



Universitat Autònoma  
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Departament de Biologia Animal, Biologia Vegetal i Ecologia  
Unitat Fisiologia Vegetal

**Morpho-physiological analysis of adaptive  
responses of common bean (*Phaseolus vulgaris* L.)  
to drought stress**

**Doctoral Thesis**

Doctoral Program of Plant Biology and Biotechnology

**JOSÉ A. POLANÍA PERDOMO**

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**Morpho-physiological analysis of adaptive responses of common  
bean (*Phaseolus vulgaris* L.) to drought stress**

Dissertation presented in fulfilment of the requirements for the degree of Doctor in  
Plant Biology and Biotechnology by

**JOSÉ A. POLANÍA PERDOMO**

Supervised by

Dr. Charlotte Poschenrieder, Dep. Biología Animal, Biol. Vegetal y Ecología (UAB)

Dr. Idupulapati M. Rao and Dr. Stephen E. Beebe

Bean Program

International Center for Tropical Agriculture (CIAT)

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José A. Polania Perdomo

---

Charlotte Poschenrieder

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Idupulapati M. Rao

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Stephen E. Beebe

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## Abstract

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in the diet of poor people in the tropics. This legume is cultivated by small farmers and is usually exposed to unfavorable conditions with minimum use of inputs. Drought and low soil fertility, especially phosphorus (P) and nitrogen (N) deficiencies, are major limitations to bean yield in smallholder systems. Beans can derive part of their required N from the atmosphere through symbiotic nitrogen fixation (SNF). Drought stress severely limits SNF ability of plants. Identification of traits associated with drought resistance contributes to improving the process of designing bean genotypes adapted to these conditions.

Field studies were conducted at the International Center for Tropical Agriculture (CIAT), Palmira, Colombia to determine the relationship between grain yield and different parameters in elite lines selected for drought resistance over the past decade. The selected traits were effective use of water (EUW), canopy biomass, remobilization of photosynthates to grain (pod partitioning index, harvest index and pod harvest index) and SNF ability. Moreover, in field trials we also validated the use of <sup>15</sup>N natural abundance in grain tissue to quantify phenotypic differences in SNF ability for its implementation in breeding programs aiming to improve SNF in common bean. Carbon isotope discrimination (CID) was used for estimation of water use efficiency (WUE) and effective use of water (EUW). A set of 36 bean genotypes belonging to the Middle American gene pool were evaluated under field conditions with two levels of water supply (irrigated and rainfed) over two seasons. Additionally, a greenhouse study was conducted at CIAT using plastic cylinders with soil inserted into PVC pipes, to determine the relationship between grain yield and different root parameters such as total root length, fine root production and visual root growth rate in same group of elite lines under drought stress.

Eight bean lines (NCB 280, NCB 226, SEN 56, SCR 2, SCR 16, SMC 141, RCB 593 and BFS 67) were identified as resistant to drought stress. Resistance to terminal

drought stress was positively associated with EUW combined with a deeper and vigorous root system, better plant growth, and superior mobilization of photosynthates to pod and seed production, but negatively associated with days to flowering and days to physiological maturity. Based on phenotypic differences in CID, leaf stomatal conductance, canopy biomass and grain yield under drought stress, the tested lines were classified into two groups, water savers and water spenders. These groups also differ in their root characteristics, water spenders with a vigorous and deeper root system and water savers genotypes with a moderate to shallow root system and more presence of fine roots.

We used  $^{15}\text{N}$  natural abundance method to compare SNF ability estimated from shoot tissue sampled at mid-pod filling growth stage vs. grain tissue sampled at harvest. The results showed a significant positive correlation between nitrogen derived from the atmosphere (Ndfa), estimated using shoot tissue at mid-pod filling, and Ndfa estimated using grain tissue at harvest. The method showed phenotypic variability in SNF ability under both drought and irrigated conditions. A significant reduction in SNF ability was observed under drought stress. We suggest that the method of estimating Ndfa using grain tissue (Ndfa-G) can be applied in bean breeding programs to improve SNF ability. Using this method of Ndfa-G, we identified four bean lines (RCB 593, SEA 15, NCB 226 and BFS 29) that combine greater SNF ability with higher grain yield under drought stress. These lines could serve as potential parents to further improve SNF ability of common bean. Better SNF ability under drought stress was related with superior presence of thick roots. Superior N uptake from the soil was associated with a large root system with more presence of fine roots. Pod harvest index, grain CID and Ndfa using grain tissue could be a useful selection criterion in breeding programs to select for drought resistance in common bean.

## Resumen

El frijol común (*Phaseolus vulgaris* L.) es la leguminosa alimenticia más importante en la dieta de las personas pobres de los trópicos. Esta leguminosa es cultivada por pequeños agricultores y por lo general se expone a condiciones desfavorables con uso mínimo de insumos. La sequía y la baja fertilidad del suelo, especialmente las deficiencias de nitrógeno (N) y fósforo, son las principales limitaciones para el rendimiento del frijol en los sistemas de pequeños productores. El frijol puede derivar parte de su requerimiento de N de la atmósfera a través de la fijación simbiótica de nitrógeno (SNF por su sigla en inglés). El estrés por sequía limita severamente la capacidad SNF de las plantas. Identificación de rasgos asociados con resistencia a la sequía contribuye a mejorar el proceso de generación de genotipos de frijol adaptados a estas condiciones.

Se realizaron estudios de campo en el Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia, para determinar la relación entre el rendimiento de grano y diferentes parámetros morfo fisiológicos tales como el uso efectivo del agua (EUW), biomasa dosel, removilización de fotosintatos a los granos (índice de partición vaina , índice de cosecha y el índice de cosecha de vaina) y la capacidad de fijación simbiótica de nitrógeno en líneas élite seleccionadas para la resistencia a la sequía durante la última década. También en los ensayos de campo se validó la metodología de abundancia natural de  $^{15}\text{N}$  usando tejido de grano para cuantificar las diferencias fenotípicas en la capacidad SNF y su aplicación en programas de mejoramiento con el objetivo de mejorar la SNF en frijol común. Se utilizó discriminación de isótopo de carbono (CID) para la estimación de uso eficiente del agua (WUE) y uso efectivo de agua (EUW). Un conjunto de 36 genotipos de frijol pertenecientes al acervo genético mesoamericano fueron evaluados en condiciones de campo con dos niveles de suministro de agua (riego y sequía) en dos temporadas. Adicionalmente, un estudio en condiciones de invernadero se llevó a cabo en el CIAT utilizando cilindros de plástico con suelo, para determinar la relación entre el rendimiento de grano y diferentes características morfo fisiológicas de raíz tales como la longitud total de las

raíces, la producción de raíces finas y la tasa de crecimiento visual de las raíces; se evaluó el mismo grupo de líneas élite bajo condiciones de estrés por sequía.

Resultados permitieron la identificación de ocho líneas de frijol (NCB 280, BCN 226, SEN 56, SCR 2, SCR 16, SMC 141, 593 y RCB BFS 67) como resistentes a la sequía. La resistencia a estrés por sequía terminal se asocia positivamente con EUW combinado con un profundo y vigoroso sistema de raíces, mejor crecimiento de las plantas, y superior movilización de fotosintatos a la formación de vaina y granos; y se asocia negativamente con días a floración y días a madurez fisiológica. Basándose en las diferencias fenotípicas obtenidas en CID, conductancia estomática de la hoja, la biomasa del dosel y el rendimiento de grano en condiciones de sequía, las líneas evaluadas se clasificaron en dos grupos, los ahorradores de agua y gastadores de agua. Estos dos grupos también se diferenciaron en sus características de raíces, los gastadores de agua con un vigoroso y profundo sistema de raíces y los ahorradores con un moderado a superficial sistema de raíces con mayor presencia de raíces finas.

Se utilizó el método de abundancia natural de  $^{15}\text{N}$  para comparar capacidad de fijar nitrógeno estimada a partir de tejido foliar muestreado en la etapa de mitad de llenado de la vaina versus el tejido granos muestreados en la cosecha. Los resultados mostraron una correlación positiva y significativa entre el nitrógeno derivado de la atmósfera (Ndfa) calculado utilizando tejido foliar en la etapa de mitad de llenado de grano y Ndfa estimado usando el tejido de grano en la cosecha. El método mostró variabilidad fenotípica en la capacidad de fijación simbiótica de nitrógeno bajo condiciones de riego y sequía y una reducción significativa en la capacidad SNF en condiciones de sequía. Se sugiere que el método de estimación de Ndfa usando tejido de grano (Ndfa-G) se podría aplicar en programas de mejoramiento de frijol para mejorar la capacidad SNF. Usando este nuevo método de Ndfa-G, se identificaron cuatro líneas de frijol (RCB 593, SEA 15, BCN 226 y BFS 29) que combinan una mayor capacidad de fijar nitrógeno con mayor rendimiento de grano en condiciones de sequía y éstas podrían servir como padres potenciales para mejorar la capacidad SNF en frijol de común. Mejor habilidad para fijar nitrógeno bajo estrés por sequía fue relacionada con superior presencia de raíces gruesas. Mayor absorción de nitrógeno desde el

suelo fue asociado con un sistema de raíces fino y profundo. El índice de cosecha vaina, discriminación de isotopo de carbono y Ndfa usando tejido de grano podría ser criterios de selección útiles en los programas de mejoramiento para seleccionar frijol común con resistencia a la sequía.

## Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in the tropics of Latin America and East, Central and Southern Africa. This plant belongs to the family Fabaceae; it has two gene pools Mesoamerican and Andean based on their centers of origin from Central and South America, respectively (Gepts and Debouck, 1991). These gene pools differ in seed size and color, protein phaseolin, and in morphological and molecular characteristics (Blair et al., 2006). There are seven races in common bean distributed in the two gene pools; in the Andean gene pool are New Granada, Chile and Peru, and the Mesoamerican gene pool are Durango, Jalisco, Mesoamerica and Guatemala (Singh et al., 1991; Beebe et al., 2000). This crop is grown by small holder farmers in Latin America and East Africa, where it is often exposed to unfavorable conditions and minimum use of inputs (Beebe et al., 2008). It is an inexpensive source of protein and calories for small farmers in countries with endemic poverty (Rao, 2014).

The bean growing season is between 80-100 days in which the crop requires between 350-500 mm of water depending on the depth of soil, climate and genotype (Beebe et al., 2013). The bean crop cycle is distributed in 10 stages of development, including five for vegetative growth and five for reproductive development. Vegetative development are: germination (Vo), Emergency (V1), Primary leaves (V2) First trifoliolate leaf (V3) and Third trifoliolate leaf (V4); and reproductive development: Pre-flowering (R5), Flowering (R6), Pod formation (R7), Pod filling (R8) and maturity (R9).

Bean yields are affected by various biotic and abiotic factors; disease is the main constraint on bean production. Among abiotic limitations, drought could reduce yields between 10% and 100% (Polania et al., 2016). About 60% of the bean production regions are affected by drought, the second most important factor in yield reduction after diseases (Thung and Rao, 1999; Rao, 2014). The development of bean varieties adapted to drought stress conditions through breeding is a useful strategy to face new challenges of climate change and to ensure food security in marginal areas. Therefore,

the implementation tools to accelerate and increase efficiency of breeding programs, such as use of molecular markers and the expansion of the selection criteria by identifying morpho-physiological characteristics of the plant that are highly related to performance, would be helpful in generating the bean varieties that are adapted to drought conditions.

In addition to drought, smallholders are often affected by declining soil fertility due to their marginalized situation and their inability to overcome production constraints (Douxchamps et al., 2010). Nitrogen (N) is considered the most limiting nutrient for agricultural production. Legumes can derive much of their required N from the atmosphere through symbiotic nitrogen fixation (SNF); a complex physiological process that can be affected by drought stress. Moreover, drought has a negative influence on both the rhizobia and on the nodulation of legumes (Devi et al., 2013), and can cause the loss of this activity in common bean, and other legume species that generally have low rates of N fixation even under well-watered conditions (Devi et al., 2013). Identification of parental genotypes to use in breeding that combine superior SNF ability under drought stress with other desirable traits could be a useful strategy to confront the new challenges of climate variability and to ensure food security in marginal areas.



## Hypothesis

The main hypothesis to be tested is if the combination of different morpho-physiological traits and mechanisms improves the performance of bean genotypes under different types and intensities of drought stress. The traits to be considered include phenology, greater root length in lower soil strata, root system size, root hydraulic conductivity, leaf area development, carbon partitioning to different plant parts, storage of carbon and nitrogen reserves, stomatal control, water use efficiency, effective use of water, symbiotic nitrogen fixation, greater mobilization of photosynthates to seed (harvest index, pod partitioning index, pod harvest index), and nutrient use efficiency under water limited conditions. The key traits identified will contribute to expansion of selection criteria to be used by bean breeding programs to improve the adaptation of common bean to drought stress.

## Objectives

### *Main objectives:*

- To identify key morpho-physiological traits that are associated with improved drought adaptation in common bean and could be useful to expand selection criteria in bean breeding
- To determine the contribution of specific morpho-physiological traits in improving bean adaptation to water-constrained environments
- To expand the selection criteria in common beans and to identify genotypes with desirable traits, that combine drought tolerance and greater symbiotic nitrogen fixation and these genotypes could serve as parents in breeding programs

### *Specific objectives:*

- To identify specific morpho-physiological traits that contribute to improved resistance to drought and that could be useful as selection criteria in breeding beans for drought resistance
- To determine the relationship between seed yield and water use efficiency using measurements of stomatal conductance and carbon isotope discrimination
- To validate a the method to estimate SNF ability using  $^{15}\text{N}$  natural abundance in grain tissue compared with  $^{15}\text{N}$  natural abundance in shoot tissue
- To quantify genotypic differences in common bean for their response of N fixation to drought stress
- To identify a few best bet genotypes with desirable traits (which combine drought resistance with greater symbiotic nitrogen fixation ability) that could serve as parents in breeding programs

## Thesis outline

**The first chapter** of this thesis consists in the identification of traits associated with drought resistance. Include the relationship between grain yield and different parameters such as effective use of water (EUW), canopy biomass and remobilization of photosynthates to grain (pod partitioning index, harvest index and pod harvest index) in elite lines selected for drought resistance over the past decade. Resistance to terminal drought stress in Mesoamerican bean lines was associated with EUW combined with superior mobilization of photosynthates to pod and seed production.

**The second chapter** provides analysis of a new and easy method to estimate phenotypic variability in SNF ability using  $^{15}\text{N}$  natural abundance in grain tissue; and also to determine the relationship between grain yield and different parameters related with N derived from the atmosphere (%Ndfa) and N derived from the soil (%Ndfs). Resulting in the report a new method to estimate SNF ability and the quantification of phenotypic variation in Ndfa among bean lines under drought stress. Results from this study showed that it is possible to identify bean lines that combine greater SNF ability with greater mobilization of photosynthates to grain under drought stress.

**The third chapter** presents analysis of root traits related with resistance to drought and SNF ability and identification of superior genotypes with desirable root traits that could serve as parents in breeding programs. Results indicate that the drought resistant lines previously identified in the chapter 1, presented deeper and vigorous root system that allows greater access to water under drought stress conditions.

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## **Chapter 1. Identification of shoot traits related with resistance to terminal drought stress in common beans**

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## Chapter 1

### 1.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in the tropics of Latin America and eastern and southern Africa, where this crop is of great importance for improving food security. This grain legume is nutritionally rich in iron and protein, and is a source of fiber and carbohydrates, essential in the nutrition of the population especially in developing countries. Beans are cultivated by small farmers in Latin America and eastern and southern Africa, where unfavorable climate conditions and minimum use of inputs frequently limit productivity (Beebe, 2012; Beebe et al., 2013). The yield of beans is affected by various constraints. Among those drought is responsible for losses between 10 and 100%. About 60% of the bean-producing regions have prolonged periods of water shortage and drought is the second most important factor in yield reduction after diseases (Thung and Rao, 1999; Rao, 2014).

The development of bean varieties resistant to drought stress conditions through breeding is a useful strategy to ensure food security in marginal areas. Breeding programs for improving resistance to drought usually select the best genotypes based on grain yield under drought stress (Rosales et al., 2012). Understanding the physiological basis of yield limitations will contribute to developing physiological selection tools in support of plant breeding (Araus et al., 2002; Girdthai et al., 2009; Mir et al., 2012). A physiological approach can increase the possibility of combining parents with complementary traits, resulting in additive gene action for improving drought resistance, provided the germplasm is characterized more thoroughly than just testing for yield (Reynolds and Trethowan, 2007; Mir et al., 2012). A useful trait must exhibit enough genetic variability, correlation with yield, higher heritability, and its evaluation must be fast, easy and cheap (Jackson et al., 1996; Araus et al., 2002).

Three key processes, among others, have been related to improved drought resistance: (i) acquiring greater amount of water by the root system from the soil profile to facilitate transpiration, (ii) acquiring more carbon (biomass) in exchange for the water transpired by the crop, and (iii) increased mobilization of accumulated carbon to the harvestable economic product (Condon et al., 2004). Previous research identified several traits that contribute to improved resistance of common bean to drought and these include earliness, deep rooting and greater ability to mobilize photoassimilates to grain production (Hall, 2004; Beebe et al., 2013; Rao, 2014).

Water use efficiency (WUE), or "more crop per drop" is the ratio between grain yield and transpired water and it is considered as an important component of drought resistance in different crops (Blum, 2009; Sinclair, 2012; Vadez et al., 2014). It has been reported that traits related with conserving water at vegetative stage (lower leaf conductance, smaller leaf canopy), would make more water available for reproductive growth and grain filling, resulting in better grain yield under terminal drought stress conditions (Zaman-Allah et al., 2011; Araújo et al., 2015). Increased WUE reduces the rate of transpiration and crop water use, processes that are crucial for carbon assimilation, biomass production and yield (Blum, 2009; Sinclair, 2012). However, the reduction in water use is generally achieved by plant traits and environmental responses that could also reduce yield potential (Blum, 2005).

WUE is a complex trait and difficult to phenotype, preventing many breeding programs from using WUE directly (Araus et al., 2002; Easlon et al., 2014). Methodologies to estimate WUE include lysimeter studies, gas exchange measurements, or stable carbon isotope composition (Easlon et al., 2014). Two widely used plant attributes for improving drought resistance are stomatal conductance and canopy temperature depression (CTD), which are integrated measures of plant water status. Also CTD has been reported as a useful parameter to assess the access to water through the deep root system or the ability to regulate stomatal opening (Araus et al., 2002; Merlot et al., 2002; Balota et al., 2007; Mir et al., 2012).

In contrast to WUE, effective use of water (EUW) implies maximal soil moisture capture for transpiration, and also involves decreased non-stomatal transpiration and minimal water loss by soil evaporation (Blum, 2009). EUW is relevant when there is still soil water available at maturity or when deep-rooted genotypes access water deep in the soil profile that is not normally available (Araus et al., 2002). Two main ideotypes of plants have been proposed for targeting in plant breeding according to agro-ecological zones and types of drought: the isohydric ('water saving') plant model and the anisohydric ('water spending') plant model. The water saving model might have an advantage in the harshest environments, whereas the water spending model will perform relatively better under more moderate drought conditions (Blum, 2015).

Another plant attribute is carbon isotope discrimination (CID), which has been used to determine genotypic and environmental responses in WUE in various species of legumes, based on the inverse relationship between CID and WUE (greater  $^{13}\text{C}$  discrimination being associated with lower values of WUE, or conversely, more water use and transpiration)(Farquhar et al., 1989). Selection for low  $^{13}\text{C}$  discrimination has been proposed as a screening method to improve WUE in breeding  $\text{C}_3$  crops (Araus et al., 2002; Khan et al., 2007; Easlon et al., 2014). CID presented some advantages by reflecting integration over long periods of gas exchange during crop development, high throughput sampling, relatively low cost, and high heritability (Easlon et al., 2014). In bush bean under non-severe droughts or non-arid environments, it has been observed that there is a positive relationship between CID, root length density and grain yield. This indicates that plants under drought stress generate deeper roots, and therefore access more water, resulting in increased stomatal conductance and thus greater  $^{13}\text{C}$  discrimination (Sponchiado et al., 1989; White et al., 1990; White, 1993; Hall, 2004; Polania et al., 2012).

Increased water use is associated with increased accumulation of carbon and plant growth. However improved harvest index (HI) or enhanced mobilization of photosynthates to grain production plays an essential role in the success of superior genotypes under stress. The success of breeding in the last century has been due to



better partitioning of biomass to grain or increase in HI (Araus et al., 2002). For example in common bean the wild ancestors show lower values of HI than their domesticated counterparts (Beebe et al., 2014). In most environments with drought, water deficit occurs at the stage of reproductive development, affecting HI (Blum, 2009). Several studies in common bean have shown that increased photoassimilate mobilization to pod and seed formation contributes to better grain yield under drought and low soil fertility stress (Rao, 2014). Therefore assimilate partitioning is an important attribute to evaluate adaptation to abiotic stress in common bean (Rosales-Serna et al., 2004; Beebe et al., 2008, 2013; Klaedtke et al., 2012; Polania et al., 2012; Rosales et al., 2012; Assefa et al., 2013; Rao et al., 2013; Rao, 2014).

Two indices have been employed to quantify biomass partitioning: pod partitioning index (PPI) which indicates the extent of mobilization of assimilates from the vegetative structures to pod formation, and pod harvest index (PHI) which indicates mobilization of assimilates from the podwall to grain formation (Klaedtke et al., 2012; Assefa et al., 2013; Beebe et al., 2013; Rao et al., 2013). Several lines of bush bean have been identified as resistant to drought stress based on greater mobilization of photoassimilates to pods and seed. These include SER 118, SEN 56, NCB 226 and SER 125 (Beebe et al., 2014); RAB 650 and SEA 23 (Rao et al., 2013); Pinto Villa (Cuellar-Ortiz et al., 2008); Pinto Saltillo (Rosales et al., 2012); SER 16, SEA 5, and SER 5 (Beebe et al., 2013); NCB 226, SER 16, SEN 56 and SEA 15 (Polania et al., 2012).

The main objectives of this study were:

- (i) To identify specific morpho-physiological traits that contribute to improved resistance to drought in lines developed over several cycles of breeding and that could be useful as selection criteria in breeding beans for drought resistance
- (ii) To identify genotypes with desirable traits that could serve as parents in breeding programs that are aimed to improve drought resistance.

## 1.2 Materials and methods

### 1.2.1 Experimental site and meteorological conditions

Two field trials were conducted during the dry season (from June to September in each year of 2012 and 2013), at the main experiment station of the International Center for Tropical Agriculture (CIAT) in Palmira, Colombia, located at 3° 29" N latitude, 76° 21" W longitude and an altitude of 965 m (Fig. 1). Basic characteristics of this field site have been previously described (Beebe et al., 2008). The soil is a Mollisol (Aquic Hapludoll) with adequate nutrient supply and is estimated to permit storage of 100 mm of available water (assuming 1.0 m of effective root growth with -0.03 MPa and -1.5 MPa upper and lower limits for soil matric potential).

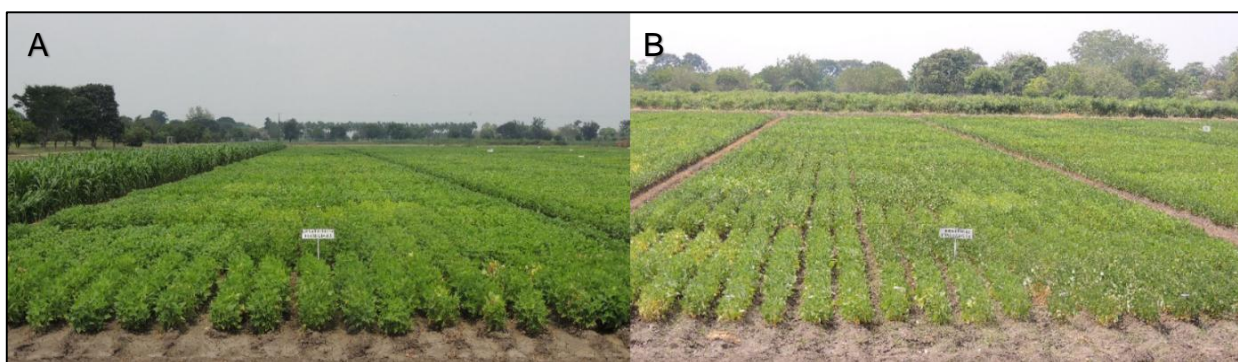


Figure 1. Phenotypic evaluation of 36 bean lines at CIAT Palmira, Colombia in 2013, under irrigated conditions (A) and drought stress conditions (B).

During the crop-growing season, maximum and minimum air temperatures in 2012 were 31.0 °C and 19.0 °C, and in 2013 were 30.2 °C and 19.2 °C, respectively. The incident solar radiation ranged from 8.8 to 24.4 MJ m<sup>-2</sup> d<sup>-1</sup> in 2012 and 8.4 to 24.5 MJ m<sup>-2</sup> d<sup>-1</sup> in 2013. Total rainfall during the active crop growth was 85.8 mm in 2012 and 87.7 mm in 2013. The potential pan evaporation was of 385.2 mm in 2012 and 351.0 mm in 2013. Two levels of water supply (irrigated and rainfed) were applied to simulate well watered (control) and drought stress treatments (Fig. 1). Trials were furrow irrigated (approximately 35 mm of water per irrigation). The drought stress treatment under rainfed conditions in 2012 received irrigations at 3 days before planting and at 5

and 23 days after planting. In 2013, irrigation was provided at 3 days before planting and at 4 and 15 days after planting. In both years, irrigation was suspended after the third irrigation to induce terminal drought stress (less water availability from flowering to physiological maturity) conditions. The irrigated control treatment received 5 irrigations in 2012 and 6 irrigations in 2013 to ensure adequate soil moisture for crop growth and development.

### 1.2.2 Plant material

For this study 36 bush bean genotypes belonging to the Middle American gene pool were selected: twenty two elite lines of common bean (BFS 10, BFS 29, BFS 32, BFS 67, MIB 778, NCB 226, NCB 280, RCB 273, RCB 593, SCR 16, SCR 2, SCR 9, SEN 56, SER 118, SER 119, SER 125, SER 16, SER 48, SER 78, SMC 141, SMC 43 and SXB 412); five interspecific lines between elite line SER 16 and *Phaseolus coccineus* (ALB 6, ALB 60, ALB 74, ALB 88 and ALB 213); one landrace of tepary bean (*Phaseolus acutifolius*) G40001 from Veracruz-Mexico, and two interspecific lines between tepary bean and common bean (INB 841 and INB 827 developed from five cycles of congruity backcrossing of tepary with ICA Pijao). SEA 15 and BAT 477 were included as drought resistant checks, and three commercial cultivars of common bean (DOR 390, Pérola and Tio Canela) as drought sensitive materials. BAT 477 NN was included as a non-nodulating bean genotype. Details of seed color and size, and growth habit are reported in the table 1.

Table 1. Characteristics of common bean genotypes used in the field studies

Genotype	Growth habit	Seed color	Seed size	Type of germplasm
ALB 6	II A	Red	M	<i>P. vulgaris</i> x <i>P. coccineus</i> interspecific line
ALB 60	II B	Red	S	<i>P. vulgaris</i> x <i>P. coccineus</i> interspecific line
ALB 74	II B	Red	M	<i>P. vulgaris</i> x <i>P. coccineus</i> interspecific line
ALB 88	II B	Red	M	<i>P. vulgaris</i> x <i>P. coccineus</i> interspecific line
ALB 213	II B	Red	S	<i>P. vulgaris</i> x <i>P. coccineus</i> interspecific line
BAT 477	III B	Cream	M	Drought resistant check
BAT 477_NN	III B	Cream	M	Non-nodulating bean genotype

Genotype	Growth habit	Seed color	Seed size	Type of germplasm
BFS 10	II A	Red	S	Inbred line
BFS 29	II A	Red	S	Inbred line
BFS 32	II A	Red	S	Inbred line
BFS 67	II B	Red	M	Inbred line
DOR 390	II A	Black	S	Commercial cultivar
G 40001	II	White	S	<i>Phaseolus acutifolius</i> line
INB 827	II B	Brown	M	<i>P. vulgaris</i> x <i>P. acutifolius</i> interspecific line
INB 841	II A	Brown	S	<i>P. vulgaris</i> x <i>P. acutifolius</i> interspecific line
MIB 778	II B	Brown	M	Inbred line
NCB 226	II B	Black	M	Inbred line
NCB 280	II A	Black	M	Inbred line
Pérola	III	Cream	M	Commercial cultivar
RCB 273	II B	Red	M	Inbred line
RCB 593	II B	Red	M	Inbred line
SCR 2	II B	Red	S	Inbred line
SCR 9	II A	Red	M	Inbred line
SCR 16	II B	Red	S	Inbred line
SEA 15	III	Purple	M	Drought resistant check
SEN 56	II A	Black	M	Inbred line
SER 16	II A	Red	S	Inbred line
SER 48	II B	Red	M	Inbred line
SER 78	II A	Red	S	Inbred line
SER 118	II A	Red	M	Inbred line
SER 119	II A	Red	S	Inbred line
SER 125	II B	Red	M	Inbred line
SMC 43	II B	Cream	S	Inbred line
SMC 141	II B	Purple	M	Inbred line
SXB 412	III B	Cream	M	Inbred line
Tío Canela 75	II A	Red	S	Commercial cultivar

M: Medium, between 25 to 40 g/100 seeds; S: small, maximum 25 g/100 seeds

### 1.2.3 Experimental design

In the two years, a 6 x 6 partially balanced lattice design with 3 replications was used. Details on planting and management of the trial were similar to those reported before (Beebe et al., 2008). Experimental units consisted of 4 rows with 3.72 m row length with a row-to-row distance of 0.6 m and plant-to-plant spacing of 7 cm (equivalent to 24 plants m<sup>-2</sup>). Trials were managed by controlling weeds with application of herbicides (Fomesafen, Fluazifop-p-butyl and Bentazon) and pests and diseases by spraying with

insecticides (Thiametoxam, Clorpirifos, Imidacloprid, Abamectina, Cyromazine and Milbemectin) and fungicides (Benomil and Carboxin) as needed.

#### *1.2.4 Yield measurements and phenological assessment*

Grain was harvested from two central rows after discarding end plants in both the irrigated and drought plots. Mean yields per hectare were corrected for 0% moisture in grain. Days to flowering (DF) and days to physiological maturity (DPM) were determined for each plot. DF is defined as the number of days after planting until 50% of the plants have at least one open flower. DPM is the number of days after planting until 50% of plants have at least one pod losing its green pigmentation.

#### *1.2.5 Shoot traits measurements*

No destructive shoot traits were determined at mid-pod filling stage; such as the stomatal conductance to water vapor was measured with a portable leaf porometer (Decagon SC-1) on a fully expanded young leaf of three different plants within each replication. Measurements were made late in the morning (10 am–12 noon) on clear, sunny day with minimal wind, on one replication per day. Leaf chlorophyll content of fully expanded leaves was measured using a nondestructive, hand-held chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co., Ltd., Japan) and is expressed as SPAD chlorophyll meter reading (SCMR). Also, at mid-pod filling, a 50 cm segment of the row (equivalent to an area of 0.3 m<sup>2</sup>) from each plot with about 7 plants was used for destructive sampling to measure leaf area index (LAI), canopy biomass (CB) and dry matter distribution between leaves, stems and pods. Leaf area was measured using a leaf area meter (model LI-3000, LI-COR, NE, USA) and the leaf area index (LAI) was calculated.

At the time of harvest, plants in 50 cm of a row from each plot were cut and dry weights of stem, pod, seed, and pod wall, seed number per area (SNA), and pod number per area (PNA) were recorded. The following attributes were determined according to (Beebe et al., 2013):

- Harvest index (HI) (%): seed biomass dry weight at harvest/total shoot biomass dry weight at mid-pod filling x 100
- Pod harvest index (PHI) (%): seed biomass dry weight at harvest/pod and seed biomass dry weight at harvest x 100
- Pod partitioning index (PPI) (%): pod and seed biomass dry weight at harvest/total shoot biomass dry weight at mid-pod filling x 100.
- Stem biomass reduction (SBR) (%): (stem biomass dry weight at mid-pod filling – stem biomass dry weight at harvest)/stem biomass dry weight at mid-pod filling x 100.

*Note:* HI and PPI were estimated using the canopy biomass value at mid-pod filling growth stage which is assumed to be the time that reflects the maximum vigor of the genotype; from this time common bean begins to lose canopy biomass through leaf fall, especially under drought stress.

One plant of each genotype from each plot (irrigated and drought) was selected for destructive sampling at mid-pod filling. The plant was cut at the soil surface, washed with deionized water and dried in the oven at 60°C for two days. A random sample of grain per experimental unit was selected, washed thoroughly and ground. The ground samples of plants at mid-pod filling and grain at harvest were sent to UC Davis Stable Isotope Facility in USA for  $^{13}\text{C}$  analysis. CID ( $\Delta^{13}\text{C}$  in ‰) was calculated according to the following equation, where  $\delta^{13}\text{C}_s$  and  $\delta^{13}\text{C}_a$  are sample and atmospheric concentrations of  $^{13}\text{C}$ , respectively, and carbon isotope composition of atmosphere is assumed to be  $-8.0\text{‰}$  (Farquhar et al., 1989). Isotopic discrimination between  $^{13}\text{C}$  and  $^{12}\text{C}$  ( $\Delta$ ) in shoot and grain was related to whole plant water use efficiency (WUE). Based

on these theoretical considerations, genotypes with lower values of CID should have higher WUE under field conditions (Farquhar et al., 1989).

$$\Delta 13C (CID) = \frac{[\delta^{13}Ca - \delta^{13}Cs]}{[1 + (\delta^{13}Cs/1000)]}$$

#### 1.2.6 *Statistical analysis*

All data were analyzed using the SAS (v 9.0) PROC MIXED and PROC CORR (SAS Institute Inc., 2008). The adjusted means for each genotype and the environment (irrigated and drought) were obtained using the mixed models theory together with the MIXED procedure considering the effects of the replications and blocks within replications as random and genotypes as fixed. Correlation coefficients were calculated by the PROC CORR. Values reported with \*, \*\* or \*\*\* are statistically significant at probability levels of 5%, 1% and 0.1%, respectively.

### 1.3 Results

The data on rainfall distribution, irrigation application, and pan evaporation in both trials indicated that the crop suffered terminal drought stress during crop development under rainfed conditions (Fig. 2). The drought trial received 190.8 mm of water (rainfall and irrigation) versus pan evaporation of 385.2 mm in 2012; while in 2013, the drought trial received 192.7 mm of water as rainfall and irrigation, compared with 351.0 mm of pan evaporation.

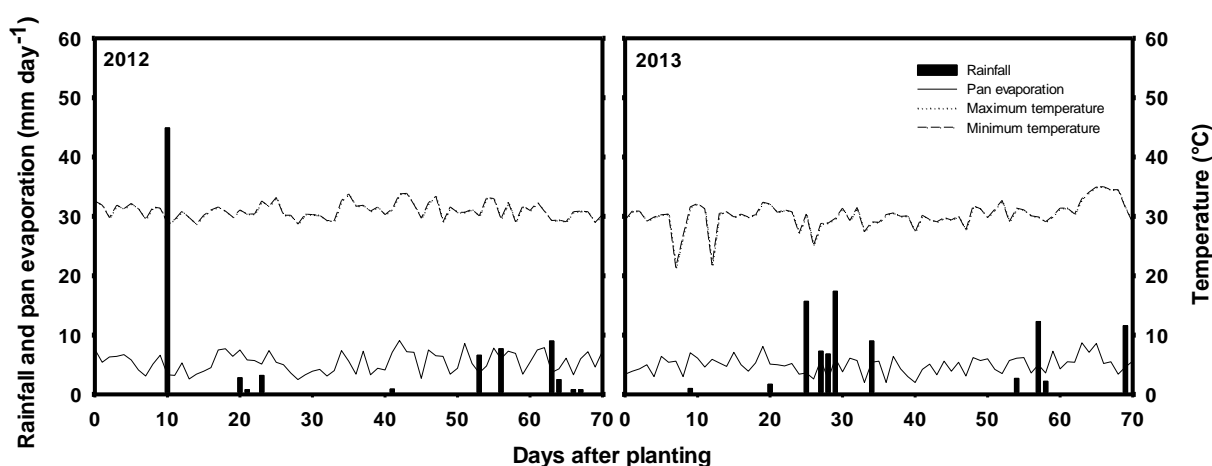


Figure 2. Rainfall distribution, pan evaporation, maximum and minimum temperatures during crop growing period at Palmira, Colombia in 2012 and 2013.

#### 1.3.1 Grain yield

The mean value of grain yield (GY) under drought conditions decreased by 56% compared with irrigated conditions (Fig. 3). Under drought stress the grain yield of 36 genotypes ranged from 59 to 1526 kg ha<sup>-1</sup> (Fig. 3). Among the genotypes tested, the lines BFS 29, NCB 280, SEN 56, BFS 10, SEA 15 and NCB 226 were outstanding in their adaptation to drought conditions. The relationship between grain yield of drought and irrigated treatments indicated that BFS 29, NCB 280, SEN 56, BFS 10 and NCB 226 were not only drought resistant but were also responsive to irrigation (Fig. 3). Among the 36 genotypes tested, the biofortified line MIB 778, was the most sensitive to drought. MIB 778 is an interspecific progeny of common bean and *P. dumosus*,



which may explain its extreme sensitivity to drought. The genotypes Pérola, DOR 390, SMC 43 and ALB 88 were also sensitive to drought stress conditions (Fig. 3).

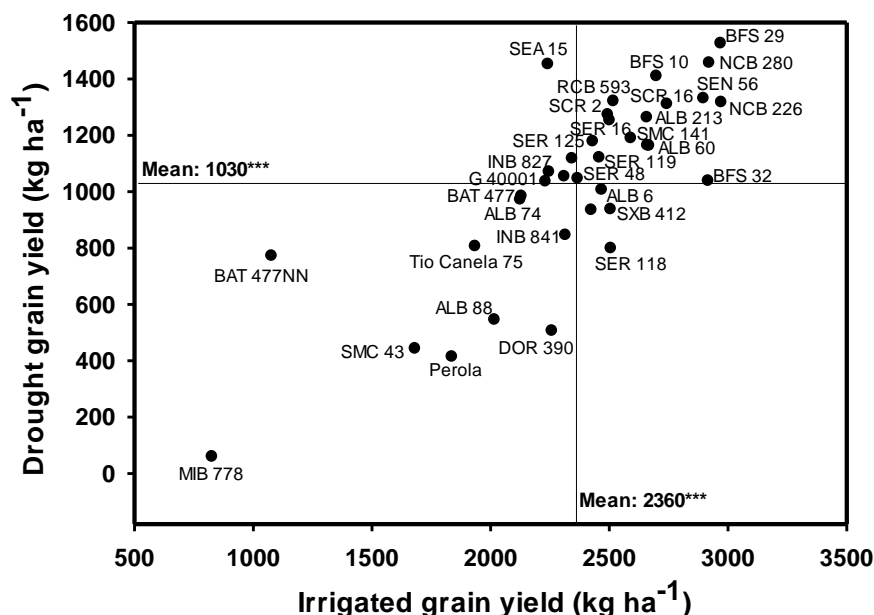


Figure 3. Identification of genotypes that are adapted to drought conditions and are responsive to irrigation on a Mollisol at Palmira. Genotypes that yielded superior with drought and were also responsive to irrigation were identified in the upper, right hand quadrant

### 1.3.2 Phenological assessment: days to flowering (DF) and days to physiological maturity (DPM)

A negative and significant correlation was observed between DF and grain yield under both irrigated and drought conditions,  $-0.51^{***}$  and  $-0.53^{***}$ , respectively (Table 2). Under irrigated conditions the DF of 36 genotypes ranged from 30 to 39 days with a mean of 34 days (Table 3); under drought stress the DF ranged from 30 to 40 with a mean of 34 days (Table 3). The lines SEA 15, INB 841, BFS 29, NCB 280, G 40001, BFS 32, SER 16, SER 125, SCR 2, ALB 60, SEN 56, RCB 273, NCB 226, SCR 9, RCB 593, ALB 74, SER 119, ALB 213, SER 48 and BFS 10 showed the shorter and similar days to flowering under both irrigated and drought conditions (Table 3). The lines BFS

29, NCB 280, SEA 15, BFS 10, SEN 56, RCB 593, NCB 226, SCR 2, ALB 213, SER 16 and SER 125 combined shorter DF with higher grain yield under drought stress conditions (Table 3). The susceptible checks Tío Canela, Pérola and DOR 390 and the lines MIB 778 and SMC 43 showed sensitivity to drought conditions with large DF under drought conditions (Table 3).

Table 2. Correlation coefficients (r) between final grain yield ( $\text{kg ha}^{-1}$ ) and other shoot attributes of 36 genotypes of common bean grown under irrigated and drought conditions in a Mollisol in Palmira.

Plant traits	Irrigated	Drought
Leaf stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	0.24***	0.31***
SPAD chlorophyll meter reading	0.29***	0.01
Leaf area index ( $\text{m}^2/\text{m}^2$ )	0.12	0.43***
Canopy biomass ( $\text{kg ha}^{-1}$ )	0.39***	0.59***
Pod partitioning index (%)	0.14*	0.29***
Harvest index (%)	0.24***	0.39***
Pod harvest index (%)	0.61***	0.48***
Stem biomass reduction (%)	-0.18**	0.14*
Days to flowering	-0.51***	-0.53***
Days to physiological maturity	-0.37***	-0.36***
Pod number per area ( $\text{no. m}^{-2}$ )	0.32***	0.55***
Seed number per area ( $\text{no. m}^{-2}$ )	0.36***	0.63***
100 seed weight (g)	0.44***	0.25***
Shoot CID (‰)	-0.12	0.15*
Grain CID (‰)	0.37***	0.36***

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

The DPM showed a negative and highly significant correlation with grain yield under both irrigated and drought treatments (Table 2). Under irrigated conditions the DPM of 36 genotypes ranged from 55 to 68 days with a mean of 60 days (Table 3); under drought stress the DPM ranged from 55 to 67 with a mean of 60 days (Table 3). The lines BFS 29, NCB 280, SEA 15, BFS 10, SEN 56 and RCB 593 showed shorter DPM with superior grain yield than the other genotypes under drought stress conditions (Table 3). A negative and significant correlation was observed between DF and canopy biomass ( $r=-0.18^{**}$  and  $r=-0.35^{***}$ ) and DPM and canopy biomass ( $r=-0.13^*$  and  $r=-0.20^{**}$ ) under irrigated and drought conditions, respectively; also between DF and SNA ( $r=-0.26^{***}$  and  $r=-0.31^{***}$ ) and DPM and SNA ( $r=-0.27^{***}$  and  $r=-0.22^{***}$ ) under irrigated and drought conditions, respectively. The lines BFS 29, SER 125, SER 16,

SER 119, SER 48, SEA 15, SER 78, RCB 593, NCB 280 and SEN 56 showed reduced number of DPM with higher values of canopy biomass and grain yield under drought stress (Table 3, Fig. 3). Widely differences in grain yield under drought stress was observed in lines with same phenology; e.g., the commercial check and susceptible to drought line DOR 390, vs. the resistant to drought lines NCB 226, SCR 2. ALB 213 and SMC 141 (Table 3, Fig. 3), and the line SMC 43 with low yielding under drought stress vs. the lines BFS 10, SEN 56 and RCB 593 with high yielding under drought stress (Table 3, Fig. 3).

Table 3. Phenotypic differences in leaf stomatal conductance, SPAD chlorophyll meter reading, days to flowering and days to physiological maturity of 36 genotypes of common bean grown under irrigated and drought conditions in 2012 and 2013 at Palmira, Colombia. Values reported are mean for two seasons.

Genotype	Leaf stomatal conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )		SPAD chlorophyll meter reading		Days to flowering		Days to physiological maturity	
	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
ALB 6	351	317	34.4	45.3	34	34	91	68
ALB 60	309	342	41.5	45.3	32	32	76	44
ALB 74	354	398	35.7	41.9	33	33	80	55
ALB 88	375	441	40.8	43.9	34	35	65	53
ALB 213	361	366	37.3	43.6	32	33	72	57
BAT 477	265	357	36.5	43.4	36	37	71	61
BAT 477 NN	373	508	33.2	37.6	37	38	99	44
BFS 10	382	408	40.7	43.4	32	33	84	66
BFS 29	410	320	41.5	45.4	31	31	87	65
BFS 32	282	399	41.9	42.3	31	32	99	67
BFS 67	311	485	37.5	43.1	35	36	74	62
DOR 390	378	346	40.4	46.4	38	39	64	32
G 40001	288	345	34.8	40.2	32	32	71	52
INB 827	384	393	36.8	44.6	35	36	64	63
INB 841	305	258	39.9	40.9	31	30	112	61
MIB 778	351	277	40.8	42.7	39	40	60	15
NCB 226	331	442	42.0	44.4	31	33	101	69
NCB 280	417	629	39.5	42.0	30	31	87	74
Pérola	360	268	39.9	47.7	39	40	87	49
RCB 273	324	321	42.6	45.9	32	33	90	57
RCB 593	357	328	41.7	44.9	31	33	74	52
SCR 2	436	395	40.7	44.2	32	32	76	66
SCR 9	447	331	40.4	45.4	32	33	90	57
SCR 16	372	506	37.3	46.5	33	34	86	74
SEA 15	271	429	37.4	42.8	32	30	92	70
SEN 56	335	492	43.4	43.7	32	32	80	74

Genotype	Leaf stomatal conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )		SPAD chlorophyll meter reading		Days to flowering		Days to physiological maturity	
	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
SER 16	383	310	41.8	42.6	31	32	84	43
SER 48	348	344	42.9	46.5	32	33	81	57
SER 78	300	363	41.8	45.6	34	35	61	73
SER 118	404	331	42.1	40.5	34	36	70	94
SER 119	364	361	38.5	45.1	33	33	80	55
SER 125	360	377	38.8	41.8	32	32	66	59
SMC 43	337	246	35.3	42.1	35	36	57	47
SMC 141	327	510	33.2	43.2	37	38	82	87
SXB 412	350	234	38.5	44.0	36	37	89	65
Tio Canela 75	315	348	40.4	44.0	37	38	70	78
<b>Mean</b>	<b>350</b>	<b>376</b>	<b>39.2</b>	<b>43.7</b>	<b>34</b>	<b>34</b>	<b>80</b>	<b>60</b>
<b>Sig. diff. P&lt;0.05</b>	<b>NS</b>	*	*	*	*	*	<b>NS</b>	*

### 1.3.3 Leaf stomatal conductance, SCMR and carbon isotope discrimination

Leaf stomatal conductance presented a significant positive correlation with grain yield under both irrigated conditions (0.24\*\*), and under drought stress (0.31\*\*\*) (Table 2). The lines NCB 280, SEN 56, SCR 16, SMC 141, NCB 226, SEA 15 and BFS 10, combined higher leaf stomatal conductance with better grain yield under drought conditions (Table 3, Fig. 4); the lines MIB 778, Pérola, SMC 43 and DOR 390 presented lower values of stomatal conductance combined with lower grain yield under drought conditions (Table 3, Fig. 4). A significant and positive correlation was observed between SPAD chlorophyll meter reading and grain yield under irrigated conditions (Table 2), but no correlation under drought stress (Table 2). The values of SCMR increase under drought stress, from an average of 39.2 SPAD units under irrigated conditions to an average of 43.7 SPAD units under drought stress (Table 3).

Shoot and grain CID showed a positive and significant correlation with grain yield under drought conditions 0.15\* and 0.36\*\*\*, respectively; the magnitude of the correlation between CID and grain yield is stronger when grain instead of shoot CID is used (Table 2). A positive and significant correlation was observed between grain CID and grain yield under irrigated (0.37\*\*\*) and drought (0.36\*\*\*) conditions (Table 2). The genotypes NCB 226, NCB 280, BFS 67, SEN 56, SCR 16 and SEA 15 combined higher grain yield with higher values of grain CID under drought stress, while MIB 778, SMC

43 and DOR 390 showed lower values of grain CID with lower grain yield under drought conditions (Fig. 4). It is noteworthy that the genotypes SCR 2, SEA 15, NCB 280, NCB 226 and SEN 56 also combined higher growth or canopy biomass with higher values of grain yield under drought conditions (Fig. 5). The accession of *Phaseolus acutifolius* G 40001 and the lines SER 16, ALB 6 and ALB 60, presented lower values of leaf stomatal conductance and grain CID (lower use of water) combined with moderate plant growth and grain yield under drought conditions (Table 3, Figs. 3, 4 and 5). The genotype ALB 88 stood out for its higher value of grain CID, but a low canopy biomass and grain yield under drought conditions compared to other genotypes (Figs. 4, 5).

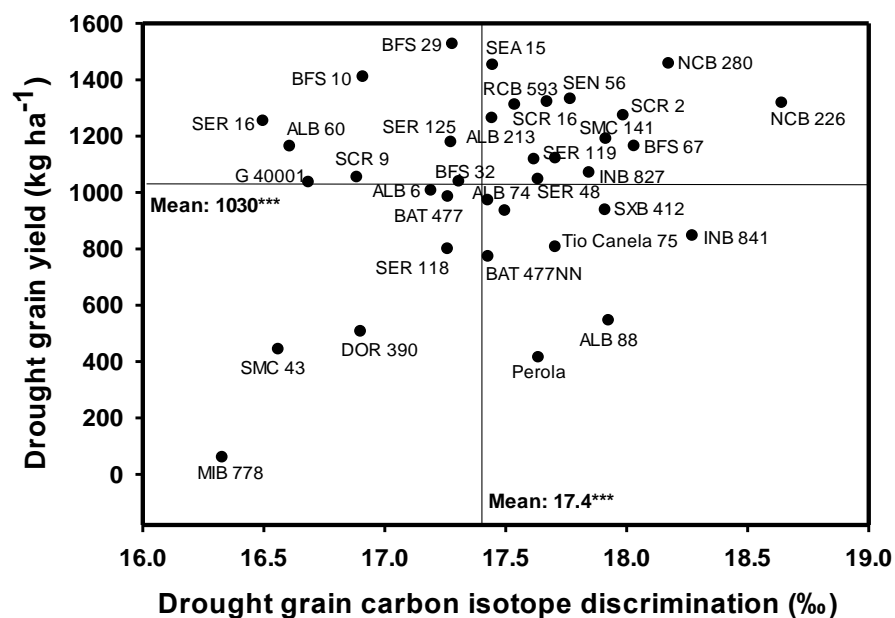


Figure 4. Identification of genotypes with greater values of grain yield and grain carbon isotope discrimination (CID) under drought conditions on a Mollisol at Palmira. Higher yielding genotypes with greater values of CID were identified in the upper, right hand quadrant.

#### 1.3.4 Canopy biomass, partitioning indices and yield components

A positive and significant correlation was observed between canopy biomass and grain yield under both irrigated and drought conditions,  $0.39^{***}$  and  $0.59^{***}$ , respectively (Table 2). The lines BFS 29, SEA 15, SCR 2, SER 16, RCB 593 and NCB 280 combined higher canopy biomass production with higher grain yield under drought stress conditions (Fig. 5), while BFS 10, SCR 16, ALB 213, ALB 60 and SMC 141 were outstanding in their grain production but with moderate plant growth (Fig. 5). The susceptible checks Pérola and DOR 390 and the lines MIB 778, SMC 43 and ALB 88 showed sensitivity to drought with lower values of both canopy biomass and grain yield under drought conditions (Fig. 5).

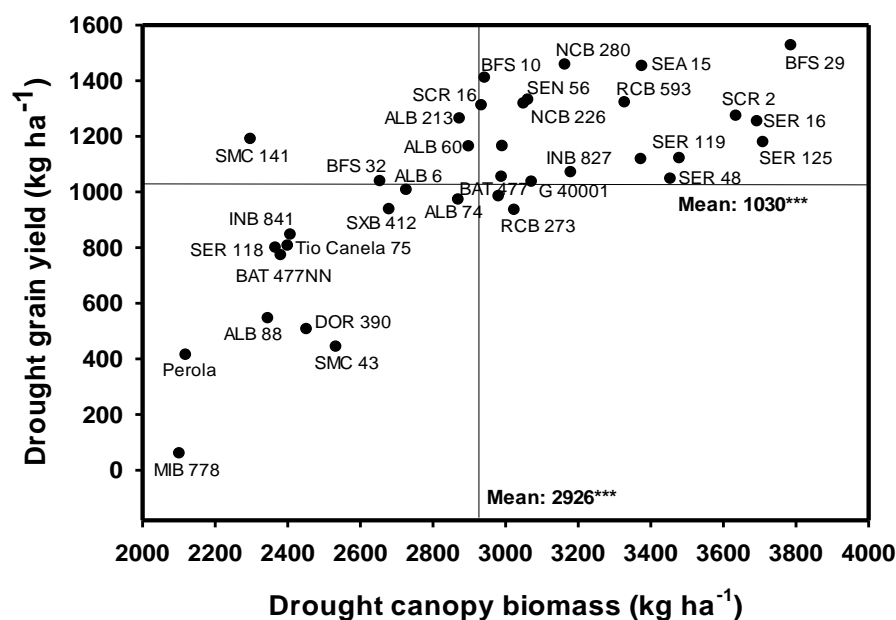


Figure 5. Identification of genotypes with greater values of grain yield and canopy biomass under drought conditions on a Mollisol at Palmira. Higher yielding genotypes with greater values of canopy biomass were identified in the upper, right hand quadrant

The pod partitioning index (PPI) reflects the biomass partitioned to pods at harvest as a proportion of the total canopy biomass at mid-pod filling growth stage. This ratio and harvest index (HI) may be overestimated because we used the canopy biomass values at mid-pod filling growth stage with the assumption that this growth stage reflects the

maximum vigor. The values of canopy biomass may be underestimated particularly under irrigated and intermittent drought conditions, because of possible additional vegetative growth after mid-pod filling to physiological maturity due to irrigation or rainfall. Correlation coefficients between grain yield and PPI were positive and highly significant under drought conditions (Table 2). The lines BFS 29, NCB 280, SEA 15, SEN 56, NCB 226, BFS 10, SCR 16 and SCR 2 combined higher value of PPI and grain yield under drought stress conditions (Fig. 6). The lines SMC 141 and SER 118 were outstanding in mobilizing photosynthates to pod formation, but the canopy biomass values of these lines were lower under drought stress (Figs. 5, 6). The genotypes ALB 88, SMC 43, Pérola and DOR 390 showed lower ability in mobilizing photosynthates to pod production under drought conditions (Fig. 6). The line MIB 778 showed very low PPI values (less than 20%) that resulted in low grain yield and sensitivity to drought stress (Fig. 6).

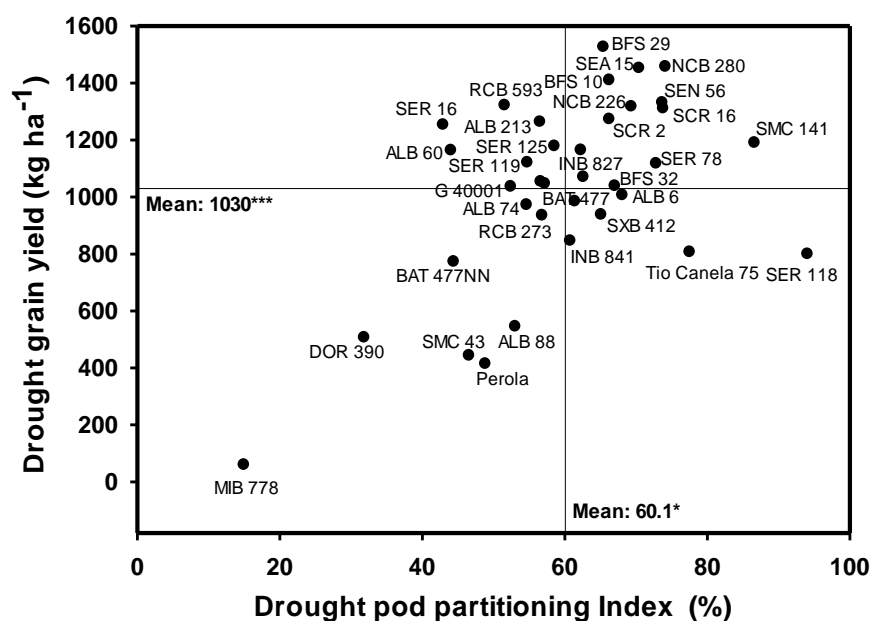


Figure 6. Identification of genotypes with greater values of grain yield and pod partitioning index (PPI) under drought conditions on a Mollisol at Palmira. Higher yielding genotypes with greater values of PPI were identified in the upper, right hand quadrant

The pod harvest index (PHI) value reflects the ability to mobilize photosynthates from pod wall to seed. A positive and highly significant correlation of PHI with grain yield under both irrigated and drought conditions were observed (Table 2). The lines BFS 29, NCB 280, SEA 15, SCR 16, SEN 56 and SER 16 were superior in PHI, resulting in a higher grain yield under drought conditions (Fig. 7). The accession of *Phaseolus acutifolius* (G 40001) and the lines INB 841 and SER 118 likewise presented higher values of PHI under drought conditions. The lines ALB 88, DOR 390, Pérola and SMC 43 combined low values of PHI with low grain yield under drought stress (Fig. 7). The biofortified line MIB 778 showed the lowest ability to mobilize photosynthates from plant structures to pod production (PPI) and from podwall to seed production (PHI) resulting in poor performance under drought stress conditions (Figs. 6, 7). A positive and significant correlation between grain yield and stem biomass reduction (SBR) was observed under drought stress (Table 2). Higher values of SBR are considered to reflect greater ability to mobilize photosynthates from stems to developing grains.

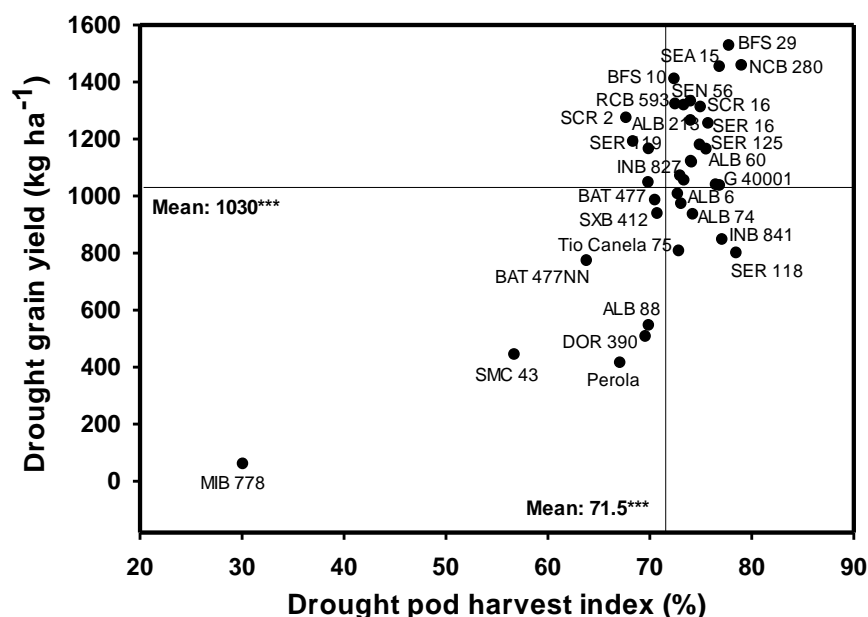


Figure 7. Identification of genotypes with greater values of grain yield and pod harvest index (PHI) under drought conditions on a Mollisol at Palmira. Higher yielding genotypes with greater values of PHI were identified in the upper, right hand quadrant.



The pod number per area (PNA), seed number per area (SNA) and 100 seed weight showed a positive and highly significant correlation with grain yield under both irrigated and drought treatments (Table 2). The lines BFS 29, BFS 10, NCB 280, SEN 56, SCR 16, SCR 2 and NCB 226 showed higher values of PNA and SNA than the other genotypes under drought stress conditions (Fig. 8). The line SEA 15 showed the highest value of 100 seed weight under drought stress followed by NCB 226, SCR 2, SCR 16, NCB 280 and BFS 10. The genotypes ALB 88, DOR 390, SMC 43, Pérola and MIB 778 produced less pods and seeds under drought stress.

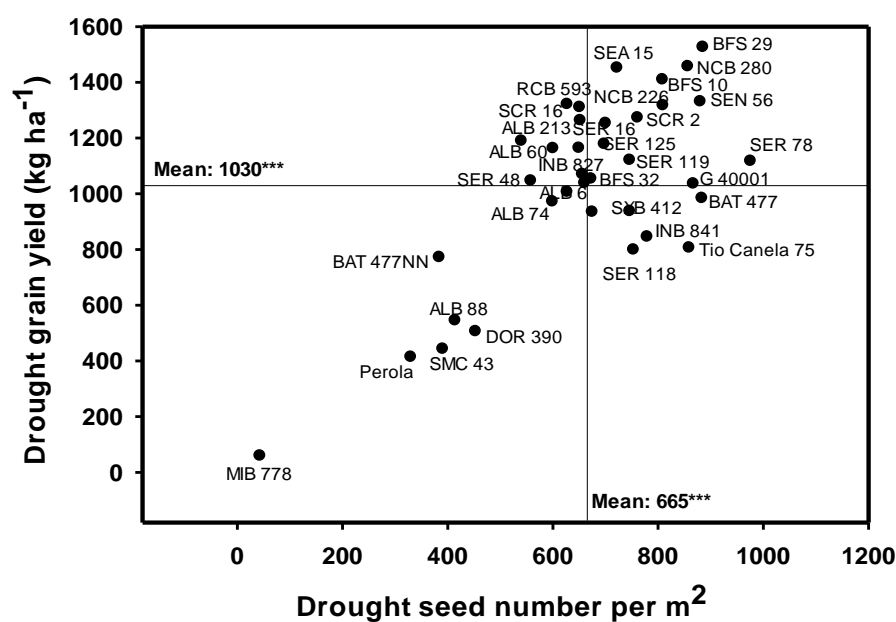


Figure 8. Identification of genotypes with greater values of grain yield and seed number per area under drought conditions on a Mollisol at Palmira. Higher yielding genotypes with greater values of SNA were identified in the upper, right hand quadrant.

## 1.4 Discussion

This study permitted evaluating a diverse set of elite common bean breeding lines recently developed for improving resistance to drought in the light of past experience with shoot traits such as CID, stomatal conductance, canopy biomass, and parameters of photosynthate remobilization of PPI, PHI and HI. The lines were derived from crosses among bean races (Beebe et al., 2008), as well as interspecific crosses with introgression from *P. coccineus* (Butare et al., 2012) or *P. acutifolius* (Beebe, 2012). The lines NCB 280, NCB 226, SEN 56, SCR 2, SCR 16, SMC 141, RCB 593, BFS 67, SER 16, ALB 60, ALB 6, BFS 10, and BFS 29 developed over several cycles of breeding to drought limitation were remarkably drought resistant, they showed values of GY that doubles the values under drought stress of three leading commercial cultivars in Latin America: DOR 390, Perola, and Tío Canela.

### 1.4.1 Grain yield and phenology

A significant negative relationship between grain yield and DF, and grain yield and DPM under both irrigated and drought stress conditions was observed. Early maturing genotypes were more adapted to drought stress and responsive to irrigation. Farmers have multiple reasons for preferring short season varieties, one of them is to minimize exposure to drought (White and Singh, 1991; Beebe, 2012). Early maturity has been a standard mechanism to confront drought in breeding programs, and it is a trait that may be more useful where terminal drought predominates (Beebe et al., 2014). However, previous results indicated that a shorter growth cycle can reduce grain yield potential per day by an estimated 74 kg ha<sup>-1</sup> (White and Singh, 1991). Contrastingly, recent results show that early maturing genotypes with superior photosynthate remobilization can compensate this effect, indicating that high yielding lines had higher grain yield per day compared with low yielding genotypes under drought (Klaedtke et al., 2012; Rao et al., 2013; Beebe et al., 2014). We also observed a negative and significant

correlation between phenology (DF and DPM) and canopy biomass and phenology and SNA under both irrigated and drought stress conditions.

#### *1.4.2 SPAD chlorophyll meter readings, stomatal conductance and CID*

A widely used trait in plant physiology is SPAD chlorophyll meter readings (SCMR), but this trait did not show relation to grain yield under drought stress. However, drought stress increased SPAD chlorophyll content in the leaves. This increase is because under drought stress conditions leaf expansion is reduced and showing a higher chlorophyll concentration per area; which can be checked with the leaf area index goes from an average of  $3.3 \text{ m}^2 \text{ m}^{-2}$  under irrigation to  $2.3 \text{ m}^2 \text{ m}^{-2}$  under drought stress. Rosales-Serna et al. 2004 found a similar response in bean genotypes evaluated under water stress; an increase of SCMR values under drought conditions in contrast to irrigated conditions, this increase was associated with the reduction of individual leaf area. The researchers conclude in the case of common bean SCMR allow discrimination between genotypes for chlorophyll content, but its use in selection for drought resistance is uncertain.

The relationship between different tissues sampled for CID and grain yield, showed that using grain is more relevant than canopy biomass at mid-pod filling in the determination of CID under drought conditions in common bean. One of the advantages of measuring CID is the integration over long periods of gas exchange and its interaction during crop development (Easlon et al., 2014). The use of grain sample for CID determination makes more sense in the case of common bean under terminal drought stress, because it is taken at maturity time, and would have more integrated effect of gas exchange during a critical and important crop growth stage which is grain filling. In our experimental conditions at this growth stage the effect of terminal drought is stronger than at mid-pod filling. A relationship was observed between stomatal conductance and grain CID under drought conditions, with differences in performance among some genotypes. Stomatal conductance and CID are useful measurements to

estimate differences in WUE, but depend on different processes. CID depends on variation in photosynthetic biochemistry, conductance of CO<sub>2</sub> to the leaf interior and the chloroplasts, or a combination of these (Seibt et al., 2008; Easlon et al., 2014).

After evaluating 36 advanced lines over two seasons, several drought resistant genotypes showed a clear evidence of superior access to soil moisture, and a few drought resistant genotypes showed a contrasting pattern using less water; these results allowed us to classify the genotypes into two groups: water savers (isohydric plants) and water spenders (anisohydric) to facilitate targeting according to agro ecological zones (Blum, 2015). These include NCB 280, SMC 141, SCR 16, SEN 56, BFS 67, NCB 226 and SEA 15, which, we classify as water spenders that are able to access more water and show better water status during their growth and grain development, resulting in higher grain yield. Our results indicate that the lines that maximize their capture of soil water for transpiration, maintain adequate gas exchange rates thus contributing to improved growth and greater proportion of biomass partitioned to grain through effective use of water (EUW) as proposed by Blum 2009. These genotypes should be useful for cultivation in areas exposed to intermittent drought in Central America, South America, and Africa, particularly in regions where rainfall is intermittent during the season and soils that store greater amount of available water deep in the soil profile.

Previous research showed that bean genotypes derived from Durango race such as SEA 5 and SEA 15, have mechanisms that can maintain a competitive level of water balance, allowing more effective use of water (EUW) under drought stress. This possibly is due to an efficient stomatal regulation, allowing these genotypes to promote grain formation and filling during stress (Rosales et al., 2012). Results with this set of bred lines under drought stress in a mollisol, indicate that EUW was more relevant for improved drought resistance than WUE.

We observed major differences among bean lines in water use under drought based on CID and stomatal conductance measurements. Based on the relationship between grain yield and grain CID under drought conditions (Fig. 4), most of the superior

performing lines can be classified as water spenders with the mechanism of EUW (higher values of grain CID and stomatal conductance) contributing to higher grain yield. However, we also identified a few genotypes that are combining lower values of grain CID and stomatal conductance with better grain yield under drought conditions, and we classified these genotypes as water savers, which are BFS 29, SER 16, ALB 6, ALB 60 and G 40001 (Fig. 4, Table 3). The water savers could be more suitable to bean farmers in semiarid to dry environments, dominated by terminal type of drought stress in Central America, Africa, northern Mexico and north-east Brazil. The tepary bean (*P. acutifolius*) evolved in the deserts of Mexico and south west United States and is well adapted to semi-arid to arid conditions (Beebe et al., 2014). The line G 40001 of tepary bean presents different traits related to drought resistance confirming its behavior as water saver with a combination of morpho-physiological characteristics such as greater ability for photosynthate remobilization, fine roots, smaller leaves for reduced water use, and reduced stomatal conductance (Mohamed et al., 2005; Butare et al., 2011; Rao et al., 2013; Beebe et al., 2014).

Our classification of the line SER 16 as water saver is consistent with a previous study conducted under greenhouse conditions where this line was characterized as responsive to soil drying by closing its stomata sooner than the other genotypes during progressive soil drying (Devi et al., 2013). The line SER 16 and its progenies (ALB lines 6, 60, 74, 88 and 213) displayed interesting contrasts in the relationship of WUE and water extraction. The ALB lines were derived from a cross of SER 16 x (SER 16 x *Phaseolus coccineus*) (Butare et al., 2012). SER 16 and two of its progenies ALB 6 and ALB 60 were outstanding in WUE under drought conditions, demonstrating that the traits of SER 16 are heritable. On the other hand, ALB 88 presented higher values of CID, indicating more water extraction, but a low canopy biomass and grain yield. This line was also identified as a line with a root system most like that of its *P. coccineus* parent (Butare et al., 2012). ALB 88 may invest biomass in roots and water extraction at the expense of shoot growth and grain yield.

### 1.4.3 *Canopy biomass, photosynthate remobilization and sink strength*

Total shoot biomass or canopy biomass can be understood in a physiological sense as the result of accumulated net photosynthesis of the crop, and it is shown to be related to yield in several crops (Araus et al., 2002). We found a positive relationship between canopy biomass at mid-pod filling and grain yield under both irrigated and drought conditions, as reported previously for grain yield under drought stress (Rosales-Serna et al., 2004; Muñoz-Perea et al., 2007; Klaedtke et al., 2012; Assefa et al., 2013; Beebe et al., 2013; Rao et al., 2013). In this study canopy biomass was reduced by 31% under drought stress compared with irrigated conditions. Water limitations decrease canopy biomass accumulation as a result of reduced transpiration and net photosynthesis (Klaedtke et al., 2012; Mir et al., 2012; Rosales et al., 2012; Assefa et al., 2013; Rao et al., 2013). The lines SCR 2, SER 119, SEA 15, RCB 593, NCB 280, SEN 56 and NCB 226 were outstanding in canopy biomass production and grain yield under drought stress. These lines were able to access more water, as reflected in the values of grain CID. This, combined with increased photosynthate mobilization (HI and PHI), resulted in better adaptation to drought (Muñoz-Perea et al., 2007; Polania et al., 2012; Assefa et al., 2013; Rao et al., 2013; Beebe et al., 2014).

The genotypes BFS 29 and SER 125 presented higher values of canopy biomass under drought stress, combined with higher values of grain yield with moderate water use based on grain CID values. This suggests that some common bean genotypes can regulate stomatal opening and transpiration and still have a better canopy development, combined with higher values of photosynthate mobilization (HI and PHI) and resulting in better grain yield under drought stress. The bred line SMC 141, was outstanding in its grain production under drought stress but it did not display greater canopy biomass compared with the other genotypes tested (Fig. 4), indicating the importance of the efficiency of mobilization of photosynthates from vegetative plant structures to pod production in this line (Fig. 5). Compared with the drought-sensitive checks DOR 390, Pérola and Tio Canela, most of the bred lines showed a higher accumulation of canopy biomass at mid-pod filling.

These results show significant progress in breeding for improved biomass accumulation and biomass partitioning towards pod formation and grain production. Previous research suggested that the drought resistance in common bean is associated with a more efficient photosynthate remobilization to pod formation and grain production (Hall, 2004; Rosales-Serna et al., 2004; Klaedtke et al., 2012; Rosales et al., 2012; Assefa et al., 2013; Beebe et al., 2013, 2014; Rao et al., 2013). Research using drought tolerant and susceptible bean genotypes and techniques such as  $^{14}\text{C}$ -labelling to quantify sugar accumulation and partitioning, showed that high grain yield under terminal drought stress is associated with an efficient carbon mobilization from leaves to pods and to seeds (Cuellar-Ortiz et al., 2008; Rosales et al., 2012). The positive and significant correlations between grain yield and biomass partitioning indices (PPI, HI, SBR and PHI) under drought stress (Table 2) highlight the importance of photosynthate mobilization from plant biomass to pod formation (PPI) and grain production (PHI) and stem reserve mobilization (SBR). The lines BFS 29, SEA 15, BFS 10, NCB 280, SEN 56, SCR 16 and SMC 141 showed greater grain yield under drought and were superior in their ability to partition greater proportion of biomass to pod and grain production (Figs. 5, 6). The lines INB 841 and SER 118 showed especially higher values of PPI and PHI across years under stress, and both are excellent parents. NCB 226, SEN 56, SER 16, SEA 5 and SEA 15 were superior in their ability to remobilize photosynthates to grain production under drought stress conditions (Klaedtke et al., 2012; Beebe et al., 2013, 2014; Rao et al., 2013).

Reduced efficiency in one of the steps of photosynthate remobilization could result in poor adaptation to drought conditions. For example, MIB 778, SMC 43, Pérola, ALB 88 and DOR 390 accumulated substantial canopy biomass but resulted in low yield, due to poor mobilization of these reserves, especially to pod formation, as noted in the low values of PPI, and from podwall to seed formation as indicated by the PHI values. Breeding programs should focus on the selection of best-performing materials that combine greater values of canopy biomass as a result of EUW, with greater mobilization of photosynthates to pod development and grain filling. PHI is a useful selection criteria for improving drought resistance because of its simplicity in

measurement and its significant correlation with grain yield under both irrigated and drought conditions (Assefa et al., 2013).

These phenotypic correlations suggest that a rapid accumulation of canopy biomass through an effective use of water, combined with an efficient remobilization of these reserves to the pod and grain formation (higher values of grain yield per day and SNA), is an important adaptive strategy of early maturing and drought resistant genotypes. Significant positive associations between grain yield and PPI, PHI and 100 seed weight under both irrigated and drought stress conditions suggest that photosynthate remobilization, or sink strength, is an important factor in determining grain yield under drought conditions (Polania et al., 2016b). Greater values of SNA and PNA likewise are consistent with the hypothesis that drought resistant lines have greater sink strength.

## **Conclusions**

The results demonstrate that drought resistance in common bean is related to effective use of water to produce greater canopy biomass, combined with the ability to remobilize photosynthates from vegetative structures to the pods and subsequently to grain production resulting in higher values of pod and seed number per area. Several lines (BFS 29, SEA 15, BFS 10, NCB 280, SEN 56, SCR 16 and SMC 141) expressed this desirable combination of traits. Resistance to terminal drought stress was found to be positively associated with effective use of water combined with superior mobilization of photosynthates to pod and seed production and negatively associated with days to flowering and days to physiological maturity. Based on genotypic differences in grain carbon isotope discrimination and leaf stomatal conductance under drought stress, the common bean lines tested were classified into two groups, water savers and water spenders. We suggest that pod harvest index could be a useful selection criterion in breeding programs to select for drought resistance in common bean.



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## **Chapter 2. Estimation of phenotypic variability in symbiotic nitrogen fixation (SNF) ability of common bean under drought stress using $^{15}\text{N}$ natural abundance in grain tissue**

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## Chapter 2

### 2.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume cultivated in the tropics of Latin America and eastern and southern Africa. It is nutritionally rich in iron and protein, and is a source of fiber and carbohydrates that are essential in the nutrition of the population, especially in developing countries. The bean crop is cultivated by small farmers and it is often exposed to unfavorable conditions with minimum use of inputs (Beebe et al., 2008, 2013). Bean yields are affected by various biotic and abiotic stress factors. Drought affects about 60% of the bean producing regions causing yield losses between 10 and 100%. Drought is the second most important factor in yield reduction after disease (Thung and Rao, 1999; Rao, 2014).

In addition to drought, smallholder bean production is often affected by low soil fertility in marginal areas (Douxchamps et al., 2010) where the majority of grain legumes are cultivated (Sinclair and Vadez, 2012; Beebe et al., 2014). About 50% of the bean production areas in Eastern and Central Africa and 60% in Southern Africa are subjected to N deficiency due to both depletion of N in the soil and application of limited N fertilizer (Beebe et al., 2014). Thus the ability of the crop to acquire N from the soil is limited. Common bean can supply at least part of its N requirement through symbiotic nitrogen fixation (SNF). However compared to other legumes, beans have lower SNF capacity (Hardarson et al., 1993; Hardarson, 2004; Peoples et al., 2009). The estimated mean value of nitrogen derived from the atmosphere (Ndfa) for common bean across different geographical regions of the world is 39% (Peoples et al., 2009). This value is in contrast with the Ndfa values of 54% to 65% observed for other widely-grown legume crops and to the values recorded for soybean and faba bean which were 68% and 75%, respectively (Peoples et al., 2009).

Abiotic and biotic stress factors such as P deficiency, drought, and pest and diseases affect SNF capacity (Ramaekers et al., 2013). Among these limitations, SNF is highly sensitive to drought (Devi et al., 2013; Beebe et al., 2014), with possible interactions among stresses. Bean genotypes with resistance to drought are affected by N

deficiency, and SNF capacity is affected by drought stress. Moreover, drought has a negative influence on both the rhizobia and the nodulation process of legumes (Devi et al., 2013). Drought even can cause the loss of SNF activity in common bean, and other legume species that generally have low rates of N fixation even under well-watered conditions (Devi et al., 2013). Identification of parental genotypes to use in breeding that combine superior SNF ability under drought stress with other desirable traits could be a useful strategy to confront with the new challenges of climate variability and to ensure food security in marginal areas.

Different techniques have been used to estimate SNF, including N<sub>2</sub> balance, N<sub>2</sub> difference, <sup>15</sup>N natural abundance, <sup>15</sup>N isotope dilution, ureide analysis, acetylene reduction assay and hydrogen evolution (Unkovich et al., 2008). The <sup>15</sup>N natural abundance method presents some advantages over other methodologies. It can be applied under both greenhouse and field conditions. Moreover, the method allows the estimation of N fixation in almost any situation where both N-fixing and non N-fixing plants are present at the same location, and it can be applied to farmers' fields, or to any field experiments where legumes and non N-fixing plants coexist (Unkovich et al., 2008; Douxchamps et al., 2010). Also, the <sup>15</sup>N natural abundance method allows to separate N derived from the atmosphere with the N derived from soil (Boddey et al., 2000). To calculate the total contribution of SNF in kg ha<sup>-1</sup> needs the estimation of shoot biomass in kg ha<sup>-1</sup> (Boddey et al., 2000; Unkovich et al., 2008).

This methodology is usually applied to shoot tissue of the plant harvested at different growth stages such as flowering or pod filling (Boddey et al., 2000; Unkovich et al., 2008). Generally, shoot tissue is used because to use the whole plant including roots is complex. However, the fact of taking shoot biomass sample, from a breeding perspective, is equally complex because of the large sample size that requires destructive sampling of the plot. This could mean significant labor costs for plant breeding programs dealing with large number of breeding lines. For these reasons, most bean breeding programs do not routinely select for better SNF ability. Developing methodologies that can estimate SNF ability using grain tissue could be easier to integrate into most breeding programs since grain is routinely harvested to estimate

yield and nutritional quality. Therefore based on the principle that common beans mobilize much of their N from vegetative structures to the grain, we propose that it would be much easier to apply the methodology of  $^{15}\text{N}$  natural abundance using the grain tissue at the time of harvest.

The main objectives of this study were to:

- (i) Test and validate the use of  $^{15}\text{N}$  natural abundance in grain to quantify phenotypic differences in symbiotic nitrogen fixation (SNF) ability for its implementation in breeding programs aiming to improve SNF in common bean; and
- (ii) Quantify phenotypic differences in SNF under drought stress to identify superior genotypes that could serve as parents.



## **2.2 Materials and methods**

### *2.2.1 Experimental site and meteorological conditions*

Two field trials were conducted during the dry season from June to September in two years (2012 and 2013), at the main experimental station of the International Center for Tropical Agriculture (CIAT) in Palmira, Colombia. Characteristics of field trials, treatments and crop management were described previously in chapter 1. The soil is a Mollisol (Aquic Hapludoll) with adequate nutrient supply, 2.3% of organic matter and no limitations of availability of iron (Fe) and molybdenum (Mo) for the process of SNF. During the crop-growing season, maximum and minimum air temperatures in 2012 were 31.0 °C and 19.0 °C, and in 2013 were 30.2 °C and 19.2 °C, respectively. Total rainfall during the active crop growth was 85.8 mm in 2012 and 87.7 mm in 2013. The potential pan evaporation was of 385.2 mm in 2012 and 351.0 mm in 2013 (Chapter 1, Fig. 2).

### *2.2.2 Plant material*

For this study, the 36 bush bean genotypes reported in the chapter 1 (Chapter 1, Table 1) were analyzed (ALB 6, ALB 60, ALB 74, ALB 88, ALB 213, BAT 477, BAT 477\_NN, BFS 10, BFS 29, BFS 32, BFS 67, DOR 390, G 40001, INB 827, INB 841, MIB 778, NCB 226, NCB 280, Pérola, RCB 273, RCB 593, SCR 2, SCR 9, SCR 16, SEA 15, SEN 56, SER 16, SER 48, SER 78, SER 118, SER 119, SER 125, SMC 43, SMC 141, SXB 412 and Tío Canela 75). BAT 477 NN was included as a non-fixing bean genotype which was used as reference plant to estimate nitrogen derived from the atmosphere (Ndfa).

### *2.2.3 Experimental design*

In the two years, a 6 x 6 partially balanced lattice design with 3 replications was used. Experimental units consisted of 4 rows with 3.72 m row length with a row-to-row

distance of 0.6 m and plant-to-plant spacing of 7 cm (equivalent to 24 plants m<sup>-2</sup>). Trials were weeded and sprayed with insecticides and fungicides as needed.

#### 2.2.4 Determination of symbiotic nitrogen fixation ability using shoot and grain

To compare and validate the method of <sup>15</sup>N natural abundance, we sampled shoot tissue at mid-pod filling and grain tissue at harvest time. We sampled a representative plant within a row of 50 cm long at mid-pod filling, and also at harvest time, for each genotype and from each plot of both irrigated and drought treatments. The plants were cut at the soil surface, washed with deionized water and dried in the oven at 60°C for two days. The dried samples were finely ground using a ball-mill and were weighed using a microbalance to pack 2.5 mg of each sample in a tin capsule. These samples in tin capsules were sent to UC Davis Stable Isotope Facility in USA for <sup>15</sup>N isotope analyses. The percentage of N derived from the atmosphere (%Ndfa) was determined for both shoot and grain samples using the <sup>15</sup>N natural abundance method (Shearer and Kohl, 1986). BAT 477 NN was used as a non-fixing reference plant.

$$\%Ndfa = \frac{\delta^{15}N \text{ non fixing reference plant} - \delta^{15}N \text{ of } N_2 \text{ fixing legume}}{\delta^{15}N \text{ non fixing reference plant} - \beta} \times 100$$

Where  $\beta$  is the  $\delta^{15}N$  value from the nitrogen fixing bean plant grown in N free medium. The isotope discrimination occurs internally within the plant so that the different plant parts differ in  $\delta^{15}N$  (Unkovich et al., 1994). Consequently, different  $\beta$  values were used to estimate %Ndfa for the shoot at mid-pod filling and grain at harvest. The  $\beta$  values used were -3.09‰ for shoot at mid-pod filling and -2.44‰ for grain at harvest for genotypes with growth habit II and -3.62‰ for shoot at mid-pod filling and -2.88‰ for grain for genotypes with growth habit III. The  $\beta$  values were generated from conducting a pot experiment in the greenhouse at CIAT, following the procedure of Unkovich et al. (1994). We used SMC 140 and GGR 18 as representative genotypes of growth habit II and III, respectively. Total shoot and seed N content per unit area (kg ha<sup>-1</sup>) were

estimated using the values of N concentration in shoot biomass and grain and dry weights of shoot biomass and grain. Total N derived from atmosphere in  $\text{kg ha}^{-1}$  (TNdfa) and total N derived from soil in  $\text{kg ha}^{-1}$  (TNdfs) were estimated (Unkovich et al., 1994). Nitrogen use efficiency (NUE) was estimated as kg of grain produced per kg of shoot N uptake at mid-pod filling growth stage.

### *2.2.5 Physiological measurements*

At mid-pod filling, a 50 cm segment of the row from each plot with about 7 plants was used for destructive sampling to measure canopy biomass (CB). Also at mid-pod filling, the roots of three plants per plot (selected randomly) of the non-fixing bean genotype (BAT 477 NN) were pulled from soil to check for the absence of nodules. At the time of harvest, plants in 50 cm of a row from each plot were cut and dry weights of stem, pod, seed, and pod wall were recorded. Grain was harvested from two central rows after discarding end plants in both the irrigated and drought plots. In order to compare shoot dry biomass with grain dry weight, mean values of grain yield per hectare were corrected for 0% moisture in grain.

### *2.2.6 Statistical analysis*

All data were analyzed using the SAS (v 9.0) PROC MIXED and PROC CORR (SAS Institute Inc., 2008). The adjusted means for each genotype and the environment (irrigated and drought) were obtained using the mixed models theory together with the MIXED procedure considering the effects of the replications and blocks within replications as random and genotypes as fixed. Correlation coefficients were calculated by the PROC CORR. In the following text, values marked with \*, \*\* or \*\*\* are statistically significant at probability levels of 5%, 1% and 0.1%, respectively.

## 2.3 Results

### 2.3.1 Estimation of Ndfa and differences in $^{15}\text{N}$ natural abundance in shoot and grain

Analysis of % N derived from the atmosphere (%Ndfa) estimates the proportional dependence of the biomass N on  $\text{N}_2$  fixation. The %Ndfa in grain was compared with %Ndfa in shoot biomass to determine their relationship. If the %Ndfa in grain is closely related with %Ndfa in shoot, legume breeders would be able to select for SNF based on the grain values without the need to harvest, dry and grind large volumes of shoot biomass. Significant and positive correlation values of  $r = 0.81^{***}$  in 2012 and  $r = 0.66^{***}$  in 2013 ( $r = 0.83^{***}$  for combined data for two seasons, Table 4) were observed between the %Ndfa values estimated with  $^{15}\text{N}$  natural abundance of shoot biomass at mid-pod filling growth stage and %Ndfa values estimated with  $^{15}\text{N}$  natural abundance in the grain under irrigated conditions. The correlation values were also significant and positive under drought conditions  $r = 0.67^{***}$  in 2012 and  $r = 0.74^{***}$  in 2013 ( $r = 0.71^{***}$  for combined data for two seasons, Table 4).

Nodule formation was observed in both irrigated and drought treatments in both years and with all the genotypes evaluated, except for BAT477 NN which was used as a non-nodulating reference plant for estimating SNF ability. Significant differences were observed in both shoot and grain  $\delta^{15}\text{N}$  between the non-fixing bean genotype and the other lines tested under both irrigated and drought conditions (Table 5). The values of  $\delta^{15}\text{N}$  for shoot for the non-fixing bean genotype (BAT 477\_NN) in 2012 were 5.6 and 8.7 under irrigated and drought conditions, respectively (Table 5); and in 2013 the values were 8.3 and 9.5 under irrigated and drought conditions, respectively (Table 5). Under irrigated conditions, the  $\delta^{15}\text{N}$  for shoot of 35 genotypes (Excluding BAT 477\_NN) ranged from -0.2 to 1.7 in 2012 and from 2.1 to 5.6 in 2013 (Table 5). Under drought conditions the  $\delta^{15}\text{N}$  for shoot of 35 genotypes ranged from 3.6 to 7.5 in 2012 and from 4.3 to 8.5 in 2013 (Table 5). The values of  $\delta^{15}\text{N}$  for grain for BAT 477\_NN in 2012 were 5.8 and 8.6 under irrigated and drought conditions, respectively (Table 5); and in 2013 the values were 6.8 and 8.3 under irrigated and drought conditions, respectively (Table 5). Under irrigated conditions, the  $\delta^{15}\text{N}$  for grain of 35 genotypes ranged from 0.0 to 1.4 in 2012 and from 1.7 to 4.8 in 2013 (Table 5). Under drought conditions, the  $\delta^{15}\text{N}$

for grain of 35 genotypes ranged from 4.5 to 8.0 in 2012 and from 4.4 to 7.0 in 2013 (Table 5).

Table 4. Correlation coefficients (r) between % nitrogen derived from the atmosphere estimated using shoot tissue (%Ndfa-SH), % nitrogen derived from the atmosphere estimated using grain tissue (%Ndfa-G), total nitrogen derived from the atmosphere in kg ha<sup>-1</sup> using grain tissue (TNdfa-G), total nitrogen derived from the soil in kg ha<sup>-1</sup> using grain tissue (TNdfs-G), nitrogen use efficiency in kg of grain produced kg<sup>-1</sup> of N uptake in the shoot (NUE), canopy biomass in kg ha<sup>-1</sup> (CB) and grain yield in kg ha<sup>-1</sup> (GY) of 36 bean genotypes of grown under irrigated and drought conditions in a Mollisol at CIAT-Palmira, Colombia. Values reported are from analysis of data collected from two seasons of evaluation (2013 and 2014).

Irrigated							
Trait	%Ndfa-SH	%Ndfa-G	TNdfa-G	TNdfs-G	NUE	CB	GY
%Ndfa-SH	1						
%Ndfa-G	<b>0.83***</b>	1					
TNdfa-G	0.65***	0.82***	1				
TNdfs-G	-0.53***	-0.69***	-0.29***	1			
NUE	0.06	0.07	0.32***	0.15*	1		
CB	0.07	0.05	0.20**	0.32***	-0.45***	1	
GY	0.16*	0.16*	0.61***	0.50***	0.51***	0.39***	1
Drought							
Trait	%Ndfa-SH	%Ndfa-G	TNdfa-G	TNdfs-G	NUE	CB	GY
%Ndfa-SH	1						
%Ndfa-G	<b>0.71***</b>	1					
TNdfa-G	0.56***	0.83***	1				
TNdfs-G	-0.22**	-0.37***	0.09	1			
NUE	0.20**	0.19**	0.45***	0.48***	1		
CB	-0.13	-0.16*	0.17*	0.57***	-0.12	1	
GY	0.05	0.05	0.51***	0.86***	0.61***	0.59***	1

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



### 2.3.2 Differences in SNF ability and genotypic response to drought

An average reduction of 70% in 2012 and 38% in 2013 in SNF ability was observed under drought stress in bush Middle American genotypes using the grain method (Table 5). The lines RCB 593, BFS 32, SER 125, SMC 141 and BFS 29 maintained a relatively higher level of SNF ability under drought stress in both years (Table 5). A weak correlation was observed between %Ndfa estimates using grain samples and grain yield under irrigated conditions (Table 4). No correlation was observed between %Ndfa estimates and grain yield under drought conditions (Table 4). However, the lines RCB 593, SEA 15, NCB 226 and BFS 29 were superior in combining high values of grain yield with greater values of %Ndfa under drought stress. The line NCB 226 was superior in %Ndfa ability under both irrigated and drought conditions (Table 5). The accumulation of N ( $\text{kg ha}^{-1}$ ) in grain was reduced by 55% due to drought stress in Middle American bush beans, being more sensitive the accumulation of total N in grain derived from the atmosphere (TNdfa-G) than N in grain derived from soil (TNdfs-G), with reduction of 78% and 43%, respectively (Figs. 9, 10).

Under irrigated conditions the lines BFS 29, SCR 16, BFS 32, NCB 280 and SEN 56 presented higher total N content in grain from both TNdfs and TNdfa values compared with the other lines tested (Figs. 9, 10). *Phaseolus acutifolius* (G 40001) was outstanding in its ability for TNdfs, and showed a drastic decrease in TNdfa under drought (Figs. 9, 10). The lines SEA 15, RCB 593 and BFS 10 maintained higher total N content in grain for both TNdfs and TNdfa values, compared with the other lines tested under drought stress (Figs. 9, 10). The three commercial varieties (DOR 390, Tio Canela 75 and Perola) presented lower N content in grain for both TNdfs and TNdfa values under both irrigated and drought conditions (Figs. 9, 10). Similar tendency was observed in the relationship between shoot biomass and total Ndfa estimated using shoot tissue (TNdfa-SH) (Fig. 11). Genotypes that stood out for a higher TNdfa-G and grain yield under both irrigated and drought conditions (Figs. 9, 10) also exhibited higher TNdfa-SH and shoot biomass at mid-pod filling under both irrigated and drought conditions (Fig. 11). Several inbred lines were superior in their shoot biomass, grain

yield and TNdfa-SH and TNdfa-G than the three commercial varieties (DOR 390, Tio Canela and Perola) under both irrigated and drought conditions (Figs. 9, 10, 11).

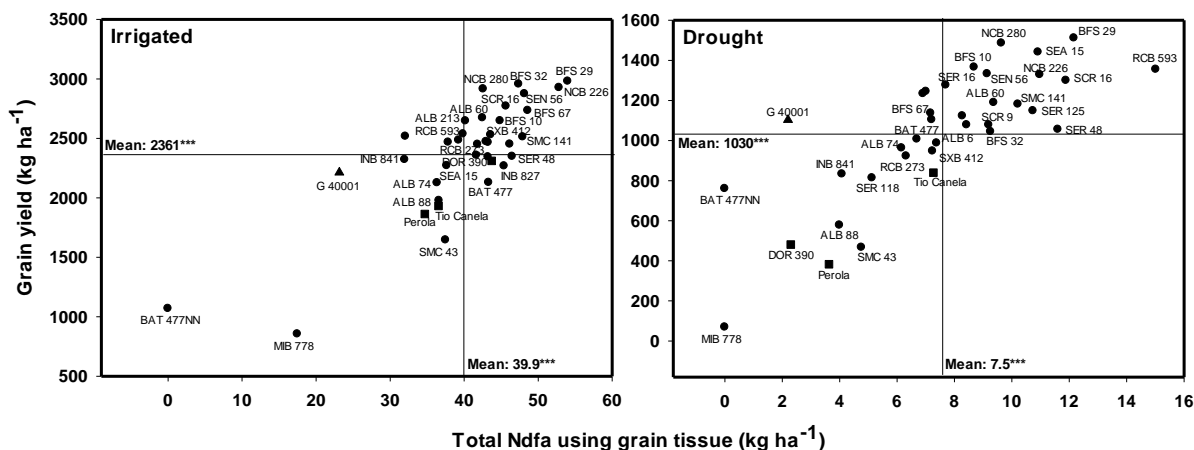


Figure 9. Identification of genotypes that combine greater total nitrogen derived from the atmosphere in kg ha<sup>-1</sup> estimated using grain tissue (TNdfa-G) with superior grain yield under irrigated and drought conditions when grown in a Mollisol at CIAT-Palmira, Colombia. Higher TNdfa-G genotypes with greater grain yield were identified in the upper, right hand quadrant. Genotypes identified with symbols of (■) are commercial varieties and with a symbol of (▲) is *P. acutifolius*

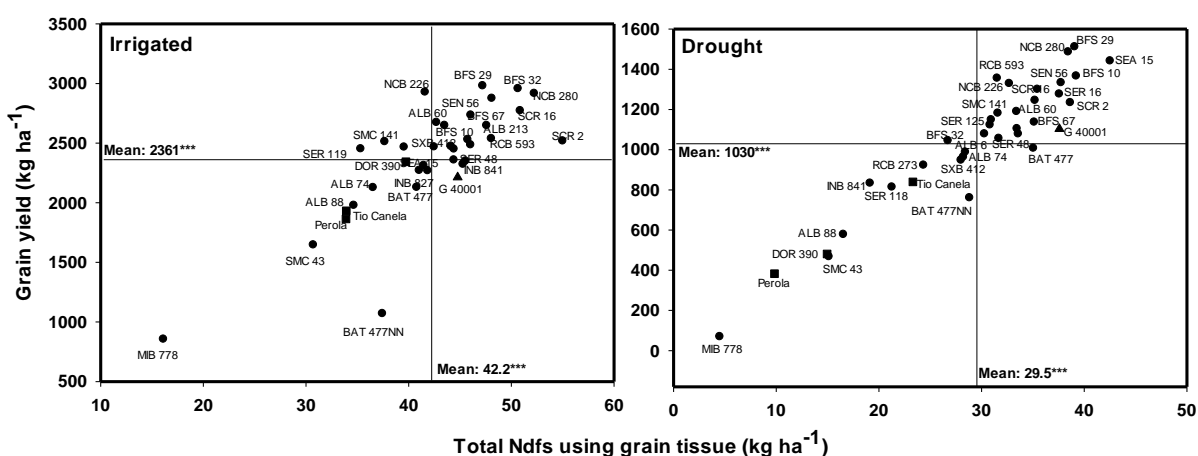


Figure 10. Identification of genotypes that combine greater total nitrogen derived from the soil in kg ha<sup>-1</sup> estimated using grain tissue (TNdfs-G) with superior grain yield under irrigated and drought conditions when grown in a Mollisol at CIAT-Palmira, Colombia. Higher TNdfs-G genotypes with greater grain yield were identified in the upper, right hand quadrant. Genotypes identified with symbols of (■) are commercial varieties and with a symbol of (▲) is *P. acutifolius*



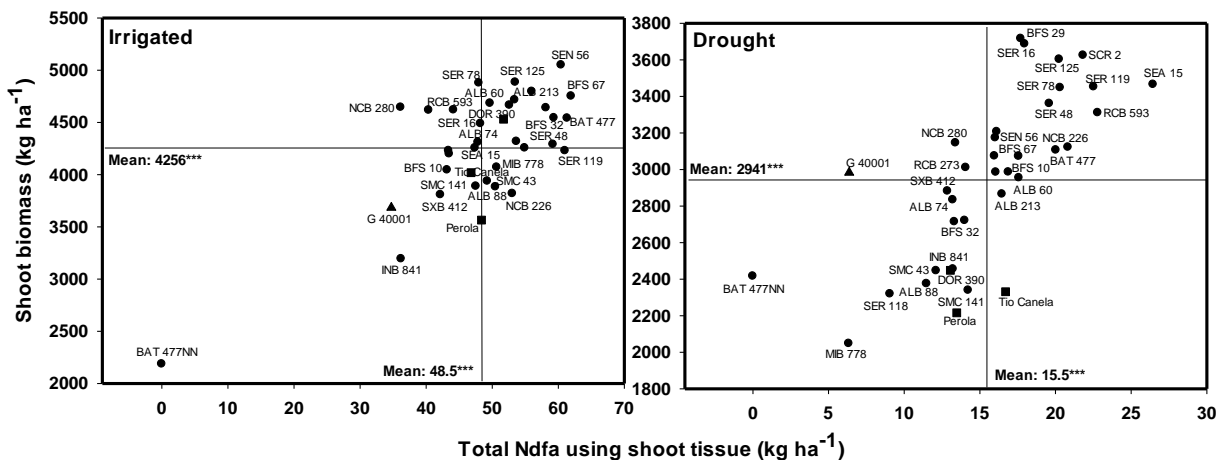


Figure 11. Identification of genotypes that combine greater total nitrogen derived from the atmosphere in kg ha<sup>-1</sup> estimated using shoot tissue (TNdfa-SH) with superior grain yield under irrigated and drought conditions when grown in a Mollisol at CIAT-Palmira, Colombia. Higher TNdfa-SH genotypes with greater grain yield were identified in the upper, right hand quadrant. Genotypes identified with symbols of (■) are commercial varieties and with a symbol of (▲) is *P. acutifolius*

Several genotypes including BFS 29, SEN 56, NCB 226, NCB 280, SCR 16, ALB 60, RCB 593 and SER 48 presented higher total N content in grain under irrigated conditions, and also maintained N levels well under drought conditions. The lines MIB 778, Pérola, SMC 43, ALB 88, Tio Canela and the non-nodulant BAT477NN showed low grain N content under both irrigated and drought conditions. The lines SEA 15, NCB 280, BFS 10, SEN 56, BFS 29, NCB 226 and SER 16 not only showed high values in grain for TNdfs, but also were outstanding in their TNdfa value using grain that resulted in greater values of grain yield under drought stress. Contrastingly, RCB 593 combined higher values of TNdfa and an intermediate level of Ndfs with greater grain yield under drought conditions (Figs. 9, 10).

A positive and highly significant correlation was observed between grain yield and nitrogen use efficiency (NUE) under both irrigated and drought conditions (Table 4). Genotypes that combined better grain yield with higher NUE under drought conditions were RCB 593, SMC 141, BFS 32, BFS 29, SEA 15, SEN 56, NCB 280 and NCB 226.

A low but significant correlation coefficient ( $r=0.19^{**}$ ) was observed between %Ndfa and NUE under drought conditions. The lines RCB 593, SMC 141, BFS 32, BFS 29, SEA 15, SEN 56, NCB 280 and NCB 226 combined higher values of NUE and %Ndfa under drought stress (Fig. 12). The commercial cultivar Pérola showed a moderate value of %Ndfa but it was poor in its NUE and was low yielding under drought (Fig. 12).

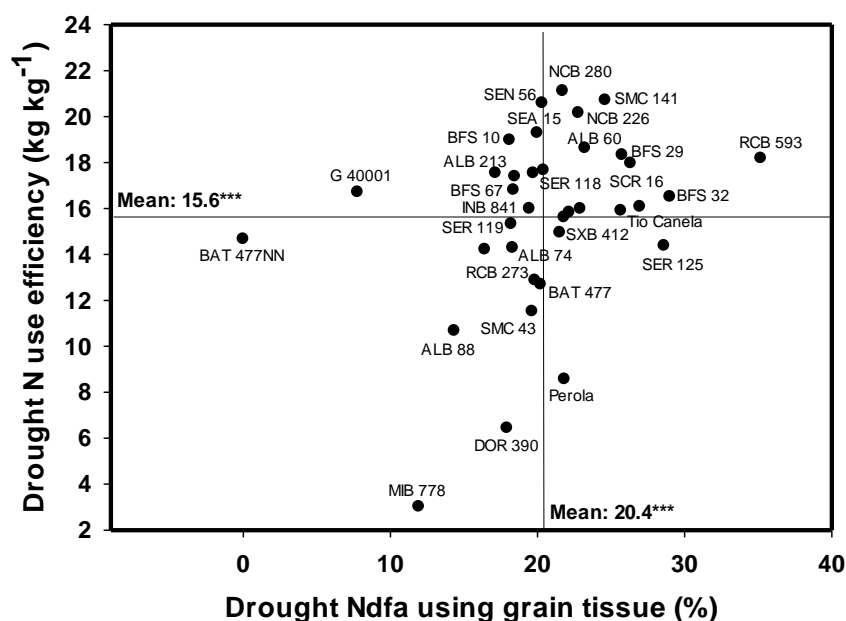


Figure 12. Identification of genotypes that combine greater values of %nitrogen derived from the atmosphere using grain tissue (%Ndfa-G) with higher values of nitrogen use efficiency (NUE) in terms of kg of grain produced  $\text{kg}^{-1}$  of shoot N uptake under drought conditions when grown in a Mollisol at CIAT-Palmira, Colombia. Higher %Ndfa-G genotypes with greater values of NUE were identified in the upper, right hand quadrant

A negative and highly significant correlation was observed between grain yield and nitrogen concentration in grain under drought stress conditions (Fig. 13). Genotypes such as NCB 280, BFS 29, SEN 56, NCB 226 and RCB 593 combined better grain yield with relative lower N concentration in grain under drought conditions. The genotypes SMC 43, Perola, DOR 390 and MIB 778 showed higher N concentration in grain than other genotypes evaluated combined with poor grain yield under drought stress conditions. Some genotypes such as BAT 477, SER 48, ALB 60, SMC 141,

SER 16 and SEA 15 were superior in their grain yield. They also presented relative higher N concentration in grain than the other genotypes evaluated under drought stress (Fig. 13).

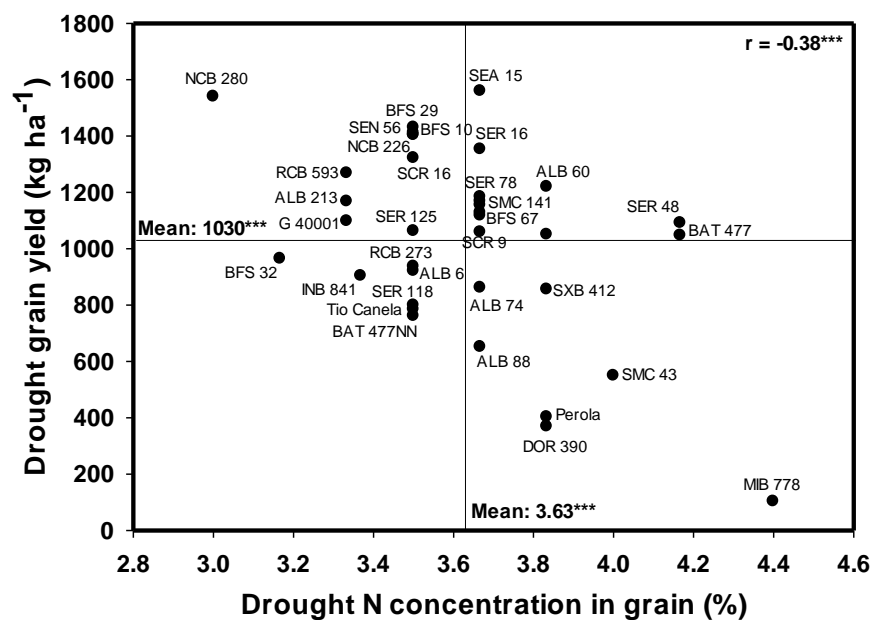


Figure 13. Identification of genotypes with greater nitrogen concentration in grain and grain yield under drought conditions on a Mollisol at Palmira, higher N concentration in grain genotypes with greater grain yield were identified in the upper, right hand quadrant.

## 2.4 Discussion

### 2.4.1 *Estimation of Ndfa and differences in <sup>15</sup>N natural abundance in shoot and grain*

This study allowed to compare the estimation of %Ndfa using shoot tissue (%Ndfa-SH) vs. grain tissue (%Ndfa-G), to quantify phenotypic differences in common bean for SNF ability under irrigated and drought stress conditions, and to test whether %Ndfa-G could be a useful trait in breeding programs. Results from two seasons with %Ndfa values estimated under field conditions, comparing the conventional methodology based on shoot tissue at the growth stage of mid-pod filling (%Ndfa-SH), and the method proposed here using grain tissue at the time of harvest (%Ndfa-G), showed that the latter methodology is feasible. The high correlation between both methods validates this statement.

We suggest that grain samples collected at harvest can be used to quantify phenotypic differences in SNF ability in common bean using the methodology of natural abundance of <sup>15</sup>N. The proposed %Ndfa-G method, from a breeding perspective, is much easier than the conventional methodology involving shoot tissue (%Ndfa-SH) which requires destructive sampling, drying of fresh tissue; i.e the conventional method requires more labor and therefore is less cost effective. With the proposed %Ndfa-G methodology, the breeder can take a sample of harvested grain, dry and grind it and analyze for the isotope ratios of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , simultaneously selecting for water use efficiency (Araus et al., 2002; Easlon et al., 2014) and SNF ability (based on higher values of %Ndfa). In a recent genomic study addressing SNF ability in common bean, Kamfwa et al. (2015) using both shoot and grain samples suggested selection for high SNF ability based on grain tissue could be easier integrated into most breeding programs. Similar results for %Ndfa using the <sup>15</sup>N natural abundance technique in grains and the whole shoots have also been reported for other legume species (Bergersen et al., 1985). Discordant results reported by others can occur when grain filling during the late stage of development is highly dependent on the contribution of N fixation rather than on the extent of remobilization of N from vegetative structures (Bergersen et al., 1992).

Previous research showed that common beans are able to translocate about 80 to 93% of its total N to the grain (Ramaekers et al., 2013). Also it has been shown that common bean accumulates preferentially the fixed N into the grain (Westermann et al., 1985; Dubois and Burris, 1986; Wolyn et al., 1991). At such high rates of N translocation in common beans, trends in %Ndfa would be similar between shoot tissue and grain tissue. Several of the lines evaluated in this study, have high mobilization of N from shoot to grain under both irrigated and drought conditions. As can be evidenced from our results, where the genotypes with greater N accumulation in shoot at mid-pod filling also showed greater values of N accumulation in grain at harvest. The contrary was true with the genotypes that accumulated less N in shoot and grain.

#### *2.4.2 Differences in SNF ability and genotypic response to drought*

This study also permitted evaluating the SNF ability in a set of elite common bean breeding lines that were recently developed for improving resistance to drought. Furthermore, we tested lines that were derived from crosses among bean races (Beebe et al., 2008), as well as interspecific crosses with introgression from *P. coccineus* (Butare et al., 2012) or *P. acutifolius* (Beebe, 2012). Several lines developed over different breeding cycles to improve drought resistance not only performed better under water shortage, but also had higher ability to fix N under these conditions. Under drought stress, both grain yield and total Ndfa in these lines doubled the values that were observed for three leading commercial cultivars grown in Latin America: DOR 390, Perola, and Tío Canela.

Under unfavorable conditions, such as drought, a decrease in the effectiveness of SNF process is expected (Devi et al., 2013). The symbiosis is based on the carbon supply from the plant to the Rhizobium, which provides fixed N to the plant. However, under drought stress, the reduced net photosynthesis decreases the supply of photosynthates to the nodules resulting in lower values of %Ndfa (Gonzalez et al., 1995; Sassi et al., 2008). Our results confirm previous reports that SNF in common bean is especially sensitive to drought stress, as drought reduced Ndfa by 57%. SNF

is a physiological process especially sensitive to soil drying (Devi et al., 2013), and the effect of drought stress on SNF varies according to the stages of development when the stress occurs. Water stress during early vegetative growth had no significant effect on SNF, but during flowering or grain filling, it significantly reduced fixed N (Chalk et al., 2010). In the two growing seasons of this study, the drought stress was imposed starting at the critical preflowering stage, resulting in high inhibition of SNF ability.

The superior performance of a few drought-adapted lines that combine greater grain yield with higher values of %Ndfa in grain might be due to greater carbon transport to both grain and the nodule. It is also noteworthy that the drought resistant bean lines with higher SNF ability had greater values of shoot biomass indicating the importance of plant vigor for supporting nodule development, as well as contributing to remobilization of both C and N to developing grains. Vigorous plants permit higher levels of N accumulation of both fixed N and non-fixed N, while maintaining or improving remobilization of this N to grain, contributing to increased grain yield in common bean (Wolyn et al., 1991). Higher shoot biomass and N accumulation before pod set could provide an advantage for drought-adapted lines (Vadez et al., 2014). The lower sensitivity of SNF ability in drought resistant lines can be explained by the expeditious removal of N products from nodules and sequestering them in the shoot of the plant to avoid N-feedback limitation on SNF (Beebe et al., 2014; Sinclair and Vadez, 2012). Better SNF ability under drought stress could be an important determining factor of yield potential, since the plant need combine biomass accumulated from photosynthesis with fixed N to form the essential components of the grain (Daryanto et al., 2015).

Tepary bean (*P. acutifolius*) has been reported to present multiple traits for drought resistance including early maturity, greater photosynthate remobilization capacity, deep rooting, small leaves, and stomatal control for improved water use efficiency (Beebe et al., 2014; Mohamed et al., 2005; Rao et al., 2013). But in spite of excellent drought resistance, our results indicate that *P. acutifolius* drastically reduces its SNF under drought, suggesting an internal control of SNF, possibly through decreased carbon supply to the nodules and/or increased uptake of mineral N from soil through

its fine root system. In early maturing genotypes, competition for photosynthates exists between nodules and developing pods/grain (Piha and Munns, 1987; Vadez et al., 2014). *Phaseolus acutifolius* is early maturing, and may preferentially channel the photosynthates into pods and grains rather than destine them to the development and maintenance of the nodules. This in part could be an evolutionary response to the hot and dry environments from where tepary bean originated.

Common beans are poor N fixers compared to other grain legumes (Hardarson, 2004; Hardarson et al., 1993; Peoples et al., 2009). We conducted our evaluation in a Mollisol with adequate soil organic matter content that can limit the expression of SNF activity. Nonetheless, in the irrigated treatment, %Ndfa-G presented a mean value of 48% for two seasons or a mean value of 40 kg ha<sup>-1</sup> of N fixed. Several genotypes presented %Ndfa-G values superior to 50%, representing more than 50 kg ha<sup>-1</sup> of N fixed under irrigated conditions. The efficiency of major food crops in the recovery of applied N is often not more than 30% (Subbarao et al., 2013); so 50 kg N fixed through SNF would be comparable to about 150 kg N applied as chemical fertilizer. Line RCB 593 suffered less inhibition of SNF ability under drought stress when compared to irrigated conditions; moreover, and it presented the highest SNF ability under drought stress. This line could be a potential parent to improve SNF capacity under drought stress in bush beans.

Other lines that were resistant to drought and showed relatively higher SNF ability included BFS 29, BFS 32, SER 48, SEA 15 and NCB 226. The drought- adapted line NCB 226 has previously been found to be superior in its SNF ability under drought stress using the methodology of acetylene reduction activity (ARA) determined with a flow-through system (Devi et al., 2013). Our results also indicated that the drought resistant check BAT 477 showed moderately high %Ndfa under drought stress. This line has previously been identified with good SNF ability under both optimal (Kipe-Nolt and Giller, 1993) and drought stress conditions (Castellanos et al., 1996). As expected, most drought sensitive lines were poor in their SNF ability, for example, DOR 390, a commercial variety used widely in at least three countries in Latin America.

This study also found that all the best N fixers under drought were drought resistant lines, confirming the tendency that was observed in previous studies with common bean (Beebe et al., 2014; Devi et al., 2013). A positive and significant correlation was observed between grain yield and TNdfa (kg ha<sup>-1</sup>) in grain under both irrigated and drought conditions, indicating that the genotypes with more N accumulation from fixation presented higher grain yield under both irrigated and drought conditions. The lines RCB 593, SEA 15, BFS 29, SCR 16, NCB 280 and NCB 226 accumulated more N from symbiotic fixation, and used it for greater grain production both under drought and irrigated conditions. Several lines that performed well under drought stress, such as SMC 141, RCB 593, BFS 32, SEN 56 and NCB 226, combined greater SNF ability with more efficient use of N to produce grain. Despite the fact that SNF ability can be drastically affected by drought stress, the more drought resistant Middle American lines overcome this limitation by using the acquired N more efficiently through greater remobilization of both C and N to grain. A higher use efficiency of the acquired N can be very relevant for crop yield in environments dominated by strong droughts where the viability of Rhizobium and in consequence SNF is severely inhibited.

## **Conclusions**

Correlations between %Ndfa using shoot tissue (%Ndfa-SH) and %Ndfa using grain tissue (%Ndfa-G) indicated that the values of %Ndfa-G can be used to quantify phenotypic differences in symbiotic nitrogen fixation (SNF) of common bean under either irrigated or drought stress conditions. Estimates of %Ndfa-G are easier to implement in a breeding program due both to less labor costs and the feasibility to determine this parameter at harvest time. Using %Ndfa-G values, we observed significant phenotypic differences in SNF ability in common bean under drought stress. We identified four bean lines RCB 593, SEA 15, NCB 226 and BFS 29 that were not only drought resistant but also were superior in their SNF ability and these lines could serve as parents in breeding programs. Our results also indicate that the drought response of the SNF ability of tepary bean (*P. acutifolius*) and common bean are different, possibly due to differences in internal regulation mechanisms of SNF.



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## **Chapter 3. Identification of root traits related with drought resistance in common bean**

## Chapter 3

### 3.1 Introduction

Drought is a main abiotic stress limitation of common bean (*Phaseolus vulgaris* L.), affecting around 60% of bean producing regions and generating losses in production from 10 to 100% (Polania et al., 2016a). It is expected that the world demand for legumes will increase in the future, not only in developing countries, but also in the developed nations given the increasing trend towards healthy diets (Daryanto et al., 2015). In order to respond to the increase of the demand, common bean production has to face the challenges brought up by climate change, such as higher temperatures and the associated increase in evapotranspiration combined with erratic and lower rainfall (Beebe et al., 2013). Different climate models predict that many drought stressed areas in Eastern and Southern Africa will become drier over the next decades (Jones and Thornton 2003; Williams et al., 2007; Rippke et al., 2016).

Different strategies must be developed to face these new challenges. A key approach is breeding of bean varieties resistant to drought stress conditions to ensure food security in marginal areas. Defining the physiological mechanisms underlying drought resistance helps to identify desirable traits and procedures for phenotyping populations and accelerates plant breeding for better yield under water shortage (Oosterom et al., 2016). Several shoot and root traits improve resistance to drought. However their contribution to superior grain yield depends on the type of drought (early, intermittent and terminal) and the agro-ecological conditions where the crop is grown (Beebe et al., 2013). According to agro-ecological zones and types of drought, breeding should target different plant ideotypes such as, the isohydric ('water saving') plant model and the anisohydric ('water spending') plant model (Polania et al., 2016b). The water saving model might have an advantage in the harshest environments, whereas the water spending model will perform relatively better under more moderate drought conditions (Blum 2015). Effective use of water (EUW), as proposed by Blum (2009), implies not only maximal soil moisture capture for transpiration but also decreased non-stomatal transpiration and minimal water loss by soil evaporation. In the water spending model,

the EUW would be the main component to consider in plant breeding program for improving drought resistance, and it is relevant to improve EUW when there is still soil water available at maturity or when there is potential for deep-rooted genotypes to access water deep in the soil profile that is not normally available (Araus et al., 2002).

Root play a vital role in the absorption of water and nutrients in plants. Phenotypic evaluations of root traits in common bean under drought stress have shown the importance of different rooting patterns; including deep rooting providing water from deeper soil layers (Sponchiado et al., 1989; White and Castillo 1992; Lynch and Ho 2005; Polania et al., 2009; Polania et al., 2012; Beebe et al., 2013; Beebe et al., 2014; Rao 2014; Burrige et al., 2016). Different ideotypes of root system have been proposed for better crop adaptation to individual and combined abiotic stress conditions (Yang et al., 2013; Rao et al., 2016). One of the root ideotypes proposed to optimize water and N acquisition is the "steep, cheap and deep - SCD" (Lynch 2013). One premise of this ideotype is that, the availability of water and nitrogen (N) is better in deeper soil strata over the growing season (Lynch 2013). This SCD ideotype includes: early root vigor, large root biomass, larger root surface area, greater N uptake capacity of root cells, greater water uptake through enhanced transpiration and greater association with organisms fixing N (Lynch 2013; Rao et al., 2016). However, it is noteworthy that some of the key root traits contributing to improved adaptation to soils with low fertility are increased fine root formation and root hairs (Eissenstat 1992; Lynch 2011; Lynch 2013; Rao et al., 2016). Fine roots and root hairs can explore a large volume of soil and have a low carbon and energy requirement for their function (Eissenstat 1992; Huang and Fry 1998; Polania et al., 2009; Butare et al., 2011; Lynch 2011; Lynch 2013; Rao et al., 2016).

Increased capacity for water and nutrient extraction and higher crop growth rate must be accompanied by an improved harvest index (HI). Better remobilization of photosynthates to the grains is essential for the success of high yielding genotypes under drought stress (Polania et al., 2016a,b). Several studies using common bean have demonstrated the importance of better plant growth, accompanied by a superior

photosynthate remobilization from plant structures to pod formation (pod partitioning index), and subsequently to grain filling and yield (pod harvest index) in improving drought resistance and adaptation to low fertility soils (Klaedtke et al., 2012; Assefa et al., 2013; Beebe et al., 2013; Rao et al., 2013; Yang et al., 2013; Beebe et al., 2014; Araújo et al., 2015; Polania et al., 2016a,b). Moreover it is possible to improve symbiotic nitrogen fixation (SNF) ability of common bean during drought stress (Devi et al., 2013; Polania et al., 2016c).

Strategic combination of different shoot and root traits seems to be the key in further improving the resistance to drought in common bean (Araújo et al., 2015). For this reason it is important to identify the role of root traits in improving drought resistance using the same group of bean genotypes and to test the relationships between root traits and shoot traits. Moreover, testing the relationships between root traits, SNF ability, and nutrient acquisition under drought stress may further contribute to improve sustainable bean production under water shortage.

The main objectives of this study were:

- (i) To identify specific root traits that contribute to improved resistance to drought and that could be useful as selection criteria in breeding beans for drought resistance
- (ii) To identify genotypes with desirable root traits that could serve as parents in breeding programs aiming to improve drought resistance and SNF ability during drought stress.

## 3.2 Materials and methods

### 3.2.1 Plant material

For this study, a set of 36 bush bean genotypes described in the chapter 1 (Chapter 1, Table 1) were used: ALB 6, ALB 60, ALB 74, ALB 88, ALB 213, BAT 477, BAT 477\_NN, BFS 10, BFS 29, BFS 32, BFS 67, DOR 390, G 40001, INB 827, INB 841, MIB 778, NCB 226, NCB 280, Pérola, RCB 273, RCB 593, SCR 2, SCR 9, SCR 16, SEA 15, SEN 56, SER 16, SER 48, SER 78, SER 118, SER 119, SER 125, SMC 43, SMC 141, SXB 412 and Tío Canela 75.

### 3.2.2 Experimental conditions

A greenhouse study was conducted at CIAT using transparent plastic cylinders (120 cm long, 7.5 cm diameter) with a Mollisol from Palmira, Colombia (Polania et al., 2009). Soil cylinders were carefully packed with soil: sand mixture (2:1), with a final bulk density of 1.4 g cm<sup>-3</sup>. The seeds were germinated in paper towels and uniform seedlings were selected for transplanting to transparent plastic cylinders, each of which was inserted into PVC sleeve-tubes (Fig. 14). Plants were grown for 45 days in these plastic cylinders/PVC sleeve-tubes with an average maximum and minimum temperature of 34 °C and 21 °C.

### 3.2.3 Experimental design

A randomized complete block design (RCB) with three replications was used. One level of water supply treatment was applied: progressive water stress (WS) with no watering after 10 days of growth in order to simulate terminal drought stress conditions. The initial soil moisture was 80% of field capacity. Plants received no water application and each cylinder was weighed at 2 day intervals for the determination of decrease in soil moisture content until the time of plant harvest.





Figure 14. Soil cylinder system used for phenotypic evaluation of 36 bean genotypes under greenhouse conditions at CIAT Palmira, Colombia (A). Bean line NCB 226 with its fine root system development under drought stress conditions (B).

#### 3.2.4 Physiological measurements

Plants were harvested at 45 days after transplanting (35 days of withholding of water application to induce water-stress treatment). Visual rooting depth was measured during the experiment at 7 day intervals using a ruler with cm scale, registering the total depth reached by the roots that were visible through the plastic cylinder. Root growth rate per day was calculated. At harvest, leaf area (LICOR model LI-3000), shoot biomass and root production were measured. The roots in each cylinder were washed free of soil and sand. The washed roots were scanned as images by a desk scanner. From the scanned images, total root length ( $\text{m plant}^{-1}$ ) and proportion of fine roots or proportion of roots (%) with diameter less than 0.5 mm, were measured through image analysis using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Total

root and shoot dry weight per plant was determined after the roots and shoots were dried in an oven at 60 °C for 48 h.

### 3.2.5 Statistical analysis

All data were analyzed using the SAS (v 9.0) PROC MIXED and PROC CORR (SAS Institute Inc., 2008). The adjusted means for each genotype were obtained using the mixed models theory together with the MIXED procedure considering the effects of the replications and blocks within replications as random and genotypes as fixed. Correlation coefficients were calculated by the PROC CORR. In the following text, values marked with \*, \*\* or \*\*\* are statistically significant at probability levels of 0.05, 0.01 and 0.001, respectively. The different root traits measured under greenhouse conditions using a soil tubes system, were correlated with grain yield and other shoot traits such as grain carbon isotope discrimination (CID), %nitrogen derived from the atmosphere using grain sample (%Ndfa-G), %nitrogen derived from the soil using grain tissue (%Ndfs-G) and shoot N uptake in  $\text{kg ha}^{-1}$  that were determined under field conditions as described in the chapters 1 and 2. Principal component analysis (PCA) with root and shoot traits was performed based on the correlation matrix using the PRINCOMP (principal components) procedure from SAS (SAS Institute Inc. 2008).

### 3.3 Results

A positive and significant correlation was observed between different root traits and grain yield under drought conditions (Table 6). Grain yield was correlated with total root biomass ( $r=0.28^{**}$ ), total root length ( $r=0.22^*$ ) and total root volume ( $r=0.22^*$ ). Also a significant and positive correlation ( $r=0.58^{***}$ ) was observed between total root length and fine root proportion (Table 6). A negative relationship was observed between grain CID and fine root proportion under drought conditions ( $r=-0.27^{**}$ ) (Table 6). Ten lines NCB 280, RCB 273, SEA 15, ALB 60, DOR 390, BFS 67, ALB 6, SMC 141, SCR 16 and SER 119 were superior in their visual root growth rate under drought conditions and these were classified as high root growth genotypes (Fig. 15). Eight genotypes Tio Canela 75, G 40001, BFS 10, SEN 56, MIB 778, INB 841, SER 48 and SCR 9 showed lower root growth rate under drought stress and these were classified as low root growth genotypes (Fig. 15). These two groups of genotypes also presented the highest and lowest visual rooting depth, respectively, at 43 days after planting under drought stress.

A wide range of diversity in total root length was observed under drought conditions (Fig. 16). Eight lines SEA 5, NCB 280, BFS 29, SER 16, SER 119, ALB 60, SMC 141 and SER 78 combined higher values of total root length with superior grain yield under drought stress (Fig. 16). Four drought sensitive genotypes ALB 88, Tio Canela 75, SMC 43 and Perola showed a poor root system with lower grain yield under drought stress (Fig. 16). In contrast with the above four genotypes, seven genotypes G 40001, SEN 56, NCB 226, SCR 16, RCB 593, SER 125 and BFS 10 were superior in their grain yield under drought stress with less root vigor compared with the other genotypes tested (Fig. 16). Contrary to this observation, two genotypes MIB 778 and DOR 390 showed vigorous root system but lower grain yield under drought stress (Fig. 16).

Table 6. Correlation coefficients ( $r$ ) between visual root growth rate in  $\text{mm day}^{-1}$  (VRGR), total root biomass in  $\text{g plant}^{-1}$  (TRB), total root length in  $\text{m plant}^{-1}$  (TRL), mean root diameter in mm (MRD), total root volume in  $\text{cm}^3$  (TRV), fine root proportion in % (FRP), canopy biomass in  $\text{kg ha}^{-1}$  (CB), grain yield in  $\text{kg ha}^{-1}$  (GY) and grain C isotope discrimination in ‰ (GCID) of 36 bean genotypes grown under drought conditions at Palmira.

	<i>VRGR</i>	<i>TRB</i>	<i>TRL</i>	<i>MRD</i>	<i>TRV</i>	<i>FRP</i>	<i>CB</i>	<i>GY</i>	<i>GCID</i>
<i>VRGR</i>	1.00								
<i>TRB</i>	0.47***	1.00							
<i>TRL</i>	0.46***	0.72***	1.00						
<i>MRD</i>	-0.11	-0.07	-0.61***	1.00					
<i>TRV</i>	0.38***	0.68***	0.38***	0.39***	1.00				
<i>FRP</i>	0.07	0.09	0.58***	-0.95***	-0.40***	1.00			
<i>CB</i>	0.00	0.29**	0.29**	-0.20*	0.09	0.20*	1.00		
<i>GY</i>	0.12	0.28**	0.22*	-0.05	0.22*	0.04	0.70***	1.00	
<i>GCID</i>	-0.02	0.08	-0.12	0.29**	0.25*	-0.27**	0.32***	0.45***	1.00

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

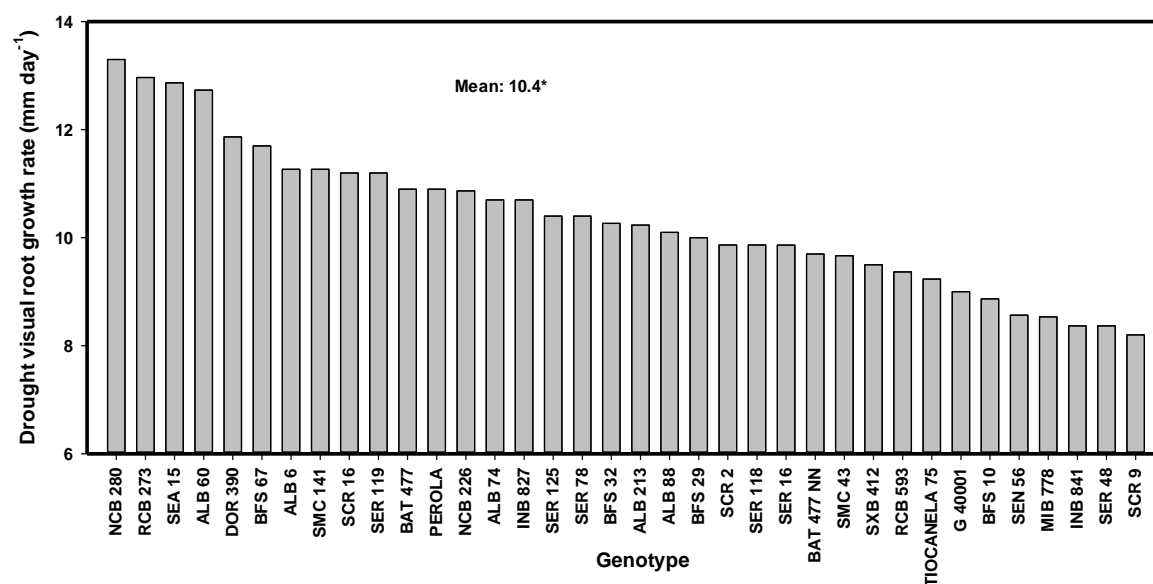


Figure. 15 Genotypic differences in visual root growth rate under drought conditions in Palmira.

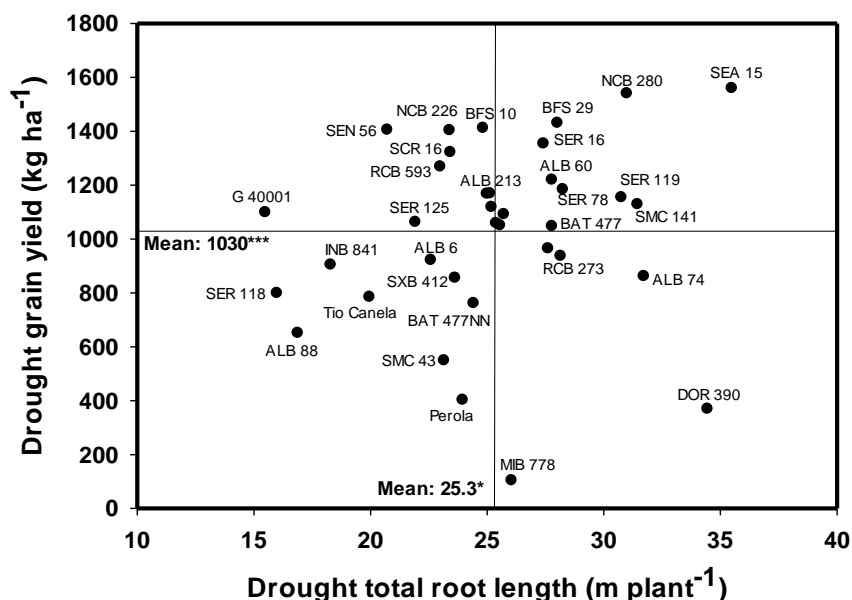


Figure 16. Identification of genotypes with greater values of grain yield (field conditions) and total root length (greenhouse conditions) under drought stress in Palmira. Higher yielding genotypes with greater values of total root length were identified in the upper, right hand quadrant.

Results on total root biomass under drought stress were similar to the results observed on total root length. The lines with superior root length also presented superior root biomass. Eight lines SEA 5, NCB 280, BFS 29, SER 16, SER 119, RCB 593, ALB 213, and SMC 141 combined higher values of total root biomass with superior grain yield under drought stress (Fig. 17). The five drought sensitive genotypes ALB 88, Tio Canela 75, SMC 43, MIB 778 and Perola showed lower values of root biomass with lower grain yield under drought stress (Fig. 17). Four genotypes G 40001, SEN 56, NCB 226 and BFS 10 were superior in their grain yield under drought stress and these also showed lower values of root biomass compared with the other lines tested (Fig. 17). The commercial variety DOR 390 presented a vigorous root system in terms of root biomass but lower grain yield under drought stress (Fig. 17).

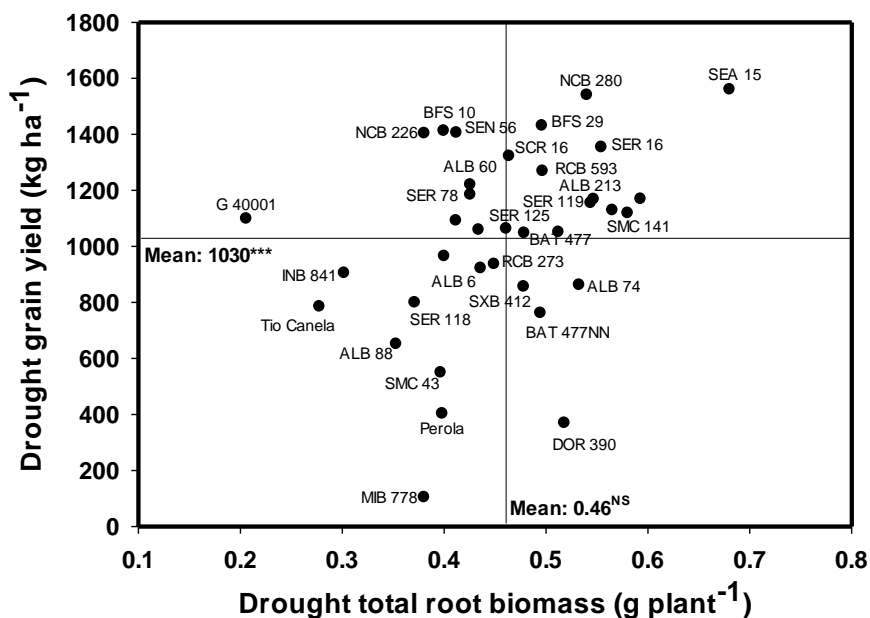


Figure 17. Identification of genotypes with greater values of grain yield (field conditions) and total root biomass (greenhouse conditions) under drought stress in Palmira. Higher yielding genotypes with greater values of root biomass were identified in the upper, right hand quadrant.

No correlation was observed between fine root proportion and grain yield under drought stress. Several lines with superior total root length showed higher proportion of fine roots under drought conditions (Fig. 18). Ten genotypes MIB 778, NCB 226, SER 78, SCR 9, RCB 273, SEA 15, NCB 280, BAT 477, G 40001 and DOR 390 showed development of fine roots under drought stress (Fig. 18). Twelve lines SMC 141, RCB 593, SER 16, BFS 10, BFS 67, SER 118, SER 125, ALB 6, ALB 213, SXB 412, ALB 88 and SEN 56 developed greater proportion of thick roots (Fig. 18). The accession of *P. acutifolius* (G 40001) and the inter-specific line between *P. acutifolius* and *P. vulgaris* (INB 841) presented a fine root system but with relatively low values of total root length and total root biomass under drought stress (Figs. 16, 17, 18).

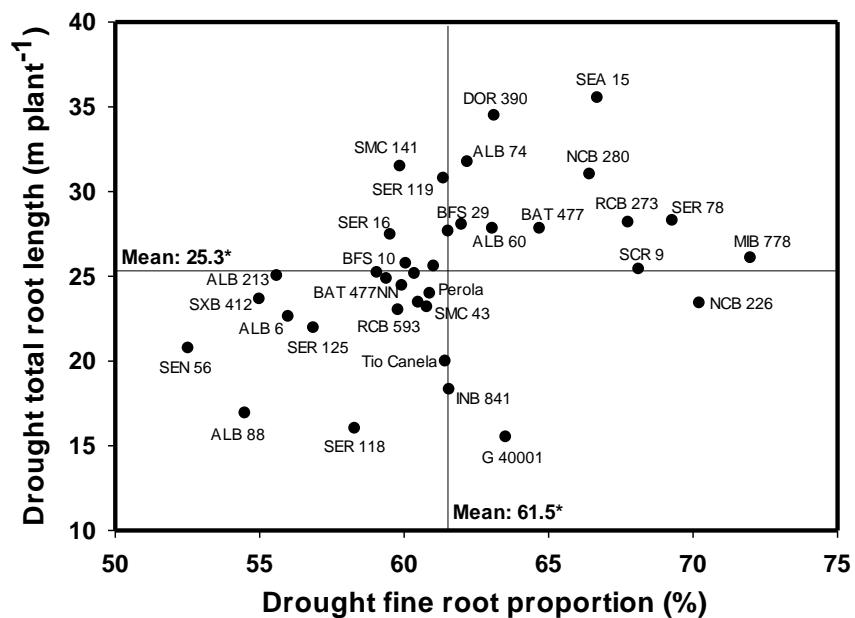


Figure 18. Identification of genotypes with greater values of total root length (TRL) and fine root proportion (FRP) under drought stress in Palmira. Higher TRL genotypes with greater values of FRP were identified in the upper, right hand quadrant.

A positive and significant correlations under drought stress conditions were observed between: mean root diameter and %Ndfa-G ( $r=0.43^{***}$ ); fine root proportion and %Ndfs ( $r=0.46^{***}$ ); fine root proportion and shoot N uptake in  $\text{kg ha}^{-1}$  ( $r=0.37^{***}$ ); total root length and shoot N uptake in  $\text{kg ha}^{-1}$  ( $r=0.39^{***}$ ). Seven lines NCB 226, SER 78, SCR 9, SEA 15, NCB 280, BAT 477 and G 40001 combined fine root system development (Fig. 18) with superior N uptake from the soil (Fig. 10 Chapter 2) under drought stress. Nine lines SMC 141, RCB 593, SER 16, BFS 10, BFS 67, SER 125, ALB 6, SXB 412 and SEN 56 combined thicker root system (Fig. 18) with better symbiotic nitrogen fixation (SNF) ability (Fig. 9 Chapter 2) under drought stress conditions. Five lines NCB 280, BFS 29, SER 16, SER 119 and BAT 477 combined higher values of total root length (Fig. 16) with higher values of shoot N uptake in  $\text{kg ha}^{-1}$  under drought stress conditions.

Multivariate analysis showed that the first three components of PC analysis explain the 61% of the variability observed in the shoot and root phenotyping of 36 bean lines under drought conditions (Table 7). In component 1, the traits with the largest contribution to variability were: grain yield, canopy biomass, pod harvest index, harvest index, seed number per area, total N uptake using grain tissue and total N fixed from atmosphere using grain tissue for estimation (Table 7). In component 2, the traits with the largest contribution to variability were: visual rooting depth at day 43 after planting, root growth rate, total root biomass, total root length and root volume (Table 7). The PC analysis suggested that under drought conditions, yield was primarily associated with canopy biomass, pod harvest index, harvest index, seed number, N derived from the atmosphere and N derived from the soil. A negative association of yield under drought was associated with days to flowering (Table 7). Yield was also associated with root traits such as visual rooting depth, root growth rate, total root length, total root biomass and root volume (Table 7). The PC analysis showed that grain yield under drought stress conditions is associated with earliness, a vigorous root system, superior plant growth, increase in partitioning of dry matter to grain and greater sink strength.

Table 7. Eigen values and percent of total variation and component matrix for the principal component axes.

<b>Principal components</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>
Eigen values	9.67	3.88	3.05	2.33	2.30	1.39	1.09
% of variance	5.79	0.83	0.72	0.03	0.91	0.30	0.37
Cumulative	0.36	0.50	0.61	0.70	0.79	0.84	0.88
<b>Component Matrix</b>							
DF	<b>-0.213</b>	0.143	0.176	0.014	0.137	<b>0.314</b>	0.237
DPM	-0.187	0.195	0.183	-0.018	0.159	<b>0.422</b>	0.163
LSCON	0.134	0.152	0.044	0.093	<b>0.295</b>	0.083	<b>-0.504</b>
CID-G	0.146	0.034	<b>0.265</b>	-0.080	0.210	0.226	-0.140
LAI	0.137	0.186	-0.012	0.226	-0.023	<b>0.429</b>	<b>0.396</b>
CB	<b>0.255</b>	0.008	-0.241	0.090	-0.186	0.122	0.085
PHI	<b>0.251</b>	-0.111	0.067	-0.023	0.142	<b>-0.315</b>	0.184
PPI	0.209	-0.094	<b>0.285</b>	-0.098	0.216	0.043	-0.087
HI	<b>0.266</b>	-0.104	0.158	-0.116	0.180	0.043	-0.060
GY	<b>0.303</b>	-0.012	-0.067	0.049	-0.032	-0.013	-0.151
100SW	0.124	0.238	0.238	-0.053	-0.204	0.212	<b>-0.376</b>
SNA	<b>0.257</b>	<b>-0.207</b>	-0.057	-0.068	0.161	0.033	0.173
PNA	0.247	-0.174	0.001	-0.010	0.192	0.150	0.208



Principal components	1	2	3	4	5	6	7
%Ndfa-G	0.152	0.033	0.232	<b>-0.328</b>	<b>-0.361</b>	-0.020	0.164
%Ndfs-G	-0.152	-0.033	-0.232	<b>0.328</b>	<b>0.361</b>	0.020	-0.164
N-Upt-SH	0.221	-0.093	<b>-0.269</b>	0.181	-0.063	0.102	0.134
N-Upt-G	<b>0.279</b>	-0.005	-0.149	0.180	0.020	0.058	-0.129
TNdfa-G	<b>0.257</b>	0.054	0.097	-0.149	<b>-0.300</b>	0.077	-0.082
P-Upt-SH	0.230	-0.036	<b>-0.296</b>	0.052	-0.054	0.222	-0.016
PSEED	<b>-0.216</b>	-0.018	-0.214	-0.008	-0.218	0.110	-0.100
VRD43	0.093	<b>0.362</b>	-0.038	-0.124	<b>0.250</b>	<b>-0.252</b>	0.192
RGR	0.095	<b>0.361</b>	-0.036	-0.125	<b>0.251</b>	<b>-0.251</b>	0.190
TRB	0.126	<b>0.396</b>	-0.054	0.198	-0.066	0.000	-0.048
TRL	0.064	<b>0.408</b>	-0.187	-0.090	-0.037	-0.088	0.004
MRD	0.026	-0.074	<b>0.345</b>	<b>0.469</b>	-0.103	-0.124	0.047
RV	0.075	<b>0.324</b>	0.104	<b>0.306</b>	-0.171	-0.196	0.016
FRP	-0.039	0.105	<b>-0.338</b>	<b>-0.442</b>	0.087	0.158	-0.119

DF: days to flowering, DPM: days to physiological maturity, LSCON: leaf stomatal conductance, CID-G: carbon isotope discrimination-grain, LAI: leaf area index, CB: canopy biomass, PHI: pod harvest index, PPI: pod partitioning index, HI: harvest index, GY: grain yield, 100SW: 100 seed weight, SNA: seed number per area, PNA: pod number per area, %Ndfa-G: % N derived from the atmosphere using grain, %Ndfs-G: % N derived from the soil using grain, N-Upt-SH: shoot N uptake, N-Upt-G: grain N uptake, TNdfa-G: grain N fixed, P-Upt-SH: shoot P uptake, PSEED: seed P content, VRD43: visual rooting depth at 43 days after planting, RGR: root growth rate, TRB: total root biomass, TRL: total root length, MRD: mean root diameter, RV: root volume, FRP: fine root proportion.

### 3.4 Discussion

This study permitted evaluating shoot and root traits related with drought resistance in advanced lines developed over several cycles of breeding. Previous research showed that deep rooting (Sponchiado et al., 1989; White and Castillo, 1992; Polania et al., 2009, 2012; Beebe et al., 2013, 2014; Rao, 2014) and increased production of fine roots (Eissenstat, 1992; Huang and Fry, 1998; Polania et al., 2009; Butare et al., 2011; Beebe et al., 2014) could contribute to greater level of drought resistance in common bean. There is a close relationship between root development and shoot development. Shoot growth provides the root with carbon and certain hormones while root growth provides the shoot with water, nutrients and hormones. In order to increase grain yield through a better plant growth under drought stress conditions, the root system must be able to supply water and nutrients to the new plant growth without sequestering too much photoassimilate from the shoot (Bingham, 2001). The identification of the root traits that are more suited to specific agroecological niches of the crop will play an important role in the development of new varieties adapted to different types of drought stress (Araújo et al., 2015).

The results from this study showed marked diversity in root system development under drought stress. Several genotypes, some of them classified as water spenders such as SEA 15, NCB 280, SCR 16, SMC 141, BFS 29, BFS 67 and SER 119 (Chapter 1) showed the ability to combine a vigorous and deeper root system with superior grain production under drought stress. The vigorous and deeper root system of these genotypes allows the plant to access greater amounts of available water, allowing the processes of gas exchange and carbon accumulation to continue and when this ability is combined with a better photosynthate partitioning towards grain, this results in a better grain yield under drought stress. Thus the strategy of these type of water spending genotypes with deeper roots and better water extraction capacity is to continue to support the rate of photosynthesis and the accumulation of water soluble carbohydrates in the stem, and their posterior remobilization to grain filling as it was observed in some wheat genotypes (Lopes and Reynolds, 2010). It is also noticeable,

that the line SER 16 and its progeny ALB 60, classified as water savers (Chapter 1), presented a deeper and vigorous root system under drought stress, indicating that the stomatal regulation is a key mechanism in the water saving strategy of these genotypes. This stomatal regulation of the line SER 16 was reported in a previous study conducted under greenhouse conditions where this line was characterized as responsive to soil drying by closing its stomata sooner than the other genotypes during progressive soil drying (Devi et al., 2013).

Several studies on root traits have demonstrated the contribution of a deep rooting system that increase water extraction from lower soil depth and its relationship with drought resistance (Sponchiado et al., 1989; White and Castillo, 1992; Lynch and Ho, 2005; Ryser, 2006; Polania et al., 2009, 2012; Asfaw and Blair, 2012; Beebe et al., 2013, 2014; Rao, 2014). These deep roots develop from the basal root that change their root angle to turn downward, or from lateral roots that develop from a tap root, or both (Bonser et al., 1996; Ho et al., 2005; Basu et al., 2007; Lynch, 2011; Miguel et al., 2013; Beebe et al., 2014).

The genotypes classified as water savers based on the values of CID and stomatal conductance (Chapter 1), such as BFS 10 and G 40001 (*P. acutifolius*) combined higher grain yield under drought stress with slow growth of a shallow root system (Figs. 15, 16). These two genotypes showed a strategy of water conservation and higher WUE, combined with a better remobilization of photosynthates to grain formation, resulting in better performance under drought stress. These genotypes could be more suitable to bean farmers in semiarid to dry environments, dominated by terminal type of drought stress in Central America, Africa, northern Mexico and northeast Brazil. The strategy of these water saving genotypes can be complemented with shoot traits related with conserving water at vegetative growth stage, such as lower leaf conductance, smaller leaf size, smaller leaf canopy, that would make more water available for reproductive development and grain filling, resulting in better grain yield under terminal drought stress conditions (Zaman-Allah et al., 2011; Araújo et al., 2015). The response of tepary bean accession, G 40001, from evaluation of root traits and

also shoot traits (Chapter 1) confirm that this species could serve as a model for improving resistance to terminal drought (Rao et al., 2013) since it combines several desirable traits such as early maturity, greater ability for photosynthate remobilization to grain, fine roots, small leaves for reduced water use, and stomatal control to minimize transpirational water loss (Mohamed et al., 2005; Butare et al., 2011; Rao et al., 2013; Beebe et al., 2014).

A poor root system can limit the optimal plant development and grain production under drought stress. Four drought sensitive lines ALB 88, Tio Canela, SMC 43 and Perola were characterized by low root production, with a low rate of root growth and shallow root development under drought conditions. Thus selection only based on root system characteristics is not enough without the proper combination of other desirable shoot traits. The results from this study indicate that the commercial line DOR 390 with its vigorous and deeper root system, appears to allocate greater proportion of carbon to root growth at the expense of grain production under drought stress. It is important to determine what size and what kind of distribution of root system across soil profile is required for a specific type of soil and specific type of drought to minimize trade-offs or any restriction to shoot growth and yield (Bingham, 2001).

The results from this work showed the relationship between root system and SNF ability and mineral N uptake from the soil under drought stress. Several genotypes showed the ability to combine superior grain production under drought stress with better SNF ability and increased presence of thicker root system. This relationship is perhaps due to an increased carbon supply to nodules under drought stress from the stored carbohydrates in thicker roots. Large root diameter is known to correlate with greater sink strength (Thaler and Pages, 1996). Previous evaluations with the drought resistant check BAT 477, showed that this line maintained a relatively higher level of SNF under drought stress; possibly due to a deep and vigorous root system that accessed water from deeper soil layers to avoid drought and to alleviate stress on SNF process (Castellanos et al., 1996; Araújo et al., 2015). Also, the positive relationship observed between fine roots proportion and mineral N uptake from the soil, highlight

the importance of fine root system to acquire mineral N from soil. The production of fine roots can be a strategy to facilitate absorption of water and mineral N when the available water in soil is limited; fine roots are "economical to build" but are essential for acquiring water and nutrients due to their high surface area per unit mass (Eissenstat, 1992; Huang and Fry, 1998). The correlations observed between grain yield, shoot N uptake in  $\text{kg ha}^{-1}$  and root traits such as fine root proportion and total root length, validate the importance of a vigorous, deeper and cheap root system to confront the challenges imposed by the combination of drought and low soil fertility stress conditions (Lynch, 2013).

A bean genotype that could combine earliness, deep rooting and better photosynthate mobilization could be more resilient for use in smallholder farm conditions minimizing risk from climate change and low soil fertility (Beebe et al., 2014; Rao, 2014; Araújo et al., 2015). A very vigorous root system contributes to greater acquisition of water and nutrients to support the vegetative growth of the shoot but if this is not combined with greater ability to partition dry matter to grain, this could lead to poor grain yield under drought stress. Thus a vigorous and deeper root system, with rapid growth rate is useful but not enough to have resistance to drought in common bean. Our results indicate that for water spender type of genotypes, a strategic combination of root and shoot traits such as deep root system combined with the ability to remobilize photosynthates from vegetative structures to the pods and subsequently to grain production could contribute to superior performance under intermittent drought stress (Beebe et al., 2014; Rao, 2014). It also appears that for water saving genotypes, a combination of development of fine root system with high water use efficiency mechanisms at leaf level will contribute to improved adaptation to prolonged or terminal drought stress (Polania et al., 2016b).

In common bean, a universal ideotype of genotype resistant to drought would not be appropriate to target to diverse agroecological niches in the tropics. There is need to develop ideotypes of bean resistant to drought according to the type of drought, climate and soil. Phenotypic evaluation of shoot traits under field conditions (Chapter 1 and 2)

and root traits under greenhouse conditions (this chapter) allow the classification of the genotypes tested into two groups, water savers and water spenders, that allows for targeting to specific agro-ecological niches. This effort also contributes to identification of morpho-physiological traits that are associated with each group. The water spenders' genotypes should be useful for cultivation in areas exposed to intermittent drought stress with soils that can store greater amount of available water deep in the soil profile. The main morpho-physiological characteristics of water spenders type of genotypes are summarized in Table 8. The water savers' genotypes can be more suitable to farmers in semiarid to dry environments dominated by terminal type of drought stress, and the specific morpho-physiological characteristics associated with these genotypes are listed in Table 8.

Table 8. Root and shoot traits related to the water saving ideotype and the water spending ideotype proposed for targeting improved common bean genotypes to drought prone agroecological zones.

<b>Ideotypes</b>	<b>Water savers' ideotype</b>	<b>Water spenders' ideotype</b>
<b>Root and shoot traits</b>	<ul style="list-style-type: none"> <li>• Intermediate to shallow rooting system</li> <li>• Intermediate root growth rate and penetration ability</li> <li>• Fine root system</li> <li>• Lower SNF ability</li> <li>• Earliness</li> <li>• High water use efficiency</li> <li>• Reduced transpiration rate</li> <li>• Less carbon isotope discrimination</li> <li>• Limited leaf area and canopy biomass development</li> <li>• Reduced sink strength</li> <li>• Superior photosynthate remobilization to pod and grain formation</li> </ul>	<ul style="list-style-type: none"> <li>• Vigorous and deep rooting system</li> <li>• Rapid root growth rate and penetration ability</li> <li>• Thicker root system</li> <li>• Moderate SNF ability</li> <li>• Earliness</li> <li>• Effective use of water</li> <li>• Moderate transpiration rate</li> <li>• More carbon isotope discrimination</li> <li>• Rapid and increased canopy biomass accumulation</li> <li>• Moderate sink strength</li> <li>• Superior photosynthate remobilization to pod and grain formation</li> </ul>
<b>Targeting to specific agroecological niches</b>	Zones with terminal drought stress and soils with lower capacity to store available water deep in the soil profile	Zones with intermittent drought stress and soils that can store greater amount of available water deep in the soil profile

## Conclusions

Results from this study demonstrate that drought resistance in common bean is related with a better developed root system that helps the plant to access water, to moderate transpiration rates and vegetative growth. Several lines of the water spender type were identified as drought resistant and their resistance was associated with effective use of water (EUW) probably resulting from a deeper root system, higher canopy biomass production and improved partitioning of photosynthates to grain. A few lines of the water saver type combined higher water use efficiency (WUE) with a relatively shallower root system and better photosynthate partitioning under drought stress. Better SNF ability under drought stress was related with superior presence of thick roots. Superior N uptake from the soil was associated with a large root system with more presence of fine roots. Seven lines SEA 15, NCB 280, SCR 16, SMC 141, BFS 29, BFS 67 and SER 119 combined the shoot and root traits of water spending ideotype characterized by superior grain production and a vigorous and deeper root system under drought stress. Four genotypes (RCB 593, SEA 15, NCB 226 and BFS 29) that were superior in their SNF ability under drought stress were also identified and these could serve as parents for further improvement of common bean in the face of climate change.

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## General Conclusions

Drought resistance is a complex trait, depending on the species, the environments where the crop is grown, and the type of drought that prevails in the target environment. In the case of common bean, physiological traits related to drought adaptation are equally diverse, and no single trait stands out for its unique and dominant contribution to drought resistance. Previous studies have reported the contribution of individual traits such as carbon isotope discrimination, canopy biomass, harvest index and pod harvest index for improved drought resistance in common bean. However, the combination of these traits and how those together contribute to a better adaptation to drought stress has not previously been explored. The results from this thesis indicate that resistance to drought in common bean is related to deep rooting helping the plant to access more water thus allowing transpiration to continue to facilitate vegetative growth. In others words, a more effective use of water (EUW), combined with the ability to remobilize photosynthates from vegetative structures to the pods and subsequently to the seed production, yields a superior number of pods and seeds per area under drought stress.

Based on phenotypic differences in grain carbon isotope discrimination, leaf stomatal conductance, canopy biomass and grain yield under drought stress, the genotypes tested were classified into two groups, water savers and water spenders. This grouping facilitates targeting genotypes to specific agro-ecological niches. Six lines NCB 280, SMC 141, SCR 16, SEN 56, BFS 67 and NCB 226 were identified as drought resistant and classified as water spenders; and their resistance was associated with superior EUW combined with a deeper and vigorous root system, higher canopy biomass and better photosynthate remobilization to pod and grain production. This important role of EUW in drought resistance implies that the understanding of the factors controlling the deep rooting and water status of the plant would be of great importance to improve drought resistance. These genotypes should be useful for cultivation in areas exposed to intermittent drought stress in Central America, South America, and Africa, particularly in agro-ecological regions where rainfall is intermittent during the season

and soils that can store greater amount of available water deep in the soil profile. A few other drought resistant genotypes were identified as water savers and these combine higher WUE with better photosynthate partitioning and shallow root system. These lines were BFS 10, SER 16, ALB 6, ALB 60 and G 40001. These can be more suitable to bean farmers in semiarid to dry environments, dominated by terminal type of drought stress in Central America, Africa, northern Mexico and north-east Brazil.

Additionally, the results from this work contributed to development of an alternative method to quantify phenotypic differences in symbiotic nitrogen fixation (SNF), having validated the application of the technique of  $^{15}\text{N}$  natural abundance using grain tissue at harvest time. Estimates of % nitrogen derived from the atmosphere using grain tissue (%Ndfa-G) are easier to implement in a breeding program due both to less labor costs and the feasibility to determine this parameter at harvest time. Using %Ndfa-G values, significant phenotypic differences were observed in SNF ability in common bean under drought stress. Also, this study found that the genotypes with more N accumulation from fixation presented higher grain yield under both irrigated and drought conditions. Four bean lines RCB 593, SEA 15, NCB 226 and BFS 29 were identified as drought resistant and with superior SNF ability under drought stress. These lines could be excellent candidates for use as parents in breeding programs.

The same set of genotypes that were evaluated for drought resistance and SNF ability were also evaluated for their differences in root system characteristics under drought stress using a soil cylinder system in the greenhouse. Differences in root characteristics were correlated with the data on shoot traits and SNF ability under field conditions. Resistance to drought stress in water spenders' genotypes was found to be related with a vigorous and deeper root system, with a rapid root growth rate and with thicker root system. In water savers' type of genotypes drought resistance was related with a moderate to shallow root system, with intermediate rate of root growth and more presence of fine root system. Better SNF ability under drought stress was found to be related with superior presence of thicker roots possibly due to greater sink strength of

these type of roots. Superior mineral N uptake from the soil under drought stress was associated with a large root system with more presence of fine roots.

Phenotypic evaluation of 36 bean lines under field and greenhouse conditions indicated that several plant traits should be considered as useful in bean breeding programs focused on improving drought adaptation. These include rooting depth, grain carbon isotope discrimination related with effective use of water, canopy biomass, % nitrogen derived from the atmosphere using grain tissue, pod partitioning index, pod harvest index, and number of pods and seeds per area. Some of these traits are easier to implement in a breeding program due to their simplicity and relatively low analytical cost. These include pod harvest index, % nitrogen derived from the atmosphere using grain tissue, carbon isotope discrimination using grain tissue, and number of seeds and pods per area. Since these parameters could be determined at harvest time, it may be easier for breeders to integrate selection for these traits into on-going breeding efforts. A major contribution of this work is identification of a few bean genotypes that combine drought resistance with SNF ability and these could serve as parents for further improvement of common bean in the face of climate variability and change.