UNDERSTANDING THE GENETIC COMPONENTS OF DROUGHT TOLERANCE IN COMMON BEAN (*PHASEOLUS VULGARIS L.*)

By

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ABSTRACT

UNDERSTANDING THE GENETIC COMPONENTS OF DROUGHT TOLERANCE IN COMMON BEAN (*PHASEOLUS VULGARIS L*.)

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Common bean (*Phaseolus vulgaris. L*.) is a cheap source of protein, energy, and micronutrients including iron, zinc, vitamins, and dietary fiber for millions in the developing world. Bean production is constrained by a number of abiotic and biotic stresses, and drought stress has become the most important abiotic stress negatively affecting subsistence farming systems particularly in Africa. The overall goal of this study was to explore additional genetic diversity for drought tolerance within the Andean gene pool, and understand the genetic control of drought responses using genomic and phenometric tools. The first objective focused on the determination of the genetic architecture of yield component and photosynthetic traits at pod filling stage under terminal drought stress using a genome-wide association study (GWAS). This study involved 256 common bean genotypes from the Andean gene pool that were evaluated for two seasons (2016 and 2017) under stress and non-stress at two locations (Kasese and Namulonge) in Uganda. Several significant marker-trait associations were identified for agronomic and photosynthetic traits under drought stress conditions. Overlapping GWAS signals were detected for yield components and partitioning traits on chromosomes Pv02, Pv06, and Pv11 respectively, while colocalized signals for photosynthetic traits were identified on Pv03, Pv04, and Pv11 under drought stress conditions. Positional candidate genes, including Phvul.006G117500 encoding, plant invertase/pectin methylesterase inhibitor (INH/PMEI) superfamily protein, a positive regulator of carbon partitioning and sink strength in the reproductive stage, and Phvul.011G210000, encoding DNAJ heat shock N-terminal domain-containing protein, were

identified. The second objective focused on identifying quantitative trait loci (QTL) associated with drought tolerance in a Portillo x Red Hawk recombinant inbred line (RIL) mapping population. 97 $F_{4:7}$ RILs were evaluated under drought stress and non-stress conditions in the field for two seasons (2016 and 2017), at Kasese and one season (2017) at Namulonge in Uganda. Thirty-two significant QTL signals were identified for phenology, yield components and partitioning traits measured under drought and non-stress conditions. Colocalized QTL signals were identified for phenology, yield component traits and partitioning traits on Pv01, Pv02, Pv03, Pv04, Pv06 and Pv11. Functional annotation of positional candidate genes identified within the colocalized peak regions were consistent with the functional similarity or pleiotropic effects of those genes on the associated traits, and could be validated for marker assisted breeding. The third objective focused on the use photosynthetic traits and visual scores for high throughput screening for drought tolerance at the seedling stage. Three hundred genotypes were evaluated under stress and non-stress conditions in the green house at Namulonge, Uganda. Drought stress was applied by withholding water 21 days after planting. Significant genetic variability was observed among accessions for photosynthetic traits during early stages of drought stress, suggesting that substantial genetic variability exists among common bean genotypes from the Andean gene pool for photosynthetic traits under drought conditions at the seedling stage. Slow wilting was positively associated with recovery and could be used to identify drought tolerant genotypes at the seedling stage. The results from GWAS, QTL analysis and high throughput screening for drought tolerance at seedling stage will facilitate the screening of germplasm, and use of marker assisted breeding for drought tolerance, to improve the genetic gain for yield under drought stress in the Andean gene pool of common bean.

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CHAPTER 1: GENERAL INTRODUCTION

Common bean (*Phaseolus vulgaris* .L*)* is the most important grain legume consumed worldwide (Beebe et al., 2014; Bellucci et al., 2014). Nutritionally, bean is a cheap source of protein, energy, and micronutrients such as iron and zinc, vitamins, and dietary fiber for millions in Africa and Latin America (Broughton et al., 2003; Akibode and Maredia, 2011). In the great lakes region of eastern Africa, bean provides up to 65% of protein, 32% of energy, and vital micro nutrients (Welch et al., 2000). Highest per capita consumption of 60kg have been reported in Rwanda and western Kenya (Blair et al., 2010; Akibode and Maredia, 2011). In Uganda, common bean is a food security crop particularly for low income families, and provides up to 25% of energy; 45% of total protein, micro nutrient and dietary fiber (Namugwanya et al., 2014). Common bean has become an important component of diets in developed countries because of the health benefits associated with disease prevention namely, diabetes, mellitus, coronary heart disease and colon cancer (Thompson et al., 2009). In 2014, the total world production of common bean was 26.5 million metric tons on 30.62 million hectares of land. In comparison to the production levels in 1990, this was a 51.2% increase in production volume and about 15% increase in area cultivated (FAOSTAT, 2014). The increase in global production over the last two decades has been attributed to genetic gains and improved management practices rather than increase in area under bean production, which has expanded to marginal areas prone to drought and soil nutrient deficiencies (Siddiq and Uebersax, 2012). Latin America and sub-Saharan Africa produce the largest volume of common beans, representing more than 60% of the world production (Beebe et al., 2013). Common bean is cultivated throughout eastern and southern Africa mainly by smallholder farmers who depend on beans for their livelihoods and household

incomes. Major producers include Kenya, Uganda, Tanzania, and Rwanda (Beebe et al., 2013; Beebe et al., 2014).

A number of abiotic and biotic stresses limit crop productivity, and drought stress is the most complex and devastating abiotic stress worldwide (Pennisi, 2008). Drought events have become recurrent due to changes in global climatic conditions. These events have threatened world agriculture and global food security (Borém et al., 2012; Cavatte et al., 2012). Drought stress affects over 60% of common bean production areas, and makes it the most important yield limiting abiotic stress of common bean (Beebe et al., 2012). Drought is particularly endemic in northeastern Brazil, the north-central highlands of Mexico, and eastern Africa (Broughton et al., 2003; Beebe et al., 2012; Beebe et al., 2014). In addition, bean production in these regions continues to expand into marginal areas with greater risk of drought stress and soil nutrient deficiencies (Beebe et al., 2014). In the semi-arid areas of western US, supplementary irrigation is required for common bean production without significant yield losses (Singh, 2007). In sub-Saharan Africa, drought events are widespread, and affect over one-third of bean production areas annually (Beebe et al., 2011). This is exacerbated by the lack of irrigation infrastructure to supplement natural rainfall (Katungi et al., 2011; Beebe et al., 2014). Furthermore, current and future predictions for climate change indicate reduced amount of rainfall in major common bean production regions worldwide (Battisti and Naylor, 2009; Lobell and Gourdji, 2012). The development of high yielding, and drought tolerant common bean cultivars has become critical for improving performance under stressed and non-stressed environments (Miklas et al., 2006; Beebe et al., 2014).

Recent genomic studies support the Mesoamerican origin of common bean (Bellucci et al., 2014) with two distinct gene pools, the Middle American and Andean gene pools following

domestication (Kwak and Gepts, 2009; Brinez et al., 2012; Schmutz et al., 2014). The two gene pools have been further categorized into races based on plant morphology, adaption range, and agronomic traits. The Middle American gene pool contain races Durango, Jalisco, Mesoamerica and Guatemala (Beebe et al., 2000). The Andean gene pool contain races Peru, Nueva Granada and Chile (Díaz and Blair, 2006; Blair et al., 2007). Race Durango from the semiarid central and northern highlands of Mexico has been identified with the highest levels of drought tolerance, followed by races Mesoamerica and Jalisco (White and Singh, 1991; Singh, 2007). Introgression between Mesoamerican (type II) and Durango races (type III) have been used as source to improve and develop drought tolerant cultivars for low land tropical environments with local adapted genetic backgrounds (Frahm et al., 2004; Beebe et al., 2008; Mukeshimana et al., 2014). Most drought tolerant cultivars from these two races have shown high photosynthate remobilization potential to grain under terminal drought (Beebe et al., 2008). Although races Durango and Mesoamerica have been a reliable source of drought tolerance, transfer of drought tolerance and other favorable genes into the large seeded Andean gene pool that is widely cultivated in Africa and South America is limited (Singh and Gutiérrez, 1984; Gepts and Bliss, 1985; Beebe et al., 2013). This is due to cross incompatibility and linkage drag between the gene pools that results in low rates of valuable recombinant progenies (Beebe et al., 2013). Additional genetic diversity for drought tolerance needs to be identified to improve the larger seeded Andean beans that are highly susceptible to drought stress.

Plant response to drought stress is physiologically complex and involves a wide range of modifications, from rapid responses to long term adjustments (Galmes et al., 2013). These responses depending on timing, duration and severity of drought stress have been broadly classified as drought escape and drought resistance (Levitt, 1972; Beebe et al., 2013; Blum,

2014). Drought resistance is further subdivied into two components namely, dehydration avoidance and dehydration tolerance (Blum, 2014).

Recent studies have reported drought escape strategies in common bean and these involved greater assimilate remobilization potential of photosynthate to pod filling. Major traits contributing to better adaption to terminal drought stress include high pod harvest index, high pod partitioning index, lower proportion of pod wall biomass and pod load (Assefa et al., 2013; Beebe et al., 2013; Mukeshimana et al., 2014). In addition, differences in photosynthetic remobilization capacity among genotypes were used to improve drought tolerance in small red and black seeded classes by selecting for improved seed filling and seed quality under terminal drought (Beebe et al., 2008). Similarly, dehydration avoidance maximizes water uptake through increased rooting depth and hydraulic conductance from the deeper soil layers while balancing water loss by reducing leaf conductance, and evaporative surface (Beebe et al., 2013). However the root to shoot biomass ratio is important to define the functional balance between water uptake by the root and photosynthesis by the shoot. In rice, alteration of root architecture using the Deeper Rooting 1 (DRO1) genes has significantly improved yield performance under drought conditions (Uga et al., 2013). Positive yield advantages associated with rooting depth under drought conditions have been reported in grain legumes such as common bean, soybean, and cowpea (Devi et al., 2010; Beebe et al., 2013; Belko et al., 2013).

Furthermore, another widely used mechanism by plants to protect cell integrity under drought stress is dehydration tolerance that involves accumulation of molecules with detoxifying capabilities or antioxidants. These molecules include superoxide dismutase, glutathione reductase and ascorbate peroxidase that detoxify and limits reactive oxygen species (ROS) that damage sensitive organelles like chloroplasts, mitochondria and peroxisomes during drought

stress (Farooq et al., 2009). Other improtant molecules include late embryonic abundant (LEA) dehydrin proteins with hydrophilic properties that protects DNA, stabilizes cytoskeletal filament, and act as molecular chaperone and anti aggregants, enhancing structural and stability to proteins (Moore et al., 2009). Another strategy involves compartmentalization of ions and compatible solutes such as soluble sugars, sugar alcohols, amino acid proline, glycinebetaine, organic acids, and potassium and calcium in the cytoplasm to maintain water potential equilibrium (Taiz and Zeiger, 2010). Osmotic adjustment in the root meristems enables plants to extract more of the tightly held water in order to maintain root turgor and root growth (Taiz and Zeiger, 2010; Blum, 2011) . These responses tend to overlap under drought stress; however, the physiological and molecular basis of these interacations is not well understood.

In order to develop common bean cultivars with improved performance under drought stress, knowledge of physiological mechanisms and genetic control of the traits contributing drought tolerance at the different stages of plant growth is critical. Previous studies identified traits associated with drought tolerance in common bean. These include deep and balanced root systems, phenology traits, biomass accumulation, accelerated partitioning of photosynthate towards seed yield components under drought stress (Acosta-Gallegos and Adams, 1991; Schneider et al., 1997; Ramirez-Vallejo and Kelly, 1998; Beebe et al., 2008). Selection for high yield potential and effective drought resistance mechanisms in the same genotype in the target environment has been the most practical way to achieve yield stability under drought stress while also maintaining high yields in higher rainfall seasons (Schneider et al., 1997; Beebe et al., 2008; Mukeshimana et al., 2014). However, this selection approach has been difficult to implement in highly unpredictable target environments, and in situations where interactions between heat and drought are difficult to separate (Boyer et al., 2013).

The sequencing of the common bean reference genome has led to recent advances in development of single nucleotide polymorphisms (SNP) marker technologies and genotyping arrays that have enhanced the possibility of generating dense genome-wide markers. This creates new opportunities for dissecting quantitative traits into their single genetic determinants through quantitative trait loci (QTL) and association mapping so that individual locus can be targeted for marker-assisted selection (MAS) with repeatability (Mukeshimana et al., 2014; Schmutz et al., 2014) .

Previous studies have identified QTL for drought tolerance traits in common bean. QTL for rooting patterns and root architectural traits have been reported in the DOR364 x BAT477 inter gene pool population (Asfaw et al., 2012; Blair et al., 2012) . In addition, a number of QTL analysis have shown the co-localization of phenology, yield and yield components, and root traits on the same chromosome regardless of mapping population and marker system used (Cichy et al., 2009; Asfaw et al., 2012; Mukeshimana et al., 2014). However, most QTL analysis studies for drought tolerance in beans have been conducted within the Middle American gene pool and inter-gene pool crosses (Schneider et al., 1997; Asfaw et al., 2012; Mukeshimana et al., 2014). In order to explore additional diversity for drought tolerance, QTL analyses for drought tolerance is critical in the Andean gene pool to identify the genetic potential and map genomic regions for populations developed from crosses within the Andean gene pool. This will provide an opportunity to analyze the effect of genetic backgrounds on QTL expression and accelerate identification of alternative sources of drought tolerance genes within the Andean gene pool. The availability of the SNP markers has enabled high throughput genotyping of populations, generating high-density maps, and precise identification of QTL that will enhance understanding of underlying genetic basis and physiological mechanism. Developing a strategy to improve

large seeded Andean types widely grown in East Africa and South America is critical (Bitocchi et al., 2013; Mukeshimana et al., 2014).

Genome wide association studies (GWAS) have become another powerful tool to identify marker allele association with quantitative traits and for gene discovery. GWAS takes advantage of early recombination events in a diverse population to identify the genetic loci underlying traits with relatively high resolution, thus reducing research time with greater allele numbers (Huang and Han, 2014). Recent GWAS studies in common beans have identified genomic regions associated with agronomic traits, phenology traits, yield traits and cooking time (Cichy et al., 2015; Kamfwa et al., 2015; Hoyos-Villegas et al., 2017). With the available and ever increasing number of SNP markers the easy of identifying genomic regions associated with important traits with high resolution is enhanced in common bean.

Additional traits associated with critical physiological processes such as photosynthesis needs to be investigated in common bean. Drought stress restricts CO_2 assimilation stimulating reduction in the photochemical Chl fluorescence quenching, photosystem II quantum yield in common bean (Dias and Brüggemann, 2010). The use of photosynthetic traits associated with drought sensitive components of photosynthetic process indicators of stress sensitivity among genotypes will improve the efficiencies of breeding for abiotic stress tolerance. The role of photosynthetic traits that relate to photosystem II quantum yield (Φ_{II}), non- photochemical excitation quenching (NPQ), total chlorophyll content, stomatal conductance, transpiration rate, leaf temperature, and leaf water potential needs to be investigated and better understood in common bean (Lizana et al., 2006; Dias and Brüggemann, 2010). The availability of versatile and robust next generation phenotyping technology such as the MultispeQ makes it possible to measure photosynthetic traits (Kuhlgert et al., 2016). Previous studies reported significant natural variation in photosynthetic

rates per unit leaf area as well as tolerance to drought (Wentworth et al., 2006). The MultispeQ can be deployed under a range of environmental conditions (temperature, humidity, CO_2 levels, intensity and quality, time, and location), which could improve phenotype-assisted breeding approach to transfer drought resistance traits into productive lines. Additionally, the importance of photosynthetic electron transport as a drought tolerance trait for common bean will be critical to breed for yield under drought conditions (Asfaw et al., 2012). Therefore, breeding for drought tolerance in common bean will require identifying critical plant tolerance responses, understanding of physiological mechanisms, and genetic control of traits contributing to critical plant developmental stages in the same environment.

This study seeks to determine the genetic architecture of drought adaptive alleles in the Andean diversity panel using genome wide association study, to identify QTL associated with drought tolerance in Portillo x Red Hawk recombinant inbred line mapping population, and determine the use of photosynthetic traits as drought sensitivity indicator of adaptive physiological response under drought conditions in common bean.

CHAPTER 2: DETERMINATION OF THE GENETIC ARCHITECTURE FOR DROUGHT ADAPTIVE ALLELES IN THE ANDEAN DIVERSITY PANEL USING GENOME WIDE ASSOCIATION STUDY

Abstract

A genome-wide association study (GWAS) was conducted to determine the genetic architecture of yield components and photosynthetic traits in 256 genotypes from the common bean (*Phaseolus vulgaris* L.) of Andean gene pool grown under drought conditions. The panel was evaluated under drought stress and non-stress conditions at two locations for two seasons in Uganda and genotyped with 5398 single nucleotide polymorphism (SNP) markers on the BARCBean6K_3 BeadChip (6K Beadchip) and 265,704 (265K) SNPs generated using genotyping by sequencing (GBS). We identified 44 significant marker-trait association signals for agronomic and photosynthetic traits using best linear unbiased predictors, 24 for agronomic traits, and 20 for photosynthetic traits under drought stress conditions with the 6K Beadchip. In addition, 56 association signals were detected for agronomic with 265K SNP data. Colocalized GWAS signals were detected for pod weight per plant and harvest index on chromosome Pv02, number of pods per plant, pod weight per plant, and seed yield per plant on Pv06, and for pod weight per plant, number of seeds per plant, and 100 seed weight on Pv11, respectively. Colocalized signals for photosynthetic traits were also identified for Phi2 and PhiNO on Pv03, for PhiNPQ and linear electron flow (LEF) on Pv04, and for PhiNPQ, LEF and Phi2 drought stress on Pv11 under drought stress conditions. Positional candidate genes, including *Phvul.006G117500* on Pv06, associated with pod weight per plant, and seed yield per plant and *Phvul.011G210000* on Pv11, associated with LEF, Phi2, and PhiNPQ, were identified. SNP ss715640054 was located 8.7kb downstream of *Phvul.006G117500*, annotated as Plant invertase/pectin methylesterase inhibitor (INH/PMEI) superfamily protein, recognized as a

positive regulator of carbon partitioning and sink strength during seed development in beans. SNP marker ss715640406 was located 28.3kb upstream of *Phvul.011G210000,* annotated as DNAJ heat shock N-terminal domain-containing protein, which acts as a molecular chaperone, protecting plants cells against drought and heat effects. This study provides insights into the genetic control of drought stress responses in Andean beans and significant genomic regions were identified that could be useful for marker assisted breeding to improve drought tolerance in the large seeded beans.

Introduction

Common bean (*Phaseolus vulgaris*. L) is the most important grain legume consumed globally (Bellucci et al., 2014). Common bean is considered a food and nutritional security crop, and provides a cheap source of proteins, energy, and micro nutrients such as iron and zinc, vitamins, and dietary fiber for millions of people in sub Saharan Africa and Latin America (Broughton et al., 2003; Akibode and Maredia, 2011). However, common bean production is constrained by a number of abiotic, and biotic stresses, and soil nutrient deficiencies (Beebe et al., 2012). Drought stress is the most important yield limiting abiotic stress of common bean, and affects over 60% of common bean production areas worldwide (Beebe et al., 2013). Drought causes significant reduction in biomass, pod set, and seed yield of common bean (Nielsen and Nelson, 1998; Ramirez-Vallejo and Kelly, 1998). These effects have become very pronounced in major bean production areas because of continued expansion of production into marginal areas with greater risk of drought stress (Beebe et al., 2011). In sub-Saharan Africa, drought events are widespread, and affect over one-third of bean production areas annually (Beebe et al., 2014). Developing high yielding, and drought tolerant common bean cultivars is the most sustainable approach to improve productivity under stressed and non-stressed environments particularly for smallholder farmers in developing countries (Miklas et al., 2006; Beebe et al., 2012).

Plant responses to drought stress are physiologically complex and involves a wide range of adaptive mechanisms, from rapid responses to long term adjustments (Galmes et al., 2013). These adaptive mechanisms depend on timing, duration and severity of drought stress and have been classified as escape, avoidance, tolerance and recovery strategies (Levitt, 1980; Blum, 2011; Beebe et al., 2013). In common bean, adaptive strategies that enhance drought tolerance have been reported and these include deeper rooting systems, efficient use of water for

photosynthesis and biomass accumulation, and efficient remobilization of photosynthate to seed under drought stress (White et al., 1994; Rao, 2001; Beebe et al., 2008; Beebe et al., 2014). Recent studies reported that greater assimilate remobilization potential of photosynthate to seed as an important strategy to escape terminal drought (Beebe et al., 2014). Major determinant traits associated with this strategy are yield related and include high seed yield, pod harvest index, pod load, and lower proportion of pod wall biomass (Beebe et al., 2013; Mukeshimana et al., 2014). Breeding and selection for greater remobilization potential of photosynthate to seed has resulted in the identification of superior landraces from race Durango and development of drought tolerant breeding lines such as SEA5, and SEA15 and a number of advanced breeding lines in small red and black bean market classes (Terán and Singh, 2002; Beebe et al., 2008).

In common bean, race Durango from the semiarid central and northern highlands of Mexico has been identified with the highest levels of drought tolerance, followed by races Mesoamerica and Jalisco (White and Izquierdo, 1991; Singh, 2007). However, moving drought tolerance and favorable genes from races Durango and Mesoamerica into the large seeded Andean gene pool has been limited (Singh and Gutiérrez, 1984; Gepts and Bliss, 1985; Beebe et al., 2011). This is due to cross incompatibility and linkage drag between the gene pools that results in low rates of recovery of valuable recombinant progeny (Beebe et al., 2013).

Additionally, in east and southern Africa, Europe and some parts of Latin America, over 70% of common beans cultivated are large seeded types of Andean origin, and are generally susceptible to many abiotic and biotic stresses (Wortmann, 1998; Beebe et al., 2013). Genetic improvement of Andean beans for yield, abiotic and biotic stress has lagged behind the Mesoamerican beans because of narrow genetic diversity and a lack of breeding efforts (Gepts and Bliss, 1988; Bellucci et al., 2014; Cichy et al., 2015). The assemblage of global Andean diversity panel

(ADP) that consists of common bean breeding lines and landraces should enhance the exploration of genetic diversity and identification of unique sources of valuable genes for different traits in Andean beans (Cichy et al., 2015).

Previous studies identified traits associated with drought tolerance in common beans of diverse genetic backgrounds (Acosta-Gallegos and Adams, 1991; Schneider et al., 1997; Ramirez-Vallejo and Kelly, 1998; Beebe et al., 2008). Additional traits associated with critical physiological processes such as photosynthesis need to be investigated in common bean. Previous studies reported significant natural variation in photosynthetic rates per unit leaf area as well as tolerance to drought (Wentworth et al., 2006). With the invention of the MultispeQ, a versatile and robust high throughput, hand-held, photosynthetic phenotyping tool, assessing photosynthetic traits under multiple environmental conditions will improve phenotype-assisted breeding by quickly identifying drought resistance traits (Kuhlgert et al., 2016). Additionally, understanding photosynthetic dynamics that relates to adaptation in multiple environments should assist decision making to improve common bean yields under drought conditions.

Furthermore, the sequencing of the common bean reference genome has led to recent advances in development of SNP marker technologies and genotyping arrays that have significantly enhanced the possibility of generating dense genome-wide markers (Schmutz et al., 2014; Song et al., 2015).

The availability of dense genome wide markers has enabled the identification of marker alleles that contribute to causative variation in quantitative traits using genome wide association studies. GWAS analysis takes advantage of early recombination events in a diverse population to identify the genetic loci underlying traits with relatively high resolution, thus reducing research time with greater allele numbers (Huang and Han, 2014). In common bean, GWAS has been used to

identity genomic regions associated with agronomic traits, phenology traits, yield traits, and cooking time in Andean and Mesoamerican gene pools (Cichy et al., 2015; Kamfwa et al., 2015; Hoyos-Villegas et al., 2017). In order to explore the genetic diversity and understand the genetic control of agronomic and photosynthetic traits contributing to drought tolerance in common bean of Andean gene pool, a GWAS was initiated to determine the genetic architecture of terminal drought tolerance in the Andean diversity panel grown under drought stress at two locations in Uganda.

Materials and Methods

Plant materials

The plant materials used in this study consisted of 256 bean genotypes from the ADP. The panel is comprised of a diverse collection of varieties, and landraces with varied seed types and market classes mainly from Africa, South America, Central America, and North America (Cichy et al., 2015). The panel is a global representative sample of the genetic diversity present in the Andean gene pool.

Field phenotyping

The ADP panel was evaluated for drought stress in the field at Mubuku Irrigation Experimental Station, Kasese and at the National Crop Resources Research Institute (NaCRRI), Namulonge in 2016 and 2017 growing seasons in Uganda. The two seasons hereafter referred to as Kasese_2016, Kasese _2017 and Namulonge_2016, Namulonge_2017, respectively. In 2016, 247 genotypes were evaluated; and the number was increased in 2017 to 256 after additional ADP lines were provided by CIAT Uganda. The Mubuku Irrigation Experimental Station is located in Kasese Western Uganda at 0^{o}16’10” N, 30^{o}6’9” E at an elevation of 1330 m above sea

level and receives 1000 mm of rainfall annually that is irregularly distributed across seasons (Figure.1). The soils were characterized as sandy loam with a pH of 5.7. The National Crop Resources Research Institute (NaCRRI), Namulonge is located in Central Uganda at 0^{o} 31'30" N, 32^{o}36'54" E at an elevation of 1160 m above sea level. The average temperatures are 21.8 ^{o}C, ranging from 15.8 ^{o}C to 27.9 ^{o}C. Namulonge receives 1300 mm of rainfall annually (Figure.1), and soils are characterized as oxisols with a pH of 5.8. The results of soil nutrient analysis indicated an average low available Phosphorus and Nitrogen where there were below the 15ppm and 0.2% respectively in both sites and NPK was applied at a rate of 50 kg/ha to enhance soil nutrient levels. The ADP lines were evaluated side by side under non-stress (NS) and drought stress (DS) conditions. In 2016, the experiments were planted in one row plots of 2 m long and 0.5 m between rows. In 2017, experiments were two rows 2 m long and 0.5 m between rows. For the two seasons, the experiments were planted in 16x16-lattice design with two replications. Planting was done during the mid-rainy to end of season to target terminal drought stress at the reproductive stage of plant growth. In Kasese, the first experiment was planted on 28 Nov 2016 and harvested on 26 Feb 2017 and the second field experiment on 15 May 2017 and harvested on 11 Sept 2017. Drip irrigation was used to irrigate both DS and NS to ensure good germination and plant establishment until flowering time. Irrigation was discontinued at flowering following emergence in the DS plots while supplemental irrigation was continued in the NS plots twice a week up to harvest maturity. The first experiment in Namulonge was planted on 19 Oct 2016 and harvested on 16 Feb 2017 and second season experiment was planted on 18 June 2017 and harvested on 30 Sept 2017. Overhead sprinklers were used to irrigate both DS and NS to ensure good germination and plant establishment until flowering time. Supplemental irrigation was continued in the NS plots twice a week up to harvest maturity while irrigation was discontinued

in the DS plots following emergence. In both locations and seasons, recommended agronomic procedures were observed. Data was collected for daily weather conditions including daily rainfall (mm) and temperature (^{0}C) during the growing seasons. Phenotypic data was collected on days to 50% flowering (DF), days to maturity (DM). In order to determine shoot biomass, yield and yield component traits, six plants were sampled from every plot for every genotype and sun dried. These were then used for measuring shoot biomass (BM), number of pods (PN), pod weight (PW), seed weight per plant (SY), Number of seeds per plant (SN), and 100 seed weight (100SWT). In both seasons, SY and 100SWT were calculated after adjusting for seed moisture to 16%. Harvest index (HI), pod harvest index (PHI), and pod partitioning index (PPI) were also computed. The severity of drought stress in each environment for yield was quantified using drought intensity index (DII) as 1– (Xds/Xns) where Xds and Xns are the mean value for yield under drought stress and non-stress, respectively. Geometric mean (GM) of seed yield as (NS x DS)$^{1/2}$ where DS and NS are seed yield for drought stress and no stress treatments, respectively (Fischer and Maurer, 1978). In addition, photosynthetic traits included linear electron flow (LEF), quantum yield of photosystem II (Phi2), ratio of incoming light that is lost via non-regulated processes (PhiNO), and ratio of incoming light that goes towards non-photochemical quenching (PhiNPQ) measured during pod filling stage using the MultispeQ (Kanazawa and Kramer, 2002; Kuhlgert et al., 2016). Data for photosynthetic traits was taken at 8-9 weeks after planting and used for analysis.

Genotyping

The ADP panel consisting of 396 accessions was genotyped using Illumina (Illumina Inc., San Diego, CA) BARCBean6K_3 Beadchip (6K Beadchip) with 5398 SNP markers distributed across the 11 the chromosomes of common bean (Song et al., 2015). The second set of marker

data consisted of 265704 (265K) SNPs generated by Oladzad and McClean (unpublished) for 325 ADP accessions using GBS.

Data Analysis

Statistical analysis for agronomic traits was performed using best linear unbiased predictors (BLUPs) which minimizes environmental, and seasonal effects, were used for GWAS analysis. The mixed model in R statistical package 'lme4'(Bates et al., 2014) was used to determine and extract the random effects (BLUPs) of the phenotypic traits. BLUPs for traits were calculated based on following statistical model;

$$Yijk = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \beta\gamma_{jk} + \varepsilon_{ijk}$$

Where Y_{ijk} is the response variable, μ is the mean, α is the effect of the i^{th} genotype, β represents the effects of the j^{th} treatment, γ corresponds to the k^{th} season and ε_{ijk} is the random error. Pearson correlation among variables was analyzed using the PROC CORR command using SAS 9.4 (Institute, 2011).

Population Structure and Marker-Trait Association Analysis

A total of 5326 SNPs remained after removing low quality and monomorphic SNPs on the 6K Beadchip. These were then filtered for minor allele frequency (MAF≥ 0.05) leaving 4393 SNPs. The 265K markers were filtered for minor allele frequency (MAF≥ 0.05) leaving 228K markers. The two data sets were later used for kinship, population structure and association analysis. Population stratification can confound GWAS results causing spurious association. Population structure in the association panel was estimated using the principal component analysis (PCA) method in TASSEL (Bradbury et al., 2007). The number of principal components used was based on the proportion of variation accounted for by the principal components. This was

determined using the Genome Association and Prediction Integrated Tool (GAPIT) (Lipka et al., 2012), and the suggested number of principal components was equal to the number of subpopulations (K). Relatedness within the panel was determined by calculating the kinship matrix using identity by descent method in TASSEL (Bradbury et al., 2007). Mixed Linear Model (MLM) (Zhang et al., 2010) was used to determine marker trait associations implemented in TASSEL (Bradbury et al., 2007) explained as;

$$Y=X\alpha +P\beta+K\mu+\varepsilon$$

Where Y is the vector of observed phenotype; X is the fixed effects of SNPs; P is the fixed effects of population structure; K is the random effects of kinship; ε is the random error. Significance threshold for p-values resulting from association analysis was determined using the false discovery rate (FDR) criteria (Benjamini and Hochberg, 1995). SNPs were declared significant after applying an FDR adjusted p value of less than 0.05 as a threshold. Candidate genes associated with significant SNPs were identified using the Jbrowse on Phytozome v12 (Goodstein et al., 2011) to browse the common bean genome v2 (Schmutz et al., 2014). TASSEL (Bradbury et al., 2007) was used to determine linkage disequilibrium (LD) among or between pairs of markers in the genomic regions associated with significant SNPs. A gene was considered a candidate gene if it was within LD block of genomic region associated with the significant SNP and the protein coded by the candidate gene plays a role in regulating drought stress response and seed yield under drought stress.

Heritability

Heritability estimate for traits was determined using mixed models in GAPIT R package (Lipka et al., 2012), and variation due to the genetic component was calculated by considering genotype

as a random effect in the model. In addition, the genetic variance for a diversity panel was calculated as the ratio of phenotypic variance associated with family relatedness (kinship) among accessions in the collection. Heritability was estimated as;

$$h^2 = \frac{{\sigma_a}^2}{{\sigma_a}^2 + {\sigma_e}^2}$$

Where $\boldsymbol{\sigma_a^2}$ is the additive genetic variance associated with variance component of the vectors **u** and **e** from

$$\mathrm{Var}\begin{pmatrix} \boldsymbol{u} \\ \boldsymbol{e} \end{pmatrix} = \begin{pmatrix} \boldsymbol{G} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{R} \end{pmatrix}$$

Where $\mathbf{G} = \boldsymbol{\sigma_a^2}\mathbf{K}$ with $\boldsymbol{\sigma_a^2}$ as the additive genetic variance and **K** as the kinship matrix, homogenous variance is assumed as the residual effect represented as $\mathbf{R} = \boldsymbol{\sigma_e^2}\mathbf{I}$, where $\boldsymbol{\sigma_e^2}$ is the residual variance (Lipka et al., 2012).

Results

Rainfall

The average temperature and daily rainfall recorded during the 2016 and 2017 growing season at Kasese and Namulonge are reported in Figure1.1. In Kasese, a total of 144 mm was recorded during the 2016 (October-February) and 180 mm of precipitation recorded for 2017 (May-September) growing season. In both growing seasons, the rainfall patterns were inconsistent and most rains were recorded during the vegetative stage of growth (122 mm and 90 mm) in 2016 and 2017 respectively, which is below the minimum 400 mm required for normal growth of common bean (Wortmann et al., 1998). In addition, only 22 mm and 16 mm of precipitation were recorded during the reproductive and pod filling stages (before 90 days after planting),

subjecting the field experiments to severe terminal drought stress (Figure 2.1 a&b). In Namulonge higher rainfall was recorded and relatively uniformly distributed during the growing season of 2017 than in 2016, a total of 160 and 216 mm of precipitation were recorded during 2016 and 2017 growing season respectively (Figure 2.1c &d). In addition, greater precipitation was recorded during the vegetative stage (129 mm and 101 mm) and only (28 mm and 52 mm) was recorded during the reproductive and pod filling stage (before 90 DAP).

Field Experiments

The overall drought severity observed in this study was moderate based on the DII for SY (0.40) measured across the two locations for the two years. More severe effect of drought on seed yield was observed in Namulonge_2016 where DII for SY was 0.58 compared to 0.30 observed in Kasese_2016. Highly significant differences ($P<0.001$) were observed among 256 genotypes for agronomic traits measured under NS and DS conditions in Kasese and Namulonge for the two seasons of 2016 and 2017 (Table 2.1 a&b). Higher mean performance was observed in 2017 at both sites compared to 2016 (Table 2.1 a&b). This was due to a severe bean fly infestation at the onset of germination that affected plant establishment in 2016 at both sites. Significant differences ($P<0.01$) were observed among 256 genotypes for photosynthetic traits such as LEF, SPAD, Phi2, PhiNO, and PhiNPQ (Table 2.2). In addition, highly significant and positive correlations were observed among yield component traits such as, PN, PW, SY, SN, and among partitioning traits such as HI, PHI, and PPI. Significant positive correlations were observed between BM, HI, PHI, PPI and yield component traits under DS conditions in Kasese_2017. Highly significant positive correlations were observed among photosynthetic traits such as Phi2 and PhiNO. However, these traits were negatively correlated with PhiNPQ. Additionally, most

yield component traits were not correlated with photosynthetic traits with exception of relative chlorophyll content under DS conditions in Kasese_2017 **(**Table 2.3**).**

Population Structure

The results of the principal component analysis showed a subpopulation structure among genotypes in the panel used in this study. The principal components were determined using the 228K markers. The first four principal components accounted for 41.2, 5.2, 4.6, and 3.5% variation observed among genotypes in the panel respectively. A plot of PC1 against PC2 indicated the presence of three clusters (Figure.2). The smallest cluster consisted of 11 individuals; seven were landraces from Africa, three from South America, and one from North America. The second cluster comprised of 23 genotypes, of which seventeen were landraces from Africa, seven from South America, and three from North America. The third cluster consisted of 222 genotypes mainly landraces, cultivars, and elite lines of diverse origin. The first three PCs accounted for 51 % of variation in the genotypes and were considered in MLM for association tests in TASSEL (Bradbury et al., 2007).

Marker-Trait Associations

Significant marker trait associations were identified for agronomic and photosynthetic traits using both the 6K Beadchip and 265K GBS marker data. The agronomic traits measured in this study included NP, PW, SN, SY, and HI, and the photosynthetic traits included LFF, Phi2, PhiNPQ, and PhiNO measured under drought stress conditions (Table1 4a & b).

Number of pods per plant (NP)

Several significant marker trait associations were detected for NP under drought stress conditions using both the 6K Beadchip and the 265K GBS marker data. Three SNPs were significantly

associated with the NP under drought stress conditions in Kasese with the 6K Beadchip. SNP (ss715641140*; P=7.5x10*$^{-06}$) at 32.72Mb on Pv10 with minor allele frequency (MAF) of 0.12 was the most significant and explained about 9% of the variation. Two significant SNPs were detected on Pv06, and marker (ss715639365; *P=4.2x10*$^{-05}$) at 31.37Mb with MAF of 0.11 was the most significant and explained 8% of the variation (Table 2.4a). In addition, 14 significant trait associations for NP were detected using the 265K SNP data in combined data under DS conditions on Pv02, Pv03, Pv06, Pv07 and Pv08 (Figure 2.3, Table 2.4b). Marker (S08_55438500; *P=7.3x10*$^{-09}$) at 55.4Mb with MAF of 0.12 was the most significant and explained 16.6% of the variation, while SNP (S07_11078838; *P=1.1x10*$^{-08}$*)* at 11.07Mb with MAF of 0.07 was the most significant on Pv07 and explained 16.2 % variation in NP under DS conditions. No significant associations for NP were identified in Namulonge under DS conditions (Figure 2.3, Table 2.4b).

Pod weight per plant (PW)

Nine SNPs were significantly associated with PW under DS conditions in Kasese and Namulonge using combined data analysis with the 6K Beadchip. Five SNPs all on Pv06 were associated with PW under drought stress in Kasese. The most significant SNP (ss715640054; *P=4.2x10*$^{-06}$) at 23.23Mb with MAF of 0.12 accounted for 10% of the variation in PW (Table 2.4a). In Namulonge, four SNPs were significantly associated with PW. These included two on Pv02 and marker (ss715641175; *P=4.1x10*$^{-06}$) located at 24.35 Mb with MAF of 0.09 was the most significant and explained 9% of the variation. SNP (ss715646672; *P=2.0x10*$^{-05}$) at 6.12Mb on Pv11with MAF of 0.10 was significant and accounted for 8% of the variation (Table 2.4a). In addition, five significant markers were detected on Pv06 for PW with the 265K SNP data using combined data under DS. The most significant SNP (S06_23021418; *P=8.6x10*$^{-08}$) at 23.02Mb

with MAF of 0.08 accounted for 14.7% of the variation in PW under DS conditions. (Figure 2.3, Table 2.4b).

Number of seeds per plant (SN)

Four SNPs were significantly associated with SN under DS conditions in Namulonge in combined data using the 6K Beadchip (Table 2.4a). These included two markers on Pv01 with marker ss715646300 located at 48.06 Mb with MAF of 0.32 being the significant and explained 12% of the variation. Two significant markers were identified on Pv11 with SNP (ss715649043; P=1.12x10^{-06}) located at 2.01 Mb with MAF of 0.17 was most significant and accounted for 12% of the variation in number of seeds per plant. No significant associations were detected for NS under drought stress in Kasese (Table 2.4a)

Seed yield per plant (SY)

Significant SNPs associated with SY were identified in Kasese under DS conditions in the combined data using the 6K Beadchip and under NS and DS using the 265K GBS data. Four markers were significant on Pv06, and two on Pv04. The most significant SNP (ss715640054; $P=2.0x10^{-05}$) on Pv06 with MAF of 0.12 was located at 23.23 Mb and explained 9% of the variation in SY. In addition, SNP (ss715640054) on Pv06 was the most significant SNP for PW. Interestingly all the significant SNPs associated with PW on Pv06 were also significant for SY. Two SNPs were identified on Pv04 and the most significant SNP (ss715645803; $P=6.0x10^{-05}$) with MAF of 0.15 located at 4.45Mb explained about 8% of the variation (Table 2.4a). In addition, 14 markers were significantly associated with SY under both NS and DS conditions using the 265K SNP data. Four markers all on Pv10 were significantly associated with SY under NS conditions using combined data. SNP (S10_27780836; $P=3.5x10^{-09}$) with a physical position of 27.7Mb and MAF of 0.08 was the most significant and explained 17.1% variation in SY under

NS conditions. An additional 10 markers were significantly associated with SY under DS conditions in Kasese on Pv02, Pv03, Pv05, Pv07, Pv08, and Pv10 (Figure 2.4, Table 2.4b). SNP (S03_51268099; $P=3.07x10^{-09}$) at 51.2Mb with MAF of 0.06 on Pv03 was the most significant and explained 16.5% variation in SY under DS conditions (Figure 2.4, Table 2.4b).

Harvest index (HI)

Significant SNPs associated with HI were identified in Kasese under DS conditions and in Namulonge NS conditions using the 6K Beadchip and 265K GBS marker data set. Two significant SNPs all on Pv02 were detected for HI under DS conditions in Kasese using the combined data analysis. The most significant SNP (ss715648530; $P= 9.1x10^{-05}$) with MAF of 0.14 and physical position of 24.23 Mb accounted for 6% of the variation in HI (Table 2.4a).

Four significant SNPs on Pv09 were detected for HI under NS conditions in combined data using the 265K SNP data. The most significant SNP (S09_28676522; $P= 5.2x10^{07}$) at 28.6Mb with MAF of 0.06 accounted for 11.1% of the variation in HI. In addition, two significant SNPs were identified for HI under NS conditions in Namulonge on Pv02 and Pv04 respectively. Marker (S02_30630638; $P=3.1x10^{-07}$) at 30.6Mb with MAF of 0.10 was the most significant and explained 13% variation in HI while SNP (S04_31117609; $P=4.6x10^{-07}$) at 31.1Mb with MAF of 0.11 on Pv04 explained 12% variation in HI under NS conditions in Namulonge (Figure 2.4, Table 2.4b).

PhiNPQ

A total of four SNPs were significantly associated with the photosynthetic trait PhiNPQ in Kasese under DS conditions in the combined data. Marker (ss715650748; $P=1.1x10^{-06}$) located at 48.77 Mb on Pv11 with MAF of 0.11 was the most significant and explained of the variation

in PhiNPQ. Other significant markers included (SNP ss715648421; $P=3.8x10^{-05}$) located at 0.54 Mb on Pv09 with MAF of 0.17, SNP (ss715648989; $P= 5.1x10^{-05}$) located at 39.78 Mb on Pv05 with MAF of 0.19 and SNP (ss715645811; $P= 2.7x10^{-05}$) with a physical position of 44.33 Mb on Pv04 with MAF of 0.10 respectively (Figure 2.6, Table 2.4a).

Linear Electron Flow (LEF)

Seven significant SNPs for LEF were identified in Kasese_2016 on Pv04 and Pv11 under DS conditions. Three SNPs were significant within a 355.2 kb region on Pv04, and the most significant SNP (ss715645811; $P=7.1x10^{-06}$) on Pv04 was located 44.40 Mb with MAF of 0.12 and explained 12 % variation in LEF. Four SNPs were significant for LEF on Pv11, and the most significant SNP (ss715650748; $P=1.6x10^{-07}$) located at 48.77 Mb with MAF of 0.11 explained 14 % of variation in LEF under DS conditions. No significant associations for LEF were identified in Kasese_2017. Additionally, SNP (ss715650748; $P=1.60x10^{-07}$) on Pv11 was most significant for both LEF and PhiNPQ (Figure 2.6, Table 2.4a).

Phi2

Significant SNPs for Phi2 were identified in both Kasese_2016 and Kasese_2017 under DS conditions. In Kasese_2016, three SNPs were significant on Pv11 and the most significant SNP (ss715640406; $P=1.35x10^{-05}$) was located at 52.21 Mb with MAF of 0.13, and explained 11% variation in Phi2 (Table 2.4a). In addition, SNP, (ss715640406; $P=1.35x10^{-05}$) was significant for both LEF and Phi2 (Table 2.4a). In Kasese_2017, five SNPs were significant, four on Pv03 and one on Pv05. The most significant SNP (ss715646087; $P=9.64x10^{-06}$) for Phi2 on Pv03 was located at 52.21 Mb with MAF of 0.13 accounted for 10% variation in Phi2. SNP (ss715649491; $P=5.26x10^{-05}$) located at 30.43 Mb on Pv05 with MAF of 0.15 explained 7% variation for Phi2 in Kasese_2017 (Figure 2.6 & 7, Table2.4a).

PhiNO

In Kasese_2017, one SNP was significantly associated with PhiNO on Pv03. Marker (ss715646087; $P=8.67x10^{-05}$) located at 52.21 Mb with MAF 0.13 was the only significant SNP detected under DS conditions, and explained for 10% variation PhiNO. Interestingly, the same marker was detected for Phi2 in Kasese_2017 (Figure 2.7, Table 2.4a).

Allelic Effects of Significant SNPs on Yield Component Traits

The allelic effects associated with significant SNPs from the 6K Beadchip for yield component traits namely PW and SY on Pv06 were assessed. Significant marker (ss715646419l; $P= 2.2x10^{-05}$) located at 24.9 Mb was significantly associated with PW, and explained 7% variation under DS conditions in Kasese. Interestingly, individuals carrying the marker allele G allele, with MAF of 0.14 had relatively higher PW under DS. Similarly, another SNP (ss715644727; $P=4.2x10^{-05}$) at 23.3 Mb on Pv06 was significantly associated with PW had the minor C allele (0.16). These two significant SNPs had substantially large positive effects of 44.9 and 44.1g on PW under DS conditions while the major alternate T allele in both SNPs had zero effect. Similarly, SNP (ss715646419; $P= 8.0x10^{-05}$) had large positive effect of 25.5g on SY and zero effects from the major T allele. In addition, 37 accessions in the panel were carrying the same G and C minor alleles for the two markers that came from diverse geographical and market classes. The red mottled cultivar PR9745-232 (ADP-432) from the Caribbean had the highest PW of 41.1g and SY of 34.7g under drought stress conditions among genotypes with these marker alleles. Interestingly, most genotypes carrying these loci have previously been reported to express drought tolerance under DS conditions. In addition, it is worth noting that the traits associated with these positive allelic effects are yield related implying that breeders have over time have

been accumulating favorable loci with positive effects on actual seed yield through indirect selection under DS conditions (Beebe et al., 2008; Assefa et al., 2013).

Discussion

The objective of this study was to use GWAS analysis to identify genomic regions and advance knowledge on genetic control of yield component traits and photosynthetic traits particularly at pod filling stage of common bean from the Andean gene pool grown under drought stress conditions. This study was conducted to explore the genetic diversity for drought tolerance and enhance genetic improvement for the trait in large seeded beans popularly cultivated in Africa. The severity of terminal drought stress observed in the current study, was moderate based on the DII of 0.4 for SY across locations and years. In addition, high variability in DII was observed across locations and years for SY and ranged from 0.30 to 0.58. The high variability in DII for SY could be attributed to the negative effects of drought stress on highly drought sensitive reproductive stage from flowering formation to seed development, with a cumulative negative effect on overall seed yield, although dependent on the severity and duration of the stress. This is consistent with previous studies that reported significant reduction in seed yield, harvest index, and pod number in common beans grown under drought stress (Schneider et al., 1997; Ramirez-Vallejo and Kelly, 1998; Rosales-Serna et al., 2004). Significant phenotypic variability was observed among the accessions for phenology, yield component, partitioning and photosynthetic traits measured during the pod filling stage with much variability observed under DS conditions (Table 1.2&3). In addition, significant positive correlations were observed among yield component and partitioning traits (Table 1.3), which is consistent with previous studies that reported significant positive correlations among yield component and partitioning traits DS (Asfaw et al., 2012; Mukeshimana et al., 2014). Such correlations imply that the ability to

remobilize photosynthate to seed yield under moderate drought conditions could be a useful for indirect selection for seed yield (Porch et al., 2009; Asfaw et al., 2012). This approach, has been used to develop and select superior common bean lines with greater assimilate remobilization potential of photosynthate to seed yield under terminal drought (Beebe et al., 2008; Klaedtke et al., 2012; Assefa et al., 2013). In this study, GM for SY was used to identify individuals that performed well under both DS and NS conditions across sites in the two locations. Accession AC Calmont (ADP-0670), a dark red kidney from Canada had the highest GM for SY (Table 1.5).

In this study, significant positive correlations were observed among photosynthetic traits such as LEF, Phi2 and PhiNO and negative correlation was observed with PhiNPQ. However, yield component and partitioning traits were not correlated with photosynthetic traits under DS conditions (Table 3), suggesting that the likelihood of using photosynthetic traits as predictors of yield trait components under DS conditions using field experiments is minimal. However, photosynthetic traits are useful for understanding the mechanistic and physiological aspects of drought stress during the critical pod filling stage.

Marker-Trait Associations

In this study, 44 significant marker-trait association signals were detected among agronomic and photosynthetic traits using the 6K Beadchip. Twenty-four significant associations for yield component and partitioning traits, such as NP, PW, SY, and HI were detected on Pv02, Pv04, Pv06, Pv09, Pv10, and Pv11. In addition, 20 significant associations were detected for photosynthetic traits on Pv03, Pv04, Pv05, Pv09 and Pv11 for PhiNPQ, LEF, Phi2 and PhiNO. Colocalized signals were detected on Pv03 for Phi2 and PhiNO, on Pv04 for PhiNPQ and LEF, and on Pv11 for PhiNPQ, LEF and Phi2 under DS conditions (Table 2.4a).

In addition, a total of 39 significant marker-trait association signals were detected for agronomic traits such as NP, PW, SY, and HI using the 265K GBS marker data (Table1.4b). Colocalized GWAS signals were detected on Pv02 for PW and HI, on Pv06 for PN, PW, and SWT (Table 2.4a) using the 6K Beadchip. This is consistent with the high significant positive correlations observed among the yield component traits, suggesting pleiotropic effects of those genomic regions on yield component traits (Table 2.3).

Yield Component Traits

Number of pods per plant

In the current study, three SNPs were significantly associated with NP on Pv06 and Pv10 under DS conditions in Kasese using the 6K Beadchip, whereas 14 significant association signals were detected for NP on Pv02, Pv03, Pv06, Pv07 and Pv08 using the 265K in combined data under DS conditions (Table 2.4a,b). The heritability for NP was 43.2% under NS and 41.5% under DS conditions respectively. This is consistent with previous studies that reported several QTL for NP across the genome of common bean. QTL for NP was previously mapped to Pv02 (Tar'an et al., 2002; Beattie et al., 2003), Pv03 (Beattie et al., 2003; Mukeshimana et al., 2014), Pv06 (Beattie et al., 2003; González et al., 2016; Diaz et al., 2017), Pv07 (Blair et al., 2006; Kamfwa et al., 2015), Pv08 and Pv10 (Koinange et al., 1996; Blair et al., 2012; Mukeshimana et al., 2014) in diverse mapping populations. However, the lack of physical positions for several markers linked to significant QTL reported in previous studies has limited comparisons of similar QTL in subsequent studies. Interestingly the most significant SNP S08_55438500; $P=7.3x10^{-09}$ at 55.4 Mb on Pv08 was detected under DS was in LD ($r^2 > 0.6$; D' =0.96) with markers S08_56044860 and S08_56048573 located at 56.0 Mb. These markers are within the same genomic region to SNP ss715648562 (56.0 Mb) associated with $NP8.1^{SC}$ identified in $F_{5:7}$ SEA5 x CAL96

population under NS conditions (Mukeshimana et al., 2014), suggesting that the genomic region associated with NP detected in the two studies is the same regardless of the population and environment. However, other significant signals detected on Pv03 at 52.3 Mb and Pv07 at 11.0 Mb and 26.0 Mb were different from those reported on Pv03 at 2.2 Mb (Mukeshimana et al., 2014) and on Pv07 at 40.0Mb (Kamfwa et al., 2015) (Table 2.4b).

Two markers on Pv10 namely ss715641139 and ss715640368 were downstream of significant SNP ss715641140 detected using 6K Beadchip were in strong LD ($r^2 > 0.8$; D' =1), and spanned 198 kb region with four genes. Positional candidate gene *Phvul.010G087800* is 2.4kb downstream of marker ss715641140, and is annotated as "GLUTAREDOXIN-RELATED (GRX)". GRXs are small molecular weight oxidoreductase glutaredoxin_related proteins that have antioxidant activity, and are associated with detoxifying reactive oxygen species (ROS) (Rouhier et al., 2006; Rouhier et al., 2008; Li, 2014), and floral organ development especially petal and anther initiation and differentiation in Arabidopsis and rice (Rouhier et al., 2008; Xing and Zachgo, 2008; Hofmann, 2009). Plants produce ROS under DS conditions, which at low concentrations are important signaling molecule mediating early plant response to drought stress (Farooq et al., 2017). However, high concentrations of ROS produced under continued drought conditions could result in oxidative damage and eventually cell death (Rouhier et al., 2006). The high expression of GRX proteins could be associated with the maintenance of cellular equilibrium through scavenging activity on ROS. The second positional candidate genes was within the 198kb region flanked by significant SNP ss715641140 and ss715640368 in LD (r2 > 0.8; D' =1) were three genes *Phvul.010G087900, Phvul.010G088000,* and *Phvul.010G088100* that encode "AGAMOUS-LIKE (AGL)" genes. These genes, namely AGL6, AGL20, and AGL14 are members of the MAD-box transcription factor family. AGL6 and AGL20 are

positive regulators of flowering time (Yu et al., 2002; Yoo et al., 2011), and are associated with floral initiation and organ development in soybean and Arabidopsis (Tapia-López et al., 2008; Wang et al., 2009; Wong et al., 2013). The functional annotation of the positional candidate gene reflects the importance of proper floral development in determining pod formation and development, and consequently overall yield under drought conditions.

Pod weight per plant

Significant association signals for PW were identified on Pv02 in Namulonge and on Pv06 in Kasese, respectively. In Kasese, the significant SNPs associated with PW were detected on Pv06 under DS conditions using both the 6K Beadchip and 265K SNP data. Interestingly, four of the significant markers (Table 2. 4b) identified using the 265K SNP data on Pv06 were in LD (r^2 > 0.88; D' >0.96), and spanned, 0.97Mb (23.0-23.9Mb) region. In addition, significant markers ss715640054 (23.2 Mb) and ss715644685 (23.1 Mb) associated with PW using 6K Beadchip were within the same genomic region. The heritability for PW was 46.6% under NS and 42.5% under DS conditions respectively. Previous studies mapped QTL associated with pod related traits on Pv06 (Beattie et al., 2003; Diaz et al., 2017) but the physical position of the markers associated with these QTL were not determined.

The most significant SNP (ss715640054; $P=4.2x10^{-06}$) located at 23.23 Mb on Pv06 with MAF of 0.12 accounted for 10% of the variation in PW (Table 1.4a). In addition, four significant SNPs (ss715640054, ss715644685, ss715645159, and ss715644727) were in strong LD (r^2 > 0.85; D' =1), and spanned 399 kb region with 45 genes on Pv06. However, marker ss715640054 is 8.7kb downstream of the positional candidate gene *Phvul.006G117500,* annotated as 'PLANT INVERTASE/PECTIN METHYLESTERASE INHIBITOR (INH/PMEI) SUPERFAMILY PROTEIN', that has been associated with the regulation of carbon partitioning and sink strength

particularly during seed development in plants (Hothorn et al., 2010; Ruan, 2014). Consistent with this finding is a recent transcriptome analysis of the drought tolerant common bean cultivar, BAT 477 that reported an up regulation of INH/PMEI at flowering and grain filling stage (de Faria Müller et al., 2014). Similarly, a recent study in soybean reported the involvement of INH/PMEI complex is determining seed size in soybean. The INH/PMEI complex was significantly up-regulated in a large seeded soybean cultivar during seed filling stage (20-22 days after flowering) than in a small seeded cultivar (Du et al., 2017). These results seems to suggest that the INH/PMEI complex is conserved in legumes and consistently expressed during pod filling and seed development stages of growth. The primary role of INH/PMEI complex appears to be related to the balancing of starch and sucrose metabolism in determining seed size, and subsequently seed weight at pod filling stage and could be targeted for marker assisted breeding to improve seed yield. In addition, four SNPs were significantly associated with PW on Pv02, Pv09, and Pv11 under drought stress conditions in Namulonge. The SNP ss715641175 located at 24.35 Mb on Pv02 was the most significant and explained 9% of the variation. The two significant markers were in complete LD (r^2 >1; D' =1), and spanned 338kb region with 18 genes on Pv02. However, within the 5 kb region upstream of ss715641175 were two genes *Phvul.002G120100,* annotated "2-OXOGLUTARATE (2OG) and FE(II)-DEPENDENTOXYGENASE SUPERFAMILY PROTEIN", and *Phvul.002G120200,* annotated as "KINESIN-LIKE CALMODULIN-BINDING PROTEIN (ZWICHEL)". The function of the two genes under drought stress remains unclear.

Number of seeds per plant (NSP)

Four SNPs were significantly associated with NSP on Pv01 and Pv11 under drought stress conditions in Namulonge. The heritability for NSP was 54.8% under NS and 43.5% under DS

conditions, respectively. SNP (ss715646300: $P=6.44x10^{-06}$) located at 48.06 Mb on Pv01 with MAF of 0.32 was the most significant SNP and explained 12 % of variation. Previous studies mapped QTL for number of seeds per pod, related to NSP on Pv01. $SP1^{PP}$ for seeds per pod was identified in PMB0225 x PHA1037 mapping population at 49.04 Mb (Yuste-Lisbona et al., 2014). QTL $NSP\text{-}1^{AM}$ for NSP was reported on Pv01 in PHA0419 x Beluga mapping population at 44.97- 45.46 Mb flanked by markers BMD045-FIN, while $NSP\text{-}1^{MA}$ was identified in Beluga x PHA0419 mapping population flanked by markers FIN (45.46 Mb) and BMC224 (48.11 Mb), respectively (Gonzalez et al., 2016). Interestingly, $NSP\text{-}1^{MA}$ colocalized with seed yield QTL $SY\text{-}1^{MA}$. In addition, marker ss715646300 (48.06 Mb) for NSP was in LD ($r^2 > 0.5$; D' =0.9) with ss715646315 (48.11 Mb) from 6K Beadchip, and in LD ($r^2 > 0.94$; D' =1) with SNPs, S01_48060395 (48.06 Mb) and S01_48119839 (48.11 Mb) from the 265K GBS data. This is consistent with previous studies that mapped QTL for seed yield in the same genomic region using Buster x Roza mapping population evaluated under multiple stress conditions (Trapp et al., 2015). However, the positional candidate gene *Phvul.001G217800* is 7.9kb upstream of ss715646300, annotated as "Ca2+ ACTIVATED OUTWARD RECTIFYING K+ CHANNEL 5", and plays a critical role in abscisic acid (ABA) mediated opening and closure of stomata during drought stress (Osakabe et al., 2014b; Cai et al., 2017).

SNP (ss715649043*; $P=1.12x10^{-06}$*) located at 2.01 Mb on Pv11 was the most significant SNP and accounted for 12% of the variation in NSP. In addition, the two significant SNPs (ss715649043and ss715649044) on Pv11 were in strong LD (r2 > 0.9; D' =1), and spanned 30.9 kb region with four genes. Two genes within this same region could be considered candidates in seed development. Marker ss715649044 is within the genic region of *Phvul.011G024100,* annotated as "SCARECROW-LIKE 3 (SCL3)". SCL3 is a member of GAI (GIBBERELLIC

ACID INSENSITIVE), RGA (REPRESSOR OF GAI) and SCR (SCARECROW), GRAS transcription factor family. In Arabidopsis, SCL3 is a positive regulator of gibberellin (GA) signaling and interacts with DELLA proteins to control GA responses and biosynthesis (Zhang et al., 2011). A recent study reported significant enrichment of SCL3 in developing seed of soybean especially late in the developmental stage, suggesting its role in seed development (Jones et al., 2010; Redekar et al., 2015). *Phvul.011G024000* is 7.3kb upstream of ss715649044, annotated as "BIFUNCTIONAL INHIBITOR/LIPID-TRANSFER PROTEIN/SEED STORAGE 2S ALBUMIN SUPERFAMILY PROTEIN". Members of this gene family have been associated with seed development and abiotic responses in plants. For instance, in rice lipid-transfer protein OsLTPL36, is essential in determining seed setting rate, 1000-grain weight, and seed quality (Wang et al., 2015). However, lipid transfer proteins have been reported to exhibit differentially expression under drought, salt, and cold conditions in Arabidopsis, wheat, and maize (Wei and Zhong, 2014; Safi et al., 2015) . In addition, seed storage 2S albumin proteins, a member of this gene complex is an important component of seed development in common bean and soybean (Parreira et al., 2016; Du et al., 2017).

Seed yield per plant (SY)

Significant signals associated with SY were detected in Kasese under DS conditions in combined data on Pv04, and Pv06 using the 6K Beadchip (Table 2.4a), and under NS and DS conditions with the 265K marker data on Pv02, Pv03, Pv05, Pv07, Pv08, and Pv10 (Figure 2.4, Table 2.4b). Heritability estimates for SY was 29.6% under NS and 22.5% under DS conditions, respectively.

Several studies reported QTL for SY across the genome of common bean. QTL for SY was mapped to Pv02 (Blair et al., 2006; Blair et al., 2012; Trapp et al., 2015; Gonzalez et al., 2016;

Diaz et al., 2017). The QTL YD2.1^{DB} tagged by marker *BMa16* (33.1 Mb) on Pv02 in 98 RILs from a cross between DOR 364 × BAT 477 was detected under low phosphorus condition (Diaz et al., 2017). This QTL was within the 3.5 Mb region flanked by the two significant markers S02_34117091 (34.1 Mb), and S02_30580034 (30.5 Mb) detected for SY in Kasese under DS. However, the genomic region described above was different from QTL SY2.1^{BR} at 11.8 Mb (SNP40055) identified in 140 $F_{7:9}$ RILs from Buster x Roza population (Trapp et al., 2015), and SY-2^{AM} flanked by markers BMB097 (13.65 Mb) and BMB080 (18.55 Mb) in $F_{2:8}$ Beluga x PHA0399 population (Gonzalez et al., 2016).

Significant SNPs for SY were identified on Pv03 under DS conditions in Kasese. The two most important significant SNPs were S03_51268099 at 51.2 Mb and S03_28782488 at 28.7 Mb. Several studies consistently reported QTL for SY on Pv03 (Beattie et al., 2003; Blair et al., 2006; Wright and Kelly, 2011; Checa and Blair, 2012; Mukeshimana et al., 2014; Kamfwa et al., 2015; Gonzalez et al., 2016). The yield QTL SY3.3^{SC} was initially reported on Pv03 at ss715649325 (39.64 Mb) in SEA5 x CAL96 population (Mukeshimana et al., 2014). Subsequent studies reported yield QTL SY3.3 flanked by markers ss715646621(39.4 Mb) and ss715639345 (39.6 Mb) in Puebla 152 x Zorro population (Heilig et al., 2017). The same QTL was reported between markers ss715647671(37.06 Mb) and ss715639244 (45.59 Mb) a nested association mapping (NAM) population consisting of three small red-seeded bean lines (S48M, S94M, and S95M) crossed to Merlot evaluated under DS conditions (Hoyos-Villegas et al., 2015). In another study using the Andean diversity panel significant marker association for seed yield was detected on Pv03 for marker ss715648538 (38.27 Mb) (Kamfwa et al., 2015). The consistency in these studies suggests the existence of stable QTL for yield on Pv03. Another seed yield QTL, SY-3^{AM} was identified in Beluga x PHA0399 population and flanked by BMD036 (46.37 Mb) and

BM189 (47.39 Mb) (Gonzalez et al., 2016). However the significant markers S03_51286099 (51.2 Mb) and S03_28782488 (28.7 Mb) associated with SY in this study were not in LD with markers previously reported, suggesting that the genomic regions detected for SY on Pv03 in this study could be different from those previously reported.

Significant signals associated SY were identified on Pv04 in Kasese under DS conditions. Previous studies reported QTL for SY on Pv04 (Blair et al., 2006; Blair et al., 2012; Checa and Blair, 2012). Four QTL for SY (yld4.1, yld4.2, yld4.3, and yld4.4) were mapped to Pv04 using advanced backcross $BC2F_{3:5}$ population from a cross between ICA Cerinza (the cultivated recurrent parent) and G24404 (the wild donor parent) (Blair et al., 2006). Another SY QTL yld4.1 on Pv04 was identified in 97 RILs from DOR 364 × BAT 477 population under DS condition (Blair et al., 2012), and HP yld4.1 on Pv04 in $F_{5:8}$ population from G2333 × G19839 evaluated under high phosphorus condition (Checa and Blair, 2012). However, validation of these seed yield QTL on Pv04 is not possible due to unknown physical positions of the prior markers tagging these QTL.

In this study, two significant SNPs were identified on Pv04 and the most significant SNP (ss715645803; $P=6x10^{-05}$) and explained about 8% of the variation (Table 2. 4a). The two SNPs (ss715645803 and ss715645801) were in strong LD ($r^2 > 0.9$; D' =0.98), and spanned 53.7kb region with four genes. Marker ss715645803 is 0.46kb upstream of *Phvul.004G163300*, annotated as "HEAT SHOCK TRANSCRIPTION FACTOR C1 (HSFC1)". HSFC1 is a member of the plant heat stress transcription factors (HSFs) that regulate the expression of stress responsive genes such as heat shock proteins under adverse environmental conditions such as heat, drought and cold stress in soybean and common bean (Li et al., 2014; Wang et al., 2014; Guo et al., 2016). A recent proteomic analysis of the stem of drought-tolerant common bean

cultivar Tiber detected increased levels of 70k dalton heat shock proteins under drought condition. This demonstrates the importance of heat shock proteins in protecting and maintaining protein conformations and cellular homeostasis during drought stress (Zargar et al., 2017). Furthermore, *Phvul.004G163600* is 1.8kb upstream of significant marker ss715645801 and annotated as "POLYOL/MONOSACCHARIDE TRANSPORTER 5 (PMT5)". PMT5 is a member of monosaccharide transporter (MST) family that are mainly involved in the long distance transport of photosynthate, primarily sugars and sugar alcohols such as mannitol, sorbitol, and xylitol in tree plants of the *Rosaceae* family (Noiraud et al., 2001; Slewinski, 2011; Reuscher et al., 2014) . In Arabidopsis AtPMT5 was partially characterized as a broad-spectrum H+symporter for polyols, cyclitols and several monosaccharaides (Klepek et al., 2005; Klepek et al., 2009; Reuscher et al., 2014). However, the physiological functions of PMT5 remain unclear because of wide specificity of substrates, and non-responsive mutant phenotype under drought stress (Klepek et al., 2005; Klepek et al., 2009; Reuscher et al., 2014).

Significant signals for SY was also detected on Pv05 at S05_18002393 (18.0 Mb). QTL for seed yield were previously mapped to Pv05 (Tar'an et al., 2002; Wright and Kelly, 2011; Trapp et al., 2015; Gonzalez et al., 2016) . QTL SY5.1^{BR} for SY at 38.7 Mb was linked to marker SNP45307 and identified in 140 $F_{7:9}$ RILs from Buster x Roza population grown under NS conditions (Trapp et al., 2015) , while QTL SY-5^{AM} flanked by IAC044(1.6Mb) and IAC010Pv05(2.7 Mb) was identified in $F_{2:8}$ RIL population from Beluga x PHA0399 cross, respectively (Gonzalez et al., 2016).

Significant QTL signals for seed yield were detected on Pv06 consistent with previous studies (Cichy et al., 2009; Blair et al., 2012).The QTL YD6.1^{DB} on Pv06 was identified from a cross between DOR 364 × BAT 477 under DS condition (Blair et al., 2012), However the physical

distance of markers tagging this particular yield QTL on Pv06 are not known. In addition, colocalized GWAS signals were detected for PW and SY on Pv06. Significant SNP (ss715640054; $P=2x10^{-05}$) with MAF of 0.12 located at 23.23 Mb on Pv06 was the most significant for PW, described earlier and explained 9% of the variation in SY. The positional candidate gene associated with this SNP is *Phvul.006G117500,* annotated as 'PLANT INVERTASE/PECTIN METHYLESTERASE INHIBITOR (INH/PMEI) SUPERFAMILY PROTEIN'.

Significant marker associations were detected on Pv07 and several QTL for SY have been reported on Pv07 (Cichy et al., 2009; Wright and Kelly, 2011; Asfaw et al., 2012; Blair et al., 2012; Hoyos-Villegas et al., 2016; Diaz et al., 2017). Interestingly, QTL for seed yield reported on Pv07 using different set of markers in previous studies appear to overlap within the same region. The QTL SY7.3 reported on Pv07 flanked by ss715650404 (4.59 Mb) and ss715640392 (5.03 Mb) detected in a NAM population grown under DS conditions (Hoyos-Villegas et al., 2015).QTL YD7.1^{DB} and YD7.2^{DB} on Pv07 flanked by BMc72 (0.28 Mb) and BMb1536 (4.6 Mb) from DOR 364 × BAT 477 population evaluated under low and medium phosphorus conditions (Diaz et al., 2017). In the current study, significant signals for SY on Pv07 were detected for markers S07_17054621 (17.0 Mb) and S07_12460824 (12.4 Mb), respectively distant from regions reported in previous studies.

Quantitative trait loci for SY were reported on Pv08 (Perez-Vega et al., 2010; Asfaw et al., 2012; Blair et al., 2012; Kamfwa et al., 2015). The QTL YLD8.1^{DB}, and YLD8.2^{DB} (Blair et al., 2012) and several SY QTL reported by Asfaw et al. (2012) on Pv08 were identified using the DOR 364 × BAT 477 population in multiple environments. However the physical positions of

the markers linked to these QTL were unknown preventing comparisons with significant marker S08_57056693 (57.0 Mb) detected in Kasese under DS conditions.

In the current study, five significant association signals were detected on Pv10 for SY under both DS and NS conditions. The four significant markers S10_27780836 (27.7 Mb), S10_27883169 (27.8 Mb), S10_28437825 (28.4 Mb) and S10_30139711 (30.1 Mb) all detected under NS conditions were in LD (r^2 > 0.65; D' =1), with exception of marker S10_39085960 (39.0 Mb) identified under DS in Kasese. QTL for SY have been consistently reported on Pv10 in previous studies (Wright and Kelly, 2011; Blair et al., 2012; Checa and Blair, 2012; Trapp et al., 2015; Hoyos-Villegas et al., 2016). The QTL, YLD10.1^{GG} on Pv10 was reported under high phosphorus condition in G2333 × G19839 population tagged by marker BM157 at 8.9 Mb (Checa and Blair, 2012). SY10.1^{BR} was identified in Buster x Roza population on Pv10 under DS linked to SNP46337 (39.9 Mb) (Trapp et al., 2015). The same QTL SY10.1 was detected between markers ss715646348 (40.1 Mb) and ss715645508 (40.95 Mb) in NAM population of three small red-seeded beans evaluated under DS conditions (Hoyos-Villegas et al., 2015).

Harvest index (HI)

Four significant SNPs were detected for HI under NS conditions in combined data on Pv09 and under DS in Namulonge on Pv02 and Pv04. The heritability estimate for HI was 48.5% under NS and 25.7 under DS. Quantitative trait loci for HI were reported on Pv02, Pv04, Pv05, Pv06, Pv09, Pv10, and Pv11 in DOR 364 × BAT 477 RIL population under DS condition in multiple environments (Asfaw et al., 2012). Two SNPs all on Pv02 were significantly associated with HI under drought stress conditions in Kasese, consistent with previous studies that reported significant QTL signal for HI and pod partitioning index QTL on Pv02 in DOR364 x BAT477 population grown under stress conditions (Asfaw et al., 2012). The most significant SNP

(ss715648530; $P=9.13x10^{-05}$) was located at 24.2 Mb on Pv02 with MAF 0.14 and explained 6% of the variation in HI. In addition, the two SNPs (ss715648530 and ss715648525) were in strong LD ($r^2 > 0.9$; D' =1) with this SNP, and spanned 87.9 kb region with seven genes. Positional candidate gene *Phvul.002G119500* is 29.7kb upstream of ss715648530, and annotated as "DIACYLGLYCEROL ACYLTRANSFERASE FAMILY (DGATs)". In soybeans, DGAT activity has been reported as the rate-limiting step in triacylglycerol (TAG) biosynthesis, the most common storage lipids in seeds, suggesting its critical role in accumulating seed oils during seed development (Lardizabal et al., 2008; Li et al., 2013; Chen et al., 2016). In addition, a recent study reported the involvement of GmDGAT2D a member of the monoacylgycerol acyltransferase (MGAT) family DGAT2, in plant response to environmental and hormonal cues in soybean (Chen et al., 2016). For instance, GmDGAT2D activity was up-regulated by plant hormone ABA under cold and heat stress, suggesting its role in responses to abiotic stresses and hormonal cues (Chen et al., 2016). Another candidate gene *Phvul.002G119000* is 6.6kb downstream of ss715648525, annotated as "NADPH-DEPENDENT THIOREDOXIN REDUCTASE A (NTRA)". In Arabidopsis and rice, NTRA activity is associated with enhanced tolerance to drought stress by acting as an antioxidant and scavenging ROS to prevent oxidative damage (Serrato et al., 2004; Cha et al., 2014; Kim et al., 2017). Recent study in wheat reported the localization of NTRs in developing and germinating wheat seed, and NTR dependent antioxidant activity in seed cells suffering from oxidative stress, suggesting that NTRs play an important role as antioxidant during reproductive stage of wheat (Pulido et al., 2009).

Colocalization of GWAS Signals for Photosynthetic Traits

In this study, significant association signals were detected for photosynthetic traits namely LEF, Phi2, PhiNPQ and PhiNO on chromosomes Pv03, Pv04, Pv05, Pv09, and Pv11 under drought stress conditions with multiple traits exhibiting colocalized signals. Heritability estimates for photosynthetic traits were LEF (22.5%), Phi2 (21.6%), PhiNPQ (14.4%), and PhiNO (8.1%) respectively. Significant QTL signals associated with photosynthetic traits listed above have not been reported previously in common bean, but QTL for relative chlorophyll content and photosynthetic efficiency (FV/FM) as fv′/fm′ (Beebe et al., 2013a) have been identified in common bean (Asfaw et al., 2012; Kamfwa et al., 2015b; Diaz et al., 2017). Previous studies reported significant QTL signals for relative chlorophyll content on Pv03, and Pv04 in DOR 364 × BAT 477 population at pod filling stage under drought and low phosphorus conditions (Asfaw et al., 2012; Diaz et al., 2017). QTL for relative chlorophyll content SCMR_f3.1^{DB} and SCMR_f3.2^{DB} were mapped to Pv03, and linked to markers BMb1008 (25.5 Mb) and BMb37 (22.2 Mb) under low phosphorus conditions, while SCMR_f4.1^{DB} mapped to Pv04 linked to X701 and flanked by BMb353 (28.6 Mb) and BMa20 (35.4 Mb) under low and moderate phosphorus conditions (Diaz et al., 2017). In addition, QTL for relative chlorophyll content were reported on Pv09 and associated with markers ss715648916 (20.0 Mb), and ss715647747 (26.6 Mb) in ADP panel evaluated under field and greenhouse conditions in Michigan (Kamfwa et al., 2015). Furthermore, QTL FVFM11.3^{DB} for photosynthetic efficiency (FV/FM) as fv′/fm′ was reported on Pv11 and linked to marker BMb10 at 40.0 Mb under low phosphorus conditions (Diaz et al., 2017).

In this study, photosynthetic traits measured exhibited colocalized QTL signals. For instance, four significant SNP associations were detected on Pv11 for LEF, Phi2 in Kasese_2016 and

PhiNPQ. Marker (ss715650748; $P=1.60x10^{-07}$) located at 48.78 Mb on Pv11 with MAF of 0.11 was the most significant SNP for LEF and PhiNPQ and in strong LD (r2 =0.65; D' =0.83) with the most significant SNP (ss715640406; $P=1.04x10^{-05}$) for Phi2 in Kasese_2016 located at 49.24 Mb with MAF of 0.13. This region spanned 462.3kb with 29 genes. Marker ss715650748 is within promoter region (0.24kb) of gene *Phvul.011G207400,* and encodes a "TRANSDUCIN/WD40 REPEAT-LIKE SUPERFAMILY PROTEIN (WD40-REPEAT)". The WD40-REPEAT proteins are key regulators of plant-specific developmental process including response to abiotic stresses such as drought (Van Nocker and Ludwig, 2003; Shaar-Moshe et al., 2015). SNP ss715640406 (most significant for Phi2 in Kasese_2016) is 1.9kb downstream of *Phvul.011G210200* and encodes "RING/U-BOX SUPERFAMILY PROTEIN", a member of ubiquitin ligase (E3) that regulate specific molecular processes by acting as target recognition sites for recruiting specific target proteins for degradation in response to various biotic and environmental cues (Yee and Goring, 2009; Lee and Kim, 2011). For instance, RING/U-BOX SUPERFAMILY PROTEINs has been found to be a negative regulator of ABA mediated responses to drought stress by modulating ABA-signaling pathway (Yee and Goring, 2009; Sadanandom et al., 2012). Another candidate gene *Phvul.011G210000* located 28.3kb upstream of marker ss715640406 encodes "DNAJ HEAT SHOCK N-TERMINAL DOMAIN-CONTAINING PROTEIN". Heat-shock proteins are molecular chaperones that protect plants cells against adverse effects of abiotic stresses such as drought and heat by maintaining stability in protein conformation and cellular homeostasis (Takayama et al., 2003). However, the activities of heat shock proteins are regulated by heat shock transcription factors (Hsf), that have been up regulated in plants overexpressing Hsf under drought stress (Li et al., 2014; Jacob et al., 2017). Recent studies in Arabidopsis and soybean showed that plants overexpressing heat shock

transcription factor had enhanced tolerance to drought stress, by activating stress responsive genes in an ABA dependent manner (Lim et al., 2006; Hwang et al., 2014; Li et al., 2014). Additionally, three SNPs were significantly associated with LEF in Kasese_2016 and PhiNPQ on Pv04. Two SNPs, ss715645808 at 44.40 Mb and ss715645811 at 44.33 Mb in strong LD (r2 =0.75; D' =0.96) spanned 71.7kb region with four genes. Candidate gene *Phvul.004G161500* is 14.4 kb downstream of ss715645811, annotated as "LEUCINE-RICH REPEAT (LRR) FAMILY PROTEIN". LRR are members of receptor-like kinase (RLK) family that acts as a signaling molecule in modulating plants response to biotic and abiotic stress (Ouyang et al., 2010; Osakabe et al., 2014; Dievart et al., 2016).

In Kasese_2017, colocalized signals were detected for Phi2 and PhiNO on Pv03. SNP (ss715646087; $P=3.5x10^{-06}$) located at 52.22 Mb with MAF of 0.11 was the most significant SNP for Phi2 and in strong LD (r2 =0.70; D' =0.93) with marker (ss715646085; $P=8.6x10^{-05}$) located at 51.73 Mb, the most significant SNP for PhiNO and for Phi2. The two markers flanked a 484.2 kb region with 56 genes. Candidate gene *Phvul.003G291500* is 5.3kb downstream of ss715646085 and encodes "GRAS FAMILY TRANSCRIPTION FACTOR FAMILY PROTEIN (GRAS)". GRAS transcription factors modulate expression of plant-specific GRAS proteins that are important in plant development (Hirsch and Oldroyd, 2009; Cenci and Rouard, 2017). A recent study in rice reported improved tolerance to drought and oxidative stress in mutants overexpressing GRAS transcription factor gene, OsGRAS23, suggesting its role in modulating plant response to abiotic stress (Xu et al., 2015). Another gene *Phvul.003G291800* is 25kb downstream of ss715646085 encoded "ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2 (AREB1/ABF2)". AREB1/ABF2 regulates the expression of ABA inducible genes that are critical in improves tolerance of plants to drought and osmotic stress,

and enhanced seed development (Fujita et al., 2005; Nakashima and Yamaguchi-Shinozaki, 2013).

Conclusion

The objective of this study was to conduct a GWAS to determine the genetic architecture of terminal drought tolerance in the Andean common bean diversity panel evaluated under drought conditions in Uganda. A substantial number of significant QTL signals associated with agronomic and photosynthetic traits were identified under both drought stress and non-stress conditions using 6K Beadchip and 265K SNP data from GBS used in this study. Genetic variance explained by the association signals ranged from 6% to 14% for 6K Beadchip, and 11.1 % to 17.1% with 265K SNP data implying that most of the genomic regions detected had relatively small but cumulative genetic effects on these traits, which is consistent with previous QTL signals identified using bi-parental mapping populations grown under drought conditions. Colocalized GWAS signals were detected for yield component traits and photosynthetic traits. Important positional candidate genes identified included *Phvul.006G117500* 8.7kb upstream of SNP ss715640054 (23.23 Mb) on Pv06, annotated as 'PLANT INVERTASE/PECTIN METHYLESTERASE INHIBITOR (INH/PMEI) SUPERFAMILY PROTEIN' that is involved in starch and sucrose metabolism during seed development under DS. *Phvul.011G024000* 7.3kb upstream of ss715649044 on Pv04, annotated as "BIFUNCTIONAL INHIBITOR/LIPID-TRANSFER PROTEIN/SEED STORAGE 2S ALBUMIN SUPERFAMILY PROTEIN", which is a seed storage protein important in seed development.

Meanwhile positional candidate genes associated with colocalized GWAS signals or photosynthetic traits include *Phvul.011G210200* located 1.9kb upstream of SNP ss715640406,

annotated as “RING/U-BOX SUPERFAMILY PROTEIN”, *Phvul.011G210000,* located at 28.3kb upstream of marker ss715640406 and encodes “DNAJ HEAT SHOCK N-TERMINAL DOMAIN-CONTAINING PROTEIN” all on Pv11 for Phi2, LEF, and PhiNPQ under DS. *Phvul.003G291800* located 25kb downstream of ss715646085, annotated as “ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING FACTOR 2 (AREB1/ABF2)” for Phi2 and PhiNO under DS. Furthermore, these candidate genes are involved in partitioning of photosynthate to seed yield and regulating plant responses to drought stress at pod filling stage. The SNPs tagging these genomic regions could be used in further studies with bi parental mapping to determine their usefulness for marker assisted breeding to improve genetic gain for seed yield under drought stress. In conclusion, this study demonstrates the effectiveness of GWAS in uncovering genomic regions that contribute to yield under drought stress in Andean common bean germplasm.

APPENDIX

Table 2.1a. Mean, minimum and maximum phenotypic values associated with agronomic traits and partitioning in Andean Diversity panel of 256 common bean genotypes grown under drought stress and non-stress conditions in Kasese for two seasons, 2016 and 2017 in Uganda

		Kasese							
		Drought stress (DS)				Non stress (NS)			
Trait	Year	Mean	Min.	Max	Variance	Mean	Min.	Max.	Variance
100SWT (g)	2016	25.8±0.5	14.3	41.7	***	27.6±0.5	12.5	64.0	**
	2017	29.6.0±0.5	16.7	61.7	***	33.2±0.5	14.4	65.4	***
Biomass per plant (g)	2016	47.3±3.2	15.0	74.2	***	61.8±3.2	26.8	93.0	***
	2017	49.1±2.9	11.5	81.7	***	68.2±2.9	16.3	99.2	***
Pod weight per plant (g)	2016	17.4±2.0	5.4	50.0	***	21.2±2.0	4.0	66.1	**
	2017	16.9±1.8	5.3	55.0	***	24.0±1.8	2.6	64.0	**
Seed yield per plant (g)	2016	11.8±1.1	2.0	48.3	**	16.9±1.1	4.5	54.0	***
	2017	12.2±1.3	2.5	50.8	***	19.2±1.3	5.5	56.2	**
Harvest index (HI)	2016	17.1±0.8	2.0	61.5	***	20.7±0.8	3.3	65.6	***
	2017	31.0±1.1	3.9	50.8	***	39.1±1.1	4.2	70.1	**
Pod harvest index (PHI)	2016	40.8±1.7	3.0	60.9	***	48.4±1.7	2.9	80.0	**
	2017	39.9±2.1	14.4	63.4	***	45.5±2.1	6.3	81.5	***
Pod Partitioning index (PPI)	2016	37.6±1.1	3.0	50.5	***	40.4±1.1	3.9	67.6	***
	2017	48.5±1.3	6.4	57.3	***	52.2±1.3	4.7	77.1	**
Days to flowering (DF)	2016	39.9±0.3	32.0	46.0	***	35.9±0.3	32.0	40.0	***
	2017	36.7±0.2	30.0	44.0	***	36.1±0.2	30.0	45.0	***
Days to maturity (DM)	2016	75.7±0.8	64.0	91.0	**	77.9±0.8	67.0	93.0	**
	2017	72.7±0.9	61.0	89.0	***	74.3±0.9	69.0	95.0	**
Pod number per plant	2016	9.6.4±1.5	2.7	15.5	**	14.5±1.5	4.0	21.5	*
	2017	10.6±1.8	2.7	16.2	*	16.1±1.8	2.0	24.0	*
Seed number per plant	2016	18.2±3.1	3.0	37.3	***	24.0±3.1	7.0	47.9	***
	2017	20.1±3.3	3.7	41.2	***	29.5±3.3	8.0	54.5	***

Table 2.1b. Mean, minimum and maximum phenotypic values associated with agronomic traits and partitioning in Andean Diversity panel of 256 common bean genotypes grown under drought stress and non-stress conditions in Namulonge for two seasons, 2016 and 2017 in Uganda

		Namulonge							
		Drought stress (DS)				Non stress (NS)			
Trait	Year	Mean	Min	Max	Variance	Mean	Min	Max	Variance
100SWT (g)	2016	24.8±0.8	12.5	45.4	***	32.8±0.8	11.5	57.6	**
	2017	26.6±0.5	15.9	47.0	***	33.1±0.5	13.3	62.7	**
Biomass per plant (g)	2016	36.6±2.9	11.0	68.1	***	51.3±2.9	14.0	88.7	***
	2017	46.3±3.5	18.6	100.2	***	58.2±3.5	20.4	113.6	***
Pod weight per plant (g)	2016	8.0±1.1	2.5	35.1	***	16.0±1.1	6.1	56.5	***
	2017	14.3±1.6	5.2	37.3	***	19.3±1.6	7.5	60.0	**
Seed yield per plant (g)	2016	5.6±1.4	1.3	23.8	***	13.6±1.4	4.0	45.2	***
	2017	9.2±1.2	2.5	29.0	***	14.9±1.2	6.3	40.3	**
Harvest index (HI)	2016	10.5±1.1	3.0	39.8	***	25.7±1.1	3.0	45.3	***
	2017	22.7±1.3	8.6	50.9	***	31.0±1.3	7.5	66.3	**
Pod harvest index (PHI)	2016	39.1±2.1	2.5	73.2	***	47.9±2.1	4.0	76.3	**
	2017	42.4±1.8	3.5	67.0	***	48.5±1.8	5.5	75.1	***
Pod Partitioning index (PPI)	2016	19.8±1.7	4.5	51.0	***	34.7±1.7	6.0	73.3	***
	2017	21.5±1.4	2.5	53.1	***	39.5±1.4	4.3	68.1	**
Days to flowering (DF)	2016	38.4±0.4	31.0	45.0	**	38.8±0.4	32.0	46.0	**
	2017	36.3±0.6	32.0	43.0	**	37.6±0.6	31.0	44.0	**
Days to maturity (DM)	2016	73.1±0.3	67.0	91.0	***	78.3±0.3	67.0	86.0	***
	2017	77.0±0.8	69.0	94.0	**	79.0±0.8	66.0	92.0	**
Pod number per plant	2016	8.1±1.0	3.0	14.4	*	12.0±1.0	4.0	19.1	*
	2017	9.2±1.3	4.0	15.5	*	13.2±1.3	5.0	22.1	*
Seed number per plant	2016	13.9±3.8	3.0	39.1	***	20.0±3.8	5.0	43.5	***
	2017	18.5±2.9	6.0	40.9	***	23.4±2.9	7.0	48.7	***

Table 2.2. Mean and Variance associated with photosynthetic traits in Andean Diversity Panel (ADP) of 256 common bean genotypes grown under drought stress and non-stress conditions in Kasese for two seasons, 2016 and 2017 in Uganda.

Trait	Kasese				
		Rainfed		Irrigated	
	Year	Mean	Variance	Mean	Variance
LEF	2016	217.7±3.2	**	206.6±3.2	*
	2017	133.7±1.5	***	140.5±1.5	***
SPAD	2016	37.9±0.3	***	36.1±0.3	***
	2017	37.7±0.4	***	39.9±0.4	***
Phi2	2016	0.35±0.004	***	0.34±0.004	**
	2017	0.39±0.003	***	0.40±0.003	***
PhiNO	2016	0.27±0.005	***	0.28±0.005	**
	2017	0.22±0.004	***	0.24±0.004	***
PhiNPQ	2016	0.38±0.007	***	0.39±0.007	***
	2017	0.39±0.006	***	0.36±0.006	***

Table 2.3. Pearson correlation coefficients among nine agronomic and three photosynthetic traits measured on 256 common bean genotypes in the Andean diversity panel grown at the Kasese, Uganda drought stress conditions in 2017.

Traits	BM	PD	PW	SD	SY	100SWT	PHI	HI	PPI	Phi2	PhiNO	PhiNPQ
DTM	0.18*	0.13*	0.16*	0.13*	0.12*	0.13*	-0.07ns	-0.02ns	0.09ns	0.01ns	-0.07ns	0.04ns
BM		0.46***	0.54***	0.46***	0.45***	-0.03ns	-0.18*	-0.09ns	-0.09ns	-0.01ns	0.08ns	-0.05ns
PD			0.84***	0.96***	0.89***	0.03ns	0.22**	0.41***	0.42***	0.01ns	0.03ns	-0.02ns
PW				0.84***	0.87***	0.19*	0.15*	0.30**	0.43***	0.03ns	0.01ns	-0.02ns
SD					0.89***	0.03ns	0.22**	0.41***	0.43***	0.01ns	0.03ns	-0.02ns
SY						0.24**	0.28**	0.40***	0.38**	-0.01ns	0.01ns	0.01ns
100SWT							0.25**	0.30**	0.27**	0.01ns	-0.04ns	0.02ns
PHI								0.60***	0.19*	-0.16*	-0.16*	0.18*
HI									0.74***	-0.11*	-0.08ns	0.09ns
PPI										0.01ns	0.02ns	-0.01ns
Phi2											0.66***	-0.88***
PhiNO												-0.93***
PhiNPQ												

BM Biomass, **PD** Pod number per plant, **PW** Pod weight per plant, **SD** Seed number per plant, **SY** Seed yield per plant.**100SWT** 100 seed weight, **PHI** Pod harvest Index, **HI** Harvest Index, **PPI** Pod Partitioning Index. Photosystem II (**Phi2**), Ratio of incoming light that is lost via non-regulated processes (**PhNO**), Ratio of incoming light that goes towards non-photochemical quenching (**PhiNPQ**), * Significant at the 0.05 probability level, ** Significant at the 0.01 probability level, *** Significant at the 0.001 probability level, † ns, not significant

Table 2.4a. Location, chromosome, position, P-values, proportion of phenotypic variation explained (R^2), and minor allele frequency of two most significant single nucleotide polymorphisms (SNPs) for five agronomic traits and four photosynthetic traits measured on 256 common bean genotypes in the Andean diversity panel grown under DS Conditions at Kasese and Namulonge, Uganda in 2016 and 2017.

Trait	Location	Chromosome	SNP	Position (bp)	P-value	R^2	MAF
Number of pods per plant	Kasese	Pv10	ss715641140	32728023	$7.5x10^{-06}$	0.09	0.12
		Pv06	ss715639365	31379011	$4.2x10^{-05}$	0.09	0.11
Pod weight per plant (g)	Kasese	Pv06	ss715640054	23234133	$4.2x10^{-06}$	0.10	0.12
		Pv06	ss715644685	23135033	$1.8x10^{-05}$	0.07	0.14
	Namulonge	Pv02	ss715641175	24351389	$4.1x10^{-06}$	0.09	0.09
		Pv02	ss715645824	24689544	$4.1x10^{-06}$	0.09	0.09
Number of seeds per plant	Namulonge	Pv11	ss715649043	2018589	$1.1x10^{-06}$	0.12	0.17
		Pv01	ss715646300	48065649	$6.4x10^{-06}$	0.12	0.32
Seed yield per plant (g)	Kasese	Pv06	ss715640054	23234133	$2.0x10^{-05}$	0.09	0.12
		Pv04	ss715645803	44509705	$6.0x10^{-05}$	0.08	0.15
Harvest index (HI)	Kasese	Pv02	ss715648530	24230627	$9.1x10^{-05}$	0.06	0.14
		Pv02	ss715648525	24142672	$9.7x10^{-05}$	0.06	0.15
PhiNPQ	Kasese	Pv11	ss715650748	48779978	$1.2x10^{-06}$	0.13	0.11
		Pv09	ss715648421	539280	$3.9x10^{-05}$	0.11	0.17
LEF	Kasese_2016	Pv11	ss715650748	48779978	$1.6x10^{-07}$	0.14	0.11
		Pv11	ss715640406	49242263	$1.0x10^{-06}$	0.11	0.13
Phi2	Kasese_2016	Pv11	ss715640406	49242263	$1.0x10^{-05}$	0.11	0.13
		Pv11	ss715649384	49159380	$8.8x10^{-05}$	0.08	0.13
Phi2	Kasese_2017	Pv03	ss715646087	52218575	$3.6x10^{-06}$	0.10	0.13
		Pv03	ss715646085	51734329	$5.4x10^{-06}$	0.11	0.13
PhiNO	Kasese_2017	Pv03	ss715646087	52218575	$8.7x10^{-05}$	0.10	0.13

Table 2.4b. Location, chromosome, position, P-values, proportion of phenotypic variation explained (R^2), and minor allele frequency of significant single nucleotide polymorphisms (SNPs) for four agronomic traits with 265K SNPs measured on 256 common bean genotypes in the Andean diversity panel grown under drought stress (DS) and non-stress (NS) conditions at Kasese and Namulonge, Uganda in 2016 and 2017.

Trait	Treatment	Location	Chromosome	SNP	Position(bp)	P_value	MAF	R^2
Number of pods	DS	Combined	8	S08_55438500	55438500	$7.30x10^{-09}$	12	16.6
Number of pods	DS	Combined	7	S07_11078838	11078838	$1.06x10^{-08}$	7	16.2
Number of pods	DS	Combined	7	S07_26035465	26035465	$1.10x10^{-08}$	22	16.2
Number of pods	DS	Combined	3	S03_52310519	52310519	$1.10x10^{-08}$	9	16.2
Number of pods	DS	Combined	3	S03_52310559	52310559	$1.10x10^{-08}$	9	16.2
Number of pods	DS	Combined	3	S03_52310595	52310595	$1.10x10^{-08}$	9	16.2
Number of pods	DS	Combined	3	S03_52310665	52310665	$1.10x10^{-08}$	9	16.2
Number of pods	DS	Combined	3	S03_52310683	52310683	$1.10x10^{-08}$	9	16.2
Number of pods	DS	Combined	3	S03_52310685	52310685	$1.10x10^{-08}$	9	16.2
Number of pods	DS	Combined	2	S02_22564824	22564824	$2.36x10^{-07}$	11	13.3
Number of pods	DS	Combined	2	S02_22564882	22564882	$2.36x10^{-07}$	11	13.3
Number of pods	DS	Combined	2	S02_22564888	22564888	$2.36x10^{-07}$	11	13.3
Number of pods	DS	Combined	2	S02_22564914	22564914	$2.36x10^{-07}$	11	13.3
Number of pods	DS	Combined	6	S06_23054750	23054750	$1.29x10^{-06}$	7	11.7
Pod weight per plant	DS	Kasese	6	S06_23021418	23021418	$8.61x10^{-08}$	8	14.7
Pod weight per plant	DS	Kasese	6	S06_23996747	23996747	$3.08x10^{-07}$	8	13.6
Pod weight per plant	DS	Kasese	6	S06_23996725	23996725	$4.92x10^{-07}$	9	13.2
Pod weight per plant	DS	Kasese	6	S06_22229432	22229432	$7.44x10^{-07}$	8	12.8
Pod weight per plant	DS	Kasese	6	S06_23996801	23996801	$8.03x10^{-07}$	8	12.8

Table 2.4b (cont'd)

Trait	Treatment	Location	Chromosome	SNP	Position(bp)	P_value	MAF	R2
Seed yield per plant	NS	Combined	10	S10_27780836	27780836	$3.55x10^{-09}$	8	17.1
Seed yield per plant	NS	Combined	10	S10_27883169	27883169	$2.24x10^{-08}$	7	15.2
Seed yield per plant	NS	Combined	10	S10_30139711	30139711	$1.46x10^{-07}$	4	13.3
Seed yield per plant	NS	Combined	10	S10_28437825	28437825	$1.57x10^{-07}$	7	13.2
Seed yield per plant	DS	Kasese	3	S03_51268099	51268099	$3.07x10^{-09}$	6	16.5
Seed yield per plant	DS	Kasese	7	S07_17054621	17054621	$1.13x10^{-08}$	6	15.3
Seed yield per plant	DS	Kasese	2	S02_34117091	34117091	$3.00x10^{-07}$	11	12.4
Seed yield per plant	DS	Kasese	5	S05_18002393	18002393	$3.37x10^{-07}$	6	12.3
Seed yield per plant	DS	Kasese	2	S02_30580034	30580034	$4.53x10^{-07}$	8	12.1
Seed yield per plant	DS	Kasese	2	S02_49146965	49146965	$4.56x10^{-07}$	34	12.1
Seed yield per plant	DS	Kasese	3	S03_28782488	28782488	$5.37x10^{-07}$	16	11.9
Seed yield per plant	DS	Kasese	10	S10_39085960	39085960	$5.50x10^{-07}$	5	11.9
Seed yield per plant	DS	Kasese	8	S08_57056693	57056693	$7.94x10^{-07}$	14	11.6
Seed yield per plant	DS	Kasese	7	S07_12460824	12460824	$8.38x10^{-07}$	10	11.5
Harvest Index	NS	Combined	9	S09_28676522	28676522	$5.21x10^{-07}$	6	11.1
Harvest Index	NS	Combined	9	S09_28676493	28676493	$5.21x10^{-07}$	6	11.1
Harvest Index	NS	Combined	9	S09_28676508	28676508	$5.21x10^{-07}$	6	11.1
Harvest Index	NS	Combined	9	S09_28676553	28676553	$5.64x10^{-07}$	5	11.1
Harvest Index	NS	Namulonge	2	S02_30630638	30630638	$3.18x10^{-07}$	10	13.0
Harvest Index	NS	Namulonge	4	S04_31117609	31117609	$4.64x10^{-07}$	11	12.6

Figure 2.1. Mean rainfall (mm) and mean temperature (°C) for Kasese_2016(a), Kasese_2017(b); Namulonge_2016(c) and Namulonge_2017(d) growing seasons**.**

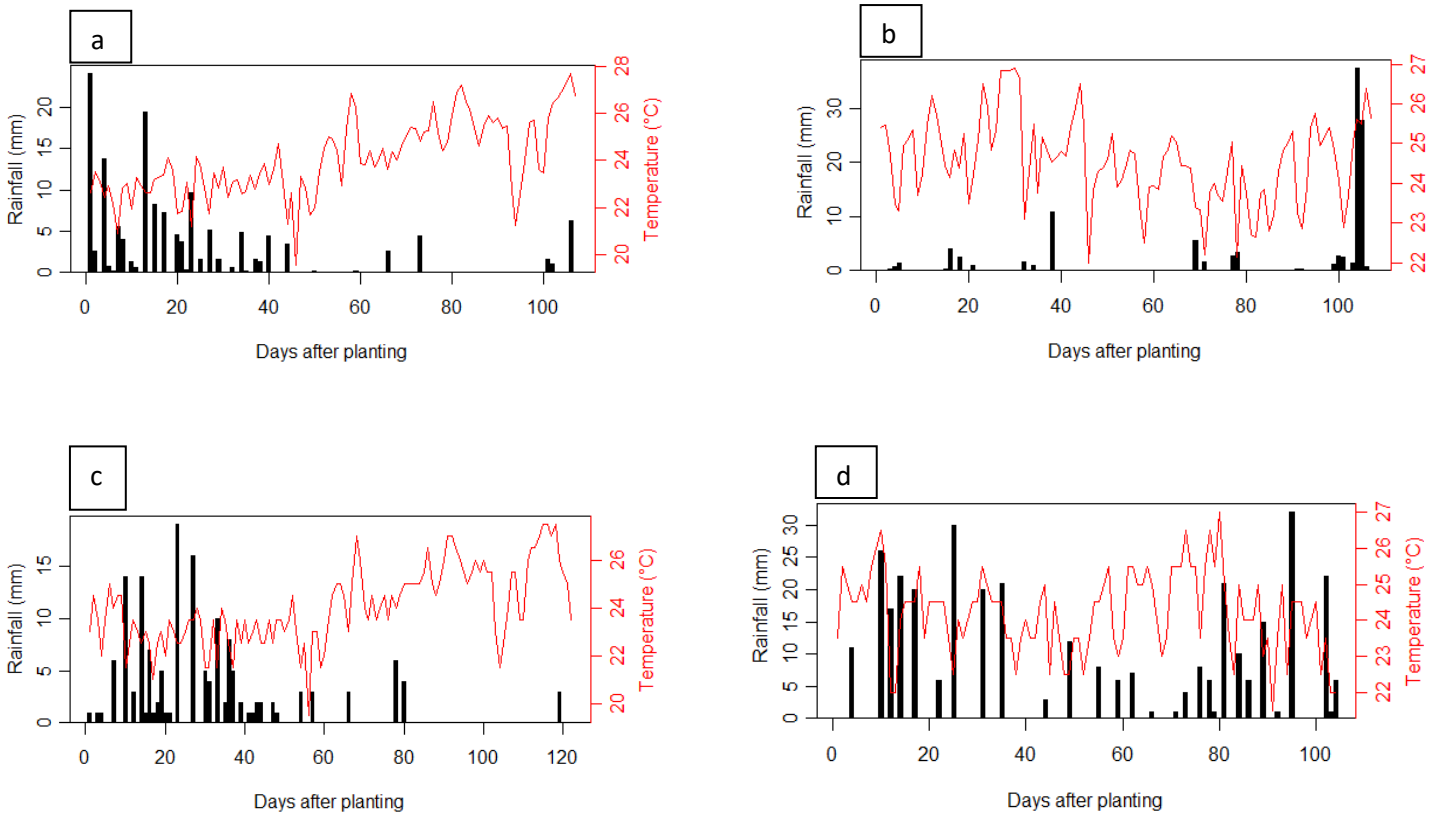


Figure 2.2. The plot of principle component analysis (PCA) PC1 against PC2, illustrating the population structure in subset of the Andean Diversity Panel used in this study with 265K SNPs

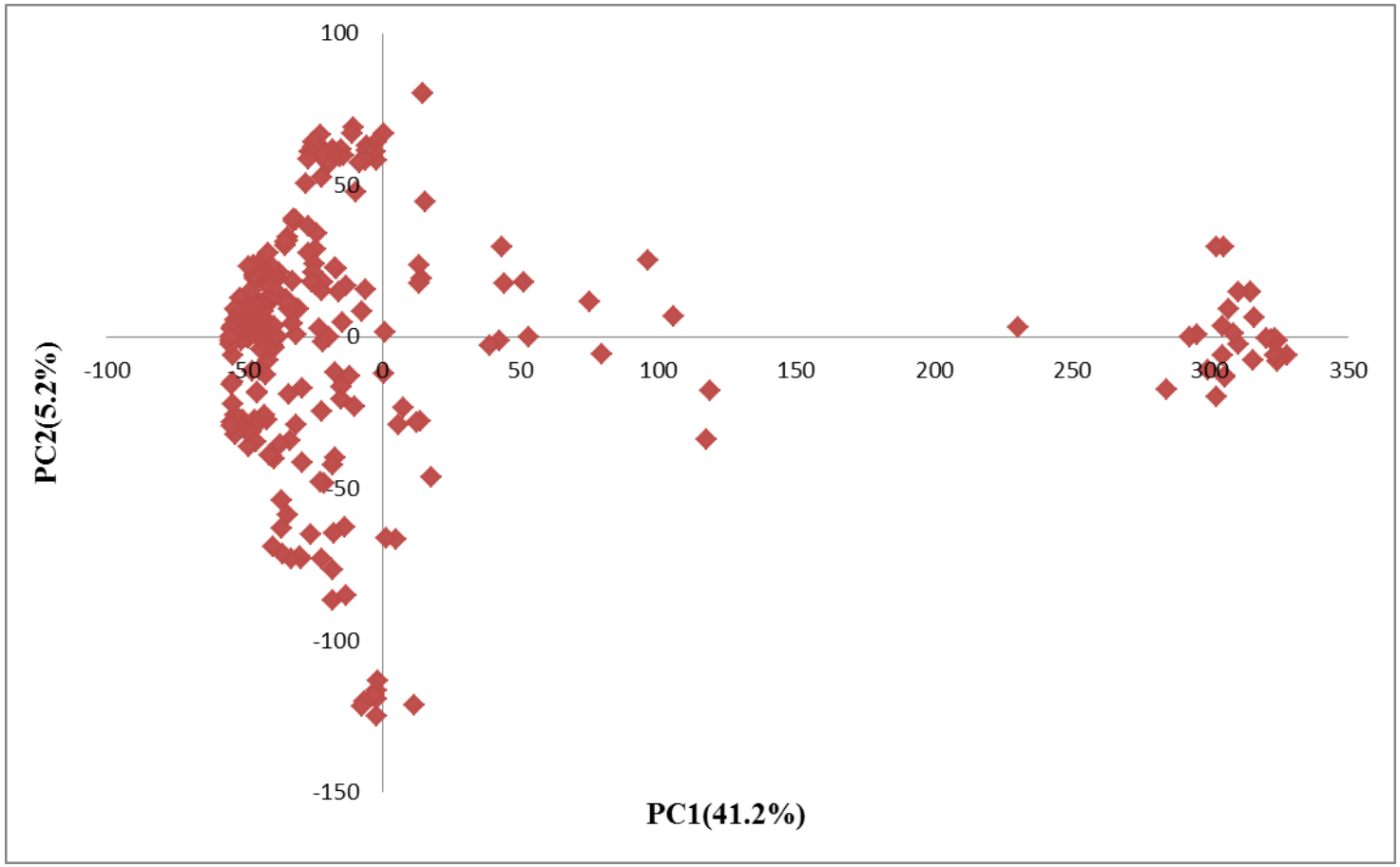


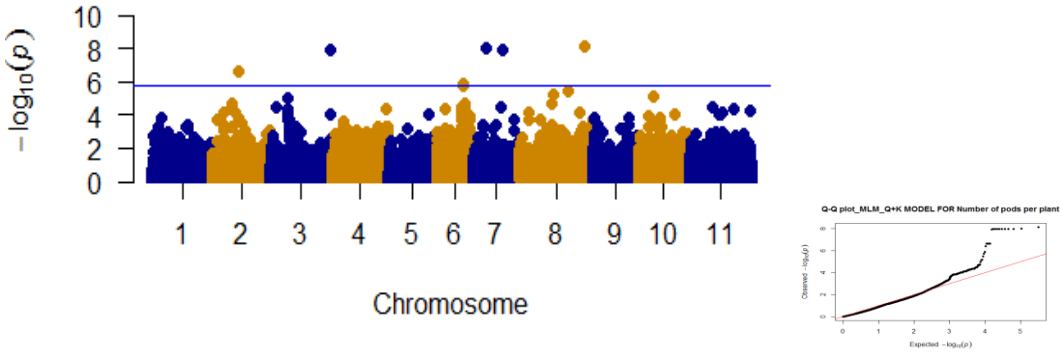
Table 2.5. Eleven genotypes with good performance based on geometric mean for seed yield from the Andean Diversity Panel evaluated under DS and NS at Kasese and Namulonge in 2016 and 2017.

ADP(ID)	Cultivar name	Country(region)	Seed color	Traits	GM	SY_DS(g)	SY_NS(g)
ADP-0670	AC Calmont	Canada	DRK		54.5	51.7	57.4
ADP-0009	Maalasa	Tanzania	Red mottled		42.6	40.2	45.1
ADP-0659	USLK-1	USA	LRK	High yield	40.5	30.0	54.7
ADP-0054	W6 16447	Tanzania	Cranberry	High yield Roza	39.7	32.9	47.8
ADP-0432	PR0637-134	Caribbean	Red mottled	HBB resistant	37.5	34.7	40.5
ADP-0119	A193	Africa	spec red		33.9	24.9	46.1
ADP-0648	Red Kloud	USA	LRK	High yield	33.5	26.4	42.4
ADP-0468	PI527538	East Africa	yellow	Rust resistant	32.3	28.6	36.4
ADP-0061	Maulasi	Africa	Cranberry	Drought Tolerant	31.0	16.7	57.1
ADP-0660	Krimson	USA	Cranberry	Drought Tolerant	30.8	20.6	46.0
ADP-0430	PR1013-3	Caribbean	Pink mottled	CBB	30.2	30.8	29.5
Grand Mean					15.5	11.5	18.3
SED					1.65	1.01	1.9

ADP Andean Diversity Panel identity, **GM** Geometric mean, **SY_DS** Seed yield under drought stress, **SY_NS** Seed yield under non-stress, **DRK** Dark red kidney, **LRK** Light red kidney, **CBB**, Common bacterial blight, **HBB**, Halo bacterial blight, **SED** standard error of difference

Figure 2.3. Manhattan plots showing candidate single nucleotide polymorphism (SNP) markers, and their *P*- values from genome wide association using mixed linear model for number of pods per plant on Pv02, Pv03, Pv06, Pv07, andPv08, pod weight per plant on Pv06 in Kasese for two seasons under DS conditions.

Number of pods per plant _Kasese



Pod weight per plant (g) _Kasese

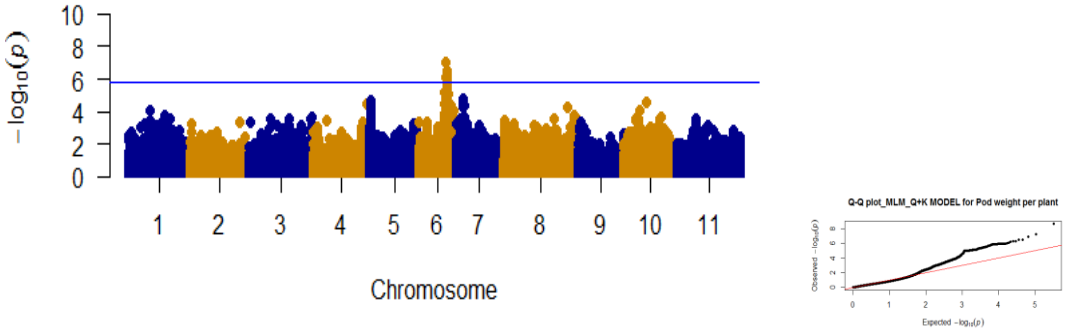


Figure 2.4. Manhattan plots showing candidate single nucleotide polymorphism (SNP) markers, and their *P*- values from genome wide association using mixed linear model for and seed yield per plant on Pv02, Pv03, Pv05, Pv07, Pv08,andPv10 under DS and harvest index on Pv02, and Pv04 under NS conditions.

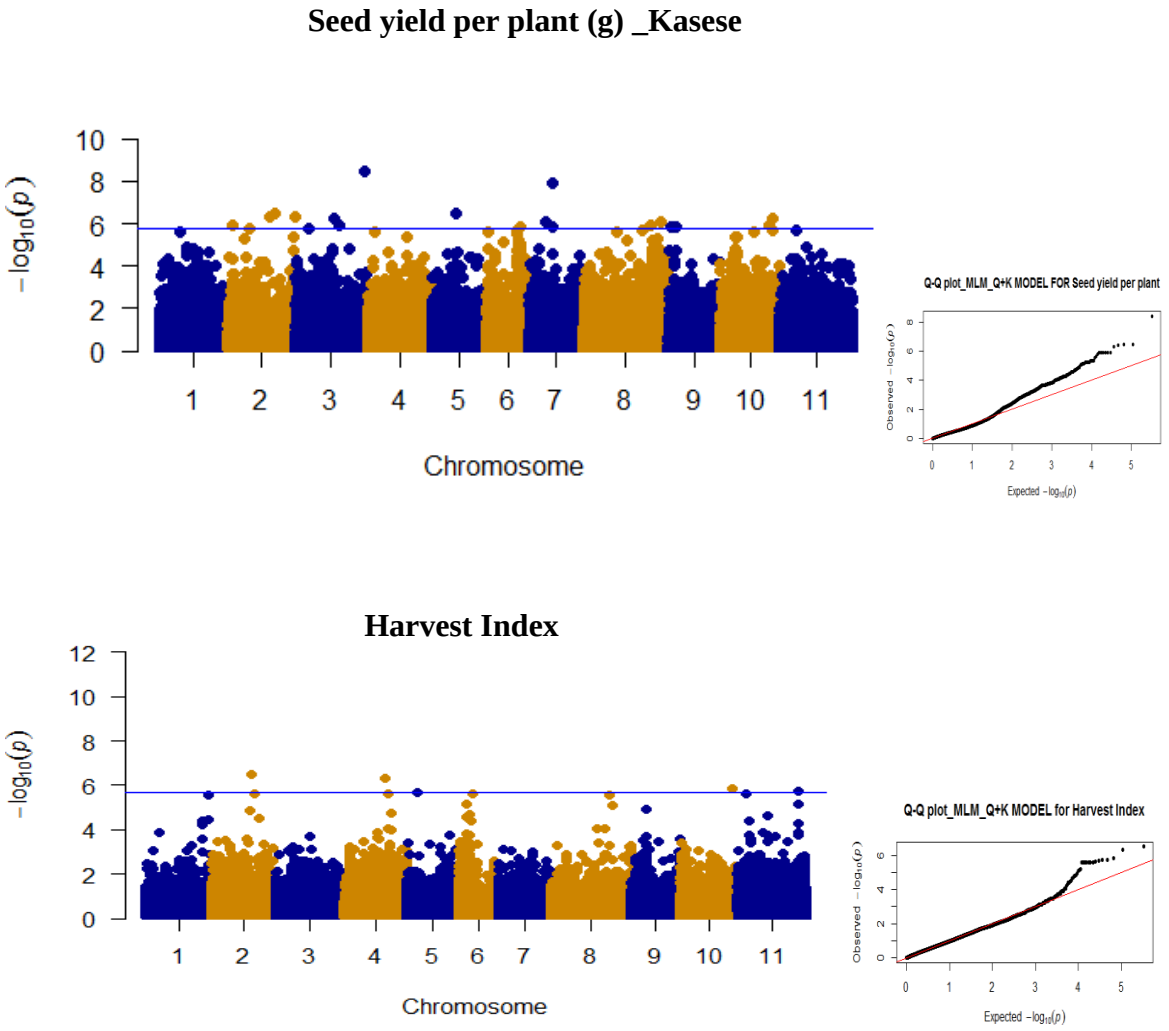


Figure 2.5. Manhattan plots showing candidate single nucleotide polymorphisms (SNPs) and their *P*- values from genome wide association using mixed linear model for LEF on Pv04 andPv011, for PhiNPQ on Pv04, Pv05, Pv09 and Pv011 and for Phi2 on Pv011 under drought conditions.

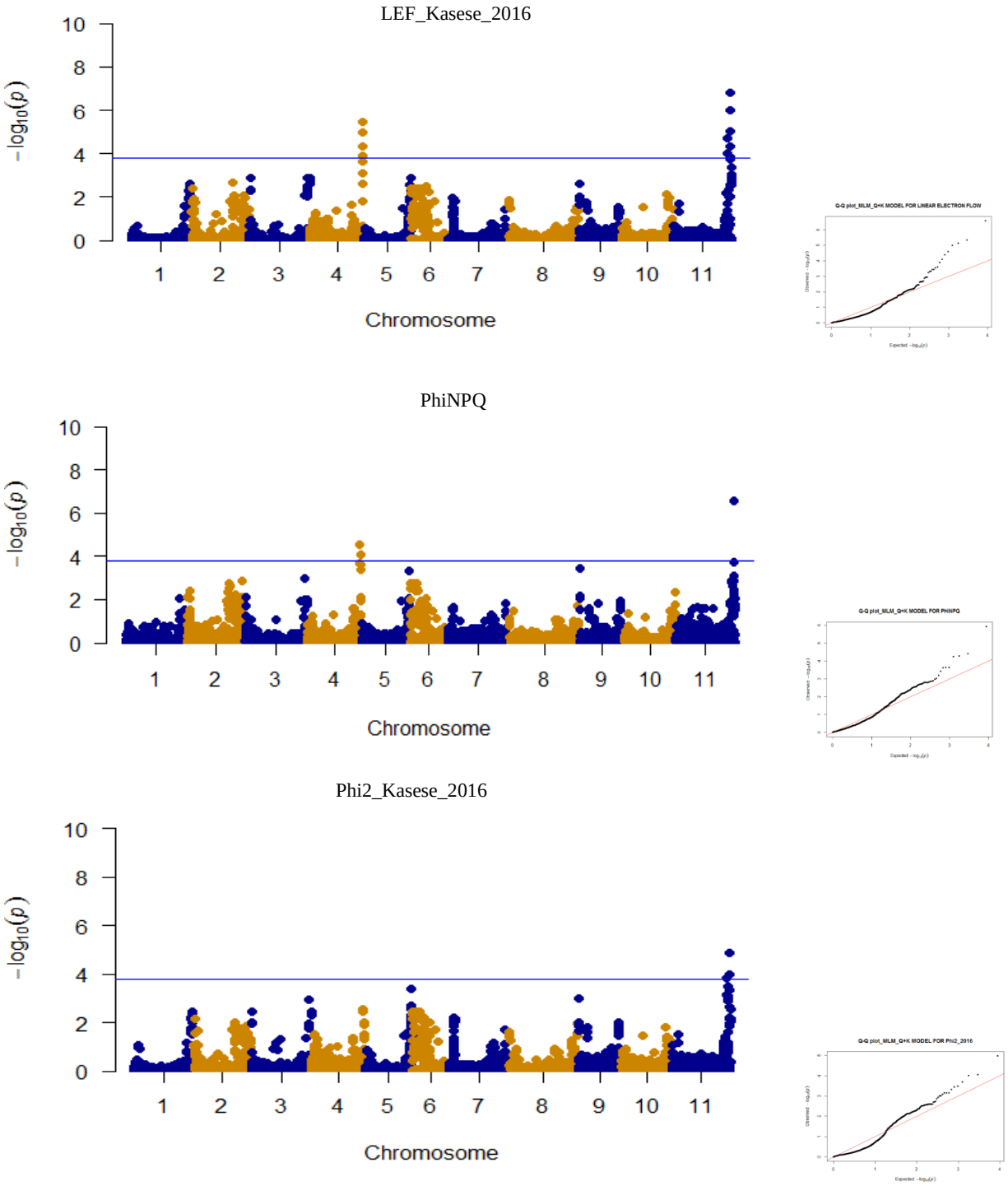
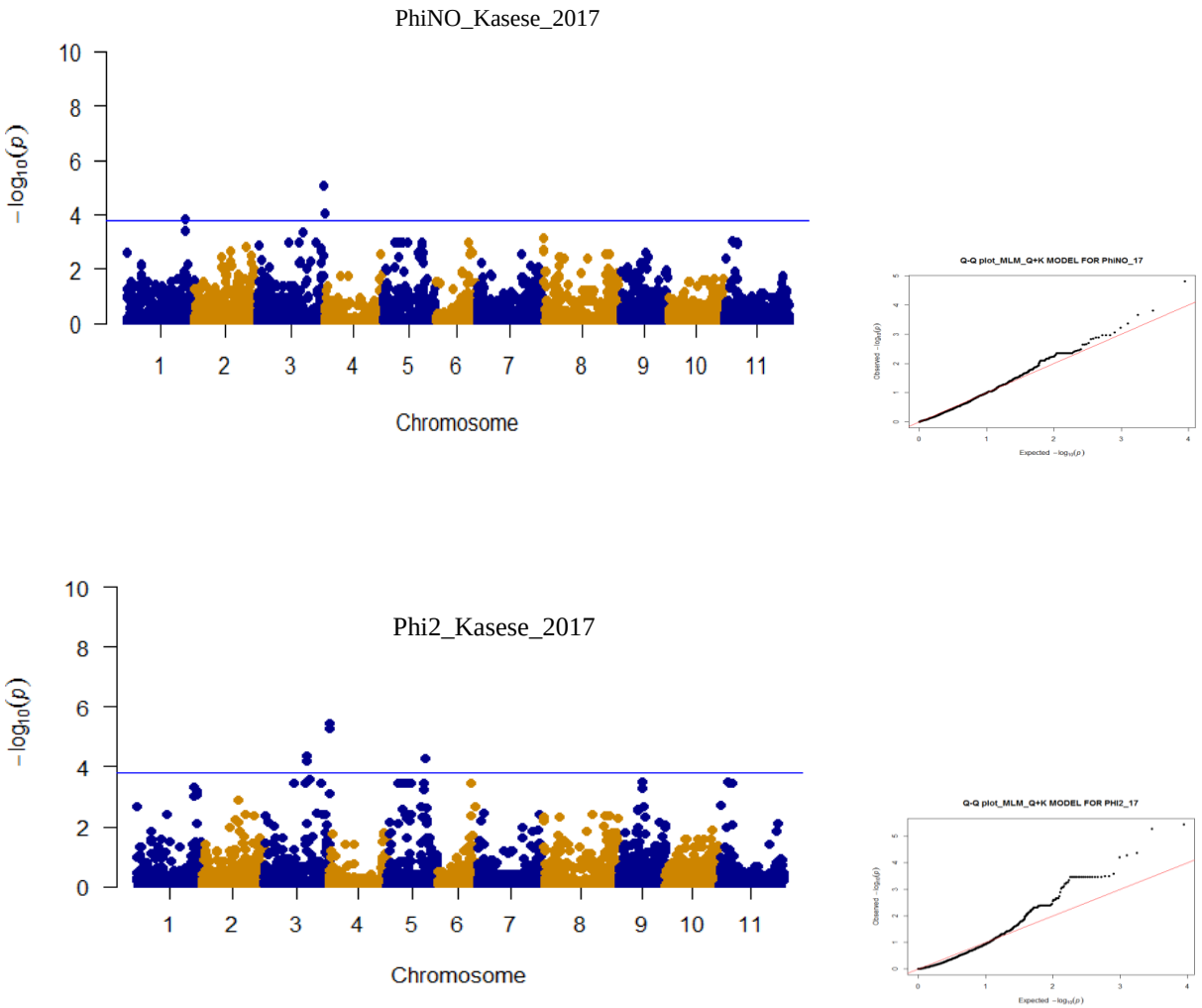


Figure 2.6. Manhattan plots showing candidate single nucleotide polymorphisms (SNPs) and their *P*- values from genome wide association using mixed linear model for PhiNO on Pv03 and for Phi2 on Pv03 and Pv05 under drought conditions.



CHAPTER 3: IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH DROUGHT TOLERANCE IN PORTILLO X RED HAWK RECOMBINANT INBRED LINE MAPPING POPULATION

Abstract

Drought stress is increasingly becoming the most important abiotic stress limiting productivity of common bean (*Phaseolus vulgaris. L*.) globally. The objective of this study was to conduct QTL analysis for drought tolerance in Portillo x Red Hawk recombinant inbred line (RIL) mapping population genotyped using single nucleotide polymorphism (SNP) markers from the BARCBean6K_3 Beadchip. The RIL population was evaluated for drought tolerance in Uganda for two seasons in Kasese and for one season at Namulonge under drought and non-stress conditions. The drought intensity index for seed yield per plant was moderate although seed yield per plant was significantly reduced under drought stress. A linkage map of the Portillo x Red Hawk mapping population was constructed with 206 markers that spanned 766.5 cM on ten of the eleven chromosomes of common bean with an average distance of 3.7 cM between markers. Thirty-two significant QTL signals were identified for phenology, yield component traits and partitioning traits measured under drought and non conditions. Colocalized QTL signals were consistently detected for phenology, yield component and partitioning traits on Pv01, Pv02, Pv03, Pv04, Pv06 and Pv11. Functional annotation of positional candidate genes identified within the colocalized peak regions were consistent with the functional similarity or pleiotropic effects of those genes on the associated traits. For instance, we identified positional candidate genes on Pv01 that were significantly associated with QTL SY1.1 and PPI1.2 under both DS and NS conditions. Gene *Phvul.001G186400*, encode "AGAMOUS-LIKE 62 (AGL62)", a type I MADS-box domain protein involved in endosperm cellularization, directly influencing seed size, sink strength and seed weigh and gene *Phvul.001G241200*, encodes

“PLASMA MEMBRANE INTRINSIC PROTEIN 1;4 (PIP1;4,PIP1E) involved with movement of water and small molecules across membrane. Therefore, validation of markers linked to these QTL could increase genetic gain for the seed yield and partitioning through marker assisted breeding

Introduction

Common bean (*Phaseolus vulgaris* L) is the most important grain legume consumed worldwide (Beebe et al., 2013). Common bean is an important food and nutritional security crop providing a cheap of source proteins (65%), energy (32%), and vital micro nutrients (Welch et al., 2000), in many developing countries. Bean is particularly important in Rwanda and western Kenya where highest global per capita consumption of 60kg have been reported (Akibode and Maredia, 2011; Namugwanya et al., 2014). Common bean production is constrained by many abiotic and biotic stresses. Drought stress is one of the most devastating cause of crop failures worldwide (Pennisi, 2008). It is estimated that over 60% of common bean production areas are prone to drought stress globally, and this makes drought the most important yield limiting abiotic stress of common bean (Beebe et al., 2013). Many bean-producing areas in sub-Saharan Africa are prone to both intermittent and terminal drought stress. In addition, common bean is cultivated by smallholder farmers who do not have irrigation infrastructure to supplement natural rainfall thus exacerbating the effect of drought (Beebe et al., 2011; Katungi et al., 2011; Beebe et al., 2014).

In recent decades, drought stress is widely recognized as an important production constraint and improving drought tolerance in common beans has been a major breeding goal for both international and national bean improvement programs worldwide (Beebe et al., 2012). However, progress towards the development of high yielding, and drought tolerant common bean cultivars has been limited because most traits that confer drought tolerance are quantitatively inherited with the low heritability, and significant genotype by environment (GxE) interactions that results in low predictability of genotype performance under variable environments (Cattivelli et al., 2008).

Developing common bean cultivars with improved tolerance to multiple stresses such as drought, diseases, and broad adaptability can be achieved by acquiring and transferring sufficient genetic diversity for the contributing traits into locally adapted genetic backgrounds. In common bean, the highest levels of drought tolerance has been identified among the race Durango from the semiarid central and northern highlands of Mexico (Singh et al., 1991; Terán and Singh, 2002; Muñoz-Perea et al., 2006), followed by races Mesoamerica and Jalisco (Singh, 2007). Under drought stress, Durango race genotypes exhibit both higher harvest index and seed yield (Singh, 2007), while the Mesoamerican race genotypes has improved seed filling traits in addition to seed yield (Beebe et al., 2008). The combination of Durango and Mesoamerican races have been a consistent genetic resource to improve and develop adapted drought tolerant germplasm for low land tropical environments (Frahm et al., 2004; Beebe et al., 2008; Mukeshimana et al., 2014). A number of breeding lines with high yield potential were selected for their ability to remobilize photosynthate more efficiently to grain under terminal drought (Beebe et al., 2008), including SEA5 that is widely used as parent (Mukeshimana et al., 2014). Although races Durango and Mesoamerica genotypes have been a reliable and widely used source of drought tolerance, introgression of drought tolerance and other favorable genes into the large seeded Andean gene pool widely cultivated in Africa and South America has been limited (Gepts and Bliss, 1985; Mumba and Galwey, 1999). This is due to cross incompatibility and linkage drag that results in low rates of recovery of valuble recombinant progenies (Beebe et al., 2013).

Recent studies identified quantitative trait loci (QTL) associated with rooting patterns and root architectural traits, phenology, seed yield and yield components in the DOR364 x BAT477 intra-gene pool RIL population. Mukeshimana et al. (2014) identified QTL associated with seed yield, and phenology traits in the SEA5 x CAL96 inter-gene pool RIL population. More recently a

number of QTL with varying size effects associated with seed yield and yield components were identified under drought stress using a nested association mapping (NAM) population (Hoyos-Villegas et al., 2016) The NAM population was developed from crossing three small red-seeded beans namely SER48, SER94, and SER95 with the common parent 'Merlot'. Interestingly, previous QTL analysis have consistently reported the co-localization of phenology, yield and yield components, and root traits on the same chromosome regardless of mapping population and marker system, thus providing evidence of functionality for specific genes contributing to complex traits such as drought (Asfaw et al., 2012; Blair et al., 2012; Mukeshimana et al., 2014).

However, most QTL analysis studies on drought tolerance in common bean have been conducted within the Middle American gene pool and inter-gene pool crosses (Schneider et al., 1997; Asfaw et al., 2012; Blair et al., 2012; Mukeshimana et al., 2014; Hoyos-Villegas et al., 2016). In order to explore additional diversity for drought tolerance alleles, QTL analyses for drought tolerance is critical in the Andean gene pool to unlock the genetic potential and map genomic regions for populations developed from crosses within the Andean gene pool. This research will provide opportunity to analyze the effect of genetic backgrounds on QTL expression and accelerate identification of alternative sources of drought tolerance genes within the Andean gene pool (Beebe et al., 2013). In addition, these efforts should facilitate developing a strategy for improving the large seeded Andean types widely grown in East Africa and South America (Mukeshimana et al., 2014). The objective of this study was to identify QTL for drought tolerance and related traits in a Portillo x Red Hawk mapping population and identify recombinant lines for use in future breeding of drought tolerant Andean genotypes for Uganda.

Materials and Methods

Plant Materials

A RIL population consisting of 97 $F_{4:7}$ lines derived from crossing Portillo a red mottled, drought tolerant variety from Ecuador with Red Hawk, red kidney bean variety, susceptible to drought developed from Michigan State University. The 97 RILs were evaluated for drought stress in the field at the Mubuku Irrigation Experimental Station in Kasese for two seasons 2016 and 2017, and at the National Crop Resources Research Institute Namulonge in 2017 off-season planting in Uganda. The two seasons hereafter referred to as Kasese_2016, Kasese_2017, and Namulonge_2017 respectively. Mubuku Irrigation Experimental Station is located in Kasese Western Uganda at 0^{o}16'10"N, 30^{o}6'9"E at an elevation of 1330 m above sea level and receives 1000mm of rainfall annually that is unevenly distributed between months. The soils are characterized as sandy loam soils with a pH of 5.68, above the 5.5 critical pH levels for common beans. The National Crop Resources Research Institute, Namulonge located in Central Uganda at 0^{o}31'30"N, 32^{o}36'54"E at an elevation of 1160 meters above sea level. The average temperatures are 21.8^{0}C ranging from 15.8^{0}C to 27.9^{0}C. Namulonge receives 1300 mm of rainfall annually and soils are characterized as oxisols with a pH of 5.8,respectively. The results of soil nutrient analysis indicated an average low available phosphorus and nitrogen below the 15 ppm and 0.2% respectively in both sites and NPK was applied at a rate of 50 kg/ha to enhance soil nutrient levels. The RIL population was evaluated side by side under non-stress (NS) and drought stress (DS) conditions respectively. The 97 RILs were planted on one row plot of 2 m long and 0.5 m between rows for two seasons in Kasese and for one season at Namulonge in 2017. The experiments were planted in 10x12lattice design with two replications. Planting was done during mid-rainy to end of season to target terminal drought stress at the reproductive stage

of plant growth. In Mubuku, the first experiment was planted on 28 October 2016 and harvested on 15 February 2017 and the second field experiment on 15 May 2017 and harvested on 11 September 2017. In Mubuku, drip irrigation was used to irrigate both DS and NS to ensure good germination and plant establishment until flowering time. Irrigation was discontinued in the DS plots at flowering while supplemental irrigation was continued in the NS plots twice a week up to harvest maturity. The field experiment in Namulonge was planted on 18 June 2017, and harvested on 30 September 2017. Overhead sprinklers were used to irrigate both DS and NS to ensure good germination and plant establishment until flowering time. Supplemental irrigation was continued in the NS plots twice a week up to harvest maturity while irrigation was discontinued in the DS plots at flowering. In both locations and seasons, recommended agronomic procedures were followed. Data was collected for daily weather conditions including daily rainfall (mm) and temperature (C) during the growing seasons. Phenotypic data was collected on days to flowering (DF), days to maturity (HM). In order to determine shoot biomass, yield and yield component traits, six plants were sampled from every plot for every genotype and sun dried and used for measuring shoot biomass, pod weight per plant (PW), seed yield per plant. In both seasons, seed yield per plant (SY) was calculated after adjusting for moisture to 14%. Partitioning traits for each genotype were estimated as a percentage as follows. Harvest index (HI) for each genotype was calculated in percentage as the ratio of total seed weight to total shoot biomass at harvest. In order to ensure uniform sample size for data collection, pod-partitioning index (PPI) was estimated using shoot biomass at harvest instead of total shoot biomass dry weight at mid-pod filling as the ratio of pod weight at harvest to total shoot biomass. The severity of drought stress in each environment for yield was quantified using drought intensity index (DII) as 1–(Xds/Xns) where Xds and Xns is the mean value for yield under

drought stress and non-stress. Geometric mean (GM) of seed yield as $(NS \times DS)^{1/2}$ where DS and NS are seed yield for drought stress and no stress treatments, respectively (Fischer and Maurer, 1978).

Phenotypic Data Analysis

Statistical analyses for field data were performed using mixed models in SAS 9.4 (SAS Institute 2002). Normality tests were conducted for each trait measured in the field using PROC UNIVARIATE command in SAS. Analysis of variance was performed to determine the effects of water treatment on the RIL population using PROC MIXED command in SAS where genotype, water treatment, location, and seasons were considered fixed effects while replication and blocks were random effects. Pearson correlations among variables were determined using the PROC CORR command and histograms using R package.

Heritability

Broad sense heritability (H^2) was calculated for days to flowering, days to maturity, shoot biomass per plant, pod weight per plant, seed yield per plant, harvest index, pod harvest index, pod-partitioning index on entry mean basis using the variance component method (Fehr et al., 1987) described as;

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{t} + \frac{\sigma_e^2}{rt}}$$

Where σ_g^2 is the genetic variance, σ_{ge}^2 variance associated with genotype x environment interaction, σ_e^2 experimental error, **t** number of environments, **r** number of replications.

Genotyping

DNA was isolated from the leaves of the F_4 RILs and parents in the greenhouse at MSU using a protocol described by (Cichy et al., 2015a). The DNA from the samples used in this study were genotyped using an Illumina BARC_Bean6K_3BeadChip with 5398 SNPs (Song et al., 2015) in the Soybean Genomics and Improvement USDA Laboratory (USDA-ARS, Beltsville Agricultural Research Center) in MD, USA. Genome Studio software from the Illumina, Inc was used to call SNPs.

Genetic Map Construction

The 5398 SNPs were filtered for polymorphism leaving 810 polymorphic markers. The SNPs were then used to construct a genetic linkage map with the JoinMap software v4.1 (Van Ooijen, 2011). In JoinMap additional filtering was conducted to remove SNPs showing severe segregation distortion and markers mapping to the same position. Markers were then assigned to linkage groups using a logarithm of odds (LOD) score threshold varying from 5 to 10. Markers were ordered within linkage groups using the regression mapping algorithm and map distances between markers was estimated using Kosambi mapping function implemented in JoinMap and linkage maps were displayed using Map Chart software (Voorrips, 2002)

Quantitative Trait Loci (QTL) Analysis

Quantitative trait loci analysis was implemented using composite interval mapping (CIM) method in Win QTL Cartographer V2.5–011 (Wang et al., 2012). The CIM mapping control parameters included model 6 (standard model) with five markers as control/ background markers. Window size of 10cM and 1 cM walk speed. Analysis was conducted using forward and backward multiple regression method for every trait. In addition, a QTL was declared

significant based on 1000 permutation test (Churchill and Doerge, 1994) for each trait in Win QTL Cartographer to determine the LOD threshold at *P=0.05* and the peak LOD score was considered the location of the QTL. The proportion of phenotypic variance accounted for by the QTL was determined using the coefficient of determination (R^2) value. Quantitative trait loci were named using guidelines provided by the Genetics Committee of Bean Improvement Cooperative for common bean QTL nomenclature (Miklas and Porch, 2010)

Results

The effect of drought stress on performance of the RIL population

The average daily precipitation and temperature recorded for 2016 growing season at Kasese, and 2017 growing season at Kasese and Namulonge were reported in Figure (2.1 a, b&c). In 2016 growing season (October-February), Kasese received a total of 150 mm of precipitation. Meanwhile during the 2017 (May-September) growing season a total of 180 mm of precipitation was recorded in Kasese. In Namulonge 2017, higher rainfall was recorded during the off-season planting with a total of 216 mm of precipitation (Figure 2.1c). In both growing seasons, the rainfall patterns were erratic and on average, most rains were recorded during the vegetative stage of growth (130 mm and 98mm) in 2016 and 2017 respectively. The results also indicated that little rains (22 mm and 16 mm) were recorded during reproductive and pod filling stages, and hence for conducive for conducting the field experiments on terminal drought stress (Figure 2.1 a&b). In addition, the total amount of precipitation recorded in this study is far below the minimum 400 mm required for normal growth of common beans.

In this study, the overall DII for SY was 0.40, which is moderate. In Kasese, DII for SY was 0.44 in 2016 and 0.40 in 2017 respectively. In Namulonge 2017, DII for SY 0.36 in 2017, implying

that drought effects on seed yield per plant among the RIL population were slightly higher in Kasese than in Namulonge.

In 2016, significant differences ($P<0.01$) were observed for drought effects in Kasese for DM, HI, PPI, PW, and SY (Table 3.1). Average HM were 73 d under DS and 75 d under NS, implying that plots under DS matured 3d earlier than in NS (Figure 3.1a). In this study, yield component traits were significantly affected by drought stress. The average PW were 21.9g and 32.9g under DS and NS resulting in 33.4% reduction in PW, while average seed SY were 9.9g and 18.1g under DS and NS conditions respectively, resulting in 45.3% reduction in SY under terminal drought (Figure 3.1b). . Similarly, partitioning indices were affected by drought stress resulting in 26% reduction in HI and PPI under DS. HI ranged from 2.1% to 37.2% under DS while it ranged 3.6 % to 52.6% under NS. Mean HI of 15.3% and 20.9% were reordered under DS and NS respectively. In addition, PPI ranged from 3.1% to 56.2%, average of 33.0% under DS while it ranged from 10.5% to 80.9 with an average of 44.6% under NS (Figure 3.1c). .

In 2017, significant differences ($P<0.01$) for drought effects were observed for DM, HI, PPI, PW, and SY, and non-significant difference were observed for DF in Kasese and Namulonge (Table 3.1). In Kasese 2017, the mean HM was 73 d under DS and 77 d under NS, implying that DS plots matured 4 d earlier than NS. Yield component traits were significantly affected by drought stress. The mean seed yield per plant was 10.5g and 17.7g under DS and NS resulting in 40.7% reduction in SY due to drought stress. Average PW were 26.2g and 36.1g under DS and NS resulting in 27.4% reduction in PW.

In Namulonge 2017, mean HM were 74 d under DS and 77 d under NS and suggesting that DS plots matured 3 d earlier than NS in this location. Significant drought effects were observed for

yield component traits such as SY and PW. The mean SY were 12.6g and 19.1g under DS and NS causing a 34 % reduction in SY due to drought stress. Average PW were 21.4g and 28.3g under DS and NS resulting in 24 % reduction in PW. Similarly, partitioning indices were affected by drought stress in Namulonge, HI were generally low with mean values of 13.2 and 16.5 under DS and NS resulting in 20% reduction in HI while average PPI were 25.5 and 28.7% under DS and NS, respectively causing 10% decrease due to DS (Table 3.1). Significant genotype by water treatments effects (GxE) across the two locations were observed for HM, HI, PW, BM and SY while non-significant GxE effects were observed for DF and PPI. This implies that the three traits namely DF and PPI were independent of the water treatments effects across the two locations.

Correlation analysis revealed high significant and positive correlations among related traits under DS. As expected positive correlations were observed among yield component traits such as SY and PW ($r=0.84$, $p<0.001$). Positive correlations were observed between HI and PPI ($r=0.82$, $p<0.001$). In addition, yield component traits were positively correlated with partitioning indices. SY was correlated with HI ($r=0.62$, $p<0.001$) and PPI ($r=0.5$, $p<0.001$). PW was moderately correlated with HI ($r=0.29$, $p<0.01$) and PPI ($r=0.23$, $p<0.05$) as in Table 3.2.

Genetic Map and QTL Analysis

The Portillo and Red Hawk parents and the RIL population were genotyped using BARC_Bean6K_3BeadChip with 5398 markers. A total of 810 SNP markers were polymorphic between the parents. Additional filtering was conducted to remove markers with severe segregation distortion and those that mapped to the same position using JoinMap 4.1. A total of 206 markers were used to build the linkage map. In this study, 10 out of 11 linkage groups representing the 10 chromosomes in common bean were constructed with exception of

chromosome Pv05 that lacked polymorphic markers between the two parents and was excluded from analysis. The Portillo x Red Hawk map spanned 766.5 cM and number of markers per linkage group ranged from 12 on Pv08 to 31 on Pv11. The largest linkage group was Pv01 with 167.3 cM and the shortest linkage group was Pv10 with 30.07 cM. The average number of SNPs per linkage group was 20 and the mean genetic distance between markers was 3.7 cM (Table 3.3). Generally the order of linkage groups and orientation agreed with those in the Stampede x Red Hawk linkage map (Song et al., 2015). In this study, 32 QTL were identified using Composite Interval Mapping (CIM) method of Win QTL Cartographer with LOD thresholds calculated using 1000 permutations. Significant QTL signals for phenology traits (DF and HM), yield component traits (PW and SY), and partitioning traits (HI and PPI) were detected on eight chromosomes namely Pv01, Pv02, Pv03, Pv04, Pv06, Pv07, Pv08, Pv10, and Pv11 (Tables 3.4, 5 &6, Figure 3.2). Quantitative trait loci associated with phenology traits mapped to Pv03, Pv06, Pv07, Pv10, and Pv11, while yield components and partitioning traits mapped to Pv01, Pv02, Pv03, Pv04, Pv06, Pv08, Pv10, and Pv11. It is worth noting that colocalized QTL signals were detected on five chromosomes namely Pv01, Pv02, Pv03, Pv04, Pv06, and Pv11.

Days to flowering

Four QTL for DF were identified in this study, three under DS and one under NS conditions on Pv03, Pv06, Pv10, and Pv11 respectively. The first QTL identified on Pv03 under NS condition, in Namulonge_2017 spanned a region of 56.5 to 69.0 cM. This QTL was flanked by markers ss715639424 (40.16 Mb) and ss715639424 (41.29 Mb) with a peak region at 67.0 cM, and the nearest marker was ss715639424 (40.16 Mb). The LOD score associated with the QTL was 3.9, additive allelic effect of 1.3 days, and explained 48% variation in DF under NS conditions. The

additive allelic effect associated with this QTL on DF was 1.3 days (Table 3.4). The second QTL for DF identified from combined analysis under DS data on Pv06 had a peak at 6.4 cM. The nearest marker associated the QTL was ss715647843 with a physical position of 6.53 Mb and accounted for 6.1% overall variation in DF under DS. The LOD score for the QTL was 3.05 and additive allelic effect on DF was -0.2 day. The third QTL signal for DF identified under DS in Kasese_2016 on Pv10 spanned a region of 18.07 to 19.7 cM, and was flanked by markers ss715649029 (37.74 Mb) and ss715639432 (38.17 Mb), with a peak position at 19.4 cM. The nearest marker was ss715646963 located at 38.09Mb. The QTL had a LOD score of 3.19 and explained 6.4% variation in DF under DS and additive allelic effect of0.1 day on DF. The fourth QTL identified under DS from combined data and in Kasese_2017 on Pv11, was detected from 10.6 to 19.69 cM with a peak at 15.7 cM, and was flanked by markers ss715649044 (1.98 Mb) and ss715646273 (2.64 Mb) in Kasese_2017. A final QTL signal for DF was detected on Pv11 in combined data and spanned 14.6 to18.6 cM interval with a peak region at 16.7 cM. The nearest marker to the peak regions was ss715646273 with a physical position of 2.64 Mb. The LOD scores associated with the QTL were 3.54 and 3.19, with R2 value of 19.4% and 16.4%, and the additive allelic effects on DF were -1.0 and -0.7 days respectively under DS conditions in Kasese_2017 and the combined analysis, respectively (Table 3.4, Figure 3.2).

Days to harvest maturity

Two significant QTL signals for HM were identified under DS and NS conditions on Pv03 and Pv07. In Namulonge_2017, a QTL for HM was detected on Pv03 under NS condition, and spanned a region from 65.0 to 67.0 cM with a peak at 65.0 cM. The nearest marker was ss715639424 with a physical position of 40.16 Mb. This QTL had a LOD score of was 3.40 and

explained 31.4% variation, with an additive allelic effect of 1.2 day on HM under NS conditions. The second QTL was identified under DS in combined data and Kasese_2016 on Pv07 at 46.7 cM between markers. ss715649609 (11.06 Mb), and ss715646773 (44.62 Mb).The nearest marker to the peak region was ss715646773 (44.62 Mb). The LOD scores associated with this QTL on Pv07 were 3.64 and 3.24, explaining 7.5% and 1.48% variation, the additive allelic effects associated with HM7.1 on HM were 0.03days and -1.2days under DS conditions in combined data analysis and Kasese_2016 respectively (Table 3.4, Figure 3.2).

Pod weight per plant

Six QTL were identified for PW on Pv01, Pv02, Pv03, Pv04, and Pv08. Significant QTL signal for PW was detected on Pv01 in Kasese_2016 under DS at 88.5 cM with a physical position of 45.15Mb. The LOD score associated with this QTL was 3.29 and explained 18.7% of the variation in PW with an additive allelic effect of -5.01g (Table 2.4).The second QTL signal was detected on Pv02 under DS in combined data, and spanned 0.01 and 4.01 cM with a peak region at 0.01cM. The nearest marker to the peak was ss715649478 with a physical position of 0.12 Mb. The LOD score for the QTL was 3.17 and explained 14.6% variation. In addition, the additive allelic effect associated with the QTL on PW was 4.11g (Table 3.4, Figure 3.2).

Another significant QTL signal for PW was detected on Pv03 in combined data under NS conditions. This QTL was located at 38.0cM tagged by marker ss715646441 at 47.81Mb, with a LOD score of 3.86, and accounted for 10.7% variation in PW. The additive allelic effect associated with this QTL signal was 3.7g. Two significant QTL signals were detected for PW on Pv04 under both DS and NS conditions in Kasese_2016. The first QTL signal was detected at 38.9 cM near marker ss715646215 at 2.67 Mb under NS conditions. This QTL had a LOD score of 3.12 with an additive allelic effect of -24.3g and explained 13.3% variation in PW. The second

QTL on Pv04 identified under DS conditions, spanning 53.7 to 54.9 cM with a peak at 53.8cM. The nearest marker to the peak region was ss715646781 (3.95Mb), with a LOD score of 4.77. The additive allelic effect associated with this signal was 1.6g and explained 5.9% variation in PW. Another QTL signal for PW was detected on Pv08 under DS conditions in combined data and spanned 32.8 to 34.7 cM with a peak at 34.3 cM tagged by marker ss715646880 with physical position of 1.21 Mb. The LOD score for this QTL was 3.33, with an additive allelic effect of 5.3g and explained 2.8% variation in PW.

Seed yield per plant

Seven QTL signals were detected for SY in this study on Pv01, Pv02, Pv03, Pv04, and Pv06. The first QTL for SY was detected on Pv01 in Kasese_2016 under both DS and NS conditions. The SY QTL detected under DS conditions spanned a region between 79.9 and 86.5 cM, and was flanked by markers ss715648564 and ss715646076, respectively. The peak region was located at 82.5cM and the nearest maker was ss715646076 with a physical position of 45.15 Mb. The LOD score for the QTL was 3.7 with an additive allelic effect of -3.21g and explained 25.1% variation in SY under DS conditions (Table 3.5, Figure 3.2). A second QTL signal for SY was detected on Pv01 under NS conditions. This QTL had a peak located at 79.9 cM and the nearest marker was ss715648564 located at 44.50 Mb. The LOD score for this peak was 3.15, and the additive allelic effect was -6.09g and explained 21.6% variation in SY under NS conditions.

Two QTL for SY were detected on Pv02 under NS and DS conditions, respectively. The first QTL was identified under DS from combined data, and spanned a region of 0.01 to 4.0 cM with a peak at 0.01 cM. The nearest marker to the peak region was ss715649478 with a physical position of 0.12 Mb. The LOD score associated the QTL was 3.3, and the additive allelic effect of 2.36g on SY, accounting for 14.03 % variation. The second QTL for SY was identified on

Pv02 under NS conditions in Kasese_2017 and spanned a region of 72.3 to 74.39 cM with a peak at 73.4 cM. The nearest marker to the peak region was ss715649647 with a physical position of 39.15Mb. The LOD score associated with the QTL was 3.27 and explained 1.9% variation in SY with an additive allelic effect of 0.61g.

Two QTL signals for SY were detected on Pv03 under both NS and DS conditions. The first QTL spanned 0.01 to 4.2 cM under NS in Namulonge_2017 and combined data under DS. The peak regions associated with these regions were 4.21 cM in and 0.01 cM respectively (Table 2.5). The QTL detected under NS conditions in Namulonge_2017 spanned 4.01cM to 4.24 cM was flanked by markers ss715648183 (0.56 Mb) and ss715649302 (0.06 Mb), the LOD score associated with this QTL signal was 3.11 and additive allelic effect of -0.07g, explaining 3.96% variation in SY. Meanwhile the QTL signal SY under DS in combined data spanned 0.01 to 4.24 cM with a peak region at 0.01 cM tagged by marker ss715648183 at 0.56 Mb. The LOD score for this peak was 11.59, with an additive allelic effect of 2.4g, explaining 13.7% variation in SY. The second QTL signal for SY on Pv03 was detected in Kasese_2017 under DS and spanned 59.5 to 73.2 cM with a peak region at 70.0 cM. The nearest marker to this peak region was ss715639424 (40.16 Mb). The LOD score associated with this QTL signal was 3.45 and additive allelic effect of 2.38g explaining 9.7% variation in SY under DS.

One QTL signal was detected for SY on Pv04 under DS in Kasese_2017 and spanned a region of 32.2 to 57.3 cM with a peak at 50.9 cM. The nearest marker to the peak region was ss715640609 located at 29.17 Mb. The LOD score associated with this QTL was 7.04 and explained 36.87% variation in SY under DS condition with additive allelic effects of 6.4g on SY (Table 3.5, Figure 3.2).

One QTL was detected for SY on Pv06 under DS conditions in Kasese_2016 and spanned a region of 8.3 to 15.9 cM flanked by markers ss715647843 (6.53 Mb) and ss715649019 (17.92 Mb) with a peak region at 8.9 cM. The nearest marker to the peak region was ss715649019 located at 17.92 Mb with a LOD score of 4.10, and a large R^2 value of 26.5% and additive effect of 2.6g on SY.

Harvest index (HI)

Six QTL signals for HI were identified in this study on Pv01, Pv03, Pv04, Pv06, Pv08, and Pv11. The first QTL for HI was detected under DS in combined data and Kasese_2017 on Pv01. In combined data, the QTL region spanned 137.4 to 151.5 cM with a peak at 148.4 cM, whereas the same QTL under DS in Kasese_2017 spanned 130.1 to 149.5 cM with a peak region at 143.4 cM. The QTL signal was flanked by markers ss715650354 (52.15 Mb) and ss715645295 (51.55 Mb), respectively, and the nearest marker to the peak regions was ss715650354 located at 52.15 Mb. The LOD scores for the QTL signals were 4.91 and 6.28 with R^2 value of 6.76% and 4.7% under DS in combined and Kasese_2017 respectively. The additive effects associated this QTL on HI were 1.06 and 1.69 respectively (Table 3.6, Figure 3.2). The second QTL detected on Pv03 in Kasese_2017 under DS conditions, spanned 71.0 to78.8 cM with a peak located at 74.8 cM. The nearest marker to this QTL was ss715649460 at 3.29 Mb, and the LOD score of 3.94 with R^2 of 5.12%. The additive allelic effects associated with the QTL on HI was -3.74.

One QTL for HI was detected on Pv04 under DS in Kasese_2016, and spanned a region of 60.9 to 62.9 cM with a peak at 61.9 cM. The nearest marker to the peak region was ss715648923 with a physical location of 45.79 Mb. The LOD score associated with the peak region was 4.22 and R^2 value of 2.53, with additive effects of -0.99 on HI. One of the QTL signal was detected on Pv06 for HI in Kasese_2016 under DS condition, and spanned a region of 8.9 to 10.9 cM with a peak

region at 8.9 cM. The nearest marker to this region was ss715649019 located at 17.92 Mb, with a LOD score of 3.01 and explained 23.3% variation in HI, the additive allelic effect of this QTL on HI was 3.09 (Table 3.6, Figure 3.2).

Another significant QTL signal for HI was detected on Pv08 under NS conditions in Kasese_2016 and spanned a region between 2.3 and 8.3 cM with a peak at 4.3 cM. The nearest marker to the peak region was ss715641300 with a physical location of 19.36 Mb. The LOD score for this QTL was 3.35 and explained 2.58% variation in HI, and additive allelic effect of 1.72 on HI. A significant QTL signal for HI was detected on Pv11 under NS conditions in Kasese_2017, located between 24.9 and 26.9 cM with a peak region at 24.9 cM. The nearest marker to the peak region was ss715645541 with a physical position of 4.31 Mb. The LOD score associated with this QTL signal was 3.31 and explained 7.05% variation in HI, with an additive allelic effect of 2.7 (Table 3.6, Figure 3.2).

Pod partitioning index (PPI)

Six QTL were detected for PPI on Pv01, Pv02, Pv04, and Pv11 under DS and NS conditions. The first QTL signal for PPI was detected in Namulonge_2017 under DS conditions and spanned a region of 59.6 to 63.1 cM, and was flanked by markers ss715648275 and ss715647963 with a peak at 61.0 cM. The nearest marker to the peak region was ss715648382 located at 6.67 Mb. The LOD score for this QTL was 3.52 and explained 11.45% variation in PPI with additive allelic effect of -4.44. The second QTL for PPI on Pv01 was identified in combined data under DS conditions and was located between 135.4 and 140.0 cM with a peak region at 138.4 cM. The nearest marker to the peak region was ss715645258 located at 50.15 Mb, and the LOD score was 3.09 with R^2 value of 3.18, and additive allelic effects of 2.92 on PPI (Table 3.6, Figure 3.2).

A third QTL for PPI was detected on Pv02 under DS in Kasese_2017 and spanned a region of 19.1 to 21.7 cM, flanked by markers ss715639436 and ss715647234 with a peak region located at 20.1 cM. The nearest marker to the peak region was ss715647234 with a physical position of 2.52 Mb. The LOD score associated was 3.04 and explained 10.44% of the variation in PPI and the additive allelic effect of 2.06 under DS conditions.

Another QTL signal was detected on Pv04 under DS conditions in Kasese_2017 and spanned a region of 59.9 to 60.9 cM, and was located between markers ss715648130 and ss715648923 with a peak at 59.9 cM. The nearby marker to the peak region was ss715648923 located at 45.79 Mb. The LOD score associated this QTL was 3.70 and R^2 value of 1.8%. The additive allelic effect associated the QTL was -3.9 (Table 3.6, Figure 3.2).

Two QTL signals were detected on Pv11 for PPI were identified under NS conditions in Kasese_2016 and Kasese_2017respectively. The first QTL was detected in Kasese_2017 and spanned a region between 20.6 and 23.4 cM flanked by markers ss715646273 and ss715648851. The peak region was 23.5 cM with marker ss715648851 located at 5.19 Mb as the nearest marker. This QTL had a LOD score of 3.68 and explained 17.56 % variation in PPI and with additive allelic effect of 4.66 on PPI. The second QTL detected on Pv11 for PPI was located at 45.63 cM as the peak region, and the nearest marker was ss715650599 with a physical position of 45.63 Mb. This QTL had a LOD score of 3.26 and explained 14% variation in PPI. The additive allelic effect on PPI was -3.42 under NS conditions (Table 3.6, Figure 3.2).

Colocalized QTL Regions

In this study, colocalized signals were detected for phenology, yield component traits and partitioning indices on Pv01, Pv02, Pv03, Pv04, Pv06, and Pv11. Interestingly, the lower arm of

chromosome Pv01 was the peak region for QTL associated with yield component traits namely PW and SY under NS conditions and for partitioning traits HI and PPI under DS. The nearest markers flanking the peak regions were ss715648564 at 44.50Mb and ss715646075 at 45.15 Mb, respectively. In addition, the upper arm of chromosome Pv02 was the peak region for significant QTL signals for PW and SY detected under DS conditions in combined analysis, and tagged by SNP ss715649478 with a physical position of 0.12 Mb.

Another colocalized QTL region was identified on Pv03 for DF and HM under NS conditions in Namulonge_2017 and for SY under DS conditions in Kasese_2017.The nearest common SNP to the peak region was ss715639424 located at 40.16 Mb. Colocalized QTL signals for partitioning traits namely HI and PPI were identified on Pv04 under DS conditions and the common marker to the peak region was ss715648923 located at 45.79 Mb. In addition, another colocalized QTL signal was detected on Pv06 HI and SY under DS conditions and the common marker associated with the peak region was ss715649019 located at 17.92 Mb (Figure 3.6, Figure 3.2).

Discussion

In the current study, a RIL mapping population was developed from a cross between Portillo a red mottled, drought tolerant Andean variety from Ecuador and Red Hawk, red kidney bean variety, susceptible to drought. Both parents from the Andean gene pool were contrasting for drought tolerance and for seed color patterns. The population was evaluated to identify QTL associated with drought tolerance traits at the pod filling stage. Significant differences ($P<0.01$) drought effects were observed for all agronomic traits except for DF. Significant genotype by water treatments effects (GxE) were also observed across the two locations except for DF and PPI. This implies that the three traits namely DF, PHI and PPI were independent of the water

treatments effects across the two locations. Mukeshimana et al. (2014) observed similar GxE effects on DF and partitioning indices in SEA5 x CAL96 mapping population evaluated in Rwanda and Colombia. As expected positive correlations were observed among yield component traits and partitioning traits, consistent with previous studies (Ramirez-Vallejo and Kelly, 1998; Mukeshimana et al., 2014), suggesting that mechanisms associated with drought responses have pleotropic effects on traits contributing to performance under drought in this population.

In this study, QTL for phenology traits mapped to chromosomes Pv03, Pv06, Pv07, Pv10 and Pv11. The QTL for DF designated as DF3.2^{PR} was detected on Pv03 NS condition in Namulonge_2017 and explained 48% variation. The nearest marker to the peak region was ss715639424 located at 40.16 Mb. Previous studies mapped QTL for DF on Pv03 (Checa and Blair, 2012; Brinez et al., 2017). The QTL DF3.1^{GG} flanked by markers BM197 and BM181B on Pv03 for DF was identified in $F_{5:8}$ derived G2333 × G19839 RIL population evaluated under high phosphorus in Darién (Checa and Blair, 2012). Another QTL DF3.1^{AS} was reported in SEA 5 x AND 277 population under NS conditions (Brinez et al., 2017), and spanned 74.5 to159.2 cM on Pv03 tagged by marker BAR3097. However, comparison of DF3.1^{GG} and DF3.1^{AS} QTL reported on Pv03 was not possible due to lack of physical positions for markers BM197, BM181B, and BAR3097. Additionally, QTL for DF mapped to Pv06, Pv10, and Pv11 under DS conditions. Previous studies reported QTL for DF on Pv06 (Blair et al., 2006; Perez-Vega et al., 2010; Blair et al., 2012), and Pv11 (Tar'an et al., 2002; Blair et al., 2006; Kwak et al., 2008; Blair et al., 2012). The QTL DF6.1^{CG} and DF6.2^{CG} on Pv06 flanked by markers BM170 (25.09 Mb)-V and V-BM187(20.25 Mb) were reported in advanced backcross a mapping population derived from Andean cultivar ICA Cerinza and G24404, a wild species of common bean (Blair et al.,

2006). Other QTL DF6.1^{DB} and DF6.2^{DB} were identified in DOR364 x BAT477 intra-gene pool mapping population under DS and NS conditions. These QTL were tagged by marker M1601 located between markers BM278 (2.69 Mb) and BMb1157 (12.11 Mb). The QTL DF6.1^{PR} identified in this study was near marker ss715647843 at 6.53 Mb under DS in combined data was within the marker interval for QTL reported by Blair et al. (2012) although the physical position of marker M1601 was not known. The QTL DF11.1^{DB} and DF11.2^{DB} for DF were detected in DOR364 x BAT477 under DS and NS conditions (Blair et al., 2012), and under moderate to low phosphorus condition (Diaz et al., 2017). These QTL were tagged by markers AB502 and P302 whose physical positions were not known but flanked by BMd43 (1.71 Mb) and BMb1074 (48.04 Mb) respectively. In addition, QTL DF11.1^{PR} for DF tagged by ss715646273 at 2.64 Mb was most likely the same QTL DF11.1^{DB} reported in previous studies (Blair et al., 2012; Diaz et al., 2017).

In this study, QTL for DM were identified on Pv03 and Pv07 under NS and DS conditions. Previous studies consistently reported QTL for DM on Pv03 (Hoyos-Villegas et al., 2015) and on Pv07 (Blair et al., 2006; Mukeshimana et al., 2014). The QTL DM3.1^{PR} was near marker ss715639424 at 40.16 Mb identified in this study was the same QTL DM3.1^{AP} linked to marker ss715646619 at 39.50 Mb reported in AN-37 x P02630 population under white mold pressure (Hoyos-Villegas et al., 2015). The second QTL DM7.1^{PR} was identified on Pv07 DS conditions and was tagged by marker ss715646773 at 44.6 Mb. The QTL DM7.1^{SC} identified on Pv07 linked to marker ss715645222 at 51.21 Mb in SEA5 x CAL96 inter-gene pool cross under NS and DS conditions (Mukeshimana et al., 2014) were 6.6 Mb apart, implying that these are the same QTL.

In addition, marker ss715646773 (44.6 Mb) on Pv07 is 1.025 kb upstream of gene Phvul.007G207300 annotated as "TETRATRICOPEPTIDE REPEAT (TPR)-LIKE SUPERFAMILY PROTEIN'. Members of TPR regulate plant hormonal responses especially ethylene biosynthesis that directly regulates transition from reproductive stage to maturity (Schapire et al., 2006; Iqbal et al., 2017). In addition, Arabidopsis, TETRATRICOPEPTIDE-REPEAT THIOREDOXIN-LIKE 1 (TTL1) has been reported as a positive regulator of ABA signaling under drought and osmotic stresses (Rosado et al., 2006).

Six QTL for PW were detected on Pv01, Pv02, Pv03, Pv04, and Pv08. Furthermore, correlation between PW and number of pods per plant in this study were positive and significant ($r=0.67$; $p<0.001$) (data not presented). Several QTL associated pod traits were previously reported on Pv01, Pv02, Pv03, and Pv04 (Beattie et al., 2003; Checa and Blair, 2012; Gonzalez et al., 2016; Diaz et al., 2017), and PW was mapped to Pv08 (Kamfwa et al., 2015). Interestingly, the marker ss715646880 at 1.21 Mb linked to QTL $PW8.1^{PR}$ on Pv08 in this study were in LD ($r2 > 0.65$; $D' > 0.85$), with two marker ss715649359 (4.74 Mb) and ss715639408 (5.15 Mb) that were significantly associated with PW in Andean diversity panel (Kamfwa et al., 2015), suggesting the same genomic region is involved.

Seven QTL signals were detected for SY in this study on Pv01, Pv02, Pv03, Pv04, and Pv06 under DS and NS conditions. This is consistent with previous studies that reported significant QTL signals for SY on the same chromosomes. In this study, QTL $SY1.1^{PR}$ was detected on Pv01 under both NS and DS condition near markers ss715646076 at 45.15 Mb and ss715648564 at 44.50 Mb respectively. SY QTL were consistently reported on Pv01 and Pv02 in Buster x Roza and DOR364 x BAT477 mapping populations evaluated under NS and DS conditions in multi-locations for several years (Asfaw et al., 2012; Trapp et al., 2015). Trapp et al. (2015)

reported SY1.1^{BR} at 47.7 Mb near marker SNP50809 in Buster x Roza mapping population under multiple stress environments, suggesting these two QTL were the same. In addition, Asfaw et al. (2012) reported the same QTL signal for SY on Pv1 under NS tagged by marker BM200 at 30.8 Mb.

The QTL SY2.1^{BR} for SY on Pv02 was identified in Buster x Roza mapping population at 11.8 Mb near marker SNP40055 (Trapp et al., 2015). This QTL was 11.7 Mb downstream of SY2.1^{PR} on Pv02 near marker ss715649478 (0.12 Mb) identified in the current study under DS in combined data. In addition, the second QTL SY2.2^{PR} for SY identified on Pv02 near marker ss715649647 (39.15 Mb) in this study was near QTL YD2.1^{DB} reported under low phosphorus condition tagged by BMa16 (33.18 Mb) in DOR364 x BAT477 mapping populations (Diaz et al., 2017). The marker ss715649647 (39.15 Mb) was not in LD with markers within the same genomic region as BMa16 (33.18 Mb) implying that SY QTL SY2.1^{BR} and YD2.1^{DB} were different as verified in this study.

Two QTL signals were detected for SY on Pv03 in the current study. This is consistent with several previous studies that reported QTL for yield on Pv03 (Wright and Kelly, 2011; Blair et al., 2012; Checa and Blair, 2012; Mukeshimana et al., 2014; Hoyos-Villegas et al., 2015; Kamfwa et al., 2015; Heilig et al., 2017). The QTL YLD3.1^{CG} linked to marker BM172 and YLD3.2^{CG} near marker BM98 for yield were detected in Popayán using Cerinza x G24404 advanced backcross population (Blair et al., 2006). Yield QTL YLD3.1^{GG}, YLD3.2^{GG} linked to markers BM197 and AG01 were reported in G2333 x G19839 mapping population in Popayán (Checa and Blair, 2012), and QTL YLD3.1^{DB}, YLD3.2^{DB} , YLD3.3^{DB} , YLD3.4^{DB} near markers AE103, Q1001, BM181, and AD1801 respectively were detected on Pv03 under NS conditions in DOR364 x BAT477 mapping population (Blair et al., 2012). However, yield QTL reported in

many of the previous studies could not verified due to unknown physical positions of markers near those QTL (Blair et al., 2006; Wright and Kelly, 2011; Blair et al., 2012; Checa and Blair, 2012). The SY3.3^{PR} for SY on Pv03 was identified under DS conditions in Kasese_2017 linked to ss715639424 at 40.16 Mb. The SY QTL on Pv03 has been consistently reported in several studies (Kelly, 2018). The QTL SY3.3^{SC} on Pv03 was initially reported in SEA5 x CAL96 mapping population under DS and NS conditions (Mukeshimana et al., 2014). A subsequent study reported significant SNP association for SY on Pv03 associated with marker ss715648538 at 38.27 Mb using Andean beans under low-N conditions in Michigan (Kamfwa et al., 2015). Interestingly, the significant marker ss715648538 was in strong LD with markers within SY3.3^{SC} interval, implying that the gene(s) underlying the QTL for seed yield reported in the two studies was the same (Mukeshimana et al., 2014; Kamfwa et al., 2015; Kelly, 2018). In addition, two separate studies mapped SY3.3 on Pv03 near markers ss715639345 (39.6 Mb) and ss715646621 (39.4 Mb) in Puebla 152 x Zorro mapping population (Heilig et al., 2017). The same QTL was mapped between markers ss715647671 (37.06 Mb) and ss715639244 (45.59 Mb) using a NAM population of Merlot with three small red-seeded bean lines (S48M, S94M, and S95M) under DS conditions (Hoyos-Villegas et al., 2016), further validating SY QTL SY3.3. The second SY QTL SY3.4^{PR} was identified on Pv03 under DS and NS conditions near marker ss715648183 at 0.56 Mb. This QTL SY3.4^{PR} at the upper arm of Pv03 could have been reported in previous studies but could not be compared due to lack of physical positions of markers linked to QTL reported in previous studies.

The QTL SY4.1^{PR} and SY6.1^{PR} for SY were identified on Pv04 and Pv06 under DS conditions in Kasese_2016,and 2017 respectively, which is consistent with previous studies (Cichy et al., 2009; Pérez-Vega et al., 2010; Asfaw et al., 2012; Blair et al., 2012). The QTL SY6.1^{PR} on Pv06

near marker ss715649019 at 17.92 Mb identified in this study was the same to YLD6.1^{DB} detected in DOR364 x BAT477 mapping population for SY under DS on Pv06 tagged by marker M501, located between flanking markers BMb342 (13.44 Mb) and IAC115 (15.45 Mb).

Significant QTL signals for partitioning traits namely HI and PPI were detected on Pv01, Pv02, Pv03, Pv04, Pv06, Pv08, and Pv11 under DS and NS conditions. Six QTL for HI were identified on Pv01, Pv03, Pv04, Pv06, Pv08, and Pv11. Asfaw et al., (2012) reported significant QTL signals for HI on Pv03, Pv04, Pv06 and Pv11 in DOR364 x BAT477 mapping population.

Interestingly, the QTL HI6.1^{PR} on Pv06 near ss715649019 at 17.92 Mb detected in the current study was within the marker interval for HI was reported on Pv06 near marker Y501 flanked by IAC115 (15.45 Mb) and BM187 (20.25 Mb) in DOR364 x BAT477 mapping population (Asfaw et al., 2012). Similarly, QTL H11.1^{PR} on Pv11 tagged by ss715645541 at 4.31 Mb was near marker BMd43 (1.73 Mb) that was flanking the peak marker AB502 in the same mapping population (Asfaw et al., 2012), implying that the QTL reported in the two studies were the same. The QTL HI6.1^{PR} on Pv06 is near ss715649019 at 17.92 Mb.

Six significant QTL signals for PPI were detected on Pv01, Pv02, Pv04, and Pv11. Several significant QTL signals for PPI were reported on Pv02, Pv04 and Pv11 using DOR364 x BAT477 mapping population (Asfaw et al., 2012). The QTL PPI11.1^{PR} and PPI11.2^{PR} detected for PPI were linked to markers ss715648851 at 5.19 Mb and ss715650599 at 45.63 Mb. The location is consistent with previous studies that detected QTL for PPI on Pv011 near marker AB502 flanked by BMd43 (1.73 Mb) and BMb1074 (48.04 Mb) in DOR364 x BAT477 mapping population (Asfaw et al., 2012), and near peak regions detected in this study.

Putative candidate genes in genomic regions associated with colocalized QTL peaks

In this study, QTL signal for phenology trait colocalized on the lower arm of Pv03. DF3.2^{PR} and DM3.1^{PR} QTL were identified under NS conditions in Namulonge_2017 and the nearest common SNP to the peak region was ss715639424 located at 40.16 Mb. Within 31kb region upstream of ss715639424 SNP were two genes that could be considered positional candidates. Gene *Phvul.003G189100* was 30.3kb upstream ss715639424 and encodes "AGAMOUS-LIKE 65 (AGL65)", a MADS-box gene family protein. AGL65 has been reported as a regulator of pollen development through activation of mature pollen genes (Verelst et al., 2007). Additional, previous studies in soybean reported several AGAMOUS-LIKE genes as key regulators of floral organ identity, flowering time and seed development (Fan et al., 2013; Chen et al., 2014). Another important gene in this region was *Phvul.003G189300,* 17.9kb upstream of ss715639424 that encodes "CYCLING DOF FACTOR 3 (CDF3)", a member of the DOF family transcriptional factor that regulates flowering time by transcriptional repressing the activity of "CONSTANS (CO)" and "FLOWERING LOCUS (FT)"(Fornara et al., 2009; Fornara et al., 2015). The significant QTL signals detected Pv03 for DF and HM may contain genes that regulate the expression of plant flowering and maturity under NS conditions.

In common bean, significant progress has been made to improve seed yield under drought stress particularly within the Middle American gene pool. However, selecting for stable seed yield under DS and NS conditions in the Andean gene pool has been a challenge. Recent studies reported the use of yield component traits and partitioning indices that positively contribute to seed yield stability under DS as surrogate traits for indirect selection of superior drought tolerant cultivars under NS and DS (Ramirez-Vallejo and Kelly, 1998; Rosales-Serna et al., 2004; Miklas et al., 2006; Beebe et al., 2008).

In addition, several studies reported that partitioning indices especially harvest index and pod harvest index as primary indicators of photosynthate remobilization potential from shoot biomass to yield under drought stress conditions (Rosales-Serna et al., 2004; Beebe et al., 2008; Assefa et al., 2013). In this study, significant and positive correlations were observed between and among yield component traits and partitioning indices (Table 7) under DS conditions, which is consistent with previous studies (Klaedtke et al., 2012; Mukeshimana et al., 2014). Significant QTL signals for yield component traits and partitioning traits colocalized on Pv01, Pv02, and Pv04 suggesting that genomic regions condition these traits were linked and could be used for indirect selection for yield in marker assisted breeding. .

The seed yield QTL SY1.1^{PR} that mapped to Pv01 was detected under NS and DS conditions in Kasese_2016. This QTL colocalized with partitioning trait QTL HI1.1^{PR} identified under DS in the combined data and in Kasese_2017 and PPI1.2^{PR} detected under DS in combined study. The significant SNPs associated with the peak QTL signals were ss715646076 (45.15 Mb) for SY1.1^{PR} ss715650354 (52.15 Mb) for HI1.1^{PR} and ss715645258 (50.15 Mb) for PPI1.2^{PR} respectively. The SNPs were not in LD and spanned 1.99Mb (45.15 to 52.15 Mb) region containing 275 genes with diverse functional annotations. In order to gain insights into the genes associated with these traits in these common genomic regions, we performed functional annotations of positional candidate genes tagged by the significant SNP ss715646076 (45.15 Mb) for SY1.1^{PR}. Two genes within 35kb region upstream of this marker were considered candidates. Gene *Phvul.001G186400* is 19.2kb upstream of SNP ss715646076, annotated as "AGAMOUS-LIKE 62 (AGL62)". AGL62 is a type I MADS-box domain protein that acts as a key regulator of endosperm cellularization, directly influencing seed size, sink strength and seed weight (Kang et al., 2008). The second gene is *Phvul.001G186300* 32kb upstream of SNP

ss715646076, annotated as “HISTONE DEACETYLASE 3 (HDA3, HD2A)” a plant specific histone that has been reportedly involved in regulating gene expression during seed development and maturation in Arabidopsis (Zhou et al., 2004). While within 60kb region upstream of the significant marker ss715645258 (50.15 Mb) associated with QTL PPI1.2^{PR} were three positional candidate genes. Gene *Phvul.001G241700* is 18kb upstream of marker s71 5645258, annotated as “ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1 (AMT1, ASA1)”, an enzyme catalyzing the rate limiting stage in the biosynthesis of tryptophan (Kreps et al., 1996; Tien Lea et al., 2016). Gene *Phvul.001G241500* is 29kb upstream of marker s715645258 and encodes “TRANSMEMBRANE AMINO ACID TRANSPORTER FAMILY PROTEIN (AUX1, MAP1)” an auxin-influx carrier protein that is associated with transport of auxin hormone (Yang et al., 2006; Basu et al., 2013). Candidate gene *Phvul.001G241200* is 59kb upstream of SNP ss715645258 and encodes “PLASMA MEMBRANE INTRINSIC PROTEIN 1;4 (PIP1;4,PIP1E)”, a member of aquaporin family protein that is associated with movement of water and small molecules across membrane (Gomes et al., 2009). However, in Arabidopsis PIP1;4 has been reported to play a dual function in water movement especially under drought stress and $C0_2$ permeability across plasma membrane hence increasing photosynthetic output (Alexandersson et al., 2005; Li et al., 2015). Ariani and Gepts (2015) recently characterized aquaporin’s in common bean into five sub families and (Hoyos-Villegas et al., 2017) reported candidate gene *Phvul.011G102700* that encodes *PvSIP1;3* gene, aquaporin on Pv11 during wilting in common bean suggesting their involvement in water transport.

A second region of interest located on Pv02 was the peak region for QTL SY2.1^{BR} and PW2.1^{PR} detected under DS conditions in the combined analysis. This region was tagged by SNP ss715649478 with a physical position of 116.65 kb. SNP ss715649478 is within the genic region

of *Phvul.002G001800* that is annotated as "CATIONIC AMINO ACID TRANSPORTER 6 (CAT6)", a putative sink-specific amino acid transporter that is involved in phloem loading of amino acids (Su et al., 2004; Hammes et al., 2006). Recent studies reported the association of CAT6 in source–sink partitioning through import of amino acids into developing seed (Rentsch et al., 2007), suggesting possible involvement in partitioning of assimilates to yield components. The upper arm of chromosome Pv04 is the peak region for QTL PW4.2 and SY4.1 detected in combined data under DS. This region was tagged by marker ss715646781 located at 3.95 Mb within genomic region containing cluster of genes that encode UDP-GLYCOSYLTRANSFERASE SUPERFAMILY PROTEIN (UGT) that are involved in glycosylation of plant compounds (Wang and Hou, 2009).

Conclusion

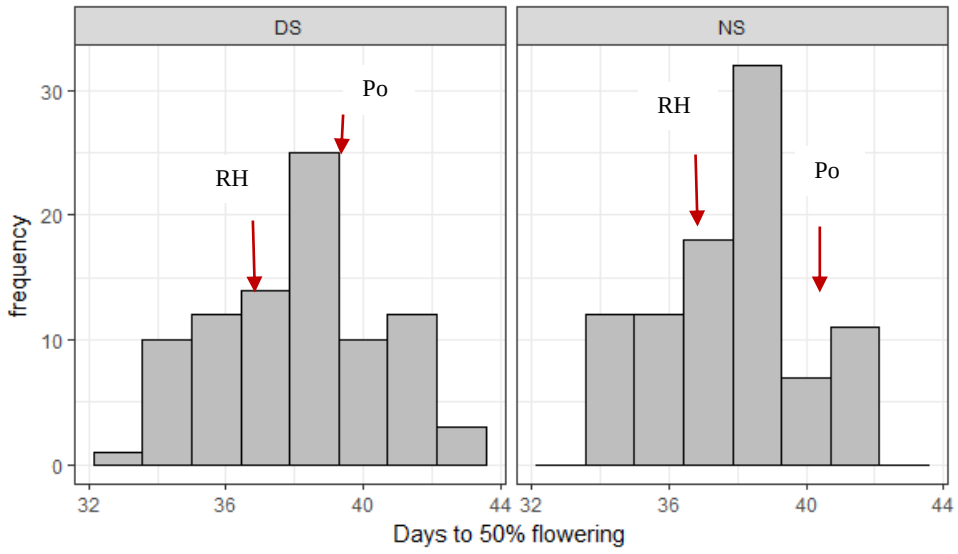
The objective of this study was to map QTL associated drought tolerance within the Andean Portillo x Red Hawk mapping population. Significant differences were observed among the mapping population for phenology, yield component and partitioning traits. Thirty-two QTL were identified using CIM method for agronomic traits measured under DS and NS conditions in Uganda for two seasons and locations. Consistent with correlation analysis for agronomic traits, colocalized QTL signals were also identified for yield component traits and partitioning traits on Pv01, Pv02, Pv03, Pv04, Pv06 and Pv11. We performed functional annotation of positional candidate genes tagged by significant SNP markers within the colocalized peak region. We report two candidate genes, *Phvul.003G189100*, 30.3 kb upstream ss715639424, and encodes "AGAMOUS-LIKE 65 (AGL65)", a MADS-box gene family protein a positive regulator of pollen development on Pv03. In addition, *Phvul.003G189300,* 17.9 kb upstream of ss715639424 and encodes "CYCLING DOF FACTOR 3 (CDF3)", a member of the DOF family

transcriptional factor which acts as transcriptional repressor of two flowering time genes namely "CONSTANS (CO)" and "FLOWERING LOCUS (FT)". These candidate genes are involved in regulating flowering time; floral organ identity and development were associated with phenology and detected in the peak region for DF3.2^{PR} and DM3.1^{PR} under NS conditions

In addition, we identified positional candidate genes on Pv01 that were significantly associated with QTL SY1.1^{PR} and PPI1.2^{PR} under both DS and NS conditions. *Phvul.001G186400*, 19.2 kb upstream of SNP ss715646076, annotated as "AGAMOUS-LIKE 62 (AGL62)", a type I MADS-box domain protein that regulates endosperm cellularization, directly influencing seed size, sink strength and seed weight. Candidate gene *Phvul.001G241500* annotated as "TRANSMEMBRANE AMINO ACID TRANSPORTER FAMILY PROTEIN (AUX1, MAP1)" is involved in the transport of auxin hormone, and *Phvul.001G241200*, annotated as "PLASMA MEMBRANE INTRINSIC PROTEIN 1;4 (PIP1;4,PIP1E) is associated with movement of water and small molecules across membrane. Interestingly the colocalized QTL signal for SY2.1^{BR} and PW2.1^{PR} on Pv02 was tagged by SNP ss715649478 (116.65 kb), within the genic region of *Phvul.002G001800* and annotated as "CATIONIC AMINO ACID TRANSPORTER 6 (CAT6)", a putative sink-specific amino acid transporter that is involved in phloem loading of amino acids into developing seed. The results from this study improve our understanding genetic control and association between seed development, sink-specific amino acid transport and biosynthesis, and water movement during reproductive stage of common bean under DS and the pleiotropic effects of genes associated with colocalized regions on the traits.

APPENDIX

Figure 3.1a Histograms of days to flowering and days to maturity for Portillo x Red Hawk common bean recombinant inbred line (RIL) population evaluated under drought stress (DS) and non-stress (NS) in Kasese and Namulonge, Uganda in 2016 and 2017



RH Red hawk, **Po** Portillo

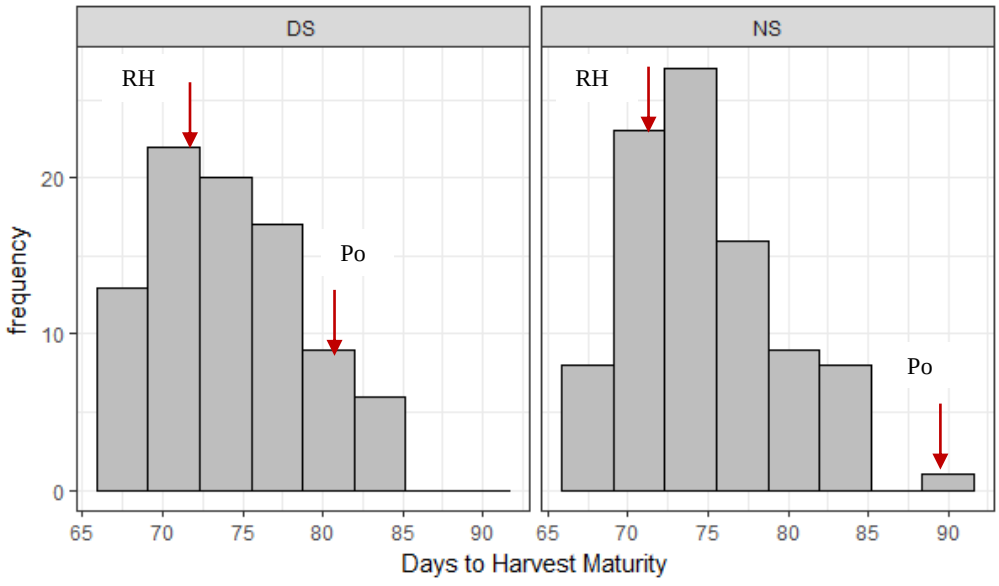
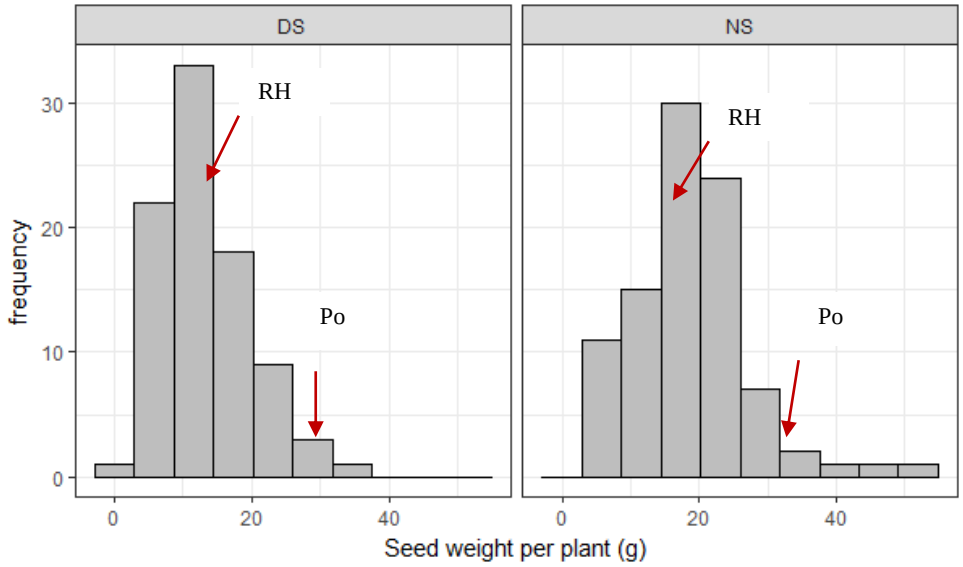


Figure 3.1b Histograms of seed yield per plant and pod weight per plant for Portillo x Red Hawk common bean recombinant inbred line (RIL) population evaluated under drought stress (DS) and non-stress (NS) in Kasese and Namulonge, Uganda in 2016 and 2017



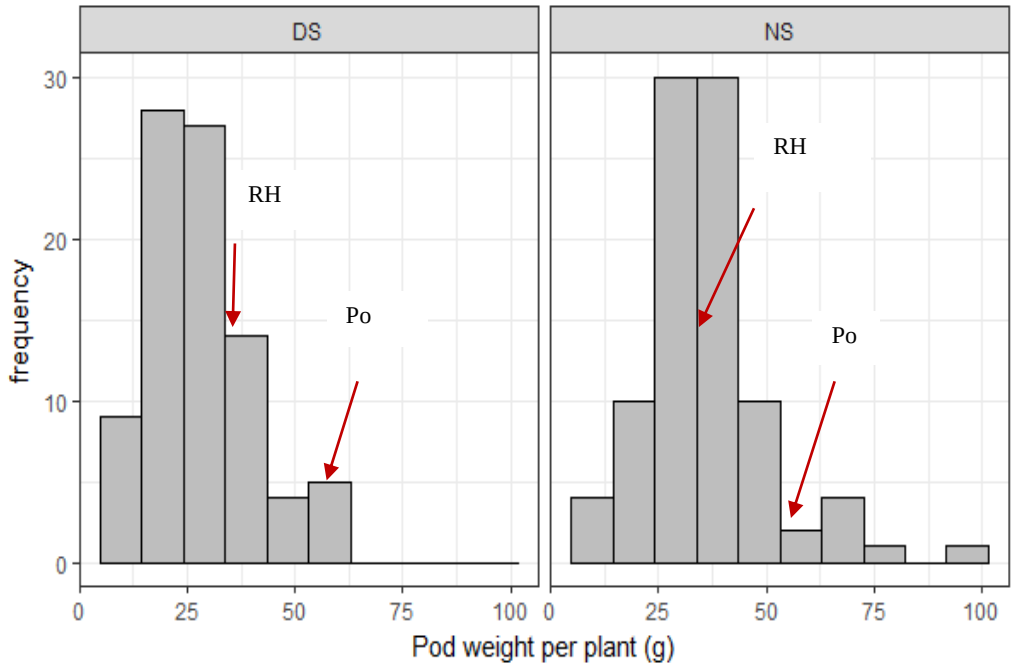


Figure 3.1c Histograms of harvest index and pod partitioning index for Portillo x Red Hawk common bean recombinant inbred line (RIL) population evaluated under drought stress (DS) and non-stress (NS) in Kasese and Namulonge, Uganda in 2016 and 2017

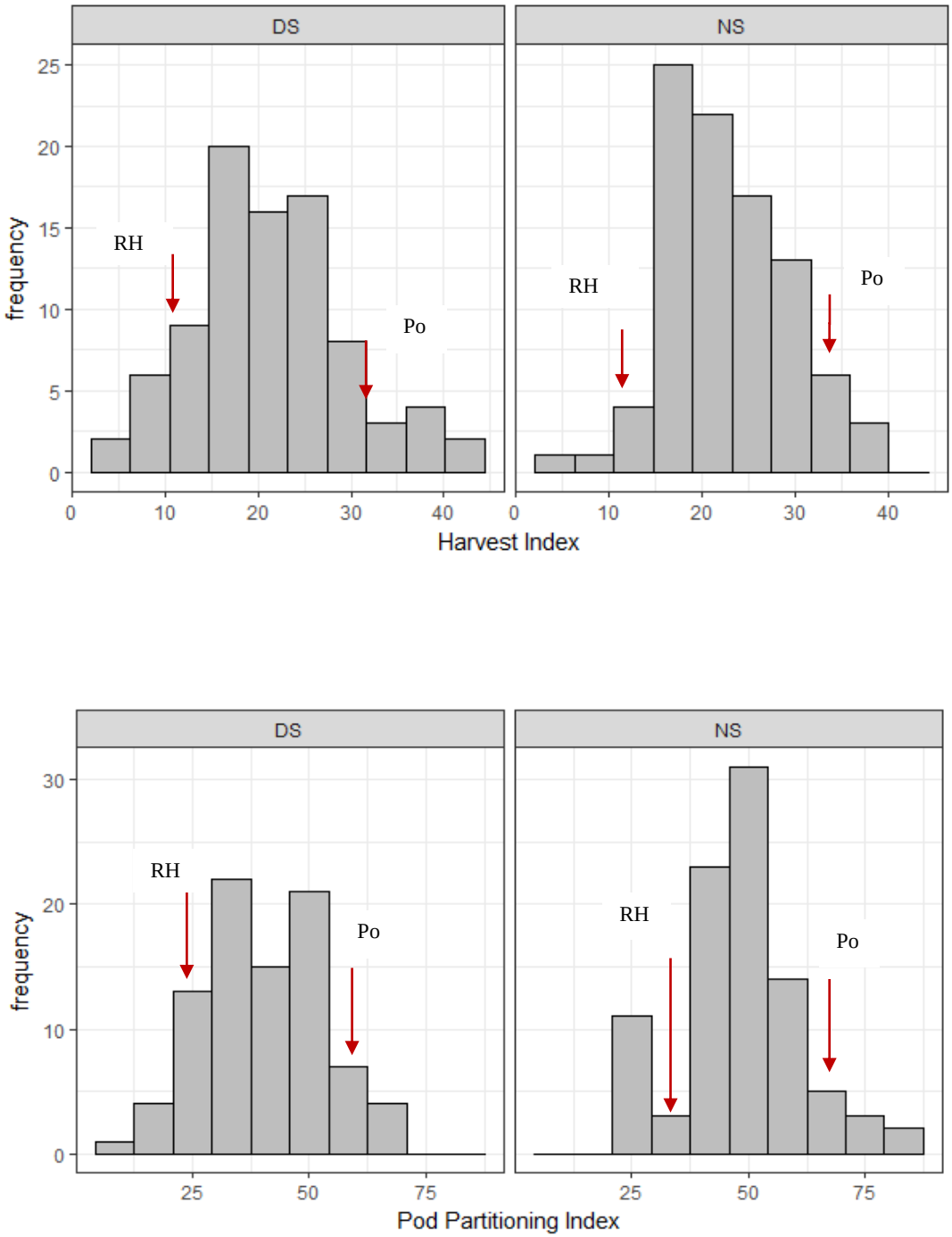


Table 3.1 Mean, range for yield component traits, phenology and portioning indices for Portillo x Red Hawk common bean recombinant inbred line (RIL) population evaluated under drought stress (DS) and non-stress (NS) in Kasese and Namulonge, Uganda in 2016 and 2017**.**

	Kasese _2016		Kasese _2017		Namulonge _2017		Treatment effects			
	DS	NS	DS	NS	DS	NS	ANOVA			
Trait	mean (range)	mean (range)	mean (range)	mean (range)	mean (range)	mean (range)	G	T	G*T	H^2
DF	36.7(33-40)	36.4(33-40)	38.7(32-43)	38.2(31-45)	38.1(33-43)	37.4(34-41)	***	ns	ns	0.87
HM	72.5(65-93)	75.5(67-91**)**	73.2(69-91)	77.5(68-85)	74.2(67-93)	77.1(70-88)	**	*	*	0.79
PW	21.9(7-70)	32.9(8-86)	26.2(5-86)	36.1(7- 45)	21.4(7-74)	28.3(7-87)	*	**	*	0.74
SY	9.9(3-32)	18.1(4-79)	10.5(4-51)	17.7(6-63)	12.6(4-60)	19.4(4-65)	***	***	**	0.61
HI	15.3(2-37)	20.9(4-53)	27.7(7-53)	30.4(12-44)	13.2(4-35)	16.5(4-29)	*	**	*	0.52
PPI	33.0(3-56)	44.6(11-83)	47.2(15-72)	59.4(28-87)	25.6(6-61)	28.7(26-64)	*	*	ns	0.63

DF days to 50% flowering(d), **HM** days to maturity (d), **PW** pod weight per plant(g), **SY** seed yield per plant(g), **HI** harvest index, **PPI** pod partitioning index, **DS** drought stress, **NS** non-stress**, G** genotype, **T** treatment, **G*T** genotype x treatment interaction**,** $\mathbf{H^2}$ broad sense heritability . * Significant at the 0.05 probability level, ** Significant at the 0.01 probability level, *** Significant at the 0.001 probability level, and **ns** not significant.

Table 3.2 Pearson correlation coefficients among mean variables for days to flowering, days to maturity, harvest index, pod partitioning index , seed yield per plant, and pod weight per plant for the Portillo x Red Hawk recombinant inbred line (RIL) population evaluated under drought stress and non-stress in Kasese and Namulonge, Uganda during 2016 and 2017 growing seasons.

	DF	DM	HI	PPI	SY	PW
DF		0.42***	-0.03ns	-0.04ns	-0.03ns	-0.11ns
DM			-0.08ns	-0.15ns	-0.05ns	0.03ns
HI				0.82***	0.62***	0.29**
PPI					0.50***	0.24**
SY						0.84***
PW						

DF days to 50% flowering(d), **HM** days to maturity(d), **HI** harvest index, **PPI** pod partitioning index, **PW** pod weight per plant(g), **SY** seed yield per plant(g). ***** Significant at the 0.05 probability level, ****** Significant at the 0.01 probability level, ******* Significant at the 0.001 probability level, and **ns** not significant.

Table 3.3 Single nucleotide polymorphism (SNP) marker distribution and distance on individual chromosomes of the genetic linkage map of Portillo x Red Hawk recombinant inbred line (RIL) population

Chromosome	No. of markers	Length	Mean distance(cM)
Pv01	20	167.33	8.4
Pv02	16	103.39	6.5
Pv03	22	86.51	3.9
Pv04	23	87.46	3.8
Pv06	14	56.89	4.1
Pv07	22	68.88	3.1
Pv08	12	46.15	3.8
Pv09	31	71.79	2.3
Pv10	20	30.07	1.5
Pv11	26	48.03	1.8
Entire genome	206	766.5	3.7

Table 3.4 Quantitative trait loci (QTL) for days to 50% flowering(DF), days to maturity(DM) and pod weight per plant(PW) in the Portillo x Red Hawk recombinant inbred line (RIL) evaluated under drought stress and non-stress in Kasese and Namulonge, Uganda during 2016 and 2017 growing seasons.

Trait	QTL	Chromosome	Site	Year	Treatment	Peak(cM)	Near marker	Peak(Mb)	LOD	Additive	R2
DF	DF3.2^{PR}	Pv03	Namulonge	2017	NS	67.01	ss715639424	40.16	3.96	1.34	48.3
	DF6.1^{PR}	Pv06	Combined		DS	6.41	ss715647843	6.53	3.05	-0.23	6.1
	DF10.1^{PR}	Pv10	Kasese	2016	DS	19.41	ss715646963	38.09	3.19	0.10	6.4
	DF11.1^{PR}	Pv11	Combined		DS	16.71	ss715646273	2.64	3.19	-0.67	16.4
	DF11.1^{PR}	Pv11	Kasese	2017	DS	15.71	ss715646273	2.64	3.54	-1.04	19.4
DM	DM3.1^{PR}	Pv03	Namulonge	2017	NS	65.01	ss715639424	40.16	3.40	1.19	31.4
	DM7.1^{PR}	Pv07	Combined		DS	46.71	ss715646773	44.62	3.64	0.03	7.5
	DM7.1^{PR}	Pv07	Kasese	2016	DS	46.61	ss715646773	44.62	3.24	-1.20	1.5
PW	PW1.1^{PR}	Pv01	Kasese	2016	DS	88.51	ss715646076	45.15	3.29	-5.01	18.7
	PW2.1^{PR}	Pv02	Combined		DS	0.01	ss715649478	0.12	3.17	4.11	14.6
	PW3.1^{PR}	Pv03	Combined		NS	38.01	ss715646441	47.81	3.86	3.74	10.7
	PW4.1^{PR}	Pv04	Kasese	2016	DS	53.81	ss715646781	3.95	4.77	1.61	5.9
	PW4.2^{PR}	Pv04	Kasese	2016	NS	38.91	ss715646215	2.67	3.12	-24.33	13.3
	PW8.1^{PR}	Pv08	Combined		DS	34.31	ss715646880	1.21	3.33	5.39	2.6

Table 3.5 Quantitative trait loci (QTL) for seed yield in the Portillo x Red Hawk recombinant inbred line (RIL) evaluated under drought stress and non-stress in Kasese and Namulonge, Uganda during 2016 and 2017 growing seasons.

Trait	QTL	Chromosome	Site	Year	Treatment	Peak(cM)	Near marker	Peak(Mb)	LOD	Additive	R2
SW	$SY1.1^{PR}$	Pv01	Kasese	2016	DS	82.51	ss715646076	45.15	3.65	-3.21	25.1
	$SY1.1^{PR}$	Pv01	Kasese	2016	NS	79.91	ss715648564	44.50	3.15	-6.09	21.6
	$SY2.1^{BR}$	Pv02	Combined		DS	0.01	ss715649478	0.12	3.30	2.36	14.0
	$SY2.2^{BR}$	Pv02	Kasese	2017	NS	73.41	ss715649647	39.15	3.27	0.61	1.9
	$SY3.3^{PR}$	Pv03	Kasese	2017	DS	70.01	ss715639424	40.16	3.45	2.38	9.7
	$SY3.4^{PR}$	Pv03	Namulonge	2017	NS	4.21	ss715648183	0.56	3.11	-0.07	4.0
	$SY3.4^{PR}$	Pv03	Combined		DS	0.01	ss715648183	0.56	11.59	2.40	13.7
	$SY4.1^{PR}$	Pv04	Kasese	2017	DS	50.91	ss715640609	29.17	7.04	6.43	36.9
	$SY6.1^{PR}$	Pv06	Kasese	2016	DS	8.91	ss715649019	17.92	4.10	2.60	26.5

Table 3.6 Quantitative trait loci (QTL) for harvest index(HI) and pod partitioning index(PPI) in the Portillo x Red Hawk recombinant inbred line (RIL) evaluated under drought stress and non-stress in Kasese and Namulonge, Uganda during 2016 and 2017 growing seasons.

Trait	QTL	Chromosome	Site	Year	Treatment	Peak(cM)	Near marker	Peak(Mb)	LOD	Additive	R2
HI	HI1.1^{PR}	Pv01	Combined		DS	148.41	ss715650354	52.15	4.91	1.06	6.8
	HI1.1^{PR}	Pv01	Kasese	2017	DS	143.41	ss715650354	52.15	6.28	1.69	4.7
	HI3.1^{PR}	Pv03	Kasese	2017	DS	74.81	ss715649460	3.29	3.94	-3.74	5.1
	HI4.1^{PR}	Pv04	Kasese	2016	DS	61.91	ss715648923	45.79	4.22	-0.99	2.5
	HI6.1^{PR}	Pv06	Kasese	2016	DS	8.91	ss715649019	17.92	3.01	3.09	23.3
	HI8.1^{PR}	Pv08	Kasese	2016	NS	4.31	ss715641300	19.36	3.35	1.72	2.6
	HI11.1^{PR}	Pv11	Kasese	2017	NS	24.91	ss715645541	4.31	3.31	2.66	7.1
PPI	PPI1.1^{PR}	Pv01	Namulonge	2017	DS	61.01	ss715648382	6.67	3.52	-4.44	11.4
	PPI1.2^{PR}	Pv01	Combined		DS	138.41	ss715645258	50.15	3.09	2.92	3.2
	PPI2.1^{PR}	Pv02	Kasese	2017	DS	20.11	ss715647234	2.52	3.04	2.06	10.4
	PPI4.1^{PR}	Pv04	Kasese	2017	DS	59.91	ss715648923	45.79	3.70	-3.90	1.8
	PPI11.1^{PR}	Pv11	Kasese	2017	NS	23.51	ss715648851	5.19	3.68	4.66	17.6
	PPI11.2^{PR}	Pv11	Kasese	2016	NS	45.61	ss715650599	45.63	3.26	-3.42	14.2

Figure 3.2 Common bean chromosomes Pv01, Pv012, Pv03, Pv04, Pv06, Pv07, Pv08, Pv010, and Pv11 showing quantitative trait loci (QTL) for phenology, yield component traits, and partitioning traits in Portillo x Red Hawk recombinant inbred line (RIL) grown under drought stress and non-stress in Kasese and Namulonge, Uganda during 2016 and 2017 growing seasons

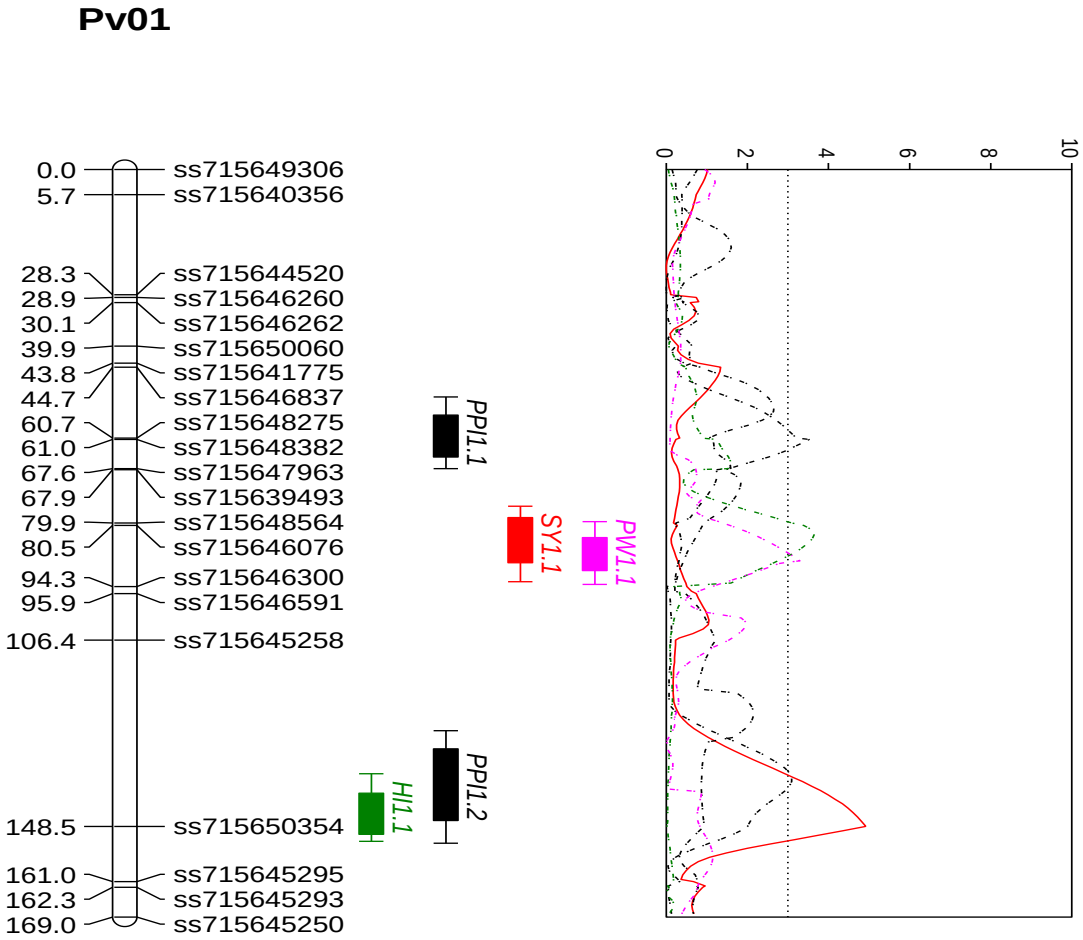
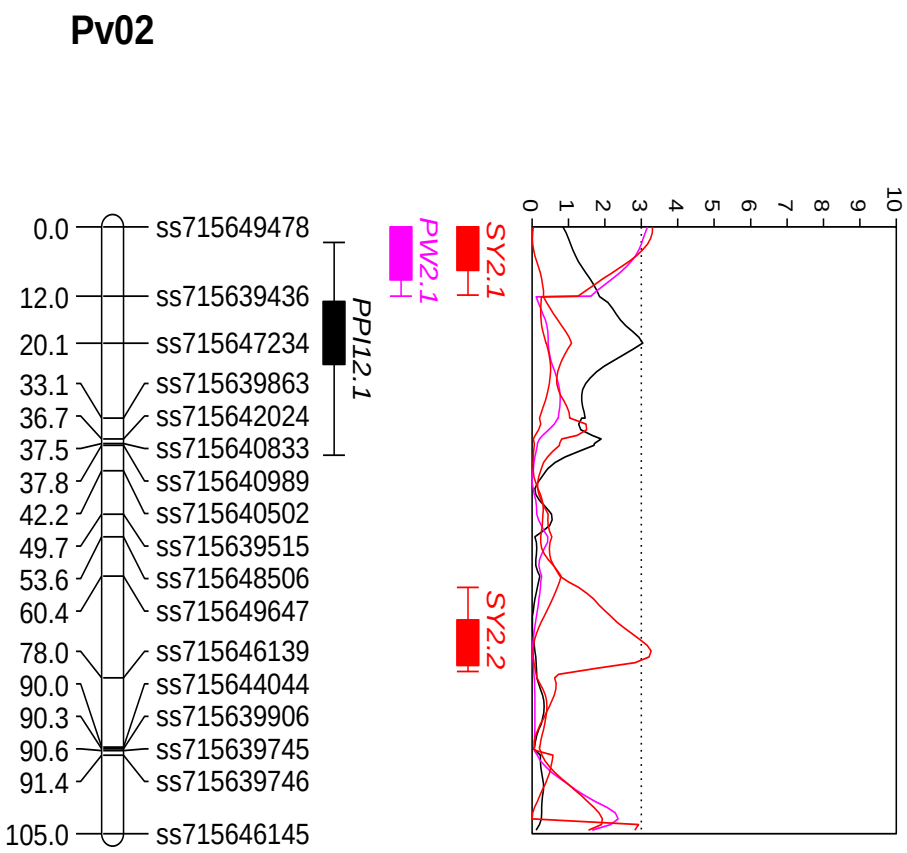


Figure 3.2 (cont'd)



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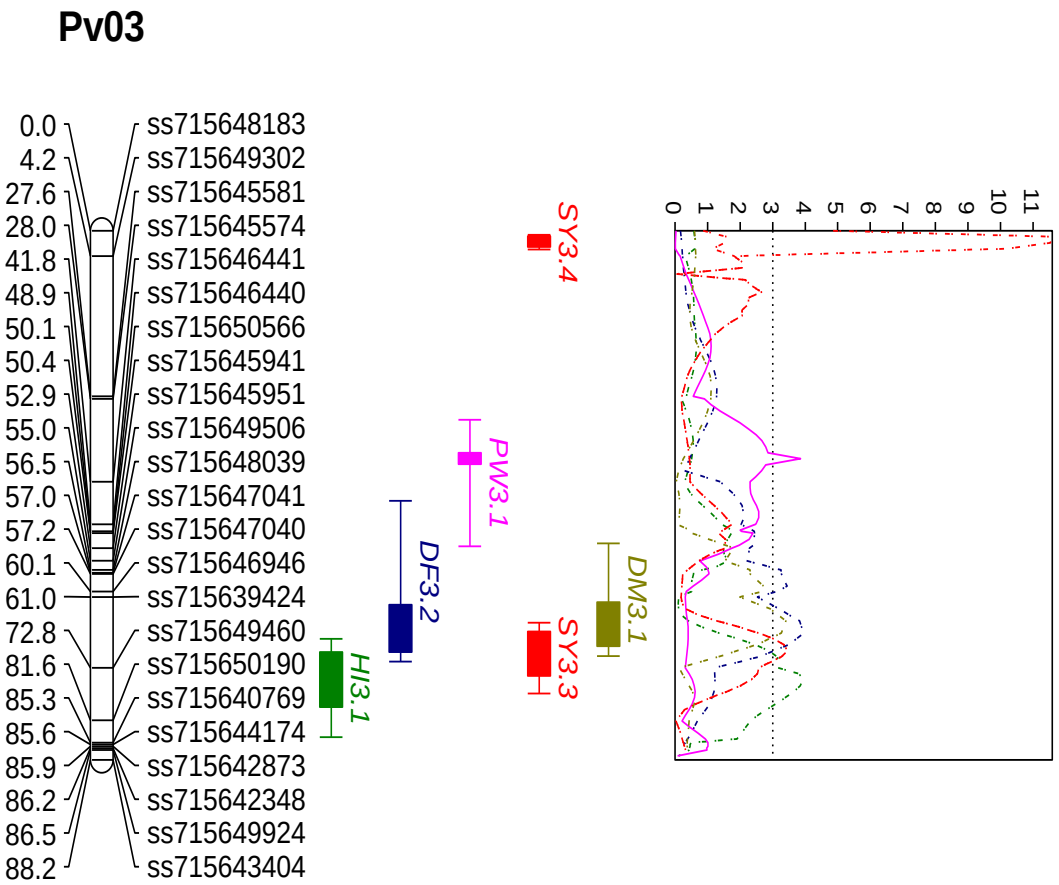


Figure 3.2 (cont'd)

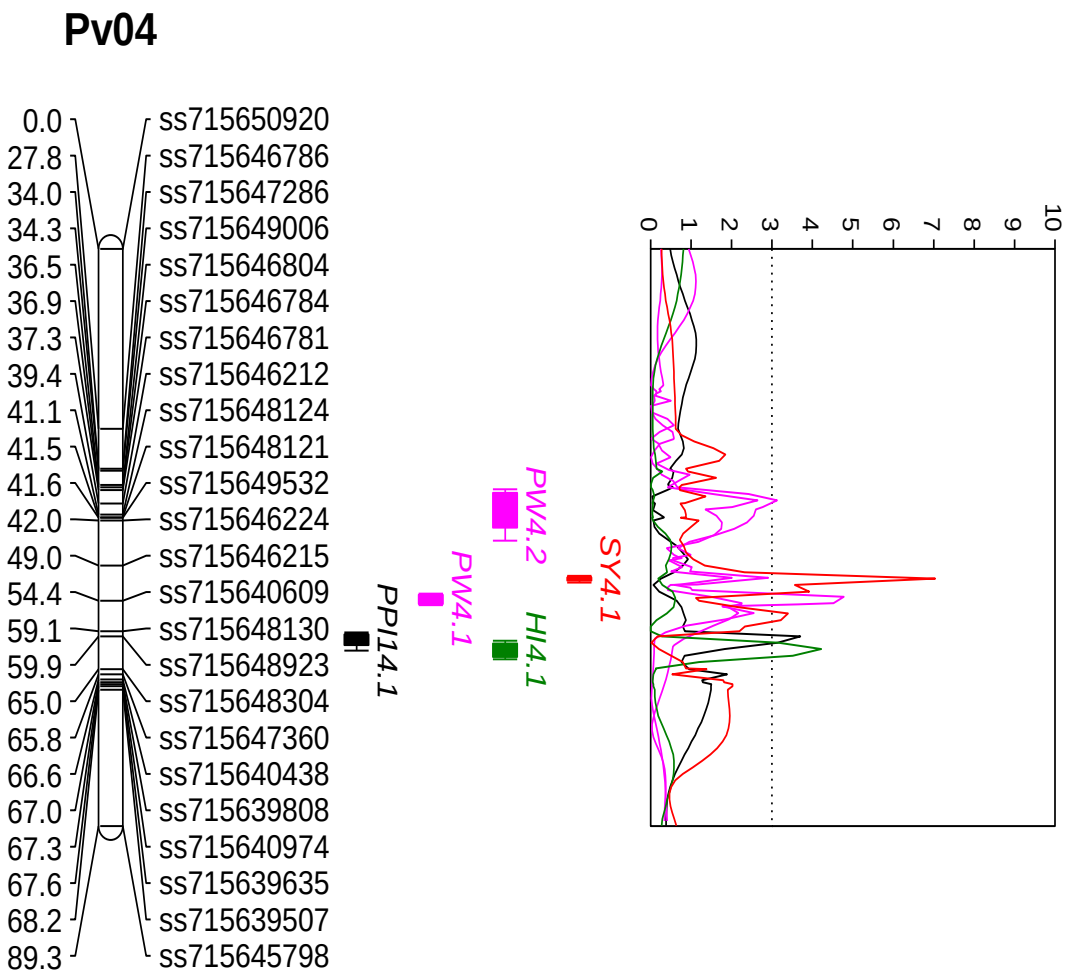


Figure 3.2(cont'd)

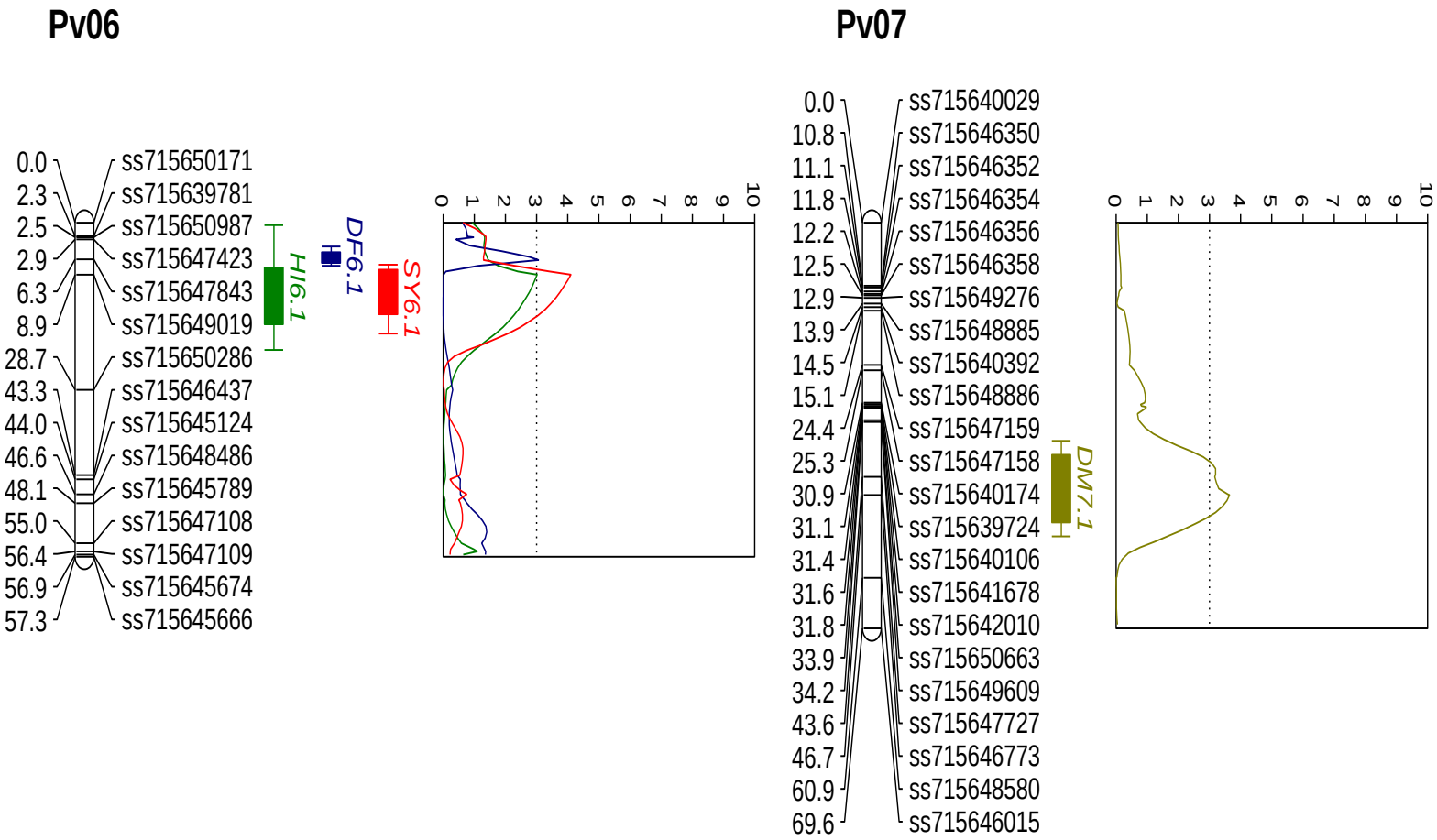


Figure 3.2(cont'd)

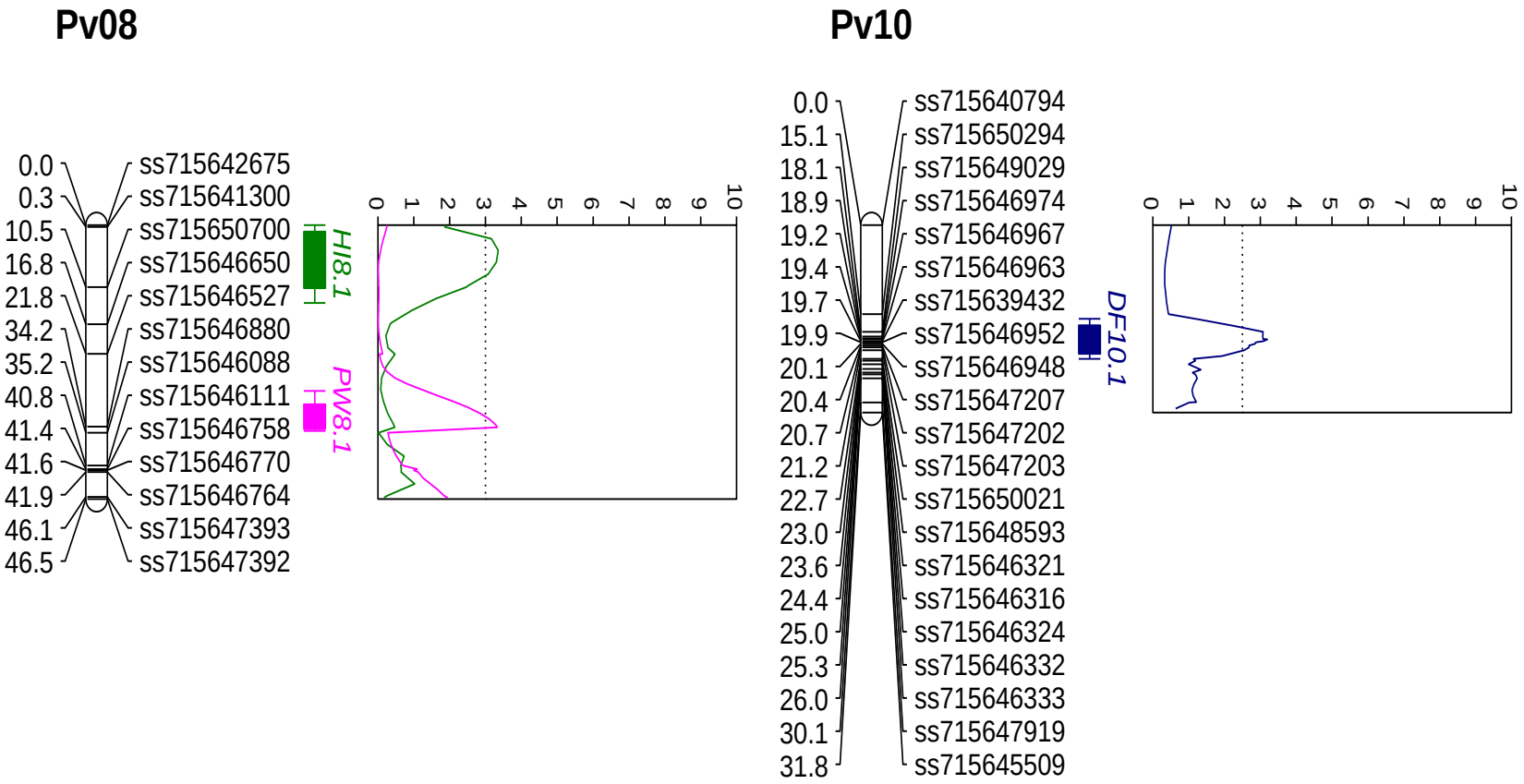
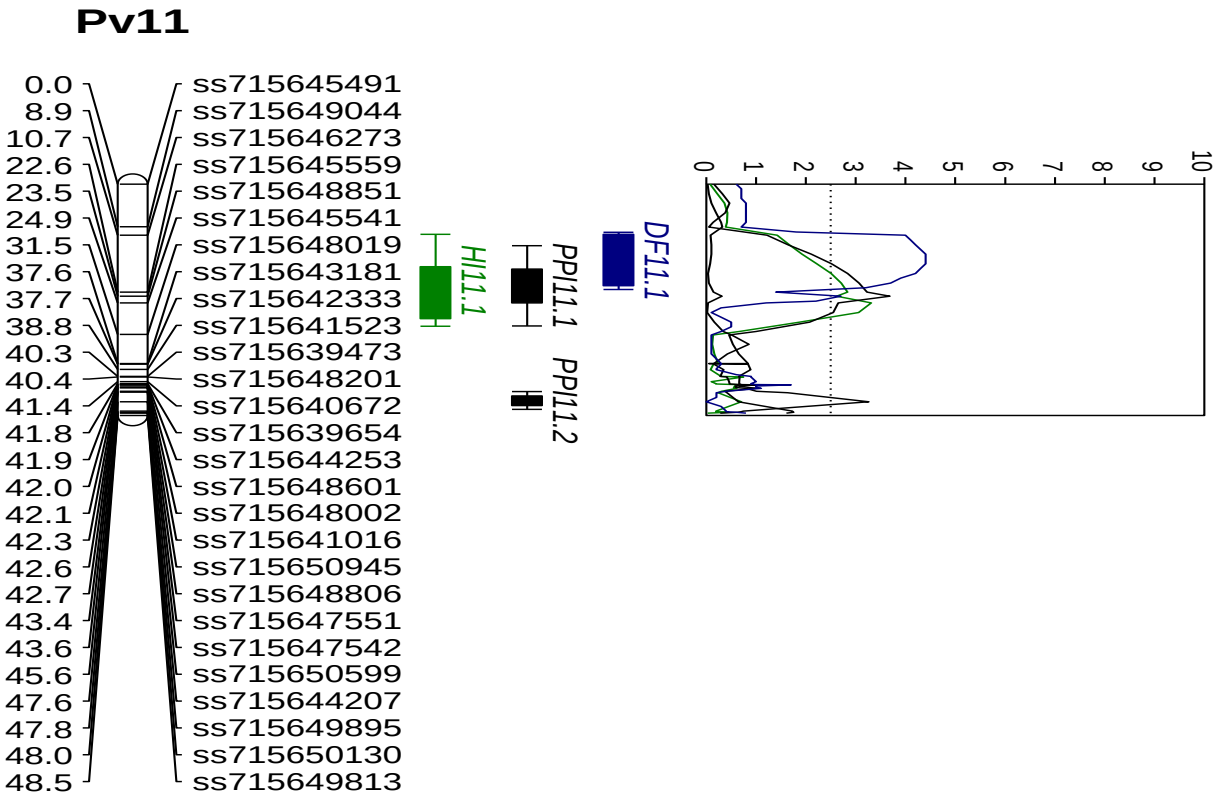


Figure 3.2(cont'd)



CHAPTER 4: DETERMINATION OF THE USE OF PHOTOSYNTHETIC TRAITS AS DROUGHT SENSITIVITY INDICATORS OF DROUGHT TOLERANCE IN COMMON BEAN

Abstract

In this study, 294 common bean genotypes from the Andean gene pool were evaluated at the seedling stage to determine the genetic variability and sensitivity of photosynthetic traits to drought stress. Drought stress was applied by withholding watering for seven days at 21 days after planting before reapplying water to determine recovery while maintaining watering in the control treatment. Photosynthetic traits namely, Phi2, PhiNPQ, and PhiNO were measured using the MultispeQ Beta device while visual scores were assessed using standard protocols. Significant differences were observed among genotypes for photosynthetic traits and visual scores under drought and control conditions, suggesting that genetic variability exists for photosynthetic traits under drought conditions at the seedling stage. Photosynthetic traits were severely affected with the progression of drought stress in the highly sensitive genotypes compared with the less sensitive genotypes, suggesting that alternative mechanisms exist that regulate plant response to drought stress. In addition, slow wilting was positively associated with recovery and could be used to identify drought tolerant common bean genotypes at the seedling stage.

Introduction

Common bean (*Phaseolus vulgaris.* L.) is a major component of food and nutrition for millions in the developing world (Broughton et al., 2003; Gepts et al., 2008). Common bean is a staple and source of income in many countries in sub Saharan Africa, and is cultivated under complex farming systems (Broughton et al., 2003). In Uganda, common bean is also an important non-traditional agricultural export crop. However, drought stress caused by erratic rainfall patterns affects over 60% of the bean production areas globally (Beebe et al., 2011), and one-third of the crop production areas in sub-Saharan Africa. The prevalence of both terminal and intermittent drought stress in major common bean production areas in sub-Saharan Africa has been widely reported by farmers, so assessing drought tolerance is an important attribute of common bean varieties (Katungi et al., 2011; Beebe et al., 2014). Therefore, identifying and developing common bean cultivars that are tolerant to both intermittent and terminal drought stress will be critical to sustain yield stability in these drought prone areas of Uganda.

Plant responses to drought stress are physiologically complex and difficult to characterize because a wide range of modifications occur within a short period of time (Galmes et al., 2013). In addition several combinations of inherent plant response factors determine the plant's abilty to sustain growth under water deficit (Farooq et al., 2009). Drought stress causes stomatal closure in plants in order to prevent excessive water loss, but this subsequently restricts diffusion of CO_2 to the sites of carboxylation inside the chloroplast (Lizana et al., 2006; Dias and Brüggemann, 2010; Ninou et al., 2013). These limitations depend on the degree of water deficit which significantly inhibits CO_2 assimilation and photosynthetic performance (Flexas et al., 2012). Previous studies have reported that photosynthetic acclimation to drought and its recovery from drought stress depends on the capacity of plants to adjust their stomatal and mesophyll

conductance to prevent photo damage from excessive light and elevated temperatures. Therefore, an efficient photo protection mechanism is an essential component of stress resistant plants (Lizana et al., 2006; Chaves et al., 2009; Flexas et al., 2012). Similar trends have been reported in common bean under drought stress resulting in decreases in photosystem II quantum yield (Φ_{II}), non-photochemical excitation quenching (NPQ), total chlorophyll content, stomatal conductance, transpiration rate, leaf temperature, and leaf water potential (Lizana et al., 2006; Dias and Brüggemann, 2010; Ninou et al., 2013; Traub et al., 2017). However, the effect of drought stress on sensitive components of the photosynthetic process are not well understood, thus limiting their use in breeding for tolerance to drought stress.

The availability of versatile and robust next generation phenotyping technologies such as the MultispeQ Beta device increases the opportunity for direct measurement of photosynthetic traits (Kuhlgert et al., 2016). MultispeQ Beta device can be deployed under multiple environmental conditions (temperature, humidity, CO_2 levels, intensity and quality, time, and location) and could boost phenotype-assisted breeding approaches designed to transfer drought resistance traits to more productive lines. Therefore, breeding for drought tolerance in common bean will require identifying critical plant tolerance responses, and better understanding of physiological mechanisms and genetic controls of contributing traits at critical plant developmental stages.

This study seeks to (i) determine genetic variability for photosynthetic traits in Andean beans evaluated under drought stress at the seedling stage; and (ii) determine the sensitivity of photosynthetic traits to drought stress and their subsequent use as an indicator of adaptive physiological responses for identifying drought tolerant common bean genotypes.

Materials and Methods

In this study a total of 294 genotypes of the Andean Diversity Panel (ADP) belonging to the Andean gene pool including five checks. These included TARS-Tep 32 (PI 666351), an improved Tepary (TB) line tolerant to drought (Porch et al., 2013; Traub et al., 2017), SEN 80, SEN 70, SCN 11, drought tolerant lines from CIAT, and NABE 14 (local check variety) were evaluated for drought stress under greenhouse conditions at the National Crop Resources Research Institute Namulonge (NaCRRI) in Uganda. The panel consisted of a global collection of Andean beans, mainly varieties, landraces, and breeding lines with different seed types and market classes from Africa, Central America, South America, and North America (Cichy et al., 2015). The experiments were set up modifying protocols previously used in common bean (Mukeshimana et al., 2014). The experiment was planted in a 7x42 incomplete block design (lattice) with two replications. The experiment was repeated twice for both drought stress and control. The genotypes were planted in a 1500 cm^3 plastic pots that were filled with a mixture of soil and lake sand in a ratio of 3:1(v/v). The pots were watered to field capacity and allowed to drain overnight before planting. Four seeds were planted in every pot for each genotype to ensure germination and seedlings were thinned to two plants per pot at 7 days after planting (DAP). Approximately 20g of NPK fertilizer was applied to every pot at 10 DAP. Drought stress was imposed at 21 DAP by withholding watering for 7 days for drought treatment while watering was continued for the control treatment. In order to assess the recovery of genotypes after stress, water was then applied to the drought treatment at 7 days (28 DAP) after drought application and recovery was evaluated at 32 DAP. In these experiments photosynthetic traits Phi2, PhiNPQ, and PhiNO were measured using the MultispeQ Beta device (Kuhlgert et al., 2016), on the middle and mature trifoliate leaf of two plants at 22, 24, and 26 DAP in every pot.

In the drought stress experiments, wilting score was determined at 24 and 26 DAP; while recovery was scored at 32 DAP. A visual rating for drought stress was performed using a wilting score on a scale of 0 to 5, where 0 shows no wilting and a 5 indicates the plant is completely wilted. The rating score for recovery was modified from the previous 0 to 1 scale used by (Mukeshimana et al., 2014), to a 0 to 5 scale, where 0 means full recovery and 5 means no recovery. Moisture content in every pot was measured in the drought stress experiment at 22, 24 and 26 DAP using soil moisture meter model (PMS-714).

Statistical Analysis

Data analysis for photosynthetic traits and visual scores was performed using SAS (Institute, 2011) and R statistical packages. Both the photosynthetic traits, Phi2, PhiNPQ, PhiNO and visual rating scores were analyzed using the PROC MIXED procedure. During the analysis for photosynthetic traits, Photosynthetically Active Radiation (PAR), and time of the day were used as covariates to account for diurnal changes in light intensity and time of the day, while the soil moisture content was used to correct for visual scores. The mean values obtained from the PROC MIXED procedure were used for subsequent analysis. The sensitivity of photosynthetic traits to drought stress was tested using a t-test by comparing the difference between the means of drought and control. A normality test was performed to check the distribution of photosynthetic traits under drought treatment at 26 DAP in SAS using the PROC MIXED procedure. In addition, two groups represented by 10% from each side of distribution designated as less and highly sensitive genotypes were used to determine the effect of drought stress on adaptive physiological responses and use for comparing and identifying drought tolerant common bean genotypes. Correlations between photosynthetic traits and visual scores were determined for the

selected group using PROC CORR procedure. Visual scores were then used to compare means of the two groups.

Results

Photosynthetic Traits

Overall, significant variation ($p<0.05$) was observed among the genotypes for photosynthetic traits, Phi2, PhiNPQ, and PhiNO at 22 and 24 DAP under drought conditions, and at 22 and 26 DAP under non-stress conditions (Table 4.1, Figure 4. 1). Under drought conditions the average Phi2 value decreased significantly from 0.48 on 22 DAP to 0.22 on 26 DAP with an overall reduction of 0.26 in photosynthetic efficiency compared to a 0.2 increase observed under control conditions during the same period (Table 4.1, Figure 4.2). Differences in PhiNO was non-significant among genotypes at 26 DAP as the drought stress progressed, and the average PhiNO decreased by 0.7 from 0.19 on 22 DAP to 0.12 on 26 DAP compared to 0.2 decrease under control conditions. Mean values for PhiNPQ increased consistently from 0.32 on 22 DAP to 0.66 on 26 DAP compared with a relatively constant PhiNPQ observed under non-stress conditions (Table 4.1, Figure 4.1). In addition, significant differences ($p<0.05$) were observed among genotypes for wilting on 24 and 26 DAP, and following recovery on 32 DAP (Table 4.2).

Determination of sensitivity of photosynthetic traits to drought stress

Significant differences ($P<0.001$) were detected among photosynthetic traits, namely Phi2 and PhiNPQ for their sensitivity to drought stress. Mean differences were compared for each photosynthetic trait on 22 DAP, 24 DAP, and 26 DAP (data not shown). Relative to the control treatment, the PhiNPQ increased significantly from 0.14 to 0.49 while Phi2 decreased from -0.05 to -0.31, and PhiNO decreased from -0.09 to -0.16 (Figure 4.3). The two photosynthetic traits considered highly sensitive to drought stress were PhiNPQ and Phi2 evaluated at 26 DAP. In

addition, the normality test for the two traits showed that both Phi2 and PhiNPQ were normally distributed at 26 DAP under drought stress (Figure 4.3).

Correlation analysis for Phi2 and PhiNPQ among the high and less sensitive genotypes

As expected, highly significant correlations were observed among photosynthetic traits under drought stress conditions using both most and least sensitive genotypes at 26DAP.

Phi2 was negatively correlated with PhiNPQ ($r = -0.93$, $p<0.001$), slow wilting at 26 DAP ($r=-0.43$, $p<0.001$), and recovery at 32 DAP ($r=-0.37$, $p<0.001$). Meanwhile, PhiNPQ was positively correlated with slow wilting at 26 DAP ($r=031$, $p<0.01$), and recovery at 32 DAP ($r=0.21$, $p<0.01$). A highly significant and positive correlation was observed between slow wilting at 26 DAP and recovery at 32 DAP ($r = 0.83$, $p<0.001$) (Table 4.3). However, it is worth noting that the correlations between photosynthetic traits and visual scores were moderate for both the highly and less sensitive genotypes (Table 4.3).

In addition, significant differences in response were observed between high and low sensitive genotypes for Phi2 and PhiNPQ as drought stress progressed from 22 DAP to 26 DAP. On 22 DAP, there were no differences between the means of high and low sensitive genotypes, while on 24 DAP, both highly and less sensitive genotypes responded in the same direction to drought stress. As expected, there was a decrease in Phi2 and an increase in PhiNPQ. The largest difference between the contrasting genotypes was observed on 26 DAP. At 24 DAP, there was a significant decrease in Phi2 and increase in PhiNPQ for highly sensitive genotypes whereas, there was no observable change for these traits among the less sensitive genotypes, implying some inherent mechanism could be responsible for keeping the functionality of the photosynthetic machinery under water deficit. A comparison of the less sensitive genotypes

identified using PhiNPQ and slow wilting at 26 DAP and recovery at 32 DAP indicated that TB1was the best candidate under drought stress (Table 4.3, Figure 4.4).

Discussion

Significant differences were observed among genotypes for photosynthetic traits namely Phi2, PhiNPQ, and PhiNO under drought and control conditions. As expected, Phi2 and PhiNO consistently decreased under the drought treatment while PhiNPQ increased significantly as stress progressed and photosynthetic traits remained stable under the control treatment. These results seem to suggest the existence of genetic variation for photosynthetic traits and drought induced differences among common bean genotypes from the Andean gene pool. These results were not, however, surprising because previous studies reported significant variation among common bean lines for photosynthetic traits that decreased significantly with increase in drought stress (Lizana et al., 2006; Traub et al., 2017). The significant decline in Phi2 from 22 DAP to 26 DAP could be associated with the limiting amounts of CO_2 diffusing into the carboxylation sites inside the chloroplast. Drought stress results in stomatal closure, which acts as a preventive measure to prevent excessive water loss. This, however, reduces the evaporative cooling ability for normal function of photosynthetic apparatus and could led to photo damage (Ninou et al., 2013). The significant increase in PhiNPQ from 22 DAP to 26 DAP under drought conditions observed here could be associated with several ways in which plants actively deal with the excessive captured light in order to prevent photo damage.

One interesting observation in this study was the difference in PhiNPQ and Phi2 between the highly and less sensitive genotypes from 24 DAP to 26 DAP. Under moderate drought stress (22 to 24 DAP), there were no observable differences between the less and highly sensitive genotypes in Phi2 and PhiNPQ suggesting that PhiNPQ was still effective in dealing with

excessive captured light and reduced the risk of photo damage without necessarily affecting Phi2. The significant decrease in Phi2 and PhiNPQ between these genotypes at 26 DAP seems to suggest that PhiNPQ is not the only process or mechanism but the involvement of other non-PhiNPQ processes in regulating drought response in less sensitive genotypes. A similar process appears to be lacking in the highly sensitive group, thus resulting in wilting that was observed in the highly sensitive group. This observation was consistent with previous studies that reported adjustments in stomatal and mesophyll conductance as an important strategy in acclimatizing the photosynthetic apparatus to drought stress in order to prevent photo damage (Lizana et al., 2006; Chaves et al., 2009; Flexas et al., 2012) . A recent study reported lower stomatal conductance for drought tolerant SER 16 and TB1 compared to the more drought susceptible Zorro and Jaguar bean varieties (Traub et al., 2017). It was unclear however, if the difference between the less and highly sensitive genotypes detected in this study was associated with adjustments in stomatal and mesophyll conductance or the involvement of inherent factors, since stomatal conductance was not measured in this study.

A highly significant ($p<0.001$) and positive correlation was observed between slow wilting at 26 DAP and recovery at 32 DAP, suggesting that genotypes that wilted slowly under drought stress had better chances of recovery following stress. A previous study consistently reported slow wilting trait was important for plant survival during drought stress at the seedling stage (Mukeshimana et al., 2014), implying that slow wilting could be a useful trait for identifying drought tolerant lines of common bean at the seedling stage. In this study, the 10 least sensitive genotypes based on low PhiNPQ ranking were generally slow wilting and exhibited higher chances of recovery after stress although correlations between PhiNPQ and the visual scores

were low. As expected, the tepary bean was the most drought tolerant genotype with an exceptional balance between photosynthetic traits under drought stress.

Conclusion

The objective of this study was to determine the genetic variability for photosynthetic traits in Andean beans, and to determine the sensitivity of photosynthetic traits to drought stress and their subsequent use as an indicator of adaptive physiological responses for identifying drought tolerant common bean genotypes. Significant differences were detected among genotypes for photosynthetic traits namely Phi2, PhiNPQ, and PhiNO under drought and control conditions, suggesting that substantial genetic variability exists among common bean genotypes from the Andean gene pool for photosynthetic traits measured under drought conditions at the seedling stage. The significant and irreversible changes in photosynthetic traits under severe drought stress is indicative that photosynthetic traits alone are not the only regulators of drought responses in plants. Genotypes that wilted slowly under drought stress had higher chances of recovery after the removal of the drought stress suggesting that slow wilting could be a useful trait for identifying drought tolerant common bean lines at seedling stage. In addition, low PhiNPQ appears to be associated with slow wilting and increased chances of recovery after stress although correlations between these traits were moderate.

APPENDIX

Table 4.1. Mean and variance for photosynthetic traits of 294 Andean common bean genotypes measured at 22, 24, and 26 days after planting (DAP) evaluated under water stress conditions.

Treatment	Drought						Control			
	22DAP		24DAP		26DAP		22DAP		26DAP	
Trait	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
Phi2	0.48±0.05	***	0.32±0.06	**	0.22±0.08	*	0.52±0.04	***	0.54±0.04	***
PhiNO	0.19±0.03	***	0.17±0.05	*	0.12±0.05	Ns	0.28±0.04	***	0.25±0.03	***
PhiNPQ	0.32±0.07	***	0.51±0.09	**	0.66±0.11	**	0.18±0.06	***	0.19±0.06	***

***** Significant at the 0.05 probability level, ****** Significant at the 0.01 probability level, ******* Significant at the 0.001 probability level, and **ns** not significant.

Table 4.2. Mean and variance for visual scores of 294 Andean common bean genotypes measured at 24, 26 and 32 days after planting (DAP) evaluated under water stress conditions**.**

Visual score (Drought)	Mean	Variance
WLT24DAP(0_5)	3.18±0.81	*
WLT26DAP(0_5)	4.31±0.67	***
RECV_32DAP(0_5)	4.17±0.84	***

WLT Slow wilting score, **RECV** Recovery , ***** Significant at the 0.05 probability level, ****** Significant at the 0.01 probability level, ******* Significant at the 0.001 probability level, and **ns** not significant.

Figure 4.1. The boxplot for the means of photosynthetic traits PhiNO, Phi2 and PhiNPQ of 294 Andean common bean genotypes measured at 22 and 26 days after planting (DAP) evaluated under water stress conditions.

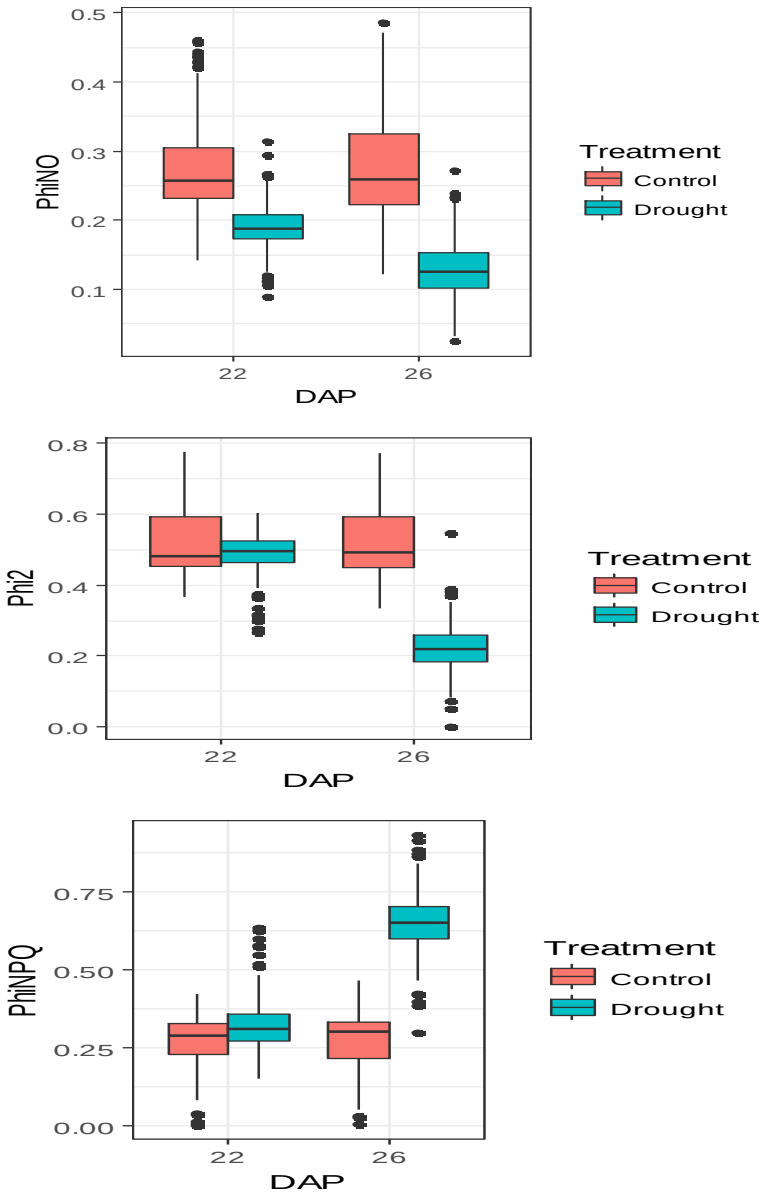
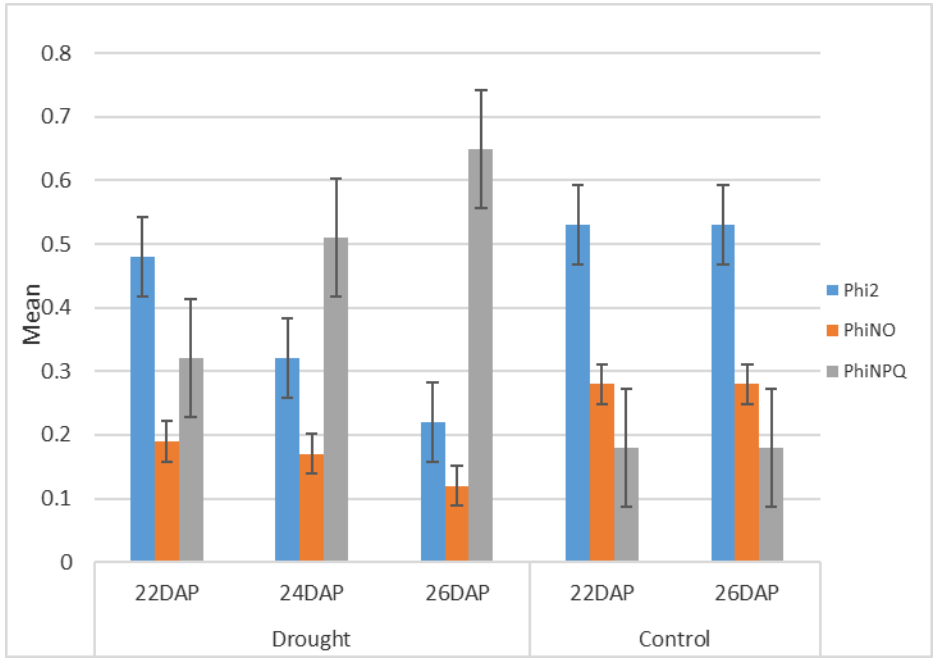


Figure 4.2. The effect of drought stress on photosynthetic traits. (A) The average Phi2, PhiNO and PhiNPQ measured drought and control conditions measured on 22, 24 and 26DAP. (B) The effect of drought stress on photosynthetic traits based on the difference between the means of drought stress and control treatments

A



B

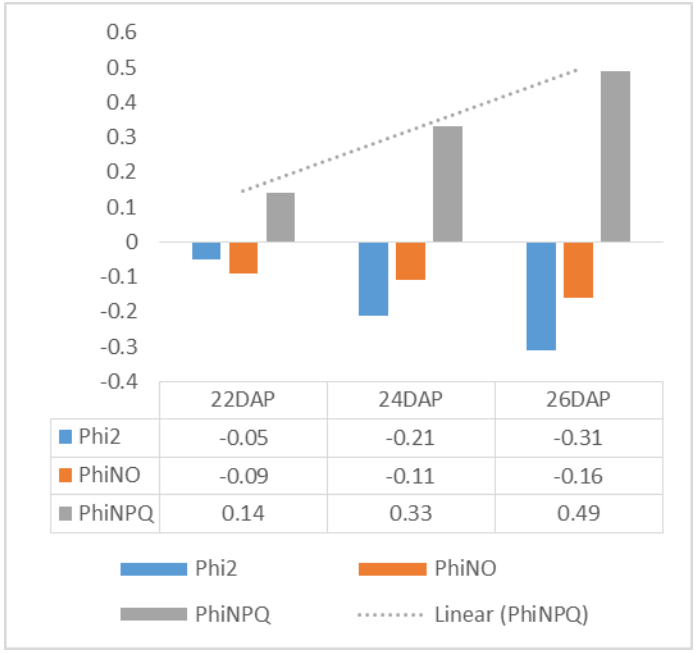
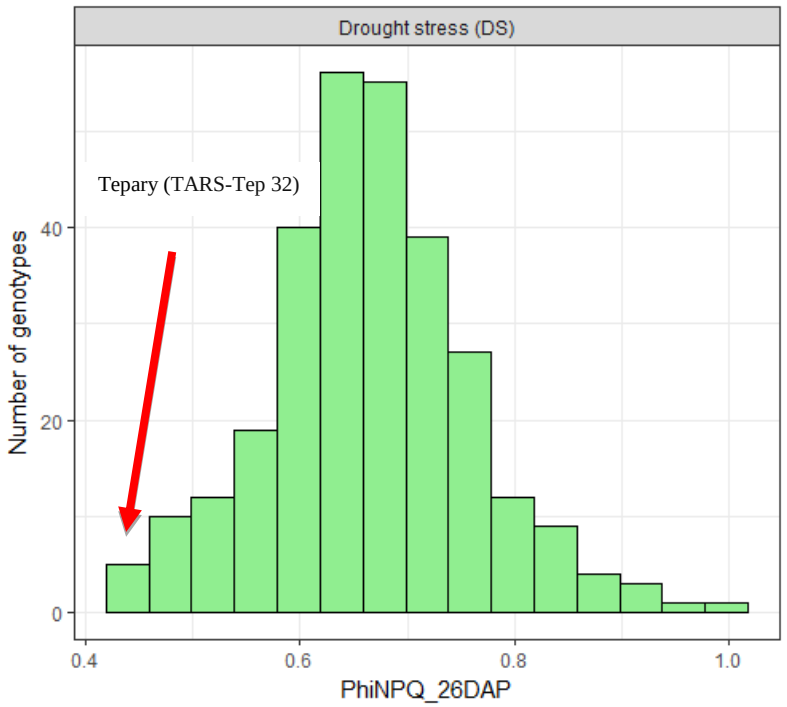


Figure 4.3. The distribution of Phi2 and PhiNPQ of 294 Andean common bean genotypes measured at 26 days after planting (DAP) under water stress conditions.



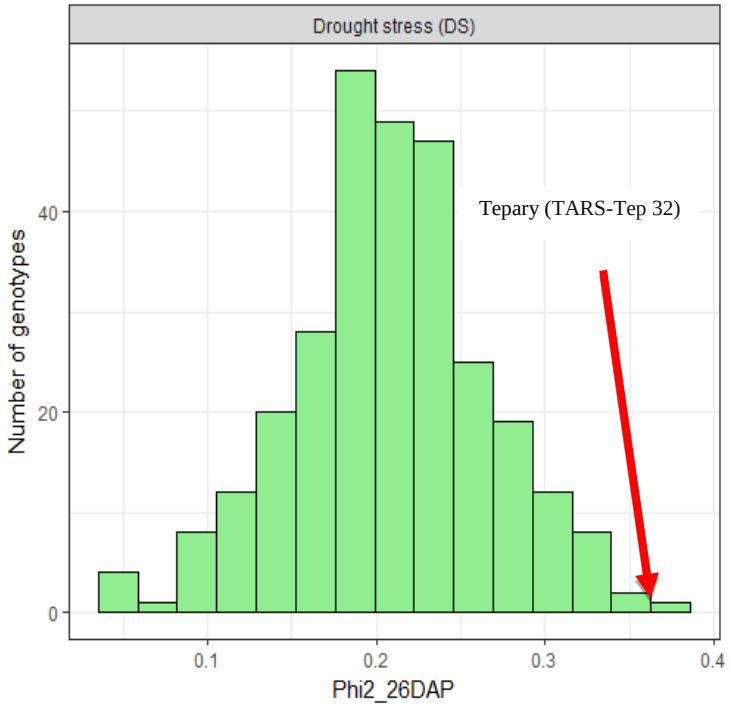
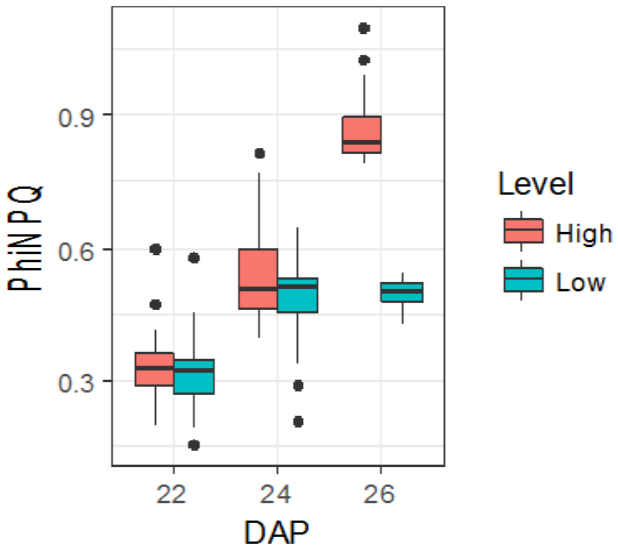


Table 4.3. Correlations among photosynthetic traits Phi2, PhiNO, PhiNPQ, slow wilting at 26 DAP and recovery at 32 DAP for high and low sensitive common bean genotypes (60) genotypes selected using PhiNPQ at 26 DAP under drought condition

Traits	Phi2_26DAP	PhiNPQ_26DAP	RECV_32DAP	WLT_26DAP
Phi2_26DAP		-0.93***	-0.37***	-0.43***
PhiNPQ_26DAP			0.21**	0.31**
RECV_32DAP				0.83***
WLT26DAP				

WLT Slow wilting score, **RECV** Recovery , ***** Significant at the 0.05 probability level, ****** Significant at the 0.01 probability level, ******* Significant at the 0.001 probability level, and **ns** not significant.

Figure 4.4. The variation in photosynthetic traits PhiNPQ, Phi2, and PhiNO among high sensitive low sensitive genotypes under drought conditions on 22, 24 and 26 DAP



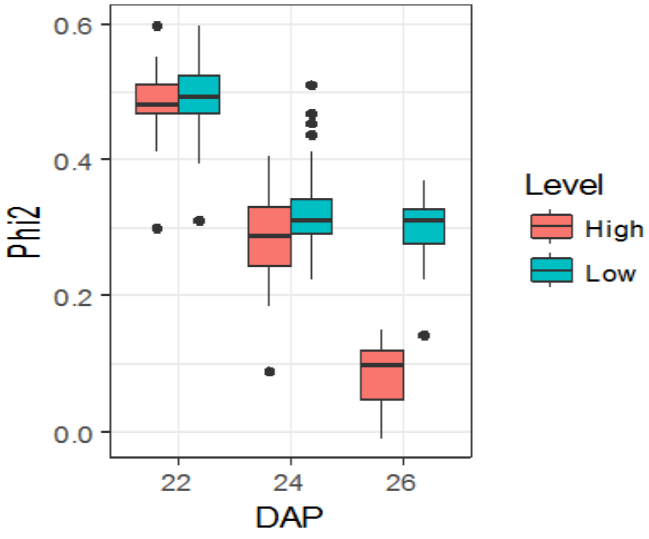


Figure 4.5. The distribution of wilting at 26 DAP and recovery on 32 DAP for genotypes evaluated under drought conditions.

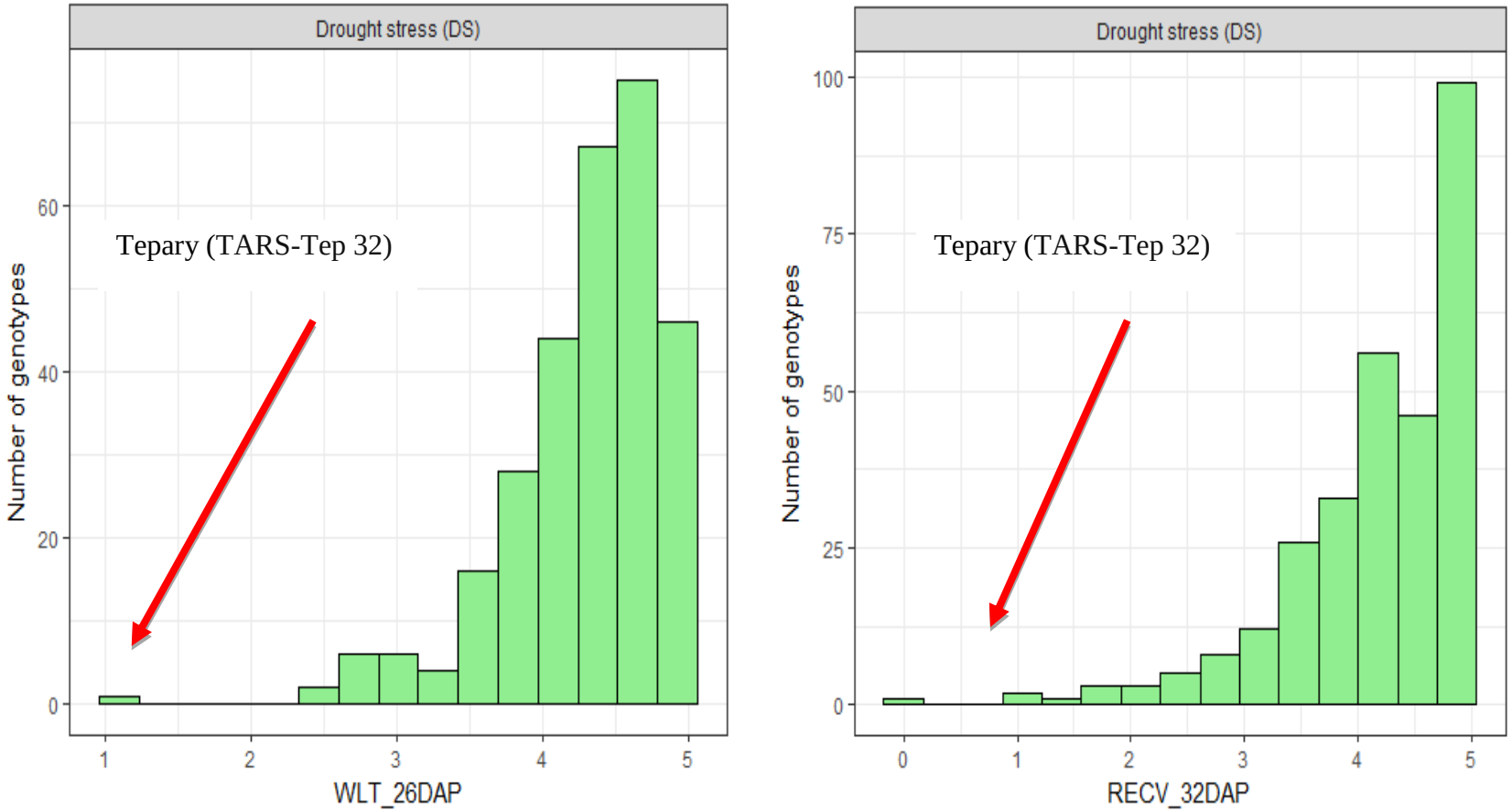


Table 4.4. Mean photosynthetic traits, visual scores, and geometric mean of seed yield for the 10 best individuals based on PhiNPQ score determined at 26 days after planting.

Genotype (ID)	Cultivar name	Country (region)	Seed color	Phi2	PhiNPQ	RECV	WLT	GM
Tepary	TARS-Tep 32	USA	Cream	0.35	0.43	0.13	1.17	
ADP 379	PI 203934	USA	Cream	0.33	0.47	1.97	4.38	11.6
ADP 654	USDK-4	USA	DRK	0.37	0.50	2.68	2.34	20.1
ADP 271	G 13167	CIAT	White	0.33	0.52	2.09	3.46	Na
ADP 445	Chijar	Caribbean	Red mottled	0.29	0.57	1.81	3.48	10
ADP 062	Maulasi	Tanzania	Red	0.27	0.59	1.66	2.69	7.2
ADP 255	G 10994	CIAT	Tan	0.28	0.60	2.63	3.79	Na
ADP 626	Badillo	Caribbean	LRK	0.23	0.61	2.97	4.26	Na
ADP 186	G 1368	CIAT core	Red	0.24	0.61	2.60	4.86	10.7
SEN 80	SEN 80	CIAT	Red	0.25	0.61	2.85	3.47	
Mean				0.29	0.55	2.14	3.39	19.8
SED				0.05	0.07	0.84	1.09	1.65

RECV= recovery at 32 DAP; **WLT**= wilting at 26 DAP; **GM**= Geometric mean of seed yield under field conditions; **ADP** Andean diversity panel identity; **LRK** Light red kidney; **DRK** Dark red kidney; **SED** Standard error of difference.

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