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Genotypic Responses of Cowpea (*Vigna unguiculata*) to Sub-Optimal Phosphorus Supply in Alfsols of Western Kenya: A Comparative Analysis of Legumes

Joseph P Gweyi-Onyango[1*], Peter Akwee[2], Christine Onyango[2] and Tsehaye Tesfamariam[3]

[1]Kenyatta University, Department of Agricultural Science and Technology, P O Box 43844-00100, Nairobi, Kenya
[2]Masinde Muliro University of Science and Technology, P.O. Box 190-50100, Kakamega, Kenya
[3]Institute of Plant Nutrition, University of Hohenheim, Stuttgart, Germany
[*]E-mail: josephonyango2002@yahoo.co.uk

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ABSTRACT Western parts of Kenya are characterized by acid soils with phosphorus [P] deficiency and aluminum [Al] phytotoxicity. Reports indicate a declining trend in yields of legumes such as cowpeas. Though legumes can fix N, starter phosphorus need to be supplied for better yields and more often plants develop adaptive strategies for better P acquisition; probably through increased Al tolerance by carboxylates exudation, improved nodulation as a result of high P and better root development. Present study examined mutual relationship between P and nodulation in explaining yield differences between Cowpea (*Vigna unguiculata*) varieties: Elanda (ELn, high yielding) and Inzeku (INz; moderate yielding), Ground nut (*Arachis hypogea*)-GN and Bambara nuts (*Vigna subterrenea* (Verdc) - BBN with proven P efficiency and Bean (*Phaseolus vulgaris* L) varieties Rosecoco (RCC) and Edwin (ED); low P efficient formed basis for comparison. The experiment was conducted at Masinde Muliro University field station from April 2009 and experimental design was CRBD having split plot with P as main plots (+P; or –P), whereas GN, BBN, ELn, INz, RCC and ED legumes were randomized within the P treatments. Legume genotypes were replicated four times. There were no differences in shoot biomass with +P and –P (INz), but ELn showed higher shoot DM with +P as compared with –P (significant $p<0.05$). Similar trends were replicated with nodulation with P supply. Shoot DM and nodulation were positively correlated for ELn ($r^2=0.99$; +P and $r^2=0.68$; -P) but not INz. Correlations were negative for beans and performance of BBN with +P and –P showed trends similar with Inz genotype. Sub-optimal P supply with concomitant enhancing nodulation in INz could not explain genotypic differences in yields, since high yielding BBN lacked this trait with additional P. Factors like high proton concentration and Al saturation need to be assessed and likely an interaction between P and Al through enhanced exudation of carboxylates may give more insights to cowpea genotypic differences.

I. INTRODUCTION

Phosphorus (P) is needed in virtually all metabolic processes such as energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis and respiration. It is, however, one of the least available and least mobile mineral nutrients to plants in many cropping environments, based on its contribution to the biomass as a macronutrient (Goldstein et al. 1988). Orthophosphate (Pi), the fully oxidized form of P is extremely insoluble in most soils because it forms Ca-salts or is complexed by constituents such as Fe or Al oxides or fixed into organic forms that render phosphate (Pi) largely inaccessible to plants (Hinsinger 2001).

There is a great disparity in distribution of Pi between plant cells (mM) and soil solution (μM) (Vance et al. 2003). Extremely low levels of available phosphorus in the rhizosphere make it one of the major growth-limiting factors in many ecosystems (Barber et al. 1963). The concentration of available (Pi) in soil seldom exceeds 10 μM (Bieleski 1973). This problem is further heightened in highly weathered and volcanic soils of the humid tropics and subtropics, and sandy soils of the semi-arid tropics (Sanchez et al. 1997). It is estimated that 5.7 billion hectares of land worldwide is deficient in Pi for achieving optimal crop production (Batjes 1997). Phosphate fixation increases significantly in acid soils, which accounts for nearly 26% of the world's soils (Eswaran et al.1997). As a consequence of organic and inorganic fixation, nearly 80% of applied Pi may be unavailable to plants (Holford 1997). This problem is especially acute in tropical regions, particularly Africa, where production of crops without fertilizer application is resulting in continuous mining of essential nutrients by plants. Furthermore, at the current worldwide rate of fertilizer application, the readily available sources of high-grade phosphate rocks

may be depleted within the next 60 to 90 years (Runge-Metzger 1995). Increasing population and extension of agriculture to low- and marginal-fertility lands will further increase the demand for the precious supply of phosphate fertilizers.

Potential symbiotic nitrogen fixation (SNF) in these heavily weathered acid soils of tropical and semi-humic tropics is limited, since P is generally deficient hence limits the nodulation (Waluyo et al. 2004). In common bean (*Phaseolus vulgaris*), soybean (*Glycine max* (L) Merr), Lupine (*Lupinus mutabilis*) and alfalfa (*Medicago truncatula*), P deficiency has been reported (i) to reduce number and biomass of nodules as well as their nitrogenase activity (Ribet and Drevon 1995; Vadez et al. 1996; Qiao et al. 2007), (ii) to increase the absorption surface and density of roots resulting in more exploration of soil volume (Vance 2001) and (iii) to acidify the rhizosphere by root exudates (Neumann and Römheld 1999) and H^+ efflux (Tang et al. 2001).

Cowpea (*Vigna unguiculata*) is considered as being more tolerant to phosphorus deficiency than soybean and common bean (Alkama et al. 2008). Thus, better tolerance has been related to three main characters; (i) a greater P use efficiency, (ii) a higher specific nodule activity and (iii) different P distributions between the plant organs (Alkama et al. 2008). Cowpea is thus considered as an excellent species in respect to low P tolerance in improvement of symbiotic nitrogen fixation programs. Ankomah et al. (1995) reported that the special capacity of P deficiency tolerance in cowpea was related to different abilities to absorb soil P or to differences in P use efficiency to fix N_2 from atmosphere, which was in agreement with similar reports for other legume species like common bean, mungbean (*Vigna radiata*) and soybean (Gunawardena et al. 1993; Vadez et al. 1999).

Genotypic variation in P use efficiency (PUE) for symbiotic nitrogen fixation (SNF) and the relation between the H^+ efflux is, however, not well- documented. Moreover, one of the options for overcoming the reliance on P-fertilizers for improved crop production in P-deficient soils is the selection of P-tolerant lines that could show a greater growth with a concomitant low proton effect.

Leguminous plants like cowpea form an integral part of African indigenous vegetable with wider acceptance amongst many communities in Kenya. A lot of work regarding its economic value, nutritive and agronomic traits has been undertaken. However, investigation underlying its adaptability to P stress is not unequivocal.

The objective of the current research was therefore, to examine the growth responses of cowpea (*Vigna unguiculata*) genotypes under sub-optimal P supply (reflecting the rate applied by peasant farmers) and under no P supply in terms of nodulation and shoot biomass accumulation. Moreover, two nuts: Groundnut (*Arachis hypogea*) and Bambara nut (*Vigna subterrenea* (Verdc), with proven ability of P efficiencies and Bean (*Phaseolus vulgaris* L) varieties with low P efficiencies formed the background for P efficiency analysis between the two cowpea genotypes selected.

II. MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the Masinde Muliro University of Science Field station (MMUST); Main campus, located 00^0 17.30' North and $34^0$45' East (GPS receiver; GARMIN RINO 130), at an altitude of about 1555 (±11m) m above sea level. The soil at MMUST is classified as an acidic Ferralsol (World Reference Base) or as a very fine, kaolinitic, *isohyperthermic Kandiudalfic Eutrudox* (Oxisol) in the USA Soil taxonomy (Hartemink et al. 2000) with pH of 4.5. This field experiment was conducted during the long rain season running from 29th March to 16th June 2009. The area is characterized by an annual rainfall of about 1000 mm distributed biannual. The mean monthly temperature was 26^0C during the growth season. The experiment was conducted on a plot previously under maize (*Zea mays* L.) cultivation.

Experimental Layout and Crop Husbandry

The experiment was designed as completely randomized block design (CRBD) with split plots having phosphorus (plus or minus P) constituting the main plot, with different legume species being part of sub-plots. Each legume species (including genotypes) were replicated four times within the block. Each plot measured 3.0 by 4.0 m (Fig. 1d). Within the subplots there were following treatments: two landrace Cowpea (*Vigna unguiculata*) varieties; Elanda (ELn, high yielding) and Inzeku (INz; moderate yielding), two

bean (*Phaseolus vulgaris* L) varieties: Rosecoco (RCC) and Edwin (ED); ground nut (Arachis *hypogea*)-GN and Bambara nuts (*Vigna subterrenea* (Verdc) – BBN.

Land was prepared to a fine tilth seedbed and furrows were made 0.5 m apart then TSP fertilizer applied at a rate of 50 kg ha^{-1} (half the recommended rate) and thoroughly mixed with the soil before seeds pre-treated with Aldrine 40% at a rate of 5 mg kg^{-1} against cutworm were sown. Each legume species was sown per hill along the furrows at an intra-row spacing of 0.1m. At second trifoliate stage (14-18 DAS- Days after sowing for most of these legumes); the stands were thinned to one plant per hill. Sampling for plant and nodule biomass were undertaken 40, 47, and 55 DAS. Ten plants per plot were sampled from 0.5 m^2 central area of each row. Sampling was not done on the outer rows to avoid boundary effects. Subsequent sampling was done on alternate row basis and was stratified to avoid creation of random gaps within the field.

Plant Dry Weight

After uprooting the plants and roots and nodules separation; different components were washed in tap water. The materials were then gently pressed between absorbent papers to remove adhering water. The nodules were counted and then rapped with aluminium foils. The leaves (including petioles) and also root nodules were dried in oven (model; Memmert UNB 500) at 72^0C for 48 hours. The dry weight measurements of stems and pods (in cases where podding had taken place) were also done by use of weighing balance (Model: Sartorus CP 224S) and values recorded.

Data Analysis

Data were analysed with the use of statistical program Sigma Stat® (Jandel Scientific). Analysis of variance between the treatments with a one-way ANOVA and a Turkey test ($p < 0.05$) was performed and significant differences are indicated with different letters.

III. RESULTS

Plant Dry Weights as Affected by P Supply

There were differences in plant growth amongst different legumes, with a characteristically poor growth in beans as compared to Bambara (*Vigna subterrenea* Verdc) nuts and cowpea (*Vigna unguiculata*) (Fig. 1).

The supply of P supply had effect in dry weight production in both cow pea genotypes, though the difference between +P and –P were not statistically significant in case of Inzeku (INz) variety but significant for Elanda (Eln) variety. The same trend was observed for root nodulations (Table 1). There were also striking variations in plant yields in terms of dry weights in case of Bambara nut under phosphorus (P) supply as compared to when P was omitted. The Bambara nut supplied with P exhibited significantly higher plant dry weight yields as compared with minus P variants (10.55 and 4.81 g $plant^{-1}$ respectively) (Table 2). These results agree closely with those of Eln cow pea genotype (Tables 1 and 2). The dry weights were similar irrespective of P supply in case of ground nut (*Arachis hypogea*). Indeed these dry weight differences in terms of P treatments were statistically insignificant for groundnut. The trends in plant biomass accumulation (progression with time) were consistently higher for P treatments as compared to minus P in case of groundnut, but the only significant statistical differences were observed at 40 DAS (Fig. 2a).

The differences in growth trends between plus and minus P were conspicuous for P treatments in case of Bambara where clear differences in P treatments were significant throughout the whole growth period (40, 47 and 55 DAS respectively) (Fig. 2a). On the contrary, although P treatment elicited higher DW (dry weight) variations within cowpea varieties (Table 1 and Fig. 2), the trends in the differences between [+P] and [-P] varied and were consistent throughout the growth period. This growth trait was particularly observable with P within Elanda (ELn) genotype, and the differences were statistically significant (Table 1; Fig 2c). The same trend was replicated with bean (*Phaseolus vulgaris* L) variety (Rosecoco- RCC), whereas Edwin (ED) had greater DW under minus P status (Table 3). Generally, bean (both genotypes) DW values were poor under the current field trials irrespective of P treatments.

Influence of P Supply on Root Nodulation

The current results reveal that nodule numbers per plant as an index nodulation was higher under P supply as opposed to when P was omit-

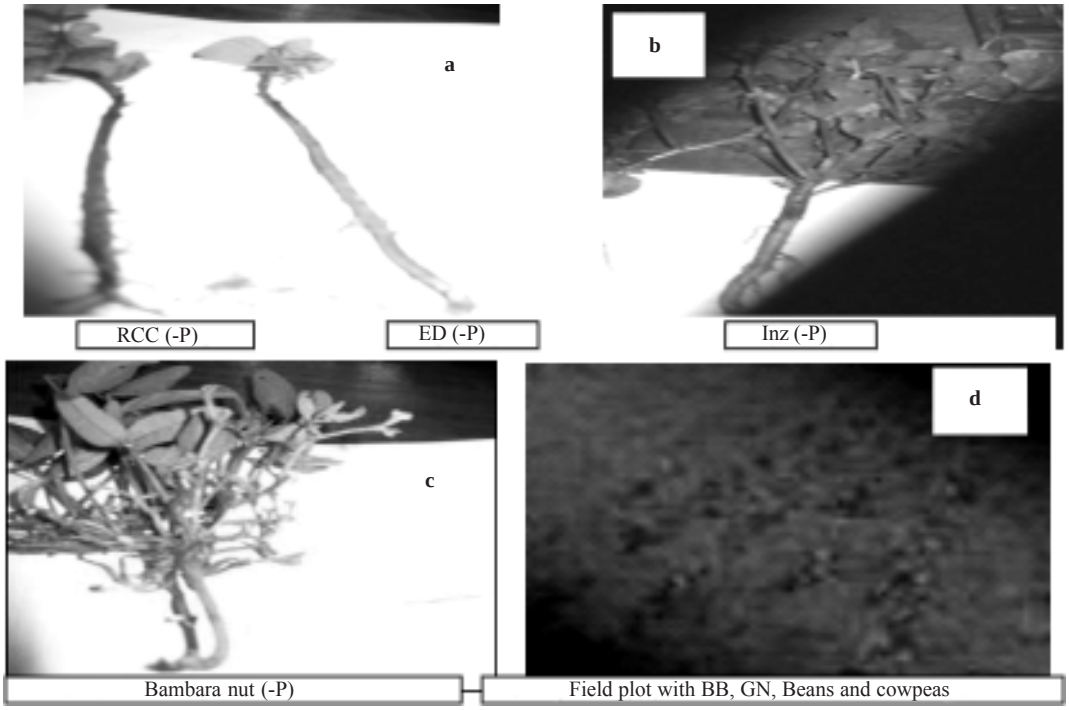

Fig. 1. Habitus of plant growth (a) Beans [Rocococo (-P) and ED (-P)], (b) cowpea [Inzeku (Inz minus P)], (c) Bambara nut (-P) and (d) Field plot showing crop growth

ted; as observed in case of bambara nut (Table 2). These differences in P treatments on nodulation were not observed with groundnut. The trends in nodulation patterns were similar in case of Bambara nut and Eln cowpea genotype, whereas the trends in these parameters were comparable in case of groundnuts and INz cowpea genotype (Tables 1 and 2).

In case of beans, there were no clear observable trends in respect to nodulation and P treatments for either variety. Nodule weights varied between 1200 – 1800 mg plant^{-1} root among nut legumes (bambara and ground nuts). The nodule weights were higher for ground nuts when P was exogenously supplied (Fig. 3a). There were significant differences in nodule weights under P

Table 1: Effects of P supply on plant dry weights and nodulation characteristics of two cowpea (*Vigna anguiculata*) varieties. The plant parameters were determined at 55 DAS

Varieties	*Plant DW (g plant^{-1})*	*Number of nodules plant^{-1}*	*Correlation between plant DW and nodules*
Inzeku (INz)			
[+P]	8.11±2.80^{ab}	11.00±1.00^{a}	r^2=0.02(+)
[-P]	6.54±0.40^{b}	9.33±0.6^{a}	r^2=0.47(+)
Elanda (ELn)			
[+P]	8.01±0.60^{a}	8.33±0.60^{a}	r^2=0.99(+)
[-P]	4.34±1.50^{c}	5.33±1.50^{b}	r^2=0.68(+)

Values followed with same letters along the column are not statistically significant at ($p<0.05$)

Table 2: Effects of P supply on plant dry weights and nodulation characteristics of Bambara nut (*Vigna subterrenea* (Verdc) and ground nut (*Arachis hypogea)*. The plants were harvested at 55 DAS (Days after sowing)

Varieties	*Plant DW (g plant^{-1})*	*Number of nodules plant^{-1}*	*Correlation between plant DW and nodules*
Bambara Nut			
[+P]	10.55±1.00^{a}	8.00±1.00^{a}	r^2=0.89(+)
[-P]	4.81±2.40^{b}	5.00±1.00^{b}	r^2=0.97(+)
Ground Nut			
[+P]	10.54±0.10^{a}	8.67±1.50^{a}	r^2=0.07(+)
[-P]	9.50±0.50^{a}	8.33±0.60^{a}	r^2=0.57(+)

Values followed with same letters along the column are not statistically significant at ($p<0.05$)

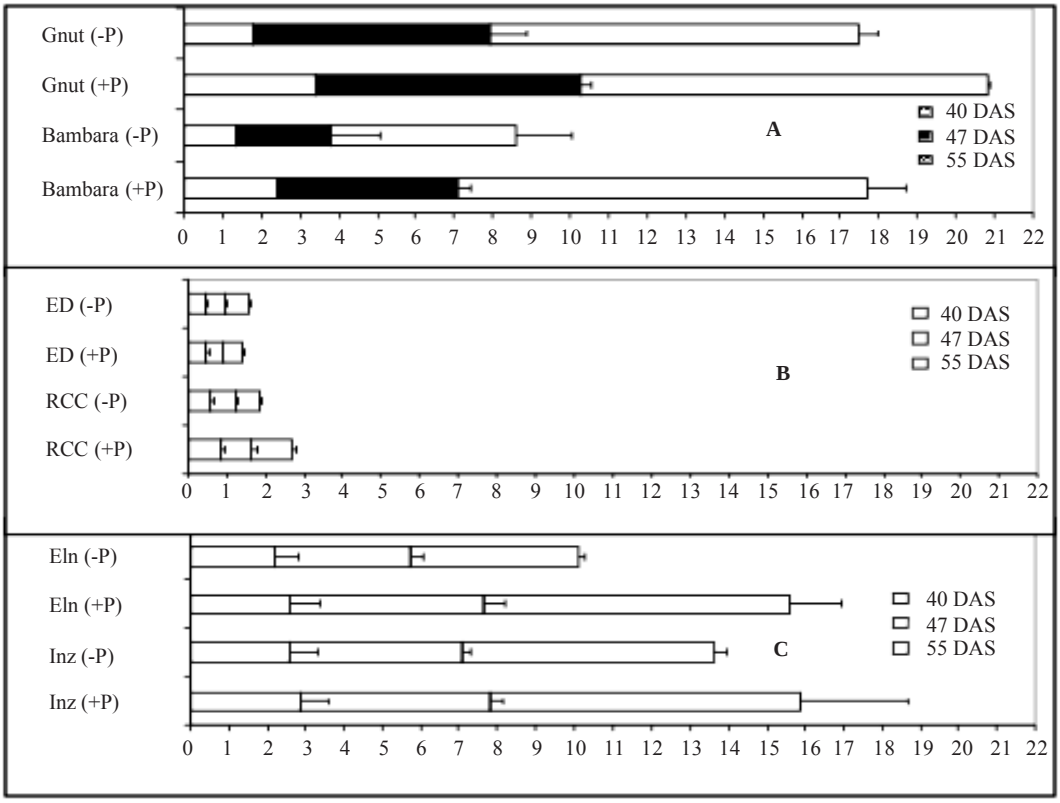

Fig. 2. Effects of sub-optimal P supply[(+P) or no P supply(-P) on plant dry weights of (a) Bambara and groundnut (Gnut) (b) Two bean varieties Rosecoco –RCC and Edwin (ED) respectively and (c) two cowpea varieties – Inzeku (Inz) and Elanda (ELn) respectively at 55 DAS

treatments for Elanda (ELn) cowpea genotype, but the differences were not significant for INz genotype between P variants (Fig.3c). There were no clear differences among bean varieties in terms of nodule weights with P supply (Fig.3b). Generally, there were striking similarity in trends of P supply and nodule numbers and weights (Tables 1, 2, 3 and Figs. 3 a, b and c).

There were also observable strong correlations between nodule numbers and plant DW under suboptimal [+P] and under no P treatments (Table 2) in case of bambara nuts; whereas the same correlations were positive and significant (R^2=0.57 at P<0.05) for ground nut when P was omitted, but there was poor or lack of correlations under P supply (Table 2) with groundnut. The trends in correlations were very similar for INz (when compared with groundnuts). Similarly correlations between Eln cowpea genotype and Bambara nuts showed corresponding correlation between dry weights and nodule numbers) (Tables 1 and 2). The apparent lack of correlations between nodule

Table 3: Effects of P supply on dry weights and nodulation characteristics of two bean (*Phaseolus vulgaris* L) varieties. The plants were harvested at 55 DAS

Varieties	*Plant DW (g plant^{-1})*	*Number of nodules plant^{-1}*	*Correlation between plant DW and nodules*
Rosecoco (RCC)			
[+P]	1.05±0.10^{a}	3.30±0.60^{a}	r^2=0.16(+)
[-P]	0.62±0.00^{b}	2.33±0.60^{ab}	r^2=0.01(+)
Edwin (ED)			
[+P]	0.52±0.10^{c}	2.67±0.60^{a}	r^2=0.90(-)
[-P]	0.63±0.10^{b}	2.33±0.10^{c}	r^2=0.60(-)

Values followed with same letters along the column are not statistically significant at (p<0.05). (+) means correlations are positive and (-) means that characters are negatively correlated

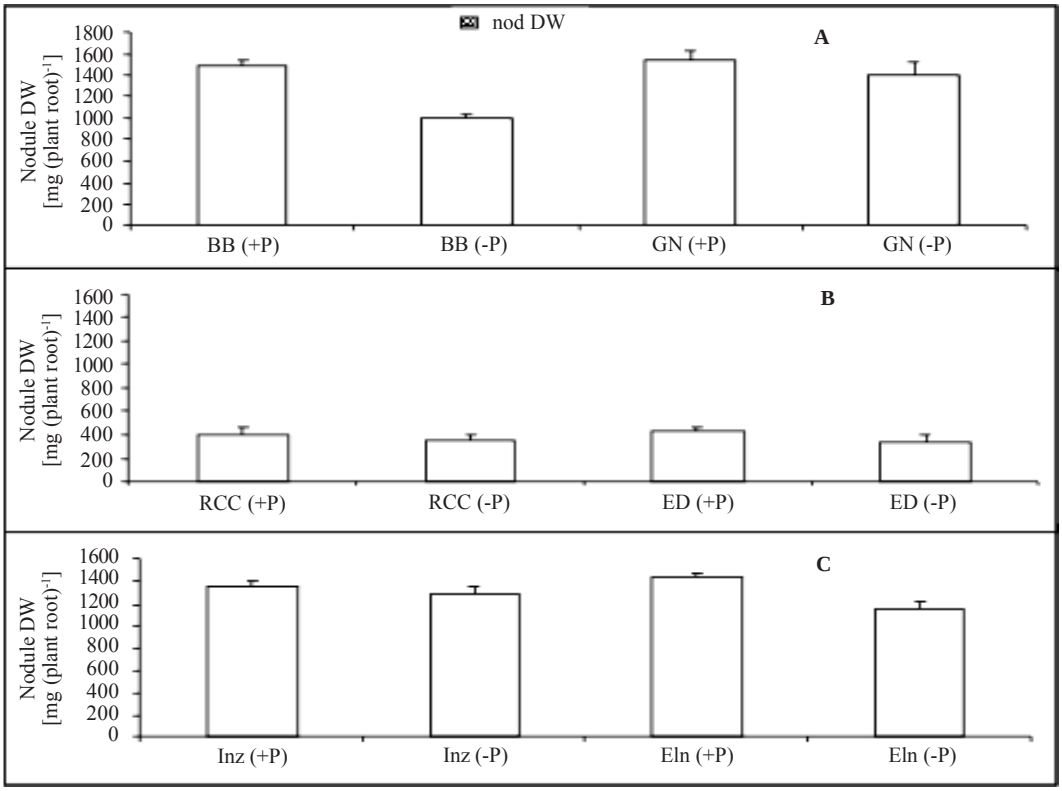

Fig. 3. Effects of sub-optimal P supply[(+P) or no P supply (-P) on nodule weights of (a) Bambara and ground nuts –BB and GN respectively (b) Two bean varieties Rosecoco –RCC and Edwin (ED) respectively and (c) two cowpea varieties –Inzeku (INz) and Elanda (ELn) respectively at 55 DAS

numbers and plant dry weights were shown in Rocecoco (RCC) bean genotype irrespective of P supply. Unexpectedly, there was a strong negative relationship between nodulation and plant dry weights in case of Edwin (ED) bean variety under the current suboptimal P supply.

IV. DISCUSSION

Crop yield on 40% of the world's arable land is limited by P availability. Paradoxically, although total P is quite abundant in many soils, it is largely unavailable for root uptake (Akhtar et al. 2007). Rock-P is a limited resource in the world and estimated to be depleted in 60–80 years (Council for Agricultural Science and Technology 1988; Runge-Metzger 1995). Developing P-efficient cultivars may be one possible way of reducing the demand for rock-P. It has been suggested that P efficiency is a multi-gene controlled quantitative trait. Exploring the genetic resource of crops is one alternative way to utilize the less available P in soils, and cope with the incoming shortage of rock phosphate (RP). In addition, water stress can reduce assimilates supply from leaves to nodules by inhibition of transpiration and phloem translocation. Work by Venkateswarlue et al. (1989) found that water stress caused severe inhibition of nitrogenase activity and nodule respiration in groundnut and cowpea, which could be reflected in biomass yield. In current experiment, Bambara nuts and Elanda (Eln) cowpea genotype showed greater yield with P supply (Fig. 2 and Tables 1 and 2). The two genotypes have preferential water requirements. Inzeku (Inz) variety is grown more preferably during short rains, while Eland is grown successfully during both long and short rainy seasons and is able to tolerate higher rainfall than Inznku (Inz). Microbial reactions, water availability and organic P are mutually related. Hence the higher biomass yield observed with

additional P to Bambara nuts could be due, though indirectly, to an association between leaf nitrogen and carbon assimilation as previously reported by Sinclair et al. (1993). Phosphorus is an important factor in symbiotic association between legumes and rhizobia as was observed in terms of biomass yield and nodule number and nodule weights (Tables 1, 2 and 3 and also Fig. 3). The improvement in plant symbiotic activity due to the presence of Vesicular-abuscular mycorrhizae (VAM) in low soil P condition was probably due to better phosphate acquisition, as had been reported by Mahdi and Atabani (1992). These responses may be species- specific as observed between two cowpea cultivars and two nuts studied (Tables 1 and 2). Bambara and Elanda (Eln) cowpea genotypes showed greater biomass improvement when P was supplied as opposed to when it was omitted. Such observations were glaringly absent in case of groundnut and Inzeku variety of cowpea where biomass yields and nodulation were comparable irrespective of exogenous P supply. Such yield variations with P supply are in harmony with the work of Sungthonwises et al. (2009) which, showed that *Vigna unguiculata* cv. 26-76 at P sufficiency, shoot growth was not significantly higher than sub-deficiency. Indeed P sufficiency did not significantly improve any biomass parameter compared to sub-deficiency for both v *unguiculata* cv Daniala and 26-73 (Sungthonwises et al. 2009). However, P deficiency reduced the number and biomass of nodule as well as their nitrogenous activity (Ribet and Drevon 1995; Vadez et al. 1996; Qiao et al. 2007). This is in consistence with part of results reported herein for Bambara and Eln (Tables 1 and 2 and Fig.3). Similar findings were also confirmed for V. *anguiculata* cultivars under no P supply (Sungthonwises et al. 2009). On the other hand, lack of biomass increment with P (Inzeku and groundnut; Tables 1and 2 and Fig.3) implies that these genotypes have other adaptive mechanisms to P deficiency. Such adaptive responses were reported by Sungthonwises et al. (2009) for cowpea as compared to beans. In addition, these results indicate same trend as one observed by Alkama et al. (2008), suggesting that cowpea is more tolerant to P deficiency than common bean. These are also partly in line with report of Ankomah (1995) where the difference in tolerance to P deficiency in cowpea was related to different ability to absorb P or to differences in P use efficiency. Poehlman (1991) pointed out that the rhizobial activity and number of nodu-les decreased at low pH (< 4.0). Rhizosphere acidification by legume roots is linked to the release of protons following excessive uptake of cation over anions during N_2 fixation (Israel and Jackson 1982; Haynes 1983; Liu et al. 1989), which is particularly under P stress. However, high soil P-deficiency tolerant lines have been shown to have a greater growth with higher proton efflux as reported by Akinrinde et al. (2006). Phosphorus deficiency generally leads to further reduction in rhizosphere pH with consequent saturation of toxic aluminium species that aggravates nodulation activity in legumes. The fact that groundnut and INz cowpea variety were able to nodulate even at lower P may imply an ameliorative potential against P stress, either by re-mobilization to more active growth parts as previously pointed out by other authors (Akinrinde et al. 2006; Smith et al. 1993).

CONCLUSION

The current experiment showed clear differences in legume responses to P stress in terms of nodulation and biomass yield. Ground nut and Inzeku genotype of cowpea showed stable yield and nodulation potential irrespective of P supply while Bambara nut and Eland cowpea genotype showed strong response to sub-optimal P supply but beans had consistently poor growth under either P treatment. It was also clear that farmers would benefit for additional P supply to Elanda (Eln) cowpea genotype and Bambara nuts since the two plant species/genotype benefited from sub-optimal P supply.

RECOMMENDATIONS

From the result of current experiment, we recommend that future work should be undertaken for selection and breeding for P efficiency, and this should call for analysis of gene components of Inzeku genotype and Groundnuts. Successful isolation and transfer of the P tolerance genes from the mentioned spp to other legume vegetables would improve productivity and food security and economic well being of farmers inhabiting these acid-prone soils.

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