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Effects of Phosphorus and Zinc levels on Total Soluble Carbohydrate and Crude Protein in Grain of Cowpea (*Vigna unguiculata* (L.) Walp) Grown in Bauchi, Nigeria

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Abstract

Two field experiments were conducted at the Faculty of Agriculture and Agricultural Engineering Research Farm of Abubakar Tafawa Balewa University, Bauchi, between 2006 and 2007 to study the effects of phosphorus (0, 25, 50 kg P/ha) and zinc levels (0, 2.5, 5 kg Zn/ha) on Total Soluble Carbohydrate and Crude Protein of six cowpea varieties namely: IT90K 277, IT93455 1, IT89KD 288, IT97K 568 18, IT90K 82 2 and Kanannado. The objectives of these experiments were to determine which levels of P, Zn and the various interactions of P, Zn, and year that had produced the highest total soluble carbohydrate (TSC) and crude protein (CP) on the grain contents of the cowpea varieties studied and to determine the best varieties in terms of TSC and CP upon the application of these nutrients (P and Zn) in Bauchi, Nigeria. The results revealed that increased levels of P from P0 to P50 significantly increased the CP and TSC contents of the cowpea grains. Similarly, higher Zn levels (2.5 and 5 kgha-1) were observed to have significant effects on TSC and CP contents of the cowpea grains. Interactions of P and Zn were equally observed to significantly affect TSC and CP contents of the cowpea grains. Of all the six (6) cowpea varieties, Kanannado recorded the highest TSC and CP in the cowpea grains. With regard to year, 2006 recorded the highest CP while 2007 had the highest TSC. Higher levels of P and Zn or their associations were recommended for improving TSC and CP contents of the cowpea grains, with emphasis on Kanannado for Bauchi farmers.

Keywords: Cowpea varieties, Total Soluble Carbohydrates and Crude Protein, *Vigna unguiculata* L., Phosphorus and Zinc Levels

INTRODUCTION

Cowpea (Vigna unguiculata L. Walp) is a widely cultivated legume (Alidu *et al*., 2020) food grown around the world (Musa *et al*., 2017) as food for human (Mfeka *et al*., 2019) and animal consumption (Alidu *et al*., 2020).The cowpea grains are highly valued for food, and the fodder and haulm used to feed livestock during the dry season (Mfeka *et al*., 2019; Langyintuo *et al*., 2003). A moderate cheap and readily available source of protein and minerals, and contains

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high concentrations of iron (Fe) and zinc (Zn) (Belar- mino *et al*., 2013; Abebe & Alemayehu, 2022). A typical ripe grain of cowpea contains excellent sources of carbohydrate (50 60%), protein (18 35%) (Addo Quaye *et al*., 2011; Stancheva *et al*., 2017) and 3.7% ash, an energy value of 340 kcal/ 100 g (Putul *et al*., 2021). Cowpea as well comprises substantial amount of micronutrients such as vitamin A, iron, zinc and calcium (Prinyawiwatkul *et al*., 1996; Quaye *et al*., 2009; Alidu *et al*., 2020; Affrifah *et al*., 2022).The crude protein from the grains and leaves is within the range of 23 to 32% (Diouf, 2011; Ddamulira *et al*., 2015; Sebetha *et al*., 2015; Abebe & Alemayehu, 2022). Cowpea leaves, grains and crop residues contain mean crude protein ranging from 32 to 34; 23 to 35 and 11 to 25%, respectively, and contains 62% soluble carbohydrates (Ahamefule &Peter, 2014; Jayathilake *et al*., 2018).

Phosphorus is the main plant nutrient concerned with energy transfer in the plant chemical reactions (Prasad. 2007; Kayoumu *et al*., 2023). Phosphorus is an essential component of numerous significant compounds in plant cells (Shen *et al*., 2011; Wieczorek *et al*., 2022). These compounds comprise the sugar phosphates implicated in respiration (Figueroa & Lunn, 2016; Fichtner & Lunn, 2021) and photosynthesis (Aluko *et al*., 2021). It is also involved in the biosynthesis of phospholipids of plant membranes (Reszczyńska and Hanaka, 2020), and in the nucleotides used in plant energy metabolism and in molecules of DNA and RNA (Taiz &Zeiger,1991; Witte &Herde, 2020; Lambers, 2022). Phosphorus is an indispensable nutrient for the biosynthesis of chlorophyll (Carstensen *et al*., 2018; Kayoumu *et al*., 2023), and in addition to being a component of cell nucleus, it plays a fundamental role in cell division and development of meristematic tissue (Razaq *et al*., 2017; Ahmed *et al*., 2018; Lambers, 2022). Phosphorus significantly increases branches, leaves, fresh and dry weight per plant in cowpea (Abobatta *et al*., 2023) at 60 kg P2 O3/ha (Namakka *et al*., 2017; Kiri *et al*., 2023). Several studies have shown increased tissue P levels with soil availability of P (Balemi & Negisho, 2012; Mathew *et al*., 2018; Wieczorek *et al*., 2022). Phosphorus plays an outstanding function in physiological processes of plants (Kalayu, 2019; Wang *et al*., 2021; Pan *et al*., 2022; Jančaitienė *et al*., 2023). Phosphorus is a key component of ATP and it plays a fundamental role in the transformation of energy in plants (Hu *et al*., 2021; Johan *et al*., 2021; Pan *et al*., 2022; Lambers, 2022; Paz Ares *et al*., 2022) and furthermore, it is crucial for storing energy and its release in living cells (Nkaa *et al*., 2014; Johan *et al*., 2021; Jančaitienė *et al*., 2023). 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 (Nkaa *et al*., 2014; Kyei Boahen *et al*., 2017; Augustine & Godfre, 2019; Sudharani *et al*., 2020; Aryal *et al*., 2021). Phosphorus was also reported to have increased the number of flower primordia (Namakka *et al*., 2017; Dangi *et al*., 2019; Sudharani *et al*., 2020) and early root development (Mohammed *et al*., 2020; Kamboj & Himanshi, 2021).

Zinc essentially functions as a structural component of quite a lot of enzymes including carb onic anhydrase (Escudero-Almanza *etal*., 2012; Lionetto *etal*., 2016; Kim *etal*., 2020), alcohol d ehydrase (Castillo-González *etal*., 2018; de Miranda *etal*., 2022), alkaline phosphatase (Suzuki *etal*., 2020; Mapodzeke *etal*., 2021), phospholipase (Dennis *etal*.,2011; Prasad & Bao, 2019; Zh ang *etal*., 2019; Yang *etal*., 2020; Rai *etal*., 2021; Saleem *etal*., 2022; Alsafran *etal*., 2022), carboxy peptidase (Coleman, 1991; Brown *etal*., 1993; Balafrej *etal*., 2020; Nandal *etal*., 2021) and RNA polymerase (Romheld & Marschner, 1991; Chanfreau, 2013; Fan *etal*., 2021; Stanton *etal*., 2022). Zinc is an essential mineral nutrient and a cofactor of over 300 enzymes and proteins invol ved in cell division, nucleic acid metabolism and protein synthesis (Marreiro *etal*., 2017; Casti llo-González *etal*., 2018; Cheng & Chen, 2021). Zinc nutrition was observed to significantly in crease yield and yield components and above ground biomass of faba bean (*Vicia faba* L.) at maturity phase (Weldua *etal*., 2012; Gerenfes &Negasa, 2021). Cakmak (2000); Subba *etal*. (20 14); Marreiro *etal*. (2017) and Benhamdi *etal*. (2021) have speculated that zinc deficiency stres s may impede the activities of a number of antioxidant enzymes, resulting into wide oxidative damage to membrane lipids, proteins, chlorophyll and nucleic acids. Zinc can impact on carbohydrate metabolism at many levels (Saleem *etal.*, 2022). Moreover, Zn is essential in the biosynthesis of tryptophan, an originator of the auxin-indole-3-acetic acid (Oosterhuis *et al.*, 1996; Ahmed *et al.*, 2012; Castillo-González *et al.*, 2018; Saleem *et al.*, 2022).

Zinc deficiency symptoms comprise small leaves, shortened internodes, and interveinal leaf chlorosis, giving the plant a stunted appearance (Hacisalihoglu, 2020; Khan *etal.*, 2022). Availability of zinc in soils and its absorption and translocation in plants is influenced by all other plant nutrients (Moreno-Lora & Delgado, 2020). Zinc in general interacts negatively with phosphorus which depends upon a number of physicochemical properties of soils (Kumar *etal.*, 2016; Prasad *etal.*, 2016; Santos *etal.*, 2021). Zinc fertilization (at higher dose of 40 kgha^{-1}) was reported to have produced highest protein content in cowpea grains than 0 and 20 kgha^{-1} (Chavan *etal.*, 2012). Similarly, Kumar *etal.* (2002), in an earlier study, observed that zinc nutrition (at 9.0 kgha^{-1}) improved nodulation, nutrient uptake, protein content and protein yield over control (0 kgha^{-1}) in a variety of (fodder) cowpea. Zinc is known to activate several enzymes associated with cell division, cell elongation, and photosynthesis. Consequently, zinc nutrition promotes zinc concentration, yield, and crude protein content of a plant (Safak *et al.*, 2009; Rudani *et al.*, 2018; Umair *et al.*, 2020; Santos et al., 2021; Saleem *et al.*, 2022). Crops treated with zinc were observed to be more robust and grow better since zinc is implicated in making RNA and DNA structurally more stable (Chanfreau *et al.*, 2013; Chakraborty & Mishra, 2020; Saleem *et al.*, 2022), in addition to its involvement in the biogenesis of auxins and gibberellins that are known for their abilities to promote growth in plants (Mousavi, 2011; Castillo González et al., 2018; Hassan *et al.*, 2020; Mapodzeke *et al.*, 2021).The objectives of this study were to examine which levels of P, Zn and the various interactions of P, Zn, and year had induced the highest total soluble carbohydrate and crude protein on the varieties and to determine the best varieties in terms of TSC and CP upon the application of these nutrients (P and Zn) in the scrub savanna of Nigeria.

MATERIALS AND METHODS

Description of the Site of the Study

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 leaf area ratio (LAR) of cowpea (*Vigna unguiculata* (L.) Walp) varieties. Bauchi, is located at 10.3010° N latitude and 9.8237° E longitude at an altitude of 109.45 m 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. Average rainfall for the 2006 and 2007 mean monthly temperature; and other meteorological data were collected during the experimental periods. The soils of the experimental site were found to be moderately well drained, deep, and tropically sandy loam. The physicochemical properties of the soil of the experimental sites for the two years were determined using the procedures described by Black (1965).

Experimental Design

A split-split plot design with a total size of the experimental area of 50 by 62 m was used. There were three (3) replicates and each replicate consisted of three sub-plots; each measuring 2.25 by 18.9 m. Each sub-plot was divided into six (6) sub-sub-plots with each measuring 2.25 by 6.30 m. A space of 1 m each was left between main plots, and replicates. Half a meter (0.5 m), and 50 cm were left between sub-plots, and sub-sub-plots respectively. Main plots were assigned to three different levels of single super phosphate (SSP) namely 0, 25, and 50 kgha^{-1} at random. Sub-plots were assigned to three (3) different levels of Zn namely 0, 2.5, and 5 kgha^{-1}. A total of fifty-four (54) treatments consisting of six varieties by three SSP levels by three Zn levels. The treatments were randomized using table of random numbers as described by Gomez & 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) 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 15-30 cm), 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 14.2 cm^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 75 cm row to row and 25 cm 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

Single Super Phosphate was incorporated into the soil before sowing as top dressing is not recommended by ITTA (2000). 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 (21 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 Crude Protein

Crude protein was determined by the Kjedahl method (Chopra and Kanweer, 1991). Two grams (2 g) of the powdered sample was placed in a Kjedahl flask and 30 ml concentrated H_2SO_4 added; 10 g of K_2SO_4 was also added. The flask was heated in a fume cupboard using heating mantle, first gently but more strongly after frothing stopped. When the solution became colourless, it was reacted for another hour and later cooled. The solution was then diluted with 200ml distilled water and transferred to 800 ml Kjedahl flask. Four pieces of granulated Zinc was put in the flask and 100 ml of 40% caustic soda was added. The flask was connected to the splash heads of the distillation apparatus and 25 ml or 0.1 N H_2SO_4 was collected in the receiving flask and distilled. When the distillation was completed, the flask was removed and the distilled titrated against 0.1N caustic soda (NaOH) solution using methyl red as indicator. The amount of protein was calculated thus: Weight of N_2 = 0.004 x volume of 0.1 NH_2SO_4 taken – volume of 0.1N NaOH used. Crude Protein=Weight of N x 6.25

Therefore, crude protein = $\frac{\text{crude protein}}{\text{weight of sample}} \times 100$

Determination of Ether (Crude Fat)
Ether extract was determined using Soxhlet extraction assembly. 2 g of sample already dried in a moisture cup was transferred into previously prepared thimble. The mouth of the thimble was plugged with fat free absorbent cotton. The clean, dry receiver flask from the Soxhlet (extract) assembly was taken and weighed accurately. The thimble with sample was introduced into the Soxhlet. The Soxhlet was filled with petroleum ether by pouring it through the condenser at the top by means of glass funnel. The apparatus was placed on a water bath at 60 ^{0}C fixed by clamps to a retort stand, and cold-water circulation in the condenser started. Extraction was done for 8 hours (about 250 times). After the extraction, the thimble with the materials were removed from Soxhlet again and heated on the water bath to recover all the ether from the flask. The outside of the flask was wiped thoroughly with a clean dry cloth to remove film of moisture and dust. It was then dried in a hot air oven at 100 ^{0}C for 1 hour, cooled in a desiccator and weighed (The extraction thimble with the material were preserved in a desiccator for crude fibre determination. Ether extract (crude fat) was calculated as: $W_2 - W/M \times 1\,X\,10$.
Where, W_2 = weight of empty oil flask; W = weight of flask after extraction; M = weight of direct material taken.

Determination of Crude Fibre
The crude fibre was determined as described by AOAC (1990) procedure. 2 g of fat free sample was transferred to 600 ml beaker, and about 1g prepared asbestos and 200ml boiling acid were added, together with a little antifoaming agent. The beaker was then heated on the digestion apparatus, with periodic rotation to keep the solids from adhering to the sides of beaker. The moisture was boiled for exactly 30 minutes and then filtered, in one operation and without breaking the suction, 50 -75 ml boiling water was added to the filter. The beaker filter mat, and residue were washed with three 50ml portions of water and the residue sucked dry. The filter mat and residue were returned to the beaker and 20ml boiling alkali added. The mixture was again boiled exactly 30 minutes and filtered, as before without breaking the suction, the filter was then washed with 25 ml boiling acid, then with three 50ml portions of boiling water and 25 ml alcohol. The filter and residue were dried for 2 hours at 130 ^{0}C, cooled in a desiccator and reweighed.
Crude fibre = loss on ignition – loss of asbestos blank

Percentage of Carbohydrate
Percentage of carbohydrate was calculated as: 100- (Moisture content + ash + crude protein + ether extract + crude fibre) = % moisture content + % ash + % crude protein + % ether extract + % crude fibre.

Determination of Moisture Content

Moisture content was determined by recording fresh weight and dry weight of the sample. The percentage moisture of sample was calculated as: Wc = wf-wd x 100/wf. Where: wc = water content (g); wf = fresh weight (g); wd = dry weight (g); % moisture = 100 – wd.

Determination of Ash

The residue remaining from burning or destruction of any biological material or organic matter in furnace at 550 ^{0}C is called ash. Percentage ash was determined as follows. 2 g of the prepared sample was placed in a crucible for determination of ash. The crucible was placed in cold muffle furnace maintained at 550 ^{0}C $\pm$ 5 ^{0}C overnight. It was kept at this temperature until white light grey or reddish ash was obtained which appeared to be from carbonaceous particles. The crucible was placed in a desiccator, allowed to cool and later weighed. The ash content was expressed as percentage on dry matter basis and was calculated as: (w + a)- a x 100/R = % ash. Where: w = weight of crucible + ash (g); a = weight of crucible(g); R = weight of sample (g).

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 & 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 & Hills (1978) in order to assess the type and magnitude of the cause-and-effect relationships among the variables.

RESULTS

Total Soluble Carbohydrate and Crude Protein of Cowpea Grain in 2006

Results in Table 1 are on the effects of Zinc and SSP levels on TSC and CP of cowpea grains in 2006. Varieties had significant effects on TSC and CP throughout sampling periods, except CP at 2 WAPS. Kanannado recorded the highest TSC throughout sampling periods. The least TSC was recorded by IT90k-277-2 at 1 and 2 WAPS, while IT89KD-288 recorded the least TSC at 3 WAPS. Kanannado recorded the highest CP at 1 and 3 WAPS.

Single Super Phosphate levels had significant effect on both TSC and CP throughout sampling period. At 1 WAPS, the highest and least TSC, were recorded by 0 and 25 and 50 SSP kgha^{-1}, respectively. However, at 2 WAPS, 25 and 50 recorded higher TSC than 0 SSP kgha^{-1}, whereas, at 3 WAPS, 50 and 25 SSP kgha^{-1} recorded the highest and least TSC. At WAPS, 0 and 50 recorded higher CP than 25 SSP kgha^{-1}, but at 2 WAPS, the highest at least CP, were recorded by 25 and 50 SSP kgha^{-1}, respectively. At 3 WAPS, the highest and least CP was recorded by 0 and 25 SSP kgha^{-1}.

Zinc levels at 0 and 5 kgha^{-1} recorded higher TSC at 1 WAPS than 2.5 kgha^{-1}. Zinc levels had no significant (p>0.05) effect on TSC at 2 WAPS. But at 3 WAPS, 2.5 and 5 Zn kgha^{-1} recorded higher TSC than the control. At 1 WAPS, the highest and least effect on Zinc levels on CP, were recorded by 2.5 and 0 kgha^{-1}. 2.5 and 0 Zn kgha^{-1} recorded the highest and least effects on CP at 2 WAPS. At 3 WAPS, the highest and least CP was recorded by the control and 2.5 Zn kgha^{-1}. Interactions between V x P, V x Zn, P x Zn, V x P x Zn had no significant (p>0.05) effect on TSC throughout sampling periods except of V x P and V x Zn at 2 WAPS. Interactions between V x P, V x Zn, P x Zn, V x P x Zn were also not significant ($p>0.05$) on CP at 1 and 2 but not at 3 WAPS (Table 1).

Total Soluble Carbohydrate and Crude Protein of Cowpea Grain in 2007

The effects of Zinc and SSP levels on TSC and CP of cowpea grain are presented in Table 2. Data indicated that varieties, SSP and Zinc levels had significant effect on TSC and CP of cowpea grains in 2007. At 3 WAPS, the highest and least TSC and CP were recorded by Kanannado and IT90K-277-2, respectively. The control (0 SSP kgha^{-1}) had higher TSC and CP than 25 and 50 SSP kgha^{-1}. 2.5 Zn kgha^{-1} recorded higher TSC than 0 and 5 Zn kgha^{-1}.5 Zn kgha^{-1}recorded higher CP than 0 and 2.5 Zn kgha^{-1}. Interactions had no significant ($p>0.05$) effect on both TSC and CP at 3 WAPS. Interactions had significant ($p>0.05$) effect on TSC at 1 WAPS but not at 2 WAPS. CP was significantly ($p>0.05$) affected by interactions at 2 WAPS. At 1 WAPS, V x Zn and V x P x Zn had significant ($p>0.05$) effects on CP but not V x P and P x Zn.

Total Soluble Carbohydrate and Crude Protein of Cowpea Grain at Combined Effects in 2006 and 2007

Data of combined effects of Zinc and SSP levels on TSC and CP of cowpea grains in 2006 and 2007 are presented in Table 3. Year had no significant ($p>0.05$) effect TSC and CP at 1 and 2 WAPS, respectively. The year 2007 recorded higher TSC at 2 and 3 WAPS. Higher CPs at 1 and 3 WAPS were recorded in 2006. Varieties had no significant ($p>0.05$) effect on TSC and CP throughout sampling periods. At 3 WAPS, Kanannado had the highest TSC and CP. The least TSC and CP at the same period, were recorded by IT89KD-288 and IT90K-277-2, respectively. SSP levels had significant ($p>0.05$) effects on TSC and CP throughout sampling periods. SSP levels did not have significant effects on TSC at 1 WAPS but at 2 and 3 WAPS. At 2 WAPS, 50 SSP kgha^{-1} had higher TSC than 0 and 25SSP kgha^{-1}, while at 3 WAPS, 25 and 50 SSP kgha^{-1} recorded higher TSC than the control. For the effect of SSP levels on CP, 50 and 0 kgha^{-1} recorded higher and least CP at 1 WAPS; at 2 WAPS, the highest and least CP were recorded by 25 and 50 kgha^{-1}, respectively. The highest and least CP at 3 WAPS was recorded by 0 and 25 kgha^{-1}. Zinc levels had no significant ($p>0.05$) effects on TSC at 1 and 3 but not at 2 WAPS, where the highest and least TSC were recorded by 0 and 2.5 kgha^{-1}, respectively. Zinc levels had significant ($p>0.05$) effects on CP at 1 and 2 but not at 3 WAPS. At 1 WAPS, the highest and least CP were recorded by 5 and 0 Zn kgha^{-1}, while at 2 WAPS, the highest and least CP were recorded by 5 and 0 Zn kgha^{-1}, respectively.

Table 1. Effect of Phosphorus and Zinc Levels on **Total Soluble Carbohydrate and Crude Protein of Cowpea Grain at** Week After Pod Setting Grown at Bauchi in 2006

Treatment	Sampling dates (WAPS)					
	1		2		3	
	TSC (%)	CP (%)	TSC (%)	CP (%)	TSC (%)	CP (%)
Varieties						
IT90K-277-2	62.09b	24.14c	63.11f	24.17	63.58a	27.29e
IT93-455-1	62.25b	24.28c	63.24d	26.33	63.67a	27.61d
IT89KD-288	62.08b	24.29c	63.16e	26.34	61.49b	27.47d
IT97K-568-18	60. 89c	24.69b	63.34c	26.67	61.72b	28.05c
IT90K-82-2	62.61b	24.84b	63.61b	26.89	64.05a	28.21b
Kanannado	63.27a	25.30a	64.36a	26.84	64.76a	29.11a
SE ±	0.589	0.028	0.014	0.388	1.227	0.017
SSP (kgha^{-1})						
0	62.43a	24.62a	63.45b	26.68b	62.92b	28.00a
25	62.43a	24.54b	63.47a	26.80a	62.77c	27.91c
50	61.73b	24.61a	63.48a	26.13c	63.95a	27.98b
SE ±	0. 417	0.020	0. 010	0.275	0.868	0.012
Zinc (kgha^{-1})						
0	62.13a	24.56b	63.47	26.20b	61.80b	28.05a
2.5	61.74b	24.61a	63.47	24.76a	63.93a	27.87c
5.0	62.44a	24.60b	63.47	26.65a	63.91a	27.96b
SE ±	0. 417	0. 020	0.010	0.275	0.868	0.012
Interactions						
V x P	n. s	n. s	*	n. s	n. s	**
V x Zn	n. s	n. s	**	n. s	n. s	**
P x Zn	n. s	n. s	n. s	n. s	n. s	**
V x P x Zn	n. s	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 2. Effect of Phosphorus and Zinc Levels on **Total Soluble Carbohydrate and Crude Protein of Cowpea Grain at** Week After Pod Setting Grown at Bauchi in 2007

Treatment	Sampling dates (WAPS)					
	1		2		3	
	TSC (%)	CP (%)	TSC (%)	CP (%)	TSC (%)	CP (%)
Varieties						
IT90K-277-2	62.00d	24.24d	63.05	25.12e	63.44e	27.20f
IT93-455-1	62.14c	24.27d	63.20	26.26d	63.63d	27.54d
IT89KD-288	62.14c	24.25d	63.25	26.27d	63.58d	27.42e
IT97K-568-18	62. 23b	24.58c	63.30	26.60c	63.79c	28.00c
IT90K-82-2	62.12c	24.74b	64.59	26.78b	64.13b	28.17b
Kanannado	63.12a	25.20a	64.14	27.93a	64.68a	29.98a
SE ±	0.029	0.039	0.640	0.045	0.046	0.064
SSP (kgha^{-1})						
0	62.26b	24.58a	63.32b	26.59b	62.95a	27.93a
25	62.39a	24.54b	63.41b	26.79a	63.84b	27.81b
50	62.38a	24.52b	74.04a	26.59b	63.83b	27.92a
SE ±	0. 021	0.027	0. 110	0.032	0.032	0.046
Zinc (kgha^{-1})						
0	62.35b	24.47c	72.98a	26.75a	63.84b	27.89a
2.5	62.32c	24.53b	63.38b	26.54c	63.92a	27.84b
5.0	63.36a	24.64a	63.41b	26.69b	63.86b	27.93a
SE ±	0. 021	0. 027	0.010	0.032	0.032	0.046
Interactions						
V x P	**	n. s	n. s	**	**	**
V x Zn	**	*	n. s	**	**	**
P x Zn	**	n. s	n. s	**	**	**
V x P x Zn	**	**	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 Effect of Phosphorus and Zinc Levels on **Total Soluble Carbohydrate and Crude Protein of Cowpea Grain at** Week After Pod Setting Grown at Bauchi in 2006 and 2007

Treatment	Sampling dates (WAPS)					
	1		2		3	
	TSC (%)	CP (%)	TSC (%)	CP (%)	TSC (%)	CP (%)
Year (Y)						
2006	62.20	24.59a	63.47b	26.54	63.21b	27.96a
2007	62.34	24.55b	66.92a	26.66	63.88a	27.89b
SE ±	0.160	0.016	2.500	0.132	0.270	0.013
Varieties						
IT90K-277-2	62.05c	24.19e	63.08b	26.15c	63.51a	27.24f
IT93-455-1	62.19c	24.27d	62.22b	26.29c	63.65a	27.58d
IT89KD-288	63.11a	24.27d	62.21b	26.30c	63.53a	27.45e
IT97K-568-18	61. 56d	24.64c	63.32b	26.64b	62.75b	28.03c
IT90K-82-2	62.51b	24.76b	74.10a	26.82b	64.09a	28.19b
Kanannado	62.20a	25.25a	64.25a	27.39a	64.72a	29.05a
SE ±	0.295	0.026	4.315	0.227	0.639	0.014
SSP (kgha^{-1})						
0	62.34	24.60c	63.39b	26.64b	63.43b	27.96a
25	62.41	24.54b	63.44b	26.80a	63.31a	27.86b
50	61.05	24.57a	68.76a	26.36c	63.89a	27.95a
SE ±	0. 209	0.017	3. 053	0.126	0.431	0.012
Zinc (kgha^{-1})						
0	62.38	24.52c	68.72a	26.47b	62.82b	27.97
2.5	62.03	24.57b	63.42b	26.65a	63.92	27.85
5.0	62.40	24.62a	63.44b	26.67a	63.89	27.94
SE ±	0. 208	0. 013	3.052	0.138	0.432	0.090
Interactions						
V x P	n. s	*	n. s	n. s	n. s	**
V x Zn	n. s	n. s	n. s	n. s	n. s	**
P x Zn	n. s	n. s	n. s	n. s	n. s	**
V x P x Zn	n. s	*	n. s	n. s	n. s	**
Y x V	n. s	n. s	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	n. s	n. s	n. s
Y x V x P	n. s	n. s	n. s	n. s	n. s	n. s
Y x V x Zn	n.s	*	n. s	n. s	n. s	*
Y x V x P x Zn	n. s	**	n. s	n. s	n. s	*
Y x P x Zn	n.s	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

DISCUSSION

Effects of Year, Phosphorus, Zinc and their Interactions on Total Soluble Carbohydrate and Crude Protein of Different Varieties of Cowpea Grain

Effects of Phosphorus and Zinc levels on Total Soluble Carbohydrate and Crude Protein in Grain of Cowpea (Vigna unguiculata (L.) Walp) Grown in Bauchi, Nigeria

The observation that year 2006 had higher CP while 2007 had higher Total Soluble TSC could be attributed to difference in meteorological factors. Temperature (Daniel et al., 2008; Moore et al., 2021), sunshine and photoperiod (Haque et al., 2015; Macioszek et al., 2021; Roeber etal., 2022) are reported to influence enzymic activity and other metabolic processes that are necessary for the synthesis of both protein (Hildebrandt et al., 2015; Rasheed et al., 2020; Trovato et al., 2021) and starch (Apriyanto et al., 2020; Tetlow & Bertoft; 2020; Yu etal., 2022). For example, temperature influences the development of cowpea varieties (Angelotti & Barbosa, 2020); heat stress during flowering may modify a sequence of physicochemical processes, comprising heat shock proteins, antioxidants, metabolites and hormones centred with sugar starvation (Liu etal. 2019). Temperature was reported to significantly affect CP content of cowpea compared to the control (Nevhulaudzi, 2020).

The observation that Kanannado recorded the highest TSC and CP in the cowpea grain planted may be attributed to its possession of large canopy, whose primary function is to intercept radiation to derive photosynthesis and other metabolic processes (Frantz et al., 2000; Fageria et al., 2006; Digrado et al., 2020; Liu et al., 2021; Sultana et al., 2023). Again, and its inherent ability to transport larger amount of photosynthate from sinks to sources than other varieties may be attributed to the recorded results. This interception is determined largely by leaf area, configuration relative to the sun, and to a lesser extent the spatial arrangements of leaves (Digrado et al., 2020). Because crop growth and yield are largely determined by photosynthesis which is generally increased by greater interception of sunlight and thus increasing grain or dry matter yield (Fageria et al., 2006; Alidu & Appiah, 2022).

The observations that each of P (0, 25, 50 kgha-1) and Zn (0, 2.5, 5.0 kgha-1) levels at one time or the other induced higher effect on TSC and CP than others could be ascribed to the fact that the effect of each of P and Zn levels on translocation and partitioning of both TSC and CP may be attributed to be determined by the duo of climatic factors and mineral nutrition. Hence, the association of rainfall, humidity, sunshine, temperature, etc. may in collaboration with mineral nutrients e.g., P and Zn, interfere with the synthesis of both protein and starch formation. However, the finding in this study is not in conformity with that of Magani and Kochinda (2009), Rathore et al. (2015), and Mohammed et al. (2021) who reported in their different studies, that higher doses of P and Zn levels improved CP content (including ether extract and ash) and TSC in cowpea than the lower doses (and the control). According to Blum et al. (1997); Paixão et al. (2019); Zhang et al. (2022); vigorous leaf growth in crop plants has generally been associated with long-term gains in photosynthetic potential. Plant size may provide substantial yield benefits (Fageria et al., 2006; Rosas et al., 2013; Tswanya et al., 2023).

Phosphorus had significant ($p>0.05$) effects on TSC and CP and it is obvious from Tables 1, 2 and 3 that increasing levels of P from P0 to P50 kgha-1 significantly ($p>0.05$) increased the CP and TSC contents of the cowpea leaf, being highest at 50 kgha 1. This may be due to the fact that P is required for synthesis of phospholipids (Zhu et al., 2022), nucleotides, ATP, glycophosphates, and other phosphate esters (Fageria et al., 2006; Kolodiazhnyi, 2021; Lambers, 2022). Phosphorus deficiency decreases photosynthetic activity for several plant species (Israel and Rufty, 1988; Carstensen et al., 2018; Meng et al., 2021; Kayoumu et al., 2023). Higher P levels (25 and 50 kgha-1) were observed to have significant effects on TSC than the control. This observation could be adduced to the cumulative effect of P in the processes of cell division and protein nutrition (Zafar, 2003; Kvakic´ et al., 2020; Bechtaoui et al., 2021).

Higher Zn levels (2.5 and 5 kgha-1) were observed to have significant ($p>0.05$) effects on TSC than the control. This may be due to the fact that Zn is important in fruiting (Khan et al., 2022), growth and metabolism of crop plants (Fageria, 2009; Andresen et al., 2018; Dobrikova et al., 2021). This observation may be ascribed to the fact that Zn is involved either directly or indirectly in starch formation, since Zn deficient plants often have been reported to have reduced starch concentrations (Fageria *et al*., 2006; Rudani *et al*., 2018; Fan et al., 2021). Zn is also vital for oxidation processes in plant cells (Cakmak, 2000; Bastakoti, 2023), involved in transformation of carbohydrates, and regulates sugars in plants. Its deficiency retards photosynthesis and N metabolism (Sadeghzadeh, 2013; Suganya *et al*., 2020).

Interactions of P and Zn were observed to significantly ($p>0.05$) affect TSC and CP by stimulating growth and development with consequent remobilization of enzymes involved in synthesis of protein and starch macromolecules. Associations of P and Zn were observed, at one period or the other, to interact with variety, year or both to significantly ($p>0.05$) affect TSC and CP. These observations could be due to the influence of each of P, and Zn, in protein synthesis, growth and metabolism in plants and thus enhancing yield and seed quality (Mousavi, 2011; Chavan *et al*., 2012; Weldua et al., 2012; Rathore et al., 2015; Santos *et al*., 2021; Gerenfes & Negasa, 2021; Sánchez-Rodríguez *et al*., 2021).

CONCLUSION AND RECOMMENDATIONS

Applications of higher levels of P and Zn or their associations were reported in this study to have improved TSC and CP contents of the cowpea grains at some period after pod setting, than lower levels. However, TSC and CP could further be augmented by ensuring an optimum environmental condition that favours growth and yield quality. This could be best achieved through best management practices such as proper weeding, thinning of plants, adequate fertilization, pests and insect control, and more importantly selecting viable seeds. Finally, for improved CP and TSC, Kananado is recommended for Bauchi farmers.

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