantly lower grain breakage rate during milling compared to the control trials and the check. This is probably linked to significantly high level of grain filling in the treatments than the control. A major pre harvest factor influencing grain breakage during milling is poor filling during reproductive growth phase which is linked to poor nitrogen management regime [22]. Poor grain filling leads to gaps or fissures in the endosperm, which in turn reduces grain resistance during milling. The low level of grain cracking in BAP-treated field in the present study could be attributed to the fact that beside 6-Bap promoting anti-senescence, it has also been reported by Criado, et al. [23] to play a pivotal role in coordinating demand for and acquisition of nitrogen as well as nitrogen remobilization. Finding adequate nitrogen management regime in rice production could result in the production of grains that are well filled, thick to resist drying and resistant to cracking during milling [22]. According to this study, besides the regular fertilization schedule, foliar application of 6 benzylamine purine (6-BAP) at a dosage of 50 ml/L during flagging improves the stay green period of the flag leaf and by extension optimizes grain yield and quality of both NERICA 3 and NERICA 8 upland rainfed rice varieties.

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Authors' Contributions: Conception of topic and principal investigator, T.K.M.; assistant Investigator, data collection field work, W.D.; data analysis and interpretation, T.E.L. All authors have read and agreed to the published version of the manuscript.

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