# EVALUATION OF COWPEA (*Vigna unguicula* L. Walp) GENOTYPES FOR ADAPTATION TO LOW SOIL PHOSPHORUS CONDITIONS AND TO ROCK PHOSPHATE APPLICATION

A Dissertation

by

# SABIOU MAHAMANE

Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of

# DOCTOR OF PHILOSOPHY

May 2008

Major Subject: Agronomy

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Approved by:

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Major Subject: Agronomy

#### ABSTRACT

Evaluation of Cowpea (*Vigna unguicula* L. Walp) Genotypes
for Adaptation to Low Soil Phosphorus Conditions and
to Rock Phosphate Application. (May 2008)
Sabiou Mahamane, B.S.; M.S., University of Arizona
Co-Chairs of Advisory Committee: Dr. William A. Payne
Dr. Richard H. Loeppert

Cowpea (*Vigna ungiculata* L. Walp) is a major food and fodder legume in poor countries, particularly Sub-Saharan Africa countries. It is generally produced in sandy, acid soils, deficient in phosphorus (P) which severely limits its production. Because processed phosphate fertilizers are expensive and poorly available to farmers, rock phosphate is viewed as a cheap alternative phosphate source. The present study evaluated 696 U.S Core Collection and IITA cowpea accessions for adaptation to low soil P environments and for response to rock phosphate application. Subsequently, organic acid exudation by selected cowpea genotypes as a mechanism for P acquisition from Fe-oxide and Ca bound P was investigated.

A low P soil from Nacogdoches pine forest was used to grow plants. There were two P treatments: 0 and 300 mg P/kg of soil as Tahoua (Niger) rock phosphate. At harvest, plant height, shoot and root dry weights were determined and total biomass and shoot-to-root ratios were computed. Shoot P contents of 100 selected accessions were measured. Sixteen accessions reflecting the wide array of responses observed were selected for the organic acid study. Plants were grown in a growth chamber hydroponically with no P and +P nutrient solutions for 3 weeks. Organic acids were collected in a CaCl<sub>2</sub>-KCl solution. The nature and quantity of the collected organic acids was determined.

Cowpea accessions were significantly different in their ability to adapt to Pdeficiency stress and to acquire P from rock phosphate. The parameters most effective in separating the accessions were shoot mass and total biomass. This data will be potentially useful in the selection of cowpea germplasm for (1) adaptation to West African soils of low P fertility, and (2) ability to utilize P from poorly soluble rock phosphate. The predominant organic acid exuded by cowpea roots was a tricarboxylic acid not yet identified. There was surprisingly more exudation of this acid under +P than under –P conditions. Exudation was more highly correlated to roots than to shoots.

#### DEDICATION

To my late sister Balki Mahamane. To my grand mother Atta, my mother Zara Harouna, my father Mahamane Ibrahim Sammy, and in-laws for providing strong family support. To my wife Hadiza Idi and my children Moubarak, Fatou and Fanna for their patience with my many absences due to school activities and for missed field trips, museum visits, and other day-to-day fun things a family should do together. I am hopeful that in years to come they would understand and forgive me for this breach.

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#### CHAPTER I

#### GENERAL INTRODUCTION

#### BACKGROUND

#### **Modern Trends in West Africa**

West Africa is one of the word's poorest regions, and most of its people live on less than \$2 per day (Payne, 2006). It is economically worse off now than in 1970, after independence from colonial powers (Sanders et al., 1996; Hall, 2001). Even Ghana, the most prosperous West African country in terms of gross domestic product per capita, has its wealth distribution heavily skewed towards a relative few, with more than 80% of its people living in abject rural poverty (Payne, 2006). Life expectancies in West Africa are much less than in developed countries, and infant mortality is much higher. Population growth is among the highest in the world, despite the presence of HIV/AIDS. Political instability is a constant threat; Côte d'Ivoire, which has long enjoyed economic and political stability, risks falling into a civil war, and Nigeria, the most populous country, continues to undergo religious, political, and ethnic strife.

Most West Africans are dependent upon agricultural production for their livelihoods, but inadequate production has lead to persistent and growing food insecurity (Payne, 2006). This worsening scenario has been attributed to inadequate rainfall, locusts, political unrest, and other site-specific perturbations, but a larger and more

This dissertation follows the style of the Agronomy Journal.

persistent cause has been the increasing demographic pressure on land and other natural resources.

Current cropping systems use little to no inputs, including fertilizers (World Bank, 1997). Fallow is practiced less and less to restore soil fertility, simply due to land shortage. Cultivation and grazing have expanded into marginal lands and sensitive forested areas, causing major ecological damage to soils and other natural resources. These trends have lead to long-term nutrient depletion and other forms of soil degradation.

It has long been recognized that addressing this situation will require sustainable intensification of West African farming systems (Pieri, 1989; Subbarao et al., 2000), but despite decades of development programs and millions of dollars of investment (Sanders et al., 1996), there has been little success in reversing the trends of inadequate production and environmental degradation.

#### The West African Sahel

The Sahel is a semiarid strip of land south of the Sahara desert that stretches from Senegal to Chad, and is nominally located between the latitudes of 11° and 15°. Rainfall in the Sahel tends to be low and erratic, and occurs mainly in high intensity storms (D'Amato and Lebel, 1998; Sivakumar, 1988; Sivakumar, 1989; Sivakumar, 1992) with a steep gradient decrease of about 1mm/km from south to north (Lebel et al., 1992).

Despite the semiarid climate and erratic rainfall, several studies have concluded that low soil fertility is an even more yield-limiting factor than rainfall in all but the driest zones of the Sahel and other parts of West Africa (Nye and Greenland, 1960; Penning de Vries and Djiteye, 1982; Payne et al., 1990, 1997). Soils tend to be sandy and to have low pH, low organic-matter content, low N, P and K availability, low waterholding capacity, and low cation-exchange capacity (CEC). Clay mineralogy is largely kaolinitic (Jones and Wild, 1975; Weil, 2000; Wong et al., 1991; Jamal et al. 1997; Casenave and Valentin, 1992; Manu et al., 1996; Rockström et al., 1998; West et al., 1984). Because of the low clay content and kaolinite's low activity, soils do not have high P fixation capacity, but they do tend to have a high ratio of free iron oxide to clay, causing much of the native P to be fixed or occluded by iron forms such as goethite (West et al., 1984).

There is a consensus among soil and agronomic scientists that the most limiting factor to crop production in soils of the Sahel and other parts of Africa is P availability (Gardiner, 1990; Payne et al., 1992; Hafner et al., 1993; Weil et al., 1991; IRAT, 1975; Bationo et al., 1985; Manu et al., 1991; Geiger et al., 1992; Buresh et al., 1997; Bekunda et al., 1997). Despite many reports of strong crop response to P fertilizer, the addition of industrial forms of mineral-P fertilizer is often not seen as economically viable (Trolove et al., 2003; Smalberger et al., 2006; Akhtar et al., 2007) because of high costs and low availability in rural areas.

#### **Rock Phosphate in West Africa**

The vast majority of farmers in this region simply do not have access to industrial fertilizers because of high cost or lack of availability (Trolove, et al., 2003), a situation that has not changed for decades (Payne, 2006). Interest has therefore grown in alternative cropping systems that can efficiently use inexpensive, slow-release forms of P such as rock phosphate (McClellan and Gremillion, 1980; Buresh et al., 1997; Clark and Duncan, 1991; Shenoy and Kalagudi, 2005; Agyin-Birikorang et al., 2007). Such systems would meet production needs while minimizing soil degradation associated with nutrient depletion.

Despite widespread soil P deficiency in West Africa, there are sedimentary deposits of rock phosphate (RP) of varying quality throughout the region (Pieri, 1990). Typically, RP contains Ca-bound P as the mineral apatite. These deposits could provide an alternative source of mineral P that is less costly, but also less soluble, than imported forms (Langyintuo et al, 2003). In principal, RP could be crushed and applied directly to fields (Sheldon, 1982), but because of low reactivity, crops do not always respond to direct application. Several factors determine the suitability of RP for direct application, including chemical reactivity, particle size, method and timing of application, soil properties, climate, crop management, and finally crop genotype (FAO, 2004; McClellan and Gremillion, 1980; van Kauwenbergh and Hellums, 1995; Zapata and Roy, 2004).

# Cowpea

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop grown throughout West Africa, often in association with pearl millet [*Pennisetum glaucum* (L.) R. Br.] and sorghum (*Sorghum bicolor* L. ). Cowpea is well adapted to the harsh growing conditions of the Sahel, including low soil fertility, high temperatures, and drought (Hiler et al., 1972 and Turk et al., 1980). Provided there is sufficient soil P availability, cowpea can fix nitrogen to improve soil fertility and cropping system productivity. Additionally, farmers feed cowpea fodder to livestock to increase income, and collect the manure produced for use in their fields. Cowpea thereby reduces farmers' reliance on commercial fertilizers and sustains soil fertility (Odion et al., 2007).

Cowpea grain and leaves provide a vital source of protein to human diets, and play a role in reducing the HIV/AIDS pandemic by retarding the onset of the destructive effects of the virus on the immune system (Widders, 2005). Cowpea grain also is rich in antioxidants that scavenge free radicals and reduce risk of cancer. Cowpea also provides an important and diverse source of household income, particularly to women, because it is widely traded for multiple uses.

Cowpea-grain yield is currently only a few hundred kg ha<sup>-1</sup> in most West African countries, largely because it is grown on sandy, infertile soils. Increased cowpea production would contribute to increased sustainability of farming systems, improve human health and nutrition, and increase income (Bado, et al., 2006).

#### **Genetic Variability in Acquisition of Phosphate by Plants**

There is a renewed interest in efficient P uptake by plants for varied reasons, including environmental remediation, e.g., where soil P levels are too high, or yield increase, e.g., where soil P levels are too low (Kowligi, 1997; Trolove et al, 2003; Raghothama, 1999). Many studies have reported that plant species and even cultivars differ in their ability to take up specific elements, including P, due to several morphological, physiological, and biochemical mechanisms (Hoffland et al., 1989; Raghothama, 1999; Neumann and Römheld, 1999; Akhtar et al., 2007).

Adaptation to low P availability results from a combination of several genetic traits related to the ability to uptake and use P efficiently, root production and growth,

root exudation of acid phosphatase and organic acids, and proton pumping into the soil media (Ming et al., 2002). Plants are known to differ in their ability to use sparingly soluble sources of P such as rock phosphate (RP). Legume species are generally more effective in utilizing P from RP because they typically have high Ca demand, which leads to a net export of protons, and can acidify the rhizosphere following N fixation (Marschner, 1995). Ankomah et al. (1995) found significant differences among genotypes of more than one species for P uptake from Gafsa RP. Generally, all mechanisms of enhanced P uptake are associated with roots, and include growth, exudation, and symbiosis (Marschner, 1995; Hoffland et al., 2006; Ohwaki and Hirata, 1992; Hocking and Jeffery, 2004; Shen et al., 2001; Penaloza, 2002; Ae et al., 1990; Lipton et al., 1987; Hocking and Jeffery, 2004; Penaloza et al., 2002; Kihara et al., 2003; Hoffland, 1992)

### **Republic of Niger**

The general trends in the West African Sahel of precarious food security, unreliable rainfall, insufficient food production, soil degradation, widespread poverty, and high population growth rate are especially acute in The Republic of Niger. Niger is the second poorest country in the world, with more than 60% of its 11 million inhabitants among the poorest of the poor that live on less than one dollar a day (OECD, 2006). Its population growth rate of 3.3% is one of the highest in the world.

Niger relies almost entirely on rain-fed cropping systems in which pearl millet [*Pennisetum glaucum* (L.) R.Br.] and sorghum (*Sorghum bicolor* L.) are monocropped or intercropped with cowpea (Manyame, 2007). The trends of less fallowing and

expansion onto marginal lands are illustrated by the fact that, in order to meet growing food demand, the cultivated area of pearl millet increased over a 25 year period from 1.7 to 3.2 million ha, while the average yield declined from 530 to 345 kg ha<sup>-1</sup> (SEDES, 1987; Vierich and Stoop, 1990).

Cowpea and livestock are the most important agricultural exports in Niger. After Nigeria, it is the world's largest cowpea producer, and it is the world's largest exporter of this crop. Langyintuo et al. (2003) indicated that some 359,000 tons are produced annually, of which 353,000 tons are exported. It is produced on sandy, acid soils typical of the Sahel, with low water retention and low fertility. Grain yields are typically only on the order of a few hundred kg/ha.

Niger has two important RP mines in Tahoua and Park-W, a wildlife preserve. The Tahoua RP is generally considered to be of higher solubility, and less environmentally sensitive. Although some agronomic work has been done with Tahoua rock phosphate (Bationo et al., 1989), suitable application rates and methods are not yet fully understood.

Based on available literature, it would seem that Niger soils are appropriate for the agronomic use of ground RP because they are acidic, but RP effectiveness would depend also upon other factors, including RP reactivity and particle size, and organic matter management. Even though it is known that P uptake from sparingly soluble sources such as RP differs among and within plant species, including cowpea, overall genetic variability has never been assessed.

# **Research Objectives**

The overall objective of this dissertation is to determine genetic variability in cowpea for P uptake and response to sparingly soluble P from 1) soils in which availability is low due to low total P and adsorption of P by Fe oxide, and 2) added Tahoua RP, which has low P availability due to low solubility of Ca-bound phosphate held mostly as apatite. Given the prominent role that organic acid exudation has played in enhancing P uptake from sparingly soluble P sources, a secondary objective was to evaluate organic acid release of cowpea cultivars grown in hydroponic culture under Pdeficient and P-sufficient conditions.

#### CHAPTER II

# SCREENING OF COWPEA IN RESPONSE TO LOW SOIL PHOSPHORUS AND TO ADDITION OF ROCK PHOSPHATE

#### **INTRODUCTION**

For decades, crop yields in West Africa have been far below their potential levels, while population growth rate has been among the world's highest (Payne, 2006). This divergence has caused increasingly common food shortages. Furthermore, current cropping systems use little to no inputs and practice fallowing to restore soil fertility less and less, leading to nutrient depletion and other forms of soil degradation. Even though much of West Africa has a semiarid climate, several studies have concluded that low soil fertility is an even more important yield-limiting factor than rainfall (Voortman and Brouwer, 2003).

Soil P availability is especially low (Sinaj et al., 2001). Smalberger et al. (2006) observed that soil P deficiency in sub-Saharan Africa was so severe that other technologies would not work without some form of P addition. Similarly, Gardiner's (1990) review of available literature found that many authors had pointed out the very low levels of P availability in Sahelian soils. Despite many reports of strong crop response to P fertilizer, the addition of industrial forms of mineral P fertilizer is often not seen as economically viable (Akhtar et al., 2007) because of high costs and low availability in rural areas. Therefore, some scientists have suggested developing cropping systems that include crops that can efficiently use inexpensive, slow-release forms of P (Clark and Duncan, 1991; Shenoy and Kalagudi, 2005; Agyin-Birikorang et al., 2007). Such systems would have sufficient production to meet food demand, while minimizing soil degradation associated with nutrient depletion.

Cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous crop grown throughout West Africa, often in association with such cereals as pearl millet (*Pnnisetum glaucum* (*L.*) *R. Br.*) and sorghum (*Sorghum bicolor* (*L.*)). Cowpea grain and leaves provide a vital source of protein to the diets of subsistence farmers and their families. Additionally, cowpea provides income, particularly to women, because its grain and fodder are widely traded in West Africa for multiple uses. Cowpea grain yield is currently only a few hundred kg ha<sup>-1</sup> in most West African countries, largely because it is grown on sandy, infertile soils that have low pH, low organic matter content, poor water-holding capacity, low cation-exchange capacity (CEC), and pronounced nutrient deficiencies, especially of P. Increased cowpea production would contribute to increased sustainability of West African cropping systems by improving diets, increasing income, and ameliorating soil properties through the addition of organic matter and fixed N (Bado, et al., 2006).

Plants possess several morphological, physiological, and biochemical mechanisms to increase acquisition and utilization of P under low soil P conditions (Ragothama, 1999; Akhtar et al., 2007). For example, white lupin, which is especially well adapted to low P conditions, develops proteoid roots that synthesize and secrete organic acids that enhance release of P from Ca, Al, Fe, and Mg compounds (Dinkelaker et al., 1995; Ragothama, 1999). Lynch (1995) reported that plants exposed to P deficiency exhibit increased root-to-shoot biomass ratios, decreased root diameter, and increased surface area of roots. Lynch and Beebe (1995) found strong positive correlations between growth of common bean genotypes and their P-use efficiency.

Paradoxically, despite widespread P deficiency of West African soils, sedimentary deposits of rock phosphate (RP) of varying quality exist throughout the region (McConnell, 1938; Pieri, 1990). These indigenous sources of RP provide an alternative source of mineral P that is less costly, but also less soluble, than imported forms (Langyintuo et al, 2003). Typically, RP contains Ca-bound P as the mineral apatite.

Several factors determine the suitability of RP for direct application to fields in terms of agronomic and economic effect (FAO, 2004; McClellan and Gremillion, 1980; van Kauwenbergh and Hellums, 1995). These include RP properties, method and timing of application, soil properties, climate, crop management, and crop genotype (Zapata and Roy, 2004):

Chemical reactivity and particle size of RP are particularly important properties for agronomic effectiveness. Direct application of high-reactivity RP increased clover yield (Rajan and Watkinson, 1993), and after four year's application, RP had as much agronomic effectiveness as conventional P fertilizer. For maize, the effectiveness of the highly reactive North Carolina RP was greater than that of less reactive Morocco RP, but neither was as agronomically effective as concentrated, highly soluble super-phosphate (Juo and Kang, 1978).

Soil properties that impact RP effectiveness include pH, organic-matter content, P adsorption, CEC, and exchangeable Ca and Mg contents. Diarra et al. (2004) found that dissolution of Tilemsi rock phosphate was mainly dependent on exchangeable soil acidity, Ca, and effective CEC. Juo and Kang (1978) found that relative effectiveness of RP depended on soil type due to the fact that P from applied Ca-P was adsorbed onto aluminum and iron oxide surfaces. Kpomblekou and Tabatabai (2003) suggested that organic acids could potentially be used to amend PR-treated soils and increase availability of P to plants. Shahandeh et al. (2004) found that pearl millet response to Ca addition from three different sources depended on both soil type and year (i.e., weather and especially rainfall).

A number of crop management factors affect RP effectiveness, including organic-matter management and cropping sequence (Weil, 2000). Cation-exchange capacity and proton availability of soils can be significantly enhanced by increasing soil organic-matter concentration and can potentially provide a sink for Ca, thereby promoting continued RP dissolution and P release (Agbenin, 2004). Poultry manure also solubilizes RP and thereby substantially enhances its agronomic effectiveness for as many as four seasons of maize and cowpea crops (Akande et al., 2005). In pot studies, Somado et al. (2003) demonstrated that residual nitrogen from a previous legume crop in combination with added RP improved subsequent rice-crop biomass production and P uptake and assimilation. They speculated that P availability would be increased if RP is added with an incorporated green manure. Kamh et al. (1999) obtained higher yields from maize when planted after legumes, and attributed this improvement to enhanced P and N nutrition made possible by the legumes.

It is well established that crop species, and genotypes within species, differ in their ability to use sparingly soluble sources of P. For example, Ankomah et al. (1995) found significant within species differences among genotypes in P uptake from Gafsa RP. Legume species are generally more effective in dissolving and utilizing P from RP because they generally have high Ca demand and acidify their rhizosphere following N fixation. Zapata and Roy (2004) also found significant differences among genotypes in P-use efficiency, and defined four classes of genotypes in terms of uptake efficiency and response as illustrated in Fig. 1. They argued that efficient and responsive and

R		
Ε	Ι	II
S		
Р	Non-Efficient	Efficient and
0	but Reponsive	Reponsive
Ν	-	-
S		
Ε		
Т	III	IV
0		
	Non-Efficient and	Efficient but
R	Non-Reponsive	Non-Reponsive
Р	_	_

#### ADAPTATION TO LOW SOIL P CONDITIONS

Fig. 1. Quadrants defining genotypic adaptation to low soil P conditions and response to applied RP for any measured parameter.

efficient and non-responsive classes were the most desirable. Fagaria and Santos (2002) found that panicle length and harvest index were major selection criteria when screening upland rice for adaptation to low P conditions and response to added P. Ming et al. (2002) viewed adaptation to low P conditions as a result of a combination of several genetic traits mainly related to the ability to uptake and use P efficiently, root production and growth, root exudation of acid phosphatase and organic acids, and proton pumping in the growth medium. Krasilnikoff et al. (2003) found that roots and root-hair length varied significantly among genotypes, and a positive correlation between P uptake and volume of soil explored by roots (Krasilnikoff et al., 2003). Kolawole et al. (2000) viewed total dry matter production as the single most important criterion for screening cowpea for tolerance to P deficiency, because it reflects the genotype's overall ability to uptake and assimilate P. They found significant response to added P fertilizer in terms of shoot, root, and grain weight among cowpea genotypes, as well as increased nodulation. For some lines, shoot response to P addition was greater than root response.

Wissuwa and Ae (2001) found a high correlation between root dry weight and P uptake in rice, and concluded that genotypes tolerant to low P conditions could be identified by large rooting systems. In their study, shoot-to-root ratio was negatively correlated with P uptake. Xiaolong et al. (1996) observed a wide range of variability in common bean varieties for adaptation to low P conditions. Superior genotypes exhibited better uptake and assimilation of commercial fertilizer P or Ca-bound P, and were not as highly correlated with the ability to mobilize P from Al or Fe oxides. The genotype with

the highest response to Ca-P addition had a greater capacity to acidify the soil root interface.

According to Mugwira and Haque (1993b), Lablab accessions varied in their response to P application in nutrient solution. Superior accessions were those yielding high shoot and root biomass with a low shoot/root ratio. Ara'ujo et al. (1998) found wide variability among diverse common bean accessions screened for P efficiency and adaptation to low soil P conditions, particularly with respect to total P and rootefficiency ratio. Greater variability was noticed when P was added than under no P conditions. Since they did not observe a significant genotype by P interaction for shoot-P concentration, they concluded that efficiency for P uptake and assimilation could be a good selection criterion when selecting for low P conditions. They also indicated that P supply influenced P uptake more than root production.

Significant relative efficiency differences were found by Akhtar et al. (2007) in Brassica accessions grown in P-deprived and P-sufficient solution media. Diversity was found for all growth parameters, including growth rate and P-utilization efficiency (PUE). Plants treated at low P concentrations exhibited increased root-shoot ratio. There were significant correlations between total biomass and its partitioning and plant P uptake and P use efficiency. Under both low and optimal P supply, efficient cultivars produced greater biomass. Low P-tolerant cultivars were able to translocate absorbed P to young tissue to sustain normal overall plant growth in a P-deficient environment.

The larger the number of genotypes involved in screening for stress tolerance, the more useful the derived information to plant breeders. For this reason, screening

methods should be simple and fast (ideally, evaluations should be made at earlier growth stages). Wissuwa and Ae (2001) reported that differences in day length sensitivity and variability in length of growth cycle among rice genotypes did not have an impact on P-deficiency tolerance. For screens related to low nutrient tolerance, soil as opposed to nutrient solution is the preferable medium because it holds the conditions under which selected accessions are expected to perform well. For example, Hayes et al. (2004) found that wheat plant-growth parameters collected from solution culture were not good criteria for selection for P efficiency. Better results for genotypic differences were obtained in soil media than in nutrient solution. Disadvantages of using soil include the variability introduced due to heterogeneous soil environments, and root extraction and washing is difficult (Gerloff, 1987).

Based on available literature, it would seem that West African soils are appropriate for the agronomic use of ground RP because they are acidic, but RP effectiveness would depend also upon other factors, including RP reactivity and particle size and organic-matter management. It is well established that P-use efficiency from sparingly soluble sources such as RP differs among and within plant species. A limited amount of data has shown genetic differences for adaptation to low P availability for cowpea, but overall genetic variability has never been assessed.

#### **OBJECTIVES**

The objectives of this investigation were to assess variability within the U.S. Cowpea Core Collection for tolerance to low P conditions in an acid, sandy soil, and for response to rock phosphate using ground rock phosphate from Tahoua, Niger.

Although it has been established by a small number of studies that cowpea cultivars respond differently to low P soil and to rock phosphate addition, assessment of the overall genetic variability for these traits within cowpea has never been made.

#### MATERIALS AND METHODS

#### **Screening Media**

The screening study in soil to evaluate genetic variability among cowpea accessions was conducted in a glasshouse at the Texas AgriLife Research Station at Bushland, Texas. The study was designed to compare cowpea accessions under both low P conditions (adaptive) and in response to RP addition (responsive). The soil used was the surface horizon (0-10 cm depth) of a Betis sand (sandy, siliceous, thermic Psammentic Paleustalf), which had been used in earlier P-response studies because of its physical, chemical, and mineralogical similarities to soils of the West African Sahel (Payne, 1990). The soil had a pH of 4.7 in a 1:1 water:soil mixture, and plant-available P content of 3 mg kg<sup>-1</sup> using the Melich III method. Other physical and chemical properties are shown in Table 1. The soil was air dried and sieved to pass a 2-mm mesh sieve to rid it of roots, leaves, and other coarse material. The soil was thoroughly mixed during drying and sieving to reduce heterogeneity, and 200 g were placed into 20-cm deep Conetainers (Ray Leach Conetainers®, Portland, Oregon) for the subsequent screening study.<sup>1</sup> A paper filter barrier was placed in each conetainer prior to addition of soil to prevent loss of soil.

<sup>&</sup>lt;sup>1</sup> Mention of trade names does not constitute an endorsement.

Analysis		Results		Units	Rating
	Composite Sample 1	Composite Sample 2	Mean		
рН	4.7	5	4.85	-	Strongly Acid
Salinity	12	20	16	ppm	None
Nitrate-N	3	3	3	ppm	Very Low
Phosphorus	3	3	3	ppm	Very Low
Potassium	15	15	15	ppm	Very Low
Calcium	102	100	101	ppm	Very Low
Magnesium	7	7	7	ppm	Very Low
Sulfur	6	6	6	ppm	Low
Sodium	136	149	142.5	ppm	Low
Iron	4.42	4.11	4.265	ppm	High
Zinc	0.33	0.03	0.18	ppm	Moderate
Manganese	6.11	6.21	6.16	ppm	High
Copper	0.11	0.07	0.09	ppm	Moderate
Boron	-	-	-	ppm	
Sand			95.0	%	
Silt			3.0	%	
Clay			2.0	%	
USDA Textura	al Class		SAND		

Table 1.	The physical and chemical properties of the Betis soil (sandy, siliceous,
	thermic Psammentic Paleustalf) used in the current study.

# **Phosphate Treatments**

Phosphate treatments were 0 added P (No P) and 300 mg P kg<sup>-1</sup> soil added as Tahoua RP from Tahoua, Niger. The Tahoua RP, previously crushed and sieved to obtain the 50 to 100 mesh per inch particle size fraction, was provided by IFDC (Muscle Shoals, Alabama). The chemical and physical properties of the Tahoua RP are summarized in Table 2. The Tahoua RP contained 15 % P (34.5% P<sub>2</sub>O<sub>5</sub>) and 8.3 % of neutral ammonium-citrate soluble P. Its relative agronomic efficiency has been evaluated to be high (Truong et al., 1978; see FAO Bulletin 13, 2004). For the P-treated samples, 400 mg of Tahoua RP was mixed with the soil of each individual pot by thoroughly

homogenizing in a zip-lock polyethylene bag.

Phosphate rocks	Total cont	tent % ore	Solubility e	xpressed as 9	% total P <sub>2</sub> O <sub>5</sub>	Substitution CO <sub>3</sub> /PO <sub>4</sub>
	P <sub>2</sub> O <sub>5</sub>	CaO	Neutral	Citric acid	Formic acid	
			NH₄ Citrate			
Arli	30.8	47.6	5.4	19.2	38.7	0.098
Kodjari	30.1	44.8	6.1	18.8	37.1	0.093
Tahoua	34.5	44.8	8.3	19.3	34	0.112
Taiba	36.5	44.8	5	19.8	38.7	0.098
Tilemsi	27.9	43.1	10.4	29.7	47.3	0.21
Hahotoe	35.4	36.4	4.3	19.1	36.7	0.088
Gafsa	30.2	31.9	20.5	37.8	78.6	0.254

Table 2.	Chemical analysis and solubility in conventional reagents of some African
	phosphate rocks.

Source: Truong et al. (1978)

#### **Cowpea Accessions**

A total of 696 cowpea accessions (681 from the U.S Cowpea Core Collection and 15 from B. B. Singh, IITA) were used. Specific accessions characteristics are summarized in Appendix A.

### **Screening Experiments**

The experimental design was a completely randomized design with accession and P as treatments, and with each of the three replications in individual blocks. The overall screening experiment is illustrated in Fig. 2. Figure 3 on the other hand, gives an idea of how P deficiency affects plant growth. The plant on the right, the P-deficient one, is stunted. Its older leaves developed chlorosis-like symptoms and will eventually dry and fall off. If P deficiency is too severe, the younger leaves might synthesize phosphatase, an enzyme which helps it remobilize P from the younger leaves to the growth tissue.

Two seeds of each accession were planted in each container at a depth of 2 cm, and plants were thinned to one plant per container immediately after germination.



### Fig. 2. Illustration of overall screening experiment.

Temperature and relative humidity in the glasshouse were recorded using Pro Series<sup>R</sup> Hobo *onset* data loggers. Day/night temperatures and relative humidity averaged approximately 32/16 °C and 60 %, respectively. Containers were watered to field capacity before planting using tap water containing negligible amounts of P to stabilize and prepare soil for sowing. After planting, water was applied once a day until germination was complete. After stand establishment, water was applied once every two days as fine droplets from a hose to bring soil back to field capacity. All nutrients were applied at every other watering at optimal rates with a Hoagland's solution with no phosphate (Table 3). About 25 to 35 mL water or nutrient solution was applied at each watering or during each nutrient solution addition, depending on the genotypes and to make sure adequate moisture was supplied to the plants in relation to their specific needs. Some genotypes required the addition of more water than others to bring soil to field capacity at each irrigation time. The nutrient solution was added using graduated cylinders.

#### **Plant Assays**

After eight weeks, plant height was measured and shoots were harvested, dried at 70 °C for 72 hr, and weighed. Roots were washed with tap water to remove soil, dried at 70 °C for 72 hr, and weighed. Prior to weighing the roots, they were inspected to ensure that no soil aggregates were adhering to the roots. If present, any adhering soil was carefully removed. Plant parameters measured were shoot dry weight (SDW), root dry weight (RDW), and plant height (Height). Shoot-to-root dry-weight ratio (STRR) was computed. Relative gain in each plant parameter was calculated to assess TRP response,  $y=(x_2 - x_1)/x_1$ ), where  $x_2$  is height or weight in the TRP treatment, and  $x_1$  is height or weight in the No P treatment.

		Stock soln.	
Salt	Stock Conc.	used	
	Macronutrients		
	$mol L^{-1}$	$mLL^{-1}$	
$Ca(NO_3)_2.4H_2O$	1	4	
KNO <sub>3</sub>	1	6	
MgSO <sub>4</sub> .7H <sub>2</sub> O	1	2	
	Micronutrients		
For 1L of	micronutrient sto	ck solution	
	$\mu$ mol L <sup>-1</sup>		
MnSO <sub>4</sub> .H <sub>2</sub> O	11.8		
$H_3BO_3$	46		
NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> 0	0.16	1 mL	
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.7		
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.32		
For 1	L of Iron stock so	lution	
	ppm		
Sequestrine 1			
330Fe-10%Fe	5	1mL	
Ferric Chel. DTPA		of either	
		one	
Sequestrine 2		We used #1	
138Fe-6%Fe	5		
EDDHA			

 Table 3. Nutrient solution used at the screening stage.



Fig. 3. Phosphorus deficiency symptoms in cowpea. left, P-sufficient plant and right, P-deficient plant.

Shoots of 100 genotypes (Appendix B), selected based on P response, were digested with concentrated  $HNO_3/H_2O_2$  (EPA method 365.1), and the solution was analyzed for P content using Method 410-3651 with the SmartChem 200 WESTCO (Brookfield, Connecticut). Concentration of P in the shoots was expressed as ppm P.

# **Data Analysis**

Data were analyzed using SYSTAT version 11 (SYSTAT Software Inc., Point Richmond, California). Gap histograms and descriptive statistics were used to assess density distribution for each parameter. Cluster analysis (K-means procedure) was used to detect natural groupings among cowpea accessions.

#### **RESULTS AND DISCUSSION**

#### **Plant Growth**

The results indicated a wide range of variability among the accessions for adaptation to the low P environment and response to added rock phosphate (e.g., the shoot data, Fig. 4). The data for SDW was normally distributed, but with some degree of skewness. If the results were due to random performance of a given plant parameter, then the distribution of that parameter should be described by a perfectly normal, bellshaped curve. But in this study, the skewness indicates that there might be two or more distinct subpopulations, especially in the case of shoot dry-matter production with no P added, possibly one subpopulation in which individuals performed well and another subpopulation in which accessions had an ordinarily low performance as would be expected under low soil P conditions. For each of these subpopulations, means and variances could be determined in support of the idea that the core collection is behaving like a bimodal population (comments from B.B. Singh).

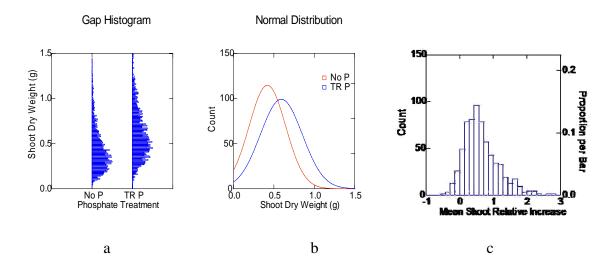


Fig. 4. Shoot dry weight (SDW) a. gap histogram; b. frequency distribution; and c. relative increases from no P to P treatments.

Our results are consistent with those of Kolawole et al. (2000) who in pot and field experiments with cowpea lines, also observed significant differences among lines for shoot, root, and total dry-matter production in response to low soil P conditions and to addition of P fertilizer. The current results also indicate that shoot response to the application of TRP was relatively greater than root response in conformity with results of Kolawole et al. (2000) and Gill et al. (2005) who reported that shoot was often the most positively affected by fertilizer-P additions. Xiaolong et al. (1996), in a study of common bean, also found that shoot represented more to the total plant response to P addition than did roots. Their results showed that shoot/root ratios increased with increasing P availability, clearly suggesting a strong response of shoots to P addition. On the other hand, the current results contrast with those of Mugwira and Haque (1993a) who found significant growth differences in alfalfa accessions for all yield components except shoots. The soil used in their study had very low pH, low P, and did not contain aluminum. They maintained that all growth parameters except shoot responded positively to P-fertilizer application and/or liming. They also concluded that adaptation of alfalfa genotypes to low soil P conditions was related to root growth and that such soils should be limed and fertilized in order to have a good fodder harvest.

In the current study (Fig. 5), there were even some cases in which plant growth in response to TRP application was negative. This negative response could have been due to the toxic effect of other elemental components contained in the Tahoua rock phosphate, such as magnesium (Mg), to which these particular accessions might be sensitive (see composition of TRP in Table 2, adapted from Truong et al., 1978). Also,

it is possible that micronutrient deficiencies, e.g., of Fe and Zn, could have contributed to yield reductions in susceptible cowpea accessions in the presence of rock phosphate.

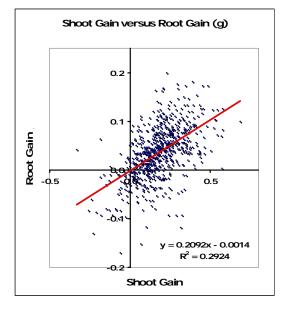


Fig 5. Gains in root mass and shoot mass of the individual accessions of cowpea as a result of the TRP treatment compared to the no P treatment.

# **Shoot Growth**

The wide ranges of shoot dry weights with both the no P and TRP treatments are illustrated in Figs. 4A and 6. The gap histogram of no added P data for SDW (Fig. 4a) tended to be more skewed towards lower SDWs and had greater kurtosis, i.e., a more peaked distribution than the TRP data which was flatter (Fig. 4b). While the mean and median increased moderately in going from no P to TRP treatments, the standard deviation increased substantially (Table 4). This trend indicates greater variability in response to TRP than to adaptation to low soil P conditions. A second implication of this observation is that with no added P most genotypes performed poorly, but that a few genotypes were still able to achieve relatively high yields by utilizing just the naturally occurring P in the soil. These genotypes were adaptive to the low soil P environment. It is suggested that these genotypes were either able to use the small amount of available P efficiently or were able to mobilize the Fe- and Al-oxide bound P in addition to the small quantity of P that was bioavailable in the non-amended soil. In contrast, under added P as TRP, many accessions exhibited a response in terms of increased shoot production

Table 4. Measures of statistical variability for shoot, root, and shoot-to-root data.

	No			No			No	
SDW	Ρ	TRP	RDW	Ρ	TRP	STRR	Ρ	TRP
Median	0.37	0.56	Median	0.2	0.24	Median	1.90	2.37
Mean	0.42	0.60	Mean	0.21	0.25	Mean	2.02	2.51
Std dev.	0.23	0.72	Stad dev.	0.09	0.09	Stad dev.	0.85	0.94
Skew (G1)	1.22	0.91	Skew (G1)	0.89	0.48	Skew (G1)	5.88	2.33
Kurtosis G2)	1.83	1.14	Kurtosis G2)	1.36	0.24			

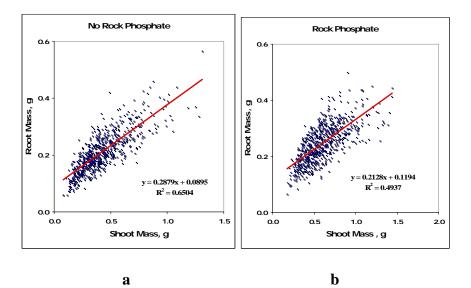


Fig. 6. Shoot mass versus root mass: (a) without TRP; (b) with TRP.

(Fig. 4 and 5). These accessions are termed responsive, because they were able to use the P from Ca-P in addition to the naturally occurring soil P.

The mean shoot relative increase histogram (Fig. 4c) showed that some plants exhibited nearly a 300 % increase in response to the addition of RP. Other accessions had even a negative relative gain compared to their performance under no P.

# **Root Growth**

The range of root dry weights for the no P and TRP treatments are illustrated in Fig. 6 and 7a. Frequency distributions and gap histograms of the root dry-weight data (Fig. 7) reflected similar trends to those observed with the shoot dry weight. Root production was highly variable between accessions. As with the shoot dry-matter data, the no P treatment root data was more skewed towards lower root dry-weight levels and had higher kurtosis than the TRP data. This trend reflects a higher degree of variability in response to added P than the case with adaptation to the low soil P environment (Table 4). Differences in root-growth pattern were observed with many genotypes, both under the no P and the added RP treatments. Significant root mass increases were observed in response to RP application as shown by the gap histogram of mean relative root increase in Fig. 5c. Some genotypes even doubled their root size, while a few others tripled it. However, there were a number of accessions that did not respond to RP addition in terms of root growth, and some exhibited a negative response (Fig. 5).

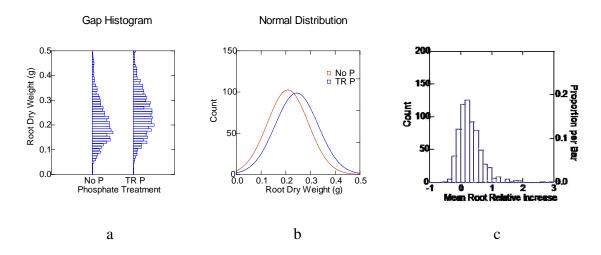


Fig. 7. Root dry weight (RDW) (a) gap histogram; (b) frequency distributions; and (c) relative increases from no P to TRP treatments.

In the current study, we did not attempt to characterize root morphology. However, depending on the specific screening objective, root characteristics might be a preferred criterion for selection. With our objective of determining root mass, we encountered some problems. Washing the roots is quite challenging, since it is difficult to remove all soil particles without loss of some roots (Gerloff, 1987). Nonetheless, we expect that root-biomass loss during washing was relatively small because it mostly affected root hairs. In spite of problems inherent in the quantitative determination of root mass from soil, we believe that with uniform procedures, e.g., as utilized in the current study it is possible to accurately compare cowpea accessions for root mass and shoot-tomass ratio.

#### **Total Dry Matter Production**

Total dry-matter gain data analysis (Fig. 8) revealed large differences among accessions in terms of response to rock phosphate addition. A few accessions had no gain at all, while some produced 3 to nearly 4 times more total biomass than others, due to the effect of RP application and their natural ability to utilize RP. Relatively few accessions exhibited a negative gain.

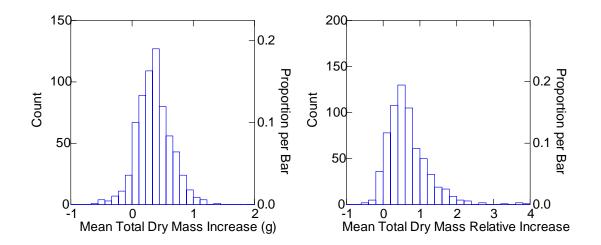


Fig. 8. Total dry matter gap and mean relative shoot-to root ratio (STRR) increase histograms.

# **Shoot-to-root Ratio**

Though characterized by a large amount of missing data as reflected in Fig. 9a, the STRR data was very informative. It revealed the variable response to TRP addition compared to the no P treatment, as illustrated by the skewness differences observed between the no P and TRP gap histograms of Fig. 8a. Some genotypes were able to achieve a STRR of up to 5:1 with the TRP treatment, while in the no P treatment, the highest STRR obtained was nearly 4:1 for only a few accessions. In both cases, very high values were observed, suggesting a superior performance. In the low soil P condition, some accessions with small root systems were able to acquire bound P and produce relatively high aboveground biomass. With the application of TRP, shoot-toroot ratio for some genotypes was almost doubled, relative to their value when P was not added. The implication of these observations is that some genotypes were able to produce larger biomass with little root growth by efficiently using P from the added RP.

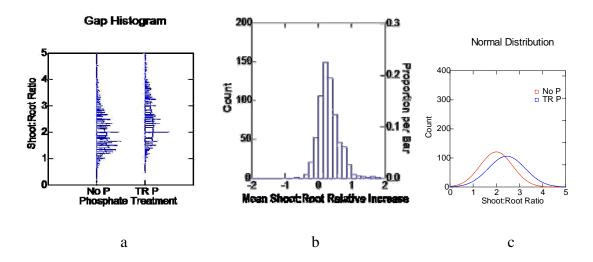


Fig. 9. Shoot/root ratio: (a) gap histograms; (b) mean relative increase; and (c) normal frequency distribution.

Our results are similar to those of Xiaolong et al. (1996), but might be in contradiction with Mugwira and Haque (1993a,b) and Wissuwa and Ae (2001) who advocated that for adaptation to low soil P, the dominant traits to select for are high shoot, high root, and low shoot-to-root ratio.

Kihara et al. (2003) reported trends similar to ours for the comparative adaptation of barley and white lupin to P starvation. They, as well as Lynch and Brown (2001) contended that decreased root/shoot ratio (i.e., increased shoot/root ratio) is the predominant evidence of plant adaptation to low soil P conditions. The results of the present study have the merit of being specific in that they show which component of the ratios contributed more to the observed increase. For instance, cowpea selection for adaptation to the P-deficient sub-Saharan Africa soils would have greater impact on production if adaptation to drought was concomitantly considered. Such programs would target those cowpea lines that exhibited the increased ratio while maintaining strong root response. Furthermore, large root biomass will help rebuild the much needed organic matter in these soils and significantly contribute to nitrogen fertilization. Hardter and Horst (1991) argued that there is superior benefit from using cowpea in a rotation rather than in intercroppin where maize yields were decreased. Whatever cowpea-based cropping system is used, enough evidence exists in support of the ultimate positive impact of cowpea on the physical and chemical characteristics of the soils, which leads to their overall enhanced impact on sustainable productivity.

Absolute weight gains indicate that some accessions had high response to TRP, as reflected by shoot and root weights. In general, shoot weight was more responsive to TRP than root weight and contributed more to the total biomass produced, as illustrated by the steeper slope of the fitting line in Fig. 10a compared to that of the line in Fig. 10b. This trend supports the suggestion that aboveground biomass production might be a better criterion than root mass for screening for response to rock phosphate application. Xiaolong et al. (1996) reported similar results, where regardless of the P sources, common bean genotypes produced comparatively more leaves and stems than root biomass. They suggested that total dry matter had a similar trend as that of aboveground biomass in response to different P sources.

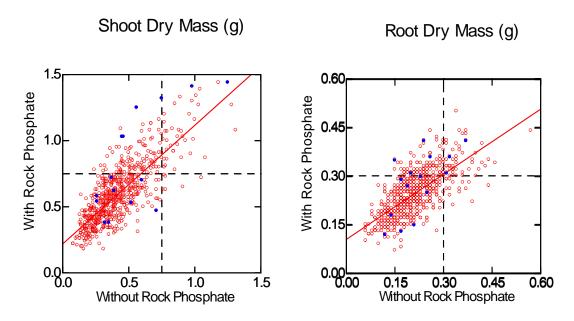


Fig. 10. Shoot and root dry mass without rock phosphate.

# **Plant Height**

The results did not show any evidence for the influence of P nutrition on plant height (Figs. 11 and 12). Gap histograms in Fig. 11 for both treatments appear to be mono-modal with no skewness, characteristic of a distinctive population. Data is normally distributed, and based on the bell-shaped curves of the respective frequency distributions, there is no substantial evidence that there are any height differences within genotypes resulting from P addition. Physiologically, significant plant height differences due to TPR would have been observed as a result of internode elongation. But internode elongation was not observed, and height differences seemed to be only dependent on inherent genetic potential of the lines, which was not modified in response to P status of the soil. Some genotypes in both soil conditions were unusually tall, simply because they produced tendrils which were taken into account when measuring height. They appeared in the data set as outliers and were removed from the analysis.

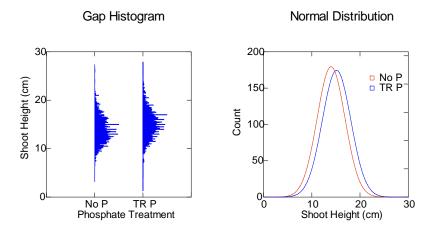


Fig. 11. Plant height gap histogram and frequency distribution.

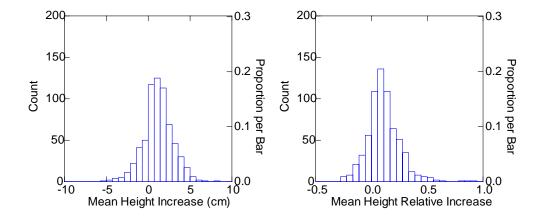


Fig. 12. Plant absolute height increase and relative height increase from no P to TRP treatments.

#### **Phosphorus: Tissue Content and Plant Uptake**

Large variabilities between genotypes for P uptake both under no P and RP conditions were observed. As expected, there was also a positive correlation between shoot dry mass and P uptake. RP addition generally favored greater P uptake and aboveground biomass production compared to the low soil P conditions, as seen in Fig. 13. With the no P treatment, few genotypes exhibited high total P uptake and were able to achieve high above-ground biomass production. This result is further confirmation of the earlier interpretation of the descriptive statistics which stated that with no added P only a few genotypes would be able to perform reasonably. There is also evidence that a few of the accessions used more P for relatively low shoot production, even if this P came from RP, which translates into poor P-utilization efficiency. Gill et al. (2005) reported similar results when comparing phosphorus efficiency of winter maize, wheat, and chickpea. Winter maize was more P-efficient than wheat and chickpea because it was able to produce 70 % of its maximum above-ground dry biomass with only 0.2 % P in its shoot, while wheat and chickpea utilized over 0.25 % P in their shoots to achieve only 30 % of their maximum shoot dry weights. It is not enough for a genotype or species to be able to uptake P from RP or any other source for that matter, but that the P must also be best utilized and assimilated for improved growth and yield. All genotypes were responsive to RP addition as far as P uptake is concerned, but some were more responsive and efficient than others.

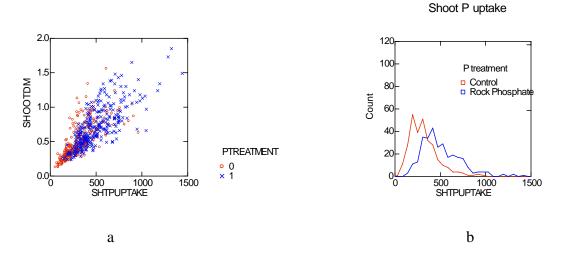


Fig. 13. (a) Shoot P uptake vs shoot dry matter and (b) frequency distribution of shoot P uptake.

Phosphorus Root uptake Efficiency as defined by Payne et al. (1995) is the amount of P taken up by a plant per unit weight of root produced. Our data reflected a high level of variability in PRE, but most genotypes were within the range of low values for both P treatments, as illustrated in Fig. 14. Within this range, the no P treatment seemed to display slightly more variability. Few accessions had very large PRE values with either treatment, although more high values were evident with the RP treatment than with the no P treatment. Payne et al. (1995) reported PRE as being potentially a more reliable indicator than PUE (phosphorus use efficiency) for selecting crops for improved yields both under low soil P and with P-fertilizer addition. Results herein indicate the possibility of screening for this trait, since high PRE values were observed with both P treatments. Shoot-P concentration also varied widely among genotypes (Fig. 12), despite the very low overall values. Most accessions had extremely low P concentrations. As in the case of total P uptake, highest shoot P concentrations were evident in a few genotypes when RP was applied.

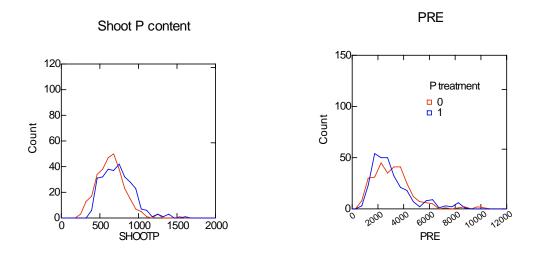


Fig. 14. Shoot P concentration and phosphorus root uptake efficiency.

Generally, results indicated a good possibility for selection of cowpea cultivars adapted to enhanced acquisition of P under low P conditions and for response to rock phosphate application. For both traits, depending on the objective of the breeder, measured parameters can be used for selection of the highest performing genotypes. Shoot dry matter appeared to be the most reliable screening criterion when grain production improvement is targeted. Total biomass, and to some degree shoot-to-root ratio and root dry biomass can be used as screening criteria to differentiate genotypes. Genotypes can also be selected on the basis of plant PRE and leaf-P content (Payne, 1995).

Since further depletion of P in already critically deficient soils would not be a sustainable solution to the enormous problems of soil degradation and low cropping-system productivity in Niger and other parts of sub-Saharan Africa, response to added rock phosphate might be a more desirable trait than adaptation to low P. Nutrient mining, when all other management alternatives such as fallow are impossible, will surely result in further soil fertility decrease to a point where even the most P-efficient genotypes will not be able to achieve their given potential.

#### **K-Means Clustering**

The K-means clustering was done using shoot, root, and dry weights, three of the most reliable screening parameters herein evaluated (Figs. 15-17). This procedure revealed important differences among accessions for their response to TRP (Fig. 17) and when grown under no P and TRP conditions (Figs. 15 and 16, respectively). For example, under no P conditions (Fig. 15), clustering revealed distinct genotypic groups with growth ranging lowest growth (cluster 1) to highest (cluster 8) for all three parameters. One accession in Cluster 3 and another in cluster 5 had a slightly high growth due almost entirely to their root growth. The high growth in total dry matter for two of the four genotypes of cluster 8 was due to higher shoot growth than to root growth, although even roots did have high growth. Cluster 6, 7, and also cluster 8 tended to have superior growth for all three parameters.

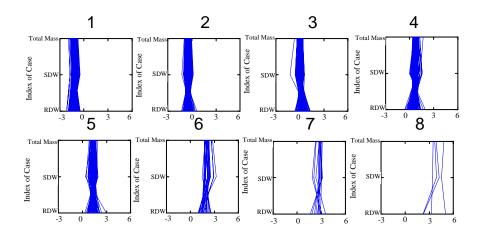


Fig. 15. No P cluster plots.

Under TRP conditions (Fig. 16), genotypes in clusters 1, 2, and 4 had a negative response for nearly all three parameters. Clusters 5 and 8 genotypes on the other hand appeared to have superior response mainly due to high shoot response in the case of cluster 5 and mainly due to high root response in the case of cluster 8. Clusters 7

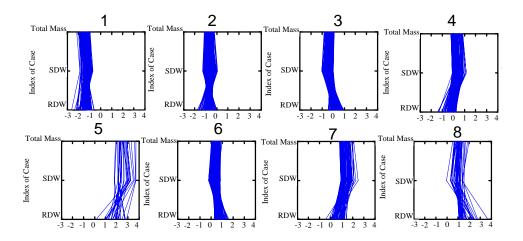


Fig. 16. TRP cluster plots.

genotypes also tended to have a high response that is largely due to their high shoot response. Overall, accessions in cluster 3 seemed to have no response in terms of total biomass, as their apparent high root response is visibly annihilated by their negative shoot response.

Clusters in Fig. 17 are particularly interesting because they indicate how the different accessions responded to TRP application. Those in cluster 4 had poor response relative to all three parameters, including some that had negative growth responses to TRP application. In contrast, those in cluster 8 had high total biomass response in which shoot contribution was greater. Genotypes in cluster 5 and also those in cluster 7 exhibited high total biomass, but unlike the accessions in cluster 8, their gain in total biomass was mainly due to superior root response compared to shoot response. Overall, accessions in cluster 8 had the highest relative response, but the single accession in cluster 8 had an unusually high root response accompanied by a negative shoot response.

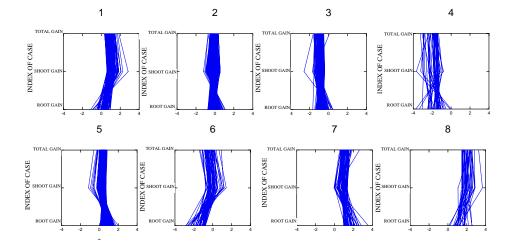


Fig. 17. Gain cluster plots.

# **Relative Rankings of Cowpea Accessions**

Tables 5-10 show the ranking of accessions relative to shoot and root mass produced with or without addition of TRP, and also according to the relative increase of these parameters. Shaded accession numbers in tables mean that the particular accession appeared in same ranking for both parameters or both P treatments levels. Some accessions were high in both parameters but had overall low ranking in terms of gains. Others have consistently high ranking shoot and root mass and are able to maintain a high ranking with regard to gain as well. Accessions at the lowest ranking level relative to gains are generally those reported as having negative gains.

	cutegories.								
	No P Ac	ddition		TRP Addition					
Serial #	Plant Inventory #		Shoot Mass, g	Serial #	Plant Inventory #		Shoot Mass, g		
431	PI	582469	1.31	677	PI	610533	1.44		
618	PI	583206	1.28	413	PI	582353	1.44		
413	PI	582353	1.25	620	PI	583209	1.41		
677	PI	610533	1.18	352	PI	307561	1.39		
412	PI	582352	1.08	202	PI	164979	1.34		
433	PI	582471	1.05	327	PI	293505	1.33		
352	PI	307561	1.04	596	PI	583182	1.32		
408	PI	582343	1.03	616	PI	583204	1.30		
202	PI	164979	1.01	565	PI	582923	1.28		
337	PI	293573	1.00	618	PI	583206	1.27		
439	PI	582523	0.99	408	PI	582343	1.27		
620	PI	583209	0.98	210	PI	170869	1.25		
616	PI	583204	0.98	683	IT79K-813	21.0	1.20		
402	PI	478622	0.97	109	PI	548784	1.20		
109	PI	548784	0.96	308	PI	292891	1.16		
111	PI	608035	0.93	337	PI	293573	1.16		
676	PI	610520	0.91	489	PI	582707	1.14		
643	PI	583241	0.91	349	PI	307556	1.13		
217	PI	175962	0.90	254	PI	205141	1.13		
685	IT97K-819	154.0	0.90	194	PI	152196	1.12		
327	PI	293505	0.89	324	PI	293477	1.09		
190	PI	148681	0.88	350	PI	307558	1.09		
615	PI	583203	0.87	305	PI	292871	1.08		
612	PI	583200	0.87	652	PI	583252	1.08		
565	PI	582923	0.85	431	PI	582469	1.08		
670	PI	583513	0.85	190	PI	148681	1.08		
505	PI	582736	0.84	685	IT97K-819	154.0	1.07		
468	PI	582670	0.84	363	PI	339565	1.07		
350	PI	307558	0.83	651	PI	583251	1.07		
336	PI	293569	0.82	566	PI	582924	1.07		
218	PI	175963	0.79	664	PI	583274	1.05		
301	PI	279845	0.79	676	PI	610520	1.03		
201	PI	163448	0.79	286	PI	250416	1.03		
363	PI	339565	0.79	368	PI	339590	1.03		
172	PI	512286	0.78	612	PI	583200	1.03		

Table 5. Shoot mass for 5% top ranking accessions with and without TRP addition.Shaded entries indicate that a given accession is in the top 5 % in bothcategories.

	TRP - SHO	OT MASS		TRP - ROOT MASS					
Serial #	Plant Invo	entory	Shoot Mass , g	Serial #	Plant Inv	entory	Root Mass , g		
677	PI	610533	1.44	41	PI	353289	0.50		
413	PI	582353	1.44	109	PI	548784	0.45		
620	PI	583209	1.41	677	PI	610533	0.44		
352	PI	307561	1.39	431	PI	582469	0.44		
202	PI	164979	1.34	566	PI	582924	0.44		
327	PI	293505	1.33	668	PI	583502	0.44		
596	PI	583182	1.32	350	PI	307558	0.43		
616	PI	583204	1.30	440	PI	582524	0.43		
565	PI	582923	1.28	295	PI	256341	0.42		
618	PI	583206	1.27	413	PI	582353	0.41		
408	PI	582343	1.27	156	PI	487497	0.41		
210	PI	170869	1.25	345	PI	300174	0.41		
683	IT79K-813	21.0	1.20	489	PI	582707	0.41		
109	PI	548784	1.20	6	PI	352765	0.41		
308	PI	292891	1.16	651	PI	583251	0.40		
337	PI	293573	1.16	617	PI	583205	0.40		
657	PI	583261	1.15	681	PI	612607	0.40		
489	PI	582707	1.14	639	PI	583237	0.40		
254	PI	205141	1.13	439	PI	582523	0.40		
349	PI	307556	1.13	682	IT98D-478	8.0	0.39		
194	PI	152196	1.12	565	PI	582923	0.39		
324	PI	293477	1.09	324	PI	293477	0.39		
350	PI	307558	1.09	20	PI	353040	0.38		
305	PI	292871	1.08	305	PI	292871	0.38		
652	PI	583252	1.08	683	IT79K-813	21.0	0.38		
431	PI	582469	1.08	111	PI	608035	0.37		
190	PI	148681	1.08	534	PI	582854	0.37		
685	IT97K-819	154.0	1.07	370	PI	339592	0.37		
363	PI	339565	1.07	422	PI	582422	0.37		
651	PI	583251	1.07	533	PI	582853	0.36		
566	PI	582924	1.07	175	PI	582429	0.36		
664	PI	583274	1.05	210	PI	170869	0.36		
676	PI	610520	1.03	530	PI	582850	0.36		
368	PI	339590	1.03	620	PI	583209	0.36		
612	PI	583200	1.03	618	PI	583206	0.36		

Table 6. Shoot and root mass for 5 % top ranking accessions with TRP addition.(See Table 5).

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Serial #	# Plant Inventory		Gain , g	Serial #	Plant Inv	entory	Gain , g
210	PI	170869	0.69	388	PI	447582	0.20
365	PI	339587	0.58	56	PI	354580	0.18
286	PI	250416	0.58	566	PI	582924	0.18
596	PI	583182	0.57	458	PI	582578	0.17
368	PI	339590	0.57	376	PI	339601	0.17
361	PI	337506	0.55	345	PI	300174	0.17
532	PI	582852	0.54	617	PI	583205	0.17
594	PI	583170	0.53	640	PI	583238	0.17
92	PI	491193	0.53	370	PI	339592	0.17
221	PI	179125	0.52	41	PI	353289	0.16
175	PI	582429	0.51	454	PI	582573	0.16
194	PI	152196	0.50	324	PI	293477	0.15
376	PI	339601	0.49	137	PI	353045	0.15
651	PI	583251	0.48	530	PI	582850	0.15
254	PI	205141	0.46	76	PI	358307	0.15
671	PI	583550	0.46	366	PI	339588	0.14
349	PI	307556	0.46	687	IT98K-589	2.0	0.14
300	PI	277786	0.46	609	PI	583197	0.14
458	PI	582578	0.45	295	PI	256341	0.13
307	PI	292890	0.45	4	PI	349852	0.13
327	PI	293505	0.44	292	PI	255782	0.13
564	PI	582913	0.44	364	PI	339572	0.13
683	IT79K-813	21.0	0.44	338	PI	293582	0.13
476	PI	582680	0.44	423	PI	582423	0.13
292	PI	255782	0.43	564	PI	582913	0.13
310	PI	292893	0.43	286	PI	250416	0.13
308	PI	292891	0.43	300	PI	277786	0.13
565	PI	582923	0.43	487	PI	582705	0.13
620	PI	583209	0.42	178	PI	115681	0.13
666	PI	583489	0.42	175	PI	582429	0.12
324	PI	293477	0.42	343	PI	300171	0.12
305	PI	292871	0.42	532	PI	582852	0.12
675	PI	610517	0.41	6	PI	352765	0.12
98	PI	527272	0.41	94	PI	517910	0.12
243	PI	194208	0.41	469	PI	582671	0.12

Table 7. Shoot and root mass gain for 5 % top ranking accessions.(See Table 5).

GAIN - SHOOT MASS

GAIN - ROOT MASS

GAIN - SHOOT MASS			GAIN - ROOT MASS					
Serial #	ial # Plant Inventory		l # Plant Inventory Gain, g		Serial #	Plant I	nventory	Gain , g
433	PI	582471	-0.33	670	PI	583513	-0.17	
164	PI	487527	-0.26	201	PI	163448	-0.15	
582	PI	583076	-0.24	431	PI	582469	-0.13	
431	PI	582469	-0.23	616	PI	583204	-0.12	
412	PI	582352	-0.21	615	PI	583203	-0.12	
428	PI	582466	-0.18	402	PI	478622	-0.10	
64	PI	354778	-0.18	417	PI	582415	-0.10	
398	PI	448760	-0.18	202	PI	164979	-0.09	
672	PI	583551	-0.16	363	PI	339565	-0.09	
405	PI	580978	-0.15	398	PI	448760	-0.09	
172	PI	512286	-0.15	595	PI	583172	-0.09	
53	PI	354518	-0.13	629	PI	583227	-0.09	
409	PI	582344	-0.13	412	PI	582352	-0.09	
658	PI	583262	-0.12	164	PI	487527	-0.08	
659	PI	583263	-0.12	416	PI	582368	-0.08	
629	PI	583227	-0.11	53	PI	354518	-0.08	
27	PI	353081	-0.09	390	PI	448096	-0.08	
87	PI	382128	-0.09	590	PI	583158	-0.08	
390	PI	448096	-0.09	87	PI	382128	-0.08	
571	PI	582941	-0.08	172	PI	512286	-0.08	
467	PI	582669	-0.08	475	PI	582679	-0.08	
402	PI	478622	-0.07	336	PI	293569	-0.07	
636	PI	583234	-0.07	61	PI	354743	-0.07	
303	PI	291139	-0.07	224	PI	183251	-0.07	
670	PI	583513	-0.06	46	PI	353362	-0.07	
416	PI	582368	-0.06	329	PI	293520	-0.06	
583	PI	583098	-0.06	301	PI	279845	-0.06	
567	PI	582930	-0.06	582	PI	583076	-0.06	
123	PI	291384	-0.06	197	PI	154134	-0.06	
130	PI	196301	-0.06	427	PI	582465	-0.06	
427	PI	582465	-0.05	313	PI	292898	-0.06	
147	PI	427093	-0.04	154	PI	487490	-0.06	
159	PI	487502	-0.04	505	PI	582736	-0.05	
73	PI	354857	-0.03	139	PI	353335	-0.05	
667	PI	583494	-0.03	679	PI	610620	-0.05	

Table 8. Shoot and root mass gain for 5 % lowest ranking accessions.(See Table 5).

GAIN - SHOOT MASS

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GAIN - ROOT MASS

TRI - SHOOT MASS			TRI - ROOT MASS					
Serial #	Plant In	ventory	Shoot Mass , g	Serial #	Plant Ir	ventory	Root Mass , g	
53	PI	354518	0.18	53	PI	354518	0.07	
624	PI	583222	0.18	390	PI	448096	0.08	
60	PI	354715	0.20	460	PI	582581	0.09	
46	PI	353362	0.20	80	PI	367863	0.10	
460	PI	582581	0.21	46	PI	353362	0.10	
66	PI	354801	0.21	214	PI	175327	0.11	
636	PI	583234	0.22	624	PI	583222	0.11	
59	PI	354708	0.22	637	PI	583235	0.11	
80	PI	367863	0.22	121	PI	255755	0.12	
19	PI	353017	0.22	78	PI	358715	0.12	
452	PI	582571	0.24	267	PI	212635	0.12	
633	PI	583231	0.25	270	PI	218123	0.12	
23	PI	353055	0.25	636	PI	583234	0.12	
121	PI	255755	0.26	231	PI	186460	0.12	
509	PI	582740	0.26	118	PI	205139	0.13	
559	PI	582879	0.26	600	PI	583188	0.13	
70	PI	354838	0.26	263	PI	211642	0.13	
526	PI	582823	0.26	298	PI	262179	0.13	
542	PI	582862	0.27	509	PI	582740	0.13	
294	PI	255815	0.27	549	PI	582869	0.13	
119	PI	205140	0.27	66	PI	354801	0.13	
17	PI	352979	0.27	294	PI	255815	0.13	
619	PI	583207	0.27	632	PI	583230	0.13	
538	PI	582858	0.28	633	PI	583231	0.13	
637	PI	583235	0.28	31	PI	353127	0.14	
270	PI	218123	0.28	2	PI	347639	0.14	
632	PI	583230	0.28	119	PI	205140	0.14	
241	PI	194206	0.28	235	PI	189374	0.14	
54	PI	354524	0.29	26	PI	353066	0.14	
47	PI	354429	0.29	114	PI	180355	0.14	
123	PI	291384	0.29	122	PI	270065	0.14	
118	PI	205139	0.29	151	PI	470274	0.14	
390	PI	448096	0.30	646	PI	583246	0.14	
529	PI	582826	0.30	59	PI	354708	0.14	
34	PI	353190	0.30	72	PI	354845	0.14	

Table 9. Shoot and root mass for 5% lowest ranking accessions with TRP addition.(See Table 5).

**TRP - SHOOT MASS** 

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**TRP - ROOT MASS** 

	No P .	Addition		TRP Addition				
Serial #	Plant Inventory		Shoot Mass , g	Serial #	Plant Ir	Plant Inventory		
59	PI	354708	0.08	53	PI	354518	0.18	
80	PI	367863	0.08	624	PI	583222	0.18	
559	PI	582879	0.10	46	PI	353362	0.20	
294	PI	255815	0.11	60	PI	354715	0.20	
121	PI	255755	0.13	66	PI	354801	0.21	
241	PI	194206	0.13	460	PI	582581	0.21	
509	PI	582740	0.13	636	PI	583234	0.22	
535	PI	582855	0.13	59	PI	354708	0.22	
318	PI	293453	0.14	80	PI	367863	0.22	
60	PI	354715	0.14	19	PI	353017	0.22	
242	PI	194207	0.14	452	PI	582571	0.24	
120	PI	250238	0.14	633	PI	583231	0.25	
237	PI	189378	0.14	23	PI	353055	0.25	
601	PI	583189	0.14	121	PI	255755	0.26	
529	PI	582826	0.14	509	PI	582740	0.26	
22	PI	353050	0.14	70	PI	354838	0.26	
263	PI	211642	0.14	526	PI	582823	0.26	
564	PI	582913	0.14	559	PI	582879	0.26	
30	PI	353125	0.15	542	PI	582862	0.27	
54	PI	354524	0.15	119	PI	205140	0.27	
72	PI	354845	0.15	294	PI	255815	0.27	
46	PI	353362	0.15	17	PI	352979	0.27	
463	PI	582665	0.16	619	PI	583207	0.27	
624	PI	583222	0.16	538	PI	582858	0.28	
70	PI	354838	0.16	270	PI	218123	0.28	
276	PI	222755	0.16	632	PI	583230	0.28	
236	PI	189375	0.17	637	PI	583235	0.28	
293	PI	255811	0.17	241	PI	194206	0.28	
452	PI	582571	0.17	47	PI	354429	0.29	
524	PI	582821	0.17	54	PI	354524	0.29	
78	PI	358715	0.17	123	PI	291384	0.29	
94	PI	517910	0.17	118	PI	205139	0.29	
537	PI	582857	0.17	390	PI	448096	0.30	
235	PI	189374	0.17	34	PI	353190	0.30	
249	PI	197056	0.17	529	PI	582826	0.30	

# Table 10. Shoot mass for 5% lowest ranking accessions with and without TRP addition. (See /table 5).

### CONCLUSIONS

Our results suggest a high degree of variability in cowpea for adaptation to low P conditions and for the ability to use Ca-bound P in ground rock phosphate. Density distributions were in each case normal, but tended to be more skewed and have higher kurtosis under no P conditions. The manner in which plants responded to low P conditions and TRP addition also varied greatly in terms of biomass, biomass partitioning, and height response. This high degree of variability suggests the need for further studies to determine physiological mechanisms of adaptation, as well as the heritability of traits that confer adaptation. Since heritability of adaptation to low P availability has been demonstrated in other species, the wide variability observed in this study suggests that breeding for improved cowpea adaptation to low P availability, and in particular to P utilization from rock phosphate, is a feasible goal that may contribute substantially to sustainable intensification of cropping systems in Sub-Saharan Africa.

#### CHAPTER III

# ORGANIC ACID EXUDATION AS PHOSPHATE DEFICIENCY STRESS ADAPTATION MECHANISM BY SELECTED COWPEA ACCESSIONS

#### **INTRODUCTION**

#### **Phosphorus Status of Sub-Saharan Africa Soils**

Sub-Saharan soils are old, highly weathered soils. They are sandy, acidic, low in organic matter, and consequently have low fertility due to low levels of most nutrients (Jones and Wild, 1975; Weil et al., 1991; Wong et al., 1991; and Jamal et al. 1997). Nearly all scientists agree that these soils are most deficient in phosphorus (P), a major limiting factor to crop production in this region (Payne et al., 1992; Hafner et al., 1993). Inherently low total P levels which result in low plant-available P (Payne, 2006 citing IRAT, 1975; Bationo et al., 1985; Manu et al., 1991; Geiger et al., 1992; Buresh et al., 1997; Bekunda et al., 1997) are commonly associated with non P-bearing parent material and the low P-fixing capacities and organic matter contents of most Sahelian soils. According to Smaling et al. (1997), decades of improper agricultural practices (mining nutrients without replenishing and removal of crop residue for fuel), harsh climate and severe wind erosion have aggravated the situation (Buresh et al., 1997). Yields are becoming lower each year, and food crises are increasingly becoming chronic even in areas once known to sustain adequate production levels. Today, the P deficiency is so severe in some places that some scientists are calling for urgent measures, before the negative impact damages soil productivity irreversibly.

#### **Phosphorus Forms and Bonding in Soils**

Generally, P exists in two forms, i.e., organic and mineral. Organic P is the fraction assimilated into organic matter or assimilated into microbial biomass. This fraction is plant available only upon mineralization. The inorganic P form is made up of soluble inorganic P (P<sub>i</sub>), which is readily available to plants, and the so called sparingly available P sources, principally CaCO<sub>3</sub>-bound P, CaPO<sub>4</sub> minerals and Fe- and Al-oxide bound P.

Sandy soils such as those of Sub-Saharan Africa have very low organic matter and hence do not contain much of the organic fraction. P retention capacity of these soils is therefore not controlled by organic matter. Calcium is also low in these soils, so they contain very little Ca-associated P in the form of Ca-P minerals and CaCO<sub>3</sub>-bound P. The dominant form of P is Fe- and Al-oxide bound P. Almost all of the potentially soluble P is adsorbed on these oxide surfaces, where it is held tightly as an inner sphere bidentate adsorption complex, and becomes difficult to release and is poorly bioavailable.

#### **Phosphorus Release from Soil Minerals**

Calcium-bound P and Fe- and Al-oxide adsorbed P can be released through two surface reactions, ligand exchange and dissolution (Johnson and Loeppert, 2006), as summarized below. Ligand exchange (Eq. 1) involves competitive adsorption of a competing anion at the mineral surface and subsequent desorption of inorganic phosphate.

Fe-oxide-HPO<sub>4</sub> + citrate 
$$\rightarrow$$
 Fe-oxide-citrate + HPO<sub>4</sub><sup>2-</sup> [1]

In the case of phosphate, ligand exchange is usually a relatively slow reaction, because of the relatively strong bonding of phosphate to Fe- and Al- oxide surfaces.

The release of phosphate by dissolution is illustrated in Eqs. 2-5 below.

Fe-oxide-HPO<sub>4</sub> + citrate  $\rightarrow$  Fe<sup>3+</sup>-citrate + HPO<sub>4</sub><sup>2-</sup> [2]

Al-oxide-HPO<sub>4</sub> + citrate 
$$\rightarrow$$
 Al<sup>3+</sup>-citrate + HPO<sub>4</sub><sup>2-</sup> [3]

$$Ca-PO_4 + citrate \rightarrow Ca^{2+}-citrate + HPO_4^{2-}$$
[4]

$$Fe-oxide-HPO_4 + e^- \rightarrow Fe^{2+} + HPO_4^{2-}$$
[5]

Eq. 2 illustrates a ligand-enhanced dissolution reaction by which citrate reacts with structural Fe<sup>3+</sup> in the Fe-oxide mineral, resulting in mineral dissolution and the subsequent solubilization of absorbed phosphate. Eq. 3 represents a similar reaction involving the release of surface-adsorbed phosphate from Al oxide. The relative importance of these reactions depends on the prevalence of the respective and Fe- and Al-oxide phases and the relative ease of dissolution of the mineral phases. The current indications for most soils of Niger are that the Fe oxides play a more important role in P retention and release. Fe oxides in soils exist in both poorly crystalline, e.g., ferrihydrite, and well crystalline, e.g., goethite and hematite, forms. The poorly crystalline minerals are more readily dissolved than the well crystalline minerals because of their overall reactivity, and hence generally play a more important role in P release and bioavailability. The soils of Niger have relatively low concentrations poorly crystalline compared to well crystalline Fe-oxide phases, so in consideration of the low P status of the soils of Niger, the overall utility of reactions illustrated by Eq. 1-3 in supplying adequate phosphate for plant growth might be questioned.

Eq. 4 illustrates the ligand-enhanced dissolution of a Ca-phosphate mineral, e.g., rock phosphate. Though Ca-phosphate minerals do not exist naturally in most agricultural soils of Niger because of their relatively low Ca status, this reaction might have relevance to the Niger situation, because of the natural occurrence of rock phosphate mineral deposits throughout the country.

Eq. 5 illustrates the reductive dissolution of Fe oxide and subsequent release of adsorbed phosphate. This reaction is important in reduced or flooded soils, but is unlikely to play an important role in the well drained, oxidized soils of Niger.

#### **Phosphorus Deficiency Stress Response of Plants**

Plants exhibit several strategies for adaptation to low soil P conditions. Vance et al. (2003) reviewed the major adaptations, which include modification of root morphology, organic acid exudation, enhanced membrane-bound phosphatase activity, and rhizosphere acidification by proton release. They reported that the nature and degree of the response depend on the plant species and to some extent also on the severity of the P-deficiency stress. Pearse et al. (2007) noted that often, plants use a combination of strategies to achieve superior performance under severe P starvation. Generally, Pdeficiency stress-response mechanisms are associated with roots and enable plants to explore larger soil volumes and to acquire P from sparingly available sources.

# **Root Morphology**

An important plant adaptation in low soil P environments is the change in root structure observed with some plant species and genotypes with enhanced P-mobilization and uptake ability. Plants having this trait typically develop roots that are more branched, finer and longer with an increased number of elongated root hairs and formation of lateral roots (Liu et al., 2004; Zhu et al., 2005; and Gahoonia et al., 2007). In addition to exploring larger areas of soil where patches of plant-available P are available, these changes offer increased root surface area and activity. In this respect, proteoid roots are the most efficient. Liu et al. (2004) attributed the better performance of a P-efficient compared to a P-inefficient maize mainly to improved root morphology both in terms of weight and lateral length, which translated into an ability to explore more soil than the inefficient counterpart.

In a summary of their research results on the mechanisms of adaptation of white lupin to low soil P conditions, Neumann et al. (2000) reported that it is the internal P status of the plant that regulates cluster-root formation. Phytohormones (both inductive and inhibitory) produced within the plant react to the internal P status to determine the response to P starvation. The dominant adaptation mechanism involves proteoid root formation, which at maturity combines organic-anion exudation with proton extrusion to mobilize sparingly available soil P. Adaptation to low P environments is also related to alteration of the citric acid cycle in the roots with subsequent adaptation of anion channels.

Hocking and Jeffery (2004) investigated conditions for cluster (proteoid) root formation and organic acid exudation by several old world lupin species and one species of a new world lupin in hydroponic culture. They specifically wanted to know how a synthetic auxin, IBA (indole-3-butyric acid) affects proteoid root formation when plants were supplied with optimal P. They also examined the potential for exudation of citrate

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and malate from roots of *L. angustifolius L.* plants grown in solution high in nitrate-N (NO<sub>3</sub>-N). The authors found that, under P starvation, some species were able to form cluster roots and exude mainly citrate and also a considerable amount of succinate. Lateral roots and root tips under any P-supply status did not exude noticeable amounts of citrate and malate. Application of IBA favored proteoid root development by certain species grown under sufficient P supply, but these roots did not exude any citrate. In one species, high NO<sub>3</sub>-N and limited P supply promoted growth of non-proteoid roots that exuded citrate. Lateral roots and root tips of these plants did not exude any organic anions. In view of the wide range of responses to diverse conditions the authors recommended that cluster root formation and organic anion exudation in lupin species be further investigated.

Zhu et al. (2005) suggested that selection for improved phosphorus efficiency by use of markers can be achieved by targeting material from genotypes that have root-hair length and plasticity, because these traits allow for a better exploration of the soil.

Barley genotypes with short root hairs tend to have positive response to added P, while long root-hair genotypes are very P-efficient and have the ability to maintain high yields under low P conditions (Gahoonia and Nielsen, 2004). Chickpea genotypes vary significantly in terms of root length, root hair length, and root hair density (Gahoonia et al., 2007).

# **Proton Release**

Phosphorus-deficient plants release protons, resulting in the lowering of rhizosphere pH (Marschner, 1995; and Kania et al., 2003). Marschner (1995) indicated

that depending on the plant and soil conditions, the difference in pH between the rhizosphere and bulk soil could reach two units. The release of the protons is intimately related to cation: anion uptake ratio. As the ratio increases, i.e., less anion uptake, proton release rate also increases. The decrease in pH favors dissolution of P from Caphosphate minerals and the potential availability of P to the plant. The release of protons is generally concomitant with exudation of organic acids, with the net effect of acidifying the rhizosphere and making more P available to the plant. Hinsinger and Gilkes (1996) observed that ryegrass and clover mobilized P from rock phosphate and from Al oxide surfaces primarily by releasing protons which acidified the rhizosphere, thereby solubilizing bound P. The magnitude of the induced pH decrease depended on the plant species, the source of P applied, and the form of nitrogen fed to the plants. While proton release seemed to be the major mechanism for phosphate mobilization from rock phosphate by both pasture species, the authors found evidence that it is the ability of a species to efficiently utilize P that most determined its performance under Al-P nutrition. Neumann and Römheld (1999) also found that under P-deficient conditions, tomato, chickpea and white lupin roots released higher amounts of protons than they normally would when sufficient P was available; however, the amount of protons released by wheat was not affected by P availability. With chickpea and white lupin, but not with wheat and tomato, large amounts of organic acid were released simultaneously with the release of protons. Some genotypes were able to acidify the rhizosphere better than others, and this characteristic seemed to be related to an increased root surface area resulting from longer root hairs in good amount (Gahoonia et al., 2007). Decrease in pH due to the extrusion of protons by *Lupinus albus* following organic acid exudation was found to enhance P-scavenging activity of the plant (Gardner et al., 1983).

### Phosphatase

Increased membrane-bound phosphatase activity is another mechanism used by some plants in P-deficient soils to retrieve sparingly soluble P from organic matter. This type of phosphatase is an enzyme attached to the root-hair membrane. Unlike organic acid exudation, the phosphatase is not released into the soil. So its activity largely depends on the ability of the root system to explore the soil for organic matter. Phosphatase is involved in the breakdown of organic matter, thereby releasing P to the soil solution, where it is available for plant uptake. The intensity of activity and amount of enzyme also depend on pH (Johnson, 1999). Maximum phosphatase activity occurs in the pH range of approximately 4 to 9. Each enzyme also has specific substrates that impact its activity. In soils high in soluble iron or other metal ions the enzyme activity seems to be inhibited (Johnson, 1999). Soil temperatures below 37 and above 40 °C, such as those occurring in sub-Saharan Africa, greatly affect enzyme production. Phosphatase will not free Fe-oxide bound P or the P from rock phosphate. Therefore, phosphatase might have less impact in soils with high Fe-oxide and low organic-matter content. The benefit derived from phosphatase might be relatively small, considering the resistance of some organic matter components to breakdown (Johnson, 1999). Phosphatase has been identified in a limited number of species, but that list does not include millet, sorghum and cowpea. Wasaki1 et al. (2003) observed increased levels of

acid phosphatase on roots of white lupin grown under low P conditions. In P-efficient maize, Liu et al. (2004) found evidence of an increased root phosphatase activity that contributed to its overall performance in a P-deficient soil. The contrasting findings indicate that the impact of phosphatase under P starvation is still not well understood. **Organic Acid Release** 

Plant Species Exhibiting Organic Acid Release. Enhanced organic acid exudation by plant roots has been observed as a plant adaptation to P deficiency in a wide range of plant species, e.g., with chick pea and white lupin (Neumann and Romheld, 1999); rice (Hoffland et al., 2006); pigeonpea (Ae et al., 1990); *Lupinus albus* (Gardner et al., 1983); *Banksia integrifolia* (Grierson, 1992); and rape (Hoffland et al., 1989). P-stressed alfalfa roots could exude as much as 182% more citric acid than phosphorus-sufficient plants (Lipton et al., 1987).

**Organic Acid Root Exudates.** The organic acid exudates identified include oxalic acid and to a lesser extent citric acid by rice (Hoffland et al., 2006); malonic, succinic, fumaric, malic, citric and t-aconitic acids to varying extents by chickpea, cowpea, soybean, pigeon-pea, groundnut and kidney bean (Ohwaki and Hirata, 1992); citric, malic and succinic acids by some lupinus species (Hocking and Jeffery, 2004); pentanedioic acid by elephantgrass (Shen et al., 2001); citric acid by white lupin (Penaloza, 2002); piscidic acid by pigeonpea (Ae et al., 1990); citric, malic, and succinic acids by alfafa (Lipton et al., 1987); citric (principally), malic and aconitic acids by Banksia integrifolia (Grierson, 1992); malic and citric acids by rape (Hoffland et al., 1989); citric, malic, malonic, succinic and piscidic acids by pigeonpea (Ishikawa et al., 2002). Between 5 and 100 times more piscidic acid than citric acid is exuded, depending on the P-starvation level, plant growth stage, and genotype of pigeonpea (Ishikawa et al., 2002). Of all identified organic acids, citric acid was the most efficient in acquiring P from alfisols (Ishikawa et al., 2002).

Localization of Organic Acid Release. Hocking and Jeffery (2004) reported enhanced cluster-root formation and significant exudation of citrate, malate, and succinate by some lupinus species grown under P-deficient conditions. Penaloza et al. (2002) observed that citrate is exuded only from cluster roots of white lupin, while malate is exuded from cluster roots as well as root tips. The proteoid roots of *Banksia integrifolia* are involved in the exudation of citric acid (Grierson, 1992). A comparative study of barley and white lupin adaptation to low P environments revealed the involvement of proteoid roots in accumulating and excreting organic anions, mainly citrate (Kihara et al., 2003). Hoffland (1992) found that rape could acquire P from rock phosphate and concluded that the only possible mechanism was organic acid exudation of mainly malic and citric acids. The rates of exudation were such that P was provided to plants more than their roots could absorb, which suggests that increased root length might not be required for P acquisition from rock phosphate unless accompanied by organic acid exudation.

**Environmental Factors Impacting Organic Acid Release.** The exudation of organic anions is dependent on other soil factors, namely the soil-N nutrition status. Other deficiency stresses might also result in the release of organic acid, e.g., oxalate and citrate exudation in response to Zn deficiency by rice (Hoffland et al., 2006).

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Physiologic Factors Impacting Organic Acid Release. Excretion and concentration of citrate in white lupin root cells were significantly correlated (Penaloza et al., 2002). They concluded that the release of citrate is dependent on the age of roots and occurs whenever citrate concentration reaches a critical level in the cluster root cells. On the contrary, Ohwaki and Hirata (1992) observed that organic acid release might be more related to leaf conditions rather than to amounts in the roots. Kihara et al. (2003) observed that the accumulation of citric acid in barley and white lupin roots occurs following an alteration of the citric-acid cycle in the roots cells. Weisskopf et al. (2006) studied isoflavonoid exudation in relation to P availability of white lupin. Genistein and hydroxygenistein have been identified as the dominant isoflavonoids exuded in larger amounts from P-stressed than from P-sufficient plants and from proteoid roots than from ordinary roots. Younger cluster roots appear to have maximum exudation, and the mature ones have decreased excretion. Seemingly, maximum flavonoid exudation occurs just before a surge of organic acid exudation. ATP-citrate lyase could potentially control citrate, and isoflavonoid exudation and phenolic compounds could positively impact the efficiency of citrate by keeping its scavengers out of the rhizosphere. Exudation of organic acid depended on the concentration and form of nitrogen as well as phytochrome release (Hocking and Jeffery, 2004).

Dong and Yang (2004) determined the activities of six root-tip produced enzymes relative to the effects of P stress on the physiological pathways of two soybeans genotypes. They stated that in early stages of P starvation there is no significant alteration of the activities of phosphoenolpyruvate carboxylase (PEPC), phosphoenolpyruvate phosphatase (PEPP), malate enzyme (ME), isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH), and pyruvate kinase (PK). However for both genotypes, a two weeks induced P stress generally enhanced the activities of PEPC, PEPP, ME and ICDH. This activity enhancement did not seem to be related to the synthesis and release of organic acid. The study concluded that the nature of organic acid exudation in soybean depends on the source of stress. Phosphorus stress specifically promoted oxalate and malate release and Al stress promoted citrate exudation. The fact that organic acid exudation and the quantity or activity of endoenzymes were not closely related implies that synthesis and exudation of organic acid by P and/or Al stresses could be imposed at different levels.

**Other Factors Impacting Phosphorus Acquisition.** Other researchers have cautioned that organic acid release is not the sole factor that impacts plant response to P deficiency. Pearse et al. (2007) observed that carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from Al-, Fe- or Ca-phosphate sources. They concluded that the ability of plants to solubilize and uptake P from slow release sources results from a combination of factors including carbohydrates, rhizosphere pH and root structure. Neumann and Romheld (1999) observed the simultaneous release of H<sup>+</sup> and organic acid, which together likely impact the mobilization of soil P.

Genetic Variability in Organic Acid Release. Significant differences in Pdeficiency stress response have been observed between species and within a species. Neumann and Römheld (1999) observed that chickpea and white lupin exuded a large amount of organic acid, principally citric acid, when grown under P-deficiency stress conditions. This phenomenon was not observed for tomato and wheat. Gahoonia et al. (2000) observed in laboratory and field studies that two barley cultivars differed considerably in their ability to mobilize and utilize P from soil-Al and -Fe oxides. They attributed this difference to the enhanced exudation of citric acid by the efficient variety, though mentioned that differences between cultivars might also be influenced by differences in P-use efficiency. Pearse et al. (2007) concluded that different plant species acquire P from different low availability sources using diverse strategies, and that no species was capable of acquiring P from all tested sources. Carboxylates released into the rhizosphere by Pisum sativum and C. arietinum did not enable them to access AIPO<sub>4</sub> or FePO<sub>4</sub>. Species accessed different forms of sparingly soluble P, but no species was superior in accessing all forms. The authors concluded that superior strategy in P acquisition from all sparingly soluble forms would be one that combines several factors including organic acid exudation, rhizosphere-pH modification, and change in root morphology. Though pigeonpea exudes a significant quantity of organic acid as a Pdeficiency stress adaptation, Ishikawa et al. (2002) concluded in their study that organic acid exudation could not alone explain genotypic differences observed for adaptation of pigeonpea to low soil P conditions. Hoffland et al. (2006) noted that exudation of low molecular weight organic anions is an adaptive mechanism for P-deficiency stress in rice genotypes, and that different genotypes released different organic anions at different rates. Shen et al. (2002) attributed the observed superior P-solubilizing activity of Andean genotypes compared to Mesoamerican genotypes to higher extrusion of organic

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acids, citrate in particular. These acids enhanced the plants ability to mobilize P from Aland Fe-bound phosphates and could be the basis for making common bean efficient in P acquisition.

Following critical assessment, Rengel (2002) noted significant differences among plants and species in their response to biotic and abiotic stresses. Different genotypes will exude diverse types of organic acids in variable quantities when faced with nutrient deficiencies, ion toxicities, and pathogen attacks. Generally, investigations have addressed the behavior of a limited number of genotypes and little assessment has been done on a large diversified group of the germplasm. Strong knowledge of the genetic mechanism for controlling organic acid exudation as a means of enhancing P acquisition under low soil P conditions is a major step towards achieving increased crop production.

Methods of Evaluation of Organic Acid Release. Scientists have evaluated Pdeficiency induced organic acid release in hydroponic culture. For example, Dong and Yang (2004) first grew seedlings normally for 6 days and then collected organic acid at 0, 2, 4, 6, 8, 10, 12, and 14 days following placement of plants in a –P 1/5 strength Hoagland's solution. Exudate was collected in a 0.5 mM CaCl<sub>2</sub> solution. Hayes et al. (2004) collected organic acid from wheat grown in hydroponic culture after initially exposing the wheat to low P concentrations (0, 1 and 10  $\mu$ M P) in a <sup>1</sup>/<sub>4</sub> strength Hoagland's solution. Organic acids were collected for 2 h in a 0.5 mM CaSO<sub>4</sub> solution and analyzed by ion chromatography. Ishikawa et al. (2002) collected root exudates from 30-45 day old pigeonpea plants initially grown in mixed nutrient solutions with 2 and 80  $\mu$ M P. Organic acids were collected from 4 seedlings grown in a 1 L container for 24 h in 1 mM CaCl<sub>2</sub>. Samples were filtered and concentrated by rotary evaporation prior to purification and concentration by an ion-exchange procedure and analysis by HPLC equipped with an ion-exclusion column. Shen et al. (2001) collected root exudates of elephant grass in 1.5 mM CaCl<sub>2</sub> for 4 h and analyzed them by capillary-electrophoresis and GC-MS. Martin et al. (2007) collected organic acids from excised apical root segments using an anion-exchange procedure and analyzed organic acids by HPLC with visible/UV detection.

#### **OBJECTIVES**

Previous studies with several plant species, including *Lupinus albus*, chickpea, barley, and soybean have shown an enhanced exudation of organic acid under Pdeficiency stress conditions. The acids identified include dicarboxylic acids (e.g., oxalic) and tri-carboxylic acids (e.g., citric, malic and succinic). These results, along with the known impact of certain organic acids on phosphate release from soil minerals, indicate the probable role of organic acid exudation by roots in phosphate acquisition by plants. The release of organic acids by cowpea has not yet been thoroughly evaluated. The objective of the current study was to evaluate organic acid release of cowpea cultivars grown in hydroponic culture under P-deficient and P-sufficient conditions. The second objective was to identify the specific organic acids released. The cultivars utilized in this study represent a range of relative adaptation to low P soil conditions and a range of responsiveness to applied rock phosphate in a previous screening study of 696 cultivars in a P-deficient soil.

# **MATERIALS AND METHODS**

# **Plant Material**

The sixteen genotypes utilized in the current study were selected from the previous screening of 696 cowpea accessions (Chapter II and Fig. 18).

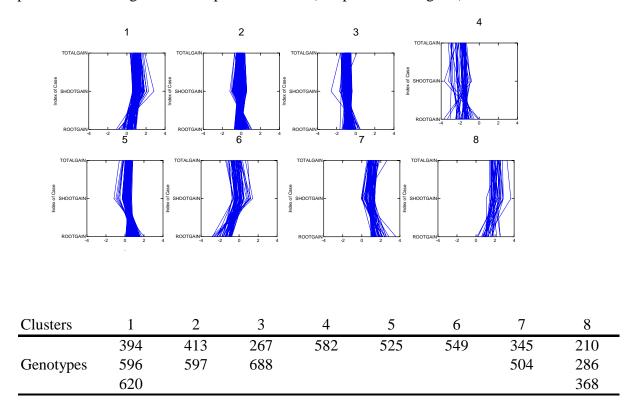


Fig. 18. Gain clusters showing the 16 genotypes selected for the organic acid exudation measurements.

		No P added			P added as TRP				GAINS					
GENOTYPE	CLUSTER #	SD		RD'		STRR	SD		RD		STRR	SDW	RDW	TOTAL
		(grms)	C.V	(grms)	C.V		(grms)	C.V	(grms)	C.V				
210	8	0.56	0.37	0.26	0.23	2.15	1.25	0.18	0.36	0.06	3.46	0.69	0.1	0.79
267	3	0.35	0.23	0.12	0.33	2.86	0.38	0.25	0.12	0.14	3.17	0.03	0.00	0.02
286	8	0.45	0.18	0.17	0.34	2.72	1.03	0.27	0.29	0.24	3.51	0.58	0.13	0.71
345	7	0.37	0.29	0.24	0.09	1.58	0.72	0.24	0.41	0.25	1.76	0.35	0.17	0.52
368	8	0.46	0.49	0.23	0.04	2.00	1.03	0.06	0.30	0.07	3.43	0.57	0.07	0.64
388	7	0.39	0.05	0.15	0.13	2.60	0.62	0.25	0.35	0.24	1.77	0.23	0.2	0.43
394	1	0.26	0.19	0.14	0.07	1.88	0.54	0.19	0.18	0.45	2.98	0.27	0.04	0.31
413	2	1.25	0.24	0.37	0.35	3.38	1.44	0.14	0.41	0.45	3.48	0.19	0.04	0.23
504	7	0.37	0.05	0.15	0.07	2.47	0.66	0.06	0.24	0.15	2.80	0.29	0.09	0.38
525	5	0.32	0.08	0.20	0.05	1.58	0.38	0.17	0.31	0.06	1.24	0.07	0.11	0.18
549	6	0.26	0.31	0.17	0.23	1.52	0.58	0.26	0.13	0.20	4.49	0.32	-0.04	0.28
582	4	0.71	0.41	0.21	0.44	3.45	0.47	0.24	0.15	0.31	3.23	-0.24	-0.06	-0.3
596	1	0.75	0.37	0.31	0.27	2.41	1.32	0.11	0.31	0.35	4.21	0.58	0	0.58
597	2	0.52	0.32	0.19	0.37	2.69	0.53	0.08	0.27	0.17	1.94	0.01	0.08	0.09
620	1	0.98	0.32	0.32	0.33	3.04	1.41	0.28	0.36	0.13	3.94	0.42	0.03	0.46
688	3	0.60	0.12	0.25	0.09	2.42	0.70	0.33	0.25	0.30	2.82	0.1	0	0.1

 Table 11. Summary of characteristics of selected genotypes including coefficient of variation of measured parameters and the absolute gains of each of these parameters.

The specific genotypes were selected based on the criteria that the coefficient of variation percentage (% c.v.) between all three replications of the experiment must be under 50%, and that each entry must have all three replications at the time of harvest. One to three lines were chosen to represent each of the eight absolute gain clusters (Fig. 16). The specific cultivars selected and their observed phenotypes are shown in Fig. 16 and Table 11, respectively.

### **Experimental Design**

There were 3 replications. Each replication consisted of 16 genotypes and two phosphate treatments, for a total of 32 individual containers, arranged in a randomized complete block design. There were two plants in each bucket. Because the space in the growth chamber was limited, the experiment was completed one replication at a time, with the same light, temperature, and relative humidity settings for all replications.

# **Plant Culture**

All stages of the plant-growth experiment were conducted in a growth chamber under the following conditions:  $400 \,\mu\text{E/m}^2$ /s light intensity; 16 hr light and 8 hr dark; temperature of 27 °C day / 25 °C night; and 50 % relative humidity.

Plants were pre-germinated in vermiculite for seven days or until a good stand was established. Sixteen seeds of each accession were used for each of the three replications. During this stage, plants were watered daily with reverse osmosis (RO) water as required, and no fertilizer was used.

The seven-day old plants were then transplanted into the hydroponic solution (Table 12) contained in 2-L polypropylene containers, each covered with a black plastic jacket and top to prevent entry of light and minimize algal growth. Plants were supported by a foam-rubber strip wrapped around the stem and placed in holes in the bucket covers. The plants destined for the plus-P treatment received a balanced Hoagland's nutrient solution (Table 2) with full strength P (100  $\mu$ mol L<sup>-1</sup>), and those plants destined for the minus-P treatment received the balanced nutrient solution with 50% of full strength P (50  $\mu$ mol L<sup>-1</sup>). This preconditioning step lasted four days. For the minus-P plants, the initial P allowed the plants to develop enough roots to sustain foliage and to prevent plants from dying from P starvation during the subsequent 0-P treatment. P treatments of 0 and 100  $\mu$ mol P L<sup>-1</sup> were begun after the initial four-day preconditioning step and were maintained for a total of 12 days. The nutrient solution in each bucket was changed every three days and was continuously aerated using small aquarium pumps. Uniform air-flow rates were maintained by creation of a back pressure by insertion of a 1-inch long 1.0-mm (ID) capillary tube inserted in the air line to each hydroponic container (Fig. 19).



Fig. 19. View of the plant culture set up for the organic acid exudation experiment.

# **Organic Acid Collection and Analysis**

After the 12 day P treatment, root exudates were collected in 500-mL Erlenmeyer flasks. The two plants from each bucket were transferred into a flask containing 500 mL of collection solution containing 5 mM CaCl<sub>2</sub> and 5 mM KCl. All flasks were covered with aluminum foil to isolate the roots and nutrient solution from light and minimize algal growth. All flasks were aerated as previously described (Fig. 20). The collection was performed for 24 hours.



Fig. 20: View of organic acid collection set up.

Nutrient	Final Conc.	Salt	Stock Soln. Conc.	Stock soln. used
				mL per 10
	$\mu$ mol L <sup>-1</sup>		$mmol L^{-1}$	L
Ca	2500	Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	2500	10
Κ	4200	KNO <sub>3</sub>	2000	20
Mg	400	MgSO <sub>4</sub> .7H <sub>2</sub> O	800	5
NO <sub>3</sub> -N	9180	MnCl <sub>2</sub> .4H <sub>2</sub> O	13.4	5
NH <sub>4</sub> -N	1200	H <sub>3</sub> BO <sub>3</sub>	40	5
Cl	13.4	$(NH_4)_2SO_4$	600	10
S	1002.3	ZnSO <sub>4</sub> .7H <sub>2</sub> O	18	1
$\mathbf{P}^*$	100	CuSO <sub>4</sub> .5H <sub>2</sub> O	5	1
Fe	60	Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> 0	3	1
HEDTA <sup>**</sup>	60	FeHEDTA***	30	20
Mn	6.7	KH <sub>2</sub> PO <sub>4</sub> *	500	2
В	20			
Zn	1.8		b	
Cu	0.5		0	
Mo	0.3			
Na	0.6			

# Table 12. Nutrient solution for growth of plants in hydroponic solution:(a) final concentration, and (b) composition of solution.

a

After 24 hours in the collection solution, the plants were removed from the flasks and blotted dry for weighing. Plants were separated into roots and shoots, each was weighed separately, and shoot/root ratios were computed. Fifty ml of solution were drawn from each flask using a syringe. The syringe was then attached to a syringe filter assembly with a  $0.2 \,\mu$ m nominal pore-size membrane filter to filter coarse impurities (large root parts and colloids) that might be contained in the collection solution. The solution was then transferred to a conical centrifuge tube and immediately placed on ice. Collected solutions were frozen immediately and thawed about one hour before the organic acid assay.

Organic acid analyses were performed by ion chromatography using a Dionex ICS 2000 (Bannockburn, Illinois). This system uses an electrical-conductivity detector and an ion-suppression method to reduce background electrical conductivity. Organic acid components were separated using an Ion Pac ICE AS1 column. Aqueous KOH was **Table 13. HPLC gradients.** 

Gradient	Time Interval (min)	Eluent (KOH) Conc. (mM)
	0 - 1	20
1	1 - 20	40
	20 - 25	90
	0 - 1	15
2	1 - 19	15
-	19 - 20	90
	20 - 25	90

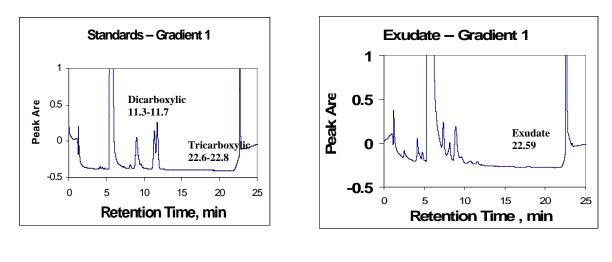
utilized as the mobile phase with elution gradients as described in Table 13. The detection limit was approximately 100 nM. The elution times of unknown organic acids

were compared with elution times of standards, including the dicarboxylic acids, oxalic, malic, succinnic and tartaric, and the tricarboxylic acids, citric, isocitric and transaconitic.

## **RESULTS AND DISCUSSION**

## **Organic Acid Exudation**

Figures 21 and 22 show chromatograms of standard organic acids and root exudates with elution gradients 1 and 2 (Table 13), respectively. Gradient 1 was more effective than gradient 2 in separating the dicarboxylic acids in a region of the chromatogram that was unaffected by inorganic anions (Figs. 21A and 22A). Gradient 2 was more effective for separation and identification of the tricarboxylic acids, though the dicarboxylic acid peaks were obscured by the inorganic anions.



a

b

Fig. 21. Chromatographic patterns of di- and tri-caboxylic acids analyzed using gradient 1: (a) standards, and (b) exudate collected from cowpea plant roots.

With both gradients, chromatograms of the root exudates indicated that a tricarboxylic acid was the predominant organic acid exuded by cowpea roots, as shown in Figs. 21B and 22B. However, this organic acid did not have an elution time that corresponded with that of any of the standard acids utilized in this study. It is possible that the exudate was piscidic acid, which was identified previously by Ae et al. (1990) and Ishikawa et al. (2002) as predominant root exudates of pigeonpea. Citric acid, which has been the most commonly observed organic acid exudate under P-deficiency stress

		GRADII	ENT
	SPECIES	1	2
	Nitrate	7.91	10.22
	Chloride	5.45	6.35
Inongonia Aniong	Sulfate	7.84	13.44
Inorganic Anions	Silicate	7.49	24.57
	Carbonate	7.63	24.77
	Phosphate	11.70	22.59
	Malate	9.10	16.46
Standard	Oxalate	9.36	17.38
<b>Dicarboxylic Acids</b>	Succinate	9.40	17.88
	Tartrate	9.07	17.10
	Malonate	8.65	15.67
	Citrate	18.01	22.97
Standard	Trans-aconitate	21.94	23.19
<b>Tricarboxylic Acids</b>	Isocitrate	19.00	23.01
	MES	None	24.74
	Unknown	8.81-14.50	15.42
	Dicarboxylic	0.01-14.30	13.42
	Unknown	14 40 15 08	22.55-24.86
	Tricarboxylic	14.40-13.98	22.33-24.80

Table 14. Elution times (in minutes) of inorganic anions, several organic acidstandards, and unknown samples collected from cowpea roots.

conditions (Grierson, 1992, with *Banksia integrifolia* L.; Penaloza, 2002, with white lupin; Neumann and Römheld, 1999, with chickpea and white lupin; Hocking and Jeffery, 2004, with old- and new-world lupins), was not observed as a root exudate of cowpea in the current study.

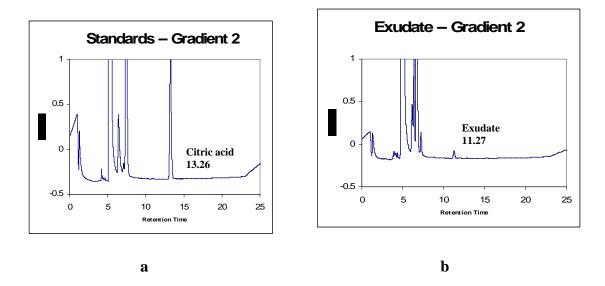


Fig. 22. Chromatographic patterns of di- and tri-caboxylic acids analyzed using gradient 2: (a) standards, and (b) exudate collected from cowpea plant roots.

# **Comparative Organic Acid Exudation between Accessions**

The results from the individual replications of root-exudate collection with the –P and +P treatments are summarized in Table 9 (sections A and B, respectively). The relative peak areas represent the relative concentrations of tri-carboxylic acid collected from the various cultivars and analyzed by ion chromatography using gradient 1 (Table 13). Values in Table 9 are expressed as relative concentration of tri-carboxylic acid in the collection solution. These values are not corrected for root or shoot mass, and thus represent the relative concentration of organic acid exuded by the whole plant. Dicarboxylic acid exudates were detected for a few of the samples (data not shown), and in these few cases the relative concentrations of di-carboxylic acid were lower than those of the tri-carboxylic acid. Relative concentrations are used instead of actual concentrations, since the predominant tri-carboxylic acid has not yet been identified, which precludes the use of any absolute concentration standard. With an asumption that the organic acid exudate was citric acid, then from the relative concentration of organic acid (Table 15; 0.017 for PI 300174) and standard curve for citrate (Fig. 23; slope of 62.7), PI 300174 following P starvation exuded a quantity of organic acid that gave a collection solution concentration of 1.07  $\mu$ M. Thus, the assumed quantity of citric acid produced by the two plants during the 24 hr collection time would be 103  $\mu$ g.

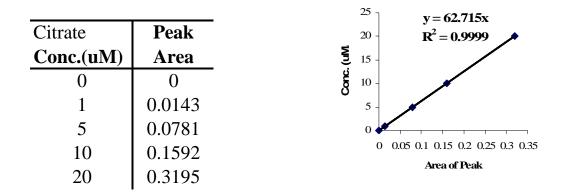


Fig. 23. Citrate standard curve generated using gradient 1 (see Table 3).

The standard deviation of relative organic acid exudation for each accession shown in Table 9 was relatively high. The exact source of this variation is uncertain, but it is likely attributable to differences in plant-growth conditions, and the resulting differences in P-deficiency stress induction that could have occurred between replications, with resulting differences in organic acid exudation. Within each replication (where all plants were grown at the same time and under identical conditions), the accessions were ranked from highest (ranking of 1) to lowest (ranking of 16) organic acid exudation. Though the quantity of organic acid exudation differed considerably between replications, the relative ranking between replications was relatively uniform. For example, with the -P treatment, PIs 256342, 300174, 582353, 582822, and 583209 had high rankings (relatively high organic acid exudation), and PIs 212635, 582869, 583076, and 583185 had low rankings (relatively low organic acid exudation), with only a few exceptions. With the +P treatment, PIs 256342, 448427, 583182, and 583209 had relatively high rankings, compared to PIs 170869, 212635, 447582, and 582869 that had relatively low rankings. PIs 256342 and 583209 are especially notable since they had relatively high organic acid exudations with both -P and +P treatments, as are PIs 212635 and 582869 that had relatively low rankings with both treatments. In no situations were the lowest ranking cultivars with the -P treatment ranked highest with the +P treatment, or vice versa. These results indicate that the rankings of the individual cultivars in relative organic acid release were relatively uniform, irrespective of initial P status. Figure 24 shows the relationship between organic acid exudation under +P versus -P conditions, which indicates that genotypes behaved differently in terms of relative organic acid release under the two different P conditions. There was an overall positive correlation, and the exudation of organic acid in the presence of P was generally considerably higher than exudation in the –P environment. This latter result contrasted

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with the previous findings of several authors, including Rengel (2002), Dong et al.

(2004), Hoffland (2006), and Pearse et al. (2007), who observed greater organic acid

Section A									
Accessions	r1		r2		<u>r3</u>			avg	
( <b>PI</b> #)	OAEx.	Rank	OAEx.	Rank	OAEx.	Rank	OAEx.	Rank	C.V
170869	0.0176	10	0.0119	3	0.0075	7	0.012	8	0.41
212637	0.0000	16	0.0036	16	0.0031	15	0.002	16	0.11
256342	0.0194	7	0.0080	6	0.0265	1	0.018	1	0.52
300174	0.0257	2	0.0182	1	0.0082	5	0.017	2	0.51
339590	0.0194	7	0.0062	10	0.0062	9	0.011	9	0.72
447582	0.0175	11	0.0039	15	0.0037	14	0.008	12	0.95
448427	0.0136	12	0.0069	9	0.0067	8	0.009	11	0.43
582353	0.0225	6	0.0179	2	0.0083	4	0.016	3	0.45
582735	0.0226	5	0.0078	8	0.0079	6	0.013	6	0.67
582822	0.0251	3	0.0086	5	0.0084	3	0.014	4	0.68
582869	0.000	14	0.0057	12	0.0026	16	0.003	15	0.53
583076	0.0109	13	0.0051	14	0.0048	11	0.007	13	0.5
583182	0.0263	1	0.0058	11	0.0061	10	0.013	6	0.92
583185	0.0000	15	0.0112	4	0.0048	11	0.005	14	0.57
583209	0.0242	4	0.0080	6	0.0089	2	0.014	4	0.66
IT99K-826	0.0187	9	0.0056	13	0.0048	11	0.010	10	0.8

Table 15.	Relative organic acid exudation (OAEx) and relative ranking of
	genotypes under –P (Section A) and +P (Section B) conditions.

# Section B

Accessions	r1		r2		r3		a	vg	
( <b>PI</b> #)	OAEx.	Rank	OAEx.	Rank	OAEx.	Rank	OAEx.	Rank	C.V
170869	0.0126	16	0.0046	14	0.0063	14	0.008	14	0.54
212637	0.0127	15	0.0037	15	0.0006	16	0.006	16	1.11
256342	0.5531	2	0.2486	2	0.5277	1	0.443	1	0.38
300174	0.3400	4	0.0506	8	0.1180	8	0.170	7	0.89
339590	0.1692	7	0.0621	7	0.3084	5	0.180	6	0.69
447582	0.0168	13	0.0206	10	0.0172	12	0.018	13	0.11
448427	0.1885	6	0.0760	5	0.4067	4	0.224	4	0.75
582353	0.0216	12	0.0153	12	0.1614	7	0.066	10	1.25
582735	0.5036	3	0.0313	9	0.0137	13	0.183	5	1.52
582822	0.0412	11	0.0154	11	0.0176	11	0.025	12	0.58
582869	0.0140	14	0.0033	16	0.0028	15	0.007	15	0.94
583076	0.0543	9	0.0149	13	0.4341	3	0.168	8	1.38
583182	0.1230	8	0.2792	1	0.5235	2	0.309	3	0.65
583185	0.2437	5	0.1221	4	0.0759	9	0.147	9	0.59
583209	0.7544	1	0.1639	3	0.1731	6	0.364	2	0.93
IT99K-826	0.0533	10	0.0686	6	0.0430	10	0.055	11	0.23

exudation under P-deficiency stress conditions. Only accession, PI 170869 in the current study exuded a higher amount of organic acid under the –P condition, indicating the possibility of a P-deficiency induced stress response.

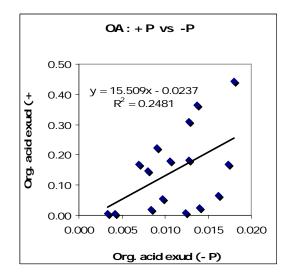


Fig. 24. Comparative organic acid exudation under -P and +P conditions.

## Fresh Biomass versus Organic Acid Exudation Relationships

The relationships between organic acid exudation and fresh shoot and root biomass produced under + P conditions are represented in Figure 23. Under P-sufficient conditions, organic acid exudation was positively correlated with root fresh weight  $(r^2 = 0.51; Fig. 25b)$ , compared to the poor correlation with fresh shoot weight  $(r^2 = 0.0002; Fig. 25a)$ . From these results, it might be inferred that root mass but not shoot mass impacted organic acid exudation. This observation was in agreement with those of Liu et al. (2004) with maize, but in contradiction with the findings of Ohwaki and Hirata (1992) who studied response of chickpea, cowpea, soybean and kidney bean to P starvation and concluded that organic acid exudation was more related to leaf mass than root mass.

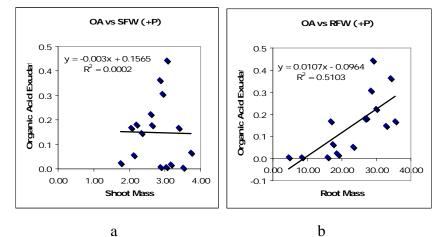


Fig. 25. Organic acid exudation under P-sufficient conditions: (a) versus shoot fresh mass and (b) versus root fresh mass.

With the –P condition, organic acid exudation was positively correlated with both shoot and root fresh weights (Fig. 26); however, better correlations were observed with roots ( $r^2 = 0.55$ ; Fig. 26b) than with shoots ( $r^2 = 0.30$ ). Root growth was the primary factor determining organic acid exudation under P-deficiency stress conditions, as was also reported by Liu et al (2004).

# **Screening Biomass versus Organic Acid Exudation Relationships**

There was a strong positive correlation ( $r^2 = 0.625$ ; Fig. 27a) between relative organic acid exudation in hydroponics under –P conditions and dry root biomass obtained during the screening experiment in soil with no added P. This result indicates that those accessions that have a tendency towards higher root mass under soil conditions, also have a tendency towards enhanced organic acid exudation. Under +P

conditions, organic acid exudation was poorly correlated (( $r^2 = 0.024$ ; Fig. 27b)) with dry root mass. With +P, genotypes varied greatly in terms of organic acid exudation and most genotypes exuded much higher organic acid amounts than they did with –P in the culture solution.

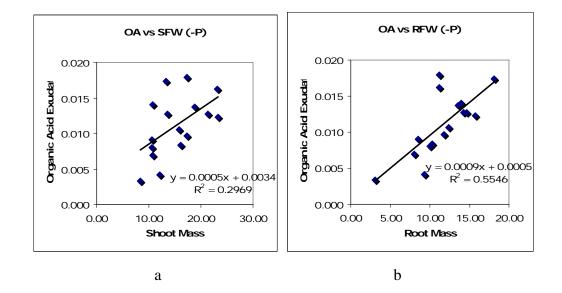


Fig. 26. Organic acid exudation under P-deficient conditions: (a) versus shoot fresh mass and (b) versus root fresh mass.

Fig. 28 indicates that in the –P situation, organic acid exudation was positively correlated with dry shoot mass. However, the correlation was not as strong as that of dry root biomass ( $R^2 = 0.33$  compared to 0.625), under the same conditions. Genotypes released considerably more organic acid in the +P treatment compared to the –P treatment, but organic acid exudation was poor correlated with shoot biomass (Fig. 28b).

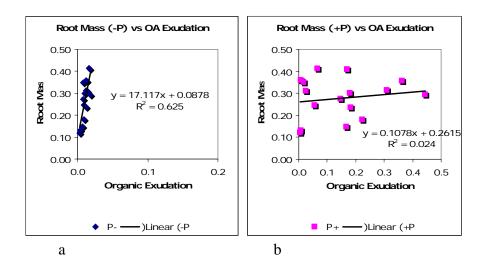


Fig. 27. Organic acid exudation versus (a) screening root mass under no added P and (b) screening root mass under added P.

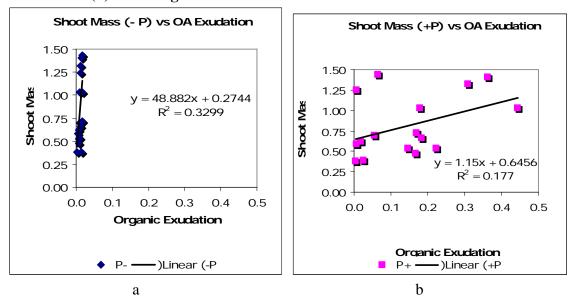
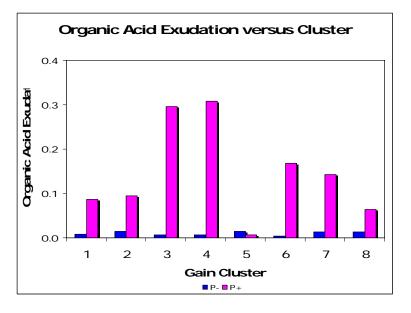


Fig. 28. Organic acid exudation versus (a) screening shoot mass under no added P and (b) screening shoot mass under added P.

Figures 25 through 28 revealed similar trends in how organic acid exudation related to shoot and root growth both from the screening experiment and during the collection experiment. In both cases, there was generally a stronger and positive relationship between organic acid exudation and root mass than there was between exudation and shoot mass. The highest correlation coefficient ( $r^2 = 0.625$ ) was observed between screening root mass and organic acid exudation under low P conditions, even though the relative amounts of exudate were highest with + P. This relationship indicates that root growth and not shoot growth determined the ability of genotypes to exude enough organic acid to help them acquire P from Fe oxides or Ca-P sources such as rock phosphate. These results support the hypothesis that adaptation to low soil P conditions and response to rock phosphate application was primarily governed by root–enhanced organic acid exudation as suggested by Penaloza et al. (2002), Liu et al. (2004), Gahoonia and Nielsen (2004), and Gahoonia et al. (2007). In contrast, Ohwaki and Hirata (1992) related this trait to larger shoot growth, and Hoffland (1992) concluded that root growth was not required for improved P acquisition under low P environments.

The fact that there was more exudation under + P than under – P conditions both in relation to the screening and collection experiments, might be indicative that organic acid exudation was not the only mechanism for adaptation to low P or for response to addition of P as rock phosphate. Pearse et al. (2007) found that plants used diverse strategies to cope with various biotic and abiotic stresses and to achieve increased biomass production. The acid exuded in the current study might have been simultaneous to proton release (pH data not shown here) as a process for achieving the necessary physiological ion balances (Marschner (1995), Hinsinger and Gilkes (1996), and Neumann and Romheld (1999).

### **Organic Acid Exudation in Relation to Screening Cluster**



# Fig. 29. Mean organic acid exudation per cluster both with and without added P.

Fig. 29 depicts the mean organic acid exudation by selected cowpea genotypes contained in each of the eight gain clusters defined in the screening phase. The clusters containing identified TRP-responsive accessions (gain clusters 8, 1, and 7; Fig. 30) did not all have consistently high organic acid exudation when grown in hydroponics culture under P starvation, although the clusters (3, 4, and 6; Fig. 30) exhibiting the lowest organic acid exudation under P starvation conditions were also the clusters that exhibited the lowest shoot and/or root responses to TRP.

The gain clusters (Fig. 30) on which accessions were selected for the organic acid exudation experiment better represented the trend of organic acid release compared to the no P or TRP clusters. The genotypes that released a relatively high amount of organic acids almost all represented the most responsive gain clusters. For example, PIs 256342, 300174, and 583209 of clusters 8, 7, and 1, respectively, which displayed higher relative shoot and/or root gain also exuded higher relative levels of organic acid under P-deficient nutrient solution and even more organic acid under P-sufficient

		<b>Organic Acid Exudation</b>								
		- P		+ P						
Geno	Clust.	Rank	H/L	Rank	H/L					
210	8	8		14	L					
267	3	16	L	16	L					
296	8	1	Н	1	Н					
345	7	2	Н	7						
368	8	9		6						
388	7	12		13	L					
394	1	11		4	Н					
413	2	3	Η	10						
504	7	6		5						
525	5	4		12						
549	6	15	L	15	L					
582	4	13	L	8						
596	1	6		3	Н					
597	2	14	L	9						
620	1	4	Н	2	Н					
688	3	10		11						

 Table 16. Rankings of selected accessions in terms of their relative organic acid exudation both under –P and + P treatments.

solution. Exudation of organic acid under P-deficient conditions might at least partially explain the ability of the plants to adapt to low P environments and the ability to

mobilize P from rock phosphate. The high exudation observed under the P-sufficient condition might be a consequence of the necessary ionic adjustment plants had to undergo in an attempt to establish ionic balance between plant internal and external environments (Hinsinger and Gilkes 1996). Also it is possible that the P-limited plants were so stressed that the organic acid exudation was limited by the –P treatment.

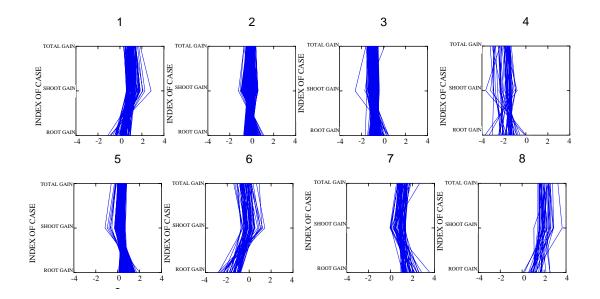


Fig. 30. Gain clusters containing ranked accessions of Table 16.

The low ranking genotypes during the screening phase with respect to biomass gain were found in clusters 3, 4, and 6 (Fig. 30) and these genotypes also classified as low in terms of organic acid exudation (Table 16). These results indicate that the genotypes in these clusters might not be able to exude enough organic acid to solubilize and utilize P from iron oxides and Ca-P, in conformity with the findings of Gahoonia et al. (2000). The current data also indicated that the highest biomass-yielding genotypes were not always the highest in organic acid exudation. This result suggests that organic acid exudation is not the only mechanism explaining the observed adaptation to low soil P conditions or response to rock phosphate application. Considering the wide variability observed under both conditions in the screening experiment, plants might be expected to have multiple P-mobilizing mechanisms. Evidently, some genotypes used other strategies, possibly including root structure modification, enhanced root-membrane phosphatase activity, proton release, or a combination of all four processes, as has been suggested by Neumann and Romheld (1999), Ishikawa et al. (2002), Pearse et al. (2007), and others.

## CONCLUSIONS

Organic acid exudation was observed under both -P and +P conditions. The predominant acid exuded was a tri-carboxylic acid that has not yet been identified. It was definitely not citric acid, but could be piscidic acid, as reported by Ae et al. (1990) and Ishikawa et al. (2002) with pigeonpea under P starvation. Results showed considerable variability among genotypes in exudation of this acid in both the absence and presence of P, but especially in the presence of P. Generally, these cowpea accessions exuded less organic acid under P-deficient conditions. This result is an indication that a maintenance level of P might be required for accelerated organic acid exudation. It might also mean that, for cowpea, organic acid exudation is not the only mechanism for adaptation to low soil P or for response to added P from sparingly available sources such as rock phosphate. However, regardless of the P-nutrition status, the data were consistent

enough between replications to facilitate the ranking of accessions in terms of their exudation ability. High exuding and low exuding genotypes have been identified.

There was a positive correlation between organic acid exudation and shoot and root biomass production. Root mass was generally more highly correlated with organic acid exudation than shoot mass, both fresh mass (exudation experiment data) and dry (screening experiment data). Correlation between organic acid exudation (hydroponic experiment) and root mass (screening experiment) was also greater in the –P treatment than with the rock phosphate amended soil. The implication of this observation is that genotypic performance under both conditions could be impacted by root growth potential which in turn had a great influence on organic acid exudation. With adequate P, roots increased growth, but shoots do not. Therefore, with adequate, shoots might be growing at maximum capacity already. However, some accessions were able under stress (-P) to still maintain enhanced root growth, which allowed them to realize enhanced shoot growth.

Exudation was also linked to k-means clustering established following the screening phase. Genotypes were ranked in terms of the relative amount of organic acid they exuded. It was noted, despite some discrepancies, that the high-exuding cultivars generally fell within identified high responsive clusters, and low-exuding cultivars within low to non-responsive clusters. This result is a confirmation that traits for adaptation to low soil P and for response to rock phosphate application did exist in the cowpea accessions, screened and that they are represented in the pool of sixteen selected genotypes. Further investigation might be required to identify the organic acid exudate.

#### CHAPTER IV

#### SUMMARY AND CONCLUSIONS

Cowpea, a major food and fodder legume, is subject to several constraints that limit its production and yields. One of the most important factors negatively impacting yields is the low soil fertility observed in most of its production areas. Phosphorus deficiency as evidenced by a strong response to commercial P-fertilizer applications during on-station experiments in Niger appeared to have the greatest influence. The high cost and scarcity of these fertilizers did not allow cowpea production improvement under farmer conditions. Given the existence of cowpea accessions capable of efficiently utilizing rock phosphate, low cost rock phosphate applied directly is viewed as a viable alternative. The experiment reported herein evaluated 696 cowpea accessions for variability in adaptation to low soil P conditions and in response to rock phosphate application.

The data collected were normally distributed with some skewness, which implied a large variability among cowpea genotypes for adaptation to low soil P environments and an even greater variability in response to rock phosphate application. The degree of variability suggested a strong possibility to select for those two traits in an attempt to improve cowpea yields. Aboveground biomass production, and to some degree total dry matter, seemed to be the best screening criteria for both traits, while plant height was the least separating parameter. Tissue-P content could also be used to separate cowpea genotypes. K-means clustering revealed details of total response in terms of biomass and its partitioning for each genotype. Some genotypes had high total biomass production under no P and/or plus P treatments. It was suggested that these accessions were able to utilize Fe oxide P and/or Ca-P.

The observed increase in total biomass was in some cases dominated by greater contribution from shoots than from roots. Genotypes possessing this trait could be of interest to breeders selecting for grain as well as fodder production. Some other accessions had higher performance, mainly due to greater root response than to shoot yield. These genotypes could be used in drought tolerance and selection for enhanced organic-matter sequestration in soil. The ultimate benefit that could be derived from this investigation would be the contribution to sustainable cowpea production systems.

Following this study, four classes of cowpea genotypes were defined. A large number of genotypes did not do well both with and without the addition of Tahoua rock phosphate. They were classified as non-adaptive and non-responsive. These are genotypes of no interest to farmers. Very few accessions performed well under low soil P conditions but not respond to TRP application. They constitute the second class of genotypes commonly termed adaptive non-responsive and could be used by farmers to improve cowpea yield. The third group of genotypes was composed of those which were not adaptive but responded well to TRP. These also could be used by farmers to improve yields. The last class of genotypes, called responsive and adaptive, by far the most desirable, was formed by varieties which produced high biomass under low soil P as well as after TRP treatment.

Sixteen accessions were selected (one to three accessions from each cluster) from the screening phase so as to represent traits portrayed in the eight gain clusters. These

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genotypes were used in a subsequent experiment aimed at investigating organic acid exudation as a possible mechanism for mobilization and acquisition of phosphorus by cowpea. Plants were grown hydroponically for three weeks in +P and –P nutrient solutions. Organic acid exudates were detected both under –P and +P conditions. The predominant acid exuded was a tri-carboxylic acid. It was not citrate, one of the most commonly exuded acids. The tri-carboxylic acid, not yet identified, was suspected to be piscidic acid.

The results also showed considerable genetic variability in organic acid exudation, especially in the presence of P. Generally, there was less organic acid exudation by cowpea under P-deficiency conditions. This result indicates that perhaps a maintenance level of P is required for accelerated organic acid exudation.

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# APPENDIX A

## LIST OF ALL GENOTYPES SCREENED

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
1	Vigna unguiculata	190191	2	SD	PI	190191	Mexico	TVu 2355
2	Vigna unguiculata	347639	2	SD	PI	347639	Ethiopia	283
3	Vigna unguiculata	349674	1	SD	PI	349674	Australia	ALOOMBA
4	Vigna unguiculata	349852	1	SD	PI	349852	Australia	PYRAMID
5	Vigna unguiculata	352762	1	SD	PI	352762	Brazil	CURUGINHA
6	Vigna unguiculata	352765	2	SD	PI	352765	Brazil	19
7	Vigna unguiculata	352872	1	SD	PI	352872	India	TVu 2724
8	Vigna unguiculata	352873	2	SD	PI	352873	India	TVu 2725
9	Vigna unguiculata	352881	2	SD	PI	352881	India	I.C. 2809
10	Vigna unguiculata	352887	2	SD	PI	352887	India	TVu 2738
11	Vigna unguiculata	352905	1	SD	PI	352905	India	UCR 1340
12	Vigna unguiculata	352907	1	SD	PI	352907	India	TVu 2757
13	Vigna unguiculata	352935	1	SD	PI	352935	India	TVu 2785
14	Vigna unguiculata	352936	1	SD	PI	352936	India	TVu 2786
15	Vigna unguiculata	352948	1	SD	PI	352948	India	TVu 2798
16	Vigna unguiculata	352956	1	SD	PI	352956	India	TVu 2806
17	Vigna unguiculata	352979	1	SD	PI	352979	India	TVu 2828
18	Vigna unguiculata	353001	1	SD	PI	353001	India	TVu 2848
19	Vigna unguiculata	353017	1	SD	PI	353017	India	TVu 2864
20	Vigna unguiculata	353040	1	SD	PI	353040	India	TVu 2886
21	Vigna unguiculata	353041	1	SD	PI	353041	India	TVu 2887
22	Vigna unguiculata	353050	1	SD	PI	353050	India	TVu 2896
23	Vigna unguiculata	353055	2	SD	PI	353055	India	TVu 2901
24	Vigna unguiculata	353059	1	SD	PI	353059	India	TVu 2905
25	Vigna unguiculata	353062	1	SD	PI	353062	India	TVu 2908
26	Vigna unguiculata	353066	1	SD	PI	353066	India	TVu 2912
27	Vigna unguiculata	353081	2	SD	PI	353081	India	TVu 2926
28	Vigna unguiculata	353087	1	SD	PI	353087	India	TVu 2932
29	Vigna unguiculata	353121	2	SD	PI	353121	India	TVu 2965
30	Vigna unguiculata	353125	2	SD	PI	353125	India	TVu 2969
31	Vigna unguiculata	353127	1	SD	PI	353127	India	TVu 2971
32	Vigna unguiculata	353129	2	SD	PI	353129	India	TVu 2973
33	Vigna unguiculata	353166	1	SD	PI	353166	India	TVu 3007
34	Vigna unguiculata	353190	1	SD	PI	353190	India	TVu 3030
35	Vigna unguiculata	353199	2	SD	PI	353199	India	TVu 3039

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
36	Vigna unguiculata	353210	1	SD	PI	353210	India	TVu 3049
37	Vigna unguiculata	353215	1	SD	PI	353215	India	TVu 3054
38	Vigna unguiculata	353236	2	SD	PI	353236	India	TVu 3073
39	Vigna unguiculata	353265	1	SD	PI	353265	India	TVu 3099
40	Vigna unguiculata	353278	1	SD	PI	353278	India	TVu 3580
41	Vigna unguiculata	353289	1	SD	PI	353289	India	PLL 282
42	Vigna unguiculata	353316	2	SD	PI	353316	India	TVu 3146
43	Vigna unguiculata	353319	1	SD	PI	353319	India	TVu 3149
44	Vigna unguiculata	353345	1	SD	PI	353345	India	TVu 3175
45	Vigna unguiculata	353352	2	SD	PI	353352	India	TVu 3182
46	Vigna unguiculata	353362	1	SD	PI	353362	India	PLL 363
47	Vigna unguiculata	354429	1	SD	PI	354429	India	TVu 1433
48	Vigna unguiculata	354464	1	SD	PI	354464	India	TVu 3246
49	Vigna unguiculata	354466	2	SD	PI	354466	India	P 636
50	Vigna unguiculata	354469	1	SD	PI	354469	India	P 640
51	Vigna unguiculata	354500	1	SD	PI	354500	India	TVu 3279
52	Vigna unguiculata	354501	1	SD	PI	354501	India	P 679
53	Vigna unguiculata	354518	1	SD	PI	354518	India	TVu 3296
54	Vigna unguiculata	354524	1	SD	PI	354524	India	P 703
55	Vigna unguiculata	354553	2	SD	PI	354553	India	Р 734
56	Vigna unguiculata	354580	1	SD	PI	354580	India	P 769
57	Vigna unguiculata	354673	1	SD	PI	354673	India	P 1127
58	Vigna unguiculata	354680	1	SD	PI	354680	India	P 1139
59	Vigna unguiculata	354708	1	SD	PI	354708	India	P 1179
60	Vigna unguiculata	354715	2	SD	PI	354715	India	TVu 3444
61	Vigna unguiculata	354743	2	SD	PI	354743	India	P 1224
62	Vigna unguiculata	354766	1	SD	PI	354766	India	P 1264
63	Vigna unguiculata	354767	1	SD	PI	354767	India	P 1265
64	Vigna unguiculata	354778	1	SD	PI	354778	India	P 1279
65	Vigna unguiculata	354782	1	SD	PI	354782	India	P 1285
66	Vigna unguiculata	354801	2	SD	PI	354801	India	P 1308
67	Vigna unguiculata	354827	2	SD	PI	354827	India	P 1343
68	Vigna unguiculata	354833	2	SD	PI	354833	India	P 1351
69	Vigna unguiculata	354837	1	SD	PI	354837	India	P 1356
70	Vigna unguiculata	354838	1	SD	PI	354838	India	P 1357
71	Vigna unguiculata	354840	1	SD	PI	354840	India	P 1359
72	Vigna unguiculata	354845	1	SD	PI	354845	India	P 1364
73	Vigna unguiculata	354857	2	SD	PI	354857	India	TVu 3552
74	Vigna unguiculata	354864	1	SD	PI	354864	India	P 1392
75	Vigna unguiculata	354881	1	SD	PI	354881	India	P 1421

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
76	Vigna unguiculata	358307	1	SD	PI	358307	Ethiopia	38 a
77	Vigna unguiculata	358715	1	SD	PI	358715	Ethiopia	22786
78	Vigna unguiculata	358716	1	SD	PI	358716	Ethiopia	22905
79	Vigna unguiculata	367863	2	SD	PI	367863	India	V. 67-05
80	Vigna unguiculata	367918	1	SD	PI	367918	Australia	CALOONA
81	Vigna unguiculata	367919	1	SD	PI	367919	Brazil	IIAM 111
82	Vigna unguiculata	367921	2	SD	PI	367921	Mozambique	NAVAJA
83	Vigna unguiculata	367927	2	SD	PI	367927	Mozambique	DR. SAUNDERS
84	Vigna unguiculata	382107	1	SD	PI	382107	Nigeria	588/2
85	Vigna unguiculata	382121	1	SD	PI	382121	Nigeria	H51-1
86	Vigna unguiculata	382124	2	SD	PI	382124	Nigeria	H64-8
87	Vigna unguiculata	382128	1	SD	PI	382128	Nigeria	H1 13-2-1
88	Vigna unguiculata	382135	1	SD	PI	382135	Nigeria	H144-4
89	Vigna unguiculata	390421	1	SD	PI	390421	Colombia	W-C 966
90	Vigna unguiculata	406290	1	SD	PI	406290	Nigeria	IFH 27-8
91	Vigna unguiculata	487518	1	SD	PI	487518	Indonesia	Bogor 1
92	Vigna unguiculata	491193	2	SD	PI	491193	Turkey	Borulce
93	Vigna unguiculata	503326	3	SD	PI	503326	Turkey	280785-11
94	Vigna unguiculata	517910	1	SD	PI	517910	Ethiopia	ILCA 6783
95	Vigna unguiculata	527259	1	SD	PI	527259	Zimbabwe	AMM 338
96	Vigna unguiculata	527263	1	SD	PI	527263	Zimbabwe	AMM 563
97	Vigna unguiculata	527267	2	SD	PI	527267	Zimbabwe	AMM 617
98	Vigna unguiculata	527272	1	SD	PI	527272	Zimbabwe	AMM 682
99	Vigna unguiculata	527277	2	SD	PI	527277	Zimbabwe	AMM 777
100	Vigna unguiculata	527282	1	SD	PI	527282	Zimbabwe	AMM 834
101	Vigna unguiculata	527286	3	SD	PI	527286	Zimbabwe	AMM 915
102	Vigna unguiculata	527289	3	SD	PI	527289	Zimbabwe	AMM 923
103	Vigna unguiculata	527299	1	SD	PI	527299	Zimbabwe	AMM 1083
104	Vigna unguiculata	527302	1	SD	PI	527302	Zimbabwe	AMM 1208
105	Vigna unguiculata	527561	1	SD	PI	527561	Burundi	IZ 29
106	Vigna unguiculata	527563	1	SD	PI	527563	Burundi	区 37
107	Vigna unguiculata	527565	1	SD	PI	527565	Burundi	IZ 112
108	Vigna unguiculata	527675	1	SD	PI	527675	Zaire	IZ 316
109	Vigna unguiculata	548784	1	SD	PI	548784	United States	CALIFORNIA BLACKEYE 46
110	Vigna unguiculata	599213	1	SD	PI	599213	United States	CALIFORNIA BLACKEYE 3
111	Vigna unguiculata	608035	1	SD	PI	608035	United States	CB27
112	Vigna unguiculata subsp. cylindrica	167284	1	SD	PI	167284	Turkey	BRABHAM
113	Vigna unguiculata subsp. cylindrica	180014	2	SD	PI	180014	India	CHOLAN
114	Vigna unguiculata subsp. cylindrica	180355	2	SD	PI	180355	India	CHAWLI
115	Vigna unguiculata subsp. cylindrica	180494	1	SD	PI	180494	India	10841

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
116	Vigna unguiculata subsp. cylindrica	189416	1	SD	PI	189416	Guatemala	51-50
117	Vigna unguiculata subsp. cylindrica	201498	1	SD	PI	201498	Mexico	7124
118	Vigna unguiculata subsp. cylindrica	205139	1	SD	PI	205139	India	
119	Vigna unguiculata subsp. cylindrica	205140	1	SD	PI	205140	India	
120	Vigna unguiculata subsp. cylindrica	250238	2	SD	PI	250238	Pakistan	K522
121	Vigna unguiculata subsp. cylindrica	255755	1	SD	PI	255755	Nigeria	
122	Vigna unguiculata subsp. cylindrica	270065	2	SD	PI	270065	Pakistan	RAMBO
123	Vigna unguiculata subsp. cylindrica	291384	1	SD	PI	291384	China	No.276
124	Vigna unguiculata subsp. cylindrica	304164	1	SD	PI	304164	Honduras	A-269
125	Vigna unguiculata subsp. dekindtiana	292883	2	SD	PI	292883	Nigeria	No. C36-258
126	Vigna unguiculata subsp. pubescens	406362	1	SD	PI	406362	Tanzania	TVnu 108
127	Vigna unguiculata subsp. pubescens	406365	1	SD	PI	406365	Tanzania	TVnu 112
128	Vigna unguiculata subsp. sesquipedalis	146618	2	SD	PI	146618	Brazil	TVu 1411
129	Vigna unguiculata subsp. sesquipedalis	147071	2	SD	PI	147071	Brazil	No.1
130	Vigna unguiculata subsp. sesquipedalis	196301	1	SD	PI	196301	Nicaragua	2925
131	Vigna unguiculata subsp. sesquipedalis	215659	1	SD	PI	215659	India	PHILIPPINE EARLY
132	Vigna unguiculata subsp. sesquipedalis	259759	2	SD	PI	259759	Hong Kong	
133	Vigna unguiculata subsp. sesquipedalis	271258	3	SD	PI	271258	India	TVu 2449
134	Vigna unguiculata subsp. sesquipedalis	286439	2	SD	PI	286439	Nepal	CLUSTER BEANS
135	Vigna unguiculata subsp. sesquipedalis	352957	1	SD	PI	352957	India	TVu 2807
136	Vigna unguiculata subsp. sesquipedalis	353006	1	SD	PI	353006	India	TVu 2853
137	Vigna unguiculata subsp. sesquipedalis	353045	2	SD	PI	353045	India	TVu 2891
138	Vigna unguiculata subsp. sesquipedalis	353074	1	SD	PI	353074	India	UCR 2576
139	Vigna unguiculata subsp. sesquipedalis	353335	1	SD	PI	353335	India	TVu 3165
140	Vigna unguiculata subsp. sesquipedalis	391658	2	SD	PI	391658	China	SHE-PAI-TOU
141	Vigna unguiculata subsp. sesquipedalis	418979	2	SD	PI	418979	China	HAN CHUI YEN
142	Vigna unguiculata subsp. sesquipedalis	418980	2	SD	PI	418980	China	LO CHIEN TAI
143	Vigna unguiculata subsp. sesquipedalis	419005	1	SD	PI	419005	China	
144	Vigna unguiculata subsp. sesquipedalis	419102	1	SD	PI	419102	China	
145	Vigna unguiculata subsp. sesquipedalis	419200	1	SD	PI	419200	China	HUNG CHO YANG
146	Vigna unguiculata subsp. sesquipedalis	419219	2	SD	PI	419219	Hong Kong	
147	Vigna unguiculata subsp. sesquipedalis	427093	1	SD	PI	427093	China	
148	Vigna unguiculata subsp. sesquipedalis	430593	1	SD	PI	430593	China	HUNG TSUI YEN
149	Vigna unguiculata subsp. sesquipedalis	430687	1	SD	PI	430687	China	HUA PI CHIA CHIANG TOU
150	Vigna unguiculata subsp. sesquipedalis	468104	1	SD	PI	468104	Suriname	PRT 4
151	Vigna unguiculata subsp. sesquipedalis	470274	2	SD	PI	470274	Indonesia	DB 26
152	Vigna unguiculata subsp. sesquipedalis	478396	1	SD	PI	478396	China	O 112
153	Vigna unguiculata subsp. sesquipedalis	487487	1	SD	PI	487487	Indonesia	CP 2
154	Vigna unguiculata subsp. sesquipedalis	487490	1	SD	PI	487490	Philippines	20-1
155	Vigna unguiculata subsp. sesquipedalis	487493	2	SD	PI	487493	Taiwan	CP 12

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156	Vigna unguiculata subsp. sesquipedalis	487497	2	SD	PI	487497	Taiwan	CP 16
157	Vigna unguiculata subsp. sesquipedalis	487499	1	SD	PI	487499	Guam	CP 18
158	Vigna unguiculata subsp. sesquipedalis	487501	1	SD	PI	487501	Thailand	China Town
159	Vigna unguiculata subsp. sesquipedalis	487502	1	SD	PI	487502	Philippines	ECO CAR POLE Sitao #2
160	Vigna unguiculata subsp. sesquipedalis	487507	1	SD	PI	487507	Philippines	Dagupan Pangasinan Collection No. 3.1
161	Vigna unguiculata subsp. sesquipedalis	487516	1	SD	PI	487516	Philippines	Bush Sitao
162	Vigna unguiculata subsp. sesquipedalis	487519	2	SD	PI	487519	Taiwan	Tainong's 1
163	Vigna unguiculata subsp. sesquipedalis	487523	1	SD	PI	487523	Taiwan	Tianong's 5
164	Vigna unguiculata subsp. sesquipedalis	487527	1	SD	PI	487527	United States	K-30
165	Vigna unguiculata subsp. sesquipedalis	487532	1	SD	PI	487532	Indonesia	CP 55
166	Vigna unguiculata subsp. sesquipedalis	487534	1	SD	PI	487534	Taiwan	CP 57
167	Vigna unguiculata subsp. sesquipedalis	487539	2	SD	PI	487539	Philippines	CP 63
168	Vigna unguiculata subsp. sesquipedalis	487544	2	SD	PI	487544	Taiwan	CP 68
169	Vigna unguiculata subsp. sesquipedalis	487549	3	SD	PI	487549	Indonesia	CP 73
170	Vigna unguiculata subsp. sesquipedalis	490770	2	SD	PI	490770	China	Huang Hua Qing Ai Jiang Dou
171	Vigna unguiculata subsp. sesquipedalis	503328	1	SD	PI	503328	Turkey	280785-0604
172	Vigna unguiculata subsp. sesquipedalis	512286	2	SD	PI	512286	China	GUILIN LONG BEANS
173	Vigna unguiculata subsp. sesquipedalis	578902	1	SD	PI	578902	China	CHANG XIAN JIANG DOU
174	Vigna unguiculata subsp. sesquipedalis	578907	2	SD	PI	578907	China	HEI ZI DANG DI JIANG DOU
175	Vigna unguiculata subsp. sesquipedalis	582429	1	SD	PI	582429	Trinidad & Tobago	LOS BANOS BUSH SITAO
176	Vigna unguiculata subsp. unguiculata	115674	1	SD	PI	115674	Sri Lanka	Hen-me
177	Vigna unguiculata subsp. unguiculata	115679	1	SD	PI	115679	Sri Lanka	HODI
178	Vigna unguiculata subsp. unguiculata	115681	1	SD	PI	115681	Sri Lanka	POLON
179	Vigna unguiculata subsp. unguiculata	115683	1	SD	PI	115683	Sri Lanka	POLON LEEMA
180	Vigna unguiculata subsp. unguiculata	121433	1	SD	PI	121433	Paraguay	4696
181	Vigna unguiculata subsp. unguiculata	141355	1	SD	PI	141355	Guatemala	TVu 1520
182	Vigna unguiculata subsp. unguiculata	142779	1	SD	PI	142779	Mexico	CUARENTANA
183	Vigna unguiculata subsp. unguiculata	145198	1	SD	PI	145198	United States	BRABHAM
184	Vigna unguiculata subsp. unguiculata	147076	1	SD	PI	147076	Brazil	TVu 1773
185	Vigna unguiculata subsp. unguiculata	147077	1	SD	PI	147077	Brazil	TVu 1807
186	Vigna unguiculata subsp. unguiculata	147561	1	SD	PI	147561	Colombia	TVu 2267
187	Vigna unguiculata subsp. unguiculata	147562	1	SD	PI	147562	Colombia	TVu 1940
188	Vigna unguiculata subsp. unguiculata	147563	1	SD	PI	147563	Colombia	TVu 1412
189	Vigna unguiculata subsp. unguiculata	148674	1	SD	PI	148674	Iran	TVu 1522
190	Vigna unguiculata subsp. unguiculata	148681	1	SD	PI	148681	Iran	TVu 2272
191	Vigna unguiculata subsp. unguiculata	151562	1	SD	PI	151562	Dominican Rep.	FRIJOLITOS (ACONI) CABEZITA BLANCO
192	Vigna unguiculata subsp. unguiculata	151563	1	SD	PI	151563	Dominican Rep.	FRIJOLITOS (ANCONI) CABEZITA NEGRA
193	Vigna unguiculata subsp. unguiculata	152194	1	SD	PI	152194	Paraguay	TAPE
194	Vigna unguiculata subsp. unguiculata	152196	1	SD	PI	152196	Paraguay	SAN FRANCISCO
195	Vigna unguiculata subsp. unguiculata	152197	1	SD	PI	152197	Paraguay	TUPI PYTA

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196	Vigna unguiculata subsp. unguiculata	152199	1	SD	PI	152199	Paraguay	TVu 1973
197	Vigna unguiculata subsp. unguiculata	154134	1	SD	PI	154134	Peru	BOCANEGRA
198	Vigna unguiculata subsp. unguiculata	162924	1	SD	PI	162924	Paraguay	XAPE 3443 II L8
199	Vigna unguiculata subsp. unguiculata	162925	1	SD	PI	162925	Paraguay	TVu 1944
200	Vigna unguiculata subsp. unguiculata	163142	1	SD	PI	163142	India	KOR
201	Vigna unguiculata subsp. unguiculata	163448	1	SD	PI	163448	Costa Rica	CHINEGRO
202	Vigna unguiculata subsp. unguiculata	164979	2	SD	PI	164979	Turkey	TVu 2287
203	Vigna unguiculata subsp. unguiculata	165493	1	SD	PI	165493	India	LOBRA
204	Vigna unguiculata subsp. unguiculata	166146	1	SD	PI	166146	India	TVu 2291
205	Vigna unguiculata subsp. unguiculata	167024	2	SD	PI	167024	Turkey	BORULCE
206	Vigna unguiculata subsp. unguiculata	170844	1	SD	PI	170844	Turkey	TVu 1527
207	Vigna unguiculata subsp. unguiculata	170861	1	SD	PI	170861	Turkey	TVu 1528
208	Vigna unguiculata subsp. unguiculata	170863	1	SD	PI	170863	Turkey	TVu 1810
209	Vigna unguiculata subsp. unguiculata	170865	1	SD	PI	170865	Turkey	TVu 1529
210	Vigna unguiculata subsp. unguiculata	170869	1	SD	PI	170869	Turkey	3151
211	Vigna unguiculata subsp. unguiculata	171891	1	SD	PI	171891	Turkey	6580
212	Vigna unguiculata subsp. unguiculata	173827	1	SD	PI	173827	Turkey	TVu 1534
213	Vigna unguiculata subsp. unguiculata	174411	1	SD	PI	174411	Turkey	TVu 2308
214	Vigna unguiculata subsp. unguiculata	175327	2	SD	PI	175327	India	LOBIA
215	Vigna unguiculata subsp. unguiculata	175332	1	SD	PI	175332	India	RONGI
216	Vigna unguiculata subsp. unguiculata	175959	1	SD	PI	175959	Turkey	TVu 1536
217	Vigna unguiculata subsp. unguiculata	175962	1	SD	PI	175962	Turkey	TVu 2312
218	Vigna unguiculata subsp. unguiculata	175963	2	SD	PI	175963	Turkey	6097
219	Vigna unguiculata subsp. unguiculata	176796	1	SD	PI	176796	Turkey	TVu 1537
220	Vigna unguiculata subsp. unguiculata	177579	1	SD	PI	177579	Turkey	TVu 1540
221	Vigna unguiculata subsp. unguiculata	179125	2	SD	PI	179125	Turkey	KARNIKARA
222	Vigna unguiculata subsp. unguiculata	179553	1	SD	PI	179553	Turkey	TVu 2320
223	Vigna unguiculata subsp. unguiculata	179554	1	SD	PI	179554	Turkey	TVu 2321
224	Vigna unguiculata subsp. unguiculata	183251	1	SD	PI	183251	Egypt	TVu 2329
225	Vigna unguiculata subsp. unguiculata	183363	1	SD	PI	183363	India	JURUNGA
226	Vigna unguiculata subsp. unguiculata	184952	1	SD	PI	184952	Ghana	TVu 1547
227	Vigna unguiculata subsp. unguiculata	185647	1	SD	PI	185647	Ghana	TVu 1549
228	Vigna unguiculata subsp. unguiculata	186360	1	SD	PI	186360	Australia	TVu 1550
229	Vigna unguiculata subsp. unguiculata	186386	1	SD	PI	186386	Uruguay	NEW ERA
230	Vigna unguiculata subsp. unguiculata	186452	1	SD	PI	186452	Nigeria	TVu 1947
231	Vigna unguiculata subsp. unguiculata	186460	1	SD	PI	186460	Nigeria	TVu 1551
232	Vigna unguiculata subsp. unguiculata	186465	1	SD	PI	186465	Nigeria	TVu 1950
233	Vigna unguiculata subsp. unguiculata	186466	1	SD	PI	186466	Nigeria	TVu 1691
234	Vigna unguiculata subsp. unguiculata	189230	1	SD	PI	189230	Congo	TVu 1881
235	Vigna unguiculata subsp. unguiculata	189374	2	SD	PI	189374	Nigeria	TVu 2200

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236	Vigna unguiculata subsp. unguiculata	189375	1	SD	PI	189375	Nigeria	TVu 1738
237	Vigna unguiculata subsp. unguiculata	189378	2	SD	PI	189378	Nigeria	TVu 1692
238	Vigna unguiculata subsp. unguiculata	194203	1	SD	PI	194203	United States	R 33
239	Vigna unguiculata subsp. unguiculata	194204	1	SD	PI	194204	United States	R 35
240	Vigna unguiculata subsp. unguiculata	194205	1	SD	PI	194205	United States	R 37
241	Vigna unguiculata subsp. unguiculata	194206	1	SD	PI	194206	United States	R 43
242	Vigna unguiculata subsp. unguiculata	194207	1	SD	PI	194207	United States	R 49
243	Vigna unguiculata subsp. unguiculata	194208	2	SD	PI	194208	United States	R 53
244	Vigna unguiculata subsp. unguiculata	194209	1	SD	PI	194209	United States	R 55
245	Vigna unguiculata subsp. unguiculata	194210	1	SD	PI	194210	United States	R 57
246	Vigna unguiculata subsp. unguiculata	194211	2	SD	PI	194211	United States	R 63
247	Vigna unguiculata subsp. unguiculata	194212	1	SD	PI	194212	United States	R 65
248	Vigna unguiculata subsp. unguiculata	194213	1	SD	PI	194213	United States	R 67
249	Vigna unguiculata subsp. unguiculata	197056	2	SD	PI	197056	El Salvador	FRIJOL DE CASTILLA
250	Vigna unguiculata subsp. unguiculata	197057	1	SD	PI	197057	Honduras	TVu 1695
251	Vigna unguiculata subsp. unguiculata	200867	1	SD	PI	200867	Myanmar	TVu 1975
252	Vigna unguiculata subsp. unguiculata	201024	1	SD	PI	201024	Guatemala	TVu 2372
253	Vigna unguiculata subsp. unguiculata	202802	3	SD	PI	202802	Peru	TVu 2373
254	Vigna unguiculata subsp. unguiculata	205141	1	SD	PI	205141	India	TVu 1567
255	Vigna unguiculata subsp. unguiculata	205240	1	SD	PI	205240	Turkey	
256	Vigna unguiculata subsp. unguiculata	205241	1	SD	PI	205241	Turkey	TVu 1414
257	Vigna unguiculata subsp. unguiculata	207527	1	SD	PI	207527	Afghanistan	TVu 1882
258	Vigna unguiculata subsp. unguiculata	208771	1	SD	PI	208771	Cuba	TVu 1696
259	Vigna unguiculata subsp. unguiculata	208845	2	SD	PI	208845	Costa Rica	TVu 2377
260	Vigna unguiculata subsp. unguiculata	209971	1	SD	PI	209971	Japan	TURU NASHI WASE SASAGE
261	Vigna unguiculata subsp. unguiculata	211109	1	SD	PI	211109	Afghanistan	TVu 1569
262	Vigna unguiculata subsp. unguiculata	211110	1	SD	PI	211110	Afghanistan	TVu 1648
263	Vigna unguiculata subsp. unguiculata	211642	1	SD	PI	211642	Afghanistan	TVu 1649
264	Vigna unguiculata subsp. unguiculata	211753	1	SD	PI	211753	Afghanistan	TVu 1818
265	Vigna unguiculata subsp. unguiculata	211754	2	SD	PI	211754	Afghanistan	TVu 2381
266	Vigna unguiculata subsp. unguiculata	211756	1	SD	PI	211756	Afghanistan	TVu 1819
267	Vigna unguiculata subsp. unguiculata	212635	1	SD	PI	212635	Afghanistan	TVu 2383
268	Vigna unguiculata subsp. unguiculata	214354	1	SD	PI	214354	India	TVu 1570
269	Vigna unguiculata subsp. unguiculata	218122	1	SD	PI	218122	Pakistan	LUBIA
270	Vigna unguiculata subsp. unguiculata	218123	2	SD	PI	218123	Pakistan	LUBIA
271	Vigna unguiculata subsp. unguiculata	220849	1	SD	PI	220849	Afghanistan	LOBIA-I-SURKH
272	Vigna unguiculata subsp. unguiculata	220851	1	SD	PI	220851	Afghanistan	LOBIA-I-SAFADE
273	Vigna unguiculata subsp. unguiculata	221730	1	SD	PI	221730	South Africa	TVu 1928
274	Vigna unguiculata subsp. unguiculata	221731	1	SD	PI	221731	South Africa	TVu 2396
275	Vigna unguiculata subsp. unguiculata	221732	1	SD	PI	221732	South Africa	TVu 1913

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276	Vigna unguiculata subsp. unguiculata	222755	1	SD	PI	222755	Iran	LOBIA-CHESHBOLBOLI
277	Vigna unguiculata subsp. unguiculata	223023	1	SD	PI	223023	Iran	LOBIA-CHESHBOLBOLI
278	Vigna unguiculata subsp. unguiculata	225922	1	SD	PI	225922	Zambia	TVu 1977
279	Vigna unguiculata subsp. unguiculata	227397	1	SD	PI	227397	Iran	LUBIA
280	Vigna unguiculata subsp. unguiculata	227829	1	SD	PI	227829	Guatemala	TVu 1953
281	Vigna unguiculata subsp. unguiculata	227830	1	SD	PI	227830	Guatemala	TVu 1577
282	Vigna unguiculata subsp. unguiculata	229551	1	SD	PI	229551	Iran	TVu 2412
283	Vigna unguiculata subsp. unguiculata	229734	1	SD	PI	229734	Iran	CHESH BOLBOLI LUBI
284	Vigna unguiculata subsp. unguiculata	229796	1	SD	PI	229796	Iran	TVu 1654
285	Vigna unguiculata subsp. unguiculata	244571	1	SD	PI	244571	Guatemala	TIQUISATE
286	Vigna unguiculata subsp. unguiculata	250416	1	SD	PI	250416	Pakistan	TVu 2417
287	Vigna unguiculata subsp. unguiculata	250587	2	SD	PI	250587	Egypt	TVu 2418
288	Vigna unguiculata subsp. unguiculata	250759	1	SD	PI	250759	Iran	TVu 2166
289	Vigna unguiculata subsp. unguiculata	251222	1	SD	PI	251222	Afghanistan	TVu 1656
290	Vigna unguiculata subsp. unguiculata	255765	1	SD	PI	255765	Nigeria	TVu 1703
291	Vigna unguiculata subsp. unguiculata	255774	2	SD	PI	255774	Nigeria	TVu 2428
292	Vigna unguiculata subsp. unguiculata	255782	1	SD	PI	255782	Nigeria	TVu 1956
293	Vigna unguiculata subsp. unguiculata	255811	1	SD	PI	255811	Nigeria	TVu 2439
294	Vigna unguiculata subsp. unguiculata	255815	1	SD	PI	255815	Nigeria	TVu 1459
295	Vigna unguiculata subsp. unguiculata	256341	1	SD	PI	256341	Afghanistan	TVu 1831
296	Vigna unguiculata subsp. unguiculata	256342	1	SD	PI	256342	Pakistan	UCR 17
297	Vigna unguiculata subsp. unguiculata	257463	1	SD	PI	257463	South Africa	TVu 1930
298	Vigna unguiculata subsp. unguiculata	262179	1	SD	PI	262179	Portugal	TVu 2443
299	Vigna unguiculata subsp. unguiculata	277784	1	SD	PI	277784	Australia	BRABHAM
300	Vigna unguiculata subsp. unguiculata	277786	2	SD	PI	277786	Australia	BAYO
301	Vigna unguiculata subsp. unguiculata	279845	1	SD	PI	279845	Mexico	FRIJOL YORIMUMI
302	Vigna unguiculata subsp. unguiculata	291094	1	SD	PI	291094	Argentina	TVu 1978
303	Vigna unguiculata subsp. unguiculata	291139	1	SD	PI	291139	Australia	MALALBAR
304	Vigna unguiculata subsp. unguiculata	291140	1	SD	PI	291140	Australia	NEGRO
305	Vigna unguiculata subsp. unguiculata	292871	1	SD	PI	292871	Israel	TVu 2459
306	Vigna unguiculata subsp. unguiculata	292889	2	SD	PI	292889	South Africa	TVu 1909
307	Vigna unguiculata subsp. unguiculata	292890	4	SD	PI	292890	South Africa	51-C-57
308	Vigna unguiculata subsp. unguiculata	292891	2	SD	PI	292891	South Africa	TVu 2463
309	Vigna unguiculata subsp. unguiculata	292892	1	SD	PI	292892	South Africa	UCR 140
310	Vigna unguiculata subsp. unguiculata	292893	1	SD	PI	292893	South Africa	BECHUANA WHITE
311	Vigna unguiculata subsp. unguiculata	292894	1	SD	PI	292894	Zimbabwe	TVu 1460
312	Vigna unguiculata subsp. unguiculata	292897	1	SD	PI	292897	Hungary	TVu 1423
313	Vigna unguiculata subsp. unguiculata	292898	1	SD	PI	292898	Hungary	TVu 1890
314	Vigna unguiculata subsp. unguiculata	292899	1	SD	PI	292899	Hungary	TVu 1596
315	Vigna unguiculata subsp. unguiculata	292908	1	SD	PI	292908	Nigeria	TVu 1893

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316	Vigna unguiculata subsp. unguiculata	292912	1	SD	PI	292912	South Africa	TVu 2483
317	Vigna unguiculata subsp. unguiculata	292913	1	SD	PI	292913	Hungary	TVu 2484
318	Vigna unguiculata subsp. unguiculata	293453	1	SD	PI	293453	United States	ARLINGTON
319	Vigna unguiculata subsp. unguiculata	293466	1	SD	PI	293466	United States	BRABHAM
320	Vigna unguiculata subsp. unguiculata	293467	1	SD	PI	293467	United States	BRABHAM K 892
321	Vigna unguiculata subsp. unguiculata	293468	1	SD	PI	293468	United States	BRABHAM VICTOR
322	Vigna unguiculata subsp. unguiculata	293470	1	SD	PI	293470	United States	BROWNEYE CREAM
323	Vigna unguiculata subsp. unguiculata	293476	1	SD	PI	293476	United States	CALHOUN CROWDER
324	Vigna unguiculata subsp. unguiculata	293477	1	SD	PI	293477	United States	CALIFORNIA BLACKEYE
325	Vigna unguiculata subsp. unguiculata	293494	1	SD	PI	293494	United States	CREAM
326	Vigna unguiculata subsp. unguiculata	293498	1	SD	PI	293498	United States	CREAM WHITE
327	Vigna unguiculata subsp. unguiculata	293505	2	SD	PI	293505	United States	EARLY RAMSHORN BLACKEYE
328	Vigna unguiculata subsp. unguiculata	293514	1	SD	PI	293514	United States	GROIT
329	Vigna unguiculata subsp. unguiculata	293520	1	SD	PI	293520	United States	IRON
330	Vigna unguiculata subsp. unguiculata	293525	2	SD	PI	293525	United States	JACKSON PURPLEHULL
331	Vigna unguiculata subsp. unguiculata	293526	1	SD	PI	293526	United States	KOREAN CROWDER
332	Vigna unguiculata subsp. unguiculata	293528	1	SD	PI	293528	United States	KUROMANDARA
333	Vigna unguiculata subsp. unguiculata	293545	1	SD	PI	293545	Unknown	PARAGUAY 12
334	Vigna unguiculata subsp. unguiculata	293557	1	SD	PI	293557		RED COWPEA
335	Vigna unguiculata subsp. unguiculata	293568	1	SD	PI	293568		SIX-WEEKS GEORGIA
336	Vigna unguiculata subsp. unguiculata	293569	1	SD	PI	293569		SPECKLED CROWDER
337	Vigna unguiculata subsp. unguiculata	293573	1	SD	PI	293573		TAYLOR
338	Vigna unguiculata subsp. unguiculata	293582	1	SD	PI	293582		VICTOR K 798
339	Vigna unguiculata subsp. unguiculata	293584	1	SD	PI	293584		WHIPPOORWILL
340	Vigna unguiculata subsp. unguiculata	293586	1	SD	PI	293586		WILT RESISTANT BLACKEYE
341	Vigna unguiculata subsp. unguiculata	293587	1	SD	PI	293587		WILT RESISTANT CHINESE RED
342	Vigna unguiculata subsp. unguiculata	297561	1	SD	PI	297561		TVu 2606
343	Vigna unguiculata subsp. unguiculata	300171	1	SD	PI	300171	South Africa	NEW ERA
344	Vigna unguiculata subsp. unguiculata	300173	1	SD	PI	300173	South Africa	BECHUANALAND WHITE
345	Vigna unguiculata subsp. unguiculata	300174	1	SD	PI	300174	South Africa	VICTOR
346	Vigna unguiculata subsp. unguiculata	300175	2	SD	PI	300175	South Africa	SAUNDERS UPRIGHT
347	Vigna unguiculata subsp. unguiculata	304150	1	SD	PI	304150	Nicaragua	TVu 1387
348	Vigna unguiculata subsp. unguiculata	305076	1	SD	PI	305076	Thailand	9
349	Vigna unguiculata subsp. unguiculata	307556	1	SD	PI	307556	United States	F.C. 31660
350	Vigna unguiculata subsp. unguiculata	307558	1	SD	PI	307558	United States	UCR 152
351	Vigna unguiculata subsp. unguiculata	307559	2	SD	PI	307559	United States	F.C. 31739
352	Vigna unguiculata subsp. unguiculata	307561	1	SD	PI	307561	United States	PURPLE HULL
353	Vigna unguiculata subsp. unguiculata	311226	2	SD	PI	311226	Guatemala	20970
354	Vigna unguiculata subsp. unguiculata	312203	1	SD	PI	312203	Mexico	FRIJOL DE CASTILLA
355	Vigna unguiculata subsp. unguiculata	312207	1	SD	PI	312207	Mexico	FRIJOL BAYO

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356	Vigna unguiculata subsp. unguiculata	312208	1	SD	PI	312208	Mexico	FRIJOL BAYITO
357	Vigna unguiculata subsp. unguiculata	312210	1	SD	PI	312210	Mexico	BAYITO CHIQUITO
358	Vigna unguiculata subsp. unguiculata	312211	2	SD	PI	312211	Mexico	FRIJOL BOLATA
359	Vigna unguiculata subsp. unguiculata	326157	1	SD	PI	326157	Uganda	TVu 1864
360	Vigna unguiculata subsp. unguiculata	337044	1	SD	PI	337044	Brazil	TVu 2644
361	Vigna unguiculata subsp. unguiculata	337506	1	SD	PI	337506	Brazil	TVu 1390
362	Vigna unguiculata subsp. unguiculata	339563	1	SD	PI	339563	Australia	C2-576
363	Vigna unguiculata subsp. unguiculata	339565	2	SD	PI	339565	Botswana	51C 295
364	Vigna unguiculata subsp. unguiculata	339572	1	SD	PI	339572	Botswana	SEKGALO
365	Vigna unguiculata subsp. unguiculata	339587	1	SD	PI	339587	South Africa	TVu 1924
366	Vigna unguiculata subsp. unguiculata	339588	2	SD	PI	339588	South Africa	TVu 1937
367	Vigna unguiculata subsp. unguiculata	339589	1	SD	PI	339589	South Africa	51C 421-2
368	Vigna unguiculata subsp. unguiculata	339590	2	SD	PI	339590	South Africa	TVu 1915
369	Vigna unguiculata subsp. unguiculata	339591	1	SD	PI	339591	South Africa	TVu 2655
370	Vigna unguiculata subsp. unguiculata	339592	1	SD	PI	339592	South Africa	TVu 2656
371	Vigna unguiculata subsp. unguiculata	339594	1	SD	PI	339594	South Africa	TVu 1641
372	Vigna unguiculata subsp. unguiculata	339595	1	SD	PI	339595	South Africa	TVu 2658
373	Vigna unguiculata subsp. unguiculata	339597	1	SD	PI	339597	South Africa	TVu 1908
374	Vigna unguiculata subsp. unguiculata	339599	1	SD	PI	339599	South Africa	EARLY BROWN EYE
375	Vigna unguiculata subsp. unguiculata	339600	2	SD	PI	339600	South Africa	EMBU BUFF
376	Vigna unguiculata subsp. unguiculata	339601	1	SD	PI	339601	South Africa	IRAN GREY
377	Vigna unguiculata subsp. unguiculata	339602	1	SD	PI	339602	South Africa	NEW ERA
378	Vigna unguiculata subsp. unguiculata	339603	1	SD	PI	339603	South Africa	PALE GREEN
379	Vigna unguiculata subsp. unguiculata	339605	1	SD	PI	339605	South Africa	SAUNDERS UPRIGHT
380	Vigna unguiculata subsp. unguiculata	339607	1	SD	PI	339607	South Africa	WITZENBORG
381	Vigna unguiculata subsp. unguiculata	339609	1	SD	PI	339609	Tanzania	TVu 1645
382	Vigna unguiculata subsp. unguiculata	339610	1	SD	PI	339610	Tanzania	TVu 1972
383	Vigna unguiculata subsp. unguiculata	339611	1	SD	PI	339611	Tanzania	TVu 2670
384	Vigna unguiculata subsp. unguiculata	339613	1	SD	PI	339613	Tanzania	TVu 2003
385	Vigna unguiculata subsp. unguiculata	339638	1	SD	PI	339638	Uganda	TVu 1395
386	Vigna unguiculata subsp. unguiculata	339709	1	SD	PI	339709	Argentina	VICTOR
387	Vigna unguiculata subsp. unguiculata	390831	1	SD	PI	390831	Peru	Chiclayo Pardo
388	Vigna unguiculata subsp. unguiculata	447582	2	SD	PI	447582	Nigeria	TVu 3834
389	Vigna unguiculata subsp. unguiculata	448060	1	SD	PI	448060	Niger	TVu 4717
390	Vigna unguiculata subsp. unguiculata	448096	1	SD	PI	448096	Niger	TVu 4757
391	Vigna unguiculata subsp. unguiculata	448112	1	SD	PI	448112	Niger	TVu 4776
392	Vigna unguiculata subsp. unguiculata	448144	1	SD	PI	448144	Niger	TVu 4811
393	Vigna unguiculata subsp. unguiculata	448192	1	SD	PI	448192	Niger	TVu 4862
394	Vigna unguiculata subsp. unguiculata	448427	1	SD	PI	448427	Niger	TVu 5115
395	Vigna unguiculata subsp. unguiculata	448499	3	SD	PI	448499	Niger	TVu 5247

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396	Vigna unguiculata subsp. unguiculata	448558	1	SD	PI	448558	Niger	TVu 5315
397	Vigna unguiculata subsp. unguiculata	448600	2	SD	PI	448600	Niger	TVu 5372
398	Vigna unguiculata subsp. unguiculata	448760	1	SD	PI	448760	Niger	TVu 5560
399	Vigna unguiculata subsp. unguiculata	448806	2	SD	PI	448806	Niger	TVu 5616
400	Vigna unguiculata subsp. unguiculata	449161	3	SD	PI	449161	Niger	TVu 6949
401	Vigna unguiculata subsp. unguiculata	449218	1	SD	PI	449218	Niger	TVu 7006
402	Vigna unguiculata subsp. unguiculata	478622	2	SD	PI	478622	Turkey	62-153-00052
403	Vigna unguiculata subsp. unguiculata	580623	1	SD	PI	580623	Nigeria	TVu 7907
404	Vigna unguiculata subsp. unguiculata	580867	3	SD	PI	580867	Nigeria	TVu 10660
405	Vigna unguiculata subsp. unguiculata	580978	1	SD	PI	580978	Nigeria	TVu 11659
406	Vigna unguiculata subsp. unguiculata	582322	1	SD	PI	582322	Burkina Faso	UCR 52
407	Vigna unguiculata subsp. unguiculata	582340	1	SD	PI	582340		PARAGUAY 1
408	Vigna unguiculata subsp. unguiculata	582343	1	SD	PI	582343	Former Soviet Union	CPI 77123
409	Vigna unguiculata subsp. unguiculata	582344	1	SD	PI	582344	Former Soviet Union	CPI 77122
410	Vigna unguiculata subsp. unguiculata	582347	1	SD	PI	582347	Myanmar	CPI 30780
411	Vigna unguiculata subsp. unguiculata	582351	1	SD	PI	582351	Argentina	CPI 11900
412	Vigna unguiculata subsp. unguiculata	582352	1	SD	PI	582352	Saudi Arabia	UCR 154
413	Vigna unguiculata subsp. unguiculata	582353	1	SD	PI	582353	Saudi Arabia	UCR 155
414	Vigna unguiculata subsp. unguiculata	582354	1	SD	PI	582354		MULLER 17G
415	Vigna unguiculata subsp. unguiculata	582366	1	SD	PI	582366	India	UCR 191
416	Vigna unguiculata subsp. unguiculata	582368	1	SD	PI	582368	India	UCR 193
417	Vigna unguiculata subsp. unguiculata	582415	1	SD	PI	582415	Mexico	GAMUSA
418	Vigna unguiculata subsp. unguiculata	582416	1	SD	PI	582416	Mexico	MALU
419	Vigna unguiculata subsp. unguiculata	582419	1	SD	PI	582419	Burkina Faso	KVu 69
420	Vigna unguiculata subsp. unguiculata	582420	1	SD	PI	582420		BUSH 1
421	Vigna unguiculata subsp. unguiculata	582421	1	SD	PI	582421		BUSH 2
422	Vigna unguiculata subsp. unguiculata	582422	1	SD	PI	582422		BUSH 3
423	Vigna unguiculata subsp. unguiculata	582423	1	SD	PI	582423		BUSH 4
424	Vigna unguiculata subsp. unguiculata	582424	1	SD	PI	582424		BUSH 5
425	Vigna unguiculata subsp. unguiculata	582425	1	SD	PI	582425		BUSH 7
426	Vigna unguiculata subsp. unguiculata	582428	1	SD	PI	582428	Trinidad and Tobago	LAURA B
427	Vigna unguiculata subsp. unguiculata	582465	1	SD	PI	582465	Mexico	UCR 342
428	Vigna unguiculata subsp. unguiculata	582466	1	SD	PI	582466	Mexico	UCR 343
429	Vigna unguiculata subsp. unguiculata	582467	1	SD	PI	582467		UCR 346
430	Vigna unguiculata subsp. unguiculata	582468	1	SD	PI	582468		UCR 347
431	Vigna unguiculata subsp. unguiculata	582469	1	SD	PI	582469	Philippines	UCR 350
432	Vigna unguiculata subsp. unguiculata	582470	1	SD	PI	582470	Botswana	UCR 776
433	Vigna unguiculata subsp. unguiculata	582471	1	SD	PI	582471	Botswana	UCR 360
434	Vigna unguiculata subsp. unguiculata	582472	1	SD	PI	582472	Botswana	UCR 1042
435	Vigna unguiculata subsp. unguiculata	582474	1	SD	PI	582474	Botswana	UCR 381

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436	Vigna unguiculata subsp. unguiculata	582512	1	SD	PI	582512	Nigeria	UCR 430
437	Vigna unguiculata subsp. unguiculata	582521	1	SD	PI	582521	Mexico	YETTOROJ
438	Vigna unguiculata subsp. unguiculata	582522	1	SD	PI	582522	Mexico	FRIJOL SARABANDO
439	Vigna unguiculata subsp. unguiculata	582523	1	SD	PI	582523	Mexico	FRIJOL TORITO
440	Vigna unguiculata subsp. unguiculata	582524	1	SD	PI	582524	Mexico	SAK XPELON
441	Vigna unguiculata subsp. unguiculata	582525	1	SD	PI	582525	China	GUILIN CHINH
442	Vigna unguiculata subsp. unguiculata	582530	1	SD	PI	582530	Ghana	SAMBRIZIE
443	Vigna unguiculata subsp. unguiculata	582531	1	SD	PI	582531	Ghana	SUMBRISOGLA
444	Vigna unguiculata subsp. unguiculata	582541	1	SD	PI	582541	Mexico	TVu 2623
445	Vigna unguiculata subsp. unguiculata	582542	1	SD	PI	582542	Mexico	TVu 2623
446	Vigna unguiculata subsp. unguiculata	582551	1	SD	PI	582551	Botswana	UCR 1004
447	Vigna unguiculata subsp. unguiculata	582554	1	SD	PI	582554	Kenya	UCR 718
448	Vigna unguiculata subsp. unguiculata	582565	3	SD	PI	582565	India	UCR 500
449	Vigna unguiculata subsp. unguiculata	582566	2	SD	PI	582566	India	PUSA DOFASLI
450	Vigna unguiculata subsp. unguiculata	582567	1	SD	PI	582567	India	UCR 504
451	Vigna unguiculata subsp. unguiculata	582569	1	SD	PI	582569	India	UCR 508
452	Vigna unguiculata subsp. unguiculata	582571	1	SD	PI	582571	Botswana	UCR 518
453	Vigna unguiculata subsp. unguiculata	582572	1	SD	PI	582572	Kenya	KVu 1-P1
454	Vigna unguiculata subsp. unguiculata	582573	1	SD	PI	582573	Kenya	KVu 23
455	Vigna unguiculata subsp. unguiculata	582574	1	SD	PI	582574	Kenya	KVu 24-P3
456	Vigna unguiculata subsp. unguiculata	582575	1	SD	PI	582575	Kenya	KVu 26
457	Vigna unguiculata subsp. unguiculata	582576	1	SD	PI	582576	Kenya	KVu 45
458	Vigna unguiculata subsp. unguiculata	582578	2	SD	PI	582578	Kenya	KVu 56 CRN
459	Vigna unguiculata subsp. unguiculata	582579	1	SD	PI	582579	Kenya	KVu 57
460	Vigna unguiculata subsp. unguiculata	582581	1	SD	PI	582581	Kenya	KVu 64
461	Vigna unguiculata subsp. unguiculata	582650	1	SD	PI	582650	Botswana	UCR 729
462	Vigna unguiculata subsp. unguiculata	582651	1	SD	PI	582651	Botswana	UCR 730
463	Vigna unguiculata subsp. unguiculata	582665	3	SD	PI	582665	Botswana	UCR 1016
464	Vigna unguiculata subsp. unguiculata	582666	1	SD	PI	582666	Botswana	UCR 1019
465	Vigna unguiculata subsp. unguiculata	582667	1	SD	PI	582667	Botswana	UCR 1021
466	Vigna unguiculata subsp. unguiculata	582668	1	SD	PI	582668	Botswana	UCR 1022
467	Vigna unguiculata subsp. unguiculata	582669	1	SD	PI	582669	Botswana	UCR 1024
468	Vigna unguiculata subsp. unguiculata	582670	1	SD	PI	582670	Botswana	UCR 1025
469	Vigna unguiculata subsp. unguiculata	582671	1	SD	PI	582671	Botswana	UCR 1026
470	Vigna unguiculata subsp. unguiculata	582672	1	SD	PI	582672	Botswana	UCR 1027
471	Vigna unguiculata subsp. unguiculata	582675	1	SD	PI	582675	Botswana	UCR 1030
472	Vigna unguiculata subsp. unguiculata	582676	1	SD	PI	582676	Botswana	UCR 1035
473	Vigna unguiculata subsp. unguiculata	582677	1	SD	PI	582677	Botswana	UCR 1037
474	Vigna unguiculata subsp. unguiculata	582678	2	SD	PI	582678	Botswana	UCR 1038
475	Vigna unguiculata subsp. unguiculata	582679	1	SD	PI	582679	Botswana	UCR 1041

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
476	Vigna unguiculata subsp. unguiculata	582680	1	SD	PI	582680	Botswana	UCR 1043
477	Vigna unguiculata subsp. unguiculata	582681	1	SD	PI	582681	Botswana	UCR 1044
478	Vigna unguiculata subsp. unguiculata	582696	1	SD	PI	582696	Botswana	UCR 1159
479	Vigna unguiculata subsp. unguiculata	582697	1	SD	PI	582697	Botswana	UCR 1176
480	Vigna unguiculata subsp. unguiculata	582698	1	SD	PI	582698	Botswana	UCR 1186
481	Vigna unguiculata subsp. unguiculata	582699	1	SD	PI	582699	Botswana	UCR 1193
482	Vigna unguiculata subsp. unguiculata	582700	1	SD	PI	582700	Botswana	UCR 1195
483	Vigna unguiculata subsp. unguiculata	582701	1	SD	PI	582701	Botswana	UCR 1203
484	Vigna unguiculata subsp. unguiculata	582702	1	SD	PI	582702	Botswana	UCR 1204
485	Vigna unguiculata subsp. unguiculata	582703	1	SD	PI	582703	Botswana	UCR 1207
486	Vigna unguiculata subsp. unguiculata	582704	1	SD	PI	582704	Botswana	UCR 1211
487	Vigna unguiculata subsp. unguiculata	582705	1	SD	PI	582705	Botswana	UCR 1220
488	Vigna unguiculata subsp. unguiculata	582706	1	SD	PI	582706	Botswana	UCR 1225
489	Vigna unguiculata subsp. unguiculata	582707	2	SD	PI	582707	Botswana	UCR 1228
490	Vigna unguiculata subsp. unguiculata	582708	1	SD	PI	582708	Botswana	UCR 1231
491	Vigna unguiculata subsp. unguiculata	582709	1	SD	PI	582709	Botswana	UCR 1232
492	Vigna unguiculata subsp. unguiculata	582718	1	SD	PI	582718	Botswana	UCR 1242
493	Vigna unguiculata subsp. unguiculata	582724	1	SD	PI	582724	Botswana	UCR 1261
494	Vigna unguiculata subsp. unguiculata	582725	1	SD	PI	582725	Botswana	UCR 1263
495	Vigna unguiculata subsp. unguiculata	582726	1	SD	PI	582726	Botswana	UCR 1264
496	Vigna unguiculata subsp. unguiculata	582727	2	SD	PI	582727	Botswana	UCR 1266
497	Vigna unguiculata subsp. unguiculata	582728	1	SD	PI	582728	Botswana	UCR 1267
498	Vigna unguiculata subsp. unguiculata	582729	1	SD	PI	582729	Botswana	UCR 1269
499	Vigna unguiculata subsp. unguiculata	582730	1	SD	PI	582730	Botswana	UCR 1270
500	Vigna unguiculata subsp. unguiculata	582731	1	SD	PI	582731	Botswana	UCR 1273
501	Vigna unguiculata subsp. unguiculata	582732	1	SD	PI	582732	Botswana	UCR 1274
502	Vigna unguiculata subsp. unguiculata	582733	1	SD	PI	582733	Botswana	UCR 1275
503	Vigna unguiculata subsp. unguiculata	582734	1	SD	PI	582734	Botswana	UCR 1278
504	Vigna unguiculata subsp. unguiculata	582735	1	SD	PI	582735	Botswana	UCR 1280
505	Vigna unguiculata subsp. unguiculata	582736	1	SD	PI	582736	Botswana	UCR 1283
506	Vigna unguiculata subsp. unguiculata	582737	1	SD	PI	582737	Botswana	UCR 1287
507	Vigna unguiculata subsp. unguiculata	582738	1	SD	PI	582738	Botswana	UCR 1290
508	Vigna unguiculata subsp. unguiculata	582739	1	SD	PI	582739	Botswana	UCR 1291
509	Vigna unguiculata subsp. unguiculata	582740	1	SD	PI	582740	Botswana	UCR 1292
510	Vigna unguiculata subsp. unguiculata	582785	1	SD	PI	582785	Kenya	KVu 479
511	Vigna unguiculata subsp. unguiculata	582789	1	SD	PI	582789	Kenya	KVu 510-1
512	Vigna unguiculata subsp. unguiculata	582805	1	SD	PI	582805	Botswana	UCR 763
513	Vigna unguiculata subsp. unguiculata	582809	1	SD	PI	582809	Botswana	UCR 772
514	Vigna unguiculata subsp. unguiculata	582810	1	SD	PI	582810	Botswana	UCR 790
515	Vigna unguiculata subsp. unguiculata	582812	1	SD	PI	582812	Botswana	UCR 794

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
516	Vigna unguiculata subsp. unguiculata	582813	1	SD	PI	582813	Botswana	UCR 796
517	Vigna unguiculata subsp. unguiculata	582814	1	SD	PI	582814	Botswana	UCR 799
518	Vigna unguiculata subsp. unguiculata	582815	1	SD	PI	582815	Botswana	UCR 804
519	Vigna unguiculata subsp. unguiculata	582816	1	SD	PI	582816	Botswana	UCR 805
520	Vigna unguiculata subsp. unguiculata	582817	1	SD	PI	582817	Botswana	UCR 809
521	Vigna unguiculata subsp. unguiculata	582818	1	SD	PI	582818	Botswana	UCR 818
522	Vigna unguiculata subsp. unguiculata	582819	1	SD	PI	582819	Botswana	UCR 825
523	Vigna unguiculata subsp. unguiculata	582820	1	SD	PI	582820	Botswana	UCR 827
524	Vigna unguiculata subsp. unguiculata	582821	1	SD	PI	582821	Botswana	UCR 830
525	Vigna unguiculata subsp. unguiculata	582822	1	SD	PI	582822	Botswana	UCR 831
526	Vigna unguiculata subsp. unguiculata	582823	1	SD	PI	582823	Botswana	UCR 832
527	Vigna unguiculata subsp. unguiculata	582824	1	SD	PI	582824	Botswana	UCR 834
528	Vigna unguiculata subsp. unguiculata	582825	1	SD	PI	582825	Botswana	UCR 839
529	Vigna unguiculata subsp. unguiculata	582826	1	SD	PI	582826	Botswana	UCR 846
530	Vigna unguiculata subsp. unguiculata	582850	1	SD	PI	582850	Botswana	UCR 927
531	Vigna unguiculata subsp. unguiculata	582851	2	SD	PI	582851	Botswana	UCR 929
532	Vigna unguiculata subsp. unguiculata	582852	1	SD	PI	582852	Botswana	UCR 935
533	Vigna unguiculata subsp. unguiculata	582853	1	SD	PI	582853	Botswana	UCR 941
534	Vigna unguiculata subsp. unguiculata	582854	1	SD	PI	582854	Botswana	UCR 945
535	Vigna unguiculata subsp. unguiculata	582855	1	SD	PI	582855	Botswana	UCR 947
536	Vigna unguiculata subsp. unguiculata	582856	1	SD	PI	582856	Botswana	UCR 948
537	Vigna unguiculata subsp. unguiculata	582857	1	SD	PI	582857	Botswana	UCR 954
538	Vigna unguiculata subsp. unguiculata	582858	1	SD	PI	582858	Botswana	UCR 966
539	Vigna unguiculata subsp. unguiculata	582859	2	SD	PI	582859	Botswana	UCR 967
540	Vigna unguiculata subsp. unguiculata	582860	1	SD	PI	582860	Botswana	UCR 968
541	Vigna unguiculata subsp. unguiculata	582861	1	SD	PI	582861	Botswana	UCR 1003
542	Vigna unguiculata subsp. unguiculata	582862	1	SD	PI	582862	Botswana	UCR 1007
543	Vigna unguiculata subsp. unguiculata	582863	1	SD	PI	582863	Botswana	UCR 1017
544	Vigna unguiculata subsp. unguiculata	582864	1	SD	PI	582864	Botswana	UCR 1018
545	Vigna unguiculata subsp. unguiculata	582865	1	SD	PI	582865	Botswana	UCR 1031
546	Vigna unguiculata subsp. unguiculata	582866	1	SD	PI	582866	Botswana	UCR 1032
547	Vigna unguiculata subsp. unguiculata	582867	1	SD	PI	582867	Botswana	UCR 1034
548	Vigna unguiculata subsp. unguiculata	582868	1	SD	PI	582868	Botswana	UCR 1051
549	Vigna unguiculata subsp. unguiculata	582869	1	SD	PI	582869	Botswana	UCR 1052
550	Vigna unguiculata subsp. unguiculata	582870	1	SD	PI	582870	Botswana	UCR 1057
551	Vigna unguiculata subsp. unguiculata	582871	1	SD	PI	582871	Botswana	UCR 1058
552	Vigna unguiculata subsp. unguiculata	582872	1	SD	PI	582872	Botswana	UCR 1060
553	Vigna unguiculata subsp. unguiculata	582873	1	SD	PI	582873	Botswana	UCR 1086
554	Vigna unguiculata subsp. unguiculata	582874	1	SD	PI	582874	Botswana	UCR 1087
555	Vigna unguiculata subsp. unguiculata	582875	1	SD	PI	582875	Botswana	UCR 1089

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
556	Vigna unguiculata subsp. unguiculata	582876	1	SD	PI	582876	Botswana	UCR 1090
557	Vigna unguiculata subsp. unguiculata	582877	1	SD	PI	582877	Botswana	UCR 1093
558	Vigna unguiculata subsp. unguiculata	582878	1	SD	PI	582878	Botswana	UCR 1100
559	Vigna unguiculata subsp. unguiculata	582879	1	SD	PI	582879	Botswana	UCR 516
560	Vigna unguiculata subsp. unguiculata	582880	1	SD	PI	582880	Botswana	UCR 1118
561	Vigna unguiculata subsp. unguiculata	582881	1	SD	PI	582881	Botswana	UCR 1126
562	Vigna unguiculata subsp. unguiculata	582894	1	SD	PI	582894	Botswana	UCR 1180
563	Vigna unguiculata subsp. unguiculata	582912	1	SD	PI	582912	Kenya	KVu 390-3
564	Vigna unguiculata subsp. unguiculata	582913	1	SD	PI	582913	Senegal	UCR 2529
565	Vigna unguiculata subsp. unguiculata	582923	1	SD	PI	582923	Senegal	BAMBEY 31
566	Vigna unguiculata subsp. unguiculata	582924	1	SD	PI	582924	Senegal	BAMBEY 28
567	Vigna unguiculata subsp. unguiculata	582930	1	SD	PI	582930	Mexico	UCR 171
568	Vigna unguiculata subsp. unguiculata	582931	1	SD	PI	582931	Malawi	UCR 173
569	Vigna unguiculata subsp. unguiculata	582934	1	SD	PI	582934	Burkina Faso	VITA 7
570	Vigna unguiculata subsp. unguiculata	582936	1	SD	PI	582936	Nigeria	UCR 289
571	Vigna unguiculata subsp. unguiculata	582941	1	SD	PI	582941	Puerto Rico	UCR 353
572	Vigna unguiculata subsp. unguiculata	582942	1	SD	PI	582942	Puerto Rico	UCR 354
573	Vigna unguiculata subsp. unguiculata	582949	1	SD	PI	582949	Mexico	XPELON
574	Vigna unguiculata subsp. unguiculata	582951	1	SD	PI	582951	Cameroon	CAMEROON-2
575	Vigna unguiculata subsp. unguiculata	582952	1	SD	PI	582952	Nigeria	UCR 452
576	Vigna unguiculata subsp. unguiculata	582965	1	SD	PI	582965	Nigeria	UCR 480
577	Vigna unguiculata subsp. unguiculata	582980	1	SD	PI	582980	Kenya	KVu 439 B
578	Vigna unguiculata subsp. unguiculata	582984	1	SD	PI	582984	Kenya	KVu 447
579	Vigna unguiculata subsp. unguiculata	583014	1	SD	PI	583014	Botswana	UCR 779
580	Vigna unguiculata subsp. unguiculata	583051	1	SD	PI	583051	Botswana	UCR 900
581	Vigna unguiculata subsp. unguiculata	583068	1	SD	PI	583068	Botswana	UCR 965
582	Vigna unguiculata subsp. unguiculata	583076	1	SD	PI	583076	Cameroon	UCR 2580
583	Vigna unguiculata subsp. unguiculata	583098	1	SD	PI	583098	Cameroon	UCR 2617
584	Vigna unguiculata subsp. unguiculata	583100	1	SD	PI	583100	Cameroon	UCR 2620
585	Vigna unguiculata subsp. unguiculata	583102	1	SD	PI	583102	Cameroon	UCR 2623
586	Vigna unguiculata subsp. unguiculata	583104	1	SD	PI	583104	Cameroon	UCR 2625
587	Vigna unguiculata subsp. unguiculata	583106	1	SD	PI	583106	Cameroon	UCR 2627
588	Vigna unguiculata subsp. unguiculata	583108	1	SD	PI	583108	Cameroon	UCR 2631
589	Vigna unguiculata subsp. unguiculata	583110	1	SD	PI	583110	Cameroon	UCR 2634
590	Vigna unguiculata subsp. unguiculata	583158	1	SD	PI	583158	Cameroon	UCR 2700
591	Vigna unguiculata subsp. unguiculata	583160	1	SD	PI	583160	Cameroon	UCR 2702
592	Vigna unguiculata subsp. unguiculata	583164	2	SD	PI	583164	Cameroon	UCR 2708
593	Vigna unguiculata subsp. unguiculata	583167	1	SD	PI	583167	Cameroon	UCR 2713
594	Vigna unguiculata subsp. unguiculata	583170	1	SD	PI	583170	Cameroon	UCR 2716
595	Vigna unguiculata subsp. unguiculata	583172	1	SD	PI	583172	Cameroon	UCR 2720

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
596	Vigna unguiculata subsp. unguiculata	583182	1	SD	PI	583182	Portugal	UCR 2742
597	Vigna unguiculata subsp. unguiculata	583185	1	SD	PI	583185	Senegal	UCR 3294
598	Vigna unguiculata subsp. unguiculata	583186	1	SD	PI	583186	Senegal	UCR 3306
599	Vigna unguiculata subsp. unguiculata	583187	1	SD	PI	583187	Senegal	UCR 3310
600	Vigna unguiculata subsp. unguiculata	583188	1	SD	PI	583188	Senegal	UCR 3313
601	Vigna unguiculata subsp. unguiculata	583189	1	SD	PI	583189	Senegal	UCR 3315
602	Vigna unguiculata subsp. unguiculata	583190	1	SD	PI	583190	Senegal	UCR 3316
603	Vigna unguiculata subsp. unguiculata	583191	1	SD	PI	583191	Senegal	UCR 3326
604	Vigna unguiculata subsp. unguiculata	583192	1	SD	PI	583192	Senegal	UCR 3332
605	Vigna unguiculata subsp. unguiculata	583193	1	SD	PI	583193	Senegal	UCR 3334
606	Vigna unguiculata subsp. unguiculata	583194	1	SD	PI	583194	Senegal	UCR 3335
607	Vigna unguiculata subsp. unguiculata	583195	1	SD	PI	583195	Senegal	UCR 3338
608	Vigna unguiculata subsp. unguiculata	583196	1	SD	PI	583196	Senegal	UCR 3341
609	Vigna unguiculata subsp. unguiculata	583197	2	SD	PI	583197	Senegal	UCR 3345
610	Vigna unguiculata subsp. unguiculata	583198	1	SD	PI	583198	Senegal	UCR 3349
611	Vigna unguiculata subsp. unguiculata	583199	1	SD	PI	583199	Senegal	UCR 3367
612	Vigna unguiculata subsp. unguiculata	583200	1	SD	PI	583200	Senegal	UCR 3372
613	Vigna unguiculata subsp. unguiculata	583201	1	SD	PI	583201	Senegal	UCR 3373
614	Vigna unguiculata subsp. unguiculata	583202	1	SD	PI	583202	Senegal	UCR 3374
615	Vigna unguiculata subsp. unguiculata	583203	1	SD	PI	583203	Senegal	UCR 3376
616	Vigna unguiculata subsp. unguiculata	583204	1	SD	PI	583204	Senegal	UCR 3378
617	Vigna unguiculata subsp. unguiculata	583205	1	SD	PI	583205	Senegal	UCR 3379
618	Vigna unguiculata subsp. unguiculata	583206	1	SD	PI	583206	Senegal	UCR 3382
619	Vigna unguiculata subsp. unguiculata	583207	1	SD	PI	583207	Senegal	UCR 3383
620	Vigna unguiculata subsp. unguiculata	583209	1	SD	PI	583209	Nigeria	TVu 2503
621	Vigna unguiculata subsp. unguiculata	583218	1	SD	PI	583218	Senegal	UCR 3284
622	Vigna unguiculata subsp. unguiculata	583219	1	SD	PI	583219	Senegal	UCR 3285
623	Vigna unguiculata subsp. unguiculata	583220	1	SD	PI	583220	Senegal	UCR 3286
624	Vigna unguiculata subsp. unguiculata	583222	1	SD	PI	583222	Senegal	UCR 3296
625	Vigna unguiculata subsp. unguiculata	583223	1	SD	PI	583223	Senegal	UCR 3300
626	Vigna unguiculata subsp. unguiculata	583224	1	SD	PI	583224	Senegal	UCR 3301
627	Vigna unguiculata subsp. unguiculata	583225	1	SD	PI	583225	Senegal	UCR 3302
628	Vigna unguiculata subsp. unguiculata	583226	1	SD	PI	583226	Senegal	UCR 3303
629	Vigna unguiculata subsp. unguiculata	583227	1	SD	PI	583227	Senegal	UCR 3307
630	Vigna unguiculata subsp. unguiculata	583228	1	SD	PI	583228	Senegal	UCR 3308
631	Vigna unguiculata subsp. unguiculata	583229	1	SD	PI	583229	Senegal	UCR 3309
632	Vigna unguiculata subsp. unguiculata	583230	1	SD	PI	583230	Senegal	UCR 3311
633	Vigna unguiculata subsp. unguiculata	583231	1	SD	PI	583231	Senegal	UCR 3314
634	Vigna unguiculata subsp. unguiculata	583232	1	SD	PI	583232	Senegal	UCR 3317
635	Vigna unguiculata subsp. unguiculata	583233	1	SD	PI	583233	Senegal	UCR 3320

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
636	Vigna unguiculata subsp. unguiculata	583234	1	SD	PI	583234	Senegal	UCR 3322
637	Vigna unguiculata subsp. unguiculata	583235	2	SD	PI	583235	Senegal	UCR 3323
638	Vigna unguiculata subsp. unguiculata	583236	1	SD	PI	583236	Senegal	UCR 3327
639	Vigna unguiculata subsp. unguiculata	583237	1	SD	PI	583237	Senegal	UCR 3336
640	Vigna unguiculata subsp. unguiculata	583238	1	SD	PI	583238	Senegal	UCR 3339
641	Vigna unguiculata subsp. unguiculata	583239	1	SD	PI	583239	Senegal	UCR 3348
642	Vigna unguiculata subsp. unguiculata	583240	1	SD	PI	583240	Senegal	UCR 3350
643	Vigna unguiculata subsp. unguiculata	583241	1	SD	PI	583241	Senegal	UCR 3353
644	Vigna unguiculata subsp. unguiculata	583242	1	SD	PI	583242	Senegal	UCR 3358
645	Vigna unguiculata subsp. unguiculata	583243	1	SD	PI	583243	Senegal	UCR 3359
646	Vigna unguiculata subsp. unguiculata	583246	1	SD	PI	583246	Senegal	UCR 3365
647	Vigna unguiculata subsp. unguiculata	583247	1	SD	PI	583247	Senegal	UCR 3368
648	Vigna unguiculata subsp. unguiculata	583248	1	SD	PI	583248	Senegal	UCR 3370
649	Vigna unguiculata subsp. unguiculata	583249	1	SD	PI	583249	Senegal	UCR 3371
650	Vigna unguiculata subsp. unguiculata	583250	1	SD	PI	583250	Senegal	UCR 3375
651	Vigna unguiculata subsp. unguiculata	583251	1	SD	PI	583251	Senegal	UCR 3377
652	Vigna unguiculata subsp. unguiculata	583252	2	SD	PI	583252	Senegal	UCR 3380
653	Vigna unguiculata subsp. unguiculata	583253	1	SD	PI	583253	Senegal	UCR 3381
654	Vigna unguiculata subsp. unguiculata	583258	1	SD	PI	583258	Senegal	UCR 178
655	Vigna unguiculata subsp. unguiculata	583259	1	SD	PI	583259	Burkina Faso	SUVITA 2
656	Vigna unguiculata subsp. unguiculata	583260	1	SD	PI	583260	Sudan	SUDAN-1
657	Vigna unguiculata subsp. unguiculata	583261	1	SD	PI	583261	Sudan	SUDAN-2
658	Vigna unguiculata subsp. unguiculata	583262	1	SD	PI	583262	Sudan	SUDAN EARLY VARIETY
659	Vigna unguiculata subsp. unguiculata	583263	1	SD	PI	583263	Nigeria	UCR 386
660	Vigna unguiculata subsp. unguiculata	583270	1	SD	PI	583270	Senegal	UCR 3293
661	Vigna unguiculata subsp. unguiculata	583271	1	SD	PI	583271	Senegal	UCR 3297
662	Vigna unguiculata subsp. unguiculata	583272	1	SD	PI	583272	Senegal	UCR 3299
663	Vigna unguiculata subsp. unguiculata	583273	1	SD	PI	583273	Senegal	UCR 3318
664	Vigna unguiculata subsp. unguiculata	583274	1	SD	PI	583274	Senegal	UCR 3333
665	Vigna unguiculata subsp. unguiculata	583487	1	SD	PI	583487	Senegal	UCR 3304
666	Vigna unguiculata subsp. unguiculata	583489	1	SD	PI	583489	Australia	UCR 3945
667	Vigna unguiculata subsp. unguiculata	583494	1	SD	PI	583494	Nigeria	UCR 4547
668	Vigna unguiculata subsp. unguiculata	583502	1	SD	PI	583502	Nigeria	UCR 4562
669	Vigna unguiculata subsp. unguiculata	583507	1	SD	PI	583507	Nigeria	UCR 4568
670	Vigna unguiculata subsp. unguiculata	583513	1	SD	PI	583513	Nigeria	UCR 4574
671	Vigna unguiculata subsp. unguiculata	583550	1	SD	PI	583550	Mali	UCR 4660
672	Vigna unguiculata subsp. unguiculata	583551	1	SD	PI	583551	Mali	UCR 4663
673	Vigna unguiculata subsp. unguiculata	593117	2	SD	PI	593117	Botswana	UCR 922
674	Vigna unguiculata subsp. unguiculata	610516	1	SD	PI	610516	Italy	UCR 5372
675	Vigna unguiculata subsp. unguiculata	610517	1	SD	PI	610517	Italy	UCR 5373

Serial No	TAXON	IVNO	IVS	IVT	ACP	ACNO	COUNTRY	PLANTID
676	Vigna unguiculata subsp. unguiculata	610520	1	SD	PI	610520	Italy	UCR 5376
677	Vigna unguiculata subsp. unguiculata	610533	1	SD	PI	610533	Italy	UCR 5389
678	Vigna unguiculata subsp. unguiculata	610604	1	SD	PI	610604	Italy	UCR 5460
679	Vigna unguiculata subsp. unguiculata	610620	1	SD	PI	610620	Italy	UCR 5476
680	Vigna unguiculata subsp. unguiculata	610621	1	SD	PI	610621	Italy	UCR 5477
681	Vigna unguiculata subsp. unguiculata	612607	1	SD	PI	612607		GC-86L-98
682					IT98D-478	8.0		
683					IT79K-813	21.0		
684					IAR	48.0		
685					IT97K-819	154.0		
686					TN256	80.0		
687					IT98K-589	2.0		
688					IT99K-826	119.0		
689					IT00K	1148.0		
690					TN-28	87.0		
691					IT97K-340	1.0		
692					IT97K-819	170.0		
693					IT98D	1399.0		
694					IT90K-277	2.0		
695					Aloka	Locale		
696					Danila	Locale		

### APPENDIX B

#### PHOSPHORUS CONTENT AND BIOMASS DATA FROM SCREENING STAGE

			ppm PO4-I	2			No Pho	osphate Shoot	Added t Dry W	eight			Root	Dry We	eight	
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD
2	2	476.41	520.80	372.07	456.43	0.17	0.23	0.34	0.30	0.29	0.19	0.13	0.14	0.11	0.13	0.12
4	7	694.01	671.79	533.83	633.21	0.14	0.23	0.30	0.36	0.30	0.22	0.12	0.20	0.21	0.18	0.28
9	7	597.45	806.67	702.84	702.32	0.15	0.28	0.28	0.42	0.33	0.25	0.14	0.17	0.21	0.17	0.20
11	5	604.50	635.08	-	619.79	0.03	0.35	0.33	0.34	0.34	0.03	0.29	0.28	0.23	0.27	0.12
16	3	462.83	762.76	692.72	639.44	0.25	0.24	0.22	0.23	0.23	0.04	0.17	0.18	0.19	0.18	0.06
84	3	687.79	971.51	581.26	746.85	0.27	0.53	0.42	0.37	0.44	0.19	0.18	0.25	0.19	0.21	0.18
92	8	687.57	534.65	764.61	662.28	0.18	0.36	0.34	0.45	0.38	0.15	0.21	0.12	0.20	0.18	0.28
109	2	382.47	441.44	786.75	536.89	0.41	0.94	0.98	0.96	0.96	0.02	0.42	0.32	0.35	0.36	0.14
118	3	419.74	518.51	471.52	469.92	0.11	0.13	0.22	0.19	0.18	0.25	0.09	0.16	0.13	0.13	0.28
122	2	707.02	1030.48	467.92	735.14	0.38	0.25	0.33	0.38	0.32	0.20	0.14	0.12	0.08	0.11	0.27
123	4	528.34	503.28	619.63	550.42	0.11	0.35	0.42	0.28	0.35	0.20	0.18	0.22	0.17	0.19	0.14
156	5	686.43	807.96	818.52	770.97	0.10	0.42	0.49	0.44	0.45	0.08	0.29	0.27	0.35	0.30	0.14
159	4	637.68	792.99	686.02	705.56	0.11	0.45	0.47	0.47	0.46	0.02	0.16	0.24	0.25	0.22	0.23
190	6	339.41	423.65	349.08	370.71	0.12	0.88	0.88	0.87	0.88	0.01	0.25	0.41	0.36	0.34	0.24
201	4	342.49	413.29	483.46	413.08	0.17	1.15	0.76	0.46	0.79	0.44	0.46	0.36	0.34	0.39	0.17
209	2	620.03	806.37	748.44	724.94	0.13	0.40	0.46	0.35	0.40	0.14	0.30	0.27	0.26	0.28	0.08
210	8	592.17	642.52	734.92	656.54	0.11	0.55	0.36	0.77	0.56	0.37	0.30	0.19	0.29	0.26	0.23
214	6	542.95	790.68	596.04	643.23	0.20	0.18	0.22	0.28	0.23	0.22	0.10	0.13	0.15	0.13	0.20
221	8	579.63	763.90	691.24	678.26	0.14	0.22	0.31	0.34	0.29	0.22	0.17	0.23	0.17	0.19	0.18
247	2	376.21	684.23	755.83	605.43	0.33	0.19	0.32	0.32	0.28	0.27	0.15	0.25	0.21	0.20	0.25
254	1	427.58	474.76	676.71	526.35	0.25	0.42	0.66	0.92	0.67	0.38	0.25	0.24	0.45	0.31	0.38
267	3	608.34	764.25	574.44	649.01	0.16	0.27	0.36	0.43	0.35	0.23	0.13	0.08	0.16	0.12	0.33
272	2	485.69	436.08	465.87	462.54	0.05	0.63	0.95	0.70	0.76	0.22	0.27	0.28	0.35	0.30	0.15
286	8	606.41	796.79	521.51	641.57	0.22	0.54	0.38	0.44	0.45	0.18	0.12	0.15	0.23	0.17	0.34
303	4	490.21	659.39	665.57	605.05	0.16	0.44	0.45	0.35	0.41	0.13	0.21	0.26	0.22	0.23	0.12

			ppm PO4-I	•			No Pho	osphate Shoo	Added t Dry W	eight			Root	t Dry We	eight	
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD
313	6	583.45	560.24	459.33	534.34	0.12	0.35	0.50	0.34	0.40	0.23	0.27	0.16	0.26	0.23	0.26
314	1	678.34	712.83	925.35	772.17	0.17	0.47	0.52	0.30	0.43	0.27	0.21	0.27	0.17	0.22	0.23
319	3	625.16	623.67	534.11	594.31	0.09	0.37	0.44	0.30	0.37	0.19	0.31	0.24	0.24	0.26	0.15
320	2	540.33	706.65	985.37	744.12	0.30	0.30	0.22	0.23	0.25	0.17	0.18	0.28	0.21	0.22	0.23
327	8	312.94	772.86	744.41	610.07	0.42	0.93	0.95	0.78	0.89	0.10	-	0.30	0.32	0.31	0.05
334	1	444.13	548.67	482.27	491.69	0.11	0.36	0.40	0.44	0.40	0.10	0.21	0.23	0.29	0.24	0.17
336	6	488.12	582.57	479.00	516.56	0.11	0.90	0.72	0.85	0.82	0.11	0.43	0.46	0.36	0.42	0.12
338	7	653.92	832.58	528.68	671.73	0.23	0.31	0.37	0.32	0.33	0.10	0.16	0.20	0.15	0.17	0.16
342	3	650.58	698.21	835.14	727.97	0.13	0.34	0.44	0.36	0.38	0.14	0.16	0.23	0.18	0.19	0.19
345	7	571.16	651.77	578.85	600.59	0.07	0.28	0.35	0.49	0.37	0.29	0.23	0.22	0.26	0.24	0.09
368	8	542.43	416.36	871.01	609.93	0.38	0.25	0.70	0.43	0.46	0.49	0.22	0.23	0.24	0.23	0.04
377	5	790.34	699.89	619.98	703.40	0.12	0.21	0.31	0.31	0.28	0.21	0.17	0.21	0.19	0.19	0.11
386	3	600.76	724.19	668.78	664.58	0.09	0.27	0.22	0.27	0.25	0.11	0.19	0.17	0.27	0.21	0.25
388	7	680.90	807.56	775.60	754.69	0.09	0.41	0.39	0.37	0.39	0.05	0.13	0.17	0.15	0.15	0.13
394	1	735.88	-	588.88	662.38	0.16	0.27	0.31	0.21	0.26	0.19	0.13	0.15	0.14	0.14	0.07
396	7	575.36	671.22	565.18	603.92	0.10	0.22	0.29	0.48	0.33	0.41	0.14	0.09	0.17	0.13	0.30
402	4	352.02	451.48	474.04	425.85	0.15	0.96	1.07	0.88	0.97	0.10	0.46	0.39	0.34	0.40	0.15
410	2	441.56	572.93	-	507.24	0.18	0.39	0.50	0.32	0.40	0.22	0.17	0.20	0.19	0.19	0.08
413	2	391.94	568.84	654.72	538.50	0.25	1.56	1.24	0.95	1.25	0.24	0.30	0.29	0.52	0.37	0.35
416	4	634.28	665.69	341.05	547.01	0.33	0.44	0.39	0.53	0.45	0.16	0.31	0.24	0.35	0.30	0.19
423	7	879.59	1008.83	508.36	798.93	0.33	0.33	0.30	0.36	0.33	0.09	0.20	0.16	0.18	0.18	0.11
426	5	645.86	641.49	667.02	651.46	0.02	0.42	0.46	0.52	0.47	0.11	0.26	0.21	0.29	0.25	0.16
427	4	298.79	523.61	565.33	462.58	0.31	0.82	0.77	0.75	0.78	0.05	0.36	0.34	0.44	0.38	0.14
435	1	1225.18	501.56	705.43	810.73	0.46	0.28	0.28	0.32	0.29	0.08	0.19	0.15	0.21	0.18	0.17
436	6	887.39	768.93	700.78	785.70	0.12	0.34	0.52	0.48	0.45	0.21	0.23	0.24	0.36	0.28	0.26
440	5	1283.03	780.82	565.35	876.40	0.42	0.42	0.55	0.71	0.56	0.26	0.30	0.35	0.43	0.36	0.18
451	1	484.34	871.16	575.34	643.61	0.31	0.31	0.25	0.33	0.30	0.14	0.17	0.22	0.23	0.21	0.16
463	2	609.59	741.91	658.41	669.97	0.10	0.14	0.17	0.16	0.16	0.10	0.14	0.16	0.21	0.17	0.21
494	3	762.89	1046.48	926.83	912.07	0.16	0.64	0.87	0.56	0.69	0.23	0.24	0.31	0.22	0.26	0.18
495	2	391.20	802.54	556.23	583.32	0.35	0.45	0.45	0.59	0.50	0.16	0.27	0.26	0.23	0.25	0.08
499	5	460.12	640.61	578.23	559.65	0.16	0.44	0.56	0.31	0.44	0.29	0.17	0.26	0.18	0.20	0.24
503	1	539.31	719.10	540.82	599.74	0.17	0.43	0.31	0.37	0.37	0.16	0.26	0.22	0.22	0.23	0.10

			ppm PO4-I	P			No Pho	osphate Shoot	Added t Dry W	eight			Root	t Dry We	eight	
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1		Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD
504	7	775.20	610.81	621.99	669.33	0.14	0.38	0.35	0.38	0.37	0.05	0.14	0.15	0.16	0.15	0.07
506	5	836.70	832.99	678.63	782.78	0.12	0.34	0.48	0.39	0.40	0.18	0.22	0.27	0.24	0.24	0.10
517	7	458.63	604.74	452.72	505.36	0.17	0.26	0.30	0.36	0.31	0.16	0.13	0.17	0.18	0.16	0.17
518	5	980.11	751.39	648.99	793.50	0.21	0.23	0.25	0.32	0.27	0.18	0.11	0.16	0.17	0.15	0.22
524	2	670.09	720.67	656.58	682.45	0.05	0.19	0.18	0.13	0.17	0.19	0.17	0.18	0.18	0.18	0.03
525	5	556.02	745.57	738.47	680.02	0.16	0.32	0.29	0.34	0.32	0.08	0.19	0.21	0.20	0.20	0.05
527	2	388.62	686.18	759.70	611.50	0.32	0.26	0.38	0.28	0.31	0.21	0.12	0.18	0.19	0.16	0.23
532	8	332.06	950.36	714.28	665.56	0.47	0.52	0.38	0.33	0.41	0.24	0.16	0.21	0.17	0.18	0.15
549	6	262.44	516.15	501.26	426.62	0.33	0.32	0.30	0.17	0.26	0.31	0.18	0.21	0.13	0.17	0.23
551	3	412.14	606.80	635.12	551.35	0.22	0.63	0.70	0.38	0.57	0.30	0.25	0.34	0.27	0.29	0.16
560	5	533.81	571.51	549.44	551.59	0.03	0.35	0.55	0.53	0.48	0.23	0.20	0.18	0.21	0.20	0.08
565	1	767.32	476.86	737.59	660.59	0.24	0.99	0.66	0.91	0.85	0.20	0.35	0.31	0.39	0.35	0.11
582	4	816.20	603.40	859.48	759.69	0.18	0.38	0.93	0.83	0.71	0.41	0.14	0.17	0.31	0.21	0.44
589	6	415.60	478.64	511.68	468.64	0.10	0.68	0.96	0.57	0.74	0.27	0.20	0.32	0.29	0.27	0.23
596	1	298.16	599.26	-	448.71	0.47	1.06	0.64	0.54	0.75	0.37	0.37	0.25	-	0.31	0.27
597	2	781.51	665.56	739.49	728.85	0.08	0.35	0.53	0.68	0.52	0.32	0.13	0.18	0.27	0.19	0.37
605	5	743.70	745.25	593.75	694.24	0.13	0.20	0.24	0.31	0.25	0.22	0.12	0.17	0.22	0.17	0.29
612	2	347.16	482.72	329.15	386.34	0.22	0.92	0.92	0.76	0.87	0.11	0.26	0.32	0.25	0.28	0.14
615	6	589.65	501.30	610.23	567.06	0.10	0.81	0.97	0.84	0.87	0.10	0.54	0.36	0.48	0.46	0.20
620	1	306.89	705.67	620.09	544.22	0.39	1.34	0.84	0.77	0.98	0.32	0.23	0.30	0.44	0.32	0.33
629	4	417.18	489.66	922.37	609.74	0.45	0.56	0.79	0.48	0.61	0.26	0.30	0.39	0.44	0.38	0.19
635	2	418.53	806.39	707.12	644.01	0.31	0.47	0.47	0.63	0.52	0.18	0.27	0.27	0.37	0.30	0.19
651	8	319.80	814.88	519.25	551.31	0.45	0.69	0.52	0.58	0.60	0.14	0.26	0.41	0.27	0.31	0.27
652	1	255.07	359.39	551.75	388.74	0.39	0.84	0.77	0.57	0.73	0.19	0.17	0.17	0.18	0.17	0.03
654	3	531.89	783.71	747.38	687.66	0.20	0.28	0.39	0.48	0.38	0.26	0.12	0.16	0.19	0.16	0.22
664	6	561.83	547.03	493.17	534.01	0.07	0.60	0.69	0.95	0.75	0.24	0.28	0.24	0.38	0.30	0.24
676	3	260.93	572.17	650.26	494.45	0.42	1.13	0.83	0.78	0.91	0.21	0.27	0.30	0.34	0.30	0.12
679	6	360.95	989.37	1079.20	809.84	0.48	0.72	0.61	0.75	0.69	0.11	0.30	0.26	0.38	0.31	0.20
682	5	484.27	494.90	699.97	559.71	0.22	0.76	0.70	0.65	0.70	0.08	0.30	0.21	0.35	0.29	0.25
683	1	778.93	1032.54	859.57	890.35	0.15	0.67	0.59	1.04	0.77	0.31	0.32	0.27	0.46	0.35	0.28
684	5	-	496.70	495.78	496.24	0.00	0.49	0.61	0.61	0.57	0.12	0.29	0.27	0.31	0.29	0.07
685	2	487.85	610.54	1067.79	722.06	0.42	0.89	1.00	0.80	0.90	0.11	0.23	0.33	0.32	0.29	0.19

			ppm PO4-I	2			No Pho	osphate Shoot	Added t Dry W	eight			Root	Dry W	eight	
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD
686	2	811.77	625.82	861.64	766.41	0.16	0.49	0.61	0.47	0.52	0.14	0.22	0.15	0.24	0.20	0.23
687	7	443.33	739.17	718.21	633.57	0.26	0.40	0.70	0.45	0.52	0.31	0.10	0.29	0.20	0.20	0.48
688	3	829.88	703.70	867.00	800.19	0.11	0.55	0.56	0.68	0.60	0.12	0.26	0.22	0.26	0.25	0.09
689	6	696.65	748.06	879.15	774.62	0.12	0.57	0.46	0.58	0.54	0.12	0.18	0.23	0.26	0.22	0.18
690	3	1251.25	936.71	1527.87	1238.61	0.24	0.30	0.74	0.63	0.56	0.41	0.13	0.26	0.37	0.25	0.47
691	2	913.88	789.79	613.45	772.37	0.20	0.52	0.61	0.73	0.62	0.17	0.11	0.19	0.27	0.19	0.42
692	3	690.67	673.64	858.98	741.10	0.14	0.42	0.64	0.75	0.60	0.28	0.12	0.19	0.20	0.17	0.26
693	1	733.33	754.29	559.30	682.31	0.16	-	0.68	0.60	0.64	0.09	-	0.25	0.26	0.26	0.03
694	1	578.10	679.08	923.80	726.99	0.24	0.75	0.58	0.42	0.58	0.28	0.24	0.28	0.27	0.26	0.08
695	8	835.24	849.96	690.14	791.78	0.11	0.23	0.42	0.66	0.44	0.49	0.08	0.17	0.25	0.17	0.51
696	6	968.65	703.20	746.89	806.25	0.18	0.30	0.34	0.68	0.44	0.47	0.24	0.20	0.22	0.22	0.09

			ppm PO4-I	2			Phospl	nate Ado Shoot	ded t Dry W	eight			Root	Dry Wo	eight	
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD
2	2	-	601.69	543.06	572.37	0.07	0.43	0.35	0.55	0.44	0.23	0.15	0.11	0.15	0.14	0.17
4	7	726.03	576.73	644.72	649.16	0.12	0.56	0.77	0.58	0.64	0.18	0.35	0.31	0.27	0.31	0.13
9	7	761.12	627.93	524.17	637.74	0.19	0.56	0.57	0.60	0.58	0.04	0.20	0.32	0.34	0.29	0.26
11	5	615.34	673.30	622.26	636.96	0.05	0.54	0.58	0.52	0.55	0.06	0.23	0.42	0.35	0.33	0.29
16	3	602.63	597.30	613.19	604.37	0.01	0.40	0.25	0.32	0.32	0.23	0.22	0.13	0.20	0.18	0.26
84	3	894.89	801.10	527.64	741.21	0.26	0.44	0.50	0.53	0.49	0.09	0.22	0.25	0.21	0.23	0.09
92	8	938.56	815.43	659.66	804.55	0.17	0.75	0.94	1.05	0.91	0.17	0.22	0.34	0.28	0.28	0.21
109	2	592.69	451.36	891.02	645.02	0.35	1.30	1.16	1.13	1.20	0.08	0.22	0.42	0.48	0.37	0.36
118	3	874.59	493.91	575.04	647.85	0.31	0.27	0.27	0.34	0.29	0.14	0.12	0.10	0.16	0.13	0.24
122	2	1359.49	1034.79	1065.89	1153.39	0.16	0.50	0.38	0.52	0.47	0.16	0.16	0.13	0.13	0.14	0.12
123	4	836.11	1141.49	945.90	974.50	0.16	0.35	0.29	0.24	0.29	0.19	0.13	0.18	0.15	0.15	0.16
156	5	1070.30	1138.17	848.65	1019.04	0.15	0.65	0.63	0.74	0.67	0.09	0.36	0.45	0.43	0.41	0.11
159	4	1250.33	736.51	688.36	891.73	0.35	0.37	0.36	0.54	0.42	0.24	0.15	0.18	0.24	0.19	0.24
190	6	520.13	400.91	444.69	455.24	0.13	1.18	1.04	1.01	1.08	0.08	0.31	0.28	0.37	0.32	0.14
201	4	444.28	451.22	534.56	476.69	0.11	1.24	0.70	0.63	0.86	0.39	0.19	0.23	0.24	0.22	0.12
209	2	805.30	715.43	751.18	757.30	0.06	0.58	0.56	0.71	0.62	0.13	0.29	0.29	0.37	0.32	0.15
210	8	477.79	639.92	741.10	619.60	0.21	1.48	1.02	1.24	1.25	0.18	0.34	0.36	0.38	0.36	0.06
214	6	963.87	649.21	586.91	733.33	0.28	0.43	0.43	0.44	0.43	0.01	0.12	0.09	0.11	0.11	0.14
221	8	872.58	819.14	679.56	790.43	0.13	0.71	1.00	0.72	0.81	0.20	0.25	0.31	0.27	0.28	0.11
247	2	1015.44	736.18	758.63	836.75	0.19	0.38	0.47	0.48	0.44	0.12	0.17	0.22	0.23	0.21	0.16
254	1	508.35	484.92	532.96	508.74	0.05	0.86	1.07	1.46	1.13	0.27	0.18	0.49	0.37	0.35	0.45
267	3	962.61	-	754.24	858.43	0.17	0.27	0.43	0.44	0.38	0.25	0.11	0.14	0.11	0.12	0.14
272	2	524.98	478.37	444.08	482.48	0.08	1.34	0.78	0.91	1.01	0.29	0.28	0.37	0.32	0.32	0.14
286	8	430.70	597.65	507.86	512.07	0.16	1.34	0.96	0.79	1.03	0.27	0.23	0.37	0.28	0.29	0.24
303	4	656.84	586.25	700.57	647.89	0.09	0.33	0.31	0.40	0.35	0.14	0.18	0.21	0.27	0.22	0.21
313	6	623.77	497.84	406.45	509.35	0.21	0.80	0.67	0.45	0.64	0.28	0.20	0.20	0.12	0.17	0.27
314	1	546.98	944.89	1393.51	961.80	0.44	0.80	0.80	0.68	0.76	0.09	0.25	0.38	0.25	0.29	0.26
319	3	860.43	890.45	726.74	825.87	0.11	0.47	0.39	0.50	0.45	0.13	0.24	0.26	0.27	0.26	0.06

			ppm PO4-I	2			Phosp	hate Ado Shoo	led t Dry W	eight			Root	Dry W	eight	
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD
320	2	1131.04	774.57	805.84	903.82	0.22	0.42	0.45	0.32	0.40	0.17	0.18	0.21	0.28	0.22	0.23
327	8	450.29	743.75	596.18	596.74	0.25	1.20	1.73	1.06	1.33	0.27	0.20	0.40	0.38	0.33	0.34
334	1	549.40	479.93	536.31	521.88	0.07	0.74	0.71	0.71	0.72	0.02	0.26	0.27	0.34	0.29	0.15
336	6	508.12	505.62	462.40	492.05	0.05	1.09	1.03	0.75	0.96	0.19	0.36	0.27	0.40	0.34	0.19
338	7	789.25	690.12	708.95	729.44	0.07	0.70	0.56	0.81	0.69	0.18	0.36	0.31	0.24	0.30	0.20
342	3	667.90	716.71	900.76	761.79	0.16	0.31	0.48	0.46	0.42	0.22	0.20	0.22	0.25	0.22	0.11
345	7	517.15	457.79	518.83	497.92	0.07	0.61	0.92	0.63	0.72	0.24	0.29	0.48	0.46	0.41	0.25
368	8	397.15	729.89	466.32	531.12	0.33	0.98	1.10	1.01	1.03	0.06	0.32	0.30	0.28	0.30	0.07
377	5	1235.65	864.01	764.91	954.86	0.26	0.38	0.53	0.34	0.42	0.24	0.27	0.34	0.22	0.28	0.22
386	3	1123.18	997.57	772.05	964.26	0.18	0.44	0.39	0.24	0.36	0.29	0.26	0.18	0.23	0.22	0.18
388	7	-	728.30	800.37	764.34	0.07	-	0.73	0.51	0.62	0.25	-	0.29	0.41	0.35	0.24
394	1	763.56	804.06	712.37	760.00	0.06	0.42	0.58	0.61	0.54	0.19	0.09	0.25	0.20	0.18	0.45
396	7	671.11	618.70	742.89	677.57	0.09	0.47	0.58	0.87	0.64	0.32	0.16	0.23	0.32	0.24	0.34
402	4	977.79	842.60	722.07	847.49	0.15	0.80	0.83	1.07	0.90	0.16	0.28	0.29	0.32	0.30	0.07
410	2	827.41	746.53	791.10	788.35	0.05	0.41	0.51	0.52	0.48	0.13	0.25	0.26	0.25	0.25	0.02
413	2	539.29	686.64	441.45	555.79	0.22	1.65	1.40	1.26	1.44	0.14	0.39	0.24	0.61	0.41	0.45
416	4	701.09	700.97	945.35	782.47	0.18	0.39	0.32	0.47	0.39	0.19	0.17	0.24	0.24	0.22	0.19
423	7	737.84	677.39	604.79	673.34	0.10	0.53	0.56	0.62	0.57	0.08	0.24	0.37	0.33	0.31	0.21
426	5	695.96	731.84	904.76	777.52	0.14	0.59	0.83	0.67	0.70	0.18	0.22	0.38	0.28	0.29	0.28
427	4	610.34	633.98	833.31	692.54	0.18	0.86	0.68	0.65	0.73	0.16	0.38	0.27	0.31	0.32	0.17
435	1	1253.17	1338.28	1002.99	1198.15	0.15	0.49	0.54	0.63	0.55	0.13	0.20	0.17	0.24	0.20	0.17
436	6	861.19	762.73	716.45	780.12	0.09	0.58	0.64	0.70	0.64	0.09	0.18	0.28	0.26	0.24	0.22
440	5	848.48	703.80	790.16	780.81	0.09	0.69	0.78	0.95	0.81	0.16	0.43	0.35	0.51	0.43	0.19
451	1	504.53	858.70	619.47	660.90	0.27	0.69	0.59	0.50	0.59	0.16	0.29	0.27	0.31	0.29	0.07
463	2	916.91	983.24	603.46	834.54	0.24	0.30	0.35	0.27	0.31	0.13	0.16	0.22	0.23	0.20	0.19
494	3	763.47	834.11	996.77	864.78	0.14	0.85	0.84	0.72	0.80	0.09	0.32	0.23	0.20	0.25	0.25
495	2	870.54	447.69	434.05	584.09	0.42	0.86	0.60	0.64	0.70	0.20	0.23	0.26	0.34	0.28	0.21
499	5	771.16	1064.59	564.22	799.99	0.31	0.68	0.63	0.57	0.63	0.09	0.30	0.28	0.36	0.31	0.13

	ppm PO4-P							Phosphate Added Shoot Dry Weight					Root Dry Weight					
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD		
503	1	777.64	781.69	757.18	772.17	0.02	0.78	0.71	0.52	0.67	0.20	0.28	0.34	0.24	0.29	0.18		
504	7	830.88	744.38	603.45	726.24	0.16	0.62	0.67	0.70	0.66	0.06	0.20	0.24	0.27	0.24	0.15		
506	5	871.67	932.80	896.05	900.17	0.03	0.67	0.66	0.57	0.63	0.09	0.33	0.24	0.32	0.30	0.17		
517	7	514.54	519.53	407.31	480.46	0.13	0.63	0.60	0.75	0.66	0.12	0.25	0.24	0.27	0.25	0.06		
518	5	588.69	772.18	577.58	646.15	0.17	0.48	0.62	0.49	0.53	0.15	0.23	0.25	0.22	0.23	0.07		
524	2	939.91	879.05	663.23	827.39	0.18	0.37	0.34	0.32	0.34	0.07	0.24	0.15	0.24	0.21	0.25		
525	5	882.42	991.06	625.97	833.15	0.23	0.35	0.46	0.34	0.38	0.17	0.30	0.33	0.30	0.31	0.06		
527	2	888.42	811.10	716.89	805.47	0.11	0.30	0.35	0.47	0.37	0.23	0.21	0.21	0.31	0.24	0.24		
532	8	559.26	719.32	503.84	594.14	0.19	1.15	0.75	0.96	0.95	0.21	0.31	0.22	0.38	0.30	0.26		
549	6	580.64	662.32	495.82	579.59	0.14	0.74	0.44	0.57	0.58	0.26	0.16	0.11	0.12	0.13	0.20		
551	3	727.55	643.38	-	685.47	0.09	0.74	0.62	-	0.68	0.12	0.37	0.29	0.38	0.35	0.14		
560	5	529.04	-	596.17	562.60	0.08	0.70	0.64	0.74	0.69	0.07	0.19	0.24	0.33	0.25	0.28		
565	1	481.35	966.77	1081.29	843.14	0.38	1.39	1.49	0.97	1.28	0.22	0.37	0.38	0.42	0.39	0.07		
582	4	854.74	882.73	737.05	824.84	0.09	0.60	0.43	0.39	0.47	0.24	0.19	0.15	0.10	0.15	0.31		
589	6	586.80	816.90	583.05	662.25	0.20	1.21	0.76	0.96	0.98	0.23	0.23	0.25	0.28	0.25	0.10		
596	1	473.58	519.75	705.54	566.29	0.22	1.38	1.43	1.15	1.32	0.11	0.19	0.40	0.35	0.31	0.35		
597	2	977.39	666.53	707.80	783.91	0.22	0.57	0.49	0.53	0.53	0.08	0.22	0.30	0.30	0.27	0.17		
605	5	884.40	654.10	626.50	721.67	0.20	0.50	0.53	0.33	0.45	0.24	0.21	0.28	0.21	0.23	0.17		
612	2	453.01	-	377.82	415.41	0.13	1.10	0.94	1.05	1.03	0.08	0.24	0.26	0.34	0.28	0.19		
615	6	564.61	579.56	674.76	606.31	0.10	0.94	1.01	0.97	0.97	0.04	0.31	0.32	0.40	0.34	0.14		
620	1	635.07	715.05	881.62	743.92	0.17	1.30	1.85	1.07	1.41	0.28	0.33	0.33	0.41	0.36	0.13		
629	4	799.42	-	651.23	725.32	0.14	0.41	0.61	0.49	0.50	0.20	0.24	0.30	0.31	0.28	0.13		
635	2	480.60	543.18	469.98	497.92	0.08	0.83	0.64	0.65	0.71	0.15	0.33	0.33	0.38	0.35	0.08		
651	8	843.74	659.57	631.47	711.59	0.16	1.06	1.21	0.95	1.07	0.12	0.37	0.50	0.34	0.40	0.21		
652	1	467.03	466.42	419.55	451.00	0.06	0.89	1.27	1.07	1.08	0.18	0.20	0.28	0.29	0.26	0.19		
654	3	878.46	797.48	830.61	835.52	0.05	0.37	0.55	0.50	0.47	0.20	0.15	0.19	0.20	0.18	0.15		
664	6	478.80	767.80	486.17	577.59	0.29	1.10	0.82	1.22	1.05	0.20	0.23	0.26	0.27	0.25	0.08		
676	3	454.00	732.84	714.27	633.70	0.25	0.96	1.18	0.96	1.03	0.12	0.26	0.31	0.34	0.30	0.13		

	ppm PO4-P							Phosphate Added Shoot Dry Weight						Root Dry Weight					
Geno	Cluster	r1	r2	r3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD	Rep 1	Rep 2	Rep 3	Mean	RSD			
679	6	821.60	684.48	803.46	769.85	0.10	0.90	0.90	0.89	0.90	0.01	0.21	0.34	0.23	0.26	0.27			
682	5	645.09	518.72	652.70	605.50	0.12	0.86	0.83	1.02	0.90	0.11	0.42	0.40	0.36	0.39	0.08			
683	1	891.45	945.45	574.60	803.83	0.25	1.34	1.24	1.03	1.20	0.13	0.42	0.37	0.34	0.38	0.11			
684	5	571.66	699.98	536.16	602.60	0.14	0.93	0.75	0.81	0.83	0.11	0.32	0.29	0.41	0.34	0.18			
685	2	694.52	830.54	547.09	690.72	0.21	1.11	1.18	0.93	1.07	0.12	0.34	0.39	0.20	0.31	0.32			
686	2	1382.21	729.57	-	1055.89	0.44	0.49	0.79	0.66	0.65	0.23	-	0.21	0.17	0.19	0.15			
687	7	999.17	1143.97	953.52	1032.22	0.10	0.62	0.66	0.78	0.69	0.12	0.21	0.32	0.48	0.34	0.40			
688	3	931.87	862.38	716.39	836.88	0.13	0.80	0.86	0.43	0.70	0.33	0.32	0.25	0.17	0.25	0.30			
689	6	837.29	603.36	-	720.32	0.23	0.58	0.85	0.94	0.79	0.24	0.13	0.16	0.26	0.18	0.37			
690	3	1108.49	1585.94	959.43	1217.95	0.27	0.33	0.66	0.77	0.59	0.39	0.18	0.35	0.26	0.26	0.32			
691	2	842.42	890.07	675.45	802.64	0.14	0.56	0.84	0.71	0.70	0.20	0.20	0.35	0.20	0.25	0.35			
692	3	1045.07	642.21	528.98	738.75	0.37	0.43	0.71	0.78	0.64	0.29	0.13	0.22	0.18	0.18	0.26			
693	1	998.01	519.07	499.52	672.20	0.42	0.84	0.85	0.96	0.88	0.08	0.41	0.29	0.31	0.34	0.19			
694	1	896.82	826.35	894.07	872.42	0.05	1.09	0.85	0.73	0.89	0.21	0.32	0.29	0.38	0.33	0.14			
695	8	739.32	892.74	931.05	854.37	0.12	0.48	1.16	0.85	0.83	0.41	0.12	0.37	0.29	0.26	0.49			
696	6	661.45	996.02	831.28	829.58	0.20	0.70	0.35	1.01	0.69	0.48	0.17	0.17	0.30	0.21	0.35			

#### VITA

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