Fitting Soybean and Cowpea Genotypes into Cropping Systems on Low-Available Phosphorus and High Aluminium Acid Soils of Southern Cameroon

Von der Naturwissenschaftlichen Fakultät
der Universität Hannover
zur Erlangung des akademischen Grades eines

Doktors der Gartenbauwissenschaften
-Dr. rer. hort.

Genehmigte
Dissertation

von
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Geboren am 28. März 1971 in Mbanga, Kamerun

2005

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Tag de Promotion 13 -07-2005

Abstract

Soil phosphorus (P) and nitrogen (N) deficiencies are major factors limiting plant production on the tropical soils of southern Cameroon (SC). On the predominantly acid soils aluminium (Al) is an additional growth-limiting factor inhibiting particularly the root growth. Field experiments over 2 years on two acid and low P soils of SC, pot experiments with the same soils and nutrient solution experiments were conducted in order to assess the genotypic variation in soybean and cowpea in P efficiency, Al resistance, and the possible contribution of P-efficient and Al resistant genotypes to positively contribute to the P use and N budget of a legume maize cropping system.

Twelve soybean and seven cowpea genotypes were grown in the field on a Typic Kandiudult (TK) and a Rhodic Kandiudult (RK) soil of southern Cameroon in 2001 and 2002 in a split block design without and with application of Togolese phosphate rock (PR) or Triple super phosphate (TSP). Shoot dry matter (DM), grain yield, N_2 fixation, and P content varied with site, genotypes, and P source. In both grain legume species clear genotypic differences in all parameters without P application and in response to P application were identified. Based on cross-classification of the genotypes in terms of P efficiency and response, genotypes were grouped as efficient responders (ER), efficient non-responder (ENR), inefficient responder (IR), and inefficient non-responder (INR). This was also reflected in their positive N balance which, however, was much lower in cowpea than in soybean.

The pot experiments showed that contributing factors to the P efficiency in soybean was enhanced by the association of the roots with arbuscular mycorrhizal fungi and/or a compensatory mechanism between shoot and root growth. In cowpea the most important mechanisms of genotypic variation in P-efficiency were attributed to an enhanced of P uptake per unit of cm root length and the efficient use of P.

In the same experiments the residual effect of the legume genotypes differing in P efficiency on subsequently grown maize was evaluated. Yields of maize after soybean genotypes TGm 1511, IT89KD-391 and cowpea genotypes IT90K-59 were significantly higher than those of other genotypes on the TK soils. The residual effect of legumes to the following maize increased on both soils when the legumes were fertilised with TSP. The capacity of some of the genotypes to better mobilise sparingly soluble soil and fertiliser P could be related to a release of the organic acid anions malate and citrate and an increased root acid phosphatase activity under P stress under controlled conditions in nutrient solution. It was concluded that the residual benefit of P to maize was enhanced with P application to the preceding legume crop thus indicating the need for legume fertilization for optimum maize yields.

Key words: Aluminium resistance - N_2 fixation – Phosphorus - Organic acids-Southern Cameroon

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Abbreviations

AAS	atomic absorption spectrophotometer
ACIAR	Australian Centre for International Agricultural Research
Al	aluminium
AMF	arbuscular mycorrhizal fungi
ANOVA	analyse of variance
cm	centimetre
CMS	Cameroon maize seed
CORR	correlation
DM	dry matter
ENR	efficient non responder
ER	efficient responder
G	genotype
GLM	general linear model
g	gram
ha	hectare
HFB	Humid forest benchmark
HPLC	high performance liquid chromatogram
HSD	honestly significant difference
ICP-OES	inductive couple plasma
IITA	International Institute of Tropical Agriculture
INR	inefficient non-responder
IPE	Institute of Plant Nutrition
IR	inefficient responder
l	litre
mg	milligram
min	minute
mM	millimolar
Mn	manganese
N	nitrogen
nM	nanomolar
n	number of observation
P	phosphorus
° C	degree Celsius
pM	picomolar
PE	pachyman equivalents
PEP	phosphoenolpyruvate
PEPC	phosphoenolpyruvate carboxylase
pNPP	para-nitrophenol phosphate
PR	phosphate rock
RCB	randomised complete block
RI	root inhibition
RK	rhodic kandiudult
RRE	relative residual effect
RUA	relative ureide abundance

SC	southern Cameroon
SD	standard deviation
µg	microgram
µM	micro molar
TCA	tricarboxylic cycle
TK	typic kandiudult
TSP	triple super phosphate
WAP	Week after planting

General Introduction

Low phosphorus (P) availability mostly due to excess removal by crops and fixation into secondary unavailable forms (Stoorvogel and Smaling, 1990; Sample et al.,1980), make P next to nitrogen (N) the most limiting nutrient for plant growth in many tropical soils, in general (Sanchez et al.,1997), and southern Cameroon (SC), in particular (Selles et al., 1995). Further limitations of the soils in SC are the high level acidity and the solubilization of aluminium and manganese into Al^{3+} and Mn^{2+} exchangeable forms toxic to plants (Menzies and Gillman, 1997) in high concentrations.

The management of the acid P-fixing agricultural soils involves the application of P fertilizers, liming to raise the soil pH, or the implementation of practices that reduce rates of acidification and Al toxicity injury. In many parts of SC, however the application of even moderate rates of P fertilizers and lime is uneconomic, because of the low-input nature of agricultural production and high P fixation to Fe and Al oxides (Selles et al., 1995). Furthermore, the cost, the availability of fertilizers, and an appropriate fertilizer technology limit the use of fertilizers by smallholder farmers in SC.

Interventions to overcome soil-P limitations and Al injury constraints are needed to improve the livelihoods of the rural poor of SC who increasingly are intensifying their cropping practices in response to increase in household population. Such approaches may require the use of P-efficient genotypes that make most efficient use of P supplied by the soil and maintenance fertiliser-P applied, plants that tolerate high levels of Al^{3+}, and a good management of factors allowing plants to generate favourable rhizosphere conditions.

Legumes improve the productivity of the cropping system in which they are grown through the process of biological N_2 fixation. Improved legume P nutrition implies increased N_2 fixation, greater organic matter input, and improved soil-N conservation (Hoshikawa, 1991). Moreover, the legume component of the cropping system is often better able to utilise P from sparingly soluble source than the cereal component (Horst et al., 2001; Kamh et al., 2001; Kamh et al., 1999). The physiological mechanisms of improved P utilisation may vary with legume species, genotypes, soils characteristics, and source of P, but generally include rhizosphere acidification (Hinsinger et al., 2003), exudation of organic acid anions (Neumann and Römheld, 1999), higher phosphatase activity (Li et al., 2003), improved uptake kinetics (Nielsen and Barber, 1978), association with arbuscular mycorrhizas fungi (AMF) (Smith and Read, 1997), higher root length, and more and longer root hairs (Gahoonia and Nielsen, 2004).

In the humid forest benchmark (HFB) area in SC the traditional annual cropping system is a mixed food crop-based system where the primary grain legume groundnut (*Arachis hypogaea* L.) is generally intercropped with other crops such as cassava (*Manihot esculenta* (Cranz)) and maize (*Zea mays* L.) (Wendt and Atemkeng, 2004; Wendt, 2002; Büttner, 1996; Mutsaers et al., 1981). In spite of its importance in humid forest cropping systems, groundnut yields are poor. Estimated average yield is 350 kg ha^{-1} (IRA, 1990), while approximated yields of 900 kg ha^{-1} and 750 kg ha^{-1} depending on the cropping season around Yaounde have been reported by Mutsaers et al. (1981). However, Mandimba and Djondo (1996) observed low levels of N_2 fixation in groundnut ranging from 14 to 15 kg N ha^{-1}, representing 36 – 39% of the N_2 derived from the atmosphere, in the Congo Basin.

In view of all the above-listed constraints, attention is now directed towards the use of other legumes to supplement or to replace groundnut as a strategy to improve yields and dietary proteins as well as soil fertility of intercropped maize systems in SC (Wendt and Atemkeng, 2004). Cowpea, soybean, and other legumes have been considered for this purpose (Hauser and Nolte, 2002; Hauser et al., 2002). Soybean (*G. max*) and cowpea (*V. unguiculata*) are important grain legumes whose integration into cropping systems of SC will be immensely beneficial for different reasons. The high protein content and quality of the grains would substantially improve nutrition of the rural poor. They are nitrogen-fixing legumes and therefore will fix substantial amounts of N_2, provide soil cover, increase soil organic matter, provide pest and disease break, suppress weeds, and can be used for animal fodder. However, the process of N_2 fixation and the legumes growth are largely restricted by P deficiency (Sanginga et al., 2002; Sanginga et al., 2001; Sanginga et al., 2000; Giller and Wilson, 1991). Furthermore, little attention has been given to the use of P-efficient genotypes, either in combination with poorly soluble P fertilizers or in soils with large P reserve but little available P, and/or plants that tolerate the Al^{3+} highly available in these soils.

For over 50 years, scientists have acknowledged the deleterious effect of P deficiencies on crop production in tropical soils. Efforts have been made to (a) assess the extent of P deficiencies in soils (Sanchez et al., 1976), (b) estimate the P requirements of major food crops, including trees and herbaceous leguminous crops (Buerkert et al., 2002; Buresh et al., 1997), and (c) evaluate the agronomic potential of various P fertilizer sources including phosphate rock from local sources (Zapata et al., 2002; Vanlauwe et al., 2000a, b). The use of legumes to supply N has been promoted to overcome soil fertility constraints. But without adequate P supply this strategy can have only limited success (Vance et al., 2001; McLaughlin al., 1990). Improving P supply to legumes would, therefore, lead to better N_2 fixation, and hence improved N nutrition in cropping systems, better yields and reduced erosion. Nevertheless, there has been little work to examine the physiological mechanisms for P efficiency observed among the promiscuous soybean and cowpea genotypes grown in the acid soils of sub-Saharan Africa.

The general forms in which P is taken up by root from soil solution to the cell are the ions $H_2PO_4^-$ and HPO_4^{2-}. Since the concentration of P in the rhizosphere is 200 to 1000 times lower than in the cell (Raghothama, 1999) and Pi ions are negatively charged, Pi needs to cross the cell wall against an electrochemical gradient (Schachtman, 1998). The entry of Pi into the cell is an energized transport that involves membrane co-transport of H^+ extrusion into the apoplast via H^+-ATPase ensuring the transfer of P_i and H^+ ions from outward to inward of the cell membrane.

The main interrelated processes governing the acquisition of soil and fertilizer P by crops are dissolution/precipitation and desorption/sorption, transport, soil/contact and biological P transformations (Horst et al., 2001). The plant may interfere with these processes either directly or indirectly through the modification of soil properties, thus enhancing P availability and uptake.

Generally, two groups of plant properties contribute to a more efficient P acquisition and use of soil and fertilizer P: those which allow efficient acquisition of P from the soil solution and are so called “rhizosphere processes” and those which make efficient use of P acquired through “above ground” processes. Both groups of plant mechanisms determine the overall P efficiency of a genotype, defined as the ability of a genotype to acquire P from the soil (uptake efficiency) and/or utilise it for the production of total plant biomass or yield, depending on the end product of interest for the farmer (utilisation efficiency, Blair, 1993). The plant properties contributing to efficient uptake and utilisation are presented in Fig. 1.

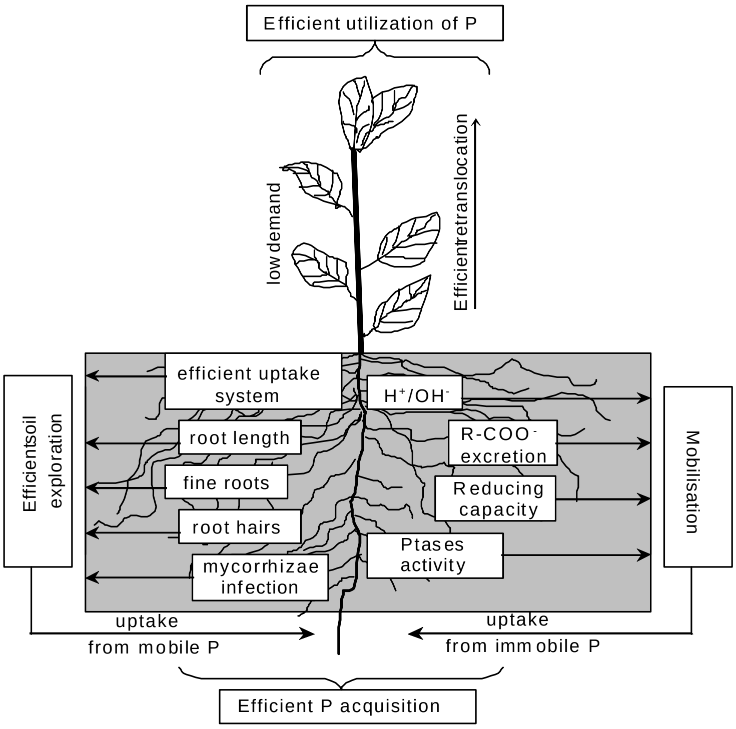


Fig.1. Plants properties contributing to efficient acquisition and use of P from soil or fertilizer-P. (Source: *Horst and Kamh, 2004).*

While differences in the ability to acquire and use P are known both at the species and genotype levels in grain legumes (Randall, 1995), there has been limited success in improving P efficiency through selection. This has been attributed partly to the lack of appropriate selection criteria (Blair, 1993) reflecting in turn the lack of understanding of key mechanisms, particularly those that have the potential for manipulation. For example, selection on the basis of root-hair length appeared to give promising improvement in P uptake efficiency of white clover, but it was later shown that the correlation broke-down in the presence of mycorrhizal fungi, which normally infect this species under field conditions (Caradus, 1981). This led to the conclusion that selection of white clover for improved performance under low-P conditions were best done in the field (Caradus, 1995). From the perspective of optimising exploration of soils by plant roots, recent progress in describing root architecture (Araujo et al., 1998; Neilsen et al., 1998) has shown promise for the identification of these attributes, which are important for P acquisition efficiency in beans. From the point of view of solubilising soil P, recent discoveries have demonstrated particularly in white lupin the role of organic acid anions, in particular citrate, as an important mechanism determining P acquisition by altering the chemistry of the rhizosphere thus solubilising P in the root rhizosphere (Gardner et al., 1983a). Similarly, in rice plants, Kirk et al. (1999) and Kirk (2002), observed the ability of the roots solubilising soil-P by the release of citrate as an important mechanism for P acquisition. The development of mechanism-based germplasm-screening procedures has been advocated for breeding of more P-efficient rice genotypes.

Other relevant studies have described the exudation of piscidic and malonic acid by pigeon pea roots which are reported to facilitate the release of P from iron-bound soil P (Fe-P), normally considered unavailable to plants (Ae et al., 1991 and 1990). In pot studies, Arihara et al. (1991) demonstrated increased P availability to subsequent maize grown in rotation with pigeon pea. This was indicative of pigeon pea’s potential to introduce otherwise unavailable P into the nutrient cycling of cropping systems, and this was related to its ability to exude specific root exudates. With white lupin, the exudation of citric acid (Gardner et al., 1983; 1982) has been shown to benefit P nutrition of the plant as well as other crops subsequently grown in rotation. Using cover-crop legumes and grain legumes including soybean and cowpea genotypes, Horst et al. (2001) and Kamh et al. (1999) found an increase in P availability to the subsequent rotational maize crop grown on soil of the derived savannah of Nigeria. Such increases in P were attributed to a high exudation of organic acids by the different legume species. However, little effort has been made to study the effect of P deficiency on the exudation of organic acid anions in cowpea and soybean grown on tropical soils with emphasis on genotypic differences (Kamh et al., 1999). Although the potential of root exudates to modify nutrient bioavailability has often been discussed, more quantitative evidence is required to evaluate the significance of root exudates on nutrient particularly P uptake. This constitutes one of the major objectives of this thesis.

Root mediated changes in pH, production of phytosiderophores and phytochelates and root-mediated microbiological changes also influence nutrient availability in the rhizosphere. For example, *Banskia spp*. has been shown to modify the soil-root environment considerably by lowering the pH and through production of organic acids and phosphatase (Grierson, 1992; Grierson and Attiwill, 1989).

The formation of proteoid roots and infection by AMF appear to be alternative strategies by plants to enhance uptake of water and nutrient (Smith and Read, 1987). The majority of plant adapted to low P soils form associations with AMF (Harrison, 1999). Mycorrhizas are thought to benefit the host plant through increase of P uptake, largely achieved by extensive proliferation of hyphae to sites well beyond those that would not be contacted by non-mycorrhizal roots (Jansa et al., 2002).

Other plant species or genotypes possess long root hairs or have rapid establishment of large root systems, which maximize uptake of P from the soil. In common bean and soybean genotypes, Yan et al. (2004) and Wang et al. (2004) observed greater root hair density and longer root hairs in P-efficient genotypes and that these root traits were responsible for their higher P uptake in comparison to the P-inefficient genotypes.

In addition to root modification, many other plants are also highly efficient in retaining P within the plant, partly through internal recycling and retention of leaves for up to several seasons. P redistribution from senescing leaves is shown to be greater in species that have developed in soil that have low P availability (Yang et al., 1995a). If the genotypes or species are to be considered for soil fertility improvement, higher accumulations of P in leaves will increase the potential of the species for their N turnover.

Besides to the problem of P deficiency, many soils in the tropic are acidic with high Al^{3+} supply. Excess of Al and P deficiency interferes and limit nutrient and water availability of the plants. Over the last decades, the symptoms of Al toxicity, the effect of Al on plant growth, and the search for efficient techniques to separate between Al sensitive and resistant genotypes have been receiving considerable attention (Kochian et al., 2002; Matsumoto, 2000; Taylor et al., 2000; Horst et al.,1997; Marschner, 1995; Kinraide et al., 1994; Horst et al.,1992; Lindberg et al.,1991; Horst and Klotz, 1990). But still the mechanisms of Al resistance are not well understood (Eticha et al., 2005; Wang et al., 2004). However, many authors have been suggesting the role of organic acid anions, the structural configuration of the plasma membrane, and the internal cell accumulation as potential mechanisms of Al resistance in plant (Ryan and Angus, 2003; Ma and Furukawa, 2003; Yang et al., 2001; Yang et al., 2000; Kollmeier et al., 2000; Ma et al., 1998; Horst et al.,1997; Marschner, 1995).

From the perspective of ameliorating or alleviating the problem of Al toxicity, other strategies have been the use of P fertilizers, as it is assumed that in the presence of P, Al forms Al-Pi complexes that precipitate in the rhizosphere. But many reports have observed considerable uptake of Al in presence of P application (Tang et al., 2004; Nian et al., 2003; Gaume et al., 2001;Tang et al., 1997; Marschner, 1995). However, little is known about the interaction between excess of Al and P deficiency, as well as the plant mechanisms involved, particularly in cowpea.

Recent studies on the Al and P interaction in soybean genotypes suggest the exudation of citric acid in presence of an excess of Al, instead of P stress as mechanism of Al detoxification (Nian et al., 2003).

This thesis addresses possible approaches to overcome the problem of P deficiency and Al toxicity in soils of SC. It is organised in four chapters. Chapters 1 and 2 examine the extent of variation for P efficiency in soybean and cowpea, particularly their ability to acquire P from sparingly soluble soil and fertilizer sources. The benefit of the P-efficient grain legumes to subsequent maize and their related P-mechanisms are investigated in Chapter 3. Chapter 4 assesses genotypic variation in cowpea in Al resistance, and studies the effect of the interaction between Al stress and P deficiency on the P nutrition and exudation of organic acid anions.

In the general discussion and conclusions, the results from the four chapters are synthesized. The beneficial role of P-efficient genotypes to subsequent maize and their related mechanisms are discussed to assist in plant selection and improvement of their impact on the overall cropping system on acid soils of SC.

Area of the study: the humid forest benchmark (HFB) area in SC

The research was conducted in the HFB area in SC. The HFB area in SC is subdivided into three blocks representing increasing levels of deforestation and land use intensity established by ASB, IRAD, and IITA (Nolte et al., 2001) and known as Yaoundé block with high land use intensity, Mbalmayo block with medium land-use intensification, and Ebolowa block with low land use intensification (Fig. 2).

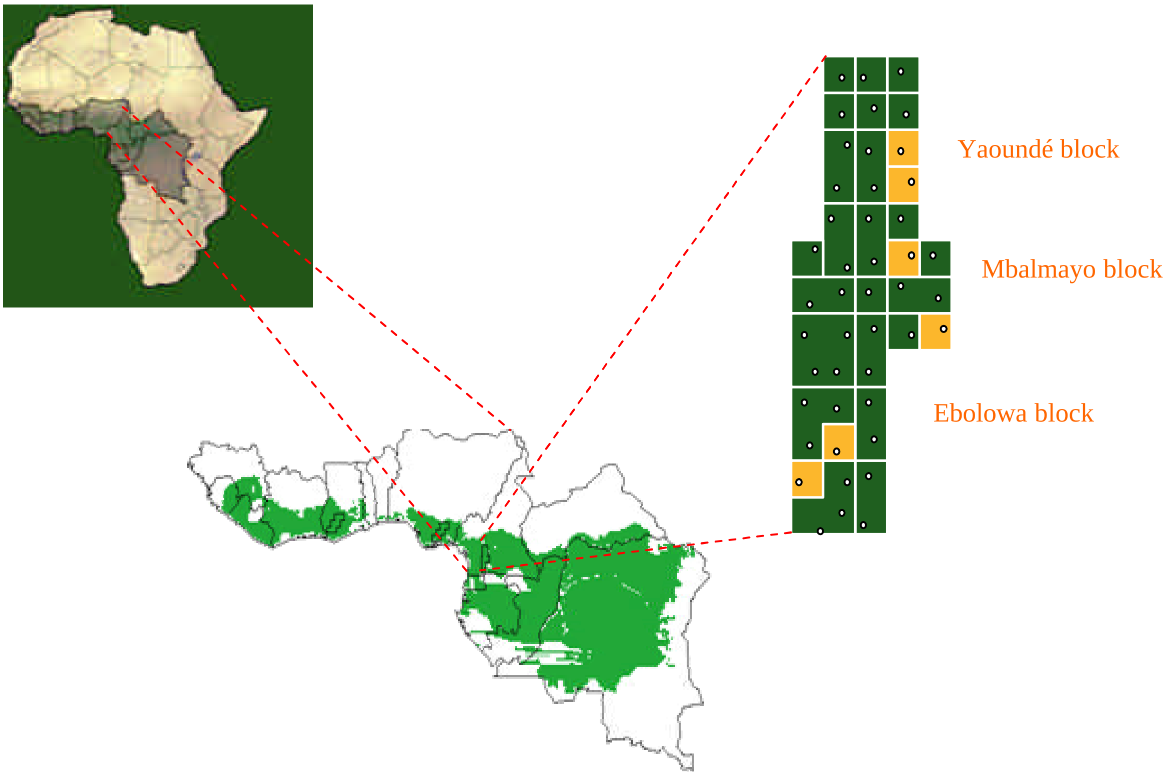


Fig.2. The humid forest benchmark (HFB) area in Southern Cameroon

Objectives, hypotheses and research approaches

Objective

The general objective of this study was to select grain legume genotypes to improve P availability and N_2 fixation in cropping system of SC.

The specific objectives were:

To determine the intra-specific variation in P acquisition and utilisation by cowpea and soybean genotypes (Chapter 1 and 2).

To investigate the benefit of P-efficient genotypes to a subsequent maize crop and the mechanisms of P efficiency observed under field conditions (Chapter 3).

To assess the genetic variation in Al resistance of cowpea and to study the interaction of Al toxicity and P deficiency on Al resistance mechanisms (Chapter 4).

Hypotheses

Selected genotypes have a superior ability to acquire P from sparingly sources that are not generally readily available to plants.

Such P-efficient genotypes make better use of sparingly soluble P fertilizer, improve the P cycling, and thus enhance the P nutrition and productivity of the cropping system, thus alleviating one of the major constraints to sustainable crop production in SC.

A better understanding of the physiological mechanisms of P efficiency will assist and provide guidelines for selecting genotypes and crops with superior P efficiency.

Soil factors such as aluminium toxicity affect the P efficiency and result in reduction of plant growth and P nutrition on acid soils.

Research approach

A proper methodology was needed to test our above-listed hypotheses.

The first approach was to identify soils with low available P (< 3 mg kg$^{-1,}$ Bray 1), and the second approach was to identify a critical P fertilizer rate. Therefore, a general survey was carried out in the humid forest area (Fig.3) and two soils were selected for a pot experiment to determine critical available P values.

Soil survey

The survey was undertaken in the HFB area in SC lying between 2° and 4° N, and 10 – 16^0 E. Based on previously work performed by Murtha and Tchienkoua (1991), five groups of soils were distinguished by their surface horizons and texture. The soils are represented in Fig. 3, with the main towns in their respective localities. Soils are classified as Typic Rodiudult, Epiaquic Kandiudult, and Typic Kandiudult for the group of Akonolinga, Ebolowa and Mbalmayo, respectively, whereas Sangmelima and Yaoundé soils are classified as Rhodic Kandiudult (USDA, 1987).

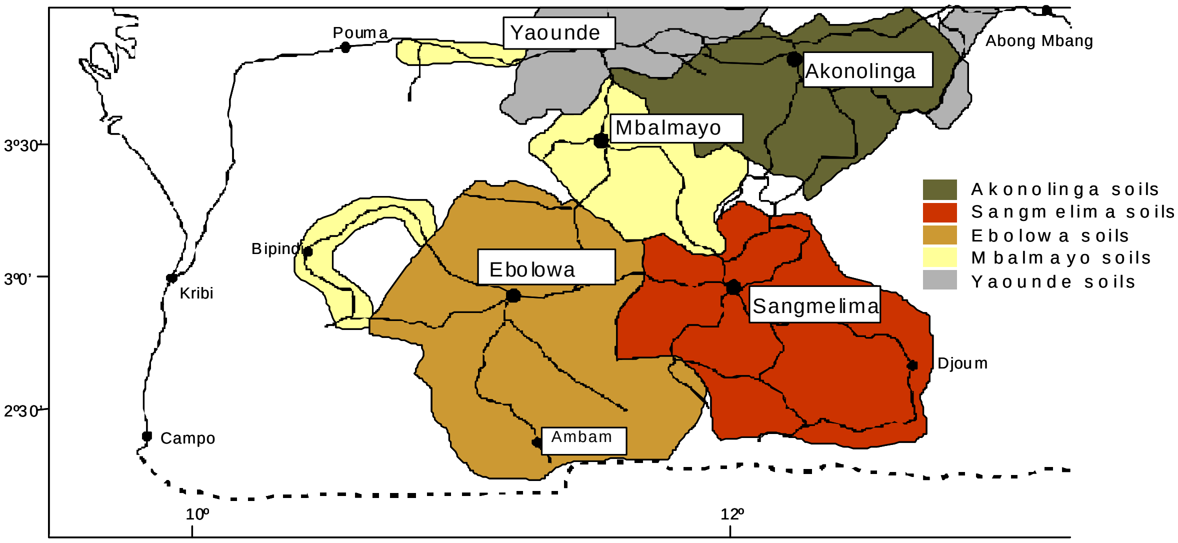


Fig.3. Reconnaissance Soil Survey in the Humid Forest benchmark of Southern Cameroon.
Note: Reference, G. G. Murtha (CSIRO) and Tchienkoua (IRAD). Field work from 19 – 25 April 1991, base map: road map of Cameroon, Scale 1:1,500 000, and soil sketch map based on field observations

Five villages in each soil class were selected and the soils were sampled at 0 – 25 cm depth and analysed for their different physical and chemical properties. Only the most important results for this study are reported (Table 1). The soil pH was measured following 1 h equilibration time in a soil: water ratio of 1:2.5. Total N was measured by the Kjeldhal digestion procedure (Bremner and Tabatabai, 1972). Exchangeable bases calcium (Ca), magnesium (Mg) and potassium (K) were measured by atomic absorption spectrophotometer (AAS). Exchangeable aluminium (Al^{3+}) was extracted in 1 *M* KCl and also measured by AAS.

Table1. Chemical properties of soils in different soil classes of southern Cameroon (mean ± standard error (s.e), n = 80).

	Akonolinga		Ebolowa		Mbalmayo		Sangmelima		Yaoundé	
Soil properties	Mean	s.e	Mean	s.e	Mean	s.e	Mean	s.e	Mean	s.e
pH (H_2O)	4.7	0.0	3.9	0.2	4.3	0.0	4.0	0.1	4.0	0.1
N (g.kg^{-1})	1.4	0.4	1.6	0.8	1.75	0.9	1.5	0.6	1.6	0.5
C (g.kg^{-1})	22.1	7.7	24	13.6	18.8	9.9	18.7	9.2	15.6	11.3
C/N	16.1	0.8	13.8	0.8	10.7	0.0	12.1	1.5	11.0	1.3
Al^{3+}cmol(+) kg^{-1})	0.9	0.3	1.8	0.7	1.9	1.2	1.5	0.2	1.1	0.5
P Bray (mg kg^{-1})	2.8	1.5	2.7	2.8	4.9	2.0	2.4	1.7	2.4	1.1

The results are presented in Table 1. All the soils were low in soil pH (H_2O) ranging from 3.9 to 4.7 in the Ebolowa and Akonolinga soils, respectively. Similarly, exchangeable Al^{3+} ranged from 0.9 to 1.9 cmol (+) kg^{-1} with the lowest and highest values in Akonolinga and Mbalmayo soils, respectively. Also the plant-available P (Bray 1) was generally low in all classes, but soils from Mbalmayo had the highest value (4.9).

Based on these results, two soils from the Yaoundé and Mbalmayo class were selected for the field trials. Prior to the set up of the field experiments, the two soils were evaluated regarding to their optimum P application rate.

Critical soil-P values

This study was carried out to assess the critical values of plant-available P in the soils to be adjusted for the field experiment. On this basis, the level of P applied for the field experiments was selected. Soils were collected at 0 – 10 cm depth on both sites; air-dried and sieved through 4 mm mesh. Five kilograms (kg) of soils were used in 5 L pots. The experiment was carried out in a greenhouse at the International Institute of Tropical Agriculture, Cameroon. The design was a RCB with 4 replications. Five levels of P were applied: 0, 7.5, 15, 30 and 45 kg P ha^{-1}. Four seeds of maize CMS8704 from the breeding program of IRAD, Cameroon, were sown. Plants were harvested 5 weeks after planting (WAP) and assessed for dry matter production, P, and N accumulation.

Fig. 4 depicts the growth of the maize plants in the two soils as influenced by the levels of P application. Growth of the maize increased with increased P application from 0 to 30 kg

P ha^{-1} on both soils. With 45 kg P ha^{-1}, the growth remained constant indicating that the P required for optimal DM growth ranged between 30 and 40 kg P ha^{-1} applied.

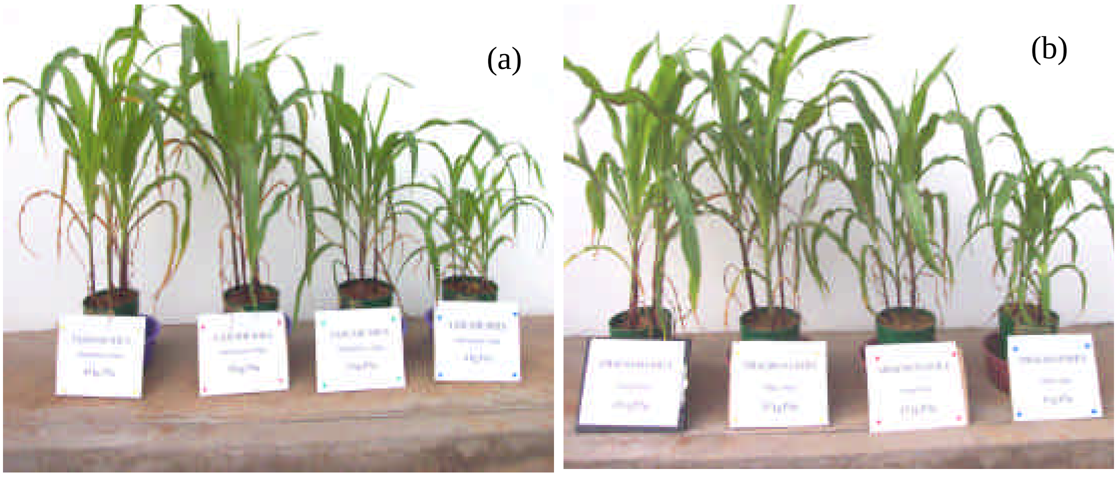


Fig.4. Response of maize to different levels (0, 15, 30, 45 kg ha^{-1}) of P application on two soils of southern Cameroon: Rhodic Kandiudult (a) and Typic Kandiudult (b).

Therefore, on the basis of the levels of P applied and the percentage of shoot DM increase, the critical soil-P values of the two soils to use for the field experiment were calculated (Fig. 5). For the Rhodic Kandiudult (RK) soil type (Yaoundé soils), the minimum amount of plant-available P required for 90% shoot DM production was 22.6 mg P kg^{-1}. With respects to the Typic Kandiudult (TK) soil of Mbalmayo, the plant needed approximately 10.6 mg P kg^{-1} for 95% of shoot dry matter production. Hence, 30 kg of P ha^{-1} representing 26.5 mg P kg^{-1} were considered sufficient for an adequate growth of the cowpea and soybean genotypes.

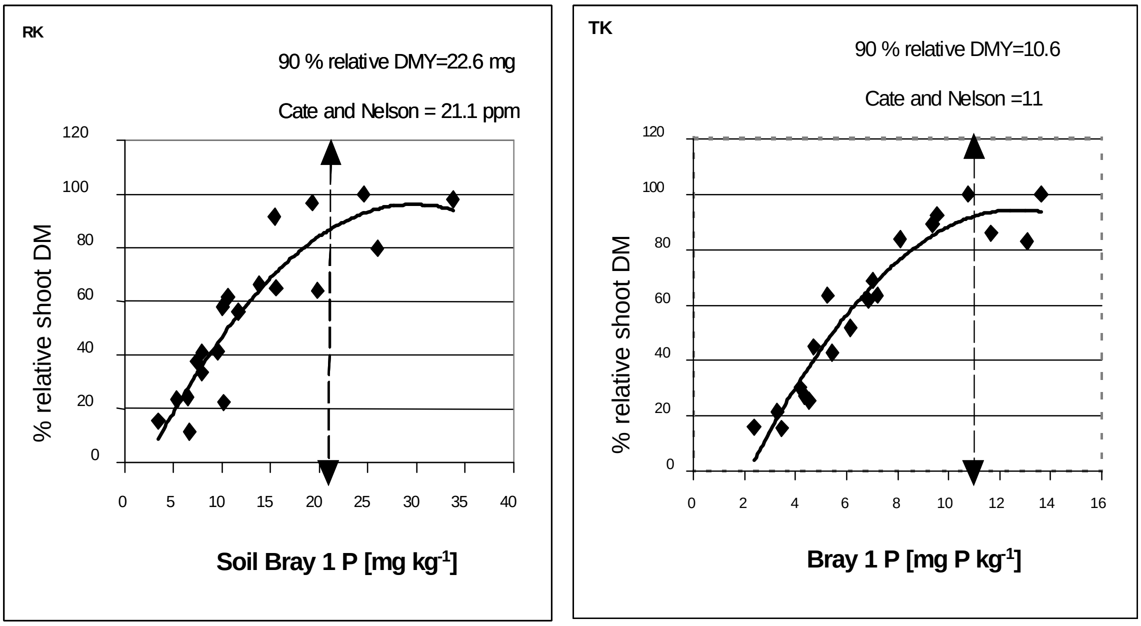


Fig.5. Critical P-values of the soils selected from the field screening experiment

Chapter 1.

Genotypic Variation in Soybean for P Uptake and Use Efficiency, and N_2 Fixation on Two Low-Available P soils of Southern Cameroon

Abstract

Efficient uptake and use of phosphorus (P) improves N_2 fixation of soybean on P-deficient soils. If a P-efficient soybean genotype is grown in rotation with a non-N-fixing crop, it can be expected that an increased P and N turnover will improve the yield of the rotational crop. This study aimed at assessing genotypic differences in soybean for P uptake from low-available P soils and the efficient use of the P applied to these soils. Out of the twelve genotypes evaluated in the field experiment, four were selected based on their P-uptake and use-efficiency potential to further investigates the mechanisms that may be responsible for the observed differences under greenhouse conditions. The soybean genotypes were grown in 2001 and 2002 in a split block design with two P sources: Togolese phosphate rock (PR) and Triple super phosphate (TSP) on Typic Kandiudult (TK) and Rhodic Kandiudult (RK) soils of SC. Shoot DM, grain yield, N_2 fixation, and P content varied with site and genotypes. On TK soil, shoot DM was significantly ($P < 0.0001$) increased by 31 and 14% with application of TSP and PR, respectively. Shoot DM also significantly ($P < 0.0001$) increased by 31 (TSP) and 17% (PR) on RK soil. However, P application significantly increased grain yield only on TK soil. Shoot P accumulation was significantly increased by 41.1 and 38.3% on TK and RK soil, respectively with TSP application. N_2 fixation ranged from 19.9 to 55 kg N ha^{-1} on TK and from 16 to 38 kg N ha^{-1} on RK soil. Based on cross classification of the genotypes in terms of P acquisition efficiency and response, only TGm 1511 and TGm 1566 were considered efficient responder, which was reflected in their positive N balance. Probable P uptake mechanism in TGm 1511 was the association of its roots with arbuscular mycorrhizal fungi, while a compensatory mechanism between shoot and root growth was observed for TGm 1566.

Key words: Arbuscular mycorrhizal fungi (AMF) – N_2 fixation – P-uptake efficiency – soybean – southern Cameroon.

Introduction

Soybean (*Glycine max* [L] Merr) is a relatively new crop for smallholder farming-communities in most African countries. In recent times its cultivation has gained popularity as a consequence of the increasing need for food and fodder (Sanginga et al., 2002). Soybean is being considered for soil-fertility improvement in cereal based cropping systems (Carsky et al., 1997), the grain contains approximately 35% dietary protein (Maesen and Somaatmadja, 1992), and it has relatively few natural pests threat. In the humid forest benchmark (HFB) area in SC, soybean is also being considered to improve the available cropping system as strategy for soil fertility restoration as well as to provide diet proteins to the small scale farmers (Wendt and Atemkeng, 2004). However, soils are generally acidic, high in exchangeable aluminium (Al), low in exchangeable cations (Ca, Mg, K) and available P (Menzies and Gillman, 1997). Growth and N_2-fixation of soybean are largely hampered by phosphorus deficiency and soil acidity (Wang et al., 2004; Guti´errez-Boem and Thomas, 1999). In initial field screening of soybean genotypes conducted in SC, Wendt and Atemkeng (2004) found significant increase in soybean yield with increase of soil-available P. Similarly Fanwoua (2001), conducting a number of field screenings of soybean genotypes in SC, observed a reduction in grain yield of 22 kg ha^{-1} while the soil available P was declining per unit.

There is increasing interest in the inclusion of grain legumes in improved cropping system of SC (Wendt and Atemkeng, 2004). However, the successful integration of such legumes will depend on the mitigation of the soil constraints such as Al toxicity and P deficiency. With respects to P, the use of P fertilizer application is generally suggested to correct the P deficiency, but this is not always possible for the mostly resource-poor farmers in SC due to high cost (Gockowski et al., 2004). Furthermore, fertilizer P is generally quickly fixed into forms unavailable to plants by Fe and Al oxide found in these soils (Sample et al., 1980). Under such circumstances, the integration of plant species or genotypes that can make most efficient use of the P supplied by the soil and/or fertiliser P applied represent a key element of sustainable cropping systems (Horst et al., 2001; Ae et al., 1990; Lynch, 1998).

Wide differences in P acquisition and use have been documented among and within many legumes including soybean (Alves et al., 2003; Sanginga et al., 2000; Vadez et al., 1999).

Up to now, there are no studies on the selection of soybean genotypes that can grow well and fix high amounts of N_2 at low soil available-P conditions or make efficient use of the P applied in SC.

Plants that are more efficient at acquiring soil P and use of P fertilizer applied may explore a larger volume of soil because they have a larger root surface area, longer and finer roots, more and longer root hairs (Gahoonia and Nielsen, 2004; Krasilnikoff et al., 2003), or infected of their roots by arbuscular mycorrhiza fungi (Smith and Read, 1997). Alternatively, they may solubilize P by modifying the chemistry of the soil adjacent to the roots (the rhizosphere) through the lowering of pH (Gahoonia et al., 1992), or they exude large amount organic acid anions that solubilize inorganic P by binding to or solubilize aluminium and iron-oxides (Raghothama, 1999; Marschner, 1991). Other plants secrete high amount of root ecto-enzymes phosphatase making P bound to organic matter available (Li et al., 1997; Marschner, 1995).

The present study was conducted: (i) to assess the genotypic differences in growth responses to P uptake and use among soybean genotypes and (ii) to assess some of the root traits that might be responsible for differences observed in P acquisition.

Materials and Methods

Experiment 1. Field Experiment

The soybean genotypes

Twelve soybean genotypes were used for the field experiment. The seeds were provided by the Legume Breeding Program of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Morphological and growth characteristics of the genotypes are presented in Table 1.1.

Table 1.1. Characteristics of the soybean genotypes used for the field experiments in 2001 and 2002.

Genotype	Mature pod colour	Lodging score	Plant Height (cm)	Days to 50% maturity	Origin geographic Code
TGX-1456-2E	Brown			86	IITA
TGm 1511	Tan	Moderate	49	86	USA
TGm 1196	Brown	Moderate	65	89	Puerto Rico
TGm 1293	Brown	Severe	70	89	Not available
TGm 1420	Brown	Slight	54	89	USA
TGm 1419	Tan	Severe	49	89	USA
TGm 1039	Brown	Slight	67	92	Taiwan
TGm1251	Brown		51	92	Not available
TGm 1566	Brown	Moderate	85	94	USA
TGm 1576	Brown	Moderate	85	94	USA
TGm 0944	Brown	Severe	72	96	Nigeria
TGm 1360	Brown	Moderate	62	96	USA
TGm 1540	Brown	Moderate	77	104	USA

Sites description

The field trials were conducted on two P-deficient soils on farmer fields at Abang and at the Institut de la Recherche Agricole pour le Développement (IRAD) research experimental station at Minkoameyos in 2001 and 2002. Abang (3° 24´N, 11° 47´E) and Minkoameyos (3° 51´N, 11° 25´E) are two localities situated in the HFB in SC located 10 km south of Mbalmayo and 7 km north of Yaoundé, respectively. The soils are Typic Kandiudult (TK) at Abang and Rhodic Kandiudult (RK) at Minkoameyos (Holland et al., 1992). The two soil types were selected on the basis of their low P availability (< 3 µg.g^{-1}) and low soil pH < 5.50. The long-term annual rainfall is 1643 mm at the Abang and 1513 mm at Minkoameyos sites, respectively, with a bimodal distribution. The selected physical and chemical properties of the topsoil (0 – 10 cm profile depth) are given in Table 1.2.

Table 1.2. Physico-chemical characteristics of the topsoil (0 – 10 cm depth) of a Typic and a Rhodic Kandiudult soil (southern Cameroon) (Means ± standard errors).

Soil type	Sand	Silt	Clay	pH	C	N	Ca	Mg	K	Al
	[%]			[H_2O]	[g kg^{-1}]		[cmol(+) kg^{-1}]			
Rhodic Kandiudult	40.8	10.2	48.9	4.5	22.5	2.1	1.1	0.33	0.08	0.63
	(5.3)	(1.8)	(7.1)	(0.1)	(1.9)	(0.1)	(0.1)	(0.01)	(0.01)	(0.13)
Typic Kandiudult	50.6	9.7	39.7	5.4	16.0	1.6	1.35	0.64	0.06	0.12
	(1.58)	(0.85)	(1.2)	(0.1)	(1.1)	(0.05)	(0.17)	(0.06)	(0.04)	(0.02)

	P sorption	FeOx	P Bray 1	NaHCO3-Pi	NaOH-Pi	HCl-P	Residual P
	[%]		[mg kg^{-1}]				
Rhodic Kandiudult	88.3	0.69	2.50	1.7	25.4	1	154.1
	(3.2)	(0.04)	(0.95)	(0.29)	(3.2)	(0.5)	(2.3)
Typic Kandiudult	77.5	2.3	5.0	3.2	29.2	0.86	190.9
	(0.3)	(0.07)	(1.20)	(0.5)	(1.2)	(0.38)	(12)

Treatments, experimental design and sowing

Experiments were laid out in a split-block design with four replications. P fertilizers were applied on the main plots at 0 kg P ha^{-1}, 30 kg P ha^{-1} Triple super phosphate (TSP) and 90 kg P ha^{-1} phosphate rock (PR), respectively. Sub plots, measuring 4 by 4 m, comprised the cowpea genotypes. No N fertilizer or bradyrhizobia inoculation was applied. Two seeds of soybean were sown per hill at 75 cm between row and 5 cm within the row and thinned to one plant, one week after emergence. Fields were established on 24 September 2001 and 15 September 2002. Fields were sprayed with thioral at 2 and 4 WAP and hand weeded at 2, 4 and 6 WAP.

Sampling

Plants were sampled at mid-pod fill, eight WAP, for shoot growth, nodule formation and N_2 fixation. Six plants were chosen in the middle rows and their shoots were cut 5 cm above ground level, then the fresh weight was recorded. A subsample of 500 g from each experimental unit was retained, and transferred to the laboratory and oven-dried at 70° C for 72 hours until constant weight. Fresh nodules were carefully removed from the root system, counted, and oven-dried at 72° C for 72 hours and their DM was determined.

At grain maturity stages (12 WAP), grain, shoot, and litter fresh weight were harvested, and dried for the dry matter measurements and also analyzed for N and P concentration.

N_2 fixation and N balance

The xylem ureide assay method of Peoples et al. (1989) was used to assess the variation in N_2 fixation among the soybean genotypes under different P sources and environment. Stem of six plants were finely ground and 0.5 g sample was used in hot water (80° C) to extract the exudates. The relative ureide-N abundance was calculated from molar concentration of ureides and nitrates with the assumption of 4 N atoms per ureide molecule following this equation:

$$\text{RUA} = \frac{4 \text{ x n (mol ureide)}}{[(4 \text{ x n (mol nitrate)} + \text{(n mol ureide)}]} \text{ x100}$$

Where n mol ureide/nitrate is the concentration of ureide/nitrate in the stem extracts expressed in n mol.

Total N and P in shoot

A sub-sample of 0.2 g was digested in concentrated H_2S0_4 at 500° C for 8 hours according to the procedure of Novozamsky et al. (1983). N and P concentrations in solution were determined following the protocols of Powers et al. (1981) and Motomizu et al. (1983), respectively.

The N balance was calculated following Peoples and Craswell (1992) formula

N balance= $Nf - Ns$, where N_f and Ns are the amount of N_2 from atmospheric fixation and N content in shoot, respectively.

Experiment 2. Greenhouse experiment

This experiment aimed at investigating some root traits that might be responsible for the variability in the P acquisition among the soybean genotypes used. A pot experiment was conducted in a greenhouse at IITA in Nkolbisson. The soil for the pot experiment was sampled at 0 – 10 cm profile depth at the Minkoameyos site, classified as Rhodic Kandiudult (RK). The soil was air-dried and sieved through 4 mm mesh and 2.5 kg portion were used in 2.5 L pot. Seeds of soybean genotypes were pre-germinated for three days and four seeds were sown in each pot. One week after planting, the seedlings were thinned to two plants per pot. The experiment was laid out in a factorial randomised complete block design where two factors were used. Factor 1 was P application with three P levels, 0 kg P ha^{-1} (0P), 90 kg P ha^{-1} as phosphate rock (PR) and 30 kg P ha^{-1} as triple super phosphate (TSP). Factor 2 comprised the four soybean genotypes selected from experiment one: two that had responded significantly to P application in 2001 and 2002, and had the highest grain yield without P, and two that had not responded and were amongst the lowest yielding at 0P. Plants were daily watered with deionised water for 8 weeks before being harvested.

Sampling

Eight WAP, soybean shoots and roots were cut at 5 cm above ground level for shoot DM yield. Nodule formation, arbuscular mycorrhizal fungi (AMF) root infection and root length were determined. P and N concentrations were measured in shoots and roots as described above.

AMF observation

A sub-sample of 1 g of fresh roots was used for the determination of AMF root colonization. Roots were washed until free of soil, preserved in 50% alcohol and stored at 4° C prior to AMF observation. Roots were later thawed and cut into 1 cm length pieces and cleared in KOH (10%) solution. The cut roots were stained with acid fuchsin in lacto-glycerine at room temperature according to Phillips and Hayman (1970) and Merryweather and Fitter (1991). Roots stained were then examined for colonization by AMF using the gridline intersects method of Giovannetti and Mosse (1980) under a stereomicroscope at 40x magnification.

Root length determination

The entire root system of the two soybean plants for each pot was harvested and washed free from soil by washing with tap water. Sub-samples were scanned using ScanJet Iicx software. Root images obtained were then converted into in Photo editor software images and the black border lines were removed from the image edges. Dt-Scan software Delta-T Devices, Cambridge, England (Webb et al., 1993) was used to measure total root length and root diameter. Scanned samples were subsequently oven-dried at 70 º C for 72 hours for dry matter determination.

Statistical analysis

Statistical analyses of the data were conducted using SAS version 8.2 (Statistic Analysis Systems, 2001). The mixed model procedure “PROC MIXED” was used for analyses of variance (ANOVA) of the field experiment and the general linear model “PROC GLM” was used to run the ANOVA of the greenhouse experiment. When the F test denoted a significant effect, the least significant difference (LSD) test was used to compare the means.

Results

Nodulation

Nodule dry mass of the soybean genotypes was significantly increased by TSP application on both soils. Average increases were 100% for TK and 178% for the RK soil compared to the 0P treatment (Table 1.3). Genotypes TGm 1511, TGm 1566, TGm 1540, TGm 1293, and TGm 0944 gave higher nodule dry mass in response to TSP application on the TK soil. With respect to the RK soil, all genotypes except TGm 1511 also had a significantly higher nodulation due to TSP application compared to 0P treatment (Table 1.3).

Application of PR resulted in lower nodule dry mass increases than TSP application compared to the 0P treatment on both soils. Mean dry-mass increases were 12% for TK and 55% for the RK soil, respectively. TGm 1540 significantly increased in its nodule dry mass in response to PR application on TK soil. On RK soil, TGm 1540 also Tgm 0944 demonstrated significantly ($P < 0.005$) higher nodulation in response to PR application.

The genotypic variation for nodule dry mass across soybean genotypes ranged from 0 (at 0 kg P ha^{-1}) to 102 mg plant^{-1} on a TK soil and from 0 to 56 mg plant^{-1} on a RK soil (Table 1.3). The soybean genotypes TGm 0944, TGm 1566, TGm 1511 and TGm 1293 were judged to be tolerant to P deficiency on the basis of their nodule dry-mass production. They resulted in a significant and higher nodule mass than other genotypes. The F test denoted significant effect of the soil in the nodule dry-mass production ($P < 0.0001$) and the soil type x genotype interaction was significant. For the 12 lines used, nodule dry mass was significantly related to the amount of nitrogen fixed on TK ($r = 0.50$, $P < 0.0001$) but not on RK soil.

Table 1.3. Nodule dry matter of soybean genotypes grown on a Typic and a Rhodic Kandiudult soil (Southern Cameroon) 8 WAP as affected by P fertilizer application (0 (0P), phosphate rock (PR), and triple super phosphate (TSP)). Means of 2001 and 2002

	Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP
Genotype	[mg.plant^{-1}]					
TGm 1196	0.0	0.7	0.1	0.1	3.1	4.3
TGm 1360	0.0	0.0	9.8	0.0	0.0	0.0
Tgm 1039	1.1	0.0	9.7	1.5	2.2	1.4
TGx 1465-2E	1.6	12.9	8.3	0.9	0.0	9.7
TGm 1251	2.7	0.5	16.4	3.6	1.5	20.8
TGm 1420	6.7	11.4	27.0	3.7	12.7	13.0
TGm 1419	9.7	5.0	25.7	7.2	16.5	49.9
TGm 1566	32.6	43.0	82.0	16.2	28.1	42.2
TGm 1511	40.2	61.6	106.3	17.2	24.1	35.2
TGm 1540	62.3	98.5	137.0	33.4	84.6	148.2
TGm 1293	69.3	72.0	105.2	50.0	31.0	112.9
TGm 0944	102.6	63.5	129.6	56.3	90.3	89.8
Mean	27.4 ^{b}	30.8 ^{b}	54.8 ^{a}	15.8^{b}	24.5^{b}	44.0^{a}
LSD (5%)						
Location (L)	10.70					
P application (P)	29.25			15.14		
Genotypes (C)	46.10			44.30		

Means following by the same letter are not significantly different at $P < 0.05$

Shoot dry matter

There were highly significant differences ($P < 0.0001$) in soybean shoot dry weights between TK and RK soil (Table 1. 4). TSP application highly and significantly increased shoot dry matter of soybean genotypes on both soils ($P < 0.0001$). All the soybean genotypes significantly increased their shoot dry weight in response to TSP application on RK soil, except TGm 1196. Similarly, shoot dry matter was notably enhanced by TSP for all but the genotypes TGm 1196, TGx 1456-2E, TGm 1251, and Tgm 1420 on TK soil. Data ranged from –5.6 for TGm 1420 to 99.9% for TGm 1293 on TK soil. On the RK soil, increases were in the ranges of 10.8% for TGm 1196 to 62.5% for the TGm 1293.

Table 1.4. Shoot dry matter (kg DM ha^{-1}) of soybean genotypes grown on a Typic and a Rhodic Kandiudult soil (southern Cameroon) 8 WAP as affected by P fertilizer application (0 (0P), phosphate rock (PR), and triple super phosphate (TSP)). Means of 2001 and 2002.

	Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP
Genotype				[kg ha^{-1}]		
TGm 1196	1239	1496	1468	1280	1425	1419
TGm 1360	1360	1771	2113	1360	1770	2114
TGm 1039	1961	2335	2374	1160	1340	1450
TGx 1456-2E	1335	1556	1525	656	750	1056
TGm 1251	1230	1320	1510	1276	1340	1476
TGm 1420	2346	2247	2214	1268	1297	1465
TGm 1419	1962	2077	2494	1417	1431	1801
TGm 1566	2362	2809	3156	1441	2032	2161
TGm 1511	2252	2276	3148	1612	1703	1850
TGm 1540	1936	2276	2767	1433	1619	1774
TGm 1293	1336	2249	2671	983	1499	1598
TGm 0944	2543	2471	3113	1522	1825	1969
Mean	1822 ^{b}	2074 ^{b}	2379 ^{a}	1284 ^{b}	1503 ^{a}	1678 ^{a}
LSD- $_{5\%}$						
Location (L)	103.4					
P application (P)	291.4			171.7		
Genotype (G)	840.3			486.5		

Means following by the same letter are not significantly different at $P< 0.05$.

The application of PR resulted in lower shoot dry matter increases on TK but not on RK soil. However, shoot dry matter of the genotypes Tgm 1360, TGm 1039, TGm 1566, TGm 1540 and TGm 1293 were significantly increased by PR on TK soil. On the RK soil, in addition to the five listed above genotypes, Tgm 0944 significantly increased shoot dry mass by PR application compared to the 0P treatment (Table 1.4).

Shoot dry matter of soybean genotypes varied between 1239 to 2543 kg ha^{-1} for TK, and from 656 to 1612 kg ha^{-1} on RK soil when the genotypes were grown under low-P conditions. The highest shoot DM yielding genotypes under low-P conditions on the TK soil were Tgm 0944 and TGm 1566. These two genotypes, along with TGm 1540 and TGm 1511 had the highest shoot dry matter production on RK soil.

Grain yield

Application of TSP fertilizer significantly increased the grain yield of soybean genotypes on both soils (Table 1.5). Values ranged from 0.23 to 58% on TK, and from –29 to 86% on

RK soil. The soybean genotypes showed lower increase in grain yield by PR application on both soils, except genotypes Tgm 1039, TGm 1420, Tgm 1511, Tgm 1566 and Tgm 1293 on a TK soil as compared to the TSP application. On RK soil, only TGm 1419 and TGm 1039 significantly increased grain yield by PR application.

Grain yield in TK soil was 2.1 times higher than in RK soil and the soil type and genotype interaction was significant ($P < 0.0001$).

At the 0P level, there were significant differences ($P < 0.0001$) in grain yield among soybean genotypes on both soils. TGm 1511, TGm 1566 produced the highest grain dry matter on TK soil while TGm 1419 and TGm 1420 were the best genotypes for grain yield production on RK soil (Table 5). Grain yield was significantly related to N_2 fixation (r = 0.49, $P < 0.0001$). Also, a significant relationship between grain yield and shoot dry matter (r = 0.40, $P < 0.0001$) was observed.

Table 1.5. Grain dry matter weight (kg DM ha^{-1}) of soybean genotypes grown on a Typic and a Rhodic Kandiudult soil (southern Cameroon) 12 WAP as affected by P fertilizer application (0 (0P), phosphate rock (PR) triple super phosphate (TSP)). Means of 2001 and 2002.

	Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP
Genotype	[kg ha^{-1}]					
TGm 1196	513	547	760	152	162	188
TGm 1360	630	749	775	291	331	399
TGm 1039	917	1171	1214	189	386	196
TGx 1456-2E	532	566	634	126	129	177
TGm 1251	552	691	695	230	250	270
TGm 1420	833	987	1001	337	278	365
TGm 1419	748	852	951	389	473	502
TGm 1566	922	1186	1234	307	338	396
TGm 1511	960	1159	1219	288	298	345
TGm 1540	421	445	514	171	118	129
TGm 1293	716	1173	1018	176	215	328
Tgm 0944	836	937	838	196	129	140
Mean	715^{b}	872 ^{a}	904 ^{a}	238 ^{a}	259 ^{a}	286 ^{a}
LSD- $_{5\%}$						
Location (L)	58.1					
P application (P)	140.6			50.2		
Genotype (G)	406.3			146.8		

Means following by the same letter are not significantly different at P< 0.05

Clustering of soybean genotypes for their efficiency and response to P application (PR or TSP) on both soils using grain yield identified the following groups: Group 1 classified as efficient responders (ER) included genotypes TGm 0944, TGm 1566, TGm 1511, TGm 1540, TGm 1039 and TGm 1419, which were high yielding under low as well as high P conditions (PR or TSP) independently on soil type (Fig 1. 1). At the other extreme, group 2 classified as inefficient non responder (INR) consisted of genotypes TGm 1251, TGm 1456, TGm 1196, TGm 1039, which grew poorly under low and high P supply. To the intermediate group belonged TGm 1293, TGm 1039 and TGm 1196. Soybean genotypes TGm 0944, TGm 1566, and TGm 1511 in the ER groups significantly responded to PR and TSP application on both of the site, while TGm 1251 and TGm1196 were consistently belonging to the INR group.

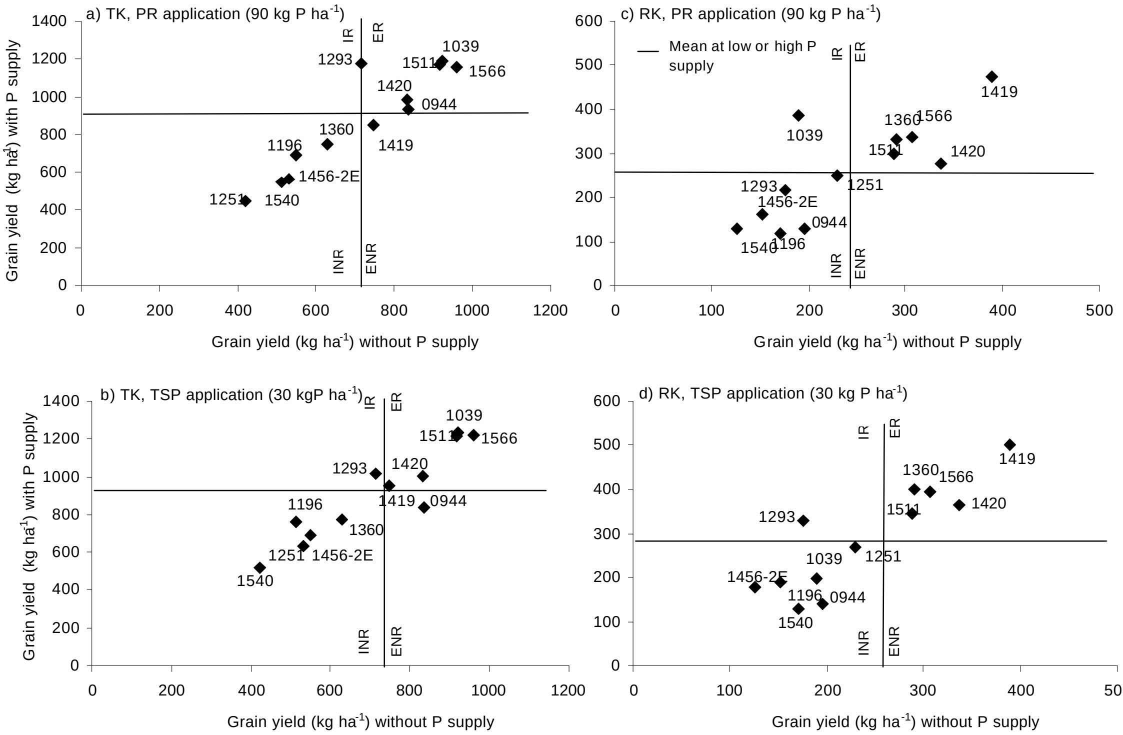


Fig 1.1. Grouping of soybean genotypes on the basis of grain yield in their P efficiency and their response to PR and TSP application on a Typic (TK) and a Rhodic Kandiudult (RK) soil (southern Cameroon) – means of 2001 and 2002. (IR = inefficient responder, INR = inefficient non-responder, ER = efficient responder, ENR = efficient non-responder).

Shoot P uptake

The application of TSP fertiliser resulted in 41% increase in shoot P accumulation of the soybean genotypes for the TK and 38% for the RK soil (Table 1.6). All the soybean genotypes significantly increased in their shoot P uptake in response to TSP application, except TGx 1456-2E and TGm 1251 on the TK soil. With respect to the RK soil, all the soybean genotypes also significantly increased in their shoot P uptake in response to TSP except TGm 1196.

The application of PR fertiliser resulted in significant increase in shoot P uptake on both soils ($P < 0.05$). The average increases were 21% for the TK and 16% on the RK soil. Out of the twelve genotypes, TGm 1419, TGm 1566, TGm 1511, TGm 1293 and TGm 0944 significantly responded to PR application on both soils (Table 1.6).

Shoot P uptake of soybean genotypes under low-P conditions varied from 3.1 to 6.0 kg ha^{-1} on TK and from 2.9 to 5.0 kg ha^{-1} on RK soil and there were significant differences among the soybean genotypes.

The shoot P uptake was significantly correlated with dry matter on TK (r = 0.81, $P < 0.0001$) and on RK soil (r = 0.80, $P < 0.0001$). Similarly, shoot P was significantly and positively related to N_2 fixation (r = 0.70, $P < 0.0001$) on both soils.

Table 1.6. Shoot P uptake (kg P ha^{-1}) of soybean genotypes grown on a Typic and a Rhodic Kandiudult soil (southern Cameroon) 8 WAP as affected by P fertilizer application (0 (0P), phosphate rock (PR) triple super phosphate (TSP)). Means of 2001 and 2002.

	Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP
Genotype	[kg P ha^{-1}]					
TGm 1196	3.3	3.5	4.4	3.4	3.3	3.8
TGm 1360	3.7	4.4	4.5	3.8	3.7	4.5
TGm 1039	3.9	3.9	6.0	4.2	5.3	6.4
TGx 1456-2E	3.2	3.5	3.5	2.9	2.2	3.9
TGm 1251	3.1	3.5	3.6	2.8	3.1	3.3
TGm 1420	3.2	4.9	5.0	3.6	3.8	5.0
TGm 1419	3.7	3.9	5.2	3.5	4.7	4.3
TGm 1566	5.3	6.1	7.7	4.4	5.7	6.6
TGm 1511	6.0	6.6	7.4	3.8	4.5	5.6
TGm 1540	4.3	4.8	5.7	4.3	5.6	6.6
TGm 1293	3.4	5.1	5.7	2.4	3.6	4.7
TGm 0944	3.3	5.8	6.8	5.0	5.6	6.4
Mean	3.87^{c}	4.67^{b}	5.46^{a}	3.68^{c}	4.26^{b}	5.09^{a}
LSD-$_{5\%}$						
Location (L)	0.35					
P application (P)	0.52			0.48		
Genotype (G)	1.48			1.35		

Means following by the same letter are not significantly different at $P< 0.05$

Total N accumulation, N_2 fixation and N balance

Total N accumulated in shoot varied from 20.2 to 138 kg ha^{-1} on TK and from 13.3 to 79.9 kg ha^{-1} on RK soil. TSP and PR application significantly ($P < 0.05$) increase the total N accumulated on both soils (data not shown).

All soybean genotypes, except TGm 0944, TGm 1540 and TGm 1251 significantly increased in N_2 fixation in response to TSP application on TK soil (Table 1.7). On the RK soil, TSP application significantly increased the N_2 fixation of all genotypes with the exception of the genotypes TGm 1196, TGm1251, TGm 1039, and TGm 1420.

The application of PR fertiliser significantly improved N_2 fixation across soybean genotypes on TK but not on RK soil. However, TGm 1293, TGm 1511, TGm 1419, and TGm 1360 significantly increased in N_2 fixation in response to PR application on the RK soil.

Intra-specific variation for N_2 fixation was observed within the 0P treatment on TK as well as RK soil with the highest N_2 fixed being recorded in genotypes TGm1511, TGm 1566 and TGm0944.

Table 1.7. N_2 fixation (kg N ha^{-1}) of soybean genotypes on a Typic and a Rhodic Kandiudult soil (southern Cameroon) as influenced by P fertilizer application (0 (0P), phosphate rock (PR), and Triple super phosphate (TSP)). Means of 2001 and 2002.

	Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP
Genotype	[kg ha^{-1}]					
TGm 1196	19.9	27.2	34.4	15.8	17.2	18.0
TGm 1360	19.1	29.7	34.4	17.5	21.4	27.8
TGm 1039	24.8	30.2	36.9	22.3	22.9	24.3
TGx 1456-2E	24.7	25.4	31.5	12.7	14.8	18.3
TGm 1251	23.7	26.6	25.5	17.9	20.3	19.2
TGm 1420	20.5	26.8	35.5	19.1	19.1	19.4
TGm 1419	20.8	24.2	30.8	16.4	22.5	25.7
TGm 1566	31.7	46.9	55.6	23.9	24.5	37.7
TGm 1511	32.9	41.2	55.4	26.7	32.0	38.1
TGm 1540	24.7	40.1	28.3	17.4	20.9	22.4
TGm 1293	20.8	32.3	52.6	15.1	29.4	32.4
TGm 0944	32.6	23.9	31.1	27.4	25.4	32.0
Mean	24.7^{c}	31.2 ^{b}	37.7^{a}	19.3 ^{b}	22.5 ^{b}	26.3 ^{a}
LSD-$_{5\%}$						
Location (L)	6.66					
P application (P)	6.30			3.72		
Genotype (G)	12.60			6.80		

The N balance of soybean genotypes ranged from –17.4 to 5.4 kg N ha^{-1} on TK and from –6.7 to 8.0 kg N ha^{-1} on RK soil (Table 1.8). However, most of the negative N balances were associated with soybean genotypes grown on the TK soil type. TSP application resulted in a significantly higher N balance for genotypes TGm 1511 and TGm 1566 than their corresponding 0P treatments on both soil types. In addition to the two genotypes listed-above, TGm 15490 and TGm 1039 had their N balances significantly increased by TSP on the RK soil type (Table 1.8).

The application of PR fertiliser resulted in lower increase of the N balance than the 0P treatment on both soils. The soybean genotypes, TGm 1039 and TGm 1511 had a significantly higher N balance in response to PR than other, except TGm 0944 and Tgm 1511 on RK soil.

Table 1.8. Nitrogen balance (kg N ha^{-1}) of soybean genotypes on a Typic and a Rhodic Kandiudult soil (southern Cameroon), as influenced by P fertilizer application (0 (0P), phosphate rock (PR), and Triple super phosphate (TSP)). Means of 2001 and 2002.

	Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP
Genotype	[kg ha^{-1}]					
TGm 1196	-15.1	-10.2	-7.7	-1.3	-1.7	-7.0
TGm 1360	-10.8	-2.4	0.1	-4.7	2.2	1.2
TGm 1039	-1.6	2.4	0.3	2.2	6.0	4.7
TGx 1456-2E	-12.7	-8.2	-7.2	-.05	0.5	1.7
TGm 1251	-1.9	-2.2	-0.2	-1.0	-3.5	3.7
TGm 1420	-10.7	-6.4	-1.5	1.1	2.7	2.1
TGm 1419	-4.2	0.1	-1.0	-6.7	0.6	4.5
TGm 1566	-3.0	0.5	5.0	0.8	2.8	4.9
TGm 1511	-3.9	-3.5	5.4	3.2	5.5	7.4
TGm 1540	-4.9	-3.5	0.0	-4.3	-0.1	6.2
TGm 1293	-17.4	-18.9	-3.1	1.5	0.4	2.0
TGm 0944	-3.6	-5.2	-0.80	-2.5	-3.6	0.2
Mean	-7.6	-4.7	-1.1	-0.4	1.6	2.5
LSD- $_{5\%}$						
Location (L)	4.25					
P application (P)	3.35			3.32		
Genotype (G)	4.80			4.75		

Means following by the same letter are not significantly different at P< 0.05

Clustering of soybean genotypes for efficiency and response to P application (PR or TSP) in both soils resulted in the following groups: Group 1 comprised TGm 0944, TGm 1566, TGm 1511, TGm 1540, TGm 1039, and TGm 1419 which fix high amount of N under low and high N conditions. These genotypes are classified as efficient responders (ER). Group 2 was made up of genotypes TGm 1251, TGm 1456, TGm 1196, TGm 1039, which poorly fixed nitrogen under low-P and high-P supply and classified as INR (Fig 1. 2). The intermediate groups comprised genotypes TGm 1293, TGm 1039 and TGm 1196. Genotypes TGm 0944, TGm 1566, and TGm 1511 in the ER groups significantly responded to PR and TSP application independently of the site, while TGm 1251 and TGm1196 were consistently belonging to the INR group.

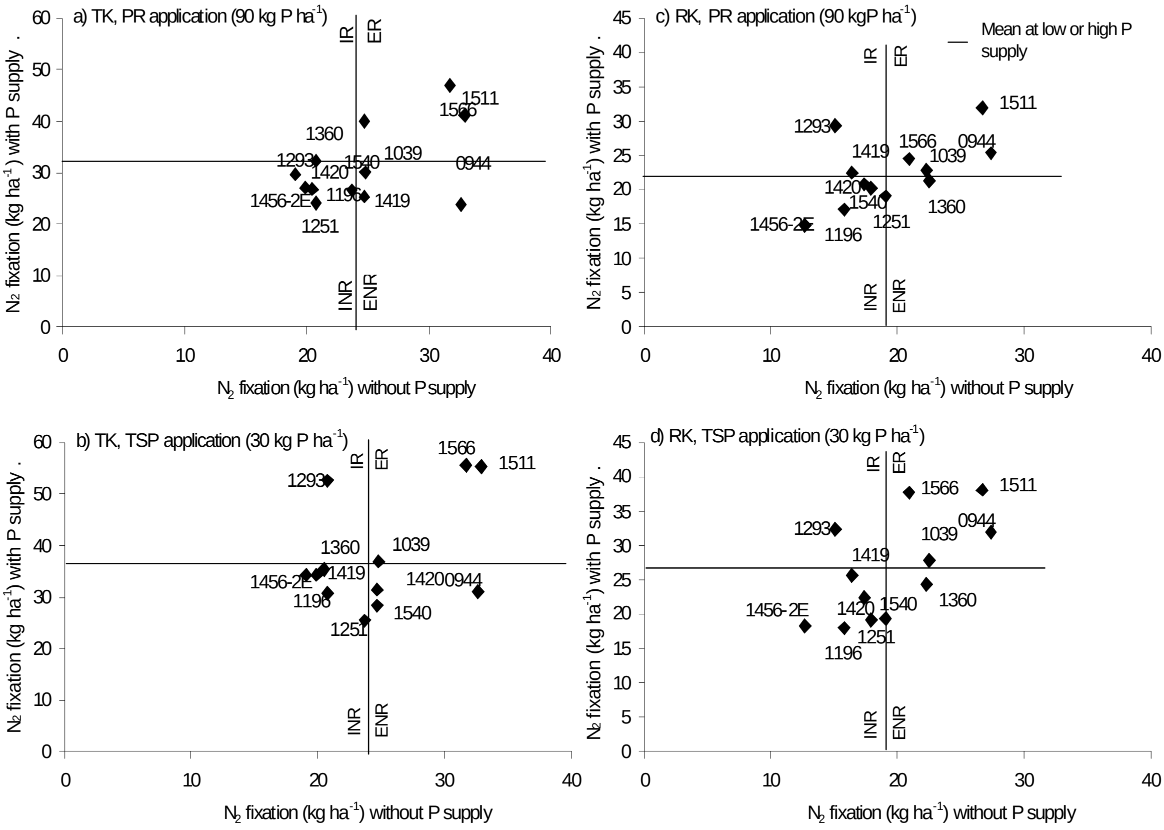


Fig.1.2. Grouping of cowpea genotypes on the basis of N_2 fixation in their P efficiency and their response to PR and TSP application on a Typic (TK) and a Rhodic Kandiudult (RK) soil (southern Cameroon) – means of 2001 and 2002 (IR = inefficient responder, INR = inefficient non-responder, ER = efficient responder, ENR = efficient non-responder).

Examination of possible P-efficiency traits

Table 1.9 shows variations in P efficiency traits of soybean genotypes belonging to different P efficiency classes. At the P level, shoot dry weight, total P in shoot and P-uptake efficiency of the genotypes in the ER class were significantly higher than the INR class.

Root/shoot ratio and P use efficiency significantly decreased with P application in the ER plants, while decreases in the INR class were non-significant for the INR genotypes. The AMF root colonization of ER genotype TGm 1511 was significantly higher than that of INR class at 0P. However, there were significant increases in the percent of root colonization by PR and TSP application in the ER class. The ER group had significant higher P uptake efficiency [µg P cm root^{-1}] than the INR plants and these were significantly increased by P application.

Table 1.9. Shoot dry matter, root shoot ratio, arbuscular mycorrhizal colonization, root length, total P uptake, P use and uptake efficiency of the efficient responder and inefficient non-responder soybean genotype grown for 8 WAP as affected by P application (0. triple super phosphate (TSP) or phosphate rock (PR)).

	Shoot DM [g plant^{-1}]	Root/shoot ratio [Dwt root.(dwt shoot)$^{-1}$]	AMF [%]	Root length [m plant^{-1}]	Total P uptake [mg plant^{-1}]	P use efficiency [g shoot dwt/mg P. in shoot]	P uptake efficiency [µ g P. cm root^{-1}]
Inefficient non-responders (INR)							
Tgm 1196							
0P	5.64	0.15	4.3	187	13.0	0.51	3.7
PR	5.98	0.15	5.3	209	13.0	0.51	5.8
TSP	6.10	0.14	4.9	194	13.7	0.53	6.3
Tgm 1251							
0P	5.53	0.14	5.7	145	12.2	0.51	4.9
PR	6.01	0.14	7.1	180	13.0	0.52	6.6
TSP	6.02	0.14	6.5	262	13.6	0.54	4.7
Efficient responder (ER)							
Tgm 1511							
0P	6.17	0.14	6.4	226	14.0	0.52	7.5
PR	6.71	0.13	8.0	166	15.5	0.47	12.5
TSP	7.49	0.12	11.7	221	17.4	0.45	11.5
Tgm 1566							
0P	6.05	0.19	3.0	251	14.1	0.49	7.4
PR	6.93	0.17	8.9	232	17.1	0.46	8.1
TSP	7.09	0.16	5.3	230	18.6	0.45	9.9
LSD_$_{1}$	0.50	0.02	1.36	90.4	1.37	0.03	2.41
LSD_$_{2}$	0.30	0.01	0.92	62.1	0.92	0.02	1.63
LSD_$_{3}$	0.58	0.028	1.73	118.2	1.36	0.04	3.09

[1] Legend: LSD-1 for comparing P treatment within group, LSD-2 for comparing group within P treatment, LSD-3 for comparing soybean genotype between group, Note:1) 0P = 0 kg P ha^{-1}, PR = 90 kg P ha^{-1} phosphate rock, TSP = 30 kg P ha^{-1} triple super phosphate

Greenhouse experiment in 2002 with Rhodic Kandiudult soil (green house experiment)

Table 1.10 presents results from the correlation matrix between the P uptake into shoots and growth, P acquisition and responses efficiency parameters of the ER and INR classes. Root length of the ER and INR plant were not significantly related to P uptake. Inversely, shoots dry matter of the ER and INR classes were significantly correlated with total P uptake. Root dry matter and the AMF of TGm 1511 were significantly related to P uptake but such relationship was insignificant for the INR genotypes and TGm 1566. Root/shoot ratio, P use and uptake efficiency were significantly related to P uptake for the ER group but in the INR genotypes.

Table 1.10. Correlation matrix between P uptake (mg P. plant^{-1}) and plant characteristics of soybean genotypes differing in P efficiency and response to P application (n = 12)
(ns: not significant, *, **, and ***, significant at 5%, 0. 01% and 0.0001% probability, respectively).

	Plant characteristics							
Genotype	Sht	Rt	Rt/Sht	TN	PUp	PUE	RL	AMF
Efficient responders								
TGm 1511	0.88 **	0.75 **	-0.69 *	0.62**	0.52 **	-0.64 **	ns	0.77 **
TGm 1566	0.85 **	Ns	-0.84 **	ns	0.53 *	-0.71 **	ns	ns
Inefficient non- responders								
TGm 1196	0.77 *	Ns	ns	ns	ns	ns	ns	ns
TGm 1251	0.89 **	Ns	ns	ns	ns	ns	ns	ns

Sht = shoot dry matter; Rt = root dry matter; Rt /Sht = shoot to root ratio; TN = total N uptake, Pup = P uptake efficiency; PUE = P use efficiency; RL = root length, AMF = Arbusuclar mycorrhizal fungi

Discussion

Our screening procedure revealed genotypic variation for N_2 fixation under P-deficient field conditions. TGm 1566, TGm 1511 and TGm 0944 fixed high nitrogen on both soils under P-limiting conditions (Table 1.7). In contrast, TGm 1196, TGm 1360, TGm 1039, TGx 1456-2E and TGm 1251 were more sensitive to P deficiency and showed lower N_2 fixation. Results, therefore, suggest the possibility to identify soybean lines adapted to low-P soils of SC. Our results compare with the findings by Sanginga et al. (2000), Vadez et al. (1999), Sanginga et al. (1997) who observed similar variation for N_2 fixation under limiting P condition by cowpea and common bean.

Average amounts of nitrogen fixed by soybean genotypes were 31 kg and 23 kg N ha^{-1}, representing 55 and 58% of total N on RK and TK soil, respectively. Estimates are within the range by N_2 fixed by soybean genotypes reported else (People and Crasswell, 1992). In SC, however, no data exist on the actual mount of N_2 fixed by soybean genotypes. An average of 91 kg N ha^{-1} has been recorded in derived Savanna soils with a pH > 6, while amounts of N_2 fixed ranging from 85 – 154 kg N ha^{-1} were recorded in Brazil using the ^{15}N isotope method. Sanginga et al. (1997) suggested an average value of 100 kg ha^{-1} representing 50% of N_2 fixed as appropriate for soybean. Thus, the soybean genotypes used in our study did not optimally fixed N_2 under the condition studied, confirming the limitation of P for N_2 fixation (Giller and Wilson, 1991). This was consistent with the application of TSP which significantly increase the amount of N_2 fixed.

The significant difference between the two soils was indicative that not only P, but also other soil factors might have interacted and consequently reduced or limited the N_2 fixation. Hardarson et al. (1989) proposed that the lack of proper rhizobia nodulating with soybean and the low soil pH are detrimental factors affecting nodulation of soybean in acid soils (O´Hara et al., 1989). The high soil acidity and high Al content observed on RK soil (Table 1.2) might have limited N_2 fixation.

The results presented in this study revealed the ability of some soybean genotypes to use P from sparingly available P sources (PR) ameliorating the symbiotic N_2 fixation (Table 1.7). Between Mucuna and Lablab genotypes, Vanlauwe et al. (2000b) also found significant increase in symbiotic properties and biomass dry yield of the plants. Although, we did not analyse the organic acid exuded, the possibility of root systems of such genotypes to mobilize P from non-Olsen P as suggested by Krasilnikoff et al. (2003) cannot be ignored. This is generally attributed to a better capacity to exude organic acid anions solubilizing Ca-P from PR (Gardner and Boundy, 1983). Such genotypes could be beneficial for small-scale farmers in the humid forest who do not have sufficient means to purchase P fertilizers.

The application of TSP fertiliser increased N_2 fixation and other parameters assessed for the soybean genotypes grown on both soils. The significant responses to TSP application denote a high P requirement of soybean genotypes for N_2 fixation. Variability in N_2 fixation among soybean genotypes at different P-levels has been reported elsewhere (Sanginga et al., 1997). Kang et al. (1995) also showed large differences in growth of woody legume species in responses to P application.

Nitrogen balance of soybean genotypes ranged from –17 to 5.5 kg N ha^{-1}on TK and -8 to 6 kg N ha^{-1} on RK soil, assuming that only seeds of lines were removed from plots. Sanginga et al. (1997) reported a range from –8 to 47 kg N ha^{-1} in soils of the derived Savanna of Nigeria in non-inoculated and inoculated fields with pH > 6.0. Although the results are based on the differences between total amount of N_2 fixed and total N content of the grain, our results indicate that contribution to N balance of the soybean genotypes are low on acid soils with P-limiting conditions of SC. This might imply that most of the N_2 fixed was translocated for grain formation. The results are indicative of that N_2 fixation is dependent on P availability since our calculation was based on the amount of N_2 fixed from the atmosphere instead of the total N content of the genotypes.

The results from this study showed that the N_2 fixation and yield were influenced by soil type. Such differences may be attributed to differential P limitation, other soil factors such as low pH and Mg contents of the soil, and high Al contents which were higher on RK soil. High soil acidity considerably limits soybean-rhizobia symbiosis, while exchangeable Al will reduce the root growth and interfere with nutrient uptake (Aune and Lal, 1997; Sanginga et al., 1996; Thompson et al., 1991).

The variability for nodule mass was related to the soil P level and especially for the TSP application on both soils, confirming the potential for detecting soybean lines with high nodulation and high N_2 fixation capacity (Araújo and Teixeira, 2000; Araújo et al., 1998; Pereira and Bliss, 1989).

The genotype x P level interaction was significant for grain yield on both soils, suggesting a wider genotypic variation of P responses in soybean. The magnitude of genotypic variation was however, higher on TK than RK soil (Table 1.5), but measurement from both soils enabled us to classify the genotypes into low-P use efficiency and P responsiveness. Some genotypes retained their classification in both soils, indicating a genotype adaptability to low and high P soil conditions of SC.

The lack of significance difference of the soil type x genotype interactions in shoot P uptake, N_2 fixation and N balance suggest that the soybean genotypes were stable for the above listed traits across different soil environment in SC. The non-significance of the genotype x P level interaction in shoot P uptake, shoot dry matter, nodule mass, N_2 fixation and N balance show a small genetic variation of such traits for P response and suggest that these plant parameters were mainly controlled by the genotype rather than by P application. These results are in agreement with those found by Araújo et al. (1998).

Different strategies for P acquisition were observed. The significant relationship between P uptake and arbuscular mycorrhizal association for TGm 1511 has been noticed (Sanginga et al., 1999) suggesting that access to soil P pools through AMF association might have been improved. The strong correlation between root/shoot ratio and P uptake (Table 1.10) suggests a compensatory mechanism between shoot and root growth may account for the fact that under P-limiting conditions TGm 1511 invest more energy in root proliferation in order to increase the soil contact. The high P uptake per unit root length observed in the plants was thus a consequence of enhanced root proliferation or the ability to form an AMF association. Similar observations have been reported by Al-Karaki (2002), Sanginga et al. (2000) and Araújo et al. (1998).

Part of the genetic variation observed in soybean genotypes in this study was due to differences in P use efficiency. The significant negative relationship between P use efficiency and P uptake suggest a better translocation of P into shoot that was not observe in the INR group (Table 1.10). This might be attributed to the ability of efficient genotype to remobilize P from senescent or relatively inactive tissue to the youngest plant organs (meristems and expanding organs) thus recycling P within the plant. Comparison of two white clover genotypes showed that the more P-responsive genotype was better able to remobilize P from senescing tissue to growing points than the less P-responsive. The leaf phosphatase activity, generally, was higher under P-limiting conditions. This has been suggested to be a useful mechanism for mobilizing polyphosphates or organic phosphates from senescent leaves to other plants organs (Yan, 1998).

Conclusion

This study identified genetic variation for P acquisition and use among the soybean genotypes included. Genetic variation for growth, N_2 fixation and N balance calls for breeding P-efficient soybean genotypes with enhanced N_2 fixation and lower N harvest indices, if the soybean genotypes are to be beneficial for a maize crop grown in rotation. The possibility to use P from sparingly available sources observed for some soybean genotypes demonstrated that some genotypes could potentially be of significance for breeders developing genotypes with lower P requirements and for small-scale farmers who can grow such genotypes using local and cheaper source of P generally available.

Chapter 2.

Genotypic Variation in Cowpea for P Uptake and Use Efficiency, and N_2 Fixation on Two Low-Available P Soils of Southern Cameroon

Abstract

Genetic variability for phosphorus (P) acquisition and use in low-available P soils is important for identifying more productive genotypes to improve the available cropping systems. Field and pot experiments were conducted to evaluate the genotypic variation in growth, P uptake, and N_2 fixation in cowpea on low-available P soils of SC. Field experiments were conducted in 2001 and 2002, and then four genotypes were selected for further investigation of probable mechanisms accounting for the variation in P acquisition and use efficiency observed under the field conditions. The field experiments were setup on two low-available P soil, a Typic (TK) and a Rhodic (RK) Kandiudult soil. Experiments were laid out in split-block design with four replications. P fertilizers were applied on the main plots with 0 kg P, 30 kg P ha^{-1} triple super phosphate (TSP) and 90 kg P ha^{-1} Togo phosphate rock (PR). Genetic variation for grain yield production was apparent at low P on both soils ($P < 0.0001$). Grain yield was, however, significantly ($P < 0.05$) higher on the TK than on the RK soil. The shoot P uptake varied significantly from 2.3 to 6.8 kg P ha^{-1} among genotypes at low P ($P < 0.0001$). The application of PR and TSP fertiliser significantly increased the shoot P uptake of the genotype IT89KD-391. The total amount of N_2 fixed increased significantly with TSP but not with PR application on both soils ($P > 0.05$). The N balance calculated as the difference between N_2 fixation and the N content of grains ranged from -6 to +6 kg ha^{-1}, with the genotypes IT90K-59, Dan´Ila and IT89KD-391 recording positive N balances. The most important mechanisms of P efficiency were an enhanced P uptake per unit of cm root length and the efficient transport of P to different plants organs. The genotypic variation for P uptake observed in this study, in combination with genotypic differences in N_2 fixation could be of significance to breeders trying to develop genotypes with a more positive N balance.

Key words: Cowpea – genotypic difference – N_2 fixation – P-sources – P-efficiency – Southern Cameroon

Introduction

Cowpea (*Vigna unguiculata* (L). Walp) is one of the most important food, fodder and cover crops in the semi- and tropical regions of Africa (Padulosi and Ng, 1990; Jackai and Adalla, 1997). The most important beneficial attribute of the legume crop is due to its contribution to the soil N budget through symbiotic N_2 fixation, thereby enhancing soil fertility and reducing the need for N fertilizer application (Martins et al., 2003). In addition, cowpea is considered to be less prone to drought and has a high yield potential especially when P fertilizers are applied. Its grain has relatively high protein content (Giami et al., 2001, Mortimore et al., 1997). Potentials yields of the crop (3000 kg ha^{-1}) have been reported (Rusoke and Rubaihayo, 1994); however, cowpea grain yields average only 200 – 400 kg ha^{-1} in Uganda (Sabiti et al., 1994), and 200 – 300 kg ha^{-1} in Nigeria (Alghali, 1992) and from 400 – 1000 kg ha^{-1} in dry area of Cameroon (Langyintuo et al., 2003).

In the humid forest benchmark (HFB) area in SC, cowpea is increasingly being used to improve the productivity of current cropping systems (Wendt and Atemkeng, 2004). However, among the factors curtailing growth and yield are soil acidity and low P availability (Menzies and Gillman, 1997; Eswaran et al., 1997; Vitousek and Farrington, 1997). Soil acidity and low soil-P availabilities are especially problematic for growth and N_2 fixation, since the nodules responsible for N_2 fixation have a high P requirement and growth is limited by P deficiency and low pH (Vance et al., 2001; Giller and Wilson, 1991). Correcting the P deficiency of cowpea in acid soils of SC through application of P fertilizer is not possible for the resource-poor farmers, because of the cost of P fertilizer (Samples et al., 1980). Also, applied P is rapidly converted into secondary and residual P-forms of low plant availability in these soils (Selles et al., 1995). The desirable, development of P-efficient genotypes requires a better understanding of the plant mechanisms controlling P efficiency and efficient field-evaluation techniques to differentiate between efficient and inefficient genotypes (Araújo et al., 1998).

Cowpea genotypes exhibit high degree of variation in growth, P-uptake as well N_2 fixation under low-P conditions (Krasilnikoff et al., 2003; Sanginga et al., 2000; Ankomah et al., 1995). To our knowledge, no attempt has so far been made to identify cowpea genotypes that grow fix N_2 well in the low-available P and acidic soils of SC.

Phosphorus efficiency can be achieved through P acquisition efficiency (PAE), defined as the ability of the plant or genotype to acquire P from soils that are low in available P, or by

P-utilization efficiency (PUE), the ability of a genotype or species to convert P into growth or yield once it is acquired from the soil (Chisholm and Blair, 1998; Blair, 1993). Plant-roots characteristics that can increase PAE include rhizosphere acidification (Marschner et al., 1987), exudation of organic acid anions (Neumann and Römheld, 1999), production of phosphatase enzymes (Kamh et al.,1999; Li et al., 1997), uptake kinetics (Nielsen and Barber, 1979), association with arbuscular mycorrhizal (Smith and Read, 1997), and root system (Gahoonia and Nielsen, 2004).

The objectives of the present study were: (i) to study intra-specific differences in growth, P-uptake and use efficiency among cowpea genotypes grown under low-P conditions of acid soils of SC and (ii) to investigate some of root traits that may account for the variability in P-efficiency observed under the field conditions.

Materials and Methods

Experiment 1. Field experiments

Site description

Field trials were conducted in two P-deficient soils in farmers' fields at Abang village and at the research station of the *Institut de la Recherche Agricole pour le Développement* (IRAD) at Minkoameyos in 2001 and 2002. Abang (3° 24´N, 11° 47´E) and Minkoameyos (3° 51´N, 11° 25´E) are both located in the forest margins in SC; Abang is located 10 km south of Mbalmayo and Minkoameyos is located 7 km north of Yaoundé. The soils are classified as Typic (TK) and Rhodic (RK) Kandiudult at Abang and Minkoameyos, respectively (Holland et al., 1992). The study sites were selected on the basis of their low-P availability (< 3 – 5 µg P g^{-1}) and high soil acidity (pH < 5.50). The long-term mean annual rainfall is 1513 and 1643 mm at Abang and Minkoameyos sites, respectively, with a bimodal distribution. Selected physico-chemical properties of the topsoil (0 – 10 cm depth) are given in Table 2.1.

Table 2. 1. Physical and chemical properties of topsoil (0 – 10 cm depth) of Rhodic and Typic Kandiudult soils (southern Cameroon), (Mean ± standard error)

Soil type	Sand	Silt	Clay	pH	C	N	Ca	Mg	K	Al
		[%]		[H_2O]	[g kg^{-1}]			[cmol (+) kg^{-1}]		
Rhodic Kandiudult	40.8	10.2	48.9	4.5	22.5	2.1	1.1	0.33	0.08	0.63
	(5.3)	(1.8)	(7.1)	(0.1)	(1.9)	(0.1)	(0.1)	(0.01)	(0.01)	(0.13)
Typic Kandiudult	50.6	9.7	39.7	5.4	16.0	1.6	1.35	0.64	0.06	0.12
	(1.58)	(0.85)	(1.2)	(0.1)	(1.1)	(0.05)	(0.17)	(0.06)	(0.04)	(0.02)

	P sorption	FeOx	P (Bray 1)	$NaHCO_3$-Pi	NaOH-Pi	HCl-P	Residual P
	[%]			[mg kg^{-1}]			
Rhodic Kandiudult	88.3	0.69	2.50	1.7	25.4	1	154.1
	(3.2)	(0.04)	(0.95)	(0.29)	(3.2)	(0.5)	(2.3)
Typic Kandiudult	77.5	2.3	5.0	3.2	29.2	0.86	190.9
	(0.3)	(0.07)	(1.20)	(0.5)	(1.2)	(0.38)	(12)

Experimental setup

Experiments were laid out in split block design with four replications. P fertilizers were applied on the main plots at 0 kg P, 30 kg P ha^{-1} Triple super phosphate (TSP) and 90 kg P ha^{-1} phosphate rock (PR). Each split also had a 0P treatment. Sub plots, measuring 4 by 4 m, comprised the cowpea genotypes. No N fertilizer or bradyrhizobia inoculation was applied. Cowpea was sown on 24 September 2001 and 15 September 2002 with two seeds per hill at 75 by 25 cm, and the seedlings were thinned to one plant, one week after emergence. The cowpea field was sprayed with thioral at 2 and 4 weeks after planting (WAP) and hand-weeded at 2, 4 and 6 WAP.

The cowpea genotypes were provided by the Grain Legume Program of IITA, Ibadan, Nigeria. Morphological and growth characteristics of the genotypes are presented in Table 2. 2.

Table 2. 2. Growth characteristics of the tested cowpea genotypes (Singh 1999, pers. comm.).

Genotype	Growth season	Growth	Resistance to disease	*PIS	Origin of germs plan	Seed type
IT82D-849	Early (70 days)	Erect broad	Major diseases, striga	PIS	Nigeria	Brown, smooth
IT 89KD-349	Early (70 days)	Semi spreading	Aphids, thrips, bruchids	PS	Nigeria, Kananandndo, Tanzania	White, rough
IT 82D-716	Medium (75 days)	Very erect	Multiple diseases	PIS	Nigeria, Tanzania	White, brown eye
IT 81D-715	Medium (75 days)	Long peduncles, Over canopy	Multiple diseases	PIS	Nigeria, Tanzania, USA	White, rough
IT 90K-59	Medium (75 days)	Semi erect	Major diseases, striga, electra	PIS	Nigeria, Tanzania	Brown, rough
IT 89KD-391	Late (80-85 days)	Semi erect	Aphids, thrips	PIS	Nigeria, Tanzania	Brown, rough
Dan 'Ila	Late (80-85 days)	Spreading	Very draught	PIS	Nigeria	White, rough

* PIS- photo- insensitive; PS- photosensitive

Plant sampling and analysis

Plants were sampled for shoot growth, nodule formation and N_2 fixation at mid-pod fill, i.e., 8 WAP. Six plants were chosen in three middle rows, their shoots were cut at 5 cm above ground level and the fresh weight was recorded. A sub-sample of 500 g of fresh shoots was retained, transferred to the laboratory and oven-dried at 70° C for 72 hours. Fresh nodules were carefully removed from the root growth, their number were determined, and was oven-dried for 72 hours and their dry matter (DM) was recorded. At the grain maturity (12 WAP), a subplot of 1.50 m x 1.50 m were harvested, the fresh weight was determined in the field and 100 g sub-samples were oven-dried at 70° C for 72 hours and the DM was measured.

The dried samples were ground to pass a 0.5 mm mesh, and their N and P concentrations were determined. Samples were digested according to Novozamsky et al. (1983). Total N and P were determined with an ammonium sensitive electrode according to Powers et al. (1981) and calorimetrically (Motomizu et al., 1983) procedures, respectively.

N_2 fixation and N balance

The xylem ureide assay method (Peoples et al., 1989) was used to assess N_2 fixation in cowpea genotypes. The entire shoot and petiole of three plants were finely ground, and 0.5 g of subsample was used to extract the xylem solutes in boiled water. The ureide and nitrate was analyzed. The relative ureide-N abundance (RUA) of the sample was calculated based on the molar concentration of ureides and nitrate with the assumption of 4 N atoms per ureide molecule using the following equation:

$$\text{RUA} = \frac{4 \text{ x n (mol ureide)}}{\left[(4 \text{ x n (mol nitrate)} + (\text{n mol ureide})\right]} \text{ x100}$$

where *n* mol ureide or nitrate is the concentration of ureide or nitrate in the stem extracts expressed in *N* mol.

The N balance of cowpea genotypes was calculated with the following formula of Peoples and Craswell (1992):

N balance = $N_f - N_{s,}$ where N_f is the amount of N_2 fixed and Ns the total N content of cowpea grain DM.

Experiment 2. Greenhouse experiment

A pot experiment was conducted in a greenhouse at IITA in Nkolbisson. The soil used was the RK, sampled at 0 – 10 cm depth at the Minkoameyos site in 2001. The soil was air-dried and sieved through a 4 mm sieve and 2.5 kg were used in a 2.5 L pot. Seeds of cowpea genotypes were pre-germinated for three days and four seeds were sown in each pot. One week after planting, the seedlings were thinned to two plants per pot. The experiment was laid out in a factorial randomised complete block design with two factors. Factor 1 was P application with three P levels: 0 kg P ha^{-1} (0P), 90 kg P ha^{-1} as phosphate rock (PR) and 30 kg P ha^{-1} as triple super phosphate (TSP). Factor 2 comprised four cowpea genotypes selected from experiment one: two that had responded significantly to P application in 2001 and had the highest grain yield under low-P (0P) conditions, and two that had not responded to P application and were amongst the lowest yielding at 0P. Plants were daily watered with deionised water and harvested 8 weeks after planting.

Sampling

Harvested plant samples were assessed for dry matter, nodule formation, arbuscular mycorrhizal fungi (AMF) colonization of roots, and root development. Total P and N concentrations were measured in shoots and roots as described above.

AMF colonization

Sub-samples of 1 g of fresh roots were washed free of soil, preserved in 50% alcohol and stored at 4° C prior to assessment of AMF infection. Roots were later allowed to attain room temperature and cut into 1 cm length pieces and cleared in KOH (10%) solution. The cut roots were stained using acid fuchsin in lacto-glycerine at room temperature according to Philips and Hayman (1970) and Merryweather and Fitter (1991) procedures. Roots were then examined for colonization by AMF using the gridline intersects method of Giovannetti and Mosse (1980) under a stereomicroscope at 40x magnification.

Root-length and root-weight determination

The entire root system of the two cowpea plants in a pot were harvested and separated from soil by washing gently with tap water. Sub-samples were spread out on a shallow plastic tray and scanned using ScanJet Iicx software. Dt-Scan software, Delta-T Devices, Cambridge, England (Webb *et al.*, 1993) was used to measure total length and diameter of roots. The scanned samples were subsequently oven-dried at 70° C for 72 hours and their dry weights determined.

Statistical analysis

Statistical analyses of the data were conducted using SAS version 8.02 (Statistic Analysis Systems, 2001). The mixed model procedure “PROC MIXED” and the general linear model “PROC GLM” were used to compute the analysis of variance (ANOVA) for the field and greenhouse experiments, respectively. When the F test denoted a significant effect, the least significant difference (LSD) test was used to compare the means. Correlation analysis was done using ``PROC CORR´´ to illustrate the relationship between a pair of variables.

Results

Experiment 1: Field experiment

Grain yield

The grain yields of cowpea genotypes did not differ significantly between 2001 and 2002 on both soils, therefore, mean data per site for both years are shown in Fig.2.1a, b. The yields of cowpea genotypes grown under low-P conditions varied significantly on both soils with yields ranging from 633 to 1250 kg ha^{-1} on TK and from 490 to 900 kg ha^{-1} on a RK soil. Grain yields obtained from TK soil were significantly higher than those from RK soil for all the cowpea genotypes. There was a marked difference between genotypes in their grain yield response to P application (Fig.2.1) and genotype x P interaction was significant ($P < 0.05$). On TK soil, only three genotypes (IT82D-849, Dan ‘Ila, and IT81D-715) had no significantly higher yields with PR application (Fig. 2.1a). The application of TSP fertiliser increased the grain yield of all genotypes compared to the 0P treatment, but only two (IT81D-715 and Dan ‘Ila) had significantly higher yields with TSP as compared to PR application. On the RK soil, there were no significant differences in grain yield by PR application (Fig. 2.1b). By contrast, TSP application significantly increased the grain yield of all genotypes, except IT82D-716 and IT81D-715 compared to 0P. The application of TSP fertiliser resulted in significantly higher grain yields of IT82D-849, IT90K-59, Dan ‘Ila, and IT89KD-391 compared to PR. Significant and positive correlation between grain yield and shoot P uptake was observed for both soils ($r = 0.49$, $P < 0.0001$).

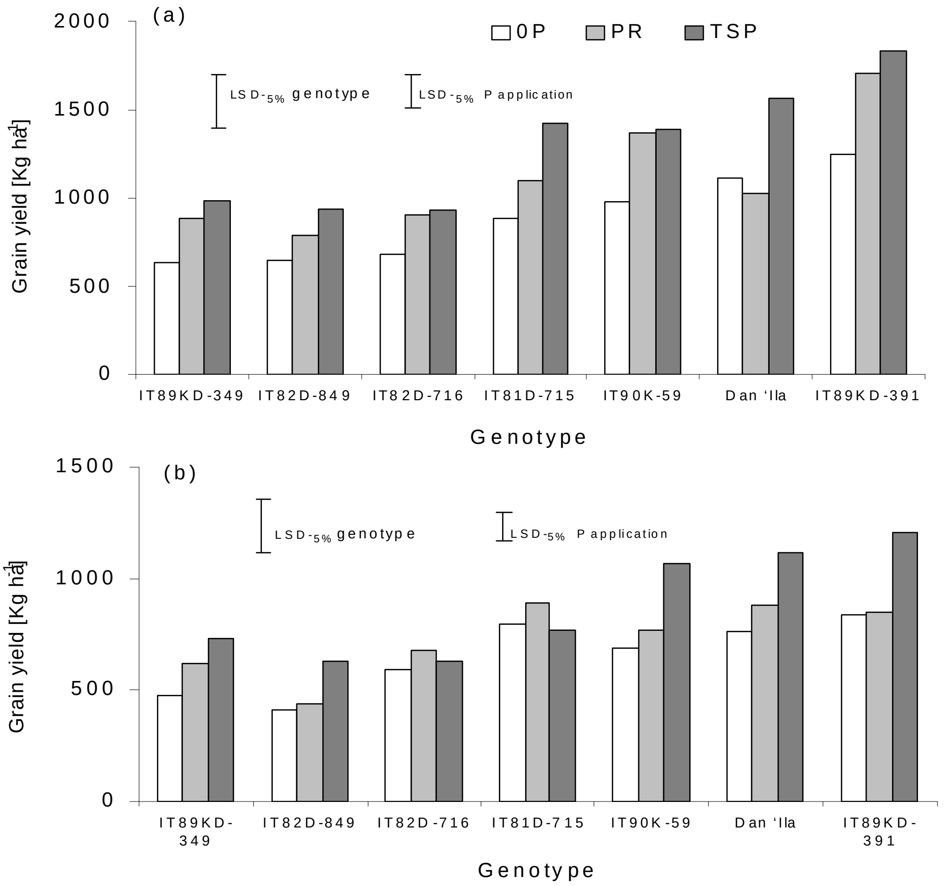


Fig.2.1. Effect of P application on dry grain yield (kg ha^{-1}) of cowpea genotypes grown on (a) a Typic and (b) a Rhodic Kandiudult soil in southern Cameroon. Means of field experiments in 2001 and 2002.

The following P efficiency groups were distinguished based on grain yield as presented in Fig. 2.2 a, b, c, d. The efficient responder (ER) group included cowpea genotypes IT90K59, IT89KD-391, IT81D-715, and Dan´Ila, which grew efficiently at low as well as responding to high P availability (PR or TSP). On the other extreme, IT89KD-349, IT82D-716 and IT82D-849 were placed in the inefficient non-responder (INR) group representing genotypes that poorly grew under both low and high-P (PR or TSP) conditions. Intermediate groups, inefficient responder (IR) and efficient non-responder (ENR) were genotypes that were inefficient or efficient at acquiring P from low P and did not or did respond to application of either PR or TSP application, respectively. Depending on P source and soil conditions IT81D-715 and Dan´Ila were classified once into the ENR group (Fig. 2.2).

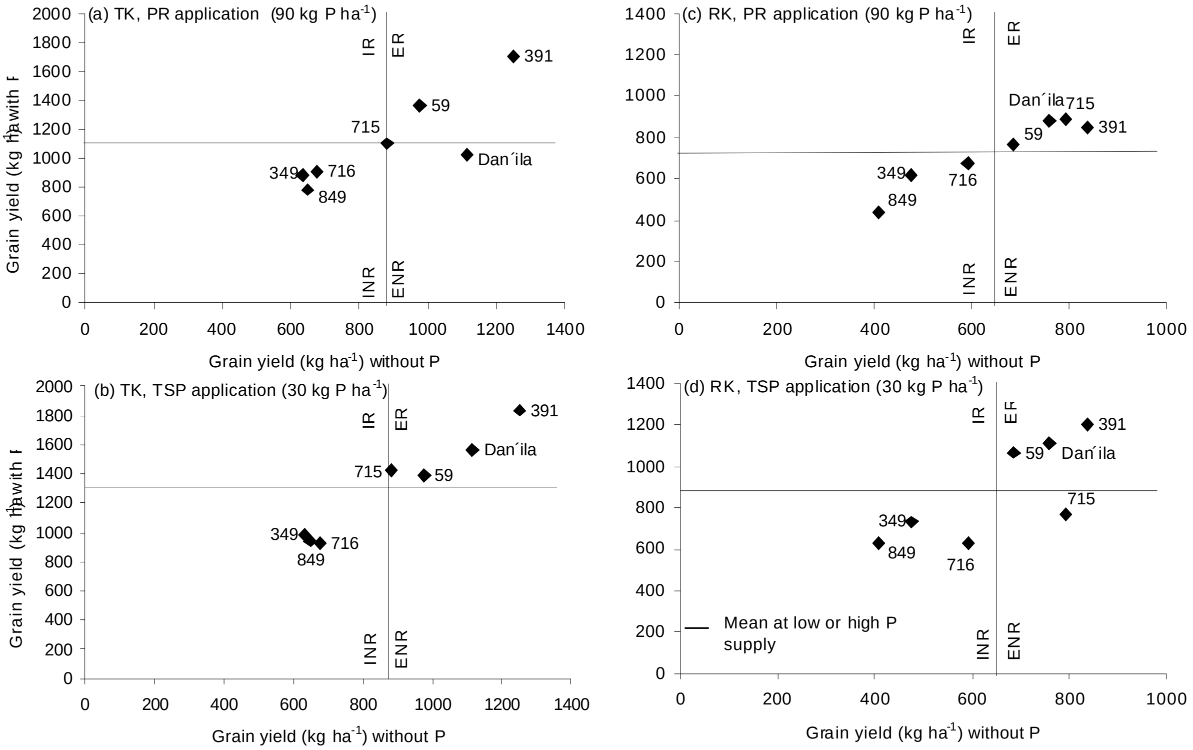


Fig.2.2. Grouping of cowpea genotypes based on grain yield for their P use and response efficiency on Rhodic Kandiudult (RK) and Typic Kandiudult (TK) soils of southern Cameroon, (IR = inefficient responder, INR = inefficient non-responder, ER = efficient responder, ENR = efficient non-responder). Data points represent mean values of 2001 and 2002.

P uptake and P uptake efficiency of cowpea

The cowpea genotypes differed significantly in their shoot P uptake under low-P conditions in both soils (Table 2.3). The genotypes by location and location by P application interactions were not significant indicating genotype adaptability across the environments used for these traits. The highest P uptake was recorded in genotypes Dan ‘Ila, IT90K-59 and IT89KD-391 on both soils. On TK soil, all but two genotypes (IT82D-849 and IT82D-716) took up significantly more P with PR application than at 0P. With TSP application, all genotypes except Dan ‘Ila increased their shoot P uptake significantly compared to 0P on RK soil. Most of the genotypes also had significantly higher P uptake with TSP than with PR application. In contrast, there were only two genotypes (IT81D-715 and IT89KD-391) on RK soil that had significantly higher P uptake after PR application compared to 0P. However, TSP application lead to significantly higher P uptake in comparison with 0P for all but three genotypes (IT89KD-349, IT82D-716 and Dan ‘Ila). The application of PR and TSP fertilisers increased significantly the P uptake of IT89KD-

391, which had the highest total P accumulations at 0P, but there was no significant difference between the fertilizer types.

Table 2. 3. Shoot P content and P uptake efficiency of cowpea genotypes grown on a Typic and a Rhodic Kandiudult soil in southern Cameroon as affected by P application. Plants were sampled 8 WAP.

	P uptake						P uptake efficiency					
	Typic Kandiudult			Rhodic Kandiudult			Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP
Genotype	[kg P ha^{-1}]						[mg P g root^{-1}]					
IT 89KD-349	2.6	3.4	3.9	2.3	2.8	3.9	65	99	109	72	101	94
IT 82D-849	2.4	3.0	3.1	2.8	3.3	4.7	66	97	105	62	92	91
IT 82D-716	3.8	4.1	5.2	3.7	3.7	4.7	60	83	72	78	64	68
IT 81D-715	3.1	4.6	5.9	2.4	4.7	4.6	111	120	135	109	98	144
IT 90K-59	4.4	5.3	6.5	4.2	4.4	6.8	112	114	132	124	93	129
Dan ‘Ila	4.9	3.9	4.6	4.5	4.5	4.7	109	123	158	120	130	106
IT 89KD-391	4.5	5.5	6.4	4.3	5.4	5.9	81	98	107	94	132	86
Mean	3.7	4.3	5.0	3.5	4.1	5.0	86	105	117	94	101	103
LSD (5%)-1	0.75			0.88				15			21	
LSD (5%)-2	1.50			1.75				32			36	

LSD-1 for comparing P levels within cowpea genotypes
LSD-2 for comparing cowpea genotypes within one P level
0P = 0 kg P ha^{-1}; PR = 90 kg P ha^{-1} phosphate rock, TSP = 30 kg P ha^{-1} triple super phosphate

Genotypes that had high shoot P content without P application had also significantly higher P uptake efficiency (mg P g root^{-1}) than genotypes with low shoot P uptake (Table 2. 3). This was consistent across locations. PR and TSP application significantly increased the P uptake efficiency of two genotypes (IT89KD-349 and IT82D-849) on both soils. However, even with P fertilizer, these genotypes took up less P per unit root weight than genotypes with high P efficiency at 0P.

In contrast, on the TK soil, only TSP application increased significantly P uptake efficiency of genotypes, with high efficiency under low conditions (0P). The P uptake efficiency of IT90K-59 and Dan ‘Ila was significantly higher with TSP as compared to PR application.

Cowpea genotypes were also placed in different efficiency classes on the basis of their shoot P contents (Fig. 2.3 a, b, c, d). The ER genotypes were IT89KD-391, IT90K-59, and Dan´Ila while the INR class comprised genotypes, IT89KD-349, IT82D-849, IT82D-716, and IT82DKD-715. The other genotypes were in the intermediate groups (IR or ENR). Genotypes IT89KD-391 and IT90K-59 on the one hand, and IT89KD-349 and IT82D-849 on the other hand retained the ranking into the ER and INR classes, irrespective of soil type and P source. These genotypes were, therefore, selected to study candidate mechanisms of P use efficiency.

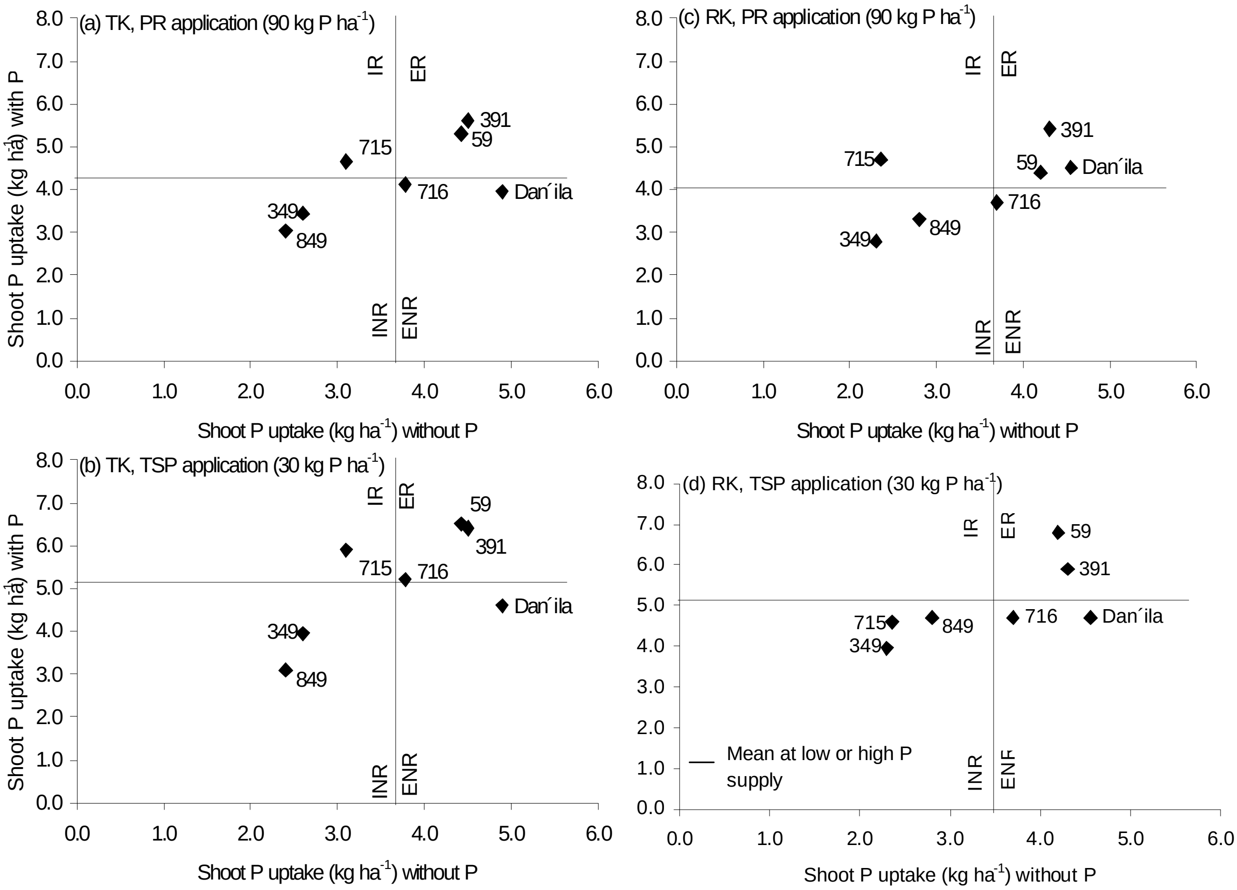


Fig.2.3. Grouping of cowpea genotypes based on shoot P uptake for their P use and response efficiency in a Rhodic Kandiudult (RK) and a Typic Kandiudult (TK) soil of southern Cameroon (IR = inefficient responder, INR = inefficient non-responder, ER = efficient responder, ENR = efficient non-responder). Data points represent mean values for 2001 and 2002.

Total N accumulation, N_2 fixation and N balance

Total N accumulated ranged from 24.4 – 55.5 kg N ha^{-1} on TK, and from 17.1 – 56.8 kg N ha^{-1} on RK soil (data not shown). Highly significant differences in total N uptake were observed between genotypes on both soils.

The application of TSP fertiliser significantly increased N_2 fixation in all the cowpea genotypes on both soils (Fig.2. 4 a, b). On TK soil, the N_2 fixation of all the cowpea genotypes, except IT81D-715 was significantly ($P < 0.05$) increased by TSP but not by PR application. The amount of N_2 fixed was significantly increased by PR for the cowpea genotypes IT89Kd-349, IT89KD-391, and IT90K-59, and on the RK soil (Fig.2.4b). In contrast, TSP application increased significantly N_2 fixation of all genotypes grown on this soil.

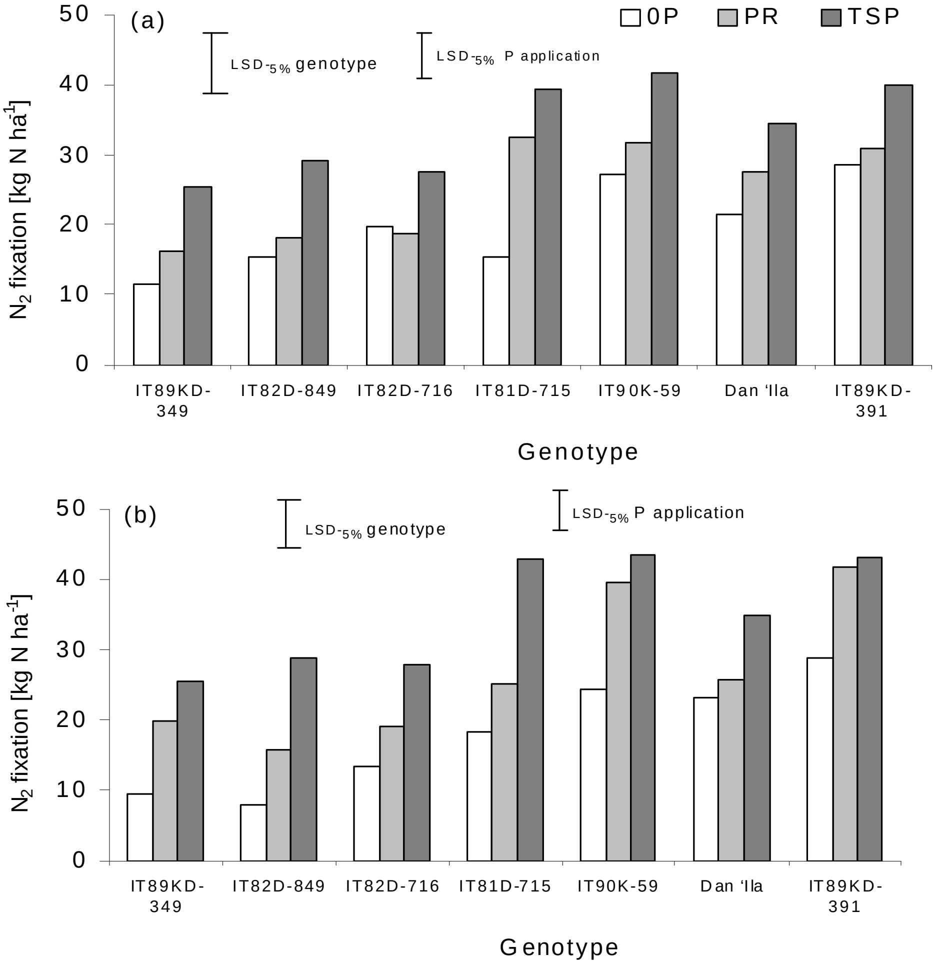


Fig.2.4. Effect of P application on N_2 fixation (kg N ha^{-1}) by cowpea genotypes grown on (a) Typic Kandiudult and (b) Rhodic Kandiudult soil (southern Cameroon). 0P = 0 kg P ha^{-1}; PR = 90 kg P ha^{-1} phosphate rock, TSP = 30 kg P ha^{-1} triple super phosphate.

The N balance of all treatments was either slightly negative or positive with most values ranging between –6 and +6 kg N ha^{-1} (Table 2.4). High-yielding genotypes IT90K-59, Dan 'Ila, and IT89KD-391 had positive N balances under low-P conditions on TK and RK soil while low-yielding genotypes IT89KD-349, IT82D-849, and IT82D-716 had significantly higher negative N balances on both soils. There was no effect of P application on the N balances with two exceptions: TSP increased significantly the positive N balance of the high-yielding genotype IT89KD-391 on RK soil and decreased significantly the negative N balance of the low-yielding genotype IT82D-849.

Table 2.4. Effect of P application on the N balance of cowpea genotypes grown on a Typic and a Rhodic Kandiudult soil in southern Cameroon

	Typic Kandiudult			Rhodic Kandiudult		
	0P	PR	TSP	0P	PR	TSP
Genotype	[kg N ha^{-1}]					
IT 89KD-349	-4.80	-6.50	-4.90	-3.50	0.20	-0.02
IT 82D-849	-1.70	-1.60	-10.40	-3.20	-2.10	-2.20
IT82D-716	1.10	-3.80	-3.01	-3.25	-0.20	2.60
IT81D-715	2.40	0.20	-5.50	-0.10	-2.25	1.95
IT 90K-59	-2.55	0.90	1.10	2.80	1.20	2.50
Dan ‘Ila	-0.90	0.75	1.45	3.30	6.85	4.40
IT89KD-391	2.70	-1.25	2.30	6.10	4.25	11.35
Mean	-0.54	-1.61	-2.71	0.31	1.14	2.9
LSD (5%)-1	3.50			4.50		
LSD (5%)-2	6.00			8.50		

LSD-1 for comparing P levels within cowpea genotypes
LSD-2 for comparing cowpea genotypes within one P level
0P = 0 kg P ha^{-1}; PR = 90 kg P ha^{-1} phosphate rock, TSP = 30 kg P ha^{-1} triple super phosphate
The N balance was calculated based on the mean amount of N_2 fixed over the two years.

Experiment2: Greenhouse experiment

A greenhouse pot experiment was conducted to further explore traits responsible for P acquisition and response of the test genotypes. Table 2.5 presents mean results for the two P efficiency groups: efficient responders (ER) and inefficient non-responders (INR). Without P application, ER genotypes had significantly higher values than INR genotypes in all parameters (i.e., shoot dry matter, AMF root colonization, root length, total P uptake and P uptake rate) measured. ER genotypes responded, on average, significantly to PR and TSP application by increasing shoot dry matter, AMF colonization and P uptake. Phosphorus fertiliser application reduced the root length of the P efficient and inefficient group, but the difference was generally not significant, except for the genotype IT89KD-391 with PR application. Phosphorus uptake rate was unaffected by PR, but increased significantly with TSP application.

Phosphorus fertilizer did not change significantly P uptake and root length of INR genotypes. However, shoot dry matter and AMF colonization were increased with PR and TSP application, while only TSP increased significantly the P uptake efficiency of this P-inefficient group. The root to shoot ratio of INR genotypes was at the 10% test level significantly (*P* = 0.095) lower than that of ER genotypes under low-P conditions (data not shown).

Table 2. 5. Effect of P fertilization on shoot dry matter, arbuscular mycorrhizal fungi (AMF) colonization, root length, P uptake, and P uptake rate of two P efficiency groups of cowpea genotypes. Pot experiment.

	Shoot dry matter [g pant^{-1}]			AMF [%]			Root/shoot ratio			Root length [m plant^{-1}]			Total P uptake [mg plant^{-1}]			P uptake efficiency [µg (cm root)$^{-1}$]		
	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP
Efficient Responders group (ER)																		
IT 90K-59	7.68	8.84	8.60	23.9	29.7	27.7	0.09	0.08	0.08	72.6	64.2	76.7	15.4	19.8	18.8	26.8	31.3	30.4
IT 89KD-391	7.70	8.75	8.70	24.5	33.5	23.8	0.10	0.07	0.07	86.8	61.1	75.0	16.0	19.6	19.9	28.8	46.3	33.8
Inefficient non responder group (INR)																		
IT 89KD-349	7.05	7.92	7.81	16.4	29.2	21.2	0.07	0.08	0.06	73.2	69.1	65.9	14.2	16.9	17.8	21.2	21.5	23.1
IT 82D-849	7.10	7.61	7.86	22.3	29.7	27.7	0.08	0.08	0.08	80.6	68.0	77.4	15.6	15.8	16.9	19.1	24.6	26.2
LSD_1		0.60			4.14			0.010			10.70			1.50			7.18	
LSD_2		0.32			2.80			0.007			7.20			0.78			4.85	

Legend: LSD-1 for comparing P treatment within genotype, LSD-2 for comparing genotype within P treatment,
0P = 0 kg P ha^{-1}, PR = 90 kg P ha^{-1} phosphate rock, TSP = 30 kg P ha^{-1} triple super phosphate

Correlation of AMF, root growth, and root-shoot ratio with P uptake

The total P uptake of ER genotypes was negatively significantly correlated with root/shoot ratio (r^2 = 0.59, n = 24) (Fig 2.5b) but the relationship was not significant for INR genotypes (r^2 = 0.09, n = 24); the difference between the regression coefficients was significant. However, the P uptake efficiency of the ER genotypes was significantly and positively related to P uptake rate (r^2 = 0.35, n = 24) (Fig. 2.5a). No such relationship was observed in the INR genotypes. The P uptake of ER genotypes was also negatively and significantly correlated (r^2 = 0.35; n = 24) with root length, while the relationship was not significant for the INR genotypes (r^2 = 0.10, n = 24). Root colonization by AMF was not significantly correlated with total P uptake (data not shown).

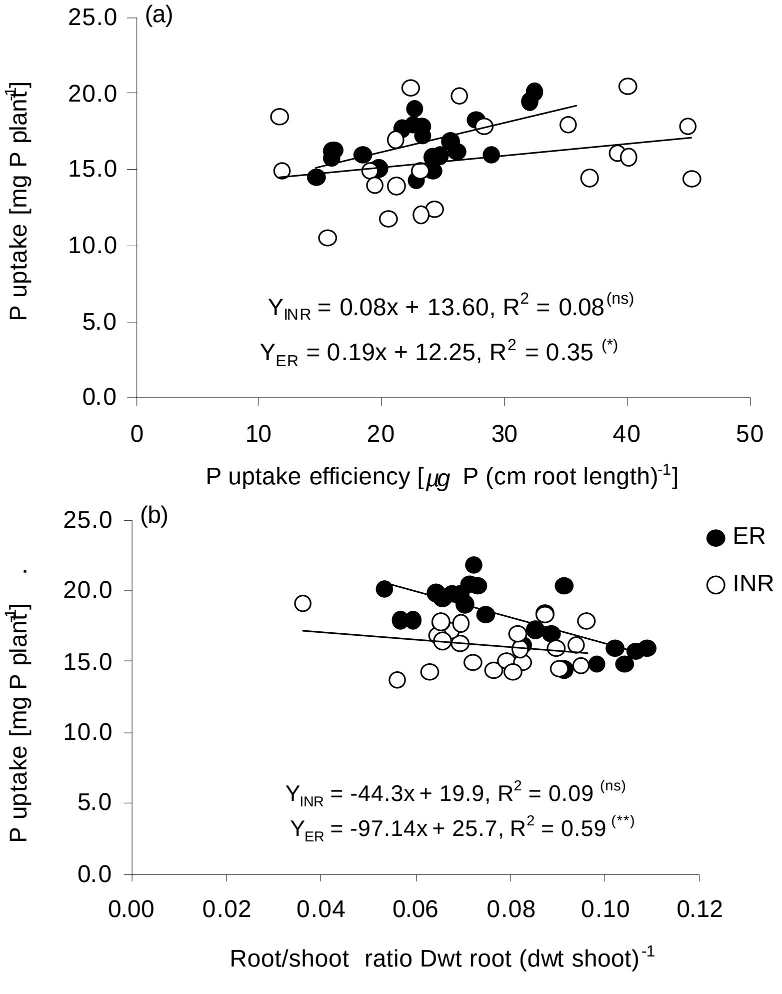


Fig.2.5. Relationship between (a) root length (b), root/shoot ratio and total P uptake of efficient responder (ER) and inefficient non-responder (INR) cowpea genotypes at 8 WAP. Pot experiment.

Discussion

The cowpea genotypes we tested showed large genotypic variation in their grain yield with and without P fertilizer application (Fig 2.1a, b). This enabled us to classify them with respect to both their P use efficiency, i.e., their potential to produce a high grain yield on low-P soil, and their response to PR or TSP supply. Sanginga et al. (2000) studying the response of cowpea genotypes to P application at Fashola (in the derived savanna, Nigeria) with a soil pH = 6.0, classified genotype IT89KD-391 as a P-responder; and genotypes IT90-59 and IT89KD-349 as non-responders based on shoot dry matter. Krasilnikoff et al. (2003) studied the ability of these cowpea genotypes to acquire P from non-Olsen-P sources and observed that IT90K-59 and IT89KD-391 exploited only a low volume of soil by their roots, but had a high P uptake rate particularly from non-Olsen-P sources. They reported that these genotypes used root-induced processes to acquire P from the strongly bound soil-P pools, and invest less in root growth. If the relative high grain yield observed for these genotypes is due to their ability to acquire P from less available sources this may be an indicator of high P efficiency, as observed by Caradus and Snaydon (1986) for white clover.

The plant traits contributing to P-efficiency of the different groups were investigated under controlled conditions (pot experiment). The importance of P acquisition per unit of root length, the root/shoot ratio and the root length were observed as the probable mechanisms for the efficient acquisition and use of P of the cowpea genotypes (Table 2.5; Figure 2.5a, b). AMF colonization of roots did not significantly relate to P uptake, although, the ER genotypes showed high levels of root colonization by AMF. AMF was, therefore, considered a less responsible for the difference in P efficiency of the two groups. Our result confirms the earlier observation made in the derived savanna of Nigeria (Sanginga et al., 2000).

The P uptake rate (µg P cm root^{-1}) of genotypes in the ER group was positively significantly related to total P uptake in shoot and roots (Fig.2.5a). Such relationships suggest that these plant traits accounted for most of the genotypic variation observed in the field. In addition, P uptake was related to root/shoot ratio, indicative of a better capacity to retranslocate P from roots to the shoot by ER than INR genotypes (Fig.2.5b). Similar findings were found by Araújo et al. (1998); Caradus and Snaydon (1986) in studies on common bean and on white clover genotypes, respectively.

Cowpea grain yield was higher on TK than on RK soil. The soil chemical analysis revealed that the RK soil had high content of exchangeable aluminium and P sorption capacity and, thus, lower P availability (Table 2.1). Exchangeable Al interferes with efficient uptake, transport, and utilization of P in plants while P sorption reduces the availability of P in soil solution (Aune and Lal, 1997; Shuman et al., 1990; Fageria et al., 1988; Rengel and Robinson, 1989). With the low levels of basic cations and higher soil acidity of the RK soil, more Al could be brought into solution through the dissolution of Al hydroxides and amorphous Al (Calba et al., 2004; Hipps et al., 2004).

Interestingly, genotypes IT90K-59 and IT89KD-391 were the most tolerant of P deficiency, and also significantly responded to PR application. The significant response to PR suggests their capability to use P from sparingly acid soluble available P sources in the soil. Krasilnikoff et al. (2003), studying the efficiency of genotype IT90K-59 and IT89KD-391 demonstrated that these genotypes were able to take up P from non-Olsen P a capacity to which long root hairs might have contributed. As shown in earlier reports (Fardeau and Zapata, 2002), most of the P contained in PR is bound to Ca, and root exudation of organic acid anions has been suggested to efficiently solubilize Ca-bound P (Jungk and Claassen, 1997; Gerke, 1992; Dinkelaker et al., 1989). In addition, the solubilization of PR by genotypes increases the soil pH, Ca and Mg content, and consequently reduces the deleterious uptake of aluminium (Fardeau and Zapata, 2002). This is generally attributed to the ability of this genotype to exude significant amount of citrate that solubilize P bound to Al and Fe most effectively (Neumann and Römheld, 1999; Gardner et al., 1983).

The genotypes were equally separated on the basis of their shoot P uptake (Fig 4 a, b, c, d), confirming the classification of these genotypes by Krasilnikoff et al. (2003). The significant relationship between cowpea grain yield and shoot P uptake confirmed that shoot P uptake could be used as a P use efficiency criterion for cowpea and is in agreement with the findings of other authors (Ankomah et al., 1995; Yan et al., 1995a, b). The lack of significant of the genotype and soil type interaction for shoot P uptake might suggest that the adaptation of genotypes to low P availability is stable across soil types and facilitates the selection for P efficiency (Araújo et al., 1998; Yan et al., 1995a). The genotype x P level interaction was not significant for P uptake rate. The rather small increase of P uptake rate with the application of P may suggest that P uptake rate is mainly under genotype control.

The increase in the amount of N_2 fixed was quite large with TSP application in both soils (Table 2.4) confirming the high P requirement of legumes in the N_2 fixation process (Vance, 2001; Hoshikawa, 1991). The variability of N_2 fixation of the genotypes at low P suggests the potential for identifying genotypes with high N_2 fixation potential under low soil-P conditions (Vadez et al., 1999). Levels of N_2 fixation recorded were similar to those reported by Sanginga et al. (2000), Eaglesham et al. (1977), and Awonaike et al. (1990), but lower than the maximum reported in cowpea in Western Australia (Peoples and Craswell, 1992). The significant relationship between N_2 fixed and total P uptake of the plant ($r = 0.51$, $P < 0.001$) suggests that the genetic differences for N_2 fixation under P deficiency were most likely due to differences in P uptake efficiency. This is agreement with the previous reports that genotypic differences in N_2 fixation under P deficiency conditions in common bean could be attributed to differences in P uptake ability (Pereira and Bliss, 1989; 1987). The differential response of N_2 fixation observed with PR application suggests the possibility to select cowpea genotypes that can grow well under P-deficient soil conditions of SC.

The low negative N balance, suggest that the bulk of N_2 fixed was only translocated into grain, rather than the shoot and therefore not likely to assure a positive balance to succeeding crop. We should point out that the N balance herein is only based in the total amount of fixed rather than total N uptake of the plant. Therefore, the potential of genetic improvement to match high yielding genotype with positive N balance exist within the cowpea genotypes as previously reported by Sanginga et al. (2000) for a location in the derived Savanna of Nigeria. Cowpea genotypes IT90K-59 and Dan´Ila had high a N_2 fixation but resulted in negative N balances. These two P-efficient genotypes would be most desirable in fertilizer-input systems where the use of P fertilizer is required to improve their N turn over. In contrast, IT89KD-391 had a high N_2 fixation and resulted in positive N balance at both locations implying that the bulk of N_2 fixed was not completely removed with the grain but remained in the crop residues. At the farmer level, this genotype combining responsiveness and efficiency characteristics can represent the best genotype to cultivate in N and P-deficient soils in SC.

Conclusion

This study has confirmed that cowpea genotypes differ in their ability to take up P from low and sparingly available soil P sources. This ability was related to efficient P uptake of the root. The P-efficient genotypes IT90K-59 and IT89KD-391 were able to acquire P from PR more readily. The improved P nutrition enhanced N_2 fixation but resulted in low N turnover on the basis of N_2 fixation. The genotypic variation for P uptake observed in this study, in combination with genotypic differences in N_2 fixation could be of significance for breeders trying to develop genotypes with a highly positive N balance and which are better adapted to conditions of low-P availability of soils in SC.

Chapter 3.

Phosphorus Benefits from Grain-Legume Crops to Subsequent Maize Grown in Acid Soils of Southern Cameroon

Abstract

Efficient uptake and use of phosphorus (P) from less available sources by grain legumes could be beneficial to subsequent rotational maize. Experiments were conducted in 2001 and 2002 to test the above hypothesis. In field experiments at two acid soil sites of SC, the effect of low-available soil P and different sources of P on grain legumes and the residual effect of P on subsequent maize were evaluated. The legumes were grown on Typic (TK) and Rhodic Kandiudult (RK) soils with 0, 90 kg P ha^{-1} as phosphate rock (PR) or 30 kg P ha^{-1} as triple super phosphate (TSP). Application of P significantly increased shoot dry weight, total N and P uptake of species and genotypes of the grain legumes on both soils. Shoot DM, P and N uptake were significantly higher on the TK than RK soil ($P < 0.05$). On the TK soil, TSP application significantly increased the shoot DM, P and N uptake by 35, 23 and 39%, respectively. Genotypes IT89KD-391, TGm 1511, TGm 1566, and IT90K-59 had higher total N and P uptake as compared to other genotypes. Shoot DM, P and N uptake were also significantly increased on RK soil, by 30, 21 and 29% relative to the control (0P), while PR application showed lower increase. Yields of maize after soybean genotypes TGm 1511, and cowpea genotypes IT89KD-391and IT90K-59 were significantly higher than those of other genotypes on the TK soil. The residual effect of legumes to the following maize increased on both soils when legumes previously received a substantial amount of TSP. The fractionation of soil P indicated the ability of genotypes TGm 1566, TGm 1511, IT89KD-391 and IT90K-59 for depleting P from NaOH fractions. In greenhouse and laboratory experiments the mechanisms responsible for the efficient uptake of P among the grain legumes were studied. The ability for P uptake in cowpea IT90K-59, IT82KD-391 and soybean genotype TGm 1511 was expressed by the high exudation of organic acid anions under P stress condition which led to high release rates of malate and citrate. High release of acid phosphatase by roots of soybean genotypes TGm 1566 under low-P condition was found as a strategy to solubilize P from less available sources. It was concluded that the residual benefit of P to maize was enhanced with P application to the preceding legume crop thus highlighting the need for legume fertilization for optimum maize yield.

Keyword*s:* Crop rotation – Organic acids – P-depletion – P-efficiency – phosphatase activity – root exudation

Introduction

Many soils in the humid forest benchmark (HFB) area in southern Cameroon with high levels of iron (Fe) and aluminium (Al) oxides have high phosphorus (P) sorption capacities that render P unavailable to plants (Eswaran et al., 1997; Menzies and Gillman, 1997; Vitousek and Farrington, 1997). On these soils, P is the most limiting nutrient for crop production (Ssali et al., 1996). However, many plant species or genotypes possess specific mechanisms allowing them to access soil P pools which are generally less available (Krasilnikoff et al., 2003; Ae and Shen, 2002; Hocking and Randall, 2001; Gardner and Boundy, 1983; Gardner et al., 1983). Earlier studies using white lupin (*Lupinus albus* L.) indicated that the release of organic acid anions from roots induced by P deficiency was an effective mechanism for the plants to acquire sparingly soluble P from soils (Gardner and Boundy, 1983). Hoffland (1992) reported that P availability of phosphate rock (PR) could be increased by rape (*Brassica napus* L.) by acidifying part of the rhizosphere through exudation of citric and malic acids. Root exudates from pigeon pea in particular piscidic acid were shown to mobilize sparingly soluble iron-bound P pools (Ae et al., 1990), while groundnut possess P-solubilizing substances in their cell wall favouring the uptake of P bound to iron oxides (Ae et al., 1996). These changes in the rhizosphere lead to enhanced P availability to the crops, and possibly to subsequent maize grown in rotation (Horst et al., 2001; Kamh et al., 1999).

In low-input cropping system of the HFB in SC, farmers usually do not apply P fertilizer to their crop because correcting the P deficiency through P fertilizer application is not practicable due to the cost of the P fertilizer necessary to overcome the high P-fixation into forms generally unavailable to plants. Therefore, the incorporation of species or genotypes that are efficient at acquiring P from less available sources may benefit subsequent maize grown in rotation (Kamh et al., 2002; Horst et al., 2001; Gardner and Boundy, 1983). In addition to a possible improvement of the P use of the cereals grown in rotation with the legume, a better N supply has been also suggested (Carsky et al., 1997; Peoples and Craswell, 1992), but also other effects including improvement of soil physical properties, nematodes suppression, high mycorrhizal infection, detoxification of aluminium (Al) may contribute to a positive rotational effect of legumes (Bagayoko et al., 2000; Horst and Hardter, 1994). However, in the acidic soils of SC, there is lack of information on the mechanisms governing efficient acquisition of P by legumes and the potential of the grain legumes to improve the P availability to crops subsequently grown in rotation.

The objective of this study was to test whether improved P nutrition of legumes lead to positive effects on maize grown in rotation and to analyse the P acquisition mechanisms of the grain legumes implicated in their P efficiency. The exploitation of genetic approaches in the acquisition of P from less available sources is emerging as alternative strategies to improve productivity in low-nutrient environments. If successful, it would, therefore, contribute to make the low-input agricultural system in the HFB in SC more sustainable.

Material and methods

Plant material

Four cowpea and soybean genotypes were used for the field experiment to evaluate the beneficial role of legumes in maize-based cropping systems in SC. Cowpea genotypes were IT82D-849, IT 89KD-349, IT 90K-59 and IT 89KD-349. For soybean, genotypes were TGm 1511, TGm 1566, TGm 1196 and TGm 125. All these genotypes were previously evaluated for their P-uptake and P use efficiency in preliminary studies (chapters 1, 2). The reference plot was maize (*Z. mays* L), cv CMS8704 obtained from the breeding program of IRAD, Cameroon. Cowpea and soybean seeds were provided from the grain-legume breeding-program of IITA, Ibadan, Nigeria

Field experiment

Field experiments were conducted between August 2001 to 2003 on two soils of SC: Abang (3° 24´N, 11° 47´E) and Minkoameyos (3° 51´N, 11° 25´E). Soils were classified as Typic (TK) and Rhodic (RK) Kandiudult for Abang and Minkoameyos, respectively (USDA, classification). The soils were similar for their silt, clay and K content but contrasted in their pH, Ca, Mg, available P and residual P, which showed lower values of all these parameters on the RK than TK soil (Table 3.1). On the other hand, the Al, OC, TN and P sorption capacity of the RK soil were higher than on TK soil. The RK soil site was intensively cropped with very short fallow periods for 30 years after forest clearing. The TK soil site was under a comparatively short intensive cropping phase after forest fallow.

Table 3.1. Physical and chemical properties of the topsoil (0 – 10 cm) of the soils of the field experimental sites in Southern Cameroon. (Mean ± SE)

Soil type	Sand	Silt	Clay	pH	C	N	Ca	Mg	K	Al
		[%]		[H_2O]	[g kg^{-1}]			[cmol (+) kg^{-1}]		
Rhodic Kandiudult	40.8 (5.3)	10.2 (1.8)	48.9 (7.1)	4.5 (0.1)	22.5 (1.9)	2.1 (0.1)	1.1 (0.1)	0.33 (0.01)	0.08 (0.01)	0.63 (0.13)
Typic Kandiudult	50.6 (1.58)	9.7 (0.85)	39.7 (1.2)	5.4 (0.1)	16.0 (1.1)	1.6 (0.05)	1.35 (0.17)	0.64 (0.06)	0.06 (0.04)	0.12 (0.02)

	P sorption	FeOx	P (Bray 1)	$NaHCO_3$-Pi	NaOH-Pi	HCl-P	Residual P
	[%]				[mg kg^{-1}]		
Rhodic Kandiudult	88.3 (3.2)	0.69 (0.04)	2.50 (0.95)	1.7 (0.29)	25.4 (3.2)	1 (0.5)	154.1 (2.3)
Typic Kandiudult	77.5 (0.3)	2.3 (0.07)	5.0 (1.20)	3.2 (0.5)	29.2 (1.2)	0.86 (0.38)	190.9 (12)

Experiments were laid out in a split block design with four replications. P fertilizers were applied on the main plots with 0 kg P, 30 kg P ha^{-1} Triple super phosphate (TSP) or 90 kg P ha^{-1} Togo phosphate rock (PR). Sub plots, measuring 4 m by 4 m, comprised the grain legumes genotypes and the maize reference plots. No N fertilizer or bradyrhizobia inoculation was applied. The legumes were sown on 24 September 2001 and 2002 with two seeds per stand, thinned to one plant one week after emergence. At flowering and grain maturity growth stages, the plants were harvested and assessed for their dry matter, grain yield, N and P were analyzed in different plants organs and grains. Only the grains were removed from the field and maize was planted on the plots. No additional P and N fertilization was applied to maize in rotation. At grain maturity, the maize plots were harvested and shoot matter, grain yield, N and P concentration were additionally analyzed. Bulk soil samples were collected from each legume and the reference plots (control maize plots) and analyzed for their available P (available-P) according to the Mehlich (1984) procedure. The relative residual effect (RRE) of grain legumes on subsequently grown maize was calculated using the following equation:

$$\mathrm{RRE} = \frac{\text{Yield maize after grain legume} - \text{control}}{\text{Yield of maize after maize} - \text{control}} * 100$$

Pot experiments

Experiment 1

The greenhouse experiment was conducted using only on a RK soil taken at 0 – 10 cm profile depth. The soil was air dried and sieve to 4 mm pore and 2.5 kg was used per pot. Only the grain legumes were used for this experiment (cowpea and soybean genotypes). Genotypes were grown in plastic pots for 8 weeks and the experiment was established in the greenhouse of the International Institute of Tropical Agriculture, Cameroon (IITA-Cameroon). Seeds of cowpea and soybean genotypes were pre-germinated for three days and four seeds were sown per pot. One week after planting, seedlings were thinned to two plants. The experiment was laid out in a factorial randomized complete block design with four replications. The treatments were genotypes and P-fertilizer application. Three P levels 0 kg ha^{-1} (0P), 90 kg P ha^{-1} as phosphate rock or 30 kg P ha^{-1} as triple super phosphate (TSP) were used. Plants were daily watered with deionised water. At harvest (8 week after planting), soil samples were taken from the bulk soil of each pot and analyzed for their P fractions. Only the two first steps of the sequential extraction procedure ($NaHCO_3$ and NaOH) described by Hedley et al. (1982) were used to assess the capability of the different grain legumes to acquire P from less available soil-P sources. Total shoot N and P in plant tissue were determined with an ammonium-sensitive electrode (Powers et al., 1981) and colorimetrically according to Motomizu et al. (1983) methods, respectively.

Experiment 2

The nutrient-solution experiment was carried out in the growth chamber at the Institute of Plant Nutrition (IPE) of the University of Hanover (Germany). This study was conducted to investigate some of the traits that might help at understanding mechanisms for acquisition of P from less available soil P sources by different legumes. The nutrient solution had the following concentration in µM: $Ca(NO_3)_2$ 1000; K_2SO_4 275; $MgSO_4$ 325; H_3BO_3 8; $ZnSO_4$ 0.4; $MnSO_4$ 0.2; $CuSO_4$ 0.2; $(NH_4)_6Mo_7O_{24}$ 0.1, FeEDDHA 40. Seeds of cowpea and soybean genotypes were pre-germinated in peat for 3 days before being transplanted into the nutrient solution. Seedlings were supplied with 0 or 100 µM P and cultured at 18/6 day/night time at the temperatures of 28/20° C. The light intensity at plant height was 270 µM.m^{-2} s^{-1}. When the first symptoms of P deficiency appeared on plants grown without P supply, root exudates were collected and analysed for their different organic acids anions. Then, the root surface acid phosphatase activity, dry matter and P concentration were determined.

Organic acid collection

Intact plants from the nutrient solution experiment were transferred to 500 µM $CaCl_2$ solution for 4 hours to collect the root exudates. The $CaCl_2$ solution containing the exudates were immediately filtered through 0.02 µm membranes and then passed first through a cation and subsequently through an anion exchange-resin which retained the organic acid anions in a cold room (4° C). The anions exchanger was eluted with 18 ml (8M) formic acid and then evaporated to dryness. The residue was then dissolved in 1 ml of 10 mM perchloric acid and 50 µl of sub-sample was injected and analysed for their different organic acids by HPLC (Kroma System 3000, Kontron Instruments, Munich, Germany) at 425 ηm.

Root surface phosphatase activity

The root surface phosphatase activity (Acid phosphomomoesterase EC 3.1.3.2) of intact roots was measured with the artificial substrate *p*-phenyl phosphate (pNPP) as described by Antibus and Lessica (1990). Fresh root sample of 0.50 g was placed into 2.0 ml of 50 mM citrate buffer (pH = 5.0) to which 0.5 ml of *p*-NPP was added. Samples were then incubated at room temperature for two hours. At the end of the incubation period, a 0.5 ml sub-sample mixed with 2 ml of 0.5M NaOH was added. Finally, the concentration of p-nitrophenol (*p*-NP) was measured at 410 *hm* in a spectrophotometer. Roots were rinsed with distilled water and oven-dried at 80° C for 72 hours for dry weight determination.

Root length determination

The root length was measured by spreading the entire root system of two plants on a plate and scanning using Winrhizo pro2003 (Win Mac, Regent Instruments Inc, Quebec, Canada). The root length was then calculated using the same software based on the Tennant Method (Tennant, 1975). Prior to root-length measurement, the scanner area was set at 21x 29 cm for standardization of the scanner areas, which generally change with samples and affect the calculation of the length.

Data analyses

Data were analyzed using SAS 8.2 (2001). The Mixed Model “Proc Mixed” and the General Linear Procedure “Proc GLM” were used to run the ANOVA for the field and pot experiments, respectively. If the F test revealed a significant effect, the Least significant difference (LSD) test was run to compare the means. Regression and correlations analysis were used to relate pairs of variables.

Results

Field experiments

Growth and development of legumes preceding maize

The shoot dry matter, shoot P and N uptake of the legumes preceding maize grown in rotation differed significantly between the sites (Table 3. 2) with higher values on TK as compared to the RK soil. P application resulted in a highly and significant increase in all the three above listed parameters ($P < 0.0001$).The shoot N-uptake also significantly differed among the grain legumes genotypes ($P = 0.02$). The soil type x genotype interaction was significant for shoot growth, P and N uptake. However, Genotypes x P level interaction was not significant ($P > 0.05$). As well, the soil type x P level interaction was not significant.

Table 3.2. F values for analysis of variance of growth, P and N uptake of legume genotypes preceding maize in rotation on Typic and Rhodic Kandiudult soils in Southern Cameroon

Source of Variance	Shoot dry weight	P uptake	N uptake
Soil type (S)	48.60 $^{(***)}$	3.64 $^{(*)}$	15.2 $^{(***)}$
Genotype (G)	14.09 $^{(***})$	6.45 $^{(***)}$	2.27 $^{(*)}$
P application (P)	25.65 $^{(***)}$	22.63 $^{(***)}$	16.5 $^{(***)}$
S x P	ns	ns	ns
S x G	2.66 $^{(*)}$	ns	4.2 $^{(**)}$
C x P	ns	ns	ns
S x G x P	ns	ns	ns

Ns: not significant, *, **, ***, significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, probability respectively.

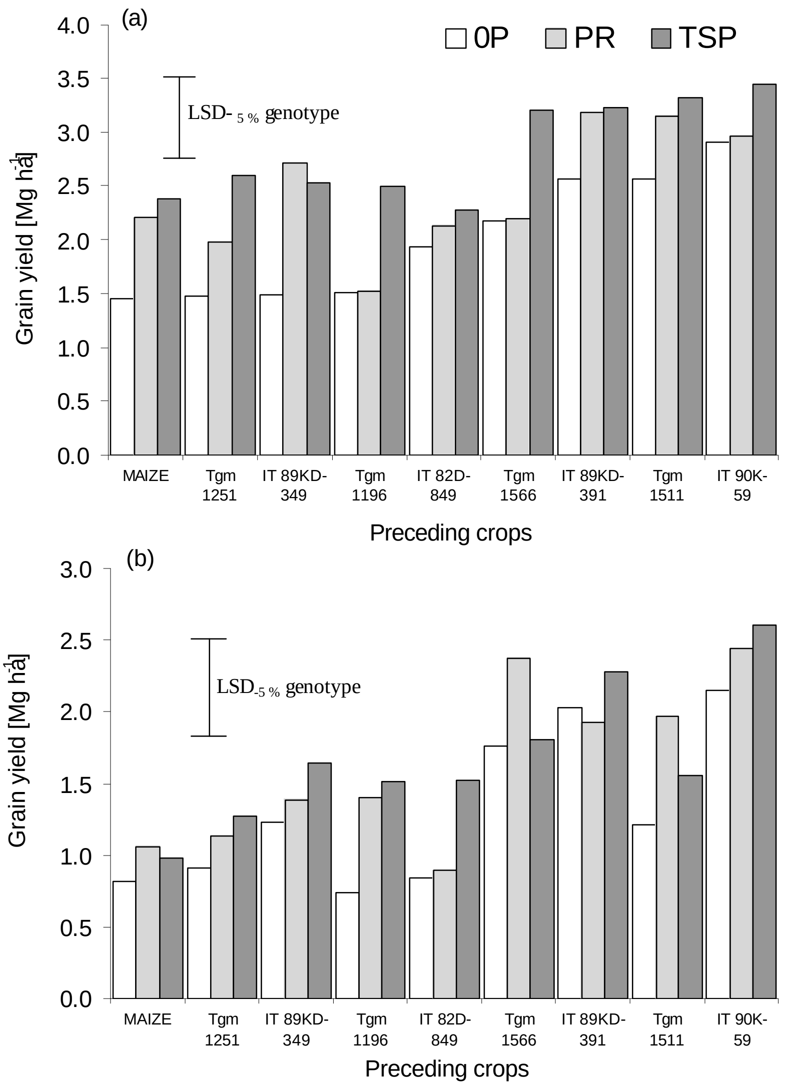
Effect of preceding grain legumes and P fertilizer application on maize grown in rotation

Maize grain yield

Maize was grown after the harvest of the grain legumes to verify whether P-efficient legumes were beneficial to the subsequently grown cereal crops. As expected from the soil properties data, maize yield was significantly lower on the RK soil indicating that P deficiency was more acute on this soil than TK soil. P application through legume residues resulted in a highly and significantly increases in maize grain yield on TK but not on the RK soil (Fig.3. 1a, b). The maize grain yield after P recycled from residues of the genotypes IT90K-59, TGm 1511 and IT90KD-391 was significantly higher than after maize as preceding crop. In contrast, low rotational effects to maize yield by genotypes TGm 1251, IT89KD-349, TGm 1196, IT82D-349 and TGm 1566 were observed.

On TK soil, except for TGm 1511, the three other genotypes: IT90K-59, TGm 1566 and IT90KD-391 showed a significantly and positively beneficial effect to maize grown in rotation. The recycling of P on plots where legumes were previously fertilized with TSP resulted in a significant increase in maize grain yield on the TK and RK soil ($P < 0.05$). Out of the eight legume genotypes, TGm 1566, IT89KD-391, TGm 1511 and IT90K-59 showed the highest and significant beneficial rotational effect to the subsequent maize on the TK soil. On RK soil, except TGm 1511, the three other genotypes listed above also had the highest and significant beneficial effect on maize grown in rotation (Fig. 3.1).

The recycling of P through grain legumes which were fertilized with PR (90 kg ha^{-1}) generally resulted in lower increase in maize grain yield on RK soil, except for TGm 1566 and IT90K-59.



(a)

F test	
Genotype (G)	4.6 (***)
P application (P)	9.9 (***)
G X P	ns
CV (%)	35

(b)

F test	
Genotype (G)	5.3 (***)
P application (P)	3.2 (*)
G X P	ns
CV (%)	36

Fig. 3.1. Effect of preceding of maize grain legumes and P fertilizer sources (0P, PR: phosphate rock, TSP: triple super phosphate) on grain yield of subsequently grown maize in rotation. Field experiment on Typic (a) and Rhodic Kandiudult (b) soils in Southern Cameroon

Yield of the subsequent maize grown in rotation significantly correlated with shoot P uptake on both soils (Fig. 3.2).

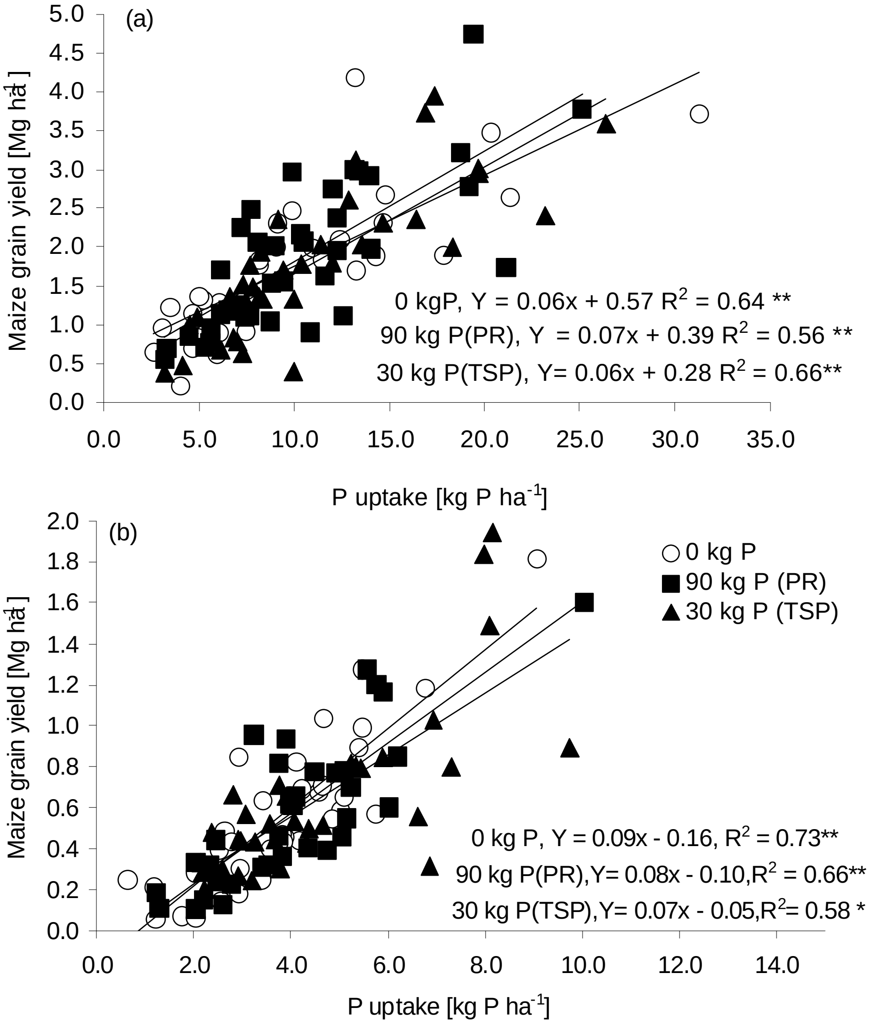


Fig. 3. 2. Relationship between total shoot P uptake and grain yield of maize grown in rotation with different grain legume genotypes on a Typic (a) and a Rhodic Kandiudult (b) soil of Southern Cameroon and fertilized with different P sources.

Shoot dry matter

Relative residual effects (RRE) of legumes on maize shoot dry matter production showed little positive rotational effects on plots on which P was recycled from grain legumes without any fertilization (Fig.3.3). On the TK soil, genotypes TGm 1566 and IT90K-59, TGm 1511, IT89KD-391 (Fig.3.3a) had a positive and significant rotational effect on shoot dry matter, whereas the rotational effect of the other legume genotypes to the maize was small as compared to the control plant maize, at the soil P level. On the RK soil, only the genotypes IT89KKD-391, TGm 1566, and TGm 1511 had higher than the control maize positive rotational effect to the subsequent maize (Fig. 3.3d). The recycling of P through crop residues of legumes fertilized with PR or TSP resulted in a significant increase in

RRE of grain legumes on shoot dry matter of maize grown in rotation on both soils. Increases were, however, higher on TK than on RK soil (Fig.3.3 b, c, e, f). Among the grain legumes, the RREs particularly from the genotypes TGm 1566, IT89KD-391, TGm 1511 and IT90K-59 were significantly enhanced when P was recycled from legume residues previously fertilized with PR and TSP on RK soil (Fig.3.3 b, c). On TK soil, the genotypes TGm 1566, IT89KD-391 and IT90K-59 consistently increased their RRE on maize grown when the legumes were pre-fertilized with PR and TSP application (Fig.3.3 e, f). For the other genotypes the RRE was small or even strongly negative (cv TGm 1251).

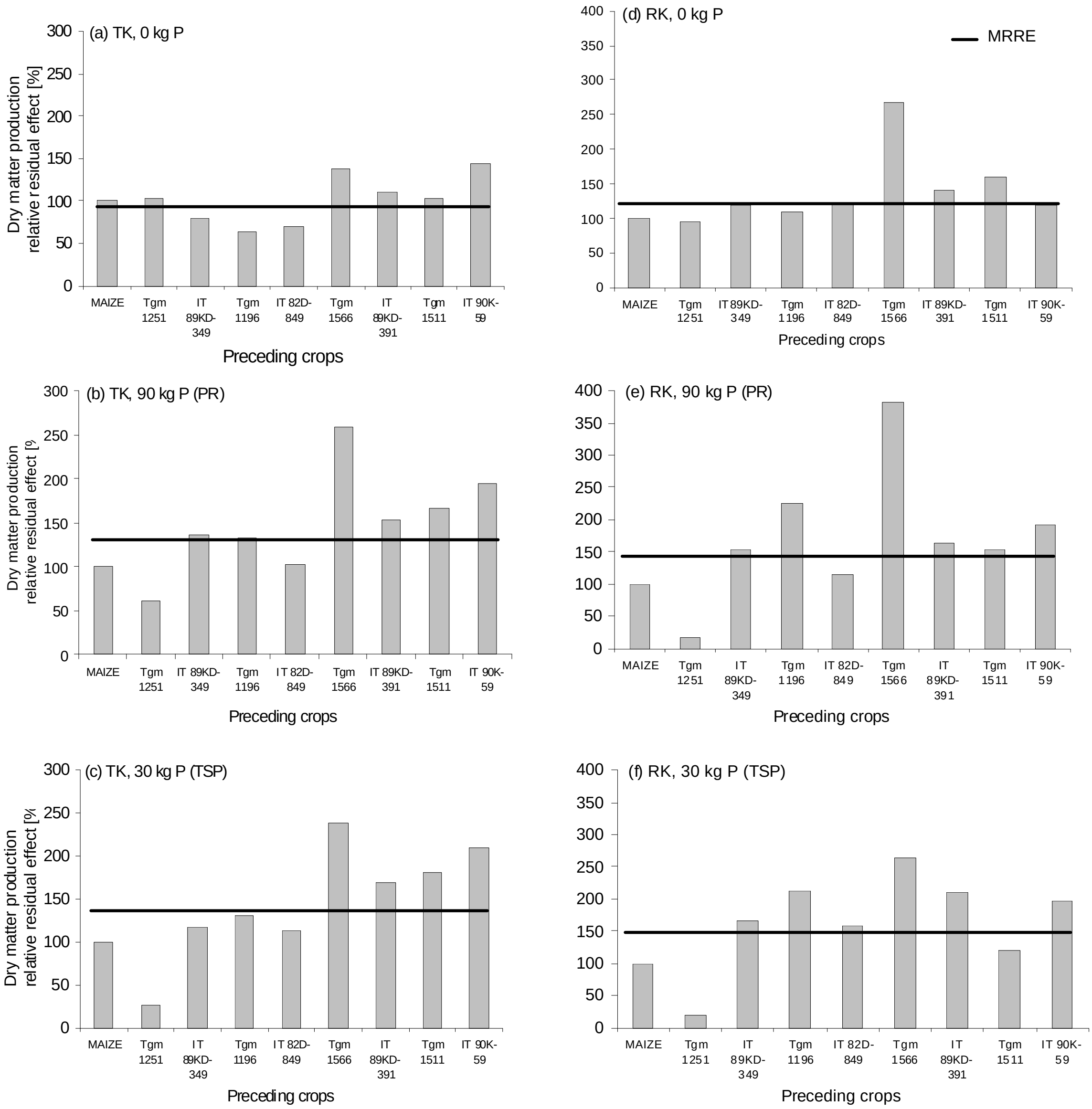


Fig. 3. 3. Relative residual effects of grain legumes on shoot dry matter increase of maize subsequently grown in rotation as affected by P fertilizer sources on a Typic (a, b, c) and a Rhodic Kandiudult (d, e, f) soil in southern Cameroon (0P, PR: phosphate rock, TSP: triple super phosphate). MRRE: mean relative residual effect.

Maize shoot P uptake

Figure 4 depicts the relationship between the maize P uptake and P application recycled from legume residues previously not or fertilized with PR or TSP. The recycling of P through legume residues, previously non-P fertilized or fertilized with PR and TSP was partly responsible for the positive effect of the legumes to the subsequently grown maize on TK soil (Fig. 3. 4a, b, c). On the RK soil, there was only a significant trend when P was recycled through legume residue fertilized with TSP (Fig. 3.4 d, e, f). However, in any case, the relationships explained less than 50% of the observed rotational effect indicating that other soil factors have influenced the rotational effect on maize dry matter production.

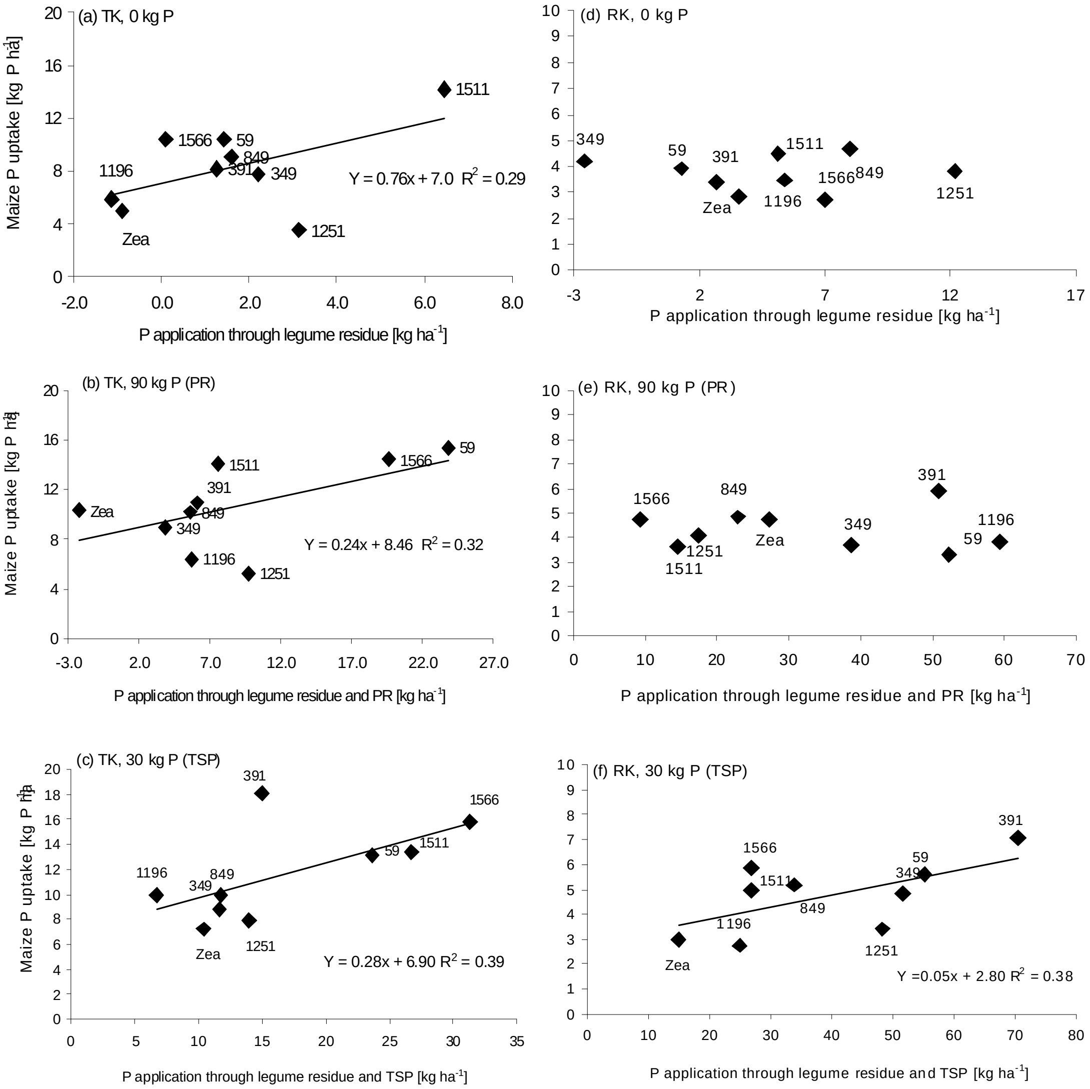


Fig.3.4. Relationships between maize P uptake and phosphate application through crop residues of grain legumes genotypes fertilized with different P fertilizer sources on a Typic (a, b, c) and a Rhodic (d, e f) Kandiudult soil of southern Cameroon.

Greenhouse experiment

Among the grain legumes, the cowpea genotypes IT89KD-391 and IT90K-59 were more P-efficient as indicated by their high P uptake (Table 3.3). This can be explained by their ability to deplete inorganic P from the NaOH_P_i fraction on the RK soil. The soybean genotypes TGm 1511and TGm 1566 were more efficient in using the labile P_i ($NaHCO_3$) fraction. In contrast, particularly genotype IT82D-849 showed a much lower depletion rate of the $NaHCO_3$ and NaOH_P_i fractions. Application of PR and TSP increased the total P uptake of most genotypes. Accordingly, the depletion of $NaHCO_3$_Pi fraction of these genotypes increased with PR and TSP application. P depletion of the NaOH fraction was unaffected with application of TSP except for TGm 1251, TGm 1511 and IT82D-849. In contrast, P depletion of NaOH P_i fraction increased with PR application especially for the genotypes TGm 1196, TGm 1566, IT82D-849, IT89KD-349, and IT89KD-391.

Table 3.3. Phosphorus uptake and depletion of inorganic extractable P fractions in the rhizosphere soil of soybean and cowpea grown in a greenhouse in pots filled with a Rhodic Kandiudult soil of Southern Cameroon) as affected by P fertilization. (0P, control; PR: phosphate rock, TSP: triple super phosphate)

	P uptake [mg plant^{-1}]			P depletion [mg kg^{-1}soil]					
				NaHCO3_Pi			NaOH_Pi		
Genotype	0P	PR	TSP	0P	PR	TSP	0P	PR	TSP
TGm 1251	12.2	13.0	13.6	4.2	0.9	2.0	3.2	3.7	4.7
TGm 1196	13.0	13.0	13.7	3.0	1.1	2.7	2.9	2.7	3.2
TGm 1511	13.9	15.5	17.4	3.9	5.0	3.4	5.3	3.8	5.3
TGm 1566	14.1	17.1	18.6	3.4	3.6	6.2	5.5	5.9	3.1
IT82D-849	14.2	16.9	17.8	-0.2	0.5	2.7	0.5	3.7	3.0
IT90K-59	15.4	19.8	19.9	2.3	6.8	6.3	6.6	5.8	5.8
IT89KD-349	15.6	15.8	16.9	3.1	2.9	5.9	3.4	4.3	3.1
IT89KD-391	16.0	19.6	18.8	2.6	3.3	3.0	7.5	8.7	7.3
LSD-1		1.09			0.39			0.98	
LSD 2		2.30			0.41			1.97	

LSD-1 for comparing P levels within cowpea genotypes
LSD-2 for comparing legume genotypes within one P level
0P = 0 kg P ha^{-1}; PR = 90 kg P ha^{-1} rock phosphate, TSP = 30 kg P ha^{-1} triple super phosphate

Nutrient solution experiment

Nutrient solution experiments were conducted to investigate whether the overall positive rotational observed by some grain legumes could be attributed to their root properties such as exudation of organic acid anions, root length, or root ecto-enzymes (phosphatase). After 10 days growth, plants in solution without P showed first symptoms of P deficiency. These were more pronounced in cowpea genotypes, which developed purple coloration of old leaves. Compared to P-treated plants P deficiency resulted in 45 and 16% reduction in shoot dry weight of cowpea and soybean genotypes, respectively (Table 3.4). The root dry matter was, however, not affected by P deficiency for most of the genotypes, which is reflected by an increase in the root /shoot ratio. The total shoot P content was significantly reduced by the P deficiency ranging from 56% for the cowpea and 91% for the soybean genotypes. Among the genotypes IT89KD-349, IT82D-849 and TGm 1196 were mostly affected by P deficiency. Under the P-limiting conditions, IT90K-59, IT89KD-391, TGm 1511 had a significantly higher P content than the other genotypes. The root/shoot ratio of plants grown under P deficiency significantly increased compared to the plant with sufficient P supply. P deficiency resulted in an increase in root length for most of the genotypes except IT90K-59, IT89KD-349 and IT82D-849.

The root surface phosphatase activity of plant without P treatment was significantly higher than that of those supplied with 100-µmol P supply except for the lines IT89KD-349 and IT90K-59 (Table 3.4). This was especially observed for the genotype TGm 1566 with high phosphatase activity under P-deficiency conditions. The relationship between P uptake and phosphatase activity was, however, weak.

Table 3.4 Shoot dry matter, P uptake, and root length and root-surface phosphatase activity of grain legume genotypes grown in nutrient solution as affected by P supply in nutrient solution after 10 days growth

	Shoot dry matter [g. Plant^{-1}]		P content [mg.plant^{-1}]		Root/shoot ratio dwt root.(dwt shoot)$^{-1}$		Root surface phosphatase activity [nmol h^{-1} (cm root)$^{-1}$]		Root length [m.plant^{-1}]	
Genotype	0µM P	100µM P	0µM P	100µM P	0µM P	100µM P	0µM P	100µM P	0µM P	100µM P
IT 89KD-349	0.27	0.66	0.90	10.4	0.19	0.09	0.90	0.20	6.6	11.0
IT82D-849	0.26	0.30	0.40	4.7	0.15	0.09	1.50	1.10	5.8	4.7
IT89KD-391	0.52	1.17	1.30	13.0	0.08	0.04	2.40	0.70	4.6	9.7
IT90K-59	0.35	0.70	2.90	6.6	0.14	0.09	0.92	0.54	8.6	9.9
TGm 1196	0.15	0.17	0.30	3.7	0.29	0.19	2.60	1.50	8.9	5.0
TGm 1251	0.16	0.18	0.70	2.7	0.19	0.11	3.15	1.4.0	7.7	4.1
TGm 1511	0.20	0.22	1.30	3.0	0.22	0.13	3.10	1.75	8.3	4.3
TGm 1566	0.16	0.24	1.00	4.6	0.21	0.12	4.80	2.80	7.1	3.7
LSD-1	0.04		0.80		0.009		0.60		1.12	
LSD-2	0.13		2.66		0.02		2.10		3.58	

LSD-1 for comparing P levels within cowpea and soybean genotypes.
LSD-2 for comparing legume genotypes within one P level

Succinate, oxalate, malate and citrate were the most abundant organic acid anions exuded from the roots (Table 3. 5). The exudation rate of other organic acid anions was lower (data not shown). In response to P deficiency, the release of malate was significantly enhanced in the genotypes ITKD-391, IT90K-59, and TGm 1511. Within the P-efficient genotypes IT82KD-391 and IT90-59 had the highest release of malate. With respect to citrate exudation, most genotypes except IT90K-59, IT82KD-391 and TGm 1511 had a significantly lower exudation rate under low P than P-treated plants. The exudation rate of malate was 10, 4, 6 and 26 times higher than that of citrate respectively for the genotypes IT90K-59, IT89KD-391, TGm 1511 and TGm 1566 in the P-deficient plants.

Exudation of succinate and oxalate were also mostly stimulated by P deficiency with the highest increases in genotypes IT89KD-391.

Table 3. 5. Exudation of organic acid anions by different grain legume genotypes as affected by P

	Malate		Citrate		Succinate		Oxalate	
	[ρmol h^{-1} (cm root)$^{-1}$]							
Genotype	0µM P	100µM P	0µM P	100µM P	0µM P	100µM P	0µM P	100µM P
IT 89KD-349	1.24	1.03	0.20	0.10	6.80	0.80	1.90	1.35
IT82D-849	1.20	0.90	0.10	1.40	2.90	0.80	1.14	0.65
IT89KD-391	7.15	1.25	1.80	0.90	16.80	4.10	10.55	1.65
IT90K-59	5.70	3.55	0.55	0.30	6.41	0.35	2.07	2.05
TGm 1196	2.40	4.03	0.04	0.20	0.90	2.00	1.70	1.45
TGm 1251	1.10	3.25	0.03	0.09	0.80	2.00	3.35	2.75
TGm 1511	5.08	4.15	0.85	0.07	3.50	0.50	3.20	1.35
TGm 1566	2.90	1.55	0.11	0.25	7.00	5.30	4.35	6.25
LSD-1	0.95		0.20		1.36		1.35	
LSD-2	3.08		0.60		4.52		4.25	

LSD-1 for comparing P levels within cowpea genotypes
LSD-2 for comparing legume genotypes within one P level
supply after 10 days growth in nutrient solution

Discussion

The results showed that P-efficient cowpea and soybean genotypes grown as pre-crop to maize produced positive rotational effects through recycling of P from their crop residues. The substantial rotational contribution of genotypes: TGm 1566, TGm 1511 for soybean and IT89KD-391 and IT90K-59 for cowpea mainly depended on their capability to take up P from sparingly available sources, especially in the NaOH-P$_i$ fractions. Our results corroborated with earlier studies done by Krasilnikoff et al. (2003), Horst et al. (2001), and

Gardner et al. (1983) who found that the high efficiency in cover crops, cowpea lines and *Lupinus albus* were related to their capability to take up P from sparingly available P sources (non-Olsen P). As with *L. albus*, and other cover-crop legumes, we showed that the capability of the efficient cowpea (IT89KD-391, IT90K-59) and soybean (TGm 1511) genotypes seem to be related to enhancement of organic acid anions exudation, mainly citrate, malate, oxalate, and succinate under P-limiting conditions. On acid soil of SC where P is strongly fixed in Fe and Al hydroxides (Menzies and Gillman, 1997), the high synthesis and release of organic acid anions, in particular tricarboxylic acids such as citrate, by the roots of P-efficient genotypes is very beneficial for several reasons (Gerke, 1992): The desorption of P bound in metal Fe/Al hydroxides will increase the soil-solution P (Jones, 1998) and the acidification of the rhizosphere due to the concomitant release of protons that can maintain a charge balance against the release of organic anions that would release and make available P to the soil solution (Kania et al., 2003; Gerke et al., 2000; Neumann and Römheld, 1999). We therefore, argue that positive rotational effects observed in genotypes IT90K-59, TGm 1511 and IT89KD-391 are attributed to their ability to desorb P from sparingly available P sources through exudation of high amounts of organic acid anions, mainly citrate.

In addition to the exudation of citrate, we observed that malate exudation was enhanced by the efficient cowpea lines suggesting that malate contributes to P mobilization in cowpea. Although, the role of malate in the solubilization of soil P is not clear, Jones and Darrah (1994) suggested that concentration at which malate can mobilize P from soils should be 10 times greater than the minimum concentration of citrate. This was clearly observed for line IT90K-59.

The exudation of succinate was high for the genotypes IT89KD-391, IT90K-59, and TGm1511 at P deficiency. In spite of the fact that succinic form weak complexes with metal ions (Nagajarah et al., 1970), the high exudation may help solubilizing P fixed by Al oxides as suggested by Gaume et al. (2001). Hue et al. (1996) observed a significant increase in the exudation of succinate in a P-deficient acid-soil tolerant maize variety compared to Al sensitive genotypes.

Interestingly, TGm 1566 a P-efficient soybean genotype exuded less citrate, but had high level of phosphatase activity and longer root length under P-limiting conditions. This genotype produced the highest relative residual effect (RRE) to the following maize at both locations. Although, we did not investigate other low-P adaptation mechanisms such as long roots hair and release of phenolics compounds, the high P accumulation observed in this genotype can be attributed to an increase in the root surface phosphatase activity and root elongation. Our results are comparable to those obtained by Kamh et al. (2002). The high RRE of this genotype on maize shoot dry matter observed at both soils for these genotypes may support the concept that the rotational benefit is due to rapid mineralization of inorganic P from legume crop residues and its high uptake rather than the direct effect of carboxylic acids (citrate) on soil P availability.

Our study revealed that maize grown in rotation might particularly benefit from P in crop residues of preceding legumes which were previously fertilized with PR. This was especially observed in the TK soil but not on the RK soil. The observed enhancement in maize yield was reflected in an improvement of the grain legume growth owing to PR application (chapter 1, 2). In the derived savanna of Africa Vanlauwe et al. (2000a) found that application of PR to *Mucuna* and *Lablab* significantly improved the yield of maize following the legumes. The positive effect of the pre-crops fertilized with PR confirms the ability of the efficient pre-crops to obtain P from sparingly available P sources through citrate exudation since most of the soil P is Ca, Al and Fe-bound and the role of citrate in the chelation of associated metals is well established (Marschner, 1995; Dinkelaker et al., 1989). The acidification effects produced in the rhizosphere of P-efficient plants as a result of high exudation of protons concomitant with the organic acid anions leads to high dissolution of the P in PR and make it available to the subsequently grown maize through crop-residue mineralization. In addition, as a consequence of the solubilization of PR, there is an increase of the Ca and Mg concentrations of the rhizosphere solution which may counteract the toxic effect of Al that may interfere with and reduce the P uptake of maize (Fardeau and Zapata, 2002). The increase of soil-available P in the rhizosphere of legumes observed at the harvest of the grain legumes is in agreement with the results from Vanlauwe et al (2000a, b), who found an increase of Olsen-P in the topsoil by cropping with mucuna and lablab. This indicates that in the presence of PR, legumes do not only increase the immediately available P pools but also the less available pool that will contribute to maize P uptake. The lack of response of maize to soluble P applied to the pre-crop on RK soil suggests that P nutrition was not the only factor controlling yield production of maize on these soils. Other soil factors such Al content might have interfered and affected the growth and P uptake of maize (Aune and Lal, 1997).

Our results clearly demonstrated that the application of water-soluble P fertilizer to the preceding grain legumes crops lead to an increase (on average 50%) in the RRE of the legumes on the maize shoot P uptake on both soils. The increase in the rotational effect observed on the subsequently grown maize in P uptake could be explained by the efficient uptake of the fertilizer P from the soil solution that was sufficiently supplied and to a better remobilization into shoot organ for dry matter production. The rapid mineralization of legume residues will release the available P into the soil solution which is then fully available to the less P-efficient maize crop. This clearly indicates the need for maintenance of application of soluble P fertilizer in addition to legumes residues for sustainable maize yield production in SC. Our results are in agreement with those reported by Horst et al. (2001) in an ultisol of northern Nigeria.

Conclusion

This present study demonstrates that efficient cowpea and soybean genotypes possess different mechanisms to acquire P from sparingly available source such as exudation of organic acid anions or the release of acid phosphatase which can solubilize P from different pools. Most importantly, improvement of P use by leguminous plants leads to a positive rotational effect on the maize grown in rotation. The incorporation of grain residues only, however, recycles only a small amount of P for the growth of the following maize especially for the TK soil. The alternative solution is the combination of water-soluble P or cheaper and sparingly available P forms such as PR with legume organic manure and this was shown on TK soil.

Chapter 4

Effects of Combined Aluminium and P-Deficiency Stress on Aluminium Resistance of Cowpea

Abstract

Plants growing in acid soils suffer both phosphorus (P) deficiency and aluminium (Al) toxicity-stress. Selection or adaptation of genotypes to either P deficiency or Al toxicity has been sometimes unsuccessful because these two soil-factors often interact. Two experiments were conducted (i) to evaluate 7 cowpea genotypes for Al resistance and (ii) to study the combined effect of P deficiency and aluminium stress on growth, uptake, and organic acid-anion exudation of two genotypes of contrasting Al resistance selected from the first experiment. The relative root inhibition by 30 µM Al ranged from 14 to 60%, and there were highly significant difference among the genotypes. Al significantly induced callose formation particularly in Al sensitive genotypes. Results from the Al stress and P deficiency interaction indicated that P accumulation was significantly reduced (50 and 80%) by Al application for both, the Al resistant (IT89KD-391) and the Al sensitive (IT89KD-394) genotype. Al supply significantly enhanced malate release of root apices of both genotypes. However, the exudation rate was significantly higher in the Al resistant genotype. Only in this genotype P deficiency also significantly increased malate exudation. Citrate exudation of the root apecies was lower than malate exudation by a factor of about 10 and was primarily enhanced by P deficiency in both genotypes, but more in the Al sensitive genotype. Al treatment only enhanced citrate exudation of P-sufficient plants of the Al resistant genotype. It is concluded that Al resistant genotype is better adapted to acid Al toxic and P-deficient soils than the Al sensitive genotype since both, malate and citrate exudation were more enhanced by combined Al and P-deficiency stress.

Keywords: Aluminium – Al x P interaction – cowpea – organic acid – P deficiency – P nutrition

Introduction

Cowpea (*Vigna unguiculata* (L) (Walp) has been increasingly introduced into the cropping system of the humid forest benchmark (HFB) area in southern Cameroon. However, many soils of the humid forest have a low inherent level of basic cations and high levels of exchangeable aluminium (Al^{3+}) (Menzies and Gillman, 1997). Exchangeable Al^{3+} concentration above 1 cmol (+) kg^{-1} in soils is often toxic to plants (Kochian, 1995; Ritchie, 1995; Andersson, 1988; Haug, 1984). Aluminium (Al) reduces root growth, alters nutrient availability in the rhizosphere, and affects nutrient uptake and translocation by plants (Matsumoto, 2000; Miyasaka et al., 1991; Rengel and Robinson, 1989; Roy et al., 1988; Taylor, 1988). In addition, Al increases P fixation by precipitation of Al-P complexes thus contributing to low P availability (Macklon et al., 1994; Foy, 1984). Soil P availability during plant seedling development is an important determinant of growth, N_2 fixation, and grain yield formation for legumes (Vance, 2001).

Generally, Al resistance in plants, including legumes, may be associated with an alteration in rhizosphere pH and release of organic acid anions (Matsumoto, 2000; Kochian, 1995; Delhaize and Ryan, 1995). Another specific mechanism exhibited by some plants to detoxify internal Al is by increasing the production of compounds that chelate Al intracellularly (Ma et al., 1998). These responses reduce Al^{3+} availability in the rhizosphere thereby reducing Al accumulation in tissues (Yang et al., 2000; Delhaize et al., 1993). However, all these beneficial changes might be affected by Al x P interaction, commonly observed on acid soils including soils of the humid forest of SC.

Earlier research work (Nian et al., 2003; Gaume et al., 2001; Tan and Keltjens, 1990a, b) showed that the supply of P in solution containing toxic levels of Al led to increased root Al concentration for the Al resistant and sensitive soybean and sorghum genotypes, respectively. However, citrate exudation was exclusively induced by Al stress, instead of P deficiency as previously suggested (Hoffland et al., 1989; Gardner et al., 1983). Furthermore, Ligaba et al. (2004), demonstrated that both P and Al application significantly induced exudation of citrate and malate from roots of rape plant. No such data is available for cowpea and on acid soils. The present study therefore aimed at (i) evaluating different cowpea genotypes for Al resistance and (ii) determining the effect of combined Al stress and P deficiency on P nutrition and organic acid anion exudation of two genotypes contrasting in Al resistance.

Materials and methods

Plant Materials

Seeds of cowpea (*V. unguiculata* L. (WALP).), genotypes with different origins were collected from the breeding program of the International Institute of Tropical Agriculture (IITA) Ibadan (Table 4.1). Seeds were surface-sterilised (3% hydrogen peroxide and 95% alcohol for 5 and 2 min, respectively), extensively washed with tap water and germinated in peat moss for 3 days at 25° C. The commercial peat used was limed to pH 5.5 – 6.0.

Table 4.1. Genotypes used and their origin

Genotype	Origin of germplasm
IT82D-849	Nigeria
IT 89KD-349	Nigeria, Kananandndo, Tanzania
IT 82D-716	Nigeria, Tanzania
IT 81D-715	Nigeria, Tanzania, USA
IT 90K-59	Nigeria, Tanzania
IT 89KD-391	Nigeria, Tanzania
Dan ‘Ila	Nigeria

Experiment 1. Screening for Aluminium tolerance

Experiments were carried out in the growth chamber at the Institute of Plant Nutrition (IPE), University of Hanover (Germany). Uniform seedlings were pre-cultured in a 22 L plastic pot containing 5 *mM* $CaCl_2$ and 20 *µM* H_3B0_3 for 2 days and adapted to low pH (4.3) at 28/18° C (days/night) 18/6 h (light/dark) cycles.

The initial pH of the nutrient solution (pH ± 6.0) was gradually decreased to 5.5, 5.0, and finally to 4.3. Thereafter, the pH was kept constant at 4.3 using an automatic titration device (0.01 N HCl and K0H). Plants were adapted to low pH for 24 hours and then treated with 30 or 0 *µM* Al ($AlCl_3$). Prior to the Al treatment, the length of the main root was measured and recorded. Twenty-four hours after Al application, the length of the main root was measured again. The relative root-growth inhibition-rate (RI) was calculated using the following formula:

$$\text{RI} \;=\frac{\text{Root length control} - \text{Root length Al treated}}{\text{Root length control}} *100$$

Callose formation

Three 1-cm length root tips were collected in 95% alcohol and kept in liquid nitrogen. Thereafter, root tips were washed with deionised water and transferred to 1.5-ml Eppendorf cups containing 95% ethanol. Roots were homogenised in 500 µL 1 *M* NaOH for two min at 20/s using a mixer mill (Retsch MM 200, Haan, Germany) and incubated in a water bath at 80^{o} C for 30 min for the extraction of callose. Callose contents in solution were determined fluorometrically (Hitachi f2000, Hitachi, Tokyo, Japan; excitation 393 nm and emission 484 nm) with decolorized aniline blue according to Kauss (1996) using Pachyman as standard.

Experiment 2. Effect of combined Al stress and P deficiency on growth and physiology of cowpea

From experiment one, two cowpea genotypes contrasting in Al resistance were selected to investigate the effect of Al stress and P deficiency on growth, P uptake and exudation of organic acid anions. The genotypes were IT89KD-391 (Al resistant) and IT89KD-394 (Al sensitive). After 3 days germination, seedlings were transplanted into 22 L pots containing the following nutrient solution (in μM) described by Horst et al. (1992): $Ca(NO_3)_2$ 1000, K_2SO_4 275, $MgSO_4$ 325, HBO_3 8, Zn SO_4 0.4, $MnSO_4$ 0.2, $CuSO_4$ 0.2, $(NH4)_6M07O_{24}$ 0.1, and FeEDDHA 40.

The experimental design was a 2x2 factorial combination in a randomized complete block design (RCB) with four replications. Factor 1 was P supply (0 and 100 μM P), and factor 2 was Al supply (0 and 30 μM Al). Two cowpea genotypes selected from experiment one were used: one Al resistant (IT89KD-391) and one Al sensitive (IT89KD-349). Plants were grown in nutrient solution for 8 days and the solution was changed twice. Prior to the

Al treatment, the pH of the nutrient solution was adjusted to 4.3 for low pH adaptation. 30 *µM* of Al ($AlCl_3$) was applied to half of the plants.

Collection of root exudates and analysis of their organic acid anion

The apical 2-cm roots of 12 intact plants were incubated in an anion resin-exchange column (16 x 14 cm) filled with 1 g of resin (AG 1-X8, 100 – 200 mesh; Bio-Rad Laboratories, Hercules, USA) and containing 2.5 ml of 500 *µM* $CaCl_3$. The incubation period was 4 hrs after which the anion exchange resin was immediately separated from the solution. The resin was then eluted in 18 ml 8*M* formic acid and elute was concentrated to dryness using a rotary evaporator (RCT 10 – 22T, Jouan, Saint Herblain, France). The residue was then dissolved in 1 ml 10 *mM* perchloric acid. The organic acids were detected by high-performance liquid chromatography (Kroma System 3000, Kontron Instruments, Munich, Germany) at 425 *hm*.

Determination of root length, Al and P concentrations

Root length was measured by spreading the entire root system of two plants on a plate and the roots were scanned using Winrhizo pro2003 (Win Mac, Regent Instruments Inc, Quebec, Canada). The scanner area was set to 21 x 29 cm for standardization of the scanner area and the root length was then calculated using the same software.

For the determination of the Al concentration and P concentrations, 0.1 g of dry mass of root or shoot (P only) was ashed overnight 480° C in an oven and dissolved in 1:3 diluted ultra pure $HN0_3$. The Al and total P concentrations were determined in solution by ICP-OES (Spektro Analytical Instruments, Kleve, Germany) after 1:20 ultra-pure water dilution.

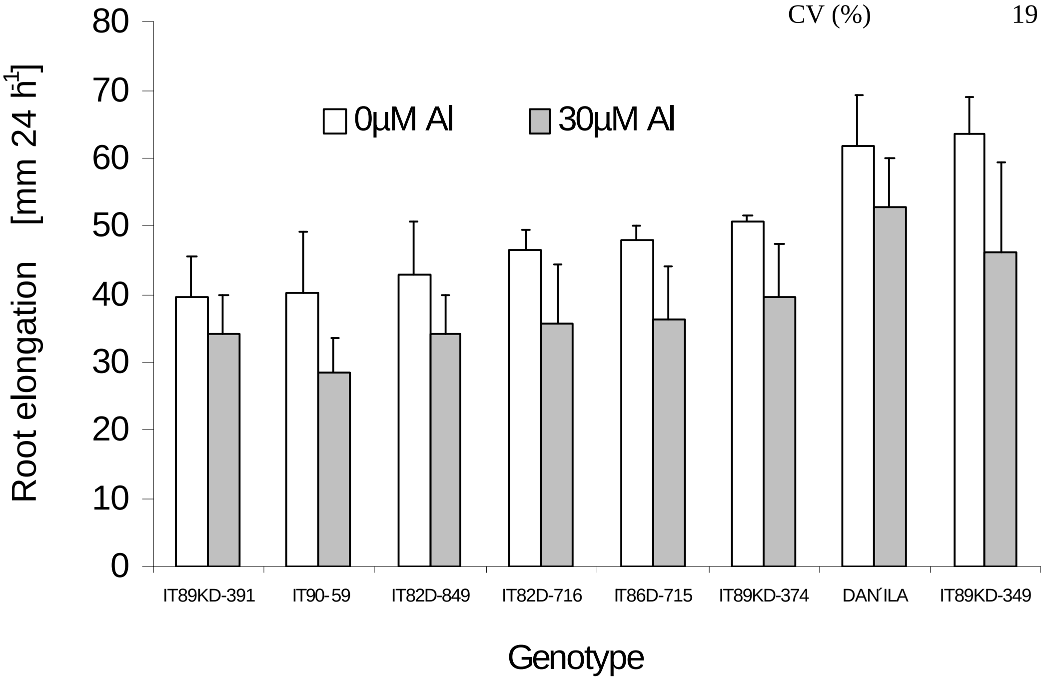
Statistical analysis

ANOVA was performed using the general linear model “PROC GLM” procedure of SAS software 8.2 (Statistical Analysis System Institute Inc, 2001). The Least significant difference (LSD) test was used for comparisons of means when the F-test showed significant treatment effects. The regression lines between pairs of variables were plotted using “PROC REG” of SAS.

Results

Screening for Al tolerance/sensitivity

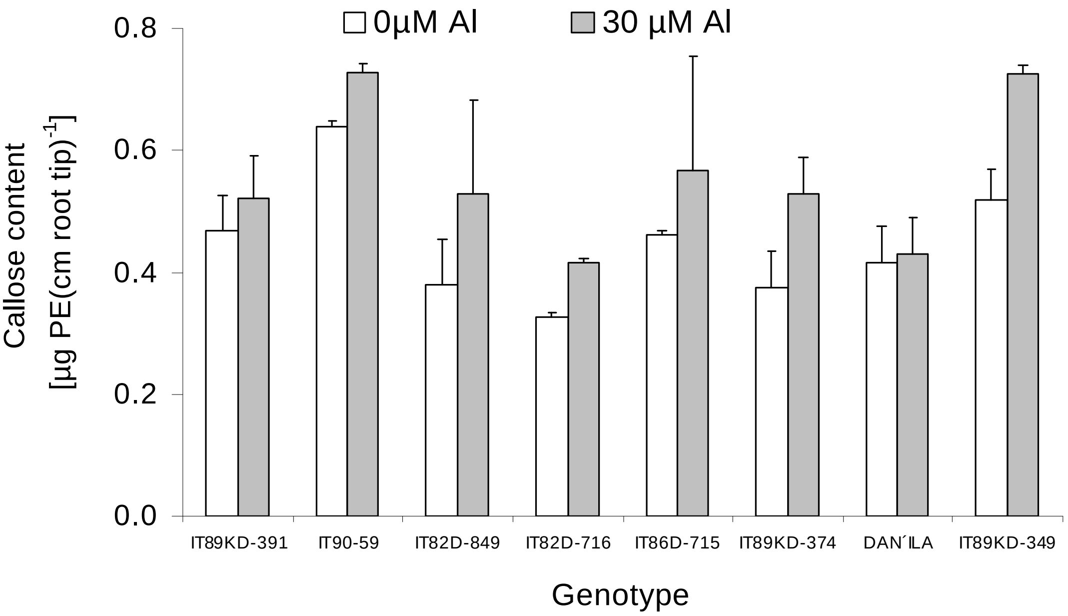
There were highly significant differences ($P < 0.0001$) in relative root-growth inhibition rate (RI) between the cowpea genotypes (Fig.4.1). Most of the genotypes showed a significant reduction in the main root growth by Al application except IT89KD-391 (Fig. 4.1). The relative Al induced inhibition of root elongation ranged from 14 (IT98KD-391) to 60% (IT90K-59 and IT89KD-349).



<u>*F value:*</u>
Al treatment (Al): 9.6 $^{(***)}$
Genotype (G) 20.3 $^{(***)}$
G x Al ns
CV (%) 19

Fig.4.1. Effect of Al supply on root elongation of different cowpea genotypes. Seedlings were subjected to 0 or 30 *µM* Al in a nutrient solution containing 0.5 *mM* $CaCl_2$ and 20 *mM* H_3BO_3 at pH 4.3. Root elongation was measured 24 hours after beginning of the Al treatment. Values are means of 12 plants. Bars show SE of 4 independent replications.

The application of 30 *µM* Al significantly induced ($P < 0.0001$) callose formation in all the cowpea genotypes tested except IT89KD391 and Dan´Ila (Fig. 4.2). The genotype x Al interaction was not significant, indicating that the formation of callose was predominantly controlled by the Al treatment rather than the genotype. In addition, there was a positive and significant relationship between callose contents of Al treated roots and the relative root-growth inhibition rate (Fig. 4.3). Based on the relative root inhibition (RI) rate and callose formation IT89KD-391 and IT89KD-349 were classified as Al resistant and Al sensitive genotypes, respectively.



<u>F value:</u>
Al treatment (Al): 9.5 $^{(***)}$
Genotype (G) 11.3 $^{(***)}$
G x Al ns
CV (%) 16

Fig.4.2. Callose content in 1-cm root tips of different cowpea genotypes. Seedlings were subjected to 0 or 30 µM Al in a nutrient solution containing 0.5 *mM* $CaCl_2$ and 20 *mM* H_3BO_3 at pH 4.3. Values are means of 12 plants. Bars show SE of 4 independent replications.

Effect of Al application and P deficiency on growth, P uptake and organic acid exudation

Shoot growth

After 10 days P deficiency symptoms appeared on old leaves of plants deprived of P in both cowpea genotypes. In genotype IT89KD-391 (Al resistant) P supply resulted in a highly significant increase in shoot dry matter ($P < 0.0004$) compared to P-starved plants (Table 2). In contrast, IT89KD-349 (Al sensitive) showed no significant increase in shoot dry matter by P application ($P > 0.05$). Comparing the two genotypes, ITK89KD-391 had a 1.4 times significantly higher shoot dry matter than IT89KD-349. Al application significantly reduced the shoot dry matter of IT89KD-349, but not of ITK89KD-391 ($P <$ 0.02). There was no significant ($P > 0.05$) Al x P interaction for shoot dry matter in both genotypes.

Table 4.2. Effect of Al and P supply on shoot growth and root length of an Al resistant and an Al sensitive cowpea genotype

		Shoot dry matter [g plant^{-1}]		Root length [m plant^{-1}]	
Main effect		IT89KD-391	IT89KD-349	IT89KD-391	IT89KD-349
	P-	0.18 b	0.16 a	1.04 a	0.63 a
	P+	0.35 a	0.23 a	1.42 a	0.61 a
	Al-	0.26 a	0.27 a	1.38 a	0.82 a
	Al+	0.30 a	0.15 b	1.08 a	0.36 b
F value					
Aluminium application (Al)		ns	14.3 (*)	ns	12.0 (*)
P application (P)		40.8 (**)	ns	ns	ns
Al*P		ns	ns	ns	ns
C.V (%)		15.4	19.0	28.12	34.14

Numbers followed by the same letter within a column are not significantly different at $P > 0.05$. (LSD test). Ns: not significant, *, **, ***, significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, probability respectively.

Root elongation

Thirty *µM* of Al application led to a significant decrease (P < 0.05) in root length of the Al sensitive IT89KD-349 (Table 4. 2). Conversely, no differences were observed for the Al resistant ITK89KD-391 confirming its high Al resistance observed in the first experiment. Phosphorus application resulted in a significant but small root-length increase for both genotypes ($P > 0.05$), although the Al resistant genotype had a significantly higher root length than sensitive. The Al x P interaction was not significant at the 5% probability level for both genotypes.

Shoot and root P uptake

Shoot P accumulation in both genotypes was significantly ($P < 0.0001$) enhanced by P application. For ITK89KD-391 and IT89KD-349, shoot P accumulation was 2.5 and 2 times higher in the P-supplied than in the P-deficient plants, respectively. Al supply significantly decreased shoot P accumulation in both genotypes (Fig. 4.4). Al supply resulted in 57 and 83% inhibition of shoot P accumulation for the Al resistant and Al sensitive genotypes, respectively (Table 4.3). Al x P interaction was not significant for both genotypes ($P > 0.05$).

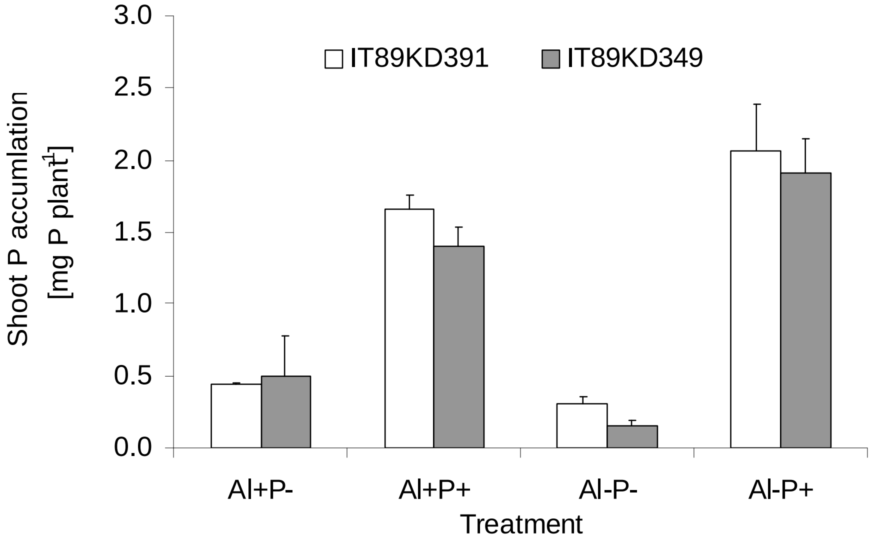


Fig. 4.4. Effect of Al and P supply on shoot P content of an Al resistant and an Al sensitive cowpea genotype. Plants were grown in complete nutrient solution for a week with 0 (P-)or 100 µM P (P+) and followed by 24 hours without and with 30 µM Al treatment. Bars show SE for 4 independents replications.

The root P accumulation was significantly increased by P application for both genotypes (Table 4.3). Al application resulted in significant increase of root P accumulation only in the Al resistant genotype (*P* < 0.05) while in the Al sensitive genotype it led to a significant decrease in root P accumulation. There was a significant Al x P interaction for root P uptake which was only observed in the Al resistant genotype.

Table 4.3. Effect of Al and P supply on shoot aluminium content and root P content of an Al resistant and an Al sensitive cowpea genotype

	Al content in shoot [*µg* Al plant^{-1}]		Root P uptake [mg P plant^{-1}]	
Main effect	IT89KD-391	IT89KD-349	IT89KD-391	IT89KD-349
P-	0.26 b	0.18 a	0.03 b	0.03 b
P+	0.45 a	0.23 a	0.10 a	0.06 b
Al-	0.26 b	0.19 a	0.05 b	0.05 a
Al+	0.48 a	0.24 a	0.10 a	0.03 b
Interaction effect				
Aluminium application (Al)	10.30 (**)	ns	6.6 (*)	8.04 (*)
P application (P)	8.04 (**)	ns	12.6 (**)	5.92 (*)
Al* P	ns	ns	4.8 (*)	ns
C.V (%)	27.1	7.8	44.6	26.1

Numbers followed by the same letter within a column are not significantly different at *P* > 0.05, LSD test, Ns: not significant, *, **, ***, significant at *P* < 0.05, *P* < 0.01 and *P* < 0.001, probability respectively.

Root aluminum accumulation

As expected Al supply lead to a significantly higher Al accumulation in the roots of Al treated plants ($P < 0.004$) (Fig. 4.5). The Al content was consistently higher in the roots of the Al resistant genotype IT89KD-391 than in the Al sensitive genotype. Phosphorus application strongly enhanced the Al accumulation in the roots particularly of the Al resistant genotype IT89KD391. In addition, there was a significant ($P < 0.05$) Al x P interaction for root Al accumulation.

The Al content of the Al resistant genotype, IT89KD-391 was however, higher than that of the Al sensitive for all treatments.

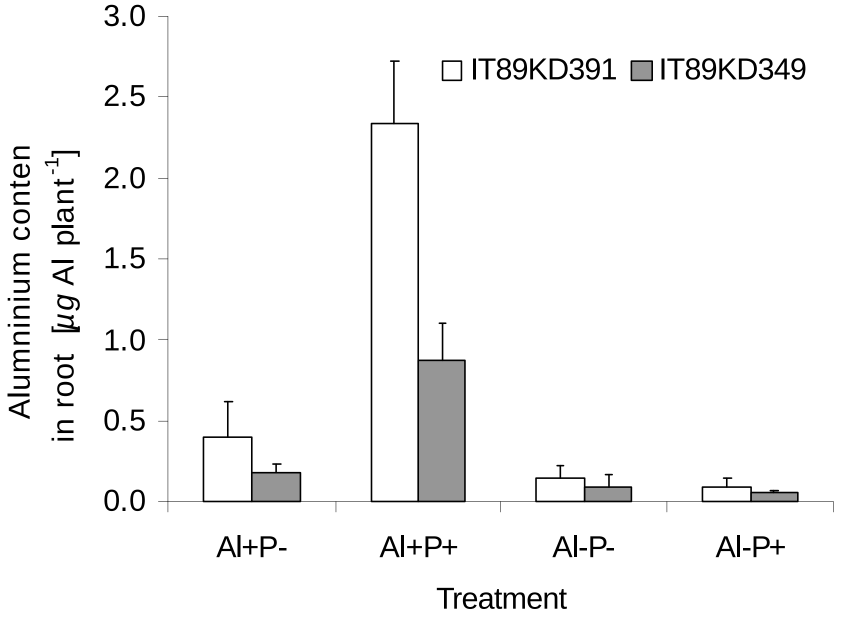


Fig. 4.5. Effect of Al and P supply on Al accumulation in roots of an Al resistant and an Al sensitive cowpea genotype. Plants were grown in complete nutrient solution for a week with 0 (P-) or 100 µM P (P+) and followed by 24 hours without and with 30 µM Al treatment. Bars show SE for 4 independents replications.

Organic acid exudation

Al supply significantly enhanced malate release of root apices of both genotypes (Fig. 4.6a). However, the exudation rate was significantly higher in Al resistant genotype IT89KD-391. In this genotype P deficiency also significantly increased malate exudation particularly in the P-deficient plants. In the Al sensitive genotype IT89KD-349 P deficiency did not significantly induced the exudation of malate.

Citrate exudation of the root apecies was lower than malate exudation by a factor of about 10 and was primarily enhanced by P deficiency in both genotypes, but more in the Al sensitive genotype IT89KD-349. Al treatment only enhanced citrate exudation of P-sufficient plants of Al resistant genotype IT89KD-391 which is reflected by a significant Al x P interaction ($P < 0.05$).

There was a high and significant difference in the citrate exudation between Al stressed plants and non-Al treated plants for the Al resistant cowpea genotype ($P < 0.0001$). The difference was 1.95 times higher in the Al treated plant as compared to the non Al treated plants.

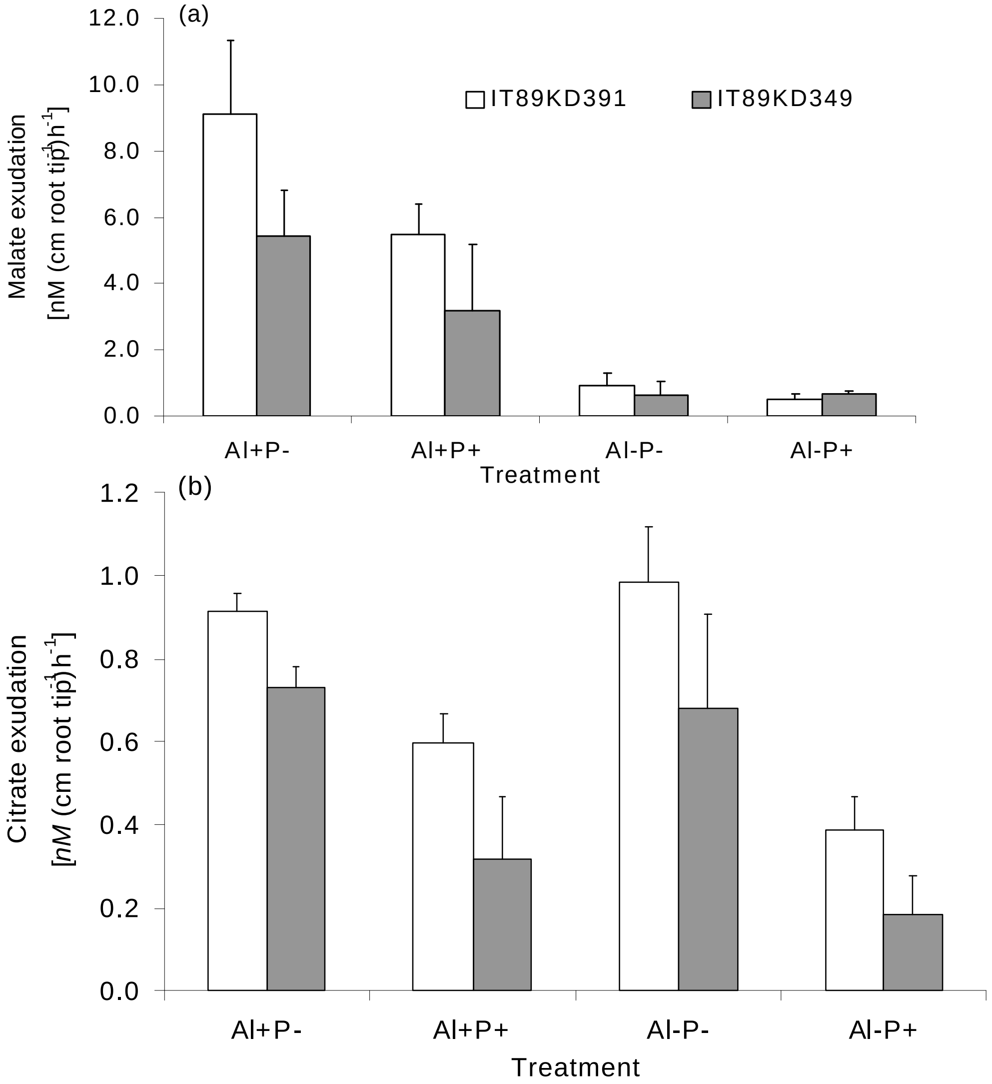


Fig. 4.6. Al and P interaction on the exudation of malate (a) and (b) citrate from root apecies. Cowpea seedlings were grown in nutrient solution with 0 or 100 μM P (KH_2PO_4) for ten days, and then treated with 0 or 30 μM Al for 4 hours.

Similarly, P deficiency induced significant exudation of citrate in response to low P tolerance ($P < 0.01$). In addition the Al x P interaction was significant ($P < 0.02$) with the highest and significant amount of citrate exuded from treatments Al+P and Al-P- (Fig. 4.6). With respect to the cowpea genotype IT89KD-349, P deficiency, but not Al application highly induced exudation of citric acid ($P < 0.03$). The Al x P interaction was however, significant indicating that both Al stress and P deficiency had significant importance for the citrate exudation. This resulted in the highest citrate exudation in treatment Al+P- (Fig. 4.6).

Discussion

The relative root inhibition and callose formation have been shown to be important parameters for screening of cowpea genotypes for Al resistance. Genotypes classified as Al resistant (IT89KD-391 and Dan´Ila, and IT90K-59) and Al sensitive (IT89KD-349), indicating the suitability of these parameters in cowpea. In maize (Collet et al., 2002; Horst et al., 1997) and soybean (Nian et al., 2003; Horst et al., 1992) root inhibition and callose formation were also found to be suitable parameters for assessing their sensitivity to Al.

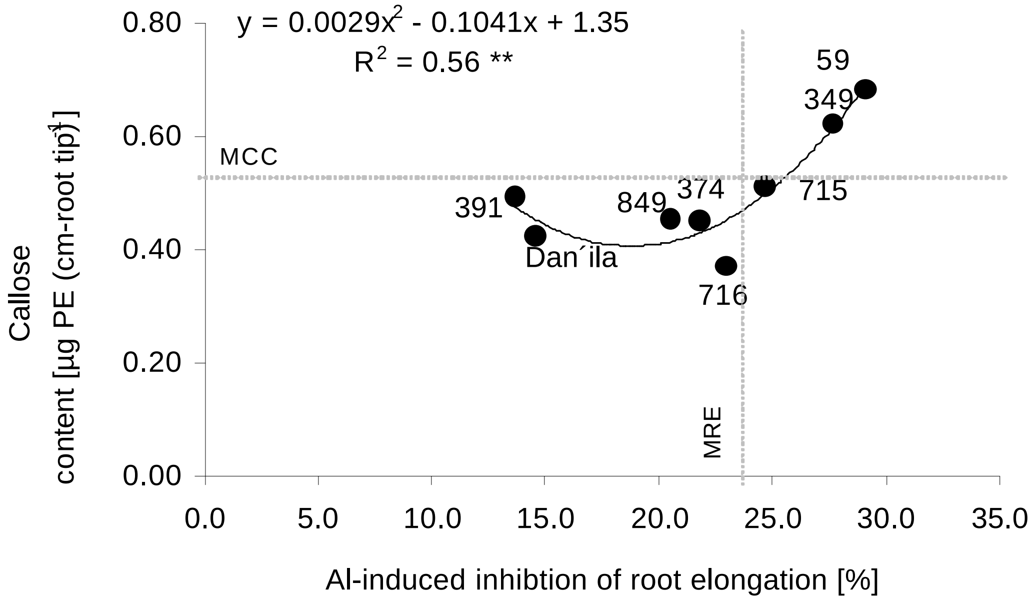


Fig.4.3. Relationship between aluminium-induced inhibition of root elongation and callose content with Al supply in root tips of 8 cowpea genotypes grown in nutrient solution with 30 μM Al supply for 24 hours. The nutrient solution contained 0.5 mM $CaCl_2$ and 20 mM H_3BO_3 at pH 4.3. (MCC = mean callose content, MRE = mean root elongation).

P uptake of cowpea was observed to be affected by Al application in both genotypes, although in the Al sensitive genotype P uptake was more severely inhibited than in the Al resistant genotype. In addition, shoot growth and root length of the Al sensitive genotype was inhibited by Al application, in contrast to the Al resistant genotype. The results compare with those found by Kolawole et al. (2000) and Tan and Keltjens (1990a; b) who found similar reduction in nutrient acquisition by Al supply in cowpea and sorghum genotypes, respectively. Indeed, on a physiological basis both short and long term exposure of plant roots to exchangeable Al^{3+} toxic concentration allow Al to penetrate into the roots thereby inhibiting root growth, cell division and cell elongation (Kochian, 1995; Ryan and Kochian, 1993). In addition to root inhibition, suppression of mitochondrial activity, disturbance of cation, and especially K^+-transport across plasma membrane, and disruption of water and nutrient uptake have been also reported (Calba and Jaillard, 1997; Kochian, 1995; de Lima and Copeland, 1994; Huang et al., 1992; Baligar et al., 1987). The Al resistant genotype IT89KD-391 was also efficient in the acquisition and use of P, as demonstrated by its response to P application. This suggests that genetic variation for Al resisatnce might be a prerequisite for achieving better selection for P efficiency of genotypes to be grown under acid conditions of SC.

Root Al accumulation for the Al resistant genotype was considerably higher than in the Al sensitive genotype (Fig.4.5) and corroborating findings by other authors (Horst et al., 1997) but contrasting with the results obtained by Delhaize et al. (1993a); Rincon and Gonzales 1992). In addition, the Al contents in leaves ranging from 0.18 to 0.45 µg was much lower compared to those found in Al includer plants species such as tea (Konishi et al., 1985; Matsumuto et al., 1976). Although this result could be attributed to the fact that we analysed Al in all the entire root system, whereas only the Al in the root apex affects root growth. In addition, Wang et al. (2004) suggested that even the total Al content in the root tip is not the determinant factor determining Al toxicity. The author rather indicated that the form of Al (Al^{3+}) in the cell apoplast or outer surface of the plasma membrane is the determinant factor for the expression of Al toxicity.

In the present investigation, both Al stress and P deficiency significantly induced the synthesis of organic acid, mainly malate and citrate in the Al resistant but only citrate in the sensitive genotype. Ryan et al. (1995), Delhaize et al. (1993b) using wheat genotypes differing in Al resistance found that the Al resistant genotype excreted 5 to 10-fold more malate than the Al sensitive genotype. Similarly, Ma et al. (1997) and Pellet et al. (1995), reported that secretion of citric acid was specifically induced by Al resistant maize and *Cassia tora*. In the present study, a lower rate of both organic acid anions were exuded in presence of both Al and P (Al+P+). This could be attributed to Al precipitation and reducing the activity of Al^{3+}, and thus resulting in low organic acid exudation (Pellet et al., 1997; Miyasaka et al., 1991).

Similar to what has been demonstrated in sorghum (Tan and Keltjens, 1990a; b), in soybean (Nian et al., 2004), and in maize (Gaume et al., 2001), our data showed that Al accumulation in root of the Al resistant cowpea genotype was significantly increased with P application, an indication that Al absorption into root cell is closely related to P nutrition. This is probably due to the fact that Al in presence of P forms insoluble complexes (Al-P) precipiting and accumulating at the surface or inside the the plants root (Taylor, 1991). The Al-P complexe is thus important to the plant root since it is inactive, and the effect of Al toxicity to the plant root is reduced (Pellet et al., 1997; Pellet et al., 1995).

The present study also revealed that the Al resistant genotype in presence of Al stress and P application had higher root Al accumulation and a lower exudation of organic acid anions (Fig. 4.4 and 4.6) than at 0 P supply. This result might imply that in presence of Al and P in solution, Al and P may form complexes thus reducing available Al^{3+} leading to a lower induction of organic acid anion release.

In conclusion, both Al stress and P deficiency may induce the release of organic acid anions. The release of malate appeared to be particularly responsive to Al supply, whereas the release of citrate was more responsive to P deficiency. Since both, malate and citrate exudation were more enhanced by combined Al and P-deficiency stress it may be assumed that the Al resistant genotype is better adapted to acid Al toxic and P-deficient soils than the Al sensitive genotype. In addition, P nutrition plays a significant role in detoxifying root Al accumulation and render the exchangeable Al^{3+} less soluble in the rhizosphere. However, it appears unlikely that such high P concentrations as supplied in the nutrient solution can be adjusted in the soil solution of the tropical acid soils of SC.

General Discussion

In the humid and sub humid tropics, the traditional approach used by the small-scale farmers to tackle the problem of nutrient depletion of their fields during cropping is to leave the land under fallow. During the fallow period, the soil nutrient capital is partially reconstituted through the fallow vegetation. The sustainability of the fallow relies mainly on their longevity varying from 6 to 15 years, which is the determinant factor for the efficiency of the nutrient-stock replenishment (Manley et al., 2002). However, population growth requires increasing land-use intensification, and thus the length of fallow is considerably shortened (less than two years). In combination with low fertilizer use this leads to nutrient mining and failure to restore soil fertility resulting in decreasing productivity of the crops (Koutika et al., 2004). Under such conditions of low/limiting nutrient supply in the soil in SC, the integration of crop species or genotypes that can make more efficient use of the P supplied by the soil and by maintenance fertilizer application represents a key element of sustainable cropping systems (Ae et al., 19990; Lynch, 1998)

In this study, several steps were taken to identify P-efficient grain legume with the objective to improve the prevailing cropping systems in SC. In the first series of activities, the target areas were characterised for their P chemistry and availability (see, introduction). Based upon the obtained information, two soils with low plant-available P (3 µg kg^{-1}) were selected and their critical P values assessed. In the second series of activities, a grain legume-maize cropping system-based technology was developed in field experiments at two major sites of SC aiming at redressing P depletion constraints (chapters 1, 2, and 3). This generated new knowledge for a management practice aiming at correcting P deficiency and increase soil and crop productivity of cropping system in SC. Thirdly, the physiological mechanisms governing efficient P acquisition of the main grain-legume crops (soybean and cowpea) to be incorporated into such cropping systems were studied under controlled conditions using soil pot and nutrient solution culture. This was necessary to base recommendations on the intensification of cropping systems and the application of the technology on a sound and stronger basis (chapter 3). In chapter 4, the cowpea genotypes were evaluated for Al resistance, and subsequently two genotypes contrasting in Al resistance were evaluated regarding their response to combined Al and P deficiency stresses.

In the following section the most important results are discussed and synthesised.

Genotypic selection for P efficiency

The present study demonstrated considerable genetic variation in phosphorus uptake and utilization among the soybean and cowpea genotypes tested on two acid low-P soils of SC (chapters 1, 2). For both species genetic variation among genotypes tested existed in grain yield, N_2 fixation and P uptake at low-available soil P. P application especially as TSP significantly increased grain yield and N_2 fixation. A superior P uptake efficiency was reflected in a higher N_2 fixation. The significant and positive correlation for both parameters underlines the importance of P for the N_2 fixation process (Vance, 2001). The results are in agreement with those found by other authors in different species (Sanginga et al., 2000; Gahoonia and Nielson, 1996; Gourley et al., 1994). However, the data also showed that genotypic differences were affected by the interaction with the environment for some of the traits studied (chapters 1, 2, 3). Although selection for P efficiency under field condition is generally difficult to achieve (Ahmad et al., 2001) because of the interaction with other biotic and abiotic soil factors, some genotypes were consistently classified as P-efficient independently of their growing environment (cowpea cultivars IT89KD-349, IT IT82D-849, IT90K-59, and IT98KD-391, and soybean cultivars TGm 1196, TGm 1251, TGm 1511 and TGm 1566). The results, therefore, clearly support the assumption that the identification of genotypes adapted to different locations in SC is possible and successful.

Genotypes studied were cross-classified for their efficiency to acquire P and for their responsiveness to P application (Chapter 1, 2). The existence of efficient responder (ER), efficient non-responder (ENR), inefficient responder (IR), and inefficient non-responder (INR) classes opens possibilities to breeders to select and develop cultivars targeting different levels of P fertilizer application.

In addition to improved P uptake, grain yield, and N_2 fixation our results also showed the possibilities to use P-efficient grain legume genotypes for slightly improving the N budget of cropping systems at low soil P as well as with small P applications (chapters 1, 2). Although negative N balances have been commonly observed for soybean (Ogoke et al., 2003; Sanginga et al., 1997), the level of N depletion decreased with P application. The selection of genotypes for more favourable soil N budgets appears to be desirable and possible.

Benefit of P-efficient legumes to rotational maize

Maize grain yield and P uptake after P-efficient grain legume genotypes increased compared to the sole-cropped maize and P-inefficient grain legume genotypes. In addition, P application to grain legumes provided more benefit to maize grown subsequently in rotation as illustrated by increasing yield and shoot biomass. The better maize growth in rotation could at least partly be attributed to an increase in soil-available P (Bray1) at grain harvest of the P-efficient legume. Relatively little research has been conducted on soils of SC on grain-legume/maize rotational systems. However, our results compare well with those obtained by Alvey et al. (2001). Enhanced cereal yields following grain legumes in rotation have been generally attributed to the amelioration of abiotic and biotic soils factors such as higher levels N_2 fixation and mineralization from legume crop residues, P availability (Vance et al., 2001; Sanginga et al., 2000; Sanginga et al., 1997; Giller and Wilson, 1991), improvement of AMF root infection (Horst et al., 2001; Bagayoko et al., 2000), but also the suppression of root nematodes. The comparison of two soil sites in our study also showed that the beneficial effect of legumes on subsequently grown maize depends on the soil and possibly other environmental conditions. For example, the yield of the grain legumes and grain yield and P uptake of maize was lower on the Rhodic Kandiudult soil with a much higher Al supply than of the Typic Kandiudult soil (Chapter 3).

Potential of non-Olsen P depletion by cowpea and soybean genotypes

Low plant-available P soils are characterised by a very low P mobility. Thus the uptake of Pi by roots creates a steep Pi depletion zone in the rhizosphere (Marschner, 1995). Hence, the ability of plants to acquire P from such soils depends on the solubilization of sparingly available P and/or to explore a large soil volume. The presented results showed that the cowpea genotypes IT89KD-391 and IT90K-59 and the soybean TGm 1566 were particularly able to acquire P from the NaOH soil-P fraction representing the P fraction that is not readily available to plant roots (chapter 3). Also Abdelgadir (1998) in soybean and Gahoonia and Nielson (1992) in barley attributed high genotypic P efficiency to the capability to access additional P pools (NaOH-Pi fraction) that could not be accessed by P-inefficient genotypes. On P-deficient soils of Nigeria, Horst et al. (2001) also described the ability of some cover-crop legumes to better use P from sparingly soluble sources, and this ability was related to root-induced rhizosphere process, such as secretion of organic acid and pH modification. For an efficient acquisition of readily available soil P the formation of many and particularly long root hairs are a most effective plant strategy (Krasilnikoff et al., 2003).

Evidence of soil-P solubilization in the rhizosphere: the role of organic acid anions

The conclusions about the ability of the P-efficient genotypes to deplete the non-Olsen-P in the rhizosphere were supported by the measured physiological changes in roots under P deficiency. P deficiency induced an enhanced release of organic acid anions mainly malate, citrate, succinate, and oxalate (chapter 3). Organic acids are low molecular weight solutes synthesised by plant roots and leaves (Jones, 1998). Although they are generally short-lived in soils because of microbial degradation, there is clear evidence of their functional role in the mobilization of nutrients, in particular of P, and in the complexation and thus detoxification of toxic metal ions such as Fe and Al (Jones, 1998; Marschner, 1995). Under P-deficient conditions, at the cellular levels, there is generally an increase in activity of a number of enzymes involved in the synthesis of organic acids such as phosphoenolpyruvate carboxylase (PEPC) and citrate synthase (Ollat et al., 2003; Lopez-Millan et al., 2000). The increases of the enzyme activity result in rapid conversion of phosphoenolpurvate (PEP) to oxaloacetate from the glycolytic pathway. The oxaloacetate is transported through the mitochondrial membrane and is then rapidly converted into malate through malate dehydrogenase. Malate is converted to pyruvate in the mitochondria and NADH is generated in the tricarboxylic acid cycle (TCA) where other organic acids are subsequently produced. The glycolytic pathway becomes dominant and the activity of PEPC activity is elevated (5-10 fold) under P deficiency. Hence more organic acid are synthesised and after transport to the cytosol may be released from the cytosol to the rhizosphere. Here, they complex Fe and Al releasing P sorbed on Fe and Al oxides representing non-Olsen or sparingly available P for plant uptake. The general process is presented in Fig. 6. Large amounts of organic acid anions have to be excreted in order to mobilize relevant quantities of P for plant uptake (Jones, 1998; Gerke, 1993, 1993).

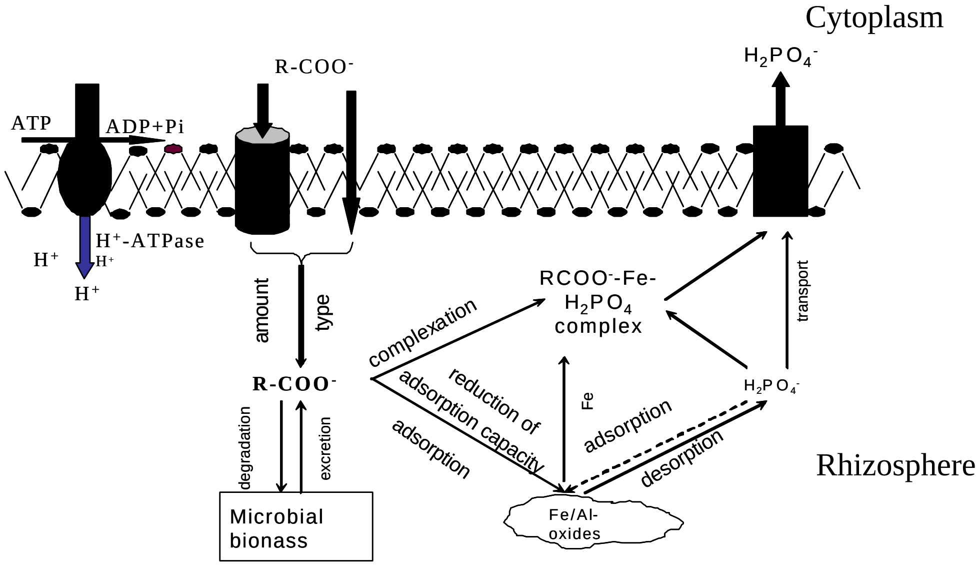


Fig.6. Effect of organic acid anions exuded from plants roots on P availability. (Root induced rhizosphere process) (Source: *Horst and Kamh, 2004*).

The role of arbuscular mycorrhizal fungi (AMF) and root-surface acid phosphatase on P availability

There is overwhelming evidence suggesting that some plant species form a symbiosis with arbusuclar mycorrhizal fungi (AMF) under conditions of P deficiency. The formations of an extensive extra-radical network of AMF mycelium in soils increase the soil volume accessible to plant roots (Kothari et al., 1991) and thus the efficiency of acquisition of soil P by inflected roots. Our data show that the P uptake of the soybean genotypes TGm 1511 was significantly related to AMF root infection (chapter 1). Phosphorus efficiency in plants is definitively a whole plant phenomenon of considerable complexity (Vose, 1990) integrating many different mechanisms. Our results are in agreement with those of different authors showing a beneficial effect of AMF root colonisation on the P efficiency of different plant species including soybean. The significant relationship between P content and AMF root colonisation might indicate the ability of such a cultivar to extend the volume of soil explored through the AMF network formation. Release of acid phosphatases to soil by plant roots have also been suggested as plant response to low-P conditions (Marschner, 1995; Helal and Dressler, 1989). The data presented in this study also showed that P-efficient genotype TGm 1511 enhanced root-surface acid phosphatase activity under low-P conditions. This indicates that, in addition to the phosphatase activity of soil microorganisms, the plant under P deficiency stress develops a higher capability to utilize organically bound P sources. The organic P fraction represents a major pool in most soils, varying from 50 – 80% of the total soil P. The association of the roots with AMF and an enhanced activity of root surface or released acid phosphatase observed in genotype TGm 1511 could be important mechanisms influencing P acquisition and transformations in the rhizosphere. This could represent important adaptation mechanisms in acid soil of SC. A few studies indicated the AMF may have better access to organic P pools, so that the available of P pool could be greater for mycorrhizal than for non-mycorrhizal plants (Tarafdar and Marschner, 1994; Jayachandran et al., 1992). If this genotype can make P available from organic P fraction, this would represent a substantial contribution to the available-P budget of the cropping system.

Genotypic variation of Al resistance and dual resistance to both P deficiency and Al toxicity in cowpea.

Considerable genetic variation was observed in cowpea genotypes for Al resistance. Al resistance was expressed as less Al induced inhibition of root elongation and callose formation in root apices. The results thus confirm earlier studies showing inhibition of root elongation and increase in callose contents in root apices when the plants are under Al stress (Horst et al., 1997). However, Rangel et al. (2004) found that callose was not the suitable parameter to differentiate genotypic variability in common bean.

It was also found that an Al resistant and P-efficient cultivar was better adapted to acid Al toxic and P-deficient soil (chapter 4), indicating the potential of selecting for Al resistance and P efficiency among the cowpea genotypes included into this study. In soybean, Dong et al. (2004) observed that the P-efficient genotype BX10 was also more resistant against Al stress compared to a P-inefficient genotype. This was particularly attributed to the release of the organic acid anions citrate and malate under both stresses. Our data clearly showed that the syntheses of both organic acids were enhanced under P deficiency and Al stress. P deficiency and Al toxicity generally co-exist on acid soils (Kochian, 1995). Plants growing under such soils may have evolved different strategies to cope with the multiple stresses such as P deficiency and Al toxicity. It has been reported that plant species or genotypes are likely to have dual resistance mechanism (Nian et al., 2003), and the exudation of organic acids has mostly been found to be involved in the dual adaptation mechanism. The exudation of organic acid under Al stress and P deficiency has been intensively documented for different plants. However, in cowpea, relatively few data are available.

Screening techniques

This study combined different screening techniques ranging from field, soil pot-culture, use of an insoluble-P form (RP), and nutrient-solution culture to address the problem of P deficiency in SC. The results were mostly consistent for most of the trait studied. For example, genotype IT89KD-391 had a high P uptake in the field as well as in soil culture and nutrient solution culture. However, in some of the genotypes specific traits expressed differently when changing the experimental environment. This was particularly applied to the root length that were genetic differences existed in the nutrient experiment but not in the greenhouse and field experiments.

Outlook

The present work clearly identified a new agronomic approach to improve the annual maize cropping system on acid soil of SC where P is strongly fixed. The higher genotypic variation in P efficiency of grain legumes soybean and cowpea cultivars can be beneficial to subsequently grown maize in rotation. The mechanisms involved in genotypic P efficiency proved to be a number of complex factors ranging from root surface phosphatase activity, exudation of organic acid anions, symbiosis with AMF, kinetics of root P uptake and root growth. It was also shown that the P efficiency of some genotypes is affected by the soil environment such as Al toxicity. Based on the accumulated knowledge, the maintenance applications of P fertilizer in combination with organic residues are recommended for sustainable soil and crop production in SC.

Field experiments were conducted only at two soil sites. It would be desirable and necessary to expand the study to other soil sites which would allow to more generally extrapolating the results across all the IITA benchmark sites of SC. The numbers of genotypes used were only seven for cowpea and twelve for the soybean. There is a need to increase the number of genotypes to better exploit the existing genetic variability in these crops. The technique used to assess the N_2 fixation capacity of the genotypes is sensitive to the growing stages of the plant at sampling time and very time consuming. The comparison of the method with other methods estimating N_2 fixation will provide information on their improvement.

The study of the mechanism of P efficiency was concentrated on greenhouse and nutrient solution experiments. Although the experiment to assess the plant mechanisms and physiology under field conditions are difficult to be conducted, focus on the observed mechanisms in this study should be towards their adaptation under field. In addition, the studies on the P efficiency mechanisms in the present study focussed on P acquisition strategies with less emphasis on P utilization efficiency by P remobilisation from leaves and stems to the roots and the grain. Using the present study as a baseline further research on P efficiency of cowpea and soybean could be concentrated on these aspects.

On the other hand, it was demonstrated that the synthesis and release of organic acids and root surface phosphatase activity are most important physiological adaptation strategies of P-efficient genotypes expressing at the plant cellular level. Using the selected genotypes in more in depth research can focus on these plant attributes as P efficiency markers in an attempt to improve the adaptation using molecular tools.

The relationship between P uptake and AMF root colonisation indicates the potential contribution of the root symbiotic micro organisms to nutrient use efficiency, especially P. On predominantly acid soils not only Al and P deficiency are limiting factors for plant growth. Mn toxicity should be integrated to have a broader understanding of all soil factors limiting the performance of such cultivars on acid soils of SC.

Summary

Phosphorus (P) and nitrogen (N) deficiencies are major factors limiting plant production on the tropical soils of Southern Cameroon (SC). On the predominantly acid soils aluminium (Al) is an additional growth-limiting factor inhibiting particularly the root growth. Field experiments over 2 years on two acids and low P soils of SC, pot experiments with the same soils and nutrient solution experiments were conducted in order to assess the genotypic variation in soybean and cowpea in P efficiency, Al resistance, and the possible contribution of P-efficient and Al resistant genotypes to positively contribute to the P use and N budget of a legume maize cropping system.

Twelve soybean genotypes were grown in the field on a Typic Kandiudult (TK) and a Rhodic Kandiudult (RK) soil of SC in 2001 and 2002 in a split block design without and with application of Togolese phosphate rock (PR) and Triple super phosphate (TSP). Shoot DM, grain yield, N_2 fixation, and P uptake varied with site, genotype, and P source. On TK as well as on RK soil, shoot DM was significantly enhanced with application of TSP and PR. However, P application significantly increased grain yield only on TK soil. Shoot P accumulation was significantly increased on TK and RK soil with TSP application. N_2 fixation ranged from 20 to 55 kg N ha^{-1} on TK and from 16 to 38 kg N ha^{-1} on RK soil. Based on cross-classification of the genotypes in terms of P efficiency and response, only TGm 1511 and TGm 1566 were considered efficient responders, which were reflected in their positive N balance. The pot experiment revealed that contributing factors to the P efficiency of TGm 1511 was the association of its roots with arbuscular mycorrhizal fungi, while a compensatory mechanism between shoot and root growth seemed to be important for TGm 1566.

Under the same field experimental conditions also seven cowpea genotypes were evaluated for growth, P uptake, and dinitrogen fixation. Genetic variation for grain yield production was apparent at low P on both soils. Grain yield was however significantly higher on the TK than on the RK soil. The shoot P uptake varied significantly from 2.3 to 6.8 kg P ha^{-1} between genotypes at low P. PR and TSP application significantly increased the shoot P uptake of the genotype IT89KD-391. The total amount of N_2 fixed increased significantly with TSP but not with PR application on both soils. The N balance calculated as the difference between N_2 fixation and the N content of grains ranged from -6 to +6 kg N ha^{-1}, with the genotypes IT90K-59, Dan´Ila and IT89KD-391 recording positive N balances.

Four genotypes were selected for further investigation in pot experiments of probable mechanisms accounting for the variation in P acquisition and use efficiency observed under field conditions. The most important mechanisms of genotypic variation in P efficiency were an enhanced P uptake per unit of cm root length and the efficient transport of P to different plants organs.

In the same field experiments the residual effect of the legume genotypes differing in P efficiency on subsequently grown maize was evaluated. Yields of maize after soybean genotypes TGm 1511, IT89KD-391 and cowpea genotypes IT90K-59 were significantly higher than those of other genotypes on the TK soil. The residual effect of legumes to the following maize increased on both soils when the legumes were fertilised with TSP. The fractionation of soil P indicated the ability of the genotypes TGm 1566, TGm 1511, IT89KD-391 and IT90K-59 for depleting P from NaOH fractions. In greenhouse and laboratory experiments the mechanisms responsible for the efficient acquisition of soil P among the grain legumes were studied. The mechanism for P mobilization in cowpea IT90K-59, IT82KD-391 and soybean genotype TGm 1511 was the high exudation of organic acid anions particularly of malate and citrate. A high release of acid phosphatase by roots under low-P conditions was found as a strategy to acquire P from less available sources by soybean genotypes TGm 1566. It was concluded that the residual benefit of P to maize was enhanced with P application to the preceding legume crop thus heightening the need for legume fertilization for optimum maize yields.

Experiments in nutrient solution were conducted to evaluate 7 cowpea cultivars for Al resistance. Subsequently the combined effect of P deficiency and aluminium stress on growth, uptake, and organic acid anion exudation of two cultivars of contrasting Al resistance selected from the first experiment were studied. The relative root inhibition by 30 µM Al ranged from 14 to 60%, and there were highly significant difference among the cultivars. Al induced callose formation particularly in roots of Al sensitive genotypes. Al stress and P deficiency interaction reduced P accumulation significantly (50 and 80%) for both, the Al resistant (IT89KD-391) and the Al sensitive (IT89KD-394) cultivar. Al supply significantly enhanced malate release of root apices of both cultivars. However, the exudation rate was significantly higher in the Al resistant cultivar. Only in this cultivars also P deficiency significantly increased malate exudation. Citrate exudation of the root apecies was lower than malate exudation by a factor of about 10 and was primarily

enhanced by P deficiency in both cultivars, but more in the Al sensitive cultivar. Al treatment only enhanced citrate exudation of P-sufficient plants of the Al resistant cultivar. It is concluded that the Al resistant cultivar is better adapted to acid Al toxic and P-deficient soils than the Al sensitive cultivar since both, malate and citrate exudation were more enhanced by combined Al and P-deficiency stress.

The results address the potential to use P-efficient and Al resistant soybean and cowpea genotypes in maize cropping systems on the acid, P-deficient soils of SC in order to improve the use of soil and fertilizer P and to enhance N_2 fixation thus contributing to a more favourable N budget of the soils. The genotypic variation for P acquisition observed in this study, in combination with genotypic differences in N_2 fixation could be of significance to breeders trying to develop genotypes with a more positive N balance. However, application of fertilizer P appears necessary to make better use of the N_2 fixing potential of the grain legumes.

Keyword: Aluminium resistance - N_2 fixation – Phosphorus

Zusammenfassung

Phosphor (P) und Stickstoff (N)-Mangel sind wichtige, die Pflanzenproduktion begrenzende Faktoren, auf den tropischen Böden in Süd-Kamerun (SK). In den vorwiegend stark sauen Böden begrenzt als weiterer wachstumsbegrenzender Faktor Aluminium (Al) das Wurzelwachstum. Über zwei Jahre wurden Feldversuche auf zwei sauren Böden mit niedriger P-Verfügbarkeit in Süd-Kamerun und ergänzend Gefäßversuche mit den gleichen Böden, und Nährlösungsversuche durchgeführt. Es sollten genotypische Unterschiede bei Sojabohne und Cowpea in der P-Effizienz und Al Resistenz charakterisiert und der mögliche Beitrag P-effizienter und Al resistenter Genotypen zur P-Nutzung und N-Bilanz von Leguminosen-Mais Anbausystemen erfaßt werden.

Zwölf Sojabohnen-Genotypen wurden in 2 Feldversuchen auf als Typic Kandiudult (TK) and Rhodic Kandiudult (RK) klassifizierten Böden in den Jahren 2001 und 2002 angebaut. Das Versuchdesign war eine geteilte Blockanlage mit den Düngungsvarianten ohne Düngung, und Düngung mit Togo Rohphosphat (PR) oder Triple-Superphosphat. Sproß-Trockenmasse, Kornertrag, N_2-Fixierung und P-Aufnahme unterschied sich in Abhängigkeit von Genotyp und P-Düngung. Auf beiden Böden führte die Düngung mit PR und TSP zu erhöhter Sproßbiomasseproduktion. Der Kornertrag wurde aber nur auf dem TK Boden erhöht. TSP-Düngung erhöhte die P-Aufnahme auf beiden Böden. Die N_2-Fixierung variierte auf TK Boden von 20 – 55, und 16 - 38 kg N ha^{-1} auf dem RK Boden. Nur die Genotypen TGm 1511 and TGm 1566 wurden aus der Gegenüberstellung der Kornerträge mit und ohne Düngung als P-effizient und auf P-Dünger reagierend eingestuft. Der Gefäßversuch zeigte, daß das zur P-Effizienz von Genotyp TGm 1511 beitragende Merkmale die Ausbildung einer arbuskulären Mycorrhiza war, während bei TGm 1566 offenbar eine kompensatorische Regulation von Sproß und Wurzelwachstum bedeutend war.

Unter den gleichen Feldversuchs-Bedingungen wurden auch 7 Cowpea-Genotypen auf Wachstum, P-Aufnahme und N_2-Fixierung verglichen. Es bestanden genotypische Unterschiede im Kornertrag bei niedrigem P-Angebot auf beiden Böden. Der Kornertrag war jedoch höher auf dem TK als auf dem RK Boden. Die Sproß P-Aufnahme variierte signifikant von 2,3 bis 6,8 kg P ha^{-1} zwischen den Genotypen. PR und TSP-Application erhöhte die P-Aufnahme von Genotyp IT89KD-391. TSP aber nicht PR-Applikation

erhöhte auch die N_2-Fixierung auf beiden Böden. Die als Differenz zwischen der N_2-Fixierung und dem N-Gehalt in den Samen berechnete N-Bilanz variierte zwischen -6 bis +6 kg N ha^{-1} mit positiven Bilanzen bei den Genotypen IT90K-59, Dan´Ila und IT89KD-391. Vier Genotypen wurden für weiterführende Untersuchungen in einem Gefäßversuch zu den Ursachen der im Feld aufgetretenen genotypischen Unterschiede in der P-Aufnahme und P-Nutzungseffizienz ausgewählt. Es zeigte sich, daß für die Unterschiede in der P-Effizienz eine erhöhte P-Aufnahmerate und eine effizientere Verteilung von P in der Pflanze verantwortlich zeichneten.

In den gleichen Feldversuchen wurde auch die Nachwirkung des Anbaus von Leguminosen mit unterschiedlicher P-Effizienz auf in der Rotation angebauten Mais untersucht. Die Maiserträge nach den Sojabohnen-Genotypen TGm 1511, IT89KD391 und dem Cowpea-Genotyp IT90K-59 waren auf TK Boden signifikant erhöht gegenüber den anderen Genotypen. Die Nachwirkung auf Mais war nach TSP-Düngung zu den Leguminosen signifikant erhöht auf beiden Böden. Die fraktionierte Extraktion von Boden-P wies auf ein besseres Vermögen der Genotypen TGm 1566, TGm 1511, IT89KD-391 and IT90K-59 hin, auch NaOH-lösliche P-Fraktionen zu verarmen. In Gewächshaus und Nährlösungsversuchen wurde die unterschiedliche Fähigkeit der Genotypen, sich Boden-P anzueignen näher untersucht. Für die Genotypen IT90K-59, IT82KD-391 und TGm 1511 erwies sich eine bei P-Mangel erhöhte Abgabe von organischen Säureanionen, besonders Malat und Citrat, als bedeutend für die P-Mobilisation. Eine verstärkte Freisetzung von saurer Phosphatase spielte offenbar für die Aneignung von P aus weniger gut pflanzenverfügbaren P-Fraktionen des Sojabohnen_Genotypen TGm 1566 eine wesentliche Rolle. Es wurde geschlußfolgert, daß für die Optimierung der positiven Nachwirkung von Leguminosen auf in der Rotation nachfolgenden Mais eine P-Düngung zu den Leguminosen erforderlich ist.

Versuche in Nährlösung wurden durchgeführt zum Screening von 7 Cowpea-Genotypen auf Al Resistenz. Anschließend wurde 2 Genotypen mit unterschiedlicher Al Resistenz bei kombiniertem Al und P-Mangel-Streß untersucht auf Wachstum und Abgabe von organischen Säureanionen. Ein Angebot von 30 µM Al hemmte genotypisch unterschiedlich das Wurzelwachstum um 14 bis 60%. Al induzierte die Kallose-Synthese besonders in den Wurzeln Al sensitiver Genotypen. Die Interaktion von Al und P-Mangel führte zu einer signifikanten Reduktion (50 und 80%) der P-Aufnahme beider Genotypen

(Al resistent, IT89KD-391 und Al empfindlich, IT89KD-394). Al Angebot führte zu einer erhöhten Malat-Abgabe der Wurzelspitzen bei beiden Genotypen, jedoch stärker beim Al resistenten Genotyp. Nur bei diesem Genotyp war die Malat-Abgabe bei P-Mangel erhöht. Die Citrat-Abgabe der Wurzelspitzen war geringer als die Malat-Abgabe um den Faktor 10, und nur bei P-Mangel verstärkt insbesondere beim Al sensitiven Genotyp. Al Angebot führte nur bei ausreichendem P-Angebot beim Al toleranten Genotyp zu einer erhöhten Citrat-Abgabe. Es wird geschlußfolgert, daß der Al resistente besser als der Al empfindliche Genotyp durch eine verstärkte Abgabe von Malat und Citrat bei kombiniertem Al und P-Mangel-Streß an saure Böden mit toxischem Al Angebot und niedriger P-Verfügbarkeit angepaßt ist.

Die Ergebnisse zeigen das Potential auf für die Integration von P-effizienten und Al resistenten Sojabohnen und Cowpea-Genotypen in Mais-Anbausystemen auf sauren Böden in SK. Dies kann dazu beitragen, die Nutzung von Boden- und Dünger-P zu verbessern und durch eine gesteigerte N_2-Fixierung die N-Bilanz der Böden positiv zu gestalten. Die beobachtete genotypische Variation in der P-Effizienz in Verbindung mit Unterschieden in der N_2-Fixierung könnte für Pflanzenzüchter, die an der Züchtung von Leguminosen-Genotypen mit positiverer N-Bilanz der Böden interessiert sind, von großer Bedeutung sein. Jedoch erscheint eine P-Düngung der Leguminosen zur erhöhten Ausnutzung des Potentials zur N_2-Fixierung erforderlich zu sein.

Aluminium Resistanze - N_2-Fixierung – Phosphor

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Acknowledgments

Prof. Dr. Walter J. Horst gave me the opportunity to work in the Institute Plant Nutrition (IPE) at the University of Hanover, Germany, providing a stimulating and enjoyable atmosphere. His close supervision and careful guidance helped me to successfully accomplish this work.

To you, Dr. R. Abaidoo (IITA Ibadan), I cannot imagine with a perfect balance, yours deeper comments on chapters and guidance during my study. This page opens to me the appropriateness occasion to acknowledge this.

I'm grateful to Prof. Dr. N. Claassen for his willingness to accept being the co-referee; and Prof. Dr. J. Böttcher and Dr. Habil K. Wydra for delightfully accepted to act as my examiners.

This project commenced with the supervision of Dr. C. Nolte, unfortunally he left the IITA-Cameroon. You taught me about doing sciences, confidence, and strictness to conduct a research work. I seize this opportunity to express to you all my sincere thanks for this opportunity you gave to me.

This project was initiated by Dr. N. Sanginga (Ciat-TSBF, Nairobi), many thanks for accepted me join the project as research fellow and gave me the opportunity to prepare a PhD program within the project.

This work also involved the personnel of the Institut de la Recherche Agricole pour le Développement (IRAD), of Yaounde, particularly M. Tchienkoua, thanks to let us share part of your experiences.

To the IITA-Cameroon national staff involved in this project, you facilitated the work to be done without many difficulties. Many thanks for this.

Many farmers in southern Cameroon allowed us to use their land to conduct the field experiments of this work. It was a wonderful time I spent with you at Minkoameyos and Abang villages, respectively. I deeply appreciated the generous hospitality given to me while being in your different villages.

I am also grateful to Ben Banful, Dr Chabi Olaye, and Dr Mosisa Worku yours different helps to read and make suggestions on this work.

A number of students from the University of Dschang, Cameroon, contributed to this project, and this also accounted for their “Mémoire”. I greatly appreciated your contribution. I am thinking especially to M. Kinfack and J. A. Messiga.

Many determinations of Aluminium and Phosphorus concentrations in plants from nutrient solutions experiment were done by Tanja Edler, at the institute of Plant Nutrition (University of Hanover), my sincere thanks for your assistances.

Frau Ingry Dusy and Andre Specht at the Institute of Plant Nutrition provided administrative and technical inputs allowing me to conduct this work. Thanks for your gratitude.

To all the staff of the Institute of Plant Nutrition, of the University of Hanover, I express all my thanks to let me passed memorable times with you and discussing other aspect related to this study.

This project was financially supported by the Australian Centre for International Agricultural Research (ACIAR). Also, the Deutscher Akadmischer Austauch Dienst (DAAD) partly supported my living expense while in Germany to carry the laboratory part of this study.

Many other people contributed to this work in different ways. My sincere thanks go to all of them, also those not mentioned explicitly.

Last but absolutely not the least; my family gave me a lot of emotional support, I´m thinking especially to Chloe Imelda and Donfack Berthe.

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