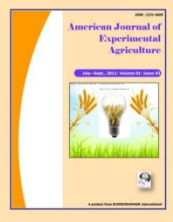

American Journal of Experimental Agriculture
4(2): 193-214, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Changes in Rhizosphere Concentration of Mineral Elements as Affected by Differences in Root Uptake and Plant Growth of Five Cowpea Genotypes Grown in Mixed Culture and at Different Densities with Sorghum

Joachim H. J. R. Makoi[1*], Samson B. M. Chimphango[2] and Felix D. Dakora[3]

[1]Faculty of Applied Science, Cape Peninsula University of Technology, Cape Town Campus, Keizergracht, P.O. Box 652, Cape Town 8000, South Africa.
[2]Botany Department, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa.
[3]Chemistry Department, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa.

Authors' contributions

This work was carried out in collaboration between all authors. Authors FDD and JHJRM were responsible for the initial idea and study design. Author JHJRM carried out the field experiment, collected data and compiled all relevant information and performed preliminary analysis. Authors FDD and SBMC worked with author JHJRM in data analysis and interpretation. Author JHJRM in collaboration with authors FDD and SBMC wrote the first draft of the manuscript. All authors contributed to the editing, revision, and final preparation of the manuscript. All authors have read and approved the final manuscript.

Original Research Article

Received 29th June 2013
Accepted 30th September 2013
Published 7th November 2013

ABSTRACT

Aims: To evaluate the effect of planting density and cropping systems on the changes in rhizosphere concentration and uptake of mineral elements of five cowpea genotypes (i.e. Bensogla, ITH98-46, Sanzie, TVu1509 and Omondaw).
Study Design: 3-factorial randomized complete block design.

**Corresponding author: Email: makoi.joachim230@gmail.com;*

Place and Duration of Study: Nietvoorbij (33º54S, 18º14E), Stellenbosch, South Africa during 2005 and 2006 summer seasons.
Methodology: A field experiment involving two cowpea plant densities (83,333 and 166,666 plants.ha^{-1}), two cropping systems (monocropping and intercropping) and five cowpea genotypes (i.e. Bensogla, ITH98-46, Sanzie, TVu1509 and Omondaw).
Results: The data for 2005 and 2006 were similar, and therefore pooled for statistical analysis. The concentrations of P, K, S, Na, Cu, and Zn were lower in rhizosphere of cowpea relative to bulk soil, while those of Ca and Mg were greater in the rhizosphere compared with bulk soil. With sorghum, only K, S, and Na were lower in the rhizosphere, in contrast to P, Ca, Mg, Cu, and Zn, which were higher in the rhizosphere. These differences in mineral concentration were due to alteration in rhizosphere pH, which was increased by cowpea but unchanged by sorghum. The data also showed that high plant density (166,666 plants.ha^{-1}) and mixed culture significantly decreased rhizosphere soil pH, resulting in low availability of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn and B in the rhizosphere of cowpea and sorghum compared with low plant density (83,333 plants.ha^{-1}) or monocropping. The results also showed significant differences in rhizosphere concentration of minerals between and among the five cowpea genotypes, with cv. Sanzie consistently indicating much lower levels of P and Ca as a result of higher root uptake, which was evidenced by the higher tissue content of P, K, Ca, Mg, Na, S, Fe, Zn, Mn and B in cv. Sanzie.
Conclusion: N_2-fixing cowpea significantly lowered the concentration and increased the uptake of mineral elements from the rhizosphere soil relative to sorghum.

Keywords: Elemental content; plant nutrients; rhizoplane; Sorghum bicolour; Vigna unguiculata.

1. INTRODUCTION

In Africa, most farmers grow two or more crops (usually legumes in mixture with cereals) simultaneously on the same field in an effort to increase crop diversity and improve food security. Such agronomic practices involving different crop components in the region, is often accompanied by the depletion of mineral elements in the rhizosphere, leading to variations in rhizosphere and tissue concentration of mineral elements. Knowing the levels of mineral concentration in the rhizosphere of crop plants could lead to better agronomic management of nutrients in cropping systems.

Root and soil interactions during plant growth induce changes that make rhizosphere soil to differ from bulk soil [1]. These changes can be caused by root uptake of nutrients, microbial activity, and/or components of root exudates [2,3,4]. Plant species differ in their uptake of soil nutrients. Legumes and cereals, for example, take up significantly different amounts of nutrients from the rhizosphere; and in so doing, legumes acidify the rhizosphere environment [5,6,7,4] through excess uptake of cations during N_2 fixation and/or reduction of N_2 to NH_4^+ and its assimilation via GIS-GOGAT pathway. Additionally, rhizosphere concentration of nutrients can be altered by agronomic practices such as cropping systems and planting patterns [8]. Stress can result into root exudation of mineral elements and organic compounds, leading to the rhizosphere modification [2,4].

Cowpea (*Vigna unguiculata* L. Walp.) is a source of high quality protein crop used by the resource poor farmers in Africa [9]. Its tolerance to moisture stress [10], its roles in nutritional

status, soil fertility improvement and weed control [11], make it a useful component of the cropping systems involving cereals such as sorghum [12].

Recent studies have shown that changes in the mineral concentration of the rhizosphere can also be caused by species differences. For example, legumes are known to secrete more acid phosphatases in the rhizosphere than cereals, often leading to greater enzyme activity and increased P availability [13]. Thus, when legumes are grown in mixtures with cereals, especially where roots are in close proximity, they can potentially enhance P supply to the associated cereal plants. In fact, the White lupin (*Lupinus albus* L.) is reported to increase P uptake by wheat (*Triticum aestivum* L.) when grown together; and pigeon pea (*Cajanus cajan* L.) also similarly improved P nutrition of sorghum in a mixed culture situation ([14,15]. Because of its ability to secrete Fe-solubilising phytosiderophores [16], maize enhanced Fe nutrition in peanut when grown in mixed culture with this legume [17]. Peanut and pigeon pea have also been suggested to increase P availability through contact reactions at the cell wall interface [18,19]. However, this mechanism still remains to be properly understood. In this study, it is hypothesized that different cropping systems and planting density can lead to stress resulting into root exudation of minerals and organic compounds. As a result, concentration of nutrients in the rhizosphere soil will be altered or modified. Similarly, it is hypothesized that different species such as those used in this study have different demand from the rhizosphere soil, resulting into changes in the rhizosphere mineral elements concentration and their respective plant uptake.

Although we have recently gained considerable insights into nutrient dynamics in the rhizosphere, little is known about the concentration of minerals in plant rhizosphere, especially when grown in different cropping systems and at different plant densities. The objectives of this study were (i) to measure and compare the mineral concentrations in the rhizosphere of five nodulated cowpea genotypes and sorghum, grown in mixed culture and at different cowpea plant densities and (ii) to study and further relate the changes in rhizosphere mineral concentrations with whole plant elemental contents and plant growth.

2. MATERIALS AND METHODS

2.1 Site Description

The study was conducted at the Agricultural Research Council (ARC) Nietvoorbij station in Stellenbosch, South Africa, during the 2005 and 2006 summer seasons. The site is located at 33º54′S and 18º14′E at an elevation of 146 m above mean sea level. The mean potential evapotranspiration (ET_O) as measured by Penman Monteith [20] during the growing season was 195.9 mm. The mean seasonal minimum and maximum temperatures were 28.2°C and 16.1ºC respectively and the mean seasonal radiation was 734.5 $MJ.m^{-2}.month^{-1}$, wind speed was 3 $m.s^{-1}$, mean annual rainfall was 98.8 $mm.month^{-1}$ and relative humidity was 59.3% (also see Table 1). The experimental sites had a previous history of table grape cultivation with a moderate application of P fertilizer (80 $kg.ha^{-1}$ maxfos, 20% P). The field soil used for this study is a sandy loam classified as skeletic leptosol in the FAO soil classification system [21].

2.2 Source of Cowpea Material Collected

Due to lack of information on cowpea genotypes which can modify the rhizosphere nutrient concentration as well as high elemental content and growth, a project funded by the

McKnight Foundation was launched in June 2003 in three African countries (namely Ghana, South Africa and Tanzania) with the aim of identifying cowpea genotypes with greater rhizosphere modification, high mineral elements uptake and growth. In order to achieve this objective, one hundred and twenty six (126) cowpea genotypes were obtained from farmers, village markets, national programmes, and gene banks, in Ghana, South Africa and Tanzania. Cowpea material was also obtained from the International Institute of Tropical Agriculture in Nigeria which has the mandate for cowpea improvement. To establish baseline data, five cowpea genotypes (randomly selected) were then grown alone or in mixture with sorghum for rhizosphere nutrient concentration, elemental contents and growth. In this study, we report the effect of cropping systems on rhizosphere nutrient concentration, elemental contents and growth of the five cowpea genotypes grown at different plant densities with sorghum.

2.3 Experimental Design

The experimental treatments used in this study included two cowpea densities (83,333 vs. 166,666 plants.ha^{-1}), two cropping systems (monocropping vs. mixed culture) and five cowpea genotypes, two of which were improved (ITH98-46 and TVu1509) and three unimproved landraces (Bensogla, Sanzie and Omondaw). The lower cowpea density was chosen to reflect farmers' practice that often has low plant stand compared with sorghum. The experimental layout was split plot with 3-factorial arrangement in a randomised complete block design with plant density as the main plot, cowpea genotypes as sub-plots, and cropping system as sub-subplots. Four replicates were used per treatment and plots measured 3.6 m x 3.2 m (11.52 m^2). After land preparation and field experimental layout, cowpea genotypes in monocropping were sown in first week of December 2005 and 2006 (i.e. a representative season in the study area) with row-to-row spacing of 60 cm, and plant-to-plant spacing of 40 cm to reflect low plant density whereas row-to-row spacing of 60 cm, and plant-to-plant distance of 20 cm was used to reflect high plant density. Sorghum plants in plots with 90 cm row-to-row spacing and 40 cm plant-to-plant spacing was used for the density of 55,555 plants.ha^{-1}. In mixed culture, cowpea was sown at a row-to-row spacing of 90 cm and plant-to-plant distances of 26.6 cm to give the lower plant density. Similarly, row-to-row spacing of 90 cm, with plant-to-plant spacing of 13.3 cm was used for high plant density cowpea in mixed culture. At planting, the cowpea seeds were inoculated with *Bradyrhizobium* strain CB756, and following germination, cowpea and sorghum seedlings were thinned out to two plants per stand. Weeding was done manually with a hoe. Plants were irrigated up to field capacity once every three days up to flowering, and once a week thereafter. The aim was to supply 650 mm of water so as to meet the crops seasonal water requirement. In this experiment, no fertilizer was used.

2.4 Collection and Preparation of Bulk and Rhizosphere Soil

At 67 days after planting (DAP), rhizosphere soil was collected from around the roots of both cowpea and sorghum plants for nutrient analysis. The soil around single plants was excavated to about 30 cm or more, and the intact soil on the roots removed for up to 16 cowpea plants per plot or 8 sorghum plants per plot. The soil adhering tightly to the roots (about 30 – 50 g) was shaken off into a plastic bag. For each rhizosphere soil collected, a corresponding non-rhizosphere (bulk) soil was collected for comparison. The bulk and rhizosphere soil samples were then taken to the laboratory, air dried, and sieved (2 mm mesh) for chemical analysis and results are shown in Table 1.

2.5 Plant Harvest and Sample Preparation

At 67 DAP, during early pod development for cowpea and taselling for sorghum, sixteen and eight plants of cowpea and sorghum were respectively harvested from the middle rows of each plot. The cowpea and sorghum plants were carefully dug out with intact root system, washed, and oven-dried at 60°C for 48 hrs and ground into fine powder (2 mm sieve) and stored, prior to analysis for mineral elements concentration on whole plant basis.

2.6 Measurement of Soil pH

The pH levels of both bulk and rhizosphere soils were measured in 0.01M $CaCl_2$ solution (1:2.5, soil to $CaCl_2$).

2.7 Determination of Plant-available Minerals in Rhizosphere Soils

Extractable P, K, Ca, Mg and Na were determined by the citric acid method as developed by [22] Dyer (1894) and modified by the Division of Chemical Services [23] and [24] Du Plessis and Burger (1964). A 20 g air-dried soil sample was extracted in 200 mL of 1% (w/v) citric acid, heated to 80°C, shaken for 2 min at 10-min intervals over 1 h period and filtered. A 50 mL aliquot was heated to dryness on a water bath, digested with 5 mL of concentrated HCl and HNO_3, evaporated to dryness on a water bath, and 5 mL of concentrated HNO_3 and 20 mL of de-ionized water added. The mixture was then heated to dissolve the dry residue, and the sample filtered. Measurement of P, K, Na, Ca and Mg were then done directly by aspiration on a calibrated simultaneous inductively coupled plasma (ICP) mass spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts USA).

The determination of S and B in the soil was done by adding 20 g of soil in 0.01M $Ca(H_2PO4)_2.H_2O$ extracting solution [25], followed by filtering. Sulphur was determined by direct aspiration on a calibrated simultaneous inductively coupled plasma (ICP) spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA).

The trace elements Cu, Zn, Mn, Fe, and Al were extracted from soil using di-ammonium ethylenediaminetetraacetic (EDTA) acid solution [26] modified by [27]. The extractants were analysed for Cu, Zn, Mn, Fe, and Al using ICP-MS spectrometry (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA).

2.8 Measurement of Mineral Elements in Plant Tissue

Measurements of macro elements (P, K, Ca, Mg, and Na) and micro elements (Cu, Zn, Mn, Fe, Al, and B) were determined by ashing 1 g ground sample in a porcelain crucible at 500°C overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and placing it in an oven at 50°C for 30 min and 35 mL of deionised water was added. The mixture was filtered through Whatman no. 1 filter paper. Mineral elements concentration in plant extracts were determined using the ICP [28]. Sulphur was determined by wet digestion procedure using 65% nitric acid. In each case, 1 g of milled plant material was digested overnight with 20 mL of 65% nitric acid in a 250 mL glass beaker. The beaker containing the extract was then placed on a sand bath and gently boiled until approximately 1 mL of the extract was left. After that, 10 mL of 4 M nitric acid was added and boiled for 10 min. The beaker was

removed from the sand bath, cooled, and the extract washed completely in a 100 mL volumetric flask and the extract filtered through Whatman no. 2 filter paper. The S in the sample was then determined [25] by direct aspiration on the calibrated simultaneous ICP-MS.

2.9 Statistical Analysis

A 3-factorial (3-Way ANOVA) analysis involving cropping systems, plant density and cowpea genotypes was used to analyse the data. Also, one-way ANOVA was used to compare nutrient concentration in the rhizosphere and uptake of cowpea and sorghum plants. The analysis was performed using the STATISTICA software of 2007 version (StatSoft Inc., Tulsa, OK, USA). Fisher's least significant difference (LSD) was used to compare treatment means at P=.05 level of significance [29].

3. RESULTS

3.1 A Comparison of Mineral Concentrations in Bulk and Rhizosphere Soils of Cowpea and Sorghum

The concentrations of P, K, S, and Na were lower in the rhizosphere of cowpea relative to bulk soil, while those of Ca and Mg were greater in the rhizosphere compared with bulk soil (Table 2). With sorghum, only K, S, and Na were decreased in the rhizosphere, in contrast to P, Ca, Mg, Cu, and Zn, which increased in the rhizosphere (Table 2). The rhizosphere pH of cowpea was also significantly lower than that of sorghum and bulk soil (Table 2).

3.2 Effect of Plant Density and Cropping Systems on Rhizosphere pH in Cowpea and Sorghum Plants

Cowpea rhizosphere pH changed significantly (P=.05) under different cowpea plant density and cropping systems. Specifically, increasing plant density from 83,333 (low) to 166,666 (high) plants.ha^{-1} significantly (P=.05) lowered the cowpea rhizosphere pH by 3.7% (Table 3 ~~2~~). Similarly, changing the cropping system from monocropping to mixed culture significantly (P=.05) lowered the cowpea rhizosphere pH by 4.2% (Table 3).

Similar results as observed for cowpea rhizosphere pH was also observed for sorghum. In this regard, the pH in the rhizosphere of sorghum grown with cowpea at high plant density was significantly lower by 1% compared with those grown in low cowpea plant density (Table 4). Intercropping also lowered the rhizosphere pH by 3.2% compared with sorghum grown alone (Table 4).

3.3 Effect of Plant Density on Rhizosphere Mineral Element Concentration, Growth and Tissue Elemental Content in Cowpea and Sorghum Plants

Increasing cowpea plant density from low to high significantly decreased (P=.05) the concentrations of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn and B in the rhizosphere of cowpea plants (Table 3), leading to reduced levels of these minerals in cowpea tissues (Table 5). Plant growth measured as whole plant biomass was similarly lower under high plant density compared with low plant density (Table 3).

Sorghum plants in mixture with cowpea at high cowpea plant density significantly (*P*=.05) lowered the concentrations of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, and B in the rhizosphere of sorghum (Table 4). Plant growth and the uptake of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, in sorghum was also significantly (*P*=.05) lowered when mixed with cowpea at high plant density compared with those mixed with cowpea at low plant density (Table 6).

3.4 Effect of Cropping Systems on Rhizosphere Mineral Element Concentration, Growth and Tissue Elemental Content in Cowpea and Sorghum Plants

Changing the cropping system significantly (*P*=.05) altered the mineral elements concentration in the rhizosphere and growth of cowpea as well as their tissue elemental content. Growing cowpea in mixture with sorghum significantly (*P*=.05) lowered the concentrations of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, and B in the rhizosphere, resulting into a markedly decreased content in tissues and plant growth compared with cowpea grown alone (Tables 3 and 5).

Similarly, intercropping sorghum with cowpea significantly (*P*=.05) lowered the concentrations of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, and B in the rhizosphere of sorghum, plant growth and the uptake of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, and B compared with sorghum grown alone (Tables 4 and 6).

3.5 Effect of Genotypes on Rhizosphere Mineral Element Concentration, Growth and Tissue Elemental Content in Cowpea and Sorghum Plants

Significant differences were found in the rhizosphere concentration of minerals elements between and among the five cowpea genotypes, with cowpea cv. Sanzie consistently showing lower levels of P and Ca compared with ITH98-46 (Table 3). The lower concentration of minerals in the rhizosphere of the cv. Sanzie was caused by higher root uptake (Table 5). Sanzie genotype showed significantly much greater accumulation of P, K, Ca, Mg, Na, S Fe, Zn, Mn and B compared with the other four cowpea genotypes i.e. Bensogla, TVu1509 and ITH98-46 but more prominently with ITH98-46 (Tables 3 and 5). The greater accumulation of mineral elements by Sanzie led to significantly increased plant growth, measured as whole-plant biomass (Tables 3 and 5). In contrast to Sanzie, cv. ITH98-46 had a much higher concentration of P, Ca, and Cu in its rhizosphere, suggesting much lower uptake and accumulation in tissues, which led to low plant growth (Tables 3 and 5).

Although there was no significant change observed in growth and rhizosphere mineral element concentration in sorghum grown in mixture with cowpea genotypes, there was a significant change on the uptake of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, and B. It was observed that the elemental content in sorghum grown with Sanzie cowpea genotype was consistently lower than those grown in mixture with ITH98-46 cowpea genotype (Tables 4 and 6).

Table 1. Climatic characteristics of the experimental area during the growing period

Month	R (mm.month^{-1})	T_{max}	T_{min}	T_{mean}	Wind (m.s^{-1})	$\%RH_{mean}$	ET_o (mm.month^{-1})	Rs (MJ.m^{-2}.day^{-1})	R_s (MJ.m^{-2}.month^{-1})
December	12.2	27.6	15.6	21.5	2.8	59.3	212.2	29.1	902.1
January	62.8	28.2	17.4	22.4	3.5	57.7	231.3	28.3	876.1
February	3.6	29.3	15.9	22.3	2.9	59.8	167.1	19.4	542.4
March	20.2	27.8	15.4	20.9	2.8	59.7	173.1	19.9	617.5
Sum	98.8	112.9	64.3	87.1	12.0	236.5	783.7	96.7	2938.1
Average	24.7	28.2	16.1	21.8	3.0	59.1	195.9	24.2	734.5

R=Rainfall, T_{max}=Maximum temperature, T_{min}=Minimum temperature, T_{mean}=Mean temperature, $\%RH_{mean}$=Mean Percent Relative Humidity, ET_o=Potential Evapotranspiration, R_s = Solar Radiation.

Table 2. Comparison of mineral element concentration between bulk soil and rhizosphere soils of cowpea and sorghum species

Treatment	pH	P	K	Ca	Mg	S	Na	Cu	Zn
		…….. mg.kg^{-1} ….....							
Bulk soil	6.4a	18.8b	137.8a	70.5b	16.6c	4.2a	90.5a	3.8b	3.4b
Rhizosphere soil									
Cowpea	5.8c	14.4c	112.5b	729.2a	186.8b	3.5b	61.7c	4.0ab	3.0b
Sorghum	6.2b	29.3a	122.5b	771.7a	210.4a	2.8c	77.8b	4.2a	5.2a
One - Way ANOVA (F-Statistic)									
	64.8**	28.8***	12.8***	534.1**	475.7**	11.5***	45.2**	6.7**	28.8***

*NS: the difference was not significant at P=.05; **: significant at P=.01; ***: significant at P=.001. Values followed by dissimilar letters in the same column for each treatment are significantly different from each other at P=.05 according to Fischer LSD. All values that were not significantly different from each other are not shown in this table.*

Table 3. Concentration of mineral elements in the rhizosphere soil of five cowpea genotypes planted under different plant densities and cropping systems

Treatments	pH	P	K	Ca	Mg	Na	S	Fe	Cu	Zn	Mn	Bo	DMY
		……					($mg.kg^{-1}$)……						(g)
Plant density ($plants.ha^{-1}$)													
83,333	5.90a	17.65a	122.28a	817.15a	207.86a	67.05a	4.92a	4.39a	3.57a	9.33a	280.03a	0.53a	28.4a
166,666	5.68b	11.15b	102.68b	641.20b	165.83b	56.38b	2.16b	3.51b	2.53b	7.39b	218.28b	0.45b	20.9b
Cropping system													
Monocropping	5.92a	17.58a	120.20a	798.55a	205.78a	66.73a	4.36a	4.18a	3.44a	9.37a	281.94a	0.54a	30.0a
Intercropping	5.67b	11.23b	104.75b	659.80b	167.90b	56.70b	2.72b	3.73b	2.66b	7.36b	216.37b	0.44b	19.3b
Genotypes													
Bensogla		13.38ab		686.25ab				3.75ab					25.6ab
ITH98-46		19.56a		826.38a				4.30a					20.8b
Sanzie		11.63b		640.13b				3.71b					28.2a
TVu1509		14.19ab		792.63ab				4.14ab					22.1b
Omondaw		13.25b		700.50ab				3.86ab					26.4ab
3 - Way ANOVA (F-Statistic)													
Main Effects													
Density	26.9***	21.8***	19.5***	23.6***	39.6***	8.9**	60.5***	61.7***	23.1***	19.2***	31.5***	15.8***	31.4***
Cropping systems	35.6***	20.9***	12.1***	14.7***	32.1***	7.9**	21.4***	16.2***	13.0***	20.6***	35.5***	23.4***	63.6***
Genotypes		3.8**		3.7**				4.2**					4.3**
Interactions													
Density*Genotypes													2.8*
CV (%)	3.3	43.2	17.6	22.2	16.0	25.9	44.8	12.7	31.7	23.6	19.7	17.4	24.4

: significant at P=.05; **: significant at P=.01; *: significant at P=.001. Values followed by dissimilar letters in the same column for each treatment are significantly different from each other at P=.05 according to Fischer LSD. (LSD: Least significance difference; DMY: Whole plant Dry matter yield). All values that were not significantly different from each other are not shown in this table.*

Table 4. Concentration of mineral elements in the rhizosphere soil of sorghum planted under different cowpea plant densities

Treatments	pH	P	K	Ca	Mg	Na	S	Fe	Cu	Zn	Mn	Bo	DMY
		..... (mg.kg^{-1}).....											(g)
Plant density (plants.ha^{-1})													
83,333	6.22a	31.78a	128.10a	801.55a	218.72a	82.68a	2.98a	4.35a	5.45a	9.09a	262.88a	0.55a	47.04a
166,666	6.16b	29.33b	122.54b	771.70b	210.37b	77.79b	2.85b	3.96b	4.93b	8.06b	228.02b	0.51b	35.51b
Cropping system													
Monocropping	6.30a	38.90a	144.00a	945.50a	273.28a	94.38a	3.60a	4.60a	7.67a	10.40a	271.80a	0.59a	46.85a
Sorghum+cowpea	6.10b	26.88b	116.98b	741.85b	202.03b	72.90b	2.71b	3.72b	2.71b	6.76b	219.10b	0.47b	35.70b
3 - Way ANOVA (F-Statistic)													
Main effects													
Density	16.0***	27.4***	18.0***	25.6***	16.4***	20.4***	14.6***	17.3***	8.5**	24.7***	12.9***	9.6**	76.7***
Cropping system	419.0***	418.1***	87.1***	381.4***	557.1***	1161.9***	168.0***	89.8***	783.3***	311.1***	29.5***	91.3***	71.7***
Interactions													
Density*Cropping systems	16.0***	27.4***	18.0***	25.6***	16.4***	20.4***	0.2	17.3***	8.5**	24.7***	12.9***	9.6**	0.9
CV (%)	10.5	14.3	2.1	8.0	8.5	7.8	14.7	10.0	15.3	10.8	17.7	10.7	14.3

: significant at P=.05; **: significant at P=.01; *: significant at P=.001. Values followed by dissimilar letters in the same column for each treatment are significantly different from each other at P=.05 according to Fischer LSD. (LSD: Least significance difference; DMY: Whole plant Dry matter yield). All values that were not significantly different from each other are not shown in this table.*

Table 5. Mineral-elements uptake in cowpea (whole plant) planted under different plant densities and cropping systems with sorghum

Treatments	P	K	Ca	Mg	Na	S	Fe	Cu	Zn	Mn	Bo
	……mg.plant^{-1}……						……µg.plant^{-1}……				
Plant density (plants.ha^{-1})											
83,333	112.5a	870.1a	591.8a	164.5a	26.2a	18.1a	53911.3a	1086.2a	2559.6a	1045.5a	1464.9a
166,666	64.7b	499.9b	321.1b	101.7b	14.6b	9.2b	17783.7b	376.6b	1468.5b	541.1b	929.5b
Cropping system											
Monocropping	116.4a	904.2a	598.0a	169.1a	26.8a	18.9a	52154.9a	1106.7a	2582.9a	1069.5a	1527.4a
Intercropping	60.8b	465.8b	314.9b	97.1b	14.0b	8.4b	19540.1b	356.2b	1445.3b	517.1b	867.0b
Genotypes											
Bensogla	95.6a	784.0ab	483.0ab	137.2ab	21.6ab	14.2ab	46182.6ab		2089.7ab	838.2ab	1258.1ab
ITH98-46	67.3b	505.2c	331.5c	107.0c	15.6c	9.3c	20542.1c		1526.7c	602.0c	947.9c
Sanzie	102.6a	821.1a	566.4a	163.1a	25.4a	16.3a	40108.2abc		2552.7a	992.6a	1427.5a
TVu1509	73.8b	592.3bc	392.1bc	116.5bc	17.4bc	10.0bc	25041.0bc		1811.1bc	696.2bc	1052.7bc
Omondaw	104.0a	722.4abc	509.3a	141.7ab	22.1ab	18.6a	47363.6a		2090.2ab	837.3ab	1299.8ab
3 - Way ANOVA (F-Statistic)											
Main Effects											
Density	49.0***	27.9***	63.7***	48.7***	54.9***	32.9***	28.9***	19.0***	50.1***	51.3***	44.8***
Cropping systems	66.5***	39.1***	69.7***	63.9***	67.6***	45.8***	23.6***	21.2***	54.5***	61.5***	68.1***
Genotypes	5.0**	2.9*	6.1***	4.8**	5.0**	5.3**	2.7*		4.9**	3.6*	4.7**
Interactions											
Density*Cropping systems	5.7*	7.6**	11.2**	5.9*	7.5**	5.2*	7.3**	8.1**	5.9*	7.3**	5.2*
Density*Genotypes	3.0*		3.6*		2.6*	2.6*			3.3*		3.1*
CV (%)	34.4	45.8	33.2	30.3	34.1	51.0	83.8	99.6	34.2	39.7	29.9

: significant at P=.05; **: significant at P=.01; *: significant at P=.001. Values followed by dissimilar letters in the same column for each treatment are significantly different from each other at P=.05 according to Fischer Least significance difference (LSD). All values that were not significantly different from each other are not shown in this table*

Table 6. Mineral-elements uptake in sorghum (whole plant) planted under different cowpea plant densities and cropping systems with different cowpea genotypes

Treatments	P	K	Ca	Mg	Na	S	Fe	Cu	Zn	Mn	Bo
	……mg.plant^{-1}……						……µg.plant^{-1}……				
Density (plants.ha^{-1})											
Sorghum in 83,333	150.6a	1063.4a	224.5a	168.4a	58.5a	36.1a	215688.4a	2379.1a	4191.8a	2068.3a	294.2a
Sorghum in 166,666	126.1b	911.9b	191.6b	145.5b	49.6b	31.0b	190822.4b	1826.2b	3474.7b	1681.5b	245.8b
Cropping system											
Mono sorghum	185.7a	1301.4a	291.0a	213.7a	67.6a	44.8a	337470.1a	2900.1a	5396.8a	2626.9a	351.2a
Sorghum+cowpea	90.9b	673.9b	125.1b	100.3b	40.5b	22.3b	69040.8b	1305.2b	2269.7b	1122.9b	188.8b
Genotypes											
Sorghum+Bensogla	139.1ab	996.5a		158.5a						1853.5ab	272.1a
Sorghum+ITH98-46	143.2a	1035.1a		164.4a						2011.1a	278.7a
Sorghum+ Sanzie	125.4b	904.8b		144.0b						1706.4b	245.8b
Sorghum+TVu1509	148.4a	1027.5a		164.4a						1951.8a	283.7a
Sorghum+Omondaw	135.6ab	974.2ab		153.6ab						1851.8ab	269.7ab
3 - Way ANOVA (F-Statistic)											
Main Effects											
Density	30.2***	33.9***	31.6***	27.7***	28.9***	30.9***	29.9***	7.6**	23.6***	44.9***	39.0***
Cropping systems	450.9***	581.7***	805.1***	680.0***	268.2***	591.9***	3485.7***	62.8***	448.6***	679.2***	439.9***
Genotypes	3.0*	3.2*		3.1*						3.2*	2.8*
Interactions											
Density*Cropping systems	30.2***	33.9***	31.6***	27.7***	28.9***	30.9***	29.9***	7.6**	23.6***	44.9***	39.0***
Cropping systems*Genotypes	3.0*	3.2*		3.1*						3.2*	2.8*
CV (%)	14.4	11.8	12.6	12.4	13.7	12.3	10.0	42.8	17.2	13.8	12.8

: significant at P=.05; **: significant at P=.01; *: significant at P=.001. There were significant interactions between density and cropping systems but they were not considered for sorghum density was not changed. Values followed by dissimilar letters in the same column for each treatment are significantly different from each other at P=.05 according to Fischer Least significance difference (LSD). All values that are not significant are not shown in this table.*

3.6 Interactive Effects

3.6.1 Interactive effects of density x cropping system on the uptake of mineral elements in cowpea

There was a significant (*P*=.05) interaction between plant density and cropping system on the uptake of mineral elements content in cowpea plants. Under Monocropping, increasing plant density from low to high consistently lowered (*P*=.05) the uptake of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn, and B in cowpea (Figs. 1 and 2). The results show that there was no effect of increasing plant density under mixed culture. Although there were significant interactions between plant density and cropping system on the elemental content in sorghum, they were not considered as density was not varied.

3.6.2 Interactive effects of density x cowpea genotypes on the uptake of mineral elements in cowpea

There was also a significant (*P*=.05) interaction between plant density and cowpea genotypes. The interaction between plant density and genotypes was significant (*P*=.05) for P, Ca, Na, S, Zn, and B contents in whole plant. For example, cvs. Sanzie, Omondaw and/or Bensogla were greatest in P, Ca, Na, S, Zn and B mineral elements in cowpea plant compared with the rest (Fig. 3).

3.6.3 Interactive effects of cropping systems x sorghum in mixture with cowpea genotypes on the elemental content in sorghum

There was also a significant (*P*=.05) interaction between cropping system and sorghum in mixture with cowpea genotypes. For example, sorghum plants grown in mixture with cvs. Sanzie and or Omondaw were consistently lowest in the contents of P, K, Mg, Mn, and B compared with sorghum plants grown in mixture with the other cowpea genotypes (Fig. 4).

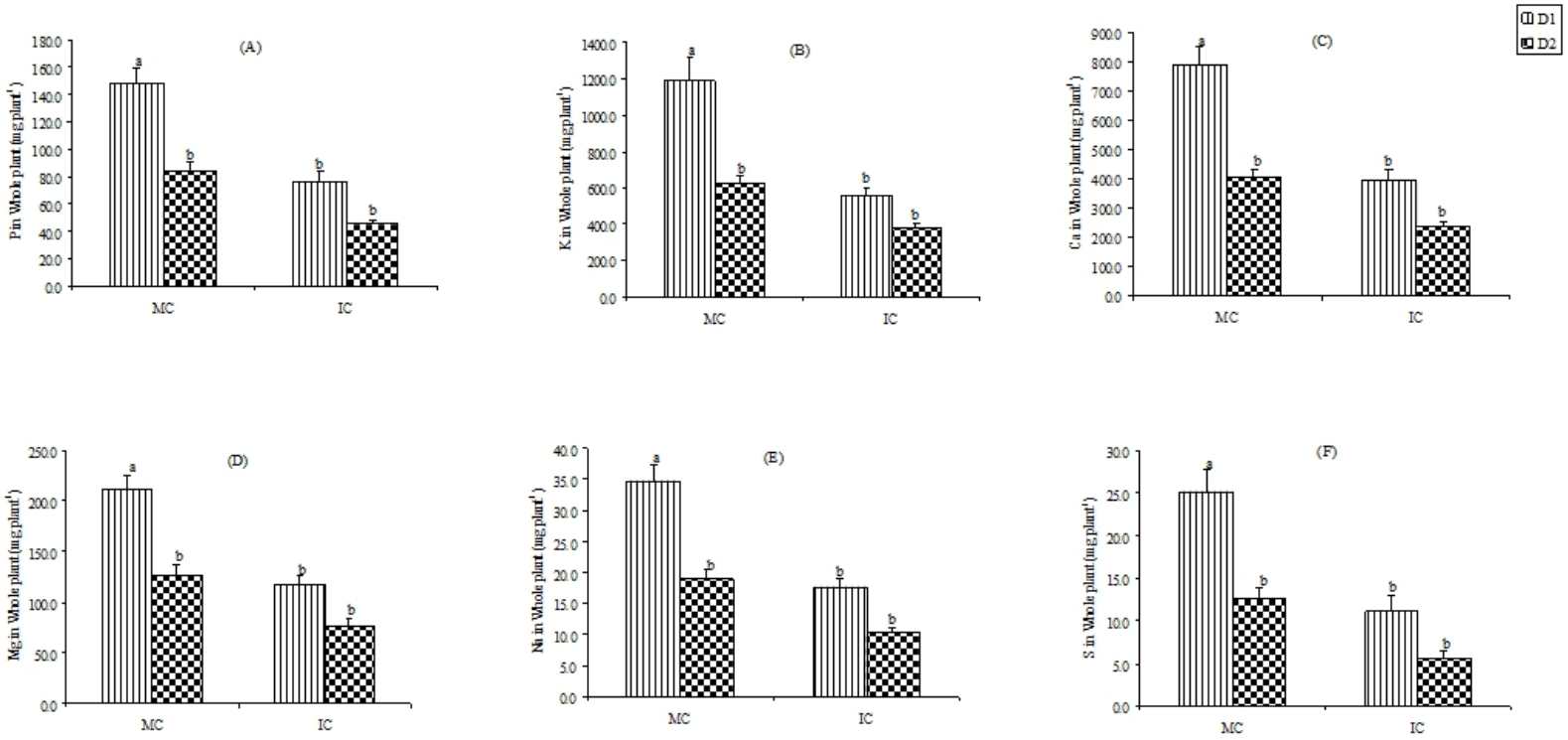

Fig. 1. Interactive effects of density and cropping systems on the contents of mineral elements in whole plant cowpea : A) P, B) K, C) Ca, D) Mg, E) Na, F) S. (D1: 83,333 plants.ha^{-1}; D2: 166,666 plants.ha^{-1}; MC: Monocropping; IC: Intercropping). Bars followed by dissimilar Letters are significantly different by Fischer LSD Test at *P*=.05

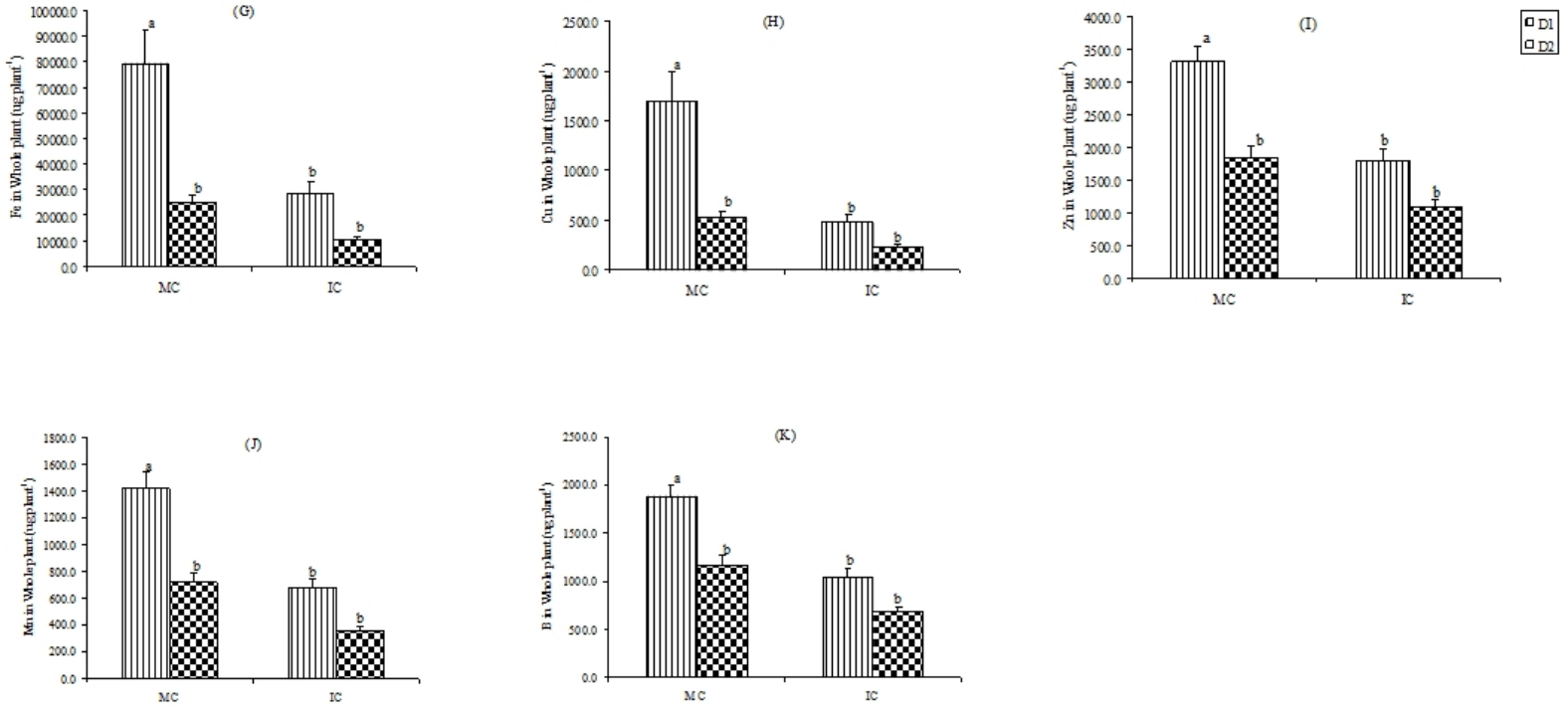

Fig. 2. Interactive effects of density and cropping systems on the contents of mineral elements in whole plant cowpea: G) Fe, H) Cu, I) Zn, J) Mn, K) B (D1: 83,333 plants.ha^{-1}; D2: 166,666 plants.ha^{-1}; MC: monocropping; IC: intercropping). Bars followed by dissimilar letters are significantly different by Fischer LSD test at *P*=.05

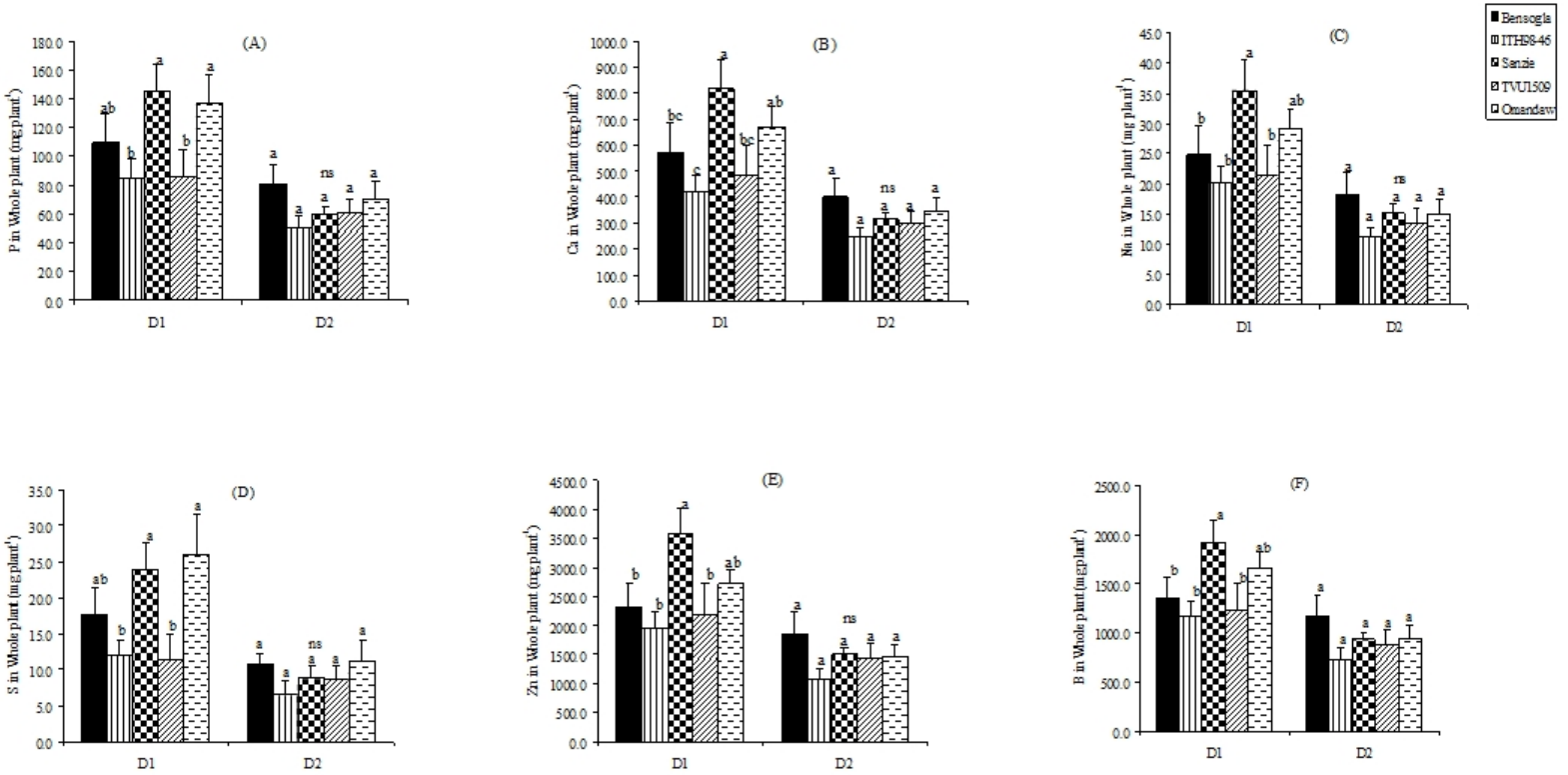

Fig. 3. Interactive effects of density and genotypes on the contents of mineral elements in whole plant cowpea: A) P, B) Ca, C) Na, D) S, E) Zn, F) B. (D1: 83,333 plants.ha^{-1}; D2: 166,666 plants.ha^{-1}). Bars followed by dissimilar letters are significantly different by Fischer LSD test at *P*=.05

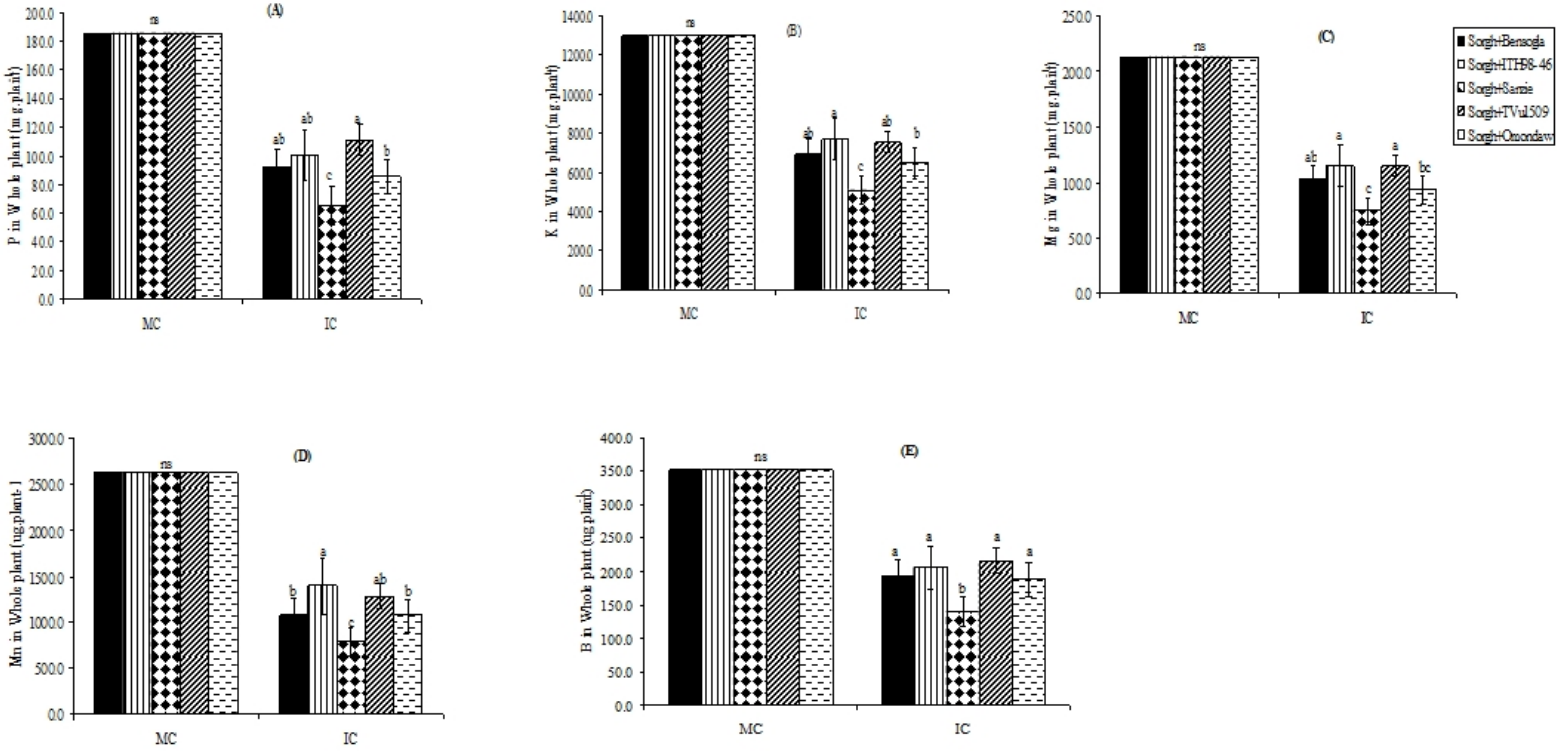

Fig. 4. Interactive effects of cropping systems and genotypes on the contents of mineral elements in whole plant sorghum: A) P, B) K, C) Mg, D) Mn, E) B. MC: Monocropping; IC: Intercropping. Bars Followed by dissimilar letters are significantly different by Fischer LSD test at *P*=.05

4. DISCUSSION

A number of factors can account for the changes in mineral concentration in the rhizosphere relative to bulk soil. For example, the lowered levels of K, S and Na in the rhizosphere of cowpea and sorghum were more likely due to uptake by plants. But Cu in the rhizosphere of cowpea was not significantly changed although there was 5% increase over the bulk soil. However, in the sorghum rhizosphere, Cu and Zn were significantly increased by 9.5% and 34.2% over the bulk soil respectively (Table 2). These positive changes could probably be attributed by specific mechanisms such as reductase stimulated by lowered pH levels in the rhizosphere or as phytometallophores which forms strong chelate of Cu ions in the soil which are soluble, less positively charged and free to diffuse towards the root in water films [30]. As shown in Table 2, there were significant differences in pH between bulk and rhizosphere soils, with cowpea exhibiting a markedly increased acidity in its rhizosphere. This increase in rhizosphere H^+ can lead to high concentration of competing polyvalent cations (e.g. Mn^{2+}, Ca^{2+}, and Mg^{2+}) in the rhizosphere, development of a steep proton gradient across the plasma membrane, and a decrease in the charge density around the plasmalemma membrane, thus, resulting in decreased uptake of Mg and Ca manifested as increased accumulation in the rhizosphere [31]. The high levels of Ca and Mg found in the rhizosphere soils of cowpea and sorghum in this study were therefore likely due to root-induced decrease in rhizosphere pH and its accompanying effects of acidification. Several authors have reported proton extrusion and release of root exudates as some of the mechanisms that modify the physico-chemical properties and biological composition of the plant rhizosphere, thus, directly influencing nutrient availability or indirectly influencing interaction with soil micro-organisms [32; 33; 34]. Our observation with Ca and Mg in this experiment is consistent with the report of [35], who also found high levels of Ca and Mg in the rhizosphere soil of millet relative to bulk soil in the sudano-sahelian savannah of West Africa.

Competition for mineral elements across different plant species and genotypes is very significant [36]. In this study, the concentrations of P and Ca were found to vary in the rhizosphere of the five cowpea genotypes. ITH98-46 and TVu1509 genotypes showed significantly high levels of P and Ca in the rhizosphere, followed by Bensogla and Omondaw, while cv. Sanzie consistently exhibited low concentrations of these minerals in its rhizosphere (Tables 3 and 4). Although the rhizosphere levels of K, Mg, Na, S, Fe, Zn, Mn and B were similar for the five cowpea genotypes, again, cv. Sanzie consistently showed the lowest level (Tables 3 and 4). To ascertain whether the low levels of P and Ca in cv. Sanzie rhizosphere was due to uptake by roots, mineral analysis was done using whole plant material. The data revealed significantly higher concentrations of P and Ca in whole plant cowpea. The levels of K, Mg, Na, Zn, Mn and B were also markedly higher in whole plant cowpea cv. Sanzie (Tables 3 and 4), suggesting that the low levels of these minerals observed in the rhizosphere of Sanzie (though not statistically significant) was due to uptake by Sanzie roots. Some mineral elements are rapidly depleted in the immediate vicinity of plant roots leading to a large gradient across the rhizosphere between bulk and the root surface [37]. In this study, the net result of the high uptake of mineral elements such as P, K, Mg, Ca, Na, S, Cu, Zn, Mn and B by cowpea cv. Sanzie was a marked increase in its growth as well as whole plant biomass (Table 3).

Survival and productivity of component crops exposed to stress such as high plant density and intercropping is dependent on their ability to adapt to such stress. Such ability include uptake of mineral elements from the rhizosphere and accumulation in plant organs for plant growth. Evidence suggests that mineral elemental status (i.e. K, Mg, Ca, Zn and B) of component crops such as those used in this study greatly affects their ability to adapt to

stress conditions [38,39]. Growing crops in mixture and at high plant density as done in this study could have resulted into a stress or competition for above- and below-ground resources for plant growth. It was interesting to note that, relative to low plant density and monocropping, high plant density and mixed culture resulted into a decrease in the concentration of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn and B in the rhizosphere of cowpea plants (Table 3), leading to decreased uptake and accumulation in plants (Table 5). [40] reported significant decrease in P and K concentration when chickpea was grown in mixed culture with wheat. Low K, Mg and Zn for example has been reported to decrease photosynthetic C metabolism and utilization of fixed C [41], massive accumulation of carbohydrates in source leaves leading to inhibition of photosynthetic C reduction, excess non-utilized light energy and photoelectrons resulting into enhanced sensitivity of plants to photo-oxidative damage [42,43], consequently low plant growth.

Although the concentration of mineral elements in the rhizosphere of both cowpea and sorghum was decreased by plant density and cropping system, the reduction was more marked in the cowpea rhizosphere, possibly as a result of higher demand by N_2-fixing bacteroids in cowpea root nodules [44,45,46,47,48,49]. There were therefore not only species differences in mineral depletion in the rhizosphere, but also cultivar differences (Tables 3 and 4). The data from other studies [50,51] have similarly shown variations in mineral depletion in the rhizosphere of various plant species and genotypes.

Interactively, it was evident that plant density in combination with cropping systems (Figs. 1 and 2), cowpea genotypes (Fig. 3) and sorghum in mixture with cowpea genotypes (Fig. 4) affected the whole plant elemental contents. Results showed that whereas change in cowpea plant density did not significantly affect any elemental contents in cowpea under mixed culture system, under monocropping system, low plant density produced greater uptake of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn and B mineral elements (Figs. 1 and 2). It was also clear that cvs. Sanzie, Omondaw and/or Bensogla were consistently greater in P, Ca, Na, S, Zn and B thus, accumulating greater biomass compared with high plant density which did not significantly affect these elemental contents. These results suggest that cvs. Sanzie, Omondaw and/or Bensogla have higher uptake efficiency at low plant density as opposed to high plant density by significantly lowering the mineral element concentration in their rhizosphere and accumulating them in their whole plant biomass. Furthermore, these data suggest that plant density rather than cropping system controls the elemental content of cowpea genotypes. Compared with sorghum plants intercropped with other cowpea genotypes, the consistently lower elemental content in sorghum grown in mixture with cv. Sanzie suggest that sorghum plants were less competitive for below ground plant growth resources than Sanzie genotypes, thus, accumulating less mineral elements in their tissues.

5. CONCLUSION

In conclusion, we found decreases in the concentration of various mineral elements in the rhizosphere of cowpea genotypes, and this was due to increased uptake by cowpea roots, which resulted in higher plant growth. The N_2-fixing cowpea plants significantly decreased the concentration of minerals in their rhizosphere relative to sorghum plants.

ACKNOWLEDGEMENTS

We are grateful to the McKnight Foundation for funding the South Africa Legumes Project, which supported this study, and provided a bursary to Joachim HJR Makoi. The Cape

Peninsula University of Technology is duly acknowledged for the continued bursary support to Joachim HJR Makoi to complete his doctoral studies.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

REFERENCES

1. Xiaoping W, Darlene Z. Nutrient composition of Douglas-fir rhizosphere and bulk soil solutions. Plant Soil. 1998;200:13-20.
2. Marschner H. Mineral nutrition in Higher Plants. Second Edition. Academic press, London. 889pp; 1995.
3. Hinsinger P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil. 2001;237:173-195
4. Dakora FD, Phillips DA. Root exudates as mediators as mineral acquisition in low-nutrient environments. Plant Soil. 2002;245:35-47.
5. Beleke T, Cino BJ, Ehlert PAI, van der Maas AA, van Diest A. An evaluation of plant borne factors promoting the solubilisation of alkaline rock phosphate. Plant Soil. 1983;75:361-378.
6. Hinsinger P, Elsass F, Jaillard B, Robert M. Root induced irreversible transformation of trioctahedral mica in the rhizosphere of rape. J. Soil Sci. 1993;44:535-545.
7. Hinsinger P, Gilkes RJ. Root-induced dissolution of phosphate rock in the rhizosphere of lupins grown in alkaline soil. Aust. J. Soil Res. 1995;33:477-489.
8. Bais HP, Loyola VM, Flores HE, Vivanco HM. Root specific metabolism: the biology and biochemistry of underground organs. In vitro Cell Develop. Biol. Plant. 2001;37:730-741.
9. Ayisi KK, Nkgapele RJ, Dakora FD. Nodule formation and function in six varieties of cowpea (*Vigna unguiculata* L. Walp.) grown in nitrogen rich soil in South Africa. Symbiosis. 2000;28:17-31.
10. Nkongolo KK. Genetic characterization of Malawian cowpea (Vigna unguiculata L. Walp) landraces: diversity and gene flow among accessions. Euphytica. 2003;129(2):219-228.
11. Aliyu BS, Emechebe AM. Effect of intra- and inter-row mixing of sorghum with two varieties of cowpea on host crop yield in a Striga hermonthica infested field. Afr. J. Agric. Res. 2006;1:24-26.
12. Blade SF, Mather DE, Singh BB, Smith DL. Evaluation of yield stability of cowpea under sole and intercrop management in Nigeria. Euphytica. 1992;61:193-201.
13. Makoi JHJR, Chimphango SMB, Dakora FD. Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere of cowpea (*Vigna unguiculata* L. Walp.) genotypes grown in mixed culture and at different densities with sorghum (Sorghum bicolor L.) Crop and Pasture Sci. 2010;61:279-286.
14. Ae N, Arihara J, Okada K, Yoshihara T, Johansen C. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Sci. 1990;248:477-480.
15. Shane MW, Lambers H. Cluster roots. A curiosity in context. Plant Soil. 2005;274:101-125
16. Römheld V. The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species. An ecological approach. Plant Soil. 1991;130:127-134.

17. Zhang F, Shen J, Li L, Liu X. An overview of rhizosphere processes related with plant nutrition in major systems in China. Plant Soil. 2004;260:89-99.
18. Ae N, Otani T, Makino T, Tazawa J. Role of cell wall of groundnut roots in solubilising sparingly soluble phosphorous in soil. Plant Soil. 1996;186:197-204.
19. Ae N, Shen RF. Root cell-wall properties are proposed to contribute to phosphorous (P) mobilization by groundnut and pigeon pea. Plant Soil. 2002;245:95-103.
20. Monteith JL. Evaporation and environment. Symp. Soc. Exp. Biol. 1965;19:205-234
21. FAO. World Soil Resources Reports, 2001;289.
22. Dyer B. On the analytical determinations of probably available “mineral plant-food in soil.” J. Chem. Soc. 1894;65:1-15.
23. Division of Chemical Services. Analytical methods. Division of Chemical Services, Department of Agriculture, Pretoria; 1956.
24. Du Plessis SF, Burger RDT. A comparison of Chemical extraction methods for the evaluation of phosphate availability of top soils. South Afr. J. Agric. Sci. 1964;8:11-13.
25. Fertilizer Society of South Africa (FSSA). Manual of soil analysis methods. FSSA Publication no 37, Pretoria; 1974.
26. Trierweiler JF, Lindsay WL. EDTA-Ammonium carbonate soil test for zinc. Soil Sci. Soc. Am. Proc. 1969;33:49-54.
27. Beyers CPDL, Coetzer FJ. Effect of concentration, pH, and time on the properties of di-ammonium EDTA as a multiple soil extractants. Agrochemophysica. 1971;3**:**49-54.
28. Giron HC. Comparison between dry ashing and wet digestion in the preparation of plant materials for atomic absorption analysis. Atomic Absorption Newsletters. 1973;12:28-29.
29. Steel RGD, Torrie JH. Principles and procedures of statistics: A biometrical approach, Second Edition. McGraw Hill, New York; 1980.
30. Graham RD, Stangoulis JCR. Trace element uptake and distribution in plants. Journal of Nutrition. 2003;133:1502S-1505S
31. Youssef RA, Chino M. Studies on the behavior of nutrients in the rhizosphere: I. Establishment of a new rhizobox system to study nutrient status in the rhizosphere. J Plant Nutr. 1987;10:1185-1195.
32. Jones DL, Nguyen C, Finlay RD. Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant Soil. 2009;321:5-33
33. Oliveira CA, Sa NMH, Gomes EA, Marriel IE, Scotti MR, Guimaraes CT, Schaffert RE, Alves VMC. Assessment of the mycorrhizal community in the rhizosphere of maize (*Zea mays* L.) genotypes contrasting for phosphorus efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE). Applied Soil Ecology. 2009;41(3):249-258
34. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil. 2009;Vol.321.
35. Bagayoko M, Alvey S, Buerkert A, Neumann G. Root-induced increases in soil pH and nutrient availability to field-grown cereals and legumes on acid sandy soils of Sudano-Sahelian West Africa. Plant Soil. 2000;225:117-127.
36. Hodge A. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytology. 2004;162:9-24
37. Tinker PB, Nye PH. Solute Movement in the Rhizosphere. Oxford University Press, New York; 2000.
38. Çakmak İ, Torun B, Erenoğlu B, Kalaycı M, Yılmaz A, Ekiz H, Braun HJ. Zinc deficiency in soils and plants in Turkey and Plant Mechanism involved in Zn efficiency. Turkish Journal of Agriculture and Forestry. 1996;20:13-23.

39. Çakmak İ. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytology. 2000;146:185-205.
40. Gunes et al., Gunes A, Inal A, Adak MS, Alpaslan M, Bagci EG, Erol T, Pilbeam DJ. Mineral nutrition of wheat, chickpea and lentil as affected by mixed cropping and soil moisture. Nutr Cycl Agroecosyst. 2007;78:83-96. DOI 10.1007/s10705-006-9075-1.
41. Mengel K, Kirkby EA. Principles of Plant Nutrition. 5th edition. Kluwer Academic publishers, Dordrecht. 2001;848.
42. Cakmak I, Kurz H, Marschner H. Short-term effects of boron, germanium and high light intensity on membrane permeability in boron-deficient leaves of sunflower. Physiol. Plantarum. 1995;95:11-18.
43. Polle A. Mehler reaction: friend or foe in photosynthesis. Botanoca Acta. 1996;109:84-89
44. Cassman KG, Munns DN, Beck DP. Phosphorus nutrition of *Rhizobium japonicum*: strain differences in phosphate storage and utilization, Soil Sci. Soc. Am. J. 1981a;45:517-520.
45. Cassman KG, Witney AS, Fox RL. Phosphorus requirements of soybean and cowpea as affected by mode of N nutrition. Agron. J. 1981b;73:17-22.
46. Ssali H, Keya SO. The effect of phosphorus on nodulation, growth and dinitrogen fixation by beans, Biol. Agric. Hortic. 1983;1:135-144.
47. Pereira PAA, Bliss FA. Selection of common bean (*Phaseolus vulgaris* L.) for N_2 fixation at different levels of available phosphorus under field and environmentally-controlled conditions. Plant Soil. 1989;115:75-82
48. Bergersen FJ, Peoples MB, Turner GL A role for poly-b-hydroxybutarate in bacteroids of soybean root nodules. Proceedings of the Royal Society of London Series B - Biological Sciences. 1991;245: 59-64
49. Makoi JHJR, Chimphango SBM, Dakora FD. Effect of legume plant density and mixed culture on symbiotic N_2 fixation in five cowpea (*Vigna unguiculata* L. Walp.) genotypes in South Africa. Symbiosis. 2009;48:57-67.
50. Marion S, Ursula F. Plant induced alteration in the rhizosphere and the utilization of soil heterogeneity. Plant Soil. 1999;209:297-309.
51. Fan TWM, Lane AN, Shenka M, Bartley JP, Crowley DE, Higashi RM. Comprehensive chemical profiling of graminaceous plant root exudates using high resolution NMR and MS. Phytochem. 2001;57:209-221.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=312&id=2&aid=2434