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Effects of Phosphorus and Zinc on Net Assimilation Rate (NAR) of Cowpea (*Vigna unguiculata* (L.) Walp) Varieties Grown in Bauchi, Nigeria

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Abstract

Plant growth analysis is today studied as it relates to change in capture and efficiency in the use of resources rather than as it relates to relative growth rate in the past. Net assimilation rate (NAR) is the rate of increase in dry matter over unit area of leaf, which represented a measurement of excess of photosynthesis overrespiratory losses of dry matter. Two field experiments were conducted at the Faculty of Agriculture and Agricultural Engineering Research Farm of Abubakar Tafawa Balewa University, Bauchi, North Eastern, Nigeria, between 2006 and 2007 to evaluate the influence of phosphorus (0, 25, 50 kg P/ha) and zinc levels (0, 2.5, 5 kg Zn/ha) on NAR of six cowpea varieties namely: IT90K 277, IT93 455 1, IT89KD 288, IT97K 568 18, IT90K 82 2 and Kanannado. Results obtained showed that NAR was not significantly influenced by P and Zn levels and therefore, 0 kg ha-1 SSP and Zn rate produced the highest NAR than higher levels. Neither applications of each of P and Zn at higher levels or their interactions, nor did year or variety were found to have significant effects on NAR; but rather NAR is being determined by number of factors such as nutrient supply, photosynthesis rate, etc and these are never fixed or constant and hence a particular factor e.g., nutrient (P or Zn) cannot dictate NAR alone.

Keywords: Cowpea varieties, Net Assimilation Rate, Phosphorus, *Vigna unguiculata* L., Zinc

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a foremost legume food grown around the world (Musa *et al.*, 2017) as food for human consumption (Mfeka *et al.*, 2019).The grains are highly valuedfor food, and the fodder and haulm used to feed livestock during the dry season (Lan gyintuo *et al.*, 2003; Mfeka *et al.*,2019). A moderate cheap and willingly available source of pr otein and minerals, and contains high concentrations of iron (Fe) and zinc (Zn) (Belarmino *et al.*, 2013; Rouault, 2015; Araújo *et al.*, 2022). An average ripe seed of cowpea contains 22% protein, 1.4% fat, 59.1% carbohydrate, and 3.7% ash, an energy value of 340 kcal/ 100g (Putul *et al.*, 2021). It is one of the most olden crops known to man. Its origin and successive cultivation are related with pearl millet and sorghum in Africa. It is an essential part of conventional cropping systems all over the continent, principally in the semi-dry area of West Africa (Steele, 1972; Mohammed *et al.*, 2021). It is nowadays a generally customized and

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extremely variable crop, domesticated around the world mainly for seed, but also as a vegetable for leafy greens, green pods, fresh shelled green peas, and shelled dried peas, and a cover crop and fodder (Quinn, 2004; Osipitan *et al*., 2021).

Phosphorus is an essential nutrient for the biogenesis of chlorophyll (Kiri *et al*., 2022), and as well as a component of cell nucleus. It is an indispensable nutrient for cell division (Siedliska *et al*., 2021) and development of meristematic tissue (Ahmed *et al*., 2018). Phosphorus significantly increases branches leaves, fresh and dry weight per plant in cowpea at 60k P2 O3/ha (Namakka *et al*., 2017; Sudharani *et al.,* 2020). Several studies have shown increased tissue P-levels with soil availability of P (Rhodes, 1980; Reddy and Sexana, 1983; Johan *et al*., 2021). The major effect of phosphorus on cowpea yield is expressed as an increase in the number of pods per plant and number of seeds per pod (Augustine and Godfre, 2019; Sudharani *et al*., 2020). Phosphorus also influences the number of flower primordial (Remison, 1980; Dangi *et al*., 2019) and early root development (Adepetu and Akapa, 1977; Mohammed *et al*., 2021). Maturity is delayed in plants which are P-deficient. Although the accumulation pattern of P is cultivar specific (Oliveira *et al*., 1982; Suzuki *et al*., 2021), it is suggested that the supply of P should be high throughout the growing season, especially during seed maturity (Jacquinot, 1967; Wahua, 1983; Sudharani *et al*., 2020; Mohammed *et al*., 2021). Pod yields were found to be greater when cowpea plants were grown with the higher level of phosphorus (Stewart and Reed, 1969; Namakka *et al*., 2017; Ibrahim *et al*., 2022). Phosphorus deficiencies result in a decrease in the rate of leaf expansion and photosynthesis per unit leaf area henceforth drop in fodder yield (Carstensen *et al*., 2018; Meng *et al*., 2021).

Crop yields are frequently limited by low soil levels of mineral micronutrients such as zinc (Zn) (Dawar *et al*., 2022). Essentiality of zinc in plants was recognized as early as 1915 by a scientist called Maze in *Zea mays (*Nielsen, 2012; Prasad et al., 2013; Kiri *et al*., 2022). Zinc is an essential mineral nutrient and a cofactor of over 300 enzymes and proteins involved in cell division (Cheng and Chen, 2021), nucleic acid metabolism and protein synthesis (Marschner, 1986; Hilal *et al*., 2016; Suganya *et al*., 2020; Stanton *et al*., 2022). Cakmak (2000), Cui and Zhao (2011) and Santos *et al*. (2021) have speculated that zinc deficiency stress may impede the activities of a number of antioxidant enzymes, leading to extensive oxidative damage to membrane lipids, proteins, chlorophyll and nucleic acids. Zinc can impact on carbohydrate metabolism at many levels. Moreover, Zn is essential in the biosynthesis of tryptophan, an originator of the auxin-indole-3-acetic acid (Oosterhuis *et al*., 1996; Garay *et al*., 2022). Zinc deficiency symptoms comprise small leaves, shortened internodes giving the plant a stunted appearance (Hacisalihoglu, 2020). Availability of zinc in soils and its absorption and translocation in plants is influenced by all other plant nutrients. Zinc in general interacts negatively with phosphorus which depends upon a number of physicochemical properties of soils (Kumar *et al*.,2016; Moreno-Lora and Delgado, 2020).

Plant growth analysis is largely stated as indexes of growth such as crop growth rate, relative growth area, net assimilation rate, leaf area ratio, and leaf area index (Fageria *et al*., 2006; Pandey *et al*., 2017; Hilty *et al*., 2021) that offer the first hint toward an understanding of discrepancy in growth rates between genotypes or species (Lambers, 1987; Pandey *et al*., 2017; Li *et al*., 2020). In contemporary years, scientists made several attempts to evaluate the probable Relative Growth Rate (RGR) in crop species, and to assess whether the differences are primarily caused by structural or by physiological characters (Ruggiero *et al*., 2012; Gleason *et al*., 2022). For herbaceous plants and grasses, variation in LAR is the major determinant of interspecific variation in Relative Growth Rate (Poorter & Remkes, 1990; Atkin *et al*., 1996; Medek *et al*., 2007; Kahiu and Hanan, 2018).

Growth differences between species can be attributed totally to a difference in NAR, whereas interspecific variation in RGR could be ascribed to differences in LAR. In other circumstances, both NAR and LAR were related with the intrinsic difference in RGR (Poorter and Remkes, 1990; Fu *et al*., 2012). NAR, also known as unit leaf rate, $g \cdot cm^{-2} \cdot day^{-1}$ is the increase in dry biomass for each unit leaf area and is a complex physiological variable related to photosynthetic and respiration rates (Caliskan *et al*., 2010; Li *et al*., 2016).

NAR is the rate of increase in dry matter over unit area of leaf, which represented a measurement of excess of photosynthesis over respiratory losses of dry matter (Fageria *et al*. 2006; Zulkarnaini *et al*., 2019). A rise in NAR may involve an improved rate of photosynthesis, which can be gathered by additional investment in the photosynthetic apparatus (Konings,1989; Poorter, 1989b; Li *et al*., 2016; Sun *et al*., 2019). It is a value that talks about plant productivity in relation to plant size. It is valuable as an extent of the photosynthetic proficiency of plants and reveals the balance of photosynthetic rate against respiration and rates of tissue loss (Quero *et al*., 2006; Li *et al*., 2016; Sun *et al*., 2019). NAR is a physiological parameter and is the primary source of variation in RGR (Li *et al*., 2016). NAR is constantly the best predictor of SGR, while specific leaf area (SLA) and the apportionment of biomass to leaves (LMR) made smaller contributions.

NAR is largely the net result of carbon gain (photosynthesis) and carbon losses (respiration, exudation, volatilization) expressed per unit leaf area (Pooter, 1990; Lundgren and Des Marais, 2020). Particularly, fast-growing plants continuously had high net assimilation rates and plants with high assimilation rates constantly matured fast (Li *et al*., 2016; Ji *et al*., 2021). The objectives of this study were to examine whether there is any disparity in the NAR of cowpea varieties under various P and Zn levels or non? And to determine the best levels of phosphorus, and Zinc, as well as the interaction of both nutrient elements that will promote NAR and consequently enhance the growth and yield of cowpea in the scrub savanna of Nigeria.

Materials and Methods

Study area

Two field experiments were conducted at the School of Agriculture Research Farm of the Abubakar Tafawa Balewa University, Bauchi, Nigeria, during the growing seasons of 2006 and 2007 to investigate the effects of phosphorus and zinc interactions on net assimilation rate (NAR) of cowpea (*Vigna unguiculata* (L.) Walp) varieties. Bauchi, is located at 10.3010° N latitude and 9.8237° E longitude at an altitude of 109.45m above sea level. It has a monsoonal climate characterized by well-defined rainy and dry seasons. Annual rainfall is mostly distributed between the months of May and October.

Experimental Design

A split-split plot design with a total size of the experimental area of 62m by 50m was used. There were three (3) replicates and each replicate consisted of three sub-plots; each measuring 18.9 m by 2.25 m. Each sub-plot was divided into six (6) sub-sub-plots with each measuring 6.30m by 2.25m. A space of 1m each was left between main plots, and replicates. Half a meter

(0.5m), and 50cm were left between sub-plots, and sub-sub-plots respectively. Main plots were assigned to three different levels of single super phosphate (SSP) namely 0kgha-1, 25kgha-1, and 50kgha-1at random. Sub-plots were assigned to three (3) different levels of Zinc (Zn) namely 0kgha-1, 2.5kgha-1, and 5kgha-1. A total of fifty-four (54) treatments consisting of six varieties by three SSP levels by three zinc levels. The treatments were randomized using table of random numbers as described by Gomez and Gomez (1984). The experiment continued up to three and half (3½) months that is, from planting to harvest period.

Experimental Plant Materials

Six cowpea varieties collected from International Institute for Tropical Agriculture (IITA) Kano station, were used in the study. The varieties are IT90K-277, IT93-455-1, IT89KD-288, IT97K-568-18, IT90K-82-2, and Kanannado.

Soil Sampling and Analysis

In each experimental year, soil samples were collected randomly from selected spots in the experimental field before land preparation. The samples were taken at two depths (0-15 and 15-30cm), using a tubular auger. The physicochemical properties of the soil were determined using procedure described by Black (1965). The following soil properties were studied: Nitrogen, phosphorus, potassium, power of hydrogen (pH), cation exchange capacity (CEC) and particle size.

Land Preparation

The land was cleared, ploughed and harrowed. It was then marked into 162 sub-sub-plots. The sub-plot size was 14.2cm^2. There were 18 sub-sub-plots in a main plot, and 3 main plots in a replicate, and 3 replicates in the whole field experiment.

Sowing of Cowpea Varieties

Sowing was done 3rd and 5th August for the years 2006 and 2007 respectively. Sowing was 75cm row to row and 25cm plant to plant, and three seeds per hill. Seedlings were thinned to one per hill two weeks later. The planting dates were considered in such a way that the varieties mature after end of the rainy season as recommended by IITA (2000).

Fertilizer Application (SSP and Zinc)

International Institute for Tropical Agriculture (2000) recommended that Single Super Phosphate (SSP) should be incorporated into the soil before sowing as top dressing is not promising. Soil application of phosphorus is more effective in increasing phosphorus content (of the soil) than foliar application (IITA, 1973). Zinc sulphate was used as the sources of Zinc and was incorporated in to soil.

Weeds and Pest Control

The first weeding (hoe weeding) was done about three weeks after sowing that is 21 days after sowing (DAS). Second weeding was at 42 DAS. For the control of insect pests, three sprays of insecticides at 30, 50 and 60 days were used, using an insecticide *dimethyl cyclopropanecarboxylate* (karate).

Determination of Net Assimilation Ratio (NAR)

The rate of increase in dry matter over unit area of leaf is referred to as net assimilation rate (NAR) and which denoted a measurement of excess of photosynthesis over respiratory losses of dry matter. NRA was calculated as

$$NAR = \left(\frac{1}{A}\right)\left(\frac{dW}{dt}\right)$$

where A is leaf area and dW/dt is the change in plant dry matter per unit time (Fageria, 1992).

Data Analysis

The results obtained were analyzed using analysis of variance (ANOVA). F test was used for a split-split-plot design using SAS software to test for significant effects of treatments as described by Snedecor and Cochran (1967), Gomez and Gomez (1984), where the observed variance ratios were compared with the table values at either 1 or 5%. Differences between means were separated by the use of Duncan multiple range test (DMRT). Correlation and path co-efficient analyses were carried out to ascertain the causes and effects of the parameters on the seed yield using the procedure described by Little and Hills (1978) in order to assess the type and magnitude of the cause-and-effect relationships among the variables.

Results

Net Assimilation Rate

Tables 4, 5, and 6 contain data on Net Assimilation Rate (NAR). Year had no significant effect on NAR. Varieties had significant effect on NAR throughout sampling periods in 2006, at combined level and 2007 except at 9 WAS. SSP and Zinc levels did not show any significant effect on NAR in both 2006 and 2007 at 3 and 5 WAS, except at 7 and 9 WAS. Similarly, SSP levels had no significant effect at the combined effect of two years on NAR throughout sampling periods, except at 5 WAS. Moreover, zinc levels were observed to have no significant effect on NAR at combined levels of two years throughout sampling periods.

Interactions had no significant effects on NAR in 2006, except of V x P at 7 WAS, V x Zn at 5 WAS, P x Zn at 7 WAS and V x P x Zn at 5 WAS. In 2007, interactions were observed to have no significant effect on NAR, except of P x Zn at 5 WAS and V x Zn and P x Zn at 7 WAS. Interactions had no significant effect on NAR at combined levels of two years, except of V x Zn, P x Zn, V x P x Zn, Y x Zn, Y x V x P x Zn and Y x V x Zn at 5 WAS; P x Zn, Y x V x Zn and Y x V x Zn at 7 WAS and V x Zn at 9 WAS (Table 3).

Table 1. Effects of Single Super Phosphate and Zinc levels on net assimilation rate of cowpea varieties grown at Bauchi, 2006

Treatment	Sampling periods (WAS)			
	3	5	7	9
Varieties				
IT90K-277-2	0.08b	0.06a	0.04a	0.03
IT93-455-1	0.12a	0.06a	0.04a	0.03
IT89KD-288	0.07b	0.06a	0.03b	0.03
IT97K-568-18	0.07b	0.05b	0.04a	0.03
IT90K-82-2	0.07b	0.05b	0.03b	0.03
Kanannado	0.08b	0.05b	0.03b	0.03
SE ±	0.012	0.002	0.001	0.001
SSP (kgha^{-1})				
0	0.08b	0.07a	0.04	0.03
25	0.09a	0.05b	0.04	0.03

50	0.08b	0.05b	0.04	0.03
SE ±	0.009	0.002	0.001	0.001
Zinc (kgha^{-1})				
0	0.10a	0.06a	0.04	0.03
2.5	0.08b	0.06a	0.04	0.03
5.0	0.08b	0.05b	0.04	0.03
SE ±	0.009	0.002	0.001	0.001
Interaction				
V x P	n.s	n.s	*	n.s
V x Zn	n.s	**	n.s	n.s
P x Zn	n.s	n.s	*	n.s
V x P x Zn	n.s	**	**	n.s

Means in a column followed by the letter(s) within treatments are not significant different at 5% level of probability using DMRT

Table 2. Effects of Single Super Phosphate and Zinc levels on net assimilation rate of cowpea varieties grown at Bauchi, 2007

Treatment	Sampling periods (WAS)			
	3	5	7	9
Varieties				
IT90K-277-2	0.09a	0.07a	0.04a	0.03
IT93-455-1	0.09a	0.07a	0.04a	0.03
IT89KD-288	0.07c	0.07ab	0.04a	0.03
IT97K-568-18	0.07c	0.06b	0.04a	0.03
IT90K-82-2	0.07c	0.06b	0.04a	0.03
Kanannado	0.08b	0.06b	0.03b	0.03
SE ±	0.002	0.002	1.001	0.001
SSP (kgha^{-1})				
0	0.008	0.07a	0.04	0.03
25	0.008	0.07a	0.04	0.03
50	0.008	0.06b	0.04	0.03
SE ±	0.001	0.001	0.001	0.001
Zinc (kgha^{-1})				
0	0.07b	0.07a	0.04	0.03
2.5	0.08a	0.07a	0.04	0.03
5.0	0.08a	0.06b	0.04	0.03
SE ±	0.001	0.001	0.001	0.001
Interaction				
V x P	n.s	n.s	n.s	n.s
V x Zn	n.s	n.s	**	n.s
P x Zn	n.s	**	*	n.s
V x P x Zn	n.s	n.s	n.s	n.s

Means in a column followed by the letter(s) within treatments are not significant different at 5% level of probability using DMRT

Table 3. Combined effects of Single Super Phosphate and Zinc levels on net assimilation rate of cowpea varieties grown at Bauchi, 2006 and 2007

Treatment	Sampling periods (WAS)			
	3	5	7	9
Year (Y)				
2006	0.08	0.06	0.04	0.03
2007	0.08	0.07	0.04	0.03
SE ±	0.004	0.001	0.000	0.000
Varieties				
IT90K-277-2	0.09b	0.06b	0.04a	0.03b
IT93-455-1	0.10a	0.07a	0.04a	0.03a
IT89KD-288	0.07c	0.06b	0.04a	0.03c
IT97K-568-18	0.07c	0.06b	0.04a	0.03c
IT90K-82-2	0.07c	0.06b	0.03b	0.03c
Kanannado	0.08c	0.06b	0.03b	0.03c
SE ±	0.008	0.002	0.001	0.001
SSP (kgha^{-1})				
0	0.08	0.07a	0.04	0.03
25	0.08	0.06b	0.04	0.03
50	0.08	0.06b	0.04	0.03
SE ±	0.004	0.001	0.001	0.001
Zinc (kgha^{-1})				
0	0.08	0.06a	0.04a	0.03ab
2.5	0.08	0.06b	0.04a	0.03a
5.0	0.08	0.06b	0.04b	0.03b
SE ±	0.038	0.034	0.026	0.023
Interaction				
V x P	n.s	n.s	n.s	n.s
V x Zn	n.s	*	n.s	*
P x Zn	n.s	**	**	n.s
V x P x Zn	n.s	**	n.s	n.s
Y x V	n.s	n.s	n.s	n.s
Y x P	n.s	n.s	n.s	n.s
Y x Zn	n.s	**	n.s	n.s
Y x V x P	n.s	n.s	n.s	n.s
Y x V Zn	n.s	n.s	*	n.s
Y x V x P x Zn	n.s	**	n.s	n.s
Y x P x Zn	n.s	*	*	n.s

Means in a column followed by the letter(s) within treatments are not significant different at 5% level of probability using DMRT

Discussion

Effects of Year, Phosphorus, Zinc and Interactions on NAR of different Varieties of Cowpea

Year had no significant effect on NAR. Similarly, Ofusu-Budu *et al.* (2007; Aikins and Afuakwa (2008) and Addo-Quaye *et al.* (2011) reported that year had no significant effect on the NAR of the cowpea varieties they have studied. Moreover, cowpea varieties were observed not to have significant effect on NAR, and this conformed with the work of Saidah *et al.* (2023), who reported that cowpea varieties did not show significant differences in net assimilation rate. However, these findings contradicted the work of Castro *et al.* (1984); Addo-Quaye *et al.* (2011), and Putul *et al.* (2021), where, varieties were observed to have significant difference on NAR. Varietal differences in NAR might be attributed to differences in the efficiencies of their leaves in producing dry matter (Addo-Quaye *et al.*,

2011). NAR was also influenced by mineral nutrition, solar radiation water supply and as well as growth habits of the varieties (Arnon, 1972; Atakora, 2020).
Effects of SSP and Zinc levels on NAR were significant at 3 and 5 WAS but not at 7 and 9 WAS in 2006, and 2007. At the combined effect of two years, SSP levels were not significant on NAR at all sampling dates except 5 WAS, where 0 SSP kgha^{-1} recorded the highest NAR values. This is in congruence with the findings of Karikari and Arkorful (2015), who reported that P at 0 kg ha^{-1} rate yielded the highest NAR. Effects of Zinc levels were found to be significant on NAR throughout sampling periods at the combined effect of two years only. These findings support those of Aikins and Afuakwa (2008), Olievera *et al*. (2008), Addo-Quaye *et al*. (2011) and Kiri *et al*. (2022). NAR of crops are determined by number of factors such as nutrient supply, photosynthesis rate, etc and these are never fixed or constant and hence a particular factor e.g., nutrient (P or Zn) cannot dictate NAR alone (Mirvat *et al*., 2006; Li *et al*., 2017; Orzech *et al*., 2022).

The results that NAR were significantly different at early periods of growth of the crops corroborated those of Mirvat *et al.* (2006) and Karikari and Arkorful (2015), who reported in their separate studies, that (NAR) for each variety reduced significantly with age of the crop. NAR declined through the growing period as more and more leaves were fully or partially dropped. Similarly, the decrease in NAR as the plant becomes older may attributed to older average leaf age which leads to decreased photosynthetic efficiency (Martín-Hernández *et al*, 2016; Putul *et al*., 2021). NAR was observed to decrease with age due to mutual shading of leaves as plants age and thus decreased assimilation rate (Fageria *et al.,* 2006; Addo-Quaye *et al.* 2011 and Muhammad, 2011; Iseki *et al*., 2022).

Interactions like Y x V, V x Zn, P x Zn, V x P x Zn, Y x Zn, Y x V x P, Y x V x P x Zn and Y x P x Zn were found to have significant effects on NAR. These findings confirmed the observations made by Castro *et al*. (1984); Mirvat (2006); Olievera (2008); Addo-Quaye (2011) and Saidah *et al*. (2023), who reported in their studies that, interactions such as the ones enumerated above had significant effects on NAR.NAR was observed to be significantly affected by P, Zn, Year, and P and Zn. Singh *et al.* (2011) reported that NAR is influenced by many factors which are complex and practically not recognizable.

Conclusion

Year was observed not to affect NAR significantly because NAR is influenced by an amalgamation of mineral nutrition, solar radiation water supply and as well as growth habits of the varieties. Effects of SSP, and Zinc levels are subject to change because factors that affect NAR, such as nutrient supply and photosynthesis rate, are not constant. However, NAR of varieties is significantly different at early phases of plant growth. NAR decreases with plant age. The more leaves are shed the less the NAR. The finding that varieties recorded significant differences in NAR might be attributed to the differences in the efficiencies of their leaves with respect to dry matter production.

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