GENETIC IMPROVEMENT OF RICE (*Oryza sativa* L.) VARIETIES FOR TOLERANCE TO PHOSPHORUS DEFICIENT SOILS IN MOROGORO, TANZANIA

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EXTENDED ABSTRACT

This work was undertaken in order to improve the performance of some selected rice varieties that are susceptible to phosphorus (P) deficiency through the introgression of P deficiency tolerant genes. This involved screening for tolerance to P deficiency of a diverse set of varieties grown in East and Southern African (ESA) countries and their molecular marker survey, in order to assess the presence or absence of the phosphorus starvation tolerance (*PSTOL1*) gene. This information served as a basis for selecting varieties for improvement and for use as donors. In this study, it was established that about 41 percent of the 96 germplasm tested lack *PSTOL1* while 59 percent of the varieties have the tolerance gene. In addition, the study revealed that among the varieties with *PSTOL1*, some were not tolerant to P deficiency as expected possibly due to the existence of inhibitors or non-functional-*PSTOL1* alleles due to mutations. On the other hand, some varieties without *PSTOL*1 performed well under P deficiency. Genetic introgression of *Pup1* QTL that contains *PSTOL*1 was successfully conducted into three selected varieties but susceptible to P deficiency namely; Pishori, TXD 88 and Tule na Bwana by markers assisted selection (MAS). The introgression lines presented yield increase between 11 to 70 percent compared to susceptible recipient parents. At the same time, four new candidate QTLs with significant effect on phenotypic expression of grain yield, tiller number, and shoot biomass under P deficiency were detected on chromosomes 4, 5 and 9 among tolerant varieties that did not have *Pup1*. The results show that, some local genotypes contain vital genes useful for genetic improvement; hence they need to be conserved. Although long term usage of varieties with high P uptake due to the presence of *Pup1* may deplete the soil P in low input systems, genetic variability for low grain P concentration as well as good P utilization efficiency (PUE) were observed and should be utilized in the genetic improvement. Also the effect of new P deficiency tolerance QTLs

detected should be further validated in different environments due to possibilities of QTL x environment interaction so as to come up with stable and reliable QTLs.

DECLARATION

I, ATUGONZA LUTA BILARO, do hereby declare to the Senate of Sokoine University of Agriculture that, this thesis is my own original work and that it has neither been submitted nor concurrently submitted for a degree award in any other institution**.**

Atugonza Luta Bilaro — Date

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The above declaration is confirmed

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DEDICATION

This work is dedicated to my parents, my mother Ma- Levina Ichwekeleza and my father, the late Mzee Titus Mugisha Bilaro who laid the foundation for my career.

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LIST OF ABBREVIATIONS

ESA	East and Southern Africa
FAO	Food and Agriculture Organization
MAB	Marker assisted breeding
MAFSC	Ministry of Agriculture Food Security and Cooperatives
MAS	Marker assisted selection
MATAB	Mixed Alkyltrimethylammonium Bromide
mM	mill Mole
MOP	Muriate of Potash
N	Nitrogen
PAE	Phosphorus acquisition efficiency
PCR	Polymerase Chain Reaction
*PSTOL*1	Phosphorus starvation tolerance
PUE	Phosphorus use efficiency
*Pup*1	Phosphorus uptake 1
QTL	Quantitative trait locus (loci)
RYMV	Rice Yellow Mottle Virus
SNP	Single Nucleotide Polymorphism
SUA	Sokoine University of Agriculture
TSP	Triple Super Phosphate
URT	United Republic of Tanzania
µl	micro litre
ng	nano gram

CHAPTER ONE

1.0 General Introduction

1.1 Importance of Rice

Rice (*Oryza sativa* L.) is one of the world's most important staple crops particularly in Asia and Africa. Recently, there has been a steady increase in Latin America as well. Rice contributes about one fifth of global calories consumed. Currently it is estimated that, more than 900 million of the world's poor depend on rice for their livelihood as producers or consumers (Pandey *et al*., 2010). In this regard, rice sector is regarded as a catalyst for economic growth with the potential to eliminate poverty.

In Africa, the rice has gained recognition as an important component of national food security and economic growth due to increase in consumption and trade (Balasubramanian *et al*., 2007; Seck *et al.*, 2012). Rice production in Africa has been expanding at an annual rate of 6% partly due to a greater investment in the rice sector. However, despite this growth, the rate of yield increase has slowed considerably, whilst production is also outpaced by the high demand as a consequence of rapid population growth and change in eating habits fuelled by urbanization (Seck *et al*., 2012) thus, resulting in rice deficit (Pandey *et al*., 2010). As a result, the continent imported 14.1 million metric tons in 2013 at a cost of US$ 7.5 billion, equivalent to 21.4% of Africa's total annual food imports.

In Africa, rice is grown under a wide range of ecologies which include upland, rainfed lowland, irrigated and deepwater ecosystem where other crops cannot grow, thus making rice the only crop with such wide adaptation (Haefele *et al*., 2013). In the East, Central and Southern African (ECSA) region, Tanzania is the second leading rice producer and consumer after Madagascar (Balasubramanian *et al*., 2007; Dawe *et al.*, 2010). In terms of food security, rice is ranked second after maize and the annual per capita consumption is

believed to have surpassed the estimated range of 25 and 30 kg per year reported by URT (2009) and Mghase *et al.* (2010). Despite the increased investments in rice sector, Tanzania remains vulnerable to hunger (FAO, 2010; FAO, IFAD and WFP, 2015). Annual rice imports range between 50 000 and 200 000 tons depending on production (URT, 2009, FAO, 2016) and this has an implication on the country's foreign currency reserve. Rice is mainly grown by small holder farmers mostly using low input and traditional (local) varieties which are low yielding. Much of the reported increase in rice production comes from expansion in the cultivated area and not the improvement in yield as yield has stagnated at around 2.0 t/ha (Global Rice Science Partnership (GRiSP), 2013; Saito *et al.*, 2015). The growth in population and standards of living both in urban and rural areas are set to increase rice demand and may trigger further rice importations unless more efforts are made to increase local production.

In Africa, about 30 million tons more rice will be needed by 2035, representing an increase of 130% in rice consumption from 2010 (Seck *et al.*, 2012). Regarding the strong competition with other socio-economic activities for land use, more intensive rice production systems should be envisaged. This will require an annual yield increase of about 1.2–1.5% to produce the additional rice needed (Seck *et al.*, 2012).

1.2 Challenges Facing Rice Production in Africa

The Asian green revolution of the 1970s was successful due to the emphasis on irrigation and the use of short statured varieties that perform better with high fertilizer dosage (Larson *et al.*, 2010). However, the same green revolution could not be replicated in Africa due a number of structural bottlenecks coupled with a diversity of biophysical and ecological constraints that hinder rice production. In many African countries; rice production is predominantly rain-fed which poses a challenge on water management.

According to Balasubramanian *et al*. (2007) only 5 percent of the potentially suitable land is planted with rice due to a number of biotic and abiotic stresses such as extreme temperatures, drought, flooding, poor soil fertility including high nutrient fixation, weeds, bacterial leaf blight, leaf blast and rice yellow mottle virus (RYMV).

Extreme conditions of too much water or too little water influence the timing of fertilizer applications which should be done at critical growth stages such as crop establishment, tillering and panicle initiation (Vinod and Heuer, 2012). Water stress also affects the movement of nutrients from the soil to the plants. In addition, much of the African soils are inherently poor in major plant nutrients, in particular nitrogen and phosphorus (Sahrawat, 2008; Nziguheba *et al.,* 2015). It is estimated that more than 95 million hectares of sub-Saharan Africa's arable land has serious soil fertility problems (Rosegrant *et al.*, 2005). Besides, there are high rates of nutrient depletion where about 41 kg N, 4 kg P_2O_5, and 30 kg K_2O ha^{-1} are lost annually through leaching and crop removal as harvests and animal feeds (Bekunda *et al.,* 2004). In areas where much of the rice is cultivated, decline in soil fertility has been singled out as a main cause for yield reduction (Smaling *et al*., 1996; Larson *et al.*, 2010). It has also been established that there are high phosphorus fixation rates due to acidic nature of tropical soil which limit the efficacy of fertilizers (Fageria, 2013). Climate change and land degradation particularly in marginal areas coupled with high rate of nutrient mining in potential areas aggravate the problem. At the same time, Africa has the lowest rate of fertilizer application compared to other continents (Wopereis *et al.*, 2008; Haefele *et al.*, 2013; Obersteiner *et al.,* 2013). Among African countries, it is only Egypt and South Africa which apply near sufficient levels of fertilizers. According to Nakamura *et al*. (2013), average P losses from African soils is 0.6 million tons against P fertilizer consumption of 0.26 million tons thus creating negative P balance. Furthermore in 2007/08 the national agricultural census in Tanzania reported that

farmers applied fertilizer only on 6.2 percent of the area planted for various crops (URT, 2010). Also available reports show that on average Tanzania consumes about 7 kg nutrients per hectare compared to South Africa's 53 kg ha^{-1} (Kamhabwa, 2014). Such low fertilizer application rates give rise to low rice yields.

In Tanzania, most soils suitable for rice have inadequate amount of major soil nutrients including soil phosphorus. Studies indicate that more than 53% of the area under rice in Tanzania is categorised as poor in terms of available plant nutrients especially phosphorus (Haefele *et al*., 2013). The problem of P deficiency in Tanzania is widespread and has been reported to affect the production of major crops such as rice (Shekiffu and Semoka, 2007; Kalala *et al.*, 2016), beans (Mourice and Tryphone, 2012; Namayanja *et al*., 2014) and Maize (URT, 2010). Furthermore, studies by Mzee (2001), Meertens (2003), Shekiffu and Semoka (2007), Kalala *et al.* (2016) in some rice growing areas of Mbeya, Mwanza, Coast region and Morogoro, reported available P content as low as 1.2 mg P kg^{-1} soil.

In rice production, soils with available P values below 10 mg P kg^{-1} soil are regarded as deficient as plants will suffer significant yield reduction (Doberman and Fairhust, 2000; Msanya *et al.*, 2001; Fairhurst *et al*., 2007; Kalala *et al.*, 2016). Thus, given the prevailing conditions and the limited resources of small-holder farmers, enhancing rice production in Tanzania will require varieties that can yield high with little fertilizer application.

1.2.1 Phosphorus as a critical nutrient in rice production

In rice, phosphorus (P) is the second most important inorganic plant nutrient after nitrogen; it is also one of the least available nutrients in the soil because of its tendency for fixation in acidic soils (Yanagihara *et al*., 2010). Phosphorus is required for early

development of strong root systems, promote tillering, early flowering and ripening (Dobermann and Fairhurst, 2000).

Plants normally take up P for metabolic activities such as photosynthesis and starch transformations; it also plays a major role in the activity of enzymes (Shen *et al*., 2011). The fact that P stimulates root growth in the process; it enhances early access to growth limiting resources such as water and of other minerals. P deficiency results in stunted growth, delayed maturity, reduced tillering ability, reduced number of panicles and few grains per panicle hence yield reduction (Fairhurst *et al*., 2007). However despite P being a critical nutrient in rice production, it gets little attention as it is difficult in the field to visually assess P deficiency on rice plants. Unlike in the other cereals, P deficiency in rice plants, do not exhibit the purple colouration of leaves. Instead, leaves of P deficient rice plants become dark green due to poor utilization of metabolites (Dobberman and Fairhust, 2000).

While the demand for P has been on the increase, the global stock for P is very limited and is likely to be depleted in the next few decades (FAO, 2008; Lynch, 2011). Because of its tendency for tight binding to other elements such as Al^{2+}, Fe^{3+} and Mn^{2+} especially in acidic soils, P is often unavailable to plants (Yanagihara *et al*., 2010). Thus, new efforts are needed to create a synergy between nutrient use and proper land use planning so as to ensure sustainable rice production for increased food security. Also sustainable use of this vital resource is fundamental for long term stable rice production. In the soil, P is less mobile than most of the other nutrients. It is absorbed by the roots through *diffusion* as opposed to *mass flow* of nitrogen and other soil nutrients. Therefore P absorption requires soil contact with the roots (Dobermann and Fairhust, 2000). This means that, a well-developed root system in rice is essential for P absorption and as shown by numerous

studies (Schachtman *et al.*, 1998; Vance *et al.*, 2003; Hu *et al.*, 2011), which ensures more P uptake hence better survival on low soil P conditions.

In their adaptation to P deficiency plants have evolved a number of mechanisms such as changing root architecture, including root morphology, topology, and distribution patterns (Wang *et al.*, 2010; Shen *et al.*, 2011) which may be manifested in lateral root formation including formation of root hairs to maximise the surface area (Hammond *et al.*, 2004; Lynch, 2011) and secretion of exudates that mobilize poorly available P into available forms (Sahrawat, 2009; Lynch, 2011). In other studies, varieties with high P uptake i.e. phosphorus acquisition efficiency (PAE) or those with high internal P utilization efficiency (PUE) have been reported (Hammond *et al.*, 2009; Rose and Wissuwa, 2012; Nziguheba *et al.*, 2015). Under PAE, varieties have high ability to extract P from the soil while in PUE varieties have the ability to utilize the little P absorbed to produce sufficient biomass for normal plant growth.

1.2.2 Improving yield in P deficient soils

Despite the existence of a wide range of approaches for improving yield such as population improvement, ideotype breeding, heterosis breeding, wide hybridization and genetic engineering (Khush, 2013), the decline in plant available nutrients has become a prominent setback in recent years (Prasetiyono *et al.*, 2010). This is aggravated by the fact that most of the improved varieties tend to yield poorly at suboptimal fertilizer application (Saito *et al.*, 2005; Aluwihare *et al.*, 2016). At the same time to be able to meet rice demand by the year 2050, rice yield has to increase by 50% or more from current levels (Khush, 2000; FAO, 2014; Van Ittersum *et al.*, 2016). This will require heavy fertilizer application which the large majority of farmers cannot afford because of their relatively high cost. Therefore, there is a need to provide farmers with more sustainable and

affordable options to avoid further land degradation due to soil nutrient mining especially in intensive crop production systems. The problem of inadequate P fertilizer application can be addressed by developing rice varieties that can survive low soil P conditions and or by providing fertilizers subsidies to farmers as to enable them purchase at much lower prices. However, since the status of P in the soil is dependent on the soil properties such as constituent of the parent material, the pH of the soil and the extent of the P fixing elements such as Al, Fe, Mn (at low soil pH) and Ca (at high soil pH) (Sahrawat, 2009), the best solution is to identify varieties that can survive low soil P, in conjunction with the adjustments in the conditions which render P unavailable in the soil.

Proper management of crop residues by retaining them in the field may provide short term solutions since it is only a fraction of P that is likely to be retained as much of the P absorbed is partitioned to the seed. Therefore combining both genetic improvements with management of pH in the soil offers the best option for improving yield in P deficient soils. This can be achieved by the use of cultivars with ability to take up more phosphorus from the soil or those with the ability to utilize the little available P in the soil to survive on low P condition while maintaining stable yield.

1.2.2 1 Soil and crop management

Soil P status is chiefly dependent on the soil properties such as constituent of the parent material. However, P dynamics in the soil is determined by soil pH and the level of P-fixing elements such as Al, Fe and Mn (at low soil pH) and Ca (at high soil pH) (Sahrawat, 2009). Thus, proper adjustment of soil conditions can increase phosphorus availability to plants. For example, when soil pH is high, organic matter or elemental sulphur (90-99% sulphur material) and other acidifying fertilizer such as ammonium sulphate are recommended to lower soil pH to desirable levels, whereas in acidic soils,

liming can help to adjust soil pH (Fageria and Baligar, 2008) thereby regulating the dynamics of soil nutrients including soil phosphorus. Furthermore, in the case of P immobilization by Al or Fe, flooding has been used to release P into the soil because flooding creates anaerobic conditions that solubilise Fe and Al through a series of oxidation and reduction reactions (Fageria, 2013). However, this is mostly limited to irrigated rice where prolonged flooding is possible and the parent rock has adequate amount of phosphorus.

Management of crop residues by retaining them in the field is a better option although it may not significantly improve P status in the short term since much of the P taken up by rice plants is located in the grains (Vandamme *et al*., 2015). According to Nziguheba *et al*. (2015), strategic application that combines small fertilizer rates and P efficient varieties may reduce fertilizer needs substantially. Also a micro-dose of P to the rice nursery bed has shown promising results but need more validation (Vandamme *et al*., 2016). In that study, application of a phosphorus dose as small as 3 kg ha^{-1} to the nursery bed doubled biomass of rice seedlings and increased grain yield by 19 to 40% in the low P field. This provides a good opportunity to address the problem of inadequate fertilizer application when soil P is limited and farmers have affordable access to fertilizers which is not the case. However, the practice is limited to irrigated rice where farmers establish nurseries of seedlings to transplant.

1.2.2.2 Breeding for low P tolerance

Several studies indicate that certain rice cultivars can tolerate low soil phosphorus better than others (Wissuwa and Ae, 2001). This can be achieved by a better ability to take up more phosphorus from the soil or to utilize more efficiently the little P taken up in producing biomass and grains (Hammond *et al*., 2009; Veneklaas *et al.*, 2012). When soil

available phosphorus is below critical levels, plants show differential response, for example the reduction of primary roots growth (Niu *et al.*, 2013), thus suggesting the possibility of a genetic control. In addition, studies by Fageria and Santos (2002) and Rose *et al.* (2012) found out that rice cultivars vary in their ability to take up more P or to tolerate P deficient conditions due to variations in gene complexes. This has been confirmed by the discovery of a genomic region on chromosome 12 where a major quantitative trait loci (QTL) associated with tolerance to P deficiency has been reported (Shimizu et *al*., 2008; Ni *et al.,* 1998; Wissuwa *et al.*, 2002; Heuer *et al*., 2009).

The QTL Phosphorus uptake 1 (*Pup1*) increases P uptake under low P conditions and the gene responsible for its effect was identified as a specific protein kinase (phosphorus starvation tolerance 1 (*PSTOL1*) (Gamuyao *et al*., 2012). *PSTOL1* was shown to induce better root growth under P deficient conditions when over-expressed in susceptible varieties such as Nipponbare and IR64, hence conferring higher P uptake and significant yield advantage (Gamuyao *et al.*, 2012). Two alleles designated as *PSTOL1* and *PSTOL2* are currently known (Pariasca-Tanaka *et al.,* 2014) the former was mapped in Kasalath rice variety (an *aus* type) and the latter in CG14 (*glaberrima*) and both are absent in the susceptible reference variety Nipponbare.

Through marker assisted selection (MAS), breeders have been able to transfer *Pup1* QTL into susceptible varieties with much precision (Chin *et al*., 2011), thereby increasing yield in P deficient prone environments. However, *Pup1* is a highly variable locus and there are reports that *PSTOL1* presence is not always associated with P deficiency tolerance when it is tested on a broad range of varieties (Mukherjee *et al.*, 2014; Aluwihare *et al.*, 2015). Therefore, before considering *PSTOL1* gene in MAS, it has been suggested to ascertain its expression and role in P deficiency tolerance in the variety intended to be used as donor

parent (Mukherjee *et al.*, 2014). Alternatively, the entire *Pup1* locus specifically from Kasalath or a breeding line produced from a Kasalath based *Pup1* introgression scheme should be used in Marker Assisted Breeding (MAB) rather than solely *PSTOL1* (Pariasca-Tanaka *et al.*, 2014).

Previous studies have shown that traditional varieties tend to have better tolerance to P deficiency than improved varieties (Wissuwa and Ae, 2001; Saito *et al.*, 2005) and they could be useful donors in breeding programs. In Tanzania, there are hundreds of local rice landraces widely grown by farmers in low input systems but no information has been documented on their potential for tolerance to P deficiency neither are the underlying genes known. Since much of the rice in Tanzania is produced on P deficient soils, the impact of P deficiency tolerance to enhance yield in such soils is potentially high. Therefore, this study was proposed to investigate the tolerance level of rice varieties widely grown by farmers in East and Southern Africa (ESA) to low P and the genetic basis of their tolerance and to improve selected rice varieties for enhanced tolerance to P deficiency. Furthermore information on the potential of local germplasm will be an incentive towards genetic resources conservation efforts.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to develop rice lines with improved yield under phosphorus deficient soil conditions in Tanzania.

1.3.2 Specific objectives

(i) To determine the tolerance level of selected rice varieties to P deficient soil conditions

(ii) To create P deficient tolerant introgression lines of sensitive widely grown rice varieties through Marker assisted backcrossing

(iii) To map new QTLs responsible for tolerance to P deficient soils among the tolerant varieties identified

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CHAPTER TWO

2.0 Genetic variability of improved and traditional rice (*Oryza sativa* L.) varieties from East and Southern Africa for phosphorus deficiency tolerance

2.1 Abstract

Phosphorus (P) is one of the most limiting mineral nutrient in rice production. In some rice production areas in Tanzania, available soil P is as low as 2.7 mg kg^{-1}. One of the solutions to ensure relatively high rice yields in P-deficient areas at reduced cost for smallholders is to develop varieties that can tolerate P deficiency. In this study, 100 rice genotypes from Tanzania, Mozambique and Malawi were assessed for tolerance to P deficiency and the presence of *Pup1* QTL as a basis for selecting recipients in the bi parental crosses. In 2013 cropping season, a field trial was conducted at Dakawa irrigation scheme, in Morogoro Region, under two P-treatments (-P = no P-fertilizer added and +P = 50 kg P ha^{-1}) applied as TSP. These same varieties were also evaluated in a pot experiment at Sokoine University of Agriculture (SUA) using an upland soil from crop museum site at SUA campus with P-treatments similar to the field trial. Soil analysis of both sites showed sub-optimal level of available P (<10 mg P kg^{-1}). This was confirmed by the reduction of crop performances in both field and pot experiments. The results showed that, average tiller number was reduced by 54% in the field and 75% in pots; flowering was delayed by six days in the field and 15 days in pots; shoot biomass was reduced by 31% in the field and 50% in pots but grain yield was reduced by only 2% in the field and 31% in the pots. Thirty two (32) varieties consistently out-yielded the best tolerant check Mudgo and eight varieties out-yielded the local check SARO 5 in both pot and field experiment hence they can be directly recommended for P deficient-prone areas or used as donors in breeding programs. Also genotyping of the varieties with *PSTOL1*-allele specific markers revealed the presence of Kasalath and CG14 allele at 49.4% and 9.6% respectively of the collection tested. Rice varieties sensitive to P-deficiency but widely grown by farmers were selected for genetic improvement of their P-deficiency tolerance.

2.2 Introduction

Phosphorus is the second most important plant nutrient after nitrogen. It is required for plant metabolism such as photosynthesis, energy transfer and transformations of starches (Vance *et al*., 2003; Yong-fu *et al*., 2006). In rice, phosphorus plays a key role in root growth and development, tillering, early maturity and yield (Dobermann and Fairhurst, 2000; Fageria, 2013). Thus when P is limiting, reduced root development and tillering is observed with significant yield reduction in severe cases (Fageria, 2013). The fact that phosphorus is non-renewable makes it the most limiting element in the soil (Cordell *et al*, 2009).

Low levels of soil phosphorus is mainly caused by insufficient replenishment through P fertilizer application coupled with removal of crop residues (Syers *et al.,* 2011), low inherent P in the parental material (Witt *et al.,* 2002; Wang *et al*., 2010; Pariasca-Tanaka *et al.,* 2014) and P fixation by particular soil elements such as Al, Fe, Mn (at low pH) or Ca (at high pH) (Shen *et al*., 2011).

In East Africa, most agricultural land generally suffers P deficiency, particularly so in western Kenya (Bekunda, 2004; Opala *et al*., 2013) and large parts of Uganda (Jama and Van Straaten, 2006; Woniala and Nyombi, 2014). In Tanzania, P deficiency is common particularly in rice growing areas (Mzee, 2001; Shekiffu and Semoka, 2007). Major soils are Ferralsols, Acrisols, Vertisols, Nitosols and Andosols which account for 24 % of all soil groups (MAFSC, 2006). These soils are either low in mineral reserve or have high content of Al, Mn and Fe. These elements affect P availability to plants through immobilization (Arai and Sparks, 2007; Fageria, 2013). Even in conditions where initial soil P was not low, soil nutrients including P have been depleted due to farmer's practices of not applying optimal dozes of fertilizers.

Phosphorus deficiency in rice production is more common in upland and rain-fed lowland ecologies compared to irrigated ecosystems (Mackill *et al.*, 1996). In Tanzania, rain-fed rice production accounts for 65 - 68% of the total rice produced (URT, 2009), thus exposing most rice producing areas to P deficiency. Regarding the fact that a large proportion of rice growers are smallholder farmers with little capital who cannot afford purchasing the required amount of P fertilizers, it is critical to identify or develop varieties that are tolerant to P deficiency for resource poor farmers.

Previous reports confirmed the existence of genetic variability in rice with regard to tolerance to phosphorus deficiency (Wissuwa and Ae, 2001; Yanagihara *et al*., 2010; Rose *et al.*, 2012). In some of the tolerant varieties, quantitative trait loci (QTL) associated with tolerance to P deficiency was identified. For instance, *Pup1* QTL and its related gene *PSTOL1* that increases P uptake under limiting P conditions was identified in Kasalathi a traditional *aus* variety from India (Wissuwa *et al*., 1998; Wissuwa and Ae, 2001; Gamuyao *et al.*, 2012). In Eastern and Southern Africa (ESA) there is a high diversity of rice germplasm which is largely unexplored. Many traditional rice varieties are still being cultivated by ESA farmers despite the availability of improved varieties (Luzi-Kihupi *et al*., 2012). In general, traditional varieties are better adapted to low-input systems hence show superior agronomic performance with low soil fertility compared to some improved varieties (Saito *et al*., 2005). For these cultivars to be adequately utilized, they need to be characterized for traits relevant to ESA rice breeding programs. Regarding the importance of P deficiency and the lack of information on their potential with regard to low P tolerance, this study was designed in order to determine the tolerance to P deficiency of selected varieties from ESA and the distribution of *PSTOL1* in these varieties. The information generated will be useful for selecting parents for genetic improvement program in order to boost rice production on low-P soils.

2.3 Materials and Methods

2.3.1 Plant material

The study involved 100 varieties from Tanzania, Mozambique, and Malawi including 4 checks from International Rice Research Institute (IRRI) (Table 2.1). Seeds of the varieties from Tanzania (72) were collected from Katrin Research Institute while varieties from Mozambique (12) and Malawi (12) were respectively obtained from Instituto do Algodão de Moçambique (IAM) and Lifuwu Research Station. Mudgo and Dular were considered as tolerant checks while Kasalathi was included as the original *Pup1* donor and IR74 as a sensitive check. The varieties used are widely grown by farmers. About 83.3 % of the selected varieties were traditional (local) varieties while 16.7% were improved varieties.

Table 2.1: Origin and classification of rice varieties used in the study

Country	Traditional	Improved	Total
Tanzania	64	8	72
Mozambique	11	1	12
Malawi	7	5	12
Checks (IRRI)	1	3	4

2.3.2 Field screening under lowland conditions

The field experiment was established at Dakawa irrigation scheme (Block 17) (06º26'S 37º32'E), a phosphorus deficient site (P< 10 mg kg^{-1} soil) on 15th March 2013. The field was divided into two blocks; one where phosphorus was applied at the rate of 50 kg P ha^{-1} (about 114.9 kg P_2O_5 ha^{-1}) and the other without any P fertilizer application. In each block, varieties were randomized following an alpha lattice (10 x 10) replicated four times. Seedlings were transplanted in the field twenty one days after nursery establishment. Transplanting was done manually at the rate of one seedling per hill. The plot size was 1.4 m x 0.6 m with spacing of 0.2 m x 0.2 m between and within rows.

Required dosage of P and K (i.e. 50 kg P ha^{-1} and 50 kg K ha^{-1}) and 1/3 of N (50 kg N ha^{-1}) were applied at transplanting by broadcasting, while the second and third doses of N were applied at active tillering and panicle initiation respectively.

2.3.3 Pot screening

Pot experiment was established in a screen house at SUA involving 97 genotypes evaluated in the field. Soils for pot experiment was collected from crop museum at SUA and filled into 5 litre pots which weighed 5 kg dry soils. The design was completely randomized design (CRD) replicated three times with sets of applied phosphorus (2.28 mg P/kg soil or 11.4 mg P/pot) and no phosphorus application, other nutrients were applied as in the field experiment. In each pot, two seeds were sown and thinned to one after germination. The pots were flooded throughout the crop growing duration.

Table 2.2: Physico-chemical characteristics of soils used in this study

Parameter	Dakawa-Block 17	SUA-Crop Museum
pH (H2O)	6.07	5.92
Sand %	68.30	66.70
Clay %	21.80	30.30
Silt %	3.48	2.92
Nitrogen %	0.04	0.22
Phosphorus (Olsen) ppm	10.00	5.80
Potassium (ppm)	195.00	472.00
Calcium (ppm)	2520.00	1460.00
Sodium (ppm)	105.00	48.20
CEC meq/100g	26.50	15.20
EC (salts) µS/cm	110.00	166.00

2.3.4 Data collection

In both the field and pot experiments, data were taken on number of tillers, number of days to flowering (50%), number of days to maturity, plant height, number of panicles per plant, grain weight and shoot biomass. In addition, seedling vigour and root weight data were collected in the pot experiment as per Standard Evaluation System for rice (SES)

(IRRI, 1996). Roots were first washed with tap water and dried to constant weight before final weighing. Grain weight data were adjusted to 14% moisture.

2.4 Genotyping with *PSTOL1* Allele-specific Makers

2.4.1 DNA extraction

Leaf samples from 14 day-old seedlings of 96 varieties (92 ESA varieties plus 4 checks) were collected from the pot experiment at SUA. DNA was extracted following the protocol described by Romero *et al.* (2014). Rice leaves (~100-150mg) were manually groud in 600 µL of extraction buffer (Tris 100mM pH 8, EDTA 20mM pH8, 1.4M of NaCl, MATAB 3%, and sodium bisulfite 0.5%) preheated at 74 ^{0}C. The homogenate was incubated for 30min in a water bath – preheated at 74 ^{0}C then the samples were left to cool at room temperature before adding 500µl of Chloroform/ isoamyl alcohol mix (24:1). This was followed by a centrifugation at 12000 rpm for 15 min and transfer of the resulting aqueous phase to clean tubes where 270 µL of cold isopropanol (-20 ^{0}C) was added to allow DNA precipitation. After incubation at -20 ^{0}C, the mixture containing the supernatant and isopropanol was centrifuged at 12000 rpm for 15 min. The supernatant was then removed and the remaining pellets washed with 100 µL of cold ethanol (70%). After a centrifugation at 12000 rpm for 10 min, ethanol was gently removed and the pellets dried at room temperature to remove any remnant alcohol. Dried DNA pellets were dissolved in 50 µL of TE buffer (0.1x) (Tris 10mM pH8, EDTA 1mM pH8) containing RNase, DNA concentration was determined using Nanodrop spectrophotometer (Thermo scientific USA) and DNA quality was assessed by gel electrophoresis on 1% agarose gel.

2.4.2 PCR reactions

Prior to PCR amplification, the DNA samples were diluted to 25ng μL^{-1}. PCR reactions comprised 20µL of mixture including 1 µL forward and reverse primers each (10 µM) specific of *PSTOL1*, 0.2 µL Taq polymerase (5U. Ml^{-1}), 2 µL PCR buffer (10x), 1.6 µL $MgCl_2$ (25mM), 1 µL dNTPs (10mM) and 2 µL of DNA. The PCR conditions were as follows: initial denaturation at 94 ^{0}C for 5min followed by 30 cycles denaturation at 94^{o}C for 30seconds, annealing at 60^{o}C for 45seconds, elongation at 72 ^{0}C for 1minute and final extension at 72 ^{0}C for 7 minutes.

2.4.3 Gel electrophoresis

Prior to electrophoresis 3.3µl of loading dye was added to the PCR samples. Agarose gel (1.5%) was prepared for 300ml TAE buffer and 15 µL of ethidium bromide added. From each sample 10 µL were loaded in the gel and migration performed at 220mV. After migration, the gels were visualised under UV light and pictures taken for scoring. *PSTOL1* alleles were scored based on ability to amplify at known specific size typical of *PSTOL1* profile.

2.5 Statistical Data Analysis

The phenotypic data obtained from pot and field experiment were subjected to analysis of variance using Genstat statistical software 14th Edition (VSN International) based on the following model:

$Y_{ijk} = \mu + \alpha_{i +} \rho_j + \beta_{jk} + \varepsilon_{ijk}$

Where:-

$Yijk$ = the observation of the line *i* in the k^{th} incomplete block within the j^{th} replicate

μ = the overall mean

α_i = the effect of the i^{th} line

ρ_j = the effect of level j^{th} replicate

β_{jk} = the effect of the k^{th} incomplete block within the j^{th} replicate

ε_{ijk} = the residual error associated with observation on the i^{th} line, j^{th} replicate and k^{th} plot

2.6 Results

2.6.1 Effect of P deficiency on traits measured

Analysis of variance showed that there were highly significant differences among genotypes ($P< 0.001$) for all characters studied. Similar significant differences between phosphorus levels were recorded for the same traits (Table 2.3). Highly significant differences were observed also on the interaction between genotypes and P levels for grain weight, 50% flowering, number of days to maturity, plant height and straw weight (shoot biomass), also number of tillers per plant and number of panicles per plant. Data for pot experiment showed that both genotypes and phosphorus levels had highly significant differences for all the traits under consideration (Table 2.3). Exceptions were on genotype x P level interaction for which seedling vigour, plant height, root weight and shoot biomass had no significant differences

Table 2.3: Mean squares for grain weight and other agronomic traits measured under field conditions at Dakawa and in the pot experiment at SUA

Field experiment (n=100)

Source of variation	df	Grain yield (g m^{-2})	Tillers $plant^{-1}$ (#)	50% flowering (days)	Days to Maturity (days)	Panicles $hill^{-1}$ (#)	Plant height (cm)	Shoot biomass (g m^{-2})
Genotypes	99	242066***	30.0***	537.3***	511.0***	39.6***	2570.9***	341031***
Phosphorus	1	315037**	6298.2***	5495.0***	10387.2***	103.6***	131691.8***	25073231***
Interaction	99	67506***	7.8*	21.2***	36.5***	3.5**	146.3***	84266***
Error	598	40887	5.9	10.4	10.1	2.4	68.1	45122

Pot experiment (n=97)

Source of variation	df	Seedling vigour	Tiller $plant^{-1}$ (#)	50 % flowering (days)	Plant height (cm)	Panicles $plant^{-1}$ (#)	Root weight $plant^{-1}$ (g)	Straw weight $plant^{-1}$(g)	Grain yield $plant^{-1}$ (g)
Genotypes	96	4.97***	20. 3***	433.7***	1363.3***	38.2***	459.6***	262.7***	83.4***
Phosphorus	1	946.9***	5786.5***	24311.4***	3962.1***	3848.5***	20543.1***	59759.2***	7246.0***
Interaction	96	2.2ns	10.2***	98.7**	181.5ns	6.9***	236.2ns	72.5ns	52.9**
Error	381	1.8	4.6	63.8	184.3	4.0	206.9	91.2	34.8

***= significant at 0.1% probability, ** = significant at 1% probability, * = significant at 5%, ns = not significant at 5% probability

The most visible effects of P deficiency in plants grown under minus -P plots were the high reduction in their tillering ability. At maximum tillering, pots with supplemental P had vigorous plant, while susceptible genotypes under minus P had fewer tiller number (Plate 2.1).

Plate 2.1: P deficiency strongly affected tillering as shown by the difference in tiller number between the plants on the right side (without P) and those on the left (with P) 60 days after sowing.

The number of tillers was reduced by 54% in the field and 74% in pot experiments. Days to flowering was reduced by six days in the field and 15 days in pots experiment. Shoot biomass was reduced by 31% in the field and 50% in the pot experiment while yield decreased by 2% in the field and 31% in pot experiment (Table 2.4). In the field, the mean yield of all genotypes was 812. 7gm^{-2} + P block and 793.8 gm^{-2} in the -P block.

In the pot experiment, the mean yield was 24.9 g/plant and 17.1 g plant^{-1} in the + P and –P pots respectively. Data on root weight varied from 3.5 to 48.3 g plant^{-1} under minus P experiment and between 4.9 to 80 g plant^{-1} under +P experiment. Similarly plant biomass ranged from 10 g plant^{-1} to 38.3g plant^{-1} under minus P plot and between 16.1 and 63.4 g plant^{-1} under plus P experiment. Tiller number and straw weight (shoot biomass) were the most affected traits by P deficiency both in the field and pot experiment while day to 50% flowering was relatively less sensitive trait across the two environments.

Table 2.4: Average growth and agronomic performance of 96 rice varieties in the field at Dakawa and in the pot experiment at SUA at two levels of P

Field experiment

Trait	Added P	No P added	Difference	Percent reduction
Grain yield (gm^{-2})	812.7	793.8	18.9	2.3
Tiller number / plant	11.0	5.0	6.0	54.5
Panicles/ plant	10	9	1	1.0
Days to 50% flowering	87.2	93.5	6.3	6.7
Shoot biomass (gm^{-2})	1130.2	776.0	354.2	31.3
Plant height (cm)	150.7	125.1	25.5	17.0

Pot experiment

Trait	Added P	No P added	Difference	Percent reduction
Grain yield/plant (g)	24.9	17.1	7.8	31.3
Tiller number/plant	8	2	6	75.0
Panicles/plant	11	6	5	45.5
Days to 50% flowering	101.7	117.1	15.4	15.1
Shoot biomass/plant (g)	38.5	19.2	19.3	50.1
Root weight /plant (g)	27.1	16.4	10.7	39.5
Plant height (cm)	113.8	108.5	5.3	4.7

2.6.2 Varieties performances in response to P deficiency

When varietal means of the 96 varieties were considered for different traits under two levels of P, the check variety Mudgo had the highest grain yield (1169.8 gm^{-2}) in the field experiment under +P, while Kalivumbula had the lowest grain yield (287 gm^{-2}). In the minus P plots, the highest yielding variety was Kisegese.

Out of the 96 genotypes evaluated in the field, sixty genotypes had higher grain yield than the tolerant check Mudgo under minus P while in the pot experiment, 45 genotypes out yielded the tolerant check and only 27 genotypes were superior under both field and pot experiments. In the pot experiment, Chencheria had the highest grain weight per plant under +P (37.3 g/plant) while Mkia wa nyumbu yielded the least (8.4 g/plant) P supplemented plots.

Under minus P, Jaribu 220 gave the highest yield of 23.7 g/plant and Dular the least 4.6 g plant^{-1}. In terms of yield reduction due to P deficiency, for Afaa Mwanza 1/159 was only 15.2% while in some varieties such as Kisegese yield reduction was 55% suggesting that such varieties are more sensitive to P deficiency. In terms of biomass yield, in pot experiment, increase of P from 0 to 50 kg P ha^{-1}; resulted in biomass increase by 71% for Mwasungo variety, 63% for Pishori, while the increase was smaller for Marista variety (20%) and Rafiki (21.8%). In the field experiment biomass yield for Themanin and Mbega did not change significantly while, variety Mkia wa nyumbu biomass weight increased by 60%.

2.6.3 Correlation between root size and other growth parameters

The variation in root size under pot experiment showed positive association with many growth parameters such as grain weight plant and shoot biomass. However this was not consistent across all genotypes in the study. Generally small root size resulted in small plant biomass as well as small grain weight per plant with few exceptions where certain genotypes with small root weight were able to produce above average biomass and or above average grain weight and vice versa. For example in this study, genotype Kalundi with the highest root weight (48.3 g plant^{-1}) had below average grain weight per plant but

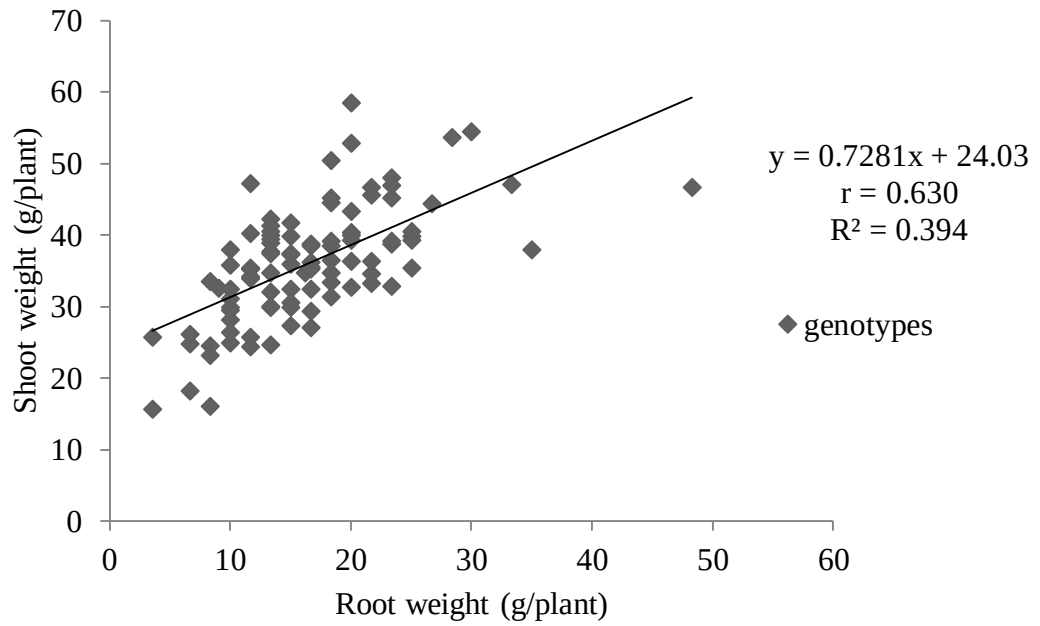
above average plant biomass. Also correlation studies between root size and other parameters consistently showed positive correlations under minus P while it had a mixed trend for same traits under +P (Table 2.5). Under both +P and -P treatments, only shoot biomass had the highest positive and significant correlation with root weight. Other traits had smaller correlations with root weight. In the P deficient treatment, all the traits showed positive correlation with root weight while under plus P, some traits were negatively related with root size.

Table 2.5: Correlation coefficients between root weight and other agronomic traits at two levels of soil Phosphorus

	Root weight	
Trait	**No P added**	**Added P**
Filled grain number	0.28***	-0.19**
Grain weight	0.26***	-0.24***
Shoot biomass	0.57***	0.68***
Total grain number	0.44***	-0.01ns
Panicle number	0.24***	0.05ns
Tiller/ plant	0.24***	0.05ns

Additionally, the relationship between root weight and shoot biomass was more apparent under P deficiency with R^2 of 39.4% compared to 26.7% when P is not limiting. A regression graph shows that, under –P, a unit increase in root size increased shoot biomass by 0.73 units compared to 0.37 units under +P (Fig. 2.1).

(a)



(b)

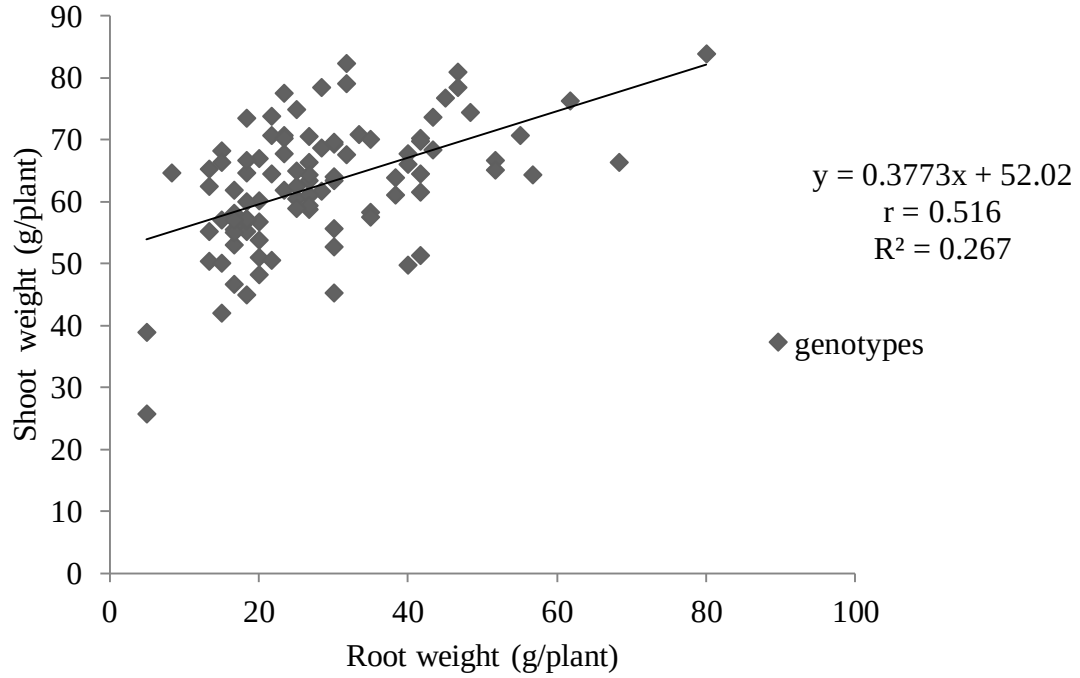


Figure 2.1: Relationship between root weight and shoot biomass under two levels of P: (a) no P application and (b) P application.

2.6.4 Distribution of *PSTOL1* among rice genotypes from East and Southern Africa

Genotyping of ESA varieties with *PSTOL1* allele-specific markers revealed that, 46 varieties out of the 93 tested (49.4%) carried the Kasalathi allele while 8 varieties (8.6%) carried CG14 allele. The rest, 39 varieties (41.9%) did not have *PSTOL1* gene (Plate 2.2). Nevertheless the grouping above did not show any significant variation for grain yield and other agronomic traits. Popular varieties such as Tule na Bwana, Pishori, Kalamata lack *PSTOL1* while Supa and SARO 5 had alleles similar to that of Kasalathi at *PSTOL1* locus. Among improved varieties the proportion of genotypes carrying the *PSTOL1* gene for each country were 44% for Tanzania, 20% for Malawi and 0% for Mozambique, while in traditional varieties, 50% (Mozambique), 57% (Malawi), 58% (Tanzania) carry *PSTOL1* gene at *Pup1* locus..

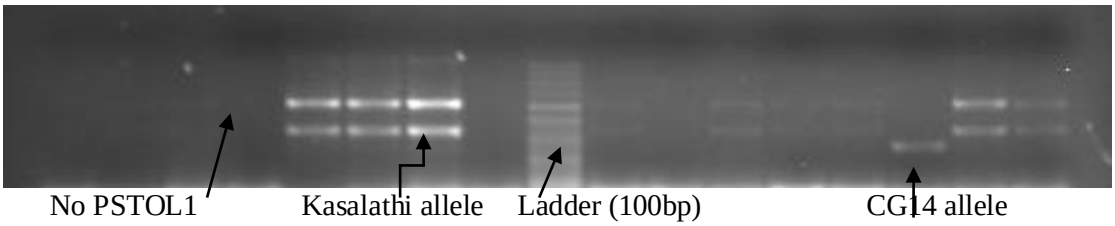


Plate 2.2: PCR amplification of *PSTOL1* in some ESA rice varieties and similarity with Kasalathi allele (double bands), CG14 allele (single band) and Nipponbare allele (no bands)

2.6.5 Relationship between varieties' performance under P deficiency and *PSTOL1* distribution

When varieties are grouped based on allelic background as either similar to Kasalathi, CG14 or Nipponbare, their performance did not show a clear pattern as to which allele has more effect on the performance of agronomic traits (Table 2.6). For example in the pot experiment shoot biomass was high (25 g/plant) for varieties with allele similar to CG14;

for the field experiment varieties with allele similar to Kasalath had the highest shoot biomass (806.3 g m^{-2}). This implies that within each grouping there are varieties with exceptional characteristics with regard to P deficiency tolerance.

Table 2.6: Average performance of the different *PSTOL1* haplotype in the field and pot experiment

	With Kasalath allele		**With CG14 allele**		**With Nipponbare allele**	
Number of varieties	46		8		39	
Traits	Field	Pot	Field	Pot	Field	Pot
Tiller number	6	2	5	2	6	2
Shoot biomass	806.3	19.4	714.4	25.0	768.3	19.0
Root biomass	-	16.6	-	17.5	-	16.7
Panicle number	9.0	6.0	8.0	6.0	10	7
Grain yieldt	779.9	16.4	628.5	18.6	844.4	16.4

A number of genotypes that showed high level of tolerance with little yield reduction relative to the tolerant check also had *PSTOL1* gene in their background while genotypes such as Pishori and TXD 88 which do not have *PSTOL1* gene had relatively high level of sensitivity to P deficiency by showing high yield reduction.

2.7 Discussion

Eastern and Southern Africa has a large diversity of unexplored rice germplasm. Traditional rice varieties that are still being cultivated by ESA farmers, despite the availability of improved varieties (Luzi-Kihupi *et al*., 2012), are likely to possess superior agronomic traits. For these cultivars to be adequately utilized, they need to be characterized for traits relevant to ESA rice breeding programs. In recent years, rice cultivation in Africa has intensified to address the growing demand driven by population growth and increased rice consumption (El-Namaky and Demont, 2013).

At the same time, soils have continued to be depleted of mineral nutrients with little or no replenishment due to the high price of fertilizers. One of the solutions to this problem is to utilize the genetic differences observed in order to develop nutrient-efficient varieties. In this study genetic differences within a rice collection from Eastern and Southern Africa with regard to P deficiency tolerance were revealed. Significant effect of P treatment was observed especially on shoot biomass, number of tillers and to some extent grain yield (at least in the pot experiment). Also significant genotype x P level interaction observed in this experiment implies that genotypes exhibited differential performance between added and no added P experiment based on G x E criteria (Walsh and Lynch, 2009). Tiller number, number of panicle, number of day to flowering and grain yield consistently showed significant genotype x P level interaction; this means the above traits in some genotypes responded differently to the environment and have been reported to be among the diagnostic keys in varieties that are susceptible to P deficiency (Fairhurst *et al*., 2007). This further confirms the existence of genetic variability for tolerance to P deficiency in these varieties and which can be exploited (Fageria *et al*., 1988; Wissuwa and Ae, 2001). Therefore, it is possible to distinguish and select tolerant genotypes from susceptible ones. However, a number of traits did not show consistent significant genotype x P level interaction in the field as well as in the pot experiment. These include shoot biomass and plant height. The possible explanation is that the pot size could have limited the free growth of vigorous varieties where the space from which to explore nutrient was limited.

The traits that showed high sensitivity under both field and pot experiment were shoot biomass and tiller number. In addition root weight which was measured only in the pot experiment showed high weight reduction between two P levels. The above results are consistent with those of Fageria and Baligar (1997), who reported that shoot biomass, and tiller number were among the most responsive traits to P application. Also, studies on barley found out that shoot growth is highly sensitive to P depletion (Veneklaas *et al.*, 2012) such sensitivity is meant to economize the P stored in leaf vacuoles. So due to this high sensitivity, it has been proposed that tiller number and shoot biomass can be used as a criteria for screening genotypes for tolerance to P deficiency (Fageria and Baligar, 1997).

Some of the varieties tested showed small yield reduction, which means that they are not very sensitive to P deficiency. Also in similar study using upland varieties, Saito *et al.* (2005) reported the existence of less sensitive rice varieties. For improved varieties, small yield reduction observed under P deficiency, would suggest tolerance because such varieties are less sensitive to P levels whilst it is common knowledge that most improved varieties are responsive to fertilizer application.

On the contrary low P response observed suggest that traditional varieties are probably adapted to farmers' practices of low-input and their yield may not be affected much when P fertilizers are not applied (Saito *et al.*, 2005). For example a number of traditional varieties such as Kalundi and Kachikope performed better under P deficiency both in the field and pot experiment but had moderate performance at adequate levels. Similar findings were also reported by Ahmad *et al.*, (2000) where some varieties showed better yield under P deficiency compared to adequate P applied plots.

For varieties that showed high reduction in yield or biomass can be considered as sensitive to P deficiency, therefore for these to be productive in P deficient soils, fertilizer application is inevitable. Alternatively genetic improvement through hybridization can be achieved by the utilization of genetic variability despite the fact that *Pup1* QTL and its related gene *PSTOL1* have been linked to better tolerance to P deficiency (Wissuwa and Ae, 2001; Chin *et al*., 2011; Gamuyao *et al.*, 2012).

In this study, some varieties had *PSTOL1* in their background but did not show high level of tolerance, there are possibilities of inhibitors or environmental factors that hinder genetic expression, thus further study is needed to understand the reason behind poor *PSTOL*1 expression in these varieties. Plant tolerance to P deficiency is usually manifested in high root growth, high biomass production and stable yield as a result of high P acquisition. In other words, expansion in root system enables plants to explore greater soil volume and absorb the little P available in the soil (Wissuwa, 2003). In a recent study by Niu *et al*. (2013), it was reported that *PSTOL1* present in tolerant varieties sends signals to trigger the growth of roots under P deficient soils and this confers tolerance to P deficiency as the plant is able to reach more P in the soil. However, in this study, a number of genotypes including TXD 85 and Rafiki did not have *PSTOL1* gene because they did not show any amplification with specific markers, yet these varieties showed some level of P deficiency tolerance. In similar study with different set of varieties also Mukherjee *et al.* (2014); Aluwihare *et al.* (2015), reported the existence of varieties with high level of tolerance but without *PSTOL1* gene. This indicates that genomic factors other than *PSTOL1* could be responsible for low P tolerance in these varieties.

Indeed, several QTLs and genes have been associated with P deficiency tolerance on chromosomes 4, 6, 9 and 11 but are yet to be characterised (Koide *et al.,* 2013). Other studies suggest that there are hundreds of genes that may be involved in plant response to P stress (Vance *et al*., 2003). Therefore further study may be conducted on these varieties to unravel the genetic basis of their tolerance to P deficiency. In the meantime, for varieties with *PSTOL1* in their background but showing poor tolerance to P deficiency suggests that the expression of *PSTOL1* is dependent on the presence or absence of certain factors that may inhibit its functions.

According to Vigueira *et al*. (2016), there is a possibility of mutation that may have resulted in loss of function. This means that there are both functional and non functional *PSTOL1* within the rice genome that can be revealed better with proper screening methods. Among the tolerant varieties, efficient P uptake tends to favour root growth, which in turn, increase the surface for nutrient absorption. However, it is possible to have varieties that are efficient in terms of nutrient uptake per unit root size (Vandamme *et al*., 2015) and this partly explains the reason for small but significant correlation values between root size and other growth parameters as observed in this study. The small correlations implies that both varieties with and without efficient P uptake were present in this study. Therefore, in order to identify and separate these two groups would require further screening.

In the field experiment, a grain yield reduction of only 2% was observed whereas it was high in the pot experiment. Such small yield difference between +P and -P treatment was quite unexpected when other traits associated with yield, particularly tiller number was strongly affected under the same conditions.

A possible explanation could be that the heavy lodging observed in a P supplemented block affected grains which in turn resulted in lower yield, as the average plant height in P supplemented plots was 150cm (about 17%) taller than no P applied plots. However it is common knowledge that tall traditional varieties tend to lodge under high input conditions (Ookawa *et al*., 2010). In this study seven varieties had above average root weight, plant biomass and grain weight, these include Sotea, Mzinga, Afaa Mwanza 1/159, Chencheria, Sindano nyeupe, Mudgo and M'finico, while genotypes including Nene, Niwiao, Dular, Mwangaza, Magonga ya wayungu, Dakawa 59, Mbega, and Mbawambili had below average for the same traits. Interestingly from the above list, all the genotypes with superior grain yield, biomass and root weight, also had *PSTOL1* gene in their background which was expected based on available literature (Kottearchchi and Wijesekara, 2013).

On the contrary, nearly half of the genotypes with below the average in all the traits carry the Nipponbare allele which is intolerant to P deficiency suggesting the role of *PSTOL*1 in root growth and its eventual effects on biomass yield (Ghassemi-Golezani and Tajbakhsh, 2012). In their study, Kottearchchi and Wijesekara (2013) reported significant variations between varieties with *Pup1* locus and those without the locus for almost similar traits. According to Wissuwa (2003), small changes in root growth related parameters such as root fineness and root P utilization efficiency increases P uptake which in turn enhances root growth, more P uptake and consequently high plant biomass. Also Gamuyao *et al.* (2012) indicated that *PSTOL1*-overexpressing lines develop more roots and absorb more nutrients hence produce relatively more biomass compared to genotypes lacking the gene.

The results above tend to suggest that *PSTOL1* expression varies to a great extent in different genetic backgrounds and therefore it implies that mere presence of the gene does not guarantee low P tolerance (Murkherjee *et al*., 2014), thus the effects of the gene or QTL must be characterised to confirm its positive expression in intolerant genotypes.

2.8 Conclusion

This is the first study on the potential of East and Southern African rice germplasm with regards to P deficiency tolerance. Based on the findings, it is evident that P deficiency is a big problem, the observed 30% yield reduction under pot experiment as a result of non-application of P needs special attention. Also the study revealed the existence of large variability between genotypes with regard to P deficiency tolerance. The study also revealed the abundance of *PSTOL1* within rice varieties tested and will therefore form a basis for genetic improvement. The study also found out the occurrence of *PSTOL*1 was in large proportion in traditional varieties compared to improved varieties thus there is a need to conserve these traditional varieties as sources of vital genes.

Some of the popular varieties such as Pishori, Tule na Bwana and TXD 88 are susceptible to P deficiency and they lack *PSTOL1* gene which is responsible for increased P uptake hence tolerance to P deficiency. By combining results of the field experiment, pot experiment and laboratory genotyping, the studied genotypes can be grouped as (i) tolerant genotypes with *PSTOL1,* (ii) susceptible genotypes with *PSTOL1,* (iii) tolerant genotypes without *PSTOL1* (iv) susceptible genotypes without *PSTOL1*. Introgression of *PSTOL1* or the entire *Pup1* QTL through marker assisted selection into these varieties could improve their tolerance level hence increase their yield in low-P soils.

Also commercial varieties such as SARO 5, Supa and Chencherisa that carry the tolerance gene can be selected as donors since they also have superior grain quality and strong aroma. At the same time it is important to note that the presence of tolerant varieties such as TXD 85 that do not have *Pup1* QTL suggest the possibility of finding new QTLs associated with P deficiency tolerance. These varieties could be used as potential donors in breeding programs for mapping new tolerance QTLs and development of new breeding lines more adapted to low P soils. In the meantime, genotypes which exhibited above average yield under P deficient condition such as TXD 306, Afaa Mwanza 1/159, Kaling'anaula, and Chencheria can be directly recommended to farmers in low P areas. Mean while, in future studies it is also important to ascertain possible ambient conditions that increase the efficiency of uptake and utilization of P and identify genes in other loci that interact with *PSTOL1* in expressing tolerance.

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CHAPTER THREE

3.0 Phosphorus use and performance of rice varieties from Eastern Africa under irrigated conditions

3.1 Abstract

Continued depletion of a non-renewable global stock of phosphorus (P) presents one of the biggest challenges in crop production especially in rice where P deficiencies result in significant yield losses. Rice genotypes with efficient P utilization are needed to ensure long term availability of soil P. In this study 20 rice genotypes selected from the previous study were evaluated for their P uptake, P utilization efficiency (PUE) including P partitioning between grains and straws under optimal and sub optimal P conditions. The experiment was conducted in 2014 at Dakawa irrigation scheme. The experimental design was an alpha lattice with three replications for each of the two blocks. Significant variation ($P<0.001$) for straw P concentration, grain P concentration, total grain P and PUE was observed between genotypes and only PUE at the two levels of P. In terms of P partitioning, generally all the genotypes tested had high proportion of grain P concentration and a few ones had sizable amount of P in straws. Genotypes such as SARO 5, Dular, Mudgo, and Paula showed high P efficiency while Shingo ya mwali, Kasalath and Tule na Bwana were non efficient. Furthermore Paula, SARO 5 and Chencheria had above average dry matter as well as PUE while Shingo ya mwali, Sotea and Tule na Bwana had below average dry matter production and PUE. Therefore genotypes with high PUE and relatively low P uptake exist and can be useful source of genes in increasing yield on P deficient fields without severe soil P depletion.

3.2 Introduction

Sustained rice production is largely dependent on the lands' capability to supply plant nutrients. This in turn is dependent on the availability and affordability of limiting input especially fertilizers (Wissuwa *et al.,* 2015). Unfortunately most agricultural lands are deficient in plant available nutrients particularly phosphorus (Fageria, 2014) which is one of the essential nutrients for plant growth and development. Phosphorus is required for optimal plant growth and reproduction and plays a vital role in virtually every plant process that involves energy transfer. Rice requires P for root development, more tiller production and grain formation, thus the role of P in rice production cannot be overemphasized (Rose *et al.,* 2012). However, P is also the most limiting plant nutrient due to its unavailability in many agricultural lands (Wang *et al.,* 2010).

In Tanzania, rice is a staple food for over 60% of the population. Estimates indicate that currently more than 1.2 million hectares are under rice cultivation (FAO, 2015). However, yields are generally low ranging between 1.5 to 3 t/ha due to poor soil fertility (Barreiro-Hurle, 2012). This low yield is partly due to high usage of traditional varieties with low yield potential or lack of traits associated with tolerance to low soil fertility. At the same time improved varieties are characterized by high fertilizer requirement thus their maximum yield cannot be attained without optimal fertilizer application which the majority of poor resource farmers cannot afford. As a result, most agricultural lands have negative nutrient balance.

In addition, the use of varieties which are inefficient in phosphorus use adds to production costs and contributes to the rapid depletion of the world's non-renewable rock phosphate supplies (MacDonald *et al*., 2011). Therefore, under limited access to fertilizer subsidies,

varieties that have the ability to adapt to low input systems will be suitable to Tanzanian and African farmers in general. In rice and other crops, two major adaptation strategies to P deficiency have been described: phosphorus acquisition efficiency (PAE) and phosphorus utilization efficiency (PUE) (DoVale and Fritsche-Neto, 2013; Rose *et al*., 2013; Wissuwa *et al.*, 2015; Vandamme *et al*., 2016a). While PAE relates to P uptake from the soil which may be a function of root size, root architecture or rhizosphere interactions that enhance P bioavailability, PUE on the other hand relates to the efficiency with which the P taken up is utilized to accumulate either grain yield or vegetative biomass (Nziguheba *et al*., 2015). From crop physiology and plant breeding perspective, PUE is defined as shoot biomass produced per unit P in shoots (Rose *et al*., 2013, Vandamme *et al*., 2015).

The major challenge is that, long term usage of varieties that have high PAE pose a threat to the sustainability of soil P pools in low-input agricultural systems particularly where crop residues are poorly managed (Veneklaas *et al.,* 2011). Therefore to be able to improve crop P efficiency, yields must increase at a given rate of P fertilizer application, or must remain stable with lower levels of P fertilizer application. In rice, more than 70% of total P contained in the above ground biomass is normally found in the grains (Rose *at al.,* 2013; Vandamme *et al*., 2016a). Much of this is not returned back in the field thus resulting in P nutrient mining (Vandamme *et al*., 2016b).

Thus, varieties with high P uptake at low soil P but able to partition substantial proportion of P taken in the straws and less in the grains without compromising the ability to germinate into healthy seedlings will be worth exploring for straw P recycling management option (Rose *et al.,* 2011; Rose *et al*., 2013; Nziguhebha *et al.,* 2015;

Vandamme *et al*., 2016a). In the previous chapters (cf. Chapter 2), several varieties with high potential to adapt well on low-P soils were identified. However detailed information on their relative P acquisition efficiency, P utilization efficiency including P partitioning between grains and straws is unknown. This study was undertaken was designed in order to determine possible presence of genetic variability in acquisition including internal utilization efficiency so as to generate information that will guide informed decision on their deployment and use in genetic improvement programmes.

3.3 Materials and Methods

3.3.1 Plant materials

Twenty (20) rice varieties including checks were purposely selected from the 96 varieties grown during 2013 cropping season. Among these varieties, some had the *PSTOL1* gene which was reported to increase P uptake (Gamuyao *et al*., 2012). A few varieties susceptible to P deficiency were included in the selection for comparison purposes. Care was taken not to select varieties that are susceptible to lodging or shattering.

3.3.2 Field evaluation under optimal and depleted P conditions

The experiment was established at Dakawa irrigation scheme block 17/1 in 2014 to assess the variation in P uptake between varieties. The experimental design was alpha lattice replicated three times. The field was divided into two adjacent blocks whereby in one block, no P fertilizer was applied while in second block, optimal P (50 kg P ha^{-1}) was applied at transplanting. The other nutrients such as N (Urea) and K (MO) were applied at the rate of 150 kg N ha^{-1} (into 3 splits) and 50 kg K ha^{-1} respectively. Each entry was planted on a 0.8 m x 1.2 m plot, at a spacing of 20 cm x 20 cm. Transplanting was done 21 days after nursery establishment. One seedling per hill was planted. Weeding and other

crop management were done as required. Data were collected on number of tillers per plant, number of days to flowering and maturity grain weight, spikelet fertility and straw weight. Relative grain yield was calculated as the ratio of grain yield without P fertilizer to grain yield with optimal P fertilizer as follows.

$$Relative\ Yield = \frac{Grain\ yield\ under\ no\ P\ applied}{Grain\ yield\ under\ P\ applied} x\ 100$$

3.3.3 Tissue P analysis

About 20 g of dried straw and seed were sent to SUA soil science laboratory for the analysis of P content in these tissues. The analysis for phosphorus concentration in plant straw and seed was done according to the procedure described by Okalebo *et al.* (2002) where; the samples were finely ground and 0.5g of tissue was transferred into Kjeldahl digestion tubes. The samples were digested in H_2SO_4 at 125 ^oC for 1 hour, before being taken off and cooled. After cooling, 5ml of H_2O_2 were added into each tube and heated at 70 ^oC on the digestion block until the reaction stopped. This reaction was repeated until the digests were colourless. The digest was then heated on the digestion block at 180 ^oC to near dryness. After cooling 10ml of 10% HNO_3 were added and the dissolved digest was transferred into 100ml volumetric flasks, which was filled to the mark with distilled water. P uptake on the basis of dry weight was determined by the following formula:

$$Uptake\ \left(\frac{mg}{plant}\right) = Concentration\ of\ nutrients\ \left(\frac{mg}{g}\right)\ x\ dry\ matter\ yield\ \left(\frac{g}{plant}\right)$$

3.3.4 Statistical data analysis

All the data were subjected to analysis of variance using Genstat statistical software VSN International 14th edition based on the following statistical model:-

$Y_{ijk} = \mu + \alpha_i + \rho_j + \beta_{jk} + \varepsilon_{ijk}$

Where:-

$Yijk$ = the observation of the line *i* in the *k*-th incomplete block within the *j*-th replicate

μ = the overall mean

α_i = the effect of the i-th line

ρ_j = the effect of level *j*-th replicate

β_{jk} = the effect of the *k*-th incomplete block within the *j*-th replicate

ε_{ijk} = the residual error

3.4 Results

3.4.1 Genotypic variation for growth and yield traits under optimal and P deficiency

Significant differences between two levels of P were observed for grain yield; shoot biomass, number of tillers, days to flowering and maturity (Table 3.1). However, the interaction between genotypes and P level was only significant for number of days to flowering, days to maturity as well as number of tillers. Between genotypes, the variation was significant for all the traits under consideration

Table 3.1: Mean squares for grain yield and other agronomic traits of 20 genotypes at two levels of P at Dakawa in 2014

Source of variation	df	Grain yield	Spikelet fertility	Straw weight	Days to maturity	Days to flowering	Number of tillers per plant
Phosphorus (P)	1	524504***	81.18	795359***	4563.3***	424.5***	23.5***
Genotype (G)	19	9764***	166.19***	14295***	343.9***	3100.8***	2717.0***
G x P	19	3668	43.9	6702	53.9*	13.1*	11.8***
Error	78	3328	41.2	4023	28.8	6.7	2.3

3.4.2 Genotypic variation for P traits under optimal and P deficiency

Phosphorus traits include grain P, straw P, phosphorus acquisition efficiency (PAE) and phosphorus utilization efficiency (PUE). In this study, genotypic variations were significant for P concentration in the grains and straws, also total P as well as PUE. Moreover PUE was highly significant (P< 0.001) between two levels of P (Table 3.2). However, no significant differences were observed for the interaction between P levels and genotypes.

Table 3.2: Mean squares for P related traits on 20 genotypes grown at Dakawa in 2014

Source of variation	df	Grain P mg/g	Straw P mg/g	P uptake mg/plant	PUE
Phosphorus (P)	1	0.27ns	0.06ns	419.0ns	50.95***
Genotype (G)	19	4.21***	1.73***	30536.1***	1959.5***
G x P	19	0.23ns	0.05ns	211.2ns	20.41ns
Error	78	0.19	0.03	315.1	12.95

3.4.3 Effect of P on grain yield and dry matter production

The mean grain yield under P applied plots was 321.17 g m^{-2} compared to 188.95 g m^{-2} when no P was applied. P deficiency resulted in grain yield reduction by 41% (Table 3.3). Meanwhile average shoot dry weight varied from 372.6 g m^{-2} in P applied plots to 209.8 g m^{-2} when no P was applied. Under P deficiency, highest shoot dry weight was recorded on Chencheria 300.3 g m^{-2} followed by Paula 245.6 g m^{-2}. The lowest shoot dry weight was recorded on Kasalath and Shingo ya mwali with 161.3 and 163.8 g m^{-2} respectively. Total P absorbed ranged between 1.74 and 2.95 mg/plant under minus P and between 2.41 to 3.69 mg/plant under +P plot. Generally much of P absorbed was located in the grain (83%) under minus P plot and 76% under +P plots. Varieties that had relatively low tissue P were TXD 85, Pishori and Sotea under P deficient conditions with 1.74, 2.14 and 2.16 mg/plant respectively while SARO 5, Rafiki and Chencheria had lowest tissue P under +P with 2.41, 2.5 and 2.51 mg/plant respectively.

Table 3.3: Variety response to P status in the soil on grain yield, shoot biomass, P uptake and partitioning at two levels of P application at Dakawa

	Grain Yield g/plot		Shoot weight g/plot		grain P mg/g		straw P mg/g		Total P mg/g	
Genotype!	**Plus P**	**No added P**	**Plus P**	**No added P**	**Plus P**	**No added P**	**Plus P**	**No added P**	**Plus P**	**No added P**
Afaa Mwanza 1/159	237.8	208.4	477.9	237.8	2.3	2	0.43	0.24	2.73	2.24
Chencheria	381.2	239.2	561.1	300.3	2	2.1	0.51	0.65	2.51	2.75
Dular	223.4	196.3	291.5	207	2.3	1.7	0.67	0.34	2.97	2.04
Faya Dume 3	328.3	188.3	321.1	214.6	2.1	1.9	0.7	0.52	2.8	2.42
IR74	422.6	201.1	347	224.3	2.6	2.5	0.71	0.4	3.31	2.9
Kalamata	338	184	202.2	171.2	2.5	2	0.73	0.3	3.23	2.3
Kasalath	193.5	98.6	407	163.8	2.4	2.2	0.45	0.25	2.85	2.45
Kisegese	306	172	337.7	188.3	2	2	0.75	0.24	2.75	2.24
Limpopo	360.5	218.7	286.3	172.9	2.4	2.3	0.61	0.52	3.01	2.82
Mudgo	306.3	191.4	327.4	237.4	2.1	1.9	0.69	0.44	2.79	2.34
Paula	323.1	220.1	336.2	245.6	2.4	2.1	0.84	0.47	3.24	2.57
Pishori	383.7	193.7	408.1	238.3	3.1	1.8	0.59	0.34	3.69	2.14
Rafiki	297.2	181.9	421.3	221.6	1.9	2	0.6	0.63	2.5	2.63
SARO 5	353	203.6	352.8	200.1	2	1.9	0.41	0.18	2.41	2.08
Shingo ya Mwali	337.5	145.2	452.1	161.3	2.3	1.7	0.49	0.37	2.79	2.07
Si Mzito	331.2	164.6	375.2	198.6	1.9	2	0.69	0.4	2.59	2.4
Sotea	270	170	411.4	220	2.8	1.8	0.74	0.36	3.54	2.16
Tule na Bwana	287.7	164.8	406.9	195.2	2.5	1.9	0.67	0.69	3.17	2.59
TXD 85	368.9	253.7	368	209.7	2.8	1.4	0.68	0.34	3.48	1.74
TXD 88	373.6	182.4	360.7	187.6	2.2	2.1	0.7	0.18	2.9	2.28
Mean	321.2	188.9	372.6	209.8	2.3	2	0.6	0.4	3	2.4
CV (%)	25.7	24.6	21.1	15.9	17	21.7	31.8	39.7	16.3	16.2
P	*	***	***	***	*	ns	ns	*	ns	**
Lsd (0.05)	114.7	63.5	136.1	81.3	0.7	0.3	0.4	0.1	0.9	0.3
SE	10.7	6	17.6	7.4	0.4	0.4	0.2	0.2	0.5	0.4

3.4.4 Variability in P uptake and PUE

In terms of P uptake, under both P applied and minus P plots, the variation between varieties were insignificant. Under P deficiency, P uptake was high in IR74 and Chencheria while under P applied plots, highest uptake was observed on TXD 85, Pishori and Limpopo, while varieties with the lowest P uptake were Rafiki and Kasalath (Table 3.4). In terms of P utilization efficiency, genotypic differences were significant under both P applied and minus P plots. Under P applied, Pishori a local variety had the higher PUE with 28 g DM/mg P absorbed. When the genotypes were evaluated under P deficient plots, Chencheria, TXD 88 and TXD 85 had the highest PUE ranging from16 to 18 g DM/mg P.

Table 3.4: Phosphorus uptake and utilization efficiency of 20 genotypes under applied and no applied phosphorus condition

	Total P taken (mg/plant)		P utilization efficiency(g/mg)	
Genotype	**plus- P**	**No added P**	**plus- P**	**No added P**
Afaa Mwanza 1/159	66.8	30.6	24.5	15.2
Chencheria	66.0	42.5	26.4	16.4
Dular	44.9	32.7	15.1	14.0
Faya Dume 3	60.0	33.2	18.8	13.7
IR74	70.5	43.3	21.0	12.8
Kalamata	72.5	28.3	22.4	13.2
Kasalath	41.0	11.5	14.2	9.0
Kisegese	53.3	27.0	19.8	12.5
Limpopo	73.8	24.6	24.6	16.2
Mudgo	55.2	28.5	20.1	11.2
Paula	67.4	39.0	20.7	15.6
Pishori	75.2	31.9	28.8	12.0
Rafiki	39.1	34.8	13.2	13.8
SARO 5	59.9	29.8	25.4	15.4
Shingo ya mwali	63.5	20.4	23.5	7.6
Si Mzito	54.7	27.2	20.9	11.3
Sotea	66.5	31.7	18.5	11.4
Tule na Bwana	62.8	30.6	19.7	12.4
TXD 85	91.6	35.6	25.7	18.4
TXD 88	69.9	32.6	24.3	16.5
Mean	62.7	30.8	21.4	13.4
CV (%)	32.1	9.7	17.4	2.1
P	ns	ns	***	*
Lsd (0.05)	33.9	33.5	6.7	9.3
SE	20.2	13.4	3.7	3.0

Generally, majority of the varieties used in this study were characterised by low P acquisition as well as P utilization efficiency regardless of the soil P status. When PUE under P deficiency is plotted against PUE under P applied plots there is a weak correlation with a very small R^2 (Fig. 3.1).

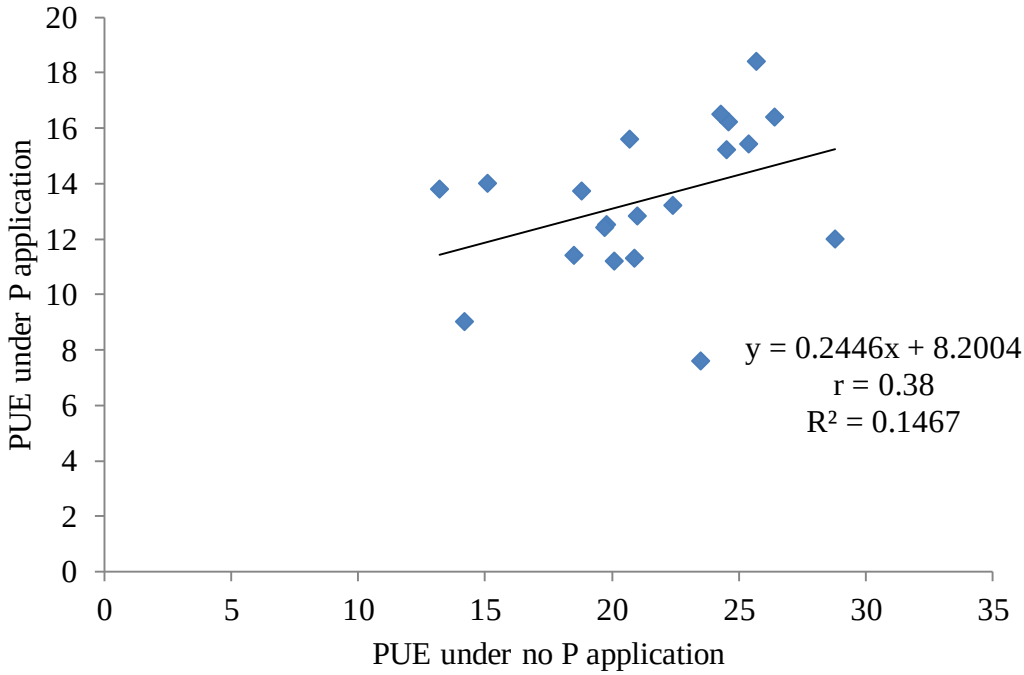

Figure 3.1: Relationship between PUE under optimal and no added P soil conditions

3.4.5 Variety tolerance to P deficiency

The sensitivity of a variety in response to P deficiency is a measure of its tolerance. A tolerant variety will normally show little response to P deficiency (i.e. less sensitivity). In this study, Dular, one of the tolerant checks along with Afaa Mwanza 1/159 and TXD 85 showed less yield reduction under P deficient soils compared to yield at adequate P application. This implies that they are less affected by P deficiency (Table 3.5). On the other hand IR74, Shingo ya mwali, TXD 88 and Simzito showed yield reduction of more than 50% meaning that these genotypes are sensitive to P deficiency.

Table 3.5: Variety response to P deficiency expressed as percent change (reduction) for grain yield and other agronomic traits for 20 genotypes grown at Dakawa.

Genotype	GYL	TN	FD	MAT	Total P	PHT	BIOM
Afaa Mwanza 1/159	13.1	66.7	9.6	8.5	16.4	22.1	50.4
Chencheria	38.1	68.5	10.4	14.0	-7.2	10.3	46.7
Dular	12.9	40	7.4	8.9	24.1	13.0	28.6
Faya Dume 3	42.7	58.4	8.5	7.5	20.7	14.8	33.5
IR74	51.7	66.7	6.3	6.1	10.9	10.4	35.3
Kalamata	45.5	70.0	8.4	-4.2	31.8	18.0	15.0
Kasalath	47.0	82.4	16.2	17.3	21.5	29.4	59.4
Kisegese	44.9	60.0	9.5	6.3	18.9	14.1	45.1
Limpopo	38.3	66.7	13.6	12.7	28.3	8.7	38.7
Mudgo	36.9	58.4	8.4	12.0	15.7	18.1	27.4
Paula	30.6	64.3	10.9	11.2	28.3	10.1	26.1
Pishori	49.6	69.3	11.1	6.9	14.0	14.6	41.4
Rafiki	38.9	73.4	5.6	6.1	-2.8	20.0	47.2
SARO 5	41.8	76.5	10.6	8.0	8.4	7.3	43.2
Shingo ya mwali	57.3	71.5	17.0	11.7	10.3	24.7	64.2
Si Mzito	51.6	75.0	12.2	8.9	11.6	18.6	47.7
Sotea	37.3	71.5	7.7	12.4	37.2	21.9	46.9
Tule na Bwana	43.5	66.7	8.1	9.3	17.5	14.4	52.4
TXD 85	31.3	64.8	9.0	4.6	47.8	8.9	43.3
TXD 88	51.5	80.0	11.7	10.7	22.7	15.0	48.3
Mean	40.2	67.5	10.1	8.9	18.8	15.7	42.0

GYL= grain yield, TN= tiller number per plant, FD = days to 50% flowering, MAT = days to maturity, TP = total phosphorus, PHT= plant height, BIOM = total biomass

In terms of biomass change, the lowest change as a result of non application of P was observed in Paula, Mudgo, Kalamata and Dular while the most sensitive varieties were Tule na Bwana, Shingo ya mwali, Kasalathi and Afaa Mwanza 1/159, showed high biomass reduction thus they are said to be sensitive to P deficiency for that trait. Of all the traits recorded, plant height and time to maturity showed less sensitivity to P deficiency in all varieties compared to other traits therefore these traits may not be good selection criteria for P deficient tolerant varieties. Although P is responsible for dry matter production, not all varieties with high total P content had higher shoot biomass.

3.5 Discussion

Given the fact that phosphorus is non renewable, efficient use of this vital resource is of paramount importance. In the previous chapter, genetic differences among the ESA assembled rice varieties with regard to P deficiency tolerance were assessed. In this study varieties' efficiency in the uptake and utilization of P was determined. Although genotypes in the current study did not show significant variation for total P in plant tissues, the maximum and minimum values shows that there is a difference between varieties. Because in this study, P was determined at maturity (after harvest), it is possible that significant differences exist at active earlier growth stages since varieties use P at different rates to develop tissues and support growth. This could be confirmed by determining PAE at different growth stages such as at vegetative and grain filling stages because similar studies in wheat revealed variation in P content at different growth stages (Zahedifar *et al*., 2011).

Despite the near similarity in P content among genotypes, clear differences for PUE were observed, varieties such as Chencheria and TXD 85 had sigh significantly high PUE than other varieties. Such genotypic variation for PUE were also reported by Mukherjee *et al*. (2014); Wissuwa and Ae (2001). Furthermore, genotypes such as Chencheria, Limpopo, TXD 88 and TDX 85 had higher PUE under P deficiency as well as P supplemented plots, this consistent efficiency regardless of soil P status is an indication that PUE is characteristic of a given variety hence it is said to be under genetic control. Therefore with such significant variation, it is easy to make effective selection for this trait. However it will be meaningful to take into consideration of other traits such yield potential under P deficiency. Under no P treatment, PUE was generally lower compared to P applied plots contrary to the results reported in Aluwihare *et al*. (2016). This discrepancy could

probably be due to variation in native soil P, as well as the genetic background of the material used. As far as P tolerance is concerned, a good variety is the one that shows little yield reduction when grown under P deficiency. However, in traditional varieties, less yield reduction may be due to adaptation to a given environment and not tolerance. Therefore under P deficiency a good variety must have above the average yield to qualify as tolerant.

According to Fageria and Baliger (1997), four categories of variety tolerance are known based on P use efficiency and dry matter yield indices as follows (i) efficient and responsive (ii) efficient and non responsive (iii) non efficient and responsive (iv) non efficient and non responsive. In this regard, a good variety is the one which have above average total dry matter (TDM) as well as above average P use efficiency which is also described as efficient and responsive i.e. produced well and responded well to P absorbed, while the reverse is said to be non responsive and non efficient where varieties have below average P use efficiency as well as below average total dry matter production.

In this study, seven varieties including Paula, SARO 5, Chencheria, Afaa Mwanza 1/159, Dular, Faya dume3 and TXD 85 were efficient and responsive. On the other hand, six varieties namely; Shingo ya mwali, Tule na Bwana, Sotea, Kisegese, Kalamata and Si Mzito were non- responsive and non-efficient therefore are ones that require high amount of fertilizer, therefore these need improvement (Fig. 3.2).

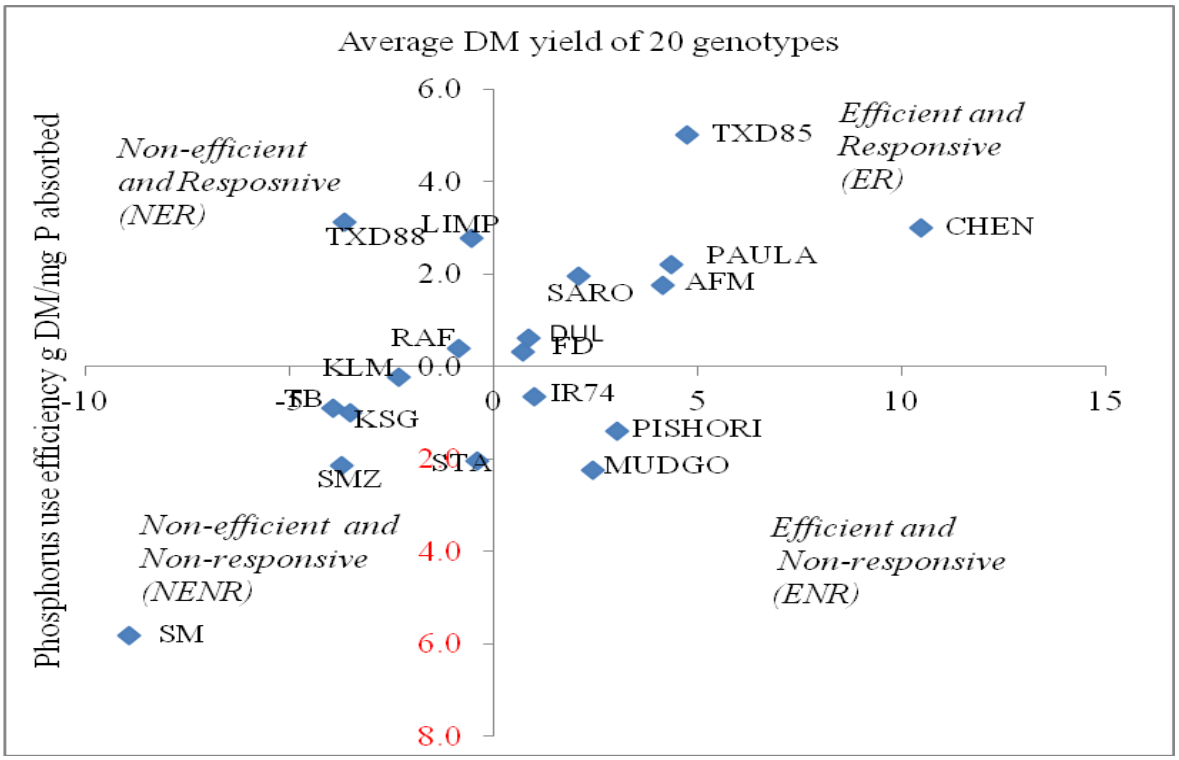

Figure 3.2: Classification of 20 rice genotypes for phosphorus use efficiency (mean deviation values have been used).

These efficient and responsive varieties can therefore be selected for use in P deficient soils because they have the ability to produce more biomass with little P uptake (Fageria and Baliger, 1997). Generally, the number of days to maturity plays a significant role in the overall total P under P deficiency because the longer it takes for a plant to reach maturity the more it was able to accumulate more P. Also studies by Banzinger *et al.* (2000), found out that, with regard to nitrogen the time available to the crop to capture N released by mineralization, governed nitrogen availability (NA), and late maturing cultivars took up more N than early maturing cultivars. This could also apply to total P in plant tissues. However in this study a few genotypes were able to absorb relatively large amount of P despite short maturity duration. This was observed in varieties such as Dular, Mugdo, Kalamata and Paula.

Additionally, some of these with exception of Kalamata had equally less yield reduction between P applied and non-P applied plots. Therefore, this means that such varieties have the ability for high P uptake compared with other varieties of equal maturity. Meanwhile, the following genotypes; Kasalathi, Kisegese, Shingo ya mwali and TXD 88 had less total P under deficiency conditions meaning they were less able to extract it from the soil or probably P absorbed is quickly converted into other organic compounds for plant growth.

In a study by Schachtman, *et al*. (1998), they reported that under P deficiency condition there is a tendency for mobilization of P stored in older leaves and retrains-location to younger leaves and growing roots. This could partly explain the reason for low P in Kasalathi which is known to have a gene associated with high P uptake under P deficient conditions. With regards to PUE, studies conducted in crops such as *Brassica oleracea*, the QTL responsible have been reported on chromosomes 3 and 7 (Hammond *et al*., 2009). This gives the possibility that even in rice the P use efficiency could be under the control of genes but whose effects whether major or minor must be quantified. At least both PAE and PUE are known to be under the control of genes (Pariasca -Tanaka *et al*., 2014; Wang *et al*., 2014) and therefore it is possible to breed for these traits.

There are also suggestions by Repalli *et al*. (2015) that, PAE and PUE could be due to presence of certain morphologically and physiologically favourable root structure for efficient P uptake in *Pup*1 bearing varieties. However, further studies on PUE are needed, since selection for tolerance based on root studies alone may not be sufficient to tell whether a particular genotype will take up more P and produce larger biomass well.

Also given that PAE may deplete the soil P where no fertilizers are applied, the priority must be breeding for low grain P and high PUE (Vandamme *et al*., 2016a). In this study, the majority of the genotypes were found to portray mixed results regarding to the response to P fertilizer application, this means there is a possibility of interactions with other factors not currently known. For example Wang *et al*. (2014), was able to distinguish PUE traits from yield traits with corresponding QTLs and suggested that there are likely different mechanisms underlying the two traits. This explained the reason why genotypes with high uptake for P did not necessarily show increased yield under P deficient conditions whilst it is known that high P content in plant tissues would enable early root establishment hence accelerate uptake of other nutrients (Hammond *et al.*, 2009).

Nutrient mining is a big challenge due to high P depletion in many agricultural lands (Senthikumar *et al.,* 2015), especially when large proportion of P absorbed is taken into the seed. Similarly, the majority of the varieties in this study had up to 90% of the P absorbed, partitioned to the seed. Only one variety (i.e. Shingo ya mwali) had 66% of P in grains which is less than the 70 percent reported in many literatures (Rose *et al*., 2013 Vandamme *et al*., 2016a). Understanding the genetic mechanism to low grain P in this variety is fundamental to designing a breeding strategy.

The fact that there are varieties with relatively low grain P regardless of soil P status means that there is genetic control (White, 2012; Wissuwa *et al*., 2015; Vandamme *et al*., 2016b). It also raises hope that it is possible to breed for low grain P therefore further screening is needed with large number of germplasm. Lower grain P has environmental benefits because P in grains exists in the form of phytate which is not normally digestible by monogastric animals, including humans thus end up polluting sewages. Other

genotypes with close to the average such as Rafiki (76.9%) and Tule na Bwana (74%) could as well reduce P removal from the fields. On the contrary TXD 88, Sotea and Kasalath had more than 90% of the total P on the grains which may contribute to rapid removal from the fields. Since some genotypes showed consistently low levels of grain P even under adequate P supply in the soil, for intensive production systems; even a 10% reduction in grain P has much economic benefits hence the need to breed for reduced grain phosphorus.

3.6 Conclusion

Varieties with efficient use of P are regarded as the best solution for long term management of P pools especially in agricultural lands with low input. In addition, low grain P is an added advantage because much of what is absorbed by plants can be retained in the field with better management of crop residues. In this study varieties with high PUE such as Chencheria and TXD 85 were identified but in general they have high to moderate grain P. However, it is an opportunity for genetic improvement of P deficiency tolerance in a sustainable way. Regarding the relevance of low grain P in reducing P mining, more varieties should be tested to identify potential donors. Also from this study it can be said that PUE efficient varieties are not necessarily high yielding but can be source of genetic improvement of varieties with poor PUE

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CHAPTER FOUR

4.0 Enhancing phosphorus deficiency tolerance of farmer-grown rice varieties through early generation selection of *Pup1* QTL

4.1 Abstract

A marker-assisted selection (MAS) was conducted to improve the tolerance to P deficiency of three Tanzanian rice varieties TXD 88, Pishori and Tule na Bwana. The donor parents were TXD 306 (SARO 5), Supa and Chencheria which have *PSTOL*1 gene responsible for increased P uptake under deficient conditions. Derived progenies were genotyped at F_1, BC_1F_1 and BC_2F_1 and lines carrying the gene of interest (*PSTOL1)* and other diagnostic genes of *Pup1* locus *(OsPupK20-2*, *OsPupK52*) were selected for further advancement. In 2015, 152 BC_1F_2 lines were evaluated in the field at Dakawa and 20 lines that showed better performances than their respective family means were selected for advancement. In 2016, two experiments involving 20 BC_1F_3, 22 BC_2F_2 lines, 6 parents and 2 checks were established to assess their performance on a P deficient soil at Dakawa irrigation scheme. The experimental design was randomized complete block (RCBD) with three replications. Data were collected on number of tillers, number of days to 50 percent flowering, shoot biomass and grain yield. The same lines were also genotyped to confirm the presence of *PSTOL1*. The results of the analysis showed that the majority of the *PSTOL1* introgression lines showed significantly higher number of tillers and grain yield compared to the recipient parents and haplotypes without the gene. Yield gain ranged from 29.5% in a cross between Tule na Bwana and Supa to 70.1% between TXD 88 and TXD 306 among BC_1F_3 lines and between 28.5 and 61% among the BC_2F_2 lines. Furthermore, 10 BC_1F_3 lines and 16 BC_2F_2 lines had grain yield above the mean of the respective experiments. These results confirm the positive effect of *PSTOL*1 in increasing grain yield under P deficiency as well as the effectiveness of MAS in varietal improvement. Best

performing introgression lines identified in this study will be valuable materials for rice breeding programs and one or two elite lines to be identified for high and stable yield potential for release in the near future.

4.2 Introduction

Rice production in Africa continues to face challenges associated with decline in soil available nutrients and rise in fertilizer prices. Unlike other continents where fertilizers are sufficiently applied to provide soil nutrient balance, Africa as a whole has suffered many years of nutrient mining coupled with poor nutrient replenishment (Nziguheba *et al*., 2015). Thus, the nutrient balance in many soils is negative, leading to low crop yields and food insecurity (MacDonald *et al*., 2011). Therefore, feeding the growing population in the next decade will be an uphill task for African governments unless more efforts are made to address the challenges associated with high fertilizer prices and their accessibility (Larson *et al*., 2010).

The case of phosphorus is unique because it is largely a non-renewable element, thus, it can mainly be replenished through inorganic as well as organic fertilizer application. However, in many smallholder farming systems in Africa, suboptimal doses of phosphorus are applied. Besides, in much of Africa, in addition to nutrient mining through harvested crops, most soils are P fixing which further affect soil P levels.

At the same time, success in breeding high yielding rice varieties is constrained by the fact that most modern varieties require high dosage of fertilizers which most farmers cannot afford. This is due to the fact that most breeding programmes focused on improving rice tolerance of biotic and abiotic constraints including diseases, insect pests and drought and

less on low soil fertility. As a result, most rice varieties were developed without paying attention to their adaptation to low soil nutrients. Cost effective practices of rice farming are needed to ensure profitable production by rice growers and affordable consumer prices (Aluwihare *et al*., 2016a). The development and use of rice varieties suitable for low input systems is one such practices and it must include varieties that are tolerant to low soil P conditions (Vigueira *et al*., 2016).

A number of genomic regions in rice have been associated with tolerance to phosphorus deficiency (Lang and Buu, 2006; Yang and Finnegan, 2010; Gamuyao *et al*., 2012; Koide *et al.,* 2013). However, so far, Phosphorus uptake QTL *Pup1* (Wissuwa *et al.,* 1998; Wissuwa and Ae, 2001), is the most well characterized QTL for P deficiency tolerance, its underlying gene *phosphorus starvation tolerance 1 (PSTOL1)* has been cloned and its function in root growth and hence increased P uptake and yield under P deficiency conditions has been demonstrated (Gamuyao *et al*., 2012). Besides, the availability of *Pup*1 markers provides a great opportunity to improve susceptible rice genotypes through molecular breeding (Chin *et al*., 2010; Prasetiyono *et al*., 2010; Chin *et al.,* 2011).

Molecular markers are being used to shorten the breeding time with enhanced precision (Collard and Mackill, 2008; Wijerathna *et al*., 2015). With molecular markers, selection for desirable traits can be done at an early stage of crop growth. This approach when combined with early generation marker assisted selection, desirable phenotypic level can be reached within a short time (Rebaut and Betran, 1999). Early generation selection also reduces the size of the breeding population to a manageable size, thus, saving resources and time (Collard *et al*., 2005). However, the success of marker assisted breeding (MAB) is much dependent on the stability of the QTL across environments and the magnitude of

the QTL effects on complex traits (Wan *et al*., 2006; Hospital, 2009). Besides, simple traits such as disease resistance are easy to handle compared to complex traits such as yield.

Using marker assisted selection, *Pup1* QTL was successfully transferred to susceptible varieties (Chin *et al*., 2011). However, the effect of *Pup*1 on P deficiency tolerance seem to be variable as it was reported that there are varieties that are tolerant to P deficiency but do not necessarily have *Pup*1. In addition, there are also incidences of partial *Pup*1 presence where tolerance is only mild despite the presence of the *Pup1* QTL (Aluwihare *et al*., 2015). Despite these variations that may depend on the genetic background or on the testing environment (Pariasca-Tanaka *et al*., 2014; Mukherjee *et al*., 2014; Aluwihare *et al*., 2015), it will be of interest to test the effects of *Pup*1 in ESA rice varieties and see if some improvement can be obtained.

In the previous study (cf. chapter 2), it was established that more than 40 percent of the most widely grown rice varieties in ESA lack the *PSTOL1* gene. These varieties could be improved through the introgression of the entire *Pup1* QTL as suggested by Pariasca-Tanaka *et al*. (2014) hence improving yields on low P soils of ESA. Therefore, this study was undertaken to introgress *Pup1* QTL into three elite rice varieties namely Pishori, Tule na Bwana and TXD 88 which were susceptible to P deficiency, using three donor varieties. The objectives were (i) to assess if *Pup1* is effective in the genetic background of ESA rice varieties and (ii) to develop breeding lines with improved tolerance to P deficiency and increased yield levels under P deficient conditions.

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4.3 Materials and Methods

4.3.1 Plant materials

Three widely grown varieties but susceptible to P deficiency TXD 88, Tule na Bwana and Pishori were crossed with three tolerant varieties namely Supa, SARO 5 and Chencheria which possess *Pup1,* a QTL associated with P deficiency tolerance. Supa and TXD 306 are from Tanzania and carry a *PSTOL1* allele similar to that of Kasalath while Chencheria is from Mozambique with a *PSTOL1* allele similar to CG14 allele (Pariaska- Tanaka *et al.,* 2014). Similarly to the recipients, donor varieties used are elite varieties that have good traits preferred by farmers.

Table 4.1: Description of parents used in the cross

Variety name	P- deficiency tolerance	Other characteristics
TXD 88	susceptible	non aromatic, high yielding under adequate P supply
Pishori	susceptible	aromatic, good yield under adequate P
Tule na Bwana	susceptible	aromatic, good yield under adequate P
TXD 306	tolerant	aromatic, good P use efficiency (PUE)
Supa	tolerant	aromatic moderate yield under adequate P
Chencheria	tolerant	high yield under no P as well as adequate P, good PUE

4.3.2 Hybridization and marker assisted selection (MAS) of *Pup1*

The crossing program started in 2014 at SUA following a scheme described in Fig. 4.1: All parents were planted in 5 litre pots filled with soil collected from the crop museum at SUA. Optimal dozes of fertilizer were applied at sowing and pots were maintained in flooded conditions till harvest. Staggered planting was used to ensure synchrony between male (donor) and female (recipient) parents. When the time was right, plants of the recipient parents were emasculated and the pollen collected from donor plants was used to pollinate them. The pollinated panicles were bagged individually to avoid cross pollination from other sources.

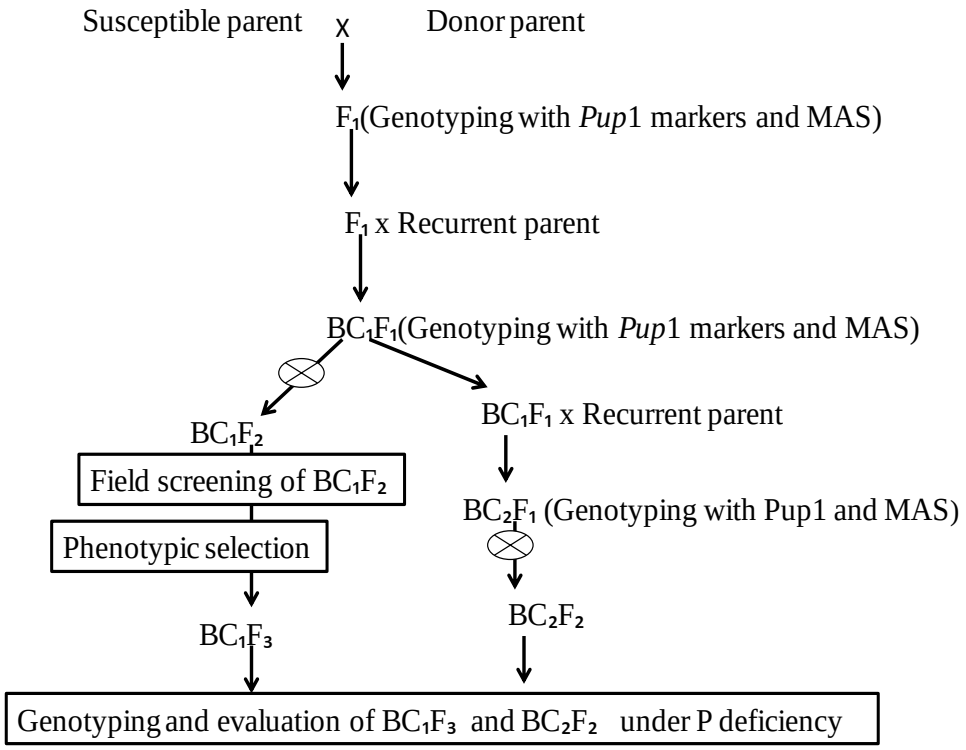

Figure 4.1: Crossing scheme to introgress *Pup*1 QTL into susceptible rice varieties

The F_1 seeds from each cross were harvested at maturity and sun dried for 1 week. They were then transferred on wet tissue papers in petri-dishes and kept in a dark room for two days to induce germination. Following germination, the seedlings were transferred in pots along with the recurrent parent for the next crossing. Fourteen days after transplanting in the pots, leaf samples were collected from F_1 plants and their respective parents to confirm *Pup1* transfer. Leaf samples were sent to Africa Rice molecular laboratory at Mikocheni for DNA extraction and genotyping as described in section 2.4. In addition to *PSTOL1* markers used in section 2.4, two other markers targeting different genes within *Pup*1 (*OsPupK20-2* and *OsPupK52*) (Chin *et al.,* 2010; Chin *et al.,* 2011) were used as diagnostic markers of the entire *Pup*1 locus.

About 22 F_1 lines with *Pup*1 were selected and crossed back to their respective recurrent parents to obtain BC_1F_1 lines. The BC_1F_1 plants were also genotyped with *Pup*1 markers and the lines without *Pup*1 were discarded. For the BC_1F_1 lines with the gene of interest, a few panicles were selfed and advanced to BC_1F_2 then to BC_1F_3 while some panicles were backcrossed to the recurrent parent to obtain BC_2F_1. The BC_2F_1 lines were genotyped to confirm *Pup*1 presence and lines without *Pup1* were discarded. The BC_2F_1 lines with *Pup*1 were advanced to BC_2F_2. Field evaluation under P deficient soil (P < 10 mg P kg^{-1} soil) was carried out for both BC_2F_2 and selected BC_1F_3 lines and *Pup1* presence was checked on phenotypically selected lines.

4.3.3 Field screening of backcross lines (BC_1F_2, BC_1F_3 and BC_2F_2)

BC_1F_2 seeds harvested from BC_1F_1 lines carrying *Pup*1 QTL were grown in 2015 at Dakawa on a P deficient field used in 2013 and 2014. Each line was planted on a single row of 5 m comprising 25 single plants spaced at 20 cm. The best 2 to 4 plants were selected based on yield and phenotypic resemblance to the recurrent parent. In 2016 BC_1F_3 seeds from phenotypically selected BC_1F_2 lines and BC_2F_2 seeds from BC_2F_1 lines selected based on marker profile were grown at Dakawa on the same field used previously. A total of 20 BC_1F_3 and 22 BC_2F_2 were evaluated including their respective parents and two checks. The experiment was laid out in RCBD with three replications. The plot size was 1 m x 1.4m. Only nitrogen (urea) and potassium (MOP) were applied at the rate of 150 kg N ha^{-1} and 50 kg K ha^{-1} respectively.

4.3.4 Data collection in the field trial and analysis

Data were collected for number of tillers, days to 50% flowering, number of panicles, grain weight, spikelet fertility, biomass yield. The collected data were analysed using

Genstat software 14th edition, (VSN International). Analysis of variance was conducted on all traits under consideration and means separation done by use of Tukey's significance test. The analysis was based on the following model:-

$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$

Y_{ijk} = the observation on i^{th} genotype on j^{th} block and k^{th} plot

μ = the overall mean of the experiment

α_i = effect of i^{th} genotype/ treatments

β_j = the effect of j^{th} the block

ε_{ijk} = random error associated with observation in the i^{th} genotype, j^{th} block and k^{th} plot

4.4 Results

4.4.1 Results of genotyping with *Pup1* specific markers

Genotyping of progenies at the different generations were to ascertain the presence of the *PSTOL1* gene throughout the generation advancement. Across all the crosses, 47 F_1 lines were genotyped out of which 26 lines had *PSTOL*1 and 26 lines carried all three target genes (Table 4.2). With regards to the high number of BC_1F_2 lines, phenotypic evaluation was carried out first and only BC_1F_3 lines derived from selected BC_1F_2 plants were genotyped and out of the 20 BC_1F_3 tested, 10 lines had all three target genes.

Table 4.2: Line selection based on marker profile at each generation

Generation	Number of lines tested	Lines with *PSTOL 1*	Lines with *OsPupK20-2*	Lines with *OsPuK52*	Lines advanced to the next generation
F_1	47	26	-	26	26
BC_1F_1	184	152	-	152	152
BC_1F_3	20	16	12	12	10
BC_2F_1	50	37	22	37	22
BC_2F_2	22	22	22	22	16

BC_1F_3 and BC_2F_2 populations

Out of the twenty BC1F3 lines which were phenotypically selected as superior in terms of yield under P deficient soils, four lines: TXD88/SARO5//TXD88-3-18, TXD88/SARO5//TXD88-1-2, TXD88/SARO5//TXD88-3-9 and PISH/CHEN//PISH -2-2, did not have *PSTOL*1 as a result of segregation (Table 4.3). The backcross population involving Pishori and Chencheria, had a *PSTOL*1 allele similar to the CG14 which means it was not tested for K46, *OsPupK20-2* and *OsPupK20-2*. On the other hand, all the 22 lines from BC_2F_2 were found to have gene of interest during genotyping.

Table 4.3: Genotypic profile of BC_1F_3 introgression lines obtained with 3 diagnostic markers of *Pup1* QTL

Entry	Entry code	Pedigree	K52	K46K	K20K	K46CG
1	B1	TXD88/ SARO5//TXD88-1-2	-	-	-	NA
2	B2	TXD88/ SARO5//TXD88-2-10	+	+	+	NA
3	B3	TXD88/ SARO5//TXD88-3-9	-	-	-	NA
4	B4	TXD88/ SARO5//TXD88-3-18	-	-	-	NA
5	B5	TB/SARO5//TB-1-13	-	+	+	NA
6	B6	TB/SARO5//TB-7-8	+	+	+	NA
7	B7	TB/SARO5//TB-10-6	+	+	+	NA
8	B8	TB/SARO5//TB-25-21	+	+	-	NA
9	B9	TB/SUPA//TB-2-13	+	+	+	NA
10	B10	TB/SUPA//TB-16-11	+	+	+	NA
11	B11	TB/SUPA//TB-12-18	-	+	+	NA
12	B12	TB/SUPA//TB-13-10	+	+	+	NA
13	B13	TB/SUPA//TB-15-4	+	+	+	NA
14	B14	TB/SUPA//TB-15-9	+	+	+	NA
15	B15	TB/SUPA//TB-2-20	+	+	+	NA
16	B16	TB/SUPA//TB-2-21	+	+	+	NA
17	B17	Pishori/Chencheria //Pishori -1-1	NA	NA	NA	+
18	B18	Pishori/Chencheria //Pishori -1-2	NA	NA	NA	+
19	B19	Pishori/Chencheria //Pishori-2-2	NA	NA	NA	-
20	B20	Pishori/Chencheria //Pishori-2-4	NA	NA	NA	+
21	IR74	Susceptible check	-	-	-	NA
22	Tule na Bwana	Recurrent parent	-	-	-	NA
23	TXD 88	Recurrent parent	+	-	-	NA
24	SARO5	Donor	+	+	+	NA
25	Pishori	Recurrent parent	-	-	-	NA
26	Chencheria	Donor	-	-	-	+
27	Supa	Donor	+	+	+	NA
28	Mudgo	Tolerant check	+	+	+	NA

(+) = amplification as expected, (-) = no amplification observed, NA = not applicable

K52: diagnostic marker of *OsPupK52*, K20-K: diagnostic marker of *OsPupK20-2* – Kasalath allele, K46-K: diagnostic marker of *PSTOL1* – Kasalath allele and K46-CG: diagnostic marker of *PSTOL1* – CG14 allele

4.4.2 Effect of P deficiency on the performance of *PSTOL* 1 introgression lines

There were significant differences for grain yield between genotypes carrying *PSTOL*1 and those without *PSTOL*1 for some crosses. In all crosses, backcross populations without

the gene had generally lower or comparable grain yield data with susceptible parents and the susceptible check. The lowest grain yield was recorded on IR74 (susceptible check) followed by TXD88/SARO5//TXD88-3-18, TXD 88 (susceptible parent) and PISHORI/CHENCHERIA//PISHORI-2-2 all of which contained no *PSTOL1* in their background. In terms of days to maturity IR74 and TXD88/SARO5//TXD88-4-18 took more days to reach maturity (136.7 and 134.3 days respectively) compared to other lines, meanwhile Mudgo (the tolerant check) reached maturity earlier than the rest at 106.1 days.

4.4.3 Yield assessment for BC_1F_3 and BC_2F_2 lines under P deficiency

BC_1F_3 populations

Because of the variation in plant type among parents, data analysis was done separately for each cross and results are presented on Table 4.4a-d. In the cross between TXD 88 and SARO 5 the resulting BC line with *PSTOL*1 i.e. TXD88/SARO5//TXD88-2-10 had the highest grain yield (945.5 g m^{-2}) and the lowest grain yield was recorded for line TXD88/ SARO5//TXD88-3-18 which is without *PSTOL1* gene (474.5 g m^{-2}). This line was also characterised by delayed flowering (99.7 days) compared to 92.7 days for TXD88/SARO5//TXD88-2-10 carrying the *PSTOL*1gene. Interestingly, two lines TXD88/SARO5//TXD88-3-9 and TXD88/SARO5//TXD88-1-2 out yielded the tolerant check despite lacking *PSTOL1*. Two backcross lines TXD88/SARO5//TXD88-2-10 and TXD88/SARO5//TXD88-3-9 had significantly high yield than the recipient parent while the remaining two were not statistically significantly different with the recipient (P=0.05)

Table 4.4a: Grain yield and other agronomic traits for BC_1F_3 lines derived from the cross between TXD 88 and SARO 5

Pedigree	Grain yield	Days to flowering	Total panicle	Days to maturity.	Tiller plant^{-2}	Straw weight
IR74 (susceptible check)	370.3a	109.3c	51.0a	137.0d	7.0ab	361.0a
TXD 88 (recipient)	554.8abc	98.3bc	56.0a	127.7c	6.0a	441.6ab
TXD88/SARO5//TXD88-4-18[a]	474.5ab	99.7c	43.0a	134.0d	9.0ab	360.4a
TXD88/SARO5//TXD88-1-2[a]	772.4a-d	97.0bc	67a.0b	125.7bc	14.0c	619.3ab
TXD88/SARO5//TXD88-3-9[a]	815.9bcd	95.3bc	81.0ab	123.3b	13.0c	610.4ab
TXD88/SARO5//TXD88-2-10	945.4d	92.7b	77.0ab	124.0bc	13.0c	805.8b
SARO 5 (donor)	863.7cd	100.0c	74.0ab	127.0bc	13.0c	736.6ab
Mudgo (tolerant check)	680.2a-d	69.7a	100.0b	105.3a	12.0bc	683.4ab
Mean	684.7	95.3	70.0	125.5	11.0	577.3
SE	25.8	1.3	8.2	0.9	2.7	15.4
CV	20.2	2.4	20.3	1.2	31.7	26.7

[a] = No *PSTOL1* in the backcross line

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

For crosses involving Tule na Bwana and SARO 5 all lines tested had *PSTOL*1 and showed higher yield than the recurrent parent and the susceptible check (Table 4.4b). Maximum yield was recorded for the line TB/SARO5//TB-25-21 (925.5 g m^{-2}) but this was not statistically different from the rest of the entries except the susceptible check IR74. The maximum tiller number (13) was recorded on SARO 5 while Tule na Bwana had the lowest number of tillers.

Table 4.4b: Grain yield and other agronomic traits for BC_1F_3 lines derived from the cross between Tule na Bwana and SARO 5

Pedigree	Grain yield	Days to flowering	Total Panicle	Days to maturity	Tiller plant^{-2}	Straw weight
IR74	370.3a	109.3d	51.0a	137.0e	7.0ab	361.8a
Tule na Bwana	715.2ab	85.7b	48.0a	121.0c	5.0a	638.8ab
TB/SARO5//TB-1-13	759.6b	86.3b	64.0ab	121.0c	9.0abc	638.8ab
TB/SARO5//TB-10-6	752.3b	87.3b	51.0a	120.3c	8.0abc	661.3ab
TB/SARO5//TB-25-21	925.5b	99.0c	65.0ab	129.0d	11.0bc	595.5ab
TB/SARO5//TB-7-8	909.3b	85.7b	68.0ab	116.3b	9.0abc	758.6b
SARO 5	863.7b	100.0c	74.0b	127d	13.0c	805.8b
Mudgo	680.2ab	69.7a	100.0c	105.3a	12.0bc	683.4ab
Mean	747.0	90.4	65.0	122.1	9.0	643.0
SE	22.4	1.0	4.4	0.8	1.1	12.4
CV	16.1	1.9	11.4	1.1	21.1	19.5

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

The backcross lines from Tule na Bwana and Supa had statistically similar grain yield. Five lines out of eight had higher grain yield than the susceptible parent Tule na Bwana (Table 4.4c). The total number of panicles was not significantly different between the lines and varieties except in the tolerant check Mudgo for which the number of panicles was much higher. However, this was not reflected in its grain weight, as it was lower than the grain weight of other lines and varieties.

Table 4.4c: Grain yield and other agronomic traits of BC_1F_3 lines derived from the cross between Tule na Bwana and Supa

Pedigree	Grain yield	Days to flowering	Total panicle	Days to maturity	Tiller plant^{-2}	Straw weight
IR74	370.3a	109.3c	51.0a	137.0c	7.0b	361.8a
TB	715.2ab	85.0b	48.0a	121.0b	5.0a	583.8ab
TB/SUPA//TB-13-10	694.9ab	80.3b	50.0a	113.0ab	6.0a	525ab
TB/SUPA//TB-12-18	709.8ab	83.7b	58.0a	118.0b	7.0ab	601.9a
TB/SUPA//TB-15-4	739.7ab	83.3b	51.0a	120.0b	8.0ab	673.2b
TB/SUPA//TB-18-6	749.0b	85.3b	63.0a	119.7b	8.0ab	695.6b
TB/SUPA//TB-2-13	831.2b	80.7b	46.0a	112.3ab	6.0a	589.6ab
TB/SUPA//TB-18-9	858.5b	85.3b	55.0a	114.0ab	7.0ab	654.6b
TB/SUPA//TB-15-9	897.0b	85.3b	50.0a	120.7b	6.0a	700.4b
TB/SUPA//TB-16-11	930.4b	83.0b	66.0a	114.0ab	7.0a	712.b
SUPA	811.8b	82.3b	58.0a	114.3ab	7.0ab	631.4b
Mudgo	680.2ab	69.7a	100.0b	105.3a	12.0b	683.4b
Mean	749.0	84.8	58.0	116.6	7.0	617.7
SE	21.4	1.1	6.3	1.9	0.94	12.8
CV	15.6	2.3	18.5	2.8	22.5	20.9

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

In the cross between Pishori and Chencheria, the backcross line PISH/CHEN//PISH-2-2 without PSTOL1 yielded less than the recurrent parent Pishori but it was not statistically significant. Only one line PISH/CHEN//PISH-2-4 outperformed the recurrent parent and it also recorded the highest grain yield among the entries in Table 4.3d with 1006.1 g m^{-2}. In all the backcross lines, the total number of panicles was less compared to the recurrent parent but their yield were comparable to that of the recurrent parent, suggesting a more effective grain filling than in the recurrent parent

Table 4.4d: Grain yield and other agronomic traits of BC_1F_3 lines derived from the cross between Pishori and Chencheria

Pedigree	Grain yield	Days to flowering	Total panicles	Days to maturity	Tiller plant^{-2}	Plant height
IR74	370.3a	109.3d	51.0abc	137.0f	7.0abc	77.6a
Pishori	752.2ab	89.7bc	77.0d	123.3de	6.0abc	136.2b
PISH/CHEN//PISH-2-2[a]	681.3ab	93.7c	71.0bcd	124.3e	10.0abc	138.0b
PISH/CHEN//PISH-1-1	735.7ab	84.7b	45.0a	120.0cde	6.0ab	132.0b
PISH/CHEN//PISH-1-2	774.4ab	84.3b	52.0abc	113.3b	7.0abc	128.6b
PISH/CHEN//PISH-2-4	1006.1b	85.0b	74.0cd	118.0bcd	12.0bc	140.6b
Chencheria	921.6b	87.3b	113.0e	116.0bc	13.0c	140.8b
Mudgo	680.2ab	69.7a	100.0e	105.3a	12.0bc	121.3b
Mean	646.2	88.0	73.0	119.7	9.0	126.9
SE	26.8	1.2	5.0	1.1	1.35	4.1
CV (%)	19.6	2.4	12.1	1.7	26.9	5.5

[a] = No *PSTOL1* in the backcross line

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

BC_2F_2 populations

Results of field evaluation under P deficient for BC_2F_2 lines are presented in Table 4.5a-d. The lines resulting from a cross between Pishori x Chencheria showed significantly higher grain weight per plot compared to the susceptible parent Pishori, but yield levels were comparable to the donor parent Chencheria and the tolerant check Mudgo (Table 4.5a). The line (PISH/CHEN)/2*PISH-1-2, had the highest grain weight and shoot biomass. It also had higher number of panicles compared to other lines but was not significant from the rest of the lines evaluated. All the lines had significantly higher grain weight compared to the recipient parent.

Table 4.5a: Grain yield and other agronomic traits of BC_2F_2 lines derived from the cross between Pishori and Chencheria

Pedigree	Grain yield	Straw weight	Days to Flowering	Total panicles	Plant height	Tiller number
IR74	427.0a	269.0a	110.0c	41.0a	99.5a	10.1b
PISHORI	500.3ab	309.8ab	88.3b	51.3ab	133.6b	7.9ab
(PISH/CHEN)/2*PISH-1-1	660.3c	520.3d	89.3b	73.0c	130.9b	9.7b
(PISH/CHEN)/2*PISH-1-2	810.3d	619.5e	89.3b	74.3c	139.4b	9.9b
(PISH/CHEN)/2*PISH - 2-1	601.0bc	368.0bc	84.7ab	62.0bc	125.6b	6.1a
CHENCHERIA	672.3c	461.0cd	91.3b	97.3d	126.8b	8.6b
MUDGO	617.5bc	458.0cd	78.3a	81.0cd	127.2b	8.2b
Mean	612.8	429.4	90.2	68.6	126.1	8.6
SE	28.9	20.75	3.9	10.37	7.12	1.1
CV (%)	11.8	12.1	4.3	15.1	5.6	13.0

Key: PISH = Pishori, CHEN = Chencheria

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

In the cross between Tule na Bwana and Supa, all the resulting backcross populations had significantly high grain yield that the recurrent parent Tule na Bwana (Table 4.5b). Also all the lines had significantly higher shoot weight than the recurrent parent. Among the lines, (TB/SUPA)/2*TB-1-1 had the highest grain yield (696 g m^{-2}) and highest number of tillers per plant.

Table 4.5b: Grain yield and other agronomic traits of BC_2F_2 lines derived from the cross between Tule na Bwana and Supa

Pedigree	Grain yield	Straw weight	Days to Flowering	Total panicles	Plant height	Tiller number
IR74	427.0a	269.0a	110.0c	41.0a	99.5a	10.1c
TULE NA BWANA	501.3ab	347.0b	88.3b	41.7a	123.3bc	5.7a
(TB/SUPA)/2*TB-1-1	696.5c	441.3c	86.3b	52.7a	119.1b	8.1b
(TB/SUPA)/2*TB-1-2	695.3c	458.0c	87.3b	51.3a	133.5c	6.3a
(TB/SUPA)/2*TB -2-1	602.0bc	384.5bc	90.0b	44.0a	126.4bc	5.9a
SUPA	644.5c	386.3bc	85.7b	53.0a	125.1bc	5.2a
MUDGO	617.5bc	458.0c	78.3a	81.0b	127.2bc	8.2b
Mean	597.8	392.0	89.4	52.1	122.0	7.1
SE	29.83	16.09	3.7	8.2	7.0	0.8
CV (%)	12.5	10.3	4.2	15.7	5.7	11.7

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

In the cross between Tule na Bwana and SARO 5 where a higher number of backcross lines were considered compared to the other cross combinations, all backcross lines had higher grain yield than the susceptible parent Tule na Bwana. Out of fourteen backcross lines, seven had significantly higher yield compared to Tule na Bwana. Maximum grain yield was recorded on line (TB/SARO5)/2*TB -2-2 (790.1 g m^{-2}) while minimum yield was recorded on (TB/SARO5)/2*TB -4-4 with 546.8 g m^{-2} (Table 4.5c) although all the lines had Pup1, out of fourteen backcross lines evaluated, seven had significantly higher yield compared to the recurrent parent Tule na Bwana.

Table 4.5c: Grain yield and other agronomic traits of BC_2F_2 lines derived from the cross between Tule na Bwana and SARO 5

Pedigree	Grain yield	Straw weight	Days to Flowering	Total panicles	Plant height
IR74	427.0a	269.0a	110.0e	41.0ab	99.5a
TULE NA BWANA	501.3ab	347.oa-d	88.3bc	41.7ab	123.3def
(TB/SARO5)/2*TB-1-1	657.8b-f	476.6a-e	86.3b	45.7ab	137.3fgh
(TB/SARO5)/2*TB -1-2	570.0a-e	423.8a-e	88.3bc	42.3ab	125.7d-g
(TB/SARO5)/2*TB -2-1	761.6ef	495.0a-e	89.0bc	50.0abc	140.1h
(TB/SARO5)/2*TB -2-2	790.1f	529.2b-e	84.7b	55.3bcd	138.1gh
(TB/SARO5)/2*TB -2-3	695.7b-f	375.0a-e	85.7b	56.0bcd	114.3bcd
(TB/SARO5)/2*TB -3-1	770.0ef	543.6cde	87.3b	64.7cd	132.6e-g
(TB/SARO5)/2*TB -3-2	572.7def	454.8a-e	87.0b	56.3bcd	124d-g
(TB/SARO5)/2*TB -4-1	655.8b-f	460.0a-e	89.7bc	48.0abc	136.1fgh
(TB/SARO5)/2*TB -4-2	765.8ef	594.0e	94.0c	43.7ab	120.5de
(TB/SARO5)/2*TB -4-3	538.1abc	325.1abc	90.3bc	46.7ab	107.1abc
(TB/SARO5)/2*TB -4-4	546.8a-d	296.3ab	87.3b	36.7a	106.1ab
(TB/SARO5)/2*TB -5-1	718.1c-f	567.3de	87.7b	69.0de	136.6fgh
(TB/SARO5)/2*TB -6-1	683.4b-f	278.6a	84.0b	48.3abc	128.1d-h
(TB/SARO5)/2*TB -7-1	739.8c-f	490.5a-e	85.3b	53.7a-d	136.1fgh
SARO 5	741.5c-f	366.3a-e	103.7d	68.0de	105.3ab
MUDGO	617.5a-f	458.0a-e	78.3a	81.0e	127.2d-h
Mean	653.0	428.3	89.0	52.9	124.4
SE	42.0	47.4	3.3	8.9	7.3
CV (%)	15.9	27.6	3.7	16.9	5.9

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

In the cross between TXD 88 and SARO 5, only two BC_2F_2 lines had *Pup*1. The grain yield of the two was higher than that of the recurrent parent and the checks although the difference was not highly significant, except with the susceptible check IR 74 (Table 4.5d). Unlike in the other crosses where a number of lines were also superior to the donor parent (Table 4-3, Table 4-5a to c); the resulting lines from this cross yielded less than the donor parent. Besides, their flowering time was significantly later than the recurrent parent which flowered at 89 days.

Table 4.5d: Grain yield and other agronomic traits of BC_2F_2 lines derived from the cross between TXD 88 and SARO 5

Pedigree	Grain yield	Straw weight	Days to Flowering	Plant height
IR74 (susceptible check)	427.0a	269.0a	110.0d	99.5a
TXD 88 (recurrent parent)	568.8ab	375.8a	89.0b	105.3ab
(TXD88/SARO5)/2*TXD88-1-1	657.5bc	393.3a	100.3c	95.6a
(TXD88/SARO5)/2*TXD88- 2-1	731.0bc	450.0a	102.7cd	99.5a
SARO 5 (donor)	741.5c	366.3a	103.7cd	123.5bc
MUDGO (tolerant check)	617.5bc	458.0a	78.3a	127.2c
Mean	624.0	385.5	97.3	108.9
SE	35.3	57.7	4.0	10.5
CV (%)	14.1	37.8	4.1	9.6

Means in the same column followed by the same letter are not significantly different at 5% probability level by Tukey's significance test

By comparing grain yield between crosses, the line with highest grain yield among BC_1F_3 was derived from a cross between Chencheria and Pishori which recorded 1006.1 g m^{-2}, while for BC_2F_2 also the line derived from cross between Chencheria and Pishori had the highest grain yield of 801.3 g m^{-2}.

4.5 Discussion

The demand for food continues to increase while the land for crop cultivation continues to decrease leading to intensive use of the available land without sufficient fallow periods. As a result, soil fertility has declined rapidly affecting crop yields. In order to overcome yield loss due to low levels of soil nutrient, farmers should apply sufficient doses of fertilizer, particularly P which is not renewable. The use of rice varieties tolerant to low-P levels can reduce the cost of fertilizer applications while providing acceptable yields. Therefore, in this study, varieties grown by farmers but susceptible to P deficiency were introgressed with *Pup*1 QTL, particularly the *PSTOL*1 gene so as to improve their performance. Since the varieties selected for improvement carry good agronomic traits preferred by farmers, some derived lines with improved P deficiency tolerance are expected to be accepted more easily than completely new varieties (Ghimire *et al*., 2015).

The results showed that, among the backcross lines carrying the *PSTOL*1 few showed significantly higher grain yield while others had less than or comparable yield values with their respective recurrent parents. At this stage it is important to note that is this stage level of segregation is still high therefore means are likely to show only small differences. In other studies, Aluwihare *et al*. (2016b) have used the extreme performance to group varieties into two categories of highly tolerant and highly susceptible, therefore the variation observed in the materials being evaluated can possibly fall into these groups as well the moderately tolerant for genotypes that fall between the two extreme values.

In this study, results also showed that, the majority the lines with *PSTOL*1 in both BC_1F_3 and BC_2F_2 populations has improved performance in terms of grain yield and other agronomic traits under P deficiency. Among the BC_1F_3 population, yield increase between

the recurrent parent and the highest yielding line in each of the crosses ranged from 29.5 in Tule na Bwana x SARO 5 to 70.1% in a cross between TXD 88 and SARO 5. Also when the average yield of all backcross populations were compared to the yield of recurrent parent; the yield increase ranged from 11 to 47%. Among the BC_2F_2, the yield increase ranged from 28.5 to 62% from a cross between TXD 88 and SARO 5 and Pishori and Chencheria respectively.

According Repalli *et al*. (2015), grain yield increase in the range of 60 to 250% as a result of *Pup1* introgression, have been reported. This increase was associated with the presence of certain morphological as well as physiological root structure that favours efficient usage of P taken. The possible explanation for the discrepancy in yield gain between the current study and those reported above could be associated with differences in the testing environment as most QTLs are affected by the environment as well.

On the contrary, backcross lines that did not have *PSTOL*1 as in the case of TXD88/SARO5//TXD88-4-18 and PISHORI / CHENCHERIA//PISHORI-2-2 had lower grain yield than haplotype lines in which the gene was present. These results further confirm that *Pup*1 plays an important role in P deficiency tolerance hence increasing yield under P deficient soils. However, it should be noted that, for some lines, despite having *PSTOL*1, had relatively low yield compared to their respective recurrent parents. Similar results were reported in previous chapters (cf. Chapter 2) also by Mukherjee *et al*. (2014), and it was suggested that these varieties may carry a non-functional *PSTOL1* (Vigueira *et al*., 2016). Another plausible explanation could be due to inhibition of gene expression arising from epistatic effects (Phillips, 2008). Therefore, it is important, as done in this

study, to use genotyping results in combination with results of phenotypic screening in order to select useful *PSTOL*1 introgession lines.

In the current study, in both BC_1F_3 and BC_2F_2 populations, the number of panicles and straw weight were in favour of the high yielding lines. Lines with high grain weight equally had relatively higher straw weight than the lines with low yield. High shoot biomass under P deficiency that emanate from good vigour at vegetative stage is considered a sign of good P uptake (Kottearachchi and Wijesekara, 2013).

In this study, the three different *Pup*1 donors used did not show much difference in yield among the resulting backcross line despite the reported variability when different donor parents are used as reported in other studies (Mukherjee *et al*., 2014). Therefore it can be said that *Pup1* present in local germplasm might have similar effect on P tolerance as Kasalath provided donor parents have been carefully selected using appropriate markers (Kottearachchi and Wijesekara, 2013). Interestingly some backcross lines between TXD 88 and SARO 5 showed higher yield than the recurrent parent despite lacking of *PSTOL1*, this suggests the presence of other factors associated with P tolerance under P deficiency also similar findings were reported by Aluwihare *et al*. (2015). Therefore further study on these lines is needed to understand the precise mechanism for their better performance under P deficiency including information on P acquisition efficiency (PAE) as well as P utilization efficiency (PUE) (Vandamme *et al.*, 2015).

4.6 Conclusion

The introgression of *Pup1* QTL in elite susceptible rice varieties was able to increase yield under P deficiency by 11% to 70% compared to the susceptible parents. This is a

confirmation that *Pup1* is involved in P deficiency tolerance thereby increasing yield. Further evidence can be drawn from the fact that backcross lines that did not have *PSTOL1* gene in their background had generally low grain yield compared to haplotypes carrying the gene. These results also suggest that *PSTOL*1 gene found in our local germpalsm is as effective as the one identified in Kasalath. Therefore, they can be used as reliable donors in genetic introgression program, besides local varieties already have traits preferred by farmers.

So far the results presented are only preliminary given the fact at BC_1F_3 and BC_2F_2 some level of segregation is still expected. Therefore further screening and selection will be needed in order to come up with better and more refined elite lines for potential release as varieties. Thus, out of the 20 BC_1F_3 and 21 BC_2F_2 lines which were evaluated under P deficiency, 10 BC_1F_3 (BC_1F_4) and 16 BC_2F_2 (BC_2F_3) had yield above their respective experimental mean. These have been selected for advancement to subsequent generation and further evaluation and selection.

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CHAPTER FIVE

5.0 Mapping of novel QTLs for Phosphorus Deficiency Tolerance among tolerant rice varieties lacking *Pup*1

5.1 Abstract

An F_3 population was developed from a cross between WITA4 and Mudgo in order to map new QTLs associated with tolerance to P deficiency. Mudgo is a tolerant variety whereas WITA4 is high yielding but sensitive to P deficiency. In this study a total of 230 $F_{2:3}$ lines were evaluated for tolerance to P deficiency based on, tiller number, grain yield data and shoot biomass at harvest. Following a selective genotyping approach, 60 extreme lines were selected out of the 230 lines, mainly based on grain yield. These were also genotyped with a total of 148 single nucleotide polymorphism (SNP) markers, well distributed across all the 12 chromosomes of rice. Based on the constructed map, a sum of four significant QTLs (LOD $\geq$ 3.0) were detected for three traits namely grain yield, tiller number and shoot biomass by composite interval mapping. Two candidate QTLs associated with grain yield were mapped on chromosome 4 and 5, while other QTL associated with tiller number and shoot biomass were mapped on chromosome 4 and 9 respectively. The QTLs for grain yield and tiller number on chromosome 4 had major effects, explaining respectively 55.8 and 32% of phenotypic variation observed and it is the first time for these QTLs to be reported under P deficiency. However, since this QTL study was based on a single experiment analysis and on selected lines, there is a need to validate the QTLs identified using the entire population and in different genetic backgrounds and environments so that their potential in genetic improvement of rice for tolerance to P deficiency can be established and utilized.

5.2 Introduction

Despite the potential of traditional rice varieties in carrying many traits of economic importance, the information on the genetic background of traits that have been discovered for African germplasm is scanty or not well documented. As a result, they are rarely used in genetic improvement programmes. Over-reliance on introduced varieties also limit adoption as most of the introduced varieties are less adapted to local conditions and often lack traits preferred by local farmers (Pandey *et al.*, 2012). Currently, rice yield is limited by low nutrient availability in many soils and requires fertilizer application to attain economic yield levels. At the same time, farmers are faced with financial constraints for purchasing fertilizers.

Efforts to break the yield barrier reached in the past decade is currently under pressure from increased costs of production because it relied much on selection of genotypes with high yield under heavy fertilizer application (Collins *et al.*, 2008). The solution to the inadequate rates of fertilizers applied by farmers requires the development of varieties which are tolerant to low soil nutrients particularly P which is the most limiting (Shen *et al.*, 2011). Availability of better screening methods backed with molecular breeding techniques offers a good opportunity to explore new sources of tolerance to low soil nutrients including P.

Despite the low yield potential of traditional varieties, a large number of them have been used as a source of essential genes in genetic improvement programs (Fujita *et al.*, 2013; Tuberosa, 2014). In addition, traditional varieties are adapted to local condition and have traits preferred by farmers and consumers. The discovery and application of molecular markers as a tool to aid plant breeders in selection, has generated a lot of information with

regard to understanding the precise location and inheritance of complex traits (Collard and Mackill, 2008). Since most agronomically useful traits existing in nature exhibit a continuous phenotypic distribution, it implies that many genes with minor effects are involved (Septiningsih *et al.,* 2003; Marathi *et al.,* 2012). This poses a challenge on successful gene transfer using conventional methods. As a result many crop improvement programs rely on indirect manipulation of these quantitative traits (Collins *et al*., 2008).

With the advent of molecular markers, the precision of gene discovery of economically important traits has been improved. Studies focusing on gene discovery has generated useful information for genetic improvement programme targeting specific production constraints as in the case of *Sub1* QTL associated with submergence tolerance (Mackill, 2006), *Saltol* for tolerance to salt affected soils (Thomson *et al*., 2010a), *Pup1* for tolerance to P deficient soils (Wissuwa *et al*., 2002; Pariasca-Tanaka *et al.*, 2014) and *qHTSF4.1* for increased spikelet fertility under heat stress (Ye *et al.,* 2015).

In Tanzania, rice is among the crops that are widely cultivated and consumed (FAO, 2015). Rapid growth in domestic and external demand coupled with heavy investment in the rice sector, will soon make the crop become number one staple. The demand is partly driven by the changing of food habit among rural and urban population and overall population growth. Besides, the fact that rice can be grown under a wide range of ecologies compared to other crops makes it special. However yields are still low due in part to poor soil fertility, and reports on low usage of fertilizer in Africa are well documented (Bekunda *et al.,* 2004; Nziguheba *et al.,* 2015). At the same time alternative strategies for improving rice yield were of variable efficiency relying much on variety introduction rather than breeding for specific stresses. Therefore rapid genetic gain to cope

with the growing demand in rice could be achieved by the use of marker assisted breeding (MAB) approach including appropriate phenotypic screening approaches (Thomson *et al*., 2010b).

Unfortunately, throughout Africa, practical application of new breeding tools that encompass molecular techniques for gene discovery and MAB are still limited. As a result, many novel genes harboured by traditional varieties in Africa, remain unknown and therefore unutilized. As production intensifies, land degradation will also increase due to the overuse of available land with limited possibilities for land fallowing which could have restored soil fertility. Tanzania and other rice growing countries in ESA have large rice germplasm which genetic potential has not been fully explored and may be at risk of disappearance due to various pressures including climate change (Rowhani *et al.,* 2011).

In order to minimize the cost of genotyping in a QTL study, three approaches are proposed namely; bulk segregation analysis (BSA) (Becker *et al*., 2011; Yadav *et al*., 2015), selective genotyping and comparative QTL mapping (Vales *et al*., 2005; Angaji, 2009; Aluwihare *et al*., 2016). These entail the use of relatively small populations to produce reliable results. The strategy is suited to breeding programs with limited resources and may enable increased discovery of new QTLs (Sun *et al*., 2010; Lee *et al.,* 2014). In a small mapping population the power to detect QTL can be increased by increasing marker density (Frisch and Melchinger, 2005; Sun *et al*., 2010).

In this study, a similar approach was used to reveal the possible existence of new QTLs associated with P deficiency tolerance in a cross between Mudgo a variety tolerant to P deficiency in different environments in Africa and Asia (Vandamme *et al.,* 2016) and

WITA4 an improved lowland variety from Africa. In previous chapters, genotypes that showed high tolerance in the absence Pup1 QTL, it was hypothesized that there are possibilities of finding new QTLs at similar or different loci. The objective of this study was to test and map these new QTLs so as enable rice breeders and diversify the range of QTLs available for genetic improvement of our local germplasm for P deficiency tolerance

5.3 Materials and Methods

5.3.1 Plant material

Two varieties WITA4 and Mudgo with contrasting response to P deficiency tolerance were used to develop a segregation population. Mudgo is tolerant while WITA4 is high yielding but moderately sensitive to P deficiency. In 2014, crosses were made and F_1 seeds obtained. Heterozygosity of the F_1 lines was confirmed using molecular markers and then they were advanced to F_2. The resulting F_2 seeds were planted in a nursery together with their parents in lowland conditions at Dakawa in 2015. Then, 400 single plants were transplanted in rows of 50 plants at a spacing of 20 cm between hills and 30 cm between rows. At maturity, individual plants were harvested separately to compose the F_3 population.

5.3.2 Field experiment

The field experiment was established at a deficient site at Dakawa, where previous experiments have been conducted since 2013. The design was an alpha lattice with three replications. A total of 230 $F_{2:3}$ lines with sufficient seed quantities and the two parents were each planted on a single row of 2 m. The spacing within rows was 20 cm and between rows was 30 cm. Only nitrogen (from urea) and potassium (from MOP) were

applied as at the rate of 150 kg N ha^{-1} and 50 kg K ha^{-1} respectively. Nitrogen was applied in three splits at transplanting, at tillering and panicle initiation stage while full doze of K was applied at transplanting. Data were collected on the number of tillers per plant, number of days to flowering, number of days to maturity, number of panicles per plant; shoot dry weight and grain yield in accordance to the standard evaluation system for rice (SES) IRRI (1996). Data were collected on each individual plant in a plot excluding the border plants then averaged to obtain the mean value.

5.3.3 Data analysis

Statistical analyses were conducted on the data collected using Genstat statistical software (VSN International) version 14, where genotypes were regarded as random effects and block as fixed effects. The model for the experiment was as shown below:-

$$Y_{ijk} = \mu + \alpha_i + \rho_j + \beta_{jk} + \varepsilon_{ijk}$$

Where:-

Y_{ijk} = the observation of the line *i* in the *k*-th incomplete block within the *j*-th replicate

μ = the overall mean

α_i = the effect of the i-th line

ρ_j = the effect of level *j*-th replicate

β_{jk} = the effect of the *k*-th incomplete block within the *j*-th replicate

ε_{ijk} = the residual error

5.3.4 Composition and genotyping of the mapping population

In 2015, leaf samples were collected from each of the 400 plants and conserved in -20°C until further use. Following a selective genotyping approach (Angaji, 2009), phenotypic

data obtained on the $F_{2:3}$ lines in 2016, particularly grain yield, was used to compose the mapping population. Out of the 230 $F_{2:3}$ lines, 30 lines from each end (i.e. high yielding and poor yielding) were selected and genomic DNA of the corresponding F_2 lines was extracted as described in section 2.4.1. The proportion of the population selected was based on the fact that for a population of 230 lines, mapping population size beyond 25 % does not increase the power to detect QTL (Sun *et al*., 2010). Prior to genotyping the F_2 lines, a polymorphism survey between the two parents WITA4 and Mudgo was conducted at LGC using 979 SNPs. Out of the 273 polymorphic SNPs, 157 well distributed across the chromosomes were selected in order to genotype the selected F_2 lines.

5.3.5 QTL analysis

The QTL analysis was carried out with the phenotypic data of the mapping population, extracted from the experiment conducted in 2016 with the 232 entries, in combination with the genotypic information from 148 SNPs. Nine SNPs that did not amplify properly were excluded from the analysis. By use of adjusted means and marker data, QTL detection was done using Genstat Breeding View (Version 3.0.1). The threshold for declaring the presence of a QTL was Logarithm of odds (LOD) equal to 3.0. SNPs located at the LOD peak of each significant QTL was used to position the QTL on the map.

5.4 Results

5.4.1 Phenotypic evaluation under P deficiency

Based on the result of analysis of phenotypic data, significant variation ($P < 0.001$) were observed for grain yield, tiller number per plant, shoot biomass, number of days to flowering, number of days to maturity and spikelet fertility. The maximum value for grain yield was 422.5 g m^{-2} while minimum was 98.9 g m^{-2}. Grain yield of both parents was

below the experimental mean. However, the difference between them was significant. WITA4 the susceptible parent had mean yield of 147 g m^{-2} while Mudgo the tolerant parent had 179 g m^{-2} (Fig. 5.1).

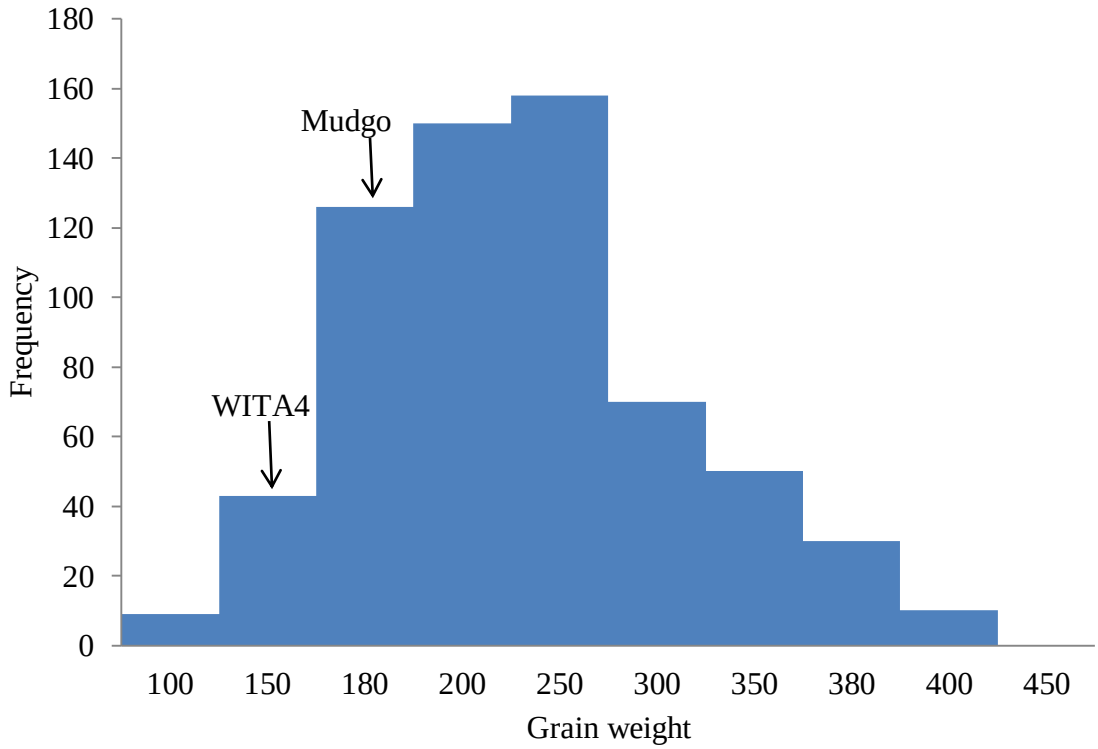

Figure 5.1: Histogram for grain weight

5.4.2 Genetic map obtained

The genetic map had a total length of 341 cM and the chromosomes are in general reasonably well covered with markers (Table 5.1). Perhaps chromosome 1 stands out as having more markers, while the opposite is for chromosome 7. The median distance between markers ranged from 1.7 to 2.6 cM. With respect to the distribution of the markers, there are no large gaps (> 20 cM) on all chromosomes as shown by the 95% percentile (ranging from 4.2 to 10.6 cM).

Table 5.1: Distribution of the SNPs markers used to genotype the mapping population on each chromosome

Chromosome	Length covered	Number of markers	Median distance between markers	95% percentile of distances
1	37.0	20	1.8	4.3
2	33.2	14	1.9	7.2
3	35.6	17	2.0	4.4
4	33.2	14	2.0	8.4
5	28.5	12	2.1	5.5
6	25.9	11	2.6	4.7
7	28.9	10	2.5	10.3
8	27.0	11	1.7	10.6
9	19.1	7	3.4	4.2
10	22.1	11	2.3	4.3
11	25.1	10	2.5	6.4
12	25.3	11	2.1	6.0
Genome	340.9	148	2.0	5.5

5.4.3 QTLs detected and their genetic effects

In the $F_{2:3}$ mapping population, two QTL candidates were detected for grain yield at chromosomes 4 and 5. Their respective markers and positions were K_id4001113 (2.5) and id5002216 (5). These two QTL candidates explained about 55.8 % and 0.0001% of the total phenotypic variance respectively (Table 5.2, Fig. 5.2). The additive value of the QTLs varied from 0.001 to 32.9g m^{-2}. Both parents contributed favourable alleles for grain yield under P deficiency. However, the QTL with the strongest additive effect came from Mudgo the tolerant donor parent while the contribution from WITA4 was negligible.

Concerning tiller number, one candidate QTL on chromosome 4 was detected. This candidate QTL explained about 32% of the total phenotypic variance and the favourable allele came also from Mudgo with a contribution of about 2 tillers per plant. This QTL candidate shared the same position as the QTL for grain yield. For shoot biomass also, one QTL candidate was detected on chromosome 9 (Table 5.2, Fig. 5.2). It explained about

13.3% of the total phenotypic variance and the favourable came from Mudgo with a contribution of 19.5 g m^{-2}.

Table 5.2: QTL detected for Grain yield shoot biomass and tiller number and their genetic effects

Trait	Chromosome	Locus Name	Marker Position	PVE*	Additive effect	High value allele	s.e.
Grain yield	4	K_id4001113	2.5	55.80	32.940	Mudgo	0.003
Grain yield	5	id5002216	3.9	0.0001	0.001	WITA4	0.002
Shoot biomass	9	id9003276	12.1	13.30	19.470	Mudgo	0.000
Tiller number	4	K_id4001113	2.5	32.0	1.79	Mudgo	0.483

PVE*: Proportion explained by the phenotypic variance

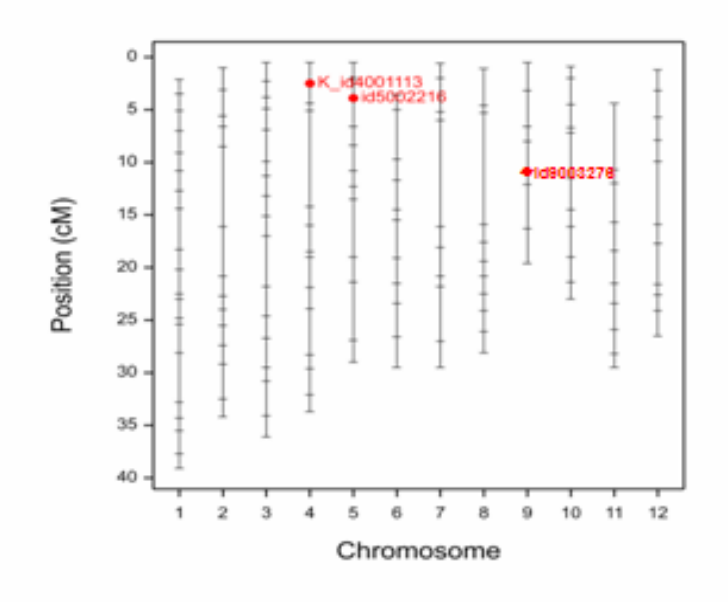

Figure 5.2: Map position of QTL candidates for grain yield, tiller number and shoot biomass detected under P deficiency conditions in a population derived from WITA4 x Mudgo

5.5 Discussion

In recent years the knowledge of QTLs effect on phenotypic variation has gained attention and hence generated a lot of interest in genetic improvement. For traits controlled by many genes where each gene contributes only a small effect, it would have been difficult to achieve substantial genetic gain in a short period using conventional breeding techniques. In the current study, both parents WITA4 and Mudgo showed relatively lower grain yield than the progenies average probably due to heterosis. In our experimental conditions, Mudgo did not show high grain yield as could be expected from a tolerant variety. Its relatively low performance in our study could be explained by some inadaptation to the soil or climatic environment at the evaluation site. This is supported by Vandamme *et al*. (2016) who confirmed its tolerance in other P deficient environments in Africa. Besides, the fact that several progenies with very high yields were observed and that all major effect QTLs (Collard *et al*., 2005) detected were from Mudgo confirmed that it is indeed a good source of P deficiency tolerance.

A total of 4 QTLs were detected for tiller number, grain yield and shoot biomass on chromosomes 4, 5 and 9 but no QTL was found for the other traits including spikelet fertility, plant height, panicle and flowering time. The presence of QTL associated with high grain yield under P deficiency on chromosome 4 has been reported in upland Nerica by Koide *et al.* (2013). However the relative position reported is quite different from the one observed in the current study, therefore it is possible that this QTL is quite novel and different from the previously reported QTLs. For tiller number the respective QTL seems to be co-localized with that of grain yield. Therefore selection of the mapping population based on grain yield led to selection for QTL for tiller number.

One of the weaknesses of selective genotyping is that, only one trait per genotyping project based on extreme data gets full attention. Genes that are less linked to the target trait may be accidentally discarded (Repalli *et al*., 2015). Since the mapping population was selected mainly on the basis of grain yield, it is possible that mapping on the entire population of 230 lines would detect more QTLs including for traits other than grain weight, tiller number and shoot biomass. The fact that tiller number and shoot biomass were strongly related to grain weight, may explain why, despite the fact that grain weight was the main target trait, QTLs with moderate to high effects were detected for these traits. According to Vales *et al*. (2005), only QTLs with major effect can be detected on a small mapping population, this means since the current study was based on a small sample size, these are major effect QTLs as well.

Grain yield is controlled by many genes because it is a function of many factors generally called yield components. A QTL that explains 56% of the variation in grain yield under P deficiency has a high potential in breeding provided that it is stable across different genotypes and environments (Collard *et al.*, 2005). In this study, the QTL for shoot biomass production were located on chromosome 9. In an independent study by Lang and Buu (2006), using a different set of parents also reported the presence of QTL associated shoot biomass production under P deficiency on the chromosome 9. Therefore such consistencies of results, is a further confirmation of the results obtained in this study. However, the precise position needs to be further studied in order to ascertain its similarity. Also in this study the size of its effect based on single environment seems to be small, and since most QTL are affected by environment (Hittalmani *et al.*, 2002), its worthiness for use in genetic improvement and must be assessed based on multi site analysis to see whether it is more stable and with large effects in other environments.

On the other hand the candidate QTL for grain yield represented by the marker id5002216; contributed very little to the total phenotypic variance despite having significant F – value. Besides, the high value allele came from WITA4 which is the susceptible parent. Based on the additive effects, the QTLs at this position contributed only 0.001g as opposed to the QTL at marker position K_id4001113 from the Mudgo which contributed 32.94 g m^{-2} of the yield increase.

Identification of the gene(s) responsible of the QTL effect is an added value as it allows design of gene-specific markers and facilitates more precise MAB. Similar work has been done with *Pup*1 QTL identified in Kasalath where many genes spanned the QTL region but not all were functional with regards to P uptake (Heuer *et al.*, 2009). Since *Pup*1 effect seems to be relatively specific, further fine mapping of the gene or set of genes involved in the QTLs identified in this study will provide additional options for targeted genetic improvement through MAB rather than relying solely on *Pup1*

5.6 Conclusion

This study revealed novel QTLs associated with tolerance to P deficiency as hypothesized. A total of 4 QTLs were detected for three traits where, two QTLs with large effect were mapped on chromosome 4 and one QTL also with major effect on chromosome 9. The following markers K_id4001113 and id9003276 were linked to QTLs with significant effect for grain yield, tiller number and shoot biomass. The results above imply there is a possibility of identifying more QTLs associated with P deficiency tolerance. However, owing to the existence of QTL x E interaction, it will be important to ascertain the stability of the candidate QTLs detected in this study in different environments and genetic background to assess its worthiness for use in breeding. For QTLs with moderate effect, it

is also important to do fine mapping using large population when resources are not limiting so as to generate a better understanding of the tolerance mechanisms. Meanwhile the results obtained in this study provide us with the information and opportunity to identify and preserve our local germplasm for use in genetic improvement and also intensify the search for new traits and genetic factors that control them.

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CHAPTER SIX

6.0 General conclusion and recommendations

6.1 General conclusion

This study was designed to address the problem of P deficiency from a genetic point of view by developing introgression lines that have improved performance on P deficient soils. Based on the results of the study yield reduction of up to 30% is very high owing to the fact that there other areas in Tanzania which have lower level of P than the one reported at the experimental site at Dakawa. This study found out that rice germplasm within ESA region have high genetic variability for tolerance to P deficiency that can be utilized in genetic improvement. The results of this study further established that, the gene associated with tolerance to P deficiency is in high proportion in traditional varieties compared to improved varieties which means they must be conserved. Also as far P uptake is concerned, long term usage of these varieties may deplete the soil therefore PUE and P partitioning are important traits for long term sustainability of P pools in the soil. However it is also important to understand that varieties with high PUE are not necessarily high yielding but could be good donors in genetic improvement programs. At the same time introgression *PSTOL1* from local genotypes showed increased yield under P deficiency compared to susceptible parents and haplotypes without the gene, this is a further confirmation that P tolerance gene in our local germplasm is affective as the *Pup1* one reported in Kasalathi. For varieties that were susceptible to P deficiency despite having *Pup1*, it possible that there are certain inhibitors in these varieties that may mask the functioning of the genes.

One notable feature is that ESA germplasm harbour new QTLs/genes associated with P deficiency tolerance other than *Pup*1 as was evidenced by the discovery of superior varieties lacking *PSTOL*1 or other *Pup*1 diagnostic genes tested. The promising QTLs that need to be validated were detected on chromosome 4, 5 and 9.

6.2 General recommendations

Based on the results of this study the following recommendations are made:

(i) The rice germplasm from east and southern Africa have a large genetic diversity which has not been well documented and utilized. Some of the popular varieties have multiple useful traits. For example Mwangaza an upland variety already known to have tolerance to RYMV also have *Pup1* QTL, SARO 5 and Supa both are aromatic also have *Pup1*. Therefore, strategic selection of donor parents that takes into consideration the presence of several traits of interest may reduce the cost of introducing individual traits separately. This study recommends further evaluation of ESA germplasm in order to identify other traits hence select the ones that have multiple resistance/ tolerance but also improve varieties that are already good in one or two traits that are essential to the target environment.

(ii) The fact that not all varieties with *Pup1* QTL are tolerant to P deficiency imply that investigation of potential donors for P deficiency tolerance must go hand in hand with a good phenotypic evaluation so as to discard varieties that may carry non-functional QTLs. This requires validating QTL effects in different environments and genetic background

(iii) Long-term use of *Pup1* bearing varieties under low-input farming condition may lead to depletion of P in the soil because of high P uptake. Varieties with good P utilization efficiency (PUE) or those which partition large proportion of P in straws and less into grains are recommended so as to ensure long term sustainability of P pools in agricultural land

(iv) The investigation on P partitioning between grain and straws indicated that genetic variability for low grain P exist in ESA rice germplasm and further screening with more varieties will be needed.

(v) New QTLs detected in this study need to be validated in different genetic backgrounds and environments in order to assess their genetic value in genetic improvement programmes.

(vi) Since some varieties with *Pup1* showed poor tolerance under P deficiency, for better breeding strategy it will be interesting to determine whether the newly detected QTLs work in a complimentary way with the already known *Pup*1 QTL or they are antagonistic to each other.

APPENDICES

Appendix 1: *Pup1* QTL specific primers and SNPs

Pup1 primers

Marker name	Expected size	Temperature	Sequence
Pup1-K20	240/243 `	55	*forward* 5'-TCAGGTGATGGGAATCATTG-3'
			reverse 5'-TGTTCCAACCAAACAACCTG-3'
Pup1-K46	523/null	58	*forward* 5'-TGAGATAGCCGTCAAGATGCT-3'
			reverse 5'-AAGGACCACCATTCCATAGC-3'
Pup1-K52	505/null	58	*forward* 5'-ACCGTTCCCAACAGATTCCAT-3'
			reverse 5'-CCCGTAATAGCAACAACCCAA-3'

Source: Chin *et al*., 2010.

List of SNPs

SNP ID	Chr.	SNP ID	Chr.	SNP ID	Chr	SNP ID	Chr	SNP ID	Chr	SNP ID	Chr
K_id1021259	1	id2012785	2	K_id4001113	4	id5000259	5	K_id8001667	8	id1000776	10
id1023854	1	id2012042	2	K_id4007212	4	id6011280	6	K_id8006792	8	id10003836	10
id1007776	1	id2008112	2	id4005526	4	K_id6002884	6	id8006485	8	id11003924	11
id1008702	1	id2009964	2	id4001817	4	id6007312	6	id8000337	8	id11006765	11
wd1001450	1	id2014452	2	ud4001019	4	id6014779	6	id8004287	8	id11004240	11
id1016322	1	id2001761	2	id4009390	4	id6010185	6	id8007896	8	K_id11011505	11
K_id1004109	1	id2009032	2	id4009900	4	K_id6006147	6	K_id8001477	8	id11009201	11
K_id1020326	1	K_id2000835	2	id4011112	4	K_id6016589	6	id8005704	8	id11001777	11
id1005271	1	ud3001808	3	id4004457	4	id6012115	6	id9000064	9	K_id11010996	11
id1006772	1	id3018268	3	K_id4011781	4	id6008704	6	id9000783	9	id11005456	11
K_id1012784	1	K_id3011233	3	id4002166	4	K_id6009055	6	id9002494	9	id11008193	11
K_id1010973	1	id3002929	3	id4005389	4	id6003446	6	id9004968	9	id11010407	11
K_id1022408	1	id3000197	3	id4000265	4	id7001003	7	id9006995	9	K_id12003862	12
id1024820	1	K_id3005145	3	id4007959	4	id7000357	7	id9003276	9	K_id12005991	12
id1014783	1	K_id3007604	3	K_id5005179	5	id7003271	7	id9001829	9	K_id12007081	12
K_id1009616	1	id3008419	3	id5014603	5	K_id7005828	7	K_id10000498	10	id12008557	12
id1001681	1	id3010173	3	id5013010	5	id7005340	7	id10006740	10	id12002490	12
id1015197	1	id3005879	3	id5004864	5	K_id7002801	7	K_id10005853	10	K_id12001321	12
id1002863	1	K_id3014650	3	id5002216	5	id7000086	7	id10004529	10	K_id12005540	12
K_id2006486	2	id3001259	3	K_id5005495	5	id7002571	7	id10002993	10	id12007672	12
id2002963	2	id3013909	3	id5003312	5	id7003593	7	K_id10001318	10	id12009798	12
id2004323	2	K_id3006808	3	K_id5004295	5	id7000740	7	id10002302	10	id12000558	12
id2003338	2	id3002060	3	id5001182	5	id8004658	8	id10000174	10	id12003141	12
ud2002021	2	ud3001370	3	K_id5007714	5	ud8001270	8	wd10001251	10		
id2011110	2	id3003854	3	K_id5009045	5	id8007144	8	id10007301	10		

Source: Generation challenge programme (GCP)

Appendix 2: Mean grain yield of top 10 and bottom 10 of the 96 varieties evaluated under P deficiency in the field and pot experiment in 2013

Field experiment

Accession	Grain yield (g m^{-2})	Tiller number / plant	Days to 50% Flowering	Days to Maturity	Straw weight (g m^{-2})
Top 10					
Kisegese	1218.6	5	88	125	682.7
Kigingi-1	1132.3	9	91.5	127.5	626
AfaaMwanza 1/159	1083.1	7	101.2	138.7	1146.6
Limota	1044.9	5	94.5	131.5	709.5
Mucandara/Redodo	1043.3	6	92.7	131.25	950.7
Chibica	1039.4	8	89.5	126.5	729.2
TXD 85	1030.2	9	104	137.5	860.6
Faya Karonga	1021.3	5	95	130.5	891.6
Katrin	1018.8	7	97	131.7	718.9
Marista	1015.2	4	94.75	134	857.7
Bottom 10					
Kachikope	521.4	5	93	132.2	821.1
Mashaka nkoge	516.4	6	90.7	128	692.5
Jambo twende	486.7	5	95.2	131.5	596.7
Meli	457.7	3	96.7	133.2	519.8
Lunyuki	416.7	3	88.2	122.5	483.1
Kalivumbula	395.3	5	103.2	136	635.6
Kasalath	394.2	5	85.7	123	556.7
Gorongosa	391.7	7	99	134.2	1131.3
Nene	355.2	3	68.7	108.5	632.5
Mwangaza	320.9	3	63.5	101	341.5
Mean	793.81	5	92.47	129.4	776.01
P(0.05)	***	**	***	***	***
CV	30	45.4	9.3	6.02	30
S.E	12.1	0.19	0.43	0.39	12.1

Pot experiment

Variety	Grain weight (g/ plant)	Tiller number /plant	Days to Floweri ng	Panicl es per plant	Straw weight (g/plant)	Root weight (g/p lant)
Top 10						
Jaribu 220	23.7	2	119.4	6	26.7	18.4
Sotea	23.5	3	124.4	8	23.4	23.4
Kigingi-2	22.2	3	116.4	6	25	11.7
Mzinga	21.8	2	130.7	4	23.4	23.4
Faya Chikuyu Manyoni	21.6	3	111.7	8	20	15
TXD 85	21.6	4	123	11	18.4	20
AfaaMwanza 1/159	21.3	4	118.7	9	26.7	23.4
Lifumba	21.3	2	106	4	20	13.4
Kilombero	21.2	2	116.4	5	23.4	18.4
Chencheria	21.1	2	126	10	31.7	20
Bottom 10						
Si mzito	12.3	2	124.7	4	15	15
Umanho	11.6	2	116.4	6	18.4	13.4
Mbawa ya Njiwa	11.6	1	109.4	4	13.4	10
Paula	11.5	2	102.7	6	11.7	8.4
Mwanza	11.1	1	109	5	13.4	11.7
Kachambo	11.1	3	99.7	7	13.4	8.4
Djisa	9.6	2	104.9	13	16.1	3.5
Nene	6.6	1	97	4	11.7	6.7
Nuwiao	6	2	97.4	5	10	8.4
Dular	4.6	1	102.9	5	11.1	3.5
Mean	17.1	2	117.1	6	19.2	16.4
P(0.05)	***	*	***	***	***	***
CV	30.7	41.0	9.1	30.8	34.4	49.6
S.E	5	0.9	10.6	1.8	6.7	8.2

Appendix 3: Genotypes that out-yielded the tolerant check under P deficiency in both pot and field experiments in 2013

With Kasalath allele	With CG14 Allele	With Niponnbare Allele
AfaaMwanza 1/159	Chencheria	AfaaMwanza
Chimdima	Kaling'anaula	Chambena
Djanibwere		Faya Chikuyu Manyoni
Faya Mafuta		Kagiha
FRX472		Katrin
Mzungu		Limota
Ngadija		Mpaka wa bibi
SARO 5		Rafiki
Sotea		Themanini
Sukari sukari		TXD 85
Supa		Wambone
Tondogoso		
Tunduru		
Chibica		