CROSS AND SOILS RESEARCH PAPER
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Evaluation of early maturing cowpea (*Vigna unguiculata*) germplasm for variation in phosphorus use efficiency and biological nitrogen fixation potential with indigenous rhizobial populations

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SUMMARY

Cowpea genotypes that efficiently utilize phosphorus (P) with high potential for biological nitrogen (N) fixation (BNF) are vital to sustainable cropping systems in West Africa. A total of 175 early maturing cowpea genotypes were evaluated in 2010 and 2011 for P use efficiency (PUE) and BNF with an indigenous rhizobial population at Shika in the Northern Guinea savanna of Nigeria. There were significant genotypic variations for all 11 variables measured. The P utilization index, percentage N derived from the atmosphere and total N fixed ranged between 2·10–4·67, 31·3–61·86% and 11·86–50 kg/ha, respectively. The 175 early maturing cowpea genotypes were divided into five categories using principal component analysis (PCA), whereby total N fixed was associated with N and P uptake and plant biomass yield. Complete linkage cluster analysis revealed a total of three distinctive clusters having remarkable correspondence with the PCA. Some genotypes were identified as potential candidates for further breeding programmes using high PUE genotypes with relatively high capacity for BNF and indigenous rhizobial populations.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume cultivated in many parts of the world (Ehlers & Hall 1997; Kimiti & Odee 2010; De Freitas *et al.* 2012), with the key production area being West Africa (Hamdy 1989). It is an important crop in low-input and traditional agricultural systems because of its ability to fix nitrogen (N) and reduce its reliance on expensive commercially produced fertilizers (Odion *et al.* 2007). Cowpea is often intercropped with cereals such as millet and sorghum in the Guinea savannah region (Mohammed *et al.* 2008) or planted in rotation with cereals and other crops, and supplements part of the cereals' N requirement through N fixation (Carsky *et al.* 2002). Yield increases of >50% have been observed in maize and millet when in rotation with cowpea compared with rotations with other cereals (Dakora *et al.* 1987; Reddy *et al.* 1994).

Phosphorus (P) is essential for growth, pod formation and N fixation in legumes. According to Adu-Gyamfi *et al.* (1989), legumes generally have a high P requirement for adequate growth, nodulation and N fixation. However, the available P content of soils in West Africa (averaging 8 mg/kg) is seldom adequate for optimal plant growth (Manu *et al.* 1991; Bationo *et al.* 2002). Consequently, production of cowpea in West Africa and many parts of sub-Saharan Africa is hindered by low soil-available P, which is also a characteristic of about 5·7 billion ha of land worldwide (Bationo *et al.* 1986; Hinsinger 2001). Furthermore, the soils of many sub-Saharan regions of Africa are characterized by low-activity clays with high P-fixing capacities, which render P less available to plants. Smalberger *et al.* (2006) observed that soil P deficiency in sub-Saharan Africa was so severe that other technologies such as optimum seeding rate, improved germplasm and inoculation would not work without some form of P addition such as fertilizers. It has therefore been suggested that crops with

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high P use efficiency (PUE) should be developed for use in P-deficient soils, as this provides benefits in both low- and high-input systems (Rose et al. 2010). Cowpea yield on farmers' fields is often low, ranging between 25 and 300 kg/ha (Ajeigbe et al. 2010); however, high yields of >1000 kg/ha are possible with application of P fertilizers (Jemo et al. 2006).

One of the major benefits of cowpea production in many parts of West Africa is its capacity for biological N fixation (BNF). Cowpea can fix between 20 and 100 kg N/ha with an estimated N fertilizer replacement value ranging from 10 to 80 kg N/ha (Horst & Hardter 1994; Carsky et al. 1999, 2002; Sanginga et al. 2000). Cowpea has therefore become important in the integrated farming system, allowing crop rotation with legumes to supply N through BNF. However, BNF in legumes can be limited or enhanced by P availability and utilization (Vance et al. 2003; Waluyo et al. 2004). For example, P deficiency has been shown to reduce the number and biomass of nodules as well as nitrogenase activity in legumes (Valdez et al. 1996; Qiao et al. 2007), which is essential for N fixation. Phosphorus concentration in nodules can be three times higher compared with that in other parts of a plant (Tsvetkova & Georgiev 2003). The ability of cowpea to use P efficiently in low-P soil may enhance nitrogenase activity to increase N fixation in soil and contribute to sustainable cowpea-based cropping systems in the savannas of West Africa.

Many studies have shown that genetic improvement for P-efficiency in crops is more economical and practical than reliance on chemical fertilizers alone (Cakmak 2002; Vance et al. 2003; Yan et al. 2004). Rose & Wissuwa (2012) suggested that breeding of P-efficient crops could give good yields with available P levels <4 mg/kg soil (Bationo et al. 2011) may reduce the impact of grain crops on the P cycle. Several breeding research studies have been conducted on legumes for PUE (Adu-Gyamfi et al. 1989; Sanginga et al. 2000; Vesterager et al. 2006; Ao et al. 2014), but most of them have focused on enhancing P uptake efficiency (Wissuwa et al. 2009). Sanginga et al. (2000) and Abayomi et al. (2008) gave a PUE range of 0·19–0·27 (measured as g shoot dry weight per mg P in shoot) in cowpea under low-P input. While the literature abounds in reported genotypic differences in PUE across a range of crops, there has been little progress in breeding crop cultivars with high PUE (Rose & Wissuwa 2012). The present study measured PUE by determining the P utilization index (PUI), which was expressed as the reciprocal of P concentration (Hammond et al. 2009) in cowpea tissue. The PUI is the factor that determines internal utilization of P in the shoot and grain.

The International Institute of Tropical Agriculture (IITA) has developed many accessions of early-maturing cowpea in its research efforts to meet the demand of farmers. There is virtually no definitive information with respect to the PUE and BNF potential of these accessions and this undermines their potential contribution to sustainable legume–cereal cropping systems in West Africa. Principal component analysis (PCA) and cluster analysis have been reliably used to screen crops for PUE when assessing multiple parameters/traits (Li et al. 2005; Pan et al. 2008). Both analyses allow for integrative effects of multiple parameters on P efficiency but unlike PCA, cluster analysis does not account for relative contributions of different parameters to P efficiency (Pan et al. 2008). The objective of the present study therefore was to screen 175 genotypes of early-maturing cowpea varieties for PUE and BNF potential for crop improvement for low-input and traditional cropping systems.

MATERIALS AND METHODS

Field preparation, soil nutrients and bradyrhizobial determination

Field experiments were conducted at Shika (11°13′N, 7°12′E) in 2010 and 2011. Shika is located in the Northern Guinea savanna of Nigeria and has a uni-modal rainfall pattern with c. 1100 mm rainfall per annum. Mean minimum and maximum temperatures vary between 17–23 and 27–35 °C, respectively, depending on the season. The soil type is a Ferric Lixisol (FAO/ISRIC/ISSS 1998). The field was previously cultivated with maize and sorghum in rotation with cowpea for 4 years. The two seasons before the trial were planted with maize and sorghum respectively with no P fertilizer application. Fields were cleared and ploughed, and ridges spaced 0·75 m apart were constructed with a tractor-mounted ridger. The plot size was 2·25 m^2 (three rows per plot, a 1 m alley between plots and 1·5 m between replicate blocks). Phosphorus was broadcast onto the plots at a rate of 20 kg P/ha triple superphosphate (TSP). Planting was done 2 days after the application of TSP in both years. The experimental design was a Randomized Complete Block Design with three replicates. Early-maturing cowpea genotypes (Table 1) used for the trial were obtained from the germplasm collection of IITA,

Table 1. *The 175 early maturing cowpea genotypes used for evaluation in 2010 and 2011*

Label	Genotype	Label	Genotype	Label	Genotype	Label	Genotype
1	86D-345	51	97K-529-4	101	IT86D-326	151	IT93K-2332-25
2	92KD-279-3	52	97K-555-6	102	IT86D-330	152	IT93K-273-2-1
3	92KD-357	53	97K-564-1	103	IT86D-347	153	IT93K-273-2-2
4	93K-2045-29	54	97K-569-9	104	IT86D-348	154	IT93K-452-1
5	93K-2271-5	55	97K-608-14	105	IT86D-371	155	IT93K-513-2
6	93K-370	56	97K-818-28	106	IT86D-378	156	IT93K-573-1
7	93K-509-16	57	97K-818-35	107	IT86D-409	157	IT93K-601-7
8	93K-619-1	58	97K-819-170	108	IT86D-422	158	IT93K-734
9	93KZ-4-5-6-1-5	59	97K-819-180	109	IT86D-446	159	IT94K-410-2
10	93KZ-8-21-23-6	60	97K-825-3	110	IT86D-470	160	IT95K-1095-4
11	93KZ-8-26-6-6	61	97K-832	111	IT86D-492	161	IT95K-364-6
12	94K-437-1	62	97K-837-8	112	IT86D-493	162	IT99K-356-1
13	94K-453-3	63	98K-1111-1	113	IT86D-520	163	TVU 1047
14	95K-1088-2	64	98K-1399	114	IT86D-633	164	TVU 11424
15	95K-1088-4	65	98K-166-4	115	IT86D-714	165	TVU 12449
16	95K-1090-5	66	98K-205-8	116	IT86D-771	166	TVU 1271
17	95K-1093-5	67	98K-249-1	117	IT86D-801	167	TVU 1272
18	95K-1384	68	98K-428-3	118	IT86D-917	168	TVU 1332
19	95K-1491	69	98K-498-1	119	IT86D-957	169	TVU 1987
20	95K-181-9	70	98K-503-1	120	IT86S-769	170	TVU 4630
21	95K-207-22	71	98K-506-1	121	IT87D-1835	171	TVU 7676
22	95K-526-2	72	98K-589-2	122	IT87D-1836	172	TVU 8337
23	95M-118	73	98K-686-2	123	IT87D-1891	173	TVX 1999-02E
24	95M-120	74	98K-697	124	IT87D-676-2	174	TVX 2724-01F
25	95M-190	75	98K-734-2	125	IT87D-941-1	175	TVX 3380-042E
26	95M-207-15	76	99K-1060	126	IT87DS-491		
27	95M-249	77	99K-1245	127	IT87S-1252		
28	95M-264-1-4	78	99K-205-9	128	IT87S-1321		
29	95M-278	79	99K-409-8a	129	IT88D-643-1		
30	95M-303	80	99K-494-6	130	IT88DM-345		
31	96D-618	81	99K-529-1	131	IT88S-524-7		
32	96D-711	82	IT00K-1150	132	IT89KD-389		
33	96D-738	83	IT00K-1217	133	IT89KD-793		
34	96D-774	84	IT00K-898-5	134	IT90K-102-6		
35	97K-1042-3	85	IT82D-849	135	IT90K-284-2		
36	97K-1042-8	86	IT85D-2682	136	IT90K-365		
37	97K-1042-8-A	87	IT85D-3318-1	137	IT91K-118-20		
38	97K-1069-6	88	IT85D-3334-1	138	IT91K-93-10		
39	97K-1075-7	89	IT85D-3428	139	IT92KD–405-2		
40	97K-1101-5	90	IT85D-3428-4	140	IT92KD-263-4-1		
41	97K-222-5	91	IT85D-3516-2	141	IT92KD-267-2		
42	97K-340-1	92	IT85F-1002	142	IT92KD-370		
43	97K-350-4-1	93	IT85F-1992	143	IT92KD-371-1		
44	97K-400-3	94	IT85F-2865	144	IT92KD-404-1		
45	97K-497-2-A	95	IT85F-3550	145	IT93-596		
46	97K-499-35	96	IT85F-958	146	IT93K-1140		
47	97K-499-38	97	IT85K-1988	147	IT93K-2045-29		
48	97K-499-39	98	IT86D-1008	148	IT93K-2046-1		
49	97K-499-8	99	IT86D-1065	149	IT93K-2046-2		
50	97K-510-2	100	IT86D-1073	150	IT93K-2309		

Table 2. *Soil chemical and physical properties of the experimental site at Shika, Nigeria in 2010 and 2011*

Soil property	2010	2011
pH (KCl)	4·65	5·6
Organic C (g/kg)	6·8	7·14
Nitrogen (g/kg)	0·6	0·6
Phosphorus (mg/kg)	2·532	2·905
Calcium (cmol/kg)	2·019	1·903
Magnesium (cmol/kg)	0·753	0·782
Potassium (cmol/kg)	0·313	0·373
Sodium (cmol/kg)	0·22	0·243
Exchange acidity	0·084	0·084
ECEC	3·389	3·384
Sand (%)	38·50	41·80
Silt (%)	44·90	41·60
Clay (%)	16·60	16·60

Nigeria. Soil physico-chemical characteristics (Table 2) were determined using standard procedures (Okalebo *et al.* 1993).

Soil sampled prior to planting was analysed for indigenous rhizobial population densities using the plant-infection technique of Vincent (1970) and growth pouch-plant cultures described by Weaver & Frederick (1972) and Somasegaran & Hoben (1994). Plants growing in the pouches were provided with adequate volumes of N-free nutrient solution (Singleton 1983) in which the micronutrient components were replaced with a micronutrient formulation proposed by Broughton & Dilworth (1970). Pouch-plant cultures were scored for the presence of nodules after 28 days from inoculation. The mean most probable number (MPN) of bradyrhizobia was obtained using the MPN enumeration system (Woomer *et al.* 1990).

Field evaluation, planting and crop maintenance

Cowpea was sown at a depth of 3–5 cm along the ridges using three seeds per hole and planting, the seeds were treated with Apron plus at a rate of 10 g per 5 kg of seeds. The plots were weeded manually using hoes at 3, 6 and 10 Weeks After Planting (WAP). Sampling was assessed at 50% flowering for BNF, at pod-filling for PUE and at maturity for grain yield. Plant samples were processed for number and dry weight of nodules, and N_2-fixation was measured using the hot water extraction method (Herridge 1982; Herridge & Peoples 1990; Herridge *et al.* 2008). The stem and petiole of cowpea shoots were ground to pass through a 1·0 mm

sieve, after which the sample was extracted with 25 ml boiling water for 2 min. The extract was filtered, made up to 50 ml volume with distilled water, and stored at –15 °C until analysis for ureides and % N derived from the atmosphere (% Ndfa).

Harvested plant samples were chopped into 10- to 20-mm pieces and sub-sampled, and *c.* 200 g fresh weight was oven-dried at 70 °C for 48 h before grinding to pass through a 0·5 mm sieve for measurement of N and P contents. At the last harvest, plant samples were separated into reproductive (grains) and vegetative parts (shoots) after oven drying. The grains were threshed from the pods and further dried to 70–80 g moisture content/kg. Plant shoots, nodules and straw were dried in the oven at 70 °C to a constant weight before weights were recorded. Total N in the grain, shoot and straw (biomass) fractions was determined by the Kjeldahl procedure (Bremner & Mulvaney 1982). Phosphorus content in tissues was determined using the procedures described by Okalebo *et al.* (1993). Phosphorus utilization index was expressed as the inverse of P concentration in the plant.

Statistical analysis

Combined analyses of variance (ANOVA) were conducted for 11 variables measured in 2010 and 2011 using GENSTAT Discovery edition package (GENSTAT 2011). Number of nodules and Ndfa (%) were transformed using logarithm to base 10 and square root transformation, respectively, before the data were subjected to ANOVA for presentation in Supplementary Table S1 (available online from: http://journals.cambridge.org/AGS). Treatment means were separated using Standard Errors of Differences of Means (S.E.D.) at significance level of $P < 0.05$. Principal component analysis based on correlation matrix was conducted on the mean values of the ten variables for each genotype using Principal Components of Multivariate Analysis of GENSTAT. Ten variables were used because PUI, which was a derived variate (reciprocal of P content), was not included in PCA and cluster analysis to avoid multi-collinearity which may confound the analyses. Variables that accounted for most of the variance in the whole set of the data were identified and grouped into axes using biplots of variates and principal components. Hierarchical cluster analysis was also performed using a complete link method to classify the cowpea genotypes into different clusters based on the plotted dendrogram. Correlation among

Table 3. *Summary of combined analysis of variance for the 11 variables of 175 early maturing genotypes evaluated in 2010 and 2011*

Parameters	Number of nodules (×10⁶/ha)	Nodule dry weight (kg/ha)	Crop biomass (kg/ha)	Grain yield (kg/ha)	Crop N content (g/100 g)	Crop P content (g/100 g)	PUI	Ndfa (%)	Total N fixed (kg/ha)	N uptake (kg/ha)	P uptake (kg/ha)
Mean	1·3	8·4	1722	718	3·0	0·34	3·1	58	30	53	5
Minimum	0·2	0·8	866	173	2·3	0·22	2·1	31	12	19	2
Maximum	3·0	27·9	2856	1468	3·8	0·49	4·7	62	50	87	8
D.F.	696	695	698	698	698	698	698	698	698	698	698
S.E.D.*											
Genotype (G)	0·37	3·26	364·5	138	0·18	0·035	0·34	2·3	11·3	6·3	1·2
Year (Y)	ns	0·35	39·0	ns	ns	ns	ns	0·3	1·2	0·7	0·1
G × Y	0·53	4·61	516	192	ns	ns	ns	3·3	16·0	8·9	1·7

N, nitrogen, P, phosphorus; PUI, phosphorus utilization index; D.F., degree of freedom; S.E.D., standard error of difference of means; ns, not significant.

* S.E.D. values significant at $P < 0.05$; ns, not significant.

variables was determined using the PROC CORR of the Statistical Analytical System (SAS 2003).

RESULTS

The soil used for the study was acidic and low in organic carbon in both years. The total N and available P in the soil were low for both 2010 and 2011 cropping seasons (Table 2). The combined ANOVA showed that genotype was significant ($P < 0.05$) for all the 11 variables tested (Table 3). Interaction between year and genotype was also significant ($P < 0.05$) for all the traits except total plant N and P contents, and PUI (Table 3). The grain yield, crop biomass, total N fixed, PUI and % Ndfa among the genotypes were in the ranges of 173–1468 kg/ha, 866–2856 kg/ha, 11·86–50 kg/ha, 2·10–4·67 and 31·3–61·86%, respectively (Table 3). The recorded ranges of the other variables were as follows: number of nodules (0.25–3.0×10^6/ha), nodule dry weight (0·84–27·85 kg/ha), biomass N content (2·24–3·78% dry matter), biomass P content (0·22–0·49% dry matter), N uptake (18·86–87·74 kg/ha) and P uptake (2·24–8·36 kg/ha).

Positive correlation (Table 4) was observed between: P uptake and total N fixed ($P < 0.05$, $r = 0.92$); P and N uptake ($P < 0.05$, $r = 0.90$); N uptake and total N fixed ($P < 0.0001$, $r = 0.99$); total N uptake and total crop biomass ($P < 0.0001$, $r = 0.95$); and crop biomass and total N fixed ($r = 0.93$). Crop N and P contents had negative correlations with other variables. There were negative correlations between PUI and crop P content ($P < 0.05$, $r = -0.97$) and PUI and crop N content ($P < 0.05$, $r = -0.63$). No significant correlation was observed between PUI and proportion of % Ndfa.

The results of the PCA (Table 5) showed that PC 1, 2, 3, 4 and 5 accounted for 0·90 of the variation with PC1 (0·45), PC2 (0·18) and PC3 (0·13) jointly contributing 0·76 of the variation. Latent vectors ⩾0·4 were identified as the logical cut-off points where each selected trait made an important contribution to the PC axis. Based on this, all the ten variables were loaded on the five PC axes with six of them on the first two axes. On PC1 were crop biomass, N and P uptake and total N fixed, while crop P and N contents were loaded on PC2. Number of nodules, % Ndfa and nodule dry weight were loaded on PC3, while % Ndfa and grain yield were loaded on PC4 and PC5, respectively.

The variate biplot of the ten variables measured for cowpea genotypes revealed the relationships among the variables (Fig. 1). The projections for number of

Table 4. *Correlation coefficients for the 11 variables measured*

	Number of nodule	Nodule dry weight	Biomass dry weight	Grain yield	Biomass N content	Biomass P content	PUI	Ndfa (%)	N uptake	Total N fixed	P uptake
Number of nodule											
Nodule dry weight	0·53 (P < 0·001)										
Biomass dry weight	0·25 (P < 0·001)	0·34 (P < 0·001)									
Grain yield	0·28 (P < 0·001)	0·27 (P < 0·001)	0·45 (P < 0·001)								
Biomass N content	−0·24 (P < 0·01)	−0·36 (P < 0·001)	−0·40 (P < 0·001)	−0·36 (P < 0·001)							
Biomass P content	−0·16 (P < 0·05)	−0·18 (P < 0·05)	−0·34 (P < 0·001)	−0·31 (P < 0·001)	0·61 (P < 0·001)						
PUI	0·15 (P < 0·05)	0·18 (P < 0·05)	0·31 (P < 0·001)	0·29 (P < 0·001)	−0·63 (P < 0·001)	−0·97 (P < 0·001)					
Ndfa (%)	0·15 (P < 0·05)	0·09	−0·01	0·002	0·17 (P < 0·05)	0·19 (P < 0·05)	−0·19 (P < 0·05)				
Nitrogen uptake	0·21 (P < 0·01)	0·29 (P < 0·001)	0·95 (P < 0·001)	0·38 (P < 0·001)	−0·22 (P < 0·01)	−0·22 (P < 0·01)	0·18 (P < 0·05)	0·13			
Total N fixed	0·23 (P < 0·01)	0·30 (P < 0·001)	0·93 (P < 0·001)	0·37 (P < 0·001)	−0·19 (P < 0·05)	−0·18 (P < 0·05)	0·14	0·21 (P < 0·01)	0·99 (P < 0·001)		
Phosphorus uptake	0·16 (P < 0·05)	0·27 (P < 0·001)	0·89 (P < 0·001)	0·32 (P < 0·001)	−0·26 (P < 0·001)	−0·07	0·04	0·16 (P < 0·05)	0·90 (P < 0·001)	0·92 (P < 0·001)	

N, nitrogen; P, phosphorus; PUI, phosphorus utilization index.

Table 5. *Latent vectors of the first five principal components (PC1, PC2, PC3 PC4 and PC5) axes for 175 early maturing cowpea genotypes evaluated for PUE and BNF*

Variables	PC1	PC2	PC3	PC4	PC5
Grain yield	–	–	–	–	−0·84
Total N fixed	0·43	–	–	–	–
Ndfa (%)	–	–	0·52	−0·74	–
Crop biomass	0·45	–	–	–	–
Crop N content	–	0·50	–	–	–
Crop P content	–	0·48	–	–	–
Nitrogen uptake	0·44	–	–	–	–
Phosphorus uptake	0·41	–	–	–	–
Nodule dry weight	–	–	−0·49	–	–
Number of nodules	–	–	−0·57	–	–
Percentage of variance accounted for by PCs	45·13	18·05	13·14	7·67	6·59
Cumulative percentage of variance accounted for by PCs	45·13	63·18	76·32	83·99	89·58

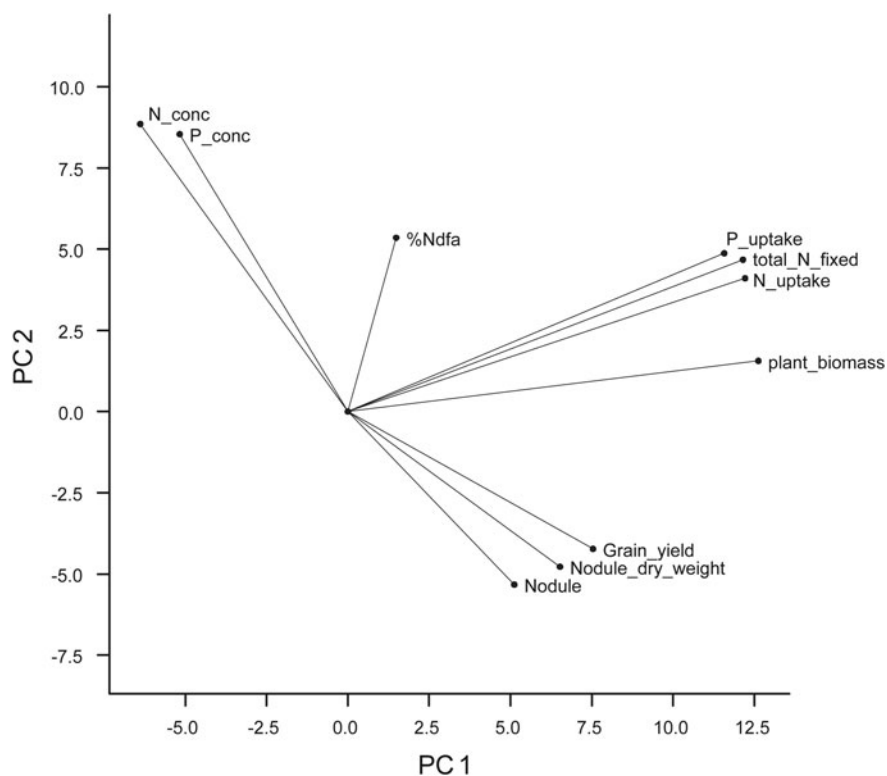PUE, phosphorus use efficiency; BNF, biological nitrogen fixation; N, nitrogen; P, phosphorus.

Fig. 1. The variate biplot (from principal component analysis) of the 11 variables that were measured for the 175 early maturing cowpea genotypes in 2010 and 2011, showing the projection of each variable on the biplot (P_conc = crop P content; N_conc = crop N content; plant biomass = crop biomass; nodule = number of nodules).

nodules, nodule dry weight and grain yield from the centre of origin of the biplot were to the positive side of PC1 (x-axis) and negative side of PC2 (y-axis), while that of crop biomass, N and P uptake, total N fixed and Ndfa (%) were to the positive sides of PC1 and PC2. Crop N and P contents were projected to the negative side of PC1 and positive side of PC2. None of the variables were projected to the negative sides of PC1 and PC2. On the PC biplot (Fig. 2), some genotypes were distinctively grouped together
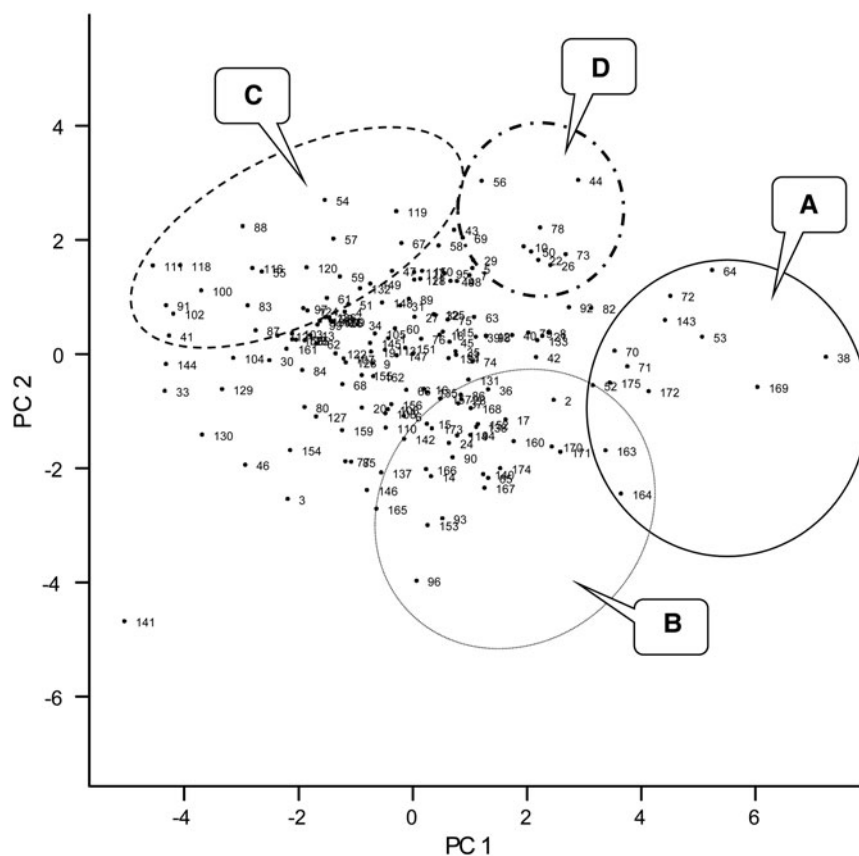
Fig. 2. Graph of PC1 and PC2 scores of 175 early maturing cowpea genotypes evaluated for phosphorus use efficiency (PUE) and biological nitrogen fixation (BNF) in 2010 and 2011. A–D are different groups of genotypes that can be identified based on their performances. Labels correspond to the genotypes as listed in Table 1.

based on their performance for the measured variables and the relationship among the variables. Group A consisted of genotypes such as 97K-1069-6 (38), 98K-1399 (64), 98K-564-1 (53), TVu 1987 (169), IT92KD-371-1 and 98K-589-2 (72), which had high values for crop biomass, N and P uptake and total N fixed as revealed on the variate biplot (Fig. 1). Group B consisted of genotypes that were most likely to be differentiated from others based on the nodulation and grain yield as loaded on the variate biplot (Fig. 1). Among the genotypes were 95M-120 (24), 92KD-370 (142), IT93K-1140 (146), IT95K-1095-4 (160), IT93K-273-2-2 (153), TVu 7676 (171) and TVx 1999-02E (173). Group C consisted of genotypes that were high in biomass N and P contents and included genotypes such as 97K-569-9 (54), IT86D-771 (116), IT86D-917 (118) and IT86D-957 (120). Group D consisted of genotypes that had high % Ndfa such as 97K-818-28 (56), 97K-350-4-1 (43) and 98K-498-1 (69). Other genotypes were not clearly differentiated based on the ten variables that were used in the study.

The complete linkage cluster analysis revealed a total of three clusters based on the normalized distance of 0·75 on the dendrogram (Fig. 3). There were a total of 14, 49 and 112 genotypes for clusters 1, 2 and 3, respectively (Fig. 3 and Table 6). The clusters showed a remarkable closeness to the PCA as most of the genotypes that were grouped as A and B the PC biplot (Fig. 2) were listed in clusters 1 and 2, respectively (Fig. 3). The genotypes in cluster 1 include 97K-1069-6 (38), 98K-1399 (64), 98K-564-1 (53), TVu 1987 (169), IT92KD-371-1 and 98K-589-2 (72), while cluster 2 includes genotypes such as 92KD-279-3 (2), 92KD-370 (142), IT93K-1140 (146), IT95K-1095-4 (160), IT93K-273-2-2 (153) and TVx 1999-02E (173). Cluster 3 was further divided into two main sub-clusters with one sub-cluster comprising just one genotype, IT92KD-267-2.

DISCUSSION

The primary objective of the present study was to screen for variation in PUE within 175 early-maturing cowpea genotypes and identify candidate genotypes
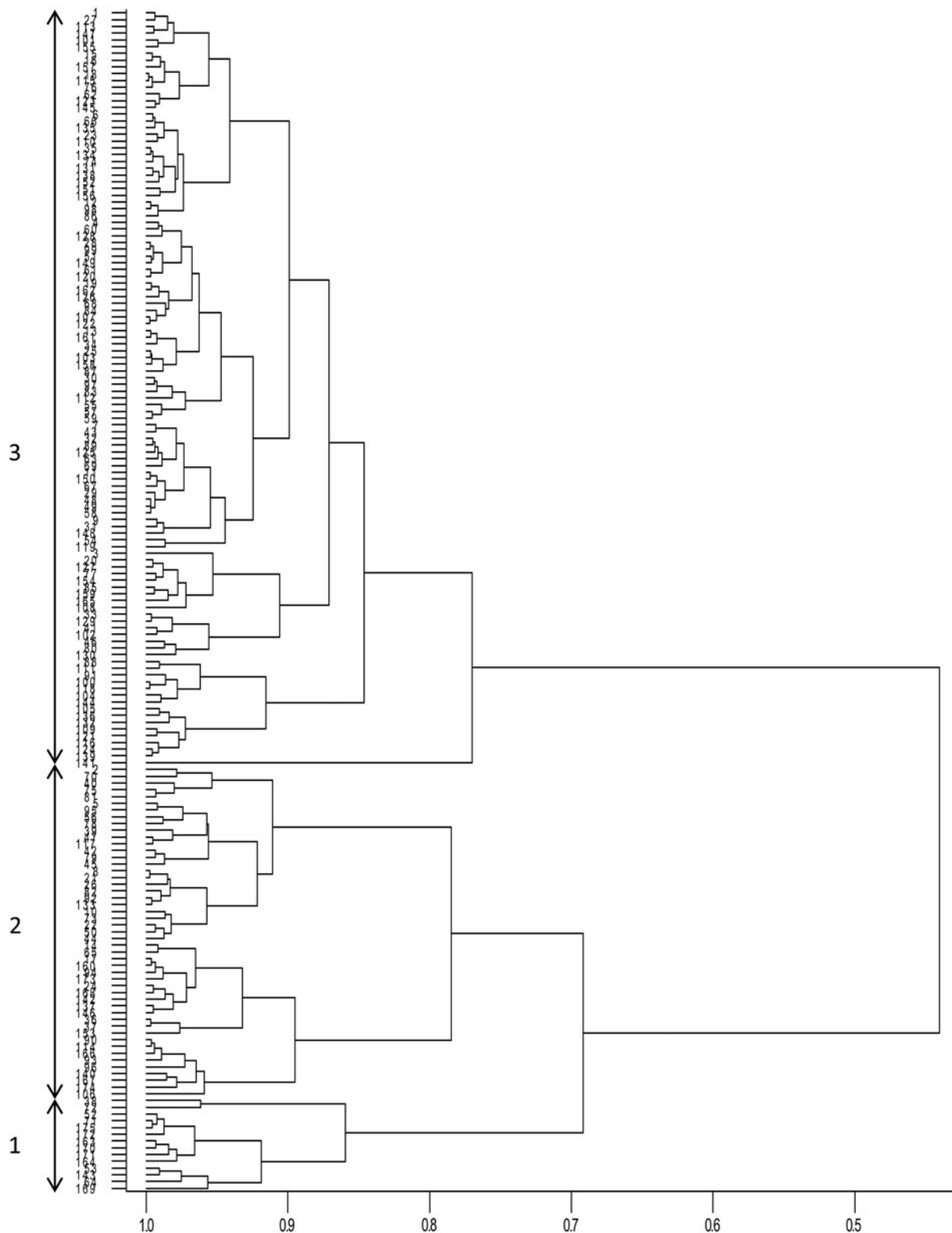
Fig. 3. The dendrogram from complete linkage cluster analysis showing the three clusters that were formed at the normalized distance of 0·75 and the cowpea genotypes in each cluster. Genotype labels (§) correspond to the genotypes as listed in Table 1. The labels are shown in Table 5 for clarity.

Table 6. *Genotype labels in each of the three clusters shown on the dendrogram*

Cluster 1	Genotype labels* Cluster 2		Cluster 3			
38	2	78	1	48	97	128
52	5	79	3	49	98	129
53	8	81	4	51	99	130
64	10	82	6	54	100	131
71	14	90	7	55	101	132
72	17	92	9	57	102	134
143	21	93	11	58	103	135
163	22	94	12	59	104	136
164	24	95	13	60	105	138
169	26	96	15	61	107	139
170	36	106	16	62	108	141
171	37	114	18	63	109	144
172	39	117	19	66	110	145
175	40	133	20	67	111	147
	42	137	23	68	112	148
	44	140	25	69	113	149
	45	142	27	74	115	150
	47	146	28	76	116	151
	50	153	29	77	118	152
	56	160	30	80	119	154
	65	166	31	83	120	155
	70	167	32	84	121	156
	73	168	33	85	122	157
	75	173	34	86	123	158
		174	35	87	124	159
			41	88	125	161
			43	89	126	162
			46	91	127	165

* Name of the genotypes for each label is provided in Table 1.

for introgression into existing farmer-preferred genotypes. Plant efficiency for nutrient uptake and utilization may improve yield potentials in situations of soil nutrient stress, reducing plant nutrient demands for a given level of crop yield. The purpose of screening such a large set of germplasm and the use of PCA and cluster analysis in identifying P-efficient genotypes was to obtain results, as previously reported for other crops (Ozturk et al. 2005; Raghothama & Karthikeyan 2005; Pan et al. 2008), that take into consideration the integrative effects and relative contributions of different parameters to PUE in early maturing cowpea.

Dry matter production is directly related to N and P supply. The positive relationship among crop biomass, N and P uptake and total N fixed was expected since N and P uptake are functions of dry matter accumulation and total N fixed was derived from N uptake and %Ndfa. Significant relationships among N and P uptake, total biomass production and grain yield have also been observed for crops such as soybean and pigeon pea in previous studies (Vesterager et al. 2006; Pan et al. 2008; Abbasi et al. 2010). Having correlated positively with PUI ($r = 31$), biomass dry weight would be an important variable when screening cowpea varieties for dual purpose of PUE and N fixation effectiveness. Pan et al. (2008) reported that shoot dry weight is an effective and simple indicator of screening for P-efficient soybean genotypes. The PUI in the present study was the reciprocal of P content in the cowpea tissue and therefore the negative correlation between two variables and also N content which had a strong positive

Table 7. *Means of 11 variables measured for the genotypes that were grouped in cluster 1*

Genotype	Number of nodules	Nodule dry weight (kg/ha)	Crop biomass (kg/ha)	Grain yield (kg/ha)	Crop N content (g/100 g)	Crop P content (g/100 g)	PUI	Ndfa (%)	Total N fixed (kg/ha)	N uptake (kg/ha)	P uptake (kg/ha)
97K-1069-6	2 736 650	27·85	2691·09	1123·83	2·65	0·37	2·81	58·57	50·09	86·74	8·25
97K-555-6	1 283 330	10·18	2326·65	817·04	2·54	0·26	3·87	57·31	36·74	64·95	6·93
97K-564-1	2 562 210	8·97	2607·54	1033·87	2·79	0·33	3·10	55·76	43·03	78·66	8·06
98K-1399	1 406 660	8·60	2855·98	893·12	2·96	0·29	3·46	56·97	46·16	81·16	8·36
98K-506-1	1 355 540	13·34	2272·87	881·89	2·64	0·28	3·57	57·55	41·02	72·56	6·71
98K-589-2	2 397 760	17·19	2587·54	688·29	2·84	0·38	2·69	58·52	41·74	73·99	8·09
IT92KD-371-1	2 004 430	8·79	2497·76	1172·43	2·93	0·34	2·96	57·15	42·70	77·07	7·08
TVU 1047	2 671 090	13·17	2216·88	790·75	2·42	0·29	3·55	55·37	35·58	66·49	6·57
TVU 11424	2 359 990	16·82	2237·76	1212·60	2·43	0·28	3·65	57·10	33·74	59·10	6·30
TVU 1987	1 786 660	15·11	2723·98	1467·68	2·44	0·33	3·04	58·04	42·74	76·39	7·89
TVU 4630	2 399 990	9·27	2189·76	791·23	2·47	0·28	3·95	56·25	34·15	62·21	5·51
TVU 7676	1 875 540	9·75	2080·88	832·07	2·37	0·24	4·19	57·58	33·70	60·13	6·21
TVU 8337	1 866 660	11·46	2496·43	863·43	2·64	0·26	3·83	56·00	42·06	76·68	6·12
TVX 3380-042E	1 799 990	12·48	2287·54	905·20	2·62	0·30	3·37	57·57	38·19	66·98	6·76
S.E.D.	373 613·8	3·256	364·5	137·97	0·17557	0·0348	0·3396	2·323	6·32	11·285	1·2126

N, nitrogen; P, phosphorus; PUI, phosphorus utilization index; S.E.D. represents standard error of difference for genotype (obtained from the analysis of variance for the 175 genotypes).

relationship with P content. The negative correlation was consistent with the work of Nwoke *et al.* (2009), who observed negative correlations between internal PUE and N and P contents when soil was treated with single superphosphate fertilizer. The PUI was used as a measure of P efficiency because it provides an understanding of the internal mechanisms associated with conservable use of absorbed P at the cellular level (Bates & Lynch 2001; Vance *et al.* 2003). A complete picture of inter-relationships between traits or variables can be revealed by PCA and cluster analyses that consider multiple traits. Badu-Apraku *et al.* (2006) reported that PCA considers underlying inter-relationships in the whole set of data, selects the best linear combination of traits that explains the largest proportion of the variance in the data set, and loads them on the first principal component axis. The outputs from the PCA revealed that the variables with the most discriminating power for the grouping of genotypes were biomass dry weight, total N fixed, and N and P uptake, since they accounted for more of the variance (0·45) in the set of data than other traits. Among the traits loaded on PC1, P uptake and biomass dry weight have been identified as important traits for selection of P-efficient genotypes (Manske *et al.* 2001; Osborne & Rengel 2002; Pan *et al.* 2008). Ding *et al.* (2006) concluded that P uptake was one of the most important indices for evaluating the ability of soybean cultivars to absorb P under deficient conditions. Since the focus of the present study was to screen for genotypes that can use P efficiently in low-P soil and are able to biologically fix adequate N, the traits listed on PC 1 are the best for selection purposes in the present study. These traits were characteristically high in *c.* 14 genotypes as revealed by group A and cluster 1 of PCA and cluster analyses, respectively. The 14 genotypes represent *c.* 8% of the total number of genotypes used for the study and can be considered to be a relatively substantial gene pool for further breeding. In addition to breeding, the information on genotype characterization is of particular use for farmers utilizing low-input/traditional cropping systems. The 14 genotypes identified in this group were; IT92KD-371-1, 97K-1069-6, 97K-555-6, 97K-564-1, 98K-506-1, 98K-589-2, 98K-1399, TVu(s) 1047, 1987, 4630, 7676, 8337, 11424 and TVx 3380-042E. Interestingly, genotype TVu 1987, TVu 11424, 97K-1069-6 and IT92KD-371-1 produced the highest grain yields (Table 7), which could also qualify them as P-efficient genotypes for the dual purpose of biomass/grain production and N fixation.

Defining P efficiency in terms of biomass production has been consistently used in most PUE studies (Pan *et al.* 2008; Rose & Wissuwa 2012). However, in legumes, there is a tendency to relate P efficiency to BNF because P is one of the components of adenosine triphosphate (ATP), which provides energy and activates nitrogenase in the atmospheric N fixation process. It would therefore be expected that genotypes with high PUI would be able to fix more N than those with low PUI. This was not observed in all cases in the present study. In fact, there was weak negative correlation between PUI and % Ndfa. One reason why the positive influence of a PUI on %Ndfa could be affected is the size of the rhizobia population and its effectiveness. Al-Niemi *et al.* (1997) reported that bacteroids can be P-limited even when plants have received otherwise adequate P. The presence of ineffective rhizobia in the nodules can also result in lower or even negligible N fixation (Cassman *et al.* 1981; Kiers *et al.* 2007) even when PUI is high in the plant. The current results revealed that even though PUI had a positive influence on crop biomass, N fixation was indirectly affected, as reflected in the total N fixed (kg/ha) in the soil. This observation was in agreement with that of Ankomah *et al.* (1996), who concluded that the P effect on N_2 fixation was mainly in the total amount of N fixed rather than on the percentage derived from the atmosphere. Therefore, incorporation of residues of dual purpose cowpea genotypes with potential for high PUI can improve the residual N content of soils.

CONCLUSION

Genotypic variation in PUE among the 175 early maturing cowpea genotypes evaluated in low-P soil was influenced by P uptake and biomass dry weight and it was demonstrated that the potential range of high P use efficient genotypes was narrow. However, early-maturing cowpea genotypes provided sources of useful gene pools for PUE and N fixation breeding programmes. A number of genotypes were identified as good candidates for further breeding programmes. It was concluded from the results that crop biomass, total N fixed, N and P uptake are effective indicators for the screening of early maturing cowpea populations for PUE and N fixation efficiency. High N fixation and P-efficient genotypes could be integrated into traditional cropping systems that are normally practiced on N and P deficient soils.

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SUPPLEMENTARY MATERIAL

The supplementary material for this paper can be found at dx.doi.org/10.1017/s002185961150015X.

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