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High soil phosphorus application significantly increased grain yield, phosphorus content but not zinc content of cowpea grains

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Abstract

Background and aims Phosphorus (P) is limiting in many soils of cowpea producing areas. To ameliorate the impact of soil P deficiency, the use of P-based fertilizers is highly recommended. However, a negative association between zinc (Zn) content and high P application has been reported in some crops. There are few reports about P-Zn interaction on cowpea. Thus, in this study, the response of cowpea to the varying amounts of P and their effect on plant P and Zn content, and yield were investigated.

Methods Thirty genotypes were grown at three P rates in screenhouse and field environments. In the screenhouse, shoot biomass, P and Zn content were measured at 55 days after sowing. In the field, grain yield, P, and Zn content were determined in the harvested tissues when plants reached full maturity.

Results Higher rates of P in the growth media led to significantly increased shoot biomass, and grain yield but were not associated with a significant change in shoot or grain Zn content. There was not a significant correlation between grain yield and Zn content in high soil P ($p < 0.05$). The site regression analysis revealed that the first two principal components explained 69.76%, and 22.30% of total variance for grain yield, and 69.01% & 24.66% of total variance for Zn content.

Conclusions The effect of higher P application on reduced shoot and grain Zn content may be genotype-dependent and could be circumvented if genotypes with high Zn content under higher P supply are identified and cultivated.

1 Introduction

Cowpea is a pulse crop providing food, nutrition and cash for its value-chain actors across sub-Saharan Africa (SSA) and beyond (Abate et al., 2012; Boukar et al., 2018). Every part of the crop including leaves, green pods, and grains are used by humans in making different dishes and its fodder is used for livestock feed (Langyintuo et al., 2003; Phillips et al., 2003). Due to its rich protein content, the crop serves as an important supplement for the carbohydrate-rich cereals (Phillips et al., 2003; Samireddypalle et al., 2017). It is a strategically important crop capable of providing millions of people with nutritional security owing to its rich nutritional value, especially high protein, iron (Fe), and zinc (Zn). This contributes to its increasing popularity (Snapp et al., 2018).

Zn is among the seven most limiting micronutrients in human diets, as over two-thirds of the global diets are deficient in one or more of iron, zinc, copper, calcium, magnesium, iodine and selenium (Philipo et al., 2020; White & Broadley, 2009). Zn is especially needed by pregnant women and children, especially because of its critical roles in pregnancy, enhanced protection from infection and maintenance of immune system functions. Its deficiency can lead to complications in childbirth, low birth weight, poor growth of children, and reduced tolerance to infectious diseases (Brown et al., 2001; Deshpande et al., 2013; Hambidge, 2000). Zn is also an important factor in the prevention and treatment of common diseases such as common cold, malaria, diarrhoea and respiratory infections (Brown et al., 2001; Deshpande et al., 2013).

Increasing the bioavailability of these nutrients in human diets has become a global priority, particularly for Fe and Zn in developing countries where many people depend on plant-based food products for nutrition (Blair et al., 2010; Manzeke et al., 2017; Snapp et al., 2018). Enhancing the bioavailability of micronutrients in food could be achieved through improved agronomic practices such as optimum application of fertilizers with micronutrients and genetic improvement of elite crop varieties (Blair et al., 2010; White & Broadley, 2009). Other ways to remedy micronutrients deficiency in food crops include biofortification, diversity of diet, mineral supplementation, and food fortification (Gaddameedi et al., 2018; Genc et al., 2005; White & Broadley, 2005, 2009).

Micronutrient deficiency is not only found in edible parts of plants, like grains, in SSA but also in most soils of growing areas of major crops including cowpeas, that are cultivated by smallholder farmers on soils with low available phosphorus (P) with minimal to no-P application (Horn et al., 2014; Magani & Kuchinda, 2009; Mohammed et al., 2020). Micronutrient deficiency of grains is further worsened because most farmers do not apply micronutrients such as Zn to crop plants, even when the application of Zn to cowpea is known to increase both grain yield and Zn content (Manzeke et al., 2017). The low P of soils further lead to low productivity of the crop (Boukar et al., 2018; ICRISAT, 2017; Olufajo & Singh, 2002).

For cowpeas, two complementary approaches have been recommended to achieve a high yield on soils with low available P; first is the use of improved agronomic approaches such as application of phosphate fertilizers, and second is the development of elite varieties with high P use efficiency (Oladiran et al., 2012; Sanginga et al., 2000; Simpson et al., 2011). Varieties with improved P efficiencies use different mechanisms including increased solubilization and mobilization of soil P, improved root architecture traits, increased ability to acquire P and accumulate it in edible tissues (Adusei et al., 2016; Gyan-Ansah et al., 2016; Saidou et al., 2012; Sanginga et al., 2000). Application of fertilizers supplying major nutrients like nitrogen-phosphorous-potassium (NPK), with little or no consideration of micronutrient requirements, may lead to a reduced amount of micronutrients in the edible tissues that serve as food for people (Zhang et al., 2012), since the micronutrient content of grains depend largely on the supply from soil nutrient pool and micronutrients-containing fertilizers (White & Broadley, 2009; Zhang et al., 2012).

Cereals are the most important source of calories globally but are deficient in Zn and Fe due to the high content of phytic acids in grains that decrease the bioavailability of Zn. Therefore, Zn deficiency is likely to be high in areas where cereals are the dominant food source (Brown et al., 2001; Genc et al., 2005). Legumes, being rich in important micronutrients like Fe and Zn, serve as good complimentary food to micronutrient deficient cereals (Gonçalves et al., 2016; Snapp et al., 2018).

P is stored in most legumes including cowpea as phytic acid which is known to limit the absorption of Zn (Cichy et al., 2009; Gonçalves et al., 2016; Zhang et al., 2012). Loneragan & Webb, (1993) reported that there is confusing literature on P-Zn interactions for crop plants and noted that the subject of P-Zn interactions remains complex, involving many phenomena in both soils and plants, such as observations that P applications can decrease, or not decrease, Zn concentrations in plant shoots. For cowpea, the application of phosphate fertilizer is required for increased productivity in areas with low soil available P. However, its influences on micronutrient availability on the grains and leaves have not adequately been investigated (Ayeni et al., 2018; Gonçalves et al., 2016; Santos & Boiteux, 2013). The application of P, especially at a high rate, may be counterproductive, if P application negatively affects Zn availability, as has been found in cereals (Ashok Kumar et al., 2013; Gaddameedi et al., 2018; Genc et al., 2005; Zhang et al., 2012). This study investigated the influence of P fertilizer applications on yield and the potential relationship between P and Zn content of cowpea grain and fodder at three levels of soil P concentrations.

2 Materials And Methods

2.1 Plant materials

A total of thirty elite cowpea genotypes from four different breeding programs in West Africa (Nigeria, Ghana, and Burkina Faso) and the USA were used. Twenty were parents for recombinant inbred line populations previously studied for mapping of important biotic and abiotic stresses (Boukar et al., 2016; Huynh et al., 2013; Muñoz-Amatriaín et al., 2017). The genotypes are presented in Online resource 1.

2.2 Description of the experiments

Two experiments were conducted: one in the screenhouse and the other under field environment. In the screenhouse experiment, river-sand was collected from the bank of a local stream "*Rafin Kudingi*" at the Ahmadu Bello University Nigeria (N11^{0}09'49.6" E007^{0}37'13.8") and was acid-washed with 1% HCl. The sand used had very low P (3.70 mg/Kg), N (0.11 g/Kg) and K (0.08 cmol/Kg) and organic carbon (0.24 g/Kg of soil contents), determined at the Department of Soil Science, Ahmadu Bello University Nigeria, and appropriately mimics major nutrients deficient soil. A Hoagland nutrient solution with three different concentrations of P (0, 1.5 and 30 mg P/kg of soil) was used to water the plants during the experiment. The experiment used a complete randomized block design in five replications. Cowpea seeds were sown and thinned to one plant per pot 10 days after sowing (DAS). The pots were watered with the dilute nutrient solution with graduated beakers as follows; 300 ml per pot at planting, and subsequently 300 ml was applied at 3 DAS, 6 DAS, 9 DAS, 16 DAS, 23 DAS, 30 DAS, 37 DAS, 44 DAS, and 51 DAS. Reverse osmosis (RO) was used to water the pots periodically to prevent wilting and prevent the accumulation of salts in the soil. Spraying of insecticide (Karate 50 g/l) was used to protect the plants against insect pests. Data on plant height (g), shoot dry weight (g), root dry weight (g), shoot P and Zn content were collected from the screenhouse plants. Plant growth continued until 55 days, at which the screenhouse experiment was terminated.

A field experiment, comprising the same set of genotypes used in the screenhouse experiment described above, was conducted in a low-P field (4.2 mg P/kg- Bray-I method) at the Institute for Agricultural Research farm (N11^{0} 10'31.7", E 007^{0}36'43.9"), Nigeria. The field used has been left uncultivated for several years and had low P (4.2 mg/kg), N (0.13 g/kg) and K (0.68 cmol/kg) and organic carbon (0.79 g/kg of soil content), as determined by the Department of Soil Science, Ahmadu Bello University Nigeria. In the field, P was applied using the single super phosphate (SSP 18% P_2O_5) fertilizer at the rate of 0, 10, and 60 kg P_2O_5 ha^{-1}. The field arrangement was a strip plot design with two replications. All the field plots received 30 kg/ha N (46% N, Urea) and K (30% K_2O, Muriate of Potash) to avoid any confounding effects of N and K deficiency on the plants. All the single fertilizers were applied as dual banding by placing them below soil surface five days after sowing. Plants were protected against insect pests by spraying insecticide (Karate 50 g/l). Data collected from the field experiment are shoot dry weight, plant height, grain yield, P and Zn content of the grains. See Table 1 for list and abbreviation of the traits measured.

2.3 Determination of Phosphorus and Zinc content from plant tissues

Samples of shoot and grains of plants were taken from the screenhouse (only shoot samples) and field experiments (in two replications) and analyzed for P and Zn content. Grain samples were milled using mortar and pestle and with an electric blender for shoot samples. 0.2 g of each sample was then weighed using an electric balance (Scout^{tm} pro SPU202, Ohaus Corporation) and digested with 30 ml mixed acids (containing nitric acid- 26 ml, perchloric- 3.2 ml and sulphuric-0.8 ml acids), then placed on a digestion block set at 230–300 ^{o}C. The digest was then transferred into a 50 ml volumetric flask and brought up to 50 ml with distilled water. Thereafter, 5 ml of the extract for each sample was pipetted into a 25 ml Volumetric flask and a 5 ml of a colour developing agent made up of 50 g ammonium molybdate dissolved in 500 ml distilled water, 2.5 g ammonium metavanadate dissolved in 500 ml distilled water, and 350 ml nitric acid in 1000 ml of distilled water which were all mixed and made up to 2.5 litres with distilled water. To determine P concentration, absorbent readings were taken using Spectrophotometer (Spectronic Unicam, Helios Gamma Spectrophotometer, Model UVG 875005) at a wavelength of 460 nm. The Zn content was determined using an atomic absorption spectrometer (Agilent Technologies, Model AA280FS). Zinc standard solutions were made using the 1000 µg/mL Zn Standard (1000µg/mL SpectraScan, Teknolab AS). The P content was determined in mg/kg and Zn was in parts per million. The formula for Zn content and P content computation are presented (Online resource 6)

2.4 Statistical analyses

2.4.1 Descriptive analysis

Data on shoot parameters, yield (shoot and grain), P concentrations and Zn content was analyzed using the linear mixed effect model to generate best linear unbiased estimator (BLUE) using *lme4* package in R. The BLUE outputs were used to visualize the phenotypic distribution of the measured traits with Box plots using *ggplot2* package in R (R Core Team). The relative difference (%) in performance between the high P and low P (control) were computed (Online resource 6).

2.4.2 Relationships among yield, phosphorous and zinc content

The relationship among yield, P and Zn content phenotypes at high soil P rate were investigated with the Pearson-product moment correlation and correlation matrices were visualized with *corrplot* package in *R* (R Core Team).

2.4.3 Interaction of the genotype main effect plus genotype by environment of the genotypes

The means of grain yield and grain Zn content were used to run a site regression analysis, also called genotype main effect plus genotype-environment interaction (GGE) (Pacheco et al., 2015). The GGE is a linear-bilinear model that removes the effect of the environment (here the rates of soil P) and expresses the output as a function of the genotype effects and its interaction with the environment. This model was used since the P rates are the main source of variation concerning the contributions of the genotypes and the interaction of the genotypes x P rates. The varying soil P rates were taken as environments for the analysis to identify the mean performance and stability of the genotypes, determined by their location and proximity to the origin of the biplot (Pacheco et al., 2015). The GGE biplot outputs were used to indicate discrimination of the environments (P rates), and the correlation coefficient between the environments. The model for the GGE analysis used is below;

$$Y_{ij} = \mu + e_j + \sum_{n=1}^{N} T_n \gamma_{in} \delta_{jn} + \varepsilon_{ij}$$

Where Y_{ij} is the Zn content/grain yield of the *i-th* genotype (i = 1,..30) in the *j-th* P rates (j = 1,..3); µ is the grand mean; e_j is the P rates deviations from the grand mean; T_n is the eigenvalue of the principal component (PC) analysis axis n; γ_{in} and δ_{in} are the genotype and P rates PC scores for axis n; N is the number of PC retained in the model and ε_{ij} is the error term.

Table 1
List of trait names with their abbreviation and definitions

Traits	Definition & abbreviations
Grain yield, kg/ha	Grain yield in kg per hectare from the low, medium, & high phosphorous treatments (yield.LP, yield.MP, & yield.HP)
Grain phosphorous content, mg/kg	Phosphorous content determined from grain samples from the low, medium, & high phosphorous treatments (grainP.LP, grainP.MP, & grainP.HP)
Grain Zinc content, ppm	The zinc content of grain samples from the low, medium, & high phosphorous treatments (grainZn.LP, grainZn.MP, & grainZn.HP)
Plant height, cm	The height of the plant measured in centimetre from the soil surface to the base of the top leaf on the main stem (average per genotype) (pht.LP, pht.MP & pht.HP)
Root dry weight, gram	Weight of dry root, per plot of low, medium and high phosphorous treatments (rdwt.LP, rdwt.MP, & rdwt.HP)
Root phosphorous concentration, mg/kg	Phosphorous content determined from root samples from the low, medium, and high phosphorous treatments (rootP.LP, rootP.MP, & rootP.HP)
Root Zinc content, ppm	The zinc content of root samples from the low, medium, and high phosphorous treatments (rootZn.LP, rootZn.MP, & rootZn.HP)
Shoot dry weight, gram	Weight of dry shoot, per plot of low, medium and high phosphorous treatments (sdwt.LP, sdwt.MP & sdwt.HP)
Shoot phosphorous concentration, mg/kg	Phosphorous content determined from shoot samples from the low, medium, and high phosphorous treatments (shootP.LP, shootP.MP, & shootP.HP)
Shoot zinc content, ppm	The zinc content of shoot samples from the low, medium, and high phosphorous treatments (shootZn.LP, shootZn.MP, & shootZn.HP)
Soil phosphorous content, mg/kg	Plant available phosphorous from the low, medium, & high phosphorous plots (soilP.LP, soilP.MP, & soil.P.HP), determined using Bray-I method

3 Results

3.1 Variable soil phosphorous influenced shoot dry matter and content of phosphorous and zinc content

Phosphorous (P) fertilization was positively associated with increased the growth and performance of the plants for most of the measured traits under the screenhouse conditions where plants were grown on sandy soil supplemented with Hoagland nutrients solution with varying P concentrations. The shoot dry weight, root dry weight, plant height, and shoot P concentration increased with increasing P supply, but the addition of P to the growth media did not result in increased Zn content of shoot and root of the genotype (Fig. 1). A similar response was observed for the plants grown in the field with varying P supplied with the addition of SSP fertilizer, where grain yield and P concentrations of plants increased as the P in the soil increased except for shoot, root, and grain Zn contents (Fig. 2, Supplementary Fig. 1). The increase in shoot growth with the addition of more P to the growth media indicates that the soils used were suboptimal in available P. The increase in growth parameters and performance indicators was maximum when the applied P was highest for all the parameters measured except for Zn content in both screenhouse and field experiments. It, therefore, means that P fertilization does not result in reduced grain Zn content. The highest level of P fertilization did slightly, but not significantly reduce shoot Zn content from the field results (Fig. 2). The Zn content of shoot and grain was relatively higher when P application was low and medium in the growth media compared with high P (Figs. 1 & 2, see Online resources 2 & 3 for means of parameters assessed for all genotypes evaluated in the screenhouse and the field environments), though the differences were not significant as determined by the Tukey honestly significant difference test.

The relative increase in plant height, shoot dry weight, shoot P concentration, and shoot zinc content in high P (HP) over the low P (control) was 34.6, 81.8, 26.1 and −3.51 %, respectively in the screenhouse (Table 2) where the Zn content in the shoot at the HP

(68.3 ppm) was slightly lower than the control (70.7 ppm), corresponding to -3.5% relative reduction in Zn content of HP plants relative to the LP. The results for the field evaluation followed a comparable pattern with the screenhouse in the relative difference in Zn content of HP compared to other P treatments. An increase in grain yield and P content followed a linear pattern with increasing P fertilizer to the soil, while Zn content was higher in plants evaluated under low and medium soil P compared to the high P which had a reduction of 17.1% in the Zn content of the HP over the LP (Table 2).

Table 2
Relative difference (RD) in performance of high phosphorous concentration relative to low phosphorous on some traits measured in the screenhouse and in field conditions.

Screenhouse					**Field**					
Phosphorous levels	**Plant height (cm)**	**Sdwt (g)**	**ShootP (mg/kg)**	**ShootZn (ppm)**	**SDWT (g)**	**ShootP (mg/kg)**	**ShootZn (ppm)**	**Yield (kg/ha)**	**GrainP (mg/kg)**	**GrainZn (ppm)**
LP	14.5	0.4	942.4	70.7	20.6	1132.4	103.9	294.1	2106.8	34.3
MP	14.3	0.5	1054.1	73.4	38.9	1162.9	96.8	558.9	2286.4	31.5
HP	22.2	2.2	1275.9	68.3	94.5	1183.7	88.7	1406.6	2368.2	29.3
RD (%)	34.6	81.8	26.1	-3.5	78.2	4.3	-17.1	79.1	11.0	-17.1

LP = low phosphorous (no-P application), MP = medium phosphorous (1.5 mg P kg^{-1}), & HP = high phosphorous (30 mg P kg^{-1}) for river sand in the screenhouse, and MP = 10 kg P_2O_5 ha^{-1} & High P = 60 kg P_2O_5 ha^{-1} for P field evaluation, where plant.ht = plant height (cm) at maturity, Sdwt = shoot dry weight (g), P = phosphorous concentration, Zn = Zinc content, & RD = relative difference (%).

3.2 Phenotypic association between yield components and Zinc content in cowpea

The relationship between several growth parameters and zinc content both in the screenhouse and field trials were not significant (r = 0.1–0.2) (Figs. 3 & 4, Online resources 4 & 5). The shoot dry weight versus shoot Zn content at HP treatment had a weak negative correlation (r = -0.1) while grain yield and grain Zn content at HP (r = 0.1) showed a weak positive significant relationship from field evaluation (Fig. 4) indicating that high yield and Zn content can be combined in some genotypes since there is no strong association between the two traits that would result in a tradeoff between high yield vs low Zn content or vice versa. The correlation between these traits measured at the vegetative stage also showed similar weak associations (Supplementary Fig. 2). This suggests selection of genotypes with high Zn content is possible when cowpeas are grown on high soil P. This was confirmed from our analysis which identified several genotypes with high Zn content and yield under high soil P treatments (Table 3). Some genotypes (IT89KD-288, IAR-48, IT82E-18, IT97K-499-35, UCR779 and DanMisra) had higher grain yield with high Zn content at HP soil environment over other soil P rates (Table 3). Similarly, grain P and Zn (r = 0.1) were not significantly correlated at all levels of soil P treatments indicating no strong association exists between these traits.

Likewise, in the screen house experiments, shoot Zn content, and shoot dry weight (shoot yield) on HP soil had higher negative association (r = -0.4) compared to the results in the field, while the shoot P and Zn content at all soil P levels had a weak and insignificant negative relationship (r = -0.1 - (0.1) (Fig. 3). Detailed correlation coefficients for these traits in both screenhouse and field experiments are presented respectively in Online resources 4 & 5. The results indicate there are suggestive negative relationships between high soil P and yield (shoot &grain) but they are not strong enough to exclude selection of outstanding genotypes with high yield and Zn content. See Supplementary Figs. 3 & 4 for scatter plots that further depicts these weak relationships.

3.3 Interaction of genotypes and phosphorous rates on grain yield and zinc content with biplot analysis

The GGE biplot analysis, where the different soil P rates were considered as distinct environments, revealed the presence of divergence between the genotypes, grain yield and Zn content at different soil P conditions. Genotypes 9, 23, 29, 13, and 8 were the top-performing in the HP trials for grain yield and the angle between their vectors and the HP (environment) vector was less than 90^0 (acute angle) while genotypes like 2, 7, 4, 6, 25 and 19 had lower grain yield below the mean and the angle of their vectors appeared to be greater than 90^0 (obtuse angle) (Fig. 5, see Online resource 1 for names of the genotypes and their corresponding entry numbers). The most stable genotypes across all the environments for the grain yield were 24, 16, 13 and 30 which are nearer to the origin of the biplots of the PC1 vs PC2 while those genotypes that are farther away from the origin are more sensitive to the varying levels of soil P (Fig. 5).

Genotypes 16, 8, and 22 were the top-performing in the HP trials with high grain Zn since the angle between their vectors and the HP vector is less than 90^0 (acute angle) while genotypes 2, 5, 17, 4, 26, and 14 had lower grain Zn below the mean under the HP soil since the angle of their vectors appeared to be greater than 90^0 (obtuse angle) (Fig. 6). The most stable genotypes across all the P environments for the grain Zn were 3, 21, 22 and 23 because there are nearer to the origin of the biplots of the PC1 vs PC2 while those genotypes that are farther away from the origin are less stable for grain Zn content across the P environments. These include genotypes 1, 4, 5, 6, 17, 25, and 29 (Fig. 6). The vectors of the environments (P levels) indicated positive correlations between grain yield and Zn content because all the vectors of the P levels formed an acute angle. The level of P at HP had the highest discriminating ability while LP had the least as shown by the length of their vectors for grain yield while for the grain Zn content, the LP and MP were more discriminating over the HP (Figs. 5 & 6).

Table 3
Mean performance of representative genotypes with higher shoot dry weight, grain yield and grain Zn content at high soil P fertilization

Genotypes	Sdwt-HP	Sdwt-MP	Sdwt-LP	Yield HP	Yield MP	Yield LP	GrainZn-HP	GrainZn-MP	GrainZn-LP
IT89KD-288	98.6	33.0	19.0	1475.0	599.2	99.9	43.7	39.2	36.2
IAR-48	116.5	32.0	21.9	1547.1	421.9	388.7	41.1	22.6	26.3
IT82E-18	90.8	49.0	21.3	1171.1	643.9	519.2	40.7	37.2	31.7
IT97K-499-35	80.3	48.0	20.2	1261.1	925.1	289.3	39.5	34.6	30.3
UCR779	93.6	13.0	19.5	1197.1	232.0	22.2	31.4	19.0	21.1
DanMisra	127.6	27.0	22.1	2105.5	459.9	571.9	31.1	24.2	39.4

LP = no-P application of P_2O_5, MP = 10 kg P_2O_5 ha^{-1} & High P = 60 kg P_2O_5 ha^{-1} for field evaluation

4 Discussion

Increased performance of cowpea, as measured by indices of high dry matter yield, increased content of phosphorous (P) in shoots, and high grain yield was observed with increasing supply of P in the growth media under both sand-nutrient solution and field environments. This corroborates several earlier reports on cowpea's response to the addition of P fertilizer under greenhouse and field conditions (Adusei et al., 2016; Gyan-Ansah et al., 2016; Kolawole et al., 2008; Krasilnikoff et al., 2003; Saidou et al., 2012; Sanginga et al., 2000). The grain Zn content of cowpea genotypes evaluated in this study showed no significant differences ($p > 0.05$) across the various P levels applied to the soil. This was further demonstrated by correlation analysis where grain Zn content had a non-significant negative association ($r = -0.2$, $p = 0.3$,) with the high rate of P application compared to the medium and minimal soil P rates. The negative and non-significant relationship between high soil P and Zn content indicates that there is no tradeoff between balancing greater yield and Zn content. Achieving greater yield with high P application and desired grain Zn content are not mutually exclusive. A similar report on two cowpea varieties with differential response to P fertilization showed that high P fertilization, in the absence of Zn application, was associated with Zn deficiency symptoms, even though there was no reduction in the Zn concentration in the shoots (Safaya & Singh, 1977). Other authors have reported that higher rates of P supplied to cowpea led to a linear increase in grain yield, P in leaves and seed, but Zn concentration was reduced in leaves (Benvindo et al.,

2014) and grains (Nyoki & Ndakidemi, 2014). Increasing P supply did not reduce Zn concentration of plant tissue in *Brassica oleracea* (Pongrac et al., 2020a), where larger shoot Zn concentration was seen in one genotype (Pongrac et al., 2020b). Earlier observations of increasing P levels in soil with no reduction in the Zn concentration of non-zinc-supplied plants have been reported in beans and potatoes (Boawn & Brown, 1968), russet Burbank potato (Boawn & Leggett, 1964), and subterranean clover (Millikan, 1963).

The association between P and Zn concentrations has been a topic of investigation by several workers. Although most agree that high P applications could lead to development of Zn deficiency symptoms in certain crop plants, but not necessarily cause reduced Zn or P content of plant tissues (Boawn & Brown, 1968; Loneragan & Webb, 1993; Pongrac et al., 2020b). The development of Zn deficiency resulting from high P application can be remedied by supplying optimum Zn nutrition in the growth medium. This phenomenon has been described in several publications as P-induced Zn deficiency (Ambler & Brown, 1969; Boawn & Brown, 1968; Loneragan & Webb, 1993). The inverse relationship between P and grain Zn concentrations when a high rate of P is applied without Zn fertilizers supplied to the growth media have been reported in maize (Bogdanovic et al., 1999; Imran et al., 2016), wheat (Ova et al., 2015; Ryan et al., 2008), common beans (Singh et al., 1988) and numerous other crops (Fageria, 2001). Though, contrary observations have been reported by Loneragan and Webb (1993) who provide evidence of a linear relationship between the Zn and P concentrations in cereals and citrus leaves treated with superphosphate fertilizers. Increased Zn concentration with high P application in plants receiving no external supply of Zn has been documented (Loneragan & Webb, 1993). However, some authors have opined that such observations may have been due to contamination of the superphosphate used. Nevertheless, in crops where antagonist relationship exists between high P rates and grain Zn content, application of Zn fertilizer/nutrients have been reported to help remedy the situation (Liu et al., 2020; Loneragan & Webb, 1993; Qaswar et al., 2017; Rose et al., 2013; Zhang et al., 2012).

In contrast to these results, we observed that a high rate of P applications had no significant effect on the Zn content of cowpea genotypes investigated or and did not caused appearance of Zn deficiency symptoms in cowpea. There are few reports on cowpea addressing the relationship between high P application and Zn content of grains or other edible parts (Ayeni et al., 2018). The few reports of P-Zn relationship on the crop are sometimes confusing. Oseni (2009) reported decreased Zn content of cowpea plants when P was applied at high levels in the soil. While a more recent study on the impact of contrasting P rates and constant level of N fertilizers on iron and Zn contents of cowpea grains made a contrary observation. Ayeni et al. (2018) observed that application of P alone at high rates do not significantly decrease the content of Zn and Fe on cowpea grains, but a combination of high P (60 kg/ha) and N was associated with reduced Zn content of the grains. The authors provided some explanation for this observation suggesting that the lack of decrease in Zn content when only high P rate was applied may be due to applied P being fixed in certain soil compounds. However, application of N, a known element that could enhance the early establishment of cowpea (Abdul Rahman et al., 2018) and uptake of P from soil (Ngwene et al., 2010), may be responsible for decreased Zn content when N and P fertilizers were applied at a high level.

Several mechanisms have been proposed to explain the P-Zn interaction in which a decrease in Zn content is observed in certain crops or genotypes when P application is at the highest level in the growth media. One of the mechanisms responsible for P induced Zn reduction in plant tissues include the following; first, increasing P supply to the growth medium stimulates the growth of crop plants sufficiently to dilute the concentration of Zn in plant tissues to levels which could induce Zn deficiency. Second, the high P supply could suppress either absorption of Zn by roots or translocation of Zn from roots to shoots (Amin et al., 2013; Fageria, 2001; Loneragan & Webb, 1993; Nishigaki et al., 2019). The lower value of grain Zn content in some genotypes at high soil P may be due to decreased diffusion of Zn in the soil colloids (Amin et al., 2013; Fageria, 2001). Another explanation for the high P – Zn reduced bioavailability is that, when both P and Zn are limiting in soil, application of high P rates tend to promote plant growth and this cause dilution in tissue Zn which may further lead to reduced availability of Zn in plant tissues (Loneragan & Webb, 1993). Such observations have led to the belief that imbalance in the ratios of P-Zn in the growth environment may be responsible for reduced Zn content observed in some crops/genotypes when P is applied at a high rate (Safaya & Singh, 1977). Some authors have also observed highest grain Zn concentration on fields where mineral N, P fertilizers and organic nutrient sources were applied (Manzeke et al., 2017).

In the present study, we observed that P induced Zn content decrease may be genotype-dependent because the relationship between the increased yield due to high P application and Zn content revealed insignificant negative association. Earlier investigations have supported this. Susceptibility to P induced Zn deficiency was attributed to genetic differences and soil conditions in cowpea (Manzeke et al., 2017; Safaya & Singh, 1977) and navy beans (Ambler & Brown, 1969), where varieties had varying efficiency to absorb and use nutrient constituents of the soil.

Various approaches have been used to achieve greater grain yield and Zn content in soils with higher P application. These include the application of Zn fertilizers either as a foliar spray (Zhang et al., 2012) or applied directly to the soil (Imran et al., 2016; Liu et al., 2020; Manzeke et al., 2017), improved agronomic practices such as integrated soil fertility management and intercropping in which cereals can increase Zn bioavailability of the soil and benefit the companion legumes (Manzeke et al., 2017; Xue et al., 2016), adopting multiple strategies such as dietary diversification (Manzeke et al., 2017) which could mean supplementing cowpea meal with high micronutrient-rich crops like common beans, a legume also rich in Zn content (Blair et al., 2010; Cichy et al., 2009) and cultivation of varieties with inherently high Zn concentration at any soil P rates. Other workers (Ayeni et al., 2018) have recommended application of medium P rates such as 40 kg P_2O_5/ha even without application of Zn fertilizer to obtain high yield and Zn content in the grains (Benvindo et al., 2014; Oseni, 2009; Saidou et al., 2012). Application of Zn containing fertilizers has been observed elsewhere to increased cowpea's grain Zn content and yield under integrated soil fertility management (Manzeke et al., 2017).

Ayeni et al. (2018) observed that some cowpea genotypes had a high content of Zn at all levels of P, a finding similar to our observation in this study where certain genotypes such as IT82E-18, UCR779, CB46, and IT97K-556-6 had increased grain Zn content with increasing supply of P in the growth environment. This was confirmed by the GGE biplots that displayed genotypes like 16 (IT89KD-288), 8 (Danila), and 22 (SAMPEA-9) with high grain Zn content under high soil P fertilization while genotypes like 3 (58–77), 21(SAMPEA-17), 22 (SAMPEA-9) and 23 (Sanzi) had stable grain Zn across all P levels. In this study, we identified 3 very stable across screenhouse and field environments and high grain Zn containing genotypes at high soil P rate and 4 high grain Zn genotypes across all the P levels, based on SREG biplot analysis (Pacheco et al., 2015). Also, genotypes with high grain yield at all P levels were identified. The use of GGE biplot analysis enabled us to visualize the genotypes and their association with the different P rates (serving as environments) using biplots which shows genotypes that are high performing for grain yield and grain Zn content at each P rate and those that are most stable across all P levels (Ayeni et al., 2018; Ukalski & Klisz, 2016). The genotypes identified with high grain Zn at high P level should be tested in more locations to further substantiate this finding and make possible recommendations for release to farmers as high Zn varieties.

5 Conclusions

There was wide variation for the Zn and P contents of cowpea genotypes investigated. Increasing P fertilization leads to a significant increase in yield and concentration of P in the shoots of the cowpea materials assessed. We conclude that since no significant relationship between increased P fertilization and grain Zn content was observed and high P fertilization is related to greater yield, there is no reason to not fertilize with P at high levels. Increased grain yield under high P supply can be achieved without sacrificing desired Zn content especially if genotypes such as IT89KD-288, IAR-48, IT97K-499-35, UCR779, CB46, IT97K-556-6, IT82E-18 and DanMisra that do not show a negative impact of decreased grain Zn content at high rates of P are cultivated. The high grain Zn genotypes at high P fertilization can be used as parents in conventional or molecular plant breeding pipeline for further improvement of grain Zn content in cowpea varieties that are popularly grown in major producing areas.

Declarations

CONFLICT OF INTEREST

The authors have no conflict of interest to declare that are relevant to the publication of this article.

AUTHORS' CONTRIBUTIONS

SBM, DKD, MFI, PBT, and VG conceived and designed the study. SBM conducted the experiments, data analysis and wrote the first draft of the manuscript. SBM, DKD, YA, MLU, MFI, PBT, and VG revised and edited the manuscript. MFI supervised the overall project. All authors read and approved the final version of the manuscript.

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Figures

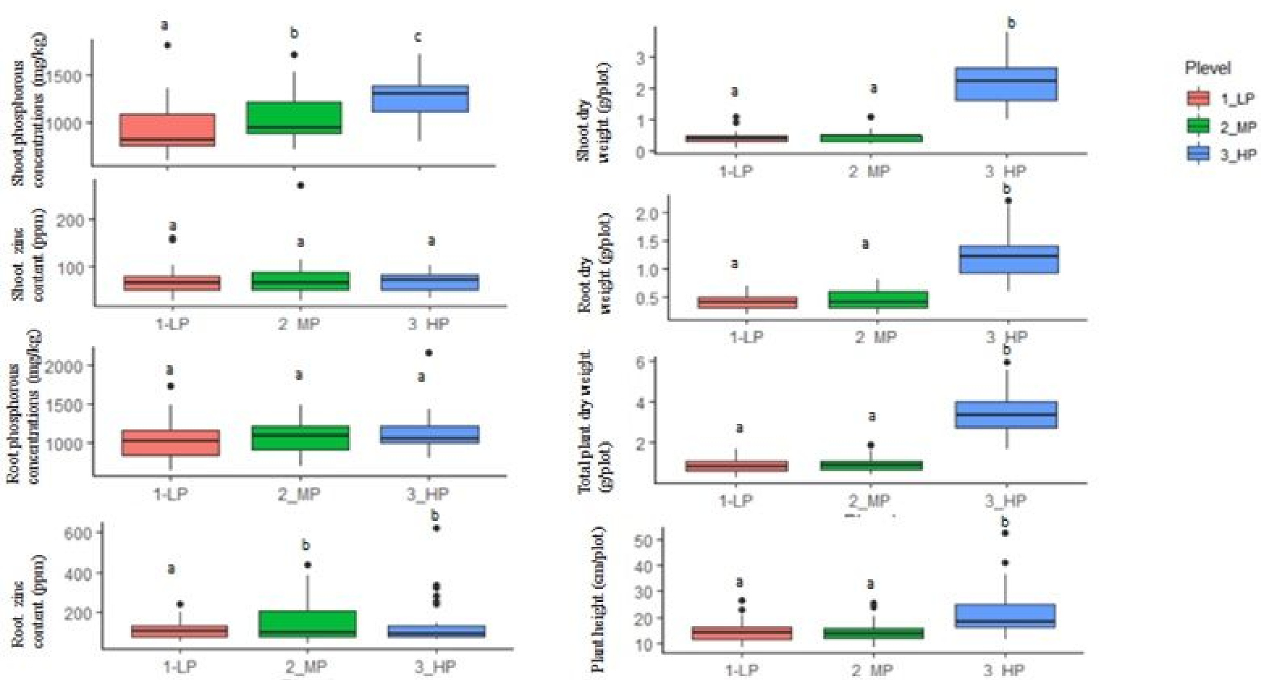

Figure 1

Phenotypic distribution of traits measured in the screenhouse under varying soil phosphorous levels. Boxplot displays the median of each group bounded by the first and third quartile where the x-axis is soil phosphorous concentrations; 1_LP = no-P application, 2_MP = 1.5 mg P kg-1 & 3_HP = 30 mg P kg-1 added to Hoagland nutrient solution used for watering the plants. Letters above each boxplot denote similarity among groups per Tukey's honestly significant difference comparison test (α ≤ 0.05). Means with the same letter are not significantly different.

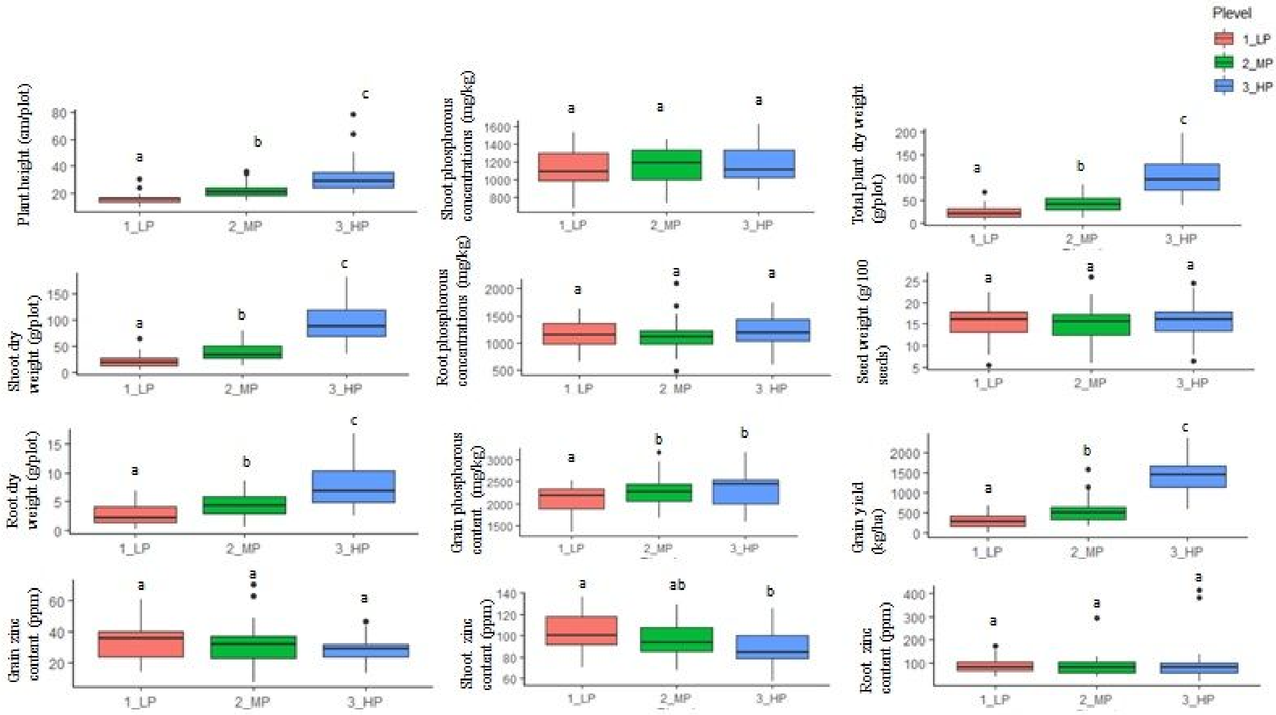

Figure 2

Phenotypic distribution of traits measured in the field under varying soil phosphorous levels. Boxplot displays the median of each group bounded by the first and third quartile where the x-axis is soil phosphorous concentrations, with 1_LP = no-P application of P2O5, 2_MP = 10 kg P2O5 ha-1 & 3_HP = 60 kg P P2O5 ha-1. Letters above each boxplot denote similarity among groups per Tukey's honestly significant difference comparison test (α ≤ 0.05). Means with the same letter are not significantly different.

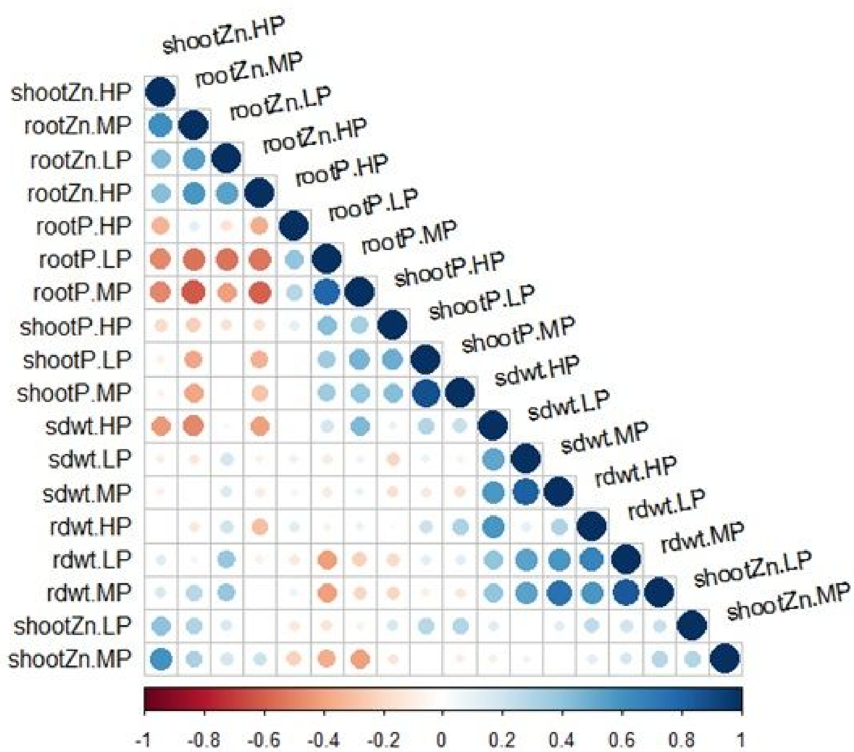

Figure 3

Correlation matrices for traits measured across varying soil phosphorus concentrations in the screenhouse (n = 30 genotypes). See Table 1 for list and abbreviations of the traits. Positive and negative associations between given traits are in blue and red, respectively. The colour intensity and the size of the circle are proportional to the correlation coefficients. On the x-axis of the correlogram, the legend colour shows the correlation coefficients and the corresponding colours.

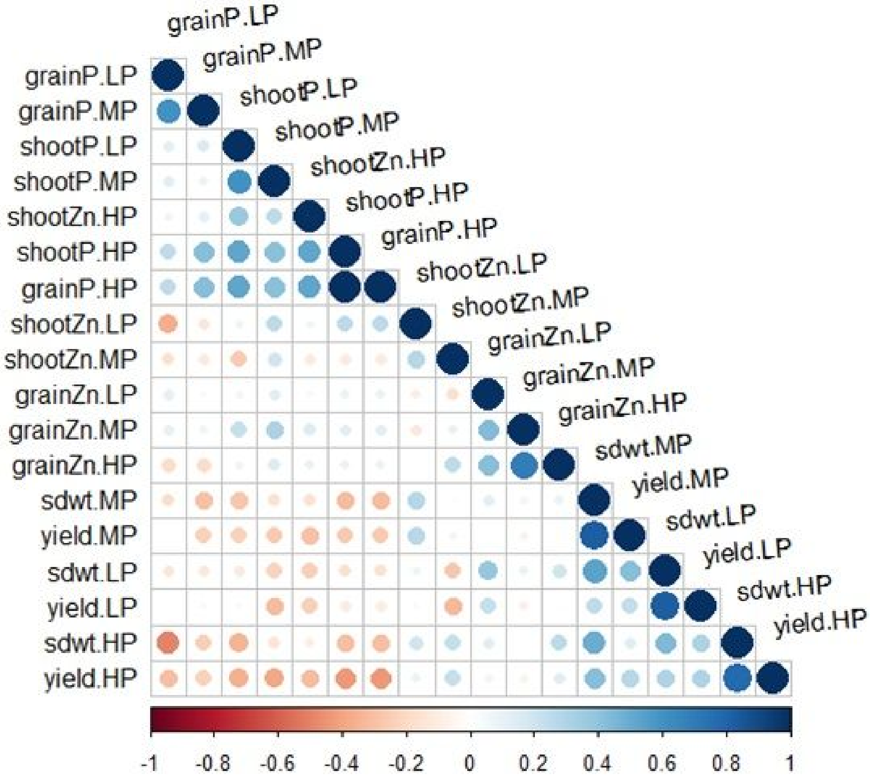

Figure 4

Correlation matrices for traits measured across varying soil phosphorus concentrations in the field (n = 30 genotypes). See Table 1 for list and abbreviations of the traits. Positive and negative associations between given traits are in blue and red, respectively. The colour intensity and the size of the circle are proportional to the correlation coefficients. On the x-axis of the correlogram, the legend colour shows the correlation coefficients and the corresponding colours. LP = no-P2O5 application, MP = 10 kg P2O5P ha-1 & High P = 60 kg P2O5 ha-1 for P field evaluation.

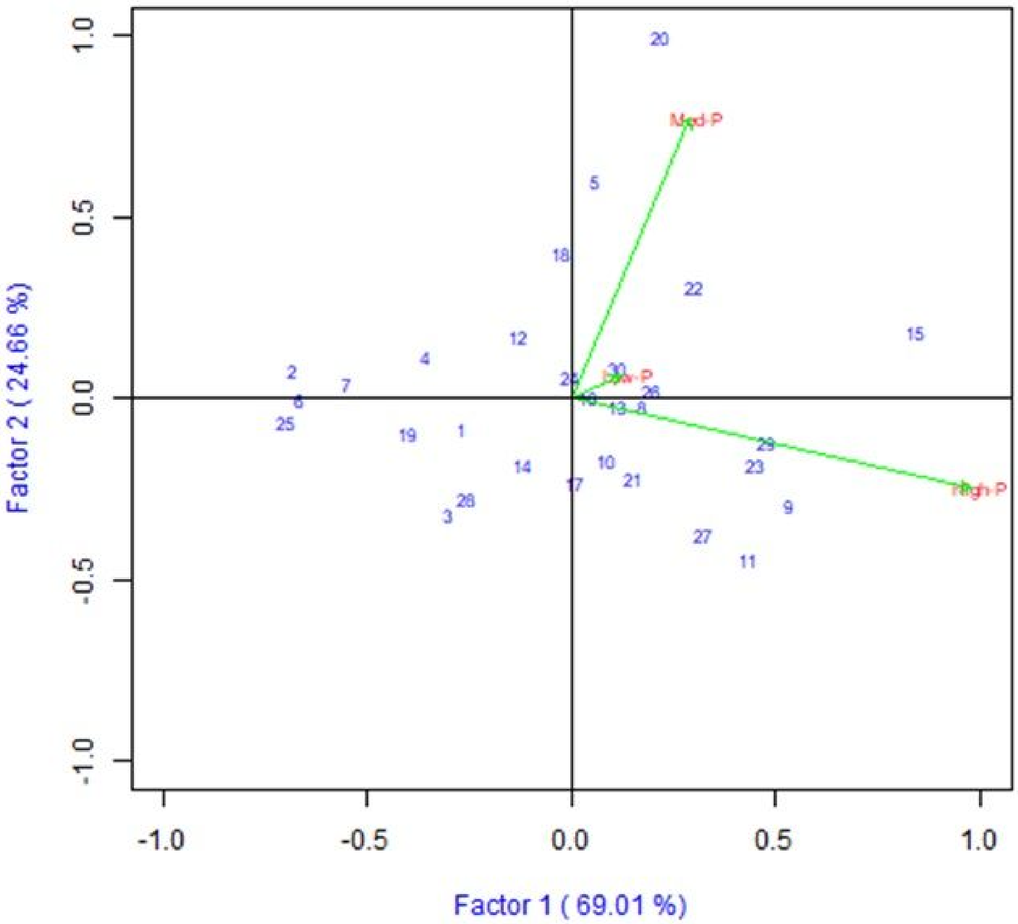

Figure 5

GGE biplot showing the grain yield of the genotypes at varying soil P levels. Factor 1 & 2 are the principal component axis. Low-P = no-P application of P2O5, Med-P = 10 kg P2O5 ha-1 & High-P = 60 kg P2O5 ha-1 for field evaluation

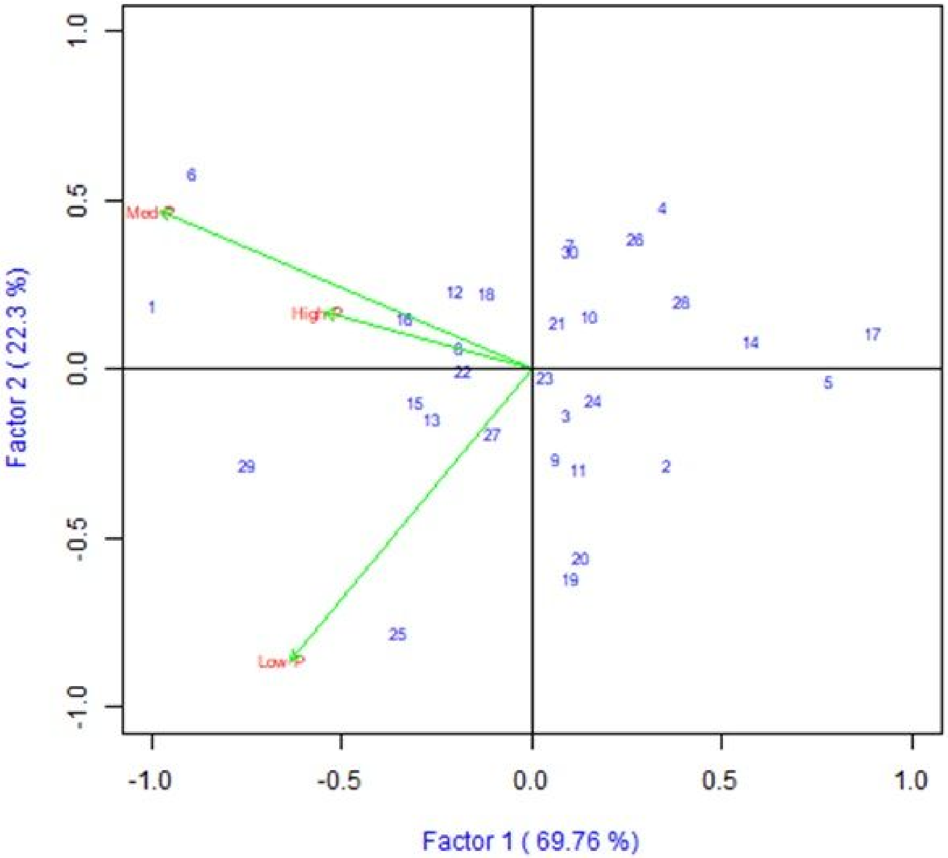

Figure 6

GGE biplot displaying the genotypes for grain Zn content at varying soil P levels. Factor 1 & 2 are the principal component axis. Low-P = no-P application of P2O5, Med-P = 10 kg P2O5 ha-1 & High-P = 60 kg P2O5 ha-1 for field evaluation

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