Cape Peninsula
University of Technology

SEED FLAVONOID CONCENTRATION IN COWPEA GENOTYPES AND THE EFFECT OF PLANT DENSITY ON GROWTH, N_2 FIXATION AND RHIZOSPHERE PHOSPHATASES AND GRAIN YIELD OF COWPEA INTERCROPPED WITH SORGHUM

by

Joachim H.J.R. Makoi

Dissertation submitted in fulfilment of the requirements for the

Doctoral Degree

in the Faculty of Applied Science

at the

CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

Supervisor: Prof. Felix D. Dakora
Co-supervisors: Assoc. Prof. Patrick A. Ndakidemi and
Dr. Samson B. M. Chimphango

Cape Town
September 2009

DECLARATION

I, Joachim H.J.R Makoi, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date** **September, 2009**

ABSTRACT

A 3-factorial experiment involving two cowpea densities (83,000 and 167,000 plants.ha^{-1}), two cropping systems (i.e. monoculture and mixed culture) and five cowpea genotypes (i.e. three farmer-selected cultivars, Bensogla, Sanzie and Omondaw and two improved varieties, ITH98-46 and TVu1509) was conducted in the field for two consecutive years in 2005 and 2006. The aim was to assess the effect of plant density, cropping system and cowpea genotypes on: (i) chlorophyll and gas-exchange, (ii) rhizosphere mineral concentration and tissue uptake of nutrients, (iii) acid and alkaline phosphatase activities in the rhizosphere, (iv) plant growth and symbiotic performance, and (v) concentration of flavonoids and anthocyanins in seed extracts and plant organs and their effect on pest infestation and diseases.

The results showed that high plant density (167,000 plants.ha^{-1}) and mixed culture significantly decreased gas-exchange parameters, leaf chlorophyll content, $\delta^{13}C$ and %C in both cowpea and sorghum plants compared with low plant density (83,000 plants.ha^{-1}) and monoculture. The data also showed significantly higher $\delta^{13}C$ and lower %C in ITH98-46 and TVu1509 compared with Bensogla, Omondaw and Sanzie genotypes with a significant correlation between $\delta^{13}C$ and water-use efficiency. At harvest, grain yield of cowpea and sorghum was significantly decreased by high plant density and mixed culture compared with low plant density and monoculture. Sanzie genotype was generally superior in grain yield (2,550 kg.ha^{-1}) followed by cvs. Omondaw and Bensogla (2,250 and 2,150 kg.ha^{-1}, respectively) compared with the improved cultivars. Sorghum plants in mixture with cv. TVu1509 or cv. ITH98-46 performed better (1,570 and 1,550 kg.ha^{-1}, respectively) compared with those in mixture with other cultivars. The results also showed greater land equivalent ratio (LER = 1.42 to 1.52), suggesting that mixed culture produced greater total yields per unit land area compared with monoculture.

High plant density and mixed culture lowered rhizosphere pH, as well as the concentrations of C, P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn and B in both cowpea and sorghum plants. The results also showed decreased N, P, Ca and Cu in the rhizosphere of the different cowpea genotypes. Relative to low plant density and monoculture, high plant density and mixed culture significantly raised the acid and alkaline phosphatase activity in the rhizosphere of cowpea and sorghum plants. The increased acid phosphatase activity in fresh roots of cowpea plants due to high plant density and mixed culture, resulted in significantly improved P nutrition, greater plant growth, and higher grain yield in cowpea, especially the cv. Sanzie.

Plant growth and symbiotic performance (%N, $\delta^{15}N$ values, %Ndfa, and N-fixed) were also significantly altered by both high plant density and mixed culture, with %Ndfa values showing a decrease where $\delta^{15}N$ values were low. Whether under low or high plant density, Sanzie genotype was found to accumulate significantly greater biomass, total N per plant, and actual amount of N-fixed, followed by Bensogla and Omondaw, and least, ITH98-46 and TVu1509.

Analysis of flavonoids and anthocyanins in seed extracts and tissue rinse revealed marked differences between genotypes, with higher levels being observed in Sanzie, Omondaw, and Bensogla relative to ITH98-46 and TVu1509. These high phenolic levels correlated with lower infestation by thrips and pod-sucking bugs. Correlation and regression analyses confirmed a direct relationship between flavonoids/anthocyanins in seed extracts which showed an inverse relationship between concentration of these phenolics and plant attack by aphids and alcidodes.

ACKNOWLEDGEMENTS

This work was carried out at the Faculty of Applied Science, Department of Chemistry, Cape Peninsula University of Technology (CPUT) during the years 2004-2009.

The financial assistance of the McKnight Foundation as well as the Cape Peninsula University of Technology towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the McKnight Foundation as well as the CPUT.

I wish to express my gratitude to my supervisor, Prof. Felix D. Dakora, for the supervision, encouragement, exceptional ideas, and tireless optimism that have kept me going. However, his shifting to TUT in 2007 in search of greener pastures caused me a delay of three years in this study program, a period which was too long and very painful both to me and my family in Tanzania. My sincere gratitude to my co-supervisors, Assoc. Prof. Patrick A. Ndakidemi of CPUT and Dr. Samson B. M. Chimphango of the University of Cape Town who tirelessly kept the going after the main supervisor has shifted to Tshwane University of Technology (TUT) in Pretoria.

My appreciation is due to the Ministry of Agriculture, Food Security and Cooperatives and Ministry of Water and Irrigation (MoWI) of the United Republic of Tanzania for granting me a study leave to carry out this programme. Thanks to the Zonal Irrigation Engineer and the staff of the Kilimanjaro Zonal Irrigation and Technical Services Unit (KZITSU) for their support during my study leave.

I also wish to express my sincere thanks to all my colleagues Dr. Maurice Dassah, Flora Pule Mulenberg, Sheku Kanu, Alfonsus Belanne for their encouragement while at CPUT and thereafter at TUT. I am also indebted to all the staff at the Faculty of Applied Science, Chemistry Department, both CPUT and TUT for generally creating inspiring conditions for my research.

Thanks to my Mother who has consistently been a foundation for my studies and carrier, for her constant encouragement and moral support during my studies, more so after the death of my father Joseph Lewando (1979) when I was still at high school. My appreciations to my young brothers and their families for accepting to take most of my family responsibilities during my studies as well as my sisters for moral support and encouragements. Sister Dr. Mary Paul (MeD) for her constant prayers and encouragement. I want to thank my mother in law Mama Marietta Thomas Mrosso, my sisters in law and close relatives for their loving and cheerful support in the course of my almost endless years of study.

To all my children, Jacqueline, Brian, Glory (Mkundee), Herieth and Thomas Boltzmann for tolerating my absence during study period.

Finally, I want to thank my dear wife, Anna, for her true love, encouragement, flexibility and absolute tolerance during these years of intense work, for taking care of our children and being the sunshine of my life.

And to Almighty GOD, Alpha and Omega, the First and Last, I AM, the source of all knowledge and wisdom, Thank you for everything.

DEDICATION

To my late Father, Joseph Lewando and to my dear Mother, Lucia Manyinda who sent me to school, who consistently encouraged me and who motivated me to aim and reach the highest education. To my lovely wife Anna and to all my lovely children, Jacqueline, Brian, Mkundee, Herieth and Boltzmann.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

A	Photosynthesis rate
AGRA	Alliance for Green Revolution in Africa
Al	Aluminium
ANOVA	Analysis of Variance
ARC	Agricultural Research Council
ATP	Adenosine triphosphate
B	Boron
BNF	Biological Nitrogen Fixation
C	Carbon
C3	Plant species that use RuBisCO to make a three-carbon compound as the first stable product of carbon fixation.
C4	Plant species of which CO_2 is first incorporated into a 4-carbon compound during carbon fixation. These plants use PEP carboxylase (PEPC) as the enzyme involved in the uptake of CO_2.
Ca	Calcium
Ca $(H_2PO_4)_2.H_2O$	Calcium biphosphate
Chl	Chlorophyll
Ci	Intercellular carbon dioxide concentration
CO_2	Carbon dioxide
Cu	Copper
$\delta^{13}C$	Stable carbon isotope signature
$\delta^{15}N$	Stable nitrogen isotope signature
DAP	Days After Planting
DMSO	Dimethyl sulphoxide
E	Transpiration rate
FAO	Food and Agricultural Organisation
Fe	Iron
Gs	Stomata conductance
H	Hydrogen
H_2O	Water
HCl	Hydrochloric acid
HCO_3	Bi-carbonate

HIV	Human Immunodeficiency Virus
HNO_3	Hydrogen nitrate
K	Potassium
LER	Land equivalent ratio
MeOH	Methanol
Mg	Magnesium
Mn	Manganese
Na	Sodium
NaOH	Sodium hydroxide
NO_3	Nitrate
OH	Hydroxyl
P	Phosphorus
PEPC	Phosphoenolpyruvate Carboxylase
PGA	Phosphoglyceric acid
RuBisCO	Ribulose-1, 5-bisphosphate carboxylase-oxygenase
S	Sulphur
SE	Standard error
USA	United States of America
UV	Ultraviolet radiation
WUE	Water Use Efficiency
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The recent global increases in food and fuel prices have added pressure on the agricultural production systems and have caught the attention of many scientists. Previous reports on the state of global food insecurity have shown that about 800 million people in developing countries have insufficient food (FAO, 2000). In sub-Saharan Africa, food crises are chronic even though high proportions (i.e. 70 - 85%) of Africans are active in agriculture (Borlaug, 1991). For example, Africa produced only 5.3% of the world's total cereal crop yield and many reports show that food imports into Africa have increased in the past decade (World Bank, 1989; von Braun and Paulino, 1990; FAO, 2000). Recently, a World Bank report estimated that the rate of cereal yield increase in Africa over the years was as low as 0.7% compared with growth rates of 1.2 - 2.3% in other developing regions of the world (AGRA, 2007). Reasons for the aforementioned trends however, have been among other factors due to low soil fertility, low grain yield and N_2 fixing cultivars, cultural practices as well as pests and disease infestations (Boserup, 1981; Cooper *et al.,* 1996; Sanchez *et al.,* 1997). To reverse these trends and increase production of these crops however, will require concerted efforts by various key players so as to improve soil fertility; identify potential high yielding and N_2 fixing genotypes of crops such as cowpea which are predominantly common in Africa, develop cultural practices which may confer insect pest resistance, and enhance yield stability by varying plant densities and cropping systems.

Cowpea (*Vigna unguiculata* L. Walp.) is among the indigenous African grain legumes grown extensively throughout Africa. It is the most important food legume, fodder and cover crop (Padulosi and Ng, 1990; Jackai and Adalla, 1997). In addition, it mature early, has wide adaptation, drought tolerant, and has broad range of local genetic diversity. Nutritionally, cowpea grain is rich in protein (20.5 - 31.7%), carbohydrates (56.0 - 65.7%); fat (1.1 - 3.0%), fiber (1.7 - 4.5%) and moisture (6.2 - 8.9%) (Onwuliri and Obu, 2002). The green leaves and young pods of cowpea contain up to 35% protein and are eaten as vegetables. Cowpea also contains other essential nutrients, such as Ca, Fe, nicotinic acid and thiamine (Platt, 1962).

Similar to other grain legumes, cowpea has been shown to contain several other important phytochemicals rich in health-related properties (Anderson *et al*., 1999). Some of the known health promoting phytochemicals in cowpea includes phytosterols, saponins, isoflavone, phenolic compounds and antioxidants (Narasinga, 1995; Warrington *et al*., 2002). Likewise, it has been reported that compounds such as flavonoids, anthroquinones, anthocyanidins and xanthones commonly present in these legumes, possess remarkable antioxidant activity (Siddhuraju *et al*., 2002). Diets rich in polyphenolic compounds have been suggested to be associated with longer life expectancy due to their richness in health-related

properties such as anticancer, antiviral, anti-inflammatory activities, effects on capillary fragility, and ability to inhibit human platelet aggregation (Stampfer *et al*., 1993; Deshpande *et al*., 1996; Hertog and Hollman, 1996). In this regard, increased dietary intake of natural flavonoids and anthocyanins through these legumes may correlate very well with increased health benefits mentioned above.

Cowpea has a potential for high grain yields of up to 3,000 $kg.ha^{-1}$ (Rusoke and Rubaihago, 1994). However, cowpea grain yields varies widely, and, are in the average of 200 - 300 $kg.ha^{-1}$ in Nigeria (Alghali, 1992); 200 - 400 $kg.ha^{-1}$ in Uganda (Sabiti *et al*., 1994); 50 - 300 $kg.ha^{-1}$ in Niger (Sivakumar *et al*., 1996); 400 – 1,000 $kg.ha^{-1}$ in Cameroon (Langyintuo *et al*., 2003); and from 1100 – 1400 $kg.ha^{-1}$ in Ghana (Adjei-Nsiah *et al*., 2008). This implies that farm yields of cowpea ranges between 1.7% and 46.7% of its potential. This low yields is due to several constraints including various biological and environmental factors, such as drought and salinity, low level of symbiotic N_2 fixation, high genotypic variation and cultural practices (Onwuliri and Obu, 2002). So, to increase production of cowpea more work is needed on, among other factors, selection of high yielding genotypes and assessing them under different plant densities and cropping systems.

Due to lack of information about genotypes that are high yielding and high N_2 fixation, a project under the support of McKnight Foundation was launched in June 2003 in three African countries i.e. Ghana, South Africa and Tanzania aimed at improving cowpea yield potential by enhancing N_2 fixation. The main activities included development of inbred high yielding cowpea populations and screening selected cowpea genotypes under field conditions for increased N_2 fixation and seed grain yield. Hundred and twenty six (126) cowpea cultivars obtained from farmers, village markets, national programmes, gene banks, and International centres in Ghana, South Africa and Tanzania were selected from preliminary observation trials of the germplasm as parental lines to be used in breeding programmes, were initiated in Ghana, South Africa and Tanzania. After data collection on various parameters such as plant growth, N_2 fixation, flavonoids concentration, pest resistance, seed yield, nutritional value of cowpea leaves and farmer preference, the test genotypes were ranked. Of the 126 cowpea landraces, 27 were selected for further field evaluation. These parental lines include 11 from Ghana, 7 from South Africa and 9 from Tanzania. It is from these selections that the five cowpea genotypes were picked and further tested against agronomical practices such as varying plant density and cropping system on a number of attributes including improvement of N_2 fixation, insect pest resistance and grain yield when grown with sorghum as cereal to mimic farmer's practices.

Growing cowpea and cereals such as sorghum and maize as intercrops for food production is popular among subsistence farmers in the tropics and sub-tropics, semi arid regions, humid tropics, Mediterranean

regions and temperate climates (Francis, 1986). For example it was estimated that 99% of cowpea and 75% of maize (*Zea mays* L.) in Nigeria are grown as mixed culture (Okigbo and Greenland, 1976). In Ethiopia, most of the beans production is from mixed culture systems (Seyoum, 1990). Likewise, in Latin America 60% of maize and 80 - 90% of beans (*Phaseolus vulgaris* L.) are produced by small scale farmers from mixed culture system (Francis *et al*., 1976). In Spain, 40% of the cultivated land is used for intercropping (MAPA, 1999). Most of these farmers have adopted this system because they want to maximise space and plant growth resources (Lie *et al.,* 2003b); crop quality and quantity (Mpairwe *et al.,* 2002). However, grain yields and N_2 fixation resulting from farmers practices in Africa has until recently been disappointing and mainly attributed to poor agronomic practices and low yielding cowpea genotypes used by farmers.

Sorghum (*Sorghum bicolor* L. Moench.) is the fifth most important small grain cereal crop after wheat, rice, maize and barley (FAO, 2005) produced in drier areas of the tropics, often grown in mixture with cowpea in low input cropping systems. In Africa, well managed sorghum crop yield ranged from 1,700 - 4,800 kg.ha^{-1} but current yields are reported to be less than 600 kg.ha^{-1} (Rohrbach *et al*., 2005). Growing sorghum in mixture or in succession with cowpea is one way of improving grain yield. For example sorghum grain yield has been reported to reach 1,620 kg.ha^{-1} following legume crop compared with 420 kg.ha^{-1} following sorghum (Ncube *et al*., 2007). Similarly, growing sorghum in mixture with peanuts (*Arachis hypogea* L.) has been shown to be more productive than monoculture crops combined (Azam-Ali *et al*., 1990). There is limited information on how different cowpea plant densities and cropping systems affects sorghum when grown with cowpea genotypes.

Plant density defines the number of plants per unit area, which in turn, determines the size of the area available to the individual plant (Wiley, 1979). Plant population is among the major cultural practices that impact on light regimes of the canopy as well as interplant competition, consequently, affecting canopy structure and light conversion efficiency (Akunda, 2001). Greater pressure on growth resources has been reported from higher plant densities compared with lower plant densities (Wiley and Osiru, 1972). For instance, in soybean (*Glycine max*. L.), high plant density may influence the extent of the fibrous root system which contributes to enhanced drought tolerance (Pantalone *et al*., 1999). Likewise, high plant density may influence foliage arrangement and increased light interception (Fisher and Wilson, 1976). In soybean-sorghum mixed culture, Akunda (2001) reported that varying plant density may be a viable alternative of manipulating the productivity of crops through their changes in physiological processes. This review seeks to assess the influence of plant density and mixed culture on photosynthesis and chlorophyll content, rhizosphere nutrients, phosphatase activities, yield, N_2 fixation and flavonoids and anthocyanins concentration in cowpea genotypes grown with sorghum.

1.2 Possible influence of varying plant densities, cropping systems and different legume genotypes on photosynthetic activities, leaf chlorophyll contents, $\delta^{13}C$ and water-use efficiency.

Photosynthetic rates and the associated parameters (i.e. stomata conductance, intercellular CO_2 concentration, and transpiration rate), chlorophyll contents, $\delta^{13}C$ and water-use efficiency (WUE) are affected by several agronomical practices. It is postulated that changes in plant population in the field, plant arrangements and type of plant species and genotypes involved in such cropping systems would affect photosynthetic parameters (Lima Filho, 2000; Li *et al.,* 2008). Since high plant densities are associated with both lower grain and dry matter yields due to decreased photosynthesis, it is evident that low plant density will possibly increase such rates (San-oh *et al.,* 2004). For example, increasing plant density has been shown to increase shading in the field, leading to limitation in light intensity, thus, lowering the photosynthetic rate and the associated parameters (Feigenbaum and Mengel, 1979; Hirose *et al*., 1988; Schieving *et al*., 1992a). Similarly, the decline in leaf area ratio was related to increased plant density as a result of competition for light (Pons *et al*., 1989). Several studies have reported photosynthetic rate variability amongst several crops such as wheat (*Triticum aestivum* L.) (Evans and Dunstone, 1970; Austin *et al*., 1982), maize (Heichel and Musgrave, 1969), faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.) (Schulze *et al*., 1999) and soybean (Buttery *et al*., 1981). This suggests that the type of crops involved in cropping systems have an important effect on the gas exchange parameters. For example, variation in C allocated to nodulated legumes and the amount of C respired has been reported to vary with species (Herridge and Pate, 1977; Atkins *et al*., 1978). Similarly, adaptation to higher C costs during N_2-fixation varies with species (i.e. faba bean, common bean, cowpea and pea). For example, faba bean has greater photosynthetic capacity compared with pea (Schulze *et al*., 1999).

Competitions for plant growth factors such as mineral elements under higher plant density and mixed culture have led to stress, differences in photosynthetic rates and chlorophyll contents (Akunda, 2001; Ghosh *et al*., 2006). For example, N deficiency due to stress caused by dense population of plants significantly decreased leaf chlorophyll concentration resulting in increased leaf reflectance (Daughty *et al.,* 2000; Zhao *et al.,* 2003; Zhao *et al.,* 2005), thus, affecting leaf photosynthetic rate (Muchow and Sinclair, 1994; Zhao *et al.,* 2005). Similarly, K deficiency has been reported to negatively affect cotton (*Gossypium hirsutum* L.*)* plants photosynthesis (Bednarz *et al.,* 1998; Zhao *et al.,* 2001). Enhancement of plant growth has been closely related to high leaf photosynthesis due to elevated CO_2 concentration which mostly depends on field plant arrangement and composition such as mixtures (Miller, 1988; Nicolodi *et al*., 1988). This is because higher CO_2 concentration can suppress RuBP oxygenase activity; decrease photorespiration and increase carbon assimilates for plant growth and development (Lawlor and Mitchell,

2000). Although chlorophyll concentration is an important physiological parameter for indicating plant photosynthesis status (Carter and Knapp, 2001), it has been reported that stress related factors may result in increased leaf reflectance due to reduced amount of chlorophyll content hence affecting its function, thus, becoming an indicator for photosynthesis status in plants (Carter and Knapp, 2001).

Plant growth (measured as biomass) is influenced by many factors, including water availability, C accumulation via photosynthesis, and the supply of mineral nutrients (Chiroma *et al*., 2006a, b). Photosynthetic CO_2 reduction by Rubisco in C3 plants such as cowpea and other legumes as well as in C4 plants such as sorghum (i.e. after PEPC have delivered CO_2 to Rubisco) is therefore the key process driving growth and agronomic yields in such crop species (Chiroma *et al*., 2006a, b). Theoretically speaking, high ^{13}C discrimination (i.e. more negative $\delta^{13}C$ value) tends to indicate low water-use efficiency, while low ^{13}C discrimination (i.e. less negative $\delta^{13}C$ value) suggests high water-use efficiency (Farquhar and Richards, 1984). As a result, the $\delta^{13}C$ values of crop plant species have been found to correlate with photosynthetic water-use efficiency estimated from gas-exchange studies (Farquhar and Richards, 1984). However, very negative $\delta^{13}C$ values in young legume leaves can also arise from the supply of ^{13}C-depleted C to shoots and other organs such as nodules, roots and developing pods by the Rubisco-operated C3 pathway (Yoneyama and Ohtani, 1983). But because these organs also fix CO_2 via phosphoenolpyruvate carboxylase (PEPC) through C4 pathway (Lawrie and Wheller, 1975; Coker and Schubert, 1981), this can shift the very negative $\delta^{13}C$ value of organs to a less negative $\delta^{13}C$ value. So, it is important to manage agronomic practices by manipulating the cropping systems, plant densities and varieties such that constraints leading to lower photosynthetic rate and related parameters are minimised so as to improve both biological and economical yields of different crop component in mixtures.

1.3 Some rhizosphere chemical reactions and mineral elements concentration as affected by plant densities, mixed culture practices and different legume genotypes.

Several studies have indicated that the rhizosphere pH is greatly influenced by the relative proportions of cations and anions absorbed by the plant root (Marschner, 1986; Haynes, 1990); the corresponding differences in net excretion of H^+ and HCO_3^- (or OH^-); excretion of organic and amino acids (Marschner *et al*., 1987) and release of CO_2 from the roots (Laurent and Eric, 1994). Legumes such as cowpea growing in mixed culture with cereals have the ability to modify soil pH in their rhizosphere (Muofhe and Dakora, 2000; Rao *et al.,* 2002; Li *et al.,* 2004b) through different mechanisms such as response to stress related to plant growth in different cropping systems. These mechanisms include net positive excess cations over anions entering the roots of N_2-fixing legumes with characteristic release of protons (Romheld, 1986;

Gahoonia *et al.,* 1992). Other mechanisms includes changes in redox potential induced by plant roots in the rhizosphere resulting into the release of proton (Ahmad and Nye, 1990); enhanced release of H^+ as a response to P-deficiency localised behind the root tip as those reported in maize and rape (*Brassica napus* L.) intercrops (Gregory and Hinsinger, 1999) and root excretion of carboxylic acids which are capable of mobilising P by ligand exchange or dissolution and occupation of P-sorption sites (Fox and Comerford, 1990; Gerke, 1995). Results from a study by Rao *et al.* (2002) concluded that rhizosphere acidification was light induced and is regulated by photosynthetic activity rather than excess cations uptake in the rhizosphere. These researchers arrived at this conclusion after a NO_3-fed non-symbiotic cowpea plants was put under illumination and significantly raised protons concentration in their rhizosphere similar to the aforementioned mechanisms. As a result of these mechanisms, mineral elements which are otherwise unavailable such as P, K, Ca, and Mg become available for plant nutrition (Vandermeer, 1989; Hauggaard-Nielsen and Jensen, 2005). To date, few studies have reported on the chemistry of rhizosphere soil, involving complex plant densities, cropping systems and genotypes. More understanding of such interactions is therefore important.

1.4 Acid and alkaline phosphatase activities in plant roots and soils as influenced by plant densities, cropping systems and legume genotypes

Phosphatase enzyme activities have been traditionally classified as being acid or alkaline (Vincent *et al.*, 1992). Acid phosphatase (AcPA) enzymes are the principal component of root exudates and occur widely in plant organs (Duff *et al*., 1994). On the other hand, alkaline phosphatase (AlkPA) activity is fungus and bacteria borne mostly found in the soil (Nakas *et al*., 1987; Tarafdar and Claassen, 1988). Accordingly, these enzymes are involved in the mobilisation of P within the rhizosphere of many cropping systems (Marschner, 1995; Strom, 1997). For example, release of acid phosphatase from roots as root exudates has been implicated as a mechanism to enhance the availability of sparingly soluble mineral elements such as P, Zn, Fe, and Cu (Marschner, 1995; Jones *et al*., 1996a). There is evidence that acid phosphatase play major roles in remobilising internal P from the plant organs (Duff *et al*., 1991; de Pozo *et al*., 1999; Baldwin *et al.*, 2001); facilitate release of P from organic P-esters by exudation of these enzymes into the rhizosphere (Lefebvre *et al*., 1990; Miller *et al*., 2001) and synthesises glycolate from P-glycolate (Christella and Tolbert, 1978) as well as glycerate from 3-PGA during photorespiration (Randall *et al*., 1971). The P released is then available for plant nutrition. Such actions could be complex and benefit the mixed culture systems

Phosphatase activity is greatly affected by soil bio-physical-chemical properties, management practices and cropping systems (Alvarez and Guerrero, 2000; Criquet *et al*., 2000). Several studies have shown that

in different agronomical settings, plants compete with each other strongly for resources (Tilman, 1988; Vandermeer, 1989). In mixed culture systems for instance, optimum intercrop yield advantage is achieved by maximising complementarity while minimising competition between component crops (Willey, 1979; Vandermeer, 1989). The reduction in competition has been suggested to be a primary reason for improved total yields in mixed culture system (Vandermeer, 1990). Apart from complementary resource use, facilitation has been suggested as a mechanism of obtaining greater total yields in intercrops as opposed to monoculture. Such beneficial interaction could be the result of increased resource availability through root induced changes in the rhizosphere including phosphatases activities (Ae *et al*., 1990; Vandermeer, 1990). There is evidence that crops that are very good at accessing sparingly available P can have a favourable effect on plants with which they are in mixture with (Horst and Waschkies, 1987; Li *et al*., 2003). Plant species and cultivars, however, possess diverse root morphological and physiological mechanisms for adapting to low P supply, with varying P mobilising processes (Gahoonia *et al*., 1997; Neumann *et al*., 1999). Although there is vast literature on acid and alkaline phosphatase activities, effect of different plant densities and cropping systems on these activities when cowpea genotypes are grown in mixture with sorghum are still lacking. Availability of such information will enable more understanding on the dynamics of acid and alkaline phosphatase activities in mixed cultures and establish their effect on the availability of plant nutrients in such agronomic systems.

1.5 Enhanced productivity and grain yield from components in mixed culture systems

Mixed culture advantage (or intercropping productivity) is commonly assessed by land equivalent ratio (LER) (Magino *et al*., 2004; Dariush *et al*., 2006). It is defined as the relative land under monoculture that is required to produce the yields achieved under mixed culture (Gocio, 2001). Total land equivalent ratio (LER_t) is obtained by the summation of LER for each crop (i.e. partial LER) in the mixture. When the $LER_t > 1$, then, mixed culture is advantageous because environmental resources are used more efficiently for plants growth. On the contrary, when the $LER_t < 1$, there is disadvantage because environmental resources are used less efficiently. However, when the $LER_t = 1$; it is considered as there is no effect by growing such crops either as monocrops or intercrops. As shown by Vandermeer (1989), competition and facilitation for growth factors takes place in mixed culture systems. As such, it is possible to obtain the net positive result whereby the $LER_t > 1$, thus indicating that in such mixed cultures, facilitation is contributing more than the competition. In their work on cowpea/sorghum mixed culture with varying number of cowpea rows, Hussain *et al*. (2000) showed that the $LER_t > 1$ was fairly high in all mixed culture treatments but the highest value of 1.89 was recorded from the sorghum-cowpea 3-rows mixed culture. This indicated that 89% yield advantage was gained due to mixed culture practice attributed to higher facilitation. Likewise, in a wheat (*Triticum aestivum* L.)/chickpea (*Cicer arientinum* L.) mixed

culture; the LER_t was highest in 4:2 rows, indicating low competition or greater complementary facilitation between the component crops (Zhang and Li, 2003; Li *et al*., 2004a; Banik *et al*., 2006). Collectively, the observed mixed culture advantage in these studies were attributed to beneficial complementarity of component crops with regard to mineral elements, light and moisture (Babu *et al*., 1988). So, mixing legumes with cereals could lead to better land use efficiency making it an important component in small scale farming in Africa and other parts of the world.

1.6 Possible influence (s) of different plant densities and cropping systems on N_2 fixation and availability to crops in mixtures

Biological nitrogen fixation (BNF) plays an important role in the nitrogen budget of cereal/legume mixed culture. For example the BNF accounted for total N accumulation of between 61 – 77% in cowpea and 58 - 78% in soybean (*Glycine max*. L.) respectively (Ofori *et al*., 1987; Adjei-Nsiah *et al*., 2008). Additionally, of the total N accumulated in the component crop such as sorghum, between 11 and 58% was directly transferred through the BNF (Fujita and Ofosu-Budu, 1996; Salvagiotti *et al*., 2008; Peoples *et al*., 2009). Legume/cereal mixed culture has been reported as potentially advantageous with increased total crop productivity compared with monoculture system. This has been ascribed to the effective use of water, mineral elements and light in such complex systems (Wiley, 1979; Midmore, 1993; Jensen, 1996). Intercropping system involves simultaneous growing of two or more crops on the same piece of land. Such cultural practices have led to increased size and stability of total grain yield compared with monoculture especially under small scale and low input farming systems (Ofori and Stern, 1987; Vandermeer, 1989). Amongst others, reasons for mixed culture practices involving legumes and cereal lies on the ability of the legume to fix N_2 which also benefits the associated cereal crop (Heichel, 1987; Dakora and Keya, 1997; Adjei-Nsiah *et al*., 2008). As a result, total grain yields, land use efficiency and efficient utilisation of the limited land resources are increased (Trenbath, 1974; Paperndick, *et. al.*, 1976; Fukai and Trenbath, 1993). A significant direct transfer of fixed N to the associated cereal crop for example, has been observed in controlled studies (Stern, 1993; Elgersma *et al.,* 2000; Chu *et al.,* 2004). Likewise, apart from the compelling evidence of increased N availability to the associated crops as a result of mineralisation from the decomposing legume roots (Schroth *et al.,* 1995; Evans *et al.,* 2001), increased total grain yield was also ascribed to less competition and greater complementarity of growth factors between the intercrops (Snaydon and Satorre, 1989; Hauggaard-Nielsen *et al.*, 2001). Legume/cereal mixed culture has been shown to use the available growth resources efficiently compared with their corresponding monoculture (Vandemeer, 1990). The efficient use of growth factors in mixed culture system however, depends on factors such as plant species, plant morphology, density of component crops,

type of management, and competitive ability of the component crops (Ofori and Stern, 1987). Improved understanding of how growth factors are used efficiently by component crops in such systems will enable proper management of soil fertility programs, thus, improved yield.

1.7 Flavonoids and anthocyanins concentrations as affected by plant densities and cropping systems

Flavonoids and anthocyanins are the major secondary metabolites which occur widely in most plants with characteristic wide range of colours (Linda, 1999; Dieter, 2006). Physiologically, they are beneficial to the plant itself by acting as effective antioxidants in photosynthetic tissues and screening harmful incident radiation (Hashimoto and Tajima, 1980; Balakumar *et al*., 1993; Rice-Evans *et al*., 1997). They also act as protectants of plants from insect pest infestations, diseases and oxidative cell injury (Hedin *et al*., 1983; Harborne, 1988). On the other hand, the accumulation of these compounds may act as a signal of nutrient limitation in a low plant/soil nutrient environment. For example, flavonoids and anthocyanins accumulation have been related to common symptom of nutrient (P, N, K, S, Mn and B) deficiency in a plant (Murali and Teramura, 1985; Close *et al*., 2000). These nutritional stresses have been reported to increase flavonoids concentration by regulating availability of substrates expression of enzymes responsible in their synthesis (Yamakawa *et al*., 1983; Plaxton and Carswell, 1999). However, some flavonoids induce spore germination and hyphae growth in the establishment of vesicular arbuscular mycorrhizal symbiosis, which is important in P acquisition, uptake and improved soil plant water relationship. Similarly, some studies have shown that flavonoids also act as chemo-attraction in the legume-rhizobium symbiosis at the onset of N_2 fixation process (Caetano *et al*., 1988; Khan and Bauer, 1988). For example, there is sufficient research evidence which established that some plant flavonoids such as genistein, daidzein and coumestrol function as signals to N-fixing microbes leading to interaction with NodD protein of the (*Brady*) *rhizobium* cells, thus, inducing expression of nodulation (*nod*) genes, consequently nodule formation and N_2 fixation (Long, 1989; Recourt *et al*., 1992; Dakora *et al*., 1993a; Dakora and Phillips, 1996; Hungria and Stacey, 1997; Philips, 2000). Furthermore, a group of flavonoids have also been identified as *haustoria* inducers that promote suicidal germination of *Striga*, a notorious parasitic crop weed in cereals and legumes (Steffens *et al*., 1982; Ndakidemi and Dakora, 2003).

Sorghum has been reported to contain flavonoids such as flavonols, flavonones, flavons and anthocyanins (Haslam, 1998; Audilakshmi *et al*., 1999; Awika *et al*., 2003; Awika and Rooney, 2004; Awika *et al*., 2004b; Dicko *et al*., 2005). The most abundant anthocyanins in sorghum grain are 3-deoxyanthocyanidins e.g. apigeninidin and luteolinidin (Awika *et al*., 2004b) which are particularly abundant in red and black

sorghum grain (Dicko *et al*., 2005) but rare or absent in other plants (Awika *et al*., 2004b). In black sorghum for example, apigeninidin and luteolinidin accounted for 50% of the anthocyanins contents (Awika *et al*., 2004a). Apigeninidin and luteolinidin (3-deoxyanthocyanidins) are of interest because they are more stable in organic solvents as well as in acidic solutions than anthocyanidins found in other cereals. Sorghum has been suggested to have a potential advantage as a viable commercial source of anthocyanins which is reported to have good antioxidant activity (Awika and Rooney, 2004a). It was recently reported that proanthocyanidins such as those found in plants may inhibit the growth of several viruses including human immunodeficiency virus 1 (HIV-1), influenza virus, and herpes simplex virus by blocking their entry in the host cells (Hamauzu *et al*., 2005). Since both cowpea and sorghum are staple food in many of the African countries, growing them in mixed culture may be the main source of natural antioxidants.

Flavonoids have also been shown to inhibit seed germination in a variety of legumes, cereal grains and toxic to seedlings of several species including weeds (Patterson, 1987; Rao, 1990). For example, the flavonoids vitexin and isovitexin which are present in the seed coat of mungbeans (*Vigna radiata* L. Wilczek) are powerful inhibitors of seed germination and seedling growth of other plant species around them (Tang and Zhang, 1986; Khalid *et al*., 2002). Similarly, tricin and some related flavonoids are considered to be responsible for phytotoxicity exhibited by quack grass (*Elytrigia repens*) residue (Rao, 1990). Therefore, it is suggested that flavonoids do not only lead to soil allelopathic effects and therefore growth problems of many crop species; but also adversely affects root growth and cause shoot bleaching, root swelling, inhibition of root hair formation and influences uptake of mineral elements such phosphate and chloride (Stenlid, 1963; Chang *et al*., 1969; Rao, 1990).

The release and accumulation of these phenylpropanoid compounds however, is dependent on such factors as plant density, cropping systems and genotypes or plant species involved in the cropping systems. It has been reported that flavonoids concentrations vary with cultural practices and varieties (Dykes *et al*., 2005). For instance, at high plant density many plants occupy the same area; relying on the same resources and may be stressed. Likewise, in mixed culture system more than one crop species grow in a unit area and rely on the same growth factors (Wiley, 1979). So, high density and mixed culture systems will definitely create competition for the growth factors leading to stress. Similarly, if one of the component crops is competitively stronger for the plant growth factors, then, stress for such growth factors will occur (Jensen, 1996). Some studies (Ampong-Nyarko *et al*., 1994; Hassan, 2009) have also reported that high plant density and intercropping practices reduced insect pest infestation in cowpea. Although several studies have shown that stress affects the release of these compounds, it is however not clear if stress resulting from high plant density and mixed culture will lead to the release of more flavonoids and anthocyanins

compounds which could then play important ecological functions such as those involving the protection of plants against insects.

A pre-requisite for a successful cowpea/sorghum intercrops is to obtain adequate plant population density (Ismail and Ali, 1996), appropriate cropping system and highly potential cowpea genotypes. For example, in areas where crop growth is constrained by limited moisture, optimising plant density is critical as high plant density may deplete the available moisture before crop matures, while low density may leave moisture unutilised in moist environment. Plant density strongly affects light interception and canopy photosynthesis (Gan, *et al.,* 2002). The efficiency of production therefore depends on several factors, including the population density of component crops, soil mineral elements status and genotype. Although some information is available, effect of plant densities and cropping systems have not been adequately studied. This study assesses the effect of plant density, cropping system and genotypes on plant growth, N_2 fixation, grain yield and plant and rhizosphere nutrition of cowpea/sorghum mixed culture.

1.8 Aims and hypothesis

The overall objective of the study was to identify cowpea genotype and agronomical practices that can maximise N_2 fixation and eventually grain yield of cowpea grown in mixture with sorghum.

1.8.1 The hypotheses

Different cowpea genotypes have varied yielding and N_2-fixing potential and may perform differently under different cropping systems.

Plant densities and cropping systems affects grain yield, N_2 fixation, and plant and rhizosphere nutrition in cowpea genotypes grown with/without sorghum.

Phosphatase activities in the rhizosphere soil and roots of cowpea genotypes is influenced by plant density and cropping systems.

Complex mixed culture systems may affect the synthesis of flavonoids and anthocyanins compounds from seed and plant organs of symbiotic cowpea genotypes.

1.8.2 The specific objectives were to:

- Define agronomic practices that improve photosynthesis, chlorophyll concentration, water-use efficiency and $\delta^{13}c$ of five cowpea genotypes grown in mixed culture and at different densities with sorghum.
- Explore agronomic practices that affect mineral element concentrations in soils, plant root uptake and growth of five cowpea genotypes grown in mixed culture and at different plant densities with sorghum.
- Assess agronomic practices that affects levels of acid and alkaline phosphatase activity in roots and rhizosphere soil of cowpea in genotypes grown in mixed culture and at different densities with sorghum
- Determine agronomic practices that will improve yield components of nodulated cowpea genotypes and sorghum grown in mixed culture.
- Examine agronomic practices that will improve symbiotic N_2 fixation in five cowpea genotypes.
- Asses flavonoids and anthocyanins concentrations in nodulated cowpea genotypes; identify genotypes with high insect pest resistance, as well as genotypes with higher levels of phenolics under different plant densities and cropping systems.

CHAPTER 2

PHOTOSYNTHESIS, WATER-USE EFFICIENCY AND $\delta^{13}C$ OF FIVE COWPEA (*VIGNA UNGUICULATA* L. WALP.) GENOTYPES GROWN IN MIXED CULTURE AND AT DIFFERENT DENSITIES WITH SORGHUM (*SORGHUM BICOLOR* L.)

2.1 Introduction

Carbon nutrition in plants via photosynthesis is the second most important physiological process after N nutrition (Drake *et al.,* 1997), as at least 90% of plant dry matter is derived from photosynthetic CO_2 reduction (Zelitch, 1982). Efficient translocation of photosynthate from source to sink organs is the key factor driving plant growth and increased crop yields (Tollenaar and Daynard, 1982; Goldberg *et al*., 1983; Dong *et al*., 1991). One of the major macromolecules important for photosynthetic functioning is chlorophyll; it is responsible for harvesting light energy for conversion to chemical energy via photoassimilation (Tanaka *et al*., 1998). Several factors affect photosynthetic C yield; these include temperature, light, CO_2 concentration, mineral nutrition and water needed for photolysis during photosynthesis (Usada *et al*., 1985; Drake *et al*., 1997; Cakmak and Engels, 1999; Balakrishnan *et al*., 2000). Photosynthesis is also reported to be affected by sink strength as increased photosynthate supply is required to meet increasing demand for photoassimilates during early vegetative growth and seed development (Richards, 2000). Agronomic practices such as intercropping (or mixed culture) and planting density also affect photosynthesis and plant growth, measured as biomass (Srinivasan *et al*., 1985; Hooper, 1998; Horton, 2000; Akunda, 2001; Andersen *et al*., 2005; San-oh *et al*., 2006). Photosynthesis is generally decreased by intercropping (mixed culture) as a result of reduced light penetration from shading. Net photosynthesis, stomatal conductance and transpiration are also reported to be reduced in *Atriplex prostrate* plants growing at high density relative to low density (Wang *et al*., 2005).

Carbon isotope discrimination during photosynthesis, measured as ^{13}C natural abundance ($\delta^{13}C$) is apparently a good indicator of WUE in C3 plants (Farquhar *et al*., 1989). The combined use of data from gas-exchange and isotopic measurements of ^{13}C permits the selection of crop genotypes with enhanced photosynthetic activity and greater water-use efficiency for increased yields. An increase in our understanding of the photosynthetic process should also permit the manipulation of source capacity in crop species for increased photoassimilate supply to sink organs in cropping systems for greater crop yields.

In Africa, cowpea is the most important food grain legume, and the crop is adapted to a wide range of soil ecologies. However, its yield is constrained by a number of factors, including drought, insect pests and diseases. African farmers depend largely on natural rainfall for crop yields, but the rainfall has become erratic often leading to poor crop yields. To increase cowpea production would require the selection of cowpea genotypes that tolerate drought and have improved water use. Because cowpea is usually cultivated as an intercrop with millet or sorghum and at high plant density, the best way to select drought-tolerant genotypes for small-scale farmers in South Africa would therefore be to assess their water use

against a background of intercropping and high plant density (the most common agronomic practices used by rural African farmers).

In this study five cowpea genotypes were evaluated for plant growth and water-use efficiency using mixed culture and high plant density as background treatments. Plant growth was measured instantaneously as photosynthesis, and as shoot C accumulated at early podding, while water-use efficiency was measured as $\delta^{13}C$, and as photosynthate produced per unit water molecule transpired.

2.2 Materials and methods

2.2.1 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. In this chapter, the 2005 and 2006 data were pooled together since they were similar. The site is located at 33°54′S and 18°14′E at an elevation of 146 m above mean sea level. The mean annual rainfall was 713.4 mm, and the potential evapotranspiration (ET_O) as measured by Penman Monteith (Monteith, 1965) was 1573 mm. The mean annual day and night temperatures were 22.6°C and 11.6°C respectively and the mean monthly radiation 544 MJm^{-2} m^{-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). According to the Soil Classification Working Group, the field soil used for this study is a sandy loam (SL) classified as Glenrosa, Hutton form (SCWG, 1991) equivalent to skeletic leptosol in the FAO soil classification system (FAO, 2001). Prior to planting in each year, 4 soil samples (0 – 20 cm soil depth) were collected from each experimental plot, pooled, and the resulting sub-samples from a total of 88 experimental plots used for chemical analysis. Before planting, the soil characteristics of the field plots are shown in Table 2.1.

2.2.2 Experimental design

The experimental treatments included five cowpea genotypes (namely, Bensogla, Sanzie, Omondaw, ITH98-46 and TVu1509), two cowpea densities (i.e. 83,000 vs. 167,000 $plants.ha^{-1}$), and two cropping systems (i.e. monoculture vs. mixed culture). Intercropping and monoculture are the two most commonly used cropping systems in Africa, with intercropping depicting high plant density, and monoculture, low density. A completely randomised block design was used with a 3-factorial arrangement. Four replicates were used for each treatment with a plot size of 3.6 m x 3.2 m. Cowpea plants in monoculture were sown with a row-to-row spacing of 60 cm, and plant-to-plant spacing of 40 cm to reflect low plant density whereas a row-to-row spacing of 60 cm, and plant-to-plant spacing of 20 cm were used for high plant

density in monoculture. Sorghum plants in plots with row-to-row spacing of 90 cm and plant-to-plant spacing of 40 cm, was maintained at a 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 spacing of 26.6 cm to give low plant density. Similarly, row-to-row spacing of 90 cm, with plant-to-plant spacing of 13.3 cm was used to obtain high plant density in mixed culture. Cowpea seeds were inoculated with *Bradyrhizobium* strain CB756 and planted together with sorghum. The seedlings were later thinned to two per stand. Weeding was done manually with a hoe. The plants were irrigated every three days up to flowering stage (50 DAP) and the frequency reduced to once every seven days until harvest. Moisture supply through irrigation was necessary as the study was done during summer season when temperature and evaporative demands are both high.

2.2.3 Chlorophyll determination in plant leaves

Chlorophyll content was extracted from each of four trifoliate leaves per plot using dimethyl sulphoxide (DMSO), as described by Hiscox and Israelstam (1979). At 67 days after planting (DAP), at 50 % flowering, each third young fully open trifoliate leaf from the tip (flag leaf) was sampled from the field for chlorophyll analysis. The trifoliate leaf was cut into small pieces, and 100 mg of leaf tissue weighed into a 15-mL vial containing 7 mL DMSO, and incubated at 4°C for 72 h. Following incubation, the extract was diluted to 10 mL with DMSO, and 3 mL of extract used to read the absorbance at 645 nm and 663 nm on a spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E) against a DMSO blank. Chlorophyll levels were calculated using equations by Arnon (1949) with unit of mg L^{-1}:

Chlorophyll a	$Chl_a = 12.7D_{663} - 2.69D_{645}$
Chlorophyll b	$Chl_b = 22.9D_{645} - 4.68D_{663}$
Chlorophyll total	$Chl_t = 20.2D_{645} + 8.02D_{663}$

2.2.4 Photosynthetic measurements in plant leaves

At 67 DAP, photosynthetic rates, stomatal conductance, intercellular CO_2 concentration and transpiration rates were measured in four young leaves (flag leaves) per plot for each species using a portable infra-red gas chromatograph (LCpro+ 1.0 ADC, Bioscientific Ltd., 12 Spurling Works, Pinder Road, Hoddesdon, Hertfordshire, EN11 ODB, UK). Because most enzymes controlling biological processes follow a diurnal rhythm, measurements were made in the morning between 8 and 11 a.m., and between 2 and 4 p.m. for each replicate plot per day. Without troubleshooting, each measurement took about 2 min. Light was maintained at 1100 µmol quanta m^{-2} s^{-1}. Water-use efficiency was calculated as (Hamid *et al*., 1990):

$$WUE = \frac{A}{E}$$

where A = photosynthesis rate (µmol CO_2 m^{-2} s^{-1}), E = transpiration rate (mmol H_2O m^{-2} s^{-1}) and WUE = water-use efficiency (µmol CO_2 $mmol^{-1}$ H_2O)

2.2.5 Plant harvest and processing for isotope analysis

During early pod development at 67 DAP, the shoots of 16 cowpea plants and 8 sorghum plants were harvested from the middle rows of each plot, pooled, oven-dried at 60°C for 48 h, weighed, and ground to fine powder (0.85 mm sieve) for analysis of ^{13}C.

2.2.6 Measurement of ^{13}C and %C in plant shoots

Plant shoot samples weighing 2 mg (cowpea) or 2.5 mg (sorghum) were transferred into tin capsules and injected into a Thermo Flash Elemental Analyser 1112 via a Thermo Conflo III devise coupled to a Thermo Finnigan Delta Plus XP Stable Light Isotope Mass Spectrometer. The ^{13}C natural abundance or $\delta^{13}C$ (‰) was calculated as (Farquhar *et al*., 1989):

$$\delta^{13}C = \frac{\left(^{13}C/^{12}C\right)_{sample} - \left(^{13}C/^{12}C\right)_{s\tan dard}}{\left(^{13}C/^{12}C\right)_{s\tan dard}} * 1000$$

where $\left(^{13}C/^{12}C\right)_{sample}$ is the isotopic ratio of the sample, and $\left(^{13}C/^{12}C\right)_{s\tan dard}$ is the isotopic ratio of PDB, a universally accepted standard from belemnite Pee Dee limestone formation (Craig, 1957).

Shoot C content per plant (g.$plant^{-1}$) was calculated as: %C x Shoot dry matter per plant

2.2.7 Statistical analysis

Data collected were analysed statistically using a 3-factorial ANOVA. The analysis was performed using STATISTICA 2007 (StatSoft Inc., Tulsa, OK, USA). One-Way ANOVA was also used to compare chlorophyll, gas exchange parameters, $\delta^{13}C$ and %C in cowpea and sorghum species. Correlation and regression analysis between $\delta^{13}C$ and photosynthetic water-use efficiency was estimated for cowpea and

sorghum. Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$ (Steel and Torrie, 1980).

2.3 Results

2.3.1 Effect of plant density and cropping system on chlorophyll levels

Analysis of data using 3-way ANOVA revealed significant differences in chlorophyll concentration of cowpea and sorghum leaves (Table 2.2). The levels of Chla, Chlb and total chlorophyll were markedly higher in plants at low density relative to high density irrespective of the plant species. Similarly, the leaves of plants under monoculture showed much greater levels of Chla, Chlb and total chlorophyll when compared with their counterparts in mixed culture (Table 2.2). The concentrations of Chla, Chlb and total chlorophyll were similar in all five cowpea genotypes.

2.3.2 Effect of plant density and cropping system on photosynthesis, stomatal conductance and transpiration

Photosynthetic rates, stomatal conductance and transpiration were all significantly higher in the leaves of plants at low density relative to those at high density, irrespective of the plant species (Table 2.3). Similarly, the same gas-exchange parameters were markedly greater under monoculture relative to mixed culture (Table 2.3). However, no differences were found in leaf photosynthesis, stomatal conductance and transpiration of the five cowpea genotypes (Table 2.3).

2.3.3 Effect of plant density and cropping system on $\delta^{13}C$ and water-use efficiency:

Water-use efficiency (calculated as photosynthate produced per unit water molecule transpired) was significantly higher in photosynthetic leaves of plants at low density relative to those of plants at high density, irrespective of the plant species (Table 2.3). Plants under monoculture also showed much higher water-use efficiency relative to those in mixed culture. There were however no marked differences in water-use efficiency between and among the five cowpea genotypes (Table 2.3).

The $\delta^{13}C$ of C3 plants is reported to be a good measure of water-use efficiency. Data from isotopic analysis revealed significant differences in $\delta^{13}C$ values of sorghum and cowpea plants under different experimental treatments. For example, the $\delta^{13}C$ values of plants were significantly higher (i.e. less negative) at low plant density relative to high plant density, irrespective of plant species (Table 2.4). Similarly, the $\delta^{13}C$ values were much higher in plants under monoculture when compared with those in

mixed culture (Table 2.4). The five cowpea genotypes also showed significant differences in $\delta^{13}C$, with Sanzie showing the most negative value and ITH98-46 the least negative value (Table 2.4).

As found with photosynthate, the concentration of C in leaves of plants at low density and under monoculture was significantly much higher, irrespective of the plant species (Table 2.4). The five cowpea genotypes also showed significant differences between and among themselves with regards to leaf C concentration. For example, Sanzie exhibited the highest C concentration in shoots, followed by Bensogla and Omondaw, while ITH98-46 and TVu1509 had the lowest concentration of C in shoots (Table 2.4). As a result, shoot C content was also significantly much higher in Sanzie, followed Bensogla and Omondaw, and lowest in cvs. ITH98-46 and TVu1509 (Table 2.4).

2.3.4 Effect of plant species on chlorophyll, photosynthesis, water-use efficiency and $\delta^{13}C$

The levels of chlorophyll a (Chla) and chlorophyll b (Chlb), as well as total chlorophyll were significantly higher in cowpea relative to sorghum. Although photosynthesis rates (A) were similar, transpiration (E), stomatal conductance (Gs) and internal CO_2 concentration (Ci) were all significantly higher in cowpea when compared with sorghum (Table 2.5). However, sorghum showed much higher water-use efficiency, $\delta^{13}C$ and %C relative to cowpea (Table 2.5).

2.3.5 Correlation and regression analysis of water-use efficiency and $\delta^{13}C$ in cowpea and sorghum

Performing regression as well as correlation analysis of $\delta^{13}C$ vs. photosynthetic water-use efficiency (Fig 2.1) showed a statistically significant relationship between the two parameters for both cowpea (r = 0.66***) and sorghum (r = 0.96***).

2.4 Discussion

In this study, the concentrations of Chla and Chlb, as well as total chlorophyll were increased in plants at low density and under monoculture possibly due to the greater N levels in photosynthetic shoots when compared with plants at high density or in mixed culture (Table 2.2). However, the higher chlorophyll concentrations in plants at low density and in monoculture could also be attributed to the observed greater shoot content of Mg (Chapter 3), a mineral element important for chlorophyll biosynthesis and a co-factor needed for the formation of enzymes involved in CO_2 fixation and energy transfer via ATP (Beale, 1999; Kaftan *et al*., 2002; Igamberdiev and Kleczkowski, 2003). Because of the role chlorophyll plays in CO_2 reduction, photosynthetic rates closely mirrored leaf chlorophyll levels in this study, with plants at low

density and in monoculture exhibiting higher photosynthetic rates relative to those at high density or in mixed culture (Table 2.3). At high plant density (or mixed culture) mineral nutrients probably became limiting from plant-to-plant competition as the concentrations of P, K, Ca, Mg, S, Fe, Cu, Zn, Mn and B were lower in the rhizosphere of plants at high density and in mixed culture relative to those at low density and in monoculture (Chapter 3). Such reductions in nutrient supply to plants were likely to lead to reduced photosynthetic functioning as found for common bean (Cornic *et al*., 1992; Lal *et al*., 1996; Wang *et al*., 2005). Analysis of cowpea shoots also revealed low concentrations of P, K, Ca, Mg, Fe, Cu, Zn, Mn and B in plants from high density and mixed culture relative to low density and monoculture (Chapter 3). These changes in mineral nutrition could have had subtle effects on plant processes in both cowpea and sorghum, as low levels of Fe, Cu and Zn were found to negatively affect the photosynthetic electron transport chain in cauliflower, which led to reduced photosynthesis and chlorophyll biosynthesis (Cakmak and Engels, 1999; Alloway, 2001; Hacisasiloglu *et al*., 2003). Additionally, Fe and Zn limitation (observed in this study, Chapter 3) was also found to decrease the levels of Chla, just as low Mg concentration (as found for plants at high density and in mixed culture in this study) also significantly reduced Chlb levels (Balakrishnan *et al*., 2000; Tang *et al*., 2006).

Tissue chlorophyll concentrations were however not different in the five cowpea genotypes. As a result, leaf photosynthetic rates were also not significantly different between and among the five cowpea genotypes tested (Table 2.3). That not withstanding, C accumulation from photosynthesis differed markedly between and among genotypes (Table 2.4). Shoot C concentration was much higher in Sanzie, followed by Omondaw and Bensogla, relative to ITH98-46 and TVu1509 (Table 2.4). As a result, the amount of C in cowpea shoots was also significantly much greater in Sanzie compared with the other four cowpea genotypes (Table 2.4). This inconsistency between photosynthetic rates and shoot C accumulated can be explained by the fact that the former is an instantaneous measure of Rubisco enzyme activity (which is affected daily by environmental factors), while the latter is a measure of the cumulative product of the enzyme's daily activity. Clearly, these are some of the dangers involved in using only instantaneous enzyme activities to measure long-term growth. Whatever the case, in this study, Sanzie emerged as the cowpea genotype with better plant growth because of its greater C accumulation against the background of high plant density and mixed culture.

The water relations of crop plants are easily affected by both mixed culture and high plant density because of plant-to-plant competition for soil water and mineral nutrients. As with photosynthetic rates, cowpea and sorghum plants at low density or in monoculture exhibited greater water-use efficiency (photosynthate produced per unit water transpired) relative to their counterparts at high density or in mixed culture (Table 2.3). Although there were no differences in photosynthetic water-use efficiency between and among the five cowpea genotypes (Table 2.3), their $\delta^{13}C$ values were markedly different (Table 2.4). The $\delta^{13}C$ of C3

plants is known to be an integrator of water-use efficiency (Farquhar *et al*., 1989), with the more negative $\delta^{13}C$ values indicating low water-use efficiency, and the less negative $\delta^{13}C$ values, high water-use efficiency. From that perspective, Sanzie exhibited high ^{13}C discrimination during photosynthesis (i.e. a more negative $\delta^{13}C$ value) and therefore showed the lowest water-use efficiency, followed by Omondaw and Bensogla, while ITH98-46 and TVu1509 had low ^{13}C discrimination (i.e. a less negative $\delta^{13}C$ value) which suggested high water-use efficiency (Table 2.4). Some earlier studies also found that low ^{13}C discrimination (or less negative $\delta^{13}C$ value) in wheat were related to high water-use efficiency (Farquhar and Richards, 1984; Ehdaie and Waines, 1993). The cowpea and sorghum plants from low density treatment or monoculture also showed greater water-use efficiency relative to those at high plant density or in mixed culture (Table 2.4). High ^{13}C discrimination during photosynthesis is generally associated with wider stomatal opening, greater CO_2 reduction and, to some extent, adequate availability of soil moisture. Thus, the increased photosynthetic C accumulation in the shoots of Sanzie is consistent with the notion of high ^{13}C discrimination. Whether measured isotopically or from gas-exchange studies (Table 2.5), sorghum (a C4 species) was found to exhibit a significantly higher water-use efficiency compared with cowpea (a C3 species), thus confirming the positive relationship between the two parameters (Figure 2.1) while also indicating the superior water-use use efficiency of C4 relative to C3 species (Farquhar and Richards, 1984; Ehdaie and Waines, 1993).

In conclusion, the decrease in chlorophyll concentration, photosynthetic rates, and C concentration/content of cowpea and sorghum shoots with intercropping or high plant density, as well as the reduction in the amounts of P, K, Ca, Mg, Fe, Cu, Zn, Mn, and B in shoots of the two species, could account for the lower crop yields under these practices in farmers' fields in Africa. This argument is re-enforced by the observation that, Sanzie, which showed a marked increase in shoot accumulation of P, K, Ca, Mg, Fe, Cu, Zn, Mn, and B, recorded the highest plant growth (Table 2.4) and grain yield. However, the farmer-selected varieties (Sanzie, Omondaw and Bensogla) showed lower water-use efficiency relative to the improved cultivars (ITH98-46 and TVu1509), just as sorghum (a C4 species) also showed a much higher water-use efficiency relative to cowpea (a C3 species).

Table 2.1: Selected soil chemical properties before planting.

	pH	C	P	K	Ca	Mg	S	Na	Fe	Cu	Zn	Mn	B
	$CaCl_2$	$g.kg^{-1}$						$mg.kg^{-1}$					
Year													
2005	6.2±0.03	18.1±0.4	18.8±1.8	137.8±4.8	70.5±1.8	16.6±0.5	4.2±0.2	90.5±2.3	253.4±9.6	3.7±0.1	3.4±0.2	8.8±0.3	0.5±0.0
2006	6.2±0.03	23.0±0.4	18.6±1.8	136.6±4.8	68.7±1.8	15.5±0.5	4.0±0.2	90.4±2.3	255.9±9.7	3.6±0.1	3.2±0.2	8.6±0.3	0.3±0.0

Each value (Mean ± SE, n = 4) is the mean of pooled soil samples from the 88 experimental plots during 2005 and 2006. Soil carbon was high in 2006 relative to 2005 possibly due to plenty of natural grasses resulting from long time fallow on the 2006 experimental plot relative to the one used in 2005.

Table 2.2: Effect of plant density and cropping system on chlorophyll level (mg.L^{-1}) in leaves of cowpea (*Vigna unguiculata* L. Walp) and sorghum (*Sorghum bicolor* L. Moench).

Treatment	Chl_a	Chl_b	Chl_{a+b}
A: COWPEA			
Density (plants.ha^{-1})			
83,000	10.9±0.4a	2.5±0.1a	13.4±0.5a
167,000	7.9±0.5b	1.7±0.1b	9.6±0.6b
Cropping System			
Monoculture	10.9±0.4a	2.4±0.1a	13.3±0.6a
Mixed culture	7.9±0.4b	1.8±0.1b	9.7±0.5b
3 - Way ANOVA (F-Statistic)			
Density	28.3***	28.1***	28.8***
Cropping system	26.8***	21.0***	26.1***
B: SORGHUM			
Density (plants.ha^{-1})			
Sorghum in 83,000	8.77±0.42a	1.58±0.08a	10.35±0.49a
Sorghum in 167,000	8.00±0.50b	1.40±0.10b	9.40±0.59b
Cropping System			
Monoculture sorghum	10.85±0.00a	1.94±0.00a	12.79±0.00a
Mixed culture (Sorghum + cowpea)	5.91±0.34b	1.05±0.07b	6.96±0.41b
3 - Way ANOVA (F-Statistic)			
Density	6.3*	7.2**	6.7*
Cropping system	261.3***	172.9***	251.1***

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *$P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. Results of different cowpea genotypes are not shown because they were not significant. Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}. Chla = Chlorophyll a; Chlb = Chlorophyll b.

Table 2.3: Effect of plant density and cropping system on photosynthesis and gas-exchange parameters of leaves of cowpea (*Vigna unguiculata* L. Walp) and sorghum (*Sorghum bicolor* L. Moench).

Treatment	A	E	Ci	Gs	WUE
	µmol $CO_2.m^{-2}.s^{-1}$	mmol $H_2O.m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ air	mmol.$m^{-2}.s^{-1}$	mmol $CO_2.mol^{-1}$ H_2O
A: COWPEA					
Density (plants.ha^{-1})					
83,000	20.4±0.7a	4.0±0.1a	234.8±5.1a	0.4±0.0a	5.1±0.1a
167,000	15.6±0.7b	3.4±0.1b	209.1±4.5b	0.3±0.0b	4.5±0.1b
Cropping system					
Monoculture	20.6±0.6a	4.0±0.1a	238.9±5.2a	0.40±0.0a	5.2±0.1a
Mixed culture	15.5±0.7b	3.4±0.1b	205.0±3.5b	0.31±0.0b	4.5±0.1b
Genotypes					
Bensogla	18.2±1.1a	3.6±0.1a	221.1±6.7a	0.3±0.0a	5.0±0.2a
ITH98-46	17.9±1.4a	3.6±0.2a	207.4±6.8a	0.3±0.0a	4.9±0.2a
Sanzie	17.5±1.3a	3.9±0.1a	232.6±8.4a	0.4±0.0a	4.4±0.2a
TVu1509	17.9±1.3a	3.7±0.1a	228.0±11.5a	0.4±0.0a	4.8±0.3a
Omondaw	18.6±1.1a	3.8±0.1a	220.7±5.8a	0.4±0.0a	4.9±0.2a
3 - Way ANOVA (F-Statistic)					
Density	36.1***	32.5***	21.0***	23.4***	16.4***
Cropping system	40.2***	37.4***	36.5***	16.8***	18.9***
Genotypes	0.2	1.9	2.3	1.4	1.8
B: SORGHUM					
Density (plants.ha^{-1})					
Sorghum in 83,000	19.9±0.4a	2.76±0.1a	142.45±6.8a	0.3±0.0a	8.7±0.2a
Sorghum in 167,000	17.7±0.7b	2.4±0.1b	117.7±5.6b	0.2±0.0b	7.7±0.4b
Cropping system					
Monoculture sorghum	21.5±0.0a	2.7±0.0a	148.3±0.0a	0.3±0.0a	9.5±0.0a
Mixed culture	16.2±0.5b	2.3±0.1b	111.7±8.2b	0.2±0.0b	6.9±0.3b
3 - Way ANOVA (F-Statistic)					
Density	31.5***	15.0***	11.0**	5.1*	22.2***
Cropping system	183.1***	26.4***	24.3***	21.0***	148.4***

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *$P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}.

Table 2.4: Effect of plant density and cropping system on shoot dry mass, ^{13}C natural abundance, %C, and C content in shoots of cowpea (*Vigna unguiculata* L. Walp.) and sorghum (*Sorghum bicolor* L. Moench).

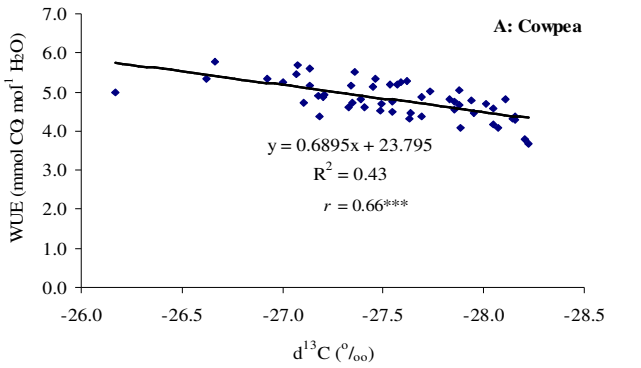
Treatment	Shoot dry mass	$\delta^{13}C$	Shoot C	Shoot C content
	g.plant^{-1}	‰	%	g.plant^{-1}
A: COWPEA				
Density (plants.ha^{-1})				
83,000	23.0±1.2a	-28.2±0.1a	41.5±0.1a	9.5±0.5a
167,000	18.8±1.0b	-28.4±0.1b	40.8±0.2b	7.8±0.4b
Cropping system				
Monoculture	23.9±1.1a	-28.2±0.1a	41.7±0.1a	9.5±0.5a
Mixed culture	17.8±1.1b	-28.5±0.1b	40.5±0.2b	7.7±0.5b
Genotypes				
Bensogla	20.2±1.9ab	-28.4±0.1b	41.4±0.2b	8.4±0.8b
ITH98-46	18.2±1.6b	-28.0±0.1a	40.6±0.3c	7.4±0.7b
Sanzie	25.4±2.1a	-28.7±0.1c	41.8±0.2a	10.6±0.9a
TVu1509	19.5±2.1ab	-28.1±0.1a	40.6±0.3c	8.0±0.9b
Omondaw	21.1±1.1ab	-28.5±0.1b	41.3±0.2b	8.7±0.5b
3 - Way ANOVA (F-Statistic)				
Density	9.5**	11.6**	22.5***	10.3**
Cropping system	20.5***	26.1***	68.7***	9.6**
Genotypes	3.3*	16.3***	11.5***	3.6*
B: SORGHUM				
Density (plants.ha^{-1})				
Sorghum in 83,000	33.5±1.2a	-12.1±0.0a	43.0±0.4a	14.5±0.6a
Sorghum in 167,000	24.1±0.7b	-12.3±0.1b	41.84±0.5b	10.2±0.4b
Cropping system				
Monoculture	33.4±1.1a	-11.91±0.0a	44.6±0.0a	14.9±0.5a
Mixed culture	24.3±0.9b	-12.54±0.1b	40.3±0.4b	9.8±0.4b
3 - Way ANOVA (F-Statistic)				
Density	95.9***	26.0***	12.2***	109.1***
Cropping system	89.2***	239.0***	168.0***	149.1***

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *$P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. Interactive effects are not shown for they were not significantly different. Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}.

Table 2.5: Comparison of chlorophyll levels, photosynthetic rates and gas-exchange parameters in cowpea (*Vigna unguiculata* L. Walp) and sorghum (*Sorghum bicolor* L. Moench).

Treatment	A	E	Ci	Gs	WUE	Chl a	Chl b	Total Chl	$\delta^{13}C$	Shoot C
	µmol CO_2 m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mmol CO_2 mol^{-1} air	mmol m^{-2} s^{-1}	mmol CO_2 mol^{-1} H_2O		mg.L^{-1}		‰	%
Cowpea	18.0±0.5a	3.7±0.1a	221.9±3.7a	0.4±0.0a	4.8±0.1b	9.4±0.4a	2.1±0.1a	11.5±0.4a	-28.3±0.1b	41.1±0.1b
Sorghum	18.8±0.4a	2.5±0.1b	130.0±4.6b	0.2±0.0b	8.2±0.2a	8.4±0.3b	1.5±0.1b	9.9±0.4b	-12.2±0.1a	42.4±0.3a
One - Way ANOVA (F-Statistic)										
	1.5	225.4***	246.8***	45.0***	238.8**	4.4*	32.0***	7.6**	63961.1**	15.9***

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *$P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$.



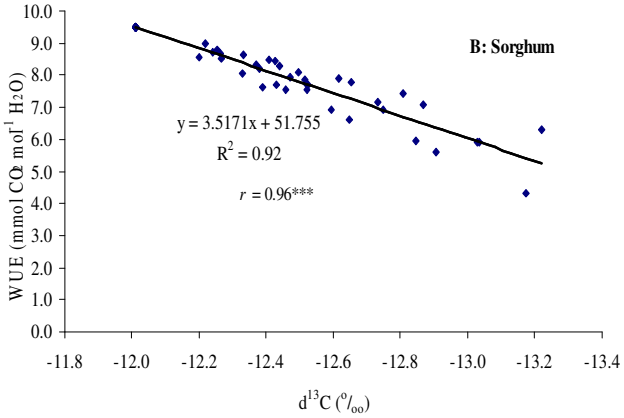


Fig.2.1: Correlation and regression analysis between $\delta^{13}C$ and WUE in A) Cowpea and B) Sorghum.

CHAPTER 3

CHANGES IN RHIZOSPHERE MINERAL CONCENTRATION CAUSED BY DIFFERENCES IN ROOT UPTAKE AND PLANT GROWTH OF FIVE COWPEA (*VIGNA UNGUICULATA* L. WALP.) GENOTYPES GROWN IN MIXED CULTURE AND AT DIFFERENT DENSITIES WITH SORGHUM (*SORGHUM BICOLOR* L. MOENCH.)

3.1 Introduction

Root and soil interactions during plant growth induce changes that make rhizosphere soil to differ from bulk soil (Xiaoping and Darlene, 1998). These changes are caused by root uptake of nutrients, microbial activity, and/or components of root exudates (Marschner, 1995; Hinsinger, 2001; Dakora and Phillips, 2002). 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 (Beleke *et al.,* 1983; Hinsinger *et al.,* 1993; Hinsinger and Gilkes, 1995; Dakora and Phillips 2002) through excess uptake of cations during N_2 fixation. Additionally, rhizosphere concentration of nutrients can be altered by agronomic practices such as cropping systems and planting patterns (Bais *et al.*, 2001). Stress is also known to lead to root exudation of minerals and organic compounds as a result of stress can further modify the rhizosphere (Marschner, 1995; Dakora and Phillips, 2002).

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 (Chapter 4). 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 (Ae *et al.*, 1990; Shane and Lambers, 2005). Because of its ability to secrete Fe-solubilising phytosiderophores (Römheld, 1991), maize enhanced Fe nutrition in peanut when grown in mixed culture with this legume (Zhang *et al.*, 2004). Peanut and pigeon pea have also been suggested to increase P availability through contact reactions at the cell wall interface (Ae *et al*., 1996; Ae and Shen, 2002). However, this mechanism still remains to be properly understood.

Although we have recently gained considerable insights into nutrient dynamics in the rhizosphere, little is known about the mineral concentrations of plant rhizosphere, especially when grown in different cropping systems and/or plant densities. This study measures and compares the mineral concentrations in the rhizosphere of five nodulated cowpea genotypes and sorghum, grown in mixed culture and at different densities. The study further relates the changes in rhizosphere mineral concentrations with uptake in shoots and whole plants, as well as with plant growth.

3.2 Materials and methods

3.2.1 Site description and Experimental design

Site description and experimental design are as reported in section 2.2.1 and 2.2.2 respectively.

3.2.2 Collection and preparation of rhizosphere soil

At 67 d after planting (DAP), rhizosphere soil was collected from around the roots of both cowpea and sorghum plants for enzyme and 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 pre-labelled plastic bag. The rhizosphere soil samples were then taken to the laboratory, air dried, and sieved (2 mm mesh) for chemical analysis.

3.2.3 Plant harvest and sample preparation

At 67 DAP, during early pod development, sixteen and eight plants of cowpea and sorghum were respectively harvested from the middle rows of each plot. The plants were carefully dug out with intact root system, washed, and separated into nodules, roots, shoots and pods in the case of cowpea, while sorghum plants were separated into only roots and shoots. The plant organs were 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 in the plant organs.

3.2.4 Measurement of soil pH

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

3.2.5 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 Dyer (1894) and modified by the Division of Chemical Services (DCS, 1956) and 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, Ca, Mg and Na 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 $Ca(H_2PO_4)_2.H_2O$ extracting solution (FSSA, 1974), 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 Fe, Cu, Zn, Mn and Al were extracted from soil using di-ammonium ethylenediaminetetraacetic (EDTA) acid solution [Trierweiler and Lindsay (1969), modified by Beyers and Coetzer, (1971)]. The extractants were analysed for Fe, Cu, Zn, Mn and Al using ICP-MS spectrometry (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA).

3.2.6 Measurement of mineral elements in plant tissue

Measurements of macro elements (P, K, Ca, Mg, and Na) and micro elements (Fe, Cu, Zn, Mn, 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 (Giron, 1973). 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 (FSSA, 1974) by

direct aspiration on the calibrated simultaneous ICP-MS. Nutrient uptake (macroelements) was then calculated from the following relationship:

$$N_{uptake}\left(mg.plant^{-1}\right) = ON_{conc.}\left(mg.g^{-1}\ DM\right) \times O_{dry\ mass}\left(g.plant^{-1}\right)$$

and for microelements,

$$N_{uptake}\left(\mu g.plant^{-1}\right) = ON_{conc.}\left(\mu g.g^{-1}\ DM\right) \times O_{dry\ mass}\left(g.plant^{-1}\right)$$

Where: N_{uptake} = nutrient uptake, ON_{conc} = organ nutrient concentration, $O_{dry\ mass}$ = organ dry mass, DM = dry mass.

Whole-plant mineral uptake (mg.plant^{-1} or µg.plant^{-1}) was calculated as the sum of the uptake of individual organs (i.e. nodules, roots, shoots and pods).

3.2.7 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 $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

3.3 Results

3.3.1 A comparison of mineral concentrations in bulk and rhizosphere soils of cowpea and sorghum

The concentrations of P, K, S, Na and Zn were lower in rhizosphere of cowpea relative to bulk soil (Table 3.1). The level of Cu was however similar, while the levels of Ca and Mg were greater in the rhizosphere compared with bulk soil (Table 3.1). With sorghum, only K, S, and Na were decreased in the rhizosphere, in contrast to P, Ca, Mg, Cu, and Zn, which showed an increase in the rhizosphere (Table 3.1). Organic matter levels were surprisingly lower in cowpea and sorghum rhizosphere soils relative to bulk soil (Fig. 3.1A). The rhizosphere pH of cowpea was also significantly lower than those of sorghum and bulk soil (Fig. 3.1B).

3.3.2 Effect of cowpea genotypes on rhizosphere mineral concentration, plant growth and elemental content

Analysis of mineral concentrations in the rhizosphere soil of five cowpea genotypes revealed significant differences among them. Cowpea genotype Sanzie consistently showed significantly much lower levels of the macronutrients P and Ca (Table 3.2), and decreased concentration of the trace element Cu (Table 3.3). Even where the rhizosphere levels of K, Mg, Na, S, Fe, Zn and Mn were not statistically different, they were nevertheless lower in magnitude compared with the other cowpea genotypes (Tables 3.2 and 3.3).

The lower concentration of minerals in the rhizosphere of the cowpea cv. Sanzie (whether significant, or only lower in magnitude), was caused by higher root uptake of these elements. As shown in Table 3.2, Sanzie showed significantly much greater amounts of P, K, Ca, Mg, Na, and S per plant compared with the other four cowpea genotypes. Even with the trace elements, the cultivar Sanzie again exhibited greater accumulation of Fe, Cu, Zn, Mn and B in tissues compared with the genotypes Bensogla, Omondaw, TVu1509 and ITH98-46 (Table 3.3). The manifestation of the greater accumulation of minerals by Sanzie was a significantly increased plant growth, measured as shoot or whole-plant biomass (Table 3.2). In contrast to Sanzie, ITH98-46, which had a much higher concentration of minerals such as P, Ca and Cu in its rhizosphere, also showed lower accumulation of these minerals in whole plants, and hence a relatively lower level of plant growth (Table 3.2).

3.3.3 Effect of plant density and cropping system on rhizosphere pH

Increasing cowpea density from 83,000 to 167,000 plants.ha^{-1} significantly decreased ($P \leq 0.05$) the rhizosphere pH of cowpea plants (Fig. 3.1C). Relative to monoculture, mixed culture also significantly decreased the rhizosphere pH of cowpea (Fig. 3.1D).

3.3.4 Effect of plant density on the mineral concentration in the rhizosphere of cowpea genotypes

Increasing cowpea plant density from 83,000 to 167,000 plants.ha^{-1} significantly decreased ($P \leq 0.05$) the concentrations of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn and B in the rhizosphere of cowpea plants (Fig. 3.2A, B), leading to reduced levels of these minerals in cowpea plants (Fig. 3.3).

3.3.5 Effect of cropping system on the mineral concentration in the rhizosphere of cowpea genotypes

Growing cowpea in mixed culture with sorghum significantly ($P \leq 0.05$) decreased the concentrations of P, K, Ca, Mg, Na, S, Fe, Cu, Zn, Mn and B in the rhizosphere of cowpea plants (Fig. 3.2C, D), leading to markedly decreased content in tissues (Fig. 3.4).

3.4 Discussion

In Africa, most farmers grow two or more crops (usually legumes in mixture with cereals) simultaneously on the same field in an effort to improve food security and their livelihoods using the available meagre resources. Such agronomic practices involving different crop components in the region, is often accompanied with the depletion of different mineral elements in their rhizosphere, thus, portraying variation in mineral elements concentration in rhizosphere and plant tissue. Understanding the status of mineral elements concentration in the rhizosphere of legumes and cereal plants (i.e. cowpea and sorghum) growing as crop components in sole or mixed culture as done in this study, is one way of improvising better soil fertility management strategies in such cropping systems. However, the range and rate of depletion of these mineral elements from the rhizosphere and ultimate uptake to plant tissues for greater grain yields will depend on plant density, cropping systems and legume cultivars or plants species involved. Although a limited number of studies have been documented on the composition and distribution pattern of a few macro-elements in the rhizosphere and plant tissues, none have addressed

legume plant density, cropping systems and cowpea cultivars as factors influencing the mineral elements concentration in the rhizosphere and the amount in plant tissues. As a result, information on the effect of these factors remains poorly understood in Africa. In the current study, rhizosphere and bulk soil and tissue mineral elements concentration was characterised and or determined, using two cowpea plant densities, two cropping systems and five cowpea genotypes grown either as sole crop or in mixture with sorghum.

Results showed that mineral elements concentration in the rhizosphere of cowpea genotypes and sorghum were significantly different from that of the bulk soil (Table 3.1) suggesting greater root uptake rates that would have exceeded their replacement through the soil solution either by mass flow and diffusion processes as shown by Yanai *et al*. (2003). Likewise, the use of different genotypes in cropping systems in this study created variation in mineral elements demand from their rhizosphere. As a result, the surrounding rhizosphere was strategically modified through root exudation and uptake and/or deposition of mineral elements. For example, due to dependency on N_2 fixation, legumes (N_2 fixing plants) such as cowpea is known to take up higher amounts of mineral elements from their rhizosphere as they are required for plant growth and N_2 fixation compared with sorghum which is a non-fixing plant (Ae *et al*., 1990; Marschner, 1995). Consequently, their rhizosphere was acidified which was not only enhanced by the release of H^+ but also by the change in cation/anion ratio (Tang *et al.,* 1997, 2001), a process which could lead to variation in mineral element concentration in the rhizosphere compared with the bulk soil (Xiaoping and Darlene, 1998; Wang *et al.*, 2001; Dakora and Phillips, 2002; Cornu *et al*., 2007).

In contrast, the data also showed increased Ca^{2+} and Mg^{2+} in the rhizosphere of cowpea and sorghum plants ascribed to several mechanisms including reduced pH (Fig. 3.1). Increased rhizosphere pH is probably a result of increased plant density and mixed culture leading to both increased root exudation and net release/uptake of H^+ by roots in response to NH_4/NO_3 uptake (Marschner, 1995) or altered cation/anion ratio in response to nutrient stress, as reported by Haynes (1990) and Xiaoping and Darlene (1998). Increased H^+ in the rhizosphere soil observed in this study suggests not only the presence of high concentration of competing polyvalent cations in the rhizosphere soil, or a steep H^+ slope across the plasma membrane which may act as a driving force for anion transport, but also decreased charge density of the root cell walls, conditions necessary for reduced Ca^{2+} and Mg^{2+} loading of the apoplast of roots. As a result, the uptake rate of these mineral elements is impaired or inhibited, consequently, leading to their accumulation in the rhizosphere soil (Youssef and Chino, 1987). Greater competing effect of Mn^{2+} in soybean species for example, is known to reduce the uptake of Mg^{2+} (Heenan and Campbell, 1981), thus, accumulating in the rhizosphere soil. Similarly, Ca^{2+} and Mg^{2+} have been reported to be higher in the rhizoplane of millet compared with bulk soil (Bagayoko *et al*., 2000). On the other hand, Inal and Gunes (2008) reported decreased shoot Na^+ concentration in peanut (*Arachis hypogea* L) indicating that this

mineral element could have been accumulated in the rhizosphere of such legume. Besides increased H^+, the data also suggest other mechanisms including mass flow driven by root water uptake and transpiration at a rate exceeding plant uptake (Barber *et. al.*, 1962; Marschner, 1998); higher ability of H^+ to easily replace Ca^{2+} and Mg^{2+} in the apoplast of root cortex due to lower exchange power (Horst, 1987; Marschner, 1989) and restriction of Ca^{2+} membrane transport in order to maintain a certain range of free Ca^{2+} concentration in the cytosol compared with K^+ (Gilroy *et al*., 1989). Overall, the result will be reduced uptake rate of these mineral elements, consequently, accumulating into the rhizosphere soil of these species as reflected in the low uptake in such plants (Table 3.1).

Results of One-Way ANOVA showed that mineral elements concentration in the rhizosphere of both cowpea and sorghum were altered by changing plant density or cropping systems (Table 3.1). However, these mineral elements were reduced more in cowpea cultivars compared with sorghum species. The higher demand of mineral elements from the rhizosphere by cowpea cultivars for plant growth and N_2 fixation and the species-to-species competition for growth factors suggest reduced mineral elements concentration in the rhizosphere of cowpea compared with sorghum species (Table 3.1) as reported in some studies (Delhaize *et al.*, 1993; Dakora and Phillips, 2002, Hinsinger *et al.*, 2003).

The data also revealed that, different cowpea genotypes significantly altered the mineral element concentration in their rhizosphere (Table 3.2) suggesting variation in mineral elements requirements for growth, N_2 fixation and grain yield formation. For example, in contrast to cv. ITH98-46, Sanzie genotype had lower mineral elements concentration in the rhizosphere soil signalling greater growth, higher uptake of mineral elements (Table 3.2), higher N-fixed (Chapter 6) and grain yield (Chapter 5). Similar to the data in this study, different depletion rates from the rhizosphere resulting from different species or cultivars have also been reported (Marion and Ursula, 1999; Fan *et al.,* 2001).

In conclusion, assessment of mineral elements concentration in the rhizosphere of cowpea and sorghum showed that high plant density and mixed culture altered mineral elements concentration in the rhizosphere soil compared with the bulk soil. In addition, results also showed that concentrations of these mineral elements were significantly decreased in the rhizosphere of cowpea compared with sorghum plants. Furthermore, the data also showed that cv. ITH98-46 was superior in the accumulation of P, Ca and Cu in their rhizosphere (lower nutrient uptake) compared with cv. Sanzie (higher nutrient uptake). In all, this result suggest that different genotypes under different agronomic practices may lead to significant variation in the concentration of mineral element in the rhizosphere and plant nutrient uptake, thus, enabling proper choice of crop components in cropping systems as well proper soil fertility management strategies for greater grain yield in Africa.

Table 3.1: Comparison between bulk soil and rhizosphere soils of cowpea and sorghum species.

Treatment	P	K	Ca	Mg	S	Na	Fe	Cu	Zn	Mn	B
						mg.kg^{-1}					
Bulk soil	18.8±1.8b	137.8±4.8a	70.5±1.8c	16.6±0.5c	4.2±0.2a	90.5±2.3a	253.4±9.6a	3.8±0.1b	3.4±0.2a	8.8±0.3a	0.5±0.0a
Rhizosphere soil											
Cowpea	14.4±0.9c	112.5±2.5b	729.2±22.2b	186.8±4.7b	3.5±0.3b	61.7±1.9c	249.2±7.7a	4.0±0.1ab	3.0±0.1b	8.4±0.3a	0.5±0.0a
Sorghum	29.3±1.2a	122.5±2.8b	771.7±21.4b	210.4±7.4a	2.8±0.1c	77.8±2.3b	245.5±6.0a	4.2±0.1a	5.2±0.3a	8.6±0.2a	0.5±0.0a
One - Way ANOVA (F-Statistic)											
	28.8***	12.8***	534.1**	475.7**	11.5***	45.2**	0.2	6.7**	28.8***	0.7	3.2

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at **: $P \leq 0.01$; ***: $P \leq 0.001$.

Table 3.2: Concentration of macro-elements in the rhizosphere soil, plant mineral content and plant growth of five cowpea genotypes planted under different plant densities and cropping systems.

Treatment	P	K	Ca	Mg	Na	S	Plant growth
Rhizosphere mineral concentration							
				$mg.kg^{-1}$			$^{x}g.whole\ plant^{-1}$
Genotypes							
Bengsogla	13.4±1.7ab	111.1±5.4a	686.3±44.0ab	184.9±10.1a	59.8±3.5a	3.3±0.5a	25.6±2.3ab
ITH98-46	19.6±3.2a	119.9±6.9a	826.4±57.3a	203.7±11.8a	68.9±5.7a	4.3±0.8a	20.8±2.0b
Sanzie	11.6±1.0b	105.3±4.2a	640.1±36.0b	174.7±8.7a	57.4±2.9a	3.1±0.4a	28.2±2.6a
TVu1509	14.2±1.2ab	116.3±5.0a	792.6±51.2ab	191.5±9.4a	62.3±3.7a	3.7±0.5a	22.1±2.3b
Omondaw	13.3±1.6b	109.8±6.3a	700.5±47.0ab	179.4±11.6a	60.3±4.4a	3.3±0.6a	26.4±2.2ab
One - Way ANOVA (F-Statistic)							
Genotypes	3.8**	1.3	3.7**	2.3	1.2	1.6	4.3**
Whole-plant mineral content							
				$mg.plant^{-1}$			$^{y}g.plant\ shoot^{-1}$
Genotypes							
Bengsogla	95.6±12.0ab	784.0±157.2ab	483.0±69.9abc	137.2±16.3ab	21.6±3.0ab	14.2±2.2abc	18.7±1.7ab
ITH98-46	67.3±9.1b	505.2±58.3b	331.5±41.8c	107.0±14.2b	15.6±2.0b	9.3±1.5c	15.9±1.3b
Sanzie	102.6±14.6a	821.1±132.3a	566.4±85.0a	163.1±18.7a	25.4±3.7a	16.3±2.8ab	21.4±1.7a
TVu1509	73.8±10.7ab	592.3±86.9ab	392.1±65.2bc	116.5±17.0b	17.4±2.9b	10.0±2.0bc	17.3±1.7ab
Omondaw	104.0±14.4a	722.4±80.7ab	509.3±60.6ab	141.7±14.7ab	22.1±2.7ab	18.6±3.6a	17.9±0.9ab
One - Way ANOVA (F-Statistic)							
Genotypes	5.0**	2.9*	6.1***	4.8**	5.0**	5.3**	3.6*

[x]: Data for whole plant dry mass, [y]: Data for shoot dry mass. Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$.

Table 3.3: Concentration of micro-elements in the rhizosphere soil and plant mineral content of five cowpea genotypes planted under different plant densities and cropping systems.

Treatment	Fe	Cu	Zn	Mn	B
Rhizosphere mineral concentration					
			$mg.kg^{-1}$		
Genotypes					
Bengsogla	239.9±13.9a	3.8±0.2ab	3.0±0.3a	8.2±0.6a	0.47±0.02a
ITH98-46	273.4±20.5a	4.3±0.2a	3.6±0.5a	9.1±0.7a	0.52±0.03a
Sanzie	232.4±15.0a	3.7±0.1b	2.8±0.2a	7.7±0.5a	0.48±0.02a
TVu1509	262.8±14.2a	4.1±0.2ab	2.9±0.2a	8.6±0.4a	0.50±0.02a
Omondaw	237.2±20.9a	3.9±0.2ab	2.8±0.2a	8.2±0.7a	0.47±0.03a
One - Way ANOVA (F-Statistic)					
Genotypes	2.1	4.2**	1.9	1.2	1.1
Whole-plant mineral content					
			$\mu g.plant^{-1}$		
Genotypes					
Bengsogla	46182.6±15623.7a	664.0±173.6b	2089.7±274.1ab	838.2±136.3ab	1258.1±150.9ab
ITH98-46	20542.1±3354.5a	461.7±99.0c	1526.7±189.3b	602.0±88.4b	947.9±110.4b
Sanzie	40108.2±7806.5a	1070.5±295.6a	2552.7±347.3a	992.6±166.6a	1427.5±167.9a
TVu1509	25041.0±6254.3a	798.2±341.6b	1811.1±309.4b	696.2±138.3ab	1052.7±153.4b
Omondaw	47363.6±12189.2a	662.7±126.9b	2090.2±222.2ab	837.3±101.0ab	1299.8±136.5ab
One - Way ANOVA (F-Statistic)					
Genotypes	2.7	1.5*	4.9**	3.6*	4.7**

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$.

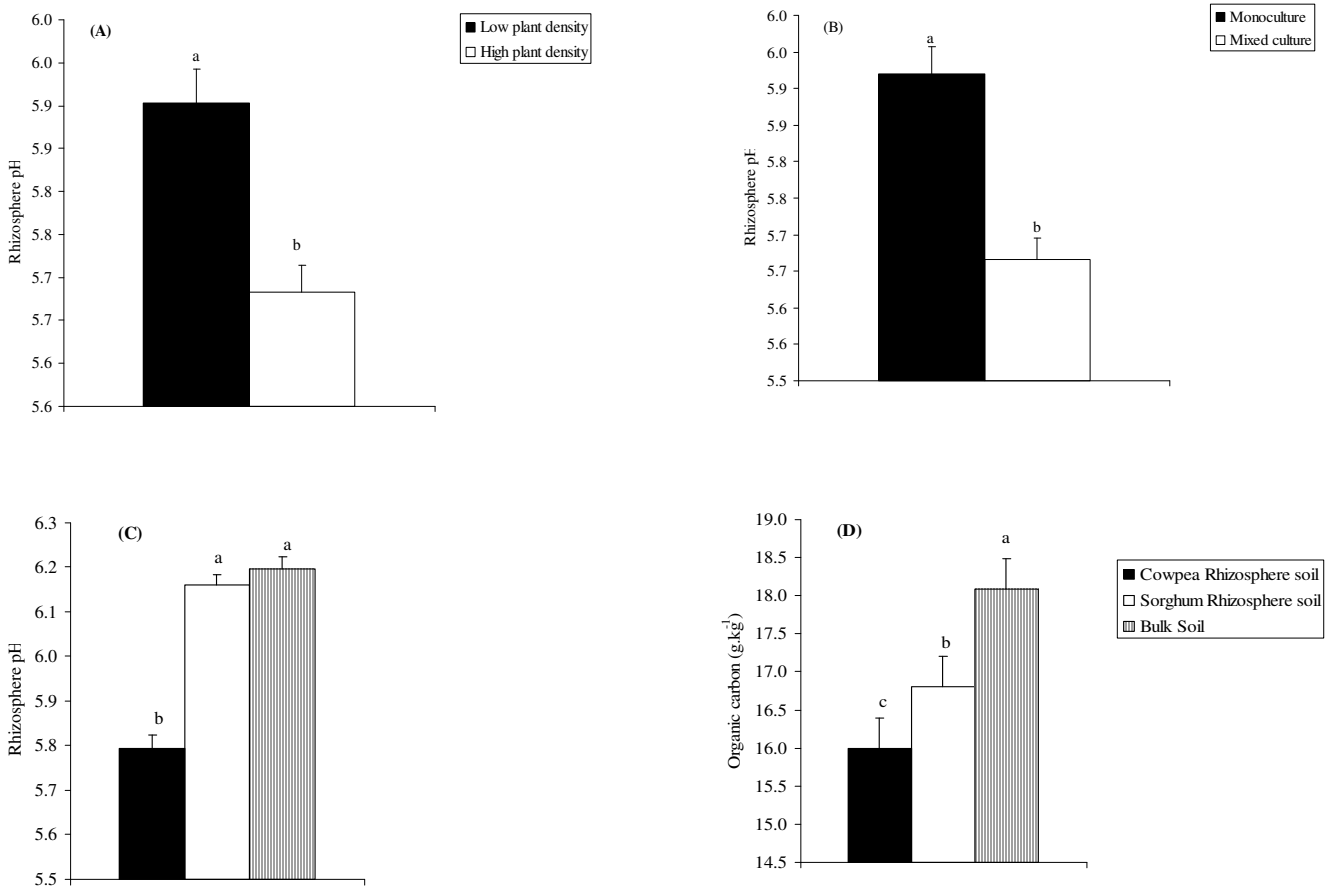


Fig. 3.1: Effect of: (A) plant density on rhizosphere pH of cowpea; (B) cropping systems on rhizosphere pH of cowpea and comparison of: (C) pH and (D) Organic carbon in the rhizosphere soils of cowpea and sorghum with bulk soil.

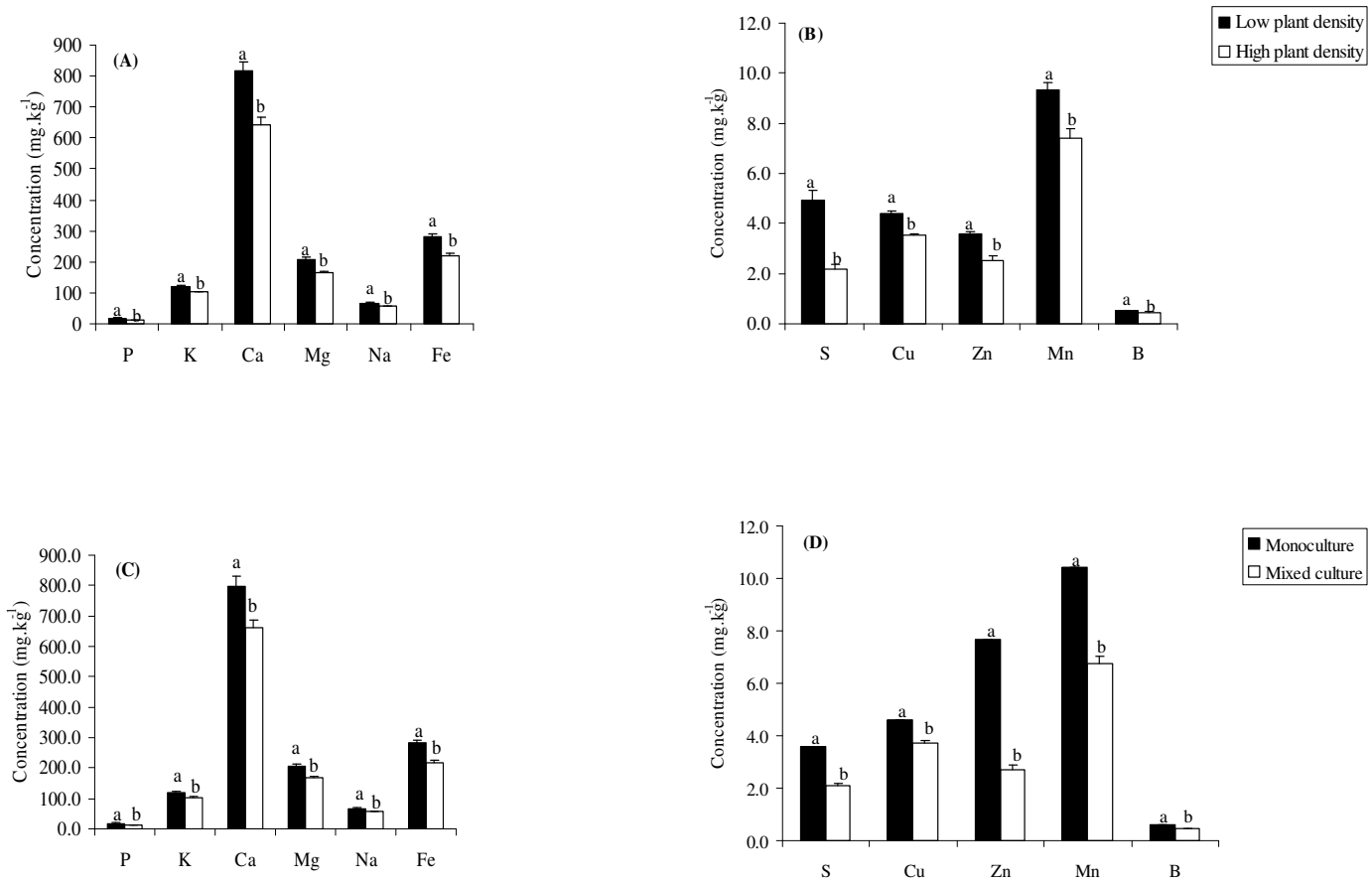


Fig. 3.2: Effect of plant density on cowpea rhizosphere concentration of mineral elements: (A) P, K, Ca, Mg, Na and Fe; (B) S, Cu, Zn, Mn and B; and cropping systems: (C) P, K, Ca, Mg, Na and Fe; (D) S, Cu, Zn, Mn and B.

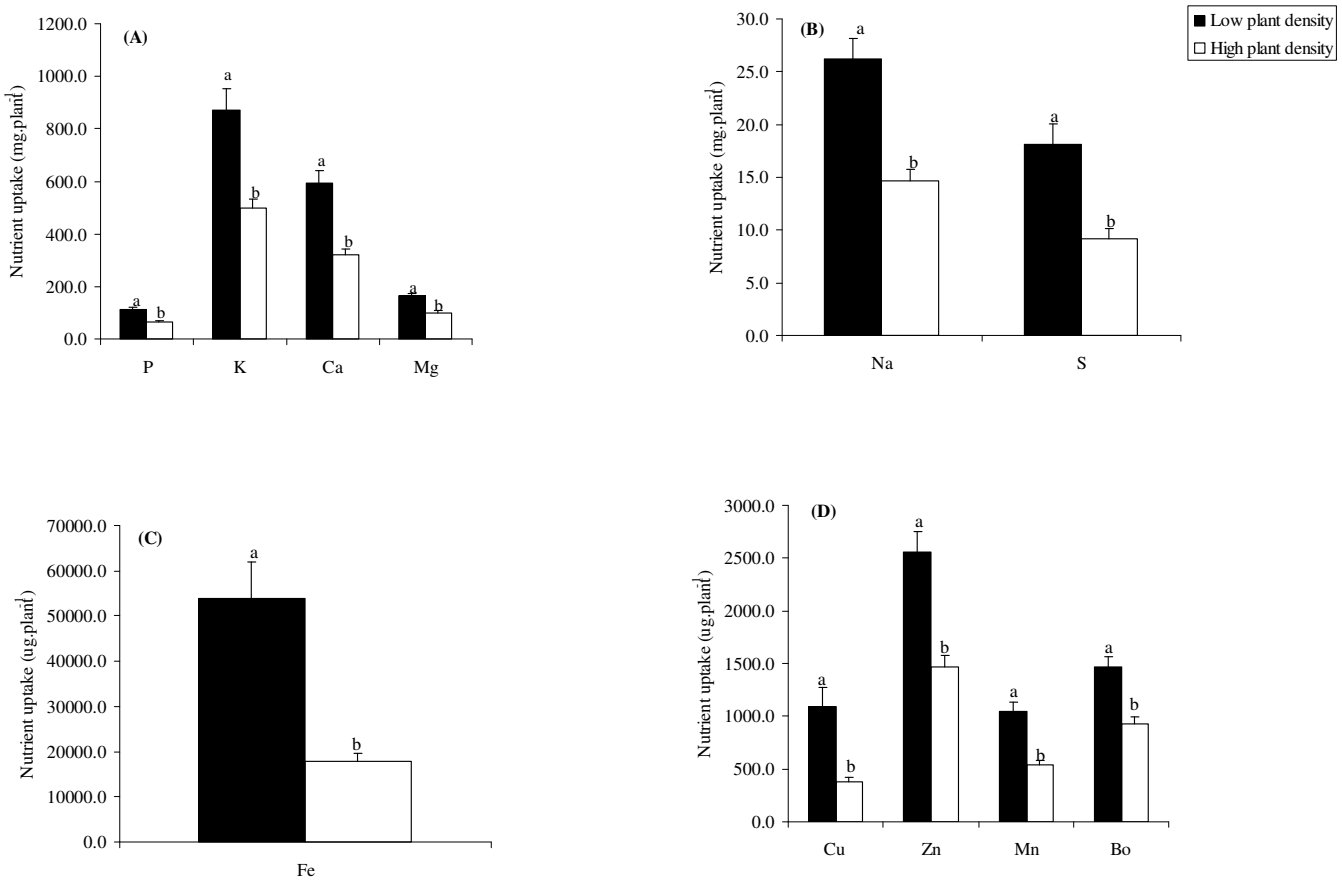


Fig. 3.3: Effect of plant density on cowpea mineral content: (A) P, K, Ca and Mg; (B) Na and S; (C) Fe; (D) Cu, Zn, Mn and B

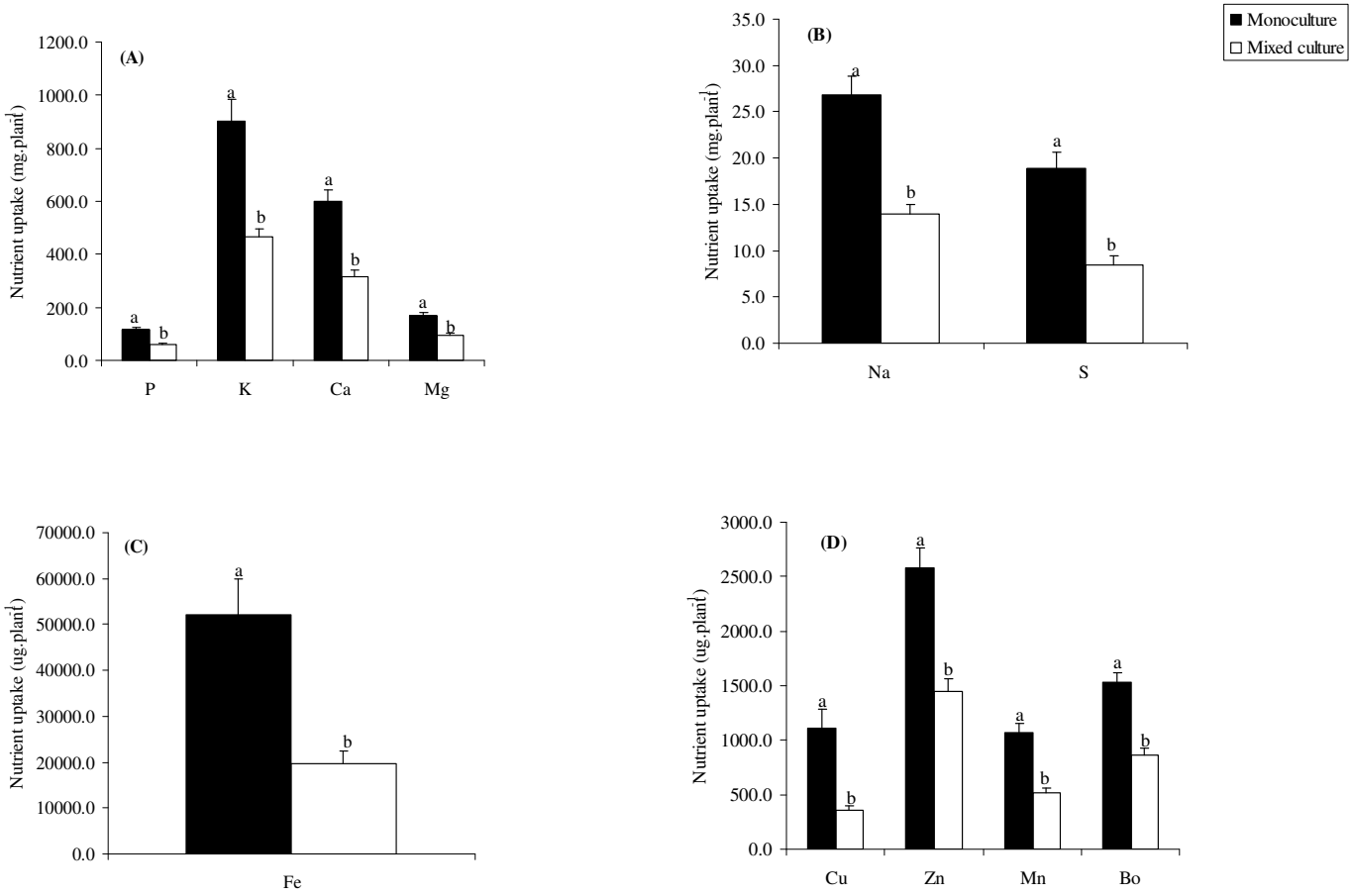


Fig. 3.4: Effect of cropping systems on cowpea mineral content: (A) P, K, Ca and Mg; (B) Na and S; (C) Fe; (D) Cu, Zn, Mn and B

CHAPTER 4

ELEVATED LEVELS OF ACID AND ALKALINE PHOSPHATASE ACTIVITY IN ROOTS AND RHIZOSPHERE SOIL OF COWPEA (*VIGNA UNGUICULATA* L. WALP.) REVEAL ENHANCED P NUTRITION, IMPROVED PLANT GROWTH AND INCREASED GRAIN YIELD IN GENOTYPES GROWN IN MIXED CULTURE AND AT DIFFERENT DENSITIES WITH SORGHUM (*SORGHUM BICOLOR* L. MOENCH)

4.1 Introduction

Plants roots secrete a variety of organic compounds, enzymes, and root border cells into the rhizosphere (Dakora and Phillips, 2002). Acid and alkaline phosphatases are some of the enzymes commonly encountered in the rhizosphere of plants (Vincent *et al*., 1992). The acid phosphatases are found in root exudates and plant organs, as well as in the rhizosphere soil of plants (Duff *et al*., 1994), while alkaline phosphatases are produced mainly by soil microbes (Tabatabai, 1994). Both acid and alkaline phosphatases are important in P availability in soils and its regulation in plant organs (Bieleski, 1973; Duff *et al*., 1994). Acid and alkaline phosphatases are therefore closely associated with organic phosphate mineralisation, liberation of inorganic P by dephosphorylation of organic P in soils, and internal utilisation of P_i reserves and other P_i-containing compounds in plant vacuoles (Dick *et al*., 2000; Richardson *et al*., 2005). The availability and uptake of inorganic P is essential for plant growth and development (Taylor *et al*., 1989), and P supply by acid and alkaline phosphatase activity depends on cultural practices as well as the plant species or genotype. Agronomic practices such as plant density and cropping system are therefore reported to affect soil phosphatase activity (Gregorich *et al*., 1997; Eivazi *et al*., 2003) leading to a correlation of enzyme activity and P stress imposed by such practices (Li *et al*., 1997; Hayes *et al*., 1999). Enhanced organic-P utilization by cereals is apparently a major factor behind increased cereal yields in mixed legume/cereal cultures (El Dessougi *et al*., 2003; Liu *et al*., 2004).

The level of secretion of acid phosphatases can vary with crop type and species (Yan *et al*., 2001; Nuruzzaman *et al*., 2006) as some genotypes possess a remarkable ability to mobilize sparingly soluble soil P (Braum and Helmke, 1995) in excess of that needed to meet metabolic requirements (Gahoonia *et al*., 1997; Neumann *et al*., 1999). There are thus differential inter-specific genetic variations in root secretion of enzymes and acid phosphatase activity (Tadano *et al*., 1993), which increases with P deficiency, and decrease with exogenous P supply (Gunes and Inal, 2008). Differences in acid phosphatase activity have therefore been found in sorghum, common bean (*Phaseolus vulgaris* L.), pigeon pea (*Cajanus cajan* L.), white lupin (*Lupinus albus* L.) and Arabidopsis (*Arabidopsis thaliana* L.) (Tadano *et al*., 1993; Subbarao *et al*., 1997; Wasaki *et al*., 1999; Haran *et al*., 2000; Nuruzzaman *et al*., 2006, Wasaki *et al*., 2008).

In Africa, cowpea is the major indigenous food legume cultivated by resource-poor farmers, usually in soils that are very poor in plant-available P. Screening cowpea genotypes for their ability to naturally promote increased P availability and uptake via the activity of acid and alkaline phosphatases in root exudates would be one way to increase cowpea yields in farmers' fields. Unfortunately, no such data currently exist for cowpea in Africa. This study assesses five cowpea genotypes grown in mixed culture

and at different densities with sorghum for their acid and alkaline phosphatase activities both in the rhizosphere and in fresh roots in order to identify cowpea genotypes with high P uptake efficiency.

4.2 Materials and methods

4.2.1 Site location, description and experimental design

Site description, location and experimental design are as reported in section 2.2.1 and 2.2.2 respectively

4.2.2 Collection and preparation of rhizosphere soil

Collection and preparation of rhizosphere soil is as described in section 3.2.2

4.2.3 Bioassay of acid and alkaline phosphatase activity in rhizosphere soil

The activity of acid and alkaline phosphatases in the rhizosphere were assayed following the method of Eivazi and Tabatabai (1977), modified by Hedley *et al*. (1982). The *p*-nitrophenyl phosphate tetrahydrate was used in the colorimetric assay of acid and alkaline phosphatases. One mL *p*-nitrophenyl phosphate tetrahydrate was dissolved in acetate buffer previously adjusted to pH 6.5 with 0.1 M HCl and to pH 11.0 with 0.1 M NaOH for acid and alkaline phosphatase, respectively. For each enzyme activity, 1.0 g of fresh rhizosphere soil in duplicates was transferred to a 50 mL Erlenmeyer flask and each treated separately with 0.2 mL of toluene and 4 mL of modified universal buffer (MUB) at pH 6.5 or 11 for acid or alkaline phosphatases respectively. For each soil sample, controls were included where *p*-nitrophenyl phosphate tetrahydrate was added after halting the reaction by adding 1 mL of 0.5 M NaOH and 4 mL of 0.5 M $CaCl_2$ immediately before filtration. Samples were mixed thoroughly and incubated at 37ºC for 1h. Following incubation, enzyme activity was halted by addition of 1 mL of 0.5 M NaOH and 4 mL of 0.5 M $CaCl_2$. The contents were mixed and filtered through Whatman No. 2 filter paper. The supernatant was transferred to vials and the absorbance of the supernatant read at 420 nm using a spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E). In order to account for non-enzymatic substrate hydrolysis, values for controls were subtracted from sample replicates. After correction for soil moisture content, the enzyme activity was expressed on soil dry wt basis as µg *p*-nitrophenol.g^{-1} soil dry weight.h^{-1}. One unit of acid phosphatases activity was defined as the activity per gram soil which produced 1 µmol *p*-nitrophenol per hour

4.2.4 Bioassay of acid phosphatase activity in root tissue

Acid phosphatase activity of fresh root tissues from cowpea and sorghum plants was determined as described by Liu *et al*. (2004). In this method 0.5 g of fresh root tissue was taken, frozen immediately in liquid nitrogen, ground in a cold mortar with acetate buffer (0.2 mM, pH 5.2) and a little quartz sand. The total volume of acetate buffer used to grind and transfer into the Eppendorf tube was 8 mL. The root extract was centrifuged at 10,000 x g for 20 min at 4°C. 0.2 mL supernatant was added to 8.8 mL acetate buffer (0.2 mM, pH 5.2) and 1 mL *p*-nitrophenyl phosphate tetrahydrate (150 mM). The reaction mix was incubated at 30°C for 30 min. After incubation, 5 mL 0.5 M NaOH was immediately added to terminate the reaction and to develop colour. The supernatant was transferred into vials and the absorbance of the supernatant read at 405 nm using a spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E). The enzyme activity in root tissue was expressed on a fresh weight (FW) basis as µg *p*-nitrophenol g^{-1} FW.h^{-1}.

4.2.5 Determination of plant-available minerals in bulk and rhizosphere soils

Determination of plant-available minerals in bulk and rhizosphere soils is as described in section 3.2.5

4.2.6 Plant harvest and sample preparation

At sixty seven days after planting, during early pod development, sixteen and eight plants of cowpea and sorghum were respectively harvested from the middle rows of each plot. The plants were carefully dug out with intact root system, washed, and separated into nodules, roots, shoots and pods in the case of cowpea, while sorghum plants were separated into only roots and shoots. The plant organs were oven-dried at 60°C for 48 h and ground into fine powder (0.85 mm sieve) and stored, prior to analysis for tissue P concentrations.

4.2.7 Measurement of P concentration in plant tissue

Measurement of P concentration in plant organs is as explained in section 3.2.6

4.2.8 Cowpea and sorghum grain yield

At physiological maturity, cowpea pods and sorghum heads were harvested from the remaining inner rows of each plot, shelled, and grain yield assessed.

4.2.9 Statistical analysis

A 3-factorial design (3-Way ANOVA) involving density, cropping systems and cowpea genotypes was used to analyse rhizosphere acid and alkaline phosphatase activities, as well as acid phosphatase activities in roots of cowpea and sorghum plants, plant growth and grain yield. Analysis of data was performed using STATISTICA program 2007 (StatSoft Inc., Tulsa, OK, USA). Where the f-value was found to be significant, Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$ (Steel and Torrie, 1980).

4.3 Results

4.3.1 Effect of cowpea genotypes on acid and alkaline phosphatase activity in roots and rhizosphere soils

Cowpea genotypes differed significantly ($P \leq 0.05$) in the acid phosphatase activity of their rhizosphere soils. As shown in Table 4.1, the rhizosphere soils of cowpea cultivars Sanzie, Bensogla and Omondaw showed significantly higher acid phosphatase activity relative to cv. ITH98-46 (which recorded the least enzyme activity). The alkaline phosphatase activity of rhizosphere soil was however similar for all the five cowpea genotypes (Tables 4.1 and 4.2). Enzyme assays using fresh roots of the five cowpea genotypes revealed significant differences in acid phosphatase activity, with cultivar Sanzie showing the highest activity when compared with the other four cowpea genotypes (Table 4.1).

4.3.2 Effect of plant density and cropping system on acid and alkaline phosphatase activity in roots and rhizosphere soils of cowpea and sorghum plants

There were significant differences in acid and alkaline phosphatase activity associated with cowpea and sorghum plants with changes in cropping system and planting density (Fig. 4.1). For example, increasing cowpea plant population from 83,000 to 167,000 plants.ha^{-1} significantly ($P \leq 0.05$) raised both the acid and alkaline phosphatase activity in the rhizosphere soil of this legume (Fig. 4.1A), just as mixed culture (or intercropping) also increased the acid and alkaline phosphatase activity of cowpea rhizosphere soil (Fig. 4.1B).

Similar results were obtained when fresh root tissues were assayed for acid and alkaline phosphatase enzyme activity. High plant density increased the acid phosphatase activity in fresh cowpea roots, just as mixed culture (or intercropping) also increased acid phosphatase activity (Figs. 4.1A and 4.1B).

As with cowpea, the acid and alkaline phosphatase activities of sorghum rhizosphere soil were significantly greater with increasing plant density (Fig. 4.1C). Mixed plant culture (or intercropping) also caused significantly increased levels of acid and alkaline phosphatase activity in the rhizosphere soil of sorghum plants (Fig. 4.1D). The acid phosphatase activity of fresh sorghum roots was increased at high plant density relative to low density (Fig. 4.1C) just as mixed culture (or intercropping) increased the acid phosphatase activity in fresh sorghum roots (Fig. 4.1D).

4.3.3 Effect of plant species on acid and alkaline phosphatase activity of fresh roots and rhizosphere soil

Acid and alkaline phosphatase activities of rhizosphere soils associated with cowpea plants were significantly higher than those of sorghum (Table 4.2). The acid phosphatase activity in fresh root tissues of cowpea was also significantly higher compared with that in sorghum roots (Table 4.2).

4.3.4 Measurement of P concentrations in rhizosphere soils and different organs of cowpea plants

The P level was lowest (and therefore highly depleted) in the rhizosphere soil of the cowpea cv. Sanzie relative to Omondaw and Bensogla, and highest in cv. ITH98-46 (Table 4.1). The tissue concentrations of P were similar in roots, but differed significantly in shoots, with cvs. Sanzie, Omondaw and Bensogla, which showed the highest P depletion in the rhizosphere, exhibiting the highest P accumulation in shoots. Total plant P concentrations were similar in pattern to shoots, as Sanzie, Omondaw and Bensogla again showed higher whole-plant P compared with the other two cowpea genotypes (Table 4.1).

4.3.5 Plant growth and grain yield as affected by cowpea genotype, plant density and cropping system

There were marked differences in plant growth, with cowpea cv. Sanzie producing significantly more dry matter than cv. ITH98-46 and TVu1509 (Table 4.1). As a result, Sanzie also produced significantly more grain yield than cv. ITH98-46 (Table 4.1). Cowpea plants showed better growth at low planting density and increased growth in monoculture than in mixed culture (Table 4.1).

4.3.6 Correlation analysis of acid and alkaline phosphatase activities with rhizosphere pH, rhizosphere soil P and organ P in cowpea plants

When acid phosphatase and alkaline phosphatase activities from the field experiments were correlated with rhizosphere pH, rhizosphere soil P, and the levels of P in nodules, roots, shoots and pods of cowpea plants, the data showed highly significant ($P \leq 0.05$) relatedness (Table 4.3). For example, acid phosphatase activity was significantly correlated with rhizosphere pH ($r = 0.76$), rhizosphere soil P ($r = 0.73$), nodule P ($r = 0.39$), root P ($r = 0.38$), shoot P ($r = 0.30$) and pod P ($r = 0.31$). Alkaline phosphatase activity, on the other hand, was only significantly correlated with rhizosphere pH ($r = 0.61$), rhizosphere soil P ($r = 0.51$), root P ($r = 0.21$) and pod P ($r = 0.19$), but not to nodule P or shoot P (Table 4.4).

4.3.7 Discussion

In Africa, low N and P supply together with inadequate water constitute the major constraints to increased crop yields. Although application of chemical fertilizers could be an option for meeting crop nutrient requirements for increased yields, the high cost and inaccessibility of these chemical inputs make them unavailable to resource-poor farmers in Africa. The inclusion of N_2-fixing legumes in cropping systems is one way to enhance N nutrition in farmers' fields (Dakora and Keya, 1997; Dakora and Phillips, 2002, Naab *et al*., 2009). Thus, selecting symbiotic legumes with dual capacity for high N_2 fixation and efficient P acquisition represents a novel approach for promoting N and P nutrition in crop plants for improved growth and increased yields. In this study, five cowpea genotypes were evaluated for high N_2 fixation and enhanced P nutrition. We report here the results on improved P nutrition as the data on N_2 fixation are detailed in chapter 6 of this thesis. Screening five cowpea genotypes (two inbred cultivars and three farmer-selected varieties) for improved P nutrition under two contrasting plant densities and cropping systems over a two-year period revealed significant differences in the activity of enzymes associated with P supply. The farmer-selected local varieties (led by Sanzie) secreted more acid phosphatase than the inbred cultivars as the former consistently exhibited much higher acid phosphatase activity in rhizosphere

soils during the two years of field experimentation (Table 4.1). These greater enzyme activities in the rhizosphere of Sanzie, suggest greater release of P from organic sources for plant uptake. Assaying cowpea roots for acid phosphatase activity again identified Sanzie as the cultivar with the highest enzyme activity in root tissues (Table 4.1).

When one combines the significantly high acid phosphatase activity in roots of cowpea cv. Sanzie with its high level of enzyme activity in the rhizosphere, a much greater P supply and significantly enhanced P nutrition would be expected in this genotype. Measuring P concentration in the rhizosphere indeed revealed a depletion of P by Sanzie roots (Table 4.1), an observation which was confirmed by tissue analysis that showed high P accumulation in shoots and whole plants of the cultivar Sanzie (Table 4.1). The effect of this improved P nutrition of the cultivar Sanzie (and also of Omondaw and Bensogla) was a greater production of plant biomass and greater grain yield by these three farmer-selected varieties (Table 4.1). Furthermore, possibly because of the enhanced P nutrition from greater P supply via higher acid phosphatase activities in roots and the rhizosphere, Sanzie also fixed significantly more N_2 in 2005 and 2006 (75.3 ± 4.2 kg $N.ha^{-1}$ and 86.5 ± 5.6 kg $N.ha^{-1}$, respectively; see chapter 6 relative to the improved cultivars (ITH98-46 and TVu1509), which had much lower acid phosphatase activity in roots and rhizosphere, and therefore accumulated less P in shoots and whole plants (Table 4.1), resulting in relatively lower amounts of N-fixed (46.3 ± 5.0 kg $N.ha^{-1}$ and 50.4 ± 5.6 kg $N.ha^{-1}$ in 2005 vs. 51.4 ± 5.0 kg $N.ha^{-1}$ and 57.1 ± 7.0 kg $N.ha^{-1}$ in 2006 for ITH98-46 and TVu1509, respectively; see chapter 6.

In addition to the secretion of acid phosphatases by plant roots (as shown in this study) and also by specialized proteoid roots (Miller *et al*., 2001), plants can additionally use other mechanisms to enhance P acquisition in low-P soils. These include the production of organic acids (Dakora and Phillips, 2002) such as piscidic acid for solubilising Fe-P (Ae *et al*., 1990), the formation of specialized root cell walls (most likely "root border cells", Hawes *et al*. (1998) for improved P nutrition (Ae and Shen, 2002), and the development of high-affinity phosphate transporters in roots (Mitsukawa *et al*., 1997; Smith, 2002) for enhancing P uptake in P-deficient soils. Of these, however, assaying for acid phosphatases in plant roots and rhizosphere soils (as done in this study) seems to be a simpler, quicker and easier technique for screening many crop genotypes for P uptake efficiency and P use efficiency. Although these acid phosphatases are also produced by other organs such as leaves, the leaf acid phosphatases probably promote internal P re-mobilization as they are reported to play no role in soil P supply to plants (Yan *et al*., 2001).

It was interesting to note the significant species differences in plant secretion of acid phosphatases, with legume roots and legume rhizosphere soil showing greater enzyme activity relative to those of the cereal (Table 4.2). This finding is consistent with the observation that legume secretion of acid phosphatases can

be 72 % higher than that of cereals (Yadav and Tarafdar, 2001). Species variation in the secretion of acid phosphatases was similarly observed by Nuruzzaman *et al*. (2006). In this study, mixed culture induced greater acid phosphatase activity in cowpea than monoculture. This implies that, in a cowpea/sorghum intercropping system, the legume is likely to facilitate improved P acquisition by the cereal through former's higher acid phosphatase activity in the rhizosphere. Li *et al*. (2004) has in fact showed that chickpea improved the P nutrition of its intercropped maize partner via higher acid phosphatase activity in the roots. In this study, higher plant density did not only stimulate greater root secretion of acid phosphatases, but also promoted microbial secretion of alkaline phosphatases (Fig. 4.1), with the acid phosphatase activity being generally higher than that of the alkaline phosphatases. Various studies (Ozawa *et al*., 1995; Miller *et al*., 2001; Nuruzzaman *et al*., 2006; Inal *et al*., 2007) have suggested that marked increases in acid phosphatase activity of plant roots is an adaptive mechanism to low P stress, and that competition for reduced P under high plant density (or mixed culture) is a major factor inducing acid phosphatase activity in the rhizosphere.

To assess whether there is a relationship between root or rhizosphere phosphatase activity and P supply in the rhizosphere, correlation analyses were done between acid/alkaline phosphatase activities and the level of P in tissues and the rhizosphere, as well as pH (Table 4.3). The data clearly showed many significant correlations between acid/alkaline phosphatase activity and P in soil as well as P in plant organs. Different studies (Sharpley, 1985; Tarafdar and Jungk, 1987; Speir and Cowling, 1991) also found similar significant correlations between acid/alkaline phosphatase activity and P levels in plant organs and in the rhizosphere. Tarafdar and Jungk (1987) found increased acid phosphatase activity in the rhizosphere of white clover (*Trifolium repens* L.) and wheat (*Triticum aestivum* L.) roots, which significantly correlated (r = 0.97 and r = 0.99) with organic P depletion around the roots. More recently, significant correlations were reported between root acid phosphatase activity, rhizosphere acid phosphatase activity and rhizosphere P concentration in a peanut (*Arachis hypogaea* L.)/maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.)/barley (*Hordeum vulgare* L.) mixed culture (Inal and Gunes, 2007). Clearly, these data all suggest a direct relationship between acid/alkaline phosphatase activity and P supply in P-deficient soils. In conclusion, the results from this study indicate that the assay of acid phosphatase activity in roots and rhizosphere of cowpea can be used as a tool to select cowpea genotypes (and other legumes) for P uptake efficiency and P use efficiency for increased crop yield in Africa.

Table 4.1: Acid and alkaline phosphatase activity in the rhizosphere, roots, rhizosphere P concentration, P concentration in organs, plant growth and grain yield of five cowpea genotypes planted during 2005 and 2006.

Cowpea genotypes	Rhizosphere phosphatase activity		Root acid phosphatase	Rhizosphere P concentration	P concentration			Plant dry matter	Grain yield
	Acid	Alkaline			Roots	Shoots	Whole plant		
	µg *p*-nitrophenol.g^{-1} soil dry wt.h^{-1}		(µg *p*-nitrophenol.g^{-1} FW.h^{-1}	mg.kg^{-1} soil	mg.g^{-1} DM			g.plant^{-1}	kg.ha^{-1}
2005									
Bensogla	0.137±0.004a	0.05±0.002a	0.244±0.004b	13.38±1.74ab	1.99±0.09a	2.86±0.12a	3.43±0.15a	25.6±2.3ab	2063.4±225.7ab
ITH98-46	0.129±0.004b	0.05±0.002a	0.242±0.004b	19.56±3.19a	1.92±0.11a	2.60±0.11b	3.14±0.16b	20.8±2.0b	1596.2±146.0b
Sanzie	0.138±0.004a	0.05±0.003a	0.259±0.005a	11.63±1.05b	2.09±0.11a	2.95±0.13a	3.41±0.16a	28.2±2.6a	2415.0±133.2a
TVu1509	0.133±0.004b	0.05±0.002a	0.241±0.003b	14.19±1.24ab	1.87±0.09a	2.76±0.12ab	3.17±0.13b	22.1±2.3b	2060.9±184.2ab
Omondaw	0.140±0.005a	0.05±0.003a	0.247±0.005b	13.25±1.61b	1.95±0.05a	2.82±0.08a	3.43±0.13a	26.4±2.2ab	2180.7±135.6a
One-way ANOVA (F-Statistic)									
	3.0*	0.6	4.3**	3.8**	2.2	3.3*	3.7*	4.3**	5.2**

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$. The data for 2005 and 2006 have been pooled together for they were similar.

Table 4.2: Comparisons of acid and alkaline phosphatase activity in the rhizosphere (µg *p*-nitrophenol.g^{-1} soil dry wt.h^{-1}) and roots (µg *p*-nitrophenol.g^{-1} FW.h^{-1}) of cowpea and sorghum species.

Treatment	Rhizosphere soil		Root tissue
	Acid phosphatase	Alkaline phosphatase	Acid phosphatase
Cowpea	0.14±0.002a	0.05±0.001a	0.25±0.002a
Sorghum	0.12±0.002b	0.04±0.001b	0.22±0.001b
One-way ANOVA (F-Statistic)			
	26.68***	112.06**	113.32**

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at **: $P \leq 0.01$; ***:$P \leq 0.001$. The data for 2005 and 2006 have been pooled together for they were similar

Table 4.3: Correlation of acid and alkaline phosphatase activity with rhizosphere soil pH, rhizosphere P and organ P in cowpea genotypes grown under different plant densities and cropping systems.

Treatments	pH		Rhizosphere P		Nodule P		Root P		Shoot P		Pod P	
	r	*t*-statistic	*r*	*t*-statistic	*r*	*t*-statistic	*r*	*t*-statistic	*r*	*t*-statistic	*r*	*t*-statistic
APA	0.76	$t_{1,78} = 49.21$***	0.73	$t_{1,78} = 12.41$***	0.40	$t_{1,78} = 7.52$***	0.38	$t_{1,78} = 9.97$***	0.30	$t_{1,78} = 9.77$**	0.31	$t_{1,78} = 8.17$**
AlkPA	0.61	$t_{1,78} = 57.53$***	0.51	$t_{1,78} = 8.68$***	0.15	$t_{1,78} = 6.94$	0.21	$t_{1,78} = 11.42$*	0.17	$t_{1,78} = 12.18$	0.19	$t_{1,78} = 9.74$*

Marked differences in bold are significantly correlated at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. APA = Acid phosphatase activity, AlkPA = Alkaline phosphatase activity.

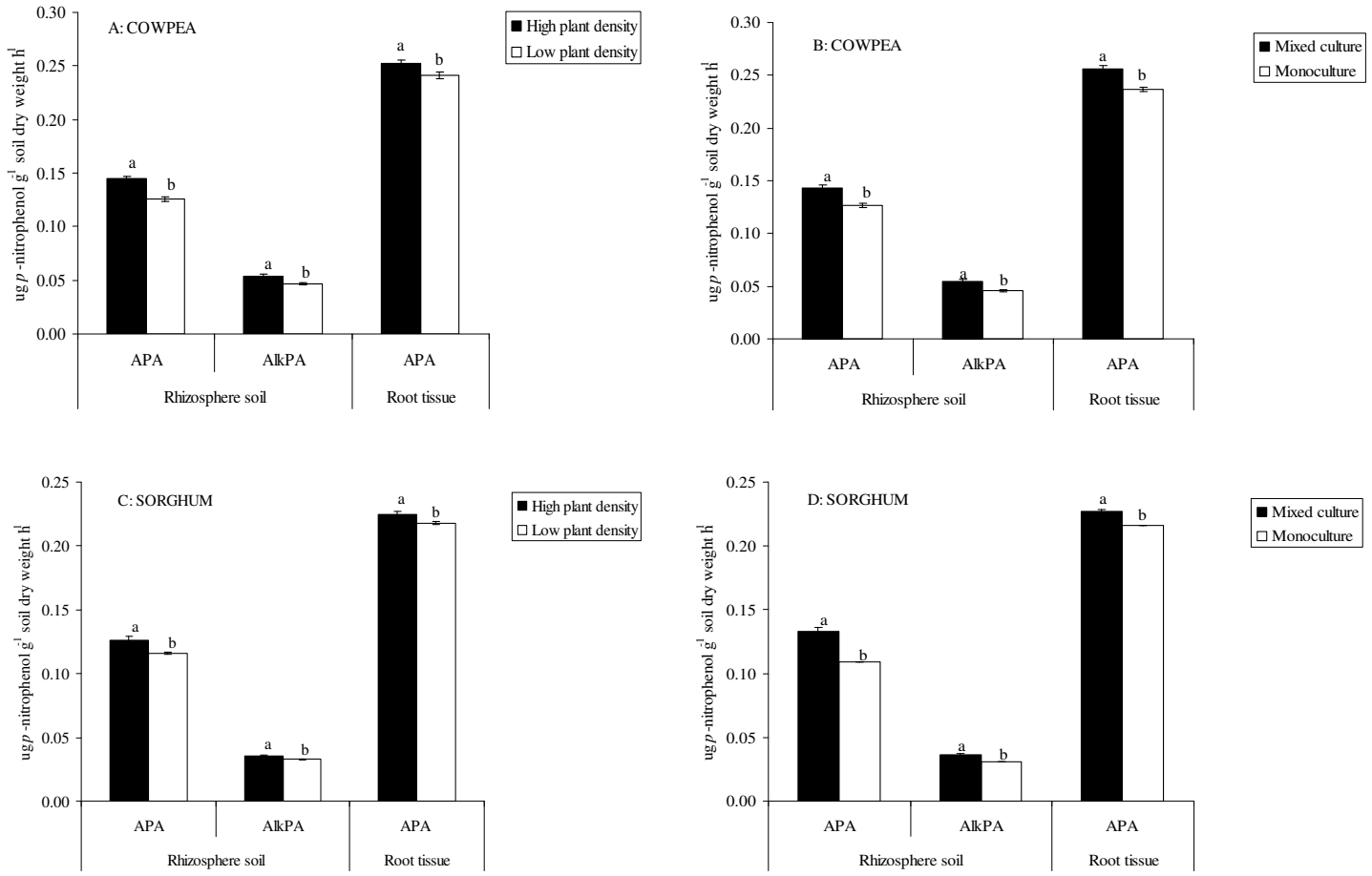


Fig. 4.1: Effect of A) plant density and B) cropping system on acid and alkaline phosphatase in rhizosphere and root tissues of cowpea; and C) plant density and D) cropping systems on acid and alkaline phosphatase in rhizosphere and root tissues of sorghum. (APA: Acid phosphatases activity; AlkPA: Alkaline phosphatases activity)

CHAPTER 5

YIELD COMPONENTS OF NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP) GENOTYPES AND SORGHUM (*SORGHUM BICOLOR* L. MOENCH) GROWN UNDER DIFFERENT PLANT DENSITIES AND CROPPING SYSTEMS.

5.1 Introduction

Cowpea is a valuable crop used by low input and small scale farmers in their traditional cropping systems in Africa (van Ek *et al*., 1997; Ayisi *et al*., 2000). Its tolerance to shading along with numerous other advantages ranging from food nutrition, soil fertility and conservation to weed control (Ayisi *et al*., 2000; Aliyu and Emechebe, 2006), makes it compatible as partner with a number of cereals (Blade et al., 1992) especially when there is no high competition for growth factors. Grain yields from cowpea and sorghum in the region have been reported to be dependent on plant stand, cropping systems and cultivars involved in such cropping systems (Haizel, 1972; Summerfield *et. al*., 1976; Chang and Shibles, 1985; Ofori and Stern, 1987; Hegstad *et al*., 1999). Due to these factors for example, cowpea seed yields have remained low, typically ranging between 100 - 400 $kg.ha^{-1}$ in traditional systems relative to 3, 000 $kg.ha^{-1}$ obtained from experimental stations (Ntare *et al*., 1993; Sivakumar *et al*., 1996). Poor planting pattern as practised in most farmers fields in Africa for instance, can lead to low plant growth due to reduced light, mineral elements, as well as other growth factors either as a result of insufficient plants or too many plants per unit area leading to plant-to-plant or species-to-species competition, thus, low seed yield (van Erk *et al*., 1997; Hauggaard *et al*., 2006). However, genotypes grown in mixture with cereals arranged in such a way that there is minimum competition and maximum complementarity between intercrops have been shown to increase grain yields over monoculture through greater land use efficiency (Agboola and Fayemi, 1972; Iragavarapu and Randall 1996; Hauggaard-Nielsen *et al*., 2001) indicating mixed culture advantage through the concept of land equivalent ratio (Willey, 1979; Clark and Myers, 1994; Silwana and Lucas, 2002; Jahansooz *et al*., 2007). Comparing grain yields between rows in a cowpea - maize (*Zea mays* L.) mixed culture system for example, Asafu-Agyei *et al*. (1997) showed that in-field arrangement such that there is two rows of cowpea after two rows of maize gave greater grain yield of both crop components and greater land equivalent ratios than a one-to-one row. Although considerable knowledge has been accumulated on mixed culture system using different cereal partners (Connolly *et al*., 1990; Reddy *et al*., 1992), data on the effect of plant density and cropping systems on yield components of different cowpea genotypes grown with or without sorghum as partner are inadequate, and thus, still needed in Africa. This study was, therefore, designed to assess the effect of plant density and cropping systems on yield components of five nodulated cowpea genotypes grown as mono crop or in mixture with sorghum.

5.2 Materials and methods

5.2.1 Site location, description and experimental design

The site location, description and experimental design are as described in chapter 2 sections 2.2.1 and 2.2.2 respectively.

5.2.2 Plant harvesting and analysis

At physiological maturity, plants were counted and harvested for yield assessment. Yield assessment was carried from the middle rows of each plot excluding the border rows. From each plot, sixteen plants of cowpea were sampled from each plot to determine number of pods per plant, number pod bearing peduncles and number of seeds per pod. From each plot of sorghum, eight plants were sampled for determination of weight of head per plant and weight of seed per plant. Both cowpea pods and sorghum heads were manually threshed and allowed to air dry up to moisture content of 13%. Grain yield of cowpea and sorghum plants was determined from each plot and 100-seed weight recorded. In order to get yield in tons per hectare, the seed weight per plant (expressed in tons) was multiplied by the number of plants per hectare.

5.2.3 Estimation of biological efficiency and productivity of cowpea-sorghum mixed culture

The biological efficiency and productivity of cowpea-sorghum mixed culture was evaluated by the land equivalent ratio (LER), defined as the total land area required under monoculture to produce the equivalent yields obtained under mixed culture as (Ofori and Stern, 1987):

$$LER_c = \frac{Y_{ICc}}{Y_{MCc}} ; LER_s = \frac{Y_{ICs}}{Y_{MCs}} ; LER_T = LER_c + LER_s$$

Where: LER_C and LER_S = Partial land equivalent ratio for cowpea and sorghum respectively; Y_{ICc} and Y_{ICs} = Mass yields per unit area of cowpea and sorghum in mixed culture respectively; Y_{MCc} and Y_{MCs} = Mass yields per unit area of cowpea and sorghum in monoculture respectively; LER_T = Total land equivalent ratio.

If LER_T is greater than 1 ($LER_T > 1$), mixed culture system has a yield advantage over monoculture. Similarly, if LER_T is greater than 1 provides evidence of complementary resource use between crop components in mixed culture. However, when LER_T is less than 1 ($LER_T < 1$), then, there is a yield disadvantage from crops under mixed culture (Beets, 1982; Willey, 1985).

5.2.4 Statistical Analysis

Mean replicate values of yield components data collected were analysed statistically using a 3-factorial analysis of variance (ANOVA). The analysis was performed using the software of STATISTICA program 2007 (StatSoft Inc., Tulsa, OK, USA). Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$ (Steel and Torrie, 1980).

5.3 Results

5.3.1 Effect of density on yield components in cowpea and sorghum plants

The number of pod bearing peduncles per plant (PBP), pods per plant, number of seeds per pod, weight of seeds plus pods per plant and 100-seed weight, were significantly ($P \leq 0.05$) reduced at high (167,000 plants.ha^{-1}) relative to low (83,000 plants.ha^{-1}) plant density during 2005 and 2006 (Table 5.1). Consequently, seed yield in cowpea expressed on per plant basis was also significantly ($P \leq 0.05$) reduced. However, when expressed on per hectare basis seed yield in cowpea was increased due to greater number of plants per hectare (Table 5.1). High plant density similarly reduced ($P \leq 0.05$) the weight of heads per plant, 100-seed weight and seed yield per plant of sorghum during 2005 and 2006 seasons (Table 5.2).

5.3.2 Effect of cropping systems on yield components in cowpea and sorghum

During 2005 and 2006 cropping seasons, the number of pod bearing peduncles per plant, pods per plant, number of seeds per pod, weight of seeds plus pods per plant, 100-seed weight and seed yield per plant were all significantly ($P \leq 0.05$) decreased by changing the cropping system from mono to mixed culture (Table 5.1). Similar trend was observed in sorghum whereby weight of heads per plant, 100-seed weight and seed yield per plant were significantly ($P \leq 0.05$) reduced in mixed culture relative to monoculture (Table 5.2).

5.3.3 Effect of genotypes on yield components in cowpea and sorghum

Cowpea genotypes performance varied ($P \leq 0.05$) with parameters. For example in 2005, shelling %, 100-seed weight, weight of seeds plus pods and seed yield per plant was higher in farmer selected cultivars (cvs. Omondaw, Sanzie and/or Bensogla) relative to the improved variety (i.e. cv. ITH98-46, Table 5.1). However, number of pod bearing peduncles per plant and number of pods per plant were significantly higher in the improved varieties (cvs. TVu1509 and ITH98-46) relative to farmer selected cultivars (cvs. Omondaw, Sanzie and Bensogla). In 2006, weight of seeds plus pods, 100-seed weight, seed yield per plant in cv. Sanzie was generally higher followed by cvs. Omondaw and Bensogla compared with cv. ITH98-46 (Table 5.1). Just as observed in 2005 season, number of pod bearing peduncles per plant and number of pods per plant were higher in the improved varieties relative to the farmer selected cultivars (Table 5.1). Sorghum plants in mixture with TVu1509 genotype was significantly higher in weight of heads per plant and seed yield per plant compared with sorghum grown with Sanzie genotype during both 2005 and 2006 cropping seasons (Table 5.2).

5.3.4 Interactions

Interaction of density x cropping system was significant ($P \leq 0.05$) on the number of seeds per pod in both 2005 and 2006 seasons (Figs. 5.1A and B). However, in 2006 season, interaction of density x cropping system was significant only for the 100-seed weight (Fig. 5.1C). These parameters were reduced ($P \leq 0.05$) by greater plant density only under monoculture in contrast to when grown in mixed culture. Cropping system x cowpea genotypes interaction was significant ($P \leq 0.05$) on seed yield only in 2005 season (Fig. 5.2). Whereas seed yield of Sanzie genotype was significantly higher relative to other cultivars under mixed culture, it was greater than only cv. ITH98-46 under monoculture (Fig. 5.2).

5.3.5 Effect of density, cowpea genotypes on land equivalent ratio.

A 2-way analysis of variance (ANOVA) involving density and cowpea genotypes showed that relative to low, high cowpea density reduced total land equivalent ratio (LER_T). In general, total land equivalent ratio values observed in 2005 season were greater than those in the 2006 cropping season (Table 5.3). Whether low or high plant density, the result show that yields of cowpea and sorghum were increased by mixed culture as shown by the LER_T values which were all greater than 1. The data also showed variation in LER_T values in different cowpea genotypes, which were all greater than 1. However, the highest LER_T value was observed in the Omondaw/sorghum mixed culture (Table 5.3).

5.4 Discussion

Traditional cropping patterns, low yield from legume genotypes used as component crops, as well as poor agronomical practises by farmers in Africa, have until recently been the reason for low grain yield. Manipulation of planting densities, cropping systems as well as proper selection of potentially high yield legume genotypes is probably one means by which low input small scale farmers in Africa can realise higher grain yields and greater profit margins. In this study, low cowpea plant density was shown to have significantly increased pod bearing peduncles per plant, pods per plant, seeds per pod, 100-seed weight, weight of seeds plus pods and consequently higher grain yield ($g.plant^{-1}$, Table 5.1). For example, the data showed that at low plant density, grain yield increased by 65.4% and 70.3% respectively in 2005 and 2006. At high cowpea plant density however, grain yield (i.e. in $kg.ha^{-1}$) was greater than at low plant density because of the larger number of plants that has to be multiplied by the grain yield. These data suggest that at low plant density, cowpea plants had a wider space which satisfied their light requirements for yield formation and relatively extensive soil volume to search for additional mineral elements and other resources for growth. This was because unlike high plant stand, in low plant density these resources are not limiting due to less pressure imposed on the growth factors between plant-to-plant and species-to-species. Increased plant population density however, has been reported to decrease 100-seed weight and grain yield in soybean (*Glycine max*. L)/maize based cropping system and attributed to the effect of shade and possibly other inter-specific competitions (Willey, 1979; Yunusa, 1989).

Similar to low cowpea plant density, growing cowpea in monoculture also significantly increased pods per plant, pod bearing peduncles per plant in cowpea relative to mixed culture during both 2005 and 2006 (Table 5.1). As a result of increased cowpea yield components under monoculture, grain yield was also significantly increased in both 2005 and 2006 relative to mixed culture where yield components were reduced and therefore grain yield (Table 5.1). In this study, seed yield was in the average of 2.53 $t.ha^{-1}$ in monoculture and 1.76 $t.ha^{-1}$ in mixed culture (Table 5.1), clearly indicating that mixed culture reduced grain yield in cowpea during 2005 and 2006. The reduced grain yield under mixed culture in this study suggest that apart from species-to-species competition for mineral elements and water for plant growth, sorghum plants which were tall, may have deprived light by imposing shade on cowpea genotypes, thus, limiting light energy reaching cowpea plant leaves necessary for CO_2 fixation and grain yield formation. In their studies involving cowpea-sorghum, black gram-sorghum and beans-maize mixed cultures, Nambier *et al*. (1983); Francis and Stern (1987); Santalla *et al*. (1999) and Gebeyehu *et al*. (2006) similarly reported lower yields in mixed culture relative to monoculture and ascribed them to shading imposed by sorghum or maize plants on the legume components. In other studies (Watiki *et al*., 1993; Dapaah *et al.,* 2003), negative effects from cowpea-maize mixed culture was ascribed to competitive

advantage of maize over cowpea for light during growth and development (Ofori and Stern, 1987; Myaka, 1995).

Weight of head per plant (g), weight of seed per plant (g), 100-seed weight (g) and grain yield (kg.ha^{-1}) of sorghum were significantly decreased at high cowpea plant density and mixed culture relative to low cowpea plant density (Table 5.1). The decreased yield components suggest interspecific competition due to cowpea genotypes and sorghum species exploring the same plant growth factors (Donald, 1958; Donald, 1963). As a result, seed yield in sorghum at high cowpea plant density was reduced by 12% in 2005 and 19% in 2006 whereas in mixed culture the reduction was 28.6% in 2005 and 31.1% in 2006. In their studies Tilman (1988) and Vandermeer (1989) similarly showed that yield components of sorghum were lowered when density of soybean as component crop was increased. Efficiency of production could therefore be improved by minimising interspecific competition while maximising complementarity for growth resources between component crops.

In this study, the cowpea genotypes were potentially different in terms of yield when subjected to altered plant density and cropping system. Of the five cowpea genotypes for example, cv. ITH98-46 was the lowest in grain yield (1.6 t.ha^{-1}) than cvs. Sanzie (2.4 t.ha^{-1}) and Omondaw (2.2 t.ha^{-1}) during 2005, and lower only to cv. Sanzie (2.7 t.ha^{-1}) in 2006 regardless of whether it was measured in g.plant^{-1} or t.ha^{-1} (Table 5.1). These results clearly showed that these genotypes are potentially different from each other and can be adapted in farming practises involving mixed culture and varying plant densities. The differences observed between genotypes grown in mixed culture systems could be attributed to differences in the ability to access and compete efficiently for growth resources. Similar trend has also been reported in mixtures involving cowpea, Andean bean, pigeon pea, maize and wheat (Ae *et al.,* 1990; Zhu *et al.,* 2001; Christiansen and Graham, 2002; Rengel, 2002 and Krasilnikoff *et al.,* 2003; Pypers *et al.,* 2006).

Results from this study have also shown significant interaction of plant density x cropping system. Regardless of the cropping system used, number of seeds per pod (Fig. 5.1A and B) and 100-seed weight (Fig. 5.1C and D) were reduced by greater plant density compared with low plant density in contrast to mixed culture where these parameters were not significantly changed indicating that plant density controls these traits. Data also showed significant interaction of cropping system x genotypes. For example, the seed yield of cv. Sanzie was greater ($P \leq 0.05$) than cvs. Bengsogla, ITH98-46 and TVu1509 under mixed culture, compared with monoculture where it was only higher than ITH98-46 (Fig. 5.2). The result suggests that selection targeting seed yield in cowpea genotypes should also be done in mixed culture rather than only under monoculture as similarly reported by Santalla *et al*. (1994); Tefera and Tana (2002); O'Leary and Smith (2004). Generally, the lowest grain yield in cv. ITH98-46 suggests that this genotype is a low yielding type regardless of cropping system. Additionally, data on the plant organ

mineral elements concentration (Chapter 3) showed that cv. ITH98-46 was the lowest, suggesting lower ability to compete and explore different resource pools for growth and consequently yield formation. This was also reflected in low amount of N-fixed in this genotype (Chapter 6). Yield components of sorghum grown with cvs. Sanzie and Bensogla showed significantly greater decrease in contrast to those grown with cv. TVu1509. The decreased yield components in sorghum plants could probably be associated to the intensified demand of growth factors by Sanzie and Bensogla genotypes which could have affected the growth of sorghum consequently poorly filled grains.

In order to determine the effectiveness of mixed culture relative to growing crops as sole culture, land equivalent ratio (LER) was used as an index to compare the yields from the component crops. Results from this study have shown that LER under mixed culture at low plant density was higher relative to high plant density (Table 5.3). However, whether growing cowpea in mixture with sorghum at high or low plant density, total LER values were always greater than 1 (1.42 - 1.52) suggesting that seed yield produced in mixed culture at low or high plant density would have required 42 - 52% more land if planted as monoculture, indicating that land and growth factors were used more efficiently. The efficient use of resources leading to total LER values greater than 1 ($LER_T > 1$) as reported in this study, are consistent with values reported from intercropping maize/soybean (Beets, 1994); mustard/pea (*Pisum sativum* L.; Waterer, 1994); soybean/wheat (*Triticum aestivum* L.; Li *et al*., 2001), maize/bean (*Phaseolus vulgaris* L.; Oljaca *et al*., 2000; Santalla *et al*., 2001) and maize/cowpea (Ndakidemi and Dakora, 2007) in mixed culture.

Improved biological efficiency and productivity was also revealed in cowpea genotypes in mixed culture as indicated by different values of total land equivalent ratio (LER_T). Total LER from the combinations of cowpea genotypes with sorghum range between 1.30 (Bensogla/sorghum) to 1.61 (Omondaw/sorghum) suggesting an overall total yield advantage from these intercrops in their mixed culture system relative to their counterparts in monoculture system. However, greatest yield advantage (61%) was obtained in Omondaw/sorghum and lowest (30%) in Bensogla/sorghum mixed culture. The greater total LER values observed in Omondaw/sorghum mixed culture could be ascribed to efficient use of both below and above ground plant growth resources in mixed culture relative to monoculture as similarly reported previously (Ofori and Stern, 1986; Fujita *et al*., 1990; Dapaah *et al*., 2003; Mazaheri *et al*., 2006, Ruginamhozi *et al*., 2006).

In conclusion, this study has shown that high cowpea plant density (167,000 plants.ha^{-1}) and mixed culture reduced yield of both cowpea genotypes (g.plant^{-1}) and sorghum species ascribed to inter- and intra-specific competition for plant growth resources. Of the five cowpea genotypes, yield of cv. ITH98-46 showed to be inferior relative to the other genotypes. Interaction results showed that yield of cv. Sanzie

was superior to all, and together with cv. Omondaw (farmer selected cultivars) their yields were not affected by mixed culture. Furthermore, sorghum yield in mixture with cv.TVu1509 or cv. ITH98-46 performed better compared to those in mixture with other cultivars. This study also showed greater total land equivalent ratio (LER_T >1) indicating that reductions in the yield of cowpea and sorghum individually were not large enough to reduce the total grain yield per unit area in mixed culture compared with those of either crops in monoculture. In all, the data suggests that for higher grain yields in our cropping systems, genotypes such as cvs. Sanzie and Omondaw (farmer selected cultivars) with ability to compete for growth factors and produce high grain yield under robust agronomical practices such as high plant density and mixed culture systems should be adopted in Africa.

Table 5.1: Effect of plant density and cropping systems on yield components of five cowpea (*Vigna unguiculata* L. Walp.) genotypes at harvesting stage planted in 2005 and 2006.

Treatment	No of PBP. plant^{-1}	No of Pods. plant^{-1}	No of Seed.pod^{-1}	Weight of seeds + pods (g.plant^{-1})	100-seed weight (g)	Shelling (%)	Seed yield (g.plant^{-1})	Seed yield (t.ha^{-1})
2005								
Density (plants.ha^{-1})								
83,000	12.5±0.6a	20.1±1.1a	11.3±0.5a	31.14±1.25a	10.0±0.3a	68.8±0.9a	21.4±0.9a	1.8±0.1b
167,000	9.1±0.5b	16.8±1.1b	8.0±0.3b	21.12±1.11b	8.7±0.3b	66.2±1.0b	14.0±0.8b	2.3±0.1a
Cropping system								
Monoculture	12.4±0.7a	20.0±1.4a	11.0±0.5a	29.72±1.35a	9.8±0.3a	68.7±0.9a	20.5±1.0a	2.4±0.1a
Mixed culture	9.2±0.5b	16.9±0.7b	8.3±0.4b	22.54±1.25b	8.9±0.3b	66.4±1.0b	15.0±0.8b	1.7±0.1b
Genotypes								
Bengsogla	8.9±0.8b	15.4±1.4b	10.4±0.9a	24.54±2.56b	10.1±0.2b	71.5±0.6a	17.7±2.0ab	2.1±0.2ab
ITH98-46	11.1±1.4ab	18.4±2.1ab	8.5±1.0a	23.70±2.45b	8.6±0.3c	59.2±1.1c	14.2±1.6b	1.6±0.1b
Sanzie	11.1±0.6ab	17.7±1.2b	10.4±0.5a	30.79±2.23a	10.2±0.3b	67.9±0.6b	20.9±1.5a	2.4±0.1a
TVu1509	13.6±1.2a	24.6±2.1a	9.5±0.6a	26.30±2.24b	6.5±0.3d	66.4±1.3b	17.5±1.6ab	2.1±0.2ab
Omondaw	9.2±0.5b	16.4±1.2b	9.5±0.7a	25.33±1.35b	11.4±0.2a	72.8±0.8a	18.4±1.0a	2.2±0.1a
3 - Way ANOVA (F-Statistic)								
Density	24.4***	5.6*	43.3***	51.614***	74.6***	12.0**	70.1***	22.3***
Cropping system	20.6***	4.9*	28.4***	26.455***	30.4***	9.1**	39.1***	30.9***
Genotypes	5.7***	5.4***	2.1	3.167*	111.6*	40.0***	5.9***	5.2**
2006								
Density (plants.ha^{-1})								
83,000	13.6±0.7a	20.9±1.2a	11.9±0.6a	31.14±1.39a	11.2±0.3a	71.0±0.9a	22.2±1.1a	1.9±0.1b
167,000	10.0±0.6b	17.5±1.2b	8.3±0.4b	23.00±1.36b	9.9±0.3b	67.8±1.0b	15.6±0.9b	2.6±0.2a
Cropping system								
Monoculture	13.5±0.7a	20.7±1.5a	11.5±0.6a	31.95±1.42a	11.0±0.3a	71.0±0.8a	22.7±1.0a	2.7±0.1a
Mixed culture	10.1±0.5b	17.6±0.8b	8.6±0.4b	22.19±1.18b	10.1±0.3b	67.9±1.0b	15.1±0.8b	1.8±0.1b
Genotypes								
Bengsogla	9.8±0.8b	15.8±1.5b	10.9±1.0a	25.47±2.72b	11.3±0.2a	73.0±0.6ab	18.8±2.1ab	2.2±0.3ab
ITH98-46	12.4±1.4ab	19.2±2.2ab	8.8±1.1a	24.04±2.72b	9.8±0.3b	61.8±1.1d	15.0±1.8b	1.8±0.2b
Sanzie	11.8±0.7b	18.3±1.2b	10.9±0.5a	31.39±2.24a	11.4±0.3a	71.3±0.5b	22.4±1.6a	2.7±0.2a
TVu1509	15.4±1.1a	25.7±2.3a	9.9±0.7a	28.41±2.47ab	7.7±0.3c	65.9±1.2c	18.8±1.8ab	2.2±0.2ab
Omondaw	9.7±0.4b	16.9±1.3b	9.9±0.8a	26.03±1.38b	12.6±0.2a	75.2±0.6a	19.6±1.1ab	2.3±0.1ab
3 - Way ANOVA (F-Statistic)								
Density	27.8***	5.3*	41.8***	25.692***	74.6***	33.1***	39.8***	28.0***
Cropping system	24.2***	4.6*	27.0***	36.977***	30.4***	29.7***	53.4***	39.4***
Genotypes	9.5***	5.6***	2.0	2.581*	111.6***	74.1***	5.3**	4.0**

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column, are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. There was significant interactive effect of density x cropping system in the number of seeds per pod and 100-seed weight in 2005 and 2006; genotypes x cropping systems and seed yield (in 2005) but are not shown in the table. (PBP= Pod bearing peduncles). Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}.

Table 5.2: Effect of plant density and cropping systems on yield components of sorghum (*Sorghum bicolor* L. Moench.) at harvesting stage planted in 2005 and 2006.

Treatment	2005				2006			
	Weight of head.plant^{-1}	Weight of seed (g.plant^{-1})	Weight of 100 seeds (g)	Yield (t.ha^{-1})	Weight of head.plant^{-1}	Weight of seed (g.plant^{-1})	Weight of 100 seeds (g)	Yield (t.ha^{-1})
Density (plants.ha^{-1})								
Sorghum in 83,000	49.40±1.21a	28.37±0.66a	2.21±0.02a	1.58±0.04a	50.23±1.23a	28.63±0.66a	2.24±0.02a	1.59±0.04a
Sorghum in 167,000	44.92±1.81b	24.95±1.13b	2.15±0.03b	1.39±0.06b	45.69±1.83b	25.11±1.14b	2.17±0.03b	1.40±0.06b
Cropping system								
Monoculture sorghum	55.63±0.00a	31.56±0.00a	2.30±0.00a	1.75±0.00a	56.55±0.00a	31.81±0.00a	2.34±0.00a	1.77±0.00a
Mixed culture (Sorghum : Cowpea)	38.69±1.14b	21.75±0.79b	2.06±0.02b	1.21±0.04b	39.38±1.16b	21.93±0.80b	2.08±0.02b	1.22±0.04b
Genotypes								
Sorghum : Bengsogla	46.62±2.55ab	26.22±1.56ab	2.19±0.04a	1.46±0.09ab	47.41±2.59ab	26.42±1.57ab	2.22±0.04a	1.47±0.09ab
Sorghum : ITH98-46	48.06±2.18ab	27.06±1.27ab	2.19±0.03a	1.50±0.07ab	48.87±2.21ab	27.26±1.28ab	2.22±0.04a	1.51±0.07ab
Sorghum : Sanzie	44.86±2.91b	25.35±1.80b	2.16±0.04a	1.41±0.10b	45.62±2.95b	25.50±1.82b	2.19±0.05a	1.42±0.10b
Sorghum : TVu1509	49.23±2.40a	27.94±1.60a	2.16±0.04a	1.56±0.09a	50.07±2.44a	28.26±1.59a	2.20±0.04a	1.57±0.09a
Sorghum : Omondaw	47.04±2.48ab	26.73±1.43ab	2.19±0.04a	1.49±0.08ab	47.84±2.52ab	26.92±1.44ab	2.22±0.04a	1.50±0.08ab
3 - Way ANOVA (F-Statistic)								
Density	33.6***	47.0***	10.3**	46.7***	32.7***	53.5***	17.0***	53.4***
Cropping system	478.7***	386.5***	181.8***	382.3***	468.3***	422.1***	241.1***	422.9***
Genotypes	3.6*	3.0*	0.5	3.1*	3.5*	3.6*	0.7	3.6*

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column, are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. Although there were significant interactions, they are not reported in this table since density and genotypes were not varied. Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}.

Table 5-3: Estimated yield advantage measured as total land equivalent ratio at different densities and cropping systems in 2005 and 2006 cropping seasons (n = 4).

Treatment	2005	2006
Density (plants.ha^{-1})		
83,000	1.52±0.05	1.45±0.04
167,000	1.42±0.06	1.38±0.06
Cropping system		
Bensogla : Sorghum	1.30±0.10	1.28±0.10
ITH98-46 : Sorghum	1.54±0.09	1.40±0.07
Sanzie : Sorghum	1.47±0.06	1.37±0.09
TVu1509 : Sorghum	1.44±0.07	1.43±0.07
Omondaw : Sorghum	1.61±0.06	1.60±0.05

Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}.

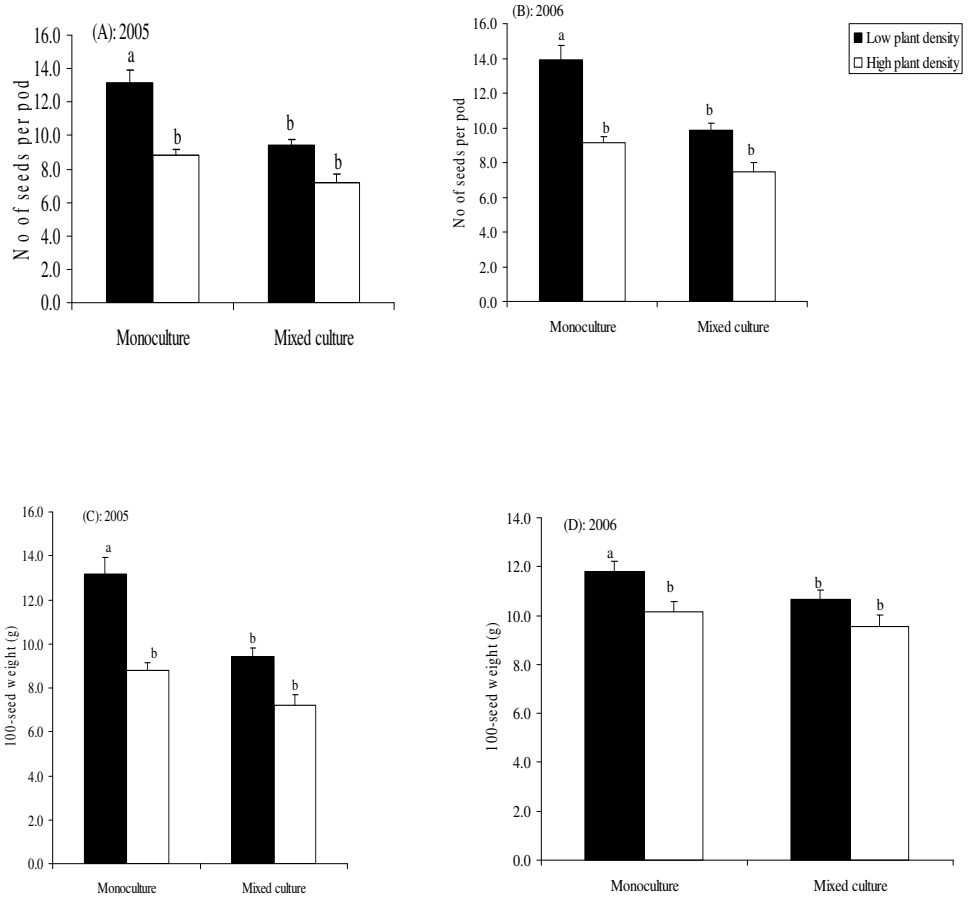


Fig. 5.1: Interactive effects of density and cropping system on A) No of seeds per pod in 2005, B) No of seeds per pod in 2006, C) 100-seed weight of cowpea in 2005 and D) 100-seed weight of cowpea in 2006.

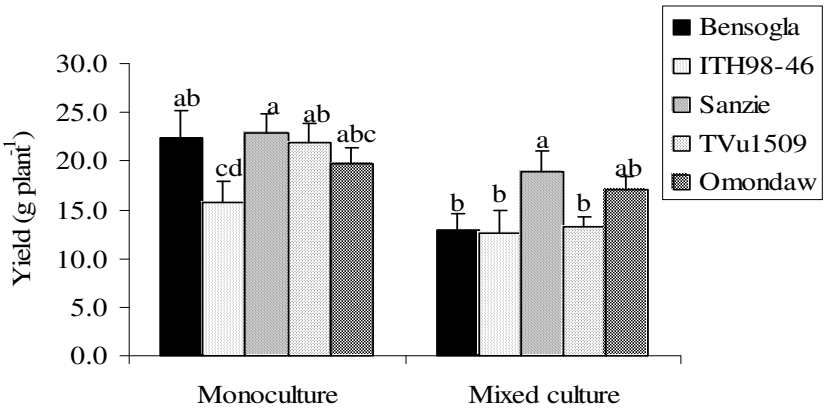


Fig. 5.2: Interactive effects of cropping system and genotypes on yield of cowpea in 2005. Data for 2006 are not shown here for they were not significant.

CHAPTER 6

EFFECT OF LEGUME PLANT DENSITY AND MIXED CULTURE ON SYMBIOTIC N_2 FIXATION IN FIVE COWPEA (*VIGNA UNGUICULATA* L. WALP.) GENOTYPES IN SOUTH AFRICA

6.1 INTRODUCTION

Symbiotic legumes are an important component of the cropping systems in tropical agriculture because of their ability to contribute fixed-N to associated cereal and other non-legume crops (Peoples *et al*., 1995). The contribution of N_2 fixation is particularly important in Africa, where N is one of the most limiting mineral nutrients for plant growth (Palm and Sanchez, 1991). Values of N contribution by various legumes, including cowpea (*Vigna unguiculata* L. Walp.) are quite high, ranging from 50 - 300 kg $N.ha^{-1}$ yr^{-1} (Peoples *et al*., 1995; Dakora and Keya, 1997). In Ghana, a local cowpea genotype has been estimated to fix as much as 201 kg $N.ha^{-1}$ $season^{-1}$, and provided about 42 kg N to a following maize (*Zea mays* L.) crop (Dakora *et al*., 1987).

The levels of N_2 fixation have however been suggested to be dependent on a number of factors, ranging from plant density, legume species, crop management to cultural systems (Kumar Rao *et al*., 1996). As a result, the amount of N-fixed has been found to vary with different cropping systems (Fujita *et al*., 1992; Jensen, 1996; Xiao *et al*., 2004). In mixed cultures, for example, reduced photosynthesis in leaves can occur in the legume due to shading by cereal, and thus, lower N_2 fixation (Wahua and Miller, 1978; Trang and Giddens, 1980). It has also been shown that in a cereal/legume mixture, planted at a density of 1:4, N contribution by legume to cereal was equivalent to 96 kg fertiliser N ha^{-1}(Walsh, 1995), indicating that greater legume density enhances nodule activity. The only critique of many of these earlier studies is that single legume genotypes were used in the density and mixed culture experiments with no chance for genotypic comparisons. This study examines the effect of plant density and cropping system on N_2 fixation in five cowpea genotypes at different levels of crop improvement.

6.2 Materials and methods

6.2.1 Site location, description and experimental design

Site location, description and experimental design are as reported in section 2.2.1 and 2.2.2 respectively.

6.2.2 Plant harvest and sample preparation

At 67 days after planting during early pod development, 16 and 8 plants of cowpea and sorghum were respectively harvested from the middle rows of each plot. The plants were carefully dug out with intact

root system, washed, and separated into nodules, roots, shoots and pods in the case of cowpea, while sorghum plants were separated into only roots and shoots. The plant organs were oven-dried at 60°C for 48 h and ground into fine powder (0.85 mm sieve) and stored, prior to analysis for ^{15}N natural abundance.

6.2.3 $^{15}N/^{14}N$ isotope analysis and determination of %Ndfa

The isotope ratio of $^{15}N/^{14}N$ and the concentration of N in cowpea plant organs weighing 1 mg (nodules), 2 mg (roots or shoots), 1.5 mg (pods) and in sorghum plant organs weighing 2.5 mg (roots or shoots) were transferred into tin capsules and injected into a Thermo Flash Elemental Analyser 1112 via a Thermo Conflo III devise coupled to a Thermo Finnigan Delta Plus XP Stable Light Isotope Mass Spectrometer. ^{15}N abundance is usually expressed in a relative, δ (delta) notation, which is the ‰ deviation of the ^{15}N natural abundance of the sample from atmospheric N_2 (= 0.36637 atom % ^{15}N) (Unkovich *et al*., 1994):

$$\delta^{15}N = \frac{atom\%\ ^{15}N\ sample - atom\%\ ^{15}N\ air}{atom\%\ ^{15}N\ air} * 1000$$

Whole plant ^{15}N natural abundance was calculated as an average of $\delta^{15}N$ in all plant organs weighted by their respective total N contents as described by Robinson *et al*. (2000):

$$\delta^{15}N_{Whole\ plant} \Rightarrow \frac{\sum\left(\delta^{15}N_{nodule} \times N_{nodule} + \delta^{15}N_{root} \times N_{root} + \delta^{15}N_{shoot} \times N_{shoot} + \delta^{15}N_{pods} \times N_{pods}\right)}{\sum\left(N_{nodule} + N_{root} + N_{shoot} + N_{pods}\right)}$$

The ^{15}N natural abundance technique was used to quantify plant reliance upon N_2 fixation for growth (%Ndfa) as follows (Shearer and Kohl, 1986):

$$\%Ndfa = \left\{\frac{\delta^{15}N_{ref} - \delta^{15}N_{fix}}{\delta^{15}N_{ref} - \delta B_{va}}\right\} \times 100$$

Where $\delta^{15}N_{ref}$ is the ^{15}N natural abundance of the non - N_2 fixing reference plant and B is the ^{15}N natural abundance of N_2 fixing plant relying on atmospheric N_2 as the sole N source. The B value is included in the equation to account for ^{15}N discrimination during the N_2-fixing process in plant (Evans *et al*., 2001). Sorghum from monoculture plots was sampled as reference plants for assessing the ^{15}N enrichment of soil. The $\delta^{15}N$ values (‰) of the reference plant material used in 2005 were: 6.077 for roots, 9.997 for shoots, pods and whole plant; in 2006 the values were: 5.912 for roots, 10.32 for shoots, whole plant, and 9.836

for pods. The B (‰) values of cowpea organs were: -1.759 for shoots, -0.94 for roots, -1.4713 for pods and -0.6333 for whole plant (S.B.M. Chimphango and F.D. Dakora, unpublished data).

The amount of N in cowpea and sorghum plant parts was calculated by multiplying the organ's dry matter by its %N.

$$Amount\ of\ N(mg.plant^{-1}) = Dry\ mass_{organ}(mg) \times \%N_{organ}$$

The proportion of N derived from fixation (Ndfa) was calculated by multiplying total N in the organ by %Ndfa from the respective plant organ. These were then added together to get the value on a whole-plant basis. This amount was then multiplied by plant density to convert to per hectare basis.

$$N_{fixed}\ (mg.plant^{-1}) = Amount\ of\ N_{organ}(mg) \times \%Ndfa_{organ}$$

$$N_{fixed}\ (kg.ha^{-1}) = N_{fixed}\ (kg.plant^{-1}) \times Density\ (plants.ha^{-1})$$

6.2.4 Cowpea grain yield

At physiological maturity during both 2005 and 2006, cowpea pods were harvested from the remaining inner rows of each plot, shelled, and grain yield assessed.

6.2.5 Statistical Analysis

A 3-factorial design involving plant density, cropping system and cowpea genotypes was used to statistically analyse the data collected. The analysis was performed using the software of STATISTICA program 2007 (StatSoft Inc., Tulsa, OK, USA). Fisher's least significant difference was used to compare treatment means at $P \leq 0.05$ (Steel and Torrie, 1980).

6.3 RESULTS

6.3.1 Effect of plant density on growth and symbiotic performance of cowpea genotypes

Increasing plant density significantly ($P \leq 0.05$) altered plant growth, $\delta^{15}N$ (^{15}N natural abundance), %N and %Ndfa in all plant organs of cowpea during 2005 and 2006. More specifically, increasing cowpea density from 83,000 to 167,000 plants.ha^{-1} reduced ($P \leq 0.05$) the dry matter of shoots, roots, pods and nodules, and therefore whole-plant biomass (Table 6.1). The %N in all organs of cowpea was similarly decreased with increasing plant density (Table 6.2), just as the amounts of N-fixed in shoots, pods and whole plants of cowpea were reduced ($P \leq 0.05$) with increasing plant density (Table 6.4), in contrast to %Ndfa which increased ($P \leq 0.05$) with greater plant density (Table 6.3). Increasing cowpea plant density significantly increased the grain yield of this legume during both 2005 and 2006 (Table 6.4).

6.3.2 Effect of mixed culture on plant growth and symbiotic performance

In all instances, growing cowpea in monoculture resulted in larger organ development and whole plant growth (Table 6.1) with significantly ($P \leq 0.05$) greater N concentrations in tissues (Table 6.2). Plants from monoculture showed greater $\delta^{15}N$ values, which indicate lower levels of N_2 fixation compared with mixed culture (Table 6.3). Consequently, the %Ndfa of monocultured plants was consistently lower than those in mixed culture (Table 6.3). As a result of the greater plant growth and %N in tissues of cowpea plants grown in monoculture, their total plant N was significantly higher compared with mixed culture (Table 6.4). The amount of N-fixed was also greater in plants under monoculture relative to those in mixed culture (Table 6.4). At physiological maturity, the grain yield of cowpea was significantly greater in monoculture compared with mixed culture during both 2005 and 2006 (Table 6.4).

6.3.3 Effect of genotypes on symbiotic performance

In general, the three unimproved farmer varieties Sanzie, Omondaw and Bensogla showed better growth compared with the two improved cultivars (Table 6.1). During 2005 and 2006, Sanzie showed much better growth compared with the other genotypes (Table 6.1). The %N of organs was also greater in Sanzie relative to the rest (Table 6.2). The $\delta^{15}N$ values of genotypes were however similar (Table 6.3). As a result, the %Ndfa data were also similar at whole-plant level in 2005, but differed significantly in 2006 with cv. Bensogla and Omondaw deriving more N from symbiotic fixation (Table 6.3). However plant

total N and N-fixed were highest in cv. Sanzie compared with the other genotypes (Table 6.4). As a result, grain yield was also significantly higher in cv. Sanzie compared with the other four genotypes during both 2005 and 2006 (Table 6.4).

6.3.4 Interactive effects of legume density and cropping system

There was a significant interaction between cropping system and plant density. Whether cultivated as monoculture or mixed culture, cowpea plants consistently showed lower levels of %N in shoots, and N content of roots, shoots, pods (Fig. 6.1), and whole plants (Fig. 6.2), as well as fixed-N in shoots and whole plants (Fig. 6.2) at high plant density compared with low plant density.

6.3.5 Interactive effects of plant density and cowpea genotypes.

There was also a significant interaction between plant density and cowpea genotypes. It was noteworthy to note that the five cowpea genotypes behaved differently under the same plant density. Compared with the other genotypes Sanzie, for example, showed greater root growth under low plant density while at high density cv. ITH98-46 developed the least root mass (Fig 6.3A). As a result of the higher root mass in 2005, the cv. Sanzie had the greatest total plant biomass (Fig 6.3B), a pattern similar to cv. ITH98-46, which showed the lowest root mass in 2005 and therefore the lowest total dry matter (Fig 6.3B). The 2006 data also showed cv. Sanzie as the genotype with the highest plant growth under low plant density (Fig 6.3C), even though its pod dry matter was significantly lower relative to cv. Omondaw under the same conditions of low plant density (Fig 6.3D). During both 2005 and 2006, the cv. Bensogla was consistently the highest producer of roots, and total biomass under high plant density (Fig 6.3A, B, C and D).

The interaction between plant density and genotypes was significant ($P \leq 0.05$) for %N in pods, and N content of shoots, pods and whole plants, as well as for fixed-N of pods and whole cowpea plants. For example, N concentration of pods from cv. Sanzie was greater than that of the cultivar Omondaw under low, but not high, plant density (Fig 6.4A and B). Shoot N was higher in cv. Sanzie than the other cultivars at low plant density; while at high density it was greater than only cv. ITH98-46 (Fig 6.5A). Similarly, the Sanzie genotype was higher in plant total N (Fig 6.5C and D) and N-fixed compared with the rest at low plant density (Figs 6.6C, D and E). However, pod-N and pod fixed-N was highest in cv. Omondaw relative to the other genotypes at low plant density (Fig 6.5B, 6.6C). A significant interaction was also observed for %Ndfa of pods, with that of TVu1509 being higher than those of cvs. Bensogla and ITH98-46, but not Sanzie or Omondaw at low plant density (Fig 6.6A and B).

6.3.6 Interactive effects of cropping system and cowpea genotypes

A significant ($P \leq 0.05$) interaction of cropping system and cowpea genotypes was observed during both 2005 and 2006. While under monoculture there were no significant differences between cowpea genotypes, the $\delta^{15}N$ of pods was lower in cultivar Sanzie compared to ITH98-46 when the two species were intercropped (Fig 6.7A). However, the %Ndfa of cv. Omondaw was greater than that of cv. Bensogla in monoculture, while in mixed culture, %Ndfa of shoots in cv. Omondaw was greater than that of cvs. Sanzie and TVu1509 (Fig 6.7B), and %Ndfa of Sanzie pods also higher than that of ITH98-46 (Fig 6.7C and D).

6.4 DISCUSSION

6.4.1 Effect of plant density and cropping system on N_2 fixation in cowpea

Farmers in Africa are frequently confronted with poor crop yields as a consequence of low soil nutrient fertility. The inclusion of symbiotic legumes in cropping systems is one approach African farmers use to improve soil nutrient fertility for increased yields. However, the level of N contribution by symbiotic legumes is dependent on the legume plant density, the cropping system and the choice of legume genotype. While a few studies have examined legume plant density and cropping systems as factors affecting cowpea yields in Africa, none has addressed symbiotic N nutrition in the species. Thus, the effect of plant density on N_2 fixation and N contribution by cowpea, in particular, remains unknown in the traditional cropping systems of Africa. In this study, cowpea N_2 fixation was evaluated, using five genotypes in two plant densities and two cropping systems. The data showed that plant growth and N_2 fixation in the five cowpea genotypes were significantly altered by treatment densities and cropping systems during both 2005 and 2006. The use of lower plant density (i.e. 83,000 plants.ha^{-1}), a population that mimicked farmer practice, significantly increased plant growth, tissue N concentration and $\delta^{15}N$ values at both organ and whole-plant level (Tables 6.1, 6.2, and 6.3). Because of the high $\delta^{15}N$ values (i.e. low N_2 fixation) of cowpea plants from the low density treatment, the %Ndfa was much lower relative to cowpea from higher plant density. However, as a result of the greater biomass produced by cowpea at low plant density, the actual amounts of N-fixed were greater at low than at high plant density during both 2005 and 2006 (Tables 6.3 and 6.4). These data suggest that, at low plant density, cowpea roots probably explored wider and bigger soil volumes and accumulated greater nutrient resources, including mineral N,

which is known to inhibit nitrogenase activity and decrease N_2 fixation (Streeter, 1988; Dakora, 1998; Ayisi *et al*., 2000). In contrast, at high plant density, intense plant-to-plant competition can decrease the uptake of various nutrients, including mineral N, and thus enhance nodule functioning, leading to significantly higher %Ndfa values as shown in Table 6.3. It would be interesting to know whether Bambara groundnut (*Vigna subterranea* L. Verdc.) and Kerstings bean (*Macroptyloma geocarpum* L.), which are reported to be tolerant of nitrate (Dakora, 1998), would also decrease their %Ndfa with low plant density.

Cultivating cowpea plants in monoculture, as opposed to mixed culture, led to better plant growth, higher nodule formation, increased tissue N concentration and greater $\delta^{15}N$ values at both organ and whole-plant level in 2005 and 2006 (Tables 6.1 and 6.2). The high $\delta^{15}N$ values of monocultured cowpea plants resulted in significantly lower %Ndfa relative to their mixed-cultured counterparts in 2005 and 2006. However, because the monocultured cowpea plants accumulated greater biomass than those in mixed culture, the actual amounts of N-fixed were also significantly higher in monoculture compared with mixed culture (Tables 6.3 and 6.4). In many ways, the effect of monoculture on cowpea growth and symbiotic performance was similar in pattern to that of low plant density. Not only would the N-starved in mixed culture with symbiotic cowpea create intense competition for soil N, and thus eliminate any inhibition of N_2 fixation by soil mineral N (Streeter, 1988), but also the transfer of fixed-N to sorghum could further induce greater nodule functioning (Eaglesham *et al*., 1981; Walsh, 1995) and hence the higher %Ndfa values in mixed-cultured cowpea plants. In other studies growing nodulated legumes in mixed culture with cereals similarly increased the values of %Ndfa relative to monoculture (Izaurralde, 1992; Senaratne *et al*., 1993; Jensen, 1996; Xiao *et al*., 2004). More recently, Fan *et al*. (2006) also showed that growing faba bean (*Vicia faba* L.) in mixed culture with wheat (*Triticum aestivum* L.) increased its %Ndfa relative to monoculture. The greater %Ndfa values observed in mixed cultures involving legumes and cereals was attributed to soil N depletion by cereals, which reduced nitrate inhibition of nodulation and nodule functioning (Streeter, 1988; Hardarson *et al*., 1988; Stern, 1993; Kerley and Jarvis, 1999). Crop plant densities and cropping patterns were found to have similar elevating effects on %Ndfa in symbiotic legumes (Fujita *et al*., 1990), as shown in this study.

Of the five cowpea genotypes (Bensogla, Sanzie, Omondaw, ITH98-46 and TVu1509) assessed for symbiotic performance in this study, Sanzie (a farmer variety) consistently showed superior plant growth, as a result of better nodulation and nodule functioning (Tables 6.1 and 6.2). Because the $\delta^{15}N$ values of organs and whole plants were not significantly different in 2005 and 2006, except for cv. TVu1509, the rest of the genotypes showed similar %Ndfa values (Table 6.3). However, due to the differences in plant biomass, symbiotic N yield was found to be significantly higher in cultivar Sanzie in both 2005 and 2006 (Table 6.4), indicating that this farmer-selected variety is better adapted for intercropping as well as

cultivation under different plant densities. As shown in this study, variation in symbiotic performance of legume genotypes is common (Kishinevsky *et al*., 1996), where many cultivars are assessed. As shown in Table 6.4, the amount of N-fixed by cv. Sanzie was 75.3 and 86.5 kg N.ha^{-1} for 2005 and 2006 respectively, while that of cv. ITH98-46 was 46.3 and 51.4 kg N.ha^{-1} for 2005 and 2006. The levels of N_2 fixation obtained for cowpea in this study are within the range (9 - 201 kg N.ha^{-1}) estimated for this legume in on-station research (Peoples and Crasswell, 1992; Herridge *et al*., 1993; Dakora and Keya, 1997), as well as in farmers' fields (15 – 267 kg N.ha^{-1}, see Peoples *et al*., 1995).

6.4.2 Interactive effects of plant density, cropping system and cowpea genotype on N_2 fixation

The data from this study also revealed significant interaction between plant density and cropping system. Whether planted as monoculture or mixed culture, N concentration of cowpea shoot was consistently higher at low plant density compared with high plant density in both 2005 and 2006 (Fig. 6.1A and B), leading to greater shoot N content in the low-density cowpea plants growing in mixed or monocultures (Figs. 6.1D and E). In 2006, the root N content of cowpea was also higher in low-density cowpea plants relative to high density in both monoculture and mixed culture (Fig. 6.1C). These results suggest that plant density rather than cropping system controls the tissue N levels of cowpea organs such as shoots and roots. However, dry matter accumulation by individual cowpea genotypes was found to depend on plant density. For example, at low plant density, cowpea cv. Sanzie produced significantly more root biomass than cvs, Bensogla and TVu1509 (Fig. 6.3A), which led to significantly greater plant total biomass in Sanzie compared to Bensogla and TVu1509 in 2005 and 2006 (Fig. 6.3B and C). At high plant density, however, there were no differences in dry matter accumulation by these genotypes at both organ and whole-plant level in 2005 and 2006 (Fig. 6.3A, B and C). The increased root growth by Sanzie (a farmer variety) at low plant density (which is similar to farmer practice) could be linked to its selection by farmers, as the trait directly influences nutrient/water uptake, and nodule formation for N_2 fixation. It is therefore not surprising that cv. Sanzie, with a much better root development, showed the highest plant growth and N_2 fixation during both 2005 and 2006 (Tables 6.1 and 6.4). As shown in this study, cv. Sanzie also accumulated more shoot and total plant N at both low and high plant densities than cvs, Bensogla and TVu1509 (Fig. 6.5A, C and D), thus indicating the species' ability to maintain a higher level of symbiotic N nutrition (Fig. 6.6D and E) despite any intense plant-to-plant competition for soil resources when planted in high densities.

6.4.3 Agronomic implications of the study

On per-plant basis, high density produced significantly reduced plant growth (15.9 vs. 20.5 g dry matter.plant^{-1} in 2005, and 18.8 vs. 23.0 g dry matter.plant^{-1} in 2006), lower N-fixed (387.2 vs. 614.0 mg.plant^{-1} in 2005, and 442.7 vs. 675.7 mg.plant^{-1} in 2006), and decreased grain yield (14.0 vs. 21.4 g.plant^{-1} in 2005, and 15.6 vs. 22.2 g.plant^{-1} in 2006) relative to low density. However, on per-hectare basis, high density produced more biomass, more fixed-N (64.5 vs. 51.2 kg N.ha^{-1} in 2005, and 73.8 vs. 56.3 kg N.ha^{-1} in 2006), and more grain yield (2,339 vs. 1787.5 kg.ha^{-1} in 2005, and 2,607.6 vs. 1850.1 kg.ha^{-1} in 2006) compared with low density. In agronomic terms, the greater biomass and larger amount of N-fixed at high plant density (Table 6.4) are more likely to enhance soil N fertility, and thus increase yields of succeeding crops. The greater grain yield per hectare with increasing plant density (Table 6.4) is also more likely to encourage farmer adoption of cowpea production in monoculture (i.e. high density), where land is readily available. The data in Table 6.4 show that cowpea cv. Sanzie fixed more N and produced more grain yield, and was therefore the best variety among the five genotypes.

In conclusion, our data showed superior plant growth and N_2 fixation in the three farmer-selected varieties (Sanzie, Bensogla and Omondaw) relative to the two improved cultivars. This implies greater potential N contribution to cropping systems. Whether under low or high plant density, the cv. Sanzie produced significantly greater symbiotic N in both 2005 and 2006, followed by the other two farmer varieties, and last the improved cultivars. Similarly, the actual amount of N derived from fixation was much greater in cv. Sanzie, followed by the other farmer varieties under both low and high plant density. Furthermore, on per-plant basis, the data also showed better growth and greater symbiotic N yield in cowpea plants cultivated in monoculture (or low plant density) relative to those in mixed culture (or high plant density). Taken together, our results suggest that, in order to optimize for higher N_2 fixation and N contribution in cropping systems for increased agronomic yields, legume genotypes must first be evaluated for their levels of symbiotic fixation under robust field conditions, as done in this study.

Table 6.1: Plant dry matter yield (g.plant^{-1}) at flowering of nodulated cowpea genotypes (*Vigna unguiculata* L. Walp.) in different cropping systems and density in during 2005 and 2006.

Treatment	2005 Shoot	Pod	Root	Nodule	Whole plant	2006 Shoot	Pod	Root	Nodule	Whole plant
Density (plants.ha^{-1})										
83,000	20.5±1.0a	6.1±0.7a	1.2±0.1a	0.6±0.1a	28.4±1.5a	23.0±1.2a	6.0±0.7a	1.3±0.1a	0.7±0.1a	30.9±1.7a
167,000	15.9±0.8b	3.5±0.4b	0.9±0.1b	0.5±0.0b	20.9±1.1b	18.8±1.0b	3.5±0.4b	1.0±0.1b	0.5±0.0b	23.9±1.3b
Cropping system										
Monoculture	21.4±1.0a	6.6±0.6a	1.3±0.1a	0.7±0.0a	30.0±1.4a	23.9±1.1a	6.5±0.6a	1.3±0.1a	0.7±0.0a	32.6±1.5a
Mixed culture	15.1±0.7b	2.9±0.4b	0.8±0.1b	0.4±0.0b	19.3±1.0b	17.8±1.1b	3.0±0.4b	1.0±0.0b	0.5±0.0b	22.2±1.3b
Genotypes										
Bensogla	18.7±1.7ab	5.4±0.8ab	1.0±0.1a	0.6±0.1ab	25.6±2.3ab	20.2±1.9ab	5.6±0.8ab	1.1±0.1a	0.6±0.1a	27.5±2.5ab
ITH98-46	15.9±1.3b	3.5±0.7b	0.9±0.1a	0.6±0.1ab	20.8±2.0b	18.2±1.6b	3.5±0.6b	1.0±0.1a	0.6±0.1a	23.5±2.1b
Sanzie	21.4±1.7a	4.9±1.0ab	1.2±0.1a	0.8±0.1a	28.2±2.6a	25.4±2.1a	4.0±0.8b	1.3±0.1a	0.8±0.1a	31.4±2.8a
TVu1509	17.3±1.7ab	3.3±0.5b	1.0±0.1a	0.5±0.1b	22.1±2.3b	19.5±2.1ab	3.3±0.6b	1.1±0.1a	0.5±0.1a	24.5±2.7ab
Omondaw	17.9±0.9ab	6.9±1.4a	1.0±0.1a	0.5±0.1b	26.4±2.2ab	21.1±1.1ab	7.3±1.4a	1.2±0.1a	0.6±0.1a	30.2±2.4ab
3-Way ANOVA (F-Statistic)										
Density	22.5***	14.5***	9.3**	7.5**	31.4***	9.5**	19.0***	15.6***	4.7*	16.9***
Cropping system	41.9***	30.5***	29.7***	40.0***	63.6***	20.5***	37.3***	26.4***	22.9***	37.6***
Genotypes	3.6*	3.8**	1.7	4.3**	4.3**	3.3*	7.1***	2.4	1.6	3.3*

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. There were significant interactive effects (not shown here) of density and genotypes on roots and whole plant biomass (in 2005); and on pods and whole plant biomass (in 2006). Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}.

Table 6.2: Effect of plant density and cropping system on N concentration (%) in five cowpea genotypes planted in 2005 and 2006.

	2005				2006			
Treatment	Nodule	Root	shoot	pods	Nodule	Root	shoot	pods
Density (plants.ha^{-1})								
83,000	4.2±0.1a	2.2±0.1a	3.6±0.1a	4.4±0.1a	4.2±0.1a	2.2±0.1a	3.6±0.1a	4.4±0.1a
167,000	3.4±0.1b	1.8±0.1b	3.0±0.1b	3.6±0.1b	3.4±0.1b	1.8±0.1b	3.0±0.1b	3.6±0.1b
Cropping system								
Monoculture	4.2±0.1a	2.1±0.1a	3.6±0.1a	4.2±0.1a	4.2±0.1a	2.1±0.1a	3.6±0.1a	4.3±0.1a
Mixed culture	3.5±0.1b	1.9±0.1a	3.0±0.1b	3.8±0.1b	3.5±0.1b	1.9±0.1a	3.0±0.1b	3.8±0.1b
Genotypes								
Bensogla	3.8±0.1ab	2.1±0.2ab	3.2±0.2b	4.0±0.1a	3.8±0.1ab	2.1±0.2ab	3.2±0.2b	4.0±0.1a
ITH98-46	3.5±0.2b	1.9±0.1ab	3.2±0.2b	4.0±0.2a	3.5±0.2b	1.9±0.1b	3.2±0.2b	4.0±0.2a
Sanzie	4.2±0.2a	2.4±0.2a	4.0±0.2a	4.1±0.1a	4.2±0.2a	2.5±0.2a	4.0±0.2a	4.1±0.1a
TVu1509	3.9±0.2ab	1.6±0.2b	3.2±0.2b	3.9±0.1a	4.0±0.2ab	1.7±0.2b	3.3±0.2ab	4.0±0.1a
Omondaw	3.7±0.2ab	1.9±0.1ab	2.9±0.2b	3.9±0.1a	3.8±0.2ab	1.9±0.1b	3.0±0.2b	4.0±0.1a
3- way ANOVA (F-Statistic)								
Density	33.0***	9.4**	15.9***	111.6***	35.9***	9.5**	15.1***	112.0***
Cropping system	23.9***	2.3	13.7***	37.0***	27.9***	2.5	12.7***	37.3***
Genotypes	3.0*	4.0**	5.2**	0.6	3.2*	4.0**	5.1**	0.6

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. There were significant interactive effects (not shown here) of density and cropping system on N concentration in shoots; of density and genotypes on N concentration in pods (in 2005 and 2006). Plant density figures have been rounded up from 83,333 to 83,000 plants.ha^{-1} and from 166,666 to 167,000 plants.ha^{-1}.

Table 6.3: Effect of plant density and cropping systems on $\delta^{15}N$ and %Ndfa of five cowpea genotypes planted in 2005 and 2006.

Treatment	^{15}N natural abundance (‰)					%Ndfa			
	Nodule	Root	Shoot	Pods	Whole plant	Root	shoot	pods	Whole plant
2005									
Density (plants.ha^{-1})									
83,000	8.9±0.2a	3.8±0.1a	2.4±0.1a	4.9±0.1a	5.3±0.1a	28.6±1.2b	64.4±0.9b	44.4±1.1b	44.4±0.8b
167,000	8.1±0.1b	3.3±0.1b	2.1±0.1b	3.7±0.1b	4.5±0.1b	39.6±1.2a	66.8±0.7a	54.9±1.0a	51.5±0.9a
Cropping system									
Monoculture	8.9±0.1a	3.9±0.1a	2.5±0.1a	4.8±0.1a	5.3±0.1a	30.7±1.4b	64.0±0.9b	45.4±1.1b	44.4±0.8b
Mixed culture	8.1±0.2b	3.2±0.1b	2.1±0.1b	3.8±0.1b	4.5±0.1b	37.5±1.3a	67.1±0.7a	54.0±1.2a	51.5±0.9a
Genotypes									
Bensogla	8.6±0.3a	3.2±0.1a	2.1±0.2a	4.5±0.3a	4.9±0.2a	38.6±1.9a	66.9±1.5a	47.9±2.2b	48.0±1.5a
ITH98-46	8.3±0.2a	3.6±0.2a	2.3±0.2a	4.5±0.2a	4.9±0.2a	31.1±1.9b	65.2±1.4a	48.0±2.1b	48.0±1.5a
Sanzie	8.8±0.2a	3.6±0.2a	2.3±0.1a	4.0±0.3a	4.9±0.2a	32.0±2.5b	65.2±0.9a	52.0±2.7a	48.4±1.8a
TVu1509	8.6±0.2a	3.9±0.2a	2.5±0.2a	4.1±0.2a	5.1±0.2a	29.4±1.7b	63.3±1.3a	51.1±1.8ab	46.2±1.4a
Omondaw	8.1±0.3a	3.3±0.2a	2.1±0.2a	4.3±0.2a	4.8±0.2a	39.4±2.6a	67.3±1.3a	49.5±1.8ab	49.2±1.7a
3 - Way ANOVA (F-Statistic)									
Density	12.8***	6.8*	4.5*	102.8***	60.5***	79.3***	4.51*	102.8***	60.5***
Cropping system	12.6***	16.8***	7.4**	68.1***	60.2***	31.1***	7.38**	68.1***	60.2***
Genotypes	1.3ns	1.8ns	1.5ns	2.5ns	1.1ns	11.1***	1.52ns	2.5*	1.1ns
2006									
Density (plants.ha^{-1})									
83,000	8.9±0.2a	3.8±0.1a	2.5±0.1a	4.9±0.1a	4.9±0.1a	31.4±1.8b	65.1±0.9b	43.3±1.1b	49.4±0.7b
167,000	8.1±0.1b	3.3±0.1b	2.2±0.1b	3.7±0.1b	4.2±0.1b	38.0±2.0a	67.5±0.7a	54.0±1.0a	55.8±0.8a
Cropping system									
Monoculture	8.9±0.1a	3.9±0.1a	2.5±0.1a	4.8±0.1a	4.9±0.1a	29.5±1.7b	64.8±0.9b	44.3±1.2b	49.4±0.7b
Mixed culture	8.2±0.2b	3.2±0.1b	2.1±0.1b	3.8±0.1b	4.2±0.1b	39.9±1.9a	67.8±0.7a	53.0±1.2a	55.7±0.7a
Genotypes									
Bensogla	8.6±0.3a	3.2±0.1a	2.2±0.2a	4.5±0.3a	4.5±0.2a	39.0±2.1a	67.6±1.4a	46.8±2.2a	53.5±1.4a
ITH98-46	8.4±0.2a	3.6±0.2a	2.4±0.2a	4.5±0.2a	4.5±0.2a	33.1±3.6a	66.0±1.4a	46.9±2.2ab	53.0±1.4a
Sanzie	8.8±0.2a	3.6±0.2a	2.4±0.1a	4.1±0.3a	4.5±0.2a	33.9±3.1a	65.9±0.9a	51.0±2.7ab	52.8±1.6a
TVu1509	8.7±0.2a	3.9±0.2a	2.6±0.2a	4.2±0.2a	4.8±0.1a	29.6±2.7a	64.1±1.3a	50.2±1.8b	50.0±1.2b
Omondaw	8.2±0.3a	3.3±0.2a	2.1±0.2a	4.4±0.2a	4.5±0.1a	38.0±3.5a	68.0±1.3a	48.5±1.8a	53.5±1.3a
3 - Way ANOVA (F-Statistic)									
Density	13.4***	6.8*	4.4*	102.1***	78.5***	6.8*	4.4*	102.1***	78.5***
Cropping system	13.3***	16.9***	7.1**	67.3***	76.1***	16.9**	7.1**	67.3***	76.1***
Genotypes	1.3ns	1.8ns	1.5ns	2.5ns	3.3ns	1.8ns	1.5ns	2.5*	3.3*

Values (Mean ± SE, n = 4) followed by dissimilar in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. There were significance interactive effects (not shown here) of density and genotypes on $\delta^{15}N$ in pods (in 2005) and cropping systems and genotypes on $\delta^{15}N$ in pods (in 2005 and 2006) and cropping systems and genotypes on %Ndfa in shoots and pods (in 2005 and 2006). Shoots and pods were considered separately. The B value (‰) of cowpea organs were: shoots = -1.759, roots = -0.94, pods = -1.4713, whole plants = -0.6333 (S.B.M. Chimphango and F.D. Dakora, unpublished data). The sorghum reference plant $\delta^{15}N$ values (‰) were: shoots = 9.997, roots = 6.077 in 2005; shoots = 10.320, roots = 5.912 in 2006.

Table 6-4: Cowpea total N, N-fixed and grain yield in different cropping systems and plant densities during 2005 and 2006.

Treatment	Total N (mg.plant^{-1})					N-fixed (mg.plant^{-1})				N-fixed (kgN.ha^{-1})	Grain yield (kg.ha^{-1})
	Nodule	Root	Shoot	Pods	Whole plant	Root	shoot	pods	Whole plant		
2005											
Density (plants.ha^{-1})											
83,000	28.0±2.5a	26.1±2.2a	766.2±55.3a	271.8±33.7a	1092.1±79.3a	7.1±0.5a	490.8±35.3a	116.1±14.5a	614.0±43.4a	51.2±3.6b	1787.5±75.0b
167,000	18.7±1.9b	16.1±1.1b	469.8±26.7b	127.5±14.7b	632.2±35.9b	6.4±0.5a	313.1±17.7b	67.7±7.4b	387.2±20.6b	64.5±3.4a	2339.0±126.6a
Cropping system											
Monoculture	31.2±2.3a	26.2±2.0a	780.3±52.2a	286.0±29.9a	1123.8±72.0a	7.6±0.5a	498.4±33.5a	124.4±12.4a	630.3±39.4a	71.9±3.1a	2387.4±107.5a
Mixed culture	15.5±1.6b	16.0±1.4b	455.7±28.6b	113.3±18.4b	600.6±40.0b	5.9±0.5b	305.4±19.1b	59.5±9.2b	370.8±23.4b	43.8±2.7b	1739.1±92.7b
Genotypes											
Bensogla	20.9±2.0b	21.1±3.2b	603.6±70.4b	220.5±37.3ab	866.1±101.2b	7.8±0.9a	396.2±41.7b	99.4±14.7ab	503.4±50.6b	60.4±6.8b	2063.4±225.7ab
ITH98-46	21.2±4.0b	17.9±2.5b	515.0±62.8b	145.6±32.2b	699.8±95.1b	5.0±0.5b	331.0±37.5b	62.2±10.8b	398.2±45.9c	46.3±5.0c	1596.2±146.0b
Sanzie	34.2±4.6a	30.3±3.4a	867.6±93.0a	214.2±50.0ab	1146.3±134.8a	8.7±0.6a	561.9±58.6a	100.5±21.0ab	671.2±71.0a	75.3±4.2a	2415.0±133.2a
TVu1509	19.7±3.5b	16.3±2.8b	567.6±77.9b	134.9±25.7b	738.5±101.2b	4.5±0.6b	360.5±51.0b	66.1±11.8b	431.1±58.8bc	50.4±5.6bc	2060.9±184.2ab
Omondaw	20.9±2.6b	20.0±1.7b	536.3±52.2b	283.1±62.8a	860.2±105.1b	7.6±0.8a	360.0±36.3b	131.4±27.9a	499.0±56.1b	56.8±4.8b	2180.7±135.6a
3 - Way ANOVA (F-Statistic)											
Density	16.7***	29.2***	69.6***	24.8***	97.7***	1.5	49.8***	12.5***	60.4***	17.0***	22.3***
Cropping system	47.6***	30.3***	83.5***	35.6***	126.5***	9.1**	58.7***	22.5***	79.1***	75.1***	30.9***
Genotypes	5.7***	7.0***	13.0***	3.5*	11.3***	8.4***	10.8***	3.5*	10.4***	9.6***	5.2**
2006											
Density (plants.ha^{-1})											
83,000	28.9±2.7a	28.9±2.1a	856.5±65.6a	266.1±32.3a	1180.5±85.1a	7.9±0.5a	557.2±44.0a	109.9±13.2a	675.7±50.4a	56.3±4.2b	1850.1±87.8b
167,000	19.2±1.9b	18.0±1.0b	550.0±31.9b	127.3±13.9b	714.5±40.1b	7.1±0.5a	369.9±21.1b	65.9±6.5b	442.7±23.5b	73.8±3.9a	2607.6±156.4a
Cropping system											
Monoculture	31.7±2.5a	27.9±2.0a	866.5±56.9	278.1±29.1a	1204.2±73.8a	8.1±0.5a	561.2±38.1a	117.7±11.6a	686.6±42.9a	79.0±3.8a	2677.8±134.6a
Mixed culture	16.4±1.6b	19.0±1.4b	540.0±43.8	115.4±17.2b	690.8±53.1b	7.0±0.5b	365.9±29.8b	58.1±7.9b	431.7±33.3b	51.1±3.5b	1779.9±105.2b
Genotypes											
Bensogla	21.3±2.6b	22.3±3.1b	643.5±67.2b	230.6±37.5ab	917.6±101.5b	8.2±0.8a	427.4±40.6b	100.2±13.9ab	535.8±51.5b	65.3±7.7b	2200.1±250.7ab
ITH98-46	20.3±3.4b	19.9±2.5b	585.5±74.8b	146.0±28.6b	771.8±96.4b	5.7±0.5a	379.6±45.1b	61.5±8.9c	447.3±49.3b	51.4±5.0c	1763.3±241.8b
Sanzie	32.9±4.6a	32.6±3.2a	1026.4±109.4a	171.6±38.8b	1263.6±144.7a	9.6±0.6a	676.9±74.4a	78.7±15.7bc	766.0±85.4a	86.5±5.6a	2654.2±217.1a
TVu1509	22.8±4.6ab	19.1±3.1b	636.9±91.3b	133.6±27.1b	812.4±113.2b	5.2±0.7a	411.0±61.3b	64.2±12.4c	480.4±68.3b	57.1±7.0bc	2210.2±199.2ab
Omondaw	23.1±3.0ab	23.3±1.8b	624.0±56.1b	301.8±62.2a	972.3±110.9b	8.9±0.8a	422.9±39.2b	134.8±25.7a	566.4±58.6b	65.0±5.3b	2316.5±147.3ab
3 - Way ANOVA (F-Statistic)											
Density	14.2***	34.7***	39.1***	30.2***	61.3***	1.7	27.5***	15.0***	34.3***	17.9***	28.0***
Cropping system	35.3***	23.2***	44.3***	41.5***	74.4***	3.7***	29.9***	27.5***	41.0***	45.9***	39.4***
Genotypes	3.1*	6.7***	11.0***	6.1***	8.5***	8.9	9.1***	5.7***	7.8***	8.3***	4.0**

Values (Mean ± SE, n = 4) followed by dissimilar letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$. There were significant interactive effects (not shown here) of density and cropping system on total N and N-fixed in shoot, pods and whole plant (in 2005), and of density and genotypes on total N and N-fixed in whole plant (in 2005).

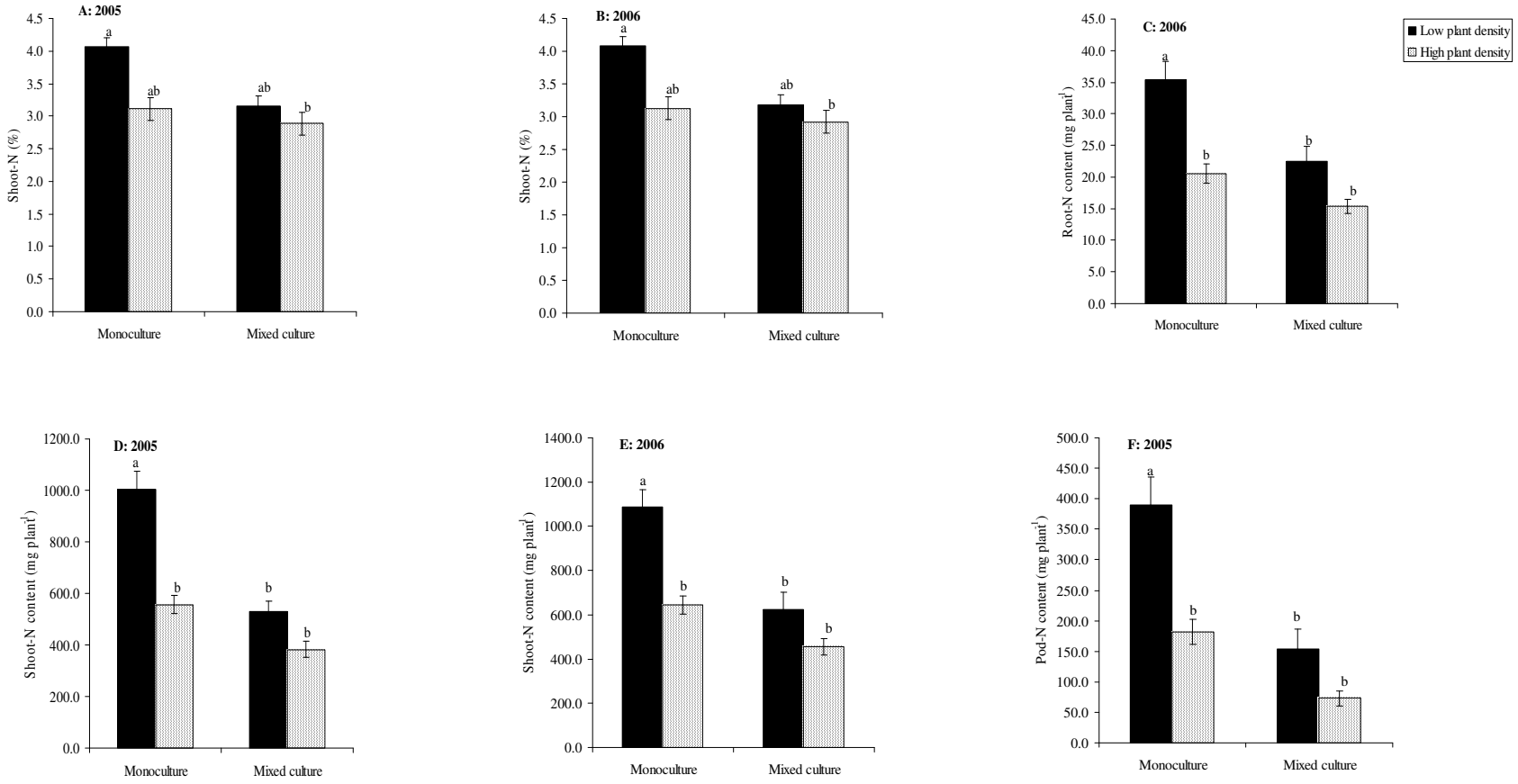


Fig. 6.1: Interactive effects of density and cropping systems on: A) %N in shoots in 2005; B) %N in shoots in 2006; C) Root-N content in 2006; D) Shoot-N content in 2005; E) Shoot-N content in 2006; F) Pod-N content in 2005. Root-N content in 2005 is not shown here as it was not significant.

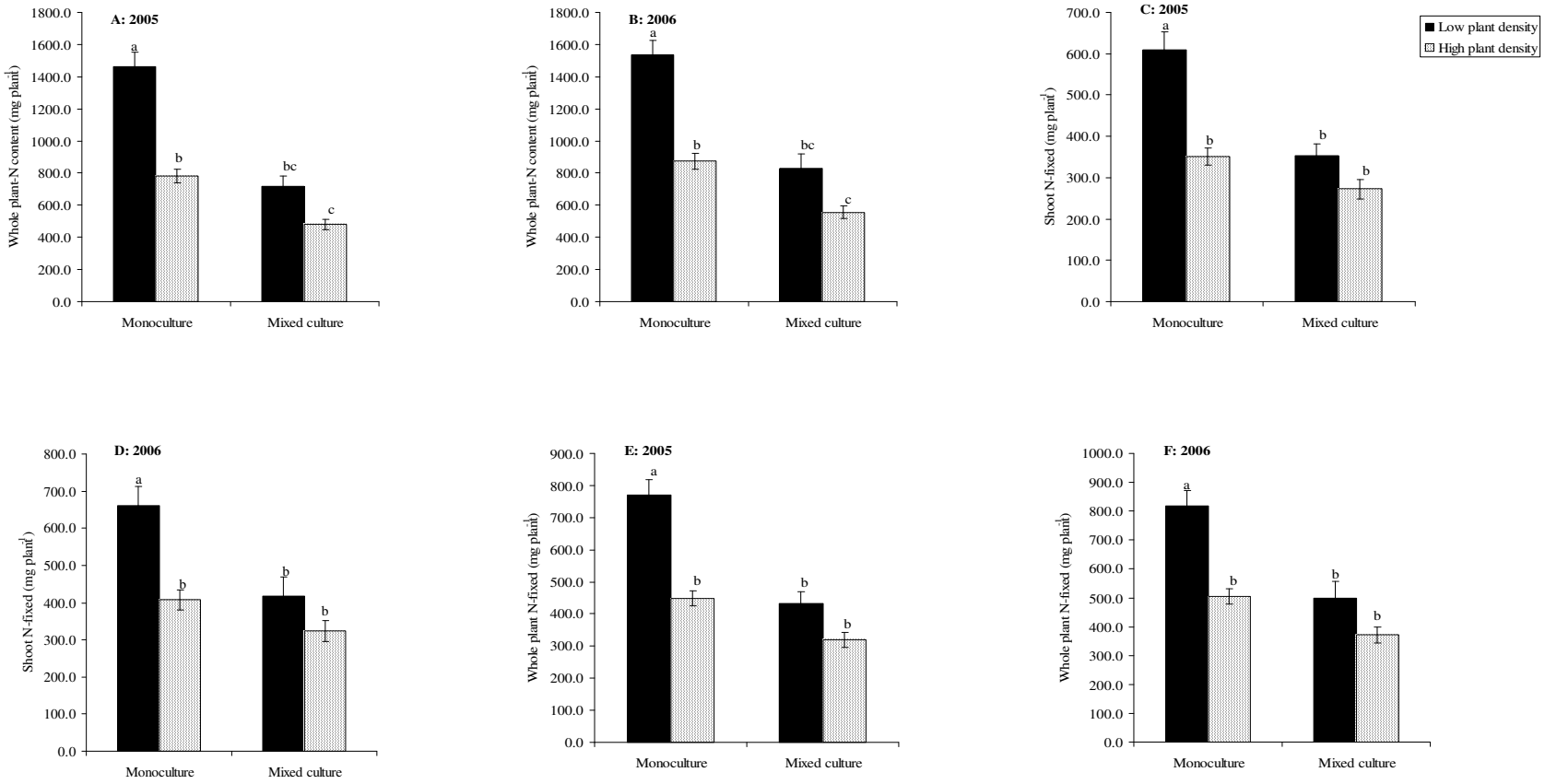


Fig. 6.2: Interactive effects of density and cropping systems on: A) Whole plant-N content in 2005; B) Whole plant-N content in 2006; C) Shoot N-fixed in 2005; D) Shoot N-fixed in 2006; E) Whole plant N-fixed in 2005; F) Whole plant N-fixed in 2006.

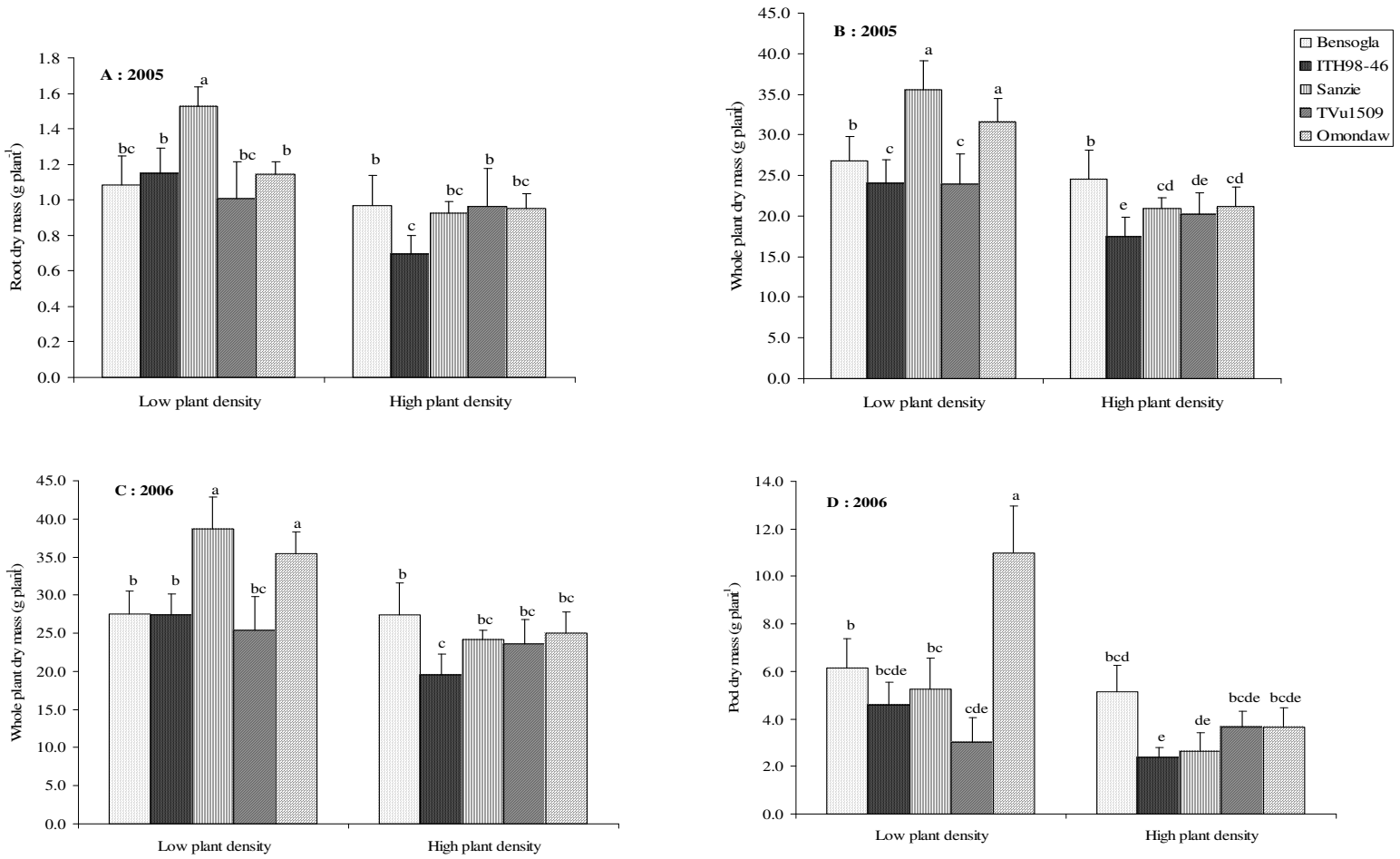


Fig. 6.3: Interactive effects of density and genotypes on crop growth: A) Root dry mass in 2005; B) Whole plant dry mass in 2005; C) Whole plant dry mass in 2006; and D) Pod dry mass in 2006. Root dry mass in 2006 and Pod dry mass in 2005 are not shown here for they were not significant.

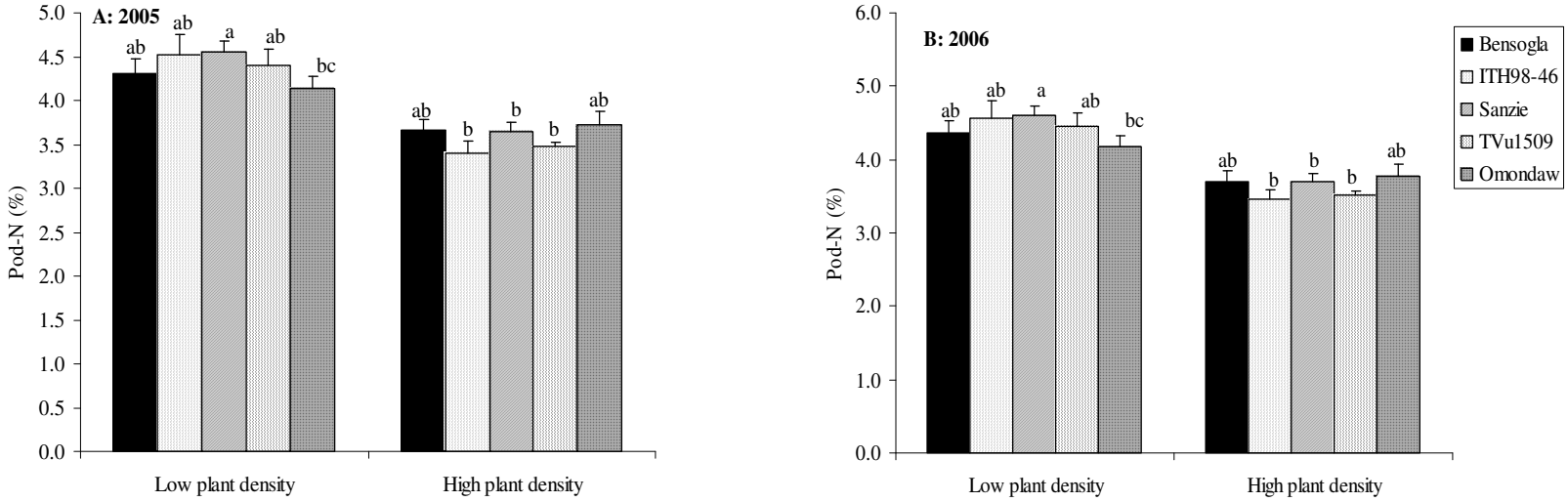


Fig. 6.4: Interactive effects of density and genotypes on: A) Pod-N (%) in 2005; B) Pod-N (%) in 2006.

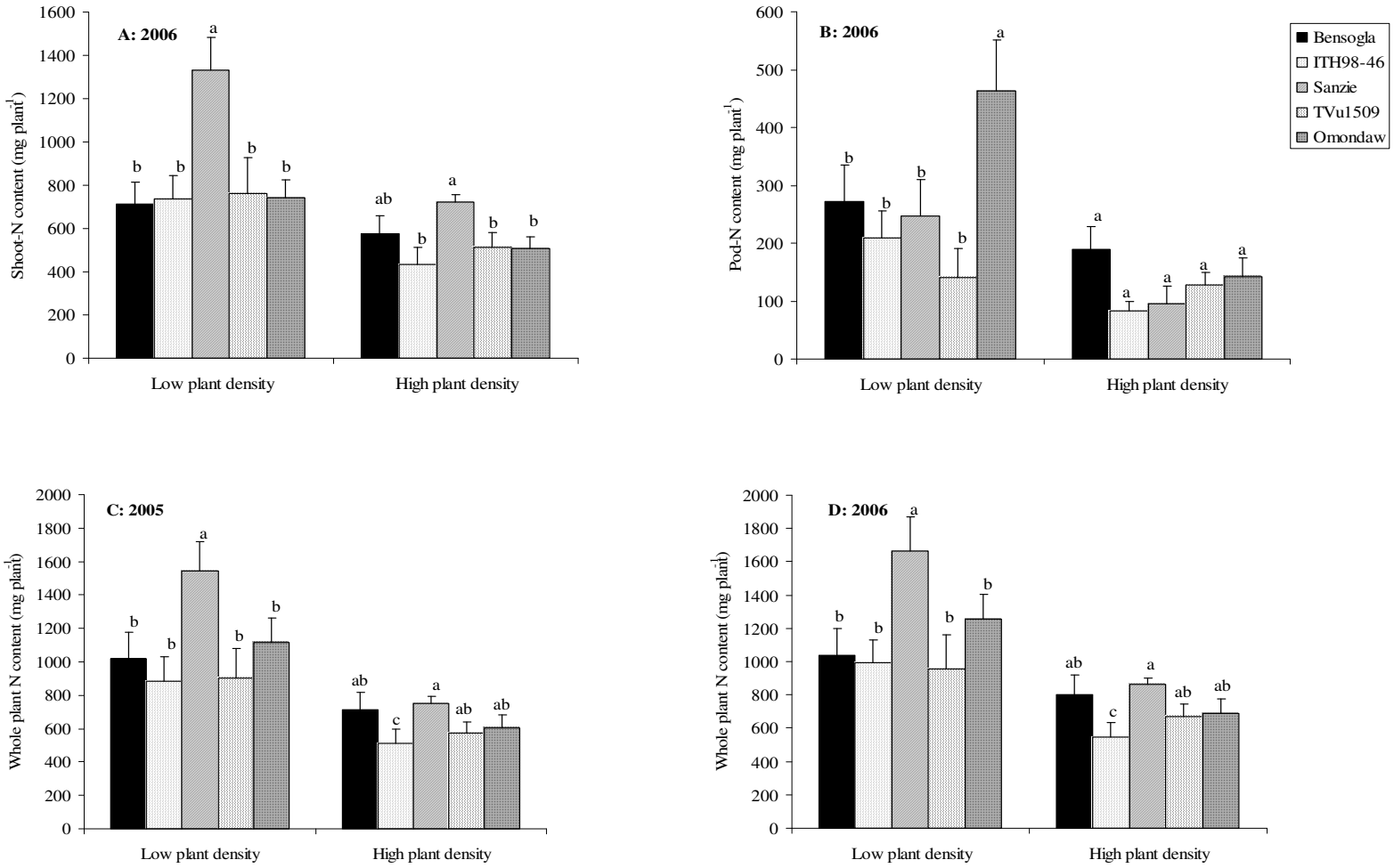


Fig. 6.5: Interactive effects of density and genotypes on: A) Shoot-N content in 2006; B) Pod-N content in 2006; C) Whole plant-N content in 2005 and D) Whole plant-N content in 2006. Shoot-N content and Pod-N content in 2005 are not shown here for they were not significantly different.

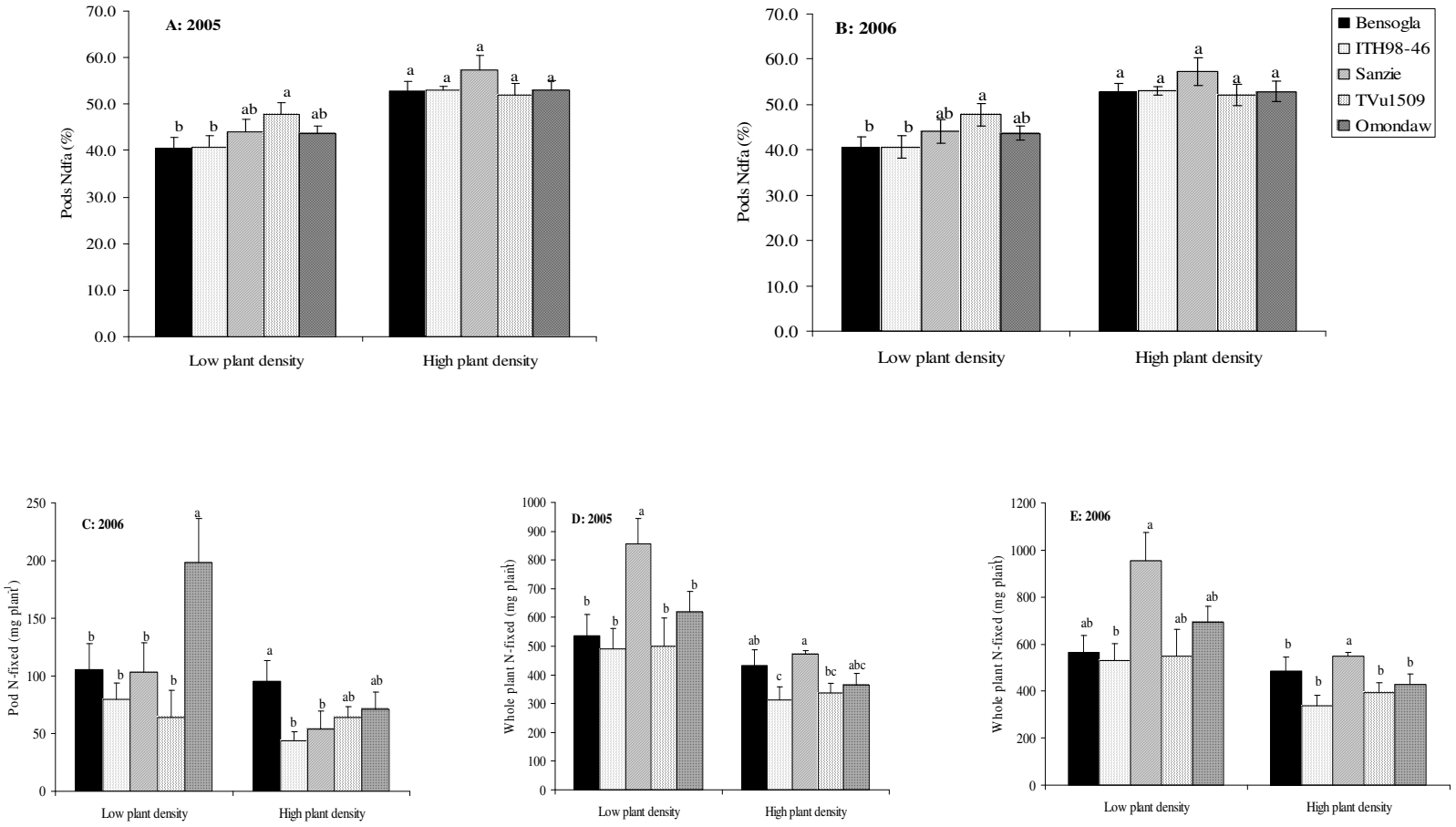


Fig. 6.6: Interactive effects of density and genotypes on: A) Pods Ndfa (%) in 2005; B) Pods Ndfa (%) in 2006; C) Pod N-fixed in 2006; C) Whole plant N-fixed in 2005 and D) Whole plant N-fixed in 2006. Pods N-fixed in 2005 is not shown here for it was not significantly different.

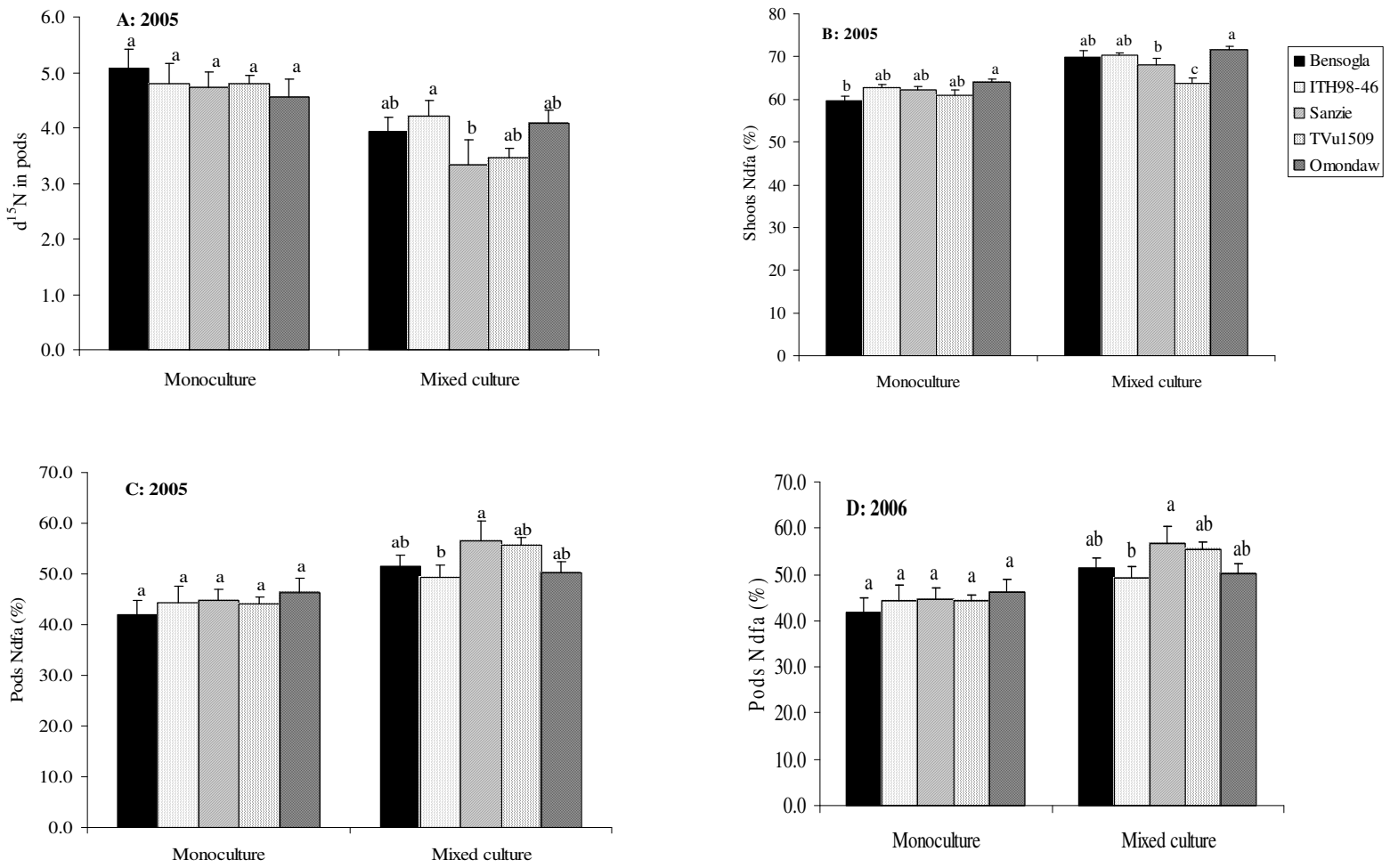


Fig. 6.7: Interactive effects of cropping systems and genotypes on: A) Pod $\delta^{15}N$ in 2006; B) Shoot-Ndfa (%) in 2005; C) Pod-Ndfa (%) in 2005; D) Pod-Ndfa (%) in 2006. Pod $\delta^{15}N$ in 2005 and Shoot-Ndfa (%) in 2006 are not shown here for they were not significantly different.

CHAPTER 7

SEED FLAVONOIDS AND ANTHOCYANINS AS MARKERS OF ENHANCED PLANT DEFENCE IN NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP.)

7.1 Introduction

Flavonoids and anthocyanins are two major classes of biologically-active secondary that are important for seedling development and plant growth (Ndakidemi and Dakora, 2003). Their synthesis and accumulation in plant tissues serve various functions ranging from acting as signals in legume symbiosis with some Rhizobiales to protectants in plant defence (Ndakidemi and Dakora, 2003), and effectors of mineral solubilisation in plant rhizospheres (Dakora and Phillips, 2002). It has been reported elsewhere that cowpea (*Vigna unguiculata* L. Walp) and Bambara groundnut (*Vigna subterranea* L. Verdc) genotypes release significantly different concentrations of flavonoids and anthocyanins when soaked in water or aqueous methanol, and that those molecules probably serve as chemical deterrents to attack by insect pests and pathogens during germination (Ndakidemi and Dakora, 2003). Earlier reports have indicated that legumes defend themselves against insect pests and diseases using isoflavonoids and anthocyanins, either as protectants phytoanticipins or directly as therapeutic phytoalexins against invading pests (Dakora and Phillips, 1996; Dakora, 2003). Cowpea, common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) respectively, use medicarpin, phaseolin, and glyceollin as phytoalexins against pathogens and insect pests (Dakora and Phillips 1996; Dakora, 2003). Other examples include *Lonchocarpus nicou, Mundelia serica,* and *Pachyrrhizus erosus*, which use the isoflavonoids rotenone, munduserone, and pachyrrizone respectively as phytoalexins and insecticides against pathogens and soil-borne insect larvae (Fukami and Nakajima, 1971; Dakora, 2003).

Under field conditions, however, legumes do not use single molecules for defence against insect pests and pathogens. Rather, they use an arsenal of biological ammunition present in tissues as well as in seed and root exudates to fight insect pests and diseases. These compounds include flavonoids, anthocyanins, alkaloids, terpenoids, peptides, amino acids, steroids and/or sugar acids (Ndakidemi and Dakora, 2003). Similarly, nodulating legumes use a potpourri of signal molecules present in seed and root exudates to induce expression of nod genes (Maxwell *et al*., 1989; Hungria *et al*., 1991; Recourt *et al*., 1991; Dakora *et al*., 1993a, b; Gagnon and Ibrahim, 1998), in order to initiate nodule formation. So far however, few studies have examined crude seed extracts as a source of both insecticides and phytoalexins for defence against seed pathogens and/or protection of young seedlings emerging from seeds. Furthermore, few data currently exist on the relationship between the mixtures of protectant molecules present in seed exudates (or tissues) and plant defence, just as little is known about the differences between farmer varieties (i.e. genotypes selected by farmers for useful biological traits) and improved varieties (i.e. cultivars bred for various agronomical traits).

The aim of this study was i) to measure the concentration of flavonoids and anthocyanins present in seed extracts of different cowpea genotypes as markers of identifying materials with effective defence potential, ii) to assess the self defence capacity of selected genotypes against insect pests under field conditions and iii) to test selected genotypes with greater defence potential in the field against agronomic practices such as intercropping and high plant density.

7.2 Materials and methods

7.2.1 Source of germplasm collected

Due to lack of information on cowpea genotypes with high insect pest and disease resistance, 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 pest resistance and grain yield. 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, seed extracts from 45 cowpea genotypes (randomly selected) were then assayed for flavonoids and anthocyanins as it is known that legume seed phenolic compounds can enhance plant defence against insect pests and pathogens. In this study, we report the role of seed flavonoids and anthocyanins in cowpea defence.

7.2.2 Experiment 1: Bioassay of seed extracts from 45 cowpea genotypes for levels of flavonoids and anthocyanins

Seeds of 45 cowpea genotypes with differing seed coat colours were randomly selected from the germplasm collected from various locations and institutions, and ground to fine powder (0.85 mm). About 10 g of ground seed powder (or 0.1 g of shoot) was weighed and mixed with 50 mL (or 10 mL in case of shoot material in centrifuge tubes) of acidified methanol prepared from a ratio of 79:20:1 MeOH H_2O HCl. The mixture was incubated for 72 h in darkness for auto extraction, filtered through Whatman paper Number 2 and absorbance of the clear supernatant measured spectrometrically at 300, 530, and 657 nm using acidified methanol as standard. Concentrations of flavonoids were measured at 300 nm and expressed as Abs $g.DM^{-1}$ (Mirecki and Teramura, 1984), while anthocyanin concentration in seed extracts was measured as Abs530 –1/3Abs657 (Lindoo and Caldwell, 1978) and expressed as Abs $g.DM^{-1}$

7.2.3 Experiment 2: Field survey for pest resistance using minimum spraying on selected cowpea genotypes at Manga, Ghana.

Of the 45 cowpea genotypes tested in Experiment 1, 25 were used in a minimum spray experiment with an insecticide under rainfed conditions at Manga in Ghana, and 33 in Tanzania in order to assess genotype susceptibility or resistance to insect pests. These experiments were conducted using a randomized complete block design with four replicates. Planting was done in January 2005 in Tanzania and in August 2005 in Ghana by placing seeds in drilled holes at the required planting distance. In Tanzania, cowpea was planted on 1.05 x 4 m^2 plots with row-to-row spacing of 35 cm and plant-to-plant spacing of 15 cm. Cowpea plots in Ghana consisted of 4 rows each 3 m long, with row-to-row spacing of 60 cm and plant-to-plant spacing of 20 cm. The treatments included protected (sprayed) plots and unprotected (unsprayed) plots. Protected plots received two sprays of lambda cyhalothrin (Karate 2.5 EC) at flowering and again at podding, while unprotected plots were only sprayed with water as control. During plant growth, records were taken on pest incidence under both protected and unprotected conditions. At early podding, further observations were done on randomly selected cowpea plants for pest resistance. In this study, thrips (*Megalurothrips sjostedti*) infestation was assessed using ten flowers randomly harvested per plot at 50% flowering (i.e. duplicate samples each consisting of 5 flowers). The number of pod-sucking bugs was also determined at early podding and at mid pod development. At harvest, the total number of pods per plot was recorded, including those damaged by pod-sucking bugs.

7.2.4 Experiment 3: Evaluating five cowpea genotypes for pest resistance and phenolics under intercropping and high plant density

7.2.4.1 Background

In this study, five cowpea genotypes (namely, Bensogla, ITH98-46, Sanzie, TVu1509 and Omondaw) were selected from Experiments 1 and 2, and further tested in the field using intercropping and high plant density as treatments to assess pest attack and tissue accumulation of flavonoids and anthocyanins. Of the five cowpea genotypes tested, three (i.e. Bensogla, Sanzie and Omondaw) consistently showed high concentrations of flavonoids in seed extracts with potential for greater pest resistance, while the other two (i.e. ITH98-46 and TVu1509) exhibited low flavonoid levels in seed extracts with potential for low pest resistance.

7.2.4.2 Site description and experimental design of Experiment 3

Site description and experimental design of experiment 3 is as reported in Chapter 2, sections 2.2.1 and 2.2.2 respectively

7.2.4.3 Plant harvest for determining tissue concentration of flavonoids and anthocyanins

At 67 DAP, 16 cowpea plants were harvested from the middle rows of each plot. The plants were carefully dug out with intact root systems, washed, and separated into nodules, roots, shoots and pods. The plant organs were oven-dried at 60°C for 48 h, weighed, ground into fine powder (0.85 mm) and stored prior to bioassay for flavonoids and anthocyanins, as described above in Experiment 1.

7.2.4.4 Statistical Analysis

A 3-factorial arrangement involving plant density, cropping system and cowpea genotypes was used to statistically analyze the data collected in this experiment. The analysis was performed using STATISTICA software (StatSoft Inc., 2007 Tulsa, OK, USA). One-Way ANOVA analysis was used to compare treatment means of the metabolites in plant organs and genotypes. Fisher's least significant difference test was used to compare treatment means at $P \leq 0.05$ (Steel and Torrie, 1980). Correlation analysis (or Student's *t*-test method) were also used to statistically determine the relationship between flavonoids/anthocyanins in seed extracts and number of insect pests (e.g. aphids, thrips or pod-sucking bugs).

7.3 Results

7.3.1 Concentrations of flavonoids and anthocyanins in seed extracts of cowpea genotypes

The flavonoids and anthocyanins (Abs.g DM^{-1}) in seeds of 45 cowpea genotypes were extracted in aqueous methanol (10 g of seed in 50 mL of acidified MeOH), and their concentrations measured spectrophotometrically. The data showed that flavonoid concentration ranged from 1.07 in cv. Ex-Zepisa to 7.45 Abs.g DM^{-1} in Sanzie (Table 7.1). The concentration of anthocyanins also ranged from 0.00 in cv. IT94D-437-1 to 1.32 Abs.g DM^{-1} in cv. Kisangata (Table 7.1). There were thus significant differences in the levels of flavonoids and anthocyanins in seeds of the different cowpea genotypes (Table 7.1).

7.3.2 Effect of minimum insecticide spraying on selected cowpea genotypes for insect pest resistance

Experiment 2 assessed insect pest infestation and the effect of minimum spraying on yield of cowpea genotypes in Ghana and Tanzania (Tables 7.2 and 7.3). The data showed that thrips and pod sucking bugs were the two most important pests of cowpea at Manga in Ghana, as the number of thrips varied from 13 to 39 per five flowers, while pod-sucking bugs ranged from 2 to 8 adults per row (Table 7.2). The main pod-sucking bugs observed on cowpea plants were *Clavigralla spp*. Stal. (Hemiptera: Coreidae), *Anoplocnemis curvipes* Fab. (Hemiptera: Coreidae), *Riptortus dentipes* Fab. (Hemiptera: Alydidae) and *Aspavia armigera.* The farmer-selected varieties, Omondaw and Bensogla, had relatively lower infestation levels of thrips and pod-sucking bugs, with these farmer-selected cultivars recording less than 4 thrips per 5 flowers relative to improved varieties, which had over 7 thrips per 5 flowers. The sprayed cowpea varieties showed higher grain yield compared with their unprotected counterparts. The farmer-selected varieties Sanzie, Omondaw and Bensogla, produced more grain yields without insecticide spray compared with the two least yielding breeder cultivars, ITH98-46 and TVu1509 (Table 7.2). As a result, yield increases from protection with chemical insecticide were lowest in the three farmer-selected varieties.

In Tanzania, the cowpea genotypes tested also showed marked differences in response to infestation by aphids and alcidodes (Table 7.3). While a genotype like Za-Kutambaa showed as high as 84% infestation by aphids, IT90K-284-2 revealed zero aphid infestation (Table 7.3). Similarly, Fahari showed 33% infestation to alcidodes, in contrast to only 2% in IT97K-499-39 (Table 7.3).

7.3.3 Effect of intercropping and plant density on pest resistance, and concentration of flavonoids and anthocyanins in organs of cowpea.

The concentration of flavonoids and anthocyanins differed significantly with cropping systems and plant density. For example, high plant density and mixed culture significantly increased the levels of flavonoids and anthocyanins in seeds and whole plant tissue (Fig. 7.1). Compared with the results obtained at Manga in Ghana, there was very little insect pest infestation in this experiment in South Africa.

To establish whether differences in pest resistance exhibited by the cowpea genotypes was caused by tissue concentration of flavonoids and anthocyanins, extracts of cowpea organs were analysed for the two phenolics. The data showed that, relative to cultivar ITH98-46, Sanzie exhibited greater concentrations of flavonoids and anthocyanins in seeds and whole plants in both 2005 and 2006 (Table 7.4). Sanzie,

Bensogla and Omondaw also showed much greater flavonoid and anthocyanin concentration in seeds compared with ITH98-46 and TVu1509 (Table 7.4).

7.3.4 Correlation and regression analyses between the levels of flavonoids and anthocyanins in seed/plant extracts vs. insect pest infestation.

Data from experiments conducted in Ghana and Tanzania were used in various correlation analyses to establish a link between flavonoid/anthocyanin concentrations in seed extracts and insect pest incidence. We found that as the concentration of seed flavonoids and anthocyanins decreased, the number of thrips per flower and the number of pod-sucking bugs per row increased significantly in plants raised from those seeds in Ghana (Table 7.5). Similarly, in Tanzania, we showed that as the levels of anthocyanins and flavonoids got lower in cowpea seed, the number of progenies attacked by aphids and alcidodes rose significantly (Table 7.5). Taken together, the data suggested an inverse relationship between the level of phenolics in seed and progeny attack by thrips, aphids, alcidodes and pod-sucking bugs.

7.4 Discussion

In this study, 45 cowpea genotypes were assayed for flavonoids and anthocyanins with the objective of quantifying the levels of these phenolics in seed extracts as markers for identifying material with effective plant defence. The results revealed significant differences in the concentration of flavonoids and anthocyanins in seed extracts, with farmer varieties such as Sanzie, Bensogla and Omondaw exhibiting much higher levels compared with improved genotypes like ITH98-46, TVu1509 and IT93K-452-1 (Table 7.1). Because seeds of grain legumes such as soybean, common bean, pea, mucuna, groundnut and chickpea store the isoflavonoids, hydroxyphaseollin, phaseollin, pisatin, dimethylhomopterocarpin, kievitone and maackiain respectively for defence against insect pests and pathogens (Keen *et al*., 1971; Sims *et al*., 1972; Naim *et al*., 1974; Keen, 1975), and common bean uses the anthocyanin, pelagonidin-3-glucoside, to fight soil-borne pests during germination (Stanton and Francis, 1966), it is likely that the variation in seed phenolic concentrations observed here could reflect different levels of resistance and susceptibility. However, the flavonoids and anthocyanins present in seed extracts (Harborne and Turner, 1984) of the 45 different cowpea genotypes could also serve as defence molecules against abiotic stresses such as UV-B radiation, drought, and low or high temperatures (Chalker-Scott, 1999).

Possibly because of the marked differences in seed phenolic metabolites (Table 7.1), cowpea plants raised from those seeds also differed in their levels of infestation by thrips, pod-sucking bugs, aphids and alcidodes when planted under field conditions in Ghana and Tanzania (Tables 7.2 and 7.3). This would be

consistent with the finding that the presence of proanthocyanins and the isoflavonoid glycitin in seeds of cowpea and soybean provided resistance to infestation by the legume weevils *Callosobruchus maculatus* and *Acanthosescelides obtechus* (Oigiangbe and Onigbinde, 1996; Reegnault-Roger *et al*., 1999). Although in this study, the grain yield was generally low due to poor rainfall, there were nevertheless marked differences between protected (with insecticide) and unprotected treatments (Table 7.2). Providing minimum protection with insecticide spray decreased insect pest infestation and increased grain yield across the board in all genotypes. However, cultivars such as Bensogla, Omondaw and IT86D-2075 (without spray) showed relatively lower infestation by thrips and pod-sucking bugs, thus indicating natural resistance of some genotypes to insect pests. Increase in grain yield from plant protection with minimum spray of insecticide was also lower in the farmer-selected genotypes, further confirming their natural resistance to insect pests (Table 7.2). It was also noteworthy that the farmer-selected varieties produced more grain yield without protection than the improved varieties. This superior pest resistance by the farmer varieties (i.e. lower infestation levels and greater grain yield from unprotected genotypes) relative to improved varieties such as ITH98-46 and TVu1509 was confirmed by the higher concentration of flavonoids and anthocyanins in seed extracts (Table 1) and in tissues of plants grown as an intercrop or at high plant density (Table 7.4, Fig. 7.1). This implies that the insect pests were deterred by the high tissue concentrations of flavonoids and anthocyanins (Hoffmann-Campo *et al*., 2001) originating from parental seed and from synthesis by progenies raised from those seeds.

Data from correlation analysis (Table 7.5) also provided a direct relationship between tissue concentration of flavonoids/anthocyanins and plant defence, in that, the higher the concentration of defence molecules in cowpea seed extracts, the lower was the insect pest incidence on plants raised from those seeds. Our suggestion that seed and tissue concentrations of flavonoids/anthocyanins were probably responsible for the suppression of insect pests on some cowpea plants (whether measured as number of thrips per flower, pod-sucking bugs per row, or percentage of plants infested by aphids and alcidodes) is consistent with earlier findings (Sutherland *et al*., 1980; Wang *et al*., 1998), which showed that isoflavonoids present in legume roots and leaves provided protection against attack by beetles (*Costelytra zealandica*) and red legged earth mites (*Halotydeus destructor*). More specifically, legumes such as lotus, cowpea, soybean, common bean, and pigeon pea are known to respectively synthesize and accumulate the isoflavonoids vestitol, medicarpin, glyceollin, phaseolin, and cajanin as insect pest deterrents (Russell *et al*., 1978; Dakora and Phillips, 1996), while species of *Lonchocarpus, Derris, Mundulea, Pachyrrhizus* and *Neoratanenia* produce the deadly isoflavones rotenone, deguelin, munduserone, pachyrrhizone and dolineone, respectively, as potent insecticides for defence against insect pests (Fukami and Nakajima, 1971).

In this study, the absence of insect attack observed under conditions of intercropping and high plant density could be attributed to the relatively increased concentration of flavonoids and anthocyanins in plant organs under those cultural conditions (Fig. 7.1A, B, C, and D). This finding could explain the data by Ampong-Nyarko *et al*. (1994) which showed a reduction in insect pest infestation with intercropping of cowpea and sorghum. In another study, the population of aphids (*Aphids craccivora* Koch.) and thrips (*Megalarothrips sjostedti* Trybom) decreased significantly with intercropping of cowpea and sorghum relative to monoculture (Hassan, 2009). As shown in this study, the finding by Hassan (2009) could be attributed to increased accumulation of flavonoids and anthocyanins when the species were planted in mixed culture. Furthermore, our data showed significantly higher concentration of flavonoids and anthocyanins in farmer-selected varieties (i.e. Sanzie, Bensogla and Omondaw) relative to improved cultivars. This could be attributed to better adaptation by the farmer varieties to intercropping and/or high plant density.

In conclusion, we have shown that the concentration of flavonoids and anthocyanins in cowpea seed extracts differed markedly among 45 genotypes tested, with farmer-selected varieties (e.g. Sanzie, Bensogla and Omondaw) showing higher levels of phenolic metabolites relative to improved genotypes. The higher concentrations of these compounds in seed extracts resulted in lower incidence of insect pests among progenies raised from those seeds. Intercropping and high plant density also increased tissue concentration of flavonoids and anthocyanins, and this could explain the lower incidence of insect pests and diseases observed under intercropped conditions (Ampong-Nyarko *et al*., 1994, Hassan, 2009). The farmer-selected varieties appeared better adapted to intercropping in terms of accumulating higher concentrations of flavonoids and anthocyanins for defence against insect pests. Correlation analysis further confirmed a direct relationship between high flavonoids/anthocyanins in seed extracts and enhanced resistance against insect pest (measured as a decrease in number of insect pests per flower, pod-sucking bugs per row or percentage of plants infested by aphids or alcidodes).

Table 7.1: Concentration of anthocyanins and flavonoids released by imbibing seeds of different cowpea varieties in aqueous methanol (10 g seed in 50 mL of acidified MeOH) during 2005.

Accession No.	Cowpea genotypes	Seed coat colour	Flavonoids	Anthocyanins
			(Abs.g DM^{-1})	
1	IT95K-1093-5	Light brown/brown	2.80hi	0.12stuv
2	IT95K-238-3	Cream	1.29zd	0.05wxyz
3	IT95K-1453-47	Cream	2.07u	0.42f
4	IT97K-497-2	Cream	2.34qr	0.04xyz
5	IT97K-1068-7	Brown	2.58m	0.31hijk
6	IT97K-499-38	Cream	2.30rs	0.41fg
7	IT97K-499-39	Cream	2.36q	0.33hij
8	IT97K-499-39	Light brown/brown	2.52n	0.15qrst
9	IT95K-1090-12	Light yellow	2.76ij	0.16qrs
10	IT95K-1156-3	Light red	1.42zb	0.30ijkl
11	IT93K-686-2	Light yellow	2.71k	0.16qrs
12	IT90K-284-2	Cream	2.93g	0.04xyz
13	IT94K-2023-3	Cream	2.07u	0.10tuvw
14	IT95K-286-4	Cream	1.77y	0.08uvwx
15	IT93K-2045-29	Cream	2.75jk	0.42f
16	IT96D-651	Cream	1.95w	0.25lmno
17	Kisangata	Cream	2.66l	1.32a
18	IT96D-618	Cream	2.19t	0.00z
19	IT94D437-1	Cream	1.79xy	0.00z
20	IT95K-627-34	Cream	1.83x	0.01z
21	IT93K-452-1	Cream	4.26e	0.60d
22	IT94K440-3	Cream	2.09u	0.26klmn
23	IT94K410-1	Cream	1.65z	0.46ef
24	Vuli-1	Dark red/purple	2.24s	0.81b
25	Line 2020	Brown	2.65l	0.13rstu
26	Kaputura	Brown	2.26rs	0.17pqrs
27	Chora	Brown	2.15t	0.13rstu
28	Ngonji	Brown	2.30rs	0.15qrst
29	Mamlaka	Brown	2.25s	0.22nop
30	Tumaini	Brown	2.27rs	0.18pqr
31	Fahari	Mixture	1.39zbc	0.02yz
32	Mchanganyiko-1	Mixture	1.47za	0.49ef
33	Mchanganyiko-2	Mixture	1.36zc	0.15qrst
34	Mchanganyiko-3	Mixture	2.43p	0.28jklm
35	Mchanganyiko-4	Mixture	2.01v	0.32hij
36	Ex-Chamwino	Mixture	2.41p	0.14rst
37	Ex-Zepisa	Mixture	1.07zg	0.45ef
38	Za-Kutambaa	Mixture	1.17zf	0.74c
39	Hombolo Makulu	Mixture	2.84hi	0.20opq
40	Mchanganyiko	Mixture	2.24s	0.36gh
41	Bensogla	Mixture	5.49c	0.24mno
42	ITH98-46	Mixture	4.93d	0.07vwxy
43	Sanzie	Mixture	7.45a	0.34hi
44	TVu 1509	Mixture	3.85f	0.04xyz
45	Omondaw	Cream	6.91b	0.14rst
One-Way ANOVA (F-Statistic)				
Reps			6328.8**	143.5**
CV (%)			1.1	13.7

Values followed by different letters in a column are significantly different at **: $P \leq 0.01$.

Table 7.2. Insect pest infestation and effect of spraying with minimum insecticide application on grain yield of 25 cowpea genotypes grown at Manga, Ghana, in 2003.

Genotype	Pest infestation		Grain yield		Yield increase from spraying
			kg.ha^{-1}		%
	No. of thrips per 5 flowers	No. of PSB per row	Protected	unprotected	
Bensogla	15.0mn	2.0e	444.4ijkl	240.7c	84.6mn
ITH98-46	24.7hi	6.3ab	370.3klmn	222.2c	66.7n
Sanzie	27.3efg	5.7abc	937.0ab	351.8a	166.3kl
TVu 1509	19.7jk	5.0cde	555.5fgh	85.2lmn	552.0c
Omondaw	17.3klm	3.0cde	592.5efg	311.1b	90.5mn
ITH98-20	18.7jk	3.3cde	388.8jklmn	181.3de	114.5lmn
ITH98-24	12.7no	4.7cde	518.5ghi	166.6def	211.2jk
ITH98-45	16.0lm	3.7cde	555.7fgh	148.1fgh	275.2ghi
ITH98-48	33.7cd	2.3de	851.8bc	185.2d	359.9ef
ITH98-49	33.0d	8.7a	814.8c	177.7de	360.0ef
TVX 3236	26.0fgh	4.0cde	907.4bc	118.5ijk	666.0b
Vita 7	35.7bc	2.3de	629.6def	74.0mn	750.0a
Bengpla	18.7jk	3.3cde	592.5efg	129.6ghij	357.2ef
Vallenga	39.34a	6.3ab	841.8bc	166.6def	405.3de
Brown eye	22.7i	2.0e	1037.0a	185.1d	460.2d
Tilli local	19.3jk	4.3cde	666.6de	133.3ghi	400.0de
IT86D-1951	13.0no	2.3de	481.4hij	111.1ijkl	333.3fg
IT90K-277-2	28.7e	3.3cde	462.9hijk	118.5ijk	290.6gh
IT87D-1951	17.7jkl	2.0e	351.8lmn	100.0klm	251.8hij
Adom	37.3ab	3.0cde	703.7d	155.5efg	352.5ef
Soronko	25.7gh	5.0cde	833.3c	122.2hijk	581.9c
IT86D-566-6	28.3ef	4.7cde	333.3mno	62.9n	429.9d
Ayiyi	20.0j	6.3ab	425.9ijklm	129.6ghij	228.6ij
IT86D-1010	19.7jk	2.7de	240.7o	103.7jkl	132.1lm
IT86D-2075	12.3p	2.0e	307.4no	137.0ghi	124.4lmn
One-Way ANOVA (F-Statistic)					
Rep	87.3**	2.0*	36.9**	48.1**	75.0**
CV (%)	6.3	54.6	10.6	10.9	11.5

Values followed by different letters in a column are significantly different at *: $P \leq 0.05$; **: $P \leq 0.01$. Grain yield values represent harvests from 3 inner rows of 3 plots per trial. PSB = pod-sucking bugs.

Table 7.3. Level of insect pest infestation among 33 cowpea genotypes grown in the field in Tanzania in 2003.

Variety	% of plants infested by	
	Aphids	Alcidodes
IT95K-1095-5	61.0c	19.0de
IT95K-238-3	18.0j	13.0f
IT95K-1453-47	6.0r	18.0de
IT97K-497-2	21.0c	7.0hij
IT97K-1068-7	17.0k	17.0e
IT97K-499-38	2.0u	7.0hij
IT97K-499-39	6.0r	2.0l
IT97K-499-39	49.0d	20.0cd
IT95K-1090-12	12.0n	6.0ij
IT95K-1156-3	3.0t	5.0jk
IT93K-686-2	15.0l	13.0f
IT90K-284-2	0.0w	3.0kl
IT94K-2023-3	13.0m	23.0b
IT95K-286-4	10.0o	13.0f
IT93K-2045-29	29.0f	19.0de
IT96D-651	2.0u	13.0f
Kisangata	17.0k	12.0f
IT96D-618	27.0g	13.0f
IT94D437-1	3.0t	3.0kl
IT95K-627-34	23.0h	12.0f
IT93K-452-1	49.0d	11.0fg
IT94K440-3	23.0h	9.0gh
IT94K410-1	9.0p	11.0fg
Vuli-1	8.0q	8.0hi
Line 2020	2.0u	6.0ij
Kaputura	6.0r	6.0ij
Chora	5.0s	13.0f
Ngonji	1.0v	24.0b
Mamlaka	6.0r	17.0e
Tumaini	8.0q	9.0gh
Fahari	33.0e	33.0a
Mchanganyiko	67.0b	22.0bc
Za-Kutambaa	84.0a	24.0b
One-Way ANOVA (F-Statistic)		
Rep	4123.8**	70.8**
CV (%)	2.9	11.4

Values followed by different letters in a column are significantly different at **: $P \leq 0.01$

Table 7.4. Plant growth and concentration of flavonoids and anthocyanins in seed and plant rinses of five cowpea genotypes grown in the field at Stellenbosch, South Africa. Concentration of flavonoids and anthocyanins were measured in aqueous methanol (0.1 g of plant sample in 10 mL of acidified MeOH).

Treatment	Plant growth g.plant^{-1}	Flavonoids Abs.g DM^{-1}		Anthocyanins Abs.g DM^{-1}	
		Seeds	Whole plant	Seeds	Whole plant
Genotypes					
Bensogla	26.6±2.4ab	8.6±0.2a	36.7±2.7ab	0.42±0.08abc	0.93±0.20a
ITH98-46	22.1±2.0b	8.2±0.3b	30.7±2.3c	0.22±0.07c	0.47±0.12b
Sanzie	29.8±2.7a	8.7±0.3a	39.1±2.5a	0.57±0.08a	1.05±0.15a
TVu1509	23.3±2.5b	8.2±0.2b	33.8±2.4bc	0.33±0.08bc	0.76±0.14ab
Omondaw	28.3±2.3ab	8.8±0.3a	37.5±3.2ab	0.49±0.10ab	0.99±0.21a
One-Way ANOVA (F-Statistic)					
Genotypes	3.8**	248.0*	3.2*	325.4**	2.7*

Values of metabolites were taken at peak light absorbance within the visible light spectrum. Values followed by different letters in a column are significantly different at *: $P \leq 0.05$, **: $P \leq 0.01$. Whole plant values are combined average values of roots, shoots and pods. These data were collected in 2005 and 2006 and combined for statistical analysis.

Table 7.5. Correlation analysis of phenolic metabolites vs. insect pest infestation in field experiments conducted in Ghana and Tanzania.

Correlation parameters	Location	Year	Significance level
Seed flavonoids vs. thrips per flower	Manga, Ghana	2003	0.12*
Seed anthocyanins vs. pod sucking bugs per row	Manga, Ghana	2003	0.38**
Seed flavonoids vs. pod sucking bugs per row	Wa, Ghana	2003	0.19*
Seed anthocyanins vs. plants infested with aphids	Morogoro, Tanzania	2003	0.13*
Seed flavonoids vs. plants infested with alcidodes	Morogoro, Tanzania	2003	0.13*

* = significant at $P \leq 0.05$, ** = significant at $P \leq 0.01$.

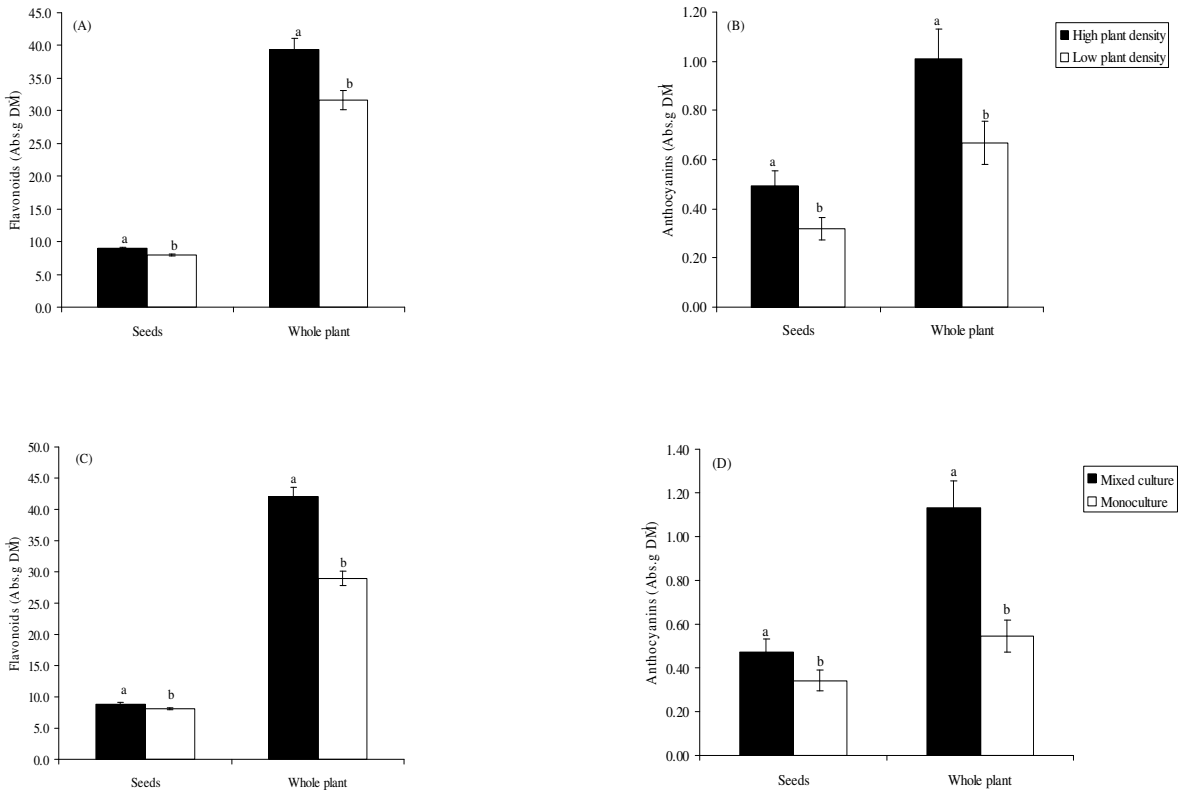


Fig. 7.1: Effect of plant density on the concentration of A) Flavonoids, B) Anthocyanins and cropping systems on C) Flavonoids, D) Anthocyanins in seed and whole plant. Data for 2005 and 2006 were combined since they were similar.

8.1 GENERAL DISCUSSION

High plant density (167,000 plants.ha^{-1}) and mixed culture (intercropping) compared with low plant density (83,000 plants.ha^{-1}) and monoculture (monocropping) system, reduced chlorophyll contents and gas-exchange parameters (Chapter 2); pH and mineral elements concentration in the rhizosphere and tissue uptake (Chapter 3) and yield components (Chapter 5) in cowpea genotypes and sorghum species. On the contrary, the data showed increased acid and alkaline phosphatases in root tissues and rhizosphere soil (Chapter 4), symbiotic performance (Chapter 6), as well as flavonoid and anthocyanin concentrations in seeds/plant organs (Chapter 7). The data also indicated species and genotype variation in response to high plant density and mixed culture system in the measured parameters. These results contribute to the body of knowledge in soil fertility and plant nutrition, plant protection, agricultural production and management, food nutrition and the environment.

Photosynthesis is the major biosynthetic process on earth, fixing about 100 billion tons of carbon annually into net primary production, the basis of all our food and fibre. Earth's surface receives 4.3 x 10^{20} J of solar energy per hour but consumes only 4.1 x 10^{20} J per year (Zhu *et al*., 2008). Despite this quantity, solar energy is diffuse, emphasising the overall efficiency of photosynthetic solar energy conversion in cropping systems. In this study, results have showed that gas-exchange parameters (i.e. photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and stomatal conductance), chlorophyll contents, $\delta^{13}C$, C and WUE were lowered at high plant density and mixed culture system. Decreased photosynthetic rate due to increased density and mixed culture suggest that there is less efficient use of light resources or photosynthetic solar energy conversion for yield formation and grain yield increase. This means that grain yield and financial gain will be adversely affected for both farmers and the general economy. By understanding photosynthesis in mixed cultures, one could design better means to improve the rate of photosynthesis, reduce respiration rate and improve the source sink capacity to sustain high photosynthesis rate or photosynthetic solar energy conversion through the choice of appropriate genotypes and cropping patterns for increased grain yield. Additionally, the higher WUE in sorghum compared with cowpea species, as well as the improved cowpea genotypes compared with farmer-selected cultivars, suggests that such species have less ^{13}C discrimination and could be used in soil moisture stressed environments.

Although high plant density and mixed culture reduced cowpea and sorghum grain yield in this study, reduction was not large enough to decrease the total grain yield of the mixture relative to those of either crop component, as the land equivalent ratio was greater than 1 (LER > 1), suggesting mixed culture (intercropping) advantage over monoculture system. Similarly, since the grain yield of cvs. Sanzie and Omondaw was unaffected by mixed cultures, this suggests that these cowpea genotypes can be included in

mixed culture to improve and increase grain yield in low input agricultural production systems in Africa. Increased cowpea grain yield will not only have implications for farmers' income but also for their nutritional and health status due to the high food value contained in both cowpea and sorghum crops. Healthy farmers are able to participate fully and efficiently in their economic activities, thus, increasing their income and reducing poverty. In addition, the natural flavonoids and anthocyanins contained in the seed grains of cowpea and sorghum species have been associated with longer life expectancy. In this regard, since cowpea and sorghum are staple foods in Africa, growing them in mixed culture may not only increase total grain yield but also will be the main source of natural antioxidants for both food nutrition and income generation in Africa.

In this study, symbiotic performance, acid and alkaline phosphatase activities as well as flavonoids and anthocyanins concentrations increased at high plant density and mixed culture. The increase in these parameters, however, reflects a high demand for plant growth factors leading to stress, thus, greater implications in cropping systems. For example, increased symbiotic performance in cropping systems would mean increased N_2-fixed to such system, thus, making it possible for less use of artificial N fertiliser for plant growth. These processes may have an implication in soil fertility, plant nutrition, and environment. For example, gross terrestrial biological nitrogen fixation (BNF) has been estimated up to 290 million tons of N per year of which about 16.6% is fixed by field agricultural crops (i.e. including nodulated cowpea genotypes) compared with 28.6% fixed industrially in fertiliser production (Cleveland *et al*., 1999; Jenkinson, 2001). Even though artificial N fertiliser contributes to increased productivity in cropping systems, it is so at the cost of environmental degradation (Jensen and Hauggaard-Nielsen, 2003). In addition, since atmospheric N_2 is a renewable resource, BNF is a sustainable source of N in cropping systems (Bohlool *et al*., 1992). So, the role of BNF on N cycling, ammonia volatilisation, N_2O emission and NO_3 leaching suggests that BNF is much more environmentally friendly compared with industrial N fertiliser applied for plant growth in cropping systems. In contrast to the large amount of fossil energy used for the industrial fertiliser N production process, the energy input for BNF is virtually free of charge synthesised from photosynthesis. Inclusion of nodulated cowpea genotypes in cropping systems will therefore contribute significantly to the supply of N through BNF with great implications for our cropping systems and the environment. Furthermore, rhizosphere acidification (i.e. low pH) observed during N_2-fixation by cowpea plants may suggest greater benefits in alkaline soils by solubilisation of mineral elements which would otherwise remain fixed, thus, increasing mineral elements availability for plant growth, as well as reducing the vagaries of salinity in salt-affected areas. Furthermore, the observed increase in acid and alkaline phosphatase activities in this study suggests a direct relationship between these activities and P supply in P-deficient soils. The assay of acid phosphatase activity in roots and rhizosphere of cowpea therefore, can be used as a tool to select cowpea genotypes (and other legumes) for

P uptake efficiency. On the other hand, increased flavonoid and anthocyanin concentrations in seed/plant organs suggest increased nutritive value in grain yield, a viable commercial source of good antioxidants, resistance to pests and diseases, control of weeds, photo-protection, stress for mineral elements and signal molecule in symbiotic nitrogen fixation.

The significant variation between farmer-selected cowpea genotypes (i.e. Bensogla, Sanzie and Omondaw) and improved cowpea genotypes (i.e. ITH98-46 and TVu1509) on the measured parameters have added to our understanding of the potential which exists in local genotypes. In this study, farmer-selected genotypes led by cv. Sanzie were among the superior cowpea genotypes which significantly had more acid and alkaline phosphatase activities, greater mineral elements content in their plant organs, greater N-fixed and higher grain yields. These observations can be interpreted as due to its genotypic potential, thus, suggesting its inclusion in mixed culture systems and in the management of low nutrient soils. Inclusion of cv. Sanzie, as well as the other farmer-selected genotypes (e.g. Bensogla and Omondaw) in cropping systems, will probably maximise the amount of mineral elements, optimise symbiotic performance and increased P uptake efficiency, thus contributing significantly to the soil nutrition in agricultural soils, and consequently, greater grain yields. In conclusion, in order to maximise plant growth, N_2 fixation, grain yield and other measured attributes from different cowpea genotypes such as those used in this study, should first be evaluated under different agronomic practices such as high plant density and intercropping systems.

9.0 REFERENCES

Adjei-Nsiah S, Kuyper TW, Leeuwis C, Abekoe MK, Cobbinah J, Sakyi-Dawson O, Giller KE. 2008. Farmers agronomic and social evaluation of productivity, yield and N_2 fixation in different cowpea varieties and their subsequent residual N effects on a succeeding maize crop. *Nutrient Cycling in Agroecosystems* **80**: 199-209.

Ae N, Arihara J, Okada K, Yoshihara T, Johansen C. 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. *Science* **248**: 477-480.

Ae N, Otani T, Makino T, Tazawa J. 1996. Role of cell wall of groundnut roots in solubilizing sparingly soluble phosphorous in soil. *Plant and Soil* **186**: 197-204.

Ae N, Shen RF. 2002. Root cell-wall properties are proposed to contribute to phosphorous (P) mobilization by groundnut and pigeon pea. *Plant and Soil* **245**: 95-103.

Agboola AA, Fayemi AA. 1972. Fixation and excretion of nitrogen by tropical legumes. *Agronomy Journal* **64**: 409-412.

AGRA. 2007. Alliance for a Green Revolution in Africa: AGRA at work. Retrieved 13th March, 2008 from www.agra-alliance.org/wor

Ahmad AR and Nye PH. 1990. Coupled diffusion and oxidation of ferrous iron in soils. I. Kinetic of oxygenation of ferrous iron in soil suspension. *Journal of Soil Science* **41**: 395-409.

Akunda EM. 2001. Intercropping and population density effects on yield component, seed quality and photosynthesis of sorghum and soybean. *The Journal of Food Technology in Africa* **6**: 96-100.

Alghali AM. 1992. Insecticide application schedules to reduce grain yield losses caused by insects of cowpea in Nigeria. *Insecticides Science Application* **13**: 725-730.

Aliyu BS, Emechebe AM. 2006. Effect of Intra- and inter-row mixing of sorghum with two varieties of cowpea on host crop yield in a Striga hermonthica infested field. *African Journal of Agricultural Research* **1**: 24-26.

Alloway BJ. 2001(ed): Zinc - the Vital Micronutrient for healthy, High Value Crops. - International Zinc Association, Brussels.

Alvarez S, Guerrero MC. 2000. Enzymatic activities associated with decomposition of particulate organic matter in two shallow ponds. *Soil Biology and Biochemistry* **32**: 1941-1951.

Ampong-Nyarko K, Reddy KV, Nyangor RA, Sexena KN. 1994. Reduction of pest attack on sorghum and cowpea by intercropping. *Entomology Experimental Application* **70**: 179-184.

Andersen MK, Hauggaard-Nielsen H, Ambus P, Jensen ES. 2005. Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops. *Plant and Soil* **266**: 273-287.

Anderson IC, Buxton DR, Hallam A. 1999. Performance of annual and perennial crops for biomass production. Department of Agronomy Working paper, Iowa State University,

Arnon DI. 1949. Copper enzymes in chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* **24**: 1-15.

Asafu-Agyei JN, Ahenkora K, Banful B, Ennin KS. 1997. Sustaining food production in Ghana. *In*: The role of cereal legume based cropping systems'(Eds Bezunch T, Emechebe AM, Sedago J, Quedraogo M.) pp 409-416 (Semi-arid grain Research and development Agency of the Scientific Technical and Research Commission of OAU: Quagadougou, Burkina Faso).

Atkins CA, Herridge DF, Pate JS. 1978. *The economy of carbon and nitrogen in nitrogen-fixing annual legumes*. Vienna: International Atomic Agency, 211-242.

Austin RB, Morgan CL, Ford MA. and Bhagwat SG. 1982. Flag Leaf Photosynthesis of *Triticum aestivum* and Related Diploid and Tetraploid Species *Annals of Botany* **49**: 177-189.

Awika JM and Rooney LW. 2004a. Sorghum phytochemicals and their potential impact on human health, *Phytochemistry* **65**: 1199-1221.

Awika JM, Rooney LW and Waniska RD. 2004a. Anthoycanins from black sorghum and their antioxidant properties, *Food Chemistry* **90**: 293-301.

Awika JM, Rooney LW and Waniska RD. 2004b. Properties of 3-deoxyanthocyanins from sorghum, *Journal of Agricultural and Food Chemistry* **52**: 4388-4394.

Ayisi KK, Nkgapele RJ, Dakora FD. 2000. Nodule formation and function in six varieties of cowpea (*Vigna unguiculata* L. Walp.) grown in nitrogen rich soil in South Africa. *Symbiosis* **28**: 17-31.

Ayisi, K.K., Nkgapele, R.J., Dakora, F.D. 2000. Nodule formation and function in six varieties of cowpea (*Vigna unguiculata* L. Walp.) grown in a nitrogen-rich field soil in South Africa. *Symbiosis* **28**: 17-31.

Azam-Ali SN, Matthews RB, Williams JH, Peacock JM. 1990. Light use, water use and performance of individual components of a sorghum/groundnut intercrop. *Experimental Agriculture* **26**: 413-427.

Babu AM, Laksmaiah K, Sekhar G. 1988. Studies on rainfed sorghum/pulse intercropping system. *Journal of Research APAU* **16**: 40-42.

Bagayoko M, Alvey S, Buerkert A, Neumann G. 2000. 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 and Soil* **225**: 117-127.

Bais HP, Loyola Vargas VM, Flores HE and Vivanco HM. 2001. Root specific metabolism: the biology and biochemistry of underground organs. *In vitro Cell Development Biology Plant* **37**: 730-741.

Balakrishnan K, Rajendran C, Kulandaivelu G. 2000. Differential responses of iron, magnesium, and zinc deficiency on pigment composition, nutrient content, and photosynthetic activity in tropical fruit crops. *Photosynthetica* **38**: 477-479.

Balakumar T, Vincent HB and Paliwal K. 1993. On the radiation of UV-B (280-315 nm) with water stress in crop plants. *Physiologia Plantarum* **87**: 217-222.

Baldwin JC, Athikkattuvalasu SK, Raghothama KG. 2001. LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiology* **125**: 728-737.

Banik P, Midya A, Sarkar BK, Ghose SS (2006). Wheat and chickpea intercropping systems in an additive series experiment: Advantages and weed smothering. *European Journal of Agronomy* **24**: 325–332.

Barber SA, Walker JM and Vasey M. 1962. Principles of ion movement through the soil to plant root. Int. Soc. Soil Sci., Trans. Comm. III Palmerston N., New Zeal. Pp. 129-124.

Beale SI. 1999. Enzymes of chlorophyll biosynthesis. *Photosynthesis Research* **60**: 43-73.

Bednarz CW, Oosterhuis DM, Evans RD. 1998. Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. *Environmental Experimental Botany* **39**: 131-139.

Beets WC. 1982. Multiple cropping and Tropical Farming systems, Westview Press, Boulder, CO.

Beets WC. 1994. Multiple cropping of maize and soybean under a high level of crop management. *Netherlands Journal of Agricultural Science* **25**: 95-102.

Beleke T, Cino BJ, Ehlert PAI, van der Maas AA, van Diest A. 1983. An evaluation of plant borne factors promoting the solubilisation of alkaline rock phosphate. *Plant and Soil* **75**: 361-378.

Beyers CPDL and Coetzer FJ. 1971. Effect of concentration, pH, and time on the properties of di-ammonium EDTA as a multiple soil extractants. *Agrochemophysica* **3:** 49-54.

Bieleski R. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Annual Review in Plant Physiology* **24**: 225-252.

Blade SF, Mather DE, Singh BB, and Smith DL. 1992. Evaluation of yield stability of cowpea under sole and intercrop management in Nigeria. *Euphytica* **61**: 193-201.

Bohlool BB, Ladha JK, Garrity DP and George T. 1992. Biological nitrogen fixation for sustainable agriculture: A perspective. *Plant and Soil* **141**: 1-11.

Borlaug NE. 1991. Reaching sub-Saharan Africa's small-scale farmers with improved technology: the Sasakawa-global 2000 experience. *In*: Agricultural issues in the 1990s Garbus I, Pritchard A, Knudsen O (eds.), World Bank, Washington D.C.

Boserup E. 1981. Population and Technology. Basil Blackwell, Oxford, UK.

Braum SM, Helmke PA. 1995. White lupin utilises soil phosphorus that is unavailable to soybean. *Plant and Soil* **176**: 95-100.

Buttery BR, Buzzell RI, Findlay WI. 1981. Relationships among photosynthetic rate, bean yield and other characters in field-grown cultivars of soybean. *Canadian Journal of Plant Science* **61**: 191-198.

Caetano-Anolles G, Crist-Estes DK, Bauer WD. 1988. Chemotaxis of Rhizobium meliloti to the plant flavone luteolin requires functional nodulation genes. *Journal of Bacteriology* **170**: 3164-3169.

Cakmak I and Engels C. 1999. Role of mineral nutrients in photosynthesis and yield formation. *In*: Rengel Z (ed): Mineral nutrition of crops. Pp. 141-168. Haworth Press, New York.

Carter GA, Knapp AK. 2001. Leaf optical properties in higher plants: Linking spectral characteristics to stress and chlorophyll concentration. *American Journal of Botany* **88**: 677-684.

Chalker-Scott L. 1999. Environmental significance of anthocyanins in plant stress response. *Photochemistry Photobiology* **70**: 1-9.

Chang C-F, Suzuki A, Kumai S, Tamura S. 1969. Chemical studies on 'clover sickness'. Part II. Biological functions of isoflavonoids and their related compounds. *Agricultural and Biological Chemistry* **33**: 398-408.

Chang JF, and Shibles RM. 1985. An analysis of competition between intercropped cowpea and maize: The effect of fertilization and population density. *Field Crops Research* **12**: 145-152.

Christeller JT, Tolbert NE. 1978. Phosphoglcolate phosphatase: purification and properties. *Journal of Biology Chemistry* **253**: 1780-1785.

Christiansen I and Graham PH. 2002. Variation in di-nitrogen fixation among Andean bean (*Phaseolus vulgaris* L.) genotypes grown at low and high levels of phosphorus supply. *Field Crop Research* **73** (2-3): 133-142.

Chu GX, Shen QR, Cao JL. 2004. Nitrogen fixation and transfer from peanut to rice cultivated in aerobic soil in an intercropping system and its effect on soil N fertility. *Plant and Soil* **263**: 17-27.

Clark KM, Myers RL. 1994. Intercrop performance of Pearl millet, *Amaranthus*, cowpea, Soybean and Guar in Response to planting Pattern and Nitrogen Fertilization. *Agronomy Journal* **86**: 1097-1102.

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedein LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A and Watson MF. 1999. Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global Biogeochemistry Cycles* **13**: 623-645.

Close DC, Beadle CL, Brown PH, Holz GK. 2000. Cold-induced photo inhibition affects establishment of *Eucalyptus nitens* (Deane and Maiden) Maiden and *Eucalyptus globules* Labill. *Trees* **15**: 32-41.

Coker GT III, Shubert KR. 1981. Carbon dioxide fixation in soybean roots and nodules I. Characterisation and comparison with N_2 fixation and composition of xylem exudates during early nodule development. *Plant Physiology* **67**: 691-696.

Connolly J, Wayne P, Murray R. 1990. Dynamic interactions of *Stellaria media* (L.) Vill. and *Poa annual Oecologia* (Berlin) **108**: 513-526.

Cooper PJM, Leakey RRB, Rao MR and Reynolds L. 1996. Agroforestry and the mitigation of land degradation in the humid and sub-humid tropics of Africa. *Experimental Agriculture* **32**: 235-290.

Cornic G, Ghashghaie J, Genty B, Briantais JM. 1992. Leaf photosynthesis is resistant to mild drought stress. *Photosynthetica* **27**: 295-309.

Cornu JY, Staunton S, Hinsinger P. 2007. Copper concentration in plants and in the rhizosphere as influenced by the iron status of tomato (*Lycopersicum esculentum* L.). *Plant and Soil* **292**: 63-77.

Criquet S, Farnet AM, Tagger S, Le Petit J. 2000. Annual variation of phenoloxidase activities in an evergreen oak litter: influence of certain biotic and abiotic factors. *Soil Biology and Biochemistry* **32**: 1505-1513.

Dakora FD and Keya SO. 1997. Contribution of legume nitrogen fixation to sustainable agriculture in sub-Saharan Africa. *Soil Biology and Biochemistry* **29**: 809-817.

Dakora FD and Phillips DA. 1996. Diverse functions of isoflavonoids in legumes transcend ant-microbial definitions of phytoalexins. *Physiology and Molecular Plant Pathology* **49:** 1-20.

Dakora FD and Phillips DA. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil* **245**: 35-47.

Dakora FD, Aboyinga RA, Mahama Y, and Apaseku J. 1987. Assessment of N_2 fixation in groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata* L. Walp.) and their relative N contribution to a succeeding maize crop in Northern Ghana. *MIRCEN Journal* **3**: 389-399.

Dakora FD, and Keya SO. 1997. Contribution of legume nitrogen fixation to sustainable Agriculture in sub-Saharan Africa. *Soil Biology and Biochemistry* **29**: 809-817.

Dakora FD, Joseph CM, and Phillips DA. 1993a. Alfalfa root exudates contain isoflavonoids in the presence of *Rhizobium meliloti*. *Plant Physiology* **101**: 819-824.

Dakora FD, Joseph CM, Phillips DA. 1993b. Common bean root exudates contain elevated levels of daidzein and coumestrol in response to *Rhizobium* inoculation. *Molecular Plant Microbe Interaction* **6**: 665–668.

Dakora FD, Keya SO. 1997. Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. *Soil Biology and Biochemistry* **29**: 809-817.

Dakora FD, Phillips DA. 2002. Root exudates as mediators as mineral acquisition in low-nutrient environments. *Plant and Soil* **245**: 35-47.

Dakora FD. 1998. Nodule function in symbiotic Bambara groundnut (*Vigna subterranea* L.) and Kerstings bean (*Macroptyloma geocarpum* L.) is tolerant of nitrate in the root medium. *Annals of Botany* **82**: 687-690.

Dakora FD. 2003. Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. *New Phytology* **158**: 39-49.

Dapaah HK, Asafu-Agyei JN, Ennin SA, Yamoah C. 2003. Yield stability of cassava, maize, soybean and cowpea intercrops. *Journal of Agricultural Science* **140**: 73-82.

Dariush M, Ahad M, Meysam O. 2006. Assessing the land equivalent ratio (LER) of two corn (*Zea mays* L.) varieties intercropping at various nitrogen levels in Karaj, Iran. *Journal of Central European Agriculture* **7**: 359-364.

Daughty CHT, Walthall CL, Kim MS, de Colstoum EB, McMurtrey III JE. 2000. Estimating leaf chlorophyll concentration from leaf and canopy reflectance. *Remote Sensing Environment* **74**: 229-239.

del Pozo JC, Allona I, Rubio V, Layva A, de la Pena A, Aragoncillo C, Paz-Area J. 1999. A type 5 acid phosphatase gene from *Arabidopsis thaliana* is induced by phosphate starvation and by some other types of phosphate mobilizing/oxidative stress conditions. *Plant Journal* **19**: 579-589.

Delhaize E, Ryan PR and Randall PJ. 1993. Aluminium tolerance in wheat (*Triticum aestivum* L.) 2. Aluminium stimulated excretion of malic acid from root apices. *Plant Physiology* **103**: 695-702.

Deshpande, S. S., U. S. Deshpande and D. K. Salunkhe. 1996. Nutritional and health aspects of food antioxidants. p. 361-469. *In*: Madhavi DL, Deshpande SS and Salunkhe DK. (eds.). Food antioxidants. Marcel Deckker, New York.

Dick WA, Cheng L, Wang P. 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biology and Biochemistry* **32**: 1915-1919.

Dicko MH, Gruppen H, Traore AS, Van Berkel WJH and Voragen AGJ. 2005a. Evaluation of the effect of germination on phenolic compounds and antioxidant activities in sorghum varieties. *Journal of Agricultural and Food Chemistry* **53**: 2581-2588.

Dieter T. 2006. Significance of flavonoids in plant resistance: a review. *Environmental Chemical Letters* **4**: 147-157.

Division of Chemical Services. 1956. Analytical methods. Division of Chemical Services, Department of Agriculture, Pretoria.

Donald CM. 1958. The interaction of competition for light and nutrients. *Australian Journal of Agricultural Research* **9**: 421-435.

Donald CM. 1963. Competition among crop and pasture plants. *Advanced Agronomy* **15**: 1-118.

Dong ST. 1991. Studies on the relationship between canopy apparent photosynthesis and grain yield in high yielding winter wheat. *Acta Agronomy Sin* **17**: 461-469.

Drake BG, Gonzàlez-Meler MA, Long SP. 1997. More efficient plants: A consequence of rising atmospheric CO_2? *Annual Review Plant Physiology Molecular Biology* **48**: 609-639.

Du Plessis SF and Burger RDT. 1964. A comparison of Chemical extraction methods for the evaluation of phosphate availability of top soils. *South African Journal of Agricultural Science* **8**: 11-13.

Duff SM, Plaxton WC, Lefebvre DD. 1991. Phosphate starvation response in plant cells: de novo synthesis and degradation of acid phosphatases. *Proceedings of the National Academy of Science USA* **88**: 9538-9542.

Duff SM, Plaxton WC. 1994. The role of acid phosphatases in plant phosphorus metabolism. *Physiologia Plantarum* **90**: 971-800.

Dyer B. 1894. On the analytical determinations of probably available "mineral plant-food in soil" *Journal Chemical Society* **65**: 1-15.

Dykes L, Rooney LW, Waniska RD and Rooney WL. 2005. Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. *Journal of Agricultural and Food Chemistry* **53**: 6813-6818.

Ehdaie B, Waines JG. 1993. Variations in water use efficiency and its components in wheat: I. Well watered pot experiment. *Crop Science* **33**: 294-299.

Eivazi F, Bayan MR, Schmidt K. 2003. Select soil enzyme activities in the Historic Sanborn Field as affected by long-term cropping systems. *Communication Soil Science Plant Analysis*. **34**: 2259-2275.

Eivazi F, Tabatabai MA. 1977. Phosphates in soils. *Soil Biology and Biochemistry* **9**: 167-172.

El Dessoug H, zu Dreeele A, Claassen N. 2003. Growth and phosphorus uptake of maize cultivated alone, in mixed culture with other crops, or after incorporation of their residues. *Journal of Plant Nutrition Soil Science* **166**: 254-261.

Elgersma A, Schelpers H, Nassiri M. 2000. Interaction between perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) under contrasting nitrogen availability, productivity, seasonal patterns of specie composition, N_2 fixation, N transfer and N recovery. *Plant and Soil* **221**: 281-299.

Evans J, Mcneill AM, Unkovich MJ, Fettell NA, Heenan DP. 2001. Net nitrogen balances for cool season grain legume crops and contribution to wheat nitrogen uptake: a review. *Australian Journal of Experimental Agriculture* **41**: 347-359.

Evans LT and Dunstone RL. 1970. Some physiological aspects of evolution in wheat. *Australian Journal of Biological Science* **23**: 725-741.

Fan F, Zhang F, Song Y, Sun J, Bao X, Guo T, and Li L. 2006. Nitrogen fixation of faba bean (*Vicia faba* L.) interacting with a non-legume in two contrasting intercropping systems. *Plant and Soil* **283**: 275-286.

Fan TWM, Lane AN, Shenka M, Bartley JP, Crowley DE and Higashi RM. 2001. Comprehensive chemical profiling of graminaceous plant root exudates using high resolution NMR and MS. *Phytochemistry* **57**: 209-221.

FAO. 2000. FAO Global Information and Early Warning System: Africa Report No. 1, April 2000.

FAO. 2001. World Soil Resources Reports, 289 pp.

FAO. 2005. FAOSTAT. http://faostat.fao.org/faostat/

Farquhar GD, Ehleringer JR, Hubick K. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review Plant Physiology Molecular Biology* **40**: 67-86.

Farquhar GD, Richards RA. 1984. Isotope composition of plant carbon correlates with water use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* **11**: 539-552.

Feigenbaum S and Mengel K. 1979. The effect of reduced light intensity and sub-optimum potassium supply on N_2 fixation and N turnover in *Rhizobium*-infected Lucerne. *Physiologia Plantarum* **45**: 245-249.

Fertilizer Society of South Africa (FSSA). 1974. Manual of soil analysis methods. FSSA Publication no 37, Pretoria.

Fisher KS, Wilson GL. 1976. Studies of grain production in (*Sorghum bicolour* L. Moench). VII: contribution of plant parts of canopy photosynthesis and grain yield in field situations. *Australian Journal Agricultural Research* **27**: 235-245.

Fox TR and Comerford NB. 1990. Low-molecular weight organic acids in selected forest soils in the southern USA. *Soil Science Society of American Journal* **54**: 1139-1144.

Francis CA, Flor CA, Temple SP. 1976. Adapting varieties for intercropping systems in the tropics. *In*: Papandick RI, Sanchez PA, Triplett GB. (Eds.), Multiple Cropping. American Society of Agronomy, Madison, pp. 235-253.

Francis CA. 1986. Multiple cropping systems. Macmillan, New York.

Francis O and Stern WR. 1987. Relative sowing time and density of component crops in a maize/cowpea intercrop system. *Experimental Agriculture* **23:** 41-52.

Fujita K, Ofosu-Budu KG, and Ogata S. 1992. Biological nitrogen fixation in mixed legume-cereal cropping system. *Plant and Soil* **141**: 155-175.

Fujita K, Ofosu-Budu KG. 1996. Significance of legumes in Intercropping Systems, Pp. 18-40. *In*: Ito O, Johansen JJ, Adu-Gyamfi K, Katayama JVDK, Kumar Rao and Rego TJ. (eds). Dynamics of Roots and Nitrogen in cropping systems of the Semi arid tropics.

Fujita K, Ogata S, Matsumoto K, Masuda T, Ofosu-Budu KG, Kuwata K. 1990. Nitrogen transfer and dry matter production in soybean and sorghum mixed cropping system at different population densities. *Soil Science and Plant Nutrition* **36**: 233-241.

Fukai S, Trenbath BR. 1993. Processes determining intercrop productivity and yields of component crops. *Field Crop Research* **34**: 247-271.

Fukami H, and Nakajima M. 1971. Rotenone and rotenoids. *In*: Jacobson, M. and Crosby, D.G., Ed. Naturally occurring insecticides. Marcel Dekker Inc., New York, p71.

Gagnon H, Ibrahim RK. 1998. Aldonic acids: A novel family of nod gene inducers of *Mesorhizobium loti*, *Rhizobium lupini*, and *Sinorhizobium meliloti*. *Molecular Plant-Microbe Interaction* **11**: 988-998.

Gahoonia TS, Care D, Nielsen NE. 1997. Root hairs and acquisition of phosphorus by wheat and barley cultivars. *Plant and Soil* **191**: 181-188.

Gahoonia TS, Claassen N and Jungk A. 1992. Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. *Plant and Soil* **140**: 241-248.

Gan Y, Stulen I, Van Keulen H and Kuiper PJC. 2002. Physiological response of soybean genotypes to plant density. *Field Crops Research* **74**: 231-241.

Gebeyehu S, Simane B, Kirkby R. 2006. Genotype x cropping system interaction in climbing beans (*Phaseolus vulgaris* L) grown as sole crop and in association with maize (*Zea mays* L.). *European Journal of Agronomy* **24**: 396-403.

Gerke J. 1995. Phosphate, aluminium and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. *Z. Pflanzenernaehr. Bodenk*. **155**: 339-343.

Ghosh PK, Manna MC, Bandyopadhyay KK, Ajay, Tripathi AK, Wanjari RH, Hati KM, Misra AK, Acharya CL, and Subba Rao A. 2006. Interspecific interaction and nutrient use in soybean/sorghum intercropping system. *Agronomy Journal* **98**: 1097-1108.

Gilroy S, Hughes WA, and Trewavas AJ. 1989. **A** comparison between Quin-2 and aequorin as indicators of cytoplasmic calcium levels in higher plant cell protoplasts. *Plant Physiology* **90**: 482-491.

Giron HC. 1973. Comparison between dry ashing and wet digestion in the preparation of plant materials for atomic absorption analysis. *Atomic Absorption Newsletters* **12**: 28-29.

Gocio M. 2001. Intercropping principles and Practises. Agronomy system guide. Fayetteville, AR 72702, Arkansas, USA pp.1-18.

Goldberg R, Prat R, Dupacq JP. 1983. In situ and IAA-Induced cell elongation is corrected to the oleoyl phosphatidylcholine content along the *Vigna radiata* hypocotyl. *Plant Cell Physiology* **24**: 541-549.

Gregorich EG, Carter MR, Doran JW, Pankhurst CE, Dwyer LM. 1997. Biological attributes of soil quality. In: C.E. Gregorich, M.R. Carter (eds.), Soil quality for crop production and ecosystem health. Development in soil science 25. Elservier, Amterdam, pp. 81-113.

Gregory PJ, Hinsinger P. 1999. New approaches to studying chemical and physical changes in the rhizosphere: An overview. *Plant and Soil* **211**: 1-9.

Gunes A, Inal A. 2008. Significance of intracellular and secreted acid phosphatase enzyme activity, and zinc and calcium interactions, on phosphorus efficiency in wheat, sunflower, chickpea, and lentil cultivars. *Australian Journal of Agricultural Research* **59**: 339-347.

Hacisasiloglu G, Hart JJ, Wang Y, Cakmak I, Kochian LV. 2003. Zinc deficiency is correlated with enhanced expression and activity of Cu/Zn superoxide dismutase and carbonic anhydrase in wheat. *Plant Physiology* **131**: 595-602.

Haizel KA. 1972. The effects of plant density on the growth, development and grain yield of two varieties of cowpea (*Vigna unguiculata* L. Walp). *Ghana Journal of Agricultural Science* **5**: 163-171.

Hamauzu Y, Yasui H, Inno T, Kume C and Omanyuda M. 2005. Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *Journal of Agricultural and Food Chemistry* **53**: 928-934.

Hamid A, Agata W, Kawamitsu Y. 1990. Photosynthesis, transpiration and water-use efficiency in four cultivars of mungbean, *Vigna radiate* (L.) Wilczek. *Photosynthetica* **24**: 96-101.

Haran S, Logendra S, Saskar M, Bratanova M, Raskin I. 2000. Characterization of *Arabidopsis* acid phosphatase promoter and regulation of acid phosphatase expression. *Plant Physiology* **124**: 615-626.

Harborne JB (ed). 1988. The flavonoids. Advances in Research since 1980. Chapman and Hall, London, pp xiii.

Harborne JB and Turner L. 1984. Plant chemosystematics. Academic press, London, pp. 562.

Hardason G, Danso SKA, Zapata F. 1988. Dinitrogen fixation measurements in alfalfa-ryegrass swards using nitrogen-15 and influence of the reference crop. *Crop Science* **28**:101-105.

Hashimoto T and Tajima M. 1980. Effects of ultraviolet irradiation on growth and pigmentation in seedlings. *Plant Cell Physiology* **21**: 1559-1571.

Haslam E. 1998. Practical polyphenolics: from structure to molecular recognition and physiological action, Cambridge University Press, Cambridge, New York, Melbourne.

Hassan S. 2009. Effect of variety and intercropping on two major cowpea (*Vigna unguiculata* L. Walp) field pests in Mubi, Adamawa State, *Nigeria. Journal of Horticultural Forestry* **1**: 14-16.

Hauggaard-Nielsen H, Ambus P, Jensen ES. 2001. Inter-specific competition N use and interference with weeds in pea-barley intercropping. *Field Crop Research* **70**: 101-109.

Hauggaard-Nielsen H, Ambus P, Jensen ES. 2001. Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops. A field study employing P-32 technique. *Plant and Soil* **236**: 63-74.

Hauggaard-Nielsen H, Andersen MK, Jørnsgaard B, Jensen ES. 2006. Density and relative frequency effects on competitive interactions and resource use in pea–barley intercrops. *Field Crop Research* **95**: 256-267.

Hauggaard-Nielsen H, Jensen ES. 2005. Facilitative root interactions in intercrops. *Plant and Soil* **274**: 237-250.

Hawes MC, Brigham LA, Wen F, Woo HH, Zhu Y. 1998. Function of root border cells in plant health: Pioneers in the rhizosphere. *Annual Review Phytopathology* **36**: 311-327.

Hayes JE, Richardson AE, Simpson RJ. 1999. Phytase and acid phosphatase activity in roots of temperate pasture grasses and legumes. *Australian Journal of Plant Physiology* **26**: 801-809.

Haynes RJ. 1990. Active ion uptake and maintenance of cation-anion balance: A critical examination of their role in regulating rhizosphere pH. *Plant and Soil* **126**: 247-264.

Hedin PA, Jenkins JN, Collun DH, White WH and Parrott WL. 1983. Multiple factors in cotton contributing to resistance to the tobacco budworm, *Heliothis virescens* F. In: *Plant resistance to insects*, Hedin PA (ed) American Chemical Society, Washington, DC, 347-367.

Hedley MJ, White RE, Nye PH. 1982. Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. III. Changes in L values, soil phosphate fractions and phosphatase activity. *New Phytology* **91**: 45-56.

Heenan DP and Campbell LC. 1981. Soybean (*Glycine max*) nitrate reductase activity influenced by manganese nutrition. *Plant Cell Physiology* **21**: 731-736.

Hegstad JM, Bollero G, and Nickel CD. 1999. Potential of using plant row yield trials of predict soybean yield. *Crop Science* **39**: 1671-1675.

Heichel GH and Musgrave RB. 1969. Varietal differences in net photosynthesis of *Zea mays* L. *Crop Science* **9**: 483-486.

Heichel GH. 1987. Legume Nitrogen: Symbiotic fixation and recovery by subsequent crops. *In*: Helsel, Z.R. (eds) Energy in Plant Nutrition and Pest Control. Elsevier Science Publication, Amsterdam pp 63-80.

Herridge DF, Pate JS. 1977. Utilisation of net photosynthate for nitrogen fixation and protein production in an annual legume. *Plant Physiology* **60**: 759-764.

Herridge DF, Rupela OP, Serraj R, and Beck DP.1993. Screening techniques and improved biological nitrogen fixation in cool season legumes. *Euphytica* **1:** 1-14.

Hertog MGL and Hollman PCH. 1996. Potential health effects of dietary flavonoid quercetin. *European Journal of Clinical Nutrition* **50**: 63-64.

Hinsinger P, Elsass F, Jaillard B and Robert M. 1993. Root induced irreversible transformation of trioctahedral mica in the rhizosphere of rape. *Journal of Soil Science* **44**: 535-545.

Hinsinger P, Gilkes RJ. 1995. Root-induced dissolution of phosphate rock in the rhizosphere of lupins grown in alkaline soil**.** *Australian Journal of Soil Research* **33**: 477-489.

Hinsinger P, Plassard C, Tang C, Jaillard B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant and Soil* **248**: 43-59.

Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant and Soil.* **237**: 173-195.

Hirose T, Werger MJA, Pons TL, Rheenen JWA van. 1988. Canopy structure and leaf nitrogen distribution in a stand of *Lysimachia vulgaris* L. as influenced by stand density. *Oecology* **77**: 145-150.

Hiscox JD, Israelstam GF. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**: 1332-1334.

Hoffmann-Campo CB, Harborne JB, McCaffery AR. 2001. Pre-digestive and post-ingestive effects of soybean extracts and rutin on *Trichoplusia ni* growth. *Entomology Experimental Application* **98**: 181-194.

Hooper DU. 1998. The Role of Complementarity and Competition in Ecosystem Responses to Variation in Plant Diversity. *Ecology* **79**: 704-719.

Horst WJ and Waschkies C. 1987. Phosphorous nutrition of spring wheat (*Tricum-aestivum* L.) in mixed culture with white lupin (*lupinus-albus* L.) *Pflanzenernahr. Bodenk*. **150**: 1-8.

Horst WJ. 1987. Aluminum tolerance and calcium efficiency of cowpea genotypes. *Journal of Plant Nutrition* **10**: 1121-1129.

Horton P. 2000. Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. *Journal of Experimental Botany* **51**: 475-485.

Hungria M and Stacey G. 1997. Molecular signals exchanged between host plants and rhizobia: basic aspects and potential application in agriculture. *Soil Biology and Biochemistry* **29**: 519-830.

Hungria M, Joseph CM, Phillips DA. 1991. Anthocyanins and flavonols, major *nod* gene inducers from seeds of a black-seeded common bean (*Phaseolus vulgaris* L.). *Plant Physiology* **97**: 751-758.

Hussain I, Jatoi SA, Sayal O, Baloch MS. 2000. Green fodder yield and land equivalent ratio of Sorghum – legume association. *Pakistan Journal of Biological Sciences* **3**: 175-176.

Igamberdiev AU, Kleczkowski LA. 2003. Membrane potential, adenylate levels and Mg^{2+} are interconnected via adenylate kinase equilibrium in plant cells. *Biochimical et Biophysica Acta.* **1607**: 111-119.

Inal A, Gunes A, Zhang F, Cakmak I. 2007. Peanut/maize intercropping induced changes in the rhizosphere and nutrient concentration in shoots. *Journal of Plant Physiology and Biochemistry* **45**: 350-356.

Inal A, Gunes A. 2007. Interspecific root interactions and rhizosphere effects on salt ions and nutrient uptake between mixed grown peanut/maize and peanut/barley in original saline-sodic-boron toxic soil. *Journal of Plant Physiology* **165**: 490-503.

Inal A, Gunes A. 2008. Interspecific root interactions and rhizosphere effects on salt ions and nutrient uptake between mixed grown peanut/maize and peanut/barley in original saline-sodic-boron-toxic soil. *Journal Plant Physiology* **165**: 490-503.

Iragavarapu TK, Randall GW. 1996. Border effect on yields in a strip intercropped soybean, corn, and wheat production system. *Journal of Production Agriculture* **9**: 101-107.

Ismail AMA and Ali AH. 1996. Plant population density effects on yields of sorghum in a dry land farming systems. *Quartar Univiversity Science Journal* **16**: 89-93.

Izaurralde RC, McGill WB, Juma NG. 1992. Nitrogen fixation efficiency, interspecies N transfer, and root growth in barley-field pea intercrop on Black Chenozemic soil. *Biology Fertility of Soils* **13:** 11-16.

Jackai LEN, Adalla CB. 1997. Pest management practices in cowpea, a review. Pp. 240-258 *in* Advances in Cowpea Research (Singh BB, Mohan Raj DR, Dashiel KE and Jackai LEN eds.). International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agricultural Sciences (JIRCAS), IITA, Ibadan, Nigeria.

Jahansooz MR, Yunusa IAM, Coventry DR, Palmer AR, Eamus D. 2007. Radiation- and water use associated with growth and yields of wheat and chickpea in sole and mixed crops. *European Journal of Agronomy* **26**: 275-282.

Jensen ES. 1996. Grain yield, symbiotic N_2-fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant and Soil* **182**: 25-38.

Jensen ES and Hauggaard-Nielsen H. 2003. How can increased use of biological N_2 fixation in agriculture benefit the environment? *Plant and Soil* **252**: 177-186.

Jenkinson DA. 2001. The impact of humans on the nitrogen cycle, with focus on temperate arable agriculture. *Plant and Soil* **228**: 3-15.

Jones DL, Prabowo AM, Kochian LV. 1996a. Kinetics of malate transport and decomposition in acid soils and isolated bacterial populations-the effect of microorganisms on root exudation of malate under Al stress. *Plant and Soil* **182**: 239-247.

Kaftan D, Brumfeld V, Nevo R, Scherz A, Reich Z. 2002. From chloroplasts to photo systems: in situ scanning force microscopy on intact thykaloid membranes. *EMBO Journal* **21**: 6246-6253.

Keen NT, Sims H, Erwin DC, Rice E, Patridge JE. 1971. 6-α-hydroxyphaseolin: an antifungal chemical induced in soybean hypocotyls by *Phytophthora megasperma* var. *sojae*. *Phytopathology* **61**: 1084-1089.

Keen NT. 1975. The isolation of phytoalexins from germinating seeds of *Cicer arietinum,Vigna sinensis, Arachis hypogaea* and other plants. *Phytopathology* **65**: 91-92.

Kerley SJ, Jarvis SC. 1999. The use of nitrogen-15 natural abundance in white clover (*Trifolium repens* L.) to determine nitrogen fixation under different management practices. *Biology Fertility of Soils* **29**:437-440.

Khalid S, Ahmad T and Shad RA. 2002. Use of allelopathy in agriculture. *Asian Journal of Plant Science* **1**(3): 292-297.

Khan MMA and Bauer WD. 1988. Chemotaxis of *Bradyrhizobium japonicum* towards flavones and isoflavones from soybean (abstract No. 760.). *Plant Physiology* **86**: S-127.

Kishinevsky BD, Zur M, Friedman Y, Meromi G, Ben-Moshe E, and Nemas C. 1996. Variation in nitrogen fixation and yield in landraces of bambara groundnut (*Vigna subterranea* L.). *Field Crops Research* **48**: 57-64.

Krasilnikoff G, Gahoonia T, and Nielsen NE. 2003. Variation in phosphorous uptake efficiency by genotypes of cowpea (*Vigna unguiculata* L.) due to differences on root and root hair length and induced rhizosphere processes. *Plant and Soil* **251**: 83-101.

Kumar Rao, JVDK, Wani SP, and Lee KK. 1996. Biological nitrogen fixation through grain legumes in different cropping systems of the Semi Arid Tropics. *In*: Ito O, Johansen C, Adu Gyamfi JJ, Katayama K, Kumar Rao, JVDK, and Rego TJ (eds). Dynamics of roots and Nitrogen in Cropping systems in the Semi Arid Tropics, (1996) JIRCAS.

Lal A, Ku MSB, Edwars GE. 1996. Analysis of inhibition of photosynthesis due to water stress in the C3 species *Hodeum vulgare* and *Vicia faba*: Electron transport, CO_2 fixation and carboxylation capacity. *Photosynthesis Research* **49**: 57-69.

Langyintuo AS, Lowenberg-DeBoer J, Lambert D, Ibro G, Moussa B, Kergna A, Kushwah S, Musa S, and Ntoukam G. 2003. Cowpea supply and demand in West and Central Africa. *Field Crop Research* **82**: 215-231.

Laurent R and Eric M. 1994. Factors of acidification of the rhizosphere of mycorrhizal plants: measurement of pCO_2 in the rhizosphere. *Acta Botanica Gall* **144**: 533-539.

Lawlor DW, Mitchell RAC. 2000. Crop ecosystem responses to climatic change: wheat in: Reddy KR, Hodges HF. (Eds). Climate change and Global productivity. CAB International, Wallingford, pp. 57-80.

Lawrie AC, Wheeler CT. 1975. Nitrogen fixation in the root nodules of *Vicia faba* L. in relation to the assimilation of carbon. II. The dark fixation of carbon dioxide. *New Phytology* **74**: 437-445.

Lefebvre DD, Duff SMG, Fife CA, Julien-Inalsingh C, Plaxton WC. 1990. Response to phosphate deprivation in *brassica nigra* suspension cells: enhancement of intracellular, cell surface and secreted acid phosphatase activities compared to increases in Pi-absorption rate. *Physiologia Plantarum* **93**: 504-511.

Li F, Meng P, Fu D, Wang B. 2008. Light distribution, photosynthetic rate and yield in a Paulownia-wheat intercropping system in China. *Agroforestry systems* **74** (2): 163-172.

Li L, Sun JH, Zhang FS, Li XL, Yang SC, and Rengel Z. 2001. Wheat/maize or wheat/soybean strip intercropping. I. Yield advantage and interspecific interactions on nutrients. *Field Crops Research* **71**: 123-137.

Li L, Tang C, Rengel Z, Zhang FS. 2004. Calcium, magnesium and microelement uptake as affected by phosphorus sources and interspecific root interactions between wheat and chickpea. *Plant and Soil* **261**:29-37.

Li L, Zhang F, Li X, Christie P, Sun J, Yang S, Tang C. 2003a. Interspecific facilitation of nutrient uptake by intercropped maize and faba bean. *Nutrient Cycling in Agroecosystem* **65**: 61-71.

Li L, Zhang FS, Li XL, Christie P, Sun JH, Yang SC, Tang C. 2003b. Interspecific facilitation of nutrient uptake by intercropped maize and faba bean. *Nutrient Cycling in Agroecosystem* **68**: 61-71.

Li M, Shinamo T, Tadano T. 1997. Distribution of exudates of lupin roots in the rhizosphere under phosphorus-deficient conditions. *Soil Science and Plant Nutrition* **43**: 237-245.

Li SM, Li L, Zhang FS, Tang C. 2004a. Acid phosphatase role in chickpea/maize intercropping. *Annuals of Botany* **94**: 297-303.

Li SM, Li L, Zhang FS, Tang C. 2004b. Calcium Magnesium and microelement uptake as affected by phosphorus sources and interspecific root interaction between wheat and chickpea. *Plant and Soil* **261**: 29-37.

Lima Filho, JMP. 2000. Physiological responses of maize and cowpea to intercropping. *Presq. agropec. bras. Brasilia* **35** (5): 915-921.

Linda C-S. 1999. Environmental significance of anthocyanins in plant stress responses. Invited Review. *Phytochemistry Photobiology* **70**: 1-9.

Lindoo SJ, Caldwell MM. 1978. Ultraviolet-B radiation induced inhibition of leaf expansion and promotion of anthocyanin production. Lack of the involvement of the low irradiation phytochrome system. *Plant Physiology* **61:** 278-282.

Liu Y, Guohua M, Fanjun C, Jianhua Z, Fusuo Z. 2004. Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. *Plant Science* **167**: 217-223.

Long SR. 1989. Rhizobium legume nodulation: life together in the underground. *Cell* **56**: 203-214.

Magino HN, Mugisha J, Osiru DSO, Oruko OL. 2004. Profitability of sorghum-legume cropping practices among households in Eastern Uganda. *Uganda Journal of Agricultural Sciences* **9**: 688-692.

MAPA. 1999. Anuario de Estadistica Agraria. Ministerio de Agricultura, Pesca y Alimentación, Spain.

Marion S and Ursula F. 1999. Plant induced alteration in the rhizosphere and the utilization of soil heterogeneity. *Plant and Soil* **209**: 297-309.

Marschner H. 1995. Mineral nutrition in Higher Plants. Second Edition. Academic press, London. 889pp.

Marschner H, Romheld V, Cakmak I. 1987. Root induced changes of inherent availability in the rhizosphere. *Journal of Plant Nutrition* **10**: 9-16.

Marschner H. 1989. Effect of soil acidification on root growth, nutrient and water uptake. *In* Internat. Congr. Forest Decline Res. State of knowledge and Perspectives. Ed. B Ulrich. pp 381-404. Friedrichshafen.

Marschner H. 1995. *Mineral nutrition in higher plants*, *2nd edn.* Boston, M.A., USA: Academic Press.

Marschner H. 1998 Soil-root interface: Biological and biochemical processes. *In* Soil Chemistry and Ecosystem Health. SSSA Special Publication no. 52. 677 S. Segoe Rd., Madison, WI 53711,

Marschner, H. 1986. Mineral Nutrition of Higher Plants. Academic Press, U.K. 674 pp.

Maxwell CA, Hartwig UA, Joseph CM and Phillips DA. 1989. A chalcone and two related flavonoids released from alfalfa roots induce nod genes of *Rhizobium meliloti*. *Plant Physiology* **91**: 842-847.

Mazaheri D, Madani A, Oveysi M. 2006. Assessing Land Equivalent Ratio (LER) of two corn (*Zea mays* L), varieties intercropping at various nitrogen levels in Karaj, Iran. *Journal of Central European Agriculture* **7**: 359-364.

Midmore DJ. 1993. Agronomic modification of resource use and intercrop productivity. *Field Crops Research* **34**: 357-380.

Miller SS, Liu J, Allan DL, Menhuber CJ, Fedorova M, Vance CP. 2001. Molecular control of acid phosphatase secretion into the rhizosphere of proteoid roots from phosphorus-stressed white lupin. *Plant Physiology* **127**: 594-606.

Miller SS, Liu J, Allan DL, Menzhuber CJ, Fedorova M, Vance CP. 2001. Molecular control of acid phosphatase secretion into the rhizosphere of proteoid roots from phosphorus-stressed white lupin. *Plant Physiology* **127**: 594-606.

Mirecki RM and Teramura AH. 1984. Effects of UV-B irradiance on soybean. V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. *Plant Physiology* **74**: 475-480.

Mitsukawa N, Okumura S, Shirano Y, Sato S, Kato T, Harashima S, Shibata D. 1997. Over expression of an *Arabidopsis thaliana* high-affinity phosphate transporter gene in tobacco cultured cells enhances cell growth under phosphate-limited conditions. *Proceedings of National Academy of Science USA* **94**: 7098-7102.

Monteith JL. 1965. Evaporation and environment. *Symposium Society of Experimental Biology* **19**: 205-234.

Mpairwe DR, Sabiiti EN, Ummuna NN, Tegegne A, Osuji P. 2002. Effect of intercropping cereal crops with forage legumes and source of nutrients on cereal grain yield and fodder dry matter yields. *African Crop Science Journal* **10**: 81-97.

Muchow RC and Sinclair TR. 1994. Nitrogen response of leaf photosynthesis and canopy radiation use efficiency in field-grown maize and sorghum. *Crop Science* **34**: 721-727.

Muofye ML, Dakora FD. 2000. Modification of Rhizosphere pH by the symbiotic legume Aspalathus linearis growing in sandy acidic soils. *Australian Journal of Plant Physiology* **27**: 1169-1173.

Murali NS, Teramura AH. 1985. Effects of ultraviolet-B irradiance on soybean. VI. Influence of phosphorous nutrition on growth and flavonoid content. *Physiologia Plantarum* **63**: 413-416.

Myaka FA. 1995. Effect of time of planting and planting pattern of different cowpea cultivars on yield of intercropped cowpea and maize in tropical sub-humid environment. *Tropical Science* **35**: 274-279.

Naab JB, Chimphango SMB, Dakora FD. 2009. N_2 fixation in cowpea plants grown in farmers' fields in the Upper West Region of Ghana, measured using ^{15}N natural abundance. *Symbiosis* **48**: 37-46.

Naim M, Gestetner B, Zikah S, Birk Y, Bond A. 1974. Soybean isoflavones: characterization, determination and anti-fungal activity. *Journal of Agricultural Food Chemistry* **22**: 806-810.

Nakas JP, Gould WD, Klein DA. 1987. Origin and expression of phosphatase activity in a semi-arid grassland soil. *Soil Biology and Biochemistry* **19**: 13-18.

Nambiar PTC, Rao MR, Reddy MS, Floyd CN, Dart PJ, Wiley RW. 1983. Effect of intercropping on nodulation and N_2 fixation by groundnut. *Experimental Agriculture* **19**: 1979-1986.

Narasinga RBS. 1995. Bioactive phytochemicals in Indian foods. *NFI bulletin* 16.

Ncube B, Dimes JP, Twomlow S, Mupangwa W, Giller KE. 2007. Raising the productivity of smallholder farms under semi-arid conditions by use of small doses of manure and nitrogen: A case of participatory research. *Nutrient Cycling in Agroecosystem* **77**: 53-67.

Ndakidemi PA and Dakora FD. 2003. Legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. A Review. *Functional Plant Biology* **30**: 729-745.

Ndakidemi PA, and Dakora FD. 2007. Yield components of nodulated cowpea (*Vigna unguiculata*) and maize (*Zea mays*) plants grown with exogenous phosphorus in different cropping systems. *Australian Journal of Experimental Agriculture* **47**: 583-589.

Neumann G, Massonneau N, Martinoia E, Romheld V. 1999. Physiological adaptations to phosphorus deficient during period of root development in white lupin. *Planta* **208**: 373-382.

Ntare BR, Williams JH, Bationo A. 1993. Physiological determinants of cowpea seed yield as affected by phosphorous fertilizer and sowing dates in intercrop with millet. *Field Crop Research* **35**: 151-158.

Nuruzzaman M, Lambers H, Bolland MDA, Veneklaas EJ. 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. *Plant and Soil* **281**: 109-120.

O'Leary N, Smith ME. 2004. Uncovering corn adaptation to intercrop with bean by selecting for system yield in the intercrop environment. *Journal of Sustainable Agriculture* **24**: 109-121.

Ofori F and Stern WR. 1987. Cereal legume intercropping systems. *Advances in Agronomy* **41**: 41-90.

Ofori F, Pate JS, Stern WR. 1987. Evaluation of N_2 fixation and nitrogen economy of a maize/cowpea intercrop system using ^{15}N dilution methods. *Plant and Soil* **102**: 149-160.

Ofori F, Stern WR. 1986. Maize/cowpea intercrops system: Effect of Nitrogen fertilizer on productivity and efficiency. *Field Crop Research* **14**: 247-261.

Ofori F, Stern WR. 1987. Cereal-legume intercropping systems. *Advanced Agronomy* **41**: 41-90.

Oigiangbe NO, Onigbinde AO. 1996. The association between some physiochemical characteristics and susceptibility of cowpea (*Vigna unguiculata* L. Walp.) to *Callosobruchus maculates* (F.). *Journal of Stored Products Research* **32**: 7-11.

Okigbo BN and Greenland DJ. 1976. Intercropping systems in tropical Africa. Pp 63-101 *In*: Multiple cropping, Paperndick RI, Sanchez PA, Triplett GB. 1976 (eds). 'Multiple cropping'. American Society of Agronomy, p. 378 special publication No. 27. American Society of Agronomy, Madison, WI.

Oljaca S, Cvetkovic R, Kovacevic D, Vasic G, and Momirovic N. 2000. Effect of plant arrangement pattern and irrigation on efficiency of maize (*Zea mays*) and bean (*Phaseolus vulgaris*) intercropping system. *Journal of Agricultural Science (Cambridge)* **135**: 261-270.

Onwuliri VA and Obu JA. 2002. Lipids and other constituents of *Vigna unguiculata* and *Phaseolus vulgaris* grown in northern Nigeria. *Food Chemistry* **78**: 1-7.

Ozawa K, Osaki M, Matsui H, Honma M, Tadano T. 1995. Purification and properties of acid phosphatase secreted from lupin roots under phosphorus deficiency. *Soil Science and Plant Nutrition* **41**: 461-469.

Padulosi S, Ng NQ. 1990. Wild *Vigna* species in Africa: their collection and potential utilization. Pp. 58–77 *in* Cowpea Genetic Resources (N.Q. Ng and L.M. Monti, eds.). IITA, Ibadan, Nigeria.

Palm CA and Sanchez PA. 1991. Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. *Soil Biology and Biochemistry* **23**: 83-88.

Pantalone VR, Rebetzke GJ, Burton JW, Thomas E. Carter TE Jr, Israel DW. 1999. Soybean PI 416937 root system contributes to biomass accumulation in reciprocal grafts. *Agronomy Journal* **91**: 840-844.

Paperndick RI, Sanchez PA, Triplett GB. 1976. 'Multiple cropping'. American Society of Agronomy, p. 378 (special publication).

Patterson DT.1987. Effects of allelopathic chemicals on growth and physiological responses of soybean (*Glycine max*). *Weed Science* **29**: 53-59.

Peoples MB, and Crasswell ET. 1992. Biological Nitrogen fixation: investments, expectations and actual contributions to agriculture. *Plant and Soil* **141**: 13-39.

Peoples MB, Brockwell J, Herridge DJ, Rochester IJ, Alves BJR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khan DF, Hauggaard-Nielsen H, Jensen ES. 2009. The contribution of N_2-fixing crop legumes to the productivity of agricultural systems. A review article. *Symbiosis* **48**: 1-17.

Peoples MB, Herridge DF, and Ladha JK. 1995. Biological Nitrogen Fixation: An efficient source of nitrogen for sustainable agricultural production. *Plant and Soil* **174**: 3-28.

Philips DA. 2000. Biosynthesis and release of rhizobial nodulation gene inducers by legumes. *In* Prokaryotic nitrogen fixation: A modal system for the analysis of a biological process (Ed. EW Triplett) pp. 349-364. (Horizon scientific press: Wymondham).

Platt BS. 1962. "Table of Representative values of food commonly used in tropical countries", *Medical Research Council, Special Rep. Series No. 302*, HMSO, London.

Plaxton WC, Carswell MC. 1999. Metabolic aspects of the phosphate starvation responses in plants: In: Lerner HR, ed. *Plant responses to enviromental stress: from phytohormones to genome reorganization.* New York, NY, USA: Marcel-Dekker, 350-372.

Pons TL, Schieving F, Hirose T, Werger MJA. 1989. Optimization of leaf nitrogen allocation for canopy photosynthesis in *Lysimachia vulgaris* (L.). *In*: Lambers H, Cambridge ML, Kinings H, Pons TL (eds). Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic, The Hague, pp 175-186.

Pypers, P., Van Loon L., Diels, J., Abaidoo, R., Smolders, E., and Merckx R., 2006. Plant-available P for maize and cowpea in P-deficient soils from the Nigerian Northern Guinea savanna – Comparison of E- and L-values. *Plant and Soil* **283**: 251-264.

Randall DD, Tolbert NE, Gremel D. 1971. 3-Phosphoglycerate in plants II. Distribution, physiological considerations and comparison with phosphoglycolate phosphatase. *Plant Physiology* **48**: 480-487.

Rao AS. 1990. Root flavonoids. *Botanical Reviews* **56**: 1–84.

Rao TP, Yano K, Iijima M, Yamauchi A, Tatsumi J. 2002. Regulation of rhizosphere by photosynthetic activity in cowpea (*Vigna unguiculata* L. Walp.) seedlings. *Annual of Botany* **89**: 213-220.

Recourt K, van Tunen AJ, Mur LA, van Brussel AAN, Lugtenberg BJJ and Kijne JW. 1992. Activation of flavonoids biosynthesis in roots of *Vicia sativa* sub-sp. *nigra* by inoculation with *Rhizobium leguminosarum* biovar *viciae*. *Plant Molecular Biology* **19**: 411-420.

Reddy KC, Visser P, and Bukner P. 1992. Pearl millet and cowpea yields in sole and intercrop systems and their after-effect on soil and crop productivity. *Field Crop Research* **28**: 315-326.

Regnault-Roger C, Hamraoui A, Bareau I, Blanchard P, Gil M, Barberan F. 1999. Isoflavonoids involvement in the non-adaptability of *Acanthoscelides obtectus* SAY (Bruchidae, Coleoptera) to soybean (*Glycine max*) seeds. *In*: Procs 16th Annual Meeting of the International Society of Chemical Ecology, p. 110. International Society of Chemical Ecology: Marseilles, France.

Rengel Z. 2002. Genetic control of root exudation. *Plant and Soil* **245**: 59-70.

Rice-Evans CA, Miller NJ, Paganga G. 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Science* **2**: 152-159.

Richards RA. 2000. Selectable traits to increase crop photosynthesis and yield grain of crops. *Journal of Experimental Botany* **51**: 447-458.

Richardson AE, George TS, Hens M, Simpson RJ. 2005. Utilization of soil organic phosphorus by higher plants. In: B.L. Turner, E. Frossard, D. Baldwin (eds.), Organic phosphorus in the environment, pp. 165-184. CABI Publishing, Wallingford.

Rohrbach DD, Mashingaidze AB, Mudhara M. 2003. The distribution of relief seed and fertilizer in Zimbabwe. Lesson derived from the 2003/04 season. FAO/ICRISAT Zimbabwe.

Römheld V. 1986. pH-Veränderungen in der Rhizosphare verschiedener Kulturpflanzenarten in Abhängigkeit vom Nährstoffangebot. *Potash Review* **55**: 1-8.

Römheld V. 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species. An ecological approach. *Plant and Soil* **130**: 127-134.

Ruginamhozi L, Murwira HK, Nyamangara J. 2006. Cotton-cowpea intercropping and its N_2 fixation capacity improves yield of a subsequent maize crop under Zimbabwean rain-fed conditions. *Plant and Soil* **287**: 327-336.

Rusoke DG, Rubaihago PR. 1994. The influence of some crop protection management practices on yield stability of cowpeas. *African Crop Science Journal* **2**: 143-148.

Russell GB, Sutherland ORW, Hutchinson RFN, Christmas PE. 1978. Vestitol: a phytoalexin with insect feeding-deterrent activity. *Phytochemistry* **33**: 1369-1371.

Sabiti A, Nsubuga ENB, Adipala E, Ngabibeki DS. 1994. Socio-economic aspects of cowpea production in Uganda. A rapid rural appraisal. *Uganda Journal of Agricultural Science* **2**: 59-99.

Salvagiotti F, Cassman KG, Specht JE, Walters DT, Weiss A, Dobermann A. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crop Research* **108**: 1-13.

Sanchez PA, Shepherd KD, Soule MJ, Place FM, Buresh RJ, Izac A-MN, Mokwunye AU, Kwesiga FR, Nderitu CG and Woomer PL. 1997. Soil fertility replenishment in Africa: an investment in natural resource capital. In: Buresh RJ, Sanchez PA and Calhoun FG (eds) Replenishing Soil Fertility in Africa, pp 1-46. SSSA Special Publication Number 51. Soil Science Society of America, Madison, WI, USA.

San-oh Y, Mano Y, Ookawa T, Hirasawa T. 2004. Comparison of dry matter production and associated characteristics between direct-sown and transplanted rice plant in a sub-merged paddy field and relationships to planting patterns. *Field Crop Research* **87**: 43-58.

San-oh Y, Tamizi S, Daisaku Y, Taiichiro O, Tadashi H. 2006. The effect of planting pattern on the rate of photosynthesis and related processes during ripening in rice plants. -*Field Crops Research* **96**: 113-124.

Santalla M, Casquero PA, de Ron AM. 1999. Yield and yield components from intercropping improved bush bean cultivars with maize. *Journal of Agronomy and Crop Science* **183**: 263-269.

Santalla M, Rodino AP, Casquero PA, de Ron AM. 2001. Interactions of bush bean intercropped with field and sweet maize. *European Journal of Agronomy* **15**: 185-196.

Santalla M, Ron de AM, Escribano MR. 1994. Effect of intercropping bush bean populations with maize on agronomic traits and their implications for selection. *Field Crops Research* **36**: 185-189.

Schieving F, Pons TL, Werger MJA, Hirose T. 1992a. The vertical distribution of nitrogen and photosynthetic activity at different plant densities in *Carex acutiformis*. *Plant and Soil* **14**: 9-17.

Schroth G, Kolbe D, Balle P, Zech W. 1995. Searching for criteria for the selection of efficient tree species for fallow improvement, with special reference to carbon and nitrogen. *Fertilizer Research* **42**: 297-314.

Schulze S, Keatinge JDH and Wells JG. 1999. Productivity and residual effects of legumes in rice-based cropping systems in a warm-temperate environment. I. Legume biomass production and N fixation. *Field Crops Research* **61**: 23-35.

Senaratne R, Liyanage NDL, and Ratnasinghe DS. 1993. Effect of K on nitrogen fixation of intercrop groundnut and the competition between intercrop groundnut and maize. *Fertiliser Research* **34**: 9-14.

Seyoum K. 1990. Haricot bean production in Kaffa Region: Current status and opportunities for the future. In: Research on Haricot bean in Ethiopia, Proceedings of Bean Research Planning National Workshop, Addis Ababa, Ethiopia, pp. 24-29.

Shane MW and Lambers H. 2005. Cluster roots. A curiosity in context. *Plant and Soil* **274**: 101-125.

Sharpley A. 1985. Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Science Society of American Journal* **49**: 905-911.

Shearer G, Kohl DH. 1986. Nitrogen fixation in field settings: estimations based on natural abundance. *Australian Journal of Plant Physiology* **13**: 699-744.

Siddhuraju P, Mohan PS, Becker K. 2002. Studies on the antioxidant activity of Indian laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chemistry* **79**: 61-67.

Silwana T and Lucas EO. 2002. The effect of planting combinations and weeding on the growth and yield of component crops of maize-bean and maize-pumkin intercrops. *Journal of Agricultural Science* **138**: 193-200.

Sims JJ, Keen NT, Keen NT, Honwad VK. 1972. Hydroxyphaseollin, an induced antifungal compound from soybeans. *Phytochemistry* **11**: 827-828.

Sivakumar MVK, Ntare BR and Roberts JM. 1996. Growth, yield and plant water relations of four cowpea (*Vigna unguiculata* L.) cultivars in the Sahel. *Journal of Agricultural Science Cambridge* **126**: 183-190.

Sivakumar MVK, Ntare BR, and Roberts JM. 1996. Growth, yield and plant water relations of four cowpea (*Vigna unguiculata* [L.]) cultivars in the Sahel. *Journal of Agricultural Science* Cambridge **126**: 183-190.

Smith FW. 2002. The phosphate uptake mechanisms. *Plant and Soil* **245**: 105-144.

Snaydon RW, Satorre EH. 1989. Bivariate diagrams for plant competition data: modifications and interpretation. *Journal of Applied Ecology* **26**: 1043-1057.

Soil Classification Working Group (SCWG). 1991. Soil classification: A taxonomic System for South Africa. *Memorandum of Natural Agricultural Resources for South Africa*. No 15.

Speir TW, Cowling JC. 1991. Phosphatase activity of pasture plants and soils: Relationship with plant productivity and soil fertility indices. *Biology and Fertility of Soils* **12**: 189-194.

Srinivasan PS, Chandrababu R, Natarajaratnam N, Sree Rangasamy SR. 1985. Leaf photosynthesis and yield potential in green gram [*Vigna radiata* (L.) Wilczek] cultivars. *Tropical Agriculture* **62**: 222-224.

Stampfer MJ, Henneekens CH, Manson JE, Colditz GA, Rosner B, and Willet WC. 1993. Vitamin E consumption and the risk of coronary disease in women. *New England Journal of Medicine* **328**: 1444-1449.

Stanton WR, Francis BJ. 1966. Ecological significance of anthocyanin in the seed coats of the Phaseoleae. *Nature* **211**: 970-971.

Steel RGD, Torrie JH. 1980. Principles and procedures of statistics: A biometrical approach, Second Edition. McGraw Hill, New York.

Steffens JC, Lynn DG, Kamat VS, Riopel JL. 1982. Molecular specificity of haustorial induction in *Agalinis purpurea* L. Raf. (Scrophulariaceae). *Annals of Botany* **50**: 1-7.

Stenlid G. 1963. The effects of flavonoid compounds on oxidative phosphorylation and on the enzymatic destruction of indoleacetic acid. *Physiologia Plantarum* **16**: 110-121.

Stern WR. 1993. Nitrogen fixation and transfer in intercrop system. *Field Crop Research* **34**: 335-356.

Streeter J. 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate *CRC Critical Review Plant Science* **7**: 1-23.

Strom L. 1997. Root exudation of organic acids: importance of nutrient availability and the calcifuge and calcicole behaviour of plants. *Oikos* **80**: 459-466.

Subbarao GV, Ae N, Otani T. 1997. Genotypic variations in iron-, and aluminium-phosphate solubilizing activity of pigeon pea root exudates under P-deficient conditions. *Soil Science and Plant Nutrition* **43**: 295-305.

Summerfield RJ, Huxley PA, Dart PJ, and Hughes AP. 1976. Some effects of environmental stress on seed yield of cowpea *(Vigna unguiculata* (L.) Walp.) cv Prima. *Plant and Soil* **44**: 527-546.

Sutherland O, Russell G, Biggs D, Lane G. 1980. Insect-feeding deterrent activity of phytoalexin isoflavonoids. *Biochemistry System Ecology* **8**: 73-75.

Tabatabai MA. 1994. Soil enzymes. In: Methods of soil analysis, Part 2, Microbiological and Biochemical properties-SSSA Book Series, No. 5. Soil Science Society of America, Madison, WI, USA, pp. 775-859, Chapter 37.

Tadano T, Ozawa K, Sakai H, Osaki M, Matsui H. 1993. Secretion of acid phosphatase by roots of crop plants under phosphorus-deficient conditions and some properties of the enzyme secreted by lupin roots. *Plant and Soil* **155/156**: 95-98.

Tanaka A, Ito H, Tanaka R, Tanaka NK, Yoshida K, Okada K. 1998. Chlorophyll a oxygenase (CAO) is involved in chlorophyll b formation from chlorophyll a. *Proceedings of National Academy of Science USA* **95**: 12719-12723.

Tang C, Hinsinger P, Jaillard B, Rengel Z, Drevon JJ. 2001. Effect of phosphorus deficiency on the growth, symbiotic N_2 fixation and proton release by two bean (*Phaseolus vulgaris*) genotypes. *Agronomie* **21**: 683-699.

Tang C, McLay CDA and Barton L. 1997. A comparison of proton excretion of twelve pasture legumes grown in nutrient solution. *Australian Journal of Experimental Agriculture* **37**: 563-570.

Tang C, Zheng SJ, Qias YF, Wang GH, Hang XZ. 2006. Interaction between high pH and iron supply on modulation and ion nutrition of *Lupinus albus* L. genotypes differing in sensitivity to iron deficiency. *Plant and Soil* **279**: 153-162.

Tang CS and Zhang B. 1986. Qualitative and quantitative determination of the allelochemical sphere of germinating mung bean. pp. 229-242. *In*: Putnum and Tang CS, Eds. The Science of Allelopathy. John Wiley and Sons, New York.

Tarafdar JC, Claassen N. 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatase produced by plant roots and microorganisms. *Biology and Fertility of Soils* **5**: 308-312.

Tarafdar JC, Jungk A. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biology and Fertility of Soils* **3**: 199-204.

Taylor BR, Parkinson D, Pearsons W. 1989. Nitrogen and lignin contents as predictors of litter decay rates: a microcosm test. *Ecology* **70**: 97-104.

Tefera T, Tana T. 2002. Agronomic performance of sorghum and groundnut cultivars in sole and intercrop cultivation under semi arid conditions. *Journal of Agronomy and Crop Science* **188**: 212-218.

Tilman D. 1988. Plant strategies and the dynamics and function of plant communities. Princeton University press, Princeton, New Jersey, USA. 360p.

Tollenaar M, Daynard TB. 1982. Effect of source sink ratio on dry matter accumulation and leaf senescence of Maize. *Canadian Journal of Plant Science* **62**: 855-860.

Trang KM, and Giddens J. 1980. Shading and temperature as environmental factors affecting growth, nodulation, and symbiotic N_2 fixation by soybeans. *Agronomy Journal* **72:** 305-308.

Trenbath BR. 1974. Biomass productivity of mixtures. *Advances in Agronomy* **26**:177-210.

Trierweiler JF and Lindsay WL. 1969. EDTA-Ammonium carbonate soil test for zinc. *Soil Science Society of American Proceedings* **33**: 49-54.

Tsubo M and Walker S. 2004. Shade effects on *Phaseolus vulgaris* L. intercropped with *Zea mays* L. under well-watered conditions. *Journal of Agronomy and Crop Science* **190**: 168-176.

Unkovich MJ, Pate JS, Sanford P, and Amstrong EL. 1994. Potential precision of the ^{15}N natural abundance method in the field estimation of nitrogen fixation by crop and pasture legumes in Southwest Australia. *Australian Journal Agricultural Research* **45**: 119-132.

Usuda H, Ku MSB, Edwards GE. 1985. Influence of light intensity during growth on photosynthesis and activity of several key photosynthetic enzymes in a C4 plant (*Zea mays* L.). *Physiologia Plantarum* **63**: 65-67.

van Ek GA, Henriet J, Blade SF, Singh BB. 1997. Quantitative assessment of traditional cropping systems in the Sudan Savanna of northern Nigeria. II. Management and productivity of major cropping systems. *Sumaru Journal Agricultural Research* **14**: 47-60.

Vandermeer J. 1989. The ecology of intercropping. Cambridge University press, Great Britain, Cambridge 237 pp.

Vandermeer JH. 1990. Intercropping. *In*: Agroecology. Eds. Carrol CR, Vandermeer JH and Rosset PM. Pp. 481-516. McGraw-Hill, New York, USA.

Vicent JM. 1970. A manual for the study of Root-Nodule Bacteria: IBP Hand-book No. 15 England: Oxford; Blackwell scientific Publications.

Vincent JB, Crowder MW, Averill BA.1992. Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. *Trends Biochemical Science* **17**: 105-110.

von Braun J, Paulino L. 1990. Food in sub-Saharan Africa: trends, policy changes for the 1990s. *Food Policy* **15**: 505-517.

Wahua TAT, and Miller EG. 1978. Effect of intercropping on soybean N_2-fixation and plant composition on associated sorghum and soybeans. *Agronomy Journal* **70**: 292-295.

Walsh KB. 1995. Physiology of the legume nodule and its response to stress. *Soil Biology Biochemistry* **27**: 637-655.

Wang LW, Showalter AM, Ungar IA. 2005. Effects of intraspecific competition on growth and photosynthesis of *Atriplex prostrate*. *Aquatic Botany* **83**: 187-192.

Wang SF, Ridsdill-Smith TJ, Ghisalberti EL. 1998. Role of isoflavonoids in resistance of subterranean clover to the red-legged earth mite. *Journal of Chemical Ecology* **24**: 2089-2100.

Wang Z, Gottlein A and Bartonek G. 2001. Effects of growth roots of Norway spruce (*Picea abies* L. Karst) and European beech (*Fagus sylvatica* L.) on rhizosphere soil solution chemistry. *Plant Nutrition and Soil Science* **164**: 35-41.

Warrington RT, Hale AL, Scheuring DC, Whitaker DW, Blessington T, and Miller Jr. JC. 2002. Variability for antioxidant activity in cowpea (*Vigna unguiculata* L. Walp) as influenced by genotype and post harvest re-hydration. *HortScience* **37**: 738.

Wasaki J, Kojima S, Maruyama H, Haase S, Osaki M, Kandeler E. 2008. Localization of acid phosphatase activity in the roots of white lupin plants grown under phosphorus-deficient conditions. *Soil Science and Plant Nutrition* **54**: 95-102.

Wasaki J, Omura M, Osaki M, Ito H, Matsui H, Shinano T, Tadano T. 1999. Structure of a cDNA for acid phosphatase from phosphate deficient lupin (*Lupinus albus* L.) roots. *Soil Science and Plant Nutrition* **45**: 439-449.

Waterer JG, Vessey JK, Stobbe EH, Soper RJ. 1994. Yield and symbiotic N_2 fixation in a pea-mustard intercrop as influenced by N fertilizer addition. *Soil Biology and Biochemistry* **26**: 447-453.

Watiki JM, Fukai S, Banda JA, Keating BA. 1993. Radiation interception and growth of maize intercrop as affected by maize plant density and cowpea cultivar. *Field Crops Research* **35**: 123-133.

Willey RW, Osiru DS. 1972. Studies on mixtures of maize and beans (*Phaseolus vulgaris* L.) with particular reference to plant population. *Journal of Agricultural Science* **79**: 517-529.

Willey RW. 1979. Intercropping, its importance and research needs. Part I. Competition and yield advantage. *Field Crop Abstracts* **32**: 1-10.

Willey RW. 1985. Evaluation and presentation of intercropping advantages. *Experimental Agriculture* **21**: 119-133.

World Bank. 1989. Sub-Saharan Africa: from crises to sustained growth. A long-term perspective study, Washington D.C.

Xiao Y, Li L, and Zang F. 2004. Effect of root contact on interspecific competition and N transfer between wheat and faba bean using direct and indirect ^{15}N techniques. *Plant and Soil* **262**: 45-54.

Xiaoping W and Darlene Z. 1998. Nutrient composition of Douglas-fir rhizosphere and bulk soil solutions. *Plant and Soil* **200**: 13-20.

Yadav RS, Tarafdar JC. 2001. Influence of organic and inorganic phosphorus supply on the maximum secretion of acid phosphatase by plants. *Biology and Fertility of Soils* **34**: 140-143.

Yamakawa T, Kato S, Ishida K, Kodama T, Minoda Y. 1983. *Agricultural Biology Chemistry* **47**: 2185-2191.

Yan X, Liao H, Trull MC, Beebe SE, Lynch JP. 2001. Induction of a major leaf acid phosphatase does not confer adaptation to low phosphorus availability in common bean. *Plant Physiology* **125**: 1901-1911.

Yanai RD, Majdi H and Park BB. 2003. Measured and modelled differences in nutrient concentrations between rhizosphere and bulk soil in a Norway spruce stand. *Plant and Soil* **257:** 133-142.

Yoneyama T, Ohtani T. 1983. Variations of Natural ^{13}C Abundances in Leguminous Plants. *Plant Cell Physiology* **24**: 971-977.

Youssef RA, Chino M. 1987. Studies on the behavior of nutrients in the rhizosphere: I. Establishment of a new rhizobox system to study nutrient status in the rhizosphere. *Journal of Plant Nutrition* **10**: 1185-1195.

Yunusa IAM. 1989. Effect of planting density and plant arrangement pattern on growth and yields of maize (*Zea mays* [L].) and soybean grown in mixtures. *Journal of Agricultural Science* (Cambridge) **112**: 1-8.

Zelitch I. 1982. The close relationship between net photosynthesis and crop yield. *BioScience* **32**: 796-802.

Zhang F, Li L. 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. *Plant and Soil* **248**: 305-312.

Zhang F, Shen J, Li L and Liu X. 2004. An overview of rhizosphere processes related with plant nutrition in major systems in China. *Plant and Soil* **260**: 89-99.

Zhao D, Oosterhuis DM, Bednarz CW. 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultra-structure of cotton plants. *Photosynthetica* **39**: 103-109.

Zhao D, Reddy KR, Kakani VG, Read JJ, Carter GA. 2003. Corn (*Zea mays* L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. *Plant and Soil* **257**: 205-217.

Zhao D, Reddy KR, Kakani VG, Reddy VR. 2005. Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. *European Journal of Agronomy* **22**: 319-403.

Zhu X-G, Long SP, and Ort DR. 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? *Current Opinion in Biotechnology* **19** (2): 153-159.

Zhu YG, Smith SE, and Smith FA. 2001. Plant growth and cation composition of two cultivars of spring wheat (*Triticum aestivum* L.) differing in P uptake efficiency. *Journal of Experimental Botany* **52**: 1277-1282.

PUBLICATIONS

Makoi, JHJR, Chimphango, SBM, Dakora, FD. 2009. 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* **48**: 57 - 67.

Makoi JHJR, Chimphango SMB, Dakora FD. 2010. Photosynthesis, water-use efficiency and $\delta^{13}C$ of five cowpea (*Vigna unguiculata* L. Walp) genotypes grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L. Moench). *Photosynthetica* (*Accepted and in press*)

Makoi JHJR, Chimphango SMB, Dakora FD. 2010. Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere soil of cowpea (*Vigna unguiculata* L. Walp) reveal enhanced P nutrition, improved plant growth and increased grain yield in genotypes grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L. Moench). *Crops and Pasture Science* (*Accepted and in press*)

Makoi JHJR, Belane A, Chimphango SMB, Dakora FD. 2010. Flavonoids and anthocyanins in seed exudates enhance nodulation and defence in symbiotic cowpea (*Vigna unguiculata* L. Walp). *Field Crop Research* (*Accepted and in press*)

Makoi JHJR, Chimphango SMB, Dakora FD. 2010. Changes in rhizosphere concentration of mineral elements caused by differences in root uptake and plant growth of five cowpea (*Vigna unguiculata* L. Walp) genotypes grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L. Moench). *Soil Science and Plant Nutrition* (*Currently under Review*).

Makoi JHJR and Ndakidemi PA. 2010. Effect of different plant densities and cropping systems on yield components of cowpea (*Vigna unguiculata* L. Walp) genotypes and sorghum (*Sorghum bicolor* L. Moench). *Journal of Tropical Agriculture* (*Accepted and in press*).

Makoi JHJR and Ndakidemi PA. 2010. Agronomic and biochemical significance of different plant densities and cropping systems involving symbiotic legumes and cereals. *Scientific Research Essays* (*Currently under Review*).