# DISSECTING THE GENETIC COMPLEXITY OF DROUGHT TOLERANCE MECHANISMS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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## ABSTRACT

**DISSECTING THE GENETIC COMPLEXITY OF DROUGHT TOLERANCE MECHANISMS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)**

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Among the abiotic stresses that limit plant growth and productivity, drought is the most complex and devastating on a global scale. With expected frequent, severe, and widespread droughts, crops with greater ability to adapt to reduced water use are needed to cope with the changing environment and more severe drought conditions.

Studies were designed to evaluate an inter-gene pool recombinant inbred line (RIL) bean population from a cross of SEA5 and CAL96 for drought resistance and identify quantitative trait loci (QTL) linked to drought resistance in the field, identify shoot traits associated with drought resistance in bean seedlings, and identify factors influencing regeneration and *Agrobacterium tumefaciens*-mediated transformation of common bean.

In the field, the SEA5/CAL96 population was evaluated for drought tolerance under rainfed and irrigated conditions in Rwanda and Colombia. Drought adaptation variables such as phenology, partitioning indices, seed yield, and yield components were evaluated. Based on a combination of partitioning indices, seed yield, and seed size, lines combining higher drought resistance than the susceptible parent with medium seed size were identified. These lines will be used to facilitate future enhancement of drought tolerance in Andean beans in Rwanda.

A genetic map of the SEA5/CAL96 population spanning 1031 cM was constructed using 92 SSR and 12 InDel markers. QTL analysis identified 41 QTL across environments. Some of these QTL mapped near previously reported QTL in different studies while others were specific to this study. A major QTL for yield and yield per day accounting for 37% of phenotypic

variation was identified on chromosome Pv11. This QTL is linked to QTL associated with 100-seed weight which would facilitate to select simultaneously for yield and seed size.

Ten bean genotypes were evaluated for shoot traits related to drought resistance at seedling stage under restricted root growth conditions in the greenhouse. After withholding watering, plants were scored for wilting, unifoliate senescence, maintenance of stem greenness, recovery, and the capacity to set pods. Stem greenness and reduced wilting were found to enhance recovery from an extended period of drought in common bean seedlings.

Various factors including media, genotypes, explants, *A. tumefaciens* strains, and transformation methods were assessed in regeneration and transformation studies of common bean. Bean embryo axes were more regenerable than other explants and there was no medium capable of inducing regeneration of non meristematic bean cells. Three strains of *A. tumefaciens* were efficient in gene delivery depending on co-cultivation time, explant type, and bean genotype. Agro-infiltration seemed to enhance gene delivery in common bean.

## DEDICATION

In memory of my family

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### Introduction

Among the abiotic stresses that limit plant growth, drought is the most complex and devastating on a global scale (Pennisi, 2008, Schiermeier, 2008; Morris, 2008). Drought is the most widespread abiotic stress threatening crop productivity worldwide (Suja et al., 2007; Vadez et al., 2011). For instance, in summer 2012, US experienced the worse drought for the last 55 years, significantly reducing crop productivity (NCDC, 2012). By the end of September 2012, most of the contiguous United States was experiencing some kind of drought, with 66% experiencing moderate drought, 42% experiencing severe drought, while 25% of the country was experiencing extreme drought conditions based on Palmer drought index (http://droughtmonitor.unl.edu/monitor.html). With expected frequent, severe, and widespread droughts (Schiermeier, 2008; Lobell et al., 2008; Dai, 2012), crops with greater ability to adapt to reduced water use are needed to cope with the changing environment and more severe drought conditions (Takeda and Matsuoka, 2008).

Drought can be classified as either terminal or intermittent. Intermittent drought is due to climatic patterns of sporadic rainfall that cause intervals of drought of varying length that can occur at any time during the growing season (Schneider et al., 1997). Terminal drought, however, occurs when plants suffer from a lack of water during later stages of reproductive growth or when crops are planted at the beginning of a dry season (Frahm et al., 2004). In both types of drought, availability of soil moisture decreases progressively and reaches a level that restricts plant growth and reduces productivity.

Drought is a multifaceted stress condition that varies in duration, severity, and affects plant at different growth stages. Since drought stress can vary significantly from year to year, plants designed for a specific type of drought may underperform when the stress changes or is

absent (Tardieu, 2012). Breeding strategies for drought prone environments have to consider the timing and intensity of the drought stress. In addition, development of crops for enhanced drought resistance, requires the knowledge of physiological mechanisms and genetic control of the contributing traits at different plant developmental stages (Farooq et al., 2009).

**Plant strategies to cope with drought stress**

Plants have evolved different strategies to avoid deleterious effects of drought. These include escape, avoidance, and tolerance mechanisms (Levitt, 1972). Plants may combine various strategies to reduce damage associated with drought.

Drought escape is expressed in genotypes with a short life cycle. These genotypes are able to complete their life cycle exclusively during the period when adequate soil moisture still exists. Times of flowering and early maturity are major traits associated with drought escape. However, earliness is usually associated with a yield penalty in most crops since higher yields generally require a longer growing period (White and Singh, 1991). In areas where terminal drought constraints production, a moderate shortening of vegetative growth period combined with a high growth rate might be advantageous. Agricultural practices that match crop growth with availability of soil moistures can significantly reduce yield losses.

Another important mechanism that maintains crop productivity under terminal drought stress is associated with better partitioning of assimilates to developing fruits or seeds. This consists of the plant's ability to store reserves in shoot and to mobilise them for growing the fruit or seed. This response exists in cereals where stem and leaf reserves are used to support grain filling (Blum, 1996; 2005; Gebbing and Schnyder, 1999; Aggarwal and Sinha, 1984; Bruce et al., 2002; Saint Pierre et al., 2010), and in legumes where remobilization of assimilates from stem, leaves, and pod wall to growing seed has been observed (Rodrigues et al., 1995; Chaves et

al., 2002; Beebe et al., 2008). The extent of assimilates partitioning depends on plant species, stage, duration, and severity of drought (Farooq et al., 2009). In common bean, greater mobilization of photosynthates to seed under terminal drought has been found to be an important trait in G21212, a bean landrace of race Mesoamerica origin (Rao, 2001). Beebe et al. (2008) were able to select high yielding bean lines in small red, small black, mulatino, and carioca classes using visual selection for pod load and normal pod maturity under terminal drought. Quantitative trait loci (QTL) associated with harvest index, pod harvest index, leaf total non structural carbohydrates, stem total non structural carbohydrates have been identified (Asfaw and Blair, 2012; Asfaw et al., 2012). Once confirmed, these QTL could facilitate the breeding for improved carbohydrate remobilization in common bean.

Drought avoidance is the plant capacity to sustain high plant water status or cellular hydration under the effect of drought (Blum et al., 2005). Plants avoid drought by maximizing water uptake or limiting water loss and by retaining cellular hydration despite the reduction in water potential (Blum et al., 2005). Growth and production can be maintained by increased water uptake from lower soil layers through deeper roots combined with an improved water conduction capacity of the root system. Water uptake in deep soil layers is of particular importance in production areas where crops are grown on stored soil moisture. Soil water uptake depends on the degree of water loss throughout the shoot (Vadez et al., 2008) or water management by the shoot. Therefore, it is important to understand the root/shoot interactions in terms of plant water management as well as the combination of both shoot and root traits interactions with the environment (Vadez et al., 2011). Patterns of water uptake during the growth cycle might be the same or different depending on crops. For instance, in lowland rice, it has been observed that water uptake by deep root was consistent throughout stress periods in drought tolerant genotypes

(Gowda et al., 2012). In legumes, the most critical component of tolerance was associated with conservative soil water use during early stages of development to allow a significant amount of water to remain for reproduction and pod filling (Devi et al., 2010; Sinclair et al., 2008; Zaman-Allah et al., 2011; Belko et al., 2012).

A number of studies have reported positive associations between yield and root depth under water deficit in cereal crops (Bernier et al., 2007, 2009; Lopes and Reynolds, 2010; Manschadi et al., 2006) especially during grain filling stages. In grain legumes such as chickpea *(Cicer arietinum* L.*),* common bean, soybean [*Glycine max* (L.) Merr*]*, and cowpea [*Vigna unguiculata* L.) Walp.], root length density, maximum root depth, and fibrous root system have been found to be associated with drought avoidance (Beebe et al., 2010; Gaur et al., 2008; Pantalone et al., 1996; Hall, 2012). Root traits associated with better water acquisition as well as their impact on yield under drought stress in peanut (*Arachis hypogea* L.) and pigeon pea (*Cajanus cajun* L.*)* are still unclear ( Vadez et al., 2008).

Shoot drought avoidance mechanisms are mainly associated with stomata closure to limit water loss through transpiration. Additional mechanisms associated with leaf movements and reduction of light absorbance such as rolled leaves, narrow leaf angles, shedding of older leaves, a dense trichome layer, leaf epicuticular wax, and leaf color have been shown to reduce water losses and improve water use efficiency (Ehleringer and Cooper, 1992; Chaves et al., 2002; Srinivasan et al., 2008).

Drought avoidance through stomatal closure received relatively little attention in crop improvement. Actually, shoot drought avoidance associated with stomatal closure is often related to the inhibition of shoot growth accompanied with reduction of leaf size. Leaf size reduction is linked to photosynthetic activity reduction which is counter-productive in intermittent drought

stress. However, under terminal drought, regulation of leaf water losses using various water saving traits during vegetative development to save water for seed development have been associated with drought tolerance in legumes such as soybean (Sinclair, et al., 2008), chickpea (Zaman-Allah et al., 2011), cowpea (Belko et al., 2012), and peanut (Devi et al., 2010). Drought tolerance in these legume crops has been characterized by restricting their transpiration rates to a certain level under high vapor pressure deficit. This management of limited water resources allows tolerant genotypes to withstand severe stress for a relatively long period. The water management in legume plants in terms of imposing a maximum transpiration rate trait is function of vapor pressure deficit. It is an interesting trait since drought tolerant genotypes are able to control water loss depending on evaporative demand. For instance, breeding for morphological traits related to limited water loss resulted in development of drought tolerant soybean with delayed wilting trait (Sinclair et al., 2008). In cereals (sorghum, rice, and maize), stay green trait characterized by delayed leaf senescence during grain filling under water limited conditions is an important drought tolerance trait (Takeda and Matsuoka, 2008).

Osmotic adjustment (OA) is another dehydration postponing trait that is expressed under soil drying conditions. It involves the accumulation of a range of compatible solutes and ions such as soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, and potassium to maintain cell water balance. Under water deficit, OA enables leaf turgor maintenance, thus supporting stomatal conductance under lower leaf water status and improves root capacity for water uptake (Blum, 2009).

Drought tolerance means that plants cannot avoid water stress. Consequently, they develop a number of adaptation mechanisms aimed to sustain and conserve plant/cell function in a dehydrated state (Blum et al., 2005). Drought tolerance mechanisms consist of accumulation of

detoxifying and cell structure stabilizing components. Drought increases reactive oxygen species (ROS) levels in the cell. Reactive oxygen species may react with proteins, lipids and nucleic acids, causing oxidative damage and impairing the normal functions of cells. Various organelles including chloroplasts, mitochondria and peroxisomes are the source as well as first target of reactive oxygen species produced under drought stress (Farooq et al., 2009).

Plants contain antioxidant enzymes such as superoxide dismutase, glutathione reductase and ascorbate peroxidase whose role is to detoxify the plants and limit ROS damage (Moore et al., 2009; Sofo et al., 2005). Structure-stabilizing proteins such as late embryonic abundant (LEA) proteins (Kavar et al., 2008; Pinheiro et al., 2008) are also synthesized during drought stress. The protective roles of LEA/dehydrin proteins range from DNA protection, cytoskeletal filament stabilization, molecular chaperone, and antiaggregants (Moore et al., 2009). Recently, a LEA protein was constitutively found in leaves and root of *Lupinus albus* while its accumulation in stem was associated with severe drought (Pinheiro et al., 2008). These authors suggested that LEA proteins might have tissue specific roles. Plants transformed with LEA genes have shown greater drought tolerance compared to wild type in various crops (Xiao et al., 2005). Other types of proteins such as heat and cold shock proteins confer drought tolerance by acting as molecular chaperones. The bacterial cold shock protein chaperone (CspB) that stabilizes mRNA confers drought tolerance in the new DroughtGard corn variety from Monsanto Inc. The ability to function under very low plant-water status and recover from dehydration is an important aspect of drought tolerance. Drought recovery in some crops has been shown to be a consistent and useful trait for selection to improve early drought adaptation where it is associated with secondary traits such green leaf area or stem greenness (Kamoshita et al., 2004; Muchero et al.,

2008). In cowpea, the 'California Blackeye No. 5' ('CB5') exhibits exceptional vegetative stage drought tolerance conferred by its capacity to recover from severe drought episodes (Hall, 2004).

**Common bean (*Phaseolus vulgaris* L.)**

Common bean is the most important grain legume for human consumption worldwide. Common bean is particularly important in Latin America and Africa where more than 300 million people depend on it as an important source of nutrients and income (Cavalieri, 2011). Beans consumption is highest in low income families in Africa where the per capita consumption reaches 60kg (in Rwanda and western Kenya) and South America where the per capita consumption can reach 36 kg (Beebe et al., 2010). Beans are considered a 'poor man meat' in low income regions of the world where they supplement energy sources such as cereal and root crops to provide a balanced diet. The importance of common bean in improving nutritional status in poor countries is associated with its richness in dietary proteins, micronutrients, fiber, and vitamins (Broughton et al., 2003). In developed countries, beans are receiving an important consideration because of their health benefits associated with disease preventive properties such as diabetes, heart diseases, and certain types of cancer (Winham et al., 2007; Lanza et al., 2006; Thompson et al., 2009).

Common beans are adapted to various ecological conditions and cropping systems and this is supported by their worldwide distribution and production. In 2010, FAO (http://faostat3.fao.org) estimates of the world dry bean production were around 23 million metric tons (MMT) of which 7 MMT are produced in South America and East Africa. In the Americas, the largest bean producers are Brazil (3.2 MMT) followed by the US (1.4 MMT) and Mexico (1.2 MMT). In Africa, the largest producers are Tanzania (0. 95 MMT), Uganda (0.45

MMT), Kenya (0.39 MMT), and Rwanda (0.33 MMT). However, these data might contain some data from other legume species and therefore, they might be overestimated in certain countries.

**Origin, gene pools, races, and market classes of common bean**

Common bean belongs to the genus of *Phaseolus* in the family of Fabaceae. *P. vulgaris* is one of the five cultivated *Phaseolus* species native to the Americas. Other cultivated species are lima bean (*P. lunatus* L.), runner bean (*P. coccineus* L.), tepary bean (*P.acutifolius* A. Gray*)*, and year bean (*P. polyanthus* Greenman).

Although the origin of common bean has been controversial (Gentry, 1968; Kaplan, 1981), the origin in Ecuador and northern Peru origin with subsequent dispersal north and southward was accepted for many years (Koenig and Gepts, 1989; Kami et al., 1995). However, recent data from molecular markers and sequence information provide increasing molecular evidence for the Mesoamerican origin of common bean (Kwak and Gepts, 2009; Bitocchi et al., 2012).

Cultivated common bean and its wild ancestral forms have a wide distribution from northern Mexico to northwestern Argentina (Koenig and Gepts, 1989; Singh et al., 1991; Chacon et al., 2005). Various diversity studies on different forms of common bean using morphological traits, seed protein, allozymes, and molecular markers demonstrated the existence of two gene pools in beans, Middle America and Andean (Gepts and Bliss, 1986; Koenig and Gepts, 1989; Singh et al., 1991; Kwak et al., 2009; Briňez et al., 2012; McClean et al., 2012). Beans in the Middle American gene pool are those which were domesticated from northern Mexico to Colombia, while those of the Andean gene pool were domesticated from Ecuador to northwestern Argentina. Following independent domestication events in these two regions,

common beans were introduced to other continents by European traders after the discovery of the new world.

Each gene pool is divided into races. Singh et al., (1991) described three races in each gene pool based on morphology and ecological adaptations. In addition, Beebe et al. (2000) suggested a fourth race in Middle America gene pool consisting of climbing beans from Guatemala based on a diversity study that used RAPD markers. The other three races of the Middle America gene pool are Mesoamerica, Durango, and Jalisco. The Mesoamerica race originates in the tropical lowlands and intermediate altitudes of Mexico and Central America and include all the small seeded beans with various color and growth habits. US commercial classes within the Mesoamerica race are black, navy, and small red beans. Race Durango from the semiarid central and northern highlands of Mexico, includes those beans that are predominantly of indeterminate and prostrate growth habit III. Beans from Durango race possess flattened seeds of medium size. Commercial classes in Durango race are pinto and great northern beans. Race Jalisco is usually characterized by indeterminate growth habit IV and medium seed size. Beans in the Jalisco race includes reds and pinks and were probably domesticated from the humid highlands of central Mexico. Although morphological differences are recognized between Durango and Jalisco races, Kwak and Gepts (2009) couldn't distinguish them at the molecular levels and suggested a recent divergence or a high gene flow between these two races.

The races in the Andean gene pool are Nueva Granada, Chile, and Peru. Race Nueva Granada consists predominantly of type I bush and climbing type IV with medium (25-40g/100 seeds) and large seeds (>40 g/100 seeds). Seed of Nueva Granada are of kidney or cylindrical shapes with variation in color. This race is distributed mostly at intermediate altitudes (<2000 m)

of Andes and Africa. It is also found in Brazil, Mexico, Caribbean, North America, and Europe. Us market classes in this race are kidney and bush cranberry beans.

Race Chile usually has a prostrate type III growth habit with round or oval seed and is not widely distributed. Race Peru is characterized by long and weak internodes with either indeterminate or determinate type IV climbing growth habit. Seeds are large and often round or oval but can also be elongated. The common types in the US are yellow canario and azufrado beans.

Compared to their wild ancestors, the genetic diversity of domesticated common beans is generally narrow (Koenig and Gepts, 1989; Chacon et al., 2005). Andean beans are genetically narrower than members of the Middle American gene pool (Kwak and Gepts, 2009; Bitocchi et al., 2012) which suggest limited progress if crosses are made between related germplasm in the same gene pool. Race structures of common bean and understanding their genetic diversity have helped to shape practical uses of bean germplasm for crop improvement (Kelly et al., 1998). Interracial, intergene pool and even interspecific crosses of common bean with its *Phaseolus sp* relatives have been exploited for bean improvement. For instance, interacial crosses between races Durango and Mesoamerica have been useful in breeding for yield and drought resistance (Singh, 1995). Co-evolution of host and pathogen has led to isolates of Andean or Middle American origin which attack beans primarily from their respective gene pool. Inter-gene pool and inter-specific crosses provided effective breeding strategies to transfer of genes for disease resistance between gene pools (Miklas et al., 2006).

**Breeding for drought tolerance in grain legumes**

Success in breeding legumes for drought resistance has been slow due to the polygenic nature of drought resistance. For many years, breeding for drought resistance has relied on empirical selection for yield in target production zones. However, yield itself is a complex trait

influenced by various factors with a high dependency on genotype/environment interaction (Sinclair, 2011). Various secondary traits have been studied and used to select drought resistant genotypes. Among these, the most widely used traits in breeding have been traits related to phenology and adaptation. For instance, breeding early maturing genotypes to escape terminal drought has led to drought tolerant chickpeas and extended the production area as well as the productivity (Gaur et al., 2008). Cowpea is recognized to be the most drought tolerant legume. Vegetative drought resistance conferred by the maintenance of higher leaf water status (Hall, 2012) is widely present in cowpea. In addition, breeding has combined vegetative drought resistance with erect earliness to produce early genotypes that flower and mature as early as 35 and 55 days, respectively in minimum rainfall areas associated with high evaporative demand (Hall, 2012). Cowpea genotypes with earliness combined with the delayed leaf senescence trait conferred by remobilization of stem reserves have been breed to provide opportunity for a second flush of production in the Sudanian savanna region of Africa and increase yield, (Hall, 2012). In addition to the phenology adjustment, breeding programs have produced soybean genotypes with delayed wilting (Sinclair et al., 2008).

In common bean, breeding for drought resistance has been built on race Durango from the semiarid central and northern highlands of Mexico (Singh et al., 1991). The combination of genes from Durango and Mesoamerica races resulted in development various drought resistant bean cultivars (Frahm et al., 2004; Beebe et al., 2008; Singh et al., 2001; Singh, 2007). Breeding for drought tolerance has progressed using various selection criteria such as biomass accumulation, seed yield traits, and pod filling under drought stress and non stress conditions (Schneider et al., 1997; Frahm et al., 2004; Beebe et al., 2008). Selection under both irrigation and drought stress allowed the selection of elite cultivars that maximize yield potential in stress

free environments but which also produce acceptable yields under drought stress. In addition, screening in both drought and non stress conditions allowed breeders to quantify yield gap caused by drought conditions so that they can be able to select cultivars with minimal yield loss under water stress.

There is an agreement among legume breeders and physiologists that root traits such as root depth, density, and biomass, are promising for terminal drought avoidance (Gaur et al., 2008; White and Castillo, 1989, 1992; Beebe et al., 2010; Manavalan et al., 2009; Kumar et al., 2012; Khan et al., 2010). However, breeding for improving water uptake using root traits has been limited due to practical difficulties associated with root sampling procedures, targeting appropriate soil properties as well as the large number of genotypes that have to be evaluated. In chickpea and common bean, PVC cylinder cultures are employed to screen root traits (Gaur et al., 2008; Beebe et al., 2010). In cowpea, a method to evaluate root length based on injecting a band of herbicide deep in the soil followed by sowing above the herbicide band, and scoring plants for sign of leaf herbicide symptoms as an indication that the roots had reached herbicide depth in the soil profile has been proposed (Robertson et al., 1985). Most of these techniques are being used for parental screening but routine incorporation of root trait selection in breeding programs is rare. In addition, root architectural tradeoffs in the presence of water or nutrient stresses have been observed (Ho et al., 2005), which highlights the importance of architectural dimorphisms in terms of distribution and structure to achieve optimum yield. Inspite of the challenges associated with breeding for root traits, the identification and use of QTL associated with root traits for better water uptake would improve breeding efficiency for drought tolerance. For instance, QTL associated with rooting patterns were identified in a bean intra-gene pool population from DOR364/BAT477 cross and most QTL showed constitutive gene mechanisms

(Asfaw and Blair, 2012). These QTL can facilitate the breeding for root traits in both in common bean irrespective of water conditions once confirmed. Other QTL for root architectural traits have been identified in phosphorus uptake studies in segregating populations of Andean origin (Cichy et al., 2009) and inter gene pool crosses (Beebe et al., 2006). Interestingly, most of these QTL linked to yield components, root traits, and plant nutrients uptake have been shown to co-localize in the genome irrespective of marker system and mapping populations used (Beebe et al., 2006; Cichy et al., 2009; Asfaw and Blair, 2012). This may provide bean breeders with an opportunity to capitalize on marker assisted selection (MAS) for chromosomal regions carrying these co-localized QTL. In addition, identification of root traits through association mapping might help overcome problems associated with repeatability of QTL identified in biparental populations. As more genomic information is becoming available as major legume crops are being sequenced, denser maps and more robust QTL will be identified and used to breed drought tolerant cultivars. The availability of cheap and abundant SNP markers will facilitate the genomewide selection (Bernardo and Yu, 2007) to enhance the selection of drought tolerant genotypes.

Another avenue to improve legumes for drought tolerance is through genetic engineering (GE) to introduce genes from other species known to have drought tolerance attributes. Many transcription factors that regulate downstream genes involved in drought tolerance have been identified and cloned (Ko et al., 2006; Torres et al., 2006; Kavar et al., 2008; Haake et al. 2002; Rodriguez-Uribe and O'Connell, 2006). These genes could be targets for legume transformation in an effort to improve drought stress tolerance. Among the candidate genes for genetic engineering to improve drought tolerance are C-repeat/DRE-binding factors, CBF/DREB and zinc-finger proteins coding genes (Medina et al., 1999; Ko et al., 2006). Peanut engineered with

DREB1A transcription factor driven by the stress responsive promoter *rd29* was shown to increase root length and water uptake after 40 days of drought stress (Vadez et al., 2008). Transgenic *Arabidopsis* plants overexpressing a Zinc finger XERICO gene (35S:XERICO), showed a marked increase in their tolerance to drought stress (Ko et al., 2006). Analysis of drought transcripts in *Arabidopsis* revealed that ABA-dependent pathways are predominant in the drought stress responses even though other plant hormones including jasmonic acid, auxin, cytokinin, ethylene, brassinosteroids, and gibberellins also affect drought-related gene expression (Huang et al., 2008). Crop engineering with components of the signal transduction network would also be important in improving abiotic stress tolerance in general and drought in particular since there is extensive cross-talk between responses to drought and other environmental stresses such as cold and salt tolerance (Xin et al., 2007).

Genetic engineering of other genes with protective roles such as LEA genes and those encoding chaperone proteins might also improve drought tolerance. A recent proteomic study in common bean reported abundance of dehydrins/LEA and heat shock proteins in a drought tolerant bean cultivar grown under drought stress (Zadraznik et al., 2012). However, the recalcitrance to regeneration and transformation in certain legume crops such as beans is still an obstacle to improvement using GE technology.

Therefore the overall goal of this study was to understand genetic and physiological mechanisms of drought resistance in common bean. Specifically, this study was conducted to:

1. Identify genomic regions associated with drought tolerance in common bean under terminal drought
2. Identify seedling shoot based drought resistance traits to be used as a green house screening method

3. Identify factors influencing regeneration and *Agrobacterium tumefaciens*-mediated transformation of common bean

## LITERATURE CITED

## LITERATURE CITED


Aggarwal, P. K. and S. K. Sinha. 1984. Effect of water stress on grain growth and assimilate partitioning in two Cultivars of wheat contrasting in their yield stability in a drought-environment. Annals Bot. 53: 329-340.

Asfaw, A. and M. W. Blair. 2012. Quantitative trait loci for rooting pattern traits of common beans grown under drought stress versus non-stress conditions. Mol. Breed. 30:681-695.

Asfaw, A., M. W. Blair, and P. C. Struik. 2012. Multienvironment quantitative trait loci analysis for photosynthate acquisition, accumulation, and remobilization traits in common bean under drought stress.*G3:* Genes|Genomes|Genetics 2: 579-595.

Beebe, S. E., M. Rojas-Pierce, X. Yan, M. W. Blair, F. Pedraza, F. Muňoz, J. Tohme, and J. P. Lynch 2006. Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Sci. 46:413-423.

Beebe, S.E, I. M. Rao, C. Cajiao, and M. Grajales, 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Sci. 48:582-592.

Beebe, S., P. W. Skroch, J. Tohme, M. C. Duque, F. Pedraza, and J. Nienhuis. 2000. Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. Crop Sci. 40:264-273.

Beebe, S. E., I. M. Rao, and M. W. Blair. 2010. Phenotyping common beans for adaptation to drought, pp. 309–334 in Drought Phenotyping in Crops: From Theory to Practice, edited by J. M. Ribaut, and P. Monneveux. Generation Challenge Program Special Issue on Phenotyping. CGIAR, Texcoco, Mexico.

Belko, N., M. Zaman-Allah, N. Cisse, N. N. Diop, G. Zombre, J. D. Ehlers, and V. Vadez. 2012. Lower soil moisture threshold for transpiration decline under water deficit correlates with lower canopy conductance and higher transpiration efficiency in drought-tolerant cowpea. Funct. Plant Biol. 39: 306-322.

Bernardo, R. and J. Yu. 2007. Prospects for genome wide selection for quantitative traits in maize. Crop Sci. 47:1082- 1090.

Bernier, J., A. Kumar, V. Ramaiah, D. Spaner, and G. Atlin, 2007. A large effect QTL for grain yield under reproductive-stage drought stress in upland rice**.** Crop Sci 47:507-518

Bernier, J., R. Serraj, A. Kumar, R. Venuprasad, S. Impa, R.P. Veeresh Gowda, R. Oane, D. Spaner, and G. Atlin, 2009. The large-effect drought-resistance QTL qtl12.1 increases water uptake in upland rice. Field Crops Res. 110: 139-146

Bitocchi, E., L. Nanni, E. Bellucci, M. Rossi, A. Giardini, P. S. Zeuli, G. Logozzo, J. Stougaard, P. McClean, G. Attene, and R. Papa. 2012. Mesoamerican origin of the common bean (*Phaseolus vulgaris L.*) is revealed by sequence data. PNAS. www.pnas.org/cgi/doi/10.1073/pnas.1108973109.

Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. Plant Grow. Reg. 20: 135-148.

Blum, A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Res. 112: 119-123.

Blum, A., P. O. Box, T. Aviv, and I. Email. 2005. Drought resistance, water-use efficiency, and yield potential - are they compatible, dissonant, or mutually exclusive ? Aust. J. Agr. Res. 56: 1159-1168.

Broughton, W. J., G. Hernandez, M. Blair, S. Beebe, P. Gepts, and J. Vanderleyden. 2003. Beans (*Phaseolus* spp.) – model food legumes. Plant Soil 252: 55-128.

Briňez, B., M. W. Blair, A. Kilian, S. A. M. Carbonell, A. F. Chiorato, and L. B. Rubiano. 2012. A whole genome DArT assay to assess germplasm collection diversity in common beans. Mol. Breed. 30:181-193.

Bruce, W. B., G.O. Edmeades, and T.C. Barker. 2002. Molecular and physiological approaches to maize improvement for drought tolerance. J. Exp. Bot.53: 13-25.

Cavalieri A., A. Merchant, and E. van Volkenburgh. 2011. Why not beans? Funct. Plant Biol. 38 : iii–vi.

Chacon, M. I., S. B. Pickersgill, and D. G. Debouck. 2005. Domestication patterns in common bean (*Phaseolus vulgaris L.)* and origin of the mesoamarican andean cultivated races. Theor. Appl. Genet.110: 432-444

Chaves, M.M., J. S. Pereira, J. Maroco, M.L. Rodrigues, S. P. P. Ricardo, M.L. Osorio, I. Calvalho, T. Faria, and C. Pinheiro. 2002. How plants cope with water stress in the field. Photosynthesis and growth. Annals Bot. 89: 907-916.

Cichy, K. A, M. W. Blair, C. H. Galeano Mendoza, S. S. Snapp, and J. D. Kelly, 2009. QTL analysis of root architecture traits and low phosphorus tolerance in an Andean bean population**.** Crop Sci. 49:59-68

Dai, A. 2012. Increasing drought under global warming in observations and models. Nature Climate Change. doi: 10.1038/nclimate1633

Devi, M. J., T. R. Sinclair and V. Vadez. 2010. Genotypic Variation in peanut for transpiration response to vapor pressure deficit. Crop Sci. 50(1): 191-196.

Ehleringer, J.R. and T.A. Cooper. 1992. On the role of orientation in reducing photoinhibitory damage in photosynthetic- twig desert shrubs. Plant Cell and Envir. 15: 301-306.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S. M. A. Basra. 2009. Plant drought stress : effects, mechanisms and management Sciences. Agron. Sustain. Dev. 29: 185-212.

Frahm, M.A, J. C.Rosas, N. Mayek-Pérez, E. Lõpez-Salinas, J. A. Acosta-Gallegos, and J. D. Kelly. 2004. Breeding Beans for Resistance to Terminal Drought in the Lowland Tropics. Euphytica 136**:** 223-232

Gaur, P. M., L. Krishnamurthy, and J. Kashiwagi. 2008. Improving drought avoidance root traits in Chickpea (Cicer arietinum L.). Current Status of Research at ICRISAT, Plant Prod. Sci. 11. 3-11.

Gebbing, T. and H. Schnyder. 1999. Pre-anthesis reserve utilization for protein and carbohydrate synthesis in grains of wheat. Plant Physiol. 121: 871-878.

Gepts, P., and F. A. Bliss. 1986. Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris* L) from Colombia. Econ. Bot. 40:469-478.

Gentry H.S. 1968. Origin of the Common Bean, *Phaseolus vulgaris.* Econ. Bot. 23: 55-69

Gowda, V.R.P, A.Henry, V. Vadez C., H. E. Shashidhar, and R. Serraj. 2012. Water uptake dynamics under progressive drought stress in diverse accessions of the OryzaSNP panel of rice (*Oryza sativa*). Funct. Plant Biol. 39: 402–411

Haake, V, D. Cook, J. L. Riechmann, O. Pineda, M. F. Thomashow, and J. Z. Zhang, 2002. Transcription factor CBF4 is a regulator of drought adaptation in Arabidopsis**.** Plant Physiol. 130: 639-648

Hall, A. E. 2004. Breeding for adaptation to drought and heat in cowpea. Europ. J. Agron. 21: 447-454

Hall, A. E. 2012. Phenotyping cowpeas for adaptation to drought. Front. Physiol. 3:155. doi: 10.3389/fphys.2012.00155

Ho, M D, J.C. Rosas, K.M. Brown and J. P. Lynch 2005. Root architectural tradeoffs for water and phosphorus acquisition. Funct. Plant Biol**.** 32: 737-748.

Huang, D., W. Wu, S. R. Abrams and A. J. Cutler, 2008. The relationship of drought-related gene expression in Arabidopsis thaliana to hormonal and environmental factors. J. Exp. Bot. 59: 2991-3007

Kami, J., V. B. Velasquez, D. G. Debouck, and P. Gepts. 1995. Identification of presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*. Proc. Natl. Acad. Sci. USA.92, pp. 1101-1104.

Kamoshita, A., R. Rodriguez, A. Yamauchi, and L. J. Wade, 2004. Genotypic variation in response of rainfed lowland rice to prolonged drought and rewatering. Plant Prod. Sci 7: 406-420

Kaplan, L. 1981. What is the origin of common bean? Econ. Bot. 35: 240-254

Kavar, T., M. Maras, J. S. Vozlič, and V. Meglič, 2008. Identification of genes involved in the response of leaves of *Phaseolus vulgaris* to drought stress. Mol. Breed. 21: 159-172.

Kelly, D. J, J. M. Kolkman, and K. Schneider. 1998. Breeding for yield in dry bean (*Phaseolus vulgaris* L.). Euphytica 102: 343-356

Khan, H. R, J. G. Paull, K. H. M. Siddique, and F. L. Stoddard. 2010. Faba bean breeding for drought-affected environments: A physiological and agronomic perspective. Field Crops Res. 115: 279-286

Ko, J.H, S.H Yang, and K.H. Han, 2006. Upregulation of an Arabidopsis Ring-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. Plant J. 47: 343-355

Koenig, R. and P. Gepts. 1989. Allozyme diversity in wild *Phaseolus vulgaris:* further evidence for two major centers of diversity. Theor. Appl. Genet. 78: 809-817

Kumar, N. J, A. S. Nandwal, R. S. Waldia, S. Singh, S. Devi, K. D. Sharma, and A. Kumar. 2012. Drought tolerance in chickpea as evaluated by root characteristics, plant water status, membrane integrity and chlorophyll fluorescence techniques. Exp. Agric. 48: 378-387

Kwak, M. and P. Gepts. 2009. Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae*).* Theor. Appl. Genet. 118:979-992

Lanza,E., T. J. Hartman, P S. Albert, R. Shields, M. Slattery, B. Caan, E. Paskett, F.Iber, J. W. Kikendall, P. Lance, C. Daston, and A. Schatzkin. 2006. High Dry bean intake and reduced risk of advanced colorectal adenoma recurrence among participants in the polyp prevention trial. J. Nutr. 136:1896-903.

Levitt, J. 1972. Responses of plants to environmental stresses. Academic Press, New York, USA, 698 pp.

Lobell, D. B., M. B. Burke, C. Tebaldi, M. D. Mastrandrea, W.P. Falcon, and R. L. Naylor. 2008. Prioritizing climate change adaptation needs for food security in 2030. Science 319: 607-610

Lopes, M. S. and M. P Reynolds. 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct. Plant Biol. 37: 147-156.

McClean, P. E, J. Terpstra, M. McConnell, C. White, R. Lee, and S. Mamidi. 2012. Population structure and genetic differentiation among the USDA common bean (Phaseolus vulgaris L.) core collection. Genet. Resour. Crop Evol. 59:499-515.

Manavalan, L. P, S. K. Guttikonda, L-S P. Tran, and H. T. Nguyen. 2009. Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell Physiol. 50: 1260-1276.

Manschadi, A. M., J. Christopher, P. deVoil, and G. L. Hammer. 2006. The role of root architectural traits in adaptation of wheat to water-limited environments. Funct. Plant Biol. 33: 823-837.

Medina, J, M. Bargues, J. Terol, M. Perez-Alonso, and J. Salinas**.** 1999. The Arabidopsis CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. Plant Physiol. 119: 463-470

Miklas, P. N., J. D. Kelly, S. E. Beebe, and M. W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147: 105-131.

Moore, J. P., N. T. Le, W. F. Brandt, A. Driouich, and J. M. Farrant. 2009. Towards a systems-based understanding of plant desiccation tolerance. Trends Plant Sci.14:110-116

Morris, E. 2008. More crop per drop. Nature 452: 273-277

Muchero, W., J. D. Ehlers, and P. A. Roberts. 2008. Seedling stage drought induced phenotypes and drought responsive genes in diverse cowpea genotypes. Crop Sci. 48: 541-552

NCDC, 2012. July 2012 drought report

Pinheiro, C., M. H. Cruz de Carvalho, D. Bartels, C. P. Ricardo, and M. M. Chaves. 2008. Dehydrins in *Lupinus albus*: pattern of protein accumulation in response to drought. Funct. Plant Biol. 35: 85-91.

Pennisi, E. 2008. The blue revolution, drop by drop, gene by gene. Science 320: 171-173

Pantalone, V.R., G. J. Rebetzke, J. W. Burton, and T. E. Carter Jr. 1996. Phenotypic evaluation of root traits in soybean and applicability to plant breeding. Crop Sci. 36:456-459

Rao, I. M. 2001. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages. p. 583-613. *In* M. Pessarakli (ed.) Handbook of plant and crop physiology. Marcel Dekker, New York

Robertson, B. M., A. E. Hall, and K.W. Foster.1985. A field technique for screening for genotypic differences in root growth. Crop Sci. 25, 1084-1090.

Rodrigues, M. L, C.M. A. Pacheco and M. M. Chaves. 1995. Soil-plant water relations, root distribution and biomass partitioning in *Lupinus albus* L. under drought conditions. J. Exp. Bot. 46. 947- 956

Rodriguez-Uribe, L. and M. A. O'Connell. 2006. A root-specific bZIP transcription factor is responsive to water deficit stress in tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris)*. ). J Exp. Bot. 57:1391-1398

Saint Pierre, C., R. Trethowan, and M. Reynolds. 2010. Stem solidness and its relationship to water-soluble carbohydrates: association with wheat yield under water deficit. Funct. Plant Biol. 37: 166-174.

Sadok, W. and T. R. Sinclair 2010. Transpiration response of "slow-wilting" and commercial soybean (Glycine max (L.) Merr.) genotypes to three aquaporin inhibitors. J. Exp. Bot. 61: 821-829.

Schiermeier, Q. 2008. A long dry summer. Nature 452:270-273

Schneider, K.A., R. Rosales-Serna, F. Ibarra-Perez, B. Cazares-Enriquez, J. A. Acosta-Gallegos, P. Ramirez-Vallejo, N. Wassimi, and J. D. Kelly. 1997. Improving common bean performance under drought stress. Crop Sci. 37:43-50

Sinclair, T. R. 2011. Challenges in breeding for yield increase for drought. Trends Plant Sci. 16. 289- 293

Sinclair, T. R., A. M. Zwienieckib and N. M. Holbrook. 2008. Low leaf hydraulic conductance associated with drought tolerance in soybean. Physiologia Plantarum 132: 446-451.

Singh, S. P. 1995. Selection for water-stress tolerance in interracial populations of common bean. Crop Sci. 35:118-124

Singh S. P. 2007. Drought resistance in the race Durango dry bean landraces and cultivars. Agron. J. 99:1219-1225

Singh, S. P., P. Gepts, and D. G. Debouck. 1991. Races of common bean (*Phaseolus vulgaris,* Fabaceae). Econ. Bot. 45:379-396.

Singh S. P, H. Terán, and J. A. Gutiérrez. 2001. Registration of SEA 5 and SEA 13 drought tolerant dry bean germplasm. Crop Sci. 41: 276-277

Sofo, A, B. Dichio B, C. Xiloyannis, and A. Masia. 2005. Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Funct. Plant Biol. 32**:** 45-53.

Suja, G., G. Venkataraman, and A. Parida. 2007. Identification of stress induced genes from the drought tolerant plant *Prosobis juliflora* (Swartz) DC through analysis of expressed sequence tags. Genome 50: 470-478

Srinivasan, S., S. M. Gomez, S. S. Kumar, S. K. Ganesh, K. R. Biji, A. Senthil, and R. C. Bab. 2008. QTLs linked to leaf epicuticular wax, physio-morphological and plant production traits under drought stress in rice (Oryza sativa L.). Plant Growth Regul. 56:245-256.

Takeda, S., and M. Matsuoka, 2008. Genetic approaches to crop improvement: responding to environmental and population changes. Nature 9:444-457

Tardieu, F. 2012. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J. Exp. Bot. 63: 25-31.

Tompson, M. D., M. A. Brick, J. N. McGinley, and H. J. Tompson. 2009. Chemical composition and mammary cancer inhibitory activity of dry bean. Crop Sci. 49: 179-186

Torres, G. A. M, S. Pflieger, F. Corres- Menguy, C. Mazubert, C. Hartmann, and C. Lelandais-Briere. 2006. Identification of novel drought related mRNAs in common bean roots by differential display RT-PCR. Plant Sci. 171:300-307

Vadez, V., S. Rao, J. Kholova, L. Krishnamurthy, J. Kashiwagi, and P. Ratnakumar. 2008. Root research for drought tolerance in legumes : Quo vadis ? J. Food Leg. 21: 77-85.

Vadez, V., J. D. Berger, T. Warkentin, S. Asseng, P. Ratnakumar, K. P. C. Rao, P. M. Gaur, N. Munier-Jolain, A. Larmure, A. S. Voisin, H. C. Sharma, S. Pande, M. Sharma, L. Krishnamurthy, and M.A. Zaman. 2011. Adaptation of grain legumes to climate change: a review. Agron. Sust. Dev. 32: 31-44.

Xiao, B, Y. Huang, N. Tang, and L. Xiong, 2005. Over expression of a LEA gene in rice improves drought resistance under field conditions. Theor. Appl. Genet. 115: 35-46

Xin, Z, A. Mandiaoka, J. Chen, R. L. Last, and J. Browse. 2007. Arabidopsis ESK1 encodes a novel regulator of freezing tolerance. Plant J. 49: 786-799

Winham, D. M, A. M. Hutchins, and C. S. Johnston. 2007. Pinto bean consumption reduces biomarkers for heart disease risk. J. Amer. College Nutr. 26: 243-249

White, J. W. and J. A. Castillo. 1989. Relative effect of root and shoot genotypes on yield of common bean under drought stress. Crop Sci. 29:360-362.

White, J. W. and S. P. Singh. 1991. Sources and inheritance of earliness in tropically adapted indeterminate common bean. Euphytica *55*: 15-19.

Zadraznik, T, K. Hollung, W. Egge-Jacobsen, V. Megli, and J. Šuštar-Vozli. 2012. Differential proteomic analysis of drought stress response in leaves of common bean (*Phaseolus vulgaris* L.). J. Proteomics. doi: 10.1016/j.jprot.2012.09.021.

Zaman-Allah, M., D. M. Jenkinson and V. Vadez. 2011. A conservative pattern of water use, rather than deep or profuse rooting, is critical for the terminal drought tolerance of chickpea. J. Exp. Bot. 62: 4239-4252.

## Chapter 2. Identification of QTL associated with drought tolerance in common bean under terminal drought


### Abstract

An inter gene pool recombinant inbred line (RIL) bean population from a cross of SEA5 and CAL96 was evaluated to identify drought resistant lines and map quantitative trait loci (QTL) associated with traits related to drought resistance. Experiments were conducted in Karama dryland research station in Rwanda for three consecutive years and in Palmira research station in Colombia for one year. The population was evaluated in rainfed and rainfed supplemented with irrigation plots in Karama and only in rainfed conditions in Palmira. Traits evaluated included number of days to flower, to maturity, to seed fill, pods per plant, seeds per pod, harvest index, pod harvest index, 100- seed weight, yield and yield per day to identify drought resistant lines and conduct QTL analysis at both sites under different water treatments.

Drought stress was moderate in Karama (DII=0.27) and was severe in Palmira (DII=0.70) based on drought intensity index (DII). Lines that yielded better than the CAL96 parent with an intermediate seed size were identified under both water conditions and these can be used as parents to further enhance drought tolerance in the Andean gene pool.

A genetic map of the SEA5/CAL96 population was constructed using 92 SSR and 12 InDel markers. The map covers all 11 linkage groups spanning 1031 cM. A total of 41 QTL were identified across environments. Some of the QTL mapped near previously reported QTL in different studies while others were specific to this study. A major QTL for yield and yield per day accounting for 37% of phenotypic variation was identified on chromosome Pv11. This QTL is linked to QTL associated with 100-seed weight suggesting that it is possible to select simultaneously for yield and seed size in this population.

## Introduction

Drought is the most important abiotic stress that limits crop productivity worldwide. It has been recognized by the Crop Science Society of America as one of its major scientific challenges of the 21st century (Lauer et al., 2012). Incidence and duration of drought in major agricultural areas are expected to increase due to climate change and negatively affect crop yields and food security (Lauer et al., 2012; McClean et al., 2011). Reduced rainfall is predicted to occur more frequently in tropics where many developing countries are located and where poverty and malnutrition are already a serious problem (Cavalieri, 2011). The lack of appropriate infrastructure and other solutions to mitigate climate change may result in a serious food security risk and its associated social and political consequences in these countries. Research directed at improving crop resistance to production stresses especially drought is needed to contribute to other efforts under way to alleviate poverty and malnutrition problems in those countries.

Plants utilize different strategies to cope with drought stresses. Among these, escape, avoidance, and tolerance mechanisms are the most important (Levitt, 1972). Each of these strategies involves different mechanisms and processes and combining these into elite genotypes is rarely achieved. Breeding for drought resistance is further complicated by the type, timing and intensity of the stress in relation to plant growth stages, which result in large environmental variations as well as numerous genotype by environment (GxE) interactions.

The most common way of breeding for drought tolerance has been to select genotypes able to maximize yield in relation to the dynamics of the drought episodes prevailing in each target environment (Tuberosa and Salvi, 2006). This is possible when high yield potential and effective drought resistance mechanisms are combined in the same genotype in the target environment. Selection for yield performance under drought and irrigation conditions across environments

optimizes the selection of lines with high yield potential in both drought and non-drought conditions, which is the best option to achieve yield stability under drought stress while maintaining high yield in higher rainfall seasons (Schneider et al., 1997a).

Common bean (*Phaseolus vulgaris* L.) is a subsistence crop with more than 300 million people relying on it for protein, micronutrients, and calories (Cavalieri, 2011). Farmers in South America and in East Africa use beans as an important source of income (Beebe et al., 2010). In Latin America and eastern and southern Africa, common bean is a small farmer crop that is generally cultivated on marginal lands prone to abiotic and biotic stresses. In these conditions, drought is the major cause of bean yield losses since bean production is dependent on natural rainfall with little access to supplement irrigation. Rainfall varies dramatically in different seasons of the year in tropical and subtropical environments. Drought can occur in different forms either throughout the season, early in the season, at mid-season or near the end of the life cycle and can have large effects on common bean growth either during early establishment, vegetative expansion, flowering, or seed filling (Rao, 2001). It is estimated that 60% of the bean crop is cultivated under either intermittent or terminal drought stress worldwide (Beebe et al., 2010). Breeding for drought resistance in areas where drought is endemic offers the most practical way of ensuring sustained bean productivity.

Common beans of the race Durango from the semiarid highlands of Mexico have been reported to possess the highest levels of drought resistance (Tarán and Singh, 2002). Bean breeders have tried to exploit race Durango germplasm to develop drought resistant bean cultivars. Better drought resistance lines such as SEA5 were derived from a double cross combining race Durango (Guanajuato 31) and race Mesoamerica BAT477 (Singh et al., 2001; Singh, 2007). Combining races Durango and Mesoamerica has been a consistent source of

improved drought resistance for lowland tropical environments, and additional cycles of breeding have served to refine these combinations, probably resulting in more effective introgression of Durango genes to lowland race MesoAmerica (Miklas et al., 2006a). In this context, a black bean line L88-63 (Frahm et al., 2004) that out-yielded SEA5 was selected from a simple cross in which one of the parents was derived from a combination of Durango and Mesoamerica. More recently, Beebe et al. (2008) developed widely adapted lines of small-seeded race Mesoamerica commercial classes (small red, small black and cream striped or carioca types) based on selection for more efficient photosynthate remobilization during terminal drought. Despite the use of drought resistance sources in Durango and Mesoamerica races as parents, little progress has been achieved in transferring drought resistance to beans in the large-seeded Andean gene pool that are widely grown in Africa and South America. Crosses have rarely been made between Durango drought tolerance sources and Andean genotypes due to gene pool incompatibilities and lack of combining ability between contrasting gene pools (Beebe et al., 2010). In the Andean gene pool, drought resistance is most needed in short-season bush beans, since long season climbing beans are usually planted at higher elevations in moister environments.

Traits associated with tolerance to drought have been identified in common bean (White and Castillo, 1985; Acosta-Gallegos and Shibata, 1989; Acosta-Gallegos and Adams, 1991; Schneider et al., 1997a, Ramirez-Vallejo and Kelly, 1998; Frahm et al., 2004; Rosales-Serna et al., 2004; Beebe et al., 2008). These include, deep and balanced root system that extracts water from deep soil profiles, days to flowering and to physiological maturity, biomass accumulation, number of pods per plant, number of seeds per pod, harvest index, pod harvest

index, 100-seed weight, yield, and yield based indices. However, the underlying genetic basis of most of these traits remains to be understood.

Molecular marker technologies offer the possibility to dissect quantitative traits into their single genetic determinants through the process of QTL mapping so that individual loci can be targeted in marker-assisted selection (MAS). In addition, co-localization of QTL provides evidence of functionality and is a powerful tool for map based cloning of specific genes contributing to complex traits.

The use of QTL in MAS has been limited in plant breeding because of the limited stability of QTL among populations, high GxE interactions for quantitative traits and epistatic interactions between QTL and genetic background. However, some successes in cultivar development have been achieved. For instance, a major QTL associated with shattering resistance in soybean was fine-mapped and MAS was used to develop shattering resistant cultivars (Funatsuki et al., 2012). Through MAS, several QTL for root depth in rice were transferred from the japonica upland cultivar Azucena, adapted to rainfed conditions, to the lowland indica variety IR64 (Steele et al., 2007), and a new drought tolerant cultivar Birsa Vikas Dhan 111, characterized by early maturity, high grain yield, and good grain quality was released in India (Steele, 2009). In common bean, MAS was used to pyramid SAP6 and SU91 QTL to develop USDK-CBB-15 dark red kidney bean line with resistance to common bacterial blight [CBB caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Miklas et al., 2006b]. In a recent study that compared the efficiency of direct disease resistance selection with MAS for the transfer of CBB resistance, MAS showed promise as a method to facilitate pyramiding CBB resistance between common bean gene pools (Duncan et al., 2012).

QTL linked to root architectural and nutrient uptake traits have been identified in common bean (Beebe et al., 2006; Cichy et al., 2009; Asfaw and Blair, 2012). Confirmation of these QTL may provide bean breeders with an opportunity to capitalize on MAS for root traits associated with drought avoidance and nutrient acquisition. Limited QTL studies for drought resistance in common bean have been conducted and these have focused largely on populations from MiddleAmerican gene pool (Schneider et al., 1997b; Blair et al., 2006; Asfaw and Blair, 2012; Blair et al., 2012; Asfaw et al., 2012). Additional QTL analyses of drought resistance in populations developed from crosses between gene pools or from crosses within the Andean gene pool are needed to explore additional diversity for drought resistance QTL alleles, and to analyze the effect of genetic backgrounds on the QTL alleles that have already been identified (Beebe et al., 2010). This study was conducted to:

a) Identify drought resistance RIL in a population derived from the cross of a Durango derived line SEA5 and an Andean bean cultivar CAL96.

b) Map QTL associated with drought tolerance mechanisms in the same common bean population.

## Materials and Methods

### Field evaluations

### Germplasm

An inter-gene pool RIL population derived from the cross of SEA5 and CAL96 consisting of 125 $F_{5:7}$ lines named as RSA, parents, and local checks were evaluated in Karama dryland research station of the Rwanda Agriculture Board (RAB). A sub-sample of 97 lines, parents, and a local check were evaluated in Palmira research station of the International Center for Tropical Agriculture (CIAT). In Rwanda local checks used were SER13, SER14, SER16, RWR2245, and RWR1668 while in Colombia ICA Quimbaya was used. SEA5 is a drought resistant line that was developed at CIAT from an inter-racial double cross between races Durango and Mesoamerica [(TR 7790 = BAT 477/'San Cristobal 83'//'Guanajuato 31'/'Rio Tibagi' (Singh et al., 2001)]. SEA5 has type III growth habit and small cream-colored seeds ranging from 22-25g per 100 seeds. SEA5 is resistant to Fusarium root rot [caused by *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyder & Hansen] and possess the *I* gene for resistance *Bean Common Mosaic Virus* (BCMV). However, it is susceptible to major bean diseases including anthracnose (caused by *Colletotrichum lindemuthianum),* CBB, and rust
*[*caused by *Uromyces appendiculatus* (Pers.:Pers.) Unger].

CAL96 is a dark red-mottled Calima type that was developed by CIAT from a cross between Calima2/Argentina1 and released in Uganda as K132. CAL96 has a type I growth habit and has large seeds with an average seed weight of 42g/100 seeds. CAL96 matures between 85 and 90 days after planting and is widely adapted in East Africa because of its high yield potential and high marketability due to its preferred seed size and color. SER13, SER14, and SER16 are small red beans that were developed by CIAT for drought resistance (Beebe et al., 2008) and

they were subsequently released in Rwanda for production under drought conditions. RWR2245 and RWR1668 are iron biofortified Andean bean cultivars that are adapted in Rwanda. ICA Quimbaya is a large red seeded drought resistant Andean bean cultivar grown in Ecuador and Colombia.

**Sites**

In Rwanda, field trials were conducted in Karama research station. Karama station is one of the semi-arid stations characterized by dry and hot climates. The annual rainfall is below 900 mm and the annual average temperatures around 21 $^{o}$C. The station is located in the region of Bugesera in the South East of Rwanda at 2$^{o}$ 16´S, 30$^{o}$ 17´ E at an altitude of 1,347 m above the sea level. Karama station is characterized by clay loam soils with a pH between 7.6 and 7.7.

In Colombia, the research was conducted in CIAT's Palmira Experiment Station located at 3$^{o}$30'N, 76$^{o}$30'W, 965 m elevation. The average temperatures are 24.3 $^{o}$C ranging from 18.8 to 28.4 $^{o}$C. Average rain fall is 896 mm and soils are fine silty, mixed, isohyper- thermic Aquic Hapludoll.

**Experimental design**

In Rwanda 125 RILs, 2 parents, and 5 local checks SER13, SER14, SER16, RWR2545, and RWR1668 making a total of 132 lines were evaluated under rainfed (RF) and rainfed supplemented with irrigation (RFS) conditions. Plots consisted of one row of 1.5 m long and 0.50 m between rows in 2010 and 2011, and two rows of 1.5 m and 0.50 m between rows in 2012. They were organized in a rectangular 12x11 lattice design with two replications. A 3m buffer zone of the drought resistant SER16 cultivar was planted between RF and RFS plots to

prevent lateral movements of water from RFS to RF plots. All experiments in Karama were planted in mid rainy seasons trying to target terminal drought during the reproductive stages. The first experiment was planted on 29 November 2010 and harvested on 1 March 2011 and this experiment is called Karama 2010 in subsequent sections. The second experiment was grown from 15 April to 15 July in 2011 (Karama 2011), and the third experiment was grown from 26 October 2011 to 1 February 2012 (Karama 2012). Overhead sprinklers were used to irrigate both RF and RFS plots to ensure good plant establishment and early growth as needed until the early pod filling. Thereafter, irrigation was discontinued in RF plots while the irrigated plots continued to receive supplemental irrigation of 20 mm of water twice a week for a total of additional 188, 263, and 211 mm in 2010, 2011, and 2012 respectively. To reduce the threat from BCMNV vectored by aphids, the plots were splayed with insecticide Dimethoate 50% at V2 stage.

In Colombia, one experiment was conducted during the dry season between 23 July and 17 October in 2011. A subsample of 97 lines, parents, and a local check ICA Quimbaya were evaluated in 10x10 lattice experimental design with three replications. Plots consisted of 2 rows of 3.72 m long and 0.6 m wide. To ensure good stand establishments, two gravity irrigations were applied six days before planting and a second at 12 days after emergence. In both locations recommended agronomic procedures were followed. All experiments were hand planted, weeded and harvested.

**Response variables**

Environmental data including daily rainfall (mm) and temperatures (C) were recorded from weather stations of the research stations. To evaluate plant response to water stress, various variables were recorded: Number of days to flowering (DF) was recorded as the number of days from planting to when 50% of plants per plot have at least one flower. Number of days to

physiological maturity (DM) was recorded as the number of days for 90% of pods to lose their green color and start to dry. Number of days for seed fill (DSF) was calculated as the number of days to physiological maturity minus number of days to flowering. Harvest Index (HI) in each plot was determined as the seed weight/total biomass at harvest. Pod Harvest Index (PHI) was calculated as the proportion between seed weight and pod weight. Seed yield (SY) per plot was measured at 14% moisture content and was estimated as $g/m^2$. Yield per day (SYD) was calculated as plot seed weight/number of days to physiological maturity and was estimated in $g/m^2$. 100-seed weight (SW) was weighted on 100 seed sample from each plot. Number of pods per plant (NP) was counted on 10 plants per plot, and the number of seed per pod (NS) was counted on a random pod/plant for ten plants per plot. Geometric seed mean yield (GM) per genotype was calculated as $\sqrt{Yd.Yp}$ where Yd is the mean yield of a genotype under rainfed treatement and Yp is the mean yield of the same genotype under rainfed supplemented with irrigation treatment. Drought intensity index (DII) calculated as 1-Xd/Xp where Xd is the mean yield averaged across genotypes in the rainfed treatment and Xp is the mean yield averaged across genotypes in the rainfed supplemented with irrigation treatment was calculated for each environment (Fischer and Maurer, 1978).

**Population genotyping**

A bulk leaf tissue sample of three plants collected from seedlings of each line and parent grown in the greenhouse at Michigan State University was used for DNA extraction following the miniprep method of Afanador et al. (1993). DNA was quantified in a Hoefer 'DNA Quant 200' fluorometer and diluted to a final concentration 40Mm for use in PCR reactions.

Simple Sequence Repeat (SSR) markers described by Hanai et al. (2010) and others listed on line (http://www.css.msu.edu/bic/PDF/Bean_SSR_Primers_2007.pdf) were first screened on parents and the polymorphic primers were screened on the entire population following the protocols for SSR markers (Blair et al., 2003). Additionally, 37 Insertion-Deletion (InDel) markers that were developed at North Dakota State University through the BeanCAP project (the author acknowledges receipt of Indel markers developed by the BeanCAP project at North Dakota State University, by S. Mafi Moghaddam and P. McClean) were used to screen the parents and those that proved to be polymorphic in parents were used to screen the population. InDel markers' PCR mixture consisted of a final concentration of 1x PCR Buffer including 0.15 mM $MgCl_2$, 0.5 mM dNTP mix, 0.252 µM Forward/Reverse primers and 1 unit of Taq polymerase within a final volume of 20 µL. PCR Cycles were 3 min at 95 $^{o}$C for one cycle, 20 sec at 95 °C, 30 sec at 55 °C, 1 min at 72 °C for 45 cycles, and a final extension of 10min at 72 °C for one cycle.

All markers were amplified in a PTC-200 (MJ research) thermocycler, resolved in 6% w/v non denaturing polyacrylamide gels (19:1; acrylamide:bis-acrylamide) using the C.B.C SCIENTIFIC Mega-Gel High Throughput electrophoresis system (Wang et al. 2003), and detected using silver staining.

**Data Analysis**

Statistical analyses for field data were conducted using SAS 9.3 (SAS institute, Cary, NC). Mixed models were used for data analysis for each year where replication and block were considered as random variables, while water treatment and genotype were considered fixed effects. PROC CORR and PROC REG commands were used to analyze Pearson correlations and

regression relations among variables. Population distributions for the variables within each water treatment were performed using the graph program of SigmaPlot version 12.0 (Systat Software, Inc., CA, USA). Heritability for all variables was estimated based on data from each year within each season under rainfed and rainfed supplemented with irrigation treatments according to Hallauer and Miranda (1981).

Mapping and QTL analysis were conducted using IciMapping V3.1 (Wang et al. 2011). For the map construction, markers were assigned to the linkage group using a LOD threshold of 3 and the maximum distance between markers of 30 cM. Commends 'order' and 'ripple' were used to organize markers on linkage groups. Linkage groups were oriented by checking markers against known marker positions on genetic maps constructed by Blair et al., (2003) and Córdoba et al., (2010). For the QTL analysis, Inclusive Composite Interval (ICM-ADD) was used. Significant QTL for individual traits were determined by the location of the peak LOD score at a genome wide based on 1000 permutation tests (Churchill and Doerge 1994), with a walking step of 1cM, probability in stepwise regression of 0.05, and the type I error of 0.05. QTLs that were consistent in more than one environment were named according to the guidelines for common bean QTL nomenclature (http://bic.css.msu.edu/_pdf/Guidelines_QTL_Nomenclature.pdf). Epistatic interactions were analyzed using ICIM-EPI with LOD threshold of 3, step of 5cM, and 0.01 probabilty in stepwise regression analysis.MapChart (Voorrips, 2002) was used to display QTL maps.

## Results

### Field results

Experiments in Rwanda experienced different levels of intermittent drought which came at various stages after flowering (Figure 2. 1). In both sites, average temperatures were suitable for the bean crop growth while rainfall was generally insufficient (Figure 2. 1). In Karama 2010, the average temperature for the growth season was 21 $^{o}$C varying from 17.4 to 23.5 $^{o}$C. Rainfall was sporadic and more than a half (79 mm) of the total rainfall (125 mm) fell after flowering subjecting the experiment to intermittent drought rather than a more typical terminal drought during the reproductive period. In the 2011, the growth season was characterized by temperatures ranging from 16.7 to 22.6 $^{o}$C and a cumulative rainfall of 222 mm. The RF plots in this season experienced sporadic drought during the reproductive stage since 82 mm of rain fell after flowering. More precipitation fell during the 2012 season with cumulative rainfalls of 256 mm. However, the RF plots received only 58 mm during the reproductive stages. Average temperature for the growing season was 21.1 $^{o}$C with a minimum of 17.8 $^{o}$C and a maximum of 23 $^{o}$C. Palmira site was characterized by higher temperatures than Karama varying between 20.4 and 26.4 $^{o}$C with an average of 24.7 $^{o}$C. The total rainfall was 119 mm most of which (106 mm) fell later in the season. However most of this rain did not impact the experiment since it came 77 days after planting while the latest line matured at 72 days. The overall drought susceptibility index (DII) was 0.27 in Karama which is a moderate drought. Drought was mild in years 2011 and 2012 with DII= 0.18, and 0.14 respectively, while it was more severe in 2010 with DII= 0.52. DII was 0.70 in Palmira based on evaluation of the same population in Palmira in 2010 (data not shown) under irrigation, which was a severe drought exposure.

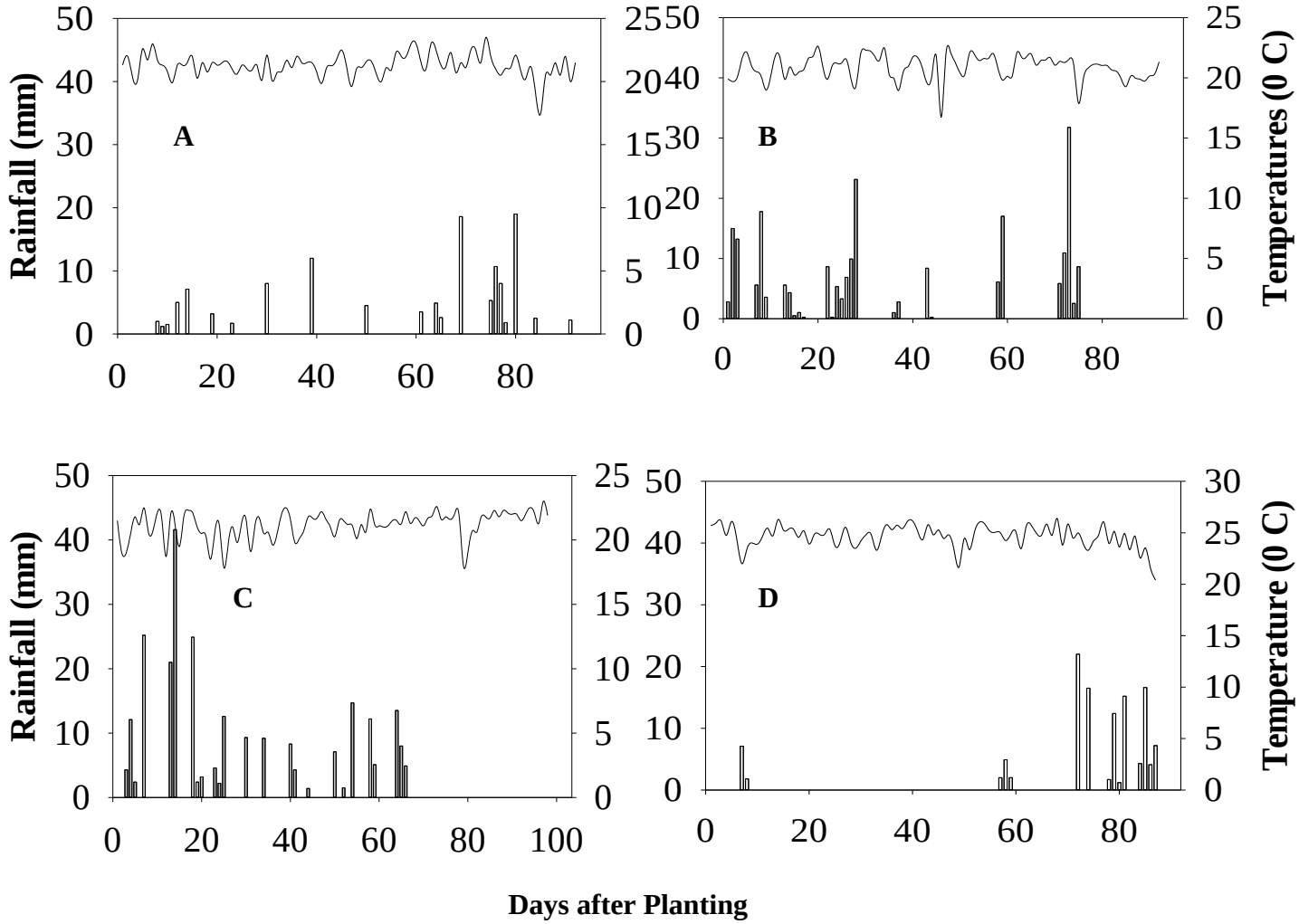



Figure 2. 1. Rainfall (mm) and Temperature ($^{O}$C) variations recorded during experiments. A. Karama 2010; B. Karama 2011; C. Karama 2012; D. Palmira 2011.

Table 2. 1. Effect of water treatment on phenology, yield, yield components, and partitioning indices traits of SEA5/CAL96 RIL population, parents, and local checks grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda during 2010-2011.

| Trait | 2010 | | | | 2011 | | | |
|---|---|---|---|---|---|---|---|---|
| | Rainfed | Rainfed supplemented | Water treatment difference | GxE† | Rainfed | Rainfed supplemented | Water treatment difference | GxE† |
| **Phenology** | | | | | | | | |
| Number of days to flowering | 41 (38-55)†† | 41 (38-51) | NS | NS | 39.7 (35-47) | 39.5 (35-45) | NS | NS |
| Number of days to maturity | 79 (71-89) | 82 (71-90) | ** | ** | 85.5 (74-92) | 85.9 (74-94) | NS | ** |
| Number of days to seed fill | 34 (22-46) | 36 (23-44) | ** | ** | 45.9 (33-53) | 46.8 (32-54) | ** | ** |
| **Yield and yield components** | | | | | | | | |
| Number of pods/plant | 4.7 (1.9-9) | 7 (1.5-14) | ** | NS | 9.2 (3-18) | 9.4 (5-20) | NS | * |
| Number of seeds/ pod | 3 (1-7) | 4 (1.5-5.5) | ** | NS | 3 (1-6) | 4 (2-6) | ** | ** |
| Seed yield ($g/m^2$) | 63.5(11-227) | 134(60-305) | ** | ** | 102 (42-228) | 124 (71-363) | ** | ** |
| Seed yield/day ($g/m^2$) | 0.8(0.14-2.35) | 1.1 (02-45) | ** | ** | 1.46 (0.15-6) | 1.5 (0.3-4.7) | ** | ** |
| Seed weight (g) | 28.5(14.1-8.5) | 28.4(15-57) | NS | ** | 24.5 (11-43.) | 24.9 (10-47) | NS | NS |
| **Partitioning indices** | | | | | | | | |
| Harvest index (%) | 33(11-59) | 38 (10-63) | ** | NS | 37 (7-59) | 38 (8-64) | NS | NS |
| Pod harvest index (%) | 62 (32-81) | 60 (31-77) | NS | NS | 54 (18-75) | 55 (17-74) | NS | ** |

*; **: significant at 0.05 and 0.01 probability levels, respectively; †GxE: genotype-environment interactions;
††: data presented as means with minimum and maximum values in the parenthesis; NS: no significant.

Table 2. 2. Effect of water treatment on phenology, yield, yield components, and partitioning indices traits of SEA5/CAL96 RIL population, parents, and local checks grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda in 2012 and under rainfed in Palmira, Colombia in 2011.

| Trait | Karama 2012 | | | | Palmira 2011 |
|---|---|---|---|---|---|
| | Rainfed | Rainfed supplemented | Water treatment difference | GxE† | Rainfed |
| **Phenology** | | | | | |
| Number of days to flowering | 39 (37-43)†† | 39.5 (36-43) | NS | NS | 32 (29-36) |
| Number of days to maturity | 78.2 (70-87) | 78.8 (70-87) | ** | ** | 65 (58-71) |
| Number of days to seed fill | 36.2 (28-46) | 36.9 (29-48) | ** | ** | 33 (30-39) |
| **Yield and yield components** | | | | | |
| Number of pods/ plant | 8 (7-15) | 10 (5-16) | ** | NS | 9 (4-15) |
| Number of seeds/ pod | 3.7 (2-6) | 4.1(3-6) | ** | NS | 3 (1-5) |
| Seed yield ($g/m^2$) | 138.6 (99-222) | 161 (6-347) | * | * | 59.7 (12-141) |
| Seed yield/ day ($g/m^2$) | 1.5 (0.4-3) | 1.6 (0.4-4) | * | * | 0.9 (0-2) |
| Seed weight (g) | 31.7 (19-58) | 30.3 (18-46) | NS | ** | 29.8 (17-50) |
| **Partitioning indices** | | | | | |
| Harvest index (%) | 42 (26-64) | 40 (24-61) | NS | NS | 37 (13-59) |
| Pod harvest index (%) | 65 (45-76) | 64 (37-89) | NS | NS | 66 (38-77) |

*; **: significant at 0.05 and 0.01 probability level, respectively; † GxE: genotype-environment interactions;
†† data presented as means with minimum and maximum values in the parenthesis; NS: no significant.

Significant differences ($p$<0.01) between genotypes were observed for almost all variables evaluated in all experiments. In 2010, all variables were significantly ($p$<0.01) affected by drought in Karama except the number of days to flowering, 100 seed weight, and pod harvest index (Table 2. 1). Genotypes in RF plots matured three days earlier than genotypes in RFS plots. The same trend was observed for the number of days to seed fill where there was a two day difference between the RFS and RF plots. RF plots had lower average number of pods per plant, number of seeds per pod than RFS plots. Average yields were 63.4 g/m$^2$ and 134 g/m$^2$ under RF and RFS conditions respectively causing a yield percentage reduction of 52.6%. The highest yielding group comprised all the local checks and SEA5. Only two lines RSA118 and RSA109 with yields of 199.6 and 183.5 g/m$^2$ respectively had yield comparable to the yields of SEA5 under RF. These two lines yielded well also under RFS. Although the yield of SEA5 was superior to the yield of CAL96 in RF conditions (135.7 vs 99.3 g/m$^2$), the yields did not differ significantly under RFS. Under RFS, the highest yielding lines were the local checks followed by parents. The same trend was observed for geometric means of yield where the highest ranking entries were the local checks (Table 2. 3). Only 3 lines RSA112, RSA109, and RSA118 with GM yield of 147.2, 168.8, and 185.5 g/m$^2$ respectively, were higher than CAL 96 parent. In both RF and RFS, average 100-seed weight (28 g) was intermediate between seed size of CAL96 and SEA5. Under RF, 100-seed weight varied between 14 to 48 g. Seed weight for RSA66, RSA158, RSA164, and RSA27 lines was not significantly different from CAL96, but some of these lines were among the lowest yielding entries. Under RFS, no line produced 100-seed weight comparable to CAL96 (56.6 g/100-seed). High yielding lines RSA118, RSA112, and RSA109 had intermediate seed size above 30g/100-seed weight (Tables 2. 3 and 2. 8)

Harvest index between the parents was not significantly different under RF while CAL 96 had higher HI than SEA5 under RFS. Only four RILs, RSA89, RSA118, RSA 87, and RSA152 had a HI above 50% under RF. PHI varied from 32 to 81% under RF with the range from 31 to 77% under RFS conditions. In this year, lines having a higher harvest index than both parents were observed. Significant genotype/water treatments (GxE) interactions were observed for number of days to maturity, number of days to seed fill, 100-seed weight, yield, and yield per day.

In Karama 2011, significant differences were observed between RF and RFS only for yield, yield per day, number of seeds per pod and the number of days to seed fill (Table 2. 1). Significant differences were observed for genotype/water treatment interactions (GxE) for all variables except harvest index and 100-seed weight. Number of days to maturity and number of days to seed fill generally were highest during this year compared to other experiments. In Karama 2011 experiments, there was a general improvement in yield, and yield components except 100-seed weight in RF. Under RF conditions, seed yield varied from 42.3 to 227.7 $g/m^2$ while in RFS plots and they ranged from 71 to 362.6 $g/m^2$. Drought stress caused a percentage yield reduction of 18%. Line RSA118 performed well under both water conditions. Geometric means for yield ranged from 56.3 to 287.4 $g/m^2$. Yield/day consistently followed the same trend as yield. Seed weight ranged from 11 to 37.54 g/100-seeds in RF while the range was from 10.4 to 41.8g/100-seeds in RFS conditions. A red-mottled line RSA120 had the highest 100-seed weight at 37.5g under RF. There were no significant differences due to water treatment between partitioning indices. Harvest index varied from 7 to 59% and 8 to 64% respectively under RF and RFS. Pod harvest index varied from 18 to 75% and 17 to 74% respectively under RF and RFS. The average number of pods per plant was 9 and 9.5 while the seeds per pod were 3 and 4 in RF and RFS respectively.

Table 2. 3. Yield and 100-seed weight of ten highest and five lowest yielding entries, parents of SEA 5/CAL 96 RIL population, and checks grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and rainfed conditions in Palmira, Colombia during 2010- 2012.

| | Yield ($g/m^2$) | | | | | | | | | | Site and year average 100-seed weight(g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Karama | | | | | | | | | Palmira | | |
| | 2010 | | | 2011 | | | 2012 | | | | | |
| Line | RF† | RFS‡ | GM§ | RF† | RFS‡ | GM§ | RF† | RFS‡ | GM§ | RF† | RF† | RFS‡ |
| **Top yielding** | | | | | | | | | | | | |
| RSA118 | 199.6 | 172.5 | 185.5 | 162.5 | 160.8 | 161.6 | 178.7 | 194.9 | 186.6 | 79.4 | 32.2 | 35.0 |
| RSA109 | 183.5 | 155.2 | 168.8 | 141.4 | 127.6 | 134.3 | 164.6 | 198.6 | 180.8 | N/A | 34.5 | 33.8 |
| RSA112 | 128.1 | 169.1 | 147.2 | 133.7 | 195.8 | 161.4 | 159.5 | 186.8 | 172.6 | 74.7 | 30.1 | 32.6 |
| RSA129 | 86.3 | 200.1 | 131.4 | 190.9 | 187.5 | 188.8 | 197.1 | 257.0 | 225.0 | 90.7 | 37.6 | 38.1 |
| RSA120 | 61.3 | 179.6 | 105.0 | 180.8 | 151.7 | 165.6 | 190.9 | 194.2 | 192.5 | 75.2 | 33.5 | 37.8 |
| RSA54 | 102.2 | 160.0 | 127.9 | 96.9 | 130.2 | 112.3 | 135.0 | 145.1 | 139.9 | 83.6 | 24.1 | 26.0 |
| RSA39 | 99.1 | 180.3 | 133.7 | 97.7 | 135.4 | 115.0 | 135.5 | 155.3 | 145.1 | 66.0 | 36.9 | 37.7 |
| RSA66 | 97.9 | 95.7 | 96.8 | 78.4 | 159.0 | 111.7 | 122.6 | 213.2 | 161.7 | 75.1 | 33.0 | 31.2 |
| RSA60 | 95.5 | 136.9 | 114.4 | 103.7 | 127.9 | 115.1 | 139.5 | 174.0 | 155.8 | 87.2 | 26.9 | 26.0 |
| RSA142 | 68.5 | 122.1 | 91.5 | 112.2 | 157.8 | 131.8 | 145.2 | 123.8 | 134.1 | 92.1 | 28.4 | 31.7 |
| **Bottom yielding** | | | | | | | | | | | | |
| RSA76 | 21.1 | 113.6 | 49.0 | 82.1 | 79.1 | 80.6 | 125.1 | 224.3 | 167.5 | 50.0 | 35.7 | 29.3 |
| RSA87 | 19.0 | 81.9 | 39.4 | 55.9 | 87.4 | 69.9 | 107.7 | 78.8 | 92.1 | 73.2 | 23.3 | 17.7 |
| RSA49 | 36.9 | 106.7 | 62.7 | 70.4 | 72.8 | 61.6 | 117.3 | 6.1 | 26.8 | 53.6 | 23.7 | 23.0 |
| RSA23 | 10.9 | 66.3 | 26.9 | 106.7 | 107.3 | 107.0 | 141.5 | 142.2 | 141.8 | 30.1 | 25.1 | 23.3 |
| RSA178 | 34.4 | 94.3 | 57.0 | 42.4 | 74.8 | 56.3 | 98.6 | 41.7 | 64.1 | N/A | 28.2 | 29.1 |

†RF: Rainfed; ‡RF: Rainfed supplemented with irrigation; § GM: Geometric means of yield

Table 2. 3 (Cont'd)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parents** | | | | | | | | | | | | |
| SEA5 | 135.9 | 206.4 | 167.4 | 202.8 | 161.4 | 180.9 | 205.5 | 173.7 | 189.0 | 140.8 | 26.2 | 24.2 |
| CAL96 | 99.3 | 220.7 | 148.1 | 127.7 | 213.2 | 165.0 | 155.5 | 173.4 | 164.2 | 87.0 | 45.5 | 49.2 |
| **Checks** | | | | | | | | | | | | |
| SER14 | 226.5 | 305.1 | 262.9 | 181.9 | 324.7 | 242.6 | 191.6 | 230.4 | 210.1 | N/A | 22.2 | 20.4 |
| SER16 | 112.9 | 251.4 | 168.5 | 174.5 | 306.5 | 231.3 | 186.7 | 347.1 | 254.6 | N/A | 25.9 | 27.4 |
| SER13 | 159.5 | 297.2 | 217.7 | 227.8 | 262.7 | 287.4 | 222.2 | 274.7 | 247.1 | N/A | 22.3 | 22.7 |
| RWR1668 | 147.7 | 297.8 | 204.3 | 157.6 | 142.4 | 149.8 | 175.4 | 311.4 | 233.7 | N/A | 49.4 | 47.0 |
| RWR2245 | 140.1 | 255.7 | 189.3 | 223.3 | 213.8 | 218.5 | 219.2 | 343.0 | 274.2 | N/A | 42.3 | 46.2 |
| ICAQuimbaya | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 79.71 | 46.6 | NA |
| Overall mean | 63.5 | 134.0 | 92.2 | 102.3 | 124.1 | 112.7 | 138.6 | 160.8 | 149.3 | 59.7 | 28.6 | 27.9 |

In Karama 2012, significant differences between water treatments were observed for the number of days to maturity, days to seed fill, number of pods per plant, number of seeds per pod, yield, and yield per day (Table 2. 2). Significant GxE interactions were observed for most of the variables except the number of pods per plant, number of seeds per pod, harvest index, and pod harvest index. Under RF, the population matured one day earlier than in RFS. A difference of one day between RF and RFS was also observed on average for the number of days to seed fill. An average of 10 pods per plant was observed under RFS while the average pod number per plant was eight in RF conditions. Drought caused a percentage yield reduction of 14%. SEA5 yielded significantly higher than CAL96 under RF while their yields were not different under RFS conditions. Yield of lines RSA120 and RSA118 (191 and 178.7g/$m^2$) did not differ significantly from the yield of the SEA5 parent (201.5 g/$m^2$). Partitioning indices were not significantly different under both water conditions with an average of 40% for harvest index in both water treatments while the mean pod harvest index was 65 and 64% respectively in RF and RFS. The average 100-seed weight ranged from 19.3 to 44.3 g under RF and 17.6 to 43.4 g under RFS. In both conditions, the best yielding lines RSA118 and RSA120 were among those with the largest seeds.

In Palmira, all lines matured earlier than in Rwanda (Table 2. 2). Days to flowering varied from 28 to 36 with an average of 32 days; days to maturity varied from 59 to 71 days with an average of 69 days; and the mean number of days for seed fill was 33 ranging from 28 to 39. Average number of pods per plant and seed per pod were nine and three respectively with ranges of four to 15 and one to five which were comparable to the ones obtained in similar conditions in Karama. Partitioning indices, harvest index and pod harvest index varied from 13 to 59% and 38 to 70%, respectively and were comparable to values obtained in Rwanda. CAL 96 did not differ

significantly from SEA5 in partitioning indexes. The average seed yield was 60 g/m$^2$ comparable to regular yields under drought conditions in Palmira

(Rao, personal communication). It is worth noting that the highest yield was that of SEA5 (140 g/m$^2$) almost double that of CAL96 and the yield of the resistant check ICA Quimbaya. Seven lines including RSA129 and RSA142 produced yields comparable to CAL96. For 100-seed weight, the average of the population at Palmira was 29.8 g/100-seed weight ranging from 17.5 to 47 g/100-seed weight.

In general this population was characterized by limited transgressive segregation beyond the upper parental values for yield and yield components (Figure 2. 2; Table 2. 8) especially under RF. Partitioning indices were not generally different between RF and RFS conditions. Limited transgressive segregation beyond the best parent values were observed for partitioning indices especially under RF conditions. Although SEA5 was the top yielder in almost all RF experiments, some lines were observed that yielded better than CAL96 under RF conditions (Table 2. 3).

The average seed size of the RILs was smaller than the seed size of CAL96 but a little bit larger than the seed size of SEA5 (Tables 2. 3 and 2. 8). Some of the high yielding RILs were among lines with the largest seed size of the population but had a significantlylower seed size than CAL96.

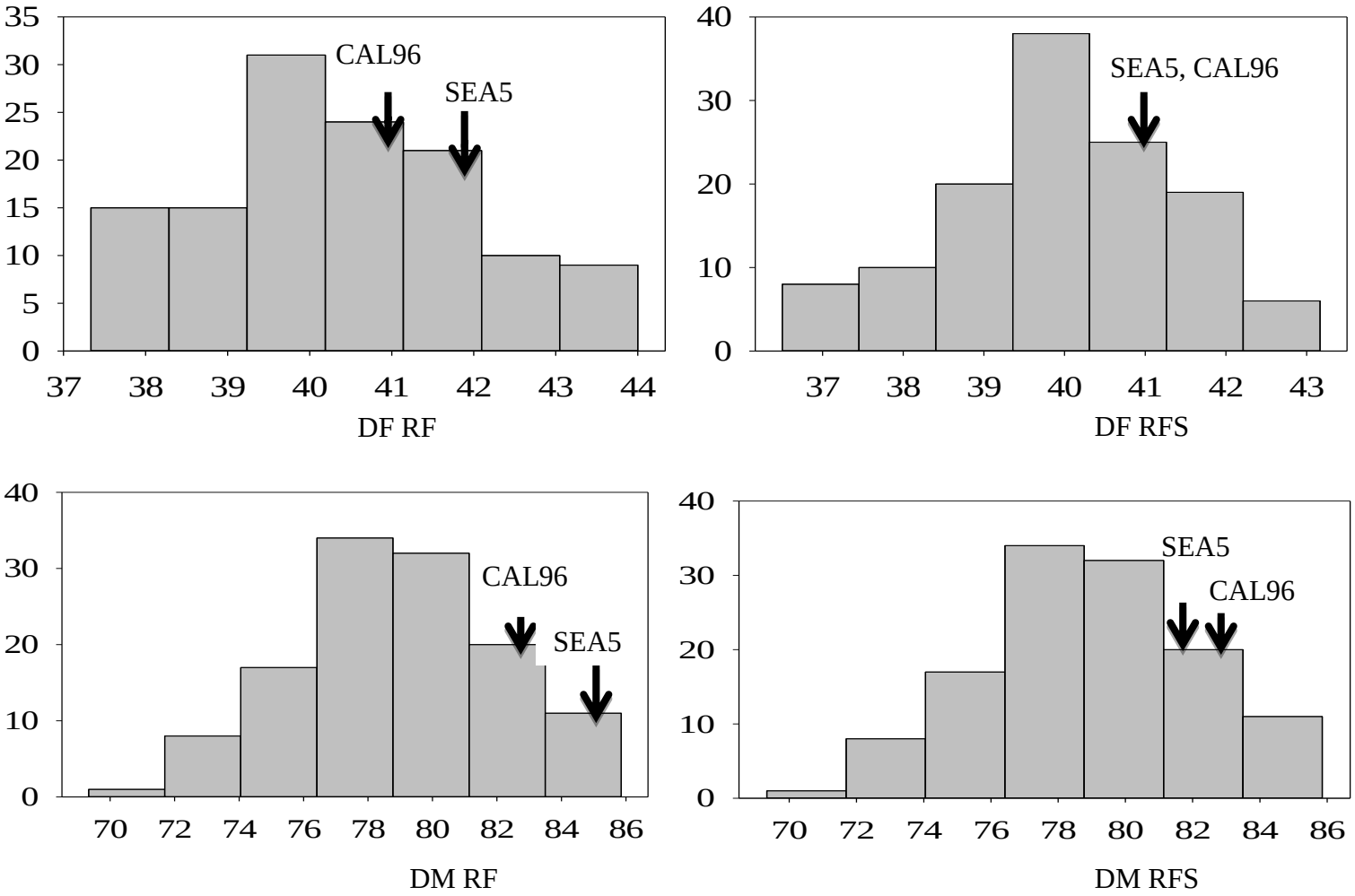



Figure 2. 2. Distribution of days to flowering (DF), days to maturity (DM), harvest index (HI), number of pods per plant (NP), number of seed per pod (NS), pod harvest index (PHI), seed weight (SW), and seed yield (SY) means of SEA5/CAL96 RIL population grown under rainfed (RF) and rainfed supplemented with irrigation (RFS) in Karama and Palmira during 2010-2012.

Figure 2. 2 (Cont'd)

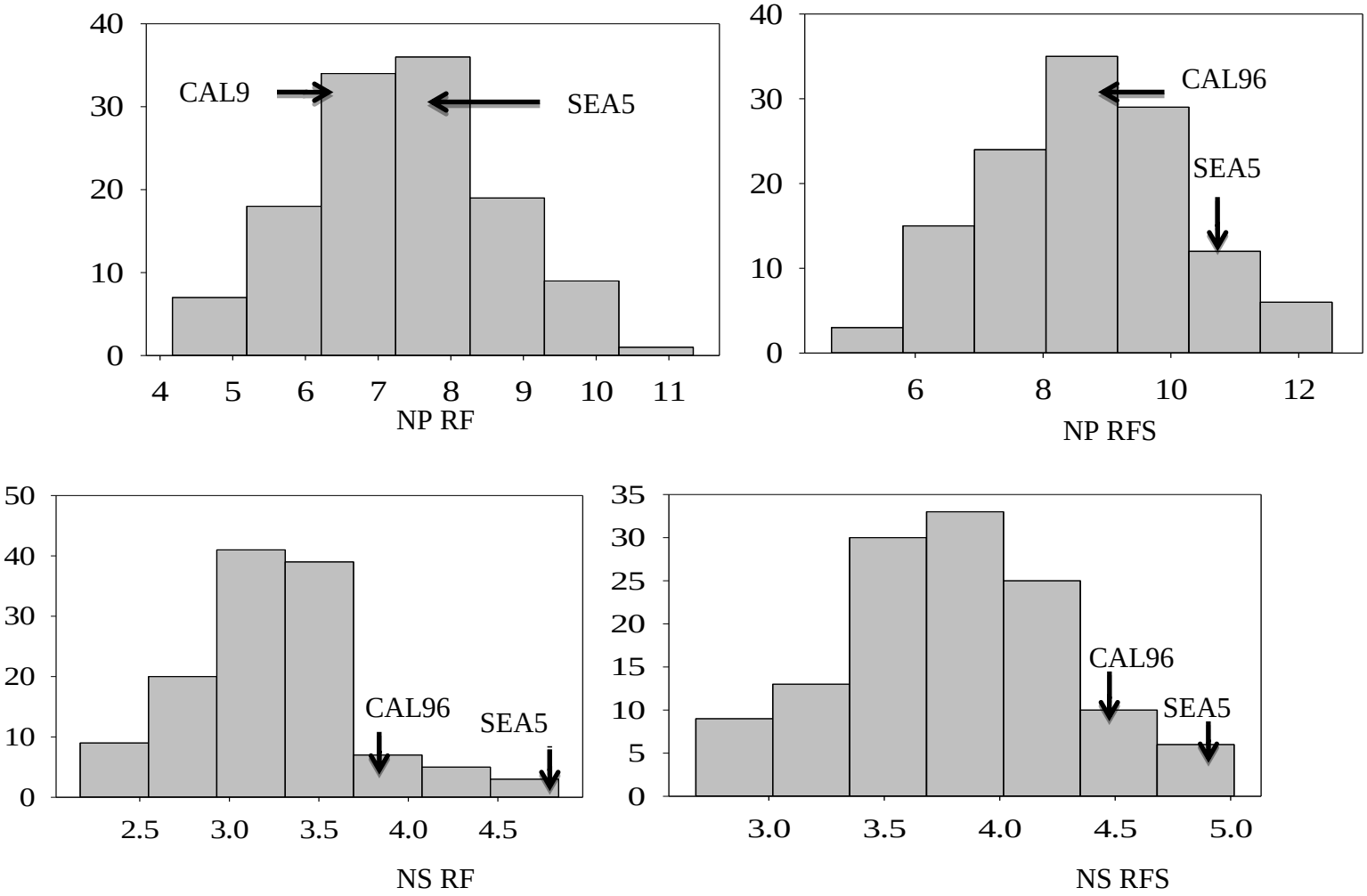
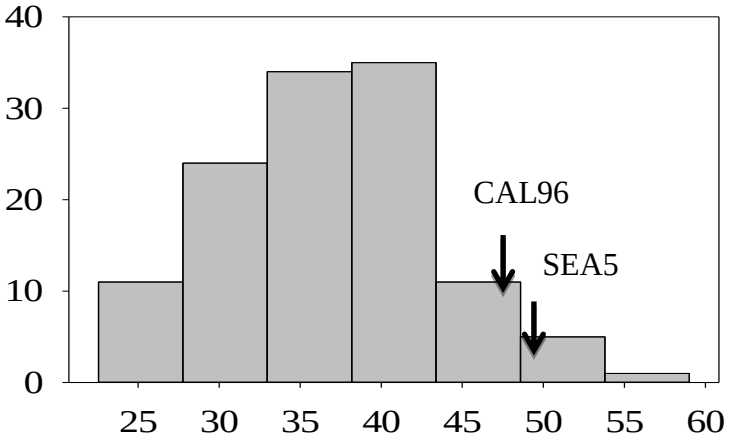
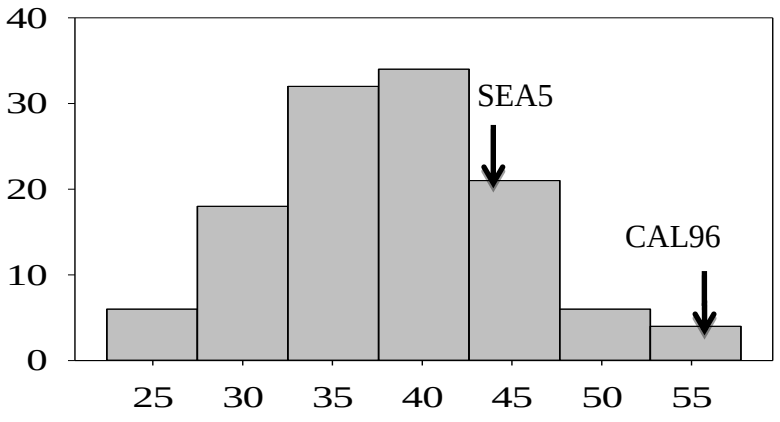


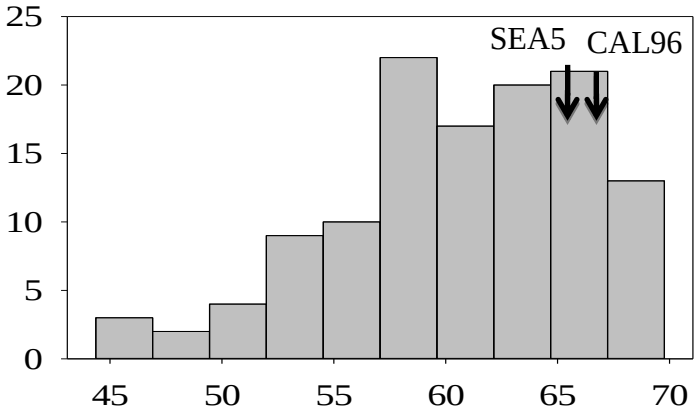
Figure 2. 2 (Cont'd)



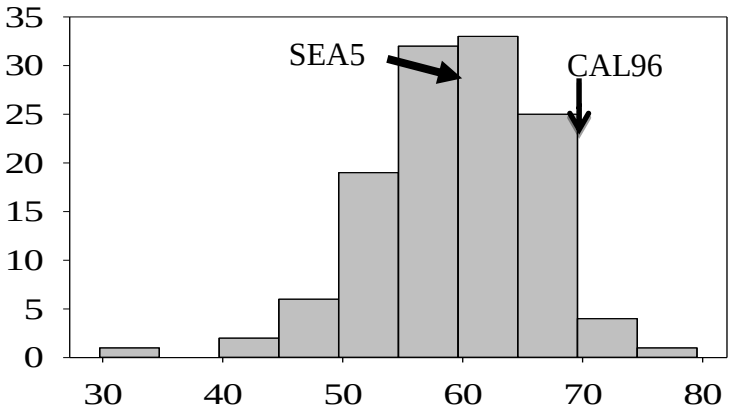

HI RF




HI RFS

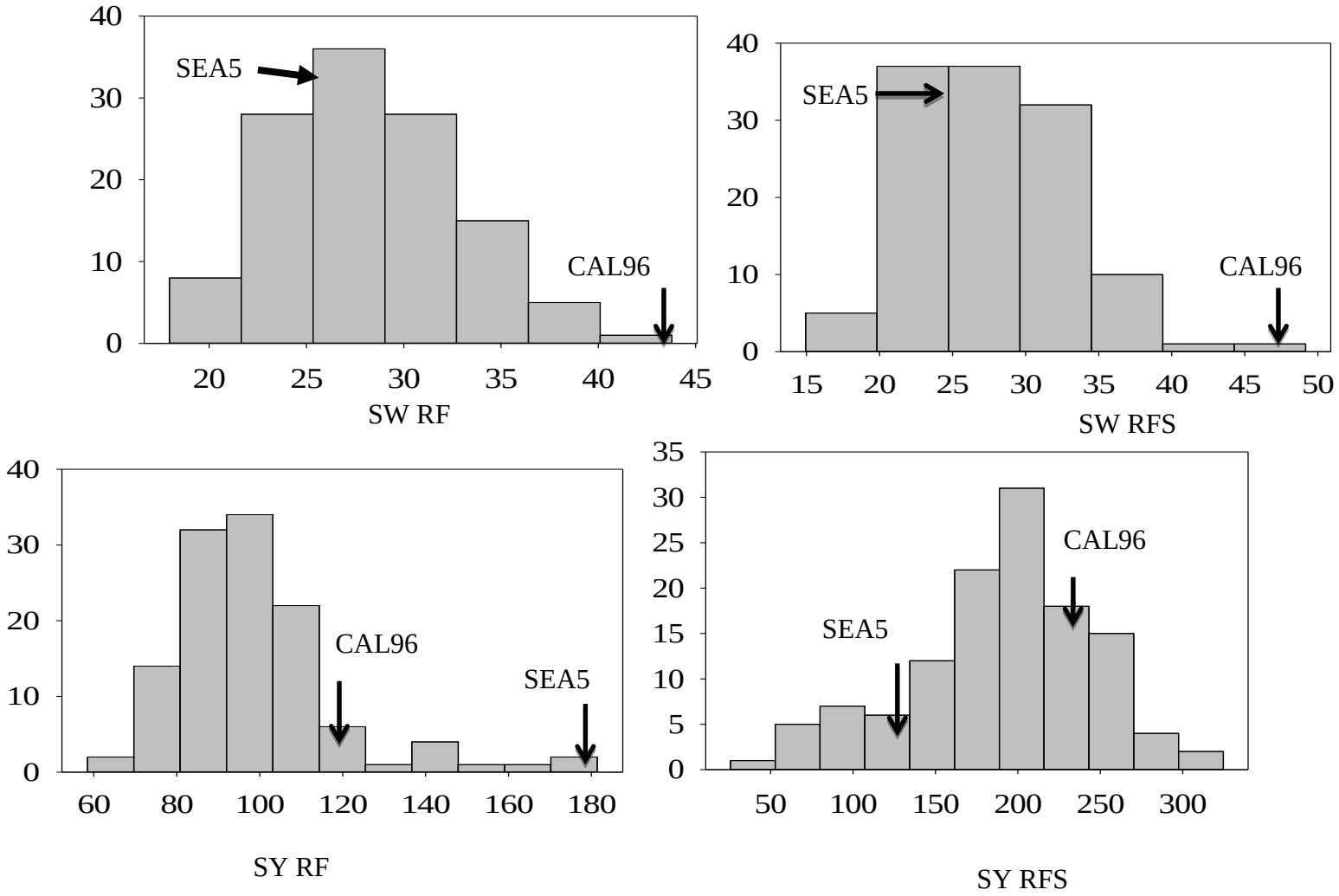



PHI RF




PHI RFS

Figure 2. 2 (Cont'd)



**Correlations among phenotypic traits across water regimes in RIL population**

Phenotypic correlations between related variables were usually strong across both water treatments and sites. For instance correlation coefficients as high as 0.77, 0.98, and 0.83 were obtained between days to maturity and days to seed fill, between yield and yield per day, and between harvest index and pod harvest index respectively (Table 2. 4). Under RF and RFS conditions, negative correlations between phenology variables and partitioning indices were observed (Table 2. 4). These negative correlation coefficients tended to be higher in RFS than in RF. For instance correlation coefficients between days to maturity and pod harvest index were -0.40 and -0.20 in RFS and RF respectively. There was no definite correlation between phenology and pod per plant or seed per pod. Positive correlations were observed between phenology and 100-seed weight regardless of water treatments especially in Karama (Table 2. 4). There was a moderate correlation between days to maturity, days to seed fill, and seed yield in Karama under RF. However, the correlation coefficients between phenology variables and seed yield were negatives in Palmira (Table A. 4). The correlation between the number of pods per plant and the number of seed per pod with partitioning indexes were less obvious especially in Karma. In Palmira, a moderate correlation between the number of seed per pod and partitioning indices were observed (Table A. 4). There was a slight correlation between seed per pod and 100-seed weight ($r=0.28^{***}$) under RFS. Under RF conditions, 100-seed weight moderately correlated to pod harvest index.Yields correlated with partitioning indices, number of pods per plant, number of seeds per pod, and 100-seed weight in both water conditions (Table 2. 4). In general, these correlations were moderate with the correlation coefficient between yield and the number of pods per plant being higher than others in Karama site (Table 2. 5). Strong positive correlations were observed between yields and yields per day regardless of water treatment.

Positive correlations under both RF and RFS were observed between yields across years and sites (Table 2. 6) with a very strong correlation coefficient between yields under RF in Karama in 2011 and 2012.

Table 2. 4. Pearson correlation coefficients among mean variables for days to maturity, day to seed fill, number of pods per plant, number of seed per pod, harvest index, pod harvest index, seed weight, seed yield, and seed yield per day within water treatments for the SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and Palmira, Colombia during 2010-2012.

| Trait | Days to maturity | Number of pods/plant | Number of seed/pod | Harvest index | Pod harvest index | 100-seed weight | Seed yield | Seed yield/day | Days to seed fill |
|---|---|---|---|---|---|---|---|---|---|
| Days to maturity | - | -0.08 | 0.19* | -0.29** | -0.27** | 0.28** | 0.37*** | 0.14 | 0.77*** |
| Number of pods/plant | 0.21* | - | 0.21* | 0.23* | 0.14 | 0.00 | 0.25** | 0.30** | 0.10 |
| Number of seeds/pod | 0.08 | 0.27** | - | 0.22* | 0.05 | -0.04 | 0.41*** | 0.36*** | 0.10 |
| Harvest index | -0.49*** | 0.11 | 0.21* | - | 0.57*** | 0.02 | 0.38*** | 0.45*** | -0.28** |
| Pod harvest index | -0.40*** | -0.02 | 0.15 | 0.83*** | - | 0.25** | 0.32*** | 0.33*** | -0.13 |
| 100-seed weight | 0.36*** | 0.16 | 0.04 | 0.04 | 0.15 | - | 0.32*** | 0.26*** | 0.33*** |
| Seed yield | 0.25** | 0.39*** | 0.29** | 0.26** | 0.22* | 0.38*** | - | 0.94*** | 0.22* |
| Seed yield/day | 0.20* | 0.52*** | 0.35*** | 0.41*** | 0.38*** | 0.48*** | 0.98*** | - | 0.10 |
| Days to seed fill | 0.75*** | 0.19* | 0.07 | -0.33*** | -0.34*** | 0.34*** | 0.14 | 0.11 | - |

Below diagonal: under Rainfed supplemented with irrigation; above diagonal: under Rainfed conditions;
*, **, ***: Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2. 5. Pearson correlation coefficients among mean trait for days to maturity, days to seed fill, number of pods per plant, number of seed per pod, harvest index, pod harvest index, seed weight, and seed yield, and seed yield per day within water treatments for the SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda during 2010-2012.

| Trait | Days to maturity | Number of pods/plant | Number of seed/pod | Harvest index | Pod harvest index | 100-seed weight | Seed yield | Seed yield/day | Days to seed fill |
|---|---|---|---|---|---|---|---|---|---|
| Days to maturity | - | 0.14 | 0.27** | -0.23* | -0.12 | 0.33*** | 0.30** | 0.12 | 0.80*** |
| Number of pods/plant | 0.14 | - | 0.43*** | 0.14 | 0.13 | 0.09 | 0.47*** | 0.47*** | 0.10 |
| Number of seeds/pod | 0.11 | 0.28** | - | 0.05 | -0.07 | -0.14 | 0.24** | 0.21* | 0.13 |
| Harvest index | -0.46*** | 0.16 | 0.20* | - | 0.52*** | -0.01 | 0.22* | 0.27** | -0.24* |
| Pod harvest index | -0.33*** | 0.03 | 0.12 | 0.83*** | - | 0.30** | 0.28** | 0.33*** | -0.02 |
| 100-seed weight | 0.35*** | 0.14 | 0.03 | 0.03 | 0.13 | - | 0.43*** | 0.38*** | 0.39*** |
| Seed yield | 0.29** | 0.31*** | 0.28** | 0.23* | 0.20* | 0.38*** | - | 0.98*** | 0.25** |
| Seed yield/day | 0.23* | 0.46*** | 0.36*** | 0.43*** | 0.37*** | 0.47*** | 0.98*** | - | 0.11 |
| Days to seed fill | 0.78*** | 0.08 | 0.06 | -0.32*** | -0.27*** | 0.36*** | 0.14 | 0.11 | - |

Below diagonal: under Rainfed supplemented with irrigation; above diagonal: under Rainfed conditions;
*, **, ***: Significant at 0.05, 0.01, and 0.001 probability levels, respectively

Table 2. 6. Correlation coefficients for yield of SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and Palmira, Colombia during 2010-2012.

| | Karama | | | | | |
|---|---|---|---|---|---|---|
| Site and water treatment | Rainfed 2010 | Rainfed supplemented 2010 | Rainfed 2011 | Rainfed supplemented 2011 | Rainfed 2012 | Rainfed supplemented 2012 |
| Karama rainfed supplemented 2010 | 0.42*** | | | | | |
| Karama rainfed 2011 | 0.41*** | 0.51*** | | | | |
| Karama rainfed supplemented 2011 | 0.38*** | 0.42*** | 0.33*** | | | |
| Karama rainfed 2012 | 0.41*** | 0.51*** | 0.99*** | 0.33*** | | |
| Karama rainfed supplemented 2012 | 0.20* | 0.30*** | 0.25** | 0.37*** | 0.25** | |
| Palmira rainfed 2011 | 0.23* | 0.37*** | 0.18 | 0.33** | 0.18 | 0.18 |

*, **, ***: Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2. 7. Stepwise regression analysis of mean yield of SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and Palmira, Colombia during 2010-2012.

| Site and water treatments | Variables in the model | Partial $R^2$ | Model $R^2$ | *p*-value |
|---|---|---|---|---|
| Palmira rainfed | Harvest index | 0.17 | 0.17 | <0.0001 |
| | Number of pods/plant | 0.07 | 0.25 | <0.0001 |
| | Number of seeds/pod | 0.07 | 0.32 | <0.0001 |
| | Pod harvest index | 0.19 | 0.5 | <0.0001 |
| Karama rainfed | Number of pods/plant | 0.22 | 0.22 | <0.0001 |
| | 100-seed weight | 0.15 | 0.37 | <0.0001 |
| | Harvest index | 0.03 | 0.4 | 0.03 |
| | Days to maturity | 0.03 | 0.43 | 0.01 |
| Karama rainfed supplemented with irrigation | 100-seed weight | 0.14 | 0.14 | <0.0001 |
| | Number of pods/plant | 0.08 | 0.21 | 0.001 |
| | Number of seeds/pod | 0.04 | 0.25 | 0.02 |

Table 2. 8. Average trait values for days to flower, days to maturity, days to seed fill, number of pods per plant, number of seed per pod, harvest index, pod harvest index, 100-seed weight, seed yield, and seed yield per day for parents, the average and ranges of these traits, and broad sense heritability of these traits for SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and Palmira, Colombia during 2010-2012.

| | | | Parents | | | RILs | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Water treatment† | Location(year) | SEA5 | CAL96 | $P_{par}$ | Mean | Min | Max | $P_{RIL}$ | $H^2_b$ †† |
| Days to flowering | RF | Karama (2010) | 48.00 | 43.00 | * | 41 | 38.75 | 45.00 | * | 0.81 |
| | RF | Karama (2011) | 40.00 | 38.00 | NS | 40 | 35.50 | 47.00 | *** | 0.71 |
| | RF | Karama (2012) | 40.00 | 40.00 | NS | 38 | 30.00 | 43.00 | *** | 0.91 |
| | RF | Palmira (2011) | 32.00 | 34.00 | NS | 32 | 28.60 | 36.35 | * | 0.91 |
| Days to maturity | RF | Karama (2010) | 80.00 | 75.00 | * | 80 | 71.00 | 90.00 | *** | 0.80 |
| | RFS | Karama (2010) | 77.00 | 82.00 | * | 83 | 74.00 | 90.00 | *** | 0.58 |
| | RF | Karama (2011) | 80.00 | 79.00 | NS | 86 | 74.00 | 91.50 | *** | 0.57 |
| | RFS | Karama (2011) | 80.00 | 79.00 | NS | 86 | 74.00 | 93.50 | ** | 0.75 |
| | RF | Karama (2012) | 78.00 | 80.00 | *** | 78 | 70.00 | 87.00 | *** | 0.90 |
| | RFS | Karama (2012) | 75.00 | 80.00 | *** | 79 | 70.00 | 87.00 | *** | 0.90 |
| | RF | Palmira (2011) | 61.00 | 66.00 | * | 66 | 58.69 | 71.04 | NS | 0.90 |
| Days to seed fill | RF | Karama (2010) | 29.02 | 32.00 | NS | 34 | 22.00 | 46.00 | NS | 0.92 |
| | RFS | Karama (2010) | 29.00 | 40.00 | *** | 36 | 26.00 | 44.00 | * | 0.86 |
| | RF | Karama (2011) | 47.95 | 46.85 | NS | 46 | 33.85 | 53.00 | *** | 0.41 |
| | RFS | Karama (2011) | 47.61 | 46.80 | NS | 47 | 35.65 | 54.61 | NS | 0.60 |
| | RF | Karama (2012) | 34.74 | 33.91 | NS | 36 | 28.33 | 46.44 | *** | 0.90 |
| | RFS | Karama (2012) | 31.97 | 34.18 | NS | 37 | 29.35 | 47.53 | ** | 0.90 |
| | RF | Palmira (2011) | 29.32 | 31.57 | NS | 33 | 28.37 | 39.62 | NS | 0.84 |

† RF: Rainfed; RFS: Rainfed supplemented with irrigation; *, **, ***, significant differences between parents (par.) or between recombinant inbred lines (RIL) at 0.05, 0.01, 0.001 probability levels respectively; NS: no significant at 0.5 probability level, †† broad sense heritability for each traits

Table 2. 8 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of pods per plant | RF | Karama (2010) | 9.00 | 3.50 | * | 5 | 1.93 | 8.51 | * | 0.36 |
| | RFS | Karama (2010) | 14.00 | 7.50 | * | 7 | 2.00 | 13.00 | * | 0.42 |
| | RF | Karama (2011) | 13.50 | 8.50 | * | 9 | 3.26 | 15.26 | NS | 0.55 |
| | RFS | Karama (2011) | 13.50 | 8.00 | NS | 9 | 5.00 | 14.51 | ** | 0.24 |
| | RF | Karama (2012) | 11.50 | 11.00 | NS | 8 | 4.50 | 14.50 | * | 0.31 |
| | RFS | Karama (2012) | 10.00 | 10.50 | NS | 10 | 4.50 | 14.50 | * | 0.37 |
| | RF | Palmira (2011) | 13.50 | 8.00 | * | 9 | 4.29 | 15.00 | NS | 0.54 |
| Number of seeds per pod | RF | Karama (2010) | 4.00 | 3.50 | NS | 3 | 1.23 | 5.50 | * | 0.18 |
| | RFS | Karama (2010) | 5.00 | 4.00 | NS | 4 | 2.00 | 6.00 | *** | 0.49 |
| | RF | Karama (2011) | 5.50 | 3.50 | * | 3 | 1.37 | 5.98 | *** | 0.14 |
| | RFS | Karama (2011) | 5.50 | 3.50 | * | 4 | 1.98 | 5.87 | * | - |
| | RF | Karama (2012) | 4.50 | 3.50 | NS | 4 | 2.50 | 5.50 | *** | 0.35 |
| | RFS | Karama (2012) | 4.50 | 3.00 | * | 4 | 2.50 | 5.50 | *** | 0.17 |
| | RF | Palmira (2011) | 3.90 | 2.80 | NS | 3 | 0.93 | 4.89 | NS | 0.41 |
| Harvest Index | RF | Karama (2010) | 0.42 | 0.42 | NS | 0.33 | 0.11 | 0.59 | NS | 0.28 |
| | RFS | Karama (2010) | 0.40 | 0.55 | NS | 0.37 | 0.10 | 0.56 | ** | 0.68 |
| | RF | Karama (2011) | 0.49 | 0.58 | NS | 0.36 | 0.07 | 0.59 | NS | 0.16 |
| | RFS | Karama (2011) | 0.47 | 0.62 | NS | 0.37 | 0.07 | 0.63 | NS | 0.50 |
| | RF | Karama (2012) | 0.55 | 0.43 | NS | 0.41 | 0.26 | 0.66 | *** | 0.37 |
| | RFS | Karama (2012) | 0.59 | 0.56 | NS | 0.40 | 0.21 | 0.64 | NS | 0.71 |
| | RF | Palmira (2011) | 0.46 | 0.52 | NS | 0.37 | 0.13 | 0.59 | NS | 0.66 |
| Pod Harvest Index | RF | Karama (2010) | 0.67 | 0.64 | NS | 0.62 | 0.32 | 0.81 | *** | 0.27 |
| | RFS | Karama (2010) | 0.54 | 0.67 | NS | 0.60 | 0.31 | 0.77 | *** | 0.37 |
| | RF | Karama (2011) | 0.67 | 0.71 | NS | 0.55 | 0.18 | 0.75 | ** | 0.38 |
| | RFS | Karama (2011) | 0.59 | 0.71 | NS | 0.54 | 0.17 | 0.76 | * | 0.48 |
| | RF | Karama (2012) | 0.69 | 0.73 | NS | 0.65 | 0.48 | 0.75 | *** | 0.62 |
| | RFS | Karama (2012) | 0.65 | 0.73 | NS | 0.63 | 0.38 | 0.91 | NS | 0.47 |
| | RF | Palmira (2011) | 0.69 | 0.72 | NS | 0.66 | 0.38 | 0.77 | *** | 0.47 |

Table 2. 8 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 100-seed weight | RF | Karama (2010) | 24.58 | 46.45 | *** | 28.66 | 15.80 | 48.45 | *** | 0.69 |
| | RFS | Karama (2010) | 26.95 | 56.65 | *** | 28.13 | 16.37 | 46.33 | * | 0.91 |
| | RF | Karama (2011) | 24.59 | 43.21 | *** | 24.53 | 11.01 | 37.54 | NS | 0.39 |
| | RFS | Karama (2011) | 20.75 | 47.39 | *** | 24.89 | 10.41 | 41.80 | ** | 0.57 |
| | RF | Karama (2012) | 28.00 | 41.70 | *** | 31.63 | 19.35 | 44.30 | * | 0.72 |
| | RFS | Karama (2012) | 25.00 | 43.45 | *** | 30.38 | 17.60 | 43.45 | * | 0.82 |
| | RF | Palmira (2011) | 27.72 | 50.57 | *** | 29.56 | 17.51 | 47.05 | NS | 0.74 |
| Yield | RF | Karama (2010) | 135.87 | 99.33 | ** | 59.51 | 10.93 | 199.60 | ** | 0.60 |
| | RFS | Karama (2010) | 206.36 | 220.73 | NS | 126.83 | 60.43 | 247.95 | * | 0.55 |
| | RF | Karama (2011) | 202.75 | 127.74 | *** | 98.16 | 42.38 | 190.09 | *** | 0.59 |
| | RFS | Karama (2011) | 161.40 | 213.15 | ** | 117.79 | 71.10 | 195.80 | *** | 0.49 |
| | RF | Karama (2012) | 205.53 | 155.52 | *** | 135.80 | 98.62 | 197.09 | *** | 0.58 |
| | RFS | Karama (2012) | 173.73 | 173.42 | NS | 156.30 | 6.14 | 278.45 | ** | 0.58 |
| | RF | Palmira (2011) | 140.88 | 87.00 | *** | 58.34 | 12.32 | 96.27 | NS | 0.62 |
| Yield per day | RF | Karama (2010) | 1.70 | 0.10 | *** | 0.74 | 0.14 | 2.35 | *** | 0.57 |
| | RFS | Karama (2010) | 2.68 | 2.69 | NS | 1.53 | 0.71 | 2.76 | NS | 0.53 |
| | RF | Karama (2011) | 2.53 | 1.62 | * | 1.14 | 0.47 | 2.18 | *** | 0.59 |
| | RFS | Karama (2011) | 2.02 | 2.70 | NS | 1.37 | 0.79 | 2.24 | *** | 0.46 |
| | RF | Karama (2012) | 2.63 | 1.94 | ** | 1.74 | 1.31 | 2.53 | *** | 0.59 |
| | RFS | Karama (2012) | 2.32 | 2.17 | NS | 1.97 | 0.00 | 3.33 | *** | 0.59 |
| | RF | Palmira (2011) | 2.34 | 1.32 | ** | 0.90 | 0.19 | 1.56 | NS | 0.63 |

**Genetic mapping and QTL analyzes.**

The SEA5 and CAL 96 parents were screened with two types of markers. In total 460 SSR markers were screened between parents of which only 148 were polymorphic, representing a polymorphism level of 32%. Of the polymorphic markers only 92 SSR were mapped in the population. Another set of 37 InDel markers selected to fill the gaps on chromosomes Pv01, Pv04, and Pv07 were screened on the parents and 12 InDel markers, representing 32% level of polymorphism were mapped in the population. As a result, 104 markers were used to generate a genetic map of SEA5/CAL96 population covering all 11 bean linkage groups. The genetic map constructed covered a total of 1031 cM (Table 2. 9). The number of markers per linkage group varied from four on Pv10 to 21 on Pv02. Markers were distributed across all chromosomes with an average distance between markers of 9.9 cM. Of the 104 mapped markers, 19 showed a deviation from expected ratios (1:1) based on Chi square ($p$=0.01). However, this distortion did not affect QTL detection since they were caused by residual heterozygosity in the population and association between marker class and phenotype remained the same.

Table 2. 9. Marker distribution and distance on the genetic linkage map of SEA5/CAL96 RIL population.

| Linkage group | Number of markers | Length (cM ) | Average distance (cM ) |
|---|---|---|---|
| Pv01 | 8 | 80.89 | 10.1 |
| Pv02 | 21 | 172.25 | 8.2 |
| Pv03 | 13 | 137.39 | 10.5 |
| Pv04 | 9 | 91.94 | 10.2 |
| Pv05 | 12 | 111.07 | 9.2 |
| Pv06 | 8 | 65.02 | 8.1 |
| Pv07 | 10 | 127.64 | 12.7 |
| Pv08 | 5 | 77.72 | 15.5 |
| Pv09 | 6 | 71.33 | 11.9 |
| Pv10 | 4 | 29.26 | 7.3 |
| Pv11 | 8 | 61.05 | 7.6 |
| Entire genome | 104 | 1031.09 | 9.9 |

Using ICIM of the ICIMAPPING, a total of 41 QTLs were identified across all 11 linkage groups (Tables 2. 10, 2. 11, and 2. 12) when all 10 traits were analyzed at single environments. When traits were averaged over the RF and RFS treatments, only 15 QTL were identified on six linkage groups (Table 2. 10, 2. 11, 2. 12). Among QTL identified in single environments analysis, 11 were found under RFS while 30 other QTL were identified in RF treatment (Figure 2. 3) suggesting that these QTL could be drought inducible. Percentage of phenotypic variance explained by single QTL varied from 8.4 to 53.8 %.

**Phenology**

Twelve QTL were identified in a single environment analysis for phenological traits (Table 2. 10). Of these, two were associated with days to flowering, two associated with both days to flowering and days to maturity, seven with days to maturity, and one associated with both days to maturity and days to seed fill. QTL for phenology mapped on all linkage groups except Pv11. Independent QTL associated with the number of days to maturity were mapped to Pv01 Pv02, Pv04, Pv09, and Pv10. On Pv03, a QTL located at 72 cM and flanked by markers BMd1 and PVat0008 was associated with both days to flowering and days to maturity in Karama 2010 but under different water treatments while another two QTL at 73 cM and at 101 cM were associated with days to flower in Palmira. The QTL for days to flowering at 73 cM found in Palmira might be the same as the QTL for days to flowering found in Karama 2010 at 72 cM since they are sharing a common marker PVat0008 and only distant of 1 cM. This region on Pv03 also showed to be associated with days to maturity in mean environment QTL analysis and explained 18.8% of the total phenotypic variation. Another QTL on Pv06 was associated with both days to flower and maturity. The same QTL on Pv05 was associated with days to maturity in RF and RFS in Karama 2010 and Karama 2012. A QTL on Pv08 flanked by markers PVBR45 and BM 153 was associated with both days to maturity and days to seed fill.

**Partitioning indices**

Twenty QTL for partitioning indices were identified on every chromosome (Table 2. 11). Of these QTL, only 5 were associated with pod harvest index. One QTL associated with harvest index located at 59 cM on Pv03 flanked by markers BM159 and PVBR21 was found in both Karama 2010 under RFS and in Karama 2012 under RF and accounted for 16 and 25% of phenotypic variation respectively. On Pv07, two QTL were associated with both harvest index and pod harvest index under RF conditions in Karama 2010. Other independent QTL associated with partitioning indices were located on Pv01, Pv03, Pv07, Pv08, and Pv11.

**Number of pods per plant and seeds per pod**

Only two QTL on Pv02 and Pv03 were associated with number of pods per plant in different environments in Karama 2011 RFS and Karama 2012 RF (Table 2. 12). The QTL under RF conditions was contributed by the SEA5 parent while the QTL under RFS was derived from CAL96. Four QTL associated with number of seeds per pod were identified in Karama 2010 RFS and 2012 RF. Those identified under RF were mapped on Pv02 while the ones identified under RFS mapped on Pv07 and Pv11. In combined environments, only the QTL associated with number of pods per plant on PV03 was found.

**Seed weight**

QTL for 100-seed weight mapped to Pv02, Pv05, Pv09, and Pv11 (Table 2. 12). QTLs for 100-seed weight were found in more than one environment except the QTL on Pv05. The QTL on Pv02 which was found both in Karama 2011, 2012 and in Palmira was also found in combined environments where it contributed 13% of the phenotypic variation. Seed weight QTL on Pv02 flanked by markers IAC51 and PVBR94 was identified under RF conditions and came from SEA5. The QTL on Pv09 was identified under RF conditions in Karama2011 and in Palmira. This QTL accounted for 14% of the phenotypic variation. On Pv11, three seed weight QTL were mapped. These seed weight QTL on Pv11, are flanked by a suite of

markers on intervals of 2 and 4 cM from each other which suggest that one gene or a suite of linked genes with small effects in this region control seed weight in common bean.

**Yield and yield per day**

QTL for yield and yield per day are found in Table 2. 12. The same QTL mapped on Pv11 associated with yield and yield per day was identified under RF conditions in Karama during the years 2011 and 2012. This QTL was flanked by markers PVM98 and InDel14, and explained respectively 37% and 50% of the phenotypic variation for yield and yield per day. This QTL came from CAL96 and increased yield by 14 and 9 $g/m^2$ respectively in 2011 and 2012. Two other yield and yield per day QTL were identified on Pv07 under RFS conditions in Karama 2011. Additional QTL for yield per day were located on Pv04 and Pv09 in Karama 2011 under RF. Yield QTL were found in mild drought conditions or under RFS conditions but no yield QTL was found for severe drought conditions of Palmira and Karama 2010.

**QTL co-localization**

Harvest index and pod harvest index in Karama 2012 and 2011 respectively colocalized on Pv01. A cluster of QTL on Pv02 associated with 100-seed weight in both Karama 2011, 2012, and Palmira 2011, the number of pods per plant in Karama 2012 under RF conditions, the number of seeds per pod, and harvest index. Two QTL clusters were identified on Pv03, a distal cluster consisting of 22 cM and associated with the pod harvest index and the number of pods per plant, and a middle cluster consisting of QTL associated with harvest indices and phenology variables days to flowering and days to maturity.

A QTL for yield per day colocalized with a pod harvest index QTL on Pv04 while a cluster of QTL for days to maturity, seed weight, and harvest index was identified on Pv05. The same QTL associated with yield, yield per day under RFS conditions in Karama 2011, harvest index, pod harvest index under RF mapped at Pv07. This QTL is a part of a 9 cM

cluster flanked by markers PVBR67 and PVBR35. The same QTL on Pv08 flanked by markers PVBR 45 and BM 153 associated with both days to maturity and days to seed fill in Karama 2011 colocalized with a harvest index QTL. A QTL on Pv09 flanked by markers PVat0007 and BM114 associated with seed weight both in Karama and Palmira colocalized with harvest index and yield per day QTL. QTL associated with yield, yield per day, number of seeds per pod, and 100-seed weight Karama site colocalized between 30 and 36 cM on Pv11. The same QTL flanked by markers InDel29 and PVM98 was associated harvest index and 100-seed weight. Another QTL at 35cM between markers PVM98 and InDel14 was associated with yields and yields per day in two different years under RF conditions while another QTL conditioning seed weight resided within less than 1cM.

Table 2. 10. Location and description of putative QTL for phenology traits in the SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama and Palmira during 2010-2012.

| Trait | Site | Year | Water treatment† | Linkage group | Peaks position (cM) | Flanking markers | LOD threshold | LOD score†† | $R^2$ (%)§ | Add¶ |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to flower | Karama | 2010 | RF | 3 | 72 | BMd1- Pvat0008 | 2.9 | 5.5 | 16.86 | -0.61 |
| | | | | 6 | 3 | PVM21- INDEL51 | | 6.4 | 19.35 | 0.63 |
| | | | | 7 | 103 | BM170- PVBR167 | | 3.1 | 8.7 | 0.42 |
| | Palmira | 2011 | | 3 | 73 | PVat0008- PVM148 | | 3 | 12.4 | -0.71 |
| | | | | 3 | 101 | PVM148- PVBR169 | | 3.2 | 20.74 | 0.87 |
| | Combined | | RFS | 2 | 52 | IAC90- IAC51 | | 3.9 | 19.69 | -1.27 |
| Days to maturity | Karama | 2010 | RF | 2 | 0 | BM165- BMd45 | 2.9 | 3.4 | 14.45 | -1.45 |
| | | | | 4 | 72 | IAC66- BM161 | | 3.4 | 12.14 | -1.5 |
| | | | | 4 | 60 | INDEL109- IAC67 | | 6.2 | 16.71 | 1.78 |
| | | | | 5 | 49 | PVBR93- PVat0006b | | 4 | 11.61 | -1.5 |
| | | | | 5 | 86 | PVBR235- PVBR131 | | 4.1 | 12.86 | 1.65 |
| | | | | 6 | 4 | INDEL51- INDEL05 | | 6 | 17.99 | 1.83 |
| | | | | 9 | 25 | PVat0007- BM114 | | 4 | 9.8 | 1.34 |
| | | | | 10 | 29 | BMd42- PVM2 | | 4 | 8.44 | -1.24 |
| | | 2011 | | 1 | 0 | BM165- BMd45 | 3.0 | 3.4 | 14.45 | -1.45 |
| | | | | 8 | 48 | PVBR45- BM153 | | 4.2 | 16.17 | 1.53 |
| | | 2010 | RFS | 3 | 72 | BMd1- Pvat0008 | 2.9 | 5.5 | 16.86 | -0.61 |
| | | 2012 | | 5 | 43 | BM175- PVBR93 | | 3.1 | 12.56 | -1.4 |
| | Combined | | RFS | 3 | 75 | PVat0008- PVM148 | | 5 | 18.84 | -1.44 |

† RF: Rainfed; RFS: Rainfed supplemented by irrigation; ††LOD: logarithm of the odds at the peak; § $R^2$: Proportion of the phenotypic variance explained by the QTL at peak LOD; ¶ Add: Effect of substituting a single allele from one parent to another. Positive values indicate allele from SEA5 and negative from CAL96.

Table 2. 10 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to seed fill | Karama | 2011 | RF | 8 | 48 | PVBR45- BM153 | 3.0 | 3.5 | 14.38 | 1.32 |
| | Combined | | RF | 5 | 43 | BM175- PVBR93 | 3.0 | 4.6 | 18.01 | -1.12 |

Table 2. 11. Location and description of identified putative QTL for partitioning indices traits in the SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and Palmira, Colombia during 2010-2012.

| Trait | Site | Year | Water treatment† | Linkage group | Peaks position (cM) | Flanking markers | LOD threshold | LOD score†† | $R^2$ (%) § | Add¶ |
|---|---|---|---|---|---|---|---|---|---|---|
| Harvest Index | Karama | 2010 | RF | 2 | 152 | IAC71- PVM11 | 2.9 | 4.7 | 13.04 | -0.03 |
| | | | | 3 | 73 | PVat0008- PVM148 | | 5.8 | 15.16 | 0.04 |
| | | | | 5 | 47 | BM175- PVBR93 | | 7.9 | 21.53 | 0.04 |
| | | | | 7 | 67 | PVBR269- PVBR35 | | 10 | 39.62 | 0.06 |
| | | | | 7 | 60 | PVBR67- InDel01 | | 9.9 | 28.72 | -0.05 |
| | | | | 10 | 11 | IAC6- PVBR185 | | 6.6 | 28.99 | -0.05 |
| | | | | 11 | 2 | BMd22- PVBR113 | | 4.2 | 11.64 | 0.03 |
| | | | | 11 | 24 | InDel11- InDel29 | | 7.7 | 25.49 | 0.05 |
| | | | | 11 | 32 | InDel29- PVM98 | | 6.1 | 17.14 | -0.04 |
| | | 2010 | RFS | 3 | 59 | BM159- PVBR21 | | 3.2 | 10.54 | -0.03 |
| | | | | 6 | 3 | PVM21- InDel51 | | 4.5 | 16.26 | -0.04 |
| | | | | 8 | 55 | BM153- PVaaat0001 | | 6.2 | 30.75 | 0.05 |
| | | 2012 | RF | 1 | 37 | PVBR250- IAC76 | 2.5 | 8.4 | 20.85 | 0.05 |
| | | | | 3 | 59 | BM159- PVBR21 | | 5.7 | 25.21 | 0.05 |
| | | | | 3 | 25 | PVBR255- BM189 | | 3.3 | 13.85 | -0.04 |
| | | | | 8 | 0 | BM181- PVBR53 | | 6.1 | 27.69 | -0.05 |
| | Palmira | 2011 | | 9 | 43 | PVBR199- BM141 | 2.8 | 3.3 | 13.82 | -0.03 |

†RF: Rainfed; RFS: Rainfed supplemented by irrigation; ††LOD: logarithm of the odds at the peak; § $R^2$: Proportion of the phenotypic variance explained by the QTL at peak LOD; ¶ Add: Effect of substituting a single allele from one parent to another. Positive values indicate allele from SEA5 and negative from CAL96.

Table 2. 11 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Combined | | RFS | 2 | 125 | IAC4- PVM127 | | 4.5 | 26.6 | -0.04 |
| | | | | 3 | 72 | BMd1- PVat0008 | | 3.9 | 15.99 | 0.03 |
| | | | | 6 | 12 | InDel05- PVBR05 | | 6.5 | 27.94 | 0.04 |
| Pod harvest index | Karama | 2010 | | 7 | 65 | PVBR269- PVBR35 | 2.8 | 7.9 | 45 | 0.06 |
| | | | | 7 | 60 | PVBR67- InDel01 | | 5.0 | 23.71 | -0.04 |
| | | 2011 | | 1 | 87 | PVM126- IAC69 | 2.9 | 3.1 | 16.6 | -0.04 |
| | | | | 3 | 3 | PVag0001- PVBR255 | | 4.1 | 27 | 0.06 |
| | | 2012 | | 4 | 23 | InDel30- InDel109 | 2.8 | 5.7 | 38.54 | 0.04 |
| | Combined | | RFS | 6 | 14 | PVBR5- PVBR20 | 3.0 | 5.1 | 22.96 | 0.03 |

Table 2. 12. Location and description of putative QTL for seed yield and yield components traits in the SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and Palmira, Colombia during 2010-2012.

| Trait | Site | Year | Water treatment† | Linkage group | Peaks position (cM) | Flanking markers | LOD threshold | LOD score†† | $R^2$(%) § | Add¶ |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of pods/ plant | Karama | 2011 | RFS | 3 | 19 | PVag0001- PVBR255 | 2.9 | 4.9 | 35.07 | -1.39 |
| | | 2012 | RF | 2 | 59 | IAC51- PVBR94 | 2.8 | 4.1 | 15.15 | 0.8 |
| | Combined | | RFS | 3 | 21 | PVag0001- PVBR255 | 3.0 | 5.8 | 27.35 | -0.85 |
| | | | | 8 | 47 | PVBR53- PVBR45 | | 11.7 | 59.64 | 1.18 |
| Number of seed/ pod | | 2010 | RFS | 7 | 58 | PVBR67- PVBR269 | 2.9 | 6.9 | 24.37 | -0.74 |
| | | | | 11 | 7 | PVBR113- InDel11 | | 8.4 | 44.28 | 0.58 |
| | | 2012 | RF | 2 | 28 | BM172- BM142 | 3.0 | 3.6 | 13.33 | 0.24 |
| | | | | 2 | 79 | PVgccacc0001-BM152 | | 3.9 | 14.9 | -0.24 |
| 100-seed weight | Karama | 2011 | RF | 2 | 60 | IAC51- PVBR94 | 2.7 | 3.8 | 15.63 | 1.91 |
| | Palmira | | | 2 | 60 | IAC51- PVBR94 | | 3 | 12.98 | 1.89 |
| | Palmira | 2011 | | 5 | 55 | PVat0006- BMd50 | | 3.8 | 18.71 | 2.28 |
| | Karama | | | 9 | 25 | PVat0007- BM114 | | 3.9 | 13.52 | 2.03 |
| | Palmira | 2011 | | 9 | 25 | PVat0007- BM114 | | 3.9 | 13.71 | 1.95 |

† RF: Rainfed; RFS: Rainfed supplemented by irrigation; ††LOD: logarithm of the odds at the peak; § $R^2$: Proportion of the phenotypic variance explained by the QTL at peak LOD; ¶ Add: Effect of substituting a single allele from one parent to another. Positive values indicate allele from SEA5 and negative from CAL96.

Table 2. 12 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Karama | | | 11 | 36 | InDel14- InDel10 | | 6.1 | 30.74 | -3.11 |
| | | 2012 | | 11 | 32 | InDel29- PVM98 | 3.0 | 3.1 | 11.59 | 1.9 |
| | | | | 2 | 64 | IAC51- PVBR94 | | 3.3 | 14.03 | 2.08 |
| | | | RFS | 11 | 30 | InDel11- InDel29 | 2.8 | 3.3 | 14.67 | 2.12 |
| | Combined | | RF | 2 | 60 | IAC51- PVBR94 | 3.0 | 3.3 | 13.03 | 1.56 |
| Seed yield | Karama | 2012 | RF | 11 | 35 | PVM98- InDel14 | 2.8 | 6.3 | 37.3 | -9.31 |
| | | 2011 | | 11 | 35 | PVM98- InDel14 | | 6.3 | 37.3 | -13.96 |
| | | | RFS | 7 | 65 | PVBR269- PVBR35 | | 8.6 | 45.9 | 20.48 |
| | | | | 7 | 60 | PVBR67- InDel01 | | 6 | 26.55 | -15.33 |
| | Combined | | RF | 5 | 86 | PVBR235- PVBR131 | 3.0 | 3.6 | 17.71 | 8.35 |
| | | | | 11 | 32 | InDel29- PVM98 | | 5.9 | 29.02 | 10.2 |
| | | | | 11 | 36 | InDel14- InDel10 | | 9.2 | 56.05 | -14.16 |
| Seed yield/day | Karama | 2011 | RF | 4 | 15 | PVBR242- BMd16 | 2.7 | 5.3 | 38.14 | 0.17 |
| | | | | 7 | 65 | PVBR269- PVBR35 | | 10.4 | 53.8 | 0.25 |
| | | | | 7 | 60 | PVBR67- InDel01 | | 7.1 | 30.82 | -0.19 |
| | | | | 9 | 64 | BM141-IAC68 | | 3.3 | 25.56 | -0.13 |
| | | | | 11 | 35 | PVM98- InDel14 | | 9.8 | 50.35 | -0.18 |
| | | 2012 | | 11 | 35 | PVM98- InDel14 | 2.9 | 8.3 | 50.6 | -0.14 |
| | Combined | | RF | 11 | 32 | InDel29- PVM98 | 3.0 | 3.2 | 15.58 | 0.09 |
| | | | | 11 | 36 | InDel14- InDel10 | | 6 | 36.66 | -0.13 |

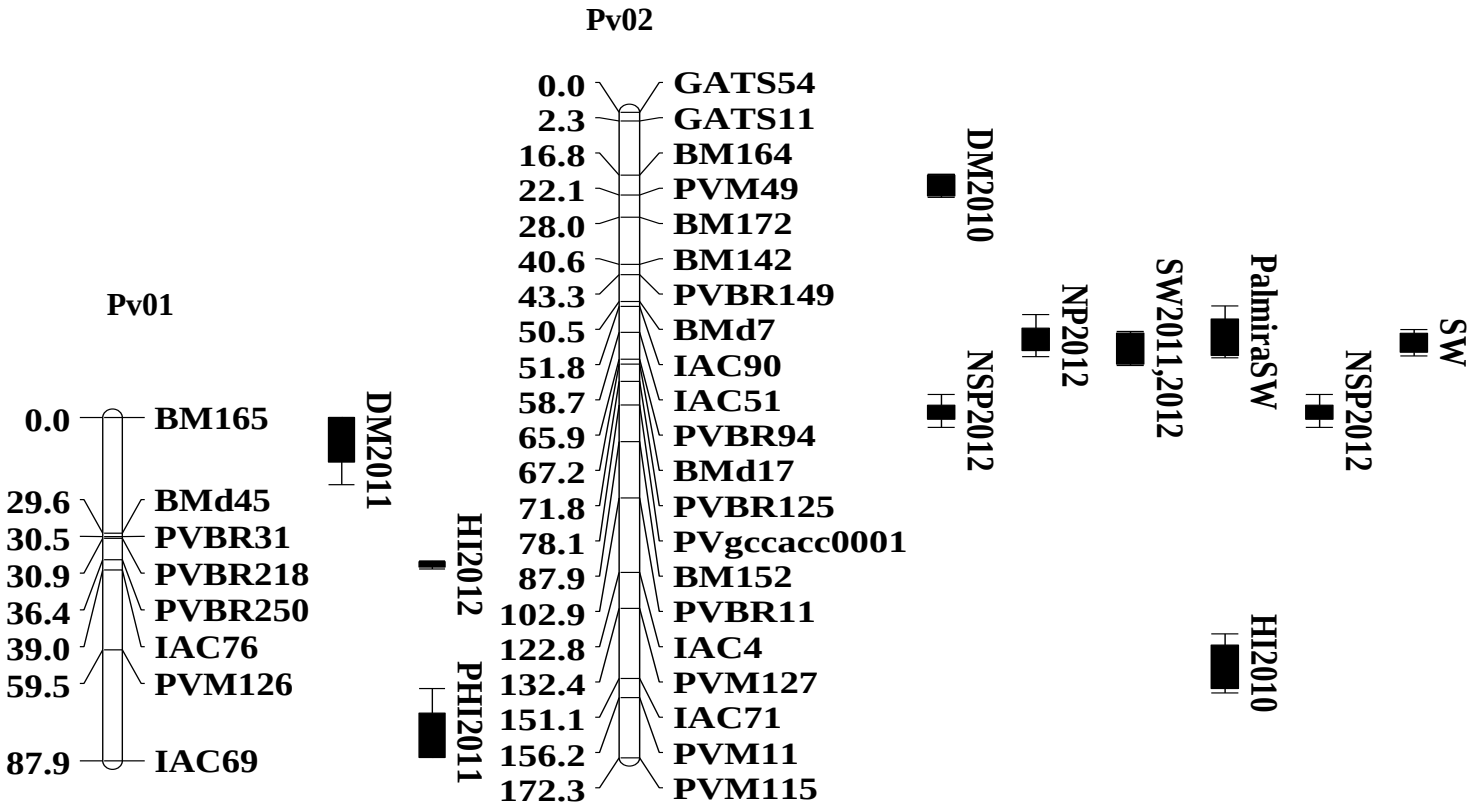



Figure 2. 3. Genetic linkage map of SEA/CAL96 RIL population and localization of QTL for days to flowering (DF), days to maturity (DM), days to seed fill (DSF), pod per plant (NP), seed per pod (NSP), Harvest Index (HI), Pod Harvest Index (PHI), seed weight (SW), yield (SY), and yield per day (SYD). QTL are further identified by year in Karama, Rwanda and by Palmira in Palmira, Colombia, and QTL with no year specified were detected in combined environment. QTL in gray cross-hatching and black were identified under rainfed supplemented with irrigation and rainfed conditions respectively.

Figure 2. 3 (Cont'd)

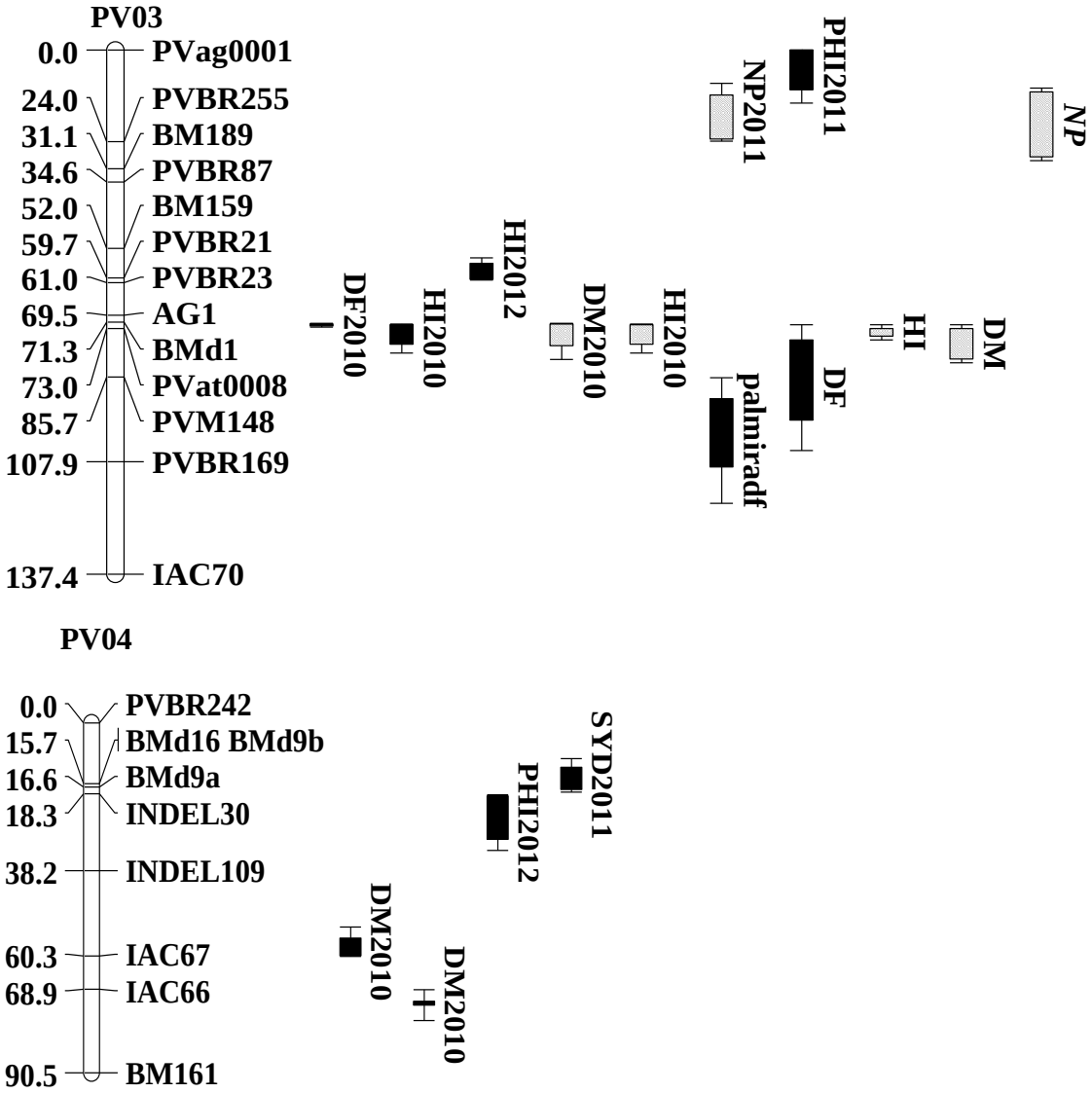


Figure 2. 3 (Cont'd)

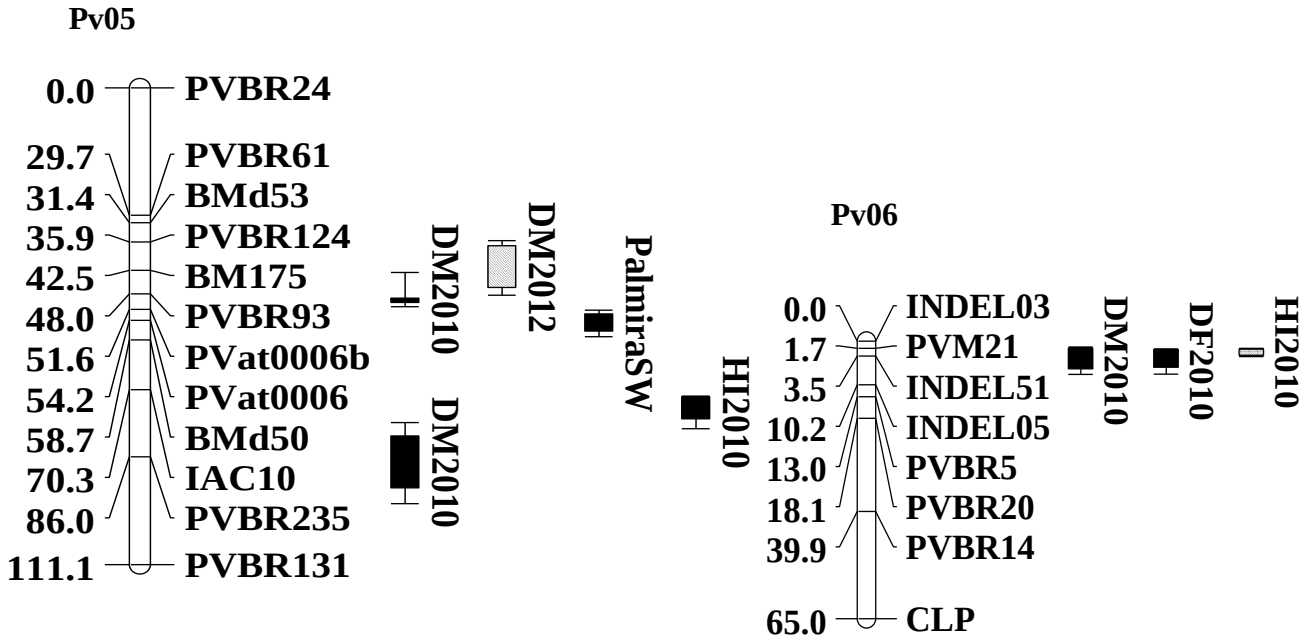


Figure 2. 3 (Cont'd)

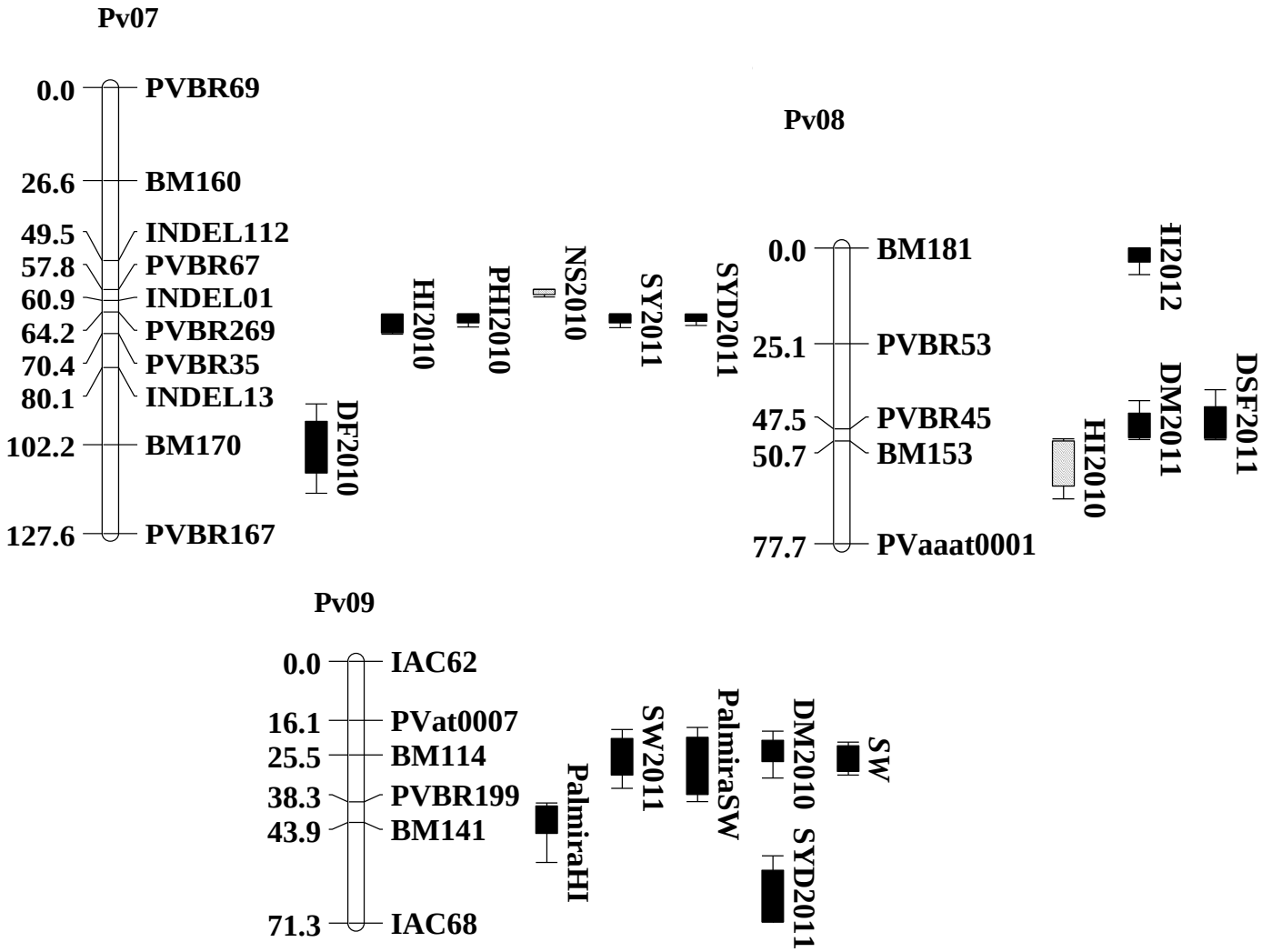
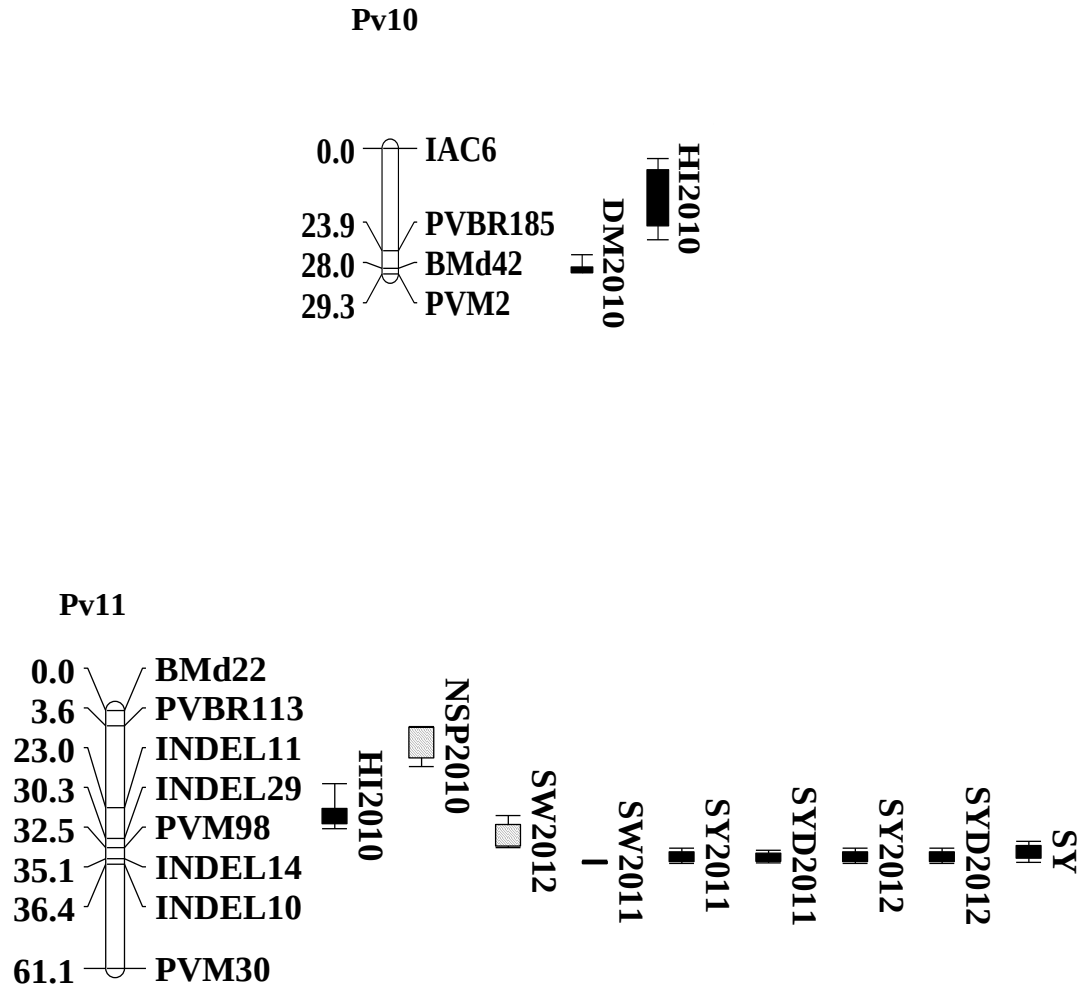


Figure 2. 3 (Cont'd)



## Discussion

### Field results

Crops with increased drought resistance are very important for maintaining yield in regions where dry growing seasons are common. Drought stress is the most devastating stress affecting the productivity of common bean in rain fed production systems (Beebe et al., 2010). In this study, a RIL population from an inter gene pool cross of a race Durango derivated genotype SEA5 and an Andean cultivar CAL96 was evaluated for drought tolerance and to map QTL for drought resistance traits in this population. The two parents were contrasting in various traits including drought resistance as well the seed size and color. Drought resistant recombinant lines with seed size superior to that of SEA5 would be more preferred in Rwanda since large seeded beans are commonly grown and consumed in East Africa. Since CAL96 is widely adapted in Rwanda and in Colombia, production of adapted lines with improved drought resistance was expected. In common bean, the physiological effects of drought stress tend to be more deleterious during reproductive development. As terminal drought is common in East Africa (Beebe et al., 2010), QTL analyzes were conducted on common bean traits associated with escape mechanisms (early flowering, maturity, and seed fill), yield and yield components (number of pod/plant, number of seed/pod, 100-seed weight, yield, and yield/per day), and partitioning indices (harvest index and pod harvest index) under RF and RFS conditions.

The rain fall amounts at the sites where the research was conducted were not sufficient for optimal bean production (Figure 2. 1) during experimental years. In addition, the rainfall patterns differed from expected typical terminal drought making it challenging to identify stable QTL across environments. However, these rainfall patterns presented the advantage of limiting the drought associated root diseases such as charcoal rot [caused by *Macrophomina phaseolina*

(Tassi) Goid] that usually occur under terminal drought in low land conditions (Frahm et al., 2004). In addition, the exposure of this population to a wide range of water stresses that represent most of conditions that bean crops are likely to encounter during the growing season, might have helped to identify a range of alleles associated with broad adaptation although this needs to be confirmed.

In general the SEA5/CAL96 population matured later in Rwanda than in Colombia. This was due to differences in temperature at the two sites. The Palmira site was characterized by higher temperatures than Karama and this impacted both flowering and maturity times. Cooler conditions have been demonstrated to cause slower growth, delayed maturity, and increased yields in common bean (Acosta-Gallegos and White, 1995; Singh et al., 2002; Welsh et al., 1995). Mean seed yields were higher in Karama than those in Palmira (Tables 2. 1; 2. 2). Although, no RIL yielded better than the SEA5 parent, some RILs with yield superior to that of CAL96 in RF conditions were obtained (Tables 2. 3 and 2. 8). These results were not unexpected since this population was from inter gene pool cross, advanced by single descent method. The absence of transgressive segregation for yield in Andean/Middle American crosses beyond the value of the highest yielding parent has been noted in other studies where the same breeding scheme was used. Welsh et al. (1995) did not find any high yielding $F_2$ derived recombinant inbred lines developed from biparental Andean/Middle American populations using the single seed descent method. Similar results were obtained by Singh et al. (2002) who selected advanced lines from inter gene pool crosses with yield superior to the ones of Andean parents but none outyielded the MiddleAmerican parents. This population designed as QTL study rather than to develop new varieties. For instance, recurrent selection, congruity backcrossing, and inbred backcrossing systems have been successfully used to select high yielding large seeded Andean

beans from Andean/Andean interracial and Andean/Middle American inter gene pool populations (Beaver and Kelly, 1994; Singh et al., 1999; Román-Avilés and Kelly, 2005).

Under the current study, none of the RIL had the same desired large seed size of the Andean parent CAL96 regardless of water treatment. The average seed size was 28.6 g/100-seed under RF and 27.9 g/100-seed under RFS conditions significantly lower than the seed size of CAL96. Fortunately, many of the highest yielding lines under drought were among lines with intermediate seed size (Table 2. 3). Unattractive seed coat colors were observed in some drought resistant RIL. This might reduce the use of these lines in regions where certain bean seed colors are preferred. However, these advanced lines could be used as parents with drought resistance component traits in future breeding efforts to enhance drought resistance of Andean gene pool.

Correlation analysis among variables showed mixed results. Positive correlations between phenology and yield variables in Karama were observed while they were negatively correlated in Palmira. This can be explained by rainfall patterns of Karama that were not as severe during reproductive growth as expected (Figure 2. 1). Instead, rainfall in Karama site had a tendency of being intermittent in the reproductive stages which might have resulted in a split pod sets and delay of the overall maturity.

The number of pods per plant, number of seed per pod, harvest index, and pod harvest index always positively correlated with yield across sites (Tables 2. 4, 2. 5; Table A. 4). These results were expected since these variables are key determinants of yield. Under RF conditions in Karama (Table 2. 5), correlation coefficients of number of seeds per pod and yield tended to be weaker than the correlation coefficient between the number of pods per plant and yield suggesting that pod abortion might have been the mechanism reducing yields in this site. Pod abortion has been recognized to be an important mechanism that reduces bean seed yield in

common bean ( Acosta Gallegos and Shibata, 1989). In addition, the number of days to maturity and the number of days to seed fill were positively correlated with yield in Karama suggesting that under moderate drought situations of this site, escaping mechanisms were not needed as drought tolerance mechanisms.

Days to maturity and days to seed fill were negatively correlated with yield in Palmira under severe drought situations. These results suggest that the escape mechanism might have been an important mechanism in drought evasion at Palmira. To better understand the contribution of different variables to yield, a stepwise regression analysis for combined data was conducted (Table 2. 7). In Karama RF, variables number of pods per plant, seed weight, harvest index, and days to maturity, showed to be most contributing to yield at 0.05 significance level. Under this model, the number of pods per plant trait was the most contributing variable to the model ($R^2$=0.22$^{***}$ ) while its contribution was small in the RFS conditions suggesting that those lines that were able to maintain a high number of pods under RF yielded better than those with lower number of pods. In Palmira, pod harvest index was the variable greatly contributing to the model ($R^2$ = 0. 19$^{***}$) followed by harvest index with ($R^2$=0.17$^{***}$). These results suggest that in addition to early maturing, lines with better partitioning indices had high yields in Palmira. Combining these results from different sites, resistance mechanisms in this population might have been different depending on drought scenario and intensity at each site. These results suggest that depending on the site and water conditions, various drought mechanisms contribute to the overall performance within the same population.

General negative correlations between partitioning indices and phenology variables were observed. The cross SEA5/CAL96 was a wide cross with expected possible phenotypic abnormalities and overall poor combining ability (Johnson and Gepts, 2002). The fact that late

maturing lines might have had lower partitioning indices might be associated with the'lazy' syndrome in some lines that stayed green longer but with fewer pods or seeds characteristic of some progenies from the inter gene pool crosses. Odd phenotypes in progeny of crosses of Andean and Middle American crosses are widely reported in common bean (Kornegay et al., 1992; Johnson and Gepts, 2002).

Positive correlations of 100-seed weight with seed yield in both RF and RFS environments can be explained by the fact that drought stress in Karama was not severe enough to affect the seed size. In fact, there was no reduction in 100-seed weight specific to this study. The intensities of the stress in RF conditions were generally mild in 2011 and 2012, but the same results were obtained in 2010 when drought was more severe than the two following years. In addition strong positive correlations ($r=0.76^{***}$) between RF and RFS treatments for seed size were observed which suggest the stability of seed size in this population. This was also reflected in the consistency of QTLs associated with 100-seed weight over years and sites. Usually, drought has been shown to reduce bean seed size in other studies (Urrea et al., 2009; Singh, 1995, 2007). However, when DII are mild, seed size has shown to be relatively stable. Schneider et al (1997a) observed the stability of the seed size in beans evaluated with similar DII varying between 0.19 and 0.49 in the Mexican highlands. The fact that 100-seed weight correlated with yield in both water treatments in this population may indicate that some genes responsible for seed yield are linked with genes controlling seed size or that they might have pleiotropic effects in this population. This was further supported by high heritability values for seed size (Table 2.8) and the identification of seed weight QTL that colocalized with seed yield QTL on Pv11 and by the fact that the best yielding lines were also among the RILs with higher seed size. The linkage

of the seed size and yield in this population might facilitate simultaneous selection of high yielding lines with acceptable seed size under drought conditions.

Harvest index and pod harvest index are indicators of the remobilization of photosynthates from shoot biomass and pod biomass respectively to the seed. It has been proposed that photo-assimilate remobilization is one of the characteristics unconsciously selected during the development of drought resistant cultivars (Rosales-Serna et al., 2004; Miklas et al., 2006a). In a previous drought study on a population from Mesoamerican cross DOR 364/BAT477, Asfaw et al. (2012) noted stability of partitioning indices across water treatments. In addition, harvest index was shown to be the most stable partitioning index under moderate levels of drought (Ramirez-Vallejo and Kelly, 1998). Since these parameters were stable in this study and highly correlated to seed yield and yield per day under both RF and RFS conditions, they can be combined with yield evaluations to identify higher drought resistant cultivars while avoiding penalties for yield potential. The usefulness of pod harvest index as a selection criterion was demonstrated by selecting plants with well filled pods in order to identify drought resistance lines for production in Colombia (Beebe et al., 2008).

**Genetic Mapping and QTL analysis**

The SEA5/CAL96 genetic map constructed in this study was 1031 cM in length. The skewed segregations of certain markers observed in this population were from the residual heterozygosity that was still present at this $F_{5:7}$ generation as detected by co-dominant SSR and InDel markers. Heterozygozity at this level of RIL populations is common in common bean even in populations deriving from parents from the same gene pool (Beattie et al., 2003).

QTL for highly correlated variables such as phenology, partitioning indexes, and yield and yield per day colocalized on the same chromosome. Most of the traits are related on a cause

effect basis which suggests that these traits may be conditioned by genes that are physically linked or have pleiotropic effects (Aastveit and Aastveit, 1993). The significant GE interactions reflected in phenotypic data in almost all traits were also reflected in QTL analysis since different QTL were identified depending on environments. In this study, with few exceptions, it is evident that detection of a QTL in one environment did not predict the presence of the same QTL in other environments. From a total of 41 QTL identified in single environment analysis, only 8 QTL were found consistently in more than one environment (Table 2.13). This suggests that each drought stress triggered different gene expression responses in the population. In combined analysis, not all the QTL in single environment analysis were identified. This was due probably to the fact that QTL explaining larger amounts of variation tended to be more constant across environments (Tanksley, 1993).

QTL for days to flowering and days to maturity co-localized on Pv03 and Pv06 in different years and conditions while days to maturity and days to seed fill were associated with the same QTL on Pv08. Mapping QTL associated with phenology variables days to flowering in different sites and days to maturity on the same region on Pv03, and the days to maturity in combined analysis suggests that there might be important gene(s) located to this linkage group associated with these traits. Another QTL linked to days to maturity in only one year analysis, was identified on Pv03 by Wright and Kelly (2011) in a black bean population derived from a cross of ‘Jaguar’ and 115M genotypes. Other studies reported the co-localization of phenology variables. Blair et al. (2012) mapped QTL for days to flowering and days to maturity on Pv05, and Pv06 in a drought study while Ender and Kelly (2005) mapped co-localized QTL for the same traits on Pv02 and Pv07 in a white mold resistance study. Tar'an et al. (2002) mapped QTLs for days to flowering and maturity on Pv09 in an agronomic study.

QTL for days to seed fill were not consistent between single and combined environments analysis. Further studies are needed to confirm days to seed fill QTL from the current study. QTL for seed fill is of particular importance since number of days to seed fill is an important escape mechanism for beans under terminal drought stress.

Table 2. 13. Location and description of named QTL identified multiple environments for SEA5/CAL96 RIL population grown under rainfed and rainfed supplemented with irrigation in Karama, Rwanda and Palmira, Colombia during 2010-2012.

| Trait | Assigned QTL[†] | Linkage group | Flanking markers | LOD[††] | $R^2$ (%)[§] | Add[¶] |
|---|---|---|---|---|---|---|
| 100-seed weight | $SW2.7^{SC}$ | 2 | IAC51- PVBR94 | 3.3 | 13.03 | 1.56 |
| Days to flowering | $DF3.1^{SC}$ | 3 | PVat0008- PVM148 | 3.0 | 12.40 | -0.71 |
| Days to maturity | $DM3.1^{SC}$ | 3 | PVat0008- PVM148 | 5.0 | 18.84 | -1.44 |
| Number of pods per plant | $NP3.1^{SC}$ | 3 | PVag0001- PVBR255 | 5.8 | 27.35 | -0.85 |
| Harvest Index | $HI3.2^{SC}$ | 3 | BM159- PVBR21 | 3.9 | 15.99 | 0.03 |
| 100-seed weight | $SW9.3^{SC}$ | 9 | PVat0007- BM114 | 3.9 | 13.71 | 1.95 |
| Yield | $SY11.1^{SC}$ | 11 | PVM98- INDEL14 | 6.2 | 37.29 | -13.90 |
| Yield per day | $SYD11.1^{SC}$ | 11 | PVM98- INDEL14 | 9.8 | 50.35 | -0.18 |

[†] DF: Days to flowering, DM: Days to maturity, HI: Harvest index, NP: Number of pods per plant, SW: seed weight, SY: Seed yield, SYD: Seed yield per day; [††]LOD: logarithm of the odds at the peak; [§]$R^2$: Proportion of the phenotypic variance explained by the QTL at peak LOD; Add: Effect of substituting a single allele from one parent to another. Positive values indicate allele from SEA5 and negative from CAL96.

Two QTL for the number of pods per plant mapped on Pv02 and Pv03 in single environment analysis. The QTL for number of pods per plant on Pv02 was contributed by SEA5 while the one on Pv03 came from CAL96. In combined analysis, the QTL for number of pods per plant on Pv03 was the only QTL for number of pods per plant that was found and accounted for 27% of the total phenotypic variation. Another QTL for this trait was mapped on Pv08 in combined analysis. Blair et al. (2006) mapped QTLs for pods per plant on Pv07, Pv09, and Pv11 in an advanced backcross population from a cross of an Andean bean ICA Cerinza with a wild bean accession G24404. In addition, Checa and Blair (2012) mapped the number of pods per plant QTLs on Pv04 and Pv10, whereas Beattie et al. (2003) mapped number of pods per plant QTL on linkage group Pv03. Since different marker technologies were used in these two studies and this linkage group has been reoriented during the process of representing bean linkage group with the short arm on top and the long arm on the bottom http://www.css.msu.edu/bic/_pdf/Standardized_Genetic_Physical_Bean_Map_2008.pdf, it is difficult to compare the current QTL with the one mapped by Beattie et al. (2003) in navy bean. The present QTL is proposed to be named NP3.1$^{SC}$. Further research is needed to confirm the QTL for number of pods per plant on Pv02.

Five QTL associated with pod harvest index were identified on Pv01, Pv03, Pv04, and Pv07 while QTL for harvest index were scattered throughout the genome (Table 2. 10). QTL for pod harvest index on Pv07 are only 5 cM apart but they do not share any marker suggesting that they might be independent. A QTL for pod harvest index mapped on Pv06 in combined environments. In common bean, only one QTL study of pod harvest index identified a QTL on Pv06 and named it Phi6.1 (Asfaw et al., 2012). The finding of a pod harvest index QTL on Pv06 in this study suggest important gene(s) conditioning pod harvest index might be residing on this

linkage group. Positive alleles for this trait were contributed by SEA5 in combined environments analysis while in single environment analysis; both QTL for pod harvest index were contributed by both parents.

The QTL on Pv03 flanked by markers BM159 and PVBR21 was associated with harvest index in RF and RFS in Karama, suggesting that it might have a constitutive effect. This QTL accounted for 25% of the phenotypic variation under RF conditions while its contribution was 10.5% in RFS conditions. Harvest index QTL have been mapped previously on Pv06 (Tar'an et al., 2002) and on Pv03 (Asfaw et al., 2012). The harvest index QTL on Pv03 Hri3.1 explained minimal phenotypic variation under both under drought stress and nonstress respectively (0.65 and 4.55 %) in DOR364/BAT477 population (Asfaw et al., 2012). Since these two maps do not share any marker, it was not possible to compare these two QTL. The finding of the current harvest index QTL ($HI3.2^{SC}$) with constitutive effect suggests that Pv03 carries genes responsible for harvest index. QTL with constitutive gene mechanisms might be of particular interest in implementing MAS in bean breeding as they can be used in the absence of the drought stress.

A QTL for 100-seed weight under RF conditions mapped to Pv02 in Palmira 2011 and in Karama for 2 years. This QTL came from SEA5 parent and decreased the seed size by 1.89, 1.91, 2.08, and 1.56 g, in Palmira, Karama 2011, Karama 2012, and mean environments, respectively. This QTL explains a mean phenotypic variance of 13%. When epistatic interactions for seed weight were analyzed, the seed weight QTL on Pv02 interacted with another seed weight qtl on Pv03 (Table B. 1). Alleles from both parents were involved in this specific interaction. Most of the other QTL interactions for seed weight in RF involved alleles from both parents and this suggests that negative allele combinations might have caused a reduced seed size in this population. Blair et al. (2006, 2012) identified several QTL for seed weight on almost

every linkage group in DOR364/BAT477 population and in an advanced backcross population from ICA Cerinza and G24404. Since some of the QTL on Pv02 in these two different populations possess the same names, the naming of QTL for this trait on this linkage group is not clear. Although limited segregation for seed weight might be expected for DOR364/BAT477 population, it is clear that important QTL might be located on this chromosome. Various other studies also have identified QTL for seed weight on Pv02 (Kolkman and Kelly, 2003; Mkwaila et al., 2011). Since the latest QTL for seed weight was named by Blair et al. (2012) was SW2.6, the current QTL is named SW2.7$^{SC}$.

Another important QTL for seed weight explaining 13% of the phenotypic variation mapped to Pv09. This QTL found under RF condition in both Karama 2011 and Palmira 2011 was contributed by the SEA5 parent and decreased 100-seed weight by 2g. Various QTL for SW have been mapped on Pv09 (Blair et al., 2006, 2012) and the current QTL is named SW9.3$^{SC}$. Other QTL for seed size were located on interval of 6 cM on Pv11 suggesting a single or suite of genes for 100-seed weight reside on this linkage group. Several other studies mapped seed weight QTL on Pv11 (Beebe et al., 2006; Blair et al., 2006; Wright and Kelly, 2011; Tar'an et al., 2002; Mkwaila et al., 2011) in different populations. In this study, QTLs for 100-seed weight were identified in both RF and RFS conditions suggesting that genes on this linkage group might have a constitutive mechanism that condition seed weight. The seed weight QTL on Pv11 is tightly linked to the QTL associated with yield and yield per day under RF conditions. This linkage was expected in this population since yield and seed size were consistently correlated. This linkage would provide the opportunity to select simultaneously for seed size and yield in cross involving materials from this study to eliminate any the loss in seed size that occurred in this cross.

Yield and yield per day QTLs were mainly identified on Pv07 and Pv11. QTL on Pv07 were associated with yield under RFS conditions while those on Pv11 were identified under RF in Karama 2011 and 2012. Both parents contributed to yield QTL on Pv07. Yield QTL have been identified before on Pv07. Blair et al. (2012) mapped a yield QTL under irrigation on Pv07 and Asfaw et al. (2012) mapped a yield QTL on the same linkage group but under drought stress in Palmira. In addition, Mkwaila et al. (2011) identified a QTL for yield on Pv07 in a white mold resistance study. The fact that many QTL studies identified yield QTL on Pv07 is indicative that yield related genes reside on this linkage group. QTL for yield on Pv07 mapped at an interval of 5 cM from each other. Since they do not share any marker, they might be independent which suggest that many genes of small effects might be responsible of yield on this linkage group. Interestingly, these yield and yield per days QTL mapped exactly at the same location with harvest index and pod harvest index from RF conditions in Karama 2010 suggesting that mechanisms associated with these partitioning indices might be responsible for yield changes in this genomic region.

Yield and yield per day QTLs on Pv11 ($SY11.1^{SC}$) were found under RF conditions in two consecutive years and were contributed by the CAL96 parent. These QTL were major QTL contributing 37% and 50% of the phenotypic variation for yield and yield per day respectively in both years. These QTL were specific to mild drought conditions since they were not identified under severe drought conditions of Palmira and Karama 2010. Markers associated with these QTL were found in seven out of ten top yielding RIL suggesting that yield can be used as a selection criterion to select for drought resistance under suitable environmental conditions. These results suggest that these QTL were beneficial in the new genetic material and this might be associated with the limited negative epistatic interactions in these lines. In addition to

independent gene actions, epistatic gene interactions play important role in determining performance of inter-gene pool crosses of common bean (Johnson and Gepts, 2002; Moreto et al., 2011)

Epistatic interaction analysis showed that yield loci on Pv11 region interacted with QTL associated with yield on Pv03 that were not significant by themselves in a combined environments analysis (Table B.1) suggesting synergic actions for yield determination. Although no significant QTL associated with yield was identified on Pv03 in this study, yield QTL on this linkage group were mapped elsewhere (Blair et al., 2012; 2006; Wright and Kelly, 2011). In addition, QTLs associated with harvest index were mapped on Pv03 which can justify these interactions since seed yield and harvest index are related traits. These results suggest that high yielding lines under RF conditions can be used as parents to further enhance drought resistance in Andean beans and that this QTL might be useful for MAS for yield and drought tolerance under mild drought conditions in Rwanda.

Usually, QTL mapping studies are a prerequisite for MAS which can be used to effectively combine several different QTLs. However, in the case of stress related QTL, the implementation of MAS is limited since some of the QTLs might be involved in complex epistatic interactions in the genetic background and do not contribute significantly in novel genetic materials (Tuberosa et al., 2002; Steele et al., 2007, 2009). The QTL on Pv11 identified in this study showed a positive interaction with other loci contributing to yield increases in this biparental cross. QTL mapping in biparental populations present the advantage of identifying QTL of major effects that can easily be used in MAS studies once confirmed. In rice, the usefulness of major QTL identified in bi-parental population was demonstrated with large-effect QTL qDTY 12.1 that confer higher grain yield under drought (Dixit et al., 2012).

## Conclusion

The evaluation of the inter gene pool RIL population from a cross of SEA5 and CAL96 was conducted in Rwanda and in Colombia to identify drought resistant recombinant lines with large seed size acceptable in Rwanda and to identify QTL associated with drought resistance in common bean. Experiments were conducted in both rainfed and rainfed supplemented with irrigation conditions. In Rwanda, the growing seasons were characterized by mild drought conditions that occurred late during reproductive stages while in Palmira, the growth season was characterized by a severe drought. Depending on the drought scenario, different resistance mechanisms contributed to the overall performance in this population. In Rwanda, retention of high number of pods per plant was an important mechanism of drought tolerance. Early maturity and efficient partitioning of total and pod biomass into seed contributed to drought tolerance in Palmira. Transgressive segregation beyond the best parental value for seed yield and yield components were limited in this population in both water conditions. The average 100-seed weight was slightly higher than the seed weight of SEA5, but significantly lower than the seed weight of the CAL96 Andean parent. Positive correlations between seed yield and seed weight were consistently found and this led to the identification of lines with yield superior to the yield of the susceptible parents CAL96 combined with medium seed sizes. These lines include RSA118 and RSA120 that consistently performed well under both RF and RFS water treatments. These lines combining high yield, red mottled seed color with medium seed sizes (> 30g) can be further evaluated in Rwanda and be released as drought resistant cultivars or used as parents to further enhance drought tolerance in Andean beans.

QTL associated with drought escape, avoidance, yield and yield components, and partitioning traits were identified. New QTL for the days to flower, days to maturity, harvest

index, and pods per plant were mapped on Pv03. Seed weight QTL were shown to be the most stable QTL mapped on Pv02, Pv09, and Pv11 near the previously mapped seed weight QTL. QTL for harvest index, and pod harvest index co-localized with QTL for seed yield and yield per day on Pv07. These traits can be combined to yield to select for genotypes with drought tolerance. Since the same QTL flanked by markers PVBR67- InDel01 were associated with harvest index, pod harvest index, seed yield, and seed yield per day, the QTL can be used in MAS to select simultaneously for these traits once confirmed.

A major seed yield QTL accounting for 37% of the phenotypic variation and linked to seed size QTL was identified on Pv11. This QTL was found to be present in most of the high yielding recombinant lines which suggests that once confirmed, this QTL might be useful for selection of drought resistance genotypes with acceptable seed size in Andean beans for Rwanda.

# APPENDICES

## Appendix A

Pearson correlation coefficients among variables for SEA5/CAL96 RIL population grown under rainfed (RF) and rainfed supplemented with irrigation (RFS) in Karama, Rwanda and Palmira, Colombia during 2010- 2012 by site and year.

Table A.1. Pearson correlation coefficients among days to flowering, days to maturity, days to seed fill, number of pods per plant , number of seed per pod, Harvest index, pod harvest index, seed weight, seed yield, and seed yield per day for SEA5/CAL96 RIL population grown under rainfed (RF) and rainfed supplemented with irrigation (RFS) in Karama 2010.

| Trait | Days to flowering | Days to maturity | Number of pods/plant | Number of seeds/pod | Harvest index | Pod harvest index | 100-seed weight | Seed yield | Seed yield/day | Days to seed fill |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to maturity | 0.41*** | - | 0.09 | 0.03 | -0.36*** | -0.35*** | 0.25** | 0.18* | 0.03 | 0.58*** |
| Number of pods/plant | -0.09 | 0.00 | - | 0.41*** | 0.24** | 0.09 | 0.32*** | 0.54*** | 0.53*** | 0.08 |
| Number of seeds/pod | 0.05 | 0.09 | 0.07 | - | 0.22* | 0.15 | 0.20* | 0.36*** | 0.36*** | 0.01 |
| Harvest index | -0.23* | -0.27* | 0.35*** | 0.18* | - | 0.79*** | 0.21* | 0.48*** | 0.54*** | -0.19* |
| Pod harvest index | -0.22* | -0.17 | -0.02 | 0.13 | 0.65*** | - | 0.21* | 0.39*** | 0.45*** | -0.25** |
| 100-seed weight | 0.08 | 0.23** | -0.07 | -0.12 | -0.03 | 0.18* | - | 0.52*** | 0.49*** | 0.16* |
| Seed yield | 0.04 | 0.20* | 0.24** | 0.12 | 0.39*** | 0.43*** | 0.41** | - | 0.99*** | 0.06 |
| Seed yield/day | -0.01 | 0.09 | 0.25** | 0.11 | 0.43*** | 0.45*** | 0.40*** | 0.99*** | - | -0.04 |
| Days to seed fill | -0.13 | 0.70*** | 0.11 | -0.02 | -0.12 | -0.02 | 0.14 | 0.25** | 0.17 | - |

Below diagonal: Rainfed; above diagonal: Rainfed supplemented with irrigation:; * Significant at α=0.05 ; ** Significant at α=0.01; *** Significant at α=0.001

Table A. 2 Pearson correlation coefficients among days to flowering, days to maturity, days to seed fill, number of pods per plant , number of seed per pod, Harvest index, pod harvest index, seed weight, seed yield, and seed yield per day for SEA5/CAL96 RIL population grown under rainfed (RF) and rainfed supplemented with irrigation (RFS) in Karama 2011.

| Trait | Days to flowering | Days to maturity | Number of pods/plant | Number of seeds/pod | Harvest index | Pod harvest index | 100-seed weight | Seed yield | Seed yield/day | Days to seed fill |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to maturity | 0.41*** | - | 0.31*** | 0.05 | -0.40*** | -0.37*** | 0.19* | 0.22* | 0.06 | 0.82*** |
| Number of pods/plant | -0.01 | 0.24** | - | 0.16 | -0.04 | -0.05 | 0.05 | 0.52*** | 0.47*** | 0.36*** |
| Number of seeds/pod | 0.20 | 0.19 | 0.32*** | - | 0.13 | 0.13 | -0.06 | 0.22* | 0.22* | 0.03 |
| Harvest index | -0.24* | -0.14* | 0.21* | 0.02 | - | 0.85*** | 0.14 | 0.30** | 0.38** | -0.27** |
| Pod harvest index | -0.16 | -0.07 | 0.12 | -0.08 | 0.83*** | - | 0.19* | 0.39*** | 0.46*** | -0.32*** |
| 100-seed weight | 0.00 | 0.27*** | 0.32*** | 0.08 | 0.27*** | 0.37*** | - | 0.33*** | 0.31*** | 0.16 |
| Seed yield | 0.09 | 0.22* | 0.56*** | 0.32*** | 0.43*** | 0.26** | 0.48*** | - | 0.99*** | 0.17 |
| Seed yield/day | 0.02 | 0.03 | 0.53*** | 0.28** | 0.46*** | 0.27** | 0.44*** | 0.98*** | - | 0.04 |
| Days to seed fill | -0.12 | 0.84*** | 0.23* | 0.08 | -0.01 | 0.01 | 0.30** | 0.17* | 0.01 | - |

Below diagonal: Rainfed; above diagonal: Rainfed supplemented with irrigation; * Significant at α=0.05 ; ** Significant at α=0.01; *** Significant at α=0.001

Table A. 3. Pearson correlation coefficients among days to flowering, days to maturity, days to seed fill, number of pods per plant, number of seed per pod, Harvest index, pod harvest index, seed weight, seed yield, and seed yield per day for SEA5/CAL96 RIL population grown under rainfed (RF) and rainfed supplemented with irrigation (RFS) in Karama 2012.

| Trait | Days to flowering | Days to maturity | Number of pods/plant | Number of seeds/pod | Harvest index | Pod harvest index | 100-seed weight | Seed yield | Seed yield/day | Days to seed fill |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to maturity | 0.33*** | - | -0.01 | 0.10 | -0.37*** | -0.15 | 0.28** | 0.31** | 0.17 | 0.85*** |
| Number of pods/plant | 0.01 | 0.11 | - | 0.28** | 0.28** | 0.17 | 0.06 | 0.26** | 0.26** | -0.10 |
| Number of seeds/pod | 0.20* | 0.10 | 0.49*** | - | 0.14 | 0.03 | -0.01 | 0.29** | 0.28** | 0.04 |
| Harvest index | 0.02 | -0.02 | 0.07 | 0.06 | - | 0.73*** | -0.04 | 0.27** | 0.33*** | -0.31*** |
| Pod harvest index | -0.21* | -0.06 | 0.23* | -0.03 | 0.19* | - | 0.11 | 0.29** | 0.32*** | -0.14 |
| 100-seed weight | -0.20* | 0.28** | 0.07 | -0.08 | -0.06 | 0.21* | - | 0.42*** | 0.40*** | 0.31*** |
| Seed yield | 0.15 | 0.24* | 0.26** | 0.10 | 0.03 | 0.21* | 0.29** | - | 0.99*** | 0.16 |
| Seed yield/day | 0.01 | -0.19* | 0.23* | 0.08 | 0.04 | 0.23 | 0.16 | 0.91*** | - | 0.05 |
| Days to seed fill | -0.10 | 0.86*** | 0.00 | -0.05 | -0.11 | -0.03 | 0.37*** | 0.11 | -0.25** | - |

Below diagonal: Rainfed; above diagonal: Rainfed supplemented with irrigation; * Significant at α=0.05 ; ** Significant at α=0.01; *** Significant at α=0.001

Table A. 4. Pearson correlation coefficients among days to flowering, days to maturity, days to seed fill, number of pods per plant, number of seed per pod, Harvest index, pod harvest index, seed weight, seed yield, and seed yield per day for SEA5/CAL96 RIL population grown under rainfed (RF) Palmira 2011.

| Trait | Days to flowering | Days to maturity | Number of pods/plant | Number of seeds/pod | Harvest index | Pod harvest index | 100-seed weight | Seed yield | Seed yield/day |
|---|---|---|---|---|---|---|---|---|---|
| Days to maturity | 0.55*** | | | | | | | | |
| Number of pods/plant | -0.02 | 0.00 | | | | | | | |
| Number of seeds/pod | -0.02 | -0.15* | -0.23*** | | | | | | |
| Harvest index | 0.01 | -0.29*** | 0.17** | 0.34*** | | | | | |
| Pod harvest index | -0.07 | -0.25*** | 0.06 | 0.29*** | 0.66*** | | | | |
| 100-seed weight | -0.01 | 0.09 | 0.03 | -0.14* | 0.15 | 0.20*** | | | |
| Seed yield | 0.01 | -0.24*** | 0.21*** | 0.27*** | 0.44*** | 0.27*** | 0.11 | | |
| Seed yield/day | -0.05 | -0.33*** | 0.21** | 0.27*** | 0.44*** | 0.28*** | 0.10 | 0.99*** | |
| Days to seed fill | -0.11 | 0.77*** | 0.03 | -0.18** | -0.35*** | -0.24*** | 0.11 | -0.30*** | -0.37*** |

* Significant at α=0.05 ; ** Significant at α=0.01; *** Significant at α=0.001

## Appendix B.

Interacting QTL from ICIM scans of the SEA5/CAL96 RIL population

Table B. 1. Significant digenic interacting QTL from ICIM scan of the SEA5/CAL96 RIL population in combined environment analysis.

| Environment† | Trait†† | LG‡‡ | Position1§ | Flanking markers1¶ | LG | Position2 | Flanking Marker2 | LOD# | $R^2$ (%)‡ | Add By Add¶¶ |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | DF | 1 | 30 | BMd45-PVBR31 | 2 | 0 | GATS54-GATS11 | 21.03 | 8.27 | -0.98 |
| RF | DF | 1 | 40 | IAC76-PVM126 | 2 | 30 | BM172-BM142 | 10.48 | 3.71 | -0.60 |
| RF | DF | 1 | 60 | PVM126-IAC69 | 6 | 65 | PVBR14-CLP | 7.40 | 1.68 | 0.37 |
| RF | DF | 1 | 85 | PVM126-IAC69 | 2 | 80 | PVgccacc0001-BM152 | 14.22 | 6.05 | 0.72 |
| RF | DF | 2 | 0 | GATS54-GATS11 | 11 | 5 | PVBR113-INDEL11 | 16.25 | 6.07 | -0.77 |
| RF | DF | 2 | 15 | GATS11-BM164 | 2 | 125 | IAC4-PVM127 | 16.87 | 7.92 | -0.84 |
| RF | DF | 2 | 30 | BM172-BM142 | 2 | 170 | PVM11-PVM115 | 6.57 | 1.86 | 0.43 |
| RF | DF | 2 | 30 | BM172-BM142 | 6 | 15 | PVBR5-PVBR20 | 6.05 | 1.86 | 0.42 |
| RF | DF | 2 | 75 | PVBR125-PVgccacc01 | 6 | 5 | INDEL51-INDEL05 | 30.75 | 21.53 | 1.33 |
| RF | DF | 2 | 80 | PVgccacc01-BM152 | 7 | 65 | PVBR269-PVBR35 | 7.13 | 1.98 | 0.45 |
| RF | DF | 2 | 125 | IAC4-PVM127 | 3 | 60 | PVBR21-PVBR23 | 16.69 | 7.02 | -1.31 |
| RF | DF | 2 | 125 | IAC4-PVM127 | 9 | 15 | IAC62-PVat0007 | 35.31 | 25.47 | -1.47 |
| RF | DF | 2 | 130 | IAC4-PVM127 | 9 | 35 | BM114-PVBR199 | 7.08 | 2.11 | -0.41 |
| RF | DF | 2 | 140 | PVM127-IAC71 | 3 | 60 | PVBR21-PVBR23 | 14.57 | 6.94 | -1.27 |
| RF | DF | 3 | 0 | PVag0001-PVBR255 | 7 | 0 | PVBR69-BM160 | 8.15 | 2.46 | -0.45 |
| RF | DF | 3 | 35 | PVBR87-BM159 | 7 | 105 | BM170-PVBR167 | 19.84 | 4.99 | -1.02 |

†RF: Rainfed; RFS: Rainfed supplemented; ‡‡Chromosome ID at scanning position; §Scanning position in cM of the QTL; ¶ Marker interval for the scanning position 1and 2, # LOD score caused by epistasis effects, ‡ Phenotypic variation explained by epistatic QTL effects; ¶¶ additive by additive effect of QTL at the two scanning positions. †† days to flowering (DF), days to maturity (DM), days to seed fill (DSF), number of pods per plant (NP), number of seed per pod (NSP), Harvest index (HI), pod harvest index (PHI), seed weight (SW), seed yield (SY), and seed yield per day (SYD)

Table B. 1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | DF | 3 | 35 | PVBR87-BM159 | 7 | 120 | BM170-PVBR167 | 10.15 | 11.31 | -1.02 |
| RF | DF | 3 | 40 | PVBR87-BM159 | 7 | 85 | INDEL13-BM170 | 5.53 | 11.58 | -1.02 |
| RF | DF | 3 | 55 | BM159- PVBR21 | 6 | 0 | INDEL03- PVM21 | 6.14 | 17.96 | 4.08 |
| RF | DF | 3 | 60 | PVBR21- PVBR23 | 3 | 70 | AG1- BMd1 | 15.99 | 9.16 | -1.27 |
| RF | DF | 3 | 60 | PVBR21- PVBR23 | 4 | 25 | INDEL30- INDEL109 | 10.37 | 4.45 | -1.39 |
| RF | DF | 3 | 60 | PVBR21- PVBR23 | 8 | 10 | BM181- PVBR53 | 15.01 | 6.61 | -1.08 |
| RF | DF | 3 | 60 | PVBR21- PVBR23 | 9 | 55 | BM141- IAC68 | 12.32 | 6.95 | -1.29 |
| RF | DF | 3 | 80 | PVat0008- PVM148 | 5 | 35 | BMd53- PVBR124 | 7.24 | 2.37 | 0.44 |
| RF | DF | 4 | 20 | INDEL30- INDEL109 | 7 | 70 | PVBR269- PVBR35 | 15.36 | 5.19 | 0.69 |
| RF | DF | 4 | 20 | INDEL30- INDEL109 | 9 | 20 | PVat0007- BM114 | 6.10 | 1.87 | 0.43 |
| RF | DF | 4 | 60 | INDEL109- IAC67 | 7 | 100 | INDEL13- BM170 | 5.86 | 1.46 | 0.37 |
| RF | DF | 4 | 75 | IAC66- BM161 | 8 | 75 | BM153- PVaaat0001 | 15.51 | 17.13 | -1.36 |
| RF | DF | 4 | 90 | IAC66- BM161 | 8 | 75 | BM153- PVaaat0001 | 31.80 | 18.05 | -1.36 |
| RF | DF | 4 | 90 | IAC66- BM161 | 11 | 60 | INDEL10- PVM30 | 10.32 | 2.97 | 0.51 |
| RF | DF | 5 | 0 | PVBR24- PVBR61 | 8 | 25 | BM181- PVBR53 | 16.92 | 6.94 | -0.76 |
| RF | DF | 6 | 0 | INDEL03- PVM21 | 9 | 35 | BM114- PVBR199 | 17.59 | 6.24 | 0.73 |
| RF | DM | 1 | 25 | BM165- BMd45 | 2 | 65 | IAC51- PVBR94 | 13.45 | 7.38 | -1.04 |
| RF | DM | 1 | 30 | BMd45- PVBR31 | 1 | 40 | IAC76- PVM126 | 10.11 | 7.61 | -1.78 |
| RF | DM | 1 | 30 | BMd45- PVBR31 | 6 | 65 | PVBR14- CLP | 17.28 | 10.76 | -1.25 |
| RF | DM | 1 | 35 | PVBR218- PVBR250 | 7 | 30 | BM160- INDEL112 | 12.03 | 5.45 | -0.98 |
| RF | DM | 1 | 85 | PVM126- IAC69 | 2 | 60 | IAC51- PVBR94 | 14.71 | 10.31 | 1.18 |
| RF | DM | 2 | 30 | BM172- BM142 | 9 | 45 | BM141- IAC68 | 25.88 | 17.65 | 1.64 |
| RF | DM | 2 | 65 | IAC51- PVBR94 | 2 | 155 | IAC71- PVM11 | 19.48 | 9.04 | -1.09 |
| RF | DM | 2 | 70 | BMd17- PVBR125 | 7 | 80 | PVBR35- INDEL13 | 12.10 | 5.72 | -0.87 |
| RF | DM | 2 | 80 | PVgccacc0001- BM152 | 4 | 90 | IAC66- BM161 | 21.57 | 15.68 | 1.58 |
| RF | DM | 2 | 80 | PVgccacc0001- BM152 | 7 | 0 | PVBR69- BM160 | 25.57 | 20.38 | 1.67 |
| RF | DM | 2 | 80 | PVgccacc0001- BM152 | 7 | 20 | PVBR69- BM160 | 8.76 | 14.94 | 1.64 |
| RF | DM | 2 | 135 | PVM127- IAC71 | 7 | 60 | PVBR67- INDEL01 | 17.52 | 8.71 | 1.11 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | DM | 3 | 50 | PVBR87- BM159 | 6 | 15 | PVBR5- PVBR20 | 17.11 | 7.85 | 1.04 |
| RF | DM | 3 | 110 | PVBR169- IAC70 | 4 | 40 | INDEL109- IAC67 | 8.66 | 2.84 | 0.61 |
| RF | DM | 3 | 110 | PVBR169- IAC70 | 5 | 0 | PVBR24- PVBR61 | 6.98 | 3.63 | -0.70 |
| RF | DM | 3 | 135 | PVBR169- IAC70 | 7 | 60 | PVBR67- INDEL01 | 23.79 | 12.71 | -1.31 |
| RF | DM | 4 | 15 | PVBR242- BMd16 | 9 | 15 | IAC62- PVat0007 | 23.24 | 29.58 | -2.18 |
| RF | DM | 5 | 0 | PVBR24- PVBR61 | 6 | 5 | INDEL51- INDEL05 | 13.81 | 6.28 | 0.93 |
| RF | DM | 5 | 60 | BMd50- IAC10 | 6 | 0 | INDEL03- PVM21 | 12.32 | 4.13 | 0.86 |
| RF | DM | 5 | 70 | BMd50- IAC10 | 8 | 75 | BM153- PVaaat0001 | 22.68 | 11.61 | -1.26 |
| RF | DM | 6 | 10 | INDEL51- INDEL05 | 7 | 55 | INDEL112- PVBR67 | 34.98 | 31.28 | 2.03 |
| RF | DM | 6 | 65 | PVBR14- CLP | 9 | 70 | BM141- IAC68 | 11.25 | 4.21 | 0.77 |
| RF | DM | 7 | 80 | PVBR35- INDEL13 | 7 | 105 | BM170- PVBR167 | 8.80 | 3.37 | 0.68 |
| RF | DM | 8 | 25 | BM181- PVBR53 | 8 | 55 | BM153- PVaaat0001 | 44.13 | 48.47 | -2.61 |
| RF | DM | 8 | 40 | PVBR53- PVBR45 | 8 | 55 | BM153- PVaaat0001 | 24.27 | 44.27 | -2.55 |
| RF | DM | 9 | 25 | PVat0007- BM114 | 9 | 40 | PVBR199- BM141 | 8.41 | 5.29 | 0.97 |
| RF | NPP | 1 | 25 | BM165- BMd45 | 4 | 0 | PVBR242- BMd16 | 16.14 | 6.20 | -0.35 |
| RF | NPP | 1 | 35 | PVBR218- PVBR250 | 4 | 40 | INDEL109- IAC67 | 35.75 | 9.48 | -0.42 |
| RF | NPP | 1 | 60 | PVM126- IAC69 | 5 | 50 | PVBR93- PVat0006b | 18.53 | 2.73 | 0.23 |
| RF | NPP | 1 | 60 | PVM126- IAC69 | 6 | 40 | PVBR14- CLP | 36.75 | 8.30 | -0.39 |
| RF | NPP | 2 | 0 | GATS54- GATS11 | 3 | 60 | PVBR21- PVBR23 | 10.55 | 1.09 | 0.15 |
| RF | NPP | 2 | 30 | BM172- BM142 | 2 | 125 | IAC4- PVM127 | 32.17 | 9.89 | -0.44 |
| RF | NPP | 2 | 30 | BM172- BM142 | 11 | 0 | BMd22- PVBR113 | 19.89 | 2.66 | 0.24 |
| RF | NPP | 2 | 65 | IAC51- PVBR94 | 7 | 105 | BM170- PVBR167 | 15.03 | 1.54 | 0.17 |
| RF | NPP | 2 | 65 | IAC51- PVBR94 | 11 | 0 | BMd22- PVBR113 | 42.07 | 10.01 | 0.44 |
| RF | NPP | 2 | 90 | BM152- PVBR11 | 7 | 25 | PVBR69- BM160 | 11.96 | 1.18 | -0.15 |
| RF | NPP | 2 | 100 | BM152- PVBR11 | 11 | 60 | INDEL10- PVM30 | 7.41 | 0.88 | -0.12 |
| RF | NPP | 2 | 120 | PVBR11- IAC4 | 7 | 70 | PVBR269- PVBR35 | 45.67 | 24.10 | 0.67 |
| RF | NPP | 2 | 150 | PVM127- IAC71 | 4 | 40 | INDEL109- IAC67 | 53.54 | 33.63 | 0.78 |

Table B. 1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | NPP | 2 | 150 | PVM127- IAC71 | 10 | 20 | IAC6- PVBR185 | 7.16 | 0.80 | 0.12 |
| RF | NPP | 2 | 170 | PVM11- PVM115 | 6 | 40 | PVBR14- CLP | 44.93 | 15.31 | -0.54 |
| RF | NPP | 3 | 0 | PVag0001- PVBR255 | 3 | 70 | AG1- BMd1 | 49.05 | 20.99 | -0.61 |
| RF | NPP | 3 | 25 | PVBR255- BM189 | 5 | 55 | PVat0006- BMd50 | 18.02 | 7.28 | -0.48 |
| RF | NPP | 3 | 25 | PVBR255- BM189 | 7 | 60 | PVBR67- INDEL01 | 12.39 | 1.26 | -0.19 |
| RF | NPP | 3 | 75 | PVat0008- PVM148 | 9 | 15 | IAC62- PVat0007 | 24.65 | 3.67 | -0.27 |
| RF | NPP | 3 | 110 | PVBR169- IAC70 | 11 | 0 | BMd22- PVBR113 | 20.29 | 2.88 | 0.23 |
| RF | NPP | 4 | 40 | INDEL109- IAC67 | 4 | 70 | IAC66- BM161 | 21.16 | 2.77 | 0.24 |
| RF | NPP | 4 | 40 | INDEL109- IAC67 | 10 | 0 | IAC6- PVBR185 | 30.70 | 6.65 | 0.34 |
| RF | NPP | 5 | 0 | PVBR24- PVBR61 | 7 | 25 | PVBR69- BM160 | 17.42 | 2.14 | 0.19 |
| RF | NPP | 5 | 30 | PVBR61- BMd53 | 11 | 25 | INDEL11- INDEL29 | 26.69 | 5.27 | 0.33 |
| RF | NPP | 5 | 35 | BMd53- PVBR124 | 5 | 55 | PVat0006- BMd50 | 19.16 | 2.37 | -0.23 |
| RF | NPP | 5 | 35 | BMd53- PVBR124 | 7 | 50 | INDEL112- PVBR67 | 15.29 | 2.43 | -0.21 |
| RF | NPP | 5 | 35 | BMd53- PVBR124 | 11 | 5 | PVBR113- INDEL11 | 13.83 | 1.95 | 0.19 |
| RF | NPP | 7 | 60 | PVBR67- INDEL01 | 7 | 75 | PVBR35- INDEL13 | 32.52 | 9.28 | -0.52 |
| RF | NPP | 7 | 125 | BM170- PVBR167 | 8 | 75 | BM153- PVaaat0001 | 17.16 | 1.81 | -0.18 |
| RF | NPP | 8 | 0 | BM181- PVBR53 | 9 | 15 | IAC62- PVat0007 | 18.98 | 2.88 | 0.23 |
| RF | NPP | 11 | 5 | PVBR113- INDEL11 | 11 | 60 | INDEL10- PVM30 | 45.09 | 25.99 | -0.67 |
| RF | NSP | 1 | 25 | BM165- BMd45 | 8 | 25 | BM181- PVBR53 | 10.33 | 7.48 | -0.13 |
| RF | NSP | 1 | 30 | BMd45- PVBR31 | 10 | 25 | PVBR185- BMd42 | 6.35 | 5.18 | -0.10 |
| RF | NSP | 2 | 40 | BM172- BM142 | 4 | 40 | INDEL109- IAC67 | 10.65 | 6.78 | 0.11 |
| RF | NSP | 2 | 80 | PVgccacc0001- BM152 | 6 | 15 | PVBR5- PVBR20 | 16.79 | 16.36 | 0.18 |
| RF | NSP | 2 | 105 | PVBR11- IAC4 | 7 | 125 | BM170- PVBR167 | 8.23 | 5.26 | 0.10 |
| RF | NSP | 2 | 135 | PVM127- IAC71 | 6 | 20 | PVBR20- PVBR14 | 7.78 | 7.65 | 0.12 |
| RF | NSP | 3 | 35 | PVBR87- BM159 | 3 | 60 | PVBR21- PVBR23 | 5.32 | 3.20 | -0.09 |
| RF | NSP | 4 | 15 | PVBR242- BMd16 | 11 | 30 | INDEL11- INDEL29 | 9.06 | 11.72 | 0.16 |
| RF | NSP | 4 | 60 | INDEL109- IAC67 | 11 | 30 | INDEL11- INDEL29 | 23.91 | 20.64 | -0.20 |
| RF | NSP | 5 | 0 | PVBR24- PVBR61 | 5 | 60 | BMd50- IAC10 | 15.90 | 14.19 | 0.17 |
| RF | NSP | 5 | 0 | PVBR24- PVBR61 | 9 | 15 | IAC62- PVat0007 | 22.68 | 19.33 | 0.19 |

Table B. 1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | NSP | 5 | 85 | IAC10- PVBR235 | 7 | 80 | PVBR35- INDEL13 | 23.99 | 25.68 | -0.23 |
| RF | NSP | 7 | 65 | PVBR269- PVBR35 | 7 | 105 | BM170- PVBR167 | 13.86 | 10.29 | -0.14 |
| RF | NSP | 7 | 75 | PVBR35- INDEL13 | 11 | 60 | INDEL10- PVM30 | 5.16 | 3.46 | -0.08 |
| RF | NSP | 8 | 45 | PVBR53- PVBR45 | 8 | 75 | BM153- PVaaat0001 | 8.96 | 6.00 | -0.11 |
| RF | HI | 1 | 0 | BM165- BMd45 | 2 | 15 | GATS11- BM164 | 18.59 | 5.77 | 0.02 |
| RF | HI | 1 | 35 | PVBR218- PVBR250 | 2 | 90 | BM152- PVBR11 | 25.53 | 9.75 | 0.02 |
| RF | HI | 1 | 35 | PVBR218- PVBR250 | 11 | 35 | PVM98- INDEL14 | 35.18 | 31.09 | -0.03 |
| RF | HI | 1 | 60 | PVM126- IAC69 | 4 | 60 | INDEL109- IAC67 | 16.40 | 2.75 | -0.01 |
| RF | HI | 1 | 60 | PVM126- IAC69 | 5 | 0 | PVBR24- PVBR61 | 11.82 | 2.16 | -0.01 |
| RF | HI | 1 | 85 | PVM126- IAC69 | 2 | 125 | IAC4- PVM127 | 10.08 | 2.27 | -0.01 |
| RF | HI | 2 | 15 | GATS11- BM164 | 9 | 70 | BM141- IAC68 | 11.02 | 2.59 | 0.01 |
| RF | HI | 2 | 40 | BM172- BM142 | 3 | 0 | PVag0001- PVBR255 | 22.43 | 6.70 | 0.02 |
| RF | HI | 2 | 45 | PVBR149- BMd7 | 7 | 25 | PVBR69- BM160 | 16.44 | 4.11 | 0.01 |
| RF | HI | 2 | 55 | IAC90- IAC51 | 7 | 70 | PVBR269- PVBR35 | 20.90 | 6.98 | 0.02 |
| RF | HI | 2 | 90 | BM152- PVBR11 | 2 | 125 | IAC4- PVM127 | 29.07 | 12.81 | -0.02 |
| RF | HI | 2 | 90 | BM152- PVBR11 | 4 | 90 | IAC66- BM161 | 12.65 | 2.26 | 0.01 |
| RF | HI | 2 | 100 | BM152- PVBR11 | 7 | 0 | PVBR69- BM160 | 12.18 | 2.22 | -0.01 |
| RF | HI | 2 | 120 | PVBR11- IAC4 | 9 | 45 | BM141- IAC68 | 34.73 | 22.04 | -0.03 |
| RF | HI | 2 | 155 | IAC71- PVM11 | 6 | 65 | PVBR14- CLP | 6.55 | 1.39 | 0.01 |
| RF | HI | 2 | 170 | PVM11- PVM115 | 5 | 0 | PVBR24- PVBR61 | 8.26 | 1.48 | 0.01 |
| RF | HI | 3 | 0 | PVag0001- PVBR255 | 7 | 0 | PVBR69- BM160 | 14.21 | 2.97 | -0.01 |
| RF | HI | 3 | 25 | PVBR255- BM189 | 9 | 40 | PVBR199- BM141 | 32.01 | 15.90 | -0.03 |
| RF | HI | 3 | 30 | PVBR255- BM189 | 5 | 70 | BMd50- IAC10 | 13.31 | 2.60 | 0.01 |
| RF | HI | 3 | 45 | PVBR87- BM159 | 7 | 90 | INDEL13- BM170 | 21.13 | 12.25 | 0.02 |
| RF | HI | 3 | 55 | BM159- PVBR21 | 7 | 105 | BM170- PVBR167 | 39.39 | 14.57 | 0.03 |
| RF | HI | 3 | 70 | AG1- BMd1 | 8 | 75 | BM153- PVaaat0001 | 38.60 | 16.92 | -0.02 |
| RF | HI | 3 | 105 | PVM148- PVBR169 | 4 | 40 | INDEL109- IAC67 | 9.54 | 2.08 | -0.01 |
| RF | HI | 3 | 135 | PVBR169- IAC70 | 4 | 20 | INDEL30- INDEL109 | 7.94 | 2.73 | 0.01 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | HI | 3 | 135 | PVBR169- IAC70 | 5 | 70 | BMd50- IAC10 | 11.89 | 2.61 | 0.01 |
| RF | HI | 3 | 135 | PVBR169- IAC70 | 8 | 25 | BM181- PVBR53 | 8.84 | 1.38 | 0.01 |
| RF | HI | 3 | 135 | PVBR169- IAC70 | 11 | 0 | BMd22- PVBR113 | 14.27 | 3.49 | -0.01 |
| RF | HI | 4 | 60 | INDEL109- IAC67 | 11 | 60 | INDEL10- PVM30 | 9.84 | 2.13 | 0.01 |
| RF | HI | 5 | 0 | PVBR24- PVBR61 | 9 | 0 | IAC62- PVat0007 | 38.11 | 17.94 | 0.03 |
| RF | HI | 5 | 45 | BM175- PVBR93 | 8 | 75 | BM153- PVaaat0001 | 11.57 | 2.22 | 0.01 |
| RF | HI | 5 | 50 | PVBR93- PVat0006b | 11 | 60 | INDEL10- PVM30 | 12.58 | 3.60 | -0.01 |
| RF | HI | 7 | 25 | PVBR69- BM160 | 9 | 25 | PVat0007- BM114 | 39.00 | 15.67 | 0.02 |
| RF | HI | 7 | 55 | INDEL112- PVBR67 | 9 | 0 | IAC62- PVat0007 | 36.78 | 18.72 | -0.03 |
| RF | PHI | 2 | 95 | BM152- PVBR11 | 7 | 50 | INDEL112- PVBR67 | 6.02 | 16.34 | -0.02 |
| RF | PHI | 3 | 85 | PVat0008-P VM148 | 11 | 60 | INDEL10- PVM30 | 6.41 | 14.33 | -0.02 |
| RF | PHI | 3 | 135 | PVBR169- IAC70 | 6 | 10 | INDEL51- INDEL05 | 11.19 | 22.84 | 0.02 |
| RF | PHI | 5 | 30 | PVBR61- BMd53 | 6 | 65 | PVBR14- CLP | 5.02 | 9.22 | -0.02 |
| RF | PHI | 9 | 40 | PVBR199- BM141 | 11 | 25 | INDEL11- INDEL29 | 8.37 | 27.67 | -0.03 |
| RF | SW | 1 | 60 | PVM126- IAC69 | 4 | 0 | PVBR242- BMd16 | 10.86 | 15.48 | 1.73 |
| RF | SW | 1 | 60 | PVM126- IAC69 | 10 | 20 | IAC6- PVBR185 | 8.41 | 8.74 | -1.31 |
| RF | SW | 2 | 70 | BMd17- PVBR125 | 3 | 70 | AG1- BMd1 | 13.77 | 17.20 | 1.85 |
| RF | SW | 3 | 85 | PVat0008- PVM148 | 4 | 20 | INDEL30- INDEL109 | 7.24 | 6.12 | 1.12 |
| RF | SW | 4 | 60 | INDEL109- IAC67 | 10 | 25 | PVBR185- BMd42 | 6.65 | 6.47 | -1.14 |
| RF | SW | 5 | 30 | PVBR61- BMd53 | 10 | 25 | PVBR185- BMd42 | 20.99 | 33.82 | 2.53 |
| RF | SY | 1 | 85 | PVM126- IAC69 | 3 | 30 | PVBR255- BM189 | 5.58 | 2.33 | -4.45 |
| RF | SY | 1 | 85 | PVM126- IAC69 | 11 | 0 | BMd22- PVBR113 | 13.77 | 9.39 | -5.77 |
| RF | SY | 1 | 85 | PVM126- IAC69 | 11 | 35 | PVM98- INDEL14 | 12.62 | 8.35 | -5.51 |
| RF | SY | 2 | 0 | GATS54- GATS11 | 2 | 25 | PVM49- BM172 | 10.28 | 5.30 | -4.83 |
| RF | SY | 2 | 45 | PVBR149- BMd7 | 2 | 150 | PVM127- IAC71 | 8.93 | 4.50 | -3.99 |
| RF | SY | 2 | 55 | IAC90- IAC51 | 3 | 50 | PVBR87- BM159 | 15.10 | 12.50 | 6.79 |
| RF | SY | 2 | 70 | BMd17- PVBR125 | 9 | 0 | IAC62- PVat0007 | 10.04 | 5.82 | 4.49 |
| RF | SY | 3 | 70 | AG1- BMd1 | 11 | 35 | PVM98- INDEL14 | 13.43 | 14.64 | -7.46 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | SY | 3 | 70 | AG1- BMd1 | 11 | 60 | INDEL10- PVM30 | 18.48 | 13.08 | -7.05 |
| RF | SY | 3 | 85 | PVat0008- PVM148 | 3 | 135 | PVBR169- IAC70 | 12.72 | 7.12 | -5.16 |
| RF | SY | 3 | 85 | PVat0008- PVM148 | 4 | 20 | INDEL30- INDEL109 | 9.48 | 5.22 | 4.60 |
| RF | SY | 3 | 85 | PVat0008- PVM148 | 11 | 60 | INDEL10- PVM30 | 9.33 | 4.64 | 4.08 |
| RF | SY | 3 | 135 | PVBR169- IAC70 | 11 | 35 | PVM98- INDEL14 | 9.86 | 6.03 | 4.63 |
| RF | SY | 4 | 40 | INDEL109- IAC67 | 5 | 45 | BM175- PVBR93 | 20.45 | 17.31 | 8.35 |
| RF | SY | 5 | 45 | BM175- PVBR93 | 6 | 0 | INDEL03- PVM21 | 19.57 | 17.56 | 8.91 |
| RF | SY | 7 | 75 | PVBR35- INDEL13 | 8 | 75 | BM153- PVaaat0001 | 5.50 | 2.80 | -3.21 |
| RF | SY D | 1 | 85 | PVM126- IAC69 | 3 | 60 | PVBR21- PVBR23 | 8.54 | 10.79 | -0.07 |
| RF | SYD | 1 | 85 | PVM126- IAC69 | 11 | 0 | BMd22- PVBR113 | 5.46 | 7.50 | -0.06 |
| RF | SYD | 3 | 70 | AG1- BMd1 | 5 | 45 | BM175- PVBR93 | 6.07 | 10.84 | -0.08 |
| RF | SYD | 3 | 105 | PVM148- PVBR169 | 7 | 60 | PVBR67- INDEL01 | 10.10 | 14.76 | -0.08 |
| RF | SYD | 4 | 0 | PVBR242- BMd16 | 6 | 15 | PVBR5- PVBR20 | 5.83 | 10.35 | -0.07 |
| RF | SYD | 5 | 45 | BM175- PVBR93 | 6 | 0 | INDEL03- PVM21 | 8.90 | 16.38 | 0.10 |
| RF | SYD | 6 | 0 | INDEL03- PVM21 | 6 | 20 | PVBR20- PVBR14 | 5.34 | 7.25 | 0.07 |
| RF | DSF | 1 | 0 | BM165- BMd45 | 3 | 70 | AG1- BMd1 | 26.52 | 24.89 | 1.34 |
| RF | DSF | 1 | 25 | BM165- BMd45 | 3 | 35 | PVBR87- BM159 | 7.05 | 3.68 | -0.65 |
| RF | DSF | 1 | 60 | PVM126- IAC69 | 4 | 15 | PVBR242- BMd16 | 14.10 | 18.80 | -1.28 |
| RF | DSF | 2 | 60 | IAC51- PVBR94 | 10 | 20 | IAC6- PVBR185 | 13.02 | 9.49 | 0.81 |
| RF | DSF | 2 | 65 | IAC51- PVBR94 | 7 | 65 | PVBR269- PVBR35 | 7.33 | 3.65 | 0.51 |
| RF | DSF | 2 | 90 | BM152- PVBR11 | 11 | 35 | PVM98- INDEL14 | 9.27 | 6.69 | -0.69 |
| RF | DSF | 2 | 130 | IAC4- PVM127 | 9 | 0 | IAC62- PVat0007 | 14.37 | 10.14 | -0.85 |
| RF | DSF | 2 | 170 | PVM11- PVM115 | 7 | 25 | PVBR69- BM160 | 13.46 | 7.33 | -0.72 |
| RF | DSF | 2 | 170 | PVM11- PVM115 | 7 | 80 | PVBR35- INDEL13 | 17.60 | 11.05 | -0.88 |
| RF | DSF | 3 | 0 | PVag0001- PVBR255 | 10 | 0 | IAC6- PVBR185 | 6.50 | 3.32 | 0.49 |
| RF | DSF | 3 | 75 | PVat0008- PVM148 | 10 | 25 | PVBR185- BMd42 | 13.07 | 8.99 | -0.82 |
| RF | DSF | 4 | 40 | INDEL109- IAC67 | 5 | 60 | BMd50- IAC10 | 16.34 | 8.55 | 0.81 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RF | DSF | 5 | 0 | PVBR24- PVBR61 | 6 | 5 | INDEL51- INDEL05 | 23.59 | 19.59 | 1.19 |
| RF | DSF | 5 | 50 | PVBR93- PVat0006b | 9 | 45 | BM141- IAC68 | 19.59 | 16.18 | -1.15 |
| RF | DSF | 5 | 50 | PVBR93- PVat0006b | 11 | 35 | PVM98- INDEL14 | 19.21 | 17.39 | -1.29 |
| RF | DSF | 6 | 0 | INDEL03- PVM21 | 10 | 0 | IAC6- PVBR185 | 22.22 | 19.10 | -1.18 |
| RF | DSF | 6 | 40 | PVBR14- CLP | 7 | 0 | PVBR69- BM160 | 14.07 | 6.37 | 0.68 |
| RF | DSF | 7 | 25 | PVBR69- BM160 | 8 | 45 | PVBR53- PVBR45 | 6.76 | 5.76 | -0.64 |
| RF | DSF | 7 | 30 | BM160- INDEL112 | 11 | 40 | INDEL10- PVM30 | 6.51 | 4.64 | -0.56 |
| RF | DSF | 7 | 45 | BM160- INDEL112 | 7 | 65 | PVBR269- PVBR35 | 6.62 | 4.78 | 0.62 |
| RF | DSF | 9 | 45 | BM141- IAC68 | 11 | 35 | PVM98- INDEL14 | 27.10 | 29.88 | 1.46 |
| RF | DSF | 10 | 0 | IAC6- PVBR185 | 11 | 40 | INDEL10- PVM30 | 9.44 | 6.34 | 0.66 |
| RFS | DM | 1 | 30 | BMd45- PVBR31 | 2 | 5 | GATS11- BM164 | 18.46 | 21.63 | 1.57 |
| RFS | DM | 1 | 30 | BMd45- PVBR31 | 6 | 40 | PVBR14- CLP | 29.56 | 50.53 | -2.61 |
| RFS | DM | 2 | 50 | PVBR149- BMd7 | 3 | 105 | PVM148- PVBR169 | 9.85 | 9.68 | -0.98 |
| RFS | DM | 2 | 55 | IAC90- IAC51 | 7 | 70 | PVBR269- PVBR35 | 11.42 | 12.70 | -1.18 |
| RFS | DM | 2 | 70 | BMd17- PVBR125 | 7 | 80 | PVBR35- INDEL13 | 12.39 | 11.40 | -1.06 |
| RFS | DM | 2 | 80 | PVgccacc0001- BM152 | 7 | 60 | PVBR67- INDEL01 | 14.96 | 13.15 | 1.17 |
| RFS | DM | 2 | 100 | BM152 PVBR11 | 9 | 0 | IAC62- PVat0007 | 5.40 | 3.02 | 0.55 |
| RFS | DM | 2 | 160 | PVM11- PVM115 | 9 | 15 | IAC62- PVat0007 | 10.11 | 7.31 | 0.87 |
| RFS | DM | 3 | 60 | PVBR21- PVBR23 | 6 | 15 | PVBR5- PVBR20 | 22.27 | 21.41 | 1.48 |
| RFS | DM | 3 | 75 | PVat0008- PVM148 | 7 | 100 | INDEL13- BM170 | 5.75 | 3.99 | 0.65 |
| RFS | DM | 4 | 40 | INDEL109- IAC67 | 7 | 50 | INDEL112- PVBR67 | 8.97 | 6.52 | 0.83 |
| RFS | DM | 5 | 110 | PVBR235- PVBR131 | 7 | 60 | PVBR67- INDEL01 | 6.14 | 4.23 | 0.66 |
| RFS | DM | 6 | 40 | PVBR14- CLP | 7 | 50 | INDEL112- PVBR67 | 23.29 | 24.32 | -1.80 |
| RFS | DM | 7 | 0 | PVBR69- BM160 | 7 | 105 | BM170- PVBR167 | 9.85 | 7.50 | 0.88 |
| RFS | DM | 7 | 100 | INDEL13- BM170 | 11 | 40 | INDEL10- PVM30 | 8.54 | 8.17 | 0.92 |
| RFS | DM | 8 | 20 | BM181- PVBR53 | 9 | 40 | PVBR199- BM141 | 5.29 | 4.73 | -0.69 |
| RFS | DM | 8 | 75 | BM153- PVaaat0001 | 9 | 0 | IAC62- PVat0007 | 16.25 | 12.46 | 1.12 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | NPP | 1 | 0 | BM165- BMd45 | 4 | 90 | IAC66- BM161 | 9.77 | 5.34 | -0.38 |
| RFS | NPP | 2 | 5 | GATS11- BM164 | 11 | 5 | PVBR113- INDEL11 | 13.55 | 15.05 | 0.61 |
| RFS | NPP | 2 | 20 | BM164- PVM49 | 7 | 100 | INDEL13- BM170 | 13.08 | 7.72 | -0.47 |
| RFS | NPP | 2 | 30 | BM172- BM142 | 9 | 15 | IAC62- PVat0007 | 19.72 | 17.07 | 0.68 |
| RFS | NPP | 2 | 40 | BM172- BM142 | 2 | 90 | BM152- PVBR11 | 7.99 | 4.85 | -0.35 |
| RFS | NPP | 2 | 50 | PVBR149- BMd7 | 7 | 70 | PVBR269- PVBR35 | 10.34 | 6.01 | -0.39 |
| RFS | NPP | 2 | 100 | BM152- PVBR11 | 3 | 0 | PVag0001- PVBR255 | 6.11 | 3.38 | -0.28 |
| RFS | NPP | 2 | 125 | IAC4- PVM127 | 8 | 50 | PVBR45- BM153 | 10.42 | 7.45 | 0.42 |
| RFS | NPP | 3 | 35 | PVBR87- BM159 | 9 | 15 | IAC62- PVat0007 | 5.14 | 2.66 | -0.29 |
| RFS | NPP | 3 | 65 | PVBR23- AG1 | 5 | 90 | PVBR235- PVBR131 | 5.34 | 3.97 | -0.32 |
| RFS | NPP | 3 | 70 | AG1- BMd1 | 4 | 0 | PVBR242- BMd16 | 7.19 | 4.46 | -0.33 |
| RFS | NPP | 3 | 105 | PVM148- PVBR169 | 5 | 50 | PVBR93- PVat0006b | 8.69 | 5.09 | -0.36 |
| RFS | NPP | 4 | 90 | IAC66- BM161 | 7 | 125 | BM170- PVBR167 | 6.10 | 4.59 | -0.34 |
| RFS | NPP | 5 | 0 | PVBR24- PVBR61 | 6 | 5 | INDEL51- INDEL05 | 10.00 | 6.04 | 0.38 |
| RFS | NPP | 5 | 45 | BM175- PVBR93 | 6 | 0 | INDEL03- PVM21 | 6.37 | 4.49 | 0.36 |
| RFS | NPP | 5 | 45 | BM175- PVBR93 | 8 | 0 | BM181- PVBR53 | 6.15 | 3.06 | -0.27 |
| RFS | NPP | 7 | 0 | PVBR69- BM160 | 11 | 35 | PVM98- INDEL14 | 10.93 | 6.82 | 0.40 |
| RFS | NPP | 7 | 55 | INDEL112- PVBR67 | 7 | 125 | BM170- PVBR167 | 8.38 | 5.48 | -0.35 |
| RFS | NPP | 7 | 55 | INDEL112- PVBR67 | 9 | 70 | BM141- IAC68 | 19.22 | 21.02 | 0.71 |
| RFS | NPP | 8 | 25 | BM181- PVBR53 | 9 | 0 | IAC62- PVat0007 | 22.95 | 15.15 | 0.59 |
| RFS | NPP | 9 | 15 | IAC62- PVat0007 | 11 | 30 | INDEL11- INDEL29 | 11.96 | 8.56 | -0.45 |
| RFS | NPP | 9 | 35 | BM114- PVBR199 | 10 | 25 | PVBR185- BMd42 | 18.37 | 24.24 | 0.75 |
| RFS | NSP | 1 | 30 | BMd45- PVBR31 | 5 | 30 | PVBR61- BMd53 | 28.42 | 17.17 | -0.23 |
| RFS | NSP | 1 | 60 | PVM126- IAC69 | 8 | 0 | BM181- PVBR53 | 58.10 | 61.13 | 0.41 |
| RFS | NSP | 2 | 5 | GATS11- BM164 | 7 | 65 | PVBR269- PVBR35 | 11.36 | 3.34 | 0.11 |
| RFS | NSP | 2 | 20 | BM164- PVM49 | 9 | 70 | BM141- IAC68 | 7.45 | 1.58 | 0.08 |
| RFS | NSP | 2 | 25 | PVM49- BM172 | 7 | 25 | PVBR69- BM160 | 30.20 | 16.44 | -0.23 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | NSP | 2 | 55 | IAC90- IAC51 | 11 | 35 | PVM98- INDEL14 | 24.64 | 13.70 | -0.20 |
| RFS | NSP | 2 | 120 | PVBR11 IAC4 | 10 | 25 | PVBR185- BMd42 | 30.36 | 20.06 | -0.23 |
| RFS | NSP | 2 | 125 | IAC4- PVM127 | 3 | 135 | PVBR169- IAC70 | 23.11 | 10.72 | -0.18 |
| RFS | NSP | 2 | 170 | PVM11- PVM115 | 7 | 25 | PVBR69- BM160 | 23.68 | 8.46 | -0.15 |
| RFS | NSP | 2 | 170 | PVM11- PVM115 | 10 | 0 | IAC6- PVBR185 | 19.35 | 6.93 | 0.13 |
| RFS | NSP | 3 | 50 | PVBR87- BM159 | 11 | 60 | INDEL10- PVM30 | 15.83 | 22.92 | 0.14 |
| RFS | NSP | 3 | 75 | PVat0008- PVM148 | 6 | 15 | PVBR5- PVBR20 | 16.12 | 4.92 | 0.12 |
| RFS | NSP | 3 | 85 | PVat0008- PVM148 | 5 | 0 | PVBR24- PVBR61 | 15.84 | 4.66 | -0.11 |
| RFS | NSP | 3 | 85 | PVat0008- PVM148 | 11 | 5 | PVBR113- INDEL11 | 16.92 | 5.98 | -0.13 |
| RFS | NSP | 4 | 15 | PVBR242- BMd16 | 11 | 0 | BMd22- PVBR113 | 8.60 | 5.51 | 0.14 |
| RFS | NSP | 4 | 20 | INDEL30- INDEL109 | 7 | 25 | PVBR69- BM160 | 22.64 | 7.79 | -0.16 |
| RFS | NSP | 4 | 90 | IAC66- BM161 | 11 | 60 | INDEL10- PVM30 | 29.85 | 13.01 | -0.19 |
| RFS | NSP | 5 | 45 | BM175- PVBR93 | 6 | 0 | INDEL03- PVM21 | 9.15 | 2.11 | 0.09 |
| RFS | NSP | 6 | 40 | PVBR14- CLP | 7 | 70 | PVBR269- PVBR35 | 27.40 | 11.24 | -0.18 |
| RFS | NSP | 6 | 40 | PVBR14- CLP | 9 | 70 | BM141- IAC68 | 11.89 | 2.46 | -0.09 |
| RFS | NSP | 7 | 55 | INDEL112- PVBR67 | 11 | 25 | INDEL11- INDEL29 | 31.86 | 24.42 | -0.25 |
| RFS | NSP | 7 | 100 | INDEL13- BM170 | 11 | 25 | INDEL11- INDEL29 | 13.86 | 4.47 | -0.11 |
| RFS | NSP | 9 | 25 | PVat0007- BM114 | 10 | 0 | IAC6- PVBR185 | 8.48 | 1.78 | -0.07 |
| RFS | HI | 1 | 35 | PVBR218- PVBR250 | 11 | 25 | INDEL11- INDEL29 | 13.06 | 1.63 | -0.01 |
| RFS | HI | 2 | 25 | PVM49- BM172 | 5 | 55 | PVat0006- BMd50 | 31.70 | 7.05 | 0.02 |
| RFS | HI | 2 | 40 | BM172- BM142 | 5 | 110 | PVBR235- PVBR131 | 14.36 | 1.61 | -0.01 |
| RFS | HI | 2 | 80 | PVgccacc0001- BM152 | 5 | 70 | BMd50- IAC10 | 29.20 | 5.57 | -0.02 |
| RFS | HI | 2 | 90 | BM152- PVBR11 | 7 | 70 | PVBR269- PVBR35 | 21.05 | 2.40 | -0.01 |
| RFS | HI | 2 | 90 | BM152- PVBR11 | 11 | 5 | PVBR113- INDEL11 | 16.42 | 1.76 | -0.01 |
| RFS | HI | 2 | 95 | BM152- PVBR11 | 5 | 30 | PVBR61- BMd53 | 15.97 | 4.09 | -0.02 |
| RFS | HI | 2 | 100 | BM152- PVBR11 | 7 | 125 | BM170- PVBR167 | 10.33 | 0.84 | -0.01 |
| RFS | HI | 2 | 120 | PVBR11- IAC4 | 3 | 85 | PVat0008- PVM148 | 27.56 | 5.85 | -0.02 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | HI | 2 | 120 | PVBR11- IAC4 | 5 | 50 | PVBR93- PVat0006b | 26.93 | 5.77 | 0.02 |
| RFS | HI | 2 | 120 | PVBR11- IAC4 | 6 | 10 | INDEL51- INDEL05 | 45.26 | 24.66 | -0.04 |
| RFS | HI | 2 | 120 | PVBR11- IAC4 | 10 | 25 | PVBR185- BMd42 | 29.59 | 7.48 | 0.02 |
| RFS | HI | 2 | 135 | PVM127- IAC71 | 9 | 35 | BM114- PVBR199 | 30.05 | 10.82 | 0.02 |
| RFS | HI | 2 | 140 | PVM127- IAC71 | 5 | 30 | PVBR61- BMd53 | 11.83 | 3.97 | 0.02 |
| RFS | HI | 2 | 170 | PVM11- PVM115 | 11 | 5 | PVBR113- INDEL11 | 12.34 | 1.43 | 0.01 |
| RFS | HI | 3 | 20 | PVag0001- PVBR255 | 5 | 30 | PVBR61- BMd53 | 16.11 | 3.82 | -0.02 |
| RFS | HI | 3 | 20 | PVag0001- PVBR255 | 7 | 70 | PVBR269- PVBR35 | 35.55 | 24.90 | -0.04 |
| RFS | HI | 3 | 25 | PVBR255- BM189 | 11 | 60 | INDEL10- PVM30 | 17.62 | 2.47 | -0.01 |
| RFS | HI | 3 | 35 | PVBR87- BM159 | 6 | 10 | INDEL51- INDEL05 | 27.21 | 3.49 | -0.02 |
| RFS | HI | 3 | 35 | PVBR87- BM159 | 8 | 10 | BM181- PVBR53 | 24.91 | 7.21 | -0.02 |
| RFS | HI | 3 | 35 | PVBR87- BM159 | 8 | 25 | BM181- PVBR53 | 35.80 | 5.93 | -0.02 |
| RFS | HI | 3 | 35 | PVBR87- BM159 | 8 | 40 | PVBR53- PVBR45 | 20.73 | 7.46 | -0.02 |
| RFS | HI | 3 | 105 | PVM148- PVBR169 | 11 | 5 | PVBR113- INDEL11 | 26.86 | 5.39 | -0.02 |
| RFS | HI | 4 | 15 | PVBR242- BMd16 | 9 | 55 | BM141- IAC68 | 7.70 | 2.62 | 0.02 |
| RFS | HI | 4 | 15 | PVBR242- BMd16 | 9 | 70 | BM141- IAC68 | 9.59 | 0.24 | 0.02 |
| RFS | HI | 4 | 40 | INDEL109- IAC67 | 5 | 35 | BMd53- PVBR124 | 11.06 | 1.19 | -0.01 |
| RFS | HI | 4 | 60 | INDEL109- IAC67 | 7 | 65 | PVBR269- PVBR35 | 27.77 | 4.38 | 0.02 |
| RFS | HI | 5 | 30 | PVBR61- BMd53 | 5 | 40 | PVBR124- BM175 | 14.44 | 3.52 | -0.02 |
| RFS | HI | 5 | 30 | PVBR61- BMd53 | 5 | 65 | BMd50- IAC10 | 18.45 | 3.56 | -0.02 |
| RFS | HI | 5 | 30 | PVBR61- BMd53 | 5 | 80 | IAC10- PVBR235 | 18.55 | 3.61 | -0.02 |
| RFS | HI | 5 | 30 | PVBR61- BMd53 | 6 | 55 | PVBR14- CLP | 14.73 | 3.86 | 0.02 |
| RFS | HI | 5 | 30 | PVBR61- BMd53 | 11 | 50 | INDEL10- PVM30 | 14.17 | 4.13 | -0.02 |
| RFS | HI | 5 | 50 | PVBR93- PVat0006b | 7 | 70 | PVBR269- PVBR35 | 19.54 | 2.73 | 0.01 |
| RFS | HI | 6 | 15 | PVBR5- PVBR20 | 9 | 15 | IAC62- PVat0007 | 6.39 | 0.65 | 0.01 |
| RFS | HI | 6 | 40 | PVBR14- CLP | 10 | 25 | PVBR185- BMd42 | 30.10 | 9.78 | 0.02 |
| RFS | HI | 6 | 65 | PVBR14- CLP | 7 | 60 | PVBR67- INDEL01 | 32.41 | 5.47 | -0.02 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | HI | 7 | 80 | PVBR35- INDEL13 | 11 | 20 | PVBR113- INDEL11 | 9.79 | 1.20 | 0.01 |
| RFS | HI | 9 | 25 | PVat0007- BM114 | 11 | 20 | PVBR113- INDEL11 | 7.23 | 0.76 | 0.01 |
| RFS | HI | 9 | 40 | PVBR199- BM141 | 11 | 0 | BMd22- PVBR113 | 6.20 | 0.58 | 0.01 |
| RFS | HI | 9 | 40 | PVBR199- BM141 | 11 | 30 | INDEL11- INDEL29 | 43.23 | 18.06 | -0.03 |
| RFS | HI | 10 | 25 | PVBR185- BMd42 | 11 | 5 | PVBR113- INDEL11 | 27.87 | 7.27 | 0.02 |
| RFS | PHI | 1 | 0 | BM165- BMd45 | 2 | 135 | PVM127- IAC71 | 23.42 | 6.89 | 0.02 |
| RFS | PHI | 1 | 0 | BM165- BMd45 | 5 | 50 | PVBR93- PVat0006b | 15.55 | 2.65 | 0.01 |
| RFS | PHI | 2 | 0 | GATS54- GATS11 | 8 | 75 | BM153- PVaaat0001 | 13.97 | 1.73 | 0.01 |
| RFS | PHI | 2 | 30 | BM172- BM142 | 3 | 60 | PVBR21- PVBR23 | 20.60 | 5.36 | -0.02 |
| RFS | PHI | 2 | 50 | PVBR149- BMd7 | 9 | 40 | PVBR199- BM141 | 26.78 | 4.73 | 0.01 |
| RFS | PHI | 2 | 60 | IAC51- PVBR94 | 8 | 55 | BM153- PVaaat0001 | 11.03 | 1.24 | 0.01 |
| RFS | PHI | 2 | 80 | PVgccacc0001- BM152 | 2 | 170 | PVM11- PVM115 | 42.50 | 19.25 | 0.03 |
| RFS | PHI | 2 | 80 | PVgccacc0001- BM152 | 10 | 0 | IAC6- PVBR185 | 20.06 | 4.22 | -0.01 |
| RFS | PHI | 2 | 120 | PVBR11- IAC4 | 4 | 15 | PVBR242- BMd16 | 15.56 | 9.50 | 0.02 |
| RFS | PHI | 2 | 125 | IAC4- PVM127 | 6 | 0 | INDEL03- PVM21 | 30.33 | 11.36 | -0.02 |
| RFS | PHI | 2 | 155 | IAC71- PVM11 | 10 | 0 | IAC6- PVBR185 | 18.56 | 2.78 | -0.01 |
| RFS | PHI | 2 | 155 | IAC71- PVM11 | 11 | 60 | INDEL10- PVM30 | 5.20 | 0.62 | -0.01 |
| RFS | PHI | 2 | 160 | PVM11- PVM115 | 3 | 50 | PVBR87- BM159 | 9.60 | 1.27 | -0.01 |
| RFS | PHI | 3 | 10 | PVag0001- PVBR255 | 11 | 40 | INDEL10- PVM30 | 9.45 | 2.55 | 0.02 |
| RFS | PHI | 3 | 10 | PVag0001- PVBR255 | 11 | 55 | INDEL10- PVM30 | 6.26 | 3.47 | 0.02 |
| RFS | PHI | 3 | 20 | PVag0001- PVBR255 | 6 | 10 | INDEL51- INDEL05 | 11.71 | 2.44 | -0.01 |
| RFS | PHI | 3 | 25 | PVBR255- BM189 | 11 | 40 | INDEL10- PVM30 | 18.67 | 0.95 | 0.02 |
| RFS | PHI | 3 | 25 | PVBR255- BM189 | 11 | 55 | INDEL10- PVM30 | 7.38 | 4.86 | 0.02 |
| RFS | PHI | 3 | 55 | BM159- PVBR21 | 5 | 25 | PVBR24- PVBR61 | 11.60 | 1.68 | -0.01 |
| RFS | PHI | 3 | 60 | PVBR21- PVBR23 | 9 | 40 | PVBR199- BM141 | 30.48 | 8.09 | 0.02 |
| RFS | PHI | 3 | 60 | PVBR21- PVBR23 | 9 | 70 | BM141- IAC68 | 15.73 | 2.43 | -0.01 |
| RFS | PHI | 3 | 60 | PVBR21- PVBR23 | 11 | 0 | BMd22- PVBR113 | 57.79 | 36.55 | 0.04 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | PHI | 3 | 90 | PVM148- PVBR169 | 7 | 55 | INDEL112- PVBR67 | 46.52 | 22.71 | -0.03 |
| RFS | PHI | 3 | 105 | PVM148- PVBR169 | 7 | 0 | PVBR69- BM160 | 22.73 | 4.08 | -0.01 |
| RFS | PHI | 3 | 105 | PVM148- PVBR169 | 7 | 125 | BM170- PVBR167 | 12.03 | 1.42 | -0.01 |
| RFS | PHI | 3 | 110 | PVBR169- IAC70 | 5 | 0 | PVBR24- PVBR61 | 30.65 | 7.68 | 0.02 |
| RFS | PHI | 4 | 60 | INDEL109- IAC67 | 6 | 0 | INDEL03- PVM21 | 19.51 | 3.22 | 0.01 |
| RFS | PHI | 5 | 35 | BMd53- PVBR124 | 6 | 10 | INDEL51- INDEL05 | 8.54 | 1.18 | 0.01 |
| RFS | PHI | 5 | 45 | BM175- PVBR93 | 11 | 40 | INDEL10- PVM30 | 8.90 | 1.22 | -0.01 |
| RFS | PHI | 5 | 50 | PVBR93- PVat0006b | 6 | 65 | PVBR14- CLP | 35.80 | 12.05 | -0.02 |
| RFS | PHI | 5 | 95 | PVBR235- PVBR131 | 6 | 15 | PVBR5- PVBR20 | 38.20 | 26.45 | -0.04 |
| RFS | PHI | 5 | 110 | PVBR235- PVBR131 | 6 | 15 | PVBR5- PVBR20 | 52.22 | 29.38 | -0.04 |
| RFS | PHI | 7 | 50 | INDEL112- PVBR67 | 8 | 50 | PVBR45- BM153 | 22.19 | 4.56 | 0.01 |
| RFS | PHI | 7 | 70 | PVBR269- PVBR35 | 8 | 25 | BM181- PVBR53 | 24.15 | 5.21 | -0.02 |
| RFS | PHI | 9 | 25 | PVat0007- BM114 | 9 | 70 | BM141- IAC68 | 31.85 | 7.31 | 0.02 |
| RFS | PHI | 9 | 25 | PVat0007- BM114 | 11 | 60 | INDEL10- PVM30 | 18.52 | 2.80 | 0.01 |
| RFS | PHI | 9 | 70 | BM141- IAC68 | 10 | 25 | PVBR185- BMd42 | 21.97 | 4.82 | -0.02 |
| RFS | SW | 1 | 35 | PVBR218- PVBR250 | 3 | 30 | PVBR255- BM189 | 6.30 | 1.86 | 0.81 |
| RFS | SW | 1 | 35 | PVBR218- PVBR250 | 5 | 60 | BMd50- IAC10 | 12.66 | 3.56 | -1.02 |
| RFS | SW | 1 | 40 | IAC76- PVM126 | 2 | 100 | BM152- PVBR11 | 8.56 | 2.65 | -0.89 |
| RFS | SW | 1 | 60 | PVM126- IAC69 | 8 | 75 | BM153- PVaaat0001 | 37.83 | 18.54 | 2.19 |
| RFS | SW | 2 | 0 | GATS54- GATS11 | 4 | 0 | PVBR242- BMd16 | 10.05 | 2.89 | 0.95 |
| RFS | SW | 2 | 0 | GATS54- GATS11 | 4 | 40 | INDEL109- IAC67 | 10.94 | 2.62 | 0.87 |
| RFS | SW | 2 | 5 | GATS11- BM164 | 9 | 70 | BM141- IAC68 | 14.28 | 5.33 | 1.25 |
| RFS | SW | 2 | 50 | PVBR149- BMd7 | 11 | 5 | PVBR113- INDEL11 | 18.75 | 7.83 | -1.46 |
| RFS | SW | 2 | 70 | BMd17- PVBR125 | 6 | 0 | INDEL03- PVM21 | 5.03 | 2.07 | 0.74 |
| RFS | SW | 2 | 70 | BMd17- PVBR125 | 10 | 0 | IAC6- PVBR185 | 10.15 | 4.33 | -1.05 |
| RFS | SW | 2 | 70 | BMd17- PVBR125 | 11 | 5 | PVBR113- INDEL11 | 17.36 | 8.48 | 1.49 |
| RFS | SW | 2 | 105 | PVBR11- IAC4 | 4 | 70 | IAC66- BM161 | 21.37 | 8.49 | 1.49 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | SW | 2 | 160 | PVM11- PVM115 | 5 | 110 | PVBR235- PVBR131 | 17.59 | 7.60 | -1.42 |
| RFS | SW | 3 | 35 | PVBR87- BM159 | 9 | 25 | PVat0007- BM114 | 41.63 | 19.28 | -2.69 |
| RFS | SW | 3 | 50 | PVBR87- BM159 | 8 | 0 | BM181- PVBR53 | 10.39 | 2.47 | -1.01 |
| RFS | SW | 3 | 50 | PVBR87- BM159 | 9 | 30 | BM114- PVBR199 | 16.29 | 16.08 | -2.62 |
| RFS | SW | 3 | 135 | PVBR169- IAC70 | 5 | 70 | BMd50- IAC10 | 13.74 | 3.38 | 0.94 |
| RFS | SW | 4 | 15 | PVBR242- BMd16 | 6 | 15 | PVBR5- PVBR20 | 11.73 | 4.98 | -1.26 |
| RFS | SW | 4 | 90 | IAC66- BM161 | 9 | 0 | IAC62- PVat0007 | 31.89 | 16.06 | 2.06 |
| RFS | SW | 5 | 0 | PVBR24- PVBR61 | 7 | 100 | INDEL13- BM170 | 9.09 | 2.28 | -0.78 |
| RFS | SW | 5 | 35 | BMd53- PVBR124 | 7 | 55 | INDEL112- PVBR67 | 37.71 | 28.44 | 2.71 |
| RFS | SW | 6 | 10 | INDEL51- INDEL05 | 7 | 0 | PVBR69- BM160 | 24.53 | 9.42 | 1.53 |
| RFS | SW | 7 | 60 | PVBR67- INDEL01 | 7 | 85 | INDEL13- BM170 | 12.83 | 4.07 | -1.01 |
| RFS | SY | 1 | 0 | BM165- BMd45 | 6 | 0 | INDEL03- PVM21 | 5.75 | 3.70 | 11.21 |
| RFS | SY | 1 | 25 | BM165- BMd45 | 3 | 75 | PVat0008- PVM148 | 16.37 | 20.57 | 30.42 |
| RFS | SY | 1 | 60 | PVM126- IAC69 | 10 | 0 | IAC6- PVBR185 | 9.37 | 7.60 | -16.01 |
| RFS | SY | 2 | 0 | GATS54- GATS11 | 5 | 30 | PVBR61- BMd53 | 16.92 | 14.79 | 24.44 |
| RFS | SY | 2 | 0 | GATS54- GATS11 | 5 | 90 | PVBR235- PVBR131 | 5.13 | 4.41 | -12.80 |
| RFS | SY | 2 | 5 | GATS11- BM164 | 11 | 40 | INDEL10- PVM30 | 7.33 | 9.15 | -18.10 |
| RFS | SY | 2 | 15 | GATS11- BM164 | 7 | 70 | PVBR269- PVBR35 | 6.49 | 5.95 | 15.28 |
| RFS | SY | 2 | 15 | GATS11- BM164 | 8 | 55 | BM153- PVaaat0001 | 9.39 | 8.82 | -18.86 |
| RFS | SY | 2 | 60 | IAC51- PVBR94 | 2 | 70 | BMd17- PVBR125 | 12.26 | 6.85 | 30.97 |
| RFS | SY | 2 | 60 | IAC51- PVBR94 | 5 | 0 | PVBR24- PVBR61 | 10.37 | 7.63 | 18.62 |
| RFS | SY | 2 | 90 | BM152- PVBR11 | 7 | 125 | BM170- PVBR167 | 7.74 | 6.48 | 15.84 |
| RFS | SY | 2 | 160 | PVM11- PVM115 | 6 | 40 | PVBR14- CLP | 5.91 | 5.45 | 13.74 |
| RFS | SY | 2 | 170 | PVM11- PVM115 | 6 | 10 | INDEL51- INDEL05 | 15.50 | 16.86 | -23.42 |
| RFS | SY | 7 | 60 | PVBR67- INDEL01 | 9 | 15 | IAC62- PVat0007 | 6.18 | 5.09 | -12.97 |
| RFS | SY | 10 | 5 | IAC6- PVBR185 | 11 | 25 | INDEL11- INDEL29 | 9.81 | 10.73 | -18.90 |
| RFS | SYD | 1 | 85 | PVM126- IAC69 | 6 | 40 | PVBR14- CLP | 19.55 | 9.25 | 0.11 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | SYD | 2 | 0 | GATS54- GATS11 | 3 | 110 | PVBR169- IAC70 | 5.50 | 1.61 | 0.05 |
| RFS | SYD | 2 | 55 | IAC90- IAC51 | 4 | 20 | INDEL30- INDEL109 | 14.78 | 8.57 | 0.12 |
| RFS | SYD | 2 | 55 | IAC90- IAC51 | 10 | 0 | IAC6- PVBR185 | 14.17 | 6.75 | -0.09 |
| RFS | SYD | 2 | 70 | BMd17- PVBR125 | 7 | 50 | INDEL112- PVBR67 | 11.67 | 5.22 | -0.09 |
| RFS | SYD | 2 | 130 | IAC4- PVM127 | 3 | 35 | PVBR87- BM159 | 21.90 | 13.89 | 0.16 |
| RFS | SYD | 2 | 145 | PVM127- IAC71 | 3 | 35 | PVBR87- BM159 | 12.63 | 13.85 | 0.15 |
| RFS | SYD | 3 | 0 | PVag0001- PVBR255 | 7 | 30 | BM160- INDEL112 | 6.49 | 2.61 | -0.06 |
| RFS | SYD | 3 | 35 | PVBR87- BM159 | 5 | 110 | PVBR235- PVBR131 | 18.49 | 8.44 | 0.13 |
| RFS | SYD | 3 | 35 | PVBR87- BM159 | 7 | 70 | PVBR269- PVBR35 | 19.18 | 3.93 | -0.15 |
| RFS | SYD | 3 | 55 | BM159- PVBR21 | 10 | 25 | PVBR185- BMd42 | 7.61 | 3.24 | 0.07 |
| RFS | SYD | 3 | 60 | PVBR21- PVBR23 | 4 | 60 | INDEL109- IAC67 | 11.06 | 3.05 | 0.07 |
| RFS | SYD | 4 | 40 | INDEL109- IAC67 | 6 | 15 | PVBR5- PVBR20 | 39.81 | 43.99 | 0.24 |
| RFS | SYD | 5 | 35 | BMd53- PVBR124 | 5 | 45 | BM175- PVBR93 | 13.71 | 7.68 | -0.12 |
| RFS | SYD | 5 | 45 | BM175- PVBR93 | 9 | 0 | IAC62- PVat0007 | 22.72 | 14.19 | -0.14 |
| RFS | SYD | 6 | 65 | PVBR14- CLP | 7 | 65 | PVBR269- PVBR35 | 21.59 | 10.08 | -0.12 |
| RFS | SYD | 7 | 25 | PVBR69- BM160 | 10 | 0 | IAC6- PVBR185 | 13.97 | 5.59 | -0.09 |
| RFS | SYD | 7 | 60 | PVBR67- INDEL01 | 10 | 0 | IAC6- PVBR185 | 7.39 | 2.46 | -0.06 |
| RFS | SYD | 9 | 70 | BM141- IAC68 | 10 | 25 | PVBR185- BMd42 | 25.05 | 20.20 | -0.17 |
| RFS | SYD | 10 | 0 | IAC6- PVBR185 | 11 | 20 | PVBR113- INDEL11 | 12.85 | 7.59 | -0.10 |
| RFS | SYD | 11 | 0 | BMd22- PVBR113 | 11 | 30 | INDEL11- INDEL29 | 8.07 | 3.14 | -0.07 |
| RFS | DSF | 1 | 0 | BM165- BMd45 | 5 | 45 | BM175- PVBR93 | 9.78 | 3.77 | 0.56 |
| RFS | DSF | 1 | 85 | PVM126- IAC69 | 7 | 125 | BM170- PVBR167 | 21.38 | 9.21 | -0.81 |
| RFS | DSF | 2 | 0 | GATS54- GATS11 | 2 | 70 | BMd17- PVBR125 | 14.13 | 4.41 | 0.64 |
| RFS | DSF | 2 | 5 | GATS11- BM164 | 7 | 80 | PVBR35- INDEL13 | 23.10 | 13.56 | -1.05 |
| RFS | DSF | 2 | 40 | BM172- BM142 | 11 | 30 | INDEL11- INDEL29 | 23.05 | 13.02 | -0.98 |
| RFS | DSF | 2 | 45 | PVBR149- BMd7 | 4 | 40 | INDEL109- IAC67 | 17.89 | 7.48 | 0.74 |
| RFS | DSF | 2 | 45 | PVBR149- BMd7 | 11 | 60 | INDEL10- PVM30 | 34.16 | 26.19 | 1.46 |

Table B.1 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| RFS | DSF | 2 | 60 | IAC51- PVBR94 | 7 | 105 | BM170- PVBR167 | 11.60 | 3.77 | -0.55 |
| RFS | DSF | 2 | 60 | IAC51- PVBR94 | 9 | 45 | BM141- IAC68 | 21.82 | 10.64 | 0.87 |
| RFS | DSF | 2 | 70 | BMd17- PVBR125 | 2 | 90 | BM152- PVBR11 | 10.57 | 4.42 | -0.89 |
| RFS | DSF | 2 | 70 | BMd17- PVBR125 | 5 | 30 | PVBR61- BMd53 | 9.45 | 4.29 | 0.55 |
| RFS | DSF | 2 | 125 | IAC4- PVM127 | 6 | 0 | INDEL03- PVM21 | 12.65 | 5.95 | 0.67 |
| RFS | DSF | 2 | 135 | PVM127- IAC71 | 2 | 160 | PVM11- PVM115 | 22.84 | 13.54 | 0.99 |
| RFS | DSF | 2 | 160 | PVM11- PVM115 | 4 | 0 | PVBR242- BMd16 | 9.04 | 5.91 | 0.67 |
| RFS | DSF | 2 | 170 | PVM11- PVM115 | 9 | 0 | IAC62- PVat0007 | 16.85 | 5.94 | 0.65 |
| RFS | DSF | 3 | 35 | PVBR87- BM159 | 5 | 70 | BMd50- IAC10 | 14.86 | 4.75 | 0.73 |
| RFS | DSF | 3 | 70 | AG1- BMd1 | 5 | 25 | PVBR24- PVBR61 | 8.55 | 3.38 | 0.52 |
| RFS | DSF | 3 | 75 | PVat0008- PVM148 | 4 | 90 | IAC66- BM161 | 10.42 | 3.89 | -0.58 |
| RFS | DSF | 3 | 105 | PVM148- PVBR169 | 11 | 5 | PVBR113- INDEL11 | 8.75 | 3.56 | 0.51 |
| RFS | DSF | 5 | 60 | BMd50- IAC10 | 8 | 45 | PVBR53- PVBR45 | 25.03 | 13.88 | -1.19 |
| RFS | DSF | 7 | 60 | PVBR67- INDEL01 | 9 | 40 | PVBR199- BM141 | 25.39 | 15.21 | 1.05 |

## LITERATURE CITED

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Aastveit, A. H. and K. Aastveit. 1993. Effects of genotype-environment interactions on genetic correlations. Theor. Appl. Genet. 86: 1007-1013.

Acosta- Gallegos, J. A. and J. K. Shibata. 1989. Effect of water stress on growth and yield of indeterminate dry bean *( Phaseolus vulgaris ).* Field Crops Res. 20: 81-93.

Acosta-Gallegos, J. A. and M. W. Adams. 1991. Plant traits and yield stability of dry bean (Phaseolus vulgaris) cultivars under drought stress. J. Agric. Sci. 117: 213-219.

Acosta-Gallegos, J. A. and J. W. White. 1995. Phenological plasticity as an adaptation by common bean to rainfed environments. Crop Sci. 35:199-204.

Afanador, L. K., S. D. Haley, and J. D. Kelly. 1993. Adoption of a "miniprep" DNA extraction method for RAPD marker analysis in common bean (Phaseolus vulgaris L.). Annu. Rep. Bean Improv. Coop. 36:10-11.

Asfaw, A. and M. W. Blair. 2012. Quantitative trait loci for rooting pattern traits of common beans grown under drought stress versus non-stress conditions. Mol. Breed. 30:681–695.

Asfaw, A., M. W. Blair, and P. C. Struik. 2012. Multienvironment Quantitative trait loci analysis for photosynthate acquisition, accumulation, and remobilization traits in common bean under drought stress.*G3*: Genes|Genomes|Genetics 2: 579-595.

Beattie, A. D, J. Larsen, T. E Michaels, and K P Pauls. 2003. Mapping quantitative trait loci for a common bean (*Phaseolus vulgaris* L .) ideotype. Genome 46: 411-422.

Beaver, J. S. and J. D. Kelly. 1994. Comparison of selection methods for dry bean populations derived from crosses between gene pools. Crop Sci. 34:34-37

Beebe, S. E., M. Rojas-Pierce, X. Yan, M. W. Blair, F. Pedraza, F. Muñoz, J Tohme, and J. P. Lynch. 2006. Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Sci. 46: 413- 423.

Beebe, S. E., I. M. Rao, C. Cajiao, and M. Grajales. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Sci. 48: 582-592.

Beebe, S. E, I. M Rao and M. W Blair. 2010. Phenotyping common beans for adaptation to drought, pp. 309–334 in Drought Phenotyping in Crops: From Theory to Practice, edited by J. M. Ribaut, and P. Monneveux. Generation Challenge Program Special Issue on Phenotyping. CGIAR, Texcoco, Mexico.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Sci. 48: 1649-1664.

Blair, M. W., F. Pedraza, H. F. Buendia, E. Gaitan-Solis, S. E. Beebe, P. Gepts, and J. Tohme. 2003. Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 107:1362-1374

Blair, M. W, G. Iriarte, and S. Beebe. 2006. QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean x wild common bean (Phaseolus vulgaris L.) cross. Theor. Appl. Genet. 112: 1149-1163.

Blair, M. W., C. H Galeano, E. Tovar, M. C M. Torres, A. V. Castrillón, S. E. Beebe, and I. M. Rao. 2012. Development of a Mesoamerican intra-gene pool genetic map for quantitative trait loci detection in a drought tolerant × susceptible common bean *(Phaseolus vulgaris* L.) cross. Mol. Breed. 29: 71-88.

Cavalieri, A., A. Merchant, and E. van Volkenburgh. 2011. Why not beans? Funct. Plant Biol. 38: iii-vi.

Checa, O.E and M.W.Blair. 2012. Inheritance of yield related traits in climbing beans *(Phaseolus vulgaris* L). Crop Sci. 52:1998-2013.

Cichy, K. A., M. W. Blair, C. H. Galeano Mendoza, S. S. Snapp, and J. D. Kelly. 2009. QTL analysis of root architecture traits and low phosphorus tolerance in an andean bean population. Crop Sci. 49:59-68

Churchill, G. A. and R. W. Doerge.1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963-971

Córdoba, J. M., C. Chavarro1, J. A Schlueter, S. A. Jackson, and M. W Blair. 2010. Integration of physical and genetic maps of common bean through BAC-derived microsatellite. BMC Genomics 11:436- 446.

Dixit, S., B. P. Mallikarjuna-Swamy, J. Bernier, P. Vikram, M. T. Sta Cruz, M. Amante, D. Atri, and A. Kumar. 2012. Increased drought tolerance and wider adaptability of qDTY12.1 conferred by its interaction with qDTY2.3 and qDTY3.2. Mol Breed. DOI 10.1007/s11032-012-9760-5

Duncan, R. W, R. L. Gilbertson, and S. P. Singh. 2012. Direct and marker-assisted selection for resistance to common bacterial blight in common bean. Crop Sci. 52:1511-1521.

Ender, M. and J. D. Kelly. 2005. Identification of QTL associated with white mold resistance in common bean. Crop Sci. 45:2482-2490.

Frahm, M. A., J.C. Rosas, N. Mayek-Perez, E. Lopez-Salinas, J. A. Acosta-Gallegos, and J. D. Kelly. 2004. Breeding beans for resistance to terminal drought in the lowland tropics. Euphytica 136: 223-232.

Funatsuki, H., M. Hajika, T.Yamada, M. Suzuki, S. Hagihara, Y. Tanaka, S. Fujita, M. Ishimoto, and K. Fujino. 2012. Mapping and use of QTLs controlling pod dehiscence in soybean. Breeding Sci. 61: 554-558.

Hallauer, A.R., and J.B. Miranda. 1981. Quantitative genetics in maize breeding. Iowa State University Press, Ames.

Hanai, L. R, L. Santini, L. E. A. Camargo, M. H. P. Fungaro, P. Gepts, S. M. Tsai, and M. L. C. Vieira. 2010. Extension of the core map of common bean with EST-SSR, RGA, AFLP, and putative functional markers. Mol. Breed. 25: 25-45.

Johnson, W. C, and P. Gepts. 2002. The Role of epistasis in controlling seed yield and other agronomic traits in an Andean × Mesoamerican cross of common bean (*Phaseolus vulgaris L* .). Euphytica 125: 69-79.

Kolkman, J. M., and J. D. Kelly. 2003. QTL conferring resistance and avoidance to white mold in common bean. Crop Sci. 43:539-548.

Kornegay, J., J. W. White, and O. Ortiz DeCruz. 1992. Growth habit and gene pool effects on inheritance of yield in common bean. Euphytica 62: 171-180.

Lauer, J. G, C. G. Bijl, M. A. Grusak, P. S. Baenziger, K. Boote, S. Lingle, T. Carter, S. Kaeppler, R. Boerma, G. Eizenga, P. Carter, M. Goodman, E. Nafziger, K. Kidwell, R. Mitchell, M. D. Edgerton, K. Quesenberry, and M. C. Willcox. 2012. The scientific grand challenges of the 21st century for the Crop Science Society of America. Crop Sci. 52:1003-1010

Levitt, J. 1972. Responses of plant to environmental stresses, Academic Press, New York.

McClean, P. E., J. Burridge, S. Beebe, I. M. Rao, and T. G. Porch. 2011. Crop improvement in the era of climate change: an integrated, multi-disciplinary approach for common bean *(Phaseolus vulgaris*). Funct. Plant Biol. 38: 927- 933.

Miklas, P. N., J. D. Kelly, S. E. Beebe, and M. W. Blair. 2006a. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147: 105-131.

Miklas, P. N., J. R. Smith, and S. P. Singh. 2006b. Registration of common bacterial blight resistant dark red kidney bean germplasm line USDK-CBB-15. Crop Sci. 46: 1005.

Mkwaila, W., K. A. Terpstra, M. Ender, and J. D. Kelly. 2011. Identification of QTL for agronomic traits and resistance to white mold in wild and landrace germplasm of common bean. Plant Breed. 130: 665-672.

Moreto, A. L, M. A. P. Ramalho, and A. T. Bruzi. 2011. Epistasis in an Andean x Mesoamerican cross of common bean. Euphytica. DOI 10.1007/s10681-011-0578-8

Ramirez-Vallejo, P. and J. D. Kelly. 1998. Traits related to drought resistance in common bean. Euphytica 99: 127-136.

Rao, I. M. 2001. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages, pp. 583–613 In Handbook of Plant and Crop Physiology, edited by M. Pessarakli. Marcel Dekker, Inc, New York.

Rosales-Serna, R., J. Kohashi-Shibata, J. A. Acosta-Gallegos, C. Trejo-Lopez, J. Ortiz-Cereceres, J. D. Kelly. 2004. Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. Field Crops Res. 85: 203-211

Román-Avilés, B and J. D. Kelly. 2005. Identification of quantitative trait loci conditioning resistance to fusarium root rot in common bean. Crop Sci. 45:1881-1890

SAS Institute, Inc. 2011. SAS version 9.3. SAS Institute Inc., Cary, NC.

Schneider, K. A, R. Rosales- Serna, F. Ibarra-Perez, B. Cazares-Enriquez, J. A. Acosta-Gallegos, P. Ramirez-Vallejo, N. Wassimi, and J. D. Kelly. 1997a. Improving common bean performance under drought stress. Crop Sci. 37:43-50.

Schneider, K. A., M. E. Brothers, and J. D. Kelly. 1997b. Marker assisted selection to improve drought resistance in common bean. Crop Sci. 37: 51-60.

Singh, S. P. 1995. Selection for water stress tolerance in interracial populations of common bean. Crop Sci. 35:118-124

Singh, S. P. 2007. Drought resistance in the race Durango dry bean landraces and cultivars. Agron. J. 99:1219-1225

Singh, S. P, H. Terán, C. G. Muňoz, and J. C. Takegami. 1999. Two cycles of recurrent selection for seed yield in common bean. Crop Sci. 39:391-397

Singh, S. P, H. Terán, and J. A. Gutiérrez. 2001. Registration of SEA 5 and SEA 13 drought tolerant dry bean germplasm. Crop Sci. 41: 276-277

Singh, S. P, H. Terán, C. G. Muňoz, and J.M. Osorno. 2002. Selection for seed yield in Andean intra-gene pool and Andean ×Middle American inter-gene pool populations of common bean. Euphytica 127: 437-444.

Steele, K. 2009. Novel upland rice variety bred using marker-assisted selection and client oriented breeding released in Jharkhand. Bangor University, India.

Steele, K. A., D. S. Virk, R. Kumar, S.C. Prasad, and J. R. Witcombe. 2007. Field evaluation of upland rice lines selected for QTLs controlling root traits. Field Crops Res. 101: 180-186.

Tanksley, S. D. 1993. Mapping polygenes. Annu. Rev. Genet. 27:205-233

Tar'an, B., T. E Michaels, and K. P. Pauls. 2002. Genetic mapping of agronomic traits in common bean. Crop Sci. 42: 544-556.

Tuberosa, R., M. C. Sanguineti, P. Landi, M. M. Giuliani, S. Salvi, and S. Conti. 2002. Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two regimes. Plant Mol. Biol. 48: 697-712.

Tuberosa, R. and S. Salvi. 2006. Genomics based approaches to improve drought tolerance of crops. Trends Plant Sci. 11: 405-412.

Urrea, C. A., C. D. Yonts, D. J. Lyon, and A. E. Koehler. 2009. Selection for drought tolerance in dry bean derived from the mesoamerican gene pool in western Nebraska. Crop Sci. 49: 2005-2010.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93: 77-78.

Wang, J., H. Li, L. Zhang, C. Li, and L. Meng. 2011. Users' Manual of QTL IciMapping v3.1. pp146

Wang, D., J. Shi, S. R. Carlson, P. B. Cregan, R. W. Ward, and B. W. Diers. 2003. A low cost, high throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop Sci. 43:1828-1832

Welsh, W., W. Bushuk, W. Roca, and S. P. Singh. 1995. Characterization of agronomic traits and markers of recombinant inbred lines from intra and interracial populations of *Phaseolus vulgaris* L. Theor. Appl. Genet. 91: 169-177.

White, J. W. and J. A. Castillo. 1985. Relative effect of root and shoot genotypes on yield of common bean under drought stress. Crop Sci. 29:360-362.

Wright, E. M. and J. D. Kelly. 2011. Mapping QTL for seed yield and canning quality following processing of black bean (*Phaseolus vulgaris* L.*).* Euphytica 179: 471-484

## Chapter 3. Identification of shoot traits related to drought resistance in common bean seedlings


### Abstract

Drought is an important abiotic stress that limits common bean (*Phaseolus vulgaris* L) productivity. The objective of this study was to determine shoot traits that are associated with drought resistance in common bean at the seedling stage. Ten bean genotypes consisting mainly of cultivars and breeding lines from Mesoamerican race of the Middle America gene pool were evaluated in the greenhouse. Genotypes were grown in shallow soil profile to limit root growth and assess shoot phenotypes under stress. Water stress was imposed by withholding watering for 24 days after planting. Traits evaluated included wilting, unifoliate senescence, stem greenness, and recovery from drought. Biomass and number of pods/plant produced after drought recovery were evaluated to quantify the effect of early drought stress on bean growth and reproduction.

In general, genotypes such as 'Jaguar', 'Phantom', and TARS-SR05 showed a slow rate of willing, maintained a green stem and had a higher recovery rate following watering. Importantly, these genotypes demonstrated a small reduction in biomass and pod number under stress compared to non stress water treatments. Genotypes such as B98311 and L88-63 known to possess drought resistance conferred by deep rooting traits performed poorly in these conditions suggesting that both root and shoot drought resistance mechanisms are needed for bean to perform in broad range of stress conditions. Since recovery from drought is a prerequisite to plant regrowth, biomass, and pod production following drought stress, factors that contribute to recovery were studied. Stem greenness was highly positively correlated to the recovery while wilting was negatively correlated to the recovery. In a regression analysis, stem greenness and slow wilting were found to be important contributors to the variability of recovery. In addition, photosynthetic rate and stomatal conductance explained variation in wilting and stem greenness.

These results suggest that wilting and stem greenness might be useful traits to screen for drought tolerance in seedlings of common bean

**Introduction**

Common bean (*P. vulgaris* L) is an important food legume. Globally, 60% of bean production is grown in drought prone areas where most farmers cannot afford the cost associated with irrigation (Beebe et al., 2010). In addition, competition with major crops continues to push beans into marginal lands that exhibit increased risk of drought stress. Bean crops are subject to erratic rainfall at different growth stages with consequence of causing substantial reduction in biomass and seed yield. Intermittent drought stress during seedling stage affects overall plant growth while terminal drought significantly reduces bean seed yield, and seed size (Singh, 2007). Seed quality is also negatively affected under prolonged periods of terminal drought. Since the incidence and duration of drought episodes are expected to increase with climate change (IPCC, 2007), breeding for drought resistance has become an important component of most bean breeding programs. Breeding programs need to screen numerous lines under repeatable conditions to identify those that are tolerant to drought. Yield screening in the field under irrigation and non-irrigation conditions facilitates the selection of drought resistant lines with minimum yield loss when subjected to drought. However, yield is a result of a combination of many factors (Sinclair, 2011) and it is sensitive to genotype/environment interactions that make it difficult to associate yield with drought resistance. In addition, field yield screening is costly in terms of resources and time. Screening of the breeding materials in controlled conditions such as those possible in greenhouses and growth chambers might improve efficiency of breeding for drought resistance. Importantly, screening for drought tolerance at seedling stage permits the efficient screening of large numbers of materials in reasonable time. Therefore, breeders need to

determine seedling stage traits than can be used to identify drought resistance lines early in the selection processes. Drought episodes occurring early in plant development can cause considerable negative impact on crop yield. The extent of recovery affects growth and development of sink size as well as source supply when plant growth resumes after vegetative stage drought. Recovery from drought stress is associated with photosynthetic recovery (Chaves et al., 2011). Slow and incomplete photosynthetic recovery can cause negative impacts on plant productivity. For instance, seedling stage drought studies in rice have shown the importance of the retaining green leaf area and the number of remaining leaves at the end of drought in the plant's ability to recover and tiller (Kamoshita et al., 2008; Mitchell et al., 1998). In soybean, drought at the seedling stages reduces the number of nodes, internode length, overall biomass and the number of flowers produced with an associated reduction of yield and yield components (Desclaux et al., 2000). In sunflower, drought during the vegetative phase reduces main stem height, stem diameter, number of nodes or leaves and leaf area affecting the final yield (Rauf, 2008). Finally, drought at seedling stage often delays developmental events because of the inhibition of growth during the water deficit period (Blum, 1996) and this can cause severe yield loss if the recovery happens late in the season.

Plant root and shoot act independently or synergistically to enable plants to cope with drought stress. For instance, drought tolerance depends on the shoot when root volume is constrained and both root and shoot factors mediated tolerance when root volume is unconstrained in cowpea (Watanabe et al., 1997). The role of abscisic acid (ABA) in root /shoot communications to trigger shoot water conserving mechanisms is recognized in plants under drought stress (Montero-Tavera et al., 2008; Sally et al., 2012).

In common bean, root length density has been recognized as an important drought avoidance trait (White and Castillo, 1989; Sponchiado et al., 1989; Frahm et al., 2004). Numerous studies to understand the role of roots in drought avoidance in common bean have been conducted. Using grafting experiments, White and Castillo (1989) demonstrated the importance of root characteristics and root characteristic/environment interactions in determining drought adaptation of beans. In addition, these experiments showed that root genotype had a large and significant effect on bean seed yield under drought conditions. Significant genetic variation has been observed in root length and other growth and architecture root traits among seedlings of common bean (Lynch and Beem, 1991). Greenhouse root phenotyping techniques using PVC tubes have been developed at CIAT (Beebe et al., 2010) and were used to map QTL associated with rooting patterns in common bean under drought and non drought stress conditions (Asfaw and Blair, 2012). Under field conditions, shovelomics (Trachsel et al., 2010) has been developed to visually score the architecture traits of root growth in corn and it has also been applied to phenotype bean root traits (http://plantscience.psu.edu/research/labs/roots/methods/field-methods/field-crop-root-crown-sampling-and-analysis). However, root studies conducted at CIAT demonstrated that deep rooting alone does not assure drought resistance and sustained yield under drought conditions (Beebe et al., 2009). Combining deep rooting systems with functional shoot traits is needed to achieve yield stability under drought stress. Combining root traits with shoot based drought resistance mechanisms in single genotypes should enhance the development of drought resistant beans.

Various shoot traits have been investigated in common bean at the seedling stage. These include physiological processes such as photosynthetic efficiency, total chlorophyll content,

stomatal conductance, transpiration rate, leaf temperature and leaf water potential (Castrillo et al., 2001; Dias, 2010; Lizana et al., 2006; Ninou et al., 2012; Wentworth et al., 2006). However, these traits showed variable results in terms of association to drought resistance and are not easily amenable to large scale screening of breeding lines generated in most breeding programs. Fast and cost effective methods to screen drought resistance in common bean at seedling stages are still needed.

In other crops, various shoot criteria are used to evaluate drought resistance at the seedling stage. For example, in wheat, sunflower, and cotton, recovery of seedling following stress is used as a criterion to assess drought tolerance (Longenberger et al., 2006; Rauf, 2008; Tomar and Kumar, 2004). In rice, tolerance to seedling leaf death and recovery have been used as a screening trait (DeDatta et al., 1988; Mitchell et al., 1998). In legumes, different shoots traits are used to select drought tolerant genotypes. For instance, maintenance of green stem was shown to be an important criterion for seedling stage drought resistance in cowpea (Muchero et al., 2008), while slow wilting trait is associated with drought tolerance in soybean (Sadok et al., 2012).

The wooden box seedling screening method was developed for phenotyping cowpea for drought tolerance (Singh et al., 1999). This method provides the advantage of limiting root growth to assess the shoot drought resistance mechanisms. This technique has been adapted to screen various other crops including cotton, wheat, and water melon (Longenberger et al., 2006; Tomar and Kumar, 2004; Zhang et al., 2011). One of the limitations of the wooden box method is that seedlings grown in the same box will compete with each other for limited moisture present. Evaluation of seedlings on a single plant basis in small pots to limit root expension would provide the benefits of the wooden box method while eliminating competition among

different seedlings. The objective of this study was to evaluate bean seedling's shoot traits to determine which traits would be most suitable to screen for drought tolerance unde greenhouse conditions.

## Materials and methods

### Plant material

Ten bean genotypes were used in this study. Eight of them were from Mesoamerican race of Middle American gene pool.These included black and small red bean cultivars widely grown in Michigan and breeding lines. These genotypes included ‘Blackhawk’, ‘Jaguar’, ‘Phantom’, ‘Zorro’, TARS-SR05, RAB 651, L88-63, and B98311. Mesoamerican race was chosen to try to maintain genetic similarity among different genotypes so that drought response can be monitored easier and not be confounded by differences in phenology and growth habit. One cultivar ‘Concepcion’ from Andean gene pool was used in early experiments, and later replaced by ‘Fuji’ cultivar that was previousely shown to be very susceptible to drought under field conditions.

Zorro is a high yielding mid-full season cultivar that was developed from a cross of B001032/X00822 (Kelly et al., 2009). Zorro exhibits the type II upright short-vine growth habit and good levels of disease resistance. Zorro combines the resistance to Bean common mosaic virus (BCMV) conferred by the presence of the *I* gene, rust *[Uromyces appendiculatus* (Pers.:Pers.) Unger] resistance, and moderate levels of resistance to the common bacterial blight [CBB caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith)] and white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. Zorro has shown good field stress tolerance tracing back to its B98311drought tolerant grandparent. It possesses a typical small opaque black bean seed averaging 21 g per 100 seed.

B98311 is a black bean breeding line that is recognized to have good levels of drought resistance conferred by a deep vigorous taproot (Frahm et al., 2004). B98311 is from Michigan State University bean breeding program and possess type II growth habit.

L88-63 black bean breeding line (Frahm et al., 2004) was produced from the cross of B98311/TLP19 and was selected as a drought resistant line after field evaluations in Honduras, Mexico and Michigan. The TLP19 parent from CIAT breeding program is recognized as a shallow rooting genotype selected to more efficiently uptake phosphorus.

Jaguar was developed from a cross of a black breeding line B90211 with navy bean breeding line N90616 (Kelly et al., 2001). Jaguar has a viny erect type II growth habit and combines high yield potential, resistance to anthracnose, virus, and white mold with good canning quality. Jaguar possesses a small seed, which averages 21 g per 100 seed and is not recognized as being drought stress tolerant.

Phantom (Kelly et al., 2000) is a midseason black cultivar that was derived from the cross of 'Raven' and navy bean breeding line N90618. Phantom combines an upright architecture with resistance to bean common mosaic virus, bean anthracnose races 7, 65 and 73, and bean rust race 53. Phantom and Jaguar are related through navy bean siblings used as their parents.

Blackhawk black bean cultivar was developed from the crosses of 'Tuscola'/CN49-242//'Black Magic'/3/'Midnight'(Ghaderi et al 1990). It has an erect, type II with a short vine growth habit and combines resistance to BCMV, Anthracnose (caused by *Colletotrichum lindemuthianum*), rust, halo blight [*Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al.], and angular leaf spot ALS [*Phaeoisariopsis griseola* (Sacc.) Ferraris]. Blackhawk has small seed size of 23 g per 100 seed and possess satisfactory color retention after processing and acceptable cooked bean texture.

TARS-SR05 small red breeding line was derived from the cross of DOR 557/XAN 176 (Smith et al., 2007). TARS-SR05 has an upright semi-determinate type II growth habit and

combines multiple root rot disease resistance with tolerance to low soil fertility. The average seed size of TARS-SR05 is 24.0 g per 100-seed and is recognized as being stress tolerant.

RAB 651 is a small red-seeded, indeterminate, Mesoamerican race breeding line from CIAT that had not previously been selected for drought resistance during its development, but was recognized to express a high level of drought resistance when evaluated as an advanced line.

Concepcion is a large seeded red-purple mottled colored bean with determinate type I growth habit which is well adapted, high yielding, and popular in Ecuador. Concepcion is not suited to temperate conditions in Michigan due to its photoperiod sensitivity and flowering was induced by reducing day length to 12 hours in the greenhouse.

Fuji (Kelly et al., 2009) is white bean that derived from a cross between 'Hime tebo', a specialty bean class from Japan and 'Matterhorn' a great northern cultivar from Michigan to introduce resistance to BCMV into Tebo. Fuji possesses a determinate type I growth habit, resistance to race 73 of anthracnose and is recognized as being sensitive to drought stress. Fuji's average seed size is 27.6 g per 100 seeds.

**Greenhouse experiments.**

Experiments were modified from the greenhouse protocol used to study drought reaction in cowpea (Muchero et al., 2008). A total of four experiments were conducted. Experiments were conducted during winter and spring seasons of 2009 and 2010. Summer seasons were avoided to limit confounding factors from excessive heat in the greenhouse. The middle bench was used to avoid microclimates associated with ventilation and cooling systems along the greenhouse perimeter. Greenhouse temperatures were kept at 22-25 $^{o}$C and 16 h of photoperiod.

Each experiment was conducted in a completely randomized design with 5 replications in 9 cm square plastic pots. In addition, an equal number of replicates were planted and used as

control maintained under irrigation. In the two first experiments pots were filled with the 200g of a mixture of Baccto potting mix (Michigan Peat Co., Houston, Texas) and coarse Perlite (3:1 v/v). In the last two experiments, 200g of Suremix Perlite (Michigan growers' product, Inc., Galesburg, MI, USA) were used as potting media. Small pots and the limited amount of soil were used to restrict root growth so that drought resistant shoot phenotypes could be assessed. All pots were watered to field capacity and excess water allowed to drain for 4 hours before planting. Each genotype was planted with three seeds visually selected for size and quality. A second and last water application was made after planting in drought stressed pots, while the controls continued to be irrigated at the field capacity on 2d intervals. Seedlings were thinned to one plant at 7d post planting. Drought stress was imposed very early to avoid confounding factors associated with plant size and vigor specific to each genotype. In drought stress experiments, individual plants were scored for wilting, unifoliate senescence, and maintenance of stem greenness. Wilting was scored at 18, 21, and 24d after planting. At 24d, watering was resumed at 2d interval for 14d when genotypes were scored for the recovery. All the pots in stress and non-stress conditions were fertilized at 25d after planting with 20 ml of 20-20-20 NPK Peters professional® water soluble fertilizer. All experiments continued to be watered to field capacity every 2d until mid-pod filling where the number of pods produced/plant was counted and dry biomass determined.

Additional measurements of stomatal conductance and photosynthetic rates were measured at 18d after planting in the third experiment. Gas exchanges were measured at a $CO_2$ reference concentration of 380 μmol $mol^{-1}$. Stomata conductance and photosynthetic rates were

measured using LI-6400XT Portable Photosynthesis System (LI-COR Biosciences, Lincoln, Nebraska, USA).

**Variables scoring**

Wilting was scored on a scale of 0 to 5, with 0 being no sign of wilting and 5 being completely wilted. Unifoliate senescing was assessed as the number of completely senescing unifoliates per pot taken when the most rapidly senescing genotypes had all the unifoliates dried. Stem greenness was scored on a scale of 0 to 5, with 0 being completely yellow and 5 being completely green. Recovery was rated on a scale of 0 to 1, 0 score was given to the genotype which did not recover, 0.5 if the recovery occurred at the basal node, and 1 if the recovery was from the top meristem.

**Statistical analyses.**

All variables were analyzed using SAS 9.3 (SAS institute, Cary, NC). Wilting was analyzed as repeated measurements using GLIMMIX procedure. Unifoliate senescence and recovery were analyzed as generalized linear models using probit link function in GLIMMIX procedure (Littell et al., 2006). Stem greenness, recovery number of pods, recovery fresh and dry biomass, stomatal conductance, and photosynthetic rates were analyzed as mixed models using MIXED procedure. Correlation and regression analysis were performed with PROC CORR and PROC REG.

## Results

### Wilting

As expected, wilting increased over time during the stress period (Figure 3. 1; Table 3. 1). The average wilting scores were 1.05, 2.21, and 4.0 respectively at day 18, 21, and 24 after planting. Correlation coefficients among the measurement times were high between adjacent measurement times. Specifically, the correlation coefficients were 0.73, 0.70, and 0.87 respectively between 18 and 21, 18 and 24, and 21 and 24 days. Significant differences ($p$=0.0016) in wilting existed among genotypes, between measurement times ($p$<0.0001), and interactions between genotype and measurement times ($p$=0.03). Genotypes Jaguar, Phantom, RAB651, Zorro, and Fuji did not show any significant wilting at day 18. However, all the genotypes wilted rapidly after day 18 except Jaguar which had a significantly lower wilting score than the other genotypes. RAB651, Zorro, and Fuji started with a low wilting score at day18, but the wilting rate from day 21 to 24 was so rapid that they did not show any difference with the faster wilting genotypes at day 24 (Figure 3.1). Wilting showed a significant positive correlation with unifoliate senescence while it was negatively correlated with stem greenness, recovery, recovery number of pods, recovery fresh and dry biomass (Table 3. 3).

### Unifoliate senescence

There was a significant difference ($p$=0.0148) for unifoliate senescence between genotypes. Jaguar and Phantom had lower average score for unifoliate senescence than the rest of genotypes while L88-63, B98311, and RAB651 had the highest unifoliate senescence scores (Table 3. 1). Unifoliate senescence was negatively correlated to other variables except wilting.

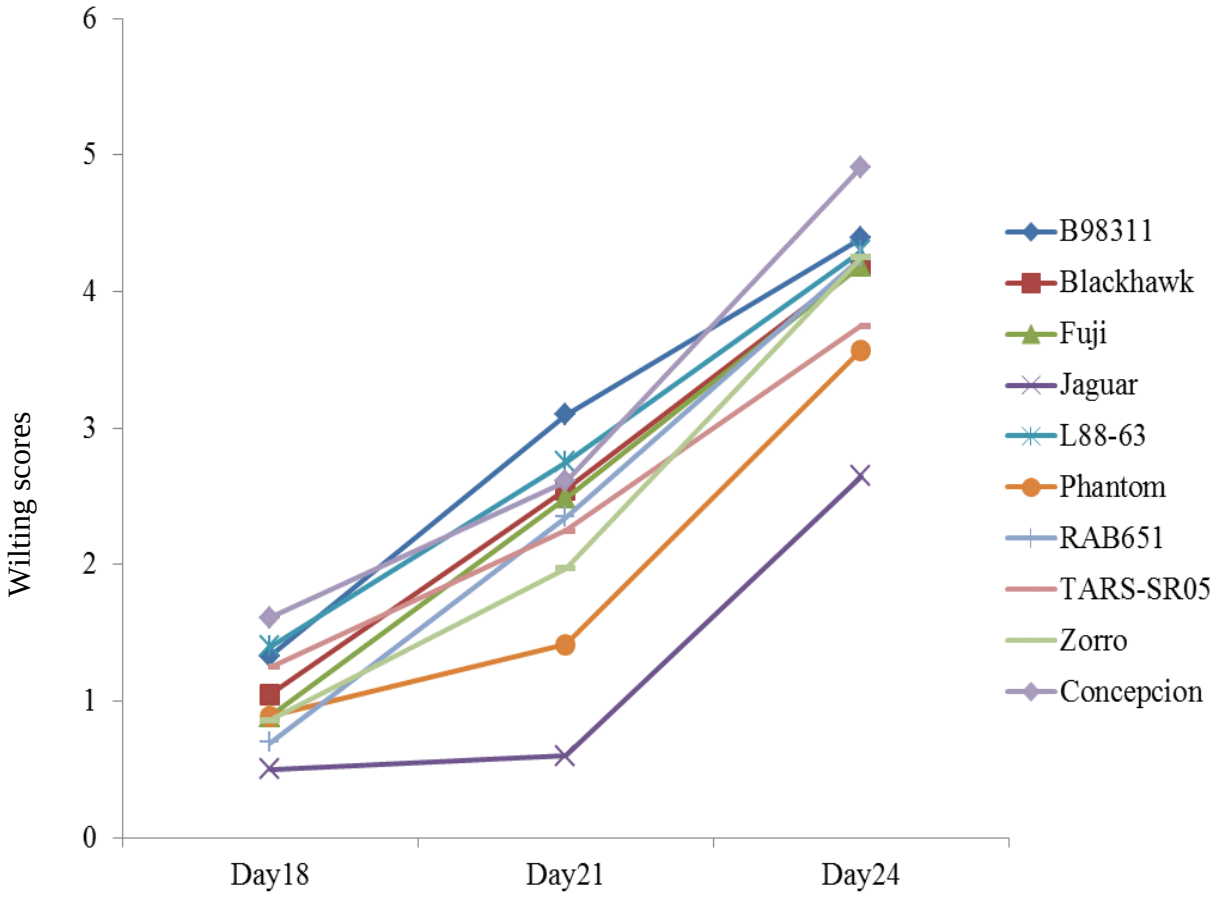


Figure 3. 1. Wilting score of 10 bean genotypes recorded at 18, 21, and 24 days after planting. Wilting was score on a scale of 0 to 5 with 0 being no sign of wilting and 5 being completely wilted.
For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation

Table 3. 1. Mean scores for wilting at 18, 21, and 24 days after planting, unifoliate senescence, stem greenness, and recovery of 10 bean genotypes evaluated under drought stress at the seedling stage.

| Genotypes | Wilting[†] | | | Unifoliate senescence[††] | Stem greenness[§] | Recovery[¶] |
|---|---|---|---|---|---|---|
| | 18 | 21 | 24 | | | |
| B98311 | 1.33a[#] | 3.10a | 4.39a | 0.87a | 2.06b | 0.60b |
| Blackhawk | 1.05a | 2.55a | 4.20a | 0.84a | 2.55b | 0.65b |
| Concepcion | 0.61ab | 2.61a | 4.91a | 0.76ab | 3.10b | 0.59b |
| Fuji | 0.89ab | 2.49a | 4.19a | 0.73ab | 3.50a | 0.48b |
| Jaguar | 0.50b | 0.60b | 2.65b | 0.42b | 3.80a | 0.97a |
| L88-63 | 1.40a | 2.75a | 4.30a | 0.96a | 2.40b | 0.60b |
| Phantom | 0.89ab | 1.41b | 3.57ab | 0.50b | 3.50a | 0.82ab |
| RAB651 | 0.70ab | 2.35ab | 4.25a | 0.87a | 2.50b | 0.68b |
| TARS-SR05 | 1.25a | 2.25ab | 3.75ab | 0.76ab | 3.90a | 0.95a |
| Zorro | 0.86ab | 1.97b | 4.25a | 0.73ab | 2.70b | 0.74b |

[†]: wilting was scored on a scale of 0 to 5 with 0 being no sign of wilting and 5 being completely wilted; [††]: unifoliate senescence was recorded as the number of senesced leave/2 when the most rapidly senescing genotype had their unifoliates dried; [§]: stem greenness scored on a scale of 0 to 5 with 0 being yellow and 5 being totally green; [¶]: recovery scored on a scale of 0 to 1 with 0 being no recovery, 0.5 when the recovery occurred from the basal meristem, and 1 when recovery occurred from the apical meristem. Seedlings were grown in shallow soil profile in small pots. [#]: Means with the same letter are not significantly different.

**Stem greenness**

Significant differences ($p$<0.0001) for stem greenness were observed between genotypes. Genotypes TARS-SR05, Jaguar, Fuji, and Phantom had high scores for stem greenness while B98311, RAB651, and L88-63 had the lowest scores (Table 3. 1). The stem greenness score of B98311 did not differ significantly from the score of Blackhawk, L88-63, RAB651, and Zorro. Stem greenness was negatively correlated to wilting and unifoliate senescence (Table 3. 3). However, stem greenness positively correlated with recovery, pod number, fresh biomass and dry biomass (Table 3.3).

**Recovery from drought**

Significant differences ($p$=0.0155) for recovery were observed among genotypes. Genotypes were classified into two groups (Table 3. 1). The first group comprised genotypes Jaguar, TARS-SR05, and Phantom with recovery rates of 0.97, 0.95, and 0.82 respectively. The rest of genotypes were grouped into the second category with low recovery rates. Recovery was negatively correlated to wilting and unifoliate senescence while it was positively correlated to stem greenness, pod number, and biomass (Table 3. 3). Since recovery from drought stress is important for subsequent plant growth and reproduction, regression analysis of recovery on wilting, unifoliate senescence, and stem greenness was used to predict variables that are likely to influence seedling recovery. As results, only two variables stem greenness and wilting fitted in the model that explained 48% of variation in recovery. Stem greenness alone accounted for 43% of the variation in recovery.

**Number of pods**

The analysis of variance for the pod number showed significant differences between stress and non-stressed treatments ($p$<0.0001), and among genotypes ($p$<0.0001). Water treatment/genotype interactions were not significant. The stressed treatment had an average of 3.3 pods while the non stress treatment had an average 7.3 pods. Drought stress significantly reduced the number of pods for all genotypes. Percentage pod number reduction was the highest in Fuji while it was lowest for Jaguar (Table 3. 2). Jaguar and Phantom did not show significant loss in pod number due to drought effects.

**Dry biomass**

Significant differences ($p$=0.0001) were observed for dry biomass between stress and nonstress treatments. The average dry biomass in stressed treatments was 4.2 g while the average biomass was 10.2 g in nonstress treatment. In addition, significant differences ($p$=0.0008) were observed for genotype and genotype/ water treatment interactions ($p$=0.0009). Under non-stress conditions, all genotypes had equivalent amounts of biomass. However, under stress conditions, genotypes Jaguar, Phantom, Blackhawk and TARS-SR05 had lower reduction of dry biomass than other genotypes (Table 3. 2).

**Photosynthesis**

Photosynthetic rates were evaluated for the experiment 3 at day 18. At this time, there were significant differences between water treatments ($p$<0.0001). However, there was no significant difference among genotypes within each water treatment or genotype/water treatment interactions. In the stress treatment, the average photosynthetic rate was 3.2 μmol $m^{-2}s^{-1}$ whereas it was 11.5 μmol $m^{-2}s^{-1}$ in non-stress treatment. Although no significant differences

among genotypes were observed, RAB651and TARS-SR05 had lower photosynthetic rates under drought stress. Certain genotypes such as Jaguar, TARS-SR05, and Phantom did not have high photosynthetic rates under non-stress but they were able to maintain relatively higher photosynthetic rates under drought conditions (Table 3. 4).

**Stomatal conductance**

Stomatal conductance was evaluated at the same time as photosynthetic activity. Photosynthesis and stomatal conductance were highly dependent on each other [$R^2 = 0.97^{**}$ and $0.82^{**}$ for stress and non stress respectively; (Figure 3. 2)]. Although the analysis of variance did not show any difference between genotypes or genotype/water treatment interaction, there was a significant difference between stress and non-stress treatments ($p$=0.0030). The average stomatal conductance was 0.0205 mmol $m^{-2}s^{-1}$ in stressed treatment whereas it was almost ten times greater (0.225 mmol $m^{-2}s^{-1}$) in non stress treatments. Genotypes RAB651 and B98311 had lower stomatal conductance in stress treatments. However, under non-stress, genotypes B98311, Fuji, and Zorro had relatively high stomatal conductance compared to the other genotypes (Table 3. 4).

**Gas exchanges in relation to wilting, stem greenness, and recovery.**

When stomatal conductance and photosynthetic rate were regressed on wilting (Figure 3. 3), stem greenness (Figure 3. 4), and recovery (Figure 3. 5), there was a strong negative relationship between wilting and photosynthesis and stomatal conductance ($R^2 = 0.65^{**}$). Variation in stem greenness variation was positively explained by photosynthetic rate and

stomatal conductance ($R^2$= 0.89$^{**}$ and 0.83$^{**}$) respectively. Similarly, recovery was positively explained by photosynthesis and stomatal conductance ($R^2$=0.53$^{*}$ and 0.57$^{*}$), respectively.

Table 3. 2. Mean values for number of pods, dry biomass along with percent reductions for 10 bean genotypes evaluated in the greenhouse under drought stress at the seedling stage.

| | Pods (number) | | | Dry biomass (g) | | |
|---|---|---|---|---|---|---|
| Genotypes | Non stress | stress | % reduction | Non stress | stress | % reduction |
| B98311 | 7.73 | 2.80 | 64$^{b}$ | 9.68 | 2.14 | 78$^{b}$ |
| Blackhawk | 7.53 | 3.66 | 51$^{b}$ | 11.34 | 5.44 | 52$^{a}$ |
| Concepcion | 2.78 | 0.90 | 68$^{c}$ | 12.34 | 2.44 | 80$^{b}$ |
| Fuji | 8.85 | 0.05 | 99$^{c}$ | 9.40 | 1.18 | 87$^{b}$ |
| Jaguar | 8.73 | 6.66 | 24$^{a}$ | 10.39 | 7.84 | 25$^{a}$ |
| L88-63 | 6.87 | 3.20 | 53$^{b}$ | 8.93 | 2.91 | 67$^{b}$ |
| Phantom | 8.38 | 5.50 | 35$^{a}$ | 10.11 | 6.50 | 36$^{a}$ |
| RAB651 | 6.93 | 2.06 | 70$^{b}$ | 9.66 | 2.49 | 74$^{b}$ |
| TARS-SR05 | 9.00 | 5.10 | 43$^{a}$ | 9.34 | 7.63 | 18$^{a}$ |
| Zorro | 6.20 | 3.20 | 49$^{b}$ | 9.94 | 3.20 | 68$^{b}$ |

Seedlings were grown in shallow soil profile in small pots. Genotypes with the same letter are not significantly different at 0.05 probability level

Table 3. 3. Spearman correlation coefficients among wilting, unifoliate senescence stem greenness, recovery, pod number, and dry biomass for 10 bean genotypes evaluated in the greenhouse under drought stress at the seedling stage.

| | Wilting | Unifoliate senescence | Stem greenness | Recovery | Pod number |
|---|---|---|---|---|---|
| Unifoliate senescence | 0.62*** | | | | |
| Stem greenness | -0.68*** | -0.52*** | | | |
| Recovery | -0.66*** | -0.42*** | 0.65*** | | |
| Pod number | -0.63*** | -0.44*** | 0.54*** | 0.72*** | |
| Dry biomass | -0.72*** | -0.57*** | 0.57*** | 0.79*** | 0.83*** |

***: Significant at 0.0001 probability level; Seedlings were grown in shallow soil profile in small pots

Table 3. 4. Mean values for photosynthetic rates and stomatal conductance for 10 bean genotypes evaluated in the greenhouse under drought stress and non stress at seedling stage.

| | Photosynthetic rate ($\mu mol/m^2/s^{-2}$) | | Stomatal conductance ($mmol/m^2/s^{-2}$) | |
|---|---|---|---|---|
| Genotype | Stress | Non stress | Stress | Non stress |
| B98311 | 2.20 | 13.42 | 0.01 | 0.27 |
| Blackhawk | 3.26 | 13.22 | 0.02 | 0.23 |
| Fuji | 3.91 | 13.32 | 0.02 | 0.29 |
| Jaguar | 4.27 | 10.88 | 0.03 | 0.20 |
| L88-63 | 2.66 | 9.96 | 0.02 | 0.19 |
| Phantom | 3.59 | 11.01 | 0.02 | 0.24 |
| RAB651 | 1.94 | 8.79 | 0.01 | 0.15 |
| TARS-SR05 | 4.04 | 9.63 | 0.03 | 0.13 |
| Zorro | 2.69 | 13.10 | 0.02 | 0.27 |

Seedlings were grown in shallow soil profiles in small pots

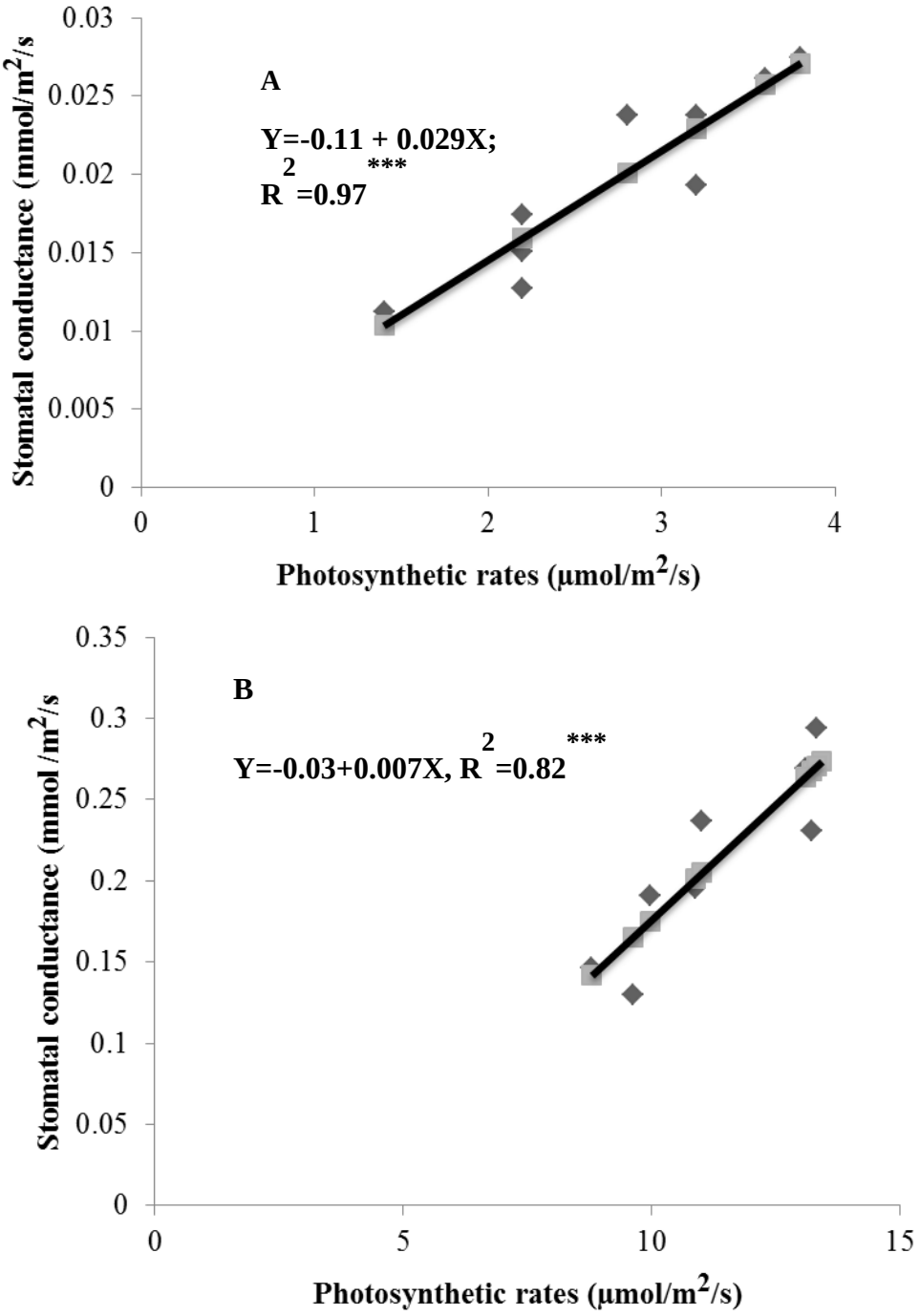


Figure 3. 2. Relationship between stomatal conductance (mmol $m^{-2}$ $s^{-1}$) and photosynthesis (µmol $m^{-2}$ $s^{-1}$) measured 18 days after planting for 10 bean genotypes evaluated in the greenhouse under drought stress at seedling stage. Seedlings were grown in shallow soil profile in small pots. A. Stress, B. Non stress

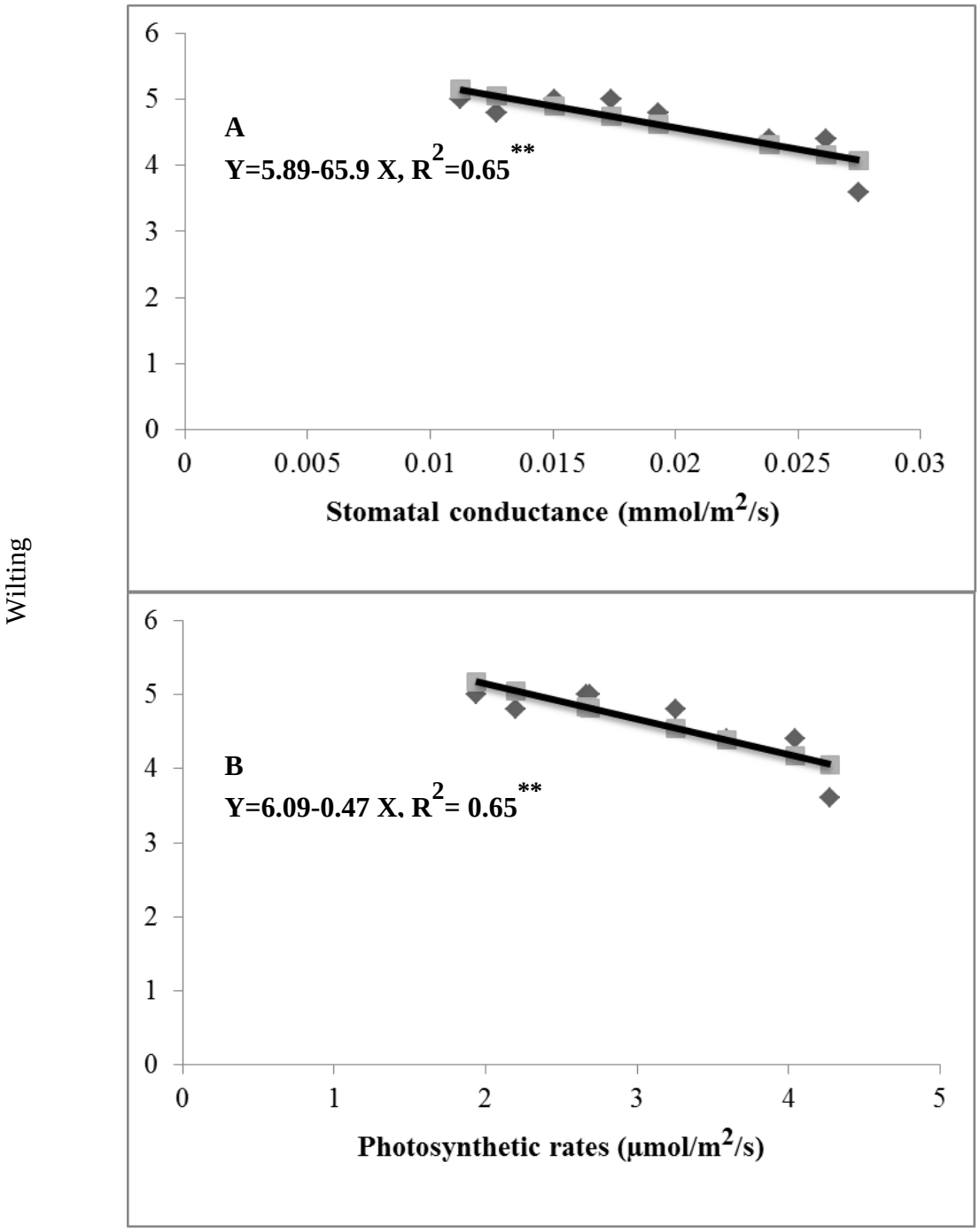


Figure 3. 3. Relationship between wilting and stomatal conductance (A) and wilting, photosynthesis (B) and wilting recorded in 10 bean genotype grown under drought stress conditions in the greenhouse. Wilting was scored at 18 days after planting on a scale of 0 to 5; 0 being no sign of wilting and 5 being completely wilted. Stomatal conductance and photosynthetic rates were measured at day18 after planting.

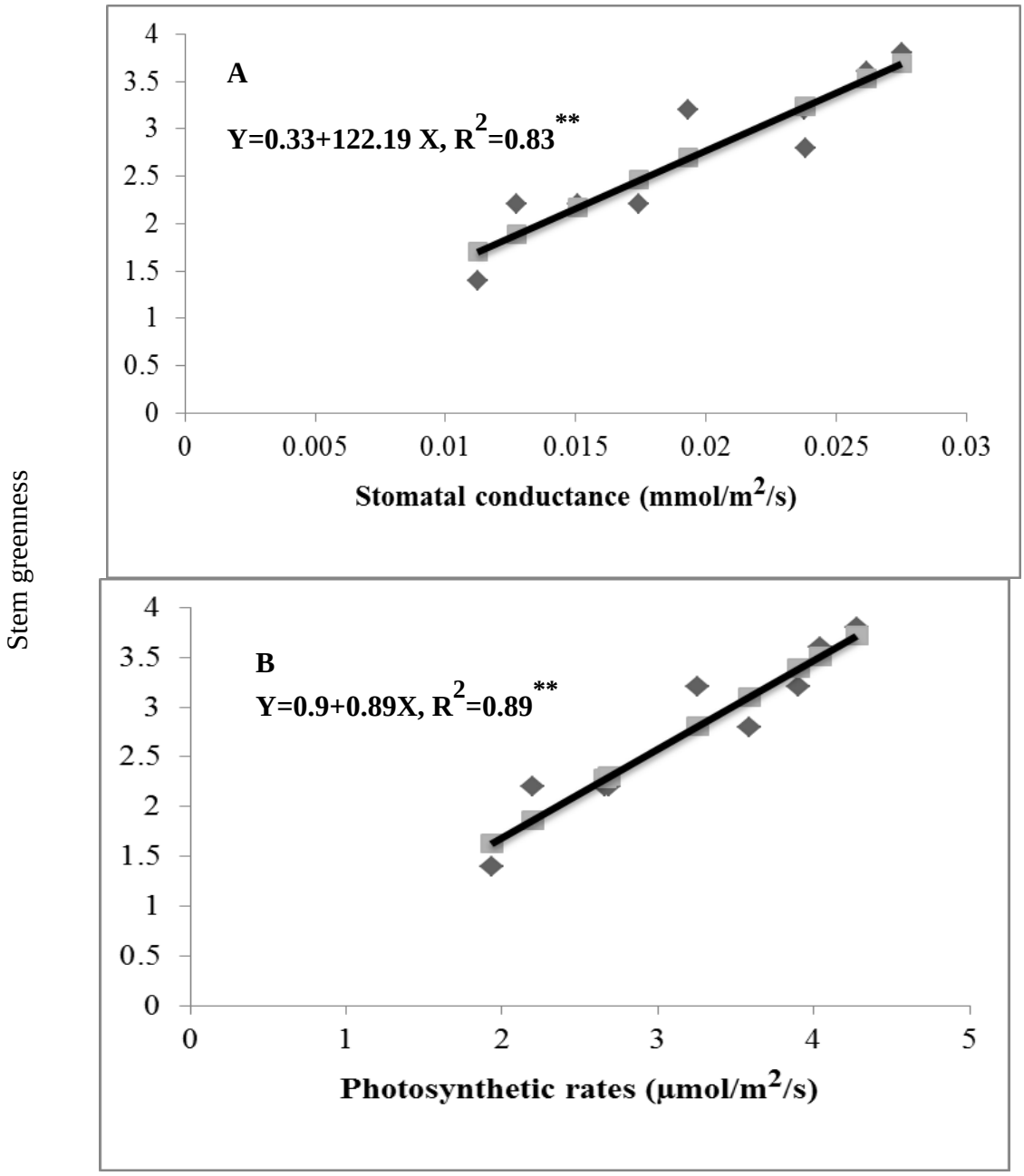


Figure 3. 4. Relationship between stem greenness and stomatal conductance (A), and stem greenness and photosynthesis (B) recorded in 10 bean genotype grown under drought stress conditions in the greenhouse. Wilting was scored at 18 days after planting on a scale of 0 to 5; 0 being yellow stem and 5 being completely green stem. Stomatal conductance and photosynthetic rates were measured at 18 days after planting

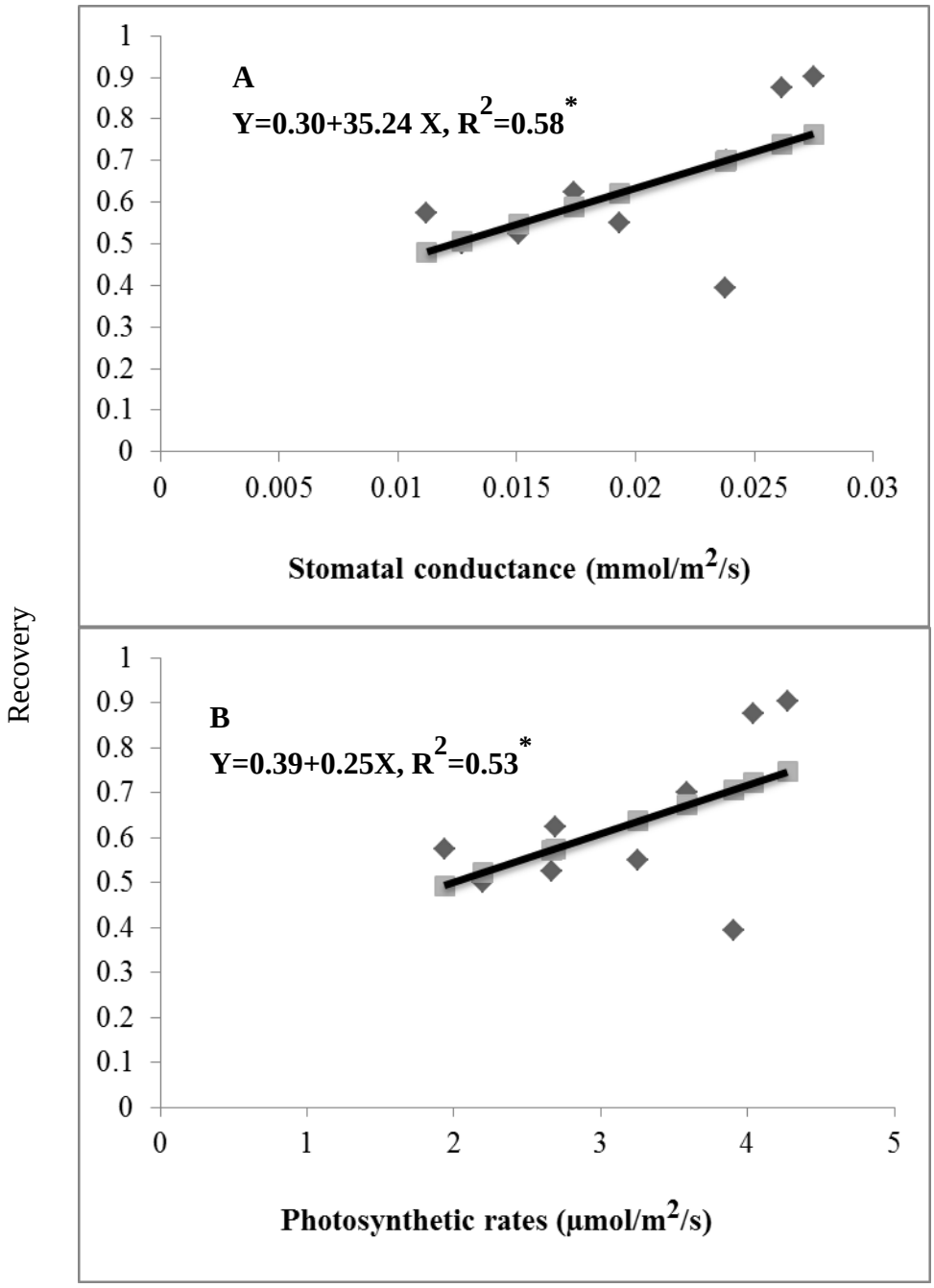


Figure 3. 5. Relationship between recovery and stomatal conductance (A), and recovery and photosynthesis (B) recorded in 10 bean genotype grown under drought stress conditions in the greenhouse. Recovery was scored on a scale of 0 to 1; 0 score given to genotypes that did not recover, 0.5 score given to genotypes with recovery from basal nodes, and 1 to genotypes that recovered from top meristems. Stomatal conductance and photosynthetic rates were measured at 18 days after planting while the recovery was recorded two weeks after resuming watering.

## Discussion

Visual observation of wilting progression under greenhouse experimental conditions identified different wilting behaviors among the ten common bean genotypes studied. Phantom and Jaguar maintained lower wilting scores compared to other genotypes whereas genotypes Concepcion, B98311, and L88-63 had higher wilting values at all observation dates. Wilting response of Jaguar, Phantom, and TARS- SR05 increased at a slower rate overtime compared to other genotypes (Figure 3. 1). At the end of experiments, those genotypes that wilted slowly were able to conserve water in leaves and stem tissues, survive the dry period, resume growth, and reproduce as evidenced by lower reduction of biomass and pod number (Table 3. 2). However, genotypes that wilted fast had a relatively lower recovery rates and reproductive capacity (Table 3. 1 and 3. 2). Genotypes Jaguar and Phantom share a common ancestry since N90616 and N90618 parents are sib lines. The similar reaction of Jaguar and Phantom to water stress might be accounted for by similar ancestry. TARS-SR05 breeding line is resistant to multiple stresses and was selected based on good performance in poor soil conditions especially compacted and water logged soils infected with root rot causing fungi (Smith et al., 2007). These three genotypes Jaguar, Phantom, and TARS-SR05 appear to possess some drought tolerance mechanisms that confer slow wilting.

Since the slow wilting trait in these experiments was not associated with deep water extraction as all genotypes were planted in shallow soil profiles, it is inferred that this trait is associated with mechanisms in the shoot. Jaguar, Phantom, and TARS-SR05 appear to conserve soil water through limited transpiration which sustained leaf turgor throughout the progression of soil drying. In soybean, a slow wilting trait was found in soybean plant introductions PI416937 and PI471938 (Sloane et al., 1990; Hufstetler et al., 2007; King et al., 2009) under drought

conditions. PI416937 stabilizes transpiration at certain threshold of the vapor pressure deficits (Fletcher et al., 2007) while the basis of the trait in PI 471938 is still unknown (Sadok et al., 2012). The limited transpiration in PI416937 was later shown to be caused by a limited hydraulic conductance of water from leaf xylem into guard cells (Sinclair et al., 2008). Another possibility would be that other leaf associated mechanisms different from stomatal closure and reduced water loss during photosynthesis might play a role in wilting avoidance observed in these bean genotypes. One possible way that these genotypes might maintain slow wilting could be through osmotic adjustment. When there is no available moisture to extract from deep soil, osmotic adjustment has been shown to result in better turgor maintenance, plant survival and recovery upon the relief of severe drought (White et al., 1992). Wilting is a trait expressed by plants that have passed the leaf water potential at turgor loss point. Leaf turgor loss point has been used to quantify plant drought tolerance levels (Bartlett et al., 2012) while visual assessment of wilting is commonly used as a measure of leaf water potential and seedling drought survival in trees (Engelbrecht et al., 2007). Plants that wilt slowly tend to maintain stomatal conductance, hydraulic conductance, photosynthesis and growth at lower soil water potential, which is especially important when drought occurs early during the growing season (Bartlett et al., 2012). Despite the absence of genotypic differences for photosynthetic rate and stomatal conductance under stress conditions, Jaguar, TARS-SR05, and Phantom had relatively higher values for these two parameters under stress and relatively lower values under non stress conditions compared to the other genotypes. These results suggest that these genotypes might have saved water which later allowed them to maintain photosynthesis under severe water deficit. In other grain legumes such as soybean, peanut, chickpea, and cowpea, maintaining maximum transpiration rates in relatively lower vapor pressure deficit has been recognized as a soil water saving strategy in

tolerant genotypes (Devi et al., 2010; Sinclair et al., 2008; Zaman-Allah et al., 2011; Belko et al., 2012). In contrast, an early study on common bean showed that all cultivars under study reached the maximum transpiration at similar values of leaf to air humidity gradient despite genotypic differences in stomatal conductance (Comstock and Ehleringer, 1993). In that study, different genotypes were grown in 1m long PVC tubes with a large volume of soil and watered every 3 days as opposed to severe drought treatment of the current study which limits a direct comparison of the two studies. Since genotypes with low wilting rates were able to maintain relatively high stomatal conductance and photosynthesis, it would be interesting to study the transpiration behavior of common bean in relation to vapour pressure deficit and determine if common bean genotypes behaves in a similar fashion to its related legume species.

Unifoliate senescence was positively correlated to wilting ($r=0.62^{***}$) but negatively correlated to stem greenness, recovery, as well as the productivity parameters of pod number and biomass. While it is possible to distinguish genotypes based on the progression of the senescing unifoliate in cowpea (Muchero et al., 2008), there were no notable differences in this trait among common bean genotypes. Bean unifoliates stayed green until their complete desiccation which eliminated the confusion between drought, aging, and nutrient deficiency.

Stem greenness was positively correlated with recovery ($r=0.65^{***}$). Stepwise regression analysis to determine which shoot variables could be used to predict recovery from drought showed that stem greenness and wilting contributed significantly to the regression model. These two variables combined explained 48% of the variation in recovery. Stem greenness alone accounted for 43% while wilting accounted only 5% of the variation in recovery. Additionally, stomatal conductance and photosynthetic rate regression to wilting, stem greenness, and recovery showed that these two variables explained more stem greenness ($R^2=0.83^{***}$ and $R^2=0.89^{***}$)

variability than wilting ($R^2=0.65^{***}$) and recovery ($R^2=0.58^{*}$ and $R^2=0.53^{*}$). These results suggest that bean genotypes need to maintain a green stem to be able to recover from drought and resume growth. However, Fuji was an exception to this trend as Fuji usually maintained a green stem. At the resumption of irrigation, instead of recovering, Fuji seedlings continued to drydown from the top and subsequently died. These results suggest that when exposed to a severe drought in seedling stage, it was not possible for seedlings of Fuji to regrow upon drought stress relief probably because of its determinant growth habit. Under severe drought stress of 2010 in the field in Michigan, Fuji showed a strange growth of producing excessive vegetative tissue instead of setting flowers and pods. Fuji must have received this drought susceptibility from the recurrent backcross Tebo parent since the Matterhorn donor parent has shown to be relatively drought tolerant (Urrea et al. 2009; Singh, 2007).

Stem greenness predicted the recovery fate for genotypes from Mesoamerican race. Genotypes such as Jaguar, Phantom, and TARS-RS05 with high stem greenness scores had higher frequency of recovery (Table 3.1). Genotypes B98311, and L88-86 not only had lower stem greenness and recovery rates, but also performed poorly in general in all experiments. B98311 is a parent of L88-63 and X00822 one of the parents of Zorro. B98311 and L88-63 were selected based on root length under terminal drought (Frahm et al., 2004) and Zorro performs relatively well under field drought stress. The fact that B98311, and L88-86 behaved poorly and similarly in constrained root growth conditions highlights the complexity associated with breeding for drought tolerance and strengthens the importance of knowing the target environment as well as the growth stage at which the crop is likely to encounter drought. In addition, these results highlight the importance of combining root and shoot drought traits to achieve stability in drought resistance over a range of soil conditions. The general prediction of

the recovery based on the stem greenness in genotypes from Mesoamerican race suggests that stem greenness can be used as a screening proxy for drought resistance at early stages of development in this bean race.

Interestingly, stem greenness was identified to be an important seedling trait associated with drought resistance in cowpea which is the closest relative of common bean (Muchero et al., 2008). Cowpea is recognized as the most drought tolerant legume species (Hall, 2012) while common bean is among the least tolerant. Sharing the same trait for drought tolerance at seedling stage suggests that this trait might be under the same genetic control in these two legume species. Common bean is generally grown in wetter environments than cowpea, and rarely drought conditions that affect stem occur during bean growth season. Since wilting was negatively correlated to stem greenness and recovery ($R^2$= - 0.68$^{***}$ and - 0.66$^{***}$) respectively, wilting might be a more practical trait to measure than stem greenness in common bean. For drought tolerance screening purposes, combining delayed wilting and stem greenness would provide more useful information about genotypic differences in terms of drought tolerance. Since this study was conducted on a limited number of genotypes, extending the number of genotypes and exercising more controlled conditions on wilting and stem greenness might provide more details on usefulness of these traits in seedling screening for drought tolerance. The usefulness of these two traits to predict the drought tolerance in the field is unclear at this point. Therefore, correlations between greenhouse and field data for these two traits need to be verified.

Pod number and biomass were significantly reduced by drought. These results suggest that severe drought in the seedling stage might have deleterious effects on yield through reduced biomass accumulation. This is important since the plants may not have sufficient time to invest in biomass production after an extended period of drought. Instead they might directly enter the

reproduction period without sufficient biomass reserves for optimum yield. This could be disastrous for bean genotypes with determinate growth habit which may not be able to initiate a second flush of pod setting when vegetative growth period has passed.

## Conclusion

Screening protocols that can be used to select drought tolerant bean cultivars in controlled environments are needed. These screening methods need to be fast and effective to be integrated into breeding programs. Seedling drought tolerance screening methods would offer these advantages by allowing the screening of numerous lines in a relatively short period at low cost compared to delaying until plant maturity to evaluate yield commonly used in field trials.

This study was conducted to determine shoot traits that are associated with drought resistance at early development stage in common bean. The study was conducted in the greenhouse using small pots and limited amount of soil to constraint root growth. Four shoot traits consisting of wilting, unifoliate senescence, stem greenness, and recovery from drought were evaluated. Stomatal conductance and photosynthetic rate were measured to understand the cause of change in these traits. In addition, the number of pods and the biomass were evaluated to quantify the impact of seedling stage drought stress on bean plant productivity. Plant growth and productivity after drought stress is dependent on the degree of recovery from stress. Wilting was negatively correlated to the recovery. Stem greenness was highly positively correlated to the recovery in bean genotypes from Mesoamerican race. Therefore stem greenness can be used to evaluate beans from this race for drought resistance. Certain bean genotypes known to have drought resistance conferred by deep rooting capacity performed poorly in shallow soil profiles used in these experiments.

These results highlighted the importance of knowing the target environment during the breeding process. In addition, these results suggested that combining root and shoot resistance mechanisms in single genotype could enhance performance under broad range of soil moisture conditions.

## LITERATURE CITED

## LITERATURE CITED


Asfaw, A. and M. W. Blair. 2012. Quantitative trait loci for rooting pattern traits of common beans grown under drought stress versus non-stress conditions. Mol. Breed. 30: 681-695

Bartlett, M. K., C. Scoffoni, and L. Sack. 2012. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecol. Letters 15: 393-405

Beebe S, I. M. Rao, M. W. Blair, and L. Butare. 2009. Breeding for abiotic stress tolerance in common bean: present and future challenges. Available at http://193.43.36.176/gipbtest/images/pdf_files/LiteratureViews/Beebe_DOC_apb09.pdf accessed 30 September 2012.

Beebe, S. E, I. M. Rao and M. W. Blair. 2010. Phenotyping common beans for adaptation to drought, pp. 309-334 in Drought Phenotyping in Crops: From Theory to Practice, edited by J. M. Ribaut, and P. Monneveux. Generation Challenge Program Special Issue on Phenotyping. CGIAR, Texcoco, Mexico.

Belko, N., M. Zaman-Allah, N. Cisse, N. N. Diop, G. Zombre, J. D. Ehlers, and V. Vadez. 2012. Lower soil moisture threshold for transpiration decline under water deficit correlates with lower canopy conductance and higher transpiration efficiency in drought-tolerant cowpea. Funct. Plant Biol. 39: 306-322.

Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. Plant Grow. Reg. 20: 135-148.

Castrillo, M., D. Fernandez, A. M. Calcagno, I. Trujillo, and L. Guenni. 2001. Response of ribulose-1, 5-bisphosphate carboxylase, protein content, and stomatal conductance to water deficit in maize, tomato, and bean. Photosynthetica 39: 221-226

Chaves, M. M, J. M. Costa, and N. J. M. Saibo. 2011. Recent advances in photosynthesis under drought and salinity. Adv. Bot. Res. 57: 50-104

Comstock, J. and J. Ehleringer. 1993. Stomata1 response to humidity in common bean (*Phaseolus vulgaris):*Implications for maximum transpiration rate, water-use efficiency and productivity. Aust. J. Plant Physiol. 20: 669-91

DeDatta, S. K., J. A. Malabuyoc, and E. L. Aragon. 1988. A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. Field Crops Res. 19: 123-134.

Devi, M. J., T. R. Sinclair and V. Vadez. 2010. Genotypic Variation in peanut for transpiration response to vapor pressure deficit. Crop Sci. 50: 191-196.

Desclaux, D, T. Huynh, and P. Roumet. 2000. Identification of soybean plant characteristics that indicate the timing of drought stress. Crop Sci. 40: 716-722.

Dias, M. C., and W. Brüggemann. 2010. Limitations of photosynthesis in phaseolus vulgaris under drought stress : gas exchange, chlorophyll fluorescence and Calvin cycle enzymes. Photosynthetica 48: 96-102.

Engelbrecht, B. M., M. T. Tyree, and T. A. Kursar. 2007. Visual assessment of wilting as a measure of leaf water potential and seedling drought survival. J. Trop. Ecol. 23: 497-500

Fletcher, A. L., T. R. Sinclair, and L. H. Allen Jr. 2007. Transpiration responses to vapour pressure deficit in well watered 'slow-wilting' and commercial soybean. Env. Exp. Bot. 61:145-151.

Frahm, M. A., J. C. Rosas, N. Mayek-Perez, E. Lopez-Salinas, J. A. Acosta-Gallegos, and J. D. Kelly. 2004. Breeding beans for resistance to terminal drought in the lowland tropics. Euphytica 136: 223–232.

Gadheri, A., J. D. Kelly, M. W. Adams, A. W. Saettler, G. L. Hosfield, G. V. Varner, M. A. Uebersax, and J. Taylor. 1990. Registration of 'Blackhawk' tropical black bean. Crop Sci. 30: 744-745.

Hall A. E. 2012. Phenotyping Cowpeas for adaptation to drought. Frontiers Physiol. 3: 2-8

Hufstetler, E. V., H. R. Boerma, T. E. Carter, and H. G. Earl. 2007. Genotypic variation for three physiological traits affecting drought tolerance in soybean. Crop Sci. 47:25-35.

IPCC, 2007. Synthesis Report.
Http://www.ipcc.ch/publications_and_data/publications_and_data_reports.shtml

Kamoshita, A, R. C. Babu, N. M. Boopathi, and S. Fukai. 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crops Res. 109: 1-23.

Kelly, J. D., G. L. Hosfield, G. V. Varner, M. A. Uebersax, and J. Taylor. 2000. Registration of Phantom black bean. Crop Sci. 40:572.

Kelly, J. D., G. L. Hosfield, G. V. Varner, M. A. Uebersax, and J. Taylor. 2001. Registration of Jaguar black bean. Crop Sci. 41:1647.

Kelly, J. D., G. V. Varner, B. Román, and B. Long. 2009. Registration of 'Fuji' Otebo Bean. J. Plant Reg. 3: 223-225

Kelly, J. D., G. V. Varner, P. O'Boyle, and B. Long. 2009. Registration of 'Zorro' Black Bean. J. Plant Registrations 3: 226-230.

King, C. A., L. C. Purcell, and K. R. Brye. 2009. Differential wilting among soybean genotypes in response to water deficit. Crop Sci. 49:290-298

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models, second edition. Cary, NC: SAS institute Inc.

Lizana, C., M. Wentworth, J. P. Martinez, D. Villegas, R. Meneses, E. H. Murchie, C. Pastenes, B. Lercari, P. Vernieri, P. Horton, and M. Pinto. 2006. Differential adaptation of two varieties of common bean to abiotic stress: I. Effects of drought on yield and photosynthesis. J. Exp. Bot. 57: 685-97.

Longenberger, P. S., C. W. Smith, P. S. Thaxton, and B. L. Mcmichael. 2006. Development of a screening method for drought tolerance in cotton seedlings. Crop Sci. 46:2104-2110

Lynch, J. and J. J Van Beem. 1993. Growth and architecture of seedling roots of common bean genotypes. Crop Sci. 33:1253-1257.

Mitchell, J. H., D. Siamhan, M. H. Wamala, J. B. Risimeri, E. Chinyamakobvu, S. A. Henderson, and S. Fukai. 1998. The Use of Seedling Leaf Death Score for Evaluation of Drought Resistance of Rice. Field Crops Res. 55: 1-2.

Montero-Tavera, V, R. Ruiz-Medrano, and B. Xoconostle-Cázares. 2008. Systemic nature of drought-tolerance in common bean: Plant Sign. Behav. 3: 663-666

Muchero, W., J. D. Ehlers, and P. A. Roberts. 2008. Seedling stage drought induced phenotypes and drought responsive genes in diverse cowpea genotypes. Crop Sci. 48: 541-552

Ninou, E., J. T. Tsialtas, C. A. Dordas, D. K. Papakosta. 2012. Effect of irrigation on the relationships between leaf gas exchange related traits and yield in dwarf dry bean grown under Mediterranean conditions. Agric. Water Manage: http://dx.doi.org/10.1016/j.agwat.2012.08.002

Rauf, S. 2008. Breeding sunflower (*Helianthus annuus* L.) for drought tolerance. Communications in Biometry and Crop Sci. 3: 29-44

Sadok, W., M. E. Gilbert, M. Aown, S. Raza, and T. R. Sinclair. 2012. Basis of Slow wilting phenotype in soybean PI 471938. Crop Sci. 52: 1261-1269

Sally, W, G. R. Kudoyarova, D. S. Veselov, T N. Arkhipova, and W. J. Davies. 2012. Plant hormone interactions: innovative targets for crop breeding and management. J. Exper. Bot. 63: 3499-3509

SAS Institute, Inc. 2011. SAS version 9.3. SAS Institute Inc., Cary, NC.

Sinclair, T. R. 2011. Challenges in breeding for yield increase for drought. Trends in Plant Sci. 16: 289- 293.

Sinclair, T. R., M. A. Zwieniecki, and N. M. Holbrook. 2008. Low leaf hydraulic conductance associated with drought tolerance in soybean. Physiol. Plant. 132:446-451.

Singh, B. B, Y. Mai-Kodomi and T. Terao. 1999. A simple screening method for drought tolerance in cowpea. Indian J. Genet. 59**:** 211-220.

Singh, S. P. 1995. Selection for water stress tolerance in interracial populations of common bean. Crop Sci. 35:118-124

Singh, S. P. 2007. Drought resistance in the race Durango dry bean landraces and cultivars. Agron. J. 99:1219-1225

Sloane, R. J., R. P. Patterson, and T. E. Carter. 1990. Field drought tolerance of a soybean plant introduction. Crop Sci. 30:118-123.

Smith, J. R., S. J. Park, J. S. Beaver, P. N. Miklas, C. H. Canaday, and M. Zapata. 2007. Registration of TARS-SR05 multiple disease-resistant dry bean germplasm. Crop Sci. 47: 457- 458.

Sponchiado, B. N., J. W. White, J. A. Castillo, and P. G. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp.Agric. 25: 249-257.

Tomar, S. M. S. and G. T. Kumar. 2004. Seedling survivability as a selection criterion for drought tolerance in wheat. Plant Breed. 123:392-394

Trachsel, S., S. M. Kaeppler, K. M. Brown, and J. P. Lynch. 2010. Shovelomics: high throughput phenotyping of maize (Zea Mays L.) Root architecture in the field. Plant and Soil 341: 75-87.

Urrea, C. A., C. D. Yonts, D. J. Lyon, and A. E. Koehler. 2009. Selection for Drought Tolerance in Dry Bean Derived from the Mesoamerican Gene Pool in Western Nebraska. Crop Sci. 49 : 2005- 2010.

Watanabe, S., S. Hakoyama, T. Terao, and B. B. Singh. 1997. Evaluation methods for drought tolerance of cowpea. P. 87-97. In B.B. Singh et al. (ed.) Advances in cowpea research. IITA, Ibadan, Nigeria.

Wentworth, M., E. H Murchie, J. E Gray, D. Villegas, C. Pastenes, M. Pinto, and P. Horton. 2006. Differential adaptation of two varieties of common bean to abiotic stress: II. Acclimation of photosynthesis. J. Exp. Bot. 57: 699-709.

White, J. W, and J. A. Castillo. 1985. Relative effect of root and shoot genotypes on yield of common bean under drought stress. Crop Sci. 29:360-362.

White, R. H., M. C. Engelke, S. J. Morton, and B. A. Ruemmele. 1992. Competitive turgor maintenance in tall fescue. Crop Sci. 32:251-256

Zaman-Allah, M., D. M. Jenkinson and V. Vadez. 2011. A conservative pattern of water use, rather than deep or profuse rooting, is critical for the terminal drought tolerance of chickpea. J. Exp. Bot. 62: 4239–4252.

Zhang, H., G. Gong, S. Guo, Y. Ren, and Y. Xu. 2011. Screening the USDA watermelon germplasm collection for drought tolerance at the seedling stage. Hort Sci. 46:1245-1248.

## Chapter 4. Factors influencing regeneration and Agrobacterium tumefaciens mediatedtransformation of common bean (*Phaseolus vulgaris* L.*)*


### Abstract

A systematic study was carried out to optimize regeneration and *Agrobacterium tumefaciens*-mediated transformation of four selected common bean (*Phaseolus vulgaris* L.) cultivars; 'Red Hawk', 'Matterhorn', 'Merlot', and 'Zorro' representing red kidney, great northern, small red, and black bean commercial classes, respectively. Regeneration capacity of leaf explants, stem sections, and embryo axes were evaluated on 30 media each containing Murashige and Skoog (MS) medium and different combinations of plant growth regulators (PGRs). For stem sections and leaf explants, none of the media enabled plant regeneration from any of the four cultivars tested; this indicated the recalcitrance of bean regeneration from these tissues. In contrast, several media enabled multiple shoot production from embryo axis explants, although optimal regeneration media was genotype-dependent. Under the optimal regeneration conditions, multiple shoots, 2.3-10.8 on average for each embryogenic explant, were induced from embryo axis explants at frequencies of 93% for Merlot, 80% for Matterhorn, 73% for Red Hawk and 67% for Zorro. Transient expression studies monitored by an intron-interrupted *gus*A on explants transformed with *A. tumefaciens* strains GV3101, LBA4404, and EHA105 indicated that all three *A. tumefaciens* strains tested were efficient in gene delivery. Gene delivery depended on parameters such as strain of *A. tumefaciens*, co-cultivation time, explant type, and bean genotype. Agroinfiltration also enhanced gene delivery. Kanamycin-resistant and GUS-positive calluses were induced from leaf, stem, and embryo axis explants. Chimeric

transformants were obtained from inoculated embryo axis explants and they showed partial GUS-staining. Lack of efficient regeneration from non-meristem containing tissues is the main limitation for stable transformation of common bean.

**Introduction**

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption (Broughton et al., 2003). As a desirable tool to complement conventional breeding techniques, genetic engineering provides the possibility to source genes from beyond the gene pool accessible only through sexual hybridization (Christou, 1997; Somers et al., 2003; Dita et al., 2006). To date, creation of stable transgenic common bean at low frequencies has been achieved using particle bombardment-mediated transformation of meristematic tissues of cv. 'Seafarer' at 0.03%, cv. 'Goldstar' at 0.05%, and cv. 'Olathe' at 0.9% (Aragão et al., 1992, 1998; Russel et al., 1993; Kim and Minamikawa, 1996; Bonfim et al., 2007). The major challenge to the production of genetically engineered beans has been the lack of a stable genetic transformation system, due to their recalcitrance to *in vitro* regeneration and low rates of *Agrobacterium*-mediated transformation (Svetleva et al., 2003; Veltcheva et al., 2005).

Common bean regeneration has been extensively studied by many groups exclusively using MS (Murashige and Skoog, 1962) medium. Some of these studies investigated the regeneration capacity of various bean explants such as cotyledonary nodes (Dang and Wei, 2009), embryo axes (Zambre et al., 1998; Delgado-Sanchez et al., 2006; Quintero-Jimenez et al., 2010), immature embryos (Geerts et al., 2000), leaf sections and petioles (Crocomo et al., 1976; Malik and Saxena, 1991) and thin cell layers (Cruz de Carvalho et al., 2000). Others have investigated the influence of different growth regulators and/or their combinations on bean regeneration (Saunders et al., 1987; Malik and Saxena, 1991; Dang and Wei, 2009; Gatica Arias

et al., 2010; Kwapata et al., 2010; Quintero-Jimenez et al., 2010). Despite these efforts, an efficient and repeatable system that can support the regeneration of transformed common bean cells does not exist.

*Agrobacterium tumefaciens* mediated transformation is the gene delivery mode most preferred by plant breeders because of its easy accessibility and tendency to produce low- or single-copy insertion of the transgene (Somers et al., 2003). Historically, large seeded legumes have been difficult to transform using *A. tumefaciens.* However, recent reports showing progress in this field suggest potential possibilities for common bean. For instance, pigeon pea (*Cajanus cajun* (L.) Millsp.) can now be easily regenerated through both organogenesis and somatic embryogenesis using various explants, and successful transformation has been attempted, although genotypic dependence still exists (Krishna et al., 2010). Chickpea (*Cicer arietinum* L.) regeneration is possible mainly through somatic embryogenesis and shoot organogenesis with varying degrees of success (Huda et al., 2003, Jayanand et al., 2003; Somers et al., 2003). Successful production of transgenic chickpea plants using *Agrobacterium*-mediated transformation has been reported (Polowick et al., 2004; Senthil et al., 2004; Indurker et al., 2010; Mehrotra et al., 2011). Regeneration and *Agrobacterium*-mediated transformation of peas (*Pisum sativum* L.) have been successful using immature cotyledons as explants (Grant and Cooper, 2006). Cotyledonary nodes at various maturity stages are being routinely utilized for *Agrobacterium*-mediated transformation of soybean (*Glycine max* (L.) Merr.; Paz et al., 2006; Dang and Wei, 2009). Peanut (*Arachis hypogaea* L.) has been easily transformable compared to other legume species (Sharma and Pooja, 2006). *Agrobacterium*-mediated transformation of cotyledonary explants has led to the generation of stable transgenic plants in cowpea (*Vigna*

*unguiculata* L.; Muthukumar et al., 1996; Popelka et al., 2006; Solleti et al., 2008; Bakshi et al., 2011).

*Agrobacterium*-mediated transformation of *Phaseolus* species has been achieved with limited success. To date, only the tepary bean (*P. acutifolius* A. Gray) has a reproducible genetic transformation system (Dillen et al., 1997; Zambre et al., 2005). Liu et al. (2005) described the successful recovery of transgenic kidney bean (*P. vulgaris*) plants using sonification and vacuum infiltration techniques to transform bean seedlings using *A. tumefaciens*. However, the transformation rate was low and no subsequent studies using this protocol have been reported.

The possibility of transformation of *Phaseolus* species using *A. rhizogenes* has been demonstrated by Estrada-Navarrete et al. (2006). Although this *Agrobacteriu*m species may be useful for the production of hairy roots to enhance nitrogen fixation and functional genomics studies of root expressed genes in common bean, the production of whole transgenic bean plants is not straight forward, since these composite plants do not transmit transgenic traits to their progenies. This reduces the utility *A. rhizogenes* for crop improvement purposes.

The present study was conducted to evaluate factors influencing transient and stable transformation of common bean using A.tumefaciens.

## Materials and methods

### Plant materials and culture media

Four common bean cultivars, Red Hawk, Matterhorn, Merlot, and Zorro, representing red kidney, great northern, small red, and black bean commercial common bean classes, respectively, were utilized in this study. These cultivars represent the racial and gene pool genetic diversity of common bean grown in North America (Broughton et al., 2003). Red Hawk belongs to race Nueva Granada in the Andean gene pool, whereas Matterhorn is race Durango, Merlot is race Jalisco, and Zorro is race Mesoamerica in the Middle American gene pool. Mature embryo axis, stem, and leaf explants were tested to determine their transformation and regeneration capacities.

### Explant preparation

Mature, dry seeds were surface-sterilized with 3% sodium hypochlorite with continuous shaking for 10 min in a 250 ml Erlenmeyer flask, followed by four rinses with sterile distilled water, and then soaked in sterile distilled water for approximately 16 h. The soaking water was then discarded; seeds were rinsed three times with sterile distilled water, and blotted dry on sterile filter paper. The seed coats were removed and the embryos were excised using a sterile scalpel. Embryo axes were obtained by cutting off radicles and leaflets.

To grow seedlings for stem and leaf explants, five sterile seeds were planted on half-strength MS medium in each Magenta® GA7 box (PhytoTechnology Laboratories, KS, USA). Seeds were germinated under a 16 h photoperiod of 30 μmol $m^{-2}s^{-1}$ from cool white fluorescent tubes at 25 $^{o}$C. Stem and leaf explants were prepared from one-week-old seedlings. Stems were cut into 6-10 mm length segments and were then cut in half longitudinally. Leaf explants of 5-7 x

5-7 mm were cut with a sterile scalpel after removing the outer leaf margins.

**Regeneration experiments**

All regeneration media contained Murashige and Skoog (1962) (MS) inorganic salts and B5 vitamins, 3% sucrose, pH adjusted to 5.6, solidified with 0.8% (w/v) Bacto agar unless otherwise mentioned, and autoclaved for 20 min.

Preliminary experiments were performed to evaluate regeneration capacity of three explant types (embryo axis, stem segments, and leaf explants) for each of the four cultivars (Red Hawk, Matterhorn, Merlot, and Zorro) on the selected media listed in Table 1. Fifteen explants were placed on each medium in Petri dishes (100 x15 mm) with 3 replications. They were cultured at 25 $^{o}$C under a 16 h photoperiod of 30 μmol $m^{-2}$ $s^{-1}$ from cool white fluorescent tubes for 4 wk. Subcultures to fresh media were carried out at 4 week intervals. The explants with multiple shoot/bud formation were documented after 8 wk. Regeneration capability of the calluses induced in some treatments were evaluated on both MS and elongation medium [herein EM: MS containing 1.45 μM gibberellic acid ($GA_3$)] after 4 more wk of culture. For embryo axis explants, regeneration refers multiple shoot/bud formation from the areas adjacent to apical shoots and auxiliary buds.

Two more experiment replications were conducted on only four selected media, including DM4 (MS+44.4 μM BAP+2.27μM TDZ), DM11 (MS+4.94μM 2ip +2.27μM TDZ), DM42 (MS+ 22 μM BAP), and DM43 (MS+44.4μM BAP) using embryo axis explants from ‘Merlot’. The regeneration frequency was calculated as the number of regenerated explants／total number of explants × 100. The number of shoots/buds per explant was counted.

**Transient transformation experiments**

*A. tumefaciens* strains GV3101 (Koncz and Schell, 1986), LBA4404 (Hoekema et al., 1983), and EHA105 (Hood et al., 1993), each harboring the pBISN1 plasmid were tested for their capacity to infect common bean. The pBISN1 binary vector is a derivative of pBI101. It contains the neomycin phosphotransferase gene (*npt II*) driven by the *nos* promoter and an intron interrupted β- glucuronidase gene (*gus*A), which is controlled by the chimeric super promoter $(Aocs)_3$AmasPmas (Ni et al., 1995). Single colonies of each strain were cultured in 10 mL liquid yeast extraction broth (YEB) (Vervilet et al., 1975) containing 100 mg $L^{-1}$ kanamycin monosulfate (Km) at 28 $^{o}$C with constant shaking for 48 h. Thirty micro-liters of the culture were then inoculated into 15 mL of the same medium and grown to an $OD_{600}$ of 0.8-1.0. Before transformation, the culture was centrifuged at 2500×g for 1 min. The bacterial pellets were resuspended to an $OD_{600}$ of 0.5 in liquid callus inducing medium (CIM) [MS + 3% sucrose + 0.45 μM thidiazuron (TDZ) + 0.25μM indole-3-acetic acid (IAA) + 100 μM acetosyringone (AS) , pH 5.6]. Explants of four cultivars (Red Hawk, Matter Horn, Merlot, and Zorro ) were incubated in the bacterial suspension for 30 min at room temperature, blotted dry on sterile filter paper, and placed on two layers of sterile filter paper saturated with liquid CIM + 100 μM AS in Petri dishes. Co-cultivation was carried out for 8 d at 25 $^{o}$C in the dark. Explants were then washed in liquid CIM containing 500 mg $L^{-1}$ timentin (Tn) for 10 min, rinsed three times in sterile water, and blotted dry on sterile filter paper.

Inoculated explants of four cultivars were either immediately assayed for the frequency

of transient GUS expression or transferred to selection CIM containing 50 mg $L^{-1}$ Km, 500 mg $L^{-1}$ Tn, and solidified with 0.8% (w/v) Bacto agar for callus induction at 25 $^{o}$C in the dark. After 2 wk, a histochemical GUS assay was performed on the entire explants and the number of explants containing GUS-positive calluses was recorded. Stem, leaf, and embryo axis explants were tested in this manner with 10 explants per dish for callus induction.

To study the effect of co-cultivation period on bean transformation, leaf explants from 'Red Hawk' were used. After infection as described above, explants were co-cultivated on sterile filter paper saturated with liquid CIM + 100 μM AS in Petri dishes at 25 $^{o}$C in the dark. Ten leaf explants were incubated in each dish and were assayed for GUS expression on days 2, 3, 4, 5, 6, 7, and 8 post-infection.

To determine the susceptibility of different genotypes and explants of common bean to *A. tumefaciens*, three different explants, including leaves and stems from 1-week old seedlings, and embryo axes, were co-cultivated with *Agrobacterium* strain GV3101 for 8 d.

In addition to the infection of explants by incubation in *Agrobacterium* solution, agro-infiltration was carried out. The *Agrobacterium* strain GV3101 harboring the pBISN1 plasmid was prepared as described above and used to infect embryo axes excised from Merlot embryos grown on half-strength MS for 2d. Explants were immersed in 15 mL of *Agrobacterium* solution in 50 mL Corning tubes (Denville Scientific Inc., NJ, USA), which were placed in a vacuum chamber at 91 kPa for 3 min. Explants were then blotted dry on sterile filter paper for 5 min, and cultured at 25 $^{o}$C in the dark on filter paper soaked with liquid medium (QL medium + 44.4 μM

BAP + 100 μM AS). After 2 wk, a histochemical GUS assay was performed on explants from each experiment.

**Stable transformation experiments**

Merlot was used for stable transformation. Whole embryos from mature seeds with seed coat and cotyledons removed after surface sterilization were used as initial explants. Five explants were transferred to each 60 x 15 mm petri dish containing about 10 ml DM4. They were cultured at 25 °C for 3 d under a 16 h photoperiod of 30 μmol $m^{-2}s^{-1}$from cool white fluorescent tubes. Sterile explants were used for transformation studies.

Preparation of GV3101: pBISN1 culture was performed as described above. The bacterial pellet was suspended to an $OD_{600}$ of 0.5 in liquid CIM containing 100 μM AS. Sterile explants were immersed in *Agrobacterium* suspension and vacuumed at 91 kPa for 3 min. The explants were then blotted dry on sterile filter paper, transferred on filter paper overlaid on solidified CIM, and cultured at 25 $^{o}$C in the dark for 8 d. After co-cultivation, the root parts and leaves of the co-cultivated explants were removed; the resulting axis parts were washed in liquid CIM three times (2 min/time) followed by one more wash in CIM supplemented with 500 mg $L^{-1}$ Tn. The axis parts were dried on sterile filter paper and subsequently cultured on selection media containing 50 mg $L^{-1}$ Km and 500 mg $L^{-1}$ Tn. Subcultures of the explants to fresh selection media were performed at 3-week intervals. For the first two subcultures, all emerged shoots were removed and subjected to histochemical GUS assays. After three subcultures, regenerants were transferred to selection EM containing 50 mg $L^{-1}$ Km, and 500 mg $L^{-1}$ Tn. The entire selection and regeneration process was carried out at 25 $^{o}$C under a 16 h photoperiod of 30

μmol $m^{-2}s^{-1}$ from cool white fluorescent tubes. Two selection media, including DM4 and DM19 [MS + 4.52 μM dichlorophenoxyacetic acid (2, 4-D)], were tested in six transformations. For each medium, 100-150 explants were used for each transformation and each experiment was repeated three times. Regeneration capability of the Km-resistant calluses induced on DM19 was evaluated on DM4 and EM, respectively. The number of explants producing either Km-resistant calluses or shoots was recorded after 16 wk selection. Histochemical GUS assays were performed on randomly selected Km-resistant transformants.

**Histochemical GUS assay**

The histochemical assay of GUS activity was carried out following Jefferson et al. (1987). Explants were incubated overnight at 37 $^{o}$C in 100 mM sodium phosphate buffer (pH 7.0) containing 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 10 mM $Na_2EDTA$, 0.5% (v/v) Triton X-100 and 5-bromo-4-chloro-3-indolyl β-D- glucuronide (X-Gluc) at 0.5 mg $L^{-1}$. Following overnight incubation, chlorophyll was removed from the tissues using 70% ethanol rinses. Transient *gus*A expression was measured by counting the number of explants and calluses with at least one blue focus. GUS assays were replicated three times with 10 or 12 explants per treatment. The frequency of transient GUS expression was the number of explants with at least one blue focus compared to the total number of explants, expressed as a percentage.

**Statistical analyses**

All experiments were arranged in completely randomized designs. Data were analyzed using PROC GLM or ANOVA of SAS 9.2 (SAS institute, Cary, NC). Means were separated by the Duncan's Multiple Range Test at $p \leq 0.05$.

## Results

### Optimization of shoot regeneration systems

The regeneration results provide one more piece of evidence that common bean cultivars are recalcitrant for regeneration from meristem-free tissues. For stem sections and leaf explants, none of the 30 media tested enabled plant regeneration from any of the four cultivars used. The DM19 induced friable calluses from stem and embryo axis explants, but the calluses could not further develop into somatic embryos or plants after they were transferred on either PGR free MS or DM43 for shoot induction from embryo axis explants.

For embryo axis explants, 20 out of 30 media enabled multiple shoot/bud production for each cultivar (Tables 4. 1, 4. 2). Both genotype and culture medium had a significant impact on regeneration frequency as well as the mean number of shoots/buds per explant. Merlot and Matterhorn were more amenable to shoot production than Red Hawk and Zorro (Figure 4.1a-d). A high level of BAP (44.4 μM), either alone (DM43) or combined with 2.27 μM TDZ (DM4), resulted in the best shoot and bud production for each cultivar. When TDZ was included, it inhibited shoot elongation but promoted more bud production (Figure 4. 1e-f). Under the optimal conditions, multiple shoots and buds, an average of 2.3-10.8 for each embryogenic explant, were induced from embryo axis explants at frequencies of 93.3% for Merlot, 80.0% for Matterhorn, 73.3% for Red Hawk and 66.7% for Zorro (Table 4. 1).

Table 4. 1 Effect of plant growth regulators on regeneration of embryo axes of four common bean cultivars.

| Plant growth regulators and MES (μM) | | | | | | | | | | Regeneration frequency (%) of different cultivars | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | BAP | 2ip | Kinetin | TDZ | 4-FA | NAA | 2, 4-D | IAA | MES | Matter horn | Merlot | Red Hawk | Zorro |
| DM41 | 88.8 | | | | | | | | | 60.0ab | 66.7bc | 40.0bcd | 33.3bcd |
| DM4 | 44.4 | | | 2.3 | | | | | | 73.3a | 93.3a* | 73.3a | 53.3ab |
| DM43 | 44.4 | | | | | | | | | 80.0a | 88.9a* | 73.3a | 66.7a |
| DM42 | 22.2 | | | | | | | | | 73.3a | 86.7ab* | 60.0ab | 46.7abc |
| DM18 | 8.88 | | | | | | | | | 46.7bc | 66.7bc | 40.0bcd | 46.7abc |
| DM9 | 8.88 | | | | | | | 2.9 | | 66.7ab | 46.7cd | 46.7bc | 33.3bcd |
| DM6 | 4.44 | | | | | | | | | 26.7cde | 26.7defg | 20.0def | 26.7bcde |
| DM1 | 4.44 | 4.9 | | | | | | | | 46.7bc | 40.0de | 40.0bcd | 26.7bcde |
| DM5 | 4.44 | | | | | 2.7 | | | | 13.3def | 20.0efgh | 6.7ef | 20.0cde |
| DM83 | 4.44 | | 4.6 | | | | | | | 33.3cd | 40.0de | 20.0def | 13.3de |
| DM21 | | | | 9 | | | | 0.6 | | 33.3cd | 46.7cd | 20.0def | 26.7bcde |
| DM20 | | | | 9 | | | | | | 26.7cde | 33.3def | 26.7cde | 20.0cde |
| DM22 | | | | 4.54 | | | | 0.57 | | 33.3cd | 40.0de | 13.3ef | 33.3bcd |
| DM2 | | | | 4.54 | | | | | | 20.0def | 26.7defg | 20.0def | 6.7de |
| DM11 | | 4.92 | | 2.27 | | | | | | 60.0ab | 84.4ab* | 46.7bc | 26.7bcde |

BAP: 6-benzyl-aminopurine; 2ip: 6-(γ,γ-dimethylallylamino) purine; TDZ: thidiazuron; 4-FA: 4-fluorophenoxyacetic acid; NAA: α-aphthaleneacetic acid; 2,4-D: 2,4-dichlorophenoxyacetic acid; IAA: indole-3-acetic acid; MES: 2-(n-morpholino)-ethanesulfonic acid. *The results of three experiments each with three replicates. Different letters in a column indicate a significant difference at $p \leq 0.05$ with Duncan's Multiple Range Test.

Table 4. 1. (Cont'd)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DM24 | | | 1.36 | | | | 0.86 | | 26.7cde | 20.0efgh | 13.3ef | 6.7de |
| DM23 | | | 1.36 | | | | | | 6.7ef | 13.3fgh | 0.0f | 0.0e |
| DM10 | 9.84 | | | | | | | | 33.3cd | 46.7cd | 53.3ab | 33.3bcd |
| DM13 | 9.84 | | | | | | 2.86 | | 33.3cd | 40.0de | 20.0def | 26.7bcde |
| DM85 | | 4.65 | | | | | 2.86 | | 20.0def | 26.7defg | 13.3ef | 13.3de |
| DM82 | | 4.65 | | | 2.69 | | | | 6.7ef | 6.7gh | 0.0f | 13.3de |
| DM84 | 4.92 | 4.65 | | | | | | | 6.7ef | 13.3fgh | 6.7ef | 13.3de |
| DM12 | 4.92 | | | | | | 2.86 | | 0.0f | 6.7gh | 0.0f | 0.0e |
| DM80 | | 2.79 | | | 2.69 | | | | 6.7ef | 0.0h | 0.0f | 0.0e |
| DM15 | | | | 5.36 | | | | 3.02 | 0.0f | 0.0h | 0.0f | 0.0e |
| DM16 | | | | 10.72 | | | | 3.02 | 0.0f | 0.0h | 0.0f | 0.0e |
| DM17 | | | | | | 9.04 | | | 0.0f | 0.0h | 0 | 0.0e |
| DM19 | | | | | | 4.52 | | | 0.0f | 0.0h | 0 | 0.0e |
| DM7 | | | | | | 9.04 | | 3.02 | 0.0f | 0.0h | 0 | 0.0e |
| DM81 | | 4.65 | | | | | | | 0.0f | 0.0h | 0 | 0.0e |

Table 4. 2 Effect of plant growth regulators on number of shoot per explant produced from embryo axes of four common bean cultivars.

| Plant growth regulators and MES (μM) | | | | | | | | | | Number of shoots per explant for each cultivar | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | BAP | 2ip | Kinetin | TDZ | 4-FA | NAA | 2, 4-D | IAA | MES | Matterhorn | Merlot | Red Hawk | Zorro |
| DM41 | 88.8 | | | | | | | | | 3.47ab | 4.87bc | 1.67cd | 1.00bcde |
| M4 | 44.4 | | | 2.3 | | | | | | 5.93a | 10.84a* | 3.40a | 1.80ab |
| DM43 | 44.4 | | | | | | | | | 3.20bc | 5.62b* | 3.87a | 2.33a |
| DM42 | 22.2 | | | | | | | | | 4.20b | 4.51bc* | 1.67cd | 1.07bcde |
| DM18 | 8.88 | | | | | | | | | 1.47def | 2.53ef | 1.27cdef | 1.73abc |
| DM9 | 8.88 | | | | | | | 2.9 | | 2.53cd | 1.80fgh | 1.87bc | 1.13bcd |
| DM6 | 4.44 | | | | | | | | | 0.93efgh | 1.00ghi | 0.60efgh | 0.93cdef |
| DM1 | 4.44 | 4.9 | | | | | | | | 1.47def | 1.33fghi | 1.27cdef | 0.80defg |
| DM5 | 4.44 | | | | | 2.7 | | | | 0.33fgh | 0.47i | 0.20gh | 0.53defg |
| DM83 | 4.44 | | 4.6 | | | | | | | 0.73efgh | 0.93ghi | 0.53fgh | 0.33defg |
| DM21 | | | | 9 | | | | 0.6 | | 1.47def | 3.27de | 0.67efgh | 1.00bcde |
| DM20 | | | | 9 | | | | | | 0.73efgh | 1.20ghi | 0.93defg | 0.67defg |
| DM22 | | | | 4.54 | | | | 0.57 | | 1.67ed | 2.13efg | 0.47gh | 1.13bcd |
| DM2 | | | | 4.54 | | | | | | 0.67efgh | 0.73hi | 0.53fgh | 0.13fg |
| DM11 | | 4.92 | | 2.27 | | | | | | 1.73ed | 3.75cd* | 1.33cde | 0.73defg |
| DM24 | | | | 1.36 | | | | 0.86 | | 1.27efg | 0.67hi | 0.53fgh | 0.27efg |
| DM23 | | | | 1.36 | | | | | | 0.20gh | 0.60hi | 0.00h | 0.00g |
| DM10 | | 9.84 | | | | | | | | 0.87efgh | 1.87fgh | 2.53b | 1.13bcd |
| DM13 | | 9.84 | | | | | | 2.86 | | 0.87efgh | 1.13ghi | 0.47gh | 0.73defg |

BAP: 6-benzyl-aminopurine; 2ip: 6-(γ,γ-dimethylallylamino) purine; TDZ: thidiazuron; 4-FA: 4-fluorophenoxyacetic acid; NAA: α-aphthaleneacetic acid; 2,4-D: 2,4-dichlorophenoxyacetic acid; IAA: indole-3-acetic acid; MES: 2-(n-morpholino)-ethanesulfonic acid
*The results of three experiments each with three replicates.
Different letters in a column indicate a significant difference at $p \leq 0.05$ with Duncan's Multiple Range Test.

Table 4.2 (Cont'd)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DM85 | | 4.65 | | | | 2.86 | | 0.67efgh | 1.00ghi | 0.53fgh | 0.60defg |
| DM82 | | 4.65 | | 2.69 | | | | 0.20gh | 0.27i | 0.00h | 0.53defg |
| DM84 | 4.92 | 4.65 | | | | | | 0.20gh | 0.40i | 0.20gh | 0.33defg |
| DM12 | 4.92 | | | | | 2.86 | | 0.00h | 0.20i | 0.00h | 0.00g |
| DM80 | | 2.79 | | 2.69 | | | | 0.13gh | 0.00i | 0.00h | 0.00g |
| DM15 | | | 5.36 | | | | 3.02 | 0.00h | 0.00i | 0.00h | 0.00g |
| DM16 | | | 10.72 | | | | 3.02 | 0.00h | 0.00i | 0.00h | 0.00g |
| DM17 | | | | | 9.04 | | | 0.00h | 0.00i | 0.00h | 0.00g |
| DM19 | | | | | 4.52 | | | 0.00h | 0.00i | 0.00h | 0.00g |
| DM7 | | | | | 9.04 | | 3.02 | 0.00h | 0.00i | 0.00h | 0.00g |
| DM81 | | 4.65 | | | | | | 0.00h | 0.00i | 0.00h | 0.00g |

**Influence of co-cultivation period on transient GUS expression**

The influence of co-cultivation period on transient GUS expression was determined on Red Hawk leaf explants (Table 4. 3). Almost no visible GUS expression was observed following 2 d of co-cultivation with all three strains. The frequency of transient GUS expression increased with increasing co-cultivation time to a maximum mean of 76.5 % for all *Agrobacterium* strains after 8 d.

**Influence of *A. tumefaciens* strains, explant type, and genotype on GUS expression**

Among the strains tested, GV3101 induced the highest level of GUS expression in all cultivars, followed by EAH105 and LBA4404 after a co-cultivation period of 8 d (data not shown).

Of the three explant types tested, the frequency of transient GUS expression was highest in leaf and embryo axis explants. Stem explants exhibited a significantly lower level of GUS expression. The average transient GUS expression frequencies for leaf, stem, and embryo axis explants were 92.5%, 21.0%, and 89.1%, respectively (Table 4. 4). When callus was induced in each explant type under selection conditions of 50 mg $L^{-1}$ of Km, the frequency of calluses expressing GUS decreased considerably for all explants (Table 4. 4).

**Effect of agroinfiltration on transient GUS expression**

Infiltration increased transgene delivery and resulted in 100% of embryo axes expressing GUS compared to 55% obtained by regular *Agrobacterium* incubation. In addition, more blue spots with intense blue color were observed in the infiltrated explants (Figure 4.2)

Table 4. 3 Effect of co-cultivation period on GUS expression in leaf explants of 'Red Hawk'.

| Percentage of GUS-positive explants after different co-cultivation time | | | | | | | |
|---|---|---|---|---|---|---|---|
| Strains | 2d | 3d | 4d | 5d | 6d | 7d | 8d |
| GV3101 | 3.8 | 18.9 | 25.3 | 56.4 | 83.3 | 88.8 | 90.2 |
| LBA4404 | 1.0 | 7.9 | 11.8 | 37.9 | 62.5 | 57.1 | 60.0 |
| EHA105 | 3.3 | 12.8 | 19.9 | 49.5 | 70.9 | 75.3 | 79.4 |
| Mean ± SD* | 2.7±0.9$^{d}$ | 13.2±3.2$^{c}$ | 19.0±3.9$^{c}$ | 47.9±5.4$^{b}$ | 72.3±6.1$^{a}$ | 73.7±9.2$^{a}$ | 76.5±8.8$^{a}$ |

*Means followed by the same letter are not significantly different (α=0.05).

Table 4. 4**.** Effect of explant type on GUS expression assayed after 8 d of co-cultivation with GV3101 and again on calluses formed after co-cultivation plus 2 wk on callus inducing medium.

| | Percent of GUS expression | | | | | |
|---|---|---|---|---|---|---|
| | Explants (8d of co-cultivation) | | | Calluses (2wk post co-cultivation) | | |
| Cultivars | Leaf | Stem | Embryo axis | Leaf | Stem | Embryo axis |
| Red Hawk | 93.8 | 19.8 | 91.4 | 4.7 | 1.3 | 4.5 |
| Zorro | 92.9 | 22.1 | 90.6 | 4.4 | 1.5 | 4.2 |
| Matterhorn | 91.6 | 24.2 | 82.1 | 3.6 | 1.2 | 3.7 |
| Merlot | 91.9 | 17.9 | 92.4 | 4.5 | 1.4 | 4.4 |
| Mean ± SD* | 92.5±0.5$^{a}$ | 21.0±1.4$^{b}$ | 89.1±2.4$^{a}$ | 4.3±0.5$^{a}$ | 1.4±0.1$^{b}$ | 4.2±0.2$^{a}$ |

*Means followed by the same letter (within explant type and within calluses) are not significantly different (α=0.05)

**Stable transformation**

Agroinfiltration followed by 8 d co-cultivation did not lead to necrosis of the explants (Figure 4.3a). After 16 wks of selection on DM19, 23.6% (150/635) of embryogenic axes produced Km-resistant calluses, of which 65% of callus clusters tested were GUS positive (Table 4.5). The calluses showed some embryogenic characteristics, but none of the calluses further developed into plants when they were transferred onto either DM4 or EM. On the selection DM4, Km-resistant shoots or buds were observed in 33% (289/876) of explants, of which 22% of explants tested had GUS positive shoots or buds (Figure 4. 3b, Table 4.5). After transfer to selection EM, 2.8% (5/174) of the explants, which had Km resistant shoots or buds, developed into plantlets after 6 wk (Figure 4. 3c). Unfortunately, these plantlets stopped growing and subsequently did not develop into normal plants after they were transplanted into soil.

Using histochemical GUS assays, blue staining was observed in some Km-resistant calluses, shoots, or buds but was absent in nontransformed tissues (Figure 4. 3 d-f). For some Km-resistant callus clusters, co-existence of blue and white cells was observed (Figure 4. 3d). Similarly, unevenly distributed blue staining was observed in leaf and root tissues from Km-resistant transformants obtained after 16 wk selection (Figure 4. 3f). These results indicate the expression of the *gus*A reporter in transgenic tissues. The variations in blue staining might be due to the uneven penetration of X-gluc or chimeric tissues. In addition, all the early induced shoots/buds obtained within 6 wk of selection were not transgenic based on GUS staining, because they either were GUS-negative or had only a few blue spots, which were similar to the pattern of transient GUS expression.

Table 4. 5 Transformation of embryo axes of common bean cultivar Merlot.

| Selection medium | Experiment | Total number of explants | Number of explants producing Km-resistant shoots | Number of explants producing Km-resistant calluses | Number of explants producing GUS-positive calluses (Number of Km-resistant calluses assayed) | Number of explants producing GUS-positive shoots | Transformation frequency (%) |
|---|---|---|---|---|---|---|---|
| DM19 | Exp 1 | 110 | 0 | 25 | 8(10) | NA | 18.2 |
| | Exp 2 | 100 | 0 | 22 | 7(10) | NA | 15.4 |
| | Exp 3 | 105 | 0 | 32 | 5(10) | NA | 15.2 |
| | Exp 4 | 100 | 0 | 29 | 6(10) | NA | 17.4 |
| | Exp 5 | 100 | 0 | 21 | 7(10) | NA | 14.7 |
| | Exp 6 | 120 | 0 | 21 | 6(10) | NA | 10.5 |
| | Total | 635 | 0 | 150 | | | 15.2 (average) |
| DM4 | Exp 1 | 150 | 43 | NA | NA | 3(21) | 4.1 |
| | Exp 2 | 145 | 55 | NA | NA | 4(26) | 5.8 |
| | Exp 3 | 133 | 38 | NA | NA | 5(20) | 7.1 |
| | Exp 4 | 150 | 48 | NA | NA | 8(30) | 8.5 |
| | Exp 5 | 150 | 69 | NA | NA | NA | NA |
| | Exp 6 | 148 | 36 | NA | NA | 5(18) | 6.8 |
| | Total | 876 | 289 | | | | 6.5 (average) |

Number in the parentheses represent the number of Km-resistant shoots assayed; *NA* not analyzed

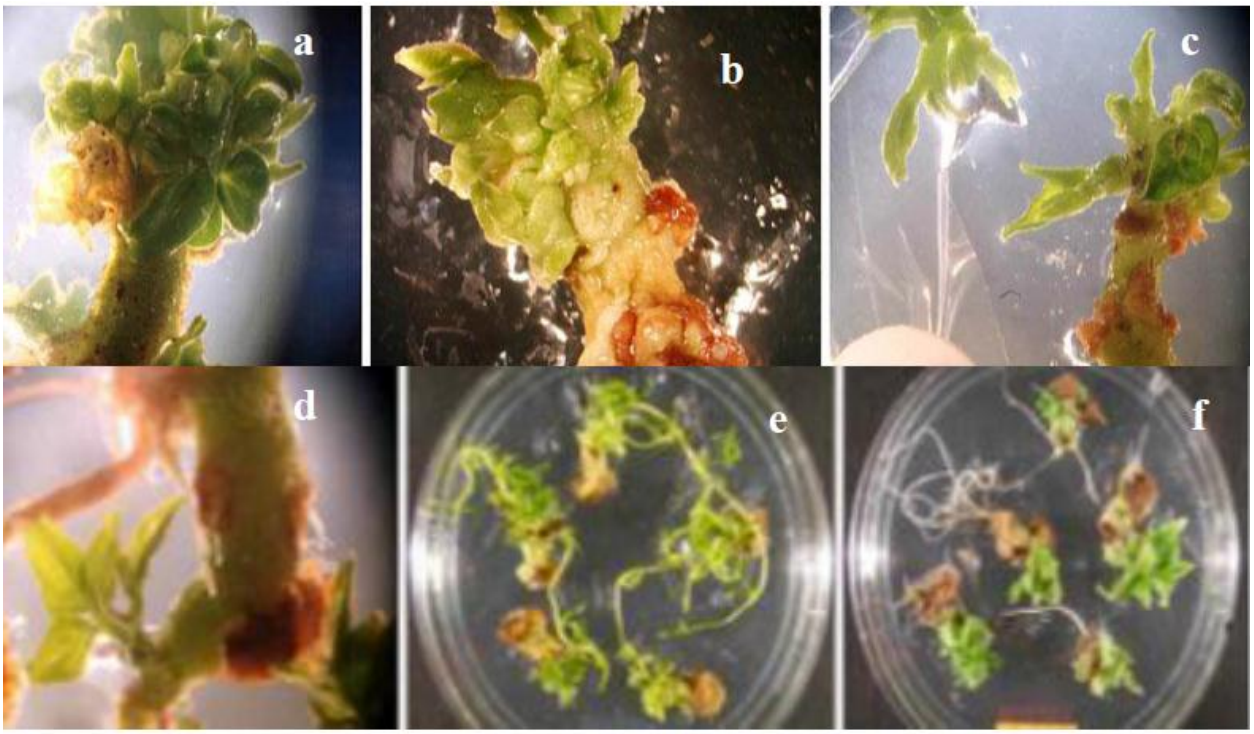


Figure 4.1**.** Shoot/bud production patterns of embryo axes of four common bean cultivars after 8 wk of culture. **a** Merlot on DM4; **b** Red Hawk on DM43; **c** Zorro on DM 43; **d** Matterhorn on DM43; **e** Merlot on DM43; **f** Merlot on DM4.


Figure 4. 2**.** Effect of agroinfiltration on bean transformation after 2 wk of co-cultivation. **a** Embryo axes transformed by incubation in *Agrobacterium* solution for 30 min; **b** Embryo axes transformed by infiltration.

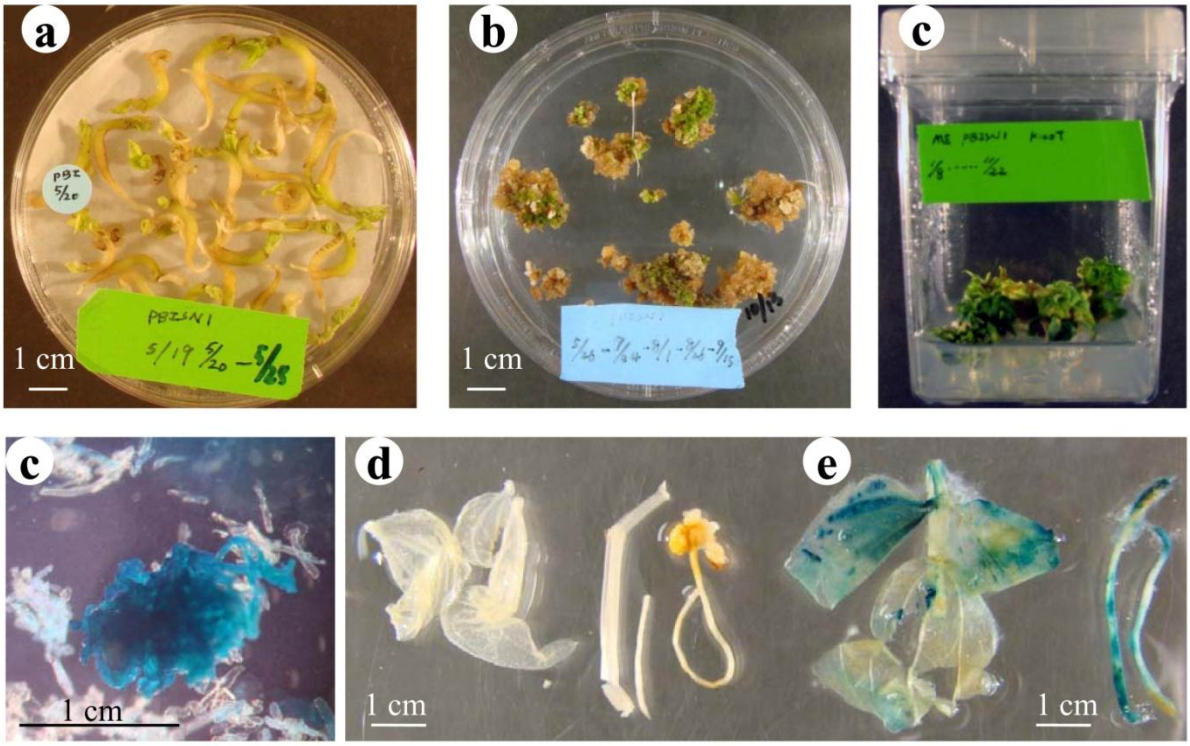


Figure 4. 3. Transformation of common bean cultivar Merlot using embryo axes as explants.**a** Explants after 8 d of co-cultivation; **b** Selection of Km-resistant shoots on selection DM4; **c** Growth of Km-resistant shoots and buds on selection EM; **d** GUS staining in the calluses induced on selection DM19; **e** GUS staining in non transformed tissues; **f** GUS staining in tissues of Km-resistant transformants.

**Discussion**

The attempt to induce plant regeneration from non-meristem containing tissues, such as leaf explants and stem sections of common bean, did not lead to any regeneration. These results are consistent with most of the previous reports, in which embryo axes of common bean were amenable for multiple shoot and bud production (Zambre et al., 1998; Delgado-Sanchez et al., 2006; Arellano et al., 2009; Kwapata et al., 2010; Quintero-Jimenez et al., 2010). However, 'shoot proliferation' instead of 'regeneration' is a more accurate term for this type of shoot production, since the shoots or buds appeared only in the adjacent areas of apical meristems or auxiliary buds (Figure 4.1a-d). It is not very clear whether the newly formed shoots are derived from a group of predetermined regenerable cells or from single cells. A single cell derived plant regeneration system is desirable for genetic transformation, since it can minimize the production of chimeric transformants. However, such a single cell-derived regeneration system from mersitem-free tissues is still lacking for common bean cultivars.

While friable calluses showing some embryogenic characteristics were induced by using 2, 4-D (Figure 4. 3d), we have not found a method that enables the conversion of these calluses to somatic embryos. However, this may indicate that it could be possible to attain common bean regeneration through somatic embryogenesis.

There is no question that common bean regeneration depends on many factors, such as genotype, explant type, and medium formula (Malik and Saxena, 1991; Delgado-Sanchez et al., 2006; Dang and Wei, 2009; Gatica Arias et al., 2010; Kwapata et al., 2010; Quintero-Jimenez et al., 2010). Most of the previous studies focused on investigating regeneration capacity of different genotypes and explants as well as different plant growth regulators. Few studies have been undertaken to evaluate the impact of other factors, such as basal salts, vitamins, and carbon

sources, on common bean regeneration. Quintero-Jimenez et al. (2010) reported that Gamborg's (1968) B5 medium resulted in a higher regeneration frequency than MS medium. More recently, we evaluated six basal media on regeneration of Merlot. Our preliminary data showed that two basal media, Lloyd and McCown's (1980) woody plant medium (WPM) and Quorin and Lepoivre medium (QL) (Quoirin and Lepoivre (1977), showed potential for further improvement of shoot production from embryo axes (data not shown).

Common bean is susceptible to *Agrobacterium* spp. (Mariotti et al., 1989; McClean et al., 1991; Lewis and Bliss, 1994; Brasileiro et al., 1996). Various factors influencing transient and stable transformation of common bean have been investigated in this study. A co-cultivation period of 2-3 d is generally considered to be suitable for *Agrobacterium*-mediated transformation in many other plant species (Hiei et al., 1994; Li et al., 1996; Cheng et al., 1997; Uranbay et al., 2005). In this study, we found that 8 d co-cultivation yielded the best transient GUS expression and did not cause necrosis of the embryo axes in the bean cultivars tested. This result is similar to some previous reports (Zhang et al., 1997; Zambre et al., 2005). Our data indicate that it is possible to improve *Agrobacterium*-mediated gene delivery by extending the co-cultivation time, especially when embryo axes are used as explants.

The virulence of *Agrobacterium* strains varies widely among host plant species depending on the interaction between the *Agrobacterium* strain and host plant (Davis et al., 1991; Zhang et al., 1997). In this study, the *Agrobacterium* strain GV3101 yielded stronger intensity of GUS staining and more GUS foci per explant than other strains under the same co-cultivation conditions for the four cultivars. This is comparable to the results obtained in other legume crops such as soybean (Margie et al., 2006). These results indicate that common beans are probably more susceptible to this nopaline type of *Agrobacterium* strain and highlight the importance of

using suitable virulent strains in bean transformation.

Explant tissues are important for both regeneration and *Agrobacterium* infection. Although leaf explants of common bean are not regenerable yet, they showed the highest susceptibility to *A. tumafaciens* in all genotypes tested (Table 4. 4). Prior studies in other legume crops such as lentil (*Lens culinaris* M.; Mahmoudian et al., 2002), showed that agroinfiltration resulted in higher transient GUS expression than regular inoculation. More importantly, agroinfiltration did not cause overgrowth of *Agrobacterium* cells during co-cultivation (Figure 4.3a). Washing of the agroinfiltrated explants following co-cultivation is necessary in order to keep *Agrobacterium* growth well controlled during the selection stage.

Despite the high 'regeneration' frequency of the embryo axis explants (Table 4.1), stable transformation of common bean is still inefficient. The main reason is that the embryo axis-based regeneration system is not desirable for genetic transformation. In this study, although 6.5% of explants had GUS-positive shoots or buds after 16 wk selection, we could not exclude the possibility that some of these were chimeric transformants. Since early formed shoots during the first 6 wk of selection were putative nontransgenics, removal of these shoots could promote the development of transformed cells and increase the chance of obtaining common bean transformants.

## Conclusion

To optimize the regeneration system for common bean cultivars, regeneration capacities of leaf explants, stem sections, and embryo axes were evaluated on 30 media containing different PGRs. Although none of the media enabled plant regeneration from leaf explants or stem sections, several media enabled multiple shoot production from embryo axes for each genotype. Under optimal regeneration conditions, *A. tumefaciens*-mediated gene delivery parameters, including strain of *A. tumefaciens*, co-cultivation time, explant type, and bean genotype, were optimized. Both agroinfiltration and an 8-d co-cultivation period enhanced gene delivery. For stable transformation, GUS-positive transformants were obtained after 16 wk selection. Removal of early formed shoots during the first 6 wk of selection could increase the chance of obtaining transformants.

In order to develop an efficient transformation protocol for common bean, more efforts are still needed to develop an efficient regeneration system using nonmeristem containing tissues as explants.

# APPENDICES

# Appendix A

## Influence of basal salt sources on regeneration of common bean

### Introduction

As the world faces the challenges of rapid population growth and climate change, the production of sufficient quantities of nutritious food from productive stress resistant crops is needed to meet these challenges. The development of such crops will require the use modern biotechnology tools such as genetic engineering to complement conventional breeding techniques, since some of the traits needed to meet these challenges may not be present in the crossable gene pool of many crops. Genetic engineering facilitates the introduction of specific traits into plants through transformation, providing the possibility to expand the sources of genes to all organisms beyond the gene pool accessible only through sexual hybridization (Christou, 1997; Somers et al., 2003; Dita et al., 2006).

Common bean (*Phaseolus vulgaris* L.) which is the most important grain legume for direct human consumption (Broughton et al. 2003) has yet to benefit from the application of these techniques. One of the major challenges to the production of genetically engineered beans is their recalcitrance to *in vitro* regeneration

Common bean regeneration has been extensively studied by many groups exclusively using MS (Murashige and Skoog, 1962) medium. However, when studying shoot organogenesis and regeneration in the common bean, Quintero-Jimenez et al. (2010) noted that MS medium was less effective in direct organogenesis of embryo axes than Gamborg's (B5) medium (Gamborg et al., 1968) regardless of growth regulator concentration. This suggests the potential importance of the source of basal salts as a factor that might enhance the regeneration of

common bean. The present study was conducted to evaluate the effect of various basal salts on the regeneration of common bean.

**Materials and methods**

To optimize the regeneration media, the effects of basal salts on regeneration of common bean were tested using embryo axis from 'Merlot' bean cultivar.

**Explant preparation**

To prepare the Merlot explant, mature, dry seeds were surface-sterilized with 3% sodium hypochlorite with continuous shaking for 10 min in a 250 ml Erlenmeyer flask, followed by four rinses with sterile distilled water, and then soaked in sterile distilled water for approximately 16 h. The soaking water was then discarded; seeds were rinsed three times with sterile distilled water, and blotted dry on sterile filter paper. The seed coats were removed and the embryos were excised using a sterile scalpel. Embryo axes were obtained by cutting off radicles and leaflets.

**Media preparation**

Six basal salts (Sigma-Aldrich, St. Louis, MO, USA), consisting of MS, KAO (Kao and Michayluk 1975), QL (Quorin and Lepoivre 1977), WPM (Lloyd and McCown 1980), Chu's (N6) (Chu et al. 1975) and White's (White 1963), were tested. Regeneration media were prepared by mixing each basal salt at recommended concentrations, 1 mg $L^{-1}$ $B_5$ vitamins, 44.4 μM 6-benzyl-aminopurine (BAP). All media contained 3% sucrose, pH adjusted to 5.6, solidified with 0.8% (w/v) Bacto agar and autoclaved for 20 min.

Two experiments were conducted. In the first experiment, 30 embryo axes were placed on each medium in Petri dishes (100 x15 mm) with 3 replications. Each dish contained 10 embryo axes. Dishes were kept in the dark for 2 wk and placed under a 16 h photoperiod of 30 μmol

$m^{-2}s^{-1}$ from cool white fluorescent tubes for 2 additional wk. Regeneration was recorded as the number of embryo axes regenerating at least one shoot. The regeneration frequency was calculated as the number of regenerated explants/ total number of explants x 100. To count the number of shoots per explant, shoots were removed from 4 randomly chosen explants in each dish per treatment, and counted.

The second experiment was conducted with MS, QL, and WPM media because these were the best performing media as determined by the first experiment. Ninety embryos axes were used for each regeneration medium in a completely randomized experimental design with 9 replications per treatment. Each Petri dish contained 10 embryo axes. Petri dishes were kept in the dark for 2 wk followed by 2 wk under a 16 h photoperiod of 30 μmol $m^{-2}s^{-1}$from cool white fluorescent tubes. Regeneration frequencies and the number of shoots per explant were determined as described above. The counts were conducted on QL and MS media only because WPM did not produce countable shoots.

**Statistical analyzes**

All experiments were arranged in completely randomized designs. Data were analyzed using SAS 9.2 (SAS institute, Cary, NC) and the means were separated by the Duncan's Multiple Range test.

**Results**

The influence of different basal salts on common bean regeneration was studied on embryo axes of cultivar Merlot using KAO, White's, WPM, Chu's, MS, and QL media. Merlot was chosen because it showed better regeneration and transformation potentials than the other cultivars in previous experiments (Mukeshimana et al., 2012). The effect of these media was evaluated in two experiments. In the first experiment, regeneration frequencies differed significantly ($p$= 0.0464) between media. The highest percentage of regeneration was observed in WPM and QL media (67% and 57%, respectively) (Table C.1). In addition to showing low regeneration frequencies, shoots regenerated on KAO, White's, and Chu's media looked unhealthy (Figure C.1) so these media were not used in the subsequent experiment. A lower regeneration frequency (20%) was observed for MS medium, but those explants that did regenerate produced strong and healthy shoots. Although WPM resulted in the highest regeneration frequency, the regenerated shoots were very small and chlorotic. The number of shoots per explant differed significantly between media ($p$=0.0006). MS, QL, and WPM resulted in higher average numbers of shoots than the other media (Table C.1)

In the second experiment, only MS, QL, and WPM media were evaluated. These media induced regeneration frequencies of 46.2%, 76%, and 69.8% respectively (Table C.1). There was a significant difference between mean shoot number per explant regenerated in MS and QL media ($p$=0.0083). Regeneration patterns observed on QL medium had more shoots compared to these from MS medium (Figure C.2). It was not possible to remove and count the number of shoots per explant on WPM medium as they were unhealthy and chlorotic.

Table C 1**.** Effect of different basal salts on regeneration of common bean embryo axes and number of shoots per explant in two experiments.

| | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| Media | Regeneration frequency (%) | Average number of shoots | Regeneration frequency (%) | Average number of shoots |
| WPM | 66.7±28.8$^{a}$ | 6.7±2.2$^{a}$ | 69.8± 22.8$^{a}$ | N/A |
| QL | 56.7±11.5$^{ab}$ | 8.0±3.8$^{a}$ | 76 ± 25.0$^{a}$ | 6.2$^{a}$ |
| KAO | 50.0±17.3$^{abc}$ | 3.2± 1.5$^{b}$ | N.A | N/A |
| White | 30.0±10$^{bc}$ | 1.5±0.57$^{b}$ | N.A | N/A |
| Chu's | 23.3±25.1$^{bc}$ | 3.0±1.41$^{b}$ | N.A | N/A |
| MS | 20.0±10$^{c}$ | 8.3±1.7$^{a}$ | 46.2±19.2$^{b}$ | 4$^{b}$ |

Numbers within the same column followed by the same letter are not significantly different (α=0.05); N/A: Not Applicable

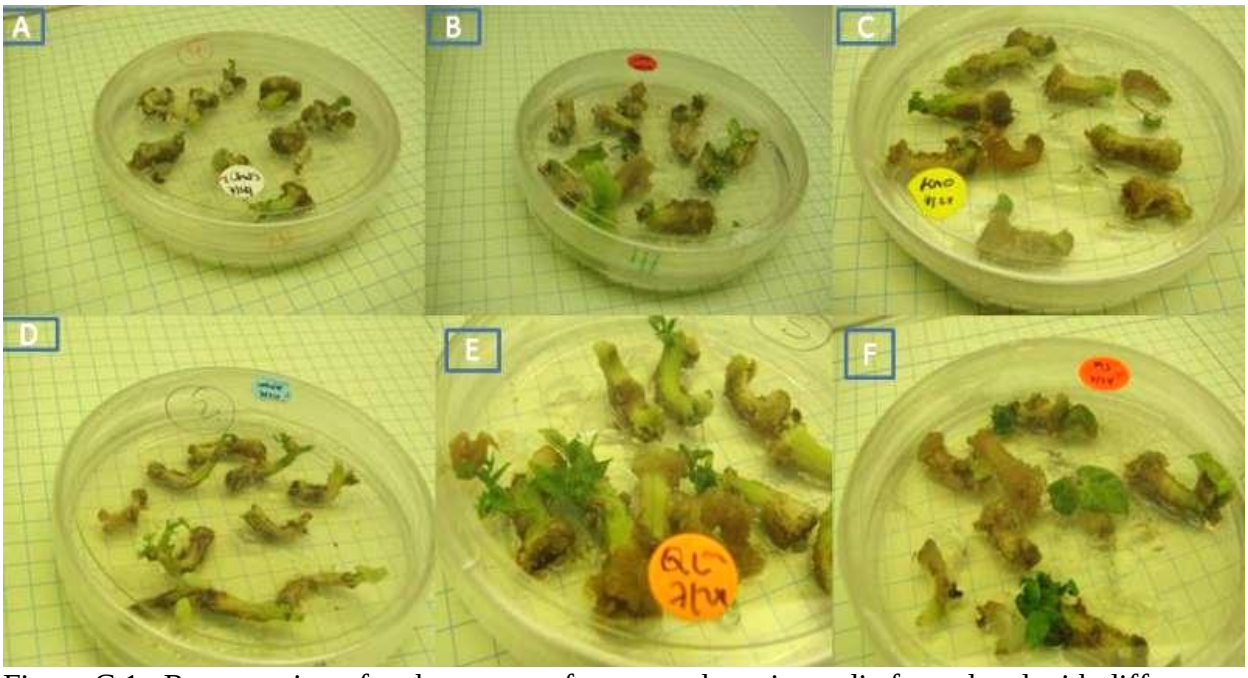

Figure C.1. Regeneration of embryo axes of common bean in media formulated with different basal salts after 2 weeks of growth. A. Chu's; B. WPM; C. KAO; D. White's; E. QL; F. MS.

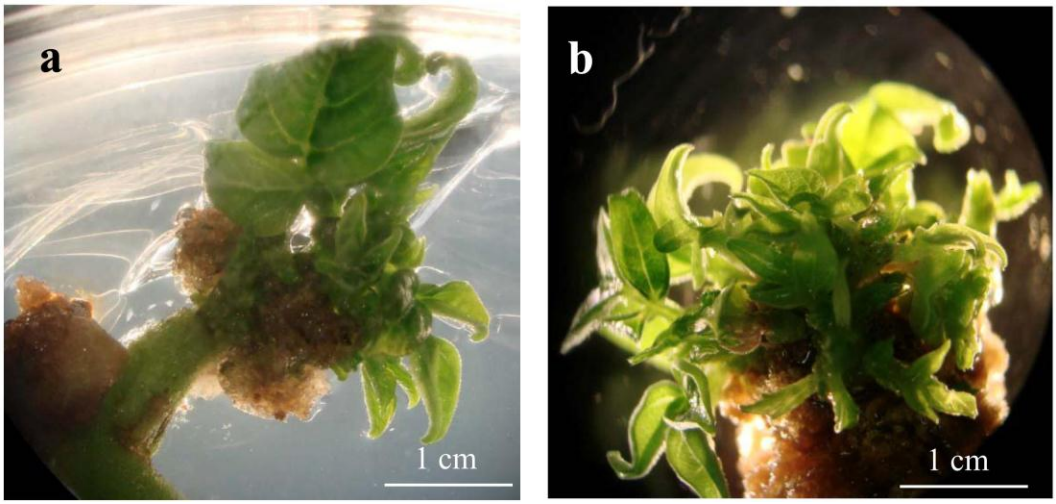

Figure C.2. Multiple shoots production from embryo axes explants of ‘Merlot’ after 6-wk culture on MS (a) and QL (b) medium each containing 44.4 μM BAP

**Discussion**

This study was conducted to determine the effect of basal salt on regeneration of common bean. Six basal salts were tested for their capacity to induce multiple shoot regeneration of common bean mature embryo axes. Two media, WPM and QL, appeared to have the greatest potential for inducing adventitious shoot regeneration despite the fact that MS has been the medium of choice in many previous studies (Dang and Wei, 2009; Delgado-Sanchez et al., 2006; Zambre et al., 1998). WPM was able to induce multiple shoot regeneration at the highest frequency, but these shoots were weak and chlorotic compared to shoots regenerated from QL and MS media (Figure C.1). The major differences in macronutrients among these media are ammonium, nitrate, and calcium ion and total ion concentrations. While WPM contains lower concentrations of both ammonium and nitrate ions than MS and QL media, MS medium has the highest concentrations of both ammonium and nitrate ions, and QL has a medium content of ammonium ions with an increased level of calcium ions. The unhealthy looking shoots regenerated from WPM medium might be due to a low concentration of nitrogen that could not support healthy bean growth. The same phenomenon was also observed on White's medium which had the lowest level of nitrogen of all tested media. The inability of WPM to sustain strong shoot growth has been reported in other plant species (Ciccoti et al., 2009; Bell et al., 2009). Bean shoots regenerated on WPM would need to be quickly transferred to a different medium when they start to regenerate to keep them alive or the WPM medium would need to be supplemented with nitrogen to produce healthy regenerated shoots. Explants cultured on MS medium produced fewer shoots than WPM and QL suggesting that an intermediate level of nitrogen content may be useful in promoting bean regeneration. The high concentration of ammonium ions in MS may have reduced the number of regenerated shoots due to the toxic

effect of ammonium ions. The toxic effect of ammonium in tissue culture might be due to pH changes and acidification of the medium or to the toxic effect of free ammonium ions (Ramage and Williams, 2002). For various crops such as cherries (Matt and Jehle, 2005), barley (Chauhan and Kothari, 2004), and plums (Nowak et al., 2007), modified ratios of $NH_4^+/NO_3^-$ in MS media have been suggested and a reduction in the amount of ammonium ions promoted higher shoot regeneration frequencies.

In common bean, the low regeneration frequency on MS medium has also been noted by Quintero-Jiménez et al. (2010) when compared to Gamborg's medium. The results of the present study in addition to the one conducted by Quintero-Jiménez indicate that MS based medium may not be the best medium for multiple shoot regeneration of beans. To promote higher regeneration frequencies, modified levels of $NH_4^+/NO_{3-}$ may be needed when using MS as a basal salt. The few shoots that regenerated on MS medium were strong, suggesting that after regeneration, enough nitrogen was present to maintain healthy growth of shoots. Further studies may be needed to identify the ideal amount of total nitrogen and ratio of $NH_4^+/NO_{3-}$ that can maintain both high regeneration frequencies and high shoot number per explant.

The high calcium ion concentration in QL medium may have been responsible for the high regeneration frequency and the number of shoots per explant observed on this medium. QL based media have shown to be superior in inducing plant regeneration in various plants such as pears, Japanese plums, and sweet cherry (Bell et al., 2009; Canli and Tian, 2009; Matt and Jehle, 2005). Among the media under study, QL has the highest level of calcium which may have enhanced the regeneration and growth of regenerated shoots. Calcium ions play an important role in cell wall and membrane formation as well as in signaling pathways (Conde et al., 2011). Additionally, calcium ions are important in young and actively growing tissues since they are

needed in mitotic spindle microtubules assembly and breakdown and thus cellular division (Hepler, 1994). Although differences in regeneration observed among these media may not be solely based on total ionic strength, it was noted that all of the poorly performing media had lower zinc content than the best performing ones, suggesting that micronutrients may also play an important role in bean regeneration.

# Appendix B

## Evaluation of drought tolerance in tobacco plants overexpressing the *XERICO* gene

Zinc finger proteins are proteins whose domains have finger-like structures held together by one or more zinc ions (Miller et al., 1985). Their zinc-binding motifs vary widely in structure as well as in function, ranging from regulating transcription through DNA and RNA binding to protein–protein interactions and membrane association (Gao et al., 2012).

Really interesting new gene (RING) fingers are members of zinc finger proteins whose zinc-finger domains are characterized by conserved cysteine and histidine residues that coordinate two zinc atoms in a "cross-brace" system, the ligation scheme of which is distinct from those of the classical zinc fingers (Freemont et al., 1991). The RING finger proteins have been classified into two main families, namely RING-HC (C3HC4) and RING-H2 (C3H2C3) depending on which amino acid (cysteine or histidine) occupies the fifth position of the motif (Freemont, 2000). RING finger proteins are known for their role predominantly in targeted protein degradation and participate in gene regulation through interaction with other regulatory proteins (Freemont, 2000). Ubiquitination of substrate proteins is conferred by the presence of domain-containing E3 ubiquitin (Ub) ligases that recruit proper substrates. RING motif containing proteins are abundant in plants. For instance, at least 477 RING motif containing proteins have been found in Arabidopsis (Vierstra, 2009) while 425 RING finger protein genes have been identified in rice (Lim et al., 2010). Increasing evidence indicates that RING class E3 enzymes play important roles in regulating ABA signaling and related abiotic stress responses in plants. For instance, overexpression of *OsRDCP1*, a rice RING domain-containing E3 ubiquitin

ligase, increased tolerance to drought stress in rice (Bae et al., 2011). The expression analysis of seven RING finger proteins from wheat showed that four of them were responsive to water deficit (Kam et al., 2007). Functional analysis of the rice *OsBIRF1* RING-H2 gene revealed a pleiotropic effect on disease resistance, ABA, and drought tolerance (Liu et al., 2008). Constitutive expression of *CaRma1H1*, an endoplasmic reticulum (ER)-localized RING E3 ubiquitin ligase from hot pepper, increases tolerance to drought and salt stresses in transgenic tomato plants (Seo et al., 2012). Expression of corn *ZmXERICO* under different abiotic stresses showed that the *ZmXERICO* was up-regulated in salt, drought, ABA, and cold stress in maize (Gao et al., 2012).

The *XERICO* gene (Ko et al., 2006) was identified as single copy in Arabidopsis and encodes a small protein (162 amino acids) with an N-terminal trans-membrane domain and a RING-H2 zinc-finger motif located at the C-terminus. Overexpression of the *XERICO* gene showed to confer hypersensitivity to salt, osmotic stress, and abscisic acid (ABA), and drought tolerance. Various experiments led to the conclusion that the overexpression of the *XERICO* gene disturbs hormonal homeostasis in the plant, leading to over accumulation of ABA, which in turn reduces transpiration by stomatal closure and therefore increase drought tolerance. This experiment was conducted to evaluate drought tolerance in tobacco seedlings overexpressing xerico gene

**Materials and methods**

**Plant materials and tobacco transformation**

Tobacco (*Nicotiana tabacum* cv. Samsun) was used to produce seedlings that provided leaf explants used in all experiments. Dry seeds of tobacco were surface-sterilized with 30 % sodium hypochlorite with continuous shaking for 10 min in a petri dish, followed by four rinses

with sterile distilled water. Sterile seeds were planted on half-strength Murashige and Skoog (MS) medium in a Magenta® GA7 box (PhytoTechnology Laboratories, KS, USA). Seedling were grown under a 16-h photoperiod of 30 µmol $m^{-2}$ $s^{-1}$ from cool white fluorescent tubes at 25 $^{o}C$.

Leaf explants were prepared from 4-week old tobacco seedlings. Explants were prepared by cutting leaves into 0. 6 to 0.8 cm square using a sterile scalpel. A*grobacterium st*rain C58 (kindly provided by Dr. Han, Horticulture department, Michigan State University) harboring pCB302-3 binary vector containing the *XERICO* gene inserted between a 35S promoter of cauliflower mosaic virus (CaMV) and a nopaline synthase terminator was used transform leaf explants. The bar gene encoding phosphinothricin acetyltransferase inside the T-DNA for transformants was used to select transformants. Infection was performed by incubating leaf explants in a bacterial suspension in a liquid tobacco regeneration medium (RM) [MS + 3 % sucrose + 2 mg/L 6-Benzylaminopurine (BAP) + 0.2 mg/L Naphthalene acetic acid (NAA) + 100 µM acetosyringone (AS), pH 5.6] at an O.D.600 of 0.2-03 for 5 minutes.

Infected tobacco explants were co-cultivated in the dark for 4 days on liquid tobacco regeneration medium. After the co-cultivation period, leaf explants were washed with MS+ 300 mg/ml timentin to remove bacteria, blot dry, and cultured on tobacco regeneration medium .02mg/L glyfosinate (GS) solidified with 8g/L of bacto-agar. After seven weeks of in vitro growth, five T0 tobacco seedlings selected based on glyfosinate resistance were transferred to pots containing Baccto professional planting mix (Michigan Peat Co.). Pots were covered with plastic bag for seedling acclimation for two weeks, and grown in the greenhouse set at 16 hour photoperiod and 25 $^{O}C$.

**Evaluation for herbicide resistance**

After 8 weeks of growth in the greenhouse tobacco plants were sprayed with glyfosinate ammonium 300mg /L.

**PCR analysis of the bar gene insertion**

DNA for PCR was extracted from each putative transgenic tobacco leaves using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA). PCR reactions were prepared for each putative transgenic line, wild type, and the bar gene line. The primers used for amplifying the bar gene fragment were forward primer 5′-ACC ATC GTC AAC CAC TAC ATC-3′ and reverse primer 5′-GAA GTC CAG CCA GAA AC-3′. PCR reaction mixture (20µl) contained 100ng of DNA, 5pmol of each primer, 0. 2mM of dNTP, 0.2mM of PCR buffer, and 1.5mM of $MgCl_2$. The PCR mixture was incubated for 4 min at 94 $^{o}$C; then 35 cycles of 60 s at 94 $^{o}$C, 50 s at 57 $^{o}$C, 90 s at 72 $^{o}$C, and a final extension of 7 min at 72 $^{o}$C using a Perkin Elmer GeneAmp PCR System 9600.

**Drought evaluation**

T1 seeds from each putative transgenic tobacco line (lines 1 to 5), wild type (line 6), and an empty vector line (line 7) were surface sterilized with 30 % sodium hypochlorite with continuous shaking for 10 min in a petri dish, followed by four rinses with sterile distilled water. Each line was grown on half strength MS. 0.2 GS medium in a petri dish for 4 weeks. Four weeks seedlings were transferred into flats potted with Baccto professional planting mix. Flats were covered with a plastic sheet for two weeks. Seedlings were grown in the growth chamber

for 16h photoperiod at 25 $^{o}$C. Seedlings were watered every 2 days from establishment until 28 days after transplanting. Each flat was divided into water stressed and non- stressed treatments using aluminum foil. Irrigation was suspended in drought stressed experiments for 17 days while non-stressed treatments continued to be watered every two days. After 17 days, irrigation was resumed in stressed treatments every two days for 10 days, after which the recovery from drought was evaluated and fresh biomass was collected and weighed. Experiments were arranged in completely randomized designs.

**Data analysis**

Data were analyzed with SAS 9.3 (SAS institute, Cary, NC, USA).using PROC MIXED procedure. Line and water treatment were analyzed as fixed effect while flat effect was considered as random effect. Least square means were compared with Tukey-Kramer at $p<0.05$

**Results and discussion**

PCR analysis of the integration of the *bar* gene resulted in a single 438-bp DNA fragment from the putative transgenic seedlings as well as that transformed with *bar* gene (Figure D.1). As expected no fragment was amplified in the wild type control. However, when tobacco plants were sprayed with glyfosinate ammonium, the level of resistance varied in putative transgenic lines. Line 1 and 2 were partially bleached after two weeks while lines 3-5 remained green. Since southern blot analysis and expression studies were not performed to confirm the copy number and quantify the expression levels of the *bar* gene in various lines, these phenotypes might be the results of various biological processes including gene inactivation or insufficient level of expression.

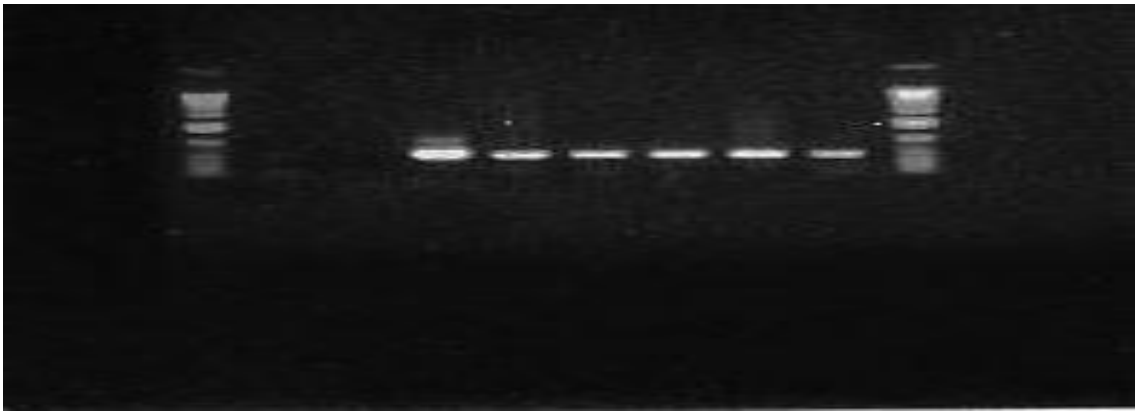

Figure D. 1. PCR detection of the *bar* gene in the wild type control, *bar* gene, and transgenic tobacco lines over expressing the *XERICO* gene. Amplified products were separated on 1% agarose gel stained with ethidium bromide and visualized under UV light. Lane L: ladder;
lane 1: water; lane 2: wild type; lane 3: bar gene; lane: 4-8: putative transgenic lines

Table D. 1. Analysis of variance for biomass fresh weight of 55 days old putative transgenic tobacco lines, wild type, and empty vector lines grown in stress and non-stress water treatments.

| Source of variation | Degree of freedom | Mean square | *F* value | *P* value |
|---|---|---|---|---|
| Flat | 3 | 3.64 | 1.14 | 0.3348 |
| Water treatment | 1 | 477.09 | 149.72 | <.0001 |
| line | 6 | 12.18 | 3.82 | 0.0017 |
| Water treatment*line | 6 | 2.75 | 0.86 | 0.5238 |
| Flat*line | 18 | 5.04 | 1.58 | 0.0772 |
| Flat*water treatment | 3 | 6.01 | 1.89 | 0.1363 |
| Flat*water treatment*line | 18 | 4.46 | 1.4 | 0.1459 |

When T1 seedlings were exposed to drought, and allowed to recover after resuming irrigation, all seedlings including wild type and those from tobacco lines transformed with empty vector recovered in a similar way. There was no distinguishable difference between the transgenic and the non- transgenic lines.

There were statistical differences in seedling's fresh weight for water treatment (Table D.1). The average fresh weight in stress conditions was 4.0g while the average fresh weight was 7.5g in non stress control treatment. These results suggest that drought caused a general growth retardation in all seedlings. In addition, statistical differences were observed between lines (Table D. 1). However, when pairwise comparisons of lines were performed, fresh weight of transformed lines did not differ significantly from that of wild type or empty vector lines under drought stress (Table D.3), which suggests that there was no drought tolerance advantage in transgenic compared to non transgenic lines. Althought not statistically different from wild type or empty vector lines, the fresh weight of line four was lower than other lines in drought conditions (Table D. 2), and this might have been the only cause of statistical difference observed among lines. Since the increased cellular levels of ABA is known to be the mechanism conferring drought tolerance in plants over expressing the *XERICO* gene (Ko et al., 2006), it is possible that when line four was exposed to severe drought stress, it over-produced ABA that resulted in low biomass. A retardation of vegetative growth under excess ABA is known in plants subject to drought stress (Sreenivasulu et al., 2012)

Under non-stress conditions, differences from pairwise comparisons of lines (Table D. 4) did not seem to favor any group of lines. In addition, there was no morphological modifications associated with over expression of the *XERICO* gene observed as opposed to the alterations of plant development that were observed in tobacco plants overexpressing a poplar RING-zinc

finger gene *PtaRHE1* (Bopopi et al., 2009). Taken together, results from stress and non-stress conditions suggest that there was no noticeable difference in drought tolerance in tobacco seedlings over expressing the *XERICO* gene. Ko et al. (2006) observed the increased drought tolerance in *Arabidopsis* seedlings over expressing xerico gene. In this study, various molecular or phenotyping related factors might provide insight in to the observed reactions. At molecular level, southern blot analysis and expression studies were not conducted; many copies of the gene may react to silence each other resulting in lack of expression. At the phenotyping level, the use of plant biomass to measure the effect of drought may not have been the most appropriate method to phenotype for the expression of drought tolerance in these transgenic tobacco lines. Other methods such as *in vitro* methods might yield more differences. Kam et al. (2007) identified RING zinc finger genes conferring drought resistance in transcripts of wheat lines subjected to drought stress and non-stress by measuring the relative water content of detached leaf and root. Various methods were used in rice to evaluate drought resistance in transgenic lines over expressing RING finger protein genes. For instance, recovery from a 15 days drought was used as a drought resistance trait in transgenic rice lines over expressing *OsRDCP1* gene (Bae et al., 2011), while drought tolerance of the *OsBIRF1* transgenic lines was studied by analyzing seed germination on PEG6000 or mannitol containing media (Liu et al., 2008). Obviously, this study failed to identify the drought resistance effect of the *XERICO* gene in tobacco under present experimental conditions. Detailed molecular studies combined with appropriate drought phenotyping are needed to confirm if the *XERICO* gene confers drought tolerance in plants other than in *Arabidopsis.*

Table D. 2. Average fresh weight and standard errors of 55 days old putative transgenic tobacco lines, wild type, and empty vector line grown in stress and non-stress water treatments.

| water treatment | line† | fresh weight (g) | standard error |
|---|---|---|---|
| drought | 1 | 4.39 | 0.52 |
| drought | 2 | 3.65 | 0.55 |
| drought | 3 | 4.06 | 0.53 |
| drought | 4 | 2.88 | 0.55 |
| drought | 5 | 4.49 | 0.55 |
| drought | 6 | 4.25 | 0.52 |
| drought | 7 | 4.28 | 0.50 |
| non stress | 1 | 7.81 | 0.52 |
| non stress | 2 | 6.86 | 0.52 |
| non stress | 3 | 6.25 | 0.52 |
| non stress | 4 | 6.26 | 0.52 |
| non stress | 5 | 8.40 | 0.52 |
| non stress | 6 | 7.63 | 0.52 |
| non stress | 7 | 8.69 | 0.52 |

†1-5: transgenic tobacco lines over expressing *XERICO* gene; line 6: wild type tobacco, line 7: tobacco line transformed with empty vector.

Table D. 3. Pairwise comparisons of 55 days old putative transgenic tobacco lines, wild type, and empty vector lines grown under drought stress conditions.

| line† | line† | difference between line estimate | standard error | *P* value |
|---|---|---|---|---|
| 1 | 2 | 0.73 | 0.75 | 0.33 |
| 1 | 3 | 0.32 | 0.74 | 0.66 |
| 1 | 4 | 1.50 | 0.75 | 0.05 |
| 1 | 5 | -0.10 | 0.75 | 0.89 |
| 1 | 6 | 0.13 | 0.73 | 0.85 |
| 1 | 7 | 0.10 | 0.72 | 0.88 |
| 2 | 3 | -0.41 | 0.76 | 0.59 |
| 2 | 4 | 0.77 | 0.78 | 0.32 |
| 2 | 5 | -0.84 | 0.77 | 0.28 |
| 2 | 6 | -0.60 | 0.75 | 0.43 |
| 2 | 7 | -0.63 | 0.74 | 0.39 |
| 3 | 4 | 1.18 | 0.76 | 0.12 |
| 3 | 5 | -0.43 | 0.76 | 0.57 |
| 3 | 6 | -0.19 | 0.74 | 0.80 |
| 3 | 7 | -0.22 | 0.73 | 0.76 |
| 4 | 5 | -1.61 | 0.77 | 0.04 |
| 4 | 6 | -1.37 | 0.75 | 0.07 |
| 4 | 7 | -1.40 | 0.74 | 0.06 |
| 5 | 6 | 0.24 | 0.75 | 0.75 |
| 5 | 7 | 0.21 | 0.74 | 0.78 |
| 6 | 7 | -0.03 | 0.72 | 0.96 |

† 1-5: transgenic tobacco lines overexpressing *XERICO* gene, 6: wild type tobacco line; 7: tobacco line transformed with empty vector.

Table D. 4. Pairwise comparisons of 55 days old putative transgenic tobacco lines, wild type, and empty vector line grown under non-stress conditions.

| line† | line† | difference between lines estimate | standard error | *P* value |
|---|---|---|---|---|
| 1 | 2 | 0.95 | 0.73 | 0.2 |
| 1 | 3 | 1.55 | 0.73 | 0.03 |
| 1 | 4 | 1.54 | 0.73 | 0.04 |
| 1 | 5 | -0.60 | 0.73 | 0.41 |
| 1 | 6 | 0.17 | 0.73 | 0.81 |
| 1 | 7 | -0.89 | 0.73 | 0.22 |
| 2 | 3 | 0.60 | 0.73 | 0.41 |
| 2 | 4 | 0.59 | 0.73 | 0.42 |
| 2 | 5 | -1.55 | 0.73 | 0.04 |
| 2 | 6 | -0.77 | 0.73 | 0.29 |
| 2 | 7 | -1.84 | 0.73 | 0.01 |
| 3 | 4 | -0.01 | 0.73 | 0.99 |
| 3 | 5 | -2.15 | 0.73 | 0.00 |
| 3 | 6 | -1.38 | 0.73 | 0.06 |
| 3 | 7 | -2.44 | 0.73 | 0.00 |
| 4 | 5 | -2.14 | 0.73 | 0.01 |
| 4 | 6 | -1.36 | 0.73 | 0.06 |
| 4 | 7 | -2.43 | 0.73 | 0.00 |
| 5 | 6 | 0.77 | 0.73 | 0.29 |
| 5 | 7 | -0.29 | 0.73 | 0.69 |
| 6 | 7 | -1.06 | 0.73 | 0.15 |

†Transgenic tobacco lines overexpressing *XERICO* gene (1-5):, 6: wild type tobacco line; 7: tobacco line transformed with empty vector.

## LITERATURE CITED

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Aragão, F. J. L, M. F. G de Sá, E. R. Almeida, E. S. Gander, and E. L. Rech. 1992. Particle bombardment-mediated transient expression of a Brazil nut methionine-rich albumin in bean (*Phaseolus vulgari*s L.). Plant Mol. Biol. 20:357-359.

Aragão, F. J. L, S. G. Ribeiro, L. M. G. Barros, A. C. M. Brasileiro, D. P Maxwell, E. L Rech, and J. C Faria.1998. Transgenic beans (*Phaseolus vulgaris* L.) engineered to express viral antisense RNAs showed delayed and attenuated symptoms to bean golden mosaic geminivirus. Mol. Breed. 4: 491-499.

Arellano, J, S. I. Fuentes, P. Castillo-España, and G. Hernández. 2009. Regeneration of different cultivars of common bean (*Phaseolus vulgaris* L.) via indirect organogenesis. Plant Cell Tiss. Organ Cult. 96:1-11.

Bae, H., S. K. Kim, S. K. Cho, B. G. Kang, and W. T. Kim. 2011. Overexpression of OsRDCP1, a rice RING domain-containing E3 ubiquitin ligase, increased tolerance to drought stress in rice (Oryza sativa L.). Plant Sci.180: 775-782

Bakshi, S, A. Sadhukhan, S. Mishra, and L. Sahoo. 2011. Improved Agrobacterium-mediated transformation of cowpea via sonication and vacuum infiltration. Plant Cell Rep. 30:2281-2292.

Bell, R. L, S. Chinnathambi, and L. Delores. 2009. Effect of nutrient media on axillary shoot proliferation and preconditioning for adventitious shoot regeneration of pears. In Vitro Cell Dev. Biol. Plant. 45:708-714.

Bonfim, K., J. C. Faria, E. O. P. L. Nogueira, É. A. Mendes, and F. J. L. Aragão.2007. RNAi-Mediated Resistance to *Bean golden mosaic virus* in Genetically Engineered Common Bean (*Phaseolus vulgaris*). Mol. Plant-Microbe Inter. 20: 717-726.

Bopopi, J.M, O. M. Vandeputte, K. Himanen, A. Mol, Q. Vaessen, M. E. Jaziri, and M. Baucher. 2009. Ectopic expression of PtaRHE1, encoding a poplar RING-H2 protein with E3 ligase activity, alters plant development and induces defense related responses. J. Exper. Bot. 61: 297-310.

Brasiliero, A. C. M., F. J. L. Aragão, S. Rossi, D. M. A. Dusi, L. M. G. Barros, and E. L. Rech.1996. Susceptibility of common beans to *Agrobacterium* ssp. Strains and improvement of *Agrobacterium* transformation using microprojectile bombardment. J. Am. Soc. Hortic. Sci. 121: 810-815.

Broughton, W. J, G. Hernandez, M. W. Blair, S. Beebe, P. Gepts, and J. Vanderleyden. 2003. Beans (*Phaseolus* spp.) -model food legumes. Plant and Soil 252: 55-128.

Canli, F. A. and L. Tian. 2009. Assessment of regeneration and transient expression factors for *Agrobacterium*-mediated transformation of *Prunus salicina* Lindl. Europ. J. Hort. Sci. 74:66-72.

Chauhan, M. and S. L. Kothari. 2004. Optimization of nutrient levels in the medium increases the efficiency of callus induction and plant regeneration in recalcitrant Indian barley (Hordeum vulgare L.) in vitro. In Vitro Cell Dev. Biol. Plant. 40:520-527.

Cheng, M, J. E. Fry, S. Pang, H. Zhou, C. M. Hironoka, D. R. Duncan, T. W. Conner, and Y. Wan.1997. genetic transformation of wheat mediated by *Agrobacterium tumefaciens.* Plant Physiol. 115: 971-980.

Christou P. 1997. Biotechnology applied to grain legumes. Field Crops Res. 53: 83-97.

Chu, C. C.1978. The N6 medium and its application to anther culture of cereal crops. In Proc. Symp. On Plant Tissue Culture. Sci. Press 43-50.

Ciccotti, A. M, C. Bisognin, I. Battocletti , and A. Salvadori , M. Herdemertens, M. Wallbraun, and W. Jarausch. 2009. Micropropagation of *Malus sieboldii* hybrids resistant to apple proliferation disease. Acta Hort. 839: 35-41.

Conde, A., M. M. Chaves, and H. Geros. 2011. Membrane transport, sensing and signaling in plant adaptation to environmental stress. Plant Cell Physiol. 52: 1583-1602.

Crocomo, O. J, J. E. Peters, and W. R. Sharp.1976. Interactions of phytohormones on the control of growth and root morphogenesis in cultured *Phaseolus vulgaris* leaf explants. Turrialba 26: 232-236.

Cruz de Caravalho, M. N., B. Van Le, Y. Zuili-Fodil, A. T. Pham Thi, and K. TranThan Van. 2000. Efficient whole plant regeneration of common bean (*Phaseolus vulgaris* L.) using thin cell layer culture and silver nitrate. Plant Sci. 159: 223-232.

Dang, W. and Z.M. Wei. 2009. High frequency plant regeneration from the cotyledonary node of common bean. Biologia Plantarum 53: 312-316.

Davis, M. E, A. R. Miller, and R. D. Lineberger.1991. Temporal competence for transformation of *Lycopersicon esculentum* (L. Mill) cotyledons by *Agrobacterium tumefaciens*. Relation to wound healing and soluble plant factors. J. Exp. Bot. 42: 359-364.

Delgado-Sánchez, P., M. Saucedo-Ruiz, S. H. Guzmán-Maldonado, E. Villordo-Pineda, M. González-Chavira, S. Fraire-Velázquez, J. A. Acosta-Gallegos, and A. Mora-Avilés. 2006. An organogenic plant regeneration system for common bean (*Phaseolus vulgaris* L.). Plant Sci. 170: 822-827.

Dillen, W., J. De Clercq, A. Goossens, M. Van Montagu, and G. Angenon.1997. Agrobacterium-mediated transformation of *Phaseolus acutifolius* A. Theor. Appl. Genet. 94: 151-158

Dita, M. A, N. Rispail, E. Prats, D. Rubiales, and K. B. Singh. 2006. Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. *Euphytica* 147: 1-24.

Estrada-Navarrete, G., X. Alvarado-Affantranger, J. E. Olivares, C. L. Díaz-Camino, O. Santana, E. Murillo, G. Guillén, N. Sánchez-Guevara, J. Acosta, C. Quinto, D. Li, P. M. Gresshoff, and F. Sánchez. 2006. *Agrobacterium rhizogenes* Transformation of the *Phaseolus* spp.: A Tool for Functional Genomics. Mol. Plant-Microbe Inter. 19: 1385-1393.

Freemont, P. S., I. M. Hanson, and J.Trowsdale. 1991. A Novel Cysteine-Rich Sequence Motif. Cell 64: 483-484

Freemont, P. S. 2000. Ubiquitination: RING for destruction? Current Biol. 10:R84-R87

Gamborg, O. L, R. A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:151-158

Gao, Y, H. Li, D. Deng, S. Chen, W. Jiang, and J. Chen. 2012. Characterization and expression analysis of the maize RING-H2 finger protein gene ZmXERICO responsive to plant hormones and abiotic stresses. Acta Physiol. Plant. 34:1529-1535.

Gatica Arias, A. M, J. M. Valverde, P. R. Fonseca, and M. V. Melara. 2010. *In vitro* plant regeneration system for common bean (*Phaseolus vulgaris*): effect of N6-benzylaminopurine and adenine sulphate. Electronic J. Biotechn. 13: 1-8.

Geerts, P, G. Mergeai, and J. P. Baudon. 2000. Succeeded plant regeneration from very immature embryos of Phaseolus vulgaris. Ann. Rep. Bean Improv. Coop. 43:206-207.

Grant, J. and P. Cooper. 2006. Peas (Pisum sativum L.) In: Wang K (ed) Methods in Molecular Biology, vol. 343: Agrobacterium Protocols, 2/e, volume 1. Humana Press Inc., Totowa, NJ. pp 337-346.

Hepler, P. K. 1994. The role of calcium in cell division. Cell Calci. 16: 322-330

Hiei, Y., S. Ohta, T. Komari, and T. Kumashiro. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J. 6: 271-282.

Hoekema, A., P. R. Hirsch, P. J. J. Hooykaas, and R. A. Schilperoort. 1983. A binary plant vector strategy based on separation of *vir*- and T-regionof the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303: 179-180.

Hood, E. A, S. B. Gelvin, L. S. Melchers, and A. Hoekema. 1993. New *Agrobacterium* helper plasmids for gene transfer to plants. Transgen. Res. 2:208-218.

Huda, S., R. Islam, M. A. Bari, and M. Asaduzzaman. 2003. Shoot differentiation from cotyledon derived callus of chickpea (*Cicer arietinum* L.). Plant Cell. Tiss. Organ. Cult. 13: 53-59.

Indurker, S., H. S. Misra, and S. Eapen. 2010. Agrobacterium-mediated transformation in chickpea (*Cicer arietinum* L.) with an insecticidal protein gene: optimization of different factors. Physiol. Mol. Biol. Plants 16:273-284.

Jayanand, B., G. Sudarsanam, and K. K. Sharma. 2003. An efficient protocol for the regeneration of whole plants of chickpea (*Cicer arietinum* L.) by using axilary meristem explants derived from *in vitro*-germinated seeds. In Vitro Cell Dev. Biol. Plant 39: 171-179.

Jefferson, D. J, T. A. Kavanagh, and M. Bevan. 1987. GUS fusions: β- glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 6:3901-3907.

Kam, J, P. Gresshoff, R. Shorter, and G. P. Xue. 2007. Expression analysis of RING zinc finger genes from *Triticum aestivum* and identification of TaRZF70 that contains four RING-H2 domains and differentially responds to water deficit between leaf and root. Plant Sci.173: 650-659

Kao N. N., M.R. Michayluk.1975. Nutrient requirements for growth of *Vicia hajastana* cells and protoplants at very low population density in liquid medium. Planta. 126: 105-110.

Kim, J. W. and T. Minamikawa. 1996. Transformation and regeneration of French bean plants by the particle bombardment process. Plant Sci. 117:131-138.

Ko, J.H., S H. Yang, and K. H. Han. 2006. Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. Plant J. 47: 343-355

Koncz, C. and J. Schell. 1986. The promoter of the TL-DNA gene 5 controls the tissue-specific expression of chimeric genes carried by a novel type of *Agrobacterium* binary vector. Mol. Gen. Genet. 204:383-396.

Krishna, G, P. S. Reddy, P. W. Ramteke, and P. S .Bhattacharya. 2010. Progress of tissue culture and genetic transformation research in pigeon pea [*Cajanus cajan* (L.) Millsp.]. Plant Cell Rep. 29:1079-1095.

Kwapata, K, R. Sabzikar, M. B. Sticklen, and J. D. Kelly. 2010. In vitro regeneration and morphogenesis studies in common bean. Plant Cell Tiss. Organ. Cult. 100:97-105.

Lewis, M. E. and F. A. Bliss.1994. Tumor formation and glucuronidase expression in *Phaseolus vulgaris* inoculated with *Agrobacterium tumefaciens*. J. Am. Soc. Hortic. Sci. 119:361-366.

Li, H. Q, C. Sautter, I. Potrykus, and J. Puonti-Kaerlas. 1996. Genetic transformation of cassava (*Manihot esculenta* Crantz). Nature Biotechnol. 14: 736-740.

Lim , S. D., W. C. Yim, J. Ch. Moon, D. S. Kim, B. M. Lee, and C. S. Jang. 2010. A gene family encoding RING finger proteins in rice: their expansion, expression diversity, and co-expressed genes. Plant Mol. Biol. 72:369–380.

Liu, Z., B. J. Park, A. Kanno, and T. Kameya. 2005. The novel use of a combination of sonication and vacuum infiltration in Agrobacterium-mediated transformation of kidney bean (*Phaseolus vulgaris* L.) with *lea* gene. Mo.l Breed.16: 189-197.

Liu, H., H. Zhang, Y. Yang, G. Li, Y. Yang, X. Wang, B. M. V. S. Basnayake, and D. Li, F. Song. 2008. Functional analysis reveals pleiotropic effects of rice RING-H2 finger protein gene OsBIRF1 on regulation of growth and defense responses against abiotic and biotic stresses. Plant Mol. Biol. 68:17-30.

Lloyd, G. and B. Mccown.1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Proc. Int. Plant Prop. Soc. 30: 421-427.

Mahmoudian, M., M. Yucel, and H. A .Oktem. 2002. Transformation of lentil (*Lens culinaris* M.) cotyledonary nodes by vacuum infiltration of *Agrobacterium tumefaciens*. Plant Mol. Biol. Rep. 20:251-257.

Malik, K. A. and P. K. Saxena.1991. Regeneration in Phaseolus vulgaris L. Promotive role of N6-benzylaminopurine in cultures from juvenile leaves. Planta 184: 148-150.

Margie, M. P, J. C Martinez, A. B. Kalvig, T. M. Fonger, and K. Wang. 2006. Improved cotyledonary node method using an alternative explant derived from mature seed for efficient Agrobacterium-mediated soybean transformation. Plant Cell Rep. 25: 206-213.

Mariotti, D, G. S. Fontana, and L. Santini. 1989. Genetic transformation of grain legumes: *Phaseolus vulgari*s L. and *P. coccineus* L. J Genet. Breed. 43:77-82.

Matt, A. and J. A. Jehle. 2005. In vitro plant regeneration from leaves and internodes sections of sweet cherry cultivars (*Prunus avium* L.). Plant Cell Rep. 24: 468-476.

McClean, P., P. Chee, B. Held, J. Simental, R. F. Drong, and J. Slightom.1991. Susceptibility of dry bean *(Phaseolus vulgaris* L.) to *Agrobacterium* infection: Transformation of cotyledonary and hypocotyl tissues. Plant Cell Tiss. Organ. Cult. 24: 131-138.

Mehrotra, M., I. Sanyal, and D. V. Amla. 2011. High-efficiency Agrobacterium-mediated transformation of chickpea (*Cicer arietinum* L.) and regeneration of insect-resistant transgenic plants. Plant Cell Rep .30: 1603-1616.

Miller, J, A., D. McLachlan, and A. Klug. 1985. Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. EMBO J. 4: 1609-1614

Mukeshimana, G, Y. Ma, A. E. Walworth , G. Song , and J. D. Kelly. 2012. Factors influencing regeneration and Agrobacterium tumefaciensmediated transformation of common bean (Phaseolus vulgaris L.). Plant Biotechnol. Rep. DOI 10.1007/s11816-012-0237-0

Murashige, T, and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant 15:473-49.

Muthukumar, B., M. Mariamma, K. Veluthambi, and A. Gnanam. 1996. Genetic transformation of cotyledon explants of cowpea (*Vigna unguiculata* L. Walp) using *Agrobacterium tumefaciens*. Plant Cell Rep. 15:980-985.

Ni, M., D. Cui, J. Einstein, S. Narasimhulu, C. E. Vergara, and S. B. Gelvin. 1995. Strength and tissue specificity of chimeric promoters derived from the octopine and mannopine synthase genes. Plant J. 7:661-676.

Nowak, B., K. Miczynski, and .L Hudy.2007. The effect of total inorganic nitrogen and the balance between its ionic forms on adventitious bud formation and callus growth of 'Wegierka Zwykła' plum (Prunus domestica L.). Acta Physiol. Plant. 29:479-484.

Paz, M. M, J. C Martinez, A. B. Kalvig, T. M. Fonger, and K. Wang. 2006. Improved cotyledonary node method using an alternative explant derived from mature seed for efficient Agrobacterium-mediated soybean transformation. Plant Cell Rep. 25: 206-213.

Polowick, P. L, D. S. Balisk, and J. D Mahon. 2004. *Agrobacterium tumefaciens*-mediated transformation of chickpea (*Cicer arietinum* L.): gene integration, expression and inheritance. Plant Cell Rep. 23: 485-491.

Popelka, J. C, S. Gollasch, A. Moore, L. Molving, and T. J. V. Higgins. 2006. Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transmission of the transgenes to progeny. Plant Cell Rep. 25: 304-312.

Quintero-Jimenez, A., E. Espinosa-Huerta, J. A. Acosta-Gallegos, H. S . Guzman-Maldonado, and M. A. Mora-Aviles. 2010. Enhanced shoot organogenesis and regeneration in the common bean (*Phaseolus vulgaris* L.). Plant Cell Tiss. Organ Cult. 102:381-386.

Quoirin, M. and P. Lepoivre. 1977. Improved media for *in vitro* culture of *Prunus* sp. Acta Hort. 78: 437-442.

Ramage, C. M. and R. R. Williams. 2002. Inorganic nitrogen requirements during shoot organogenesis in tobacco leaf discs. J. Exp. Bot. 53: 1437-1443.

Russel, D. R., K. M. Wallace, J. H. Bathe, B. J. Martinell, and D.E McCabe. 1993. Stable transformation of *PhaseoIus vulgaris* via electric-discharge mediated particle acceleration. Plant Cell Rep. 12:165-169.

Saunders, J. W., G. L. Hosfield, and A. Levi. 1987. Morphogenetic effect of 2, 4-dichlorophenoxyacetic acid on pinto bean (*Phaseolus vulgaris* L.) leaf explants in vitro. Plant Cell Rep. 6: 46-49.

Senthil, G., B. Williamson, R. D. Dinkins, and G. Ramsay. 2004. An efficient transformation system for chickpea (*Cicer arietinum* L.). Plant Cell Rep. 23: 297-303.

Seo, Y.S, J. Y. Choi, S. J. Kim, E. Y. Kim, J. S. Shin, and W. T. Kim. 2012. Constitutive expression of CaRma1H1, a hot pepper ER-localized RING E3 ubiquitin ligase, increases tolerance to drought and salt stresses in transgenic tomato plants. Plant Cell Rep. 31:1659-1665.

Sharma, K. K. and B. M. Pooja. 2006. Peanut (*Arachis hypogaea* L.) In: Wang K (ed) Methods in Molecular Biology, vol. 343: Agrobacterium Protocols, 2 edn, vol.1. Humana Press Inc., Totowa, NJ. pp 347-358.

Solleti, S. K., S. Bakshi, J. Purkayastha, S. K. Panda, and L. Sahoo. 2008. Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean *a*-amylase inhibitor 1 confer resistance to storage pests, bruchid beetles. Plant Cell Rep. 27:1841-1850.

Somers, D. A., D. A. Samac, and P. M. Olhoft. 2003. Recent Advances in Legume Transformation. Plant Physiol. 131: 892-899.

Sreenivasulu, N., V. T. Harshavardhan, G. Govind, C. Seiler, and A. Kohli. 2012. Contrapuntal role of ABA: Does it mediate stress tolerance or plant growth retardation under long-term drought stress? Gene 506: 265-273

Svetleva, D., M. Velcheva, and G. Bhowmik. 2003. Biotechnology as a useful tool in common bean (*Phaseolus vulgaris* L.) improvement. Euphytica 131: 189-200.

Uranbey, S., C. S. Sevimay, M. D. Kaya, A. Ipek, C. Sancak, D. Başalma, C. Er, and S. Ozcan. 2005. Influence of different co-cultivation temperatures, periods and media on *Agrobacterium tumefaciens*-mediated gene transfer. Biologia Plantarum 49: 53-57.

Veltcheva, M., D. Svetleva, S. P. Petkova, and A. Perl. 2005. In vitro regeneration and genetic transformation of common bean (*Phaseolus vulgaris* L.) Problems and progress. Scientia Horticulturae 107: 2-10.

Vervliet, G., M. Holsters, H. Tecuchy, M. van Montagu, and J. Schell. 1975. Characterization of different plaque-forming and defective temperate phages in *Agrobacterium* strains. J. Gen. Virol. 26:33-48.

Vierstra, R.D. 2009. The ubiquitin-26S proteasome system at the nexus of plant biology. Nat. Rev. Mol. Cell Biol. 10, 385-397.

White, P. R.1963. The cultivation of animal and plant cells. 2nd Ed. The Ronald Press Co., NewYork, NY.

Zambre, M. A, J. De Clercq, E. Vranová, M, Van Montagu, G. Angenon, and W. Dillen. 1998. Plant regeneration from embryo-derived callus in *Phaseolus vulgaris* L.(common bean) and *P. acutifolius* A. Gray (tepary bean). Plant Cell Rep. 17: 626-630.

Zambre, M., A. Goossens, C. Cardona, M. Van Montagu, N. Terryn, and G. Angenon. 2005. A reproducible genetic transformation system for cultivated *Phaseolus acutifolius* (tepary bean) and its use to assess the role of arcelins in resistance to the Mexican bean weevil. Theor. Appl. Genet. 110: 914-924.

Zhang, Z., D. P. Coyne, and A. Mitra. 1997. Factor affecting Agrobacterium-mediated transformation of common bean. J. Amer. Soc. Hort. Sci. 122:300-305.