

Variation in Growth and P Uptake of Maize Cultivars Colonized by Arbuscular Mycorrhizas on Acid Soil of Southern Cameroon

¹S.N Tchameni, ¹L. Nana Wakam ²M. Jemo, ¹R. Fokom, ³C. Thé, ⁴D. Nwaga and

¹Department of Biochemistry, Faculty of Sciences, University of Yaoundé 1,
PO Box.812, Cameroon;

²International Institute of Tropical Agriculture (IITA), Humid Forest Ecoregional Center (HFC),
PO Box 2008 Messa, Yaoundé;

³Institute of Agronomic Research for Development (IRAD), PO Box 2067,
Yaoundé', Cameroon;

⁴Laboratory of Soil Microbiology, Biotechnology Centre & Department of Plant Biology,
Faculty of Science, University of Yaoundé1, P.O. Box 812, Yaoundé, Cameroon.

¹Department of Biochemistry, Faculty of Science, University of Yaoundé1, P.O. Box 812,
Yaoundé, Cameroon.

Abstract: Two maize cultivars contrasting on acid stresses, ATP S4.syn Y (acid soil tolerant) and CMS8501 (acid soil sensitive) were grown on aluminium toxic soil with low-P available soil P of southern Cameroon. The objectives were to assess maize cultivar differences for plant growth, P uptake, root colonisation by fungi, and the activity of root phosphatases following inoculation of arbuscular mycorrhizal fungi (AMF). The experiment was conducted in a 2 x 2 factorial arrangements in a complete randomised block design (RBD). The main factor one was the maize varieties (ATP S4.syn Y and CMS8501) and the second factor, the AMF inoculation consisting of four different levels of AMF application: M0 (no AMF inoculation), M1 (*Gigaspora margarita* inoculation), M2 (*Glomus intraradices* inoculation), and M3 (the mixture of M1 and M2). Data were collected 14, 28, 42 and 63 days after planting. Significant differences ($P < 0.05$) were observed between the two maize cultivars for shoot growth, P uptake, the percentage of root colonisation by AMF, and the activity of root phosphatases, ATP S4.syn Y the tolerant cultivar for Al toxicity generally exhibited higher values than CMS8501. AMF inoculation significantly increased all the measured plant parameters. Significant relationships between, shoot dry matter and P uptake, and the acid phosphatase activity, respectively observed particularly for ATP S4.syn Y imply that the induction of specific acid phosphatase exuded by roots, and the efficient P remobilisation for biomass production may account as potential candidate mechanisms for adaptation of a such cultivar into acid and low available P soil of southern Cameroon.

Key words: acid phosphatase; arbuscular mycorrhizal fungi (AMF); phosphorus uptake efficiency; *Zea mays*.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals extensively grown worldwide for food and as a source of raw material for manufacturing several products such as corn sugar, corn flakes, corn oil, and corn protein [6]. In Cameroon, maize is grown in all the five ecological zones, mostly for its high carbohydrates content and is now a cash crop for many farmers [30]. In southern part of the country, maize cultivated area has expanded over the region, although the estimated average yield is still considerably low (1.13 to 2.50 Mg

ha⁻¹) compared to the world average yield which is 4.3 Mg ha⁻¹ [7]. There is a potential to increase the maize production in Cameroon, particularly in the humid forest zone. However, among the factors curtailing growth and yield of maize in this area are insect damages [3], low soil fertility [10], and acid soil stress due to aluminium and / or manganese toxicity [32].

Acid soils cover up to 75% of arable lands in the humid forest of southern Cameroon, and yield lost attributed to this stress is estimated at 40 to 80 % [29]. The primary limitations on acid soils are toxic levels of exchangeable aluminum (Al³⁺) soluble into soil solution

that impaired with root growth^[14,15]. Correcting maize yield through liming is not feasible for the small resources poor farmers due to the cost needed to meaningfully overcome such soil constraint.

On the predominantly acid soils of southern Cameroon, low P availability is an additional limiting factor that hampers maize growth^[4,18]. Its bioavailability is low due to binding to soil mineral surface and fixation into organic forms. Maize cultivation in the humid forest zone of Cameroon suffers from both acid soil stress and suboptimal levels of P^[13]. Though, Al resistant maize cultivars for the humid forest soils have been developed, their adaptation to low P availability have however been rarely tested^[30,32]. The development of soil acidity resistant and P-efficient cultivar could significantly increase maize yield at the farmers' level in the Southern Humid zone of Cameroon. However, this will require a better understanding of the plant mechanisms controlling resistance to both acid and P stresses and an efficient evaluation technique to differentiate for acid tolerant and P-efficient/inefficient cultivars^[2].

Significant variation for tolerance to acid stress, P uptake has been observed in maize under low-P conditions. In spite of its ecological significance, the interaction of acid soil and P deficiency stresses on genotypic difference of crops species for growth and P nutrition have been addressed in only a few studies to date, especially in the humid forest zone of the tropic^[12,30]. Plants generally respond to both acid stress and P deficiency through exudation of organic acid^[15], or efficient root symbioses with arbuscular fungi which extended the hyphae network beyond the depleted P root zone for its P acquisition^[26]. In addition, the activation of the metabolism of root phosphates under P deprivation and acid soils stresses resulting in higher synthesis of phosphatase will improve the P nutrition and reduce the effect of acid stresses^[5].

The present study was thus, aimed at evaluating two contrasting maize cultivars on acid soils of southern Cameroon for growth, P uptake efficiency, root colonisation by AMF root phosphatases activity after inoculation of different AMF species. This is an effort to develop strategies for managing maize acid soil with low P content for sustainable maize production in the humid forest zone of southern Cameroon.

MATERIALS AND METHODS

Plant Materials: Seeds of maize cultivars ATP S4.syn Y and CMS8501 were obtained from the cereal breeding program of the Institut of Agricultural Research for Development (IRAD), Cameroon. Some of the varieties characteristics are presented in Table 1.

CMS8501 is an acid soil sensitive while ATP S4.syn Y an acid soils tolerant^[32]. Seeds of both cultivars were sterilised in 5 % Sodium hypochlorite (NaOCl) and thoroughly washed with distilled water and pre-germinated for five days.

Table 1: Characteristics of the two maize varieties used for experimentation (IRAD, 1999).

Characteristics	Variety	
	CMS 8501	ATP S4. Syn Y
Color	White	Yellow
Cycle (days)	115	115
Type	Composite	Synthetic
Grain form	Arc	Large
Percentage of germination (%)	100	96
Humidity (%)	13.5	13
Acidity	Sensible	Tolerant
Potential yield (t ha-1)	6.0-7.5	6.5 -9.0

Experiment Set up: Pot experiment was conducted in greenhouse of the Department of Plant Biology, of the University of Yaoundé 1, Cameroon using soils collected at the farmers' field around Yaoundé. The soils were classified as Rhodic kanduidlut (USDA classification) and were sampled at depths 0 – 10 cm depth. The chemical and physical compositions of the soils are presented in table 2. The soil was air-dried, sieved through a 4 mm sieve 3 kg of this soil were mixed to 1 kg of sterilized sand quartz substrate, and filled into 4 liters pots. Uniform seedlings were then transplanted into each pot containing fields soils and substrate. Thereafter, one week after planting, seedlings were thinned to two plants per pot. Plants were then supplied with 20 ml of nutrient solution containing urea (0.5 g/l), MgSO₄ 7H₂O (0.246 g/l), CaCl₂ 2H₂O (0.147 g/l) and 1 g of chemical fertiliser NPK, 20/10/10 once a week. The experimental design was a 2 x 2 factorial combination in a randomized complete block with three replicates. Factor one was maize cultivars (CMS8501 and ATP syn 4) and factor two, AMF species inoculation: M0 (without AMF inoculation), M1 (*Gigaspora margarita* inoculation), M2 (*Glomus intraradices* inoculation); M3 (the mixture of the M1 and M2 inoculum).

Table 2: Chemical and physical properties of the soil use for the experimentation

N (meg/100g)	1056
Ca (meg/100g)	1.04
Mg (meg/100g)	0.25
K (meg/100g)	0.21
Na (meg/100g)	0.08
Al (meg/100g)	2.43
Mn (meg/100g)	0.02
pH (H2O)	5.0
P available (mg kg-1)	6.5
Organic matter (%)	3.03
Clay (%)	51.9

Plants were daily watered with deionised water to 60 % of water holding capacity (WHC), and were harvested at 14, 28, 42 and 63 days after planting (DAP) for dry matter production, root colonisation by AMF, determination of P concentration, and the analyses acid phosphatase activity of roots.

Plant Sampling and Analysis: Shoots were cut at 5 cm above ground level and the fresh weight recorded. They were then transported to the laboratory and oven-dried at 70° C for 72 hours and dry matter was recovered. The whole plants were crushed and sub-samples used for the determination of P concentration in shoot. Analysis of P in the shoots was done using the vanadomolybdate yellow method Motomizu et al.^[21] after the samples were ashed at 480 ° C in concentrated sulfuric acid.

AMF inoculation: The AMF isolate were obtained in greenhouse by chopping into 0.5 – 1 cm piece root segments. The root segments of maize previously infected either by the spores of *G. margarita*, *Gl. intraradices*, or the mixture of the two AMF species. The AMF isolates had been collected from different sites in Southern Cameroon and screened using method proposed by Nwaga *et al.*,^[22] Ten grammes of soil, containing spores of the same fungi isolates were placed into the seedbed at the seedlings transplantation.

Assessment of AMF Root Colonization: The maize root section was harvested at 14, 28, 48, and 63 DAP and the percentage of root colonization by AMF was estimated. Sub-samples of 1 g 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 get back to room temperature were cut into 1 cm length pieces and cleared in KOH (10%) solution. The cut roots were stained with acid fuchsin in lacto-glycerin at room temperature according to Phillips and Hayman^[24] and Merryweather and Fitter^[19]. They were then examined for colonization by AMF using the grid-line intersection method of Giovannetti and Mosse (1980) under a microscope at 100x magnification.

Acid Phosphatase Specific Activity: The acid phosphatase-activity (acid phosphomonoesterase EC 3.1.3.2) of fresh root was measured with the artificial substrate paranitro-phenyl phosphate (pNPP) as described by Tarafdard and Marschner^[28]. Fresh roots were ground in a mortar in 0.4 M CH₃COONaOH (pH = 5) and centrifuged for 20 minutes at 5000 rpm at 4° C, and the acid phosphatase activity was estimated in the supernatant after incubating at room temperature for 15 minutes. At the end of the incubation period, 0.5 ml

of sub-sample was mixed with 2 ml of 0.5M NaOH and the concentration of *p*-NP was measured at 410 nm in a spectrophotometer. The concentration of soluble protein in the supernatant was determined using the Bradford reactive procedure and the specific activity of the root acid phosphatase was estimated per $\mu\text{mol per min}^{-1} \text{ mg}^{-1}$ of protein.

Statistical Analyses: Statistical analyses of the data were carried out using the (SAS) Statistical Analysis System software version 9.1 (2001). Analysis of variance (ANOVA) was performed using the General Linear Procedure "Proc GLM". The LSMEANS/PDIFF option was used to test the significance between levels of factors. Levels of significance in tables and graphs are given by ns, *, **, *** for not significant, significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$. Values in columns followed by the same letter are not significantly different at $P < 0.05$ (LSMEANS/PDIFF option). Relationship between pair of variables was done using the Proc REG of SAS.

RESULTS AND DISCUSSION

Shoot Dry Matter: Significant differences were observed in shoot dry matter between the two maize cultivars at 14, 28, 42 and 63 DAP (Table 3). Shoot dry matter increase with increasing data of harvest. At 14 and 28 DAP, no significant dry matter were obtained for the two cultivars between inoculated and non inoculated plant. However, ATP exhibited better dry matter production than CMS 8501. These results suggested that AMF inoculation plant was inactive at early stage (less than 28 days) but become very efficient in increasing dry matter at later stage (more than 28 days). In addition, the AMF strains used produced similar dry matter on both varieties. ATP S4 syn Y showed significantly and higher shoot dry matter production than CMS8501. With respect to ATP S4.syn Y, such significant differences were also observed at 42 and 63 DAP with early apparition at 28 DAP following inoculation by AMF ($P < 0.05$). The interactions between M x Cv were significant at 42 and 63 DAP, indicating that AMF symbiosis association with roots occurred at a given plant growing development period and the association was preferential for a particular cultivar.

P uptake: Significant differences in P uptake were also observed between the maize cultivars at the different harvest stage, except at 63 DAP, with cv ATP S4.syn Y generally had higher uptake than CMS8501 (Table 4). Significant P uptake increases were observed between maize inoculated with AMF and non-inoculated maize. ATP S4 syn Y exhibited higher P

uptake increase (18 %) compared to CMS8501 (15 %). P uptake increased with increasing date of harvest for the 2 cv. However, at early stage of growth (14 days) only the Al sensitive CMS8501 inoculated exhibited better P uptake than the control (non-inoculated plants). This suggested that AMF inoculation did not produce any additional P uptake with the acid tolerant cultivar. AMF inoculation of both AMF species and the mixture highly and significantly increased the P uptake of both maize cultivars at 14, 28, 42, and 63 DAP, respectively ($P < 0.001$). All the two maize cultivars significantly and highly responded to AMF inoculation at the different plant harvest stage. The significant interaction of cv x M indicated that, the response of the two maize cultivars were different. Inoculation with *Gi margarita* and the mixture of the two strains were significant on ATP S4 syn Y while *Gl intraradices* had better response for P uptake in CMS8501. The individually calculated regression between the P uptake and shoot dry matter were highly significant for both maize cultivars (Figure 1a).

A unit increase in P uptake resulted in 135 % and 60 % increase in shoot dry matter for ATP S4 syn Y and CMS8501 respectively. This seems to suggest that ATP S4 syn Y was better P use efficient than CMS8501.

Root Colonisation by AMF: Highly and significantly differences in the percentage of root colonisation by AMF ($P < 0.001$) were observed between the two maize cultivars at 14, 28, 42, and 63 DAP (Table 5). The percentage of root colonisation increased with increase date of harvest ATP S4 syn Y. except for 63 DAP, ATP S4 syn Y, the Al tolerance cv, generally exhibited higher colonisation percentage than CMS8501, the Al sensitive cv. On plot with no AMF inoculation, no root was colonized by AMF, indicating the non presence of reactive AMF on soil used. Inoculation with M2 exhibited higher percent root colonisation than M1 and M3 on both cultivars. The M x cv interaction were significant at 28, 42 and 63 DAP ($p < 0.05$). This indicated that the two cultivars responded differently to the different AMF species used. In fact, M1 showed better root colonisation with ATP S4 syn Y than with CMS8501. With M2 and M3, CMS8501 had more root colonized than ATP S4 syn Y. The relationships between the percentage of root colonisation by AMF and shoot dry matter were also positive and significant for the cv ATP S4.syn Y and CMS8501 (Figure 1b). However, there no was significant differences between the two coefficients of determination of the two cultivars.

Root Acid Phosphatases: Highly and significant cultivar differences in acid phosphatase activity of

crushed roots were observed at 14, 28, 42, and 63 DAP (Table 6). Significant root acid phosphatase were observed between root maize inoculated with ATP S4 syn Y compare to the non inoculated for the two contrasting cultivar. When cultivar did not receive any AMF, root acid phosphatase activity was higher in ATP S4 syn Y than in the CMS8501. Except for 14 days, the root acid phosphatase activity generally increases significantly with increase date of harvest. These above results screening of genotype for phosphatase activity would not result in meaningful results if done at early stage (less than 14 days). With respect to AMF inoculation, the acid phosphatase activity of plants inoculated with *Gi margarita* was significantly higher than *G. intraradices* ($P < 0.05$). The interaction between cv x date of harvest indicate that, CMS8501 the Al sensitive cultivar produced higher rate of root acid phosphatase activity than the Al tolerant cultivar as tolerance mechanism. The interaction between cultivar and AMF inoculation was highly significant at 14, 28, 42, and 63 DAP ($P < 0.001$). The mixture of the two inoculums did not produce a synergist effect on root acid phosphatase activity suggesting this activity should be measure, with only one strain of AMF. Shoot dry matter and acid phosphatase activity were significantly related for the both cv and ATP S4.syn Y had significantly higher coefficient of correlation than CMS8501 (Figure 1c). The same relation was observed between P uptake and phosphatase activity. This indicated that, even though the activities were lower in ATP S4 syn Y than CMS8501. The response in dry matter production and P uptake was higher in ATP S4 syn Y than in CMS8501.

Discussion: Results from the present study show the superior ability of maize cultivar ATP S4.syn Y to adapt on acid soil of southern Cameroon compared to CMS 8501 for their growth, P uptake and acid phosphatase. Furthermore, except for 14 and 28 days for dry matter production, significant increases in shoot dry matter P uptake and root colonisation were observed following AMF inoculation by *Gi margarita* and *Gl intraradices* at the different growth and harvest stages. Generally ATP S4 syn Y exhibited higher values than CMS8501 for the above parameters. This could partly explain why ATP S4 syn Y is an Al tolerant than CMS8501. Our results corroborate and confirmed previous findings of Thé *et al.*,^[29] and Thé *et al.*,^[31] who identified ATP S4.syn Y as acid soil-tolerant cultivar while CMS8501 was acid soil sensitive with AMF inoculation. Significant increase for all parameter measured, was observed on both maize Cv on compared to non-inoculated maize. The role of AMF in nutrient uptake particularly P has been

Table 3: Dry matter production of the maize CMS8501 and ATP S4.syn Y at 14, 28, 42 and 63 days after inoculation with *Gigaspora margarita* (M1), *Glomus intraradices* (M2), and M3, the mixture of M1 and M2 on acid soil of southern Cameroon. M0=control, n=3.

Treatment	CMS8501				ATP S4.syn Y			
	14	28	42	63	14	28	42	63
M0	0.15 aC	0.70 aB	1.50 bB	6.00 bA	0.20 aD	1.00 aC	1.70 bB	6.50 bA
M1	0.13 aD	1.00 aC	2.45 aB	9.80 aA	0.20 aD	1.09 aC	2.20 aB	10.50aA
M2	0.14 aD	0.85 aC	2.70 aB	9.75 aA	0.20 aC	1.06 aB	2.55 aB	10.35aA
M3	0.15 aD	1.00 aC	2.50 aB	9.10 aA	0.20 aC	1.04 aB	2.15 aB	11.30aA
<i>F test</i>								
Cultivar (Cv)	22.9 ***	7.0 *	15.6 **	31.1 ***				
Mycorrhizal (M)ns	ns	ns	811 ***	34.8 ***	ns	7.1 *	194.3**	152.1 ***
M x Cv	ns	ns	15.5 ***	8.8 **				
Cv (5%)	13.2	15.2	3.6	4.20				

Note 1. Numbers followed by the same lower case letter between Treatments within different harvest stage are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note 2. Numbers followed by the same upper case letter between different harvest stages within Treatment are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note ns: not significant, *, **, ***, significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

Table 4: P uptake (mg plant⁻¹) of maize cultivars CMS8501 and ATP S4.syn Y at 14, 28, 42 and 63 days after inoculation with *Gigaspora margarita* (M1), *Glomus intraradices* (M2), and the mixture of M1 and M2 on acid soil of southern Cameroon. M0=control, n=3.

Treatment	CMS8501				ATP S4.syn Y			
	14DAP	28DAP	42DAP	63DAP	14DAP	28DAP	42DAP	63DAP
M0	0.12 cC	0.70 bB	1.00 dB	5.90 cA	0.21 aC	0.73 cB	0.80dB	3.50 dA
M1	0.16 abD	1.15 aC	3.00 aB	14.30 bA	0.20 aD	1.70 aC	3.05aB	14.90aA
M2	0.17 aD	1.15 aC	2.75 bB	17.10 aA	0.13 bD	1.20 bC	2.55bB	13.30bA
M3	0.15 bC	1.25 aB	1.60 cB	14.70 bA	0.19 aC	1.30 bB	2.10cB	17.20 cA
<i>F test</i>								
Cultivar (Cv)	25.8 ***	157.0 ***	ns	7.2 *				
Mycorrhizal (M)	128.8 ***	138.5 ***	319.4 ***	784.0 ***	7.4 *	484 ***	1787 ***	116.4 ***
M x Cv	17.2 ***	69.1 ***	34.9 ***	11.3 ***				
Cv (5%)	9.9	3.0	3.40	5.60				

Note 1. Numbers followed by the same lower case letter between Treatments within different harvest stage are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note 2. Numbers followed by the same upper case letter between different harvest stages within Treatment are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note ns: not significant, *, **, ***, significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

Table 5: Percentage of root colonisation of maize cultivars CMS8501 and ATP S4.syn Y at 14, 28, 42 and 63 days after inoculation with *Gigaspora margarita* (M1), *Glomus intraradices* (M2), and (M3) the mixture of M1 and M2 on an acid soil of southern Cameroon. M0=control, n=3.

Treatment	CMS8501				ATP S4.syn Y			
	14DAP	28DAP	42DAP	63DAP	14DAP	28DAP	42DAP	63DAP
M0	0 bD	0 cA	0 cA	0 bA	0 bA	0 bA	0 bA	0 cA
M1	10aC	25bC	30 b	100 aA	15aB	50 aA	55 aA	65 bA
M2	10aB	50 aA	60 aA	100 aA	15aB	50 aA	60 aA	80 aA
M3	10aC	50 aB	50 aB	100 aA	15aB	50 aA	60 aA	60 bA
<i>F test</i>								

Table 5: Continue

Cultivar (Cv)	819 ***	12.5 ***	24.5 ***	89.5 ***				
Mycorrhizal (M)	ns	55.0 ***	84.0 **	267.0 ***	6.8 *	300 ***	205 .5 ***	362.3 ***
M x Cv	ns	12.5 ***	11.2 ***	54.3 ***				
Cv (5%)	37.7	12.6	11.0	7.10				

Note 1. Numbers followed by the same lower case letter between Treatments within different harvest stage are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note 2. Numbers followed by the same upper case letter between different harvest stages within Treatment are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note ns: not significant, *, **, ***, significant at $P ? 0.05$, $P ? 0.01$ and $P ? 0.001$, respectively.

Table 6: Root phosphatases activity [$\mu\text{Mol (min}^{-1} \text{ mg protein}^{-1})$] of the maize cultivar CMS8501 and ATP S4.syn Y at 14, 28, 42 and 63 days after inoculation with *Gigaspora margarita* (M1), *Glomus intraradices* (M2), and M3, the mixture of M1+M2 on acid soil of southern Cameroon. M0= control, n= 3.

Treatment	CMS8501				ATP S4.syn Y			
	14DAP	28DAP	42DAP	63DAP	14DAP	28DAP	42DAP	63DAP
M0	1.30 aA	2.00 cA	1.50 cA	2.85 cA	0.25 cC	2.00 cB	3.75 bA	4.90 bA
M1	0.85 bC	3.85 bB	5.85 bA	7.40 bA	0.35bC	2.65 bB	5.70 aA	6.70 aA
M2	1.60 aC	4.65 aB	9.35 aA	11.00 aA	0.65 aB	5.35 aA	5.30 aA	6.25 aA
M3	0.09 cC	2.80 bB	4.10 bA	6.00 bA	0.30cB	3.95 bA	4.40 bA	5.35 abA
<i>F test</i>								
Cultivar (Cv)	83.2 **	230.9 ***	66.6 ***	29.6 ***				
Mycorrhizal (M)	25.8 **	6.68 *	3612.2 ***	85.6 ***	87.0 ***	246.2 ***	103.5 **	19.8 ***
M x Cv	19.2 ***	72 ***	648.5 ***	46.5 ***				
Cv (5%)	23.70	4.3 0	2.50	7.85				

Note 1. Numbers followed by the same lower case letter between Treatments within different harvest stage are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note 2. Numbers followed by the same upper case letter between different harvest stages within Treatment are not significantly different at $P > 0.05$ (LSMEANS /PDIFF test).

Note ns: not significant, *, **, ***, significant at $P ? 0.05$, $P ? 0.01$ and $P ? 0.001$, respectively.

generally well documented, and attributed to that, successful establishment of mycorrhizal association enhance P uptake through the hyphal network extended beyond the P-depletion zone around the plants roots [26]. A number of studies have also suggested that AMF will induce the excretion of higher amount of organic acids into the plants rhizosphere rendering soluble inorganic P bound to Fe and Al oxide and hydroxides [23]. On the predominantly acid soils of southern of Cameroon, availability of P is very low making P one of the most limiting nutrient to crops growth [19], all the mechanisms exerted by the AMF lead to improve the P uptake. Under agricultural conditions of southern Cameroon [13,27], where farmers do not apply P fertilizers to crops, efficient root colonization by AMF which was highly related to root acid phosphatase activity would help the plant improve its growth and P uptake and thus reduce the need for P fertilizers application.

Plant adaptation to low P environment generally possesses various efficient P mechanisms. These include the enhancement of P uptake and use capability and/or mobilization of inaccessible P such as sparingly soluble inorganic P and organic P. It was somewhat

showed in this study that both maize cultivars were well colonized by AMF and that ATP S4.syn Y had significantly high P uptake, P use efficiency. Such significant differences in P use might account for their adaptation on acid and low P soil of southern Cameroon. Generally, plant sensitive to acid stresses is associated to aluminum toxicity due the Al^{3+} toxic form [11], depletion of pH around the rhizosphere and inhibition of P uptake by the roots [12]. ATP S4.syn Y would therefore cope with the P limitation by increasing its P uptake and remobilization capacity.

The results presented herein indicated the induction of the root phosphatase activity of the acid resistant maize cultivar (ATP Syn 4 y) as potential candidate mechanisms for their adaptation in high acid of southern Cameroon. The ATP S4.syn Y cultivar responded to low P adaptation by exuding significant amount of phosphatase that was related to shoot growth and P uptake at different growth stage of the plant, and the response was cultivar dependant (significant interaction Cv x M). Recent evidence on the role of AMF on plant adaptation to low P soils indicate that the fungal partner activates a part of the adaptation to low P-adaptation through phosphatases secretion and

improve the overall efficiency of P uptake [5]. These authors recently identified a specific phosphatase *TpPAP*, specific to AMF and further classified in the super family of phosphatase, with their gene expression regulated by P nutrition. The transcription levels of *TpPAP1* increased in response to P deficiency and decreased under P-sufficient conditions. It assumed that AMF symbiosis with plant roots improve P nutrition, and this may results in decrease in PA gene [9,16,20]. However, the molecular mechanism involved in the interaction is of interest and remains un-clarified.

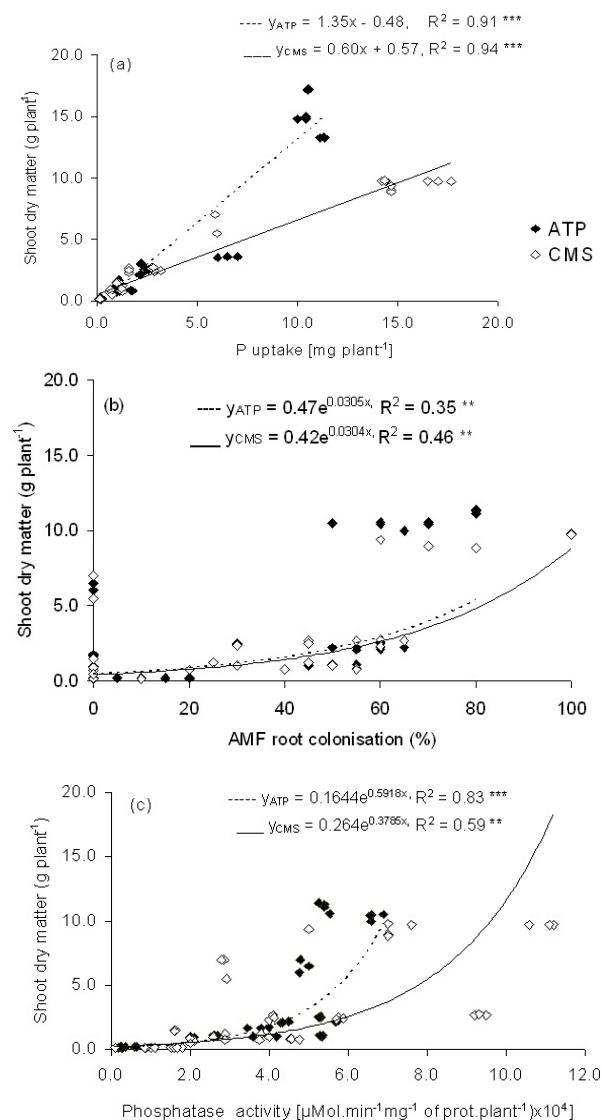


Fig. 1: Relationship between shoot dry matter and (a) P uptake, (b) percentage of root colonisation by AMF, and (c) phosphatase activity of maize cultivar ATP s4.syn Y (filled symbols) and CMS8501 (open symbols) grown on acid and low available P soil of southern Cameroon. Filled

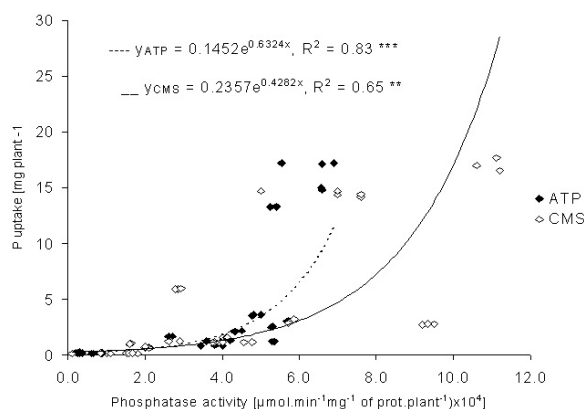


Fig. 2: Relationship between the P uptake and the activity of phosphatase in roots of maize cultivar ATP s4.syn Y (filled symbols) and CMS8501 (open symbols) grown on acid and low available P soil of southern Cameroon.

At the present stage of this work, conclusions about the growth and yielding capacity under field conditions on acid soils with high Al supply and low P availability may be misleading, because under such conditions the performance of maize also depend on many others soil factors such as nitrogen supply, light availability, the total rainfall, and the competition with indigenous AMF populations present in soils.

ACKNOWLEDGMENTS

The research work was supported by Biotechnology Centre of the University of Yaounde 1 and the International Institute of Tropical Agriculture of Cameroon. The Cereal breeding program of IRAD Cameroon is acknowledged for the supply of the maize seeds.

REFERENCES

1. Sample, E.C., R.J. Soper and G.J. Racz, 1980. Reactions of phosphate in soils. *In* The role of phosphorus in agriculture, Eds F E Khasawneh, E C Sample and E J Kamprath. Am. Soc. Agron., Madison, Wisconsin, USA., pp: 263-310.
2. Araújo, A.P., M.G. Teixeira and D.L.D. Almeida, 1998. Variability of traits associated with phosphorus efficiency in wild and cultivated genotypes of common bean. *Plant and Soil.*, 203: 173-182.
3. Chabi-Olaye, A., C. Nolte, F. Schulthess and C. Borgemeister, 2005. Effects of grain legumes and cover crops on maize yield and plant damage by *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in the humid forest of southern Cameroon. *Agriculture, Ecosystems and Environment*, 108: 17-28.

4. Eswaran, H., R. Almaraz, Van den E. Berg and P. Reich, 1997. An assessment of the soil resources of Africa in relation to productivity. *Geoderma*, 77: 1-18.
5. Ezawa, T., M. Hayatsu and M.A. Saito, 2005. new hypothesis on the strategy for acquisition of phosphorus in arbuscular mycorrhiza: up-regulation of secreted acid phosphatase gene in the host plant. *Molecular Plant and Microbes Interactions*, 18: 1046 -1053.
6. FAO., Food, 1993. and Agriculture Organisation of United Nations. Maize in human nutrition. Rome, 160.
7. FAO., 2001. FAOSTAT Data Query Crops www.apps.fao.org.
8. Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol*, 84: 489- 500.
9. Haran, S., S. Logendra, M. Seskar, M. Bratanova and I. Raskin, 2000. Characterization of arabidopsis acid phosphatase promoter and regulation of acid phosphatase expression. *Plant Physiol*, 124: 615-626.
10. Hauser, S., J. Henrot, and A. Hauser, 2002. Maize yields in mulched and burned *Mucuna pruriens* var *utilis* and *Pueraria phaseoloides* relay fallow systems in southern Cameroon. *In Biological Agriculture and Horticulture*, 243 - 256.
11. Horst, W., A.k. Puschel and N. Schmohl, 1997. Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. *Plant Soil*, 192: 23-30.
12. Jemo, M., R.C. Abaidoo, C. Nolte and W.J. Horst, 2007. Aluminium resistance of cowpea as affected by phosphorus-deficiency stress. *Journal of Plant Physiology*, 164: 442—451.
13. Jemo, M., R.C. Abaidoo, C. Nolte, M. Tchienkoua, N. Sanginga and W.J. Horst, 2006. Phosphorus benefits from grain-legume crops to subsequent maize grown on acid soils of southern Cameroon. *Plant and Soil*, 284: 385 - 397.
14. Klugh, K.R. and J.R. Cumming, 2007. Variations in organic acid exudation and aluminum resistance among arbuscular mycorrhizal species colonizing *Liriodendron tulipifera*. *Tree Physiology*, 27: 1103 - 1112.
15. Kochian, L., O. Hoekenga and M. Pineros, 2004. How do crop plants tolerate acid soils? - Mechanisms of aluminium tolerance and phosphorous efficiency. *Annual review of Plant biology*, 55: 459-493.
16. Li, D., H. Zhu, K. Liu, X. Liu, G. Leggewie, M. Udvardi and D. Wang, 2005. Purple acid phosphatases of *Arabidopsis thaliana*. Comparative analysis and differential regulation by phosphate deprivation. *J. Biol. Chem.*, 277: 27772-27781.
17. Marschner, H., 1991. Mechanisms of adaptation of plant to acid soils. *In Plant-soil interaction at low pH*, Dordrecht, The Netherlands, Eds R J Wright, V C Baligar and R P Mrrmann, pp: 683-720.
18. Menzies, N.W. and G.P. Gillman, 1997. Chemical characterization of soils of a tropical humid forest zone: a methodology. *Soil Sci. Soc. Am. J.*, 62: 1355–1363.
19. Merryweather, J.M. and A.H.A. Fitter, 1991. modified method for elucidation the structure of the fungal partner in a vesicular mycorrhiza. *Mycological Research*, 95: 1435-1437.
20. Miller, S.S., J. Liu, D.L. Allan, C.J. Menzhuber, M. Fedorova and C.P. Vance, 2001. Molecular control of acid phosphatase secretion into the rhizosphere of proteoid roots from phosphorus-stressed white lupin. *Plant Physiol*, 127: 594-606.
21. Motomizu, S., P. Wakimoto and K. Toei, 1983. Spectrophotometric determination of phosphate in river waters with molybdate and malachite green. *Analyst (London)* 108: 361-367.
22. Nwaga, D., M.E.L. Ngonkeu, M.M. Oyong, A. Ngakou, M.P. Abelong and J.S. Foko, 2000. Soil beneficial micro-organisms and sustainable agricultural production in Cameroon: current research and perspectives., Eds The Tropical soil Biology and fertility (TSBF). pp. UNESCO-TSBF, Nairobi, Kenya, pp: 62-65.
23. Ouahmane, L., J. Thioulouse, M. Hafidi, Y. Prin, M. Ducouso, A. Galiana, C. Plenchette, M. Kisa and R. Duponnois, 2007. Soil functional diversity and P solubilization from rock phosphate after inoculation with native or allochtonous arbuscular mycorrhizal fungi. *Forest Ecology and Management*, 241: 200–208.
24. Phillips, J.M. and D.S. Hayman, 1970. Improved procedure for clearing roots and staining parasitic and Vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 5: 158- 161.
25. SAS., 2001. Statistical Analysis System Institute SAS/STAT User's Guide, Cary NC, USA.
26. Schachtman, D.P., R.J. Reid and S.M. Ayling, 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiol*, 116: 447–453.
27. Selles, F., C.A. Campbell and R.P. Zentner, 1995. Effect of cropping and fertilization on plant and soil phosphorous. *Soil Sci. Soc. Amer. J.*, 59: 140-144.
28. Tarafdar, J.C. and H. Marschner, 1994. Dual inoculation with *Aspergillus fumigatus* and *Glomus mosseae* enhances biomass production and nutrient uptake in wheat (*Triticum aestivum* L.) supplied with organic phosphorus as Na-phytate. *Plant Soil*, 173: 97–102.

29. Calba, C.H., W.J. Horst and C. Zonkeng, 2001. Three- year performance of a tolerant and a susceptible maize cultivars on non-amended and amended acid soil. *In* Plant nutrition: Food security and sustainability of agro-ecosystems through basic and applied research, International Plant Nutrition Colloquium. 14, 2001-07-27/2001-08-03, Hanovre, Allemagne, Eds Horst et al. 2001, 980- 985.
30. Thé, C., H. Calba, C. Zonkeng, E.L.M. Ngonkeu, V.O. Adetimirin, H.A Mafouasson, S.S. Meka and W.J. Horst, 2006. Responses of maize grain yield to changes in acid soil characteristics after soil amendments. *Plant and Soil.*, 284: 45–57.
31. Thé, C., M.E.L. Ngonkeu, D. Nwaga, N.J. Oloumane, C.G. Zonkeng, and N.S. Tchameni, 2004. Response of two contrasting maize cultivars to mycorrhiza inoculation and fertilizer amendments on acid soil. p 130-131. *In* Bationo A, Kimetu J and Kihara J Abstract of the international symposium of the African Network for soil Biology and Fertility (AfNet) of TSBF Institute of CIAT, Yaoundé, Cameroon, May, pp: 17-21.
32. Thé, C., W.J. Horst, H. Calba, C. Welcker and C. Zonkeng, 1997. Identification and development of Maize genotypes adapted to acid-soil of the tropic. *In* Plant Soil Interaction at low pH: sustainable agriculture and forestry production, Campinas, SP Brazil., 1997. Eds A C M e al.. 17 - 24 March, pp: 265.