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Exploiting Interspecific Genetic Variability for Improving Common bean for higher productivity on soils presenting biotic and abiotic stresses

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Dissertation originale présentée en vue de l`obtention du grade de docteur en sciences agronomiques et ingénierie biologique

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General Abstract :

Butare L. [2015] Exploiting Interspecific Genetic Variability for Improving Common bean for higher productivity on soils presenting biotic and abiotic stresses (PhD Thesis). Université de Liège - Gembloux Agro - Bio Tech, 210p., 28 tabl., 37 fig.

Biotic and abiotic stresses often occur in the same field of rural poor farmer households in tropical countries resulting in heavy losses of common bean yields. To improve resistance of common beans, sensitive *Phaseolus vulgaris* (SER16) was crossed to resistant *P. coccineus* (G35346-3Q) to create 94 F5:6 recombinant inbred lines (RILs) of the pedigree SER16♀ x (SER16♀ x G35346-3Q♂). The objectives of this study were to (i) identify potential parents for resistance to Al, drought and *Fusarium* root rot among 11 bean genotypes, (ii) to evaluate 94 F5:6 Recombinant Inbred Lines (RILs) of the cross SER 16♀ x (SER 16♀ x G35346-3Q♂) both for their resistance to Al and /or drought, (iii) to evaluate RILs for resistance to *Fusarium* root rot, and (iv) to identify QTL for resistance to these stresses. RILs were characterized in greenhouse for resistance to Al using a hydroponic screening employing a nutrient solution with or without 20 µM Al , to Al-toxic acid soil with high Al (HAl) and low Al (LAl) saturation, to terminal drought simulation with and without progressive soil drying, to combined stresses of Al and terminal drought in 80 cm long soil cylinder system, and to *Fusarium* root rot using inoculated perlite soil and sand (2:1). Two field studies were also carried on in Colombia under rainfed and irrigated conditions in Palmira, and high Al saturated acid soil in Santander of Quilichao. Our studies confirmed the superiority in Al response of Andean common beans in greenhouse trials compared to Middle American type for several root traits. Each screening method of our Al greenhouse experiments permitted an evaluation of different aspects of root traits. The two parents were virtually equal for tap root elongation rate at 24 h in the 20 µM Al treatment at about 1.4 mm h^{-1} while progenies ranged from less than 1-1.75 mm h^{-1}. The correlation between leaf area and total root length was highly significant under high Al saturation (r = 0.70***) for HAl-acid soil. Two genotypes (ALB88 and ALB 91) emerged as strong multiple trait lines for the two abiotic stresses. *Fusarium* root rot induced root growth inhibition as high as 80.8% for the susceptible ALB 5, while resistant RILs (ALB45, ALB41, ALB126, ALB84, ALB49, ALB34, ALB88 and ALB85) didn`t show any inhibition . Seed yield under drought stress conditions was positively associated to 100-seed weight both under irrigated field (r = 0.28**) and rainfed field (r = 0.36***), and negatively associated to days to maturity (DTM) (r = -0.36***) in field evaluation in Al-toxic acid soil in Quilichao (Colombia). QTLs for important traits including root characteristics under high Al , grain yield and yield components for drought and high Al saturation soil were identified. The use of both soil and hydroponic system, and field could contribute to evaluation of breeding materials to identify genotypes that combine Al resistance with acid soil tolerance, drought and root rot tolerance.

Keywords : Common bean, *Phaseolus vulgaris*, abiotic stress, acid soil, aluminium resistance, water stress, root growth, leaf area, biotic stress, *Fusarium* root rot, disease severity, screening methods.

Résumé Général :

Butare L. [2015] Exploitation de la Variabilité génétique pour l`Amélioration Interspécifique du Haricot commun pour une grande productivité en condition de stress biotique et abiotique du sol (Thèse de Doctorat). Université de Liège - Gembloux Agro - Bio Tech, 210p., 28 tabl., 37 fig.

Les contraintes biotiques et abiotiques du haricot commun agissent souvent ensembles dans des exploitations des fermiers pauvres des pays tropicaux, et entrainent de lourdes pertes de rendements. Dans le but d`améliorer la résistance à ces stresses, l`hybridation interspécifique en backcross de SER16♀ x (SER16♀ x G35346-3Q♂) a été réalisé pour produire 94 lignées hybrides de la génération BC $F_{5:6}$. Les objectifs de ce travail consistaient à (i) identifier les parents potentiels parmi 11 genotypes de *Phaseolus*, (ii) évaluer la performance de ces hybrides pour la résistance à la toxicité aluminique et/ou à la sécheresse, (iii) à la pourriture racinaire causée par *Fusarium solani*, et (iv) identifier des gènes QTLs responsables de la résistance à ces stresses. L`évaluation de ces hybrides pour la résistance à la toxicité aluminique a été réalisée en serre en hydroponie avec présence ou absence des ions $Al^{+3}$ (20 µM Al) et en tube cylindrique avec sol acide à saturation faible et élevée d`Al, à la sécheresse, à la combinaison du stress hydrique et d`Aluminium, et enfin pour la résistance aux maladies racinaires dues au *Fusarium*. Des essais en champs ont été réalisés pour la sélection des hybrides en structure irriguée et non-irriguée à Palmira, et en sol acide et saturation elevée en Al à Santander de Quilichao. Notre étude confirme la supériorité de la résistance à la toxicité aluminique observée chez le haricot du pool génique Andine par rapport au pool Mésoaméricain pour la plupart des caractéristiques des racines. Les essais en serre ont démontré que chaque méthode d`évaluation permet d`étudier un aspect différent du système racinaire. Alors que leur hybrides ont montré une vitesse de croissance de la racine principale (en 24h sous 20µM) qui varie de 1 à 1.75 mm h^{-1}, les 2 parents restent á un même niveau (à environ 1.4 mm h^{-1}). Une bonne corrélation a été observée entre la surface foliaire et la longueur totale des racines en sol acide avec saturation élevée en Al (r = 0.70***). Les hybrides (ALB88 et ALB 91) ont été sélectionnés comme des lignées aux caractéristiques multiples vis-à-vis des deux stresses abiotiques. L`inhibition de la croissance des racines due aux maladies racinaires a été très élevé pour l`hybride sensible ALB5 (80.8 %) alors que aucune inhibition n`a été observée chez les hybrides résistant (ALB45, ALB41, ALB126, ALB84, ALB49, ALB34, ALB88 and ALB85). Le rendement en graines se retrouve positivement associé au poids de 100 graines en structure non-irriguée (r = 0.36***) et négativement associé avec le nombre de jours á la maturité en sol acide et saturé en Al (r = -0.36^{***}). Des gènes QTLs pour certains caractères important pour les racines, le rendement en graines et quelques caractéristiques qui lui sont associés ont été identifiés. Les essais en serre et en champs devraient contribuer á l`identification des génotypes résistant á la toxicité aluminique, á la sécheresse, et aux maladies racinaires.

Mots clés : Haricot commun, *Phaseolus vulgaris*, stress abiotique, sol acide, résistance, toxicité aluminique, stress hydrique, croissance des racines, surface foliaire, stress biotique, *Fusarium*, pourriture des racines, sévérité, méthodes d`évaluation.

Abbreviations:

- ANOVA: Analyse of variance
- AFLP: Amplified fragment length polymorphism
- ASP: Aluminium saturation percent
- BC: Backcross
- CAPS: Cleavable amplified polymorphic sequence
- CIAT: International Center for Tropical Agriculture
- CTAB: Cetyl trimethyl ammonium bromide
- DF: Degree of freedom
- DII: Drought intensity index
- DSI: Drought susceptibility index
- DTF: Days to flowering
- DTM: Days to maturity
- DTZ: Distal region of the transition zone
- FAO: Food and agriculture organization of the United nations
- FRP: Fine root proportion
- GFI: Grain filling index
- GPS: Global positioning system
- HAl: High aluminium soil saturation
- ILM: Inoculum layer method
- LA: Leaf area
- LAl: Low aluminium soil saturation
- LIM: Liquid inoculum method
- LSD: Least significant difference
- MRD: Mean root diameter
- NO: Nitric oxide
- NRT: number of root tips
- PC: Percentage of control
- PCR: Polymerase chain reaction
- PDA: Potato dextrose agar
- PHI: Pod harvest index
- PVC: Polyvinyl chloride-synthetic plastic polymer
- PWBP: Pod wall biomass proportion
- OA: Organic acids
- QTL: Quantitative trait loci
- RAPD: Random-amplified polymorphic DNA
- RDW: Root dry weight
- REGWQ: Ryan-Einot-Gabriel-Welsh Q multiple comparison test
- RFLP: Restriction fragment length polymorphism
- RGR: Relative growth rate
- RILs: Recombinant inbred lines
- R:S: Root to shoot ratio
- RSA: Root surface area
- SAS: Statistical analysis system
- SDW: Shoot dry weight
- SPAD: Soil plant analysis development chlorophyll meter
- SRL: Specific root length
- SSCP: Single-strand conformation polymorphism
- SSD: Single seed descent
- SSR: Single sequence repeats
- SSRP: Single sequence repeat polymorphism or Microsatellites
- TRL: Total root length
- TRER: Tap root elongation rate
- TRER 24h: Tap root elongation rate between 0-24h
- TRER 24-48h: Tap root elongation rate between 24-48h
- TRER 48h: Tap root elongation on rate between 0-48h
- TPRL48h: Tap root length at 48 h of exposure to with and without aluminium in solution
- TPRL120h: Tap root length at 120 h of exposure to with and without aluminium in solution
- VRD29d: Visual rooting depth at 29 days
- VRD33d: Visual rooting depth at 33 days
- VRD34d: Visual rooting depth at 34 days
- WS: Water stress
- WW: Well watered

Dedication

This thesis is dedicated to my beloved wife Mary RUCIBIGANGO and childrens for all you endured during my absence. Your prayers, inspiration and support have comforted me through out all the journey.

To my parents, brothers and sisters for your words of wisdom, I dedicate to you too this work.

"I know that you can do all things; no plan of yours can be thwarted". Job 42:2

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INTRODUCTION

The genus *Phaseolus* contains five domesticated species, of which common bean (*P. vulgaris* L.), is economically the most important in the world. That species is involved in more than 90% of all *Phaseolus* bean production as a source of dietary protein in many cropping systems of developing countries (Santalla et al., 1998). Common bean is the most important food legume for direct human consumption. Wordwide annual production, including both dry and snap bean, exceeds 21 million metric tons (Miklas et al., 2006). Beans provide an important source of proteins (~22%), vitamins (folate), and minerals (Ca, Cu, Fe, Mg, Mn, Zn) for human diets, especially in developing countries (Broughton et al., 2003). The annual consumption per person in Rwanda and Burundi exceeds 40 kg. Beans are produced by resource-poor farmers in Latin America and Africa and are more susceptible to be attacked by diseases, insects and abiotic stresses (Miklas et al., 2006). In this part of the world plant growth and productivity are severely limited by the environmental stresses including heat, drought, waterlogging, low soil fertility and soil acidity. Bean root health is an essential component in managing abiotic stresses as root pathogens aggravate problems of drought or phosphorus acquisition by restricting root systems (Miklas et al., 2006).

P. coccineus L., scarlet runner bean, is a perennial species which is usually grown as an annual crop in temperate climates for its attractive flowers and fleshy, fiber-free immature pods (Santalla et al., 1998; 2004). The cross between *P. vulgaris* and *P. coccineus* remained the most common interspecific cross (Smartt, 1981), and *P. coccineus* could be a potential source of high yield for common bean (Wilkinson, 1983; Acosta-Gallegos et al., 2007). *P. vulgaris* would benefit from some of the desirable traits that have been identified in *P. coccineus*, including resistance to aluminum (Butare et al., 2011), and *Fusarium* root rot (Wallace and Wilkinson, 1965; Beebe et al., 2013), and its deep and tuberous root system associated with drought tolerance.

Aluminum toxicity is one of the most deleterious factors for plant growth in acid soil. An estimated 30-40% of the world's arable soils have a pH below 5.5 (Von Uexküll and Mutert, 1995). Aluminum is one of the most abundant minerals in the soil comprising approximately 7% of soil mass. At neutral or weakly acidic pH, it exists in insoluble forms of alumino-silicate or oxide; however, when the soil becomes more acid, it is solubilised into a phytotoxic form. The primary effect of Al is to inhibit root growth in Al-sensitive genotypes with subsequent effects on nutrient and water uptake (Foy, 1983; Rangel et al., 2007).

Common bean is particularly sensitive to Al toxicity; and development of genotypes with better root growth in Al-toxic soils is a priority (López-Marin et al., 2009). Treatment of roots with micromolar concentrations of Al^{3+} at pH $\leq$ 4.5 inhibits the root elongation within hours. Mechanisms of Al phytotoxicity are complex and models to explain it still remain unclear (Zheng and Yang, 2005; Ma, 2007; Zhang et al., 2007; Silva et al., 2010). Toxic Al levels damage roots, restrict plant size, and lower yield in most crops (Villagarcia et al., 2001). Root stunting is a consequence of Al-induced inhibition of root elongation and such roots are inefficient in absorbing both nutrients and water (Mossor-Pietraczewska, 2001). The toxic effects of Al in soil can be overcome by adding to acid soil appropriate amendments (Pandey et al., 1994; Villagarcia et al., 2001). The addition of lime to acidic soils can significantly relieve Al toxicity by increasing soil pH, but the energy cost for application or actual cost of lime often prohibits widespread adoption of this practice (Zhou et al., 2007). Significant genotypic differences in Al resistance in common bean were reported based on Al-inhibited root elongation in nutrient solution (Butare et al., 2011; Foy, 1988; Massot et al., 1999; Rangel et al., 2005; Manrique et al., 2006; Rangel et al., 2007); and under Al-toxic acid soil in polyethylene cylinders inserted into PVC pipes (Butare et al., 2011).

Among the different abiotic stresses, drought is by far the most complex and devastating on a global scale (Pennisi, 2008). Drought stress is a worldwide production constraint of common bean (Fairbain, 1993; Wortmann et al., 1988). The importance and urgency of developing high yielding drought resistant cultivars which use water efficiently, reduce dependence on irrigation water and associated production costs, increase and stabilize yield in drought-prone environments, and increase profit margins for producers can never be over emphasized (Muñoz-Perea et al., 2006). Intermittent or terminal drought affects more than 60% of dry bean production worldwide (White and Singh, 1991). Rooting pattern, especially greater root length in lower soil strata, is an important drought resistance mechanism (Sponchiado et al., 1989). Selection for drought resistance based on yield alone may not bring the required genetic shift in specific physiological attributes as different mechanism would have different opportunities for expression under different conditions (Subbarao et al., 1995). Root systems show considerable architectural variation among species, among genotypes of given species, and even with different parts of a single root system (Lynch, 1995). Drought tolerant bean genotypes could extend their roots to 1.2 m depth in drought environments, whereas the sensitive genotypes could not extend their roots beyond 0.8 m; and these differences in rooting depths were reflected in overall shoot growth and yield (White and Castillo, 1988). Wild relatives in many legumes possess deep rooting capability that could be transferred to cultivated

legumes. A number of *Phaseolus species*, such as *P. acutifolius*, *P. retensis*, and *P. coccineus*, have deep and/or tuberous primary root attributes (Singh and White 1988).

Root rot, caused by *Fusarium solani* f. sp. *Phaseoli*, is one of the main root diseases impacting production of common bean throughout the world (Chaudhary et al., 2006). Yield losses range from a trace to 80%, especially when adverse environmental conditions persist after planting and through flowering (Dryden and Van Alfen, 1984; Miller and Burke, 1986; O'Brian et al., 1991; Park and Tu, 1994). *Fusarium* root rot is often aggravated by the presence of a compacted soil, because the pathogen becomes dispersed and primarily confined to the plow layer (Burke et al., 1972a, b). Disease management options include crop rotation, correcting soil fertility levels and reducing soil compaction. However, the disease cannot be completely eliminated from fields because the pathogen can survive between dry bean crops as dark thick-walled resting chlamydospores (Abawi and Pastor-Corrales, 1990, Burke and Hall, 1991).
Moderate levels of resistance for *Fusarium* root rot from the scarlet runner bean (*Phaseolus coccineus* L.) have successfully been introgressed into common bean (Wallace and Wilkinson, 1965). Improving the levels of root rot resistance is a key element in the successful development of drought tolerance in beans (Miklas et al., 2006).

The objective of this research work was to improve resistance of common bean genotype SER16 to Al, drought, and *Fusarium solani* f. sp. using *P. coccineus* accession G35346-3Q as a source of resistance.

CHAPTER I. BIOTIC AND ABIOTIC STRESSES IN COMMON BEANS

I.1. Common bean (*Phaseolus Vulgaris*, L.)

Common bean (*Phaseolus vulgaris* L.) is the most important food legume consumed worldwide. It is one of the five cultivated species from the genus *Phaseolus* and the first in direct human consumption (Broughton et al., 2003). The genus *Phaseolus* (2n = 2x = 22) is the most import with around 70 species (Freytag and Debouck, 2002) and has contributed to human welfare with five cultigens domesticated in pre-Columbian times: the common bean (*P. vulgaris*), the year bean (*P. dumosus* Macfad.), the runner bean (*P. coccineus* L.), the tepary bean (*P. acutifolius* A. Gray), and the lima bean (*P. lunatus* L.) (Acosta-Gallegos et al., 2007).

I.1.1. Genetic diversity of Common beans

Cultivated common bean originated from two centers of diversity or two gene pools: Middle American from Central America and Mexico, and Andean from the Andes mountains of South America (Gepts et al., 1986; Koenig and Gepts, 1989; Singh et al., 1991a, b, c; Becerra et al., 1994; Tohme et al., 1996; Beebe et al., 2000, 2001). The existence of the Andean and Middle American gene pools in both the wild and the cultivated gene pools is supported by diversity analysis for phaseolin seed proteins (Gepts and Bliss, 1986; Gepts, 1988, Broughton et al., 2003, Blair et al., 2010), isozymes (Koenig and Gepts, 1989; Singh et al., 1991b), and mitochondrial and genomic RFLPs (Becerra-Velazquez and Gepts, 1994; Khairallah et al. 1992) and simple sequence repeat based DNA markers that are detected with silver stain gels or fluorescently (Broughton et al., 2003, Blair et al., 2010).

Seven races of cultivated common bean have been described and are grouped according to which of two gene pools they belong to. The Middle American gene pool has been subdivided into four genetic groups or races (Beebe et al., 2000), while three races have been defined in the Andean gene pool based on plant morphology and adaptation range (Singh et al., 1991a). The genetic resources of common bean are sub-classified into races that are made up of morphologically-similar cultivars that share equivalent agro ecological adaptation and some agronomic characteristics such as growth habit and seed type (Singh et al. 1991a; Beebe et al., 2001). The Andean gene pool is organized into three races comprising Nueva Granada, Peru, and Chile, while the Middle American gene pool constitute four races: Durango, Guatemala, Jalisco, and Mesoamerica (Blair et al., 2006; Diaz and Blair, 2006).

I.1.2. Importance of Common beans

Beans provide an important source of protein (~22%), vitamins (folate), and minerals (Ca, Cu, Fe, Mg, Mn, Zn) for human diets, especially in developing countries (Broughton et al., 2003; Acosta-Gallegos et al., 2007). Recently, some bean seed properties have been associated to major health issues, such as the control of type II diabetes were found. In this regards, the presence of essential minerals, such Fe and Zn, and high fiber and polyphenolic content played a key role in nutritional well being. Typically, consumption of bean seeds leads to lower glycemic and cholesterolemic indices and lower incidence of certain types of cancer (Andersen et al. 1984; Hangen and Bennick, 2003). Per capita consumption varies with each producing and consuming country and also among regions within a country depending on consumer preferences but can be as high as 66 kg/capita/year in Rwanda and parts of western Kenya (Broughton et al. 2003, Beebe et al., 2013). Averages in the Americas are from 4-5 kg/capita/year in the United States, to more than 10 kg/capita/year in Brazil to as much as 35 kg/capita/year in Nicaragua (CIAT, 2009).

I.1.3. Constraints of bean production

A majority of the bean production occurs under low input agriculture on small-scale farms in developing countries. Beans produced by these resource-poor farmers are more vulnerable to attack by disease and insect pests and to abiotic stresses including drought and low soil fertility (Butare et al., 2012). The major production constraints to common bean production include biotic (fungal, bacterial, and viral diseases; insect pests) and abiotic (drought, heat, nitrogen [N] and phosphorus [P] deficiency; acid soil) stress factors (Beebe et al., 2013). Intense cultivation under increasing population pressure, without fallow periods or adequate crop rotations, results in declining soil fertility or soil compaction, or both, and in build-up of pathogen inoculum in the soil (Wortmann et al., 1998).

I.1.4. Bean Productivity and production

Two general types of common beans are grown including bush beans, as a short-season crop, and climbing beans, as a long-season crop (Schoonhoven and Voysest, 1991). Bush beans produce a crop in as little as 65 days and may yield up to 2.5 t/ha per season, although average yields in Latin America are between 600 and 800 kg/ha (Blair et al., 2013), and even lower in some countries of eastern and southern Africa. Climbing beans, on the other hand, have a slightly longer growing season (100−120 days; some even up to 240 days) and have a yield potential of 4.5 t/ha (Broughton et al., 2003). One advantage of climbing beans over bush beans is that they fix larger amounts of

nitrogen (Schoonhovern et al., 1991) and yield over three time more than bush bean under similar condition.

Annual production, including both dry and snap bean, exceeds 21 million metric tons (MT), and the production is over an area of 18 million hectares with large amounts of production in developing countries of Latin America and Eastern and Southern Africa (Broughton et al. 2003). Most important producers of dry beans in the World are India leading, followed by Brazil and then Myanmar (FAO, 2010). In Africa, Tanzania leads other countries. For snap beans (or green beans), 4 important producers as China, Indonesia, India, and Turkey (FAO, 2011). Major producing countries for national consumption are Brazil and Mexico, whereby the United States, Canada, Argentina, and China are all exporting countries (Blair et al., 2013).

I.2. Effects of biotic and abiotic stresses

Plant survival, development, biomass production, and crop yield are negatively influenced by biotic and abiotic stresses. Around 60% of cultivated soils world wide have plant growth limiting problems caused by mineral nutrient deficiencies and toxicities (Cakmak, 2002). Environmental factors may affect the incidence of biotic stress, and a more challenging situation is that plants face a combination of stresses at a time of their development. Common bean (*Phaseolus vulgaris* L.) yields are severely limited by abiotic stresses, including drought and aluminum toxicity; and are also affected by an array of diseases and pests. *Fusarium* root rot caused by the soil borne pathogen of the same name, is a major limiting disease of common bean (Abawi, 1989; Naseri and Marefat, 2011). The disease may interfere with absorption and translocation of water nutrients from soil to various parts of the plant, reduce photosynthetic efficiency of plant parts or translocation of photosynthetic products through the plant and their storage, or may interfere with flowering or fruit and seed formation (Mohan, 2006).

Plants have developed and elaborated mechanisms to perceive external signals and to manifest adaptive responses with proper physiological and morphological changes to survive these challenges (Bohnert et al., 1995). In common bean, the main stem derives from the axis of the seed embryo, and the number of branches and branching pattern may vary greatly depending upon the genotype and environment (Miklas and Singh, 2007). Selection of deep and extensive root system has been advocated to increase productivity of food legumes under moisture-deficit (Subbarao et al., 1995; Serraj et al., 2004; Sarker et al., 2005). Root architecture is very important in plant productivity, and root growth requires nutrients that are absorbed from the soil and also the

photosynthates that are transported from the shoot (López-Busio et al., 2003). The plant's first defense mechanism against abiotic stress is located in its roots (Ghosh et al., 2014). The common bean root system is composed of several types of root (e.g. tap, basal, and lateral roots) whose physiological functions may be of great difference (Shen et al., 2004). López-Bucio et al. (2003) have identified three major processes that affect the overall architecture of the root system. First, cell division at the primary root meristem that enables indeterminate growth by adding new cells to the root; second, lateral root formation that increases the exploratory capacity of the root system; and third, root-hair formation that increases the total surface of primary and lateral roots. Alterations of one of these three processes by plant stresses will affect first the root system and architecture; and then plant growth and productivity. Thicker roots are proven to have greater ability to penetrate soil (Materechera et al., 1992), thus greatly influencing soil physical properties (Bengough et al., 2006). Fine roots are of great importance in the uptake of water and nutrients, and input of carbon to the soil (Liu et al., 2010).

Lack of adequate screening methodologies and novel sources of resistance for varietal improvement remain obstacles for breeding for abiotic stress resistance in common bean. Genotypic variation in adventitious root formation has been observed in several crops including common bean (Miller et al., 2003), and changes in root architecture affect plant capacity to acquire nutrients and water. Hybrids between wild and cultivated beans are fully fertile and no major barriers exist for introgression and exchange of favorable alleles and Quantitative Trait Locus (QTL) (Singh et al., 1995; Koinange et al., 1996; Zizumbo-Villarreal et al., 2005). Researchers have successfully introgressed from *P. coccineus* to common bean moderate levels of resistance for *Xanthomonas* (Miklas et al., 1994a, 1994b), for *Fusarium* root rot (Wallace and Wilkinson, 1965; Naseri and Marefat, 2011) and for white mold in dry bean (Miklas et al., 1998) as well as in snap bean (Abawi et al., 1978; Lyons et al., 1987).

Breeding for resistance to biotic and abiotic stresses constitute a key opportunity to improve yield in common bean. The improvement of crop yield has been possible through the indirect manipulation of quantitative trait loci (QTLs) that control heritability of the traits and physiological mechanisms that determine biomass production and its partitioning (Collins et al., 2008).

I.3. Aluminum stress

I.3.1. Aluminum toxicity

Al is the most abundant metallic element in the earth's crust, and considered as the most important limiting factor for plant growth in acid soil. The most productive soils in the world are already under cultivation, and those available for agricultural expansion, particularly in Latin America and Africa, are often strongly acid, possessing toxic levels of soil Al (Kamparath, 1984). Different forms of Al that occur in soil solution are $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ at pH 4-5, Al^{3+} at pH 5.5-7, and $Al(OH)_4^{-}$ at pH 7-8 (Rout et al., 2000). At acid pH, the phytotoxic Al^{3+} cation is released into the soil solution, where it inhibits root growth, hindering the ability of plants to acquire water and nutrients (Collins et al., 2008). In wheat the most toxic ion is Al^{3+}, while in dicots the more toxic ions appear to be $Al(OH)_2^{+}$ and $Al(OH)^{2+}$ (Delhaize and Ryan, 1995; Kochian, 1995). Most plants contain no more than 0.2 mg Al g^{-1} dry mass (Matsumoto et al., 1976). Plants in acid soils also suffer from deficiencies in phosphorus, nitrogen, calcium, magnesium and potassium (Samac and Tesfaye, 2003). For legumes, acid soils pose an additional challenge because their symbiotic rhizobia are acid sensitive (Hartel and Bouton, 1989).

Aluminum does not affect the seed germination but compromises new root development and seedling establishment (Nosko et al., 1988). A first symptom of Al toxicity is an inhibition of root elongation (Stass et al., 2007). In common beans, excess Al will result in a rapid inhibition of root elongation and enhanced callose synthesis in the root tips; both are sensitive indicators of Al injury in roots (Delhaize and Ryan, 1995; Rangel et al., 2007; Staß and Horst, 2009). Symptoms of Al toxicity in beans include the production of shortened roots with the presence of thickened, but fragile roots that undergo browning (Mossor Pietraszewska, 2001).

Toxic Al levels damage roots, restrict plant size, and lower yield in most crops (Villagarcia et al., 2001). Al not only causes a reduction in the length of the main root, but also changes the entire root architecture (Doncheva et al., 2005). Aluminum-injured roots become stubby and frequently acquire a brownish coloration; and fine branching roots and root hairs are also reduced. In the root apex, cracks can easily be observed in the epidermis (Viterello et al., 2005). Although symptoms of Al toxicity are also manifested in the shoots, these are usually regarded as a consequence of injuries to the root system (Viterello et al., 2005). Some authors reported that Al toxicity may decrease directly shoot growth (Ryan et al., 1993; Ali et al., 2008). Others argued that shoot growth reduction may be a consequence of root damages or a strategy to reduce transpiration rates (Silva et al., 2010). The most common responses of shoots to Al toxicity are cellular and ultrastructural

modifications in leaves, reduced stomatal opening, decreased photosynthetic activity, chlorosis and foliar necrosis (Viterello et al., 2005).

Long term exposure to Al and inhibition of root growth generally lead to nutrient deficiencies, mainly P, K, Ca and Mg (Hang and Viterello, 1996). A number of studies have shown that inhibition of root growth occurs rapidly (minutes to hours) after exposure to Al, while inhibition of cell division requires 6-24 h to occur (Čiamporová, 2002). The ultimate consequence is reduced plant biomass (Viterello et al., 2005). One well known Al-induced cell response is the synthesis of callose which is frequently used as an indicator for Al induced stress (Horst et al., 1997). Al induces also the expression of several genes (Snowden and Gardener, 1993; Snowden et al., 1995) and the synthesis of several proteins (Basu et al., 1994). In general, the genes expressed in response to Al and the proteins synthetized appear to be general stress- or wound- response genes and proteins (Sugimoto et al., 2004). But there are also other genes which may be induced by Al and which are promising for resistance, particularly transporters (like Al activated malate transporter TaALMT1, the first Al tolerant gene cloned) for organic acids (Sasaki et al., 2004).

I.3.2. Interaction between Al and other minerals

The mutual interactions of metals are very important for plant growth and development and determine the availability of metal ions under different soil conditions, such as pH or redox potential (Mossor-Pietraszewska, 2001). Aluminium induced changes in the uptake of most macroelement cations by plant roots, including reductions in the uptake of Ca (Clarkson and Sanderson, 1971; Liu and Luan, 2001; Bhalerao and Damador, 2013), Mg (Huett and Menary, 1980; Mariano and Keltjens, 2005) and K (Cumming et al., 1985; Giannakoula et al., 2008). Al interferes with uptake or transport of nutrients such as Ca, Mn, P, Mg, B, Fe, Cu or K (Keltjens and Tan, 1993, Keltjens, 1995; Lukaszewski and Blevins, 1996; Slaski et al., 1996; Taylor et al., 1998; Lidon et al., 2000; Liu and Luan, 2001; Guo et al., 2003, 2007; Olivares et al., 2009; Bhalerao and Damador, 2013). Interaction between Al and Ca are probably the most important factors affecting Ca uptake and transport in plants grown in acid soils (pH<5.5) (Mossor-Pietraszewska, 2001). It has been suggested that Al tolerance in some varieties of wheat, barley, soybean, and snapbean is associated with the ability of these species to resist Al-induced Ca deficiency or Al-induced inhibition of Ca^{2+} transport (Foy et al., 1978). The root elongation in common bean increased with increasing pH or Ca concentration (Nian et al., 2009). Ca uptake in Al-sensitive wheat lines was significantly more inhibited than in Al-tolerant lines (Huang et al., 1992). Affected root tips are stubby due to inhibition of cell elongation and cell division; and the resulting restricted root system

is impaired in nutrient and water uptake, making the plant more susceptible to drought stress (Samac and Tesfaye, 2003). With increased Al levels Ca concentration in shoots and roots in wheat decreased dramatically (Jones et al., 1998). There is information indicating that Ca and Mg accumulation in plants is depressed by Al much more significantly than the uptake of other important mineral nutrients (Rengel and Robinson, 1989; Sharma and Dubey, 2005; Huang et al., 2007). Such roots are inefficient in absorbing both nutrients and water (Mossor-Pietraczewska, 2001); and young seedlings are more susceptible than older plants.

Trim (1959), Foy (1978), and Bhalerao and Damador (2013) reported that Al is known to form strong complexes to precipitate nucleic acids. Intensification of the process of Al compounds solubilisation is connected with the degree of soil acidification caused by the washing out of alkaline metals ions (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}) from the soil and a decrease in the pH of soil solutions (Rout et al., 2001). The ions translocate very slowly to the upper parts of plants (Ma et al., 1997a; Bhalerao and Damador, 2013). Previous studies indicated that some bean genotypes of the Andean origin had superior P efficiency (Yan et al., 1995a; 1995b; Beebe et al., 1997). The large-seeded Andes genotypes were superior to the small-seeded Mesoamerican genotypes in P efficiency, and the root structure of common beans was closely related to P uptake efficiency under field conditions (Liao and Yan, 2000) but the induction of a major leaf acid phosphatase did not confer adaptation to low P availability (Yan et al., 2001). Also P-efficient soybean genotype adapted better to acid soil than P-inefficient genotype (Liang et al., 2013), and aluminum tolerance in certain pea cultivars has been associated with higher P concentrations in plant roots.

Al negatively interferes with P, Mg and K in Wheat and Al interactions with some nutrients depend on the level of plant tolerance (Silva et al., 2010). High Al concentrations in nutrient solution influenced the uptake of minerals; uptake of divalent cations particularly Ca and Mg was often disturbed by Al (Delhaize and Ryan, 1995; Foy et al., 1978, Rout et al., 2001). It was found that low concentration of Mg (200 μmol l^{-1}) improved soybean root elongation in the presence of toxic Al^{3+} concentrations, but this protective effects was not observed for wheat roots (Silva et al., 2001). Mg added to a subsurface solution compartment promotes root elongation in both the presence and absence of Al in Soybean (Silva et al., 2001). Aluminum interference with P uptake might result in P deficiency in plants grown on acid soils or in nutrient solutions (Jan and Pettersson, 1989; Rout et al., 2001; Liang et al., 2013). Keltjens and Tan (1993), Rout et al. (2001), and Gupta et al. (2013) reported that Mg was more effective than Ca in alleviating Al stress in monocotyledons whereas the reverse occurred for the dicotyledons.

I.3.3. Aluminum tolerance

Aluminum-tolerant plants may be grouped according to how Al accumulates within their tissues (Foy et al., 1978). In one group, Al concentrations in the shoots are not consistently different from those of Al-sensitive plants, but in the root Al concentrations are lower in certain tolerant cultivars of wheat, barley, soybean and pea (Klimashevskii et al., 1976). In such cases, Al tolerance apparently involves an exclusion mechanism (Rout et al., 2000). In a second group of plants, Al tolerance is associated with less Al in plant shoots, entrapment of more Al in roots or both in wheat, barley, potato (Foy et al., 1978), grass and cabbage (Huett et al., 1980). In a third group, Al tolerance is directly associated with Al accumulation by the tops; such plants have high internal tolerance to Al, particularly pine trees, tea and mangroves (Foy et al., 1978).

In many plants Al tolerance appears to be closely associated with phosphorus-use-efficiency (Mossor-Pietraszewska, 2001). Al binding by organic acids prevents the formation of P-Al complexes, which results in an increased availability of P in the root cell. Therefore, Al-tolerant plants have a lower demand for P (Mossor-Pietraszewska, 2001). The tolerant cultivars efficiently took up and utilized Ca and P in the presence of Al (Rout et al, 2001). The susceptible (Al-sensitive) and intermediate cultivars exhibited less Ca and P uptake and utilization (Sivaguru and Paliwal, 1993).

Preliminary evaluation indicated significant genotypic variation in grain yield among bean genotypes grown on Al-toxic soils (Rangel et al., 2005). These genotypic differences could be related to differences in Al resistance (Thung and Rao, 1999; CIAT, 1999). Significant genotypic differences in Al resistance in common bean were also reported on Al-induced root elongation in nutrient solution (Massot et al., 1999; Rangel et al., 2005; Manrique et al., 2006, Butare et al. 2011, 2012). Differential tolerance of plant genotypes to Al stress is a promising approach to increase our understanding of Al resistance in plants.

I.3.4. Mechanisms of Al tolerance

Numerous hypotheses for the mechanism of Al toxicity have been advanced in the literature (Kochian, 1995; Richards et al., 1998; Barcelo and Poschenieder, 2002; Illés et al., 2006), and have been broadly classified as those which prevent the entrance of Al into the plant (Al exclusion) and those that detoxify or sequester Al internally (Al tolerance) (Delhaize et al., 2007; Rengel, 1996, Kochian, 1995). Many potential mechanisms by which plants may tolerate Al are not to date supported by experimental evidence (Tang et al., 2002). Mecanisms that prevent Al from crossing

the plasma membrane, entering the symplast and reaching sensitive intracellular sites (Al exclusion) are mechanisms of Al resistance while those conferring the ability of plants to tolerate Al in the root (and/or shoot) symplast (internal resistance) are mechanisms of Al tolerance (Taylor, 1991; Kochian, 1995). The most commonly documented mechanism of Al tolerance is the Al-activated exclusion of Al-chelating anions such as malate and citrate from the root tips and subsequent formation of nontoxic Al complexes in the apoplast or rhizosphere (Collins et al., 2008). Of the two principal strategies (tolerance and avoidance) of plants for adaptation to adverse soil conditions, the strategy of avoidance is more common for adaptation to acid mineral soils (Marschner, 1991).

I.3.4.1. Aluminum exclusion

Aluminum tends to form strong complexes with oxygen donor ligands (Bhalerao and Prabhu, 2013). Extensive experimental evidence has shown that complexation with chelating root exudates or binding to mucilage play a main role in the prevention of the accumulation of phytotoxic Al^{3+} in both apoplast and symplast (Barcel`O et al., 2002). In plants, Al^{3+} makes complexes with phosphate and carboxylates secreted from the root apex, but strong complexes can also be formed with phenolic substances, pectates, mucopolysaccharides or siderophores (Winkler et al., 1986). Resistance here is achieved by exclusion of Al^{3+} from root apex. One exception is exclusion of Al^{3+} from the root tip, achieved by Al-induced release of organic acids that chelate Al ($Al^{3+)}$ and thus prevent its entry into the root apex (Miyasaka et al., 1991; Delhaize et al., 1993; Pellet et al., 1995; Ryan et al., 1995). When the mucilage was periodically removed from the root tips of cowpea with brush, root elongation was considerably more inhibited by Al, indicating a protective function of the mucilage against Al toxicity (Horst et al., 1982). The Al^{3+} in rhizosphere is bound to mucilage and blocks the entry of Al into the root. Mucilage has various protective functions against toxic metals in the soil.

Organic acids (OA) have been shown to have a central role in exclusion of Al^{3+}, although additional exclusion mechanisms have been identified (Samac and Tesfaye, 2003). A wealth of studies provide very strong evidence that Al-tolerant genotypes of wheat, corn, sunflower, soybean and common bean, among others, exclude Al from roots by excretion of organic acids that chelate Al (Jones, 1998; López-Bucio et al., 2000; Ma, 2000; Ma et al., 2001; Ryan et al., 2001; Kochian et al., 2002; Watanabe and Osaki, 2002). Of the organic acids, citrate has the highest binding activity for Al followed by oxalate, malate and suscinate (Hue et al., 1986). Negatively charged cell surfaces of the root accumulate the toxic cations, and amelioration is affected by treatment that

reduces the negativity of the cell-surface electrical potential by charge screening or cation binding (Matsumoto, 2000). The binding of Al to the fixed negative charges of the wall is followed by the precipitation of $Al(OH)_3$ and aluminium phosphate.

The most powerful candidate of Al chelating substance was an organic acid which can be synthesized in a large amount through photosynthesis (Larsen et al., 1998). The first example of a chelating substance was the citrate from snap bean under Al stress (Miyasaka et al., 1991). The root of the Al-resistant cultivar released 70 times as much citrate as in the absence of Al, and citrate exuded was 10 times that of Al-sensitive cultivars. Much of current evidence argues that Al-stimulated efflux of organic acids such as citrate, malate and oxalate from roots is an important Al resistance mechanism, although some reports demonstrated that organic acid efflux plays a minor role such as in signal grass (Wenzl et al., 2001) and spinach (Yang et al., 2005) or is not the only mechanism for Al resistance such as in maize (Piñeros et al., 2002) and buckwheat (Zheng et al., 2005). The enhanced exudation of citrate in response to Al stress has been reported in common bean (Miyasaka et al., 1991; Mugai et al., 2000; Rangel et al., 2010; Shen et al., 2002; Stass et al., 2007), maize (Pellet et al., 1995; Kollmeier et al., 2001) and soybean (Glycine max L., Silva et al., 2001; Yang et al., 2000, 2001). Citric acid is known to chelate Al strongly and to reverse its phytotoxic effects also; citric acid has been shown previously to enhance the availability of P from insoluble Al phosphates (Miyasaka et al., 1991). Al treatment enhanced the exudation of citrate from the root tips of both Quimbaya (Al resistant) and VAX-1 (Al sensitive) bean genotypes (Rangel et al., 2009). Constitutively higher OA contents of the Al-resistant common bean genotype Dade compared with the Al-sensitive Romano have been suggested to contribute to a higher potential for Al chelation and detoxification (Lee and Foy, 1986; Miyasaka et al., 1991). Many plant species are able to release organic acids in response to Al stress, such as malate in wheat (Ryan et al., 1997), oxalate in buckwheat and taro (Ma et al., 1997a, Ma and Miyasaka, 1998), citrate in maize, snapbean, soybean, Cassia tora and Triticale (Pellet et al., 1995; Ma et al., 1997b; Li et al., 2000; Yang et al. 2001).

Al-induced callose formation is restricted to the rhizodermal and root cap cells (Wissemeir et al., 1987). Callose (β-1,3-glucane) formation at plasma membrane is a very sensitive phenomenon under Al stress (Wissemeier et al., 1992). The inhibition of Ca, Mg, and water uptake seems to be possible because callose can be considered as a sealing system in plants (Eschrich, 1975; Yim and Bradford, 1998). Induction of callose (β-1,3-glucan) formation is a sensitive marker for genotypic differences in Al toxicity (Host et al., 1997). Callose may cause the blockage of cell-to-cell transport by blocking plasmodesmata (Sivaguru et al., 2000). Callose is accumulated in the cell

wall around plasmodesmata in response to the damage caused by Al in the roots of various plants (Mossor-Pietraczewska, 2001).

I.3.4.2. Internal Aluminum detoxification

Although exclusion from root tips and restriction of Al^{3+} transport to upper plant parts seems to be the most important mechanism in Al resistance, there are numerous species that tolerate relatively high Al concentrations that is based on the complexation and detoxification of Al^{3+} after its entry into the plant (Bhalerao and Prabhu, 2013). It is involve intracellular tolerance by sequestration of Al^{3+} in the symplasm. Transfer of Al^{3+} into cells and sequestration in the vacuoles might be an Al-tolerance mechanism (Vazquez et al., 1999). In internal tolerance mechanisms, absorbed Al is detoxified by the organic acids in the cytosol (Singh and Chauhan, 2011). About 100 plant species accumulate Al in their stem and leaves without showing symptoms of Al toxicity (Barcelo and Poschenrieder, 2002). The formation of a non-toxic Al complex with organic acids or other chelators, and sequestering these complexes in the vacuoles play an important role in internal detoxification of Al in Al-accumulating plants (Ma, 2006). Foy (1984) defines Al accumulator plants as those with more than 1000 mg kg^{-1} of Al in the leaves. Al tolerance is associated with Al accumulation in plant shoot as seen in *Arnica montana*, *Deschampsia flexuosa* L. (Pegtel, 1987), *Melastona malabathricum* (Watanabe et al., 1998) and *Camellia sinensis* (Matsumoto et al., 1976).

It is suggested that inhibition of cell elongation rather than cell division plays a role in short-term response to Al supply, whereas over longer periods of time (more than 24h), both elongation and division of cells are inhibited (Matsumoto, 2000). The cell wall and the cell membrane (under normal metabolic conditions) probably function as an important barrier to the passive movement of Al^{3+} into symplastic compartment (Wagatsuma, 1983). The selective permeability to Al^{3+} and negative charges on the surface of the plasma membrane are important characters which determine the passage of Al^{3+} into the symplasm (Wagatsuma and Akiba, 1989; Wagatsuma et al., 1991).

Many investigators have described that the major portion of absorbed Al^{3+} is localized in the apoplast ranging from 30 to 90% of the total Al (Tice et al., 1992; Rengel, 1996). Many studies have been done to find proteins related to Al tolerance. However, the role of these proteins or peptides on Al tolerance *in vivo* is not clear (Matsumoto, 2000). The stimulatory effect of Al on ferrous ion [Fe(II)]-induced lipid peroxidation has been proposed as one of the possible mechanisms of Al toxicity (Cakmak and Horst, 1991; Yamamoto et al., 1997a).

Because the solubility of Al is strongly pH-dependent, maintenance of a high solution pH may have reduced the solubility and toxicity of Al. Blamey et al. (1983) found that an increase in the pH of dilute nutrient solutions from 4.5 to 4.6 caused a 26% decline in solubilized Al concentration.

I.3.5. Screening methods for identifying Al resistant plants

Several methods are available to study roots of different crops in field, pot, and rhizotron, but none of them is without shortcomings (Girdthai et al., 2010). A number of screening methods have been used for identifying Al-resistant plants. These include nutrient solution culture assays based on inhibition of root growth or measurement of Al accumulation within roots, or assays that evaluate biomass accumulation (Samac and Tesfaye, 2003). Methods have been also categorized as destructive, and non-destructive that allow many observations on roots during experimental period. Laboratory-and greenhouse-based techniques for screening for Al resistance are widely used because they are quick, highly accurate, non-destructive, and can be applied at early stages of plant development (Hede, 2001). Field-based techniques have been also used; but are more laborious (Carver and Ownby, 1995).

I.3.5.1. Hydroponics

Hydroponics is an attractive alternative to soil-based screening for Al resistance (Villagarcia et al., 2001). It allows evaluation of a large number of genotypes quickly and has been used to identify parental stock for soybean breeding (Spehar, 1994; Bianchi-Hall et al., 2000; Silva et al., 2001a). Solution-culture techniques allow studying the effects of one factor of the soil-acidity complex without affecting others, and provide the adaptation of plant roots to low pH (Edmeades et al., 1995). In grain legumes, hydroponic screening has been reported as a rapid method for evaluating root elongation in common bean (Rangel et al. 2005, 2007, 2009); cowpea [*Vigna unguicuata* (L.) Walp.] (Paliwal et al., 1994 ; Ogbonnaya et al., 2003), and soybean (Villagarcia et al., 2001).
Methods assessing root growth in nutrient solutions are easy to manage and more accurate than soil assays. They provide controlled forms of Al stress, are easily repeatable, and cost and time effective. The outcome of results and evaluations, however, depends on multiple factors, such as pH, temperature, interaction of Al with other nutrients in the solution, and the sensitivity of the biological specimen (Narasimhamoorthy et al., 2007). Hydroponic screening has identified VAX-1 as Al-sensitive bean cultivar while it had been found acid soil tolerant in soil-based and field experiments at CIAT (Santander of Quilichao soil). The hydroponic system for the evaluation of genetic materials provides a strict control of nutrient availability and is widely used in genetic studies. However, breeders have not adopted hydroponic screening, because it is usually limited to seedling assays, and there is a question of how well rankings of seedling Al tolerance apply to the field (Villagarcia et al., 2001). The method is commonly used in specific genetic research work

such the expression of selected candidate genes and their roles in Al resistance (Eticha et al., 2010), and understanding mechanism of Al tolerance (Rangel et al., 2010). More recently it has been used in breeding to identify and characterize tolerance to Al toxicity, and physiologically assess root architectural traits in recombinant inbred lines (RIL) population that contrasts for Al resistance (Rangel et al., 2007; Lopez-Marin et al., 2009; Butare et al., 2012).

1.3.5.2. Soil-based screening

Screening experiments that are based on growth of plants in acid soil with higher levels of exchangeable Al have been used to identify tolerance in alfalfa (Devine et al., 1976), barley (Foy, 1996a), in beans (Butare et al., 2012), sorghum (Foy et al., 1993), soybean (Foy et al., 1992) and wheat (Foy, 1996b). Plants are grown in pots (or transparent cylinders) with acid soil for approximately 1 month or in the field for a growing season and then root dry matter, shoot dry matter and Al concentration in plant tissues is compared to plants grown in the same soil limed to a non-toxic pH and Al saturation (Samac and Tesfaye, 2003). The advantage of these soil based techniques is that they allow plant evaluation at young stage when root growth is important for plant establishment and also at older stages in which nutrient deficiency and/or drought stress can affect plant growth (Samac and Tesfaye, 2003). Foy et al. (1993) found that unlimed Al-toxic Tatum soil could not separate sorghum varieties, although tolerant varieties were later identified when soil was limed to pH 4.3. A similar situation was observed for durum wheat (Foy, 1996b).

Villagarcia et al. (2001) found that soil-based rankings for Al tolerance may be soil-type dependent and not easily reproducible across wide geographical areas, and leading to the use of sand-culture, a potentially viable alternative, as a supplement to hydroponic screening for evaluating Al resistance. According to their research sand-culture method allows for easy excavation of intact roots from pots for quantification of root characters using relative root surface area (RRSA). The sand-culture developed by Villagarcia et al. (2001) does not require access to acid soil and the amount of Al and other minerals delivered to plants can be controlled. However the method is time consuming; plants are treated twice a day, once with an acidic Al solution and once with an acid nutrient solution. A modified screening media that involves a soil-on-agar assay was described by Voigt et al. (1997) for small seeded plants that would have difficulty germinating in an acid soil (Samac and Tesfaye, 2003). This screening method requires only a small layer of acid soil to be placed on top of an agar layer. Recently, at CIAT, a technique using transparent plastic tubes with high Al saturation soil was developed to rank bean genotypes for Al resistance based on differences in root development

and distribution (Butare et al., 2011). The screening conditions are similar to that in field and allow evaluation of both root and shoot development under Al stress.

1.3.5.3. Field screening

The ultimate and most direct method of evaluating for Al tolerance is by measuring economic yield under field conditions. Field screening for stress response often involves growing germplasm lines in contrasting conditions. Difference on grain yield in an unamended plot, a lime-amended plot, and potential yield without acid soil stress (Carver and Ownby, 1995; Johnson et al., 1997) has been used in Al-toxic acid screening. Aluminum toxic field in Santander of Quilichao in Colombia is used for evaluation of bean genotypes for tolerance to Al-toxic acid soil conditions. Two most important problems when evaluating for Al tolerance in field are the presence of fungal pathogens (Hede et al., 2001), and spatial variability of pH in the surface and subsurface soil layers (Carver and Ownby, 1995).

I.3.6. Identification of genes associated with aluminum tolerance

Major loci and QTLs controlling Al tolerance have been identified in alfalfa (*Medicago sativa*; Narasimhamoorthy et al., 2007), beans (López-Marín et al., 2009), soybean (Bianchi-Hall et al., 2000), rice (Xue et al., 2007), sorghum (Magalhaes et al., 2007), maize (Ninamango-Caedenas et al., 2003), barley (Wang et al., 2007), wheat (Raman et al., 2005), oat (*Avena sativa*; Wight et al., 2006), and rye (*Secale cereale*; Matos et al., 2005). In a study on Al-tolerance genes in a F4-derived population of soybean from hybridization of Al-susceptible Young and Al-tolerant PI416937, Bianchi-Hall et al. (2000) found that while most RFLP alleles for Al tolerance were derived from the PI 416937, an allele from susceptible Young (for marker EV2-1) was associated with Al tolerance expressed as per cent of control (PC).

An Al resistance gene from wheat, *ALMT1,* has been cloned, and identified as a gene encoding an Al-activated malate transporter, and expression of this gene in other genotypes increased malate efflux and enhanced Al resistance (Delhaize et al., 2004; Sasaki et al., 2004; Hoekenga et al., 2006). Recently, López-Marín et al. (2009), in a work on identification of QTL for root architectural traits related to Al resistance, showed that common bean has polygenic inheritance of tolerance to Al and that some Al resistance QTL co-localize with QTL for tolerance to low

phosphorous (P) (Liao et al., 2004; Yan et al., 2004; Beebe et al., 2006). They concluded that there are cross-links between different mechanisms of abiotic stress adaptation in common bean.

I.4. Drought stress

Drought is a major abiotic stress in many parts of the world (Johansen et al., 1994). Drought originates from a deficiency of precipitation over an extended period of time, resulting in a water shortage. Drought or low moisture availability lead to poor development, wilting and death of plants; and is responsible for heavy production losses in cool-season food legumes (Saxena, 1993; Singh et al., 1994; Subbarao et al., 1995). Intermittent and terminal droughts both occur during periods of water shortage for plant development. These two distinct kinds of drought are both associated with limited rainfall. Intermittent drought usually occurs in highland regions, whereas terminal drought is a lowland phenomenon. Intermittent drought is due to climatic patterns of sporadic rainfall that causes intervals of drought and can occur at any time during the growing season (Schneider et al., 1997), and affect the yield potential. Terminal drought occurs when plants suffer lack of water during later stages of reproductive growth or when crops are planted at the beginning of a dry season (Frahm et al., 2004) without irrigation facilities. Terminal drought stress which occurs during the pod-filling phase of crops is common and could act as yield reducer for crops growing with current rainfall (Nageswara Rao et al., 1985a, b) but is even more critical for crops grown during a post-rainy season and reliant on stored soil moisture (Subbarao et al., 1995). Drought, whether intermittent or terminal, can be confounded with high temperatures in certain locations or aggravated by shallow soils and root rotting pathogens (Ramirez-Vallejo and Kelly, 1998). Maintenance of water status under water limitation can be partially attributed to rooting depth and root length density (Turner, 1986; Subbaro et al., 1995). The stimulation of drought conditions may be useful in eliminating other confounding factors, since it is not always possible to separate these effects from those due to drought (Ramirez-Vallejo and Kelly, 1998). Selection for drought resistance based solely on grain yield may not bring genetic improvement in the desirable physiological traits and may not detect when different mechanisms have similar outcomes (Subbarao et al., 1995).

Bean plants receiving less than 300 mm of total precipitation during crop growing season could be considered as being under water deficit. More than 60% of common bean grown in the developing countries of Latin America, Africa, and Asia suffers from water stress at some stage of crop growth

(White and Singh, 1991). Limited water availability to the bean crop can be caused by physical and climatic factors of the environment, the soil-precipitation relationship, the soil-plant relationship, the atmosphere-plant relationship, excessive demand by the plant, or any combination of these (Nilson and Orcutt, 1996). A study of bean distribution by environment in Latin America showed that, in 93% of common bean-growing areas, the physiological water requirements of the plant are not fulfilled (Fairbairn, 1993). In tropical and subtropical Latin American bean growing environments, drought is often intermittent (Acosta-Gallegos et al., 1999; Schneider et al., 1997b) and complete crop failures are not uncommon.

For common bean, the highest levels of drought resistance were found in race Durango, followed by race Jalisco cultivars in field tests conducted at CIAT, Palmira, Colombia (Terán and Singh, 2002). Breeding for drought resistance is an important strategy in alleviating the problem and offers the best long-term solution (Songsri et al., 2008). The degree of tolerance of plants to environmental stress varies greatly not only between species but in different varieties of the same species (Wentworth et al., 2006). Root architecture has been linked with plant acquisition of water in many publications.

I.4.1. Effects of drought on beans

The effects of drought on common bean are dependent on the intensity, type and duration of the stress (Muñoz-Perea et al., 2006, Beebe et al., 2013). Under drought stress, bean plants may decrease their vegetative growth initially, leaves change their direction and turn away from the sun to reduce water loss through transpiration. If the stress continues plants may wilt early, loose leaves, and the productivity is severely reduced (Osakabe et al., 2014). Drought stress increases root shrinkage that consequently affects nutrient transport to the root surface due to reduced contact between root and soil (North and Nobel, 1997). Moderate to severe drought stress in common bean is known to reduce canopy biomass and seed yield, harvest index, number of pods and seeds, seed weight, and days to maturity (Nunez-Barrios et al., 2005; Beebe et al., 2013). Most plants have developed morphological and physiological mechanisms which allow them to adapt and survive (Ludlow, 1989). The mechanism mainly comprise a reduction of the leaf size, leaf rolling, dense leaf pubescence deeply developing (Bosabalidis and Kofidis, 2002).

Drought is one of the most common environmental stresses that affects growth and development of plants through alterations in metabolism and gene expression (Ceccarelli and Grando, 1996). Plant responses to water stress will lead first to acclimation and later, as water stress become more severe, to functional damage and loss of plant parts (Chaves et al., 2003). Drought stress may

reduce P uptake (Guida dos Santos et al., 2004) and concentration of N, its partitioning and fixation in dry bean (Ramos et al., 1999; Serraj and Sinclair, 1998). Excessive abortion of flowers, young pods, and seeds occurs because of drought stress during pre-flowering (10 to 12 days before anthesis) and reproductive periods (Muñoz-Perea et al., 2006). Despite great effort made in breeding during many years yield losses due to drought stress in common bean are still high. They can range from 41 to 92% (Foster et al. 1995; Castellanos et al., 1996; Terán and Singh, 2002). This reveals that progress in transferring morphological, physiological and biochemical traits with potential impact in drought tolerance to bean cultivars has been rather poor (Lizana et al., 2006).

I.4.2. Mechanisms of tolerance to drought

In genetic sense, the mechanisms of drought tolerance can be grouped into three categories: drought escape, drought avoidance and drought tolerance (Levitt, 1972; Mitra, 2001). Conveniently for other authors the three categories are represented by phenological, morphological, and physiological mechanisms. Crop plants use however more than one mechanism at a time to resist drought (Gaff, 1980; Mitra, 2001).

Drought escape is defined as the ability of the crop to complete its life cycle before serious soil and crop water deficits develop (Beebe et al., 2013). Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where growing season is short and terminal drought stress predominates (Turner, 1986). Drought escape plays an important role in early maturing varieties of wheat, sorghum, maize, and rice. The mechanism help these crop to be less affected by severe drought than late maturing ones.The success of increasing legume production in drier regions prone to terminal drought largely depends on the development of short season varieties that enable the crop to escape severe soil-water deficits (Erskine et al., 1994; Siddique et al., 2001; Berger et al., 2003). In common beans, this mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water deficit), and remobilization of photosynthates to the grain (Mitra, 2001; Beebe et al., 2013). The effects of water deficits on phenological development are usually small (Blum, 2005).

Drought avoidance is defined as the ability of the plant to maintain relatively high tissue water potential, despite a shortage of soil moisture (Passioura, 1987; Beebe et al., 2013). In other words, plants which avoid drought retain high water contents in their tissues (Beebe et al., 2013). Root

responses when soil moisture dries out are important mechanisms for drought avoidance (Ketring, 1984; Songsri et al., 2008). In cereals, drought avoidance operates during vegetative phase, while tolerance operates during reproductive phase (Agrinfo.in, 2011). Root traits such as biomass, length density and depth have been proposed as the main drought avoidance traits to contribute to seed yield under terminal drought environments in chickpeas (Ludlow and Muchow, 1990; Subbarao et al., 1995; Turner et al., 2001; Kashiwagi et al., 2005). In common beans, drought avoidance is achieved through increased rooting depth, an efficient root system and increased hydraulic conductance, and by reduction of water loss through reduced leaf conductance, reduced absorption of radiation by leaf movement/rolling, and reduced evaporation surface (leaf area) (Beebe et al., 2013). Turner et al. (2001) identified rooting depth and density as a main drought avoidance trait in grain legumes for use in terminal-drought environments.

The ability of stomata to regulate water loss provides an important mechanism for reducing water loss during drought. In beans, the mechanisms of drought avoidance include principally the development of an extensive root system and an efficient stomatal closure (Haterlein, 1983; Trejo and Davies, 1991; Barradas et al., 1994). Stomatal closure is one of the first steps in adaptation to drought stress and it relates to dehydration avoidance by reducing water loss and maintaining water status during unfavorable conditions (Stoddard et al., 2006). Blum (2005) concluded that the design of dehydration-avoidant genotypes based on moderate water use cannot consider only one physiological factor or one gene without understanding the full spectrum of interactions among plant development, phenology, water use, penalty in yield potential, and the specific dry land ecosystem.

Drought tolerance is defined as the ability of plants to withstand water-deficit with low tissue water potential (Turner, 1986; Mitra, 2001). It is achieved through maintenance of turgor through osmotic adjustment (a process which induces solute accumulation in the cell), increase in cell elasticity and decrease in cell size, and desiccation tolerance by protoplasmic resistance (Beebe et al., 2013). When drought stress becomes persistent or severe and when other mechanisms of adaptation fail or have been exhausted, the ability of tissues to withstand dehydration becomes important. Under conditions of unsecured soil resources, a potentially large root is required to ensure capture of resources under erratic conditions (Blum, 2005). Root mass rarely increases under stress. However, root length and depth may increase in a drying soil even at a reduced total root mass; but it is not absolutely clear whether the capacity for developing longer roots under stress is compatible with a high yield potential phenotype (Blum, 2005).

In cereals, drought tolerance generally operates during reproductive phase. Tolerant cultivars exhibit better germination , seedling growth and photosynthesis (Agrinfo.in, 2011). In sorghum, a drought resistant line exhibited higher photosynthetic rate at lower leaf water potential than a less drought resistant line (Agrinfo.in, 2011). Under drought stress, the content of abscisic acid (ABA), which is associated with stomatal closure, increased in peas, more in the tolerant genotype than in the susceptible (Upreti and Murti, 1999). In a range of faba bean germplasm, however, ABA content did not correlate significantly with drought tolerance (Riccardi et al., 2001). Stoddard et al. (2006) showed that nitric oxide (NO) was able to induce 65% stomatal closure in faba bean, indicating that exogenous NO might increase tolerance to severe drought. In common beans, genetic variation for leaf angle movement, which has been implicated as a drought resistance mechanism, has also been demonstrated in genotypes of Andean origin (Kao et al., 1994). A large root system can be an important character for drought tolerance (Girdthai et al., 2010). Shoot/root dry matter ratio increases under drought stress, not because of an increase in root mass but due to a relatively greater decrease in shoot mass (Blum, 2005). Reduced plant size, leaf area, and leaf area index (LAI) are a major mechanism for moderating water use and reducing injury under drought stress (Mitchell et al., 1998). The maintenance of relatively higher leaf water content with increasing water deficit plays an important role in terms of higher pod setting (Omae et al., 2005), pod retention and seed yield in snap bean (Kumar et al., 2006).

Many of the traits that explain plant adaptation to drought such as phenology, root size and depth, hydraulic conductivity and the storage of reserves are associated with plant development and structure and are constitutive rather than stress induced (Chaves et al., 2003).

I.4.3. Bean adaptability and Strategies to improve drought tolerance

Plants exposed to heat and drought or water stress have evolved a series of morphological and physiological adaptations, which confer tolerance to these stresses (Kumar et al., 2006). Bean plants may react to the stress through short-term strategies, like changes in hydraulic signals or stomatal adjustment. And whenever bean plants experience a rapid water deficit, bean leaves are known to orient themselves parallel to the incident light, and also alarm the biochemical systems. Ramirez-Vallejo and Kelly (1998) revealed that yield components which exhibited the largest differential genotypic responses to drought stress were pod and seed number, whereas seed size was more stable. Drought susceptibility of a genotype is also measured as a function of the reduction in yield under drought stress (Blum, 1988) but Ramirez-Vallejo and Kelly (1998) have showed that the values are confounded with differential yield potential of genotypes.

Kelly (1998) suggested that using differences in growth habit to indirectly select for root architecture as superficial root systems of type III genotypes (indeterminate growth, semi-climber bush bean with a prostrate vine) are better suited to intermittent drought where as the deep tap root of type II genotypes (determinate growth bush type bean with upright short vine, narrow plant profile, and three to four branches) sustains plants through short periods of drought by mining the lower soil profiles for moisture. Adequate root density throughout the soil profile may increase the diffusion area, thereby improving water availability and uptake. Maintenance of water status under water limitation can be partially attributed to rooting depth and root length density (Turner, 1986; Subbarao et al., 1995).

Beaver and Rosas (1995) found that selection for earlier flowering, a greater rate of partitioning and a shorter reproductive period permitted the selection of small red bean breeding lines having one week earlier maturity without sacrificing yield potential. Water stress during the flowering and grain filling periods reduced seed yield and seed weight and accelerated maturity of dry bean (Singh, 1995). Lines with earlier maturity would be less vulnerable to terminal drought, but caution needs to be exercised, as an association between early maturity and lower yields exists.

I.4.4. Bean genotypic differences in drought resistance

Genetic variation exists in potential root length (maximum root length measured under non-stress and non-restrictive soil conditions) in many crops. However, when plants are exposed to a drying soil, root morphology and growth can change to the extent that the potential root length, whether it is short or long, becomes irrelevant (Blum, 2005). Abebe et al. (1998), Acosta-Gallegos et al. (1999), and Terán and Singh (2002) reported genotypic differences for drought resistance in common bean. Selection for deep and extensive root system has been advocated to increase productivity of food legumes under moisture-deficit conditions as it can optimize the capacity to acquire water (Subbarao et al., 1995; Sarker et al., 2005). A unique study, in which root and shoot systems of contrasting common bean genotypes were separately evaluated using grafted plants, demonstrated that root characteristics can be much more important than shoot characteristics in conferring adaptation to drought (White and Castillo, 1989).

Parsons and Howe (1984) compared common bean (*Phaseolus vulgaris*) with tepary bean (*Phaseolus acutifolius*) for their water relations, and concluded that tepary beans were more drought tolerant than common beans due to the higher osmotic adjustment potential they possessed. They, thus, suggested a transfer of osmotic gene from tepary beans to common beans to improve drought resistance. Large genotypic differences were found in plant water status in snap bean,

which were correlated to crop productivity under drought conditions in a high temperature environment (Omae et al., 2004).

Decrease in soil water caused a decline in leaf water status. The high yielding cultivars displayed a smaller reduction in leaf water content but a larger reduction in leaf water potential than the poor yielder (Kumar et al., 2006). Morphologically, loss of leaf area is the most important drought response of common bean and can be the result of reduced number of leaves, reduced size of younger leaves, inhibited expansion of developing foliage, or leaf loss accentuated by senescence, all of which result in decreased yields (Acosta-Gallegos, 1988). Leaf water content is an important physiological trait for heat and drought tolerance in snap bean (Kumar et al., 2006). The only traits that have proven to be valuable in both terminal and intermittent drought are earliness and partitioning towards reproductive structures, resulting in greater harvest index (Acosta-Gallegos and Adams, 1991; Foster et al., 1994; Beaver et al., 2003).

I.4.5. Screening techniques for drought Tolerance

Different techniques of screening for drought tolerance have been used including infrared thermometry for screening for efficient water uptake (Blum et al., 1982); psychrometric procedure for evaluating osmotic adjustment (Morgan, 1980, 1983); diffusion porometry for leaf water conductance (Gay, 1986); use of carbon isotope discrimination for selecting for increased water-use efficiency (Farquhar and Richards, 1984); pots (Ogbonnaya et al., 2003; Read and Bartlett, 1972; Sarker et al., 2005).

The simulation of drought conditions may be useful in eliminating other confounding factors, although it is not always possible to separate these effects from those due to techniques for drought tolerance that were realized in the past (Levitt, 1964). Rooting pattern, especially greater root length in lower soil strata, is an important drought resistance mechanism (Sponchiado et al., 1989). Phenotypic selection has been practiced with considerable success to improve drought tolerance in common beans (Miklas et al., 2006). Drought reduces bean plant biomass (Abebe and Brick, 2003; Muñoz-Perea et al., 2006; Padilla-Ramírez et al., 2005; Ramirez-Vallejo and Kelly, 1998). Selection for deep and extensive root system have been advocated to increase productivity of food legumes under moisture-deficit condition as it can optimize the capacity to acquire water (Subbarao et al., 1995; Serraj et al., 2004a; Sarker et al., 2005). Acosta-Gallegos et al. (1995) had suggested that selection for drought tolerance under local conditions may enhance root resistance.

The direct measurement of seed yield is the most practical way to screen for drought resistance (Acosta-Gallegos and Adams, 1991; Terán and Singh, 2002; White and Singh, 1991). Field

screening for stress response often involves growing germplasm lines in contrasting conditions and estimating a susceptibility index from the relative yield in the two environments, as initially proposed by Fischer and Maurer (1978) for drought purposes. Drought tolerance can only be estimated by comparing the performance of breeding lines under stress and non-stress (irrigated) conditions. During field experimental setting, a susceptible check is usually included at frequent intervals in the lines for easy comparison (Stoddard et al., 2006). Singh et al. (1997) considered that the lack of efficient screening techniques impeded efforts to breed for drought resistance in food legumes. Two main methods for controlling water supply are line-source sprinkler irrigated, which provides a gradient of water deficits (Hanks et al., 1976), and rainout shelters (Serraj et al., 2003).

I.4.6. Breeding and gene(s) involved in controlling bean tolerance to drought

Three major factors thought to be involved in improving drought tolerance in common bean are: (1) maximizing water capture through deep and ample rooting, (2) maximizing water use efficiency for growth and development through low transpiration, and (3) directing photosynthate into harvestable grain through efficient mobilization. Because seed yield is the most important economic trait of common bean, the most practical method to improve performance is through the direct measurement of yield-related characteristics (Acosta-Gallegos and Adams, 1991). Drought tolerance is found in diverse bean races suggesting possible diversity in genes and mechanisms (White, 1987; Rao, 2001; Teran and Singh, 2002). Marker-assisted selection in the Sierra/AC1028 population was found to be effective in Michigan under severe stress and ineffective in Mexico under moderate stress (Schneider et al., 2001). Comparative genomics among the legumes could be used to integrate drought QTL studies conducted for common bean (Blair et al., 2002; Schneider et al., 1997) and soybean (Mian et al., 1996, Mian et al., 1998, Specht et al., 2001). Recent advances in comparative mapping among the legumes has clarified the genetic relationship of model and crop legumes as well as linked the genomes of the tropical and temperate legumes that represent the major clades of the legume family (Choi et al., 2004). This will allow aligning drought QTLs between legume species and determining the most important regions for saturated mapping.

For other crops, a number of studies have reported QTLs for root architecture and have investigated their effects on yield under varying moisture regimes in rice (MacMillan et al., 2006; Steele et al., 2006, 2007; Yue et al., 2006) and maize (Tuberosa et al., 2002, 2003; Landi et al., 2007). In maize, a major QTL originally reported for leaf ABA concentration (Tuberosa et al., 1998) was later shown to affect root size and architecture (Giuliani et al., 2005b) and grain yield (Landi et al., 2007). In sorghum, four major QTLs that control stay-green and grain yield (Stg1-Stg4) have been

identified (Harris et al., 2007). Major QTLs for seed weight and grain yield across diverse moisture conditions have also been identified in rice (Wang et al., 2006; Bernier et al., 2007) and durum wheat (Triticum durum; Maccaferri et al., 2008).

I.5. Root health in common bean

I.5.1. Bean root rot

Root rot occurs in all bean-growing areas in the world. Root rot of common beans is a soil-borne disease that may be incited by several fungal pathogens including *Fusarium solani* f. sp. *phaseoli*, *F. oxysporum*, *Pythium ultimum*, and *Rhizoctonia solani* (Sippel and Hall, 1982). The root rot pathogens are favored by moderate to high soil moisture, various soil temperature regimes, soil compaction, poor drainage, continuous or frequent cropping to beans, and other factors that cause plant stress (Kraft et al., 1981; Schwartz et al., 2001). Root rots are economically important in most bean production areas (Snapp et al., 2003) but are particularly problematic in regions characterized by low soil fertility, limited crop rotation and intensive seasonal bean production (Miklas et al., 2006). They are also particularly severe under water-stressed or compacted soil conditions (Burke and Hall, 1991; Thung and Rao, 1999). Bean root health is an essential component in managing drought stress as root pathogens aggravate problems of water and nutrient acquisition by restricting root systems. Yield losses range from a trace to 80%, especially when adverse environmental conditions persist after planting and though flowering (Dryden and Van Alfen, 1984; Miller and Burke, 1986; O'Brian et al., 1991; Park and Tu, 1994). Charcoal rot (*Macrophomina phaseolina* (Tassi) Goid.) is a major problem under condition of terminal drought (Frahm et al., 2004), whereas Rhizoctonia root rot (*Rhizoctonia solani*) and Fusarium root rot (*Fusarium solani* f. *phaseoli*) are major root pathogens in the regions where intermittent drought occurs (Navarrete-Maya et al., 2002). Improving the levels of root rot tolerance is a key element in the successful development of drought tolerance in beans.

I.5.2. *Fusarium* root rot

Fusarium root rot, caused by *Fusarium solani* f. sp. *phaseoli*, is one of the main root diseases impacting production of common beans throughout the world (Chaudhary et al., 2006). The widespread nature and importance of *F. solani* as the predominant root rot pathogen in common

bean emphasizes the need for effective control through the development of resistant cultivars (Boomstra and Bliss, 1977; Schneider et al., 2001; Chowdbury et al., 2002; Navarro et al., 2003). *Fusarium* root rot can persist for many years in previously infected bean debris and infected soil by producing chlamydospores, its survival structures. In common bean (*Phaseolus vulgaris* L.) *Fusarium* root rot (caused by *Fusarium solani* f. sp. *Phaseoli*) disease severity is increased by environmental factors that stress the plant (Cichy et al., 2007). Complex inheritance combined with genetic incompatibility have limited attempts to transfer *Fusarium* root rot resistance into Andean bean cultivars, despite extensive information on sources of resistance in the Middle American gene pool (Beebe et al., 1981; Wallace and Wilkinson, 1973, 1975).

I.5.3. Symptoms of *Fusarium* root rot.

Fusarium root rot is characterized by reddish-brown lesions along the tap roots (Fig. 1) and lower hypocotyls (Chaudhary et al., 2006). Lateral roots may develop from the hypocotyl above the initial infection site if sufficient soil moisture is available (Abawi, 1980; Hall, 1991; Abawi and Pastor-Corrales, 1991; Schwartz et al., 2001). Symptoms may extend from the main root, into the stems of older plants, and hypocotyls to the soil surface. In older lesions, longitudinal cracks may develop, and severely infected primary and secondary roots are killed. Aboveground symptoms include leaf chlorosis, stunting of stems, and reduction in pod production (Cichy et al., 2007; Schneider and Kelly, 2000; Burke and Barker, 1966).

Infected plants are frequently stunted, grow slowly compared with healthy plants, and are light green to yellow. The root system is unable to ensure nutrient and water uptake, and yield is strongly reduced as a result of the poor root function.

Figure 1: Root rot (a) and vascular reddish-brown lesion (b) on infected bean caused by *Fusarium* root rot disease.

I.5.4 Epidemiology of *Fusarium* root rot

Fusarium root rot is caused by the pathogen *Fusarium solani* (Mart.) Appel and Wollen V. f.sp. phaseoli (Burk.) Snyd. and Hans. It has an ascomycetous sexual state, *Nectria haematococca* Berk. and Br. (Abawi and Pastor Corrales, 1990). The fungus is morphologically similar to all other pathogenic and saprophytic members of the species *F. solani* (Booth, 1971). Hower, it is distinguish by its physiological and pathological adaptation to beans (Abawi and Pastor Corrales, 1990). *Fusarium* root rot is favored by warm temperatures of 72 to 90 ^{o}F (22 to 32°C) on a high soil moisture and acid soils (Hagedorn and Inglis,1986). Like *Fusarium oxisporum, F.solani* also produces macroconidia, microconidia, and chlamydospores. The dark, thick-walled chlamydospores are the long-term survival structures in soil. These overwintering spores germinate readily in response to plant root exudates and infect plants through stomata and wounds (Harveson, 2011). The macroconidia of *F. solani* differ in shape from those of *Fusarium* wilt pathogen by being less curved, having one blunt end, and being some what larger (Abawi, 1990). *Fusarium* root rot pathogen also survive in soil by colonizing roots of nonhost crops without causing disease.

The pathogen is capable of directly penetrating bean tissues or through wounds and natural openings (Babadoost, 1989). *Fusarium solani* can be spread by drainage or irrigation water, or by any means which moves infested soil from field to field (Hagedorn and Inglis,1986). It may also spread in bean straw or organic manure. It is not a seed borne disease, but possibly it is transmited in soil adhering to seed surfaces (Hagedorn and Inglis,1986). Soil compaction, nematodes *Pratylenchus penetrans* or *Meloidogyne spp*. and the fungus *Pythium ultimum* also contribute to the disease severity(Hagedorn and Inglis,1986) .

I.5.5. Screening for resistance to *Fusarium* root rot

Field trials are conducted in a field previously identified as infected with *Fusarium solani* f. sp. *phaseoli*; seeds are planted in a randomized complete block design, and standard agronomic practices are applied to ensure good crop growth and development. Different greenhouse screening methods have been used for screening common bean for resistance to *Fusarium* root rot (Boomstra et al., 1977; Tu and Park, 1993; Park and Tu, 1994). Liquid inoculum method (LIM) (Schneider and Kelly, 2000; Chaudhary et al., 2006) and inoculum layer method (ILM) (Schmitthenner and Bhat, 1994; Chaudhary et al., 2006) are two greenhouse techniques that have been used for screening for resistance to *Fusarium* root rot. Root rot pathogens grown on cereal substrate (e.g.

Sorghum) and mix with soil (Burgess et al., 1994) have been used also in greenhouse for the evaluation of resistance to *Fusarium solani* f. sp. *phaseoli*. For the liquid inoculum method, ten days after planting , when the primary leaves are fully expanded, 1ml of spore suspension (adjusted to 10^6 conidia/ml with a haemocytometer) is applied by pipette around the hypocotyl of each plant. And for the inoculum layer method, fungal culture including the agar disc is carefully removed from the petri dish and placed, inoculum side up, on the vermiculite, the inoculum layer is covered with 1 cm of vermiculite and seeds are put on the surface, and then covered with an additional 2 cm of vermiculite before watering. All those greenhouse screening methods are able to reduce the influence of environments factors.

I.5.6. Resistance to *Fusarium* root rot

Sixteen QTL for *Fusarium* root rot resistance were identified using a $F_{4:5}$ RIL population (recombinant inbred lines) derived from a cross between the susceptible large-seeded red kidney 'Montcalm' and the root rot resistance snap bean breeding line FR266 (Schneider et al., 2001). Interval mapping revealed two QTL for *Fusarium* root rot resistance using an $F_{2:6}$ RIL population derived from across between 'A.C. Compass', a root rot susceptible navy bean, and NY2114-12, a highly resistant root rot germplasm (Chowdbury et al., 2002). In beans, several defense responses co-localize with resistance QTL suggesting a functional relationship between the QTL and the defense response genes (Geffroy et al., 2000). Another cross between the root rot susceptible snap bean 'Eagle' and 'Puebla 152', a small black seeded root rot resistant dry bean (Navarro et al., 2004) revealed six QTL for resistance to *Fusarium* root rot from the derived RIL population. Most of these QTL were located on LGs B2 and B3 of the integrated bean map (Freyre et al., 1998) close to a region where defense response genes *Pgip*, and *ChS* and pathogenesis related proteins, *PvPR-1* and *PvPR-2*, have been identified (Schneider et al., 2001). QTL for white mold have been previously mapped to regions close to *ChS*, *Pgip*, and the *PVPR-2* on B2, suggesting that physiological resistance to *Fusarium* root rot and white mold [*Sclerotinia sclerotiorum* (Lib.) de Bary] is associated with a generalized host defense response (Schneider et al., 2001; Kelly and Vallejo, 2005). QTL associated with root rot resistance were located on linkage group (LGs) B2 and B5 of the integrated bean map close to previously identified QTL for resistance on B2 (Román-Avilés and Kelly, 2005). Another resistance factor previously mapped to B5 (Kelly et al., 2003) include the lipoxygenase gene, *Lox-1*, required during development of bean plants under desiccation stress (Porta et al., 1999). Despite the obvious connection between root health and

water stress, the potential role of lipoxygenase in root rot resistance is speculative in the absence of direct evidence for co-localization of the Lox-1 gene and QTL for root rot (Román-Avilés and Kelly, 2005).

I.6. Development of variability and Identification of superior genotypes

I.6.1. Development of variability

Crop variability for desired traits is obtained by crossing parents that possess specific traits that breeders intend to transfer into new or improved varieties. Progenies of these crosses segregate genetically in successive generations of selfing (self pollination) , and new genotypes are formed. Various selection methods are used for identification of these progenies that possess the most useful combinations of the desired traits.

I.6.2. Method of selection in segregating populations.

Pedigree method, bulk method, and single seed descent method are commonly used to manage segregating populations of self-pollinated crops including beans. (1) Breeders of self-pollinated crops usually apply the pedigree method of selection to handle segregating populations. Pedigree selection involves visual selection of best-appearing families in each generation, followed by within-family selection of one or more plants to advance to the next generation (Miladinović et al. 2011). A long felt disadvantage of this method is the limitation it imposes on the number of crosses a breeder can handle, the volume of record keeping, and the need for more land and labor. (2) The bulk method was developed to avoid the book keeping (free from pedigree record) required by pedigree method and consists of harvesting all plants in bulk every generation and planting a random sample of seed to propagate the next generation (Meena and Kumar, 2012). The population is advanced in bulk with no artificial selection until later generations when nearly homozygous lines are selected for yield testing (Orf et al., 2004; Miladinović et al. 2011). The pedigree record is not maintained, and the natural selection may also work against desirables traits. (3) To minimize the limitations of the bulk method and also to reduce the generations, Goulden (1941) proposed the single seed descent method (SSD) which was later on modified by Grafius (1965) and Brim (1966). This method consists of advancing segregating populations by taking a single seed from each plant to grow the next generation. The nearly homozygous lines developed by single seed descent still

preserve most of the original genetic variation in a population (Miladinović et al. 2011). The population size does not increase with successive generations. Extensive field trials are not required, little record keeping, and natural selection has little influence. The method is suitable for the evaluation of large numbers of inbred lines to find superior ones.

I.7. Genome mapping and QTLs for abiotic and biotic stress

Crop performance is the end result of the action of thousands of genes and their interactions with environmental conditions and cultural practices (Collins et al., 2008). Molecular markers offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait (Mohan et al., 1997, El-Nahas et al., 2011). These markers are not environmentally regulated and are, therefore, unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth (Mohan et al., 1997; Francia et al., 2005). The use of molecular techniques has made possible to hasten the transfer of desirable genes among varieties and to introgress novel genes from wild relative species to local and popular genotypes (Mohan et al., 1997; Preetha and Raveendren, 2008). Only by the joint analysis of segregation of marker genotypes and of phenotypic values of individuals or lines, it is possible to detect and locate the loci affecting quantitative traits (Quantitative trait loci or QTL) (Semagn et al., 2010). Once QTLs have been identified, the next challenge is to identify the gene (Salvi and Tuberosa, 2005; Angaji, 2009). To date many candidate genes or linked markers have been identified but few of them have been successfully verified and transferred into practical uses (Zhu and Zhao, 2007).

I.7.1. Genome mapping

Mapping and sequencing of plant genomes would help to elucidate gene function, gene regulation and their expression (Mohan et al., 1997). Polymorphism in the nucleotide sequence usually is sufficient for it to function as a molecular marker in mapping (Mohan et al., 1997; Angaji, 2009). Polymorphic markers are particularly useful because they reveal differences between individuals of the same or different species. These polymorphisms are revealed by molecular techniques such as restriction fragment length polymorphism, RFLP (Becerra and Gepts, 1994; Becerra et al., 2011); amplified fragment length polymorphism, AFLP (Tohme et al., 1996; Papa and Gepts, 2003; Rosales-Serna et al., 2005); microsatellite or simple sequence repeat polymorphism, SSRP (Gaitán-

Solís et al., 2002; Masi et al., 2003; Blair et al., 2003, 2006; Benchimol et al., 2007; Grisi et al., 2007; Hanai et al., 2007; Zhang et al., 2008), random-amplified polymorphic DNA, RAPD **(**Beebe et al., 2000; Maciel et al., 2001), cleavable amplified polymorphic sequence (CAPS) and single-strand conformation polymorphism (SSCP) (Mohan et al., 1997; Galeano et al., 2009; Ince and Karaca, 2011).

Genetic maps have been constructed in many crop plants using these markers on a single segregating population (Mohan et al., 1997; Wu et al., 2014). The recent comprehensive map of disease resistance genes constructed in common bean revealed numerous resistance gene clusters (Kelly et al., 2003; Gonçalves-Vidigal et al., 2011; Sousa et al., 2014; Campa et al., 2014). Efforts in breeding for abiotic stress resistance will generate more new QTLs. The most important use for linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest (Collard et al., 2005). Genes or markers that are close together or tightly-linked will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart (Collard et al., 2005). Common bean is self-pollinating species, and the mapping populations for the species are originated from parents that are both highly homozygous (inbred).

F2 populations derived from F1 hybrids, and backcross (BC) populations, derived by crossing F1 hybrid to one of parents, are the simplest types of mapping populations developed for self pollinating species (Collard et al., 2005). They are easy to construct and require only a short time to produce. Inbreeding from individual F2 plants allows the construction of recombinant inbred lines (RILs), which consist of a unique combination of chromosomal segments from the original parents (Collard et al., 2005). The production of RIL populations required usually six to eight generations, and RIL populations have advantage of producing homozygous or true-breeding lines that can be multiplied and reproduced without genetic change occurring. The ability to detect QTLs or the information contained in F2 or F2 derived populations and RILs are relatively higher than others (Tomar et al., 2010). Another advantage of RILs is the ability to perform larger experiments at several locations and even in multi-location while this seems not possible with population of F2:3 families due to lack of seeds (Tomar et al., 2010). Final step of the construction of a linkage map involves coding data for each DNA marker on each individual of a population and conducting linkage analysis using computer programs. Ideally, mapping populations should consist of a minimum of 50 individuals for constructing linkage maps (Young, 1994; Semagn et al., 2006).

I.7.2. Quantitative trait loci (QTL) analysis

Most of the important agronomic characters like yield and yield components (grain number, grain weight), plant height and days to flowering are controlled by several genes (Mohan et al., 1997). QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers (Collard et al., 2005). Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Tanksley, 1993; Young, 1996). The closer a marker is from a QTL, the lower the chance of recombination occurring between marker and QTL (Collard et al., 2005). The QTL and marker will be usually inherited together in the progeny, and the mean of the group with the tightly-linked marker will be significantly different to the mean of the group without the marker (Collard et al., 2005). Molecular markers linked to quantitative traits in grain legumes such as Al-resistance, drought resistance, and resistance to *Fusarium* root rot have been reported (Serraj et al., 2003; Beebe et al., 2013). Many QTLs have been identified by using DNA markers in different crop plants e.g., in beans using Map QTL 4.0 computer software (Van Ooijen, 2000), and QTL cartographer V2.0 (Basten et al., 2001; Oblessuc et al., 2013). Using the first software, it resulted in the identification of new candidate genes and markers closely linked to a major ALS disease resistance QTL, which can be used in marker-assisted selection fine mapping and positional QTL cloning in common beans (Oblessuc et al., 2013). In another study, QTL associated with both root traits and P efficiency parameters identified under high P conditions was also located in the same region, suggesting that this is an important QTL which might have great commercial potential for future genetic improvement of soybean P efficiency (Liang et al., 2010). On the basis of a linear regression model with the second software analysis, a combination of five markers associated with QTL on two different LGs accounted for 73 % of the phenotypic variation for root rot resistance (Roman-Aviles and Kelly, 2005). Data from the study provide to breeders an opportunity to combine through marker-assisted backcrossing large-effect QTL identified on different LGs to enhace root rot resistance in Andean beans.

CHAPTER II. OBJECTIVES AND HYPOTHESIS

The objectives of this study were to (i) identify potential parents among 11 genotypes (6 breeding lines of *Phaseolus vulgaris*, 4 accessions of *P. coccineus*, and 1 accession of *P. acutifolius*) to improve resistance to Al and drought stress using hydroponic and soil tubes screening methods and improve the tolerance of common bean (SER 16, a Mesoamerican small red, Al and root rot sensitive and drought tolerant bush bean line); (ii) screen 94 $F_{5:6}$ Recombinant Inbred Lines (RILs) of the cross SER 16♀ x (SER 16♀ x G35346-3Q♂) both for their resistance to Al and /or drought based on phenotypic differences under greenhouse conditions using hydroponic and soil tube systems, and for their tolerance under rainfed and irrigated fields in Palmira, Colombia; and high Al saturated acid soil in Santander of Quilichao, Colombia; (iii) screen RILs for resistance to *Fusarium* root rot in greenhouse using isolates from infected bean plants collected in Rwanda; and (iv) identify QTL for resistance to these three stresses.

In the process of screening the progenies of this Al sensitive line (SER16) and an Al resistant *Phaseolus coccineus* (G 35346-3Q) in greenhouses, popular bean varieties were included in each trial as control in the screening for resistance to Al toxicity and drought (VAX 1, Tio-Canela 75, DOR 390, G 21212, ICA Quimbaya, and G 40159); and for *Fusarium* root rot (VAX 1, Tio-Canela 75, DOR 390, G 21212, ICA Quimbaya, G 40159 and ALB 252).

Working hypotheses were that among bean species, differences exist in aluminum resistance, drought resistance, combined stress of Al and drought, and for root rot diseases. Such differences are expressed in phenotypic variability in root and shoot traits in response to these abiotic and biotic stresses. Any factor which contributes in inducing root injury and reduction of root growth might increase the susceptibility of bean to *Fusarium* root rot. It is well known that Al-induced inhibition of root growth in common beans, and Al-injured roots are susceptible to bean root rot on the one hand, and and on the other hand the disease is particularly severe under water stress conditions. When the two stresses occurs in an environnement where drought prevails, losses for the crop will be severe. For more efficiency in controlling these two abiotic stresses (Drought and Al toxicity), root pathogen that agravates their effects on bean production, will be addressed. When an improved root system increases access to soil moisture, shoot biomass and yield will increase too. We need to understand what root traits (including root architecture, deployment and vigor) are most resistant to biotic stress (root health) and soil acidity (Al tolerance). Deploying genotypes with vigorous vegetative growth and /or great root systems will be almost of no value if they are not efficient in translocating/remobilizing soil nutrients into seeds (yield).

CHAPTER III. MATERIALS AND METHODS

III.1. Materials

For the identification of potential parents among *Phaseolus* species, and improvement of common bean for resistant to biotic (*Fusarium* root rot) and abiotic (Aluminum toxicity and drought) stresses ten trials were realized in different sites (Table 1). The table describe in each trial, which plant materials were evaluated, under eight greenhouse trials for different stresses with a strict control of nutrient (Al), vertical soil cylinders with a medium similar to field conditions (Al, drought, combined stress of Al and drought), and right inoculum concentration of pathogen (*Fusarium* root rot); and finally screening directly under field conditions for abiotic stresses (under rainfed, irrigated, and high Al saturation soil). Trial codes will be used within the text when describing each experiment.

Table 1: Summary description of trials realized in this study

Trial number	Materials	Site	Medium	Stress	Code
1	**Potential parents (P)**	Greenhouse (G)	Hydroponic (H)	Aluminum (A)	**T1.PGHA**
2	**Potential parents (P)**	(G)	Soil (S)	(A)	**T2.PGSA**
3	**Potential parents (P)**	(G)	Soil (S)	Combined Al and drought (C)	**T3.PGSC**
4	**RILS (R)**	(G)	Hydroponic (H)	Aluminum (A)	**T4.RGHA**
5	**RILS (R)**	(G)	Soil (S)	(A)	**T5.RGSA**
6	**RILS (R)**	(G)	(S)	Drought (D)	**T6.RGSD**
7	**RILS (R)**	(G)	(S)	(C)	**T7.RGSC**
8	**RILS (R)**	(G)	(S)	*Fusarium* root rot (Fs)	**T8.RGSFs**
9	**RILS (R)**	Field (F)	(S)	Drought (D)	**T9.RFSD**
10	**RILS (R)**	(F)	(S)	Aluminum (A)	**T10.RFSA**

III.1.1 Plant Materials

Eleven bean genotypes were selected for the identification of parental genotypes (T1.PGHA, T2.PGSA, and T3.PGSC). These genotypes included 4 *P. coccineus* accessions (G35066-1Q, G35346-2Q, G35346-3Q and G35464-5Q); 6 common bean genotypes comprising 4 lines of the Middle American gene pool (VAX1, VAX3, VAX6, SER16) and 2 large seeded beans of the Andean gene pool (ICA Quimbaya and IJR, Indeterminate Jamaica Red); and one *P. acutifolus* accession (G40159). The *P. coccineus* accessions had been identified in a field screening of 155

entries of *P. coccineus* and *P. polyanthus* (= *P. dumosus*) in an Al toxic field site in Santander of Quilichao, Colombia, based on vegetative vigor. *P. acutifolius* is a drought resistant desert species and one of its accessions, G40159 had been identified as especially drought tolerant. The VAX lines had been selected for common bacterial blight resistance in Santander of Quilichao during their development, and VAX 1 had expressed good shoot vigor in Al toxic soils.

Recombinant Inbred Lines (RILs) in the $F_{5:6}$ generation of SER 16 x (SER 16 x G35346-3Q) were developed by single seed descent from the BC1:F2 to the BC1 F5:6 generation used in this study (Fig. 2). After backcross with the recurent parent (SER16) to F1 of the simple cross with G35346-3Q, the BC1F1:2 was planted. The single seed descent (SSD) method, as per the method of Grafins (1965) and Brim (1966), was used. One seed was collected from each BC1F1:2 plant (one pod) to produce the BC1F2:3 generation. Similarly in BC1F2:3 and subsequent generations one random seed (selfed seed) was selected from every plant, to produce the next generation. We followed this procedure until the BC1F5:6 generation when plants became nearly homozygous. Visually selection was also applied to remove plants with signs of genetic incompatibility.

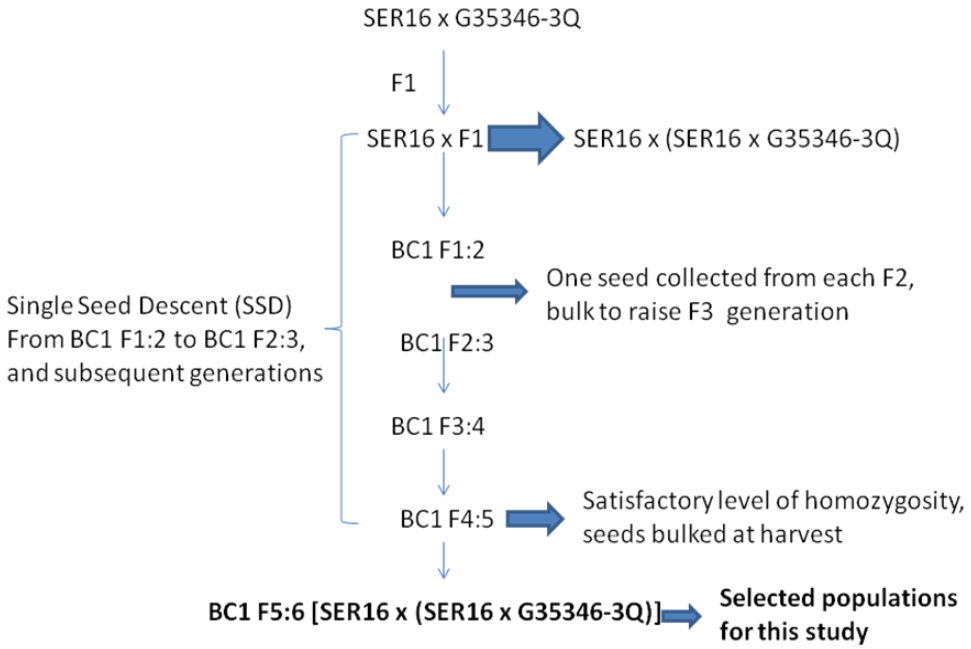

Figure 2: Outline of generation advancemennt in interspecific backcross population from the hybridization SER16 x (SER16 x G35346-3Q) using single seed descent (SSD) method

For characterising progenies of the interspecific crosses, all greenhouse studies for individual stress of Al (T4.RGHA and T5.RGSA), and drought (T6.RGSD) resistance were conducted using 102 bean genotypes including 94 F5:6 RIL, both parents (SER16 and G35346-3Q), and six checks

including VAX 1, Tio-Canela 75, DOR 390 and G 21212; an elite Andean cultivar (ICA Quimbaya); and one *Phaseolus acutifolius* accession (G 40159). For *Fusarium* root rot (T8.RGSFs) resistance evaluation, one more line ALB 252 was added (Table 2). For combined stress of Al and drought (T7.RGSC), twenty five bean genotypes were used. These genotypes were twenty one Recombinant Inbred Lines (F5:6 RILs) selected from individual stress from an interspecific backcross between SER 16 and G35346-3Q (SER 16♀ x (SER 16♀ x G35346-3Q♂)); both parents and two popular bean varieties in Colombia (ICA Quimbaye, an Andean large seeded bean genotype resistant to Aluminum; and Tio-Canela 75, a commercial Middle American bean landrace).

Five to ten replications were used for evaluation in all experiments using hydroponic system; while for soil tube, the screening was conducted using three replications.

Table 2: Characteristics of different bean genotypes for screening of potential parents and selection of recombinant inbred lines (RILs) evaluated both under greenhouse (hydroponic and soil tube) and field condition (Palmira and Satander del Quilichao CIAT research stations

No	Bean lines						Seed color	Seed size	Hydrolic Al	Al Soil tube	Drought Soil tube	Combined Al & drought	F. root rot	Field Palmira	Field Quilichao
1	ALB	1	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	CrSt	M	X	X	X	X	X	X	X
2	ALB	2	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
3	ALB	3	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Re	M	_	_	_	_	_	X	X
4	ALB	4	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
5	ALB	5	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
6	ALB	6	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	X	X	X	X
7	ALB	7	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Bl	M	_	_	_	_	_	X	X
8	ALB	8	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	ReOp	M	_	_	_	_	_	X	X
9	ALB	9	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Bl	M	X	X	X	_	X	X	X
10	ALB	10	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
11	ALB	13	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	CrSt	M	X	X	X	_	X	X	X
12	ALB	15	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	PiSt	M	X	X	X	X	X	X	X
13	ALB	16	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Bl	M	_	_	_	_	_	X	X
14	ALB	17	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
15	ALB	18	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
16	ALB	19	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	PiSp	S	X	X	X	_	X	X	X
17	ALB	20	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
18	ALB	21	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Re	P	X	X	X	_	X	X	X
19	ALB	22	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-2Q-1C-1C-MC-MC	Re	P	_	_	_	_	_	X	X
20	ALB	23	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-3Q-1C-1C-MC-MC	Re	S	X	X	X	X	X	X	X
21	ALB	24	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-4Q-1C-1C-MC-MC	Re	S	X	X	X	X	X	X	X
22	ALB	25	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-5Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
23	ALB	26	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-6Q-1C-1C-MC-MC	PiSt	S	X	X	X	_	X	X	X
24	ALB	27	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-7Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
25	ALB	28	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-8Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
26	ALB	29	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-9Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_
27	ALB	30	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-10Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
28	ALB	31	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-11Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
29	ALB	32	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-12Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_
30	ALB	33	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-13Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_

31	ALB	34	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-14Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
32	ALB	35	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-15Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
33	ALB	36	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-16Q-1C-1C-MC-MC	Re	S	X	X	X	X	X	X	X
34	ALB	37	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-17Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
35	ALB	38	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	X	X	X	X
36	ALB	40	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	CrSt	M	X	X	X	_	X	X	X
37	ALB	41	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
38	ALB	42	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	X	X	X	X
39	ALB	43	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_
40	ALB	44	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
41	ALB	45	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	PuSp	S	X	X	X	_	X	X	X
42	ALB	46	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Bl	S	X	X	X	X	X	X	X
43	ALB	48	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
44	ALB	49	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
45	ALB	50	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	S	_	_	_	_	_	X	X
46	ALB	52	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_
47	ALB	53	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	_	_
48	ALB	54	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
49	ALB	55	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	_	_	_	_	_	X	X
50	ALB	56	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
51	ALB	57	SER	16 X (SER	16xG	35346-3Q)F1/-6Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	X	X	X	X
52	ALB	58	SER	16 X (SER	16xG	35346-3Q)F1/-7Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
53	ALB	59	SER	16 X (SER	16xG	35346-3Q)F1/-8Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
54	ALB	60	SER	16 X (SER	16xG	35346-3Q)F1/-9Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
55	ALB	61	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
56	ALB	62	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_
57	ALB	63	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
58	ALB	64	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	_	_	_	_	_	X	X
59	ALB	65	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
60	ALB	67	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Bl	M	X	X	X	_	X	X	X
61	ALB	69	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Bl	M	_	_	_	_	_	X	X
62	ALB	70	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Bl	M	X	X	X	X	X	X	X
63	ALB	71	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X

64	ALB	72	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_
65	ALB	74	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	X	X	X	X
66	ALB	76	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Bl	M	_	_	_	_	_	X	X
67	ALB	77	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Bl	M	X	X	X	_	X	X	X
68	ALB	78	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	L	X	X	X	X	X	X	X
69	ALB	79	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	CrSt	M	X	X	X	X	X	X	X
70	ALB	80	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	_	_	_	_	_	X	X
71	ALB	81	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	_	_
72	ALB	83	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	_	_
73	ALB	84	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
74	ALB	85	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
75	ALB	86	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	S	_	_	_	_	_	X	X
76	ALB	87	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	X	X	X	X
77	ALB	88	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	X	X	X	X
78	ALB	89	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	LiPi	M	X	X	X	_	X	X	X
79	ALB	90	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
80	ALB	91	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	X	X	X	X
81	ALB	92	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
82	ALB	93	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	_	_
83	ALB	94	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	CrSt	M	X	X	X	_	X	X	X
84	ALB	95	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
85	ALB	96	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
86	ALB	99	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	M	_	_	_	_	_	X	X
87	ALB	101	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	S	_	_	_	_	_	X	X
88	ALB	102	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Pi	S	X	X	X	X	X	X	X
89	ALB	103	SER	16 X (SER	16xG	35346-3Q)F1/-6Q-1Q-1C-1C-MC-MC	Re	P	X	X	X	_	X	X	X
90	ALB	104	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	P	X	X	X	_	X	X	X
91	ALB	105	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	PiSt	S	X	X	X	_	X	X	X
92	ALB	106	SER	16 X (SER	16xG	35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Pi	M	X	X	X	_	X	X	X
93	ALB	108	SER	16 X (SER	16xG	35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
94	ALB	109	SER	16 X (SER	16xG	35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
95	ALB	110	SER	16 X (SER	16xG	35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	X	X	X	X
96	ALB	111	SER	16 X (SER	16xG	35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X

97	ALB 112	SER 16 X (SER 16xG 35346-3Q)F1/-6Q-1Q-1C-1C-MC-MC	ReSp	M	X	X	X	_	X	X	X
98	ALB 113	SER 16 X (SER 16xG 35346-3Q)F1/-7Q-1Q-1C-1C-MC-MC	PiSp	M	_	_	_	_	_	X	X
99	ALB 117	SER 16 X (SER 16xG 35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	CrSt	M	X	X	X	_	X	X	X
100	ALB 118	SER 16 X (SER 16xG 35346-3Q)F1/-5Q-1Q-1C-1C-MC-MC	PiSt	M	X	X	X	_	X	_	_
101	ALB 119	SER 16 X (SER 16xG 35346-3Q)F1/-6Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
102	ALB 120	SER 16 X (SER 16xG 35346-3Q)F1/-7Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
103	ALB 121	SER 16 X (SER 16xG 35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Br	S	X	X	X	_	X	X	X
104	ALB 122	SER 16 X (SER 16xG 35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re, Bl	S	X	X	X	X	X	X	X
105	ALB 123	SER 16 X (SER 16xG 35346-3Q)F1/-1Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
106	ALB 124	SER 16 X (SER 16xG 35346-3Q)F1/-1Q-2Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
107	ALB 125	SER 16 X (SER 16xG 35346-3Q)F1/-2Q-1Q-1C-1C-MC-MC	Re	S	X	X	X	_	X	X	X
108	ALB 126	SER 16 X (SER 16xG 35346-3Q)F1/-3Q-1Q-1C-1C-MC-MC	Re	P	X	X	X	X	X	X	X
109	ALB 127	SER 16 X (SER 16xG 35346-3Q)F1/-4Q-1Q-1C-1C-MC-MC	Re	M	X	X	X	_	X	X	X
110	ALB 252	((VAX1 x BRB 191)F1xG 21212)F1x(RAB655xG 22041)F1/-MQ-MQ-2Q-MC-MQ-MC	Re,Cr,Bl	L	_	_	_	_	X	X	X
111	DOR 390	DOR 390	Bl	S	X	X	X	X	X	_	_
112	I.Quimbaya	ICA Quimbaya	Re	L	XX	XX	X	X	X	_	_
113	I.J.R.	Indeterminate Jamaica Red (IJR)	ReMo	S	X	X	_	X	_	_	_
114	G 21212	G 21212	Bl	S	X	X	X	_	X	_	_
115	G35066-1Q	G35066-1Q	_	L	X	X	_	X	_	_	_
116	G35346-2Q	G35346-2Q	_	L	X	X	_	X	_	_	_
117	**G 35346-3Q**	**G 35346-3Q**	DPuBl	L	XX	XX	X	XX	X	_	_
118	G35464-5Q	G35464-5Q	_	L	X	X	_	X	_	_	_
119	G 40159	G 40159	Wh	S	XX	XX	X	X	X	_	_
120	**SER 16**	**SER 16**	Re	S	XX	XX	X	XX	X	X	X
121	Tio-Canela 75	Tio-Canela 75	Re	S	X	X	X	X	X	X	X
122	VAX 1	VAX 1	PiSt (Ca)	M	XX	XX	X	X	X	X	X
123	VAX 3	VAX 3	Re	S	X	X	_	X	_	_	_
124	VAX 6	VAX 6	Re	S	X	X	_	X	_	_	_
Total number of bean genotypes per experiment for RILs evaluation					102	102	102	25	103	100	100
Total number of bean genotypes per experiment for potential parents screening					11	11		11			

Bl (Black), Br (Brown), CrSt (Cream striped), DPuBl (Dark purple mottled with white), LiPi (Light pink), Pi (Pink), PiSp (Pink speckled), PiSt or Ca (Pink striped or carioca), ReOp (Red opaque), ReSp (Red speckled), Re,Bl (Red,Black), Re,Cr,Bl (Red, Cream, Black), ReMo (Red mottled); X (Bean Genotype screened for the identification of potential parents and experiments where they were involved).

III.1.2 Fungal Isolates

III.1.2.1.Isolate collection

The isolate of *F. solani* f.sp. *phaseoli* used in this study was collected on infected bean plants from individual fields across all the 11 agro-ecological zones of Rwanda. The origins of all isolates are indicated on the map (Fig. 3) based on location areas (provinces, districts and sectors), and geographic data such as latitude/longitude and altitude reading from a GPS (Global Positioning System). Total number of 92 isolates was obtained after culture on media, purification, and single spore subcultivation.

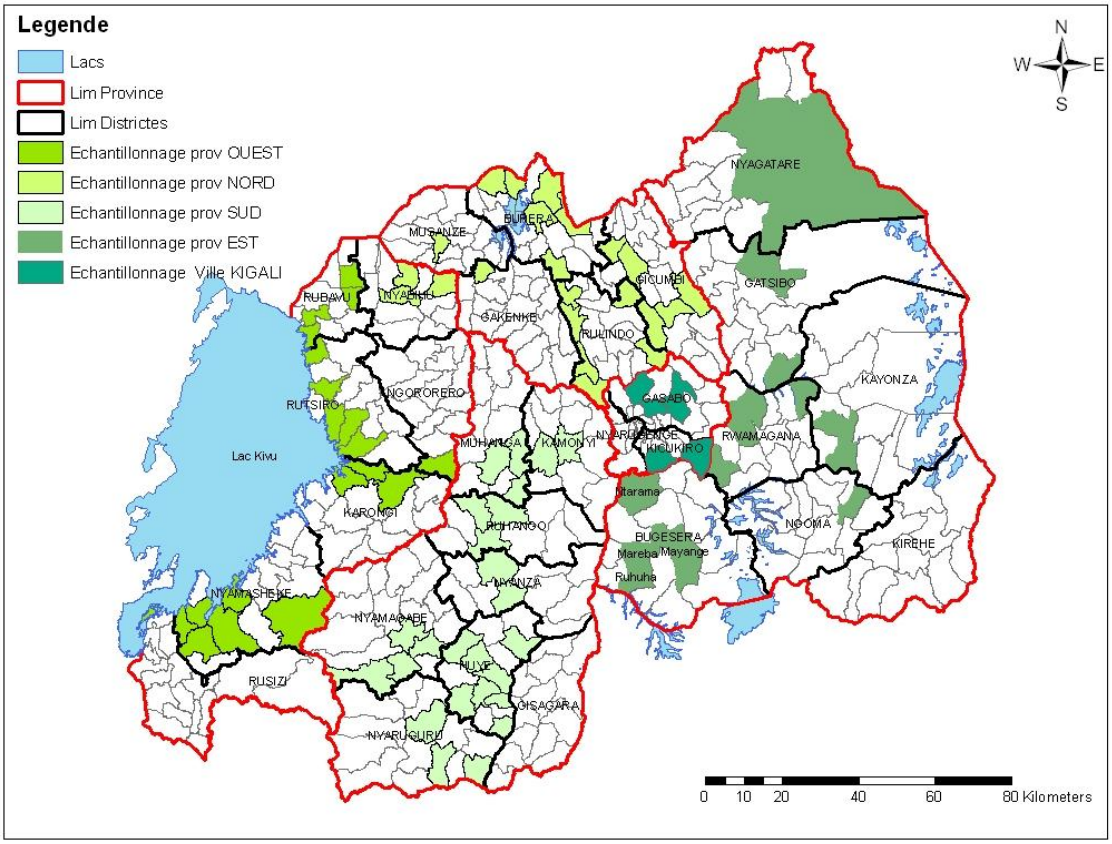

Figure 3: Geographic representation of *Fusarium f. sp*. isolate collection on infected bean plants across all the 11 agro-ecological zones of Rwanda distributed in five provinces.

III.1.2.2. Fungal isolation and culture conditions

Fusarium f. sp. isolates regeneration and test of virulence

Isolates were maintained at -40°C at Gembloux plant pathology laboratory (ULg, Gembloux Agro-Bio Tech) and were regenerated on potato dextrose agar (PDA, Difco**,** 213400; 39 g L-1 distilled water). PDA cultures were incubated for 14 days at 22 to 24°C. Morphological identification of *Fusarium solani* f. sp. *phaseoli* was realized using a microscopy (Fig.4). Fresh PDA media have been used to produce single spore colonies. Test of virulence of all *Fusarium* root rot isolates was made on susceptible bean variety, RWR1668; and allowed the identification of three pathogenic isolates to be used in our study. These isolates were from 3 different agro-ecological zone of Rwanda (North, South and West) for diversification of their pathogenicity, and identification of strong resistance among bean genotypes and recombinant inbred lines evaluated. The inoculum mixture of three isolates of *F. solani* f. sp. *phaseoli* was used to produce inoculum on sorghum substrate for this study. The evaluation of resistance to *Fusarium* root rot (T8.RGSFs) among the recombinant inbred lines (RILs) from a back cross SER16♀ x (SER16♀ x G35346-3Q♂) was realized on four-week old plants.

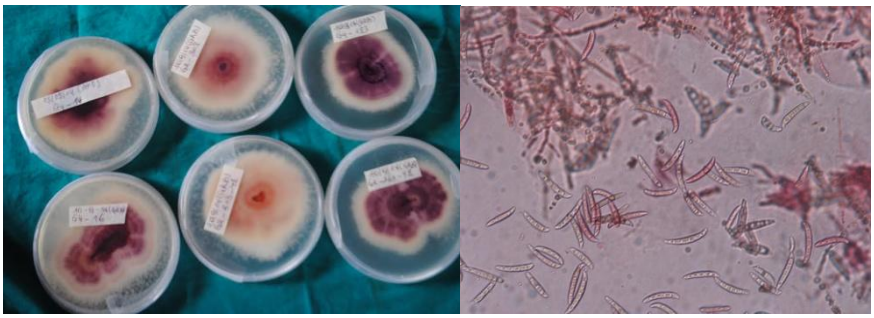

Figure 4: *Fusarium solani* f. sp. *phaseoli* grown on potato dextrose agar media (photo a) and observation of conidia (photo b) under microscopy

Inoculum production and inoculation

Inoculum of *Fusarium f. sp. solani* was produced on sorghum substrate using 20 day old single spore colonies. Sorghum seed was washed 3 times with tap water and placed in cleaned bottles (half full) before autoclaving for 20 minutes at 120°C, and re-autoclaving two days after. Bottles with sterile sorghum seed were cooled under laminar flow for 6 h. Three single colonies of Fusarium from 20 days old PDA media were macerated in 100 ml of autoclaved distilled water, and distributed to 1 kilo of sorghum. Lastly, the bottles were covered with cotton and aluminum paper, and agitated to mix inoculum solution and sorghum substrate, and incubated at 20-22°C to allow

Fusarium solani f. sp. *phaseoli* to grow (Fig.5). After five days incubation, the bottles were opened to evaporate the excess of moisture in the bottles; incubated again, and emptied after 3 week of incubation. The inoculated sorghum substrates were allowed to dry slowly on a bench in greenhouse, which ensured the maturation of the fungal resting spores.

Figure 5: Growth of *Fusarium solani* f.sp. *phaseoli* on sorghum seed substrates

III.2. Greenhouse Screening

Three greenhouse trials were conducted at CIAT headquarters in Palmira (Lat. 3°29' N; Long. 76°21' W, Altitude 965 m) using hydroponic and soil cylinder systems. For purposes of these studies, ‘‘Al resistance’’ refers to the response of a genotype to toxic Al in the hydroponic system (T1.PGHA and T4.RGHA), and tolerance to Al-toxic acid soil conditions refers to tolerance to high Al saturation (HAl) in acid soil together with low availability of nutrients (T2.PGSA and T5.RGSA). A single hydroponic screening (Fig. 6; T1.PGHA and T4.PGHA) employed a low ionic strength nutrient solution to evaluate root traits of seedlings grown with or without 20 µM Al in a basal nutrient solution (Rangel et al. 2005, 2007; Butare et al. 2011). One soil cylinder experiment (Fig. 6;T2.PGSA and T5.PGSA) carried to compare plant response in two oxisols with high Al (HAl) and low Al (LAl) saturation (Polanıa et al. 2009; Butare et al. 2011). A second soil cylinder experiment (T3.PGSC and T7.RGSC) was conducted to evaluate plant response to individual and combined stress factors of acid soil (HAl and LAl saturation) and two levels of soil moisture (well watered (WW) and water stress (WS) induced by progressive soil drying in a factorial design (Polanıa et al. 2009; Butare et al. 2011). The three greenhouse studies were conducted twice, first for selection of potential parents, and then for evaluation of recombinent inbred lines (RILS) (Fig. 6). A separate soil tube greenhouse experiement for screening to drought tolerance (T6.RGSD) was carried out with the RILs using Darien soil, and two level of water treatment.

Figure 6: Evaluation for Al resistance using hydroponic (T1.PGHA and T4.RGHA) and Al tolerance using soil tube screening method in greenhouse at CIAT headquarter in Palmira-Colombia (T2.PGSA and T5.RGSA)

III.2.1. Evaluation for Al resistance using hydroponic system

The hydroponic experiment (T1.PGHA and T4.RGHA) was conducted during November and December 2007. Plants were grown in a greenhouse with an average temperature of 31.1/22.3°C (day/night), a relative humidity of 48.0/67.3% (day/night), under natural light and photoperiod (natural daylengths of 12.5 hour) and with a maximum photosynthetic active radiation of 1,100 µmol $m^{-2}s^{-1}$ photon flux density at noon. Seedlings with uniform root length (5–7 cm) were selected for evaluation with nutrient solution composed with 5 mM $CaCl_2$, 0.5 mM KCl and 8 µMH_3BO_3 at pH 4.5, with or without 20 µM $AlCl_3$ (Rangel et al., 2007; Lopez-Marın et al., 2009; Butare et al., 2011). Twenty liter plastic tanks were filled with 16 l of nutrient solution. Each seedling was placed in an individual compartment in a tray floating on the solution, and the nutrient solution was permanently aerated with a compressor during the evaluation. Acclimation to low pH before applying Al treatment was made by adjusting the solution pH to 5.5 for 0 h, followed by pH 4.9 for 18 h and lastly by pH 4.5 for 24 h (Rangel et al.2007; Butare et al., 2011). Nutrient solutions were renewed every third day. Plants with the same root length were distributed in pairs in each treatment after measuring the length of tap root with a ruler. The experimental design was a randomized complete block with five to ten replications. Root morphological attributes were evaluated. Tap root length (TPRL) at 48 h (TPRL48h) and at 120 h (TPRL120h) was recorded. Tap root elongation rate (TRER) was determined at 48 h (TRER48h) and after 120 h (TRER120h) with and without Al stress based on the initial measurement of tap root length. TRER was defined as the difference between the initial and final tap root length during the treatment period; and Al-induced inhibition of TRER was calculated according to Rangel et al. (2005) and Butare et al. (2011):

Inhibition of root elongation (%): $[(TRER_{control} - TRER_{Al})/TRER_{control}] \times 100$

At harvest, roots were separated from the rest of the plants, saved in plastic bags and refrigerated at 4°C while proceeding to analyze images using a flatbed colour scanner, Epson Expression1680 Scanner. Differences in root morphological attributes among genotypes including total root length (TRL), mean root diameter (MRD), and number of root tips (NRT) were analyzed using WinRhizo® software program. Specific root length (SRL, root length per unit dry weight) was calculated, and the root dry weight (RDW) was determined by drying roots at 65°C in an oven for 48 h.

III.2.2. Soil-based study

For the evaluation for individual (T2.PGSA) and combined (T7.RGSC) stress of Al and drought in soil two soil cylinder experiments were carried out, each arranged as randomized complete blocks. The first soil cylinder experiment (T2.PGSA) compared plant response in Oxisols with LAl and HAl saturation, and was conducted during June–July 2007 in a greenhouse in Palmira (CIAT/Colombia) with an average temperature of 29.4/23.1°C (day/night), relative humidity of 57.2/79.4% (day/night), under natural light and photoperiod (natural daylengths of 12.5 hour), and maximum photosynthetic active radiation of 1,100 μmol $m^{-2}s^{-1}$ photon flux density at noon. Stress of Al toxic acid soil was simulated using soils collected from Santander de Quilichao, Cauca Department (3°06' N lat., 76°31'W long; 990 m altitude), Colombia (Butare et al. 2011, 2012). All soil tube experiements were planted as a randomized complete block design with three replications. Soil used in the Al stress treatment (HAl) was characterized by a pH of 4.11 and 76% Al soil saturation (Aluminium saturation percent, ASP = Exchangeable aluminium/ Cation Exchange capacity x 100) (0–10 cm) for top-soil (top 10 cm of the cylinder) and 83% Al saturation for subsoil (10–75 cm) with pH 4.14 (Table 3). This treatment did not receive any additional fertilizer application to simulate HAl with low nutrient availability soil conditions that are typical of Al-toxic acid soils. Root and shoot growth of bean genotypes under this treatment was visually (based on symptoms) restricted by both Al-toxicity and low availability of P. Soil used for LAl treatment was characterized by a pH of 4.45 and 28% Al saturation (0–10 cm) for top-soil and 58% Al saturation for subsoil (10–75 cm) with pH 4.29.

III.2.2.1. Al-toxic acid soil in tube experiment

The soil cylinders for low Aluminum (LAl) treatment (T2.PGSA and T5.RGSA) were packed with Quilichao soil (described in Table 3), previously fertilized with adequate amendments (g kg^{-1}soil) for top soil (0–10 cm): 3.69 N (urea), 5.30 P (triple superphosphate), 5.30 Ca (triple superphosphate), 4.08 K (KCl), 6.36 Ca ($CaCO_3$), 6.36 Mg ($MgCO_3$ or dolomite lime), 0.49 S (elemental sulphur), 0.09 Zn ($ZnCl_2$), 0.11 $CuCl_2$ $2H_2O$, 0.01 B (H_3BO_3) and 0.1 Mo ($NaMoO_4$ $2H_2O$); and for subsoil (10–75 cm) 14.76 N (urea), 21.2 P (triple superphosphate), 21.21 Ca (triple superphosphate), 16.32 K (KCl), 25.45 Ca ($CaCO_3$), 25.45 Mg ($MgCO_3$ or dolomite lime), 1.97 S (elemental sulphur), 0.36 Zn ($ZnCl_2$), 0.46 $CuCl_2$ $2H_2O$, 0.05 B (H_3BO_3) and 0.02 Mo (Na-MoO_4 $2H_2O$). This level of fertilizer application was designed to provide adequate supply of nutrients, and it did not affect Al saturation and pH of the amended soil. The polyethylene cylinders (Fig. 7) were inserted into PVC pipes and were maintained at 80% field capacity by weighing each cylinder every 3 days and applying water to the soil at the top (Polanıa et al. 2009; Butare et al. 2011).

Table 3: Characterization of two soils from Santander del Quilichao (plot number D6-1) used for evaluating acid soil tolerance with their chemical characteristics.

Al saturation	Soil depth (cm)	pH	Al	Ca	Mg	K	Al sat.	SOM[1]	Available P
			(cmol kg^{-1} soil)				(%)	(%)	(mg kg^{-1})
High	0-10	4.11	4.60	0.94	0.30	0.18	76	5.96	8.80
High	10-20	4.14	4.40	0.69	0.16	0.07	83	4.94	3.30
Low	0-10	4.45	1.65	3.32	0.89	0.26	28	5.38	9.70
Low	10-20	4.29	3.02	1.63	0.25	0.28	58	4.56	4.30

[1] SOM = soil organic matter.

Shoot and root attributes were evaluated on plants. Total chlorophyll content (SPAD) was measured every week using SPAD-502 Chlorophyll meter (Minolta camera Co., Ltd, Japan). Visual rooting depth (VRD) was determined at 29 days after planting. At the time of harvest (29 days after planting), leaf area (LA) was determined by scanning leaves of each genotype using a LI-3100 Area meter (LI-COR Biosciences). Shoot dry weight (SDW) was measured after drying leaves, stems and pods in an oven at 70° C for 72 h. Each soil cylinder was sliced into six layers representing different soil depths (0–5, 5–10, 10–20, 20–40, 40–60, 60–75 cm), soil and roots were collected, and roots washed and cleaned to separate living plant roots from organic debris before

scanning. Root length and biomass distribution were determined for each profile, but cutting of soil cylinders at different depths did not permit measuring NRT.

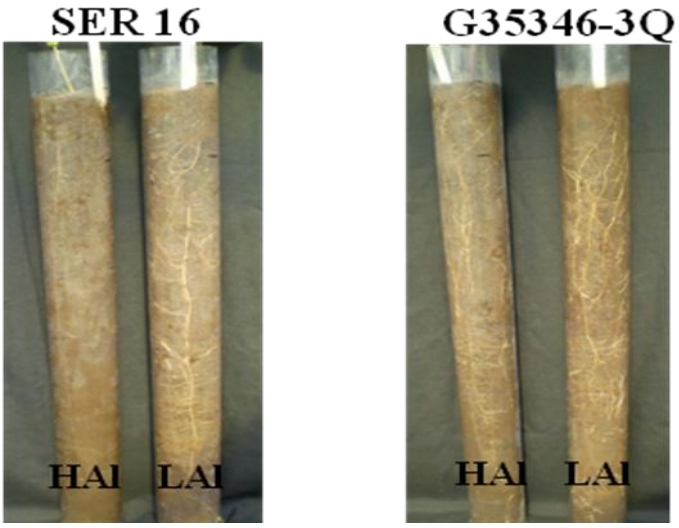

Figure 7: Soil cylinders with high aluminum (HAl) and low aluminium (LAl) treatment, and root development of two parents (SER 16 and G35236-3Q) of interspecific recombinamt inbred lines (RILs)

III.2.2.2. Greenhouse drought simulation in soil tube

This soil tube experiment (T6.RGSD) was conducted during July-September 2008 in greenhouse in Palmira (CIAT/Colombia) with an average temperature of 28.8/22.3°C (day/night), a relative humidity of 52.2/69.8% (day/night) and an average of photosynthetic active radiation of 814 µmol m^{-2} s^{-1} photon flux density at noon under natural light and photoperiod (natural daylengths of 12.5 hour). Soil for the experiment was collected from Darién, Valle del Cauca, department of Colombia; located at 3°55' N; 76°28' W; 1523 m for mean elevation; average annual precipitation are 1650 mm and 20 °C of mean temperature. Soil was mixed with river sand in a proportion soil by sand (2:1). This proportion increased soil drainage and made easy water stress induction. Soil-sand was fertilized with the following levels of nutrients (en g per 150 kg of sandy soil) to promote vigorous plant development: 12.648 Urea (N); 18.182 Triple Superphosphate (P); 18.182 Superphosphate (Ca); 13.986 KCl (K); 21.818 Dolomite lime (Ca); 21.818 Dolomite lime (Mg); 1.691 Elemental Sulphur (S); 0.309 $ZnCl_2$ (Zn); 0.392 $CuCl_2.2H_2O$ (Cu); 0.042 H_2BO_3 (B); and $Na_2MoO_4.2H_2O$; and mixed thoroughly using a concrete mixer. Cylinders were packed until soil-sand mix reached up to 75 cm length the top of cylinder using, mean 3.5 kg.

The experiment was planted as a Randomized Complete Block design with two treatments (well watered: WW, and water stress: WS) and 3 replications. For drought the trial was set up with two levels of water supply maintaining the well-watered soil tubes at 80% field capacity and withholding water supply to the terminal drought stress tubes. Water stress was imposed seventeen days after plants emerged from the soil. A number of shoot attributes including plant vigor and height, and number of leaves per plant was determined. Leaf chlorophyll content was measured using a non-destructive hand held Chlorophyll meter (SPAD-502 chlorophyll Meter, Minolta Camera Co. Ltd., Japan). Leaf canopy temperature was also determined with an infrared thermometer (Telatemp model AG-42D, Telatemp CA, USA). Stomata conductance was measured with a steady-state porometer (Model Li-COR, Lincoln, NE). Root depth was measured each three days by ruler targeting the deepest visible root and registering the length reached.

Shoot and roots of 45 days old plants were separated at harvest time in order to determine the leaf area and shoot biomass. Total leaf area of each plant was determined using a Leaf area meter (LICOR model Li-3000). Shoot dry biomass was determined by drying in an oven at 70° C for 72 hours and weighing. Each soil tube sliced into different soil profiles (0-5, 5-10, 10-20, 20-40, 40-60, 60-75 cm); soil and roots collected; roots washed and cleaned before scanned for root images analysis using WinRHIZO software to measure root attributes (root biomass, root length, specific root length, mean root diameter, root volume).

III.2.2.3. Individual and combined stress of Al and drought

Individual and combined stress of Al and drought (T3.PGSC) was evaluated in September 2008 under greenhouse conditions at an average temperature of 30.7/23.3°C (day/night), a relative humidity of 49/68.3% (day/night), and at an average photosynthetic photon flux density of 820 µmol m^{-2} s^{-1} during the day under natural light and photoperiod (natural daylengths of 12.5 hour). Plants were grown in transparent tubes inserted in PVC pipes as previously described for Al screening with same soil type from Santander de Quilichao. The individual and combined stress trial was planted with four treatments including two levels of aluminum saturation in soil (high Al and low Al) and two levels of water supply (Well watered and terminal drought). The low Al saturation treatment was fertilized as described above. The experimental design was a randomized complete block with two levels of Al saturation in soil respectively for 0-10 cm and 10-75 cm, high Al saturation (HAl): 76% and 83% and low Al saturation (LAl): 28% and 58%, and two levels of water supply (well watered and terminal drought simulation) and all into 3 replications. Each cylinder was packed with two types of soil (top-soil and sub-soil); and maintained at 80% of field

capacity by weighing every three days (4780 g for high Al saturation treatment tubes and 4910 g for the low saturation). Water stress (WS) was imposed to simulate progressive soil drying after 10 days in the terminal drought treatment while for the well watered (WW) treatment; water was applied to the top of cylinders to maintain them at 80% of field capacity. At harvest time, shoots and roots of 33 day-old plants (23 days without water application in the terminal drought treatment) were separated, and leaf area measured by scanning leaves. Shoot biomass was determined after drying leaves and stems in an oven. Roots were processed in the same way as for previous soil tube trial with Al-toxic acid soil alone and the same parameters were determined.

III.2.3. Tolerance to *Fusarium* root rot

Resistance of common beans to root rot is a quantitative trait that is strongly influenced by environmental factors. Reproducible methods of screening bean plants for resistance to root rot are critical to the selection (Chaudhary et al., 2006). For the evaluation of tolerance to *Fusarium solani* f. sp. among RILs population, a screening method using infested sorghum substrate was used. Experiments (T8.RGSFs) were carried out at Gembloux in controlled conditions in greenhouse maintained at 14 hours of light and 10 hours of darkness with a day to night temperatures ranging from 20 to 22 ^{o}C, and relative humidity of 92.5 to 95.6 %. Genotypes were sowned in 1.5 l pots with the infested sorghum substrate mixed of commercial garden soil and vermiculite (2:1). The 5 kg of the substrate was mixed to soil-vermiculite volume equal to 100 plastic pots. Seeds of the 103 bean genotypes including 94 RILs, two parents (SER16 and G35346-3Q) and 7 checks (ALB252, DOR390, ICA Quimbaya, G21212, G40159, Tio-Canela75, and VAX1) were sown. The experimental design was a randomized complete block (2 plants per pot to be scored) and 3 replications. From the 3 seeds sown in each pot and 2 seedlings were maintained for scoring after emergence from the soil. Disease severity was determined using a rating scale adapted from Schneider and Kelly (2000) by Chaudhary et al. (2006) for bean root rot screening through observation of vascular discolation using the inoculum layer method (ILM) developed by Schmitthenner and Bhat (1994). Bean plants were uprooted carefully, roots and hypocotyls were cleaned properly with tap water. Then after cleaning, harvested plants (from the greenhouse) was visually scored by assessing tap root and lower hypocotyl discoloration using a rating scale of 1-9 (Table 4) as discribed by Chaudhary et al. (2006) for bean root rot.

Table 4: Description of disease rating scale based on severity of lesion on tap root and lower hypocotyl under screening for resistance to root rot (*Fusarium solani* f. sp. *phaseoli*) on beans (adapted from Schneider and Kelly (2000) by Chaudhary et al. (2006)).

Disease score	Phenotypic description
1	no apparent infection
2	0.1-0.5 cm reddish brown lesion
3	0.5-1.0 cm reddish brown lesion and covering half of the stem
4	1.0-1.5 cm reddish brown to brown lesion
5	1.5-2.0 cm brown to dark brown lesion, lesion girdling the stem
6	2.0-2.5 cm brown to dark brown lesion, lesion often associated with increasing intensity
7	2.5-3.0 cm brown to dark brown lesion, lesion often associated with increasing intensity
8	3.0-3.5 cm brown to dark brown lesion, lesion often associated with increasing intensity
9	Dead plant

III.3. Field study of the RIL population

III.3.1. Evaluation in drought stressed and non-stressed field in Palmira

The 96 F5:7 families from RILs (SER 16♀ x (SER 16♀ x G35346-3Q♂)) were evaluated both under water-stressed and non stressed experiments (T9.RFSD) in Palmira (3°29'N; 76°21'W). The four checks (SER16, *P.vulgaris* parent; Tio-Canela75, VAX1, and ALB252) were included in each trial. Moisture stress and non-stress treatments were applied through control of irrigation. Both water-stressed (terminal drought) and non stressed plots received a pre-germination irrigation. Rainfed plots (water stress) received two additional irrigations (of 30 mm each) during sixteen days after sowing and one week before initiation of flowering (for 36 days old plants). Total amount of watering including rainfall was 197.7 mm. For the irrigated plots, rainfall was short (81.8 mm), and six additional irrigations of 40 mm each were applied; and the total amount of water received during the season was 321.8 mm. Among collected data were days to flower and maturity, 100 seeds weight and grain yield (kg per ha). Several yield parameters were calculated : yield per day (kg/day/ha), grain filling index (GFI = 100 seed weight of rainfed/100 seed weight of irrigated) and pod harvest index [PHI (%) = seed biomass dry weight at harvest/pod biomass dry weight at harvest x 100], and also the pod wall biomass proportion [PWBP (%): pod wall biomass dry weight

at harvest/pod biomass dry weight at harvest x 100]. To predict the performance of each RIL under stressed and non-stressed conditions, we calculated the drought susceptibility index [DSI = (1–Yds/Yns)/DII, where Yds and Yns are mean yields of a given genotype in drought stress and no stress environments, respectively (Fischer and Maurer, 1978), and DII (drought intensity index = 1–Xds/Xns, where Xds and Xns are the mean of all genotypes under drought stress and no stress treatments, respectively).

The irrigation treatments were considered as main plots and genotypes as subplots. The experimental design was a 10 by 10 balanced lattice but with four replications (10 X 10 X 4). For the square lattice design used here, the number of treatment must be a perfect square ($t=k^2$), and plots per blocs (k) are equal (s=k), and are the square root of the number of treaments (t). This type of design increase precision and make comparisons under more uniform condition. The trial (T9.RFSD) was repeated in two different cropping seasons (January to March as season A, and June to August, as season B).

III.3.2. Evaluation in Quilichao Al-toxic acid-soil

The same number of RILs and checks were evaluated also in Quilichao (T10.RFSA), CIAT experimental site (Latitude and longitude: 3°06' N; 76°40' W) with 990 m elevation, mean temperature 24.1°C annual rainfall of 1756 mm. The soil type is an oxisol (very fine, kaolinitic, isohyperthermic Plinthidic Kandiudox) with high Al saturation (70.9-75.1%) and low pH (4-5.5). Field preparation and sowing were also made by machine. Weeds, diseases and pests were controlled with all recommended agronomic practices. Plots were two rows of 3.72 m long each spaced by 60 cm and 10 cm within row. Soil amendments were applied just after plant germination (six days after sowing) and were made by dolomite calcium (0.5 ton per ha), 200 kg of $MgSO_4$ (20% MgO and 3% P_2O_5) and 50 kg of Agrimins as source diverse microelements. The field was under rain fed conditions with two irrigations (25 mm each) : one for pre-germination and the other one at pod filling. The total amount of water received by crop during the season was 594 mm. In Quilichao, the experimental design was also a 10 by 10 balanced lattice but with four replications (10 X 10 X 4). Genotypes were considered as main plots and replications as subplots. Plots were hand harvested to calculate yield. Data were collected on yield and yield components: days to flower and maturity, 100 seed weight, yield (kg per ha) and Yield per day per ha). The trial was also repeated in two different cropping seasons

III.4. Statistical analysis for greenhouse and field trials

Analysis of variance was performed using SAS computer program, SAS 9.1 (SAS institute Inc. SunOS 5.9 platform). Analysis of variance was pursued using the ANOVA procedure of statistical program and means were compared using the Duncan test. Three probability levels (0.001, 0.01 and 0.05) have been considered as statistically significant. Correlation coefficients were calculated (PROC CORR) for all genotypic means among replications and treatments.

III.5. QTL development for root traits, yield and yield components of recombinant inbred lines from backcross BC1 F5:6 SER 16 x (SER16 x G35346)

III.5.1. Plant material and mapping population

The mapping population was developed by a cross between SER16 and G35346-3Q. *P. coccineus*, G 35346-3Q, is a sister species of common bean which is characterized by a very aggressive vine with great biomass and low harvest index; and SER 16 is a Mesoamerican small red drought tolerant common bean line. They were identified as contrasting parents (Butare et al., 2011). A high level of Al resistance had been observed in G35346-3Q compared to SER 16 (CIAT, 2007; Butare et al., 2011). A backcross of the F_1 hybrid to the recurrent parent was pursued to create recombinant ibred lines, to recover the desirable plant traits, and seed type of *P. vulgaris*. The mapping population has been phenotypically evaluated both in greenhouse, hydroponics (T4.RGHA) and soil tubes (T5.RGSA); and field under irrigated and non-irrigated fields in Palmira (T9.RFSD) and Al-toxic acid soil in Quilichao (T10.RFSA).

III.5.2. DNA extraction and Molecular marker analysis

III.5.2.1. DNA extraction, SSR primers, PCR conditions and electrophoresis

Microsatellites, known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are repeating sequences of 2-5 base pairs of DNA. They are typically co-dominant, and are also single-locus markers that are specific to given places in the genome. They can serve as highly informative genetic markers.

For each F5:6 families, leaves were harvested from 2 plants grown for 7 weeks in greenhouse; leaves bulked, freeze-dried, and milled to a fine powder. The isolation of total DNA was performed

using a CTAB extraction method as described by Afanador and Hadley (1993). A total of 64 common bean microsatellite were used in this study. They were selected from 63 BM markers (Gaitán-Solis et al., 2002); 55 BMd (Blair et al., 2003); and others including BMy (Yu et al., 2000), GA, CA, CAC and BMa using both parents. The final matrix was constructed with a total of 64 selected markers taking into account all missing data per line, these include 35 BM markers, 19 BMd markers, 7 BMa markers, and 3 individual markers (CAC1, CA5, and GA16).

Polymorphisms between the mapping parents were determined on parental survey gels. Standard microsatellite conditions as defined by Blair et al. (2003) were used. For further screening any primer that didn`t amplify parental DNA under these conditions will not be considered. After amplification, a maximum volume of 3 µl of formamide, containing 0.4% bromophenol blue and 0.25% w/v xylene cyanol FF, was added to each PCR reaction and the mixture was denatured at 92ºC for 2 min. Four microliter of the mixture were then loaded onto 4% denaturing polyacrylamide (29:1 acrylamide:bis-acrylamide) gels that contained 5 M urea and 0.5 x TBE, and run in Sequi-Gen GT electrophoresis units (Biorad, Hercules, Calif., USA) at a constant power of 120 W. Detection of PCR amplification products was carried out with silver staining (Blair et al. 2003). The sizes of the parental alleles were estimated on 10-bp and 25-bp molecular-weight ladders. Microsatellites that were polymorphic for the parents of the population were amplified on the RILs.

III.5.2.2. SSR allele scoring

Alleles of the progenies were scored based on the parental bands amplified as controls along with the RILs. Size ladders were added with the load to confirm the allele sizes observed in the parental survey. First a matrix was created using raw data where the allele of *P. vulgaris* parent was represented by A and *P.coccineus* parent by B, and heterozygous allele by H. For this matrix missing data was calculated for all lines both for BM and BMd markers. Using this information a final matrix with a total of 64 markers was constructed with 89 lines. The frequency of each allele per marker was calculated, and the Chi test determined on ratio of 3:1 to understand the behavior of all markers. The test was made by taking into account only the total amplification excluding missing data.

III.5.2.3. Construction of the linkage map

Each F5:6 individual plant was scored with A, B or H code (A: Homozygous for parent 1 allele, B: Homogozygous for parent 2 allele, and H: Heterozygous containing one allele from each parent). Mapdisto v.7 was used for the genetic mapping (Lorieux, M., 2012). SSR markers data were subjected to the chi-square test (P=0.05) to verify the adjustment to the expected segregation in the recombinant inbred lines (RILs) of 3:1 ratio, considering the codominant nature of SSR markers.
The best marker order for each linkage group was determined with the "ripple function" at Lod >3. Genetic distance was based on recombination fraction using the Kosambi function to estimate map distances from recombination values. The x^2 test was applied to identify markers with a distorted (P<0.01) segregation from the expected ratios. Total map length for the base map was 156.91 CM. The best marker order of the linkage groups was checked with the best plausible positions in maximum likelihood mapping algorithm set to 1000 permutations. For the QTL analysis for all traits, simple interval mapping in QTL Cartographer (V. 2.5) software was used (Basten et al., 2001).

CHAPTER IV. RESULTS AND DISCUSSION

IV.1. Identification of new sources of resistance in *Phaseolus* species to individual and combined stress factors of Aluminum and drought

To identity sources of resistance to Al and drought, eleven bean genotypes were selected for the study (Table 2), including 4 *P. coccineus* accessions (G 35066-1Q, G 35346-2Q, G35346-3Q and G-35464 5Q); 6 common bean genotypes including 4 from Mesoamerican lines (VAX1, VAX3, VAX6, SER16) and 2 from Andean large seeded beans (ICA Quimbaya and I.J.R, Indeterminate Jamaica Red); and one *P. acutifolus* accession (G40159).

IV.1.1. Effect of Al stress on root development and architecture

Phenotypic characterization of 11 bean genotypes for Al resistance in nutrient solution (T1.PGHA) revealed that all genotypes were affected by Al toxicity (Table 5). Results on total root length (TRL), tap root elongation at 48 h (TPRL48h) and 120 hours (TPRL120h), root biomass dry weight (RDW), mean root diameter (MRD), specific root length (SRL) and number of root tips (NRT) showed considerable architectural variation in response to Al stress (20µM Al) among bean species and varieties (Table 5).

IV.1.1.1. Total root length and tap root elongation

In our study (T1.PGHA), genotypic differences were highly significant ($P < 0.001$) for all root traits except for root length at 48h where the significance was at $P < 0.05$. Three *P. coccineus* accessions (G35464-5Q, G35346-2Q, and G35346-3Q) showed a high level of resistance to Al whereas three Mesoamerican bean genotypes (VAX6, VAX3 and SER16) were found more sensitive. Our results indicated that 20 µM Al significantly affected root system of sensitive bean genotypes compared to Al resistant *P. coccineus* accessions. Resistance to Al-toxic stress was associated with some primary root traits (Table 5). Al stress effect revealed significant genotypic differences ($P < 0.001$) for TRL, TPRL120 h, RDW, MRD, SRL and NRT. Similar results were found by López-Marín et al. (2009) for Genotype, and Genotype x Al treatment interaction. In contrast, in our study Genotype x Al treatment was significant only at $P < 0.05$ for TRL and MRD and highly significant for NRT ($P < 0.01$) and SRL ($P < 0.001$) (but not for TRER) indicating that the 11 genotypes showed differential response to Al stress. Total root length was highly correlated with root length at

48 h (r = 0.77), with root length after 120 hours (r = 0.92), with root biomass dry weight (r = 0.91) and with number of root tips (r = 0.96). Correlation was also found between TPRL48 h and TPRL120 h (r = 0.74, P < 0.01); and between TPRL48 h and NRT (r = 0.91, P < 0.001) (Table 6). These positive and high correlations suggested a strong relationship between NRT, TRL, and TPRL120h for evaluation of beans in the hydroponic system.

High range of diversity among the 11 bean genotypes was observed with and without Al in hydroponics for these three root characteristics confirming their genetic diversity as they are from three different genepools (primary, secondary and tertiary genepools). Root vigor as reflected in root system size (TRL) and tap root length (TPRL) of Al-stressed plants compared to non-stressed plants revealed changes in root growth and function, and were interpreted as indicators of Al resistance (Manrique et al., 2006). In contrast López-Marín et al. (2009) found that while bean genotype G19833 showed a higher tap root elongation than DOR364 in both control and Al treatment solutions, differences in TRL between the two genotypes were less notable. In wheat also, clear differences in tap root length was observed between Al-tolerant and Al-sensitive wheat seedlings at 20 µM Al, with 50% inhibition of root growth (Delhaize et al., 1993).

Table 5: Influence of Al-stress with aluminum (20 µM Al) and without aluminum (0 µM Al) on total root length (TRL), Tap root length (TPRL) at 48 h and at 120 h Root dry weight (RDW), mean root diameter (MRD), Specific root length (SRL) and number of root tips (NRT) of 11 bean genotypes from three *Phaseolus* species (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) grown in hydroponic system.

Genotypes	TRL (m)		TPRL48h (cm)		TPRL120h (cm)		RDW (g)		MRD (mm)		SRL (m g^{-1})		NRT (no.)	
	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al	20µM Al	0µM Al
G 35464-5Q	8.4	11.7	22.4	22.3	30.8	31.1	0.13	0.12	0.57	0.5	68.2	91.4	926.3	1924.2
G 35346-2Q	8.1	11.0	17.3	20.0	26.4	28.7	0.15	0.16	0.69	0.61	53.6	72.8	668.4	1307.4
G 35346-3Q	5.5	9.7	16.7	19.6	24.0	27.1	0.11	0.14	0.62	0.57	49.3	70.5	472.6	1395.0
G 35066-1Q	4.2	8.8	16.0	18.4	20.9	22.6	0.1	0.12	0.7	0.55	41.2	75.3	374.3	1325.6
ICA Quimbaya	3.2	5.9	17.4	19.2	20.2	24.2	0.07	0.09	0.63	0.54	44.3	62.6	364.9	1221.2
I.J.R.	2.8	4.7	16.4	18.6	18.9	23.9	0.06	0.09	0.6	0.55	53.7	61.6	396.8	965.3
VAX 1	2.5	6.4	16.4	21.0	17.0	26.2	0.04	0.06	0.57	0.45	59.8	114.6	284.0	1110.6
G 40159	2.4	4.0	16.6	20.5	17.8	23.5	0.03	0.04	0.47	0.42	86.1	111.4	306.9	707.0
SER 16	2.0	3.7	15.8	18.0	17.0	23.0	0.03	0.05	0.58	0.51	64.1	75.9	228.8	561.1
VAX 3	2.0	4.9	16.1	20.3	18.2	26.5	0.03	0.06	0.56	0.48	63.2	91.4	218.9	802.9
VAX 6	1.9	3.3	13.0	15.9	14.3	19.7	0.04	0.06	0.63	0.58	49.9	56.0	177.3	422.1
Mean	3.5	6.3	16.5	19.4	19.6	24.8	0.07	0.08	0.6	0.52	56.3	80.5	360.7	1028.8
$LSD_{0.05}$	0.3	0.4	0.6	0.7	0.8	0.8	0.01	0.02	0.03	0.02	15.3	25.7	4.8	7.0

Table 6: Correlation coefficients and mean squares for total root length (TRL), tap root length (TPRL) at 48 hour and 120 h, mean root diameter (MRD), specific root length (SRL), and number of root tips (NRT) for 11 bean genotypes (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) under hydroponics screening with two level of Al (20 µM Al and 0 µM Al)

Traits/Source	Al level/DF	TRL	TPRL48h	TPRL120h	RDW	MRD	SRL	NRT
TRL	0	1						
	20	1						
TPRL48h	0	0.55 (ns)	1					
	20	0.77**	1					
TPRL120h	0	0.78**	0.74**	1				
	20	0.92***	0.74**	1				
RDW	0	0.85***	0.13 (ns)	0.58 (ns)	1			
	20	0.91***	0.59 (ns)	0.83**	1			
MRD	0	0.14 (ns)	-0.59 (ns)	-0.04 (ns)	0.61*	1		
	20	0.29 (ns)	-0.13 (ns)	0.24 (ns)	0.57 (ns)	1		
SRL	0	0.17 (ns)	0.72*	0.26 (ns)	-0.34 (ns)	-0.84***	1	
	20	-0.23 (ns)	0.07 (ns)	-0.20 (ns)	-0.46 (ns)	-0.87***	1	
NRT	0	0.95***	0.43 (ns)	0.66*	0.86***	0.16 (ns)	0.04 (ns)	1
	20	0.96***	0.78**	0.91***	0.84***	0.20 (ns)	-0.11 (ns)	1
Level of Al	1	12.34***	4.54*	10.93***	0.0062***	0.0494***	11894.35***	5158.2***
Rep. (Al level)	40	0.099 (ns)	0.46 (ns)	0.68 (ns)	0.0002 (ns)	0.0008 (ns)	518.5 (ns)	34.97 (ns)
Genotype	10	4.93***	0.86*	2.33***	0.0157***	0.0184***	5894.5***	525.5***
Gen. X Al level	10	2.23*	0.1 (ns)	0.45 (ns)	0.0001 (ns)	0.0015*	1713.6***	73.77**
Error	236	0.12	0.37	0.53	0.0002	0.0007	377.5	30.3

*, ** and ***: significant at the1% and 0.1% level of probability, respectively.
ns: no significant

IV.1.1.2. Root growth rate

Phenotypic characterization of resistance to Al-toxicity (T1.PGHA) stress based on root growth rate, inhibition of root growth and increase of mean root diameter revealed differences in root growth per treatment period and in Al-induced changes on root morphology (Fig.8). The tap root elongation rate (TRER) of three *P. coccineus* accessions (G35464-5Q, G35346-2Q and G35346-3Q) and ICA Quimbaya (an Andean genotype) was high compared to other genotypes (rate > 0.06 $mm.h^{-1}$), with no significant difference between the four genotypes. Among these Al resistant bean genotypes, G35464-5Q and G35346-2Q showed significant differences at $P < 0.05$ (REGWQ test)

with two susceptible genotypes (VAX1 and VAX6) (Fig. 8). Clarkson (1965) considered that a reduction in root growth rate was the most obvious consequence of Al treatment. The highest root elongation rate in this study was found in three Al resistant *P. coccineus* accessions (G35464-5Q, G35346-2Q and G35346-3Q) and ICA Quimbaya compared to other genotypes, confirming the variability revealed between the two bean species observed before using hydroponic system (CIAT, 2005), and the level of Al resistance reported in ICA Quimbaya (Rangel et al., 2007).

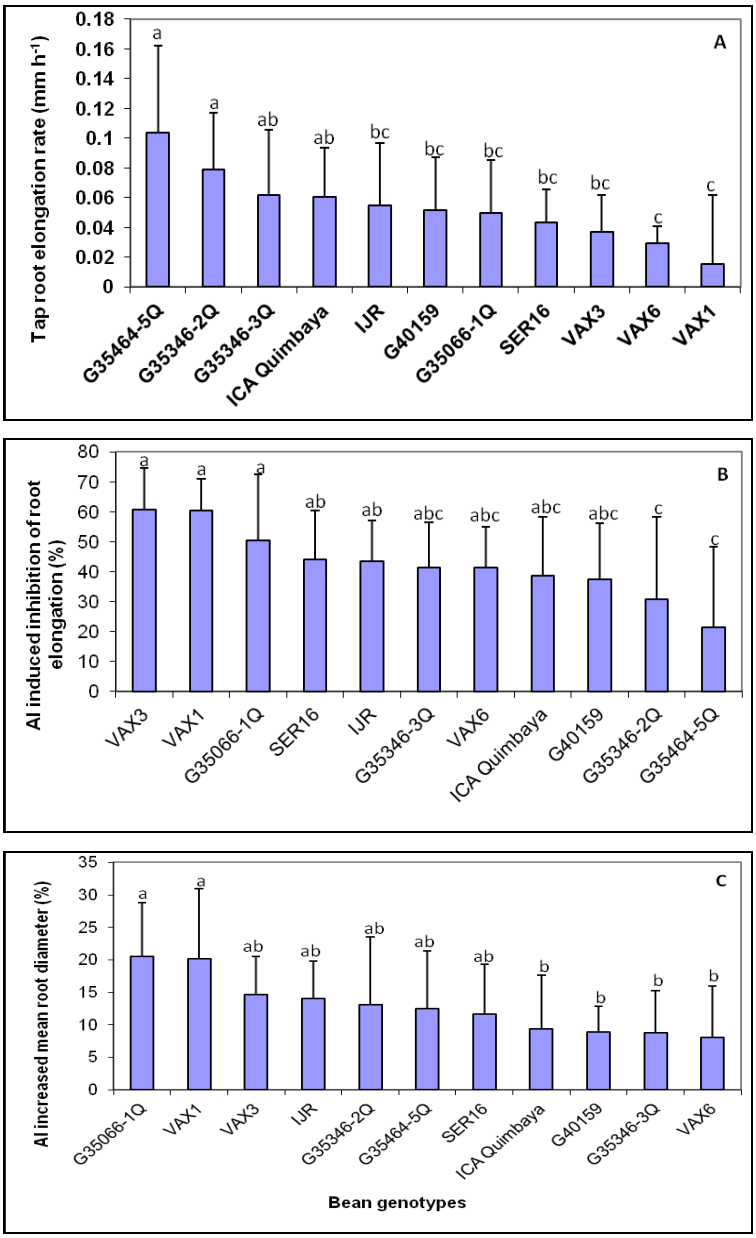

Figure 8: Root growth rate under high Al (20 µM Al) (A), percent Al-induced inhibition of root elongation (B) and increase of root mean diameter (C) of 11 bean genotypes (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) under hydroponic screening with two levels of Al (20 µM Al and 0 µM Al) at pH 4.5. Bars represent means ± SD, with 4 replicates. Different letters indicate statistical significant differences at P < 0.05 (REGWQ test).

Growth rates of roots recovering from Al treatment are initially faster than normal suggesting that the early phases of recovery may involve growth stimulation (Bennet and Breen, 1991). Comparing ICA Quimbaya to Al-sensitive genotype VAX1 in hydroponics, Rangel et al. (2007) found that the recovery continued in genotype Quimbaya (Al-resistant) until the root elongation rate reached the level of the control (without Al). Quimbaya was also less severly inhibited by Al in the elongation zone (EZ) than VAX1. We found similar results where high root growth rate (after 120 h) found in G35464-5Q and G35346-2Q was associated to lesser root growth inhibition. Kopittke et al. (2004) indicated that the higher growth rate of mungbean (*Vigna radiata* L.) roots between days 3 to 6 resulted in the accumulation of detectable quantities of Al_{13} during the exposure time.

IV.1.1.3. Inhibition of root elongation

The major Al toxicity symptom observed in plants is inhibition of root growth (Delhaize and Ryan, 1995; Marschner, 1991; Rhue and Grogan, 1977; Ryan et al., 1993). Our results (T1.PGHA) showed that at 48 h there was no difference for tap root elongation between the 11 genotypes, therefore the Al exposure time was extended to 120 h to detect genotypic variability among *P. coccineus* accessions, and root growth was inhibited by 42.7%. Three genotypes (VAX3, VAX1 and G35066-1Q) were sensitive to Al with an inhibition superior to 50%; six genotypes including SER16, Indeterminate Jamaica Red (IJR), G35346-3Q, VAX6, ICA Quimbaya, and G40159 were intermediate with a root elongation inhibition ranging between 37.5% and 44.01%, whereas only two *P. coccineus* accessions (G35346-2Q and G35464-5Q) were resistant with a root elongation inhibition of 30.8% and 21.3%, respectively. These two genotypes were significantly different at $P < 0.05$ (REGWQ test) from sensitive lines VAX3, VAX1 and G35066-1Q (Fig. 8b).Using the same method with two common bean genotypes (ICA Quimbaya and VAX1), Rangel et al. (2007) showed that root elongation was greatly inhibited at 36 h. Genotypic ranking for Al resistance in the current study (T1.PGHA) indicated that G35464-5Q and G35346-2Q were the most Al resistant with less inhibition of root growth (Fig. 8b). G35346-3Q and ICA Quimbaya were found to be intermediate in their level of Al resistance based on Al-induced inhibition of root growth but were among the best in developing fine roots (with a low increase of mean diameter) (Fig.8b). These genotypes grown in presence or absence of Al (0 and 20 µM Al) after 120 h showed also differential response among genotypes to Al for the increase of mean root diameter (Fig. 8c). Thin roots are believed to be more important than thick roots in nutrient and water absorption, and therefore more important in terms of Al resistance (Eisenstat, 1992; Villagarcia et al., 2001). Two

contrasting parents used by López-Marín et al. (2009) to generate recombinant inbred lines (RIL) presented mean root diameter significantly different, DOR364 with thinner roots than G19833. According to Liu et al. (2010) in a study on pulse crops, classification of thin roots needs to be further refined. In our work we found that the increase of average root diameter was associated with root growth inhibition ($r = 0.33$, $P < 0.0001$; data not reported). Similar results were found during an evaluation of Al resistance among 52 genotypes of common bean (CIAT, 2005). In our case, some genotypes (VAX6, G35346-3Q, G40159 and ICA Quimbaya) formed a group with less increase of root diameter (< 9.43%) and were significantly different ($P < 0.05$, REGWQ test) from G35066-1Q and VAX1 that were classified as sensitive (> 20.23 %) (Fig. 8c). Root growth inhibition was detected 2-4 days after the initiation of seed germination (Bennet et al., 1991). Significant genotypic differences in Al resistance in common bean were reported based on Al-inhibited root elongation in nutrient solution (Massot et al., 1999; Rangel et al., 2005; Manrique et al., 2006).

IV.1.1.4. Root vigor, number of tips, and branching

Genotypes combining both lower inhibition of root elongation and lower increase of root diameter were identified based on data from the hydroponics experiment (T1.PGHA) with or without Al-stress (Fig. 9). Five genotypes from different bean species and gene pools (G35464-5Q, G35346-3Q, G40159, ICA Quimbaya and VAX6) were outstanding for minimizing root growth inhibition and increase of root diameter, while another *P. coccineus* accession (G35346-2Q) presented inhibition of root elongation of 30.87% and was only slightly superior to the mean for increase of root diameter (13.1%). Using a hydroponic system, Narasimhamoorthy et al. (2007) concluded that genotypic vigor was an important factor to consider while applying selection for Al tolerance. In our study, Al sensitive line VAX1 presented inhibition of root growth and increase of root diameter of 60.28% and 20.2%, respectively, but is relatively tolerant in the field. The highest increase of root diameter under exposure of 20 µM Al was shown by G35066-1Q while root elongation of VAX3 was the most inhibited among all 11 genotypes. Higher Al contents were observed in root tips of Al-sensitive common bean compared with Al-resistant after 1 d or 3 d of Al treatment (Mugai et al., 2002; Shen et al., 2002; and Rangel et al., 2007). This could explain strong root inhibition observed in sensitive genotypes. In a study of Al-induced inhibition of root development, Villagarcia et al. (2001) reported that number of basal roots and branching from taproot were clearly reduced in all genotypes. We found similar results for number of root tips (NRT, root branching) and for specific root length (SRL) which is affected by number of fine branches. As

expected, SRL exhibited a strong negative correlation with average root diameter. However, ranking the 11 bean genotypes by specific root length values as a trait for determining Al-resistance did not correspond to ranking with any other root characteristics (Table 5). Plants with high SRL build more roots for a given dry-mass investment, it is better to consider SRL together with other morphological parameters of root thickness. High SRL can result from having a low diameter or low tissue density.

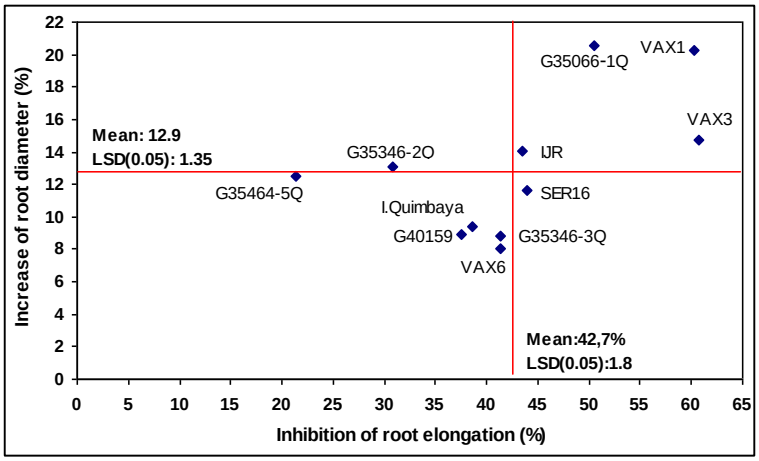

Figure 9: Relationship between Al induced inhibition of root elongation and increase of mean root diameter of 11 bean genotypes (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) grown for 48 h in a nutrient solution containing 5 mM $CaCl_2$, 0.5 mM KCl and 8 µM H_3BO_3 at pH 4.5 under hydroponic screening with two levels of Al (20 µM Al and 0 µM Al).

IV.1.2. Al-resistance and acid soil tolerance

IV.1.2.1. Root development and distribution in Al-toxic soil

The effects of Al level (T2.PGSA) were highly significant ($P < 0.001$) for all root parameters except for mean root diameter (MRD) (Table 8). Average values for low and high Al treatments respectively were: total root length (TRL), 59 and 27.4 m; mean root diameter (MRD), 0.30 and 0.29 mm; vision root depth (VRD), 67.1 and 56.7 cm; and specific root length (SRL), 106.0 and 78.7 $m.g^{-1}$ (Table 7). Genotypic differences were highly significant ($P < 0.001$) for TRL, MRD, SRL and VRD at 29 d. Al resistant genotypes G35346-2Q, G35346-3Q and G353464-5Q maintained good root structure under Al stress. Genotype x Al level interaction was significant for SRL (0.05) and VRD at 29 d (0.01). The genotype ranking based on variation of TRL, MRD, VRD and SRL was different under Al treatment and control (Table 7). Total root length was correlated (P

< 0.01) with only two root traits, VRD at 29 d (r = 0.78) and R:S ratio (r = 0.81); whereas MRD was highly correlated with VRD of 29 day-old plants (r = 0.92, P < 0.001) (Table 7).

Table 7: Influence of acid soil stress (high aluminum saturation, HAl; low aluminum saturation, LAl) on total root length (TRL), mean room diameter (MRD), specific root length (SRL) and visual root depth (VRD) for 29 days-old plants of 11 bean genotypes (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) from three *Phaseolus* species grown in soil tubes under well watered conditions.

	TRL (m)		MRD (mm)		VRD (cm) at 29d		SRL ($m.g^{-1}$)	
Genotypes	HAl	LAl	HAl	LAl	HAl	LAl	HAl	LAl
G 35346-2Q	62.0	76.8	0.33	0.35	74.3	71.3	88.8	88.6
G 35346-3Q	40.1	73.0	0.35	0.37	75.0	75.0	84.8	79.3
G 35464-5Q	40.0	66.1	0.34	0.33	68.0	66.7	96.1	89.8
G 35066-1Q	24.6	47.1	0.30	0.29	57.0	64.3	65.8	111.8
VAX 1	23.0	56.1	0.24	0.24	40.8	62.7	61.1	126.2
ICA Quimbaya	22.8	55.1	0.31	0.31	62.0	65.7	63.1	90.4
G 40159	21.3	55.5	0.25	0.27	53.0	69.0	134.3	135.7
VAX 6	19.1	50.0	0.28	0.27	55.0	57.0	71.6	106.5
I.J.R.	18.1	57.2	0.32	0.31	55.8	75.0	61.9	113.0
SER 16	15.4	53.1	0.26	0.28	37.7	66.3	71.3	109.5
VAX 3	15.0	59.5	0.26	0.27	45.0	64.7	67.1	114.9
Mean	27.4	59.0	0.29	0.30	56.7	67.1	78.7	106.0
$LSD_{0.05}$	22.6	32.0	0.09	0.07	24.2	17.02	45.7	35.1

Table 8: Correlation coefficients and mean squares for total root length (TRL), mean root diameter (MRD), specific root length (SRL), and vision root depth (VRD) of 29 days-old plants, leaf area (LA), shoot dry biomass weight (SDW) and root:shoot ratio (R:S) for 11 bean genotypes (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) under high and low aluminum saturated soil in a soil tube evaluation.

Traits/Source	Al level/DF	TRL	MRD	SRL	VRD 29d	LA	SDW	R:S
TRL	LAl	1						
	HAl	1						
MRD	LAl	0.53 (ns)	1					
	HAl	0.58 (ns)	1					
SRL	LAl	-0.39 (ns)	-0.79**	1				
	HAl	0.33 (ns)	0.27 (ns)	1				
VRD 29d	LAl	0.65*	0.77**	-0.33 (ns)	1			
	HAl	0.78**	0.92***	0.34 (ns)	1			
LA	LAl	0.15 (ns)	0.35 (ns)	0.02 (ns)	0.64μ	1		
	HAl	0.52 (ns)	0.14 (ns)	0.22 (ns)	0.31 (ns)	1		
SDW	LAl	0.64*	0.57 (ns)	-0.26 (ns)	0.82**	0.69*	1	
	HAl	0.50 (ns)	0.38 (ns)	0.46 (ns)	0.47 (ns)	0.81**	1	
R:S	LAl	0.47 (ns)	0.68*	-0.79**	0.24 (ns)	-0.29 (ns)	-0.02 (ns)	1
	HAl	0.81**	0.70*	0.05 (ns)	0.71*	0.15 (ns)	0.11 (ns)	1
Level of Al	1	16524.7***	0.0007 (ns)	12236.7***	1774.25***	811056.2***	19.58***	0.34***
Rep. (Al level)	4	28.77 (ns)	0.0009 (ns)	299.3 (ns)	420.9 (ns)	2991.6 (ns)	0.19 (ns)	0.008 (ns)
Genotype	10	776.2***	0.009***	1342.06***	3747.87***	3235.02 (ns)	0.28*	0.06***
Gen. x Al level	10	100.38 (ns)	0.0003 (ns)	986.2**	1815.5μ	8193.2*	0.18 (ns)	0.012*
Error	40	138.97	0.0012	301.16	79.23	3773.3	0.119	0.004

*, **, and ***: significant respectively at $P \leq 0.05$, 0.01 and 0.001
ns: non significant

IV.1.2.2. Relationships between Hydroponics and soil tube screening

Our results from the hydroponic system (T1.PGHA) showed that differences in Al resistance among the eleven bean genotypes to high Al in solution was associated with four root traits, TRL, SRL, number of root tips and MRD (Table 5); whereas tolerance to Al-toxic acid soil was associated with two root traits, SRL and VRD (Table 7). When we compared the results from the hydroponic system (T1.PGHA) with soil based evaluation (T2.PGSA) of these bean genotypes we found a strong relationship between Al resistance and acid soil tolerance for some specific root traits. Both screening methods were highly correlated ($r = 0.85$, $P<0.001$) for total root length in soil with high Al saturation and nutrient solution with 20 µM Al (Fig. 10c). A significant but lower

correlation (r = 0.62, P < 0.05) was found between total root length in low Al saturation soil (T2.PGSA) and nutrient solution without Al in hydroponic (T1.PGHA) system (Fig. 10d). Three *P. coccineus* accessions (G35346-2Q, G35346-3Q, and G35464-5Q) were found to be outstanding in their level of Al resistance under both hydroponic (T1.PGHA) and soil tube systems (T2.PGSA). Our results were similar insomuch as we found significant correlation between three root traits from hydroponic evaluation (TRL, TPRL 120 h and SRL) and three other root traits from soil tube evaluation (TRL, VRD at 29 d and MRD). No significant correlation found between Specific root length (SRL) in hydroponics and soil tube experiments indicating that the effects of Al-toxic level on SRL of bean genotypes grown in nutrient solution and soil could be distinct resulting in a failure to detect any relation between the two methods. Narasimhmoorthy et al. (2007) compared three methods including hydroponics, soil, and root staining for evaluation of Al tolerance in *Medicago truncatula* (Barrel Meic) germplasm found a weak correlation, suggesting that each technique is distinct and cannot be substituted for each other. A large discrepancy between hydroponics-based ratings of seedlings and sand-culture-based ratings of soybean plants was found when Al tolerance was expressed as percentage of controls, and correlations between sand culture and hydroponics-based results were found to be low (Villagarcia et al., 2001). Horst and Klotz (1990) compared 31 soybean genotypes using hydroponics and soil systems have detected a positive though non-significant relationship (r = 0.79). Recent research on peanut indicated that root characteristics of peanut grown in hydroponics were closely related with those of peanut grown with soil in small pot conditions (Girdthai et al., 2010). Although genotypic ranking based on SRL in nutrient solution (with 20 µM Al) did not agree with the ranking in Al-toxic acid soil. Hydroponic evaluation identified the soybean cultivar Perry as Al sensitive even though it had been found to be tolerant in soil-based assays with older plants (Armiger et al., 1968; Devine et al., 1979; Sapra et al., 1982; Horst and Klotz, 1990; Foy et al., 1969 and 1992). VAX1 which was found to be Al sensitive in hydroponic system was found to be acid soil tolerant under field conditions because of its abundant adventitious root system. To avoid misleading conclusions it is better to consider the two methods separately.

IV.1.2.3. Discrepancy and complementarily between screening methods

Total root length was analyzed at two levels of Al treatment both in soil tube (T2.PGSA) and nutrient solution (T1.PGHA) screening. There was a strong linear relationship between total root length per plant under 20 µM Al and the control with 0 µM Al in hydroponic system (R^2 = 0.89 for A) (Fig. 10a). For soil tube system, the linear relationship between root length density under low Al

and high Al saturation was also strong (R^2 = 0.71) (Fig. 10b). Villagarcia et al. (2001) found that despite the imposition of stress to approximately the same degree in hydroponic and sand culture systems, the genotypic variation in Al tolerance of soybean was much greater in hydroponics as evidenced by the following: much greater Al x genotype interaction, increased genotypic variation in response to stress, a much wider range in Al tolerance expressed as PC, a lower correlation between genotype means for Al-free and Al-stress treatments, and a greater correlation between ratings under Al-stress conditions and PC. We found some differences in genotype ranking between hydroponic evaluation for Al resistance and soil based evaluation for acid soil tolerance revealing their complementarily.

The response of root attributes (root length, mean root diameter, specific root length and root depth) to Al stress was used to assess resistance of beans to Al toxicity. A significant variation ($P < 0.001$) among the 11 genotypes was found in this study using both hydroponics (T1.PGHA) and soil tube screening (T2.PGSA) systems. High variability in root architecture was observed between genotypes except for root length at 48 h of treatment with 20µM Al in nutrient solution. In the hydroponics system (T1.PGHA) *P. coccineus* accessions did not show any difference after 48h of Al exposure in comparison with no exposure to Al but at after 120 h of Al exposure significant genotypic variation ($P < 0.001$) in Al resistance was observed.

Four *P. coccineus* accessions (G35464-5Q, G35346-2Q, G35346-3Q, and G35066-1Q) showed greater values of total root length in both conditions (with and without Al in nutrient solution). Similar observations were made for soil tube (T2.PGSA) studies where three *P. coccineus* (G35346-2Q, G35346-3Q, and G35464-5Q) accessions maintained good root development in soil with either low or high Al saturation.

In addition, each screening method demonstrated some particularity, as use of hydroponic system (T1.PGHA) enabled quantification of number of root tips (most Al-sensitive part of the root) that was not possible with soil tubes (T2.PGSA) after cutting the tubes at different soil depths, and soil based screening revealed rooting ability to penetrate Al-toxic soil. Narasimhamoorthy et al. (2007) concluded that a combination of soil-based screening and hydroponics could be essential to identify Al tolerant genotypes possessing multiple Al tolerance mechanisms. Our results are consistent with this suggestion.

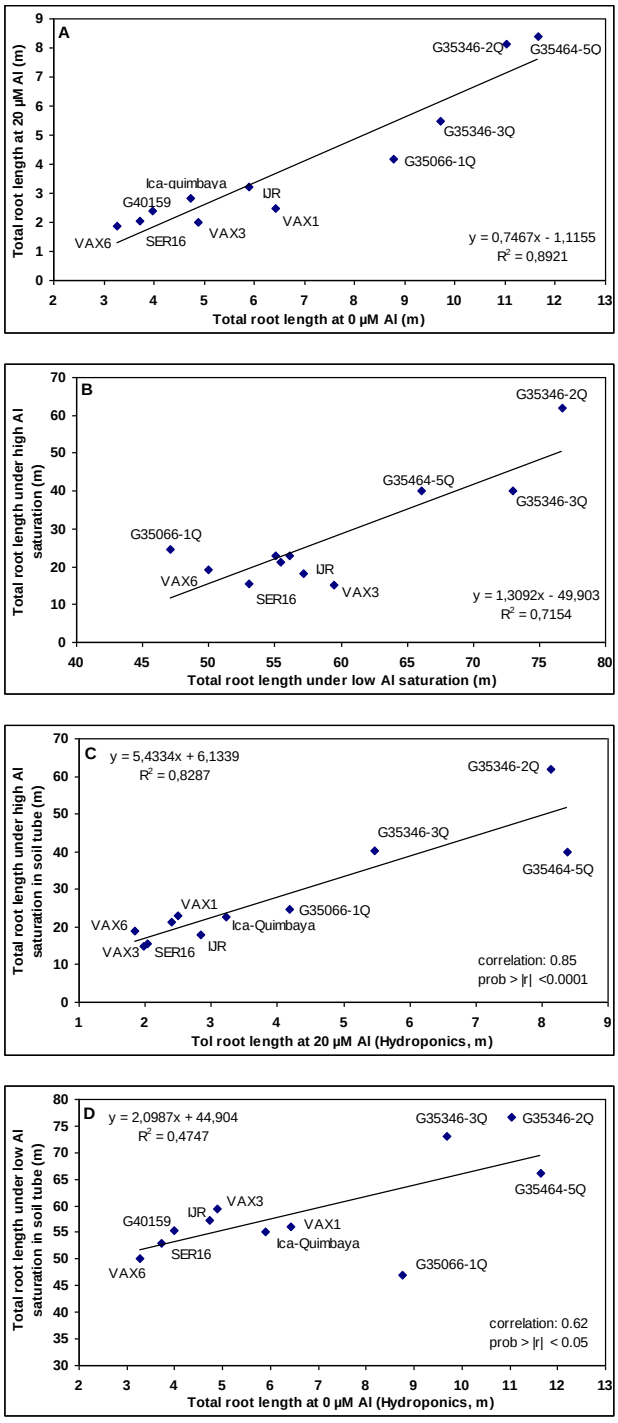

Figure 10: Relationship between total root length of 11 bean genotypes (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) in Al treatment in hydroponics and soil tube evaluation, and controls for the two screening methods. The Al treatment was characterized by a soil with 76% Al soil saturation (pH = 4.1) for top-soil of the cylinder (0-10 cm), and 83% saturation for subsoil (10-75 cm) (pH = 4.14). The low Al saturation was made by tubes packed for topsoil with soil (28 % Al saturation, pH = 4.45) and for subsoil (58 % Al saturation, pH = 4.29). Hydroponic evaluation was done in a nutrient solution containing 20 µM Al (with 0 µM Al for the control). The roots of seedlings were harvested after 5 days of Al treatment.

IV.1.3. Tolerance to combined stress of Al-toxic and drought

Improving resistance to two complex stresses such as Al toxicity and water stress (WS) in common bean requires identifying new sources of resistance among *P. vulgaris* accessions and in sister species including *P. coccineus* and *P. acutifolius* (Butare et al., 2011). Soil based systems offer a medium that is more similar to field conditions. Improving the characterisation of combined Al toxicity and drought stress on root growth, and the understanding of Al/drought interaction on common bean is key in the identification of new sources of these abiotic stresses.

Treatment effects of Al levels x water regimes were highly significant ($P < 0.001$) for TRL and SRL, and significant ($P < 0.05$) for MRD and VRD. Combined stress of Al-toxic soil and drought (T3.PGSC) was the most inhibitory to TRL, followed by drought alone, and then by Al alone. However, as expected, Al-stress alone was more inhibitory than drought to specific root length and rooting depth. Genotypes were significantly different ($P < 0.001$) for all root traits considered (TRL, MRD SRL, and VRD) (Table 10). Two sister lines of *P. coccineus*, G35346-3Q and G35346-2Q, were the most tolerant to combined stress, presenting the highest values of TRL while maintaining a deeper root system (Table 9). They were also the two best for their TRL under Al-stress alone but showed difficulties to develop a deep rooting system under water stress alone. Other relatively tolerant genotypes in combined stress were G35066-1Q and ICA Quimbaya.

The ability of roots to sense and respond to stress largely determines how successful they can be in adaptation to a changing soil environment (Wolters and Jürgens, 2009). Acid soils with high levels of Al impede root growth, causing increased crop sensitivity to drought and decreased nutrient acquisition (Bianchi-Hall et al., 2000). Compared to the control with low Al saturation and well watered soil, the means of shoot attributes such as leaf area, shoot biomass dry weight and root:shoot ratio showed not only the effects of the stress but also genetic variability under Al alone, drought alone, and combined stress of Al and drought (Table 9). Interactions of genotype x treatment (Al and water stress) were significant for total root length and specific root length ($P < 0.01$), and highly significant for root depth ($P < 0.001$) (Table 10). Rankings of genotypes under Al stress alone, and drought alone, versus combined stress of Al and drought were different, suggesting that Al-resistant or drought resistant lines are not necessarily tolerant to combined stress.

Larger leaves are expected to require disproportionately greater fractions of biomass in support to counterbalance the bending moments exerted by leaf elements (Niinements et al., 2007). Our results confirmed this assumption insomuch as extensive leaf area was accompanied by strong

shoot biomass investment in stems, branches and petioles. The genotypes G35346-2Q, G35464-5Q and ICA Quimbaya were outstanding under Al stress by maintaining certain acceptable level of shoot development while sensitive genotypes were highly affected. A different tendency was identified in Al sensitive genotypes which showed better shoot growth under drought stress (Table 11) than Al tolerant genotypes that had a deeper root system (Table 9). These genotypes were SER16, G40159 and VAX1. Our results (T3.PGSC) revealed that genotype x (Al and water regime) interaction was highly significant for root:shoot (R:S) ratio and leaf area, and SBD.

Mimmo et al. (2009) showed that R:S ratio of translocated-Al increased significantly with Al concentration in *Phaseolus vulgaris* L. and *Phaseolus lunatus* L. At low Al concentrations as 25 and 50 µM 1/10 of Al is translocated into shoots, whereas at high Al concentrations as 100 µM translocation is further reduced down to 1/20. In another study, shoot weight under Al-stress conditions in 18 day-old plants was significantly correlated to seedling ratings of Al tolerance (r = 70*), while root weight and Relative root surface area (RRSA) were associated to a lesser degree (r = 0.50 and 0.45, respectively) (Villagarcia et al., 2001). In our experiment with Al-toxic soil, total root length of 33 day-old plants correlated with R:Sh ratio (r = 0.81**).

When the 11 bean genotypes experienced water deficit in addition to Al-toxicity stress we found that physiological parameters varied from those in either unstressed conditions or under individual stress factors. In response to individual and combined stress of aluminum and drought some genotypes such G35346-2Q and G35346-3Q maintained high R:S ratio whereby Mesoamerican bean genotypes SER 16 and VAX6 showed low R:Sh ratio (Table 11). Except for injury to the roots, a reduction root-shoot ratio is a response to more favorable growing conditons (Harris, 1992). The two coccineus accessions tolerate the stress conditions while for the two Mesoamerican beans conditions were not favorable. The effect of water stress combined with Al-toxicity in soil lead to higher reduction of root density, leaf area, and biomass accumulation. Samac and Tesfaye (2003) found that root tips affected by Al were stubby due to inhibition of cell elongation and cell division, and concluded that the restricted root system impaired nutrient and water uptake making the plant more susceptible to drought stress.

Table 9: Influence of individual and combined stress factors of acid soil (HAl, high aluminium; LAl, low aluminium) and drought (WW, well watered; WS, water stress) on total root length (TRL), mean root diameter (MRD), Specific root length (SRL) and visual root depth (VRD) for 33 days-old plants of 11 bean genotypes (*P. coccineus*: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) from three *Phaseolus* species grown in soil tubes.

Genotypes	TRL (m)				MRD (mm)				VRD at 33d (cm)				SRL (m g^{-1})			
	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS
G 35346 3Q	73.7	30.4	38.7	42.5	0.38	0.35	0.42	0.39	72.2	56.6	66.8	75.0	68.6	67.6	56.6	57.4
G 35346 2Q	56.9	28.6	66.5	37.7	0.36	0.36	0.37	0.37	62.9	59.0	68.0	73.0	76.7	63.4	70.6	61.7
G 35066 1Q	25.6	18.8	31.7	27.8	0.35	0.34	0.38	0.38	45.8	59.0	67.8	62.0	58.7	51.9	54.5	58.6
I. Quimbaya	32.4	17.2	29.2	22.5	0.36	0.41	0.39	0.39	68.1	65.5	66.0	69.0	66.3	54.9	57.7	53.7
I.J.R.	44.0	28.4	22.7	21.9	0.38	0.38	0.41	0.4	75.0	75.0	72.0	71.0	64.9	56.6	52.6	47.1
G 35464 5Q	42.3	26.7	35.7	19.9	0.35	0.37	0.43	0.4	60.5	61.4	69.2	59.8	66.3	59.3	47.9	43.5
VAX 1	34.0	25.4	25.4	16.4	0.33	0.33	0.35	0.34	58.5	71.6	59.5	65.7	82.6	75.9	73.2	64.1
G 40159	54.1	22.9	20.5	15.1	0.32	0.34	0.33	0.33	75.0	71.5	64.5	57.0	101.9	83.5	86.9	84.1
VAX 3	42.7	19.7	15.3	12.2	0.36	0.34	0.34	0.35	67.7	68.2	46.9	49.0	83.1	75.4	56.9	59.0
SER 16	58.9	21.9	14.6	11.9	0.36	0.34	0.35	0.37	70.3	72.5	60.0	48.3	80.4	72.4	59.7	59.0
VAX 6	29.9	16.7	12.1	11.8	0.35	0.35	0.3	0.41	68.2	66.7	44.2	42.9	68.8	69.4	59.2	59.6
Mean	45.0	23.3	28.4	21.8	0.35	0.36	0.37	0.37	65.8	66.1	62.3	61.2	74.4	66.4	61.4	58.9
$LSD_{0.05}$	45.7	11.4	14.0	9.4	0.07	0.05	0.07	0.08	22.6	10.0	14.9	18.1	13.0	15.7	10.6	12.7

Table 10: Correlation coefficient and mean squares for total root length (TRL), mean root diameter (MRD), specific root length (SRL), and root depth at 33 days-old plants (VRD at 33 d), leaf area (LA), shoot dry biomass weight (SDW), root:shoot ratio for 11 bean genotypes (P. coccineus: G35464-5Q, G35346-3Q, G35346-2Q, G35066-1Q; *P. acutifolius*: G40159; *P. vulgaris*: ICA Quimbaya, Indeterminate Jamaica Red (IJR), VAX1, VAX3, VAX6, SER 16) (23 days under drought), under screening for individual and combined stress of Al and drought.

Traits/Source	df	TRL	MRD	VRD 23d	SRL	LA	SDW	R:S
TRL	_	1						
MRD	_	0.23**	1					
VRD at 33d	_	0.59***	0.28**	1				
SRL	_	0.22*	-0.57***	0.14^{ns}	1			
LA	_	0.75***	0.04 (ns)	0.54***	0.32***	1		
SDW	_	0.77***	0.17*	0.56***	0.25**	0.80***	1	
R:S	_	0.04 (ns)	0.38***	-0.08 (ns)	-0.54***	-0.31***	-0.48***	1
Al & Water reg.	3	3716.93^{***}	0.003^{*}	206.45^{*}	1533.36^{***}	331.127^{***}	8.629^{***}	0.5226^{***}
Rep. (Al.&Wr.)	8	167254 (ns)	0.001 (ns)	61.58 (ns)	115.38^{**}	2312.9 (ns)	0.123 (ns)	0.05 (ns)
Gen.	10	1023.53^{***}	0.005^{***}	313.86^{***}	1244.17^{***}	13.549^{**}	0.435^{*}	$0,355^{***}$
Gen. x (Al.&Wr.)	30	240.33^{**}	0.001 (ns)	208.53^{***}	65.93^{**}	17.211^{***}	0.432^{**}	$0,355^{***}$
Error	80	113.45	0.0008	52.59	31.4	5234.6	0.22	0.032

*, ** and ***: significant at the 5%, 1% and 0.1% level of probability; respectively
ns: non significant

In this study differences in resistance to combined stress of Al and drought were associated with three root traits, VRD at 33 d ($P < 0.001$), TRL ($P < 0.05$) and SRL ($P < 0.05$) (Table 10). Leaf expansion continued under stress as shown by higher leaf area and shoot biomass values of *Phaseolus vulgaris* genotypes. However, as they were strongly affected by Al-toxic acid soil they were more sensitive under combined stress factors of Al and drought (Table 9), indicating that their capacity to acquire nutrients and water for shoot growth was reduced.

A combination of environmental stresses can alter plant metabolism in a novel manner that may be different from that caused by each of the different stresses applied individually, and may require a new type of response that would not have been induced by each of the individual stresses (Rizhsky et al., 2002). Combined stress of Aluminum and drought is more damaging than each single stress considered separately. When combined stress of Aluminum and drought (T3.PGSC) to each of these stress alone were compared, *P. coccineus* genotypes presented unusual response. *P. coccineus* (G35346-3Q, G35346-2Q, G35066-1Q) and to lesser extent ICA Quimbaya developed more and deeper roots under combined stress than with drought alone (Table 9). Al stress was able to ameliorate the effects of drought in these genotypes, while in others the added stress of Al on top of drought was deleterious as expected. Al stress alone showed less effect on total root length than combined Al and drought stress for all bean genotypes except for *P. coccineus*, G35346-3Q. Similarly, for all common bean genotypes with exception of ICA Quimbaya (Al resistant

genotype), TRL was less affected by drought stress alone compared to combined stress. Total root length was correlated with mean root diameter ($r = 0.23$, $P < 0.01$), with root depth ($r = 0.59$, $P < 0.001$), and with specific root length ($r = 0.22$, $P < 0.05$) (Table 10). Total root length of all *P. coccineus* were more affected by water stress alone than Al toxic stress alone. Weak correlation between MRD and VRD was found ($r = 0.28$, $P < 0.01$), and between MRD and R:S ratio ($r = 0.38$, $P < 0.001$), whereby MRD was negatively correlated with SRL ($r = -0.57$, $P < 0.001$).

Table 11: Leaf area (LA), shoot dry biomass weight (SDW) and root-shoot ratio (R:S) under Al soil tube experiment and individual and combined stress of Al and drought experiment for 11 bean genotypes including 4 *P. coccineus* accessions (G 35066-1Q, G 35346-2Q, G 35346-3Q, G 35464-5Q) , 1 *P. acutifolius* (G 40159) and 6 *P. vulgaris* (I.J.R., ICA Quimbaya, SER 16, VAX 1, VAX 3, VAX 6) under soil tube greenhouse screening.

	Individual stress of Al						Individual and combined stress of Al and drought											
Genotypes	LA (cm^2)		SDW (g)		R:S		LA (cm^2)				SDW (g)				R:S			
	HAl	LAl	HAl	LAl	HAl	LAl	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS	LAl-WW	LAl-WS	HAl-WW	HAl-WS
G 35346-3Q	66.6	399.1	0.67	2.25	0.71	0.42	356.6	78.0	132.0	136.6	1.69	0.52	0.96	0.92	0.75	0.99	0.72	0.8
G 35346-2Q	212.2	300.3	1.4	1.90	0.52	0.48	317.4	93.2	152.8	106.8	1.66	0.72	0.86	0.6	0.45	0.75	1.08	1.03
I.J.R.	67.8	375.6	0.68	2.22	0.45	0.25	365.2	91.1	108.4	95.1	1.97	1.17	0.82	0.56	0.34	0.43	0.53	0.85
G 35464-5Q	136.4	304.9	1.13	2.10	0.56	0.35	164.5	123.1	189.8	91.0	1.21	0.91	1.19	0.77	0.49	0.51	0.63	0.62
G 35066-1Q	110.8	330.2	0.7	1.54	0.48	0.28	125.9	153.6	137.5	83.8	0.96	0.36	0.88	0.75	0.44	1.18	0.65	0.66
VAX 1	87.0	290.4	0.55	1.37	0.49	0.29	169.9	150.7	127.1	69.8	0.98	0.77	0.63	0.35	0.45	0.46	0.55	0.73
SER 16	73.1	341.7	0.73	1.86	0.33	0.25	564.1	157.4	60.9	62.3	3.13	0.98	0.6	0.36	0.24	0.32	0.4	0.58
I. Quimbaya	123.5	245.3	0.83	1.58	0.43	0.37	178.3	36.3	98.9	56.6	1.24	0.52	0.87	0.59	0.4	0.61	0.57	0.74
G 40159	92.2	333.4	0.82	2.07	0.19	0.19	418.7	136.7	109.5	43.1	2.49	1.14	0.69	0.44	0.2	0.25	0.34	0.42
VAX 6	61.5	291.7	0.55	1.76	0.44	0.25	260.5	105.1	55.2	40.2	1.57	0.68	0.62	0.32	0.29	0.36	0.34	0.62
VAX 3	69.7	326.8	0.5	1.91	0.37	0.24	350.2	134.9	70.9	29.6	1.89	0.67	1.09	0.32	0.3	0.39	0.5	0.68
Mean	100.1	321.8	0.78	1.87	0.45	0.31	297.4	114.6	113.0	74.1	1.71	0.77	0.84	0.54	0.4	0.57	0.57	0.7
$LSD_{0,05}$	148.6	140.0	0.75	0.9	0.18	0.12	318.7	84.1	56.6	61.6	1.9	0.57	0.92	0.25	0.3	0.6	0.29	0.37

IV.1.3.1. Root length density across experiments

There is considerable variability in Al resistance within some species and this has been useful to breeders in developing Al-tolerant cultivars of various crops (Delhaize and Ryan, 1995). In our study, comparing total root length between the two treatments (with and without Al) used in hydroponic evaluation (T1.PGHA) for Al resistance (Fig.10a) we found a close and high relationship between them (R^2 = 0.89) with a highly significant correlation ($P < 0.001$). This suggests that the effect of Al resulted in much less TRL was not an effect for which lines expressed resistance. In soil tube system (T2.PGSA), although the effects of genotype x Al interaction for TRL were significant, the relationship of TRL with and without Al stress for soil-based screening (Fig. 10b) was strong (R^2 = 0.71), and three resistant *P. coccineus* accessions (G35346-2Q, G35346-3Q, G35464-5Q) form a separate group from all the other genotypes. Urrea-Gomez et al. (1996) suggested that constitutive morphological characteristics such as vigorous rooting could be advantageous in the breeding of Al-resistant cultivars. Four *P. coccineus* accessions (G35464-5Q, G35346-2Q, G35346-3Q, and G35066-1Q) showed greater values of total root length both under with and without Al in nutrient solution (T1.PGHA). Comparing the relationship between the two methods of Al resistance (T1.PGHA and T2.PGSA) screening (Fig.10c) we found that the relation was most consistent between total root length values for both systems of evaluation for Al resistance. Correlation between Al-toxic soil system and hydroponic system with 20 µM Al in nutrient solution was highly significant ($r = 0.85$; $P < 0.001$) whereas the correlation of the two controls (Fig.10d) was lower but still significant ($r = 0.62$, $P < 0.05$). This suggests that in addition to the genetic effect, higher difference was due to treatment effects. Villagarcia et al. (2001) revealed that hydroponics-based assay of Al tolerance with seedlings and the sand-media nutrient-solution-based assays with somewhat older plants may both have a role in breeding. They concluded that the implication was that some genetic sources will lend themselves well to hydroponics-based screening while others may not (Villagarcia et al., 2001). Our strategy was to correlate data from hydroponic evaluation with Al-toxic soil evaluation to select best parental sources of Al resistance.

IV.1.3.2. Root and shoot attributes

Genotype ranking based on plant vigor has been made based on leaf area and shoot biomass dry weight under individual and combined stress of Al and drought (T3.PGSC). Genotype x Al level interaction was significant only for LA and R:S ratio ($P < 0.05$) but a highly significant interaction was observed for genotype x (Al and water regime) for LA and R:S ratio ($P < 0.001$), and SDW ($P < 0.01$) (Table 10). Roots have very little direct control over the rate of carbon import from the leaves however they do exert indirect control of leaf growth which depends on the supply of cytokinins and water from roots (Lambers et al., 1995). High root length is an important characteristic for the acquisition of nutrients at low availability (Ryser and Lambers, 1995). Identification of a bean genotype that combines mechanisms for better biomass partitioning and an extensive root system to explore the soil volume more effectively will be an important achievement for this study. The relationships between shoot traits (LA and SDW) and TRL in Al stress alone, and in combined stress of Al and drought in soil tube system were analyzed. Linear regression of LA on TRL in Al stress for the 11 genotypes showed positive relationship ($R^2 = 0.65$; $P < 0.05$) (Fig.11a), and a similar relationship was observed between SDW and TRL ($R^2 = 0.69$) (Fig.11b) in Al stress. Relationship between SDW and root length density in combined stress of Al and drought also showed a strong positive relationship ($R^2=0.66$) (Fig.10c). At low nutrient availability in short-term experiments some species with a high potential root growth rate (RGR) still grow faster than those with low potential RGR and have greater capacity to acquire nutrients (Chapin, 1980; Lambers and Poorter, 1992; Ryser and Lambers, 1995).

Individual stress of Al on 29 day-old plants in soil tube experiment in this study revealed strong relationships between total root length and leaf area, $R^2 = 0.65$ (Fig.11a); and between TRL and SDW, $R^2 = 0.69$ (Fig.11b). All *P. vulgaris* cultivars and the *P. acutifolius* accession performed poorly in root development and leaf area with the exception of an Andean bean type Indeterminate Jamaica Red (IJR) that was intermediate. *P. coccineus* genotypes, G35346-3Q favoured root growth at the expense of shoot growth while G35464-5Q was intermediate. G35346-2Q showed a pattern of high biomass allocation in leaves, stems and roots; confirming this ability for Al resistance in beans. Combined stress of Al and drought (Fig.11c) showed also a high relationship between shoot biomass dry weight and total root length ($R^2 = 0.66$). G35346-3Q showed superior ability in biomass allocation in the whole plant.

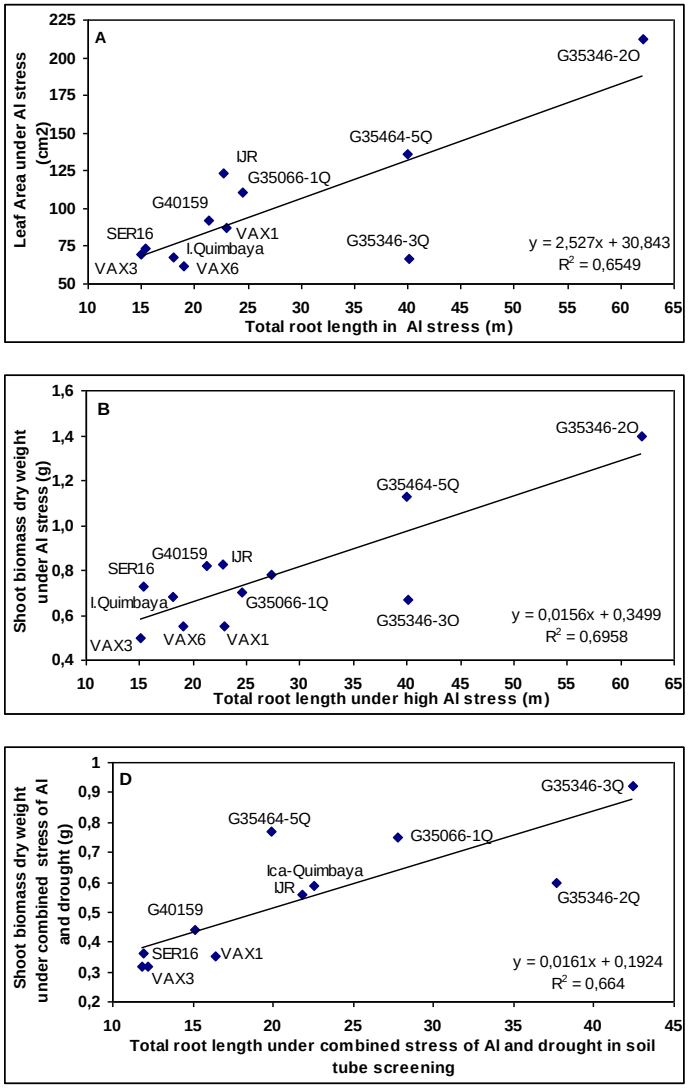

Figure 11: Relationship between total root length and shoot attributes of 11 bean genotypes including 4 *P. coccineus* accessions (G 35066-1Q, G 35346-2Q, G 35346-3Q, G 35464-5Q) , 1 *P. acutifolius* (G 40159) and 6 *P. vulgaris* (I.J.R., ICA Quimbaya, SER 16, VAX 1, VAX 3, VAX 6). under individual and combined stress of Al and drought.

IV.1.4. Breeding implications: identification of aluminum and drought tolerant bean genotypes

In our experience with the hydroponic system (T1.PGHA) and in Al-toxic acid soil (T2.PGSA), each screening method permitted evaluation of different aspects of root behaviour. For example, the hydroponic system enabled quantification of NRT (the most Al-sensitive part of the root) which was not possible with soil cylinders after cutting the cylinders at different soil depths, whereas soil based screening revealed rooting ability to penetrate Al-toxic soil. In this sense the two methods were complementary (Table 12).

These studies also confirmed the superiority in Al response of Andean common beans compared to Middle American types for several important traits under Al toxicity in the hydroponic system (e.g., TPRL120h, or TRL) or acid soil stress (e.g., TRL or VRD) (Table 12).

Table 12: Root result summary of experiements on high aluminium stress (HAl), water stress (WS), combined stress of HAl for 11 bean genotypes including 4 *P. coccineus* accessions (G 35066-1Q, G 35346-2Q, G 35346-3Q, G 35464-5Q) , 1 *P. acutifolius* (G 40159) and 6 *P. vulgaris* (I.J.R., ICA Quimbaya, SER 16, VAX 1, VAX 3, VAX 6) under hydroponic and soil tube greenhouse screening.

Experiement and some root key traits	HAl under Hydroponic system			HAl acid soil tube system		WS acid soil tube system		Combined HAl & WS acid soil tube system	
Genotype	TRL (m)	TPRL 120h (cm)	NRT (nb)	TRL (m)	VRD at 29d (cm)	TRL (m)	VRD at 23d (cm)	TRL (m)	VRD at 23d (cm)
G 35066-1Q	4.2	20.9	374.3	24.6	57.0	18.8	59	27.77	62
G 35346-2Q	8.1	26.4	668.4	62.1	74.3	28.6	59	37.66	73
G 35346-3Q	5.5	24.0	472.6	40.1	75.0	30.4	56.6	42.48	75
G 35464-5Q	8.4	30.8	926.3	40.0	68.0	26.7	61.4	19.88	59.75
G 40159	2.4	17.8	306.9	21.3	53.0	22.9	71.5	15.14	57
I. Quimbaya	2.8	18.9	396.8	22.8	62.0	17.2	65.5	22.53	69
I.J.R.	3.2	20.2	364.9	18.1	55.8	28.4	75	21.85	71
SER 16	2.0	17.0	228.8	15.4	37.7	21.9	72.5	11.9	48.33
VAX 1	2.5	17.0	284.0	23.0	40.8	25.4	71.6	16.42	65.67
VAX 3	2.0	18.2	218.9	15.0	45.0	19.7	68.2	12.22	49
VAX 6	1.9	14.3	177.3	19.1	55.0	16.7	66.7	11.84	42.87

In this study, root phenotyping for Al resistance indicated that total root length (TRL) and tap root length (TPRL) could be considered as most important root characteristic when identifying Al resistant bean genotypes using hydroponics (T1.PGHA), while total root length (TRL) and visual root depth (VRD) are the most useful root traits to be considered for tolerance to Al-toxic acid soil (T2.PGSA). These root traits had shown genotype sensitivity to toxic-Al stress through root growth inhibition. They predicted how roots of resistant genotypes continue to branch themselves under Al stress and maintained their exploratory capacity of soil. There is considerable genetic variability in Al and drought tolerance within the three bean species and this will be useful to breeders in developing tolerant cultivars for these plant stresses individually or combined.

The results from hydroponics (T1.PGHA) and soil tube (T2.PGSA) studies indicated that both methods of evaluation were effective in screening for resistance to Al stress, or combined stress factors of Al and drought (T3.PGSC) in the case of soil. The greater level of Al-resistance found in *P. coccineus* accessions (G35346-2Q and G35464-5Q) offers the opportunity to obtain much better Al resistance in common bean through interspecific crosses. Another *P. coccineus* accession

G35346-3Q identified in this study showed the ability to tolerate combined stress factors of Al and WS. Given that the two abiotic stresses often co-occur in farmers fields in association with bean root rot diseases, the genotype G35346-3Q was used to improve resistance of sensitive common bean SER16 to abiotic (Al and drought) and biotic (*Fusarium* root rot) stresses.

Summary statement:

Identification of source of resistance in *Phaseolus* species to individual and combined stress of Al and drought

Bean species and genotypes show wide phenotypic variability in relation to aluminium (Al) resistance and progressive soil drying. One experiment on hydroponic screening of Al resistance was carried out using a basal nutrient solution with and without 20 μM Al. Two experiments were carried out using two oxisols in 80 cm long soil cylinders with high Al (HAl) and low Al (LAl) saturation treatments. The three experiments showed an average of 36.9–53.5% inhibition of root growth with HAl compared with LAl treatments. Differences in root development and distribution were observed among genotypes and species. Two accessions of *P. coccineus* (G35346-2Q, G35464-5Q) and one Andean common bean genotype (ICA Quimbaya) were outstanding in root and shoot growth in the HAl treatments. On the other hand, *P. coccineus* accession (G35346-3Q) was outstanding under combined stress of Al-toxic acid soil and progressive soil drying. Accessions of *P. coccineus* may represent unique sources of Al resistance for the improvement of common bean through interspecific crosses.

IV.2. Phenotypic evaluation of interspecific recombinant inbred lines (RILs) of *Phaseolus* species for aluminium resistance and root and shoot growth response to aluminium stress under greenhouse conditions

The other important objective of this study was to conduct phenotypic evaluation of a population of recombinant inbred lines (RILs) of *Phaseolus* species using hydroponic (T4.RGHA) and soil cylinder (T5.RGSA) systems to identify superior progenies with traits of the Al-resistant parent, and to make a comparative analysis of these two experimental methods to identify Al resistant and acid soil tolerant genotypes based on root and shoot traits. This study was conducted using 102 bean genotypes including the 94 RILs, both parents (SER16 and G35346-3Q), and four checks from the Middle American gene pool (VAX 1, Tio-Canela 75, DOR 390 and G 21212); an elite Andean cultivar (ICA Quimbaya); and one *Phaseolus acutifoius* accession (G 40159). A combination of hydroponics and soil-tube screening has been used to assess Al-resistance among these bean genotypes.

IV.2.1. Phenotypic evaluation of Al resistance in hydroponic system

The 102 bean genotypes described above were evaluated under hydroponic system (T4.RGHA). After germination, seedlings with well developed uniform roots were transferred to a tray floating in nutrient solution (López-Marín et al. 2009; Butare et al. 2011) before applying Al treatment (0 or 20 µM Al as $AlCl_3$).

IV.2.1.1. Root growth and aluminum resistance

Al resistance screening using hydroponic system (T4.RGHA) with two levels of Al (0 and 20 µM Al) was used to detect genotypic differences in Al resistance among 102 bean genotypes. Seedlings of all genotypes (except the donor parent G35346-3Q) under Al stress in nutrient solution showed that root elongation rates were affected by the supply of 20 µM Al-stress (Data not shown). López-Marín et al. (2009) have shown with a mapping population on bean in hydroponic screening that genotype (RIL) effects, Al treatment, and the interaction RIL x treatment from the analyses of variance were highly significant for tap root elongation rate, total root length, average root diameter, number of root tips, root dry weight and specific root length. We found similar results

also in our experiment except that Genotype x Al interaction for TRL was not significant (Table 13).

Table 13: Correlation between root characteristics of bean genotypes grown with 20 µM Al and mean squares (from combined ANOVA) of tap root elongation rate at 0-24 hours, at 0-48 hours, and between 24 and 48 hours; total root length (TRL), mean root diameter (MRD), number of root tips (NRT), and specific root length (SRL) for 102 bean genotypes including 94 RILs, 2 parents, 6 checks grown using hydroponic system.

Variable/Source	df	TRER 24h (mm)	TRER 48h	TRER 24-48h	TRL (m)	MRD (mm)	NRT (nb)	SRL (m g^{-1})
TRER 24h (mm)	_	1						
TRER 48h	_	0.83***	1					
TRER 24-48h	_	0.49***	0.88***	1				
TRL (m)	_	-0.31***	-0.17**	-0.02 (ns)	1			
MRD (mm)	_	0.37***	0.25***	0.07 (ns)	-0.80***	1		
NRT (nb)	_	-0.24***	-0.09(ns)	0.04 (ns)	0.89***	-0.81***	1	
SRL (m g^{-1})	_	-0.19**	-0.08 (ns)	0.03 (ns)	0.66***	-0.80***	-0.72***	1
Level of Al	1	49.95***	53.64 ***	57.57***	504.1***	1***	28955056***	661867***
Rep. (Al level)	4	2.73 ***	0.91***	0.25***	78.3***	0.32***	1882195***	54286***
Genotype	95	0.12 ***	0.15***	0.21***	2.53***	0.01***	119760***	2464***
Genotype X Al level	95	0.03***	0.05***	0.11***	0.43*	0(ns)	33006***	645 (ns)
Error	376	0.02	0.01	0.02	0.31	0.24	14936	704

*** Significant at the 0.001 probability level,
** Significant at the 0.01 probability level,
* Significant at 0.05 probability level,
ns: no significant.

IV.2.1.2. Root Growth Rate and Al resistance

In a more detailed examination, the distal region of the transition zone (DTZ) was shown to be the most Al-sensitive root apical region (Sivaguru and Horst, 1998). Some of the differences observed in Al uptake in roots can be explained by differences in root growth since Al-tolerant roots continue to grow in the presence of Al and would effectively dilute the Al in apices (Delhaize et al., 1993). Mean tap root growth rate in Al-toxic stress (T4.RGHA) in the present study implied that for each hour there are more new primary root cells in resistant RIL than in sensitive lines. The Al-resistant parent (*P. coccineus*) and some RILs were outstanding in Al-resistance by maintaining a lower level of inhibition of tap root growth rates (Fig.12). These were G35346-3Q, ALB32, ALB41, ALB45, ALB23, ALB43, ALB87, ALB78, ICA Quimbaya and ALB34 with root growth rate inhibition of -15.7%, 2.6%, 3.5%, 5.4%, 8.1%, 10%, 10.8%, 11.5%, 12.5% and 14.1%, respectively. SER 16 presented an inhibition of TRER of 31.1 %. Rangel et al. (2007) found that

the maximum rate of relative root elongation was 3.9 mm (31% h^{-1}) in Quimbaya and 3.3 mm (41% h^{-1}) behind the root tip in VAX 1. We found that across all 102 genotypes the range of tap root elongation rate was between 0.57 and 1.8 mm h^{-1} at 3 cm behind the root tip. In another experiment, Doncheva et al. (2005) concluded that Al-induced inhibition of root elongation rate, measurable after 45 min in the Al-sensitive variety HS 16X36, must be attributed to an Al-induced decrease of root cell expansion rather than to fast inhibition of root cell division.

Aluminum tolerance ranking based (T4.RGHA) on mean Tap root elongation rate (TRER) between 0-24h and 24-48h classified as most tolerant genotypes, ALB43, ICA Quimbaya, ALB45, ALB84, ALB106, ALB87, ALB23, ALB2, ALB32, ALB24, ALB56 and G35346-3Q (Fig.12). A positive but mid-range correlation was found between TRER at 0-24h and TRER at 24-48h (r = 0.67***), suggesting that measurements of TRER at 0-24h may not be fully representative of TRER at 24-48h. The range of TRER decreased from 0.96-1.98 mm h^{-1} at 24h to 0.03-1.67 mm h^{-1} at 24-48h. Genotype ranking varied between both periods of time indicating that different lines are responding differently. ICA Quimbaya was second for TRER at 0-24h and 6th for TRER at 24-48h, while G34346-3Q was 53rd for TRER at 24h but 5th for TRER at 24-48h. G36346-3Q was the only genotype with tap root growth recovery higher than Al-induced inhibition of root growth, 1.43mm h^{-1} at the beginning (0-24h) and reaching 1.53mm h^{-1} between 24 and 48h. At pH 4.3, Rangel et al., (2005) showed that root-elongation rates of SEA-5 and VAX-1 were reduced respectively by 74% and 85%. When comparing the Al-treatment to the control (without Al) in this study at pH 4.5, we found similar results whereby tap root growth of sensitive genotypes was affected by 79.5% (ALB79), 69% (ALB77), and 60.5% (ALB18). Mean value for root growth rate in nutrient solution screening was 1.18 mm h^{-1} for Al treatment (20 µM Al) and 1.71 mm h^{-1} for the control (0 µM Al) treatment.

The relationship between TRL and TRER (T4.RGHA) at 24h (Fig.12) was very weak (R^2 = 0.0084). ICA Quimbaya was the only genotype which combined high TRL (extensive root system) and high TRER at 24h while ALB18 and ALB104 were very sensitive to Al stress both with low TRL and TRER at 24h. ALB43 and ALB106 were observed to have high TRER accompanied by low TRL. In their work on root development in *Zea mays* L., Bennet and Breen (1991) concluded that Al acts by releasing the root meristem from growth

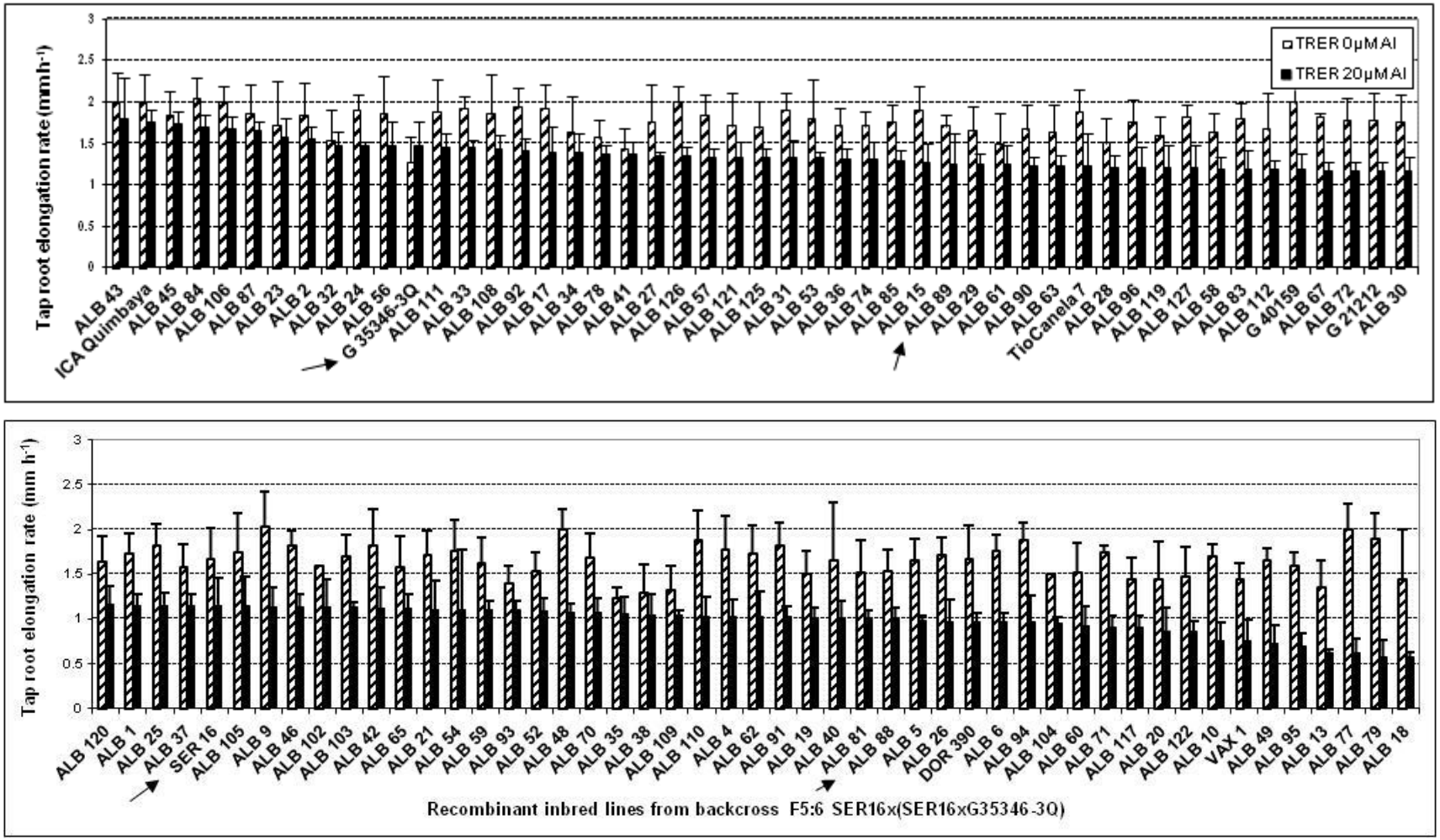

Figure 12: Tap root elongation rate (mm h^{-1}) at 0-48 hours for 102 bean genotypes including 94 recombinant inbred lines, 2 parents (G35346-3Q and SER16), 6 checks (VAX1, DOR390, G21212, G40159, Tio canela75, and ICA Quimbaya) grown in nutrient solution with or without 20 µM Al.

inhibition originating in the root cap. This could be an explanation of the high TRER of G35346-3Q at 24-48h that showed increase of root growth rate in the Al resistant parent.

IV.2.1.3. Superior root vigor and Al resistance in RILs

A highly significant genotype effect was found on total root length (TRL), but genotype x Al treatment interaction was not significant (Table 13). Relationships between TRER at 0-24h and TRER at 24-48h (Fig.13a), and TRER at 0-24h and TRL (Fig.13b) in hydroponic system (T4.RGHA) were analysed. Figure 12a showed that eight genotypes (ALB43, ICA Quimbaya, ALB45, ALB84, ALB87, ALB2, ALB23 and ALB106) were Al resistant by maintaining high TRER across these two separate time points. ALB13, ALB18, ALB79 and ALB77 were found to be Al sensitive. Tap root growth rate at 0-24h significantly correlated to tap root growth at 24-48h ($r = 0.49$; $P<0.001$) (Table 13).

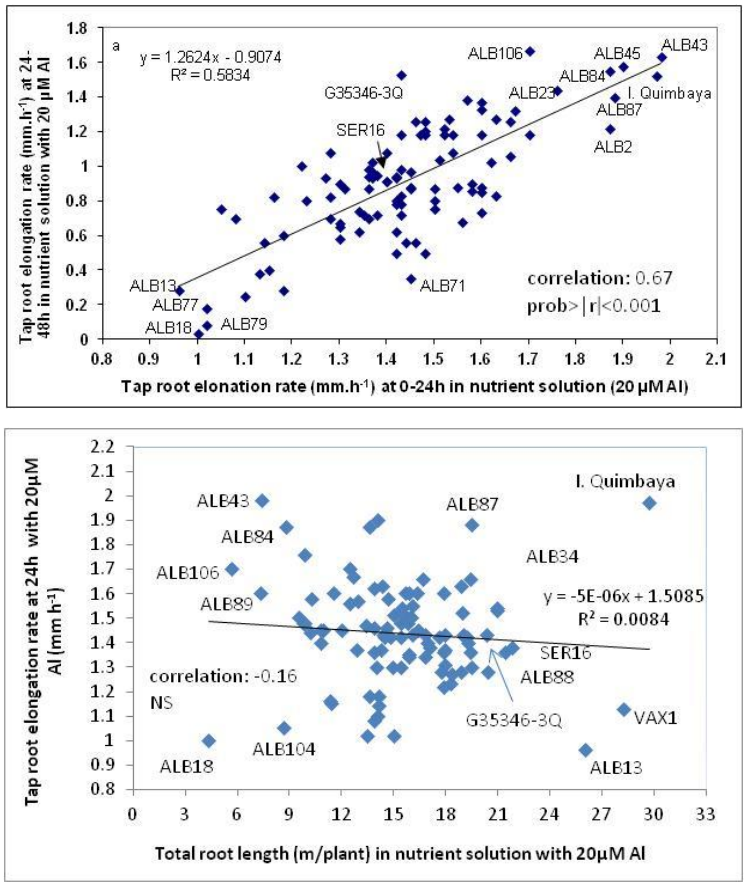

Figure 13: Relationship between tap root elongation rate at 0-24h and tap root elongation rate at 24-48h, and total root length (m) and tap root elongation rate at 0-24h of 98 bean genotypes including 90 recombinant inbred lines, two parents (G35346-3Q and SER16), and six checks (VAX1, DOR390, G21212, G40159, Tio canela75, and ICA Quimbaya) grown in nutrient solution containing 20µM Al.

The relationship between total root length (TRL) and number of root tips (NRT), and between TRL and mean root diameter (MRD) is illustrated in Fig. 14 for 102 bean genotypes including 94 RILs, two parents and six check genotypes. TRL and NRT were highly correlated (r = 0.83***). ICA Quimbaya, VAX1, ALB13, ALB88, SER16, ALB34 and ALB46 maximized the expression of these two traits and were characterized by an extensive root system with greater number of root tips (Fig.14a). Although inhibition of tap root growth could be associated to the crop's sensitivity to the stress, no association was found between TRER and TRL. Our results (T4.RGHA) did not suggest that TRER has any effect on overall root system development (TRL) in general, and therefore may not be useful for screening for acid soil tolerance. Although no association was found between TRER at 24h and TRL (20 µM Al), ICA Quimbaya showed Al resistance based on both root traits (Fig.13b). A weak correlation was found between tap root growth rate at 24 hour and average root diameter (r = 0.37***), suggesting that differences in genotype ranking of the 94 RILs could be due to differences among genotypes in maintaining thin root (low average root diameter) development and high root growth rate as components of Al-resistance. Despite this correlation, similar significant correlation was also found between TRER at 24-48h, or TRER at 0-48h and MRD, respectively 0.07 (ns) and 0.2539***. Other investigators suggested that inhibition of cell elongation rather than cell division plays a role in short-term response to Al supply, whereas over longer periods of time (more than 24 h), both processes of cell elongation and cell division are inhibited (Matsumoto, 2000; Stass et al., 2007). Bennet and Breen (1991) reported that the recovery of root growth rates from Al treatment are initially faster than normal suggesting that the early phases of recovery may involve growth stimulation.

IV.2.1.4. Root Architecture and Aluminum Resistance

Al-toxicity is known to induce morphological changes on root architecture of sensitive genotypes. Our study (T4.RGHA) revealed a range of increase in average root diameter from resistant to sensitive, 6.81% (ALB 38) to 31.87% (ALB89) over 48h. A clear difference in root length was observed between Al-tolerant and Al-sensitive seedlings of wheat at 20 µM Al (Delhaize et al., 1993). Villagarcia et al. (2001) showed that basal roots and branches from the taproot were clearly reduced in all genotypes of soybean. Root elongation of genotypes was inhibited as early as 1 h after the beginning of the Al treatment (Rangel et al., 2007).

Inhibition of tap root growth rate was accompanied by an increase of average root diameter on Al-sensitive RIL (data not shown). Fine roots are considered to be more important than thick roots in nutrient and water absorption, and therefore, more important in terms of Al tolerance (Eisenstat,

1992; Villagarcia et al., 2001; Liu et al., 2010). Two parents used in a mapping population on bean, DOR364 and G19833 revealed a contrasting average root diameter in response to Al-stress in nutrient solution (López-Marín et al., 2009), DOR364 with fine root system and G19833 with thick roots. Blancaflor et al. (1998) showed with maize that Al induced increase in root diameter over longer periods (> 4h). Doncheva et al., (2005) detected twelve QTL associated to Al resistance for six variables, and showed that Al caused not only a reduction in the length of the main root, but also changes to the entire root architecture. We identified four RILs (ALB41, ALB45, ALB87 and ALB43) with thin roots and less inhibition of root growth under Al-toxic stress in hydroponic system (T4.RGHA). However none of them exhibited superior total root length (TRL) combined with number of root tips (NRT) for more extensive root system (Fig.14a), or TRL combined with lower mean root diameter (MRD) for better exploitation of soil profiles (Fig.14b).

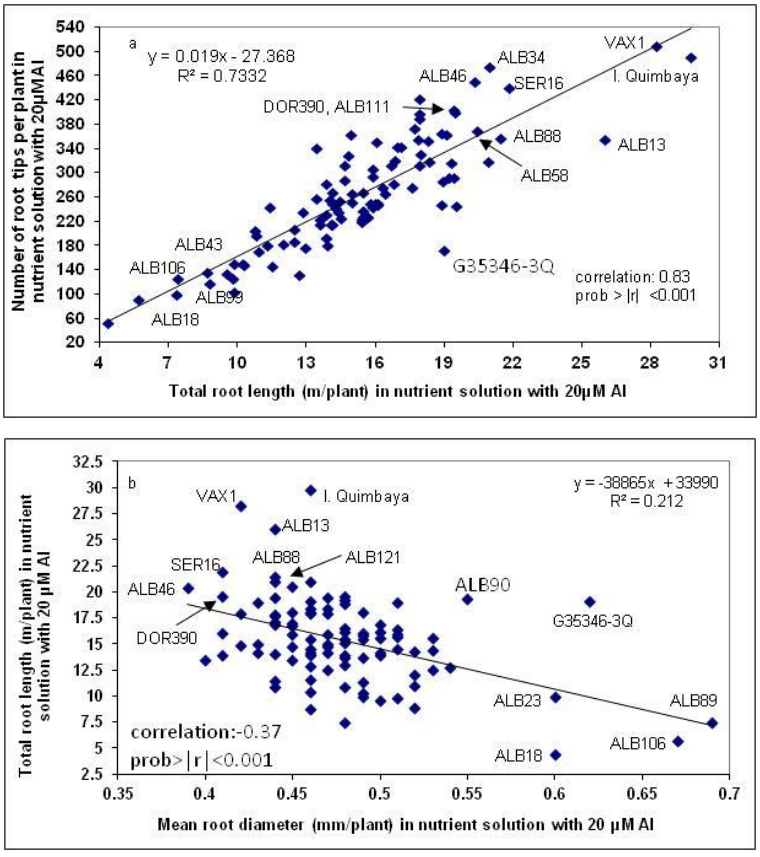

Figure 14: Relationship between total root length and number of root tips per plant, and total root length and mean root diameter per plant for 102 bean genotypes including 94 recombinant inbred lines, two parents (G35346-3Q and SER16), and six checks (VAX1, DOR390, G21212, G40159, Tiocanela75, and ICA Quimbaya) grown in nutrient solution containing 20µM Al.

The primary site of Al toxicity is the root tip (Ryan et al., 1993; Chen et al., 2012). Working with wheat, Rincon and Gonzales (1992) observed the effects of Al toxicity particularly in root tips and

in lateral roots. In our study, similar interaction was found with NRT; but not TRL. The number of root tips was strongly reduced by exposure to Al for all genotypes in our experiment (T4.RGHA). Root tips represent a significant part of total root surface area which increases contact of roots with soil solution, and are responsible for water and nutrient up-take. In seedlings, it is clear that the root tip is the most sensitive part of the root to Al toxicity (Samac and Tesfaye, 2003). With RILs from DOR364 x G19833 (López-Marín et al., 2009), genotype effects and genotype x treatment interaction were highly significant for total root length and number of root tips. The relationships between total root length and number of root-tips have been used to identify extensive, well branched root systems. Wagatsuma et al. (1987) reported that Al concentration was high in the roots and generally low in the tops, and was largely deposited in the root tips in Al sensitive plants. This suggests that root tips are a key site of Al-resistance. However, our results showed that resistant parent G35346-3Q was characterized by low number of root tips and large total root length. In contrast, the susceptible recurrent parent SER16 showed a favorable combination of both root traits. Best lines in nutrient solution for the combination of these traits were ICA Quimbaya, VAX1, ALB34, ALB46, SER16, ALB88, DOR390, ALB111, ALB58 and ALB13 (Fig.14a). The lines that combined high TRL with low MRD were VAX1, ICA Quimbaya, ALB13, SER16, ALB46, ALB88, ALB121, and DOR390 (Fig.14a). Ironically, the Al sensitive parent SER16 and the sensitive control genotype VAX1 appear in both groups of elite lines, making it difficult to conclude about these traits as criteria to identify Al resistant genotypes.

Total root length and number of root tips per plant (T4.PGHA) were highly correlated ($r = 0.83^{***}$; Fig.14a) in 20 µM Al. This explains why injuries on root tips have direct effect on whole development of root system. Mean root diameter was more correlated to number of root tips than to total root length, both correlations being negative, respectively $r = -0.65^{***}$ and $r = -0.37^{***}$. Root diameter and tips influence the size of root, as the number of root tips increased the mean root diameter decreased simultaneously. Liu et al. (2010) concluded that roots of larger (>0.4 mm) diameters only contributed a small proportion to the total root length for the oil seeds. This seems to be an explanation of the high relationship between total root length and number of root tips (and corresponding small roots) found in our study. ALB88 emerged as the best RIL under hydroponic screening. It is a line with multiple traits combining higher TRL with small MRD (many thicker roots), and also with high number of root tips, meaning high potential of water and mineral uptake.

IV.2.2. Phenotypic differences in Al-toxic acid soil tolerance in soil tube system

After characterising the 94 RILs to Al resistance under hydroponic system with 0 and 20 μM Al in nutrient solution, their tolerance to Al-toxic acid soil (T5.RGSA) was accessed using a soil tube cylinder system. The effects of Al toxicity on bean shoot growth, the identification of superior RILs with deep penetration of primary roots and the distribution of roots in Al-toxic acid soil will be determined.

IV.2.2.1. Rooting depth in soil

Differences in rooting depth among 102 genotypes were identified in the soil tube experiment with low and high Al saturation (T5.RGSA). Effects on rooting depth in soil cylinders were highly significant for Al-stress, for genotypes, and genotypes x Al treatment interaction (Table 14).

Table 14: Correlation between root and shoot characteristics of bean genotypes grown with high Al saturation and mean squares (from combined ANOVA) of total root length (TRL), mean root diameter (MRD), specific root length (SRL), and visual root depth (VRD) of 34 days-old plants, leaf area (LA), shoot dry biomass weight (SDW) and root:shoot ratio (R:S) for 102 bean genotypes including 94 RILs, 2 parents, 6 checks grown using soil tube system.

Variable/Source	Df	TRL	MRD	SRL	VRD at 34d	LA	SDW	R:S
TRL	_	1						
MRD	_	-0.43***	1					
SRL	_	0.40***	-0.69***	1				
VRD at 34d	_	0.53***	-0.16**	0.15*	1			
LA	_	0.70***	-0.32***	0.26***	0.39***	1		
SDW	_	0.66***	-0.25***	0.19**	0.34***	0.72***	1	
R:S	_	0.22***	0.06 (ns)	-0.20***	0.17**	-0.09 (ns)	-0.42***	1
Level of Al	1	324648***	0,89***	100314***	100142***	2611014***	1080***	3.17***
Rep. (Al level)	4	3532***	0,16**	10947***	644***	58744***	2.27***	0 (ns)
Genotype	95	332***	0***	268***	124***	17383***	0,8***	0,03***
Genotype X Al level	95	197***	0(ns)	197***	127**	15992***	0,59***	0,01***
Error	380	125	0	109	84.4	8.427	0.27	0.01

*** Significant at the 0.001 probability level
** Significant at the 0.01 probability level.
* Significant at 0.05 probability level
ns: no significant

Visual Root depth (VRD) of all bean genotypes were reduced under Al-stress except for G35346-3Q which showed root penetration into deeper soil layers in high Al saturation soil tubes ($P<0.001$) than in low Al control (Fig. 15). Villagarcia et al. (2001) have shown in sand culture that tap root length was not affected greatly by Al treatments.

The major Al toxicity symptom observed in plants is inhibition of root growth that affects directly the distribution of roots in soil profiles. VRD of 34 day old plants (VRD34d) showed significant differences between the two levels of Al treatment (T5.RGSA), replications (Al level), genotype effects, and Genotype x Al interaction (Table14). Mean value of VRD34d in soil cylinders was 63.9 cm in low Al treatment and 37.2 cm for high Al treatment. Sensitive genotype DOR390 reached only 25.2 cm. Al-induced root-growth inhibition (Rangel et al., 2005; 2007). The best RIL (ALB 70) reached 55.3 cm. At 38 days G35346-3Q actually increased root penetration in high Al saturation soil cylinders by 12% compared to the low Al-saturation treatment (data not shown). VRD34d was reduced by 46% for recurrent parent SER16, and by as much as 65% in RIL. The deepest rooting genotype was G35346-3Q penetrating to 64.3 cm deep while sensitive genotype DOR390 in this study reached only 25.2 cm. The most deep-rooted genotypes in high Al saturation were (in cm) G35346-3Q (64.3), ICA Quimbaya (60.2), ALB70 (57.7), ALB65 (56.8), ALB72 (56.8), ALB77 (54.33) and ALB91 (53.33). (Fig. 15) and were the most Al-resistant and acid soil tolerant by this criterion. More than 50% of the RILs were deeper rooted than SER16 which is considered as Al sensitive parent (REGWQ ranking). Considering VRD as classification parameter, the Al-toxic acid soil tolerance ranking allowed to classify the following genotypes as Al sensitive in acid soil: ALB13, DOR390, ALB60, ALB21, ALB95, ALB117, ALB106, ALB90, ALB56 and ALB110 with a decrease in root depth ranging between 52.2% and 64% when mean value for low and high Al saturation was compared. This confirms that the major Al toxicity symptom observed in plants is inhibition of root growth an affects directly the distribution of roots in soil profiles.

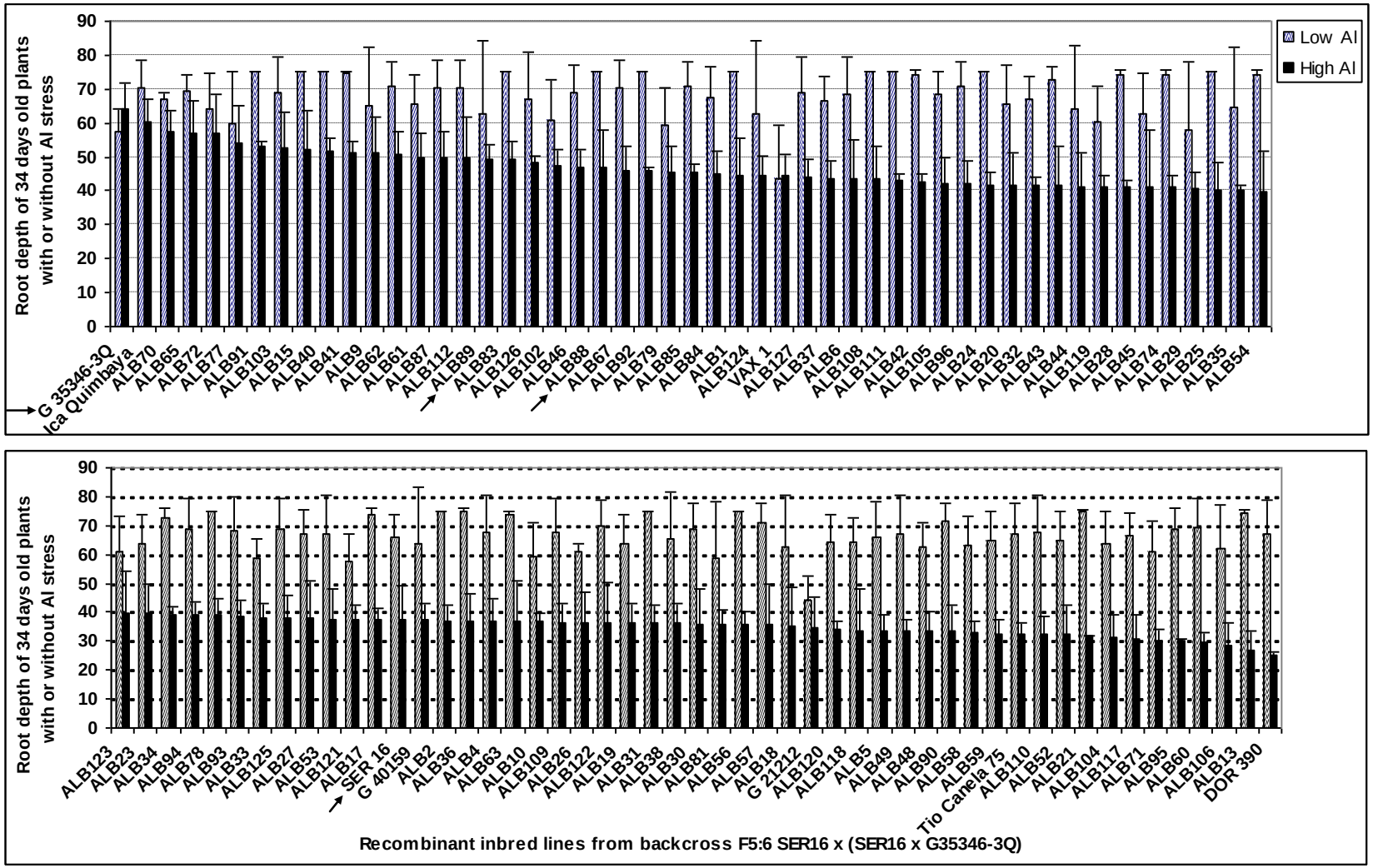

Figure 15: Visual root depth of 34 days old plants(VRD34d) in low and high Al saturation in acid-toxic soil for 102 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 6 checks (VAX1, DOR390, G21212, G40159, G40159, Tio canela75, and ICA Quimbaya) grown using soil tube system.

IV.2.2.2. Root growth and acid soil tolerance

A close relationship ($R^2 = 0.84$) was found between total root length with and without Al treatment in nutrient solution (T4.RGHA) of hydropony (Fig.16a). This relationship showed that genotypes with high total root length without Al (0 µM Al), maintained such characteristic in presence of Al (20 µM Al) . Genotypes with such characteristics were ICA Quimbaya, VAX1, ALB13, DOR390, and ALB88. Contrasting observation was made with the relationship between total root length in low Al and high Al saturation (T5.RGSA) in soil tubes (Fig.16b) with a very weak (r = 0.29***) relationship. Few genotypes were outstanding in Al stress and exhibit root vigor by showing good root development under both stress and non-stress conditions. Significant differences among RILs reflected introgression of genes from G35346-3Q into SER16. VRD34d was highly correlated with some plant traits (Table14) of the soil cylinder experiment with high Al saturation soil: TRL (r = 0.53***), LA (r = 0.39***), and SDW (r = 0.34***). There were also significant correlations with MRD (r = -0.16**) and with SRL (r = 0.15*). Al not only causes a reduction in the length of the main root, but also changes the entire root architecture (Doncheva et al., 2005). As free Al concentration in acid soils generally increases with depth, Al-resistant cultivars having roots in the more toxic subsoil might be able to obtain soil resources such as water and nutrients from that layer (Bushamuka and Zobel, 1998).

Effects on LA and SDW were highly significant for genotype, and genotype x Al treatment interaction (Table 14). Correlations between these two shoot traits and TRL under high Al saturation soil (T5.RGSA) were highly significant, r = 0.70*** for LA and r = 0.66*** for SDW (Table 14). Rating based on the combination of TRL and SDW revealed not only the effects of Al stress in the soil but biomass accumulation in shoot that could translate into yield in the reproductive phase. Genotypes characterized by an extensive and deep root system, enhanced exploratory capacity, and superior SDW were G35346-3Q, and ALB15, ALB 40, ALB 77, ALB 88, ALB 91, and ALB 119. In this regard ALB91 was the RIL that most closely approximated the behavior of G35346-3Q (Fig. 16b,c).

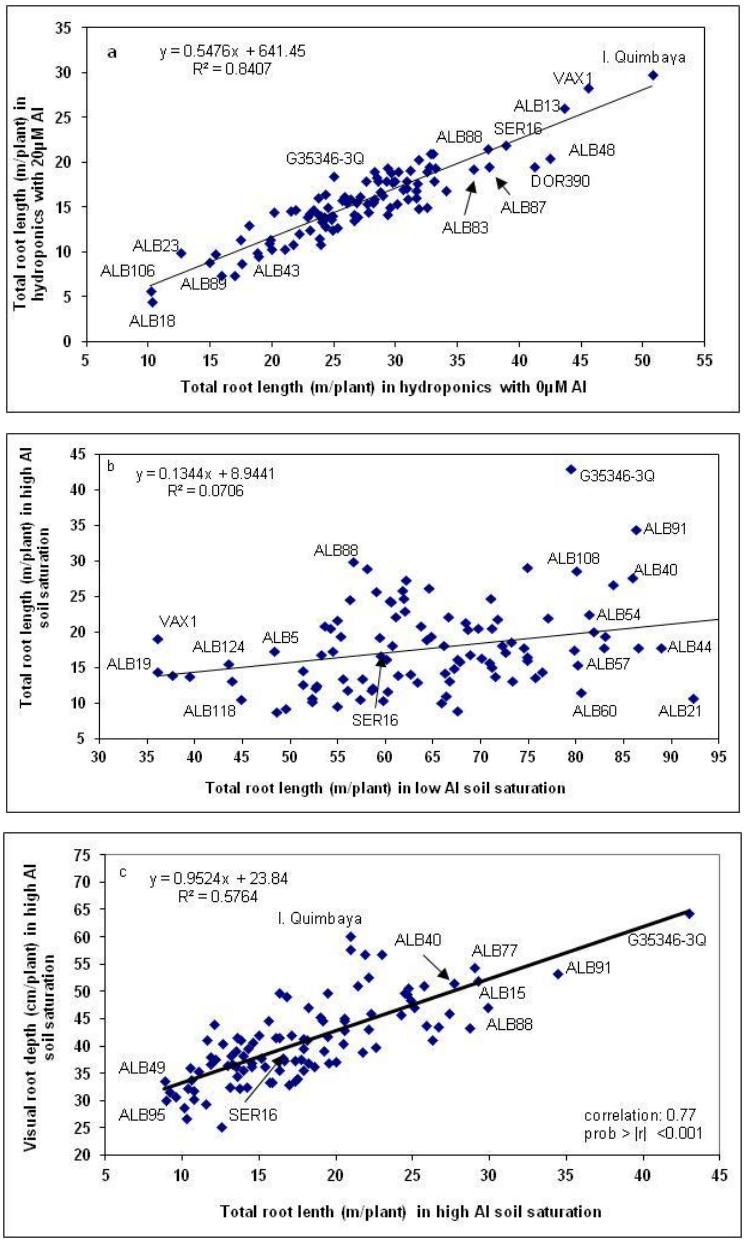

Figure 16: Relationships between total root length under hydroponics (0 and 20 µM Al), and between total root length in soil tube experiment with two level of Al saturation (low and high), total root length and root depth in soil with high Al saturation using soil tube system for 94 recombinant inbred lines, with 2 parents, and with 6 checks.

IV.2.2.3. Segregation of resistance among RIL

Mean for some specific root and shoot traits in both Al treatment and control for the two parents G 35346-3Q and SER 16 have been compared, and the implication for inheritance of these traits from each parent was identified (Table 15). The range of progeny means exceeded that of their parents both in hydroponics (with and without Al (T4.RGHA)) and soil tube screening (with high and low

Al saturation in soil (T5.RGSA)) suggesting transgressive segregation for these plant phenotypes. Both positive and negative transgressive segregation for TRER was observed, both with and without Al in solution (Table15), showing strong genetic variability among the two parents. The two parental lines were virtually equal for TRER 24 h in the 20 µM Al at about 1.4 mm h^{-1} but the progenies ranged from less than 1 mm h^{-1} to nearly 2 mm h^{-1} (T4.RGHA). The range of TRER among RILs in 20 µM Al increased from 0.88 to 1.75 mm h^{-1} at 24 h to 0.18–1.69 mm h^{-1} at 24–48 h. TRER at 0–24 h correlated to TRER at 24–48 h (r = 0.49***; Table 14), although this correlation was lower than might have been expected, considering that the data were taken on the same plants in the same environment. This suggests that measurements of TRER at 0–24 h may not be fully representative of TRER at 24–48 h.

Table 15: Trait values of parents of interspecific populations (G35346-3Q and SER16)evaluated under 0 and 20 µM Al treatments in hydroponics and in low and hight Al saturation in soil cylinder screening, and range of their recombinant inbred lines for specific plant traits.

	0 µM Al treatment				20 µM Al treatment			
Hydroponics	G35346-3Q	SER16	Range	LSD0.05	G35346-3Q	SER16	Range	LSD0.05
TRER 24h	1.52	1.82	1.42-**2.25**	0.21	1.41	1.38	0.88-**1.75**	0.2
TRER 24-48h	1.49	1.64	1.15-**1.86**	0.26	1.46	1.04	0.18-**1.69**	0.24
TRER 48h	1.5	1.73	1.31-**2.04**	0.19	1.44	1.21	0.55-**1.72**	0.18
TRL	4.22	4.1	1.48-**5.51**	1.13	2.82	2.3	0.84-**2.90**	0.57
MRD	0.47	0.32	0.29-0.47	0.03	0.54	0.4	0.35-0.54	0.04
NRT	720	962	394-**1316**	259	457	441	172-**513**	105
SRL	121.1	245.9	121-**281**	37.2	95.3	170.5	95-**279**	48
	Low Al saturation soil				High Al saturation soil			
Soil cylinder	G35346-3Q	SER16	Range	LSD0.05	G35346-3Q	SER16	Range	LSD0.05
TRL	79.4	64.8	36.1-**97**	24.2	42.9	16.6	8.81-42.9	7.8
MRD	0.43	0.32	0.3-0.43	0.03	0.46	0.4	0.34-**0.47**	0.07
SRL	60.2	98.1	60.2-**123**	18.1	62.1	72.1	51.3-**89.1**	15.5
VRD34d	53.6	61.5	50-75	17.6	53.6	33.2	22.6-**55.3**	11.2
LA	444	548	269-**854**	189	256	106	48.8-**271**	88
SDW	2.6	3.46	1.62-**5.2**	1.12	1.25	0.81	0.26-**1.43**	0.37
R:S	0.54	0.21	0.13-0.54	0.08	0.59	0.29	0.14-**0.79**	0.18

TRER, Tap root elongation rate (mm h^{-1}); TRL, total root length (m $plant^{-1}$); MRD, mean root diameter (mm), NRT, number of root tips; SRL, specific root length (m g^{-1}); VRD34d , visual rooting depth at 34 days (cm); LA , leaf area (cm^2 $plant^{-1}$); SDW, shoot dry biomass weight (g $plant^{-1}$); R:S, root:shoot ratio.

High transgression segregation for tap root elongation was also observed in the control solution with a common bean population generated from the cross DOR364 x G19833 (López-Marín et al., 2009). Other traits for which the RIL presented transgressive segregation were TRL in 20 µM Al, TRL in 0 µM Al (T4.RGHA), and TRL in low Al saturation soil (T5.RGSA). López-Marín et al. (2009) found that percentage of negative transgression was high in both control and Al treatment

for TRL while positive transgressive segregation was low. Mapping gene controlling aluminum tolerance in rice, Nguyen et al. (2002) found that the range of progeny means appreciably exceed that of their parents for root length in stress and control and root length ratio, suggesting transgressive variation among genotypes.

The genetic dissection of the quantitative traits controlling the adaptive response of crops to abiotic stress is a prerequisite to allow cost-effective applications of genomics-based approaches to breeding programs aimed at improving the sustainability and stability of yield under adverse conditions (Collins et al., 2008). RILs showed high percentage of transgression (formation of extreme phenotypes) in control and Al treatment for NRT (López-Marín et al., 2009). Differences among RILs, and between RILs and the recurrent parent SER16 reflect introgression from the *coccineus* parent (Table 15).

Segregation of most traits suggested quantitative inheritance and often transgressive segregation, implying that both parents possessed complementary genes for some traits. This was not a surprise considering that SER16 was quite good for TRL and NRT in the hydroponic system (T4.RGHA). Screening with hydroponic system F2 plant from the cross of Young x PI 416937 in soybean, Bianchi-Hall et al. (2000) found that progeny exhibited significant ($P < 0.05$) transgressive segregation for tap root extension under 0µ M Al.

IV.2.3. Benefits from evaluation in both hydroponic and soil tube systems

IV.2.3.1. Correlations among treatments, traits and evaluation methods

Several weak correlations were identified between parameters of RILs for the two methods (Table 16). TRL in acid soil (T5.RGSA) correlated with NRT in Al toxic hydroponic (T4.RGHA) solution ($r = 0.24^{***}$). SRL in soil presented negative correlations with TRER and MRD in hydroponics, and positive correlations with TRL ($r = 0.23^{*}$), NRT ($r = 0.25^{*}$), and SRL ($r = 0.41^{**}$). MRD in high Al saturation soil gave a response that was opposite to that of SRL, presenting negative correlations with TRL ($r = -0.36^{***}$) and with NRT ($r = -0.31^{**}$) in nutrient solution with 20 µM Al.

The relationship between total root length in Al-toxic acid soil (T5.RGSA) and total root length in hydroponics (T4.RGHA) with Al (20 µM Al) was poor, and the correlation not significant. G35346-3Q, ALB88, ALB108, and ALB46 (data not shown) were among few genotypes that developed extensive root system in both evaluations. Several RILs and especially ALB88 presented

low MRD in soil cylinders and maintained high TRL and NRT in nutrient solution. NRT could not be evaluated effectively in soil due to cutting the soil cylinders. Among the genotypes that showed good values of TRL in hydroponics and fine root (low MRD) system in acid soil conditions were ALB88, ALB46, ALB67, ALB34 and ALB58. Based on correlations between root characteristics under Al stress in both hydroponics and soil cylinder systems, the response of RILs and two parents in terms of MRD and SRL was similar (r = 0.36*** and 0.41**, respectively) (Table 16).

Table 16: Correlation between root and shoot characteristics of bean genotypes including 94 RILs, 2 parents, 6 plant controls under Al-stress both hydroponics (20 µM Al) and soil tube screening (high Al saturation soil): TRER, Tap root elongation rate (mm h^{-1}); TRL, total root length (m $plant^{-1}$); MRD, mean root diameter (mm), NRT, number of root tips; SRL, specific root length (m g^{-1}); VRD34d , visual rooting depth at 34 days (cm), LA , leaf area (cm^2 $plant^{-1}$), SDW shoot dry weight (g $plant^{-1}$), R:S ratio, root:shoot ratio.

Hydroponics / Soil tubes	TRL (m)	MRD (mm)	SRL (m g^{-1})	VRD at 34d (cm)	LA (cm2)	SDW (g)	R:S
TRER 24h (mmh^{-1})	0.22*	0.01 (ns)	-0.14	0.15 (ns)	0.06 (ns)	0.07 (ns)	0.21*
TRER 48h (mmh^{-1})	0.19 (ns)	0.16 (ns)	-0.21*	0.06 (ns)	0.09 (ns)	0.08 (ns)	0.20 (ns)
TRER 24-48h (mmh^{-1})	0.15 (ns)	0.23*	-0.22**	0.002 (ns)	0.08 (ns)	0.07 (ns)	0.13 (ns)
TRL (m)	0.29**	-0.36***	0.23*	0.12 (ns)	0.14 (ns)	0.17 (ns)	0.03 (ns)
MRD (mm)	-0.11 (ns)	0.36***	-0.42***	0.02 (ns)	-0.04 (ns)	-0.19 (ns)	0.09 (ns)
NRT (nb)	0.24*	-0.31***	0.25*	0.09 (ns)	0.16 (ns)	0.20*	-0.025 (ns)
SRL (m g^{-1})	0.04 (ns)	-0.31**	0.41**	0.02 (ns)	-0.007 (ns)	0.054 (ns)	-0.09 (ns)

*** Significant at the 0.001 probability level
** Significant at the 0.01 probability level.
* Significant at 0.05 probability level
ns: no significant

Genotypes that combined high TRL and number of root tips in hydroponics (T4.RGHA) and thin root system (MRD) in soil tubes included ALB46, ALB34, ALB54, ALB88, ALB67, and ALB58; several of which were identified based on other traits.

In our study, a close relationship (R^2 = 0.84) was found between total root length with and without Al treatment in nutrient solution (Fig.16a). This suggests that whatever effect of Al that resulted in much less TRL, was not an effect for which lines expressed resistance. In contrast, in soil tube system (T5RGSA), the effects of genotype x Al interaction for TRL were significant, and the relationship between TRL under high and low Al saturation treatment was weak (Fig.16b). TRL discriminates genotypes for their Al resistance in acid soil while in hydroponics it did not. In contrast, Villagarcia et al. (2001) found that despite the imposition of stress to approximately the same degree in hydroponics and sand culture, the genotypic variation in Al tolerance was much greater in hydroponics as evidenced by the following: much greater Al x genotype interaction, increased genotypic variation in response to stress, a much wider range in Al tolerance expressed as

percentage of control (PC), a lower correlation between genotypic means for Al-free and Al-stress treatments, and a greater correlation between ratings under Al-stress conditions and PC.

In their work on root hair of barley, Gahoonia and Nielsen (1997) did not find significant differences between soil and solution culture, and concluded that this could be due to using a nutrient solution with ionic strength similar to that in the soil solution. However, Noble et al. (1982, 1984, 1987) reported in a series of studies that ratings in greenhouse pots did not agree with solution culture results. Large discrepancy between hydroponics-based rating of seedlings and sand-culture-based ratings of plants was also observed when Al tolerance was expressed as per cent of control (PC) (Villagarcia et al., 2001). Sartain and Kamprath (1978) and Sapra et al. (1982) compared shoot growth in the greenhouse with root growth in solution culture and found no association between the two. We found similar results in our study when comparing results from soil tube system (T5.RGSA) and hydroponics system (T4.RGHA) for TRL, correlating results in low Al saturation soil with 0µM Al nutrient solution, and high Al saturation soil and 20 µM Al solution.

Several weak correlations were observed between the two methods in controls or in Al treatments (Table 16) suggesting that each technique is distinct, and cannot substitute to each other but are complimentary. ALB88 was very good both for TRL under stress with the two methods. Horst and Klotz (1990) compared 31 soybean genotypes for Al resistance in solution and sand culture and found a low but significant positive correlation ($r = 0.43$) between genotypic means of the two methods. In our study, a few weak correlations were identified between the two methods: mean root diameter (MRD) in high Al saturation soil, with TRL ($r = -0.36^{***}$) and NRT ($r = -0.31^{**}$) in nutrient solution with 20µM Al. Urrea-Gomez et al. (1996) suggested that constitutive morphological characteristics such as vigorous rooting could be advantageous in the breeding of Al–tolerant cultivars.

One important difference between the two systems used in our study is the tolerance to Al-toxic acid soil (T5.RGSA) through root depth evaluation but not with hydroponics system (T4.RGHA). Other potential differences could be in the nutritional status of the plants. The ability of roots to adjust their growth and development to environmental factors (Lopez-Bucio et al., 2003; Forde and Lorenzo, 2001) could be an explanation of these differences. Seedlings in nutrient solution are evaluated for a short period (for several hours or days) while for soil tube, plants are grown for several days or even months. P diffuses freely to the root surface in solution culture (Clarkson, 1991), whereas in soil, diffusion of P to the root surface is rate limiting and the zone of soil close to

the root is depleted uniformly due to the geometrical arrangement of root hairs on root (Gohoonia and Nielsen, 1991).

To successfully select Al-resistant lines that perform well in acid soil, Al-resistance could not be deduced based only on the inhibition of root growth alone but must take into account the effects of Al on the entire plant. Ryan et al. (1994) found that low concentrations of Al could inhibit root growth and Ca uptake. Screening methods and Al concentrations that reveal differences in shoot development could probably improve correlations with performance in acid soil, and selection for Al resistance. The hydroponic system (T4.RGHA) used in our study is suitable for selection for Al resistance alone based only on root development and distribution, while soil tube system (T5.RGSA) revealed differences in root architecture, acquisition of soil resources and pattern of biomass allocation into leaves, steams, and roots. The use of both soil and hydroponic systems could contribute to evaluation of breeding materials to identify genotypes that combine Al resistance with acid soil tolerance.

IV.2.3.2. Interaction between root and shoot traits

Root growth requires nutrients that are absorbed from the soil and photosynthates that are transported from the shoot (Lopez-Bucio et al., 2003). Effects of leaf area (LA) and shoot dry biomass weight (SDW) were highly significant (T4.RGSA) for genotype, and genotype x Al treatment interaction (Table 14). Relationships between shoot attributes (leaf area and shoot dry biomass weight) and root length density under high Al saturation in the soil tube experiment and the hydroponics screening were determined. Our results on shoots in soil tube screening (Table 16) indicate significant genotype, and genotype x Al level effect ($P < 0.001$). Villagarcia et al., (2001) found also a significant Al x genotype interaction indicating a genotypic variation in response to the imposition of Al stress for shoot dry weights.

Both total root length and shoot biomass showed genotypic variation in response to the Al-toxic acid soil stress (T5RGSA). Rating based on the combination of total root length and shoot dry biomass weight revealed not only the effects of Al-stress in the soil but also the biomass accumulation in shoot that could translate into yield in the reproductive phase. Interesting lines that maintain a high capacity for biomass accumulation above and below ground with an extensive root system are ALB119, ALB77, G35346-3Q, ALB89, ALB88 and ALB91 (Butare et al. 2012).

Among these lines ALB88 was able to produce more leaf area with less biomass which is an advantage in a competitive situation, and it has also high numbers of root tips.

The relationship of shoot dry biomass accumulation in soil tubes (T5.RGSA) and a fine root system in hydroponics (T4.RGHA) was evaluated, and although there was no correlation (Butare et al., 2012). ALB77, ALB88, ALB117, ALB58, ALB15, and ALB38 were found to be superior lines that combine high shoot biomass accumulation and fine root (≤0.45mm of MRD) development in nutrient solution. Correlations between two important shoot traits and total root length under high Al saturation soil were highly significant, r = 0.70*** for leaf area and r = 0.60*** for shoot dry biomass weight (Table 12).

IV.2.4. Conclusions on methods and selection of interesting phenotypes for resistant to Al

In conclusion, our data reaffirm that the runner bean genotype, G353466-3Q, is an Al resistant material and could be a very good source for improving acid soil tolerance in common bean. Regarding a number of root and shoot traits evaluated using soil tube system (T5.RGSA); several root parameters expressed well in the best lines for shoot development and may contribute to shoot biomass accumulation of those lines. Higher values of total root length and rooting depth found with ALB91, ALB88, ALB77 and ALB 89 contributed to greater shoot development. RILs that emerged as superior for several traits could be candidates for crossing with each other or with other lines. The use of both hydroponic and soil tube systems could contribute to evaluate breeding materials to identify genotypes that combine Al resistance with acid soil tolerance. However, as results obtained from hydroponic system were not highly correlated with the data from Al-toxic acid soil tolerance from soil tube system indicating that use of either one system alone can eliminate some useful genotypes. Different concentrations of Al in hydroponic system and different levels of Al saturation in soil system could be tested to further test the relationship of root traits with shoot traits. This knowledge will be useful to understand the physiological basis of differences in ranking of genotypes under acid soil field conditions across seasons and years. The results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

Summary statement:

Phenotypic evaluation of Interspecific recombinant inbred lines (RILs) of *Phaseolus* species for Al resistance, and root and shoot growth response to Al stress under greenhouse conditions.

Aluminium (Al) toxicity limits common bean productivity in acid soil regions of the tropics. To improve Al resistance of common bean, Al-sensitive *Phaseolus vulgaris* (SER16) was crossed to Al resistant *P. coccineus* (G35346-3Q) to create 94 F5:6 recombinant inbred lines (RILs) of the pedigree SER16 x (SER16 x G35346-3Q). RILs were characterized for resistance to Al in a hydroponic system with 0 and 20 μM Al in solution, and for shoot and root growth response to Al-toxic infertile acid soil in 75 cm long soil cylinder system using an oxisol of low Al- (12.5%; pH 4.6; fertilized) and high Al-saturation (77%; pH 4.1; unfertilized). G35346-3Q increased its taproot elongation rate by 3.5% between 24 and 48 h under 20 μM Al in solution, while the best RIL, Andean genotype ICA Quimbaya, and sensitive genotype VAX1 expressed reductions of 2.6, 12.5, and 69.5%, respectively. In the acid soil treatment the correlation between leaf area and total root length was highly significant under high Al saturation (r = 0.70***). Genotypes that were Al resistant in the hydroponic system were not necessarily tolerant to Al-toxic acid soil conditions based on shoot and root growth responses. Phenotypic evaluation using both systems allows the identification of genotypes with Al resistance combined with acid soil adaptation. Two genotypes (ALB88 and ALB91) emerged as lines with multiple traits. Results suggest that inheritance of Al resistance and acid soil tolerance in G35346-3Q is complex. Results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

IV.3. Phenotypic evaluation of interspecific Recombinant Inbred Lines (RILs) of *Phaseolus* species for their tolerance to individual and/or combined stress of aluminum and drought

Two soil studies were conducted in greenhouse at CIAT headquarters in Palmira (Altitude 965 m; Lat. 3°29'N; Long. 76°21'w). 94 RILs populations from an interspecific backcross between *P. vulgaris* and *P. coccineus*; SER16 x (SER16 x G35346-3Q) (Butare et *al*., 2012) were screened for drought resistance in the first experiment and compared to both parents (SER16, G35346-3Q) and 6 checks (*P. acutifolius*, G 40159; ICA Quimbaya; Tio Canela 75; G21212; VAX1 and DOR39 (T6.RGSD). For the second experiment (T7.RGSC), twenty five bean genotypes including twenty one Recombinant Inbred Lines (RILs) selected from individuals of the same interspecific backcross between SER 16 and G35346-3Q; the two parents and two popular bean varieties (ICA Quimbaya, an Andean large seeded bean genotype resistant to Aluminum and Tio-Canela 75, a commercial Mesoamerican bean landrace) were screened for individual and combined stress.

IV.3.1. Phenotypic differences in terminal drought in soil tube system

In agriculture, drought resistance refers to the ability of a crop plant to produce its product with minimum loss in a water-deficit environment relative to the water-constraint-free management (Mitra, 2001). Beans revealed several mechanisms that enable the crop to adjust its system and adapt to limited water supply. In our study (T6.RGSD), the primary plant traits with highly significant genotype (RIL) effects and treatment (Drought) effects were total root length (TRL), root surface area (RSA), specific root length (SRL), fine root proportion (FRP), and shoot dry biomass weight (SDW) (Table 17). In cowpea genotypes selection for tolerance to drought, Ogbonnaya et al. (2003) found significant genotypic differences in plant height, collar diameter, leaf area, shoot and root biomass, root volume, and root-Shoot ratio.

Significant variations among genotypes were observed for all the morphological traits. Root depth at 34 days (VRD34d) was significant for genotype and treatment effects, respectively ($P < 0.01$) and ($P < 0.001$). Mean root diameter (MRD) was significant also for genotype and drought treatment but at $P < 0.05$. Leaf area (LA) and shoot dry biomass weight (SDW) were both highly significant for drought treatment; but for genotype treatment effects, LA was significant at ($P < 0.05$) and SDW at ($P < 0.001$).

In our study (T6.RGSD), genotype x drought interaction was significant only for specific root length (SRL) ($P < 0.05$) (Table 17). Significant interactions for water regimes and genotypes were also observed for collar diameter, root biomass, root:shoot ratio, and root volume (Ogbonnaya et al., 2003).

Table 17: Correlation between root and shoot characteristics of bean genotypes grown in water stress and mean squares (from combined ANOVA) of total root length (TRL), root surface area (RSA), mean root diameter (MRD), specific root length (SRL), and visual root depth (VRD34d) of 34 days-old plants, fine root proportion (FRP) leaf area, shoot dry biomass weight (SDW) and root:shoot ratio (R:S) for 102 bean genotypes including 94 RILs, 2 parents, 6 checks grown using soil tube system.

Variable/Source	WS/DF	TRL	RSA	MRD	SRL	VRD	FRP	LA	SDW	R:S
TRL	WS	1								
	WW	1								
RSA	WS	0.90***	1							
	WW	0.93***	1							
MRD	WS	0.22*	0.46***	1						
	WW	0.37***	0.56***	1						
SRL	WS	0.27**	0.09 (ns)	-0.12 (ns)	1					
	WW	0.16 (ns)	0.02 (ns)	0.03 (ns)	1					
VRD34d	WS	0.56***	0.44***	0.31**	0.42***	1				
	WW	0.60***	0.60***	0.54***	0.26**	1				
FRP	WS	0.03 (ns)	-0.26**	-0.68***	0.57***	-0.03 (ns)	1			
	WW	-0.06 (ns)	-0.29**	-0.50***	0.66***	-0.17 (ns)	1			
LA	WS	0.35***	0.33***	0.28**	0.10 (ns)	0.23*	0.008 (ns)	1		
	WW	0.68***	0.65***	0.31**	0.02 (ns)	0.64***	-0.17 (ns)	1		
SDW	WS	0.53***	0.38***	0.04 (ns)	0.32***	0.52***	0.21*	0.61***	1	
	WW	0.51***	0.46***	015 (ns)	0.06 (ns)	0.44***	-0.09 (ns)	0.65***	1	
R:S	WS	0.14 (ns)	0.29**	0.22*	-0.60***	-0.21*	-0.49***	-0.25*	-0.52***	1
	WW	0.19 (ns)	0.27**	0.13 (ns)	-0.48***	-0.08 (ns)	-0.32***	-0.10 (ns)	-0.50***	1
Drought	1	9836.2***	3283784.3***	0.02*	7037880.2***	75420.06***	700.8***	7044133.5***	244.06***	0.007 (ns)
Rep. (Dr.)	4	2556.7***	431096.6***	0.01**	16186.8 (ns)	968.13***	85.67***	111016.6***	4.17***	0.02 (ns)
Gen.	101	431.7***	39547.4***	0.006*	37332.9***	185.5**	39.08***	13329.3*	0.704***	0.01 (ns)
Gen. x Dr.	101	245.7 ns	21575.4 ns	0.004 ns	20661.4*	116.2 (ns)	17.12 (ns)	10015.5 (ns)	0.425 (ns)	0.01 (ns)

*, **, *** represent significant respectively at 0.05, 0.01, and 0.001 probability level; and ns: no significant.

IV.3.1.1. Segregation of drought resistance among RILs

Transgressive segregation is particularly attractive as a mechanism for large and rapid evolutionary transitions because hybridization generates variation at many genes simultaneously and the variant alleles have already been tested by selection (Rieseberg et al., 2003). Transgressive individuals observed in early generations (F2, F3) may be heterozygous and their superiority will not be maintained in successive generations (Kuczyńska et al., 2007). Genotypic differences for drought resistance in root development and distribution were observed in this greenhouse study (T6.RGSD). When comparing segregating hybrids from backcross F5:6 SER16♀ x (SER16♀ x G35345-3Q♂) to their parents, we found extreme phenotypes for some plant traits (Table 18). The most noticeable morphological response to controlled moisture deficit is the stimulated root growth (Read and Bartlett, 1972). Among root traits that showed transgressive segregations in water stress and well watered soil tubes were total root length (TRL), specific root length (SRL), and visual root depth (VRD). Mean root diameter (MRD) was extreme when considering the importance of fine roots among segregating hybrids. Some RILs have more proportion of thicker roots than their parents both under drought stress and well watered conditions.

Table 18. Parents of interspecific populations (G35346-3Q and SER16) evaluated under drought stress and control test in greenhouse soil tube screening, and range of recombinant inbred lines for specific plant traits.

Treatment and plant traits	Water stress treatment				Well watered treatment			
	G35346-3Q	SER16	Range	$LSD_{0.05}$	G35346-3Q	SER16	Range	$LSD_{0.05}$
TRL	67.8	38.5	28.1-**74.6**	32.8	40.4	37.9	33.9-**95.2**	51.2
RSA	756.12	290.8	232.7-756.12	271.7	531.8	339.5	295.4-**868.1**	500.6
MRD	0.35	0.22	0.19-0.35	0.07	0.38	0.19	0.17-**0.59**	0.24
SRL	492.7	772.9	492.7-**1042.9**	343.35	289.7	404.5	289.7-**857.9**	324.1
VRD	71.9	58.4	41.0- **75.0**	27.1	38.7	38.5	32.7-**64.2**	33.1
LA	311.5	269.2	121.2-**367.3**	189.54	412.8	481.5	268.6-**692.1**	333.8
SDW	1.57	1.65	1.02-**2.45**	1.04	2.78	2.83	1.65-**4.26**	2.24
R:S	0.09	0.035	0.02-**0.1**	0.04	0.05	0.034	0.019-**0.699**	0.39

( ) Total root length (TRL), root surface area (RSA), mean root diameter (MRD), specific root length (SRL), and visual root depth (VRD34d) of 34 days-old plants, fine root proportion (FRP) leaf area (LA), shoot dry biomass weight (SDW) and root:shoot ratio (R:S).

Stomatal aperture and leaf area determine the rate of both photosynthesis and transpiration. Therefore, there is an inherent contradiction between biomass accumulation and stress avoidance via a reduction of transpiration (Colins et al., 2008). White and Castillo (1989) have suggested that shoot characteristics are of less importance. In our experiment (T6.RGSD), transgressive segregations were observed for shoot traits (leaf area and shoot dry biomass weight) both under

water stress and well watered treatments suggesting that the two traits constitute a source of novel adaptation to drought observed in some hybrids.

IV.3.1.2. Root surface area and tolerance to drought stress

Some species have evolved large surface root systems to quickly absorb rainfall, while other species grow deep root systems to get access to deep water. Water stress (T6.RGSD) markedly decreases the root surface area (RSA), the range was 232.68 to 756.12 cm^2 $plant^{-1}$ for drought stress treatment and 295.4 to 868.06 cm^2 $plant^{-1}$ for well watered treatment (Table 18). Root hairs form an important part of surface area of roots through which plant absorbs most of water and nutrients. Root hair can contribute up to 67% of the total root surface area (Nielsen et al., 2001). Gene effects toward increasing root growth (Vadez et al., 2008) were reported in common bean, these are about gene components that control the expression of root dry weight and root surface area (Araújo et al., 2004).

Eleven genotypes including G35346-3Q, ICA Quimbaya, ALB28, ALB43, Tio-Canela 75, ALB49, ALB122, ALB125, ALB118, ALB60, and ALB20 were found to be outstanding in maintaining high root surface area in drought stress treatment (Fig.17) compared to the value in well watered treatment. The average of RSA for drought stress and well watered conditions was respectively 412.8 and 559.3 cm^2 $plant^{-1}$. A number of genotypes maintained a greater RSA, probably through more fine roots, and could lead to better water up-take. A root system that effectively occupies more the soil, may delay the development of water stress (Markhart, 1985). Water uptake is maximized by adjusting the allocation pattern, namely increasing investment in the roots (Jackson et al., 2000). The majority of genotypes were superior compared to SER16, the parent from *P. vulgaris* with an average RSA of 290.76 and 339.46 cm^2 $plant^{-1}$; respectively for drought stress and well watered treatment. Some RILs such as ALB17, ALB26, ALB104, ALB117, and ALB56 were highly sensitive to drought and were characterized by a low RSA. In our study, total root length was highly correlated to RSA both in water stress ($r = 0.93^{***}$) and well watered ($r = 0.90^{***}$). RSA was also highly and positively correlated to root mean diameter (MRD) and root depth at 34days, respectively $r = 0.46^{***}$ and 0.44^{***} in drought stress, and $r = 0.56^{***}$ and 0.60^{***} for well watered treatment (Table 16). In their work on drought resistant and agronomic traits in peanut, Painawadee et al. (2009) found that root surface area was positively and significantly correlated to root length ($r = 0.98^{**}$), root volume ($r = 0.97^{**}$), and root dry weight ($r = 0.71^{**}$). Population differences for root surface area and length were mainly attributed to variation in the root mass rather than to differences in root thickness (Araújo et al. 2005). Root hairs are thought to increase the adsorptive

capacity of the root by increasing the surface area (Clarson, 1985), the root-soil contact that increase absorption area. Root characters were also positively and significantly correlated with biomass production (Painawadee et al., 2009). We found similar results both with water stress and well watered treatment respectively between RSA and leaf area ($r = 0.33^{***}$, and $r = 0.45^{***}$); RSA and shoot biomass ($r = 0.38^{***}$, and $r = 0.46^{***}$). But in contrast, RSA was negatively correlated with thin root proportion both under stress and no stress conditions ($r = -0.26^{**}$, and $r = -0.29^{**}$).

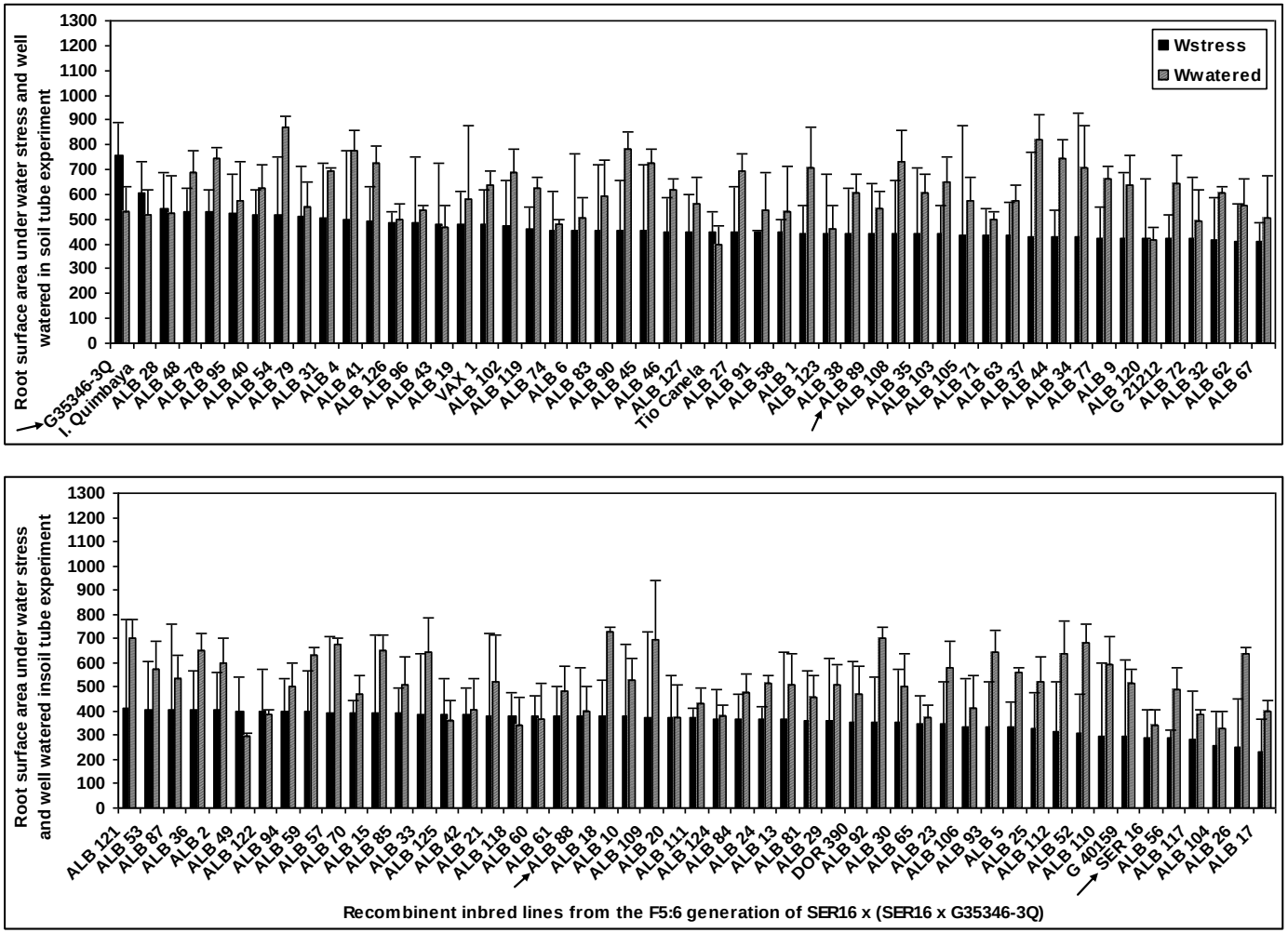

Figure 17: Root surface area (RSA) in water stress and well watered soil for 102 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 6 checks (VAX1, DOR390, G21212, G40159, G40159, Tio-canela75, and ICA Quimbaya) grown using soil tube system.

IV.3.1.3. Interaction between root growth traits

Selection for deep and extensive root has been advocated to increase productivity of food legume under moisture-deficit conditions as it can optimize the capacity to acquire water (Subbarao et al., 1995; Serraj et al., 2004a; Sarker et al., 2005; Stoddard et al., 2006). Genotypes that revealed extensive root system (total root length and specific root length) both under terminal drought stress and well watered treatment were characterized in this study (T6.RGSD). Mean of total root length ranged respectively from 28.12 to 74.6 m plant^{-1}, and 33.9 to 95.2 m plant^{-1} in water stress and well watered treatment (Table 18).

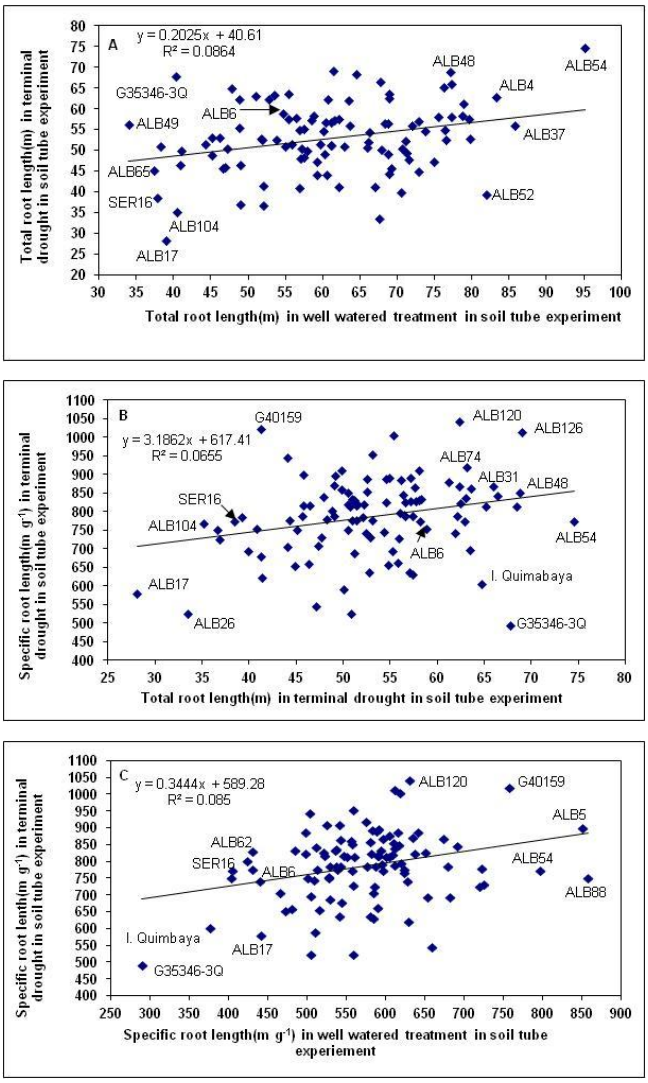

Figure 18: Relationship between total root length and specific root length for 102 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 6 checks (VAX1, DOR390, G21212, G40159, G40159, Tio-canela75, and ICA Quimbaya) with or without simulation of terminal drought in a greenhouse soil tube experiment.

Water stress (T6.RGSD) markedly affects total root length with mean of 53 m plant^{-1} for drought treatment and 61 m plant^{-1} for well watered. Merrill et al. (2002) showed that soybean and dry bean had the greatest root growth in the driest year of their study and the least root growth in the wettest year. The relationship between total root length in terminal drought treatment and the control (with well watered treatment) was positive but weak ($r = 0.28^{**}$)(Fig.18a). ALB54, ALB4, ALB34, and ALB48 are among genotypes that maintain extensive root system both into drought and control. Gardner (1961) has demonstrated the importance of effective root length as a determinant of rate of water uptake from soil.

Selections with more extensive root systems could extract more soil water from greater soil volumes than selections with limited root system (Painawadee et al., 2009). In our study (T6.RGSD), the range of specific root length both under drought stress and well watered treatment was respectively, 492.69-1042.9 and 289.66-857.9 m g^{-1}. These findings agree with those of Huang and Fry (1998) that specific root length increased with soil drying. Genotypes that combined extensive and well branched root system as revealed respectively by TRL and SRL were identified. They include as examples ALB126, ALB120, ALB48, ALB31, and ALB 78. High SRL both into drought stress and control was found with *P. acutifolius* accession G40159, while SRL was low for both treatments in G35346-3Q. A weak but positive relationship was found between SRL under water stress and well watered treatment ($r = 0.30^{**}$)(Fig.18c). Specific root length and total root length were poorly associated ($r = 0.26^{*}$)(Fig.18b).

IV.3.1.4. Interaction between root distribution and total root length

Several researchers (Pandey et al.1994, Sponchiado et al., 1980; Benjamin and Nielson, 2006) have suggested that the ability of a plant to change its root distribution in the soil is an important mechanism for drought avoidance. Our results (T6.RGSD) on the relationship between root distributions in two deep soil profiles (40-60 cm, and 60-75 cm) showed that greater proportion of the roots was found in the deeper soil layers under drought stress soil tubes than tubes under well watered treatment (Fig.19). In our study, variability in root distribution was found between drought treatment and control, and between soil profiles (40-60 cm and 60-75 cm). Root distribution decreases with soil depth both under water stress and well watered treatment, but root density in the soil profile increases with water stress. The range of root distribution in well watered was 2.4-33.8 m plant^{-1} and 0-12.9 m plant^{-1}, respectively for 40-60 and 60-75 cm soil profile; while for water stress, the range was higher, from 7.14 to 82.07 m plant^{-1}, and 1.45 to 20.6 m plant^{-1}. Similar

conclusions have been made by Benjamin and Nielsen (2006) for pea grown in irrigated and non-irrigated conditions and found that about 20% of the roots were in the 0.23-0.46m soil layer under non irrigation condition compared with about 12% of the total roots in this layer under irrigation. Turner et al. (2001) identified rooting depth and density as a main drought avoidance trait in grain legumes for use in terminal drought environments. Genotypic differences have been reported also in common bean for root distribution along the soil profile by Sponchiado et al. (1989) and Guimarães et al. (1996).

A strong linear relationship exists between root distribution at 40-60 cm and total root length both in well watered and drought treatments, respectively $R^2 = 0.60$ (Fig.19a) and $R^2 = 0.64$ (Fig.19c), but for the root distribution in the 60-75 cm soil profile, the relationship between the two root traits was weak ($R^2 = 0.14$)(Fig.19b) in the control and strong in drought stress ($R^2 = 0.55$) (Fig.19d). Recombinant inbred lines ALB54, ALB4, and ALB37 have been identified on the basis of their deep and extensive root system, and selected as the most drought resistant RILs. Significant gains in crop productivity due to plant breeding for semi-arid regions were resulted from enhancements in rooting depth (Fisher and Turner, 1978; Blum, 1984).

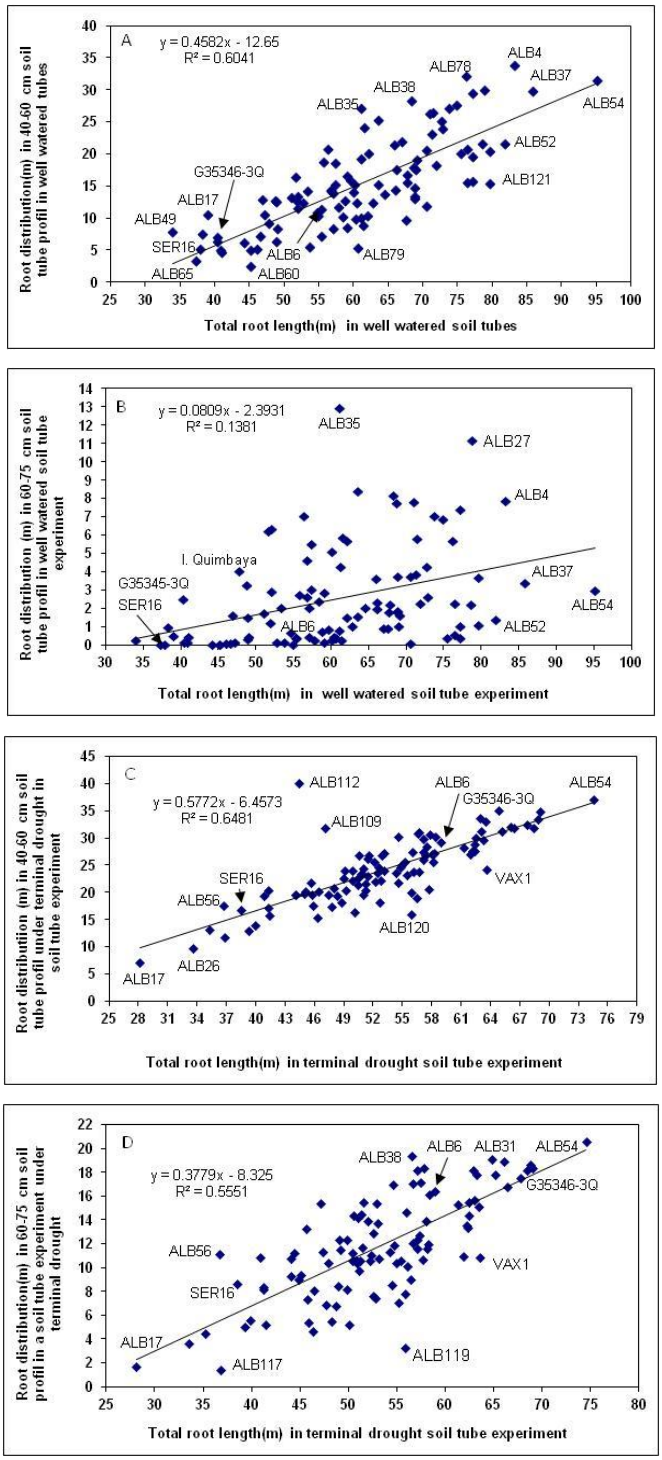

Figure 19: Relationship between root distribution per profile and total root length density for 102 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 6 checks (VAX1, DOR390, G21212, G40159, G40159, Tio-canela75, and ICA Quimbaya) with or without simulation of terminal drought, total root length and specific root length in terminal drought in a greenhouse soil tube experiment.

IV.3.1.5. Interaction between root and shoot attributes

Morphologically, loss of leaf area is the most important drought response of common bean and can be the result of reduced number of leaves, reduced size of younger leaves, inhibited expansion of developing foliage, or leaf loss accentuated by senescence, all of which result in decreased yields (Acosta-Gallegos, 1988). In our study (T6.RGSD) we found that leaf area (LA) and shoot dry biomass weight (SDW) varied relatively little with total root length. LA ranged from 268.6 to 692.1 cm^2, and 121.2 to 367.3cm^2; SDW from 1.65 to 4.26g, and 1.02 to 2.45g; respectively for well watered and water stress treatments (Table 18). Read and Bartlett (1972) showed with soybean that relative growth rate (RGR) of root and shoot change quite differently in relation to soil water potential. They found that the root RGR increases with decreasing soil water potential but observed a corresponding decline in RGR of the shoot (Read and Bartlett, 1972). Leaf area ratio decreases uniformly with fall of water potential (Read and Bartlett, 1972). Working on lentil landraces, Sarker et al. (2005) found that mean tap root length ranged from 11.6 to 47.2 cm and lateral root number showed a wide range (from 16 to 50).

Large root systems and deep growth of root systems into lower soil profile can permit more water absorption to support plant growth and yield (Ludlow and Muchow, 1990; Turner et al., 2001). In our study (T6.RGSD), the relationship between LA and total root length was weak ($R^2 = 0.14$) but the correlation was significant ($r = 0.37^{***}$) (Fig. 20b); similar relationship was observed for SDW with $R^2 = 0.24$, and $r = 0.48^{***}$ (Fig. 20d). The total root dry mass showed a significant linear relationship with total shoot dry matter and total leaf area of chickpea plants under drought conditions (Serraj et al., 2004b; Stobbard et al., 2006). McPhee (2005) also observed positive correlation between total root characters and biomass. Plant dry weight and leaf area were reduced by water stress in both *P. vulgaris* and *P. acutifolius* (Markhart, 1985).

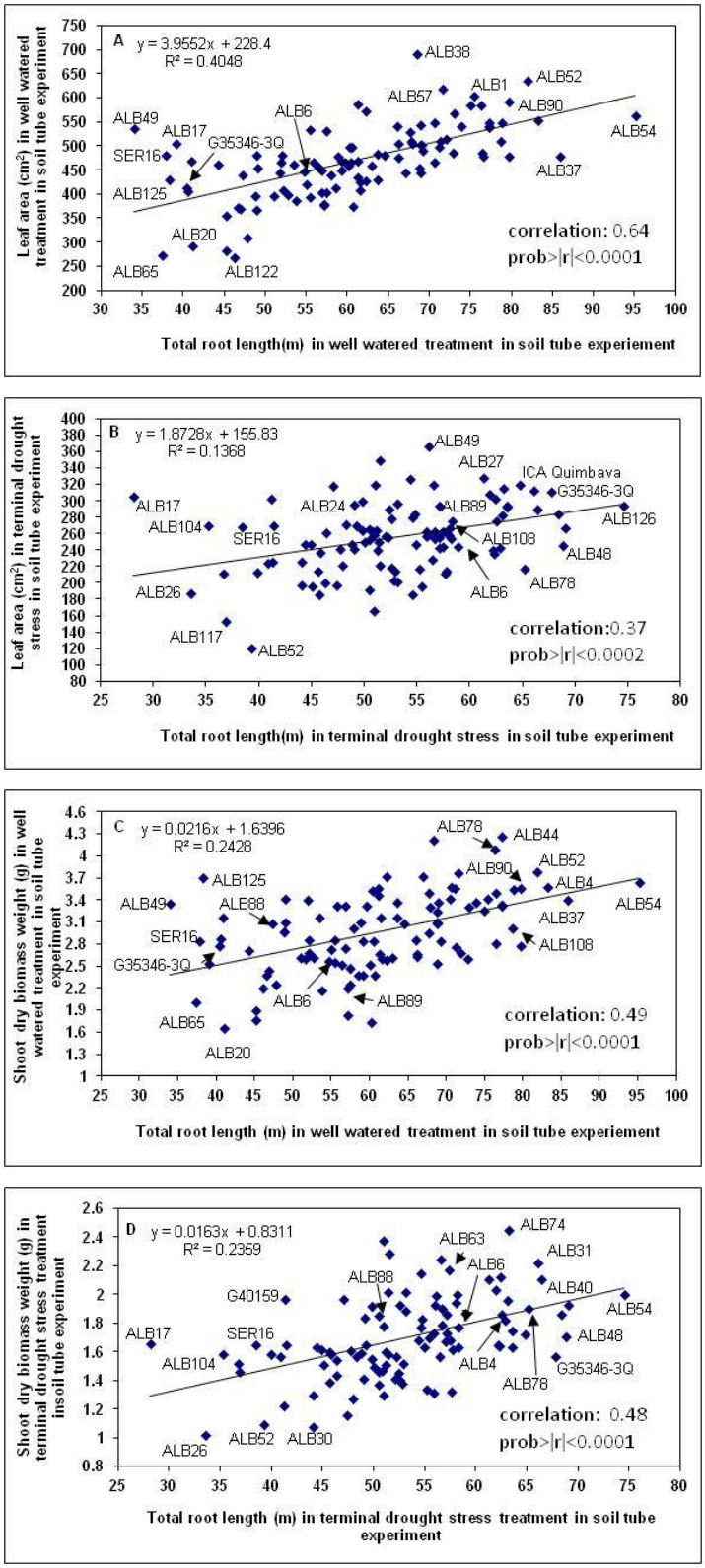

Figure 20: Relationship between total root length and shoot attributes (shoot biomass dry weight and leaf area surface) for 102 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 6 checks (VAX1, DOR390, G21212, G40159, G40159, Tio-canela75, and ICA Quimbaya) in a greenhouse soil tube experiment using Darien soil.

IV.3.2. Phenotypic differences in combined stresses of Al and drought in soil tube system

Root development and distribution play a major role in bean response and adaptation to water stress environment. However, because of aluminum-induced inhibition of root development, combined stress of aluminum and drought will aggravates problems of water and nutrient acquisition by restricting root systems. By improving Al tolerance in common bean, we indirectly improve drought tolerance.

In our study (T7.RGSC), genotype differences were significant (P<0.05) for TRL (Table 19), and highly significant (P<0.001) all other root traits (Table 19) including root surface area (RSA), mean root diameter (MRD), visual root depth (VRD), specific root length (SRL), fine root proportion (FRP) leaf area (LA), shoot dry biomass weight (SDW) and root:shoot ratio (R:S) under combined stress of Al-toxic soil and water stress (Table 19). We found similar reaction while screening the parents for the RILs population with highly significant difference among genotypes for TRL, MRD, VRD, and SRL under the same condition (Butare et al., 2011). A deep and thick root system is generally considered to be useful trait towards maintaining yield under stress in a broad range of conditions and ecosystems. Many traits in plants are quantitatively inherited, showing a continuous variation in phenotype among a population of individuals (Guzmán-Maldonado et al., 2003).

Table 19: Mean squares of root attributes of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75) under individual and combined stress of Al and drought in soil tube experiment

Source	df	TRL (m)	RSA (cm^2)	MRD (mm)	VRD (cm)	RDW (g)	SRL (m g^{-1})	FRP (%)
Genotypes	24	217.6^{*}	34235.9^{***}	0.007^{***}	235.06^{***}	0.08^{***}	923.3^{***}	94.2^{***}
Treat	3	20653.5^{***}	2222121.7^{***}	0.035^{***}	4400.2^{***}	1.93^{***}	9957.8^{***}	494.08^{***}
Rep	2	1385.1^{***}	102542.97^{**}	0.0005 (ns)	4.1 (ns)	0.027 (ns)	3616.3^{***}	2.67 (ns)
Error	270	128.6	14779.6	0.0009	100.6	1.23	355.6	10.47
CV%	_	36.3	36.9	8.35	23.5	70.96	23.6	3.88

( ) Total root length (TRL), root surface area (RSA), mean root diameter (MRD), visual root depth (VRD), root dry biomass weight (RDW), specific root length (SRL), and), fine root proportion (FRP).

Breeding for drought resistance must be combined with Al resistance to ensure that drought resistance is expressed adequately in crops grown on soils with acid Al-toxic suboils (Yang et al., 2013). In our study (T7.RGSC), we found that genotype variation (Table 20) was highly significant (P<0.001) for root:shoot ration (R:S), and significant (P<0.01) for leaf area (LA). No significant difference found for shoot dry biomass weight (SDW). In our previous study with three Phaseolus species (*P. vulgaris, P. coccineus,* and *P. acutifolius*), we found similar results for significant for

R:S ratio (P<0.001) and for LA (P<0.01) (Butare et al., 2011). Excess may strongly reduce root growth without affecting shoot growth (Kochian et al., 2004; Yang et al., 2009). But, as the primary response of plants to drought stress is the inhibition of shoot growth (Yang et al., 2013), we should expect more effects on the vegetative part of plants with the combination of these two abiotic stresses.

Table 20: Mean squares of shoot attributes of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75) under individual and combined stress of Al and drought in soil tube experiment

Source	df	LA (cm^2)	SDW (g)	R:S
Genotypes	24	11779.9**	1.68 (ns)	0.03***
Treat	3	941906.7***	89.7***	0.54***
Rep	2	1046.1 (ns)	0.12 (ns)	0.0076 (ns)
Error	270	6058.9	1.23	0.007
CV%	_	43.3	70.97	27.8

( ) Leaf area (LA), shoot dry biomass weight (SDW) and root:shoot ratio (R:S).

IV.3.2.1. Root growth in individual and combined stress of Al and drought

Resistance among recombinant inbred lines of the cross SER16♀ x (SER16♀ x G35346-3Q♂) to individual and combined stress of Al and drought (T7.RGSC) was evaluated in Al-toxic soil. Genotypic variation in root development when genotypes (T7.RGSC) were grown under Al stress alone, drought alone, and combined stress has been identified. Aluminium alone affected more root length density than combined stress of Al and drought or drought alone (Fig. 21). Low Al saturation associated to well watered treatment showed that TRL ranged from 33.8 to 70.1 m with average TRL of 51.12 m. TRL ranged from 18.19 to 52.13 m for water stress treatment alone. Comparing the four treatments of our study, we found that Al-stress alone was more inhibitory than combined stress of Al-toxic soil and WS, and WS alone. Simular observations were made in our study for identification of potential parents resistant to Al and /or drought stresses (Butare et al. 2011).

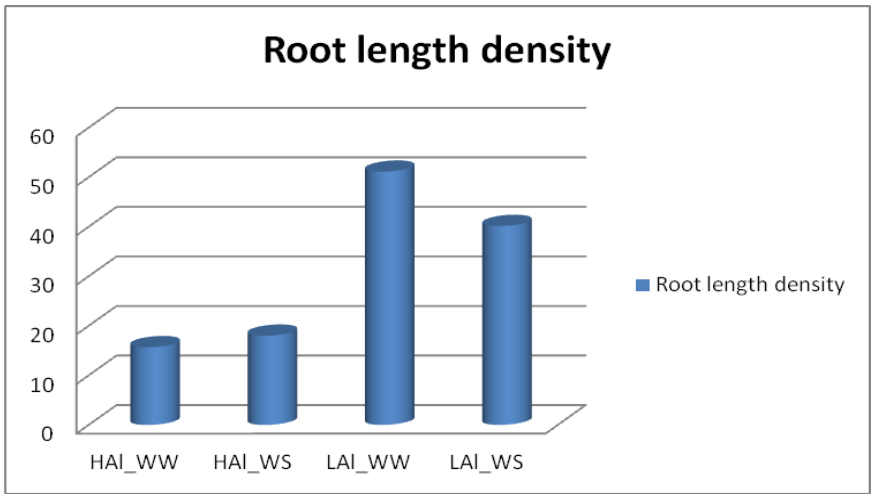

Figure 21: Effects of individual and combined stress of Al and drought in soil tube experiment (4 treatments: HAl_WW, HAl_WS, LAl_WW, and LAl_WS) on root length density of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75)

Combined stress of Al and drought, and Al stress alone showed that TRL ranged from 10.6 to 40.14 m, and 8.87 to 26.58 m respectively. The mean of TRL in these two treatments was respectively 18.01 and 15.69 m. Total root length was highly correlated to RSA ($r = 0.98^{***}$), RDW ($r = 0.94^{***}$), SDW ($r = 0.90^{***}$), LA ($r = 0.86^{***}$), SRL ($r = 0.64^{***}$), FRP ($r = 0.37^{***}$), VRD ($r = 0.28^{***}$), but the correlation was negative with R:S ration ($r = -0.34^{***}$) and MRD ($r = -0.22^{***}$) (Table 21). Under conditions of unsecured soil resources, a potentially large root is required to ensure capture of resources under erratic conditions (Blum, 2005). Root-soil contact is an important factor for uptake of a less mobile soil nutrient such as phosphorus (P) by crop plants (Gahoonia et al., 1997). Miguel (2004) found a positive correlation between root hair length and density when plants grow under low phosphorus availability.

ALB 91 emerged as superior genotype in root length density under combined stress of Al and drought even compared to resistant parent (G35346-3Q), a *P.coccineus* accession (Fig. 21). The line was also good under individual stress of Al, and drought. Among the other 21 RILs, most of them showed high root length density compared to the recurent parent SER16. Potential RILs for indiviual and combined stress of Al-toxic soil and water stress were ALB91, ALB6, ALB78, ALB70, and ALB42 (Fig. 22).

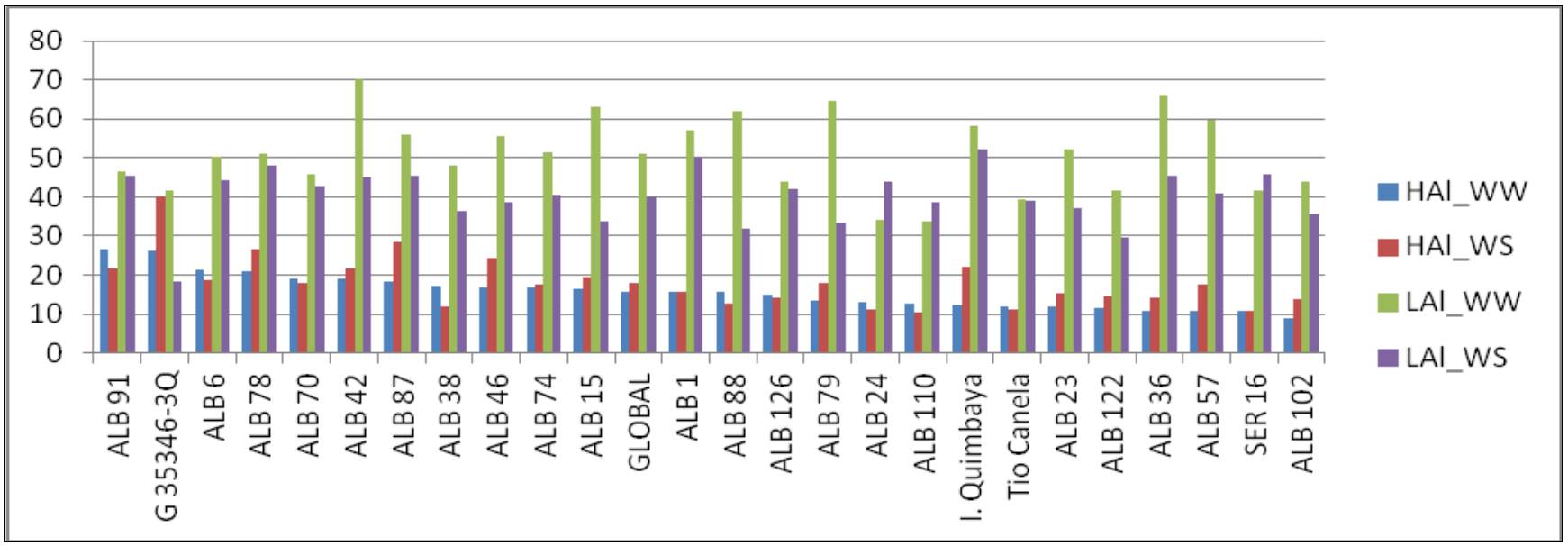

Figure 22: Differences in root length density of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75) under individual and combined stress of Al and drought in soil tube experiment

Table 21: Correlation between root and shoot characteristics density of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75) in combined stress of Al and drought in soil tube experiment

Traits	TRL	RSA	MRD	SRL	VRD34d	FRP	LA	SDW	R:S
TRL	1								
RSA	0.98***	1							
MRD	-0.22***	-0.09 (ns)	1						
SRL	0.64***	0.55***	-0.45***	1					
VRD34d	0.28***	0.27***	-0.32*	0.004 ns	1				
FRP	0.37***	0.24***	-0.82***	0.66***	0.12*	1			
LA	0.86***	0.87***	-0.04 (ns)	0.48***	0.15*	0.23***	1		
SDW	0.90***	0.91***	-0.07 (ns)	0.55***	0.09 (ns)	0.26***	0.90***	1	
R:S	-0.34***	-0.33***	-0.06 (ns)	-0.48***	36***	-0.17**	-0.47***	-0.63***	1

*, **, *** respectively Significant at 0.05, 0.01, and 0.001 probability level; and ns: no significant.

( ) Total root length (TRL), root surface area (RSA), mean root diameter (MRD), specific root length (SRL), visual root depth (VRD34d) of 34 days-old plants, fine root proportion (FRP), leaf area (LA), shoot dry biomass weight (SDW) and root:shoot ratio (R:S).

IV.3.2.2. Interaction between thin root and total root density

Roots are a common denominator of tolerance to several abiotic stress factors such as drought, poor soil fertility, and Al toxicity. Different classes of roots for the same genotype can exhibit differential elongation responses to rhizotoxic Al (Armando et al., 2000). The relationships between root parameters (T7.RGSC) including total root length (TRL) and mean root diameter (MRD), and total root length and specific root length (SRL) showed weak association between them (Fig. 23). TRL was markedly affected by combined stress of Al and drought with TRL ranged from 34.3 to 70.1 m, and 10.6 to 40.12 m, respectively for non-stressed tubes and for Al and drought combined stresses. SRL was also highly affected by these two abiotic stress, the mean was 93.25 m.g $^{-1}$ in control without stress and 68.48 m.g $^{-1}$ for combined stress of al and drought. Under low Al-toxic stress and well watered, TRL and MRD were not correlated, whereas a negative and highly significant correlation (-0.22***) was observed between these root traits under combined stress of Al and drought. There was a positive correlation between root hair length and density when plants grow under low phospholus availability (Miguel, 2004). Vieira et al. (2007) also found a significant correlation between root hairs in basal root and root hairs in primary root (r = 0.54, P = 0.0057).

Fiscus (1981) reported that two-thirds of the total area of *Phaseolus vulgaris* L. roots were in a root class with an average diameter of 0.2 mm while about one-fifth of total area was in roots averaging 0.5 mm. In our experiment, MRD ranged between 0.32 and 0.47 mm with average MRD of 0.36 mm in low Al saturation and well watered treatment. Under combined stress of Al and drought,

MRD did not increase and maintained an average to 0.34 mm. This could be the inheritance from the donor parent G35346-3Q that showed more thin roots under combined stresses of Al and drought than in total absence of stress. In general for RILs, drought alone was associated with thin roots, followed by combined stresses of Al and drought, and then the control (without stresses) while Al alone was associated to thick roots. An opposite behavior with the *coccineus* parent characterized by less TRL with higher MRD (0.47 mm) in absence of stress. G35346-3Q develop more thin roots under Al stress alone than without stresses or under water stress alone. Its roots were even more thin (mean of 0.41 mm) under combined stresses of Al and drought (T7.RGSC).

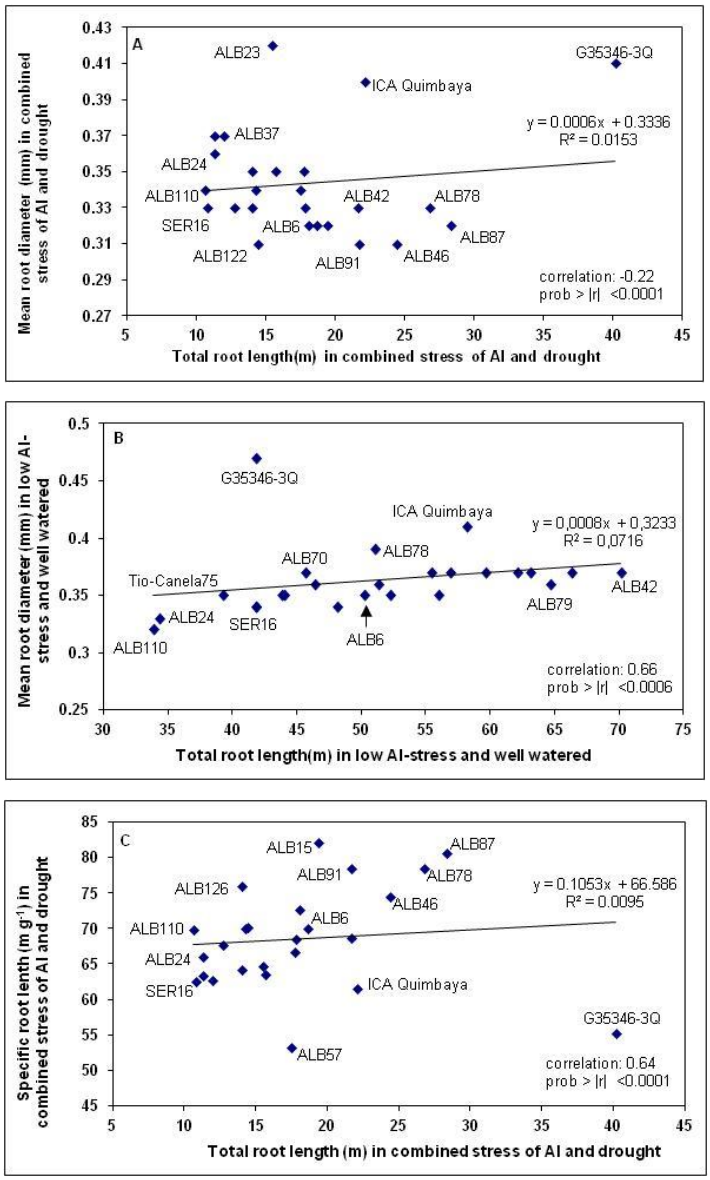

Figure 23: Relationship between total root length and mean root diameter/specific root length of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75) in combined stress of Aluminum and drought in a greenhouse soil tube experiment.

IV.3.2.3. Interaction between total root density and rooting depth

The capacity of plants to absorb both water and mineral nutrients from the soil depends on the development of deep and extensive root system. Genotypic variation in rooting depth has been reported in several legumes (Kaspar et al., 1984; While and Castillo, 1988). Deep-rooted genotypes tend to be more drought-tolerant. We found an unusual scenario where combined stress of Al and drought affected TRL less than Al-toxic acid soil alone (T7.RGSC). This may be explained by the greater energy the RILs invest to overcome drought stress through development of deeper and more extensive root systems, thus counteracting in part the effects of Al. Genotypes with total root length less affected by Al alone are ALB91, G35346-3Q, ALB6, ALB78, ALB70, and ALB42; while a different ranking was observed of genotypes less affected by the combined stress of Al and drought that included G35346-3Q (40.4 m, near double of TRL of the second RIL), ALB87 (28.32 m), ALB78, ALB46, ICA Quimbaya, ALB42, ALB15, etc. For visual root depth (VRD), the mean VRD was 51.57 cm for drought treatment, 46.65 cm for combined stress of Al and drought, and for Al alone 38.3 cm. In the control without stress, the mean of VRD was 34.49 cm, meaning that the plant didn't deploy its resistance mechanism of deep roots. Mean VRD ranged from 33.4 to 69.85 cm in combined stress of Al and drought, and 24.13 to 46.2 cm for non-stressed treatment. Strong relationship was found between VRD and TRL (R^2 = 0.68) under combined stress of Al and drought. Root allocation and distribution may depend on plant growth strategies and their general response to water deficits and distribution of available soil water (Comas et al., 2013).

The correlation between the two root traits was respectively $r = 0.28^{***}$ and $r = 0.54^{**}$ for combined stress of Al and drought (Fig.24), and for control treatment without stress. A general reduction in root xylem diameter can reduce total plant hydraulic conductance under well-watered conditions and limit plant maximum growth potential, therefore, when breeding these traits, programs have targeted their expression specifically in roots that function in water uptake primarily under dry conditions (Passioura, 1983). Selection of genotypes with more extensive and deeper root systems could extract more soil water and nutrients from subsoil and greater occupation of the soil volume.

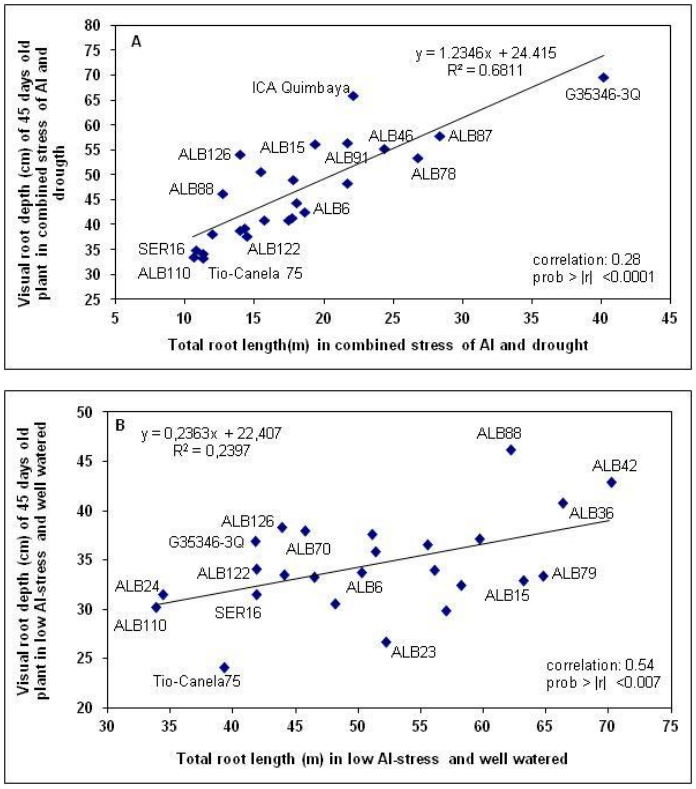

Figure 24: Relationship between visual root depth and total root length of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75) in a greenhouse soil tube experiment under combined stress of Aluminum and drought.

IV.2.3.2.4. Interaction between root and shoot

When moisture deficit develops slowly, crops tend to adjust their transpiring surface area through reducing leaf growth and enhancing senescence of older leaves to balance transpirational demand against reduced water uptake (Hsiao, 1982). We found results confirming that drought stress alone (T7.RGSC) reduce leaf area (LA) where the mean was 215.89 cm^2 compared to 328.88 cm^2 for the control without drought stress. Markhart (1985) concluded that leaf area was significantly reduced by water stress in both tepary and common beans. Morphologically, loss of leaf area is the most important drought response of common bean and can be the result of reduced number of leaves, reduced size of younger leaves, inhibited expansion of developing foliage, or leaf loss accentuated by senescence (Acosta-Galegos, 1988). Tepary produced more total dry matter and leaf area under both well-watered and water-stressed conditions (Markhart, 1985). Our results were similar with the coccineus parent G35346-3Q that maintained high LA under individual and combined stress of Al and drought (Fig. 25). Plants often maintain higher root length density than is required by the surface area of the shoot, mainly to minimize effects of other stress factors such as pests, and nutrient deficiency (Passoura, 1983).

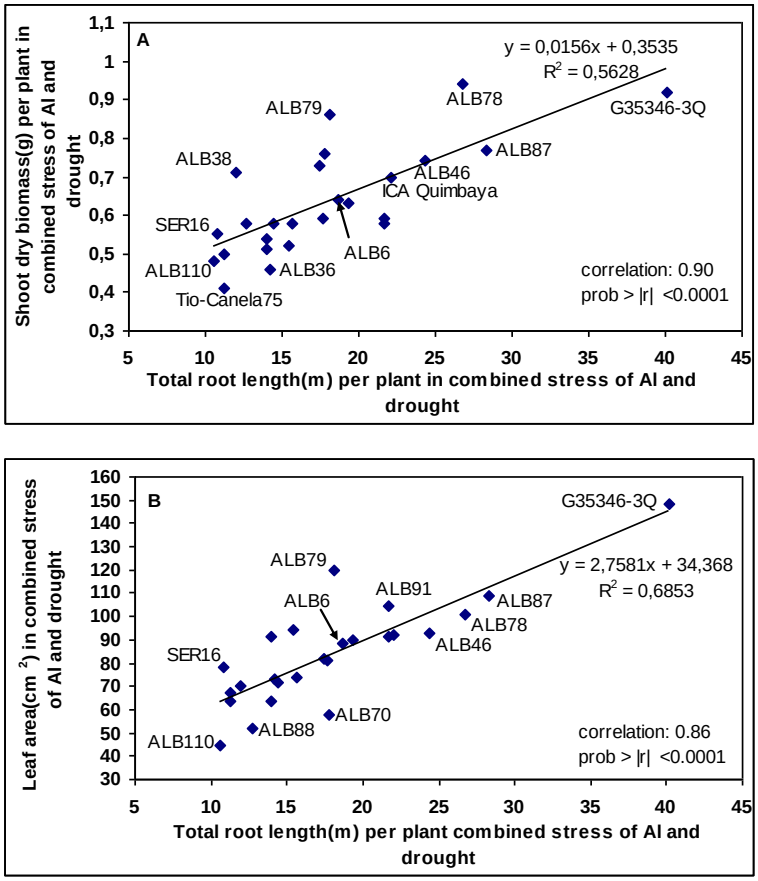

Figure 25: Relationship between total root length and shoot attributes (shoot biomass dry weight and leaf area surface) of 25 genotypes including 21 RILs, 2 parents (G35346-3Q and SER16) and 2 controls (ICA Quimbaya and Tio-Canela75) in combined stress of Aluminum and drought in a greenhouse soil tube experiment

Both direct and indirect effects can affect root and subsequent shoot development of the plant (Schenck, 1976). Under combined stress of Al and drought (T7.RGSC), we found highly significant correlation between total root length and two shoot traits (SDW, r = 0.90***; and LA, r = 0.86***). Amede et al. (1999) showed that in faba beans, drought sensitivity increases with increasing plant height and the correlation was very high (r = 0.93).

Combined stress of Al and drought affected LA and SDW more than each stress considered individually or the control without stress. The range of means for LA were 103.74-323.42 cm^2, 45.93-225.2 cm^2, and 44.35-148.23 cm^2; respectively for drought stress alone, Al alone, and combined stress of Al and drought. The same order in range was observed with shoot dry biomass weight (SDW) showing SDW more affected by combined stress than each stress considered individually. Mean SDW were 0.63g, 0.73g, 1.85g and 3.04g respectively for combined stress of Al and drought, Al-stress, drought, and the control without these two stresses. A plant with a large mass of leaves in relation to the root system is prone to drought stress because the leaves may lose water faster than the roots can supply it.

Summary statement:

Phenotypic evaluation of Interspecific Recombinant Inbred lines (RILs) of *Phaseolus* species for tolerance to individual and/or combined stress of Al and drought

Drought stress and Aluminum toxicity are two major abiotic constraints for bean production in regions prone to drought and toxic levels of subsoil Al. Two greenhouse studies were conducted with two different types of soil. 94 RILs were evaluated in soil tube using clay soil with sand. Individual and combined stresses were established with two levels of Al saturation in soil (HAl and LAl) and two levels of water regime (WW and WS) using an oxisol collected from Quilichao. Eleven genotypes including G35346-3Q, ICA Quimbaya, ALB28, ALB43, Tio Canela 75, ALB49, ALB122, ALB125, ALB118, ALB60, and ALB20 maintained high root surface area under drought stress. Transgressive segregations were observed for shoot traits both under water stress and well watered treatments. Total root length was highly correlated to RSA both in water stress ($r = 0.93^{***}$) and well watered ($r = 0.90^{***}$), highly and positively correlated to mean root diameter (MRD) and root depth at 34days, respectively $r = 0.46^{***}$ and 0.44^{***} in drought stress. ALB54, ALB4, ALB34, and ALB48 maintained an extensive root system both in drought and control. High root distribution into deep soil layers under water stress supported the role of roots as a drought avoidance mechanism among RILs. Under combined stress of Al and drought, a highly significant correlation was found between total root length and two shoot traits (SDW, r = 0.90***; and LA, r = 0.86***). ALB 91 emerged as superior genotype in root length density under combined stress of Al and drought, even more resistant than the donor parent (G35346-3Q), a *P.coccineus* accession. An unusual scenario was observed with combined stress of Al and drought affecting less TRL than Al-toxic acid soil alone. Al tolerance in common bean indirectly improves drought tolerance. Selection of genotypes with more extensive and deeper root system that extract more soil water and nutrients from subsoil, and greater occupation of the soil volume will enhance bean productivity under abiotic stresses.

IV. 4. Greenhouse screening for resistance to *Fusarium* root rot of interspecific Recombinant Inbred Lines (RILs) of *Phaseolus* species

Improvement in resistance to multiple stresses simultaneously should be a concern for the greater goal of improving seed yield and its sustainability in common beans by plant breeders. After characterizing RILs for abiotic stress resistance, our integrated strategy was also to evaluate their resistance to *Fusarium* root rot the severity of which has been shown to increase with soil stress factors. For resistance to the pathogen (T8.RGSFs), the two parents (G35346-3Q and SER16), 94 RILs and 7 control cultivars (VAX1, DOR390, G21212, G40159, G40159, Tio canela75, ICA Quimbaya, and ALB252) were evaluated for their reaction to *Fusarium solani* f. sp. *phaseoli*. Infested sorghum substrate mixed with soil:vermiculite mixture (2:1) was used for artificial inoculation.

IV.4.1. Segregation for *Fusarium* root rot resistance among RILs

All roots parameters (root dry biomass weight of inoculated and uninoculated plants (T8.RGSFs), Fusarium root rot scores of inoculated plants, and root growth inhibition) were highly significant for genotypes (Table 22). Coefficient of variation (CV) for *Fusarium* root rot scores of inoculated of bean genotypes was 12.87 % in our study whereby the CVs for root dry biomass weight (RDW) of uninoculated bean genotypes, root dry biomass weight inoculated bean genotypes, and root growth inhibition were respectively 5.96 %, 11.22 %,and 22.88 % (Table 22). Coefficients of variation for mean root rot scores from previous work on root rot evaluations conducted on commercial bean cultivars ranged from 14.5 to 24.3% (Schneider and Kelly, 2000); and on two RIL populations developed from FR266 and Montcalm, CVs remained relatively constant over experiments and ranged from 14.9 to 25.6% (Schneider et al., 2001).

Table 22: Mean squares of root dry biomass weight inoculated and uninoculated plants, disease score of inoculated plants, and reduction of root growth of inoculated plants compared to uninoculated control for 103 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 7 checks (VAX1, DOR390, G21212, G40159, G40159, Tio canela75, ICA Quimbaya, and ALB252)in a greenhouse

	Treatments			
Source	**RDW of inoculated plants**	**RDW of uninoculated control**	**Disease score of inoculated plants**	**RGI**
Genotypes	0.00066***	0.002***	5.96***	1455.06***
Rep	0.0000086 (ns)	0.0000003 (ns)	0.41 (ns)	10.22 (ns)
Error	0.0000224	0.000014	0.35	41.23
CV%	11.22	5.96	12.87	22.88

In general the disease severity is assessed in greenhouse between 10 and 31 days after planting (DAP) (Schneider and Kelly, 2000; Chaudhary et al., 2006; Bilgi et al., 2008). In our greenhouse study (T8.RGSFs) using a disease rating scale of 1-9 (Table 5), *Fusarium* root rot severity rates attributed to the103 bean genotypes ranged from 1.7 to 7, and the average was 4.57. The evaluation was done on 28 days old plants, and scores of the two parents were 3.33 and 6, respectively for G35346-3Q and SER16, suggesting transgressive segregation in two ways. 19 RILs were more resistant to *Fusarium* root rot compared to the resistant parent with disease scores ranging from 1.7 to 3. Root rot disease rating using severity of lesion on lower hypocotyls for common bean with LIM (liquid inoculum method) with the same disease scale (1-9) showed a range disease rating between 2.3 to 6.5, with general mean of 3.99, using the same *Fusarium* root rot (FRR) rating scale (1-9) (Chaudhary et al., 2006). In their experiment with the same disease reaction scale, Nicoli et al. (2012) found that FRR ratings varied from 5.2 to 6.7 at the R5 stage. On the other hand, black beans were among the most resistant genotypes with FRR ratings varying from 3.4 to 5.6 (Nicoli et al., 2012). Disease rating on a scale of 1 to 7, bean genotype FR266 scored from 2.0 to 4.5, whereas Montcalm scored from 1 to 2 points higher than FR266 in all experiments carried by Schneider et al. (2001). Among our RILs, the most resistant were ALB28, ALB40, ALB9, ALB42, ALB52, ALB90, ALB94, ALB123, and ALB125; and the most sensitive to *Fusarium solani* f. sp. *phaseoli* were ALB74, ALB70, ALB57, ALB45, ALB37, ALB36, ALB61 and ALB32.

Root dry biomass weight (RDW) of inoculated plants ranged from 0.0117 g (ALB5) to 0.118 g (G35346-3Q) whereby the susceptible parent SER16 showed RDW of 0.043 g, slightly higher than the average 0.042 g. Compared to RDW of uninoculated plants, the inhibition of root growth revealed high range (0-80.8 %). ALB45, ALB41, ALB126, ALB84, ICA Quimbaya, ALB49, ALB34, ALB88, and ALB85 did not showed inhibition of root growth due to *Fusarium* root rot. The inhibition of root growth for both parents was 1.88 and 19.4 % respectively for resistant parent G35346-3Q, and SER16 suggesting more negative transgressive segregation for the trait.

IV.4.2. Relationship between root mass of inoculated and uninoculated plants and *Fusarium* root rot score

Detecting genetic differences in root growth patterns and architecture between genotypes may offer unique selection criteria for tolerance to root diseases enhanced by drought, flooding, and stressful root zone temperatures (Leskovar and Stoffella, 1995). The relationships of root dry biomass

weight (RDW) of inoculated and uninoculated bean genotypes, and with inhibition of root growth induced by Fusarium root rot (T8.RGSFs) were very weak (Fig.26). In this study, the relation between root dry biomass weight of inoculated and uninoculated bean genotypes was positive (R^2 = 0.14), but negative (y = -315.65x + 64.073) between root dry biomass weight of inoculated bean genotypes and the inhibition of root growth by *Fusarium* root rot (T8.RGSFs). Pathogen infection acts to reduce root density by killing roots and may attenuate the functional efficiency of the remaining infected roots (Román-Avilés et al., 2003). Using root dry mass we detected genetic differences in root growth pattern both in inoculated and uninoculated soil treatments. More than 90 % of the bean genotypes showed reduction of root growth due to *Fusarium* root rot severity.

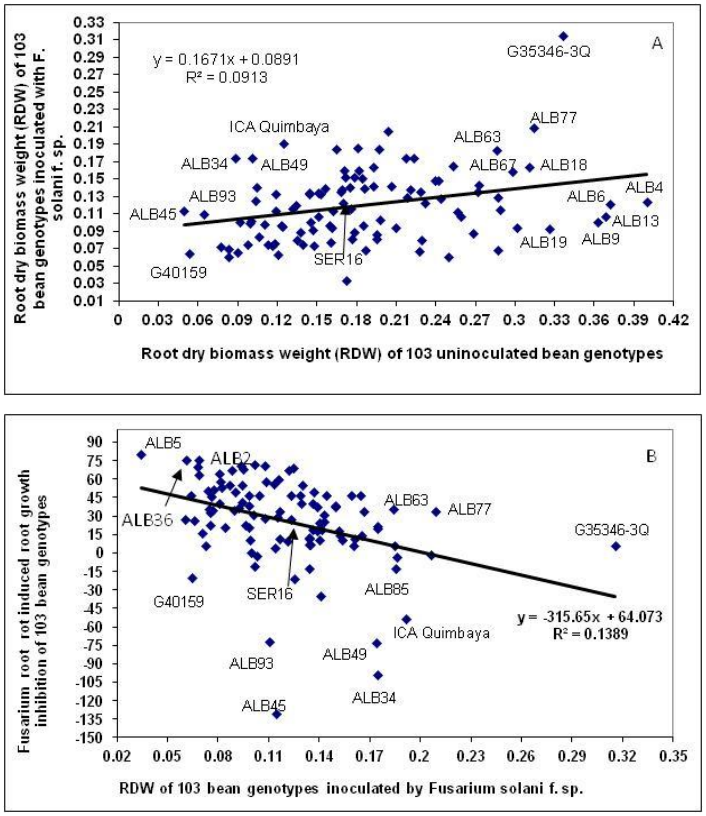

Figure 26: Relationship between root mass of inoculated and uninoculated plants; and *Fusarium* root rot score for 103 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 7 checks (VAX1, DOR390, G21212, G40159, G40159, Tio canela75, ICA Quimbaya and ALB252) in a greenhouse experiment.

Among bean genotypes that showed greater RDW both under inoculated and uninoculated soil conditions were G35346-3Q, ALB77, ALB67, ALB63, and G21212 (Fig.26a). Another group of bean genotypes was characterized by high RDW and less inhibition of root growth including G35346-3Q, ALB44, ALB85, and ALB88 (Fig.26b).

Integrated management strategies that combine vigorous rooting systems with bio-control seed treatment may be the most profitable and environmental appropriate approach to controlling root rot in bean (Estevez de Jensen et al., 2002).

IV.4.3. Relationship between *Fusarium* root rot scores and root dry biomass weight of inoculated plants; and inhibition of root growth

Adventitious roots are likely to persist under irrigation, and continue to function and contribute to the long-term development of root system in soils infested with soil borne pathogens (Johnson et al., 2000). The relationships between *Fusarium* root rot scores (T8.RGSFs) and both root dry biomass weight (RDW) and the inhibition of root growth by *Fusarium solani* were analysed in this study (Fig.27).

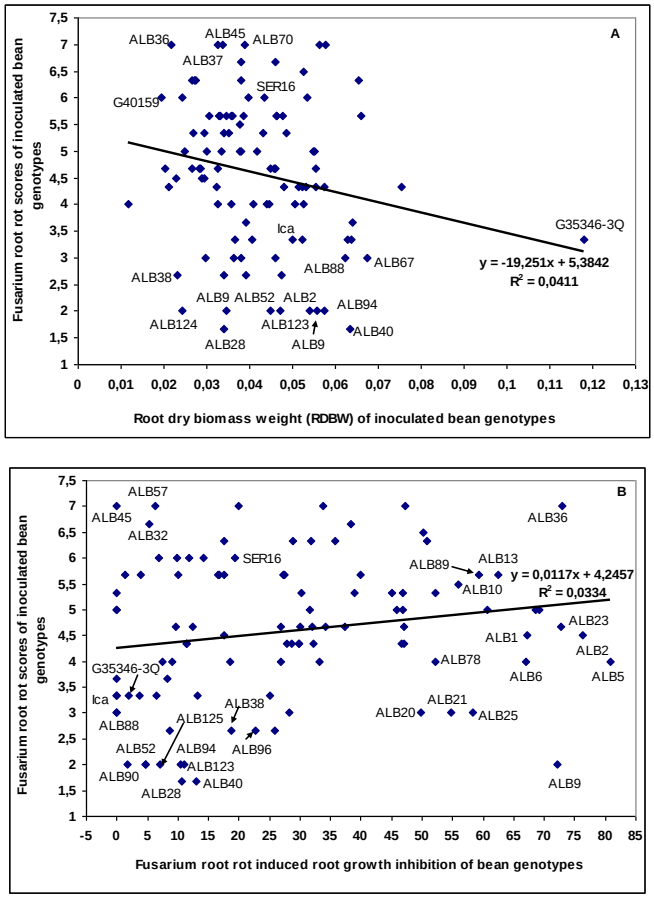

Figure 27: Relationship between *Fusarium* root rot scores and root dry biomass weight of inoculated plants; and root growth inhibition for 103 bean genotypes including 94 RILs, 2 parents (G35346-3Q and SER16), 7 checks (VAX1, DOR390, G21212, G40159, G40159, Tio canela75, ICA Quimbaya and ALB252) in a greenhouse experiment.

The relation between Fusarium root rot scores and RDW of inoculated bean genotypes (T8.RGSFs) was very weak (R^2 = 0.04) with linear regression y = -19.25x + 5.38 (Fig.27a). Similarly the relation between root scores and the inhibition of root growth was also very weak (R^2 = 0.03) with y = 0.0112x + 4.24 (Fig.27b). The highest score in our study was 7 for the most susceptible RILs (ALB36, ALB37, ALB45, ALB57, ALB70, and ALB74). The susceptible bean genotypes (Red Hawk, Montcalm, and C97407) had significantly higher scores too (4.1-6.5) (Román-Avilés and Kelly, 2005). Similar *Fusarium* root rot scores were observed in an evaluation of Montcalm x FR266 population where the susceptible parent FR266 scored up to 6.5 (Schneider et al., 2001). Román-Avilés and Kelly (2005) found that root vigor was significantly and positively correlated with root rot scores for the cranberry IBL population in MRF03 (r = 0.24; P < 0.001). The difference between RDW of inoculated resistant parent G35346-3Q and RDB of RILs was wide. RDW of the coccineus parent was 0.118 g whereby the highest RDB of hybrids was 0.075 g (ALB77). *Fusarium* root rot induced root growth inhibition was high as 80.8 % for the susceptible RIL, ALB5. The widespread nature and importance of *F. solani* as the predominant root rot pathogen in common bean emphasizes the need for effective control through the development of resistance cultivars (Boomstra and Bliss, 1977; Schneider et al., 2001; Chowdbury et al., 2002; Navarro et al., 2003). Resistant RILs identified from this study will be further evaluated for their resistance to other bean root rot diseases both in grenhouse and field screening.

Summary statement:

Greenhouse screening for resistance to *Fusarium* root rot of Interspecific Recombinant Inbred Lines (RILs) of *Phaseolus* species

Fusarium root rot (*Fusarium solani* f.sp. *phaseoli*) is one of the most important soil born disease affecting common bean worldwide. Recombinant Inbred lines (RILs) were developed from a backcross with the recurrent parent SER16: SER16 x (SER16 x G35346-3Q). 103 bean genotypes including 94 F5:6 RILs, the two parents, and seven control checks [VAX 1, Tio-Canela 75, DOR 390, ALB252, G 21212, ICA Quimbaya, and one *Phaseolus acutifolius* accession (G 40159)] were assessed for the introgression of *Fusarium* root rot resistance using isolates collected from Rwanda. A mixture of three virulent isolates of *F. solani* f. sp. *phaseoli* was used to produce inoculum on sorghum substrate for the study. Resistance to *Fusarium* root rot was assessed on 28 days old plants. Segregation for *Fusarium* root rot resistance among RILs, relationship between root parameters (root dry biomass, *Fusarium* root rot scores, and root grow inhibition both for inoculated and uninoculated plants) were determined. *Fusarium* root rot resistance as indicated by disease reaction score and root grow inhibition were successful transferred to progenies. Genotype x Treatment was highly significant ($P < 0.001$). Very weak relationship was found between *Fusarium* root rot scores and root dry biomass weight. Disease severity ranged from 1.7 to 7, with an average of 4.57. Disease severity was 3.33, and 6, respectively for G35346-3Q and SER16; suggesting the presence of a two way transgressive segregation. 19 RILs were more resistant than the *P. coccineus* parent (G35346-3Q). Our findings are consistent with the hypothesis that interspecific improvement could enhanced *Fusarium* root rot resistance.

IV.5. Evaluation of Phenology and Yield Components of interspecific Recombinant Inbred Lines (RILs) on soils presenting abiotic stresses

Many of the traits that explain plant adaptation to drought such as phenology, root size and depth, hydraulic conductivity and the storage of reserves are associated with plant development and structure and are constitutive rather than stress induced (Chaves et al., 2003). Different root systems will be required for different soil environments: shallow roots to maximize P acquisition in a P-poor soil (Ho et al., 2005; Nord and Lynch, 2009); deep roots for water acquisition under drought; greater number of tips for calcium absorption; exudation of organic acids as a defense mechanism against aluminum toxicity or as a mechanism of P acquisition (Beebe et al., 2009). Aluminum resistance could be a key strategy in improving drought tolerance particularly in species such as common bean that appear to use for better performance under stresses, shallow root system in low phosphorus soils and deep roots in terminal drought (Ho et al. 2005). We have identified physiological traits to be used as criteria of selection in greenhouse screening for biotic and abiotic stresses experiments. Field studies where bean crops are grown in suboptimal environmental conditions with presence of these environmental stresses interacting will enhance our understanding toward developing high adaptability and stability in grain yields of new resistant bean genotypes.

At Palmira (T9.RFSD), 100 genotypes including 96 RILs, *P. vulgaris* drought tolerant parent SER16 (*P. coccineus* parent, "G35346-3Q" was not included in field trials due the fact that *P. vulgaris* and *P.coccineus* are closely related and isolating mechanisms between them are not completely effective in preventing gene flow. *P. coccineus* showed high degree of allogamy, and might led to hybridization *P. coccineus*♀ x *P. vulgaris*♂), and 3 control plants (VAX 1, Tio-Canela 75, and ALB252) were evaluated in drought-stress and non-stressed environments (Table 23). The same number of genotypes was evaluated for tolerance to Al-toxic acid soil in Quilichao (Table 23). The objective of these field experiments was to evaluate how the introgression of root characteristics from *P. coccineus* (root depth and vigorous root growth) to *P. vulgaris* has contributed to improve yield under drought and/or Al-toxic acid soil environments.

Grain yield is a converted function of biomass accumulation, which is linearly related to cumulative transpiration (Tanner and Sinclair, 1983). Our results revealed that drought stress reduced seed yield by 40.8% and 100-seed weight by 11.1% (i.e. 3 g) (Table 23). For Terán and Singh (2002a), drought stress reduced seed yield by an average of 60%. They found also that drought stress reduced 100-seed weight by 13% and days to maturity by 3%**.** Castellanos et al.

(1996) and Foster et al. (1995) also reported yield reductions between 41 and 95% due to drought stress. Drought stress in other research showed that mean 100-seed weight was reduced by 5 g (i.e. 14%) (Muñoz-Perea et al., 2006; Singh, 1995). The reduction in 100-seed weight due to drought stress reached 22% for cultivar Bill Z (Singh, 2007). All theses conclusions are supporting our findings. Seed yield of all genotypes in drought-stressed was significantly lower than in non-stressed environment.

Under terminal drought stress, number of days to maturity is often shortened (Terán and Singh, 2002a, 2002b). We found different results where days to maturity increased in drought-stressed field by 4 d compared to non-stressed field (Table 23). The discrepancy could be due to RILs under evaluation, derived from interspecific cross with coccineus (a species not likely to be drought tolerant) could well respond differently than standard common bean lines. Sporadic rain that occurred at grain filling stage could have influenced plant recovery and delay in pod setting and filling. Muñoz-Perea et al. (2006) arrived at a similar conclusion when they showed that number of days to maturity was delayed in drought stress (DS) compared to non-stress (NS) in 2003. In contrast to our findings and in a normal scenario the mean days to maturity was reduced by 4 d in DS compared with NS with mean reduction in seed yield due to drought stress ranging from 32% at Kimberly to 88% at Parma in 2001; i.e. 90 d for Common Pinto landrace to 96 d for great northern cultivar UI 425 in non-stressed (Singh, 2007). Early maturity may help to escape terminal drought while late maturity, especially in determinate cultivars, may facilitate partial recuperation from a mild drought stress during flowering (Nleya et al., 2001).

The range of days to flower was 32-42 d (data not shown) showing possibility to later flowering genotypes to escape early seasonal drought if water stress is followed by rains.

Table 23: Seed color, size, days to maturity, 100-seed weight, seed yield, and mean yield for 100 genotypes including 96 recombinant inbred line (RIL) population of common bean from SER16 x (SER16 x G35346-3Q), one parent (SER16), and 3 checks (VAX1, Tio canela 75, ALB252)

No	Genotypes	Seed color	Seed size	Water-stressed (WS) in Palmira			Well watered (WW) in Palmira			DSI	High Al saturation in Quilichao			Mean seed yield
				Days to maturity	100-seed weight	Seed yield	Days to maturity	100-seed weight	Seed yield		Days to maturity	100-seed weight	Seed yield	
1	ALB 6	Red	M	78	28	2513	71	32	3607	0.7	64	27	907	2253
2	ALB 91	Red	M	76	28	2074	69	33	3267	0.9	64	26	735	2088
3	SER 16	Red	S	67	26	2364	65	28	3339	0.7	58	21	647	2028
4	ALB 58	Red	S	67	26	2134	65	28	3191	0.8	59	23	727	1924
5	ALB 60	Red	S	64	23	1749	66	28	3539	1.2	58	23	791	1921
6	ALB 4	Red	S	77	27	2135	69	31	3046	0.7	63	24	674	1899
7	ALB 36	Red	S	77	23	2384	68	24	3453	0.8	64	21	753	1898
8	ALB 27	Red	M	69	23	1641	67	30	3313	1.2	62	22	647	1879
9	ALB 88	Red	M	75	28	1997	68	33	3229	0.9	64	27	679	1871
10	ALB 8	Red opaque	M	67	25	1855	65	29	3203	1.0	61	23	565	1859
11	ALB 63	Red	M	67	26	2120	66	28	3177	0.8	60	22	599	1850
12	ALB 15	Pink striped	M	72	25	1815	67	27	3101	1.0	62	24	757	1818
13	VAX 1	Pink striped	M	69	20	1383	68	24	3705	1.5	67	21	649	1790
14	ALB 34	Red	M	68	25	1951	68	27	3113	0.9	60	21	652	1767
15	ALB 95	Red	S	67	21	1564	69	25	3162	1.2	63	17	582	1764
16	ALB 42	Red	S	68	24	1901	67	26	2927	0.9	61	25	643	1758
17	ALB 9	Black	M	73	23	1757	68	26	2790	0.9	63	25	910	1738
18	ALB 38	Red	M	77	26	1791	70	30	2948	1.0	64	27	989	1734
19	ALB 61	Red	S	71	21	1384	66	25	2923	1.3	63	20	685	1732
20	ALB 90	Red	M	73	27	1648	69	32	3016	1.1	64	25	659	1714
21	ALB 74	Red	M	69	29	1842	66	31	2743	0.8	62	25	630	1711
22	ALB 7	Black	M	67	28	1932	66	32	2803	0.8	62	25	824	1709
23	TIO CANELA 75	Red	S	72	21	2101	68	24	2721	0.6	63	19	660	1701
24	ALB 86	Red	S	73	26	1584	68	31	2691	1.0	63	26	569	1691
25	ALB 56	Red	S	68	25	1832	67	28	2945	0.9	62	24	617	1687

26	ALB 102	Pink	S	69	21	1683	68	27	2732	0.9	61	17	622	1679
27	ALB 89	Light pink	M	73	23	1733	68	29	3177	1.1	67	23	535	1673
28	ALB 46	Black	S	72	24	1773	67	27	2989	1.0	63	21	563	1670
29	ALB 117	Cream striped	M	66	22	1438	66	27	3183	1.3	61	26	612	1668
30	ALB 78	Red	L	77	30	1943	69	33	2919	0.8	61	25	705	1663
31	ALB 31	Red	M	71	22	1483	70	26	3052	1.3	64	19	443	1663
32	ALB 101	Red	S	67	27	1797	66	28	2429	0.6	59	22	568	1660
33	ALB 108	Red	S	71	25	2008	67	28	2552	0.5	63	22	514	1634
34	ALB 59	Red	S	66	22	1743	66	25	2730	0.9	59	22	583	1617
35	ALB 252	Red,Cream,Black	_	74	25	1822	69	28	2792	0.8	65	24	647	1601
36	ALB 24	Red	S	74	25	1824	69	27	3042	1.0	63	22	587	1594
37	ALB 122	Red, Black	S	66	26	1536	65	27	2785	1.1	60	22	589	1589
38	ALB 109	Red	S	74	23	1542	68	28	2931	1.2	63	24	638	1585
39	ALB 57	Red	M	66	26	1730	66	30	2640	0.8	62	24	613	1555
40	ALB 84	Red	M	68	24	1654	66	27	2936	1.1	62	23	544	1546
41	ALB 110	Red	S	69	24	1710	66	24	2524	0.8	63	21	493	1543
42	ALB 10	Red	M	70	26	1459	67	26	2821	1.2	63	23	637	1534
43	ALB 121	Brown	S	73	21	1485	67	25	2645	1.1	66	18	581	1533
44	ALB 26	Pink striped	S	75	19	1581	69	23	2706	1.0	65	22	666	1509
45	ALB 25	Red	S	70	23	1339	67	26	2493	1.1	63	23	484	1492
46	ALB 87	Red	M	67	27	1615	66	31	2475	0.8	62	24	550	1491
47	ALB 80	Red	M	77	30	1738	69	37	2430	0.7	63	27	390	1489
48	ALB 113	Pink speckled	M	66	26	1506	64	26	2471	1.0	58	23	586	1481
49	ALB 77	Black	M	74	23	1493	69	26	2285	0.8	63	22	723	1479
50	ALB 76	Black	M	71	23	1374	66	24	1956	0.7	64	24	862	1467
51	ALB 44	Red	M	76	28	1623	69	29	2708	1.0	62	24	681	1459
52	ALB 111	Red	M	66	22	1575	65	29	1957	0.5	61	24	720	1447
53	ALB 79	Cream striped	M	69	23	1523	68	28	2754	1.1	63	25	666	1442
54	ALB 85	Red	M	67	23	1148	66	29	2920	1.5	62	23	572	1437
55	ALB 13	Cream striped	M	70	20	1558	65	22	2584	1.0	64	21	405	1424
56	ALB 1	Cream striped	M	73	25	1616	69	26	2209	0.7	63	23	633	1416
57	ALB 23	Red	S	75	20	1354	66	25	2526	1.1	66	22	720	1413

58	ALB	92	Red	M	78	28	1319	70	32	2358	1.1	64	24	592	1401
59	ALB	22	Red	S	75	19	1314	72	25	2508	1.2	66	20	564	1397
60	ALB	103	Red	S	74	24	1685	69	27	2387	0.7	63	21	461	1388
61	ALB	126	Red	S	66	19	1533	65	23	2331	0.8	60	18	709	1386
62	ALB	55	Red	S	68	25	1392	66	27	2306	1.0	63	22	499	1377
63	ALB	64	Red	S	75	26	1293	69	32	2448	1.2	63	25	559	1371
64	ALB	104	Red	S	75	24	1341	66	25	2565	1.2	65	22	434	1367
65	ALB	21	Red	S	77	23	1499	68	26	2531	1.0	65	23	565	1343
66	ALB	112	Red speckled	M	72	25	1534	69	30	2471	0.9	63	23	392	1322
67	ALB	35	Red	M	80	26	1394	81	29	2036	0.8	68	28	762	1318
68	ALB	40	Cream striped	M	81	27	1616	74	27	2099	0.6	67	26	542	1311
69	ALB	2	Red	M	80	29	1789	71	29	1992	0.2	64	27	582	1274
70	ALB	54	Red	M	69	26	996	67	28	2423	1.4	62	24	540	1269
71	ALB	16	Black	M	79	34	1328	76	37	1968	0.8	65	29	767	1268
72	ALB	3	Red	M	76	31	1213	67	30	2103	1.0	63	27	388	1247
73	ALB	17	Red	M	77	24	1551	70	27	2229	0.7	67	26	343	1247
74	ALB	28	Red	S	77	19	972	74	26	2298	1.4	69	21	651	1208
75	ALB	94	Cream striped	M	66	21	1268	66	25	2030	0.9	59	23	646	1197
76	ALB	96	Red	M	70	21	1213	70	24	2199	1.1	64	20	514	1193
77	ALB	18	Red	S	75	23	1237	68	24	2443	1.2	67	21	371	1191
78	ALB	125	Red	S	73	21	1002	66	25	2270	1.4	64	22	553	1191
79	ALB	105	Pink striped	S	79	20	1141	71	23	2036	1.1	68	21	524	1181
80	ALB	71	Red	S	66	20	972	66	27	2325	1.4	61	21	514	1159
81	ALB	106	Pink	M	68	22	999	65	26	1981	1.2	63	20	550	1109
82	ALB	48	Red	M	75	28	1033	67	31	2138	1.3	64	26	370	1100
83	ALB	70	Black	M	75	27	1096	67	32	2007	1.1	67	29	485	1096
84	ALB	69	Black	M	72	25	887	66	26	2013	1.4	62	23	537	1083
85	ALB	127	Red	M	78	20	1076	72	24	2129	1.2	71	19	331	1080
86	ALB	124	Red	M	77	20	746	71	28	2267	1.6	66	23	375	1068
87	ALB	119	Red	M	79	20	1454	78	23	1978	0.6	74	22	491	1066
88	ALB	5	Red	M	75	28	885	66	26	1565	1.1	63	24	509	1021
89	ALB	99	Red	M	80	28	1082	69	29	1510	0.7	64	27	461	1009

90	ALB 50	Red	S	68	20	798	65	26	1923	1.4	63	22	525	1002
91	ALB 120	Red	M	72	26	850	65	31	2012	1.4	64	25	564	980
92	ALB 20	Red	S	73	17	1096	67	19	1454	0.6	67	19	613	943
93	ALB 37	Red	S	80	21	1311	73	25	1695	0.6	73	23	410	931
94	ALB 123	Red	S	80	23	1050	71	25	1325	0.5	65	22	434	927
95	ALB 45	Purple speckled	S	72	25	752	67	27	1689	1.4	64	25	524	920
96	ALB 30	Red	S	80	15	1237	79	17	1272	0.1	74	18	343	836
97	ALB 67	Black	M	70	22	558	69	32	2371	1.9	67	26	200	824
98	ALB 41	Red	S	77	17	926	78	19	1324	0.7	67	22	504	790
99	ALB 49	Red	M	78	26	399	66	30	1462	1.8	70	29	148	695
100	ALB 19	Pink speckled	S	78	19	898	84	21	1232	0.7	74	21	263	643
	Mean			72	24	1486	68	27	2510		64	23	569	1185
	CV (%)			4	6	20	69	4	12		2	4	32	20
	LDS (5%)			4.14	2.33	490.1	3.17	1.73	473.5		1.9	1.39	251	381

IV.5.1. Evaluation of RILs for drought resistance in Palmira (Colombia)

Drought is the most severe abiotic stress reducing bean yield in a rainfed drought prone environment. Ramirez-Vallejo and Kelly (1998) concluded that the most effective approach to breed beans for resistance to drought would be based first on selection for high geometric mean seed yields followed by selection for low Fischer Maurer drought susceptibility index values. Understanding the relationships between target agronomic traits and grain yield (GY) responses to drought constitute a priority in developing high yield genotypes under different water conditions (Li et al. 2011). Table 24 (T9.RFSD) showed that there were significant differences among genotypes for days to maturity, 100-seed weight, and seed yield both for drought-stress and non-stressed yield. For Rao et al. (2013), the analysis of grain yield (YLD) showed significant differences (0.01) for all source of variance except the year x environment (Y x E) interaction. Traits including grain yield (YLD), 100 seed weight (SW), days to physiological maturity (DPM) showed significant differences for G x E, G x Y, and G x E x Y interactions (Rao et al., 2013). In Karama, Rwanda in 2011, significant differences were observed for genotype x water interactions (G x E) for most variables except number of days to flower and harvest index (Mukeshimana et al. 2014).

Table 24: Mean squares of days to maturity, 100-seed weight, and seed yield of 100 bean genotypes including 96 RILs from F5:6 SER16 x (SER16 x G35346-3Q) evaluated under water-stressed (WS) and non-stressed (NS) environments at CIAT-Palmira, Colombia in 2008.

Source of Variance	Df	Non-stressed field			Drought-stressed field		
		Days to maturity	100-seed weight	Seed yield	Days to maturity	100-seed weight	Seed yield
Replications	2	7.86	7	932665.5	181.76	50.52	4122808.2
Treatments							
- Undjusted	99	37.71***	34.26***	900288.5***	61.63***	33.02***	490838.6***
- Adjusted	99	_	34.19***	916542.9***	59.53***	33.54***	491132.6***
Blocks within Reps (adj.)	27	2.53	2.08	320259.3	18.4	4.43	200109.2
Error							
- Effective	171	_	1.16	86317.8	6.61	2.1	92467.1
- RCB							
Design	198	3.88	1.22	111254.9	7.74	2.29	101373.8
- Intrablock	171	4.1	1.09	78254.2	6.06	1.95	85784

*** Significant at the 0.001 probability level

Bean plants exposed to water stress for days or weeks may develop long term physiological strategies such as altering the leaf area, and modifying root to shoot ratio. Drought reduces biomass

and seed yield, harvest index, number of pods and seeds, seed weight, and days to maturity (Abebe and Brick, 2003; Muñoz-Perea et al., 2006; Padilla-Ramírez et al., 2005; Ramirez-Vallejo and Kelly, 1998; Singh, 2007). In our field experiments, genotypes were evaluated across water regimes using drought susceptibility index (DSI) and DII (drought intensity index) calculated for the two experiments. DII based on seed yield of all genotypes in non-stressed and drought-stressed fields was low (0.41). DSI ranged from 0.1 to 1.8, whereby mean DSI was 0.98 (all genotypes), Tio Canela 75 (0.6), SER16 (0.7), and VAX1 (1.5). Ramirez-Vallejo and Kelly (1998) observed genotypic variation for DSI in two years, finding that it ranged from 0.46 to 1.24 with a moderate level of drought stress (DII = 0.63). Limitations in the use of the DSI have been reported in common bean (Schneider et al., 1997; White and Singh, 1991). DSI does not differentiate between potentially drought-resistant genotypes and those genotypes with low yield potential due to other causes (Ramirez-Vallejo and Kelly (1998).

A positive association was found between seed weight under non-stressed and drought-stressed treatments in two years (Muñoz-Perea et al., 2006). In Palmira, we found that seed yield, 100-Seed weight, days to maturity, and days to flower from a drought-stressed field were highly correlated ($P < 0.001$) to the same variables in the non-stressed field, respectively $r = 0.73^{***}$, $r = 0.78^{***}$, $r = 0.76^{***}$ and $r = 0.84^{***}$(Table 25).

Table 25: Simple correlation coefficients between seed yield and yield components of 100 bean genotypes including 96 RILs from F5:6 SER16 x (SER16 x G35346-3Q) evaluated under water-stressed (WS) and non-stressed (NS) environments at CIAT-Palmira, Colombia in 2008.

Yield and yield components	Seed yield		100-Seed weight		Days to maturity		Days to flower	
	NS	WS	NS	WS	NS	WS	NS	WS
Seed yield NS	1							
Seed yield WS	0.73***	1						
100-Seed weight NS	0.25**	0.28**	1					
100-Seed weight WS	0.16 (ns)	0.36***	0.78***	1				
Days to maturity NS	-0.16 (ns)	-0.07 (ns)	-0.02 (ns)	-0.07 (ns)	1			
Days to maturity WS	-0.18 (ns)	-0.21*	0.01 (ns)	0.10 (ns)	0.76***	1		
Days to flower NS	-0.36***	-0.31**	-0.23*	-0.29**	0.69***	0.71***	1	
Days to flower WS	-0.32**	-0.30**	-0.13 (ns)	-0.18 (ns)	0.65***	0.76***	0.84***	1

*, **, *** represent significant at 0.05, 0.01, and 0.001 probability level respectively; and ns: no significant.

Seed yields under drought stress conditions were associated to 100-seed weight both in non-stressed field ($r = 0.28^{**}$) and drought-stressed field ($r = 0.36^{***}$). 100-Seed weight in WS was also negatively correlated to days to flower in NS ($r = -0.29^{**}$). DTF in WS was highly correlated to

DTF in NS (r = 0.84***)(Table 25). Terán and Singh (2002a) found that the mean of 100-seed weight of 81 bean genotypes in the drought-stressed environment was slightly lower than in the non-stressed environments. There was a moderate correlation between phenology traits and seed yield in Karama, Rwanda under DS while the correlation coefficients between phenology variables and seed yield were negatives in Palmira. Strong positive correlations were observed between yields and yield per day regardless of water treatment (Mukeshimana et al. 2014).

IV.5.1.1. Grain yield and yield components

IV.5.1.1.1.. Relationship between biomass yield and seed yield

Yield under water-limited conditions can be determined by the genetic factors controlling yield potential, and/or drought resistance, and/or WUE (Blum, 2005). Field evaluation of 100 bean genotypes including 96 RILs (T9.RFSD) has been used to analyze the association between seed filling and biomass production in pod biomass. In this study, many high yielding genotypes showed a high ratio between seed biomass weight per pod biomass weight. Pod harvest index (PHI) under drought-stressed conditions ranged from 61.2% (ALB67) to 83.2% (ALB48) with a mean of PHI of 76.24%. PHI was high in the non-stressed field with a range of 71.5% (ALB67) to 85.9% (ALB6). Genetically for some genotypes such as ALB67, ALB49, and ALB120 biomass translocation to seed is low both under drought-stressed and non-stressed conditions. On the other hand, genotypes like ALB6 and SER16 have high translocation to seed biomass from pod wall biomass.

Slim and Saxena (1993) found that higher grain yield in legumes was positively correlated with higher plant biomass but negatively with drought resistance. In our study (T9.RFSD), PHI in the drought-stressed treatment in Palmira was highly correlated with both seed yield in the non-stressed treatment (r = 0.38***)(Fig.28a) and in the drought-stressed treatment (r = 0.49***)(Fig.28b). Muñoz-Perea et al. (2006) found that biomass yield under drought-stress was positively correlated to harvest index and seed weight under drought stress in 2003, but negatively correlated with harvest index under non-stressed and drought-stressed in 2004.

Biomass yield under drought-stressed was positively correlated to seed yield under non-stressed and drought-stressed (Muñoz-Perea et al., 2006). Single stressed yield (Yd) and non stressed yield (Yp) were both highly correlated with biomass. Correlation coefficients ranged from 0.41*** (Sierra/Lef-2RB) to 0.65*** (Sierra/AC1028) between Yd and biomass under non-stress, whereas correlation coefficients between Yd and Yp were 0.52*** and 0.80*** for Sierra/Lef-2RB and

Sierra/AC1028, respectively (Schneider et al., 1997). Scully et al. (1991) found that harvest index had the lowest correlations with seed yield.

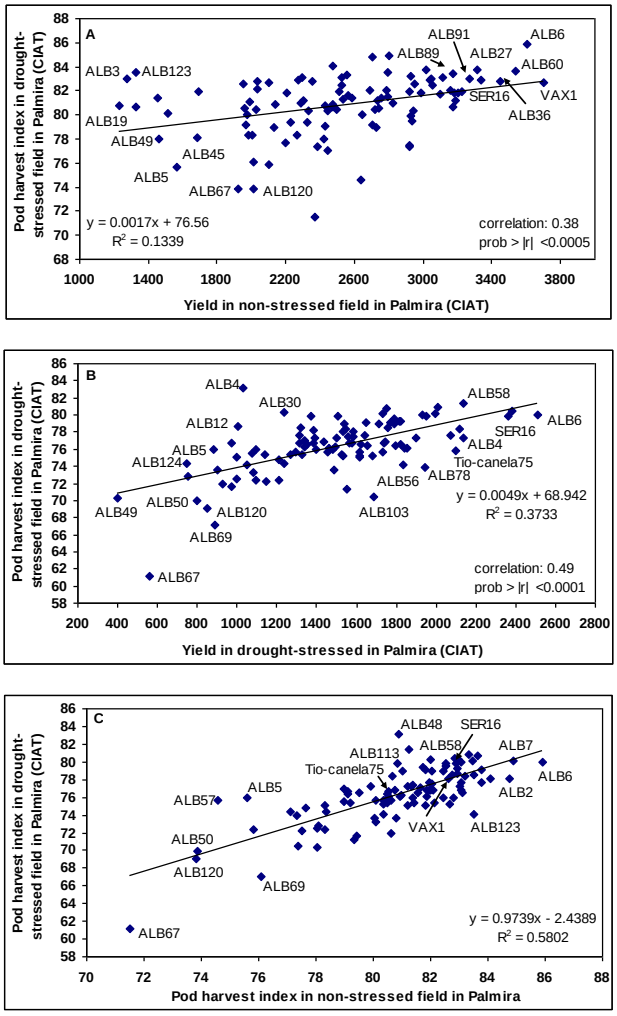

Figure 28: Relationship between seed yield in drought-stressed and non-stressed field and pod harvest index in drought-stressed field, and days to maturity in drought-stressed and non-stressed field of 100 bean genotypes including 96 RILs, 1 parents (SER 16), 3 checks (VAX 1, Tio-Canela 75, and ALB 252) in field in Palmira.

In our study (T9.RFSD) we found also a high relationship between pod harvest index (PHI) under drought-stressed treatment and PHI under non-stressed treatment with $R^2 = 0.58$ (Fig.28c). Muñoz-Perea et al. (2006) found that harvest index under non-stressed was positively correlated to harvest index under drought-stressed.

IV.5.1.1.2.. Relationship between phenology and seed yield

Among various morphological, physiological, phenological, yield, and yield-related traits to identify drought-resistant genotypes, mean seed yield (arithmetic and geometric) of drought-stressed and non-stressed environments was found to be the most effective selection criterion (White et al., 1994a; Abebe et al., 1998; Ramirez-Vallejo and Kelly, 1998; Terán and Singh, 2002b). Drought stress in our trials (T9.RFSD) strongly reduced seed yield. Yield under non-stressed field ranged from 1232.46 kg ha^{-1} (ALB19) to 3705.3 kg ha^{-1} (VAX1) with the mean of seed yield of 2510.05 kg ha^{-1}. Some RILS performed even better than the *P. vulgaris* parent SER16 (3338.8 kg ha^{-1}): ALB36 (3452.8 kg ha^{-1}), ALB60 (3538.67 kg ha^{-1}), and ALB6 (3606.7 kg ha^{-1}).
The effect of drought stress on seed yield was high as shown by the range of the seed yield decreasing from 2512.8 to 399.04 kg ha^{-1} with mean seed yield of 1485.9 kg ha^{-1}. Comparing the RILs to controls (SER16, 2364.2 kg ha^{-1} and Tio Canela75, 2100.6 kg ha^{-1}) for drought resistance, two elites genotypes were outstanding under stress: (in kg ha^{-1}) ALB6 (2512.8) and ALB36 (2384.1). Among other prominent drought resistant RILs were ALB4 (2134.8), ALB58 (2134.23), ALB63 (2119.6), ALB91 (2073.6), and ALB108 (2007.5).
When the mean of seed yield in drought and non-stressed treatments in Palmira were compared, we found a strong relationship, although the relationship between yield and days to maturity in the drought-stressed field was very weak. Some RILS (among them ALB6, ALB36, ALB63, ALB58, and ALB4), and SER16 and Tio-canela 75) yielded well in both drought-stressed and non-stressed conditions in Palmira. Many RILs were intermediate in yield in the two environments, however, RILs such as ALB49 yielded poorly in both drought-stressed and non-stressed field.
Yield in these two treatments in our study were positively and highly correlated ($r = 0.73^{***}$) (Fig. 29a). A negative but non-significant correlation was found between seed yield and days to maturity in the drought-stressed field. In their research, Muñoz-Perea et al. (2006) found that seed yield in non-stressed was positively correlated to seed yield under drought-stressed.They found also that seed yield under drought-stress was negatively correlated to harvest index under the same conditions.
Drought escape through early flowering and/or short growth duration is advantageous in environments with terminal drought stress and where physical or chemical barriers inhibit root growth (Blum, 1988; Turner, 1986; Blum et al., 1989). In our study (T9.RFSD), under terminal drought stress, number of days to maturity was delayed for 4d compared to DTM in non-stressed field, with 73 d under drought-stressed field and 69 d under non-stressed field. However, DTM

ranged from 64-81 d, and 65-85 d, respectively for drought-stressed and non-stressed treatments. Terán and Singh (2002) found that mean days to maturity were reduced by 4 d in drought-stressed compared with non-stressed. In 2003, all genotypes except UI 259 took longer to mature in DS than NS environment (Muñoz-Perea et al. (2006). SER16, ALB58, and ALB63 were the best genotypes characterized by a high yield and early maturity.

We found also negative correlations between phenology (DTF, DTM) and seed yield both in WS and NS; correlations were significant only between seed yield and DTM in WS ($r = -0.21^{*}$), and DTF both in WS ($r = -0.30^{**}$) and NS ($r = -0.31^{**}$)(Table 24). Days to maturity were positively correlated with seed yield in non-stressed and early maturity helped escape terminal drought (White and Singh, 1991). Singh (2007) found no association between seed yield and days to maturity in either non-stressed or drought-stressed field.

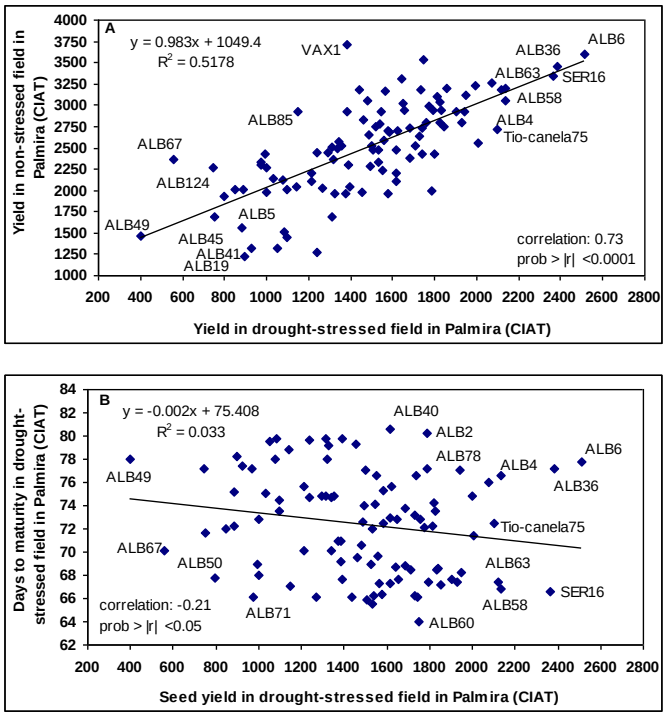

Figure 29: Relationship between seed yield in drought-stressed and non-stressed environments, seed yield and days to maturity in drought-stressed field of 100 bean genotypes including 96 RILs, 1 parent (SER 16), 3 checks (VAX 1, Tio-Canela 75, and ALB 252) in field in Palmira.

Days to maturity were positively correlated with seed weight in both non-stress and drought-stress (T9.RFSD) suggesting that later maturing cultivars and landraces, on average, had higher seed weight and early maturity reduced seed weight under both non-stressed and drought-stressed conditions in race Durango dry bean cultivars and landraces (Singh, 2007). Maturity under non-stressed was positively associated to maturity under drought-stressed (Muñoz-Perea et al., 2006).

This was also confirmed by our findings that DTM in WS was highly associated to DTM in NS with a positive correlation ($r = 0.76^{***}$).

IV.5.1.2. Relationship between root architecture and yield

In rainfed environments, rooting and the ability to sustain an uninterrupted supply of water are important factors (Gregory, 1988). Given the importance of roots in confronting abiotic stress, we expected that root vigor should favor better yield. However, the relationship between seed yield in non-stressed field and total root length in well watered soil tube experiment in our study was very weak. This could be explained that for some RILs as Beebe et al. (2009) mentioned, by stimulating more partitioning of photosynthates to roots, better yield may not necessarily result. Total root length ranged from 33.9 m (ALB49) to 95.16 m (ALB54). TRL of SER16 was 37.87 m, while its seed yield was 3338.8 kg ha^{-1}, and was ranked among the best. TRL of the other control (Tio-Canela 75) was 48.79 m with a seed yield of 2720.7 kg ha^{-1}. TRL in greenhouse was affected by water stress.

A larger root system, or a root system that more effectively occupies the soil, may delay the development of water stress (Markhart, 1985). Among RILs, some like ALB78, ALB4, ALB6, and ALB34 showed extensive root system accompanied by high yield. No association was found between total root length and seed yield with or without stress (Fig. 30a,b). However, all 7 best high yielding genotypes including ALB6, ALB36, ALB4, ALB58, ALB63, Tio Canela75, ALB91 and ALB108 have more root than SER16 (*P. vulgaris* drought tolerant parent) in drought-stressed field. Here also, the maintenance of water status under water limitation can be partially attributed to rooting depth and root length density (Turner, 1986; Subbarao et al., 1995).

Work at CIAT with interracial parental material and combining the deep rooting trait with improved seed filling also produced lines yielding as much as 50% more than SEA 5 (Ishitani et al., 2004). The relationship between root length in two deep soil profiles in WS treatment in a soil tube experiment in greenhouse and seed yield under drought-stressed field showed how the depth of root was associated to seed yield. Root length of genotypes in 40-75 cm and 60-75 cm soil profiles ranged from 7.14m to 40.14m and 1.45m to 20.61m respectively.

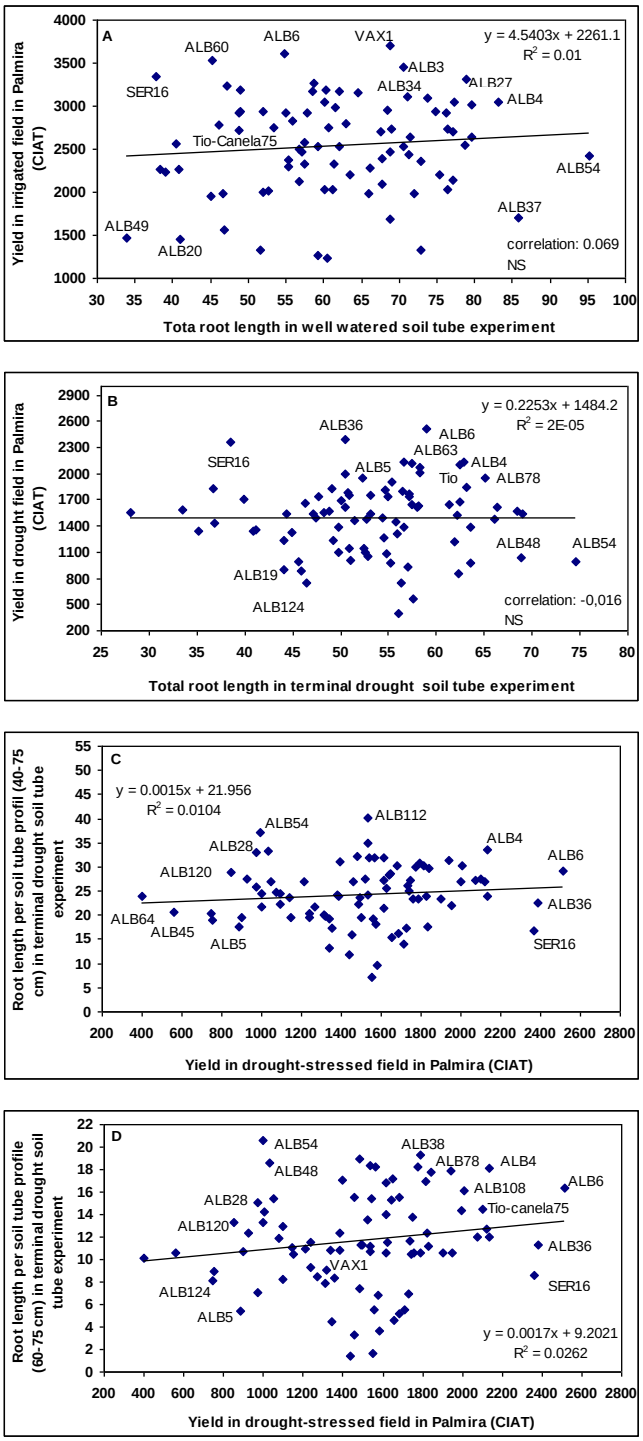

Figure 30: Relationship between seed yield in drought-stressed and non-stressed environments, total root length, and root length by soil profile (40-75 and 60-75 cm) in terminal drought and well watered treatments in soil tube experiment of 100 bean genotypes including 96 RILs, 1 parents (SER 16), 3 checks (VAX 1, Tio-Canela 75, and ALB 252) in Palmira.

Mean root length was respectively for 40-75 and 60-75m soil profiles, 16.8 and 8.62 m. In this study, we found that deep rooting genotypes are not necessary high yielding. However, SER16 and some RILs including ALB6, ALB4, ALB36, and ALB108 were high yield and ranked among the

deeper rooting genotypes. Root depth could be considered as an alternative trait for screening drought resistant lines, but a selection based on deep rooting alone will not assure proper outcome from breeding program. Results on root density at various level of the soil profile suggested that deep rooting genotypes are not always the best yielding materials (CIAT, 2007; CIAT, 2008). These CIAT report showed that drought resistance appears to result from a combination of mechanisms including a deeper root system, stomatal control and improved photosynthate remobilization under stress. Root length and depth may increase in a drying soil even at a reduced total root mass.

IV.5.2. Evaluation of RILs Al-toxic field in Quilichao (Colombia)

When the subsoil layers are acidic, plant roots will not be able to penetrate the acid layer and reach critical water and nutrient supplies below it, as a consequence plant development and yield are considerably reduced on this acid Al-toxic soil. Selection and development of genotypes with enhanced tolerance to acid soils and toxic levels of Al is the only reasonable solution to this problem (Hede et al., 2001). Mean squares (T10.RFSA) for days to flower (DTF), days to maturity (DTM), 100-seed weight, and seed yield were highly significant (Table 26). In common bean genotypes, highly significant ($P<0.001$) differences were also observed for grain yield, hundred seed weight and harvest index in both lime treated and untreated soils (Legesse et al., 2013).

Table 26: Mean squares of days to maturity, 100-seed weight, and seed yield of 100 bean genotypes including 96 RILs from F5:6 SER16 x (SER16 x G35346-3Q) Al-toxic soil environments at CIAT-Quilichao, Colombia in 2008.

Source of variance	Df	Days to flower	Days to maturity	100-seed weight	Seed yield
Replications	3	3.3	19.3	4.22	4122808.2
Treatments					
- Unadjusted	99	38.59***	38.85***	27.84***	490838.6***
- Adjusted	99	38.57***	38.76***	27.63***	491132.6***
Reps (adj.)	36	2.36	2.83	2.01	200109.2
Error					
- Effective	261	2.05	1.9	0.99	92467.1
- RCB Design	297	2.06	1.95	1.06	101373.8
- Intrablock	261	2.01	1.83	0.93	85784

*** Significant at the 0.001 probability level

IV.5.2.1. Relationship between seed yield and yield components

Preliminary evaluation indicated significant genotypic variation in grain yield among bean genotypes grown on Al-toxic soils (Rangel et al., 2005). These genotypic differences could be related to differences in Al resistance (Thung and Rao, 1999; CIAT, 1999). Compared to SER16 (Al sensitive parent) and VAX1 (Al sensitive genotype in greenhouse hydroponic experiment that expressed good vigor in Al toxic soils), our field experiment in Quilichao (T10.RFSA) clearly indicating presence of genetic variation for tolerance to Al-toxic acid soil. Days to maturity (DTM) ranged from 58 d to 74 d (days), with a mean of 64 d. DTM of SER16 and VAX1 were respectively 58 d and 67 d. Apparently Al-toxic acid soil did not influence the DTM.

Al-resistant and Al-sensitive bean genotypes can be differentiated by their yield potential under Al-toxic soil. The range of seed yield in Quilichao was 148 to 910 kg ha^{-1} while the general mean was 569 kg ha^{-1}. The mean of SER16 and VAX1 were respectively 647 and 649 kg ha^{-1} (data not shown). Days to maturity (DTM) was positively and highly associated ($r = 0.77^{***}$) to days to flower (DTF), in field evaluation in Al-toxic acid soil in Quilichao (Colombia) (Table 27). No correlation was found between 100-Seed weight and seed yield, DTM, and DTF. Assessing differential response of common bean genotypes to soil acidity, Legesse et al. (2013) have found a positive and signicant correlation between days to flowering and days to maturity in both lime treated and untreated soils. But, the correlation between days to maturity and grain yield in soil not treated with lime was strong and negative.

Table 27: Simple correlation coefficients between seed yield and yield components of 100 bean genotypes including 96 RILs from F5:6 SER16 x (SER16 x G35346-3Q) evaluated under Al-toxic soil environments at CIAT-Quilichao, Colombia in 2008.

Yield and yield components	Days to flower	Days to maturity	100-Seed weight	Seed Yield
Days to flower	1			
Days to maturity	0.77***	1		
100-Seed weight	-0.12 (ns)	0.005 (ns)	1	
Seed Yield	-0.33***	-0.36***	0.14 (ns)	1

*** Significant at the 0.001 probability level, and ns: no significant.

The relationship between seed yield and yield components (days to maturity and 100-seed weight) have been analyzed in this study (Fig. 31). Seed yield and DTM showed a weak association, and their correlation was great and highly significant, but negative (r = -0.36***). DTF was also negatively correlated to seed yield (r = -0.33***) (Table 27). Under high Al saturation soil in Quilichao field, some RILs (ALB21, ALB1, ALB63, ALB25, and ALB5) were relatively less sensitive to Al-toxic acid soil stress, as opposed to SER16 and Tio canela75 that showed greater sensitivity and low yield (Fig 31a).

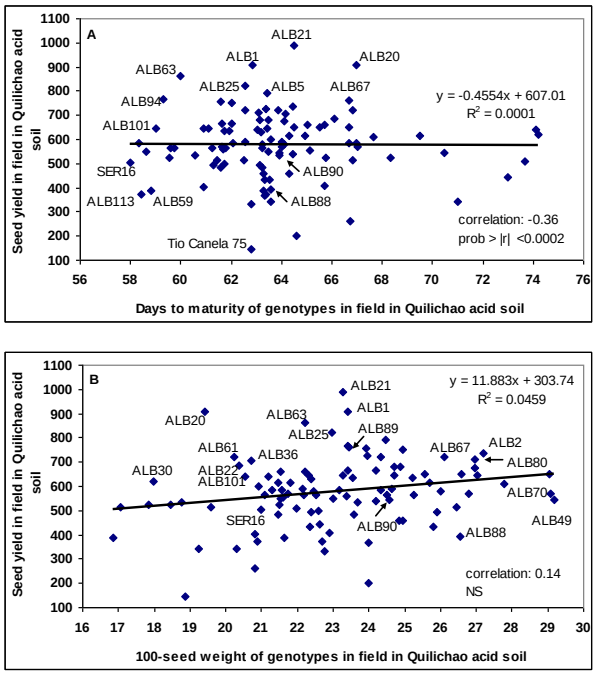

Figure 31: Relationship between seed yield and yield components (days to maturity and 100-seed weight) in Al-toxic soil environments at CIAT-Quilichao, Colombia of 100 bean genotypes including 96 RILs, 1 parents (SER 16), 3 checks (VAX 1, Tio-Canela 75, and ALB 252).

Similar observation was made for the relationship between seed yield and 100-seed weight. No association found between these two components of the yield (Fig. 31b). In both lime treated and untreated soils, 100 seed weight was strongly and positively correlated with pod length and weakly correlated with the number of pods in lime un treated soil (Legesse et al., 2013).

IV.5.2.2. Relationship between seed yield and total root length

The root system as a whole is affected by Al toxicity, with many stubby lateral roots and no fine branching (Hede et al., 2001). Such roots are inefficient in absorbing nutrient and water (Foy et al., 1978). Genotype yield potential is strongly reduced in such conditions. The range of total root length in soil tube (T5.RGSA) and nutrient solution (T4.RGHA) experiments was respectively, 8.8-29.9 m and 43.71-218.62 m. Seed yield under Al-toxic acid soil in Quilichao (T10.RFSA) correlate with total root length both in high Al saturation soil tube (r = 0.27*) and in hydroponics with 20 µM Al.

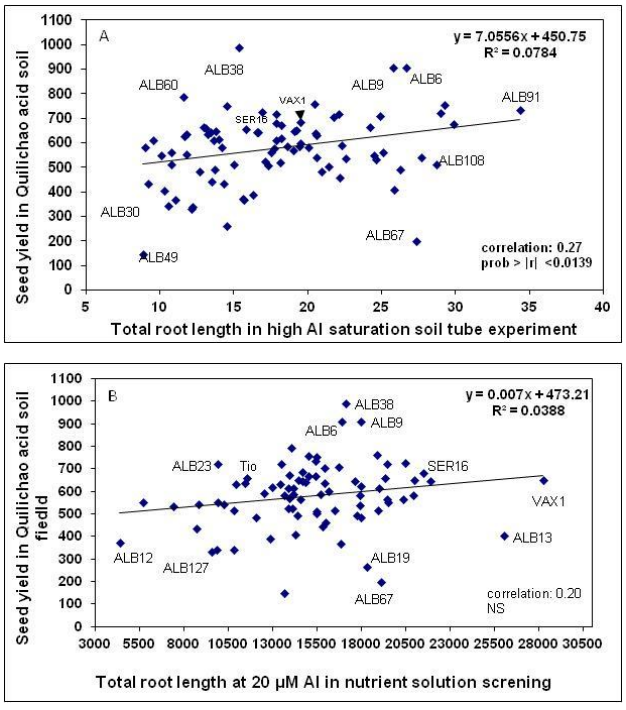

Figure 32: Relationship between seed yield in Al-toxic soil environments at CIAT-Quilichao, Colombia; and total root length both in nutrient solution and soil tube experiments in greenhouse of 100 bean genotypes including 96 RILs, 1 parents (SER 16), 3 checks (VAX 1, Tio-Canela 75, and ALB 252).

An examination of seed yield in Quilichao (T10.RFSA) and total root length in nutrient solution (T4.RGHA) or in the soil tube experiment (T5.RGSA) showed that RILs such as ALB6 and ALB9

have extensive roots in greenhouse and high yield in field. ALB38, another genotype that revealed high yield in Al-toxic acid soil has more root in nutrient solution than in soil tube experiment (Fig.32). Bean genotypes sensitive to Al-toxic acid soil have greatly reduced yield as a result of high aluminium saturation injuring and stunting their root system.

IV.5.3. Interaction of Al-drought stresses on bean yield and yield components

A possible breeding strategy for developing crops for superior adaptation to combined stress conditions of soil acidity and drought could involve screening germplasm under well watered and drought-stressed conditions on an acid soil and make selections based on superior performance (yield) under both conditions (Yang et al., 2013). As we do not find an acid-toxic soil field where it is easy to simulate terminant drought stress, two separate fields were used for the experiment, high Al saturation soil in Quilichao/Colombia (T10.RFSA) and terminal drought prone field in Palmira (T9.RFSD). The most direct method of evaluating the interaction of Al and drought stresses is by measuring economic yield (grain or forage) under field conditions (Yang et al., 2013). The field studies conducted on both Al and drought stresses respectively in Palmira and Quilichao indicated that yield was strongly reduced by these abiotic stresses. The yield on the irrigated plot in Palmira ranged from 1132 to 3705 kg ha^{-1} while the range of the yield on rainfed plot (under water stress) was 399 to 2512.8 kg ha^{-1} (T9.RFSD), and was mostly affected at Quilichao (high Al-toxic soil) where yield ranged from 145.3 to 898.6 kg ha^{-1} (T10.RFSA). The growth of roots in the acidic subsoil will then subsequently suffer from Al toxicity and the Al-impeded root growth will further restrict the exploitation of deeper subsoil for water (Yang et al., 2013). Similar to acid soil stress, yield under drought stress with reference to yield under non-stressed conditions, has normally been employed as the primary phenotypic selection criterion in improving drought resistance in crops (Blum, 2010). The best lines selected as drought tolerant were ALB6 and ALB 36 with higher yield than SER16, followed by ALB4, ALB58, ALB63, ALB91, ALB108, and ALB88. Al-tolerant lines selected in Quilichao field were ALB38, ALB9, ALB16, ALB28, ALB58, ALB6, ALB35, ALB76, ALB91, and ALB252. Across the two environement, the best lines that require more attention in our future selection were ALB6, ALB58, ALB36, ALB91, ALB78, ALB7, ALB76, and ALB56.
Breeding for drought resistance must be combined with Al resistance, to assure that drought resistance is expressed adequately in crops grown on soils with acid Al-toxic subsoils (Yang et al., 2013).

Summary statement:

Evaluation of Phenology, Yield and Yield Components of Interspecific Recombinant Inbred Lines (RILs) on soils presenting abiotic stresses

Drought stress and Aluminum (Al) toxicity are two major constraints for bean production in low input agriculture in the tropics. 96 F5:7 families from a backcross SER 16 x (SER 16 x G35346-3Q) were evaluated both under irrigated and non-irrigated fields in Palmira. Two additional irrigations (of 30 mm each) after sowing and one week before initiation of flowering (for 36 days old plants) were applied to the drought plot. Total amount of watering including rainfall was 197.7 mm. For the irrigated plots, as rainfall was short (81.8 mm), six additional irrigations of 40 mm each were applied. The total amount of water received during the season was 321.8 mm. The same number of RILs and checks were evaluated in Quilichao. Results revealed that drought reduced seed yield by 40.8 % and 100-seed weight by 11%. Days to maturity when compared under drought and irrigated field increased by 4 days in water stress. This unusual observation was due to sporadic rain that occured at grain filling and influence the recovery of plants. Under drought, drought susceptibility index (DSI) ranged from 0.1 to 1.8; the pod havest index (PHI) correlated to seed yield (r = 0.49***), and seed yield was associated to 100-seed weight. Mean yield under non-stressed was 2510.05 $kg.ha^{-1}$, whereby under drought stress was 1485.9 $kg.ha^{-1}$. Deep rooting genotypes are not necessary high yielding. Two elite RILs were the best (in $kg\ ha^{-1}$): ALB6 (2512.8) and ALB36 (2384.1). The range of seed yield in Quilichao was 148 to 910 $kg\ ha^{-1}$. Seed yield and DTM showed a weak association, and their correlation was great and highly significant, and negative (r = -0.36***). Under high Al saturation soil in Quilichao field, some RILs (ALB21, ALB1, ALB63, ALB25, and ALB5) were relatively less sensitive to Al-toxic acid soil stress. The relationship between seed yield in Quilichao and total root length in nutrient solution or soil tube experiment showed that RILs such as ALB6 and ALB9 have extensive roots in greenhouse and high yield in field. A few of the RILs such as ALB6 expressed superior yield potential accross environements.

IV.6. Simple Sequence Repeats (SSR), and Quantitative trait loci (QTL) mapping in RILs resulting from interspecific improvement of Common vean.

IV.6.1. Source of Microsatellite markers and Polymorphism survey

All of the 64 microsatellite markers described above were screened for amplification using two parents (SER16 and G35346-3Q) of Recombinant inbred lines (RILs) from the interspecific population developped from backcross BC1 F5:6 SER 16 x (SER16 x G35346-3Q); and G19833. An example of screening is presented on Fig. 33. G19833 is a Peruvian landrace and parent of the principal mapping population used at CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia), DOR364 x G19833 (Blair et al., 2003).

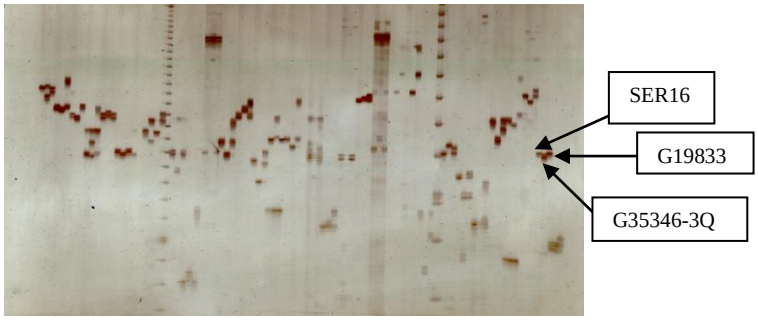

Figure 33: High polymorphism found using BM SSR group of markers in selection of SSR Markers (eg. BM) using the two parents (SER16 and G35346-3Q) of Recombinant inbred lines (RILs) and the known check G19833.

Sixty-four markers were used that generated at least one polymorphic band among the family. The amplification pattern of the polymorphic microsatellites on 94 recombinants inbred-line progenies**,** the 2 parents (SER16, G35346-3Q) and several checks using 64 primers were performed. These 64 primers yielded 6479 polymorphic amplification products (bands). A majority of the microsatellites produced single bands for the two parents, 5784 bands for the recurrent parent (A) and 560 for the donor parent (B). The number of heterozygous loci was 135, and in general the polymorphism rates for the population was high. Figure 33 shows an example of polymorphism using the SSR marker BM212 on 94 RILs, 2 parents (SER16, G35346-3Q) and 2 checks (DOR364, a Mesoamerican bean variety and G19833, an Andean Peruvian landrace).

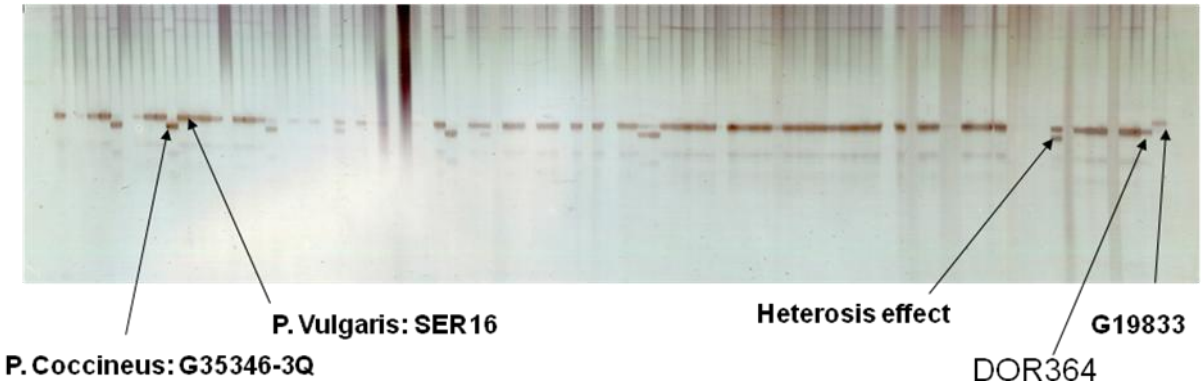

Figure 34: Polymorphism with BM212 among 94 RILs, 2 parents (SER16, G35346-3Q) and 2 checks (DOR364, a Mesoamerican bean variety and G19833, an Andean Peruvian landrace).

IV.6.2.Introgression

Introgression is an important source of genetic variation in common beans and it results in a complex mixture of parental genes. The introgression of genes from *P. coccineus* to *P. vulgaris* will allow the study of quantitative traits loci, and also the breeding of recombinant inbred lines from these interspecific crosses. The percentage of introgressed lines per marker has been calculated, and varied from 0 to 35.2 % with an average performance of introgression of about 10.5 %. These percentages were organized in a decreasing order (Fig. 35).

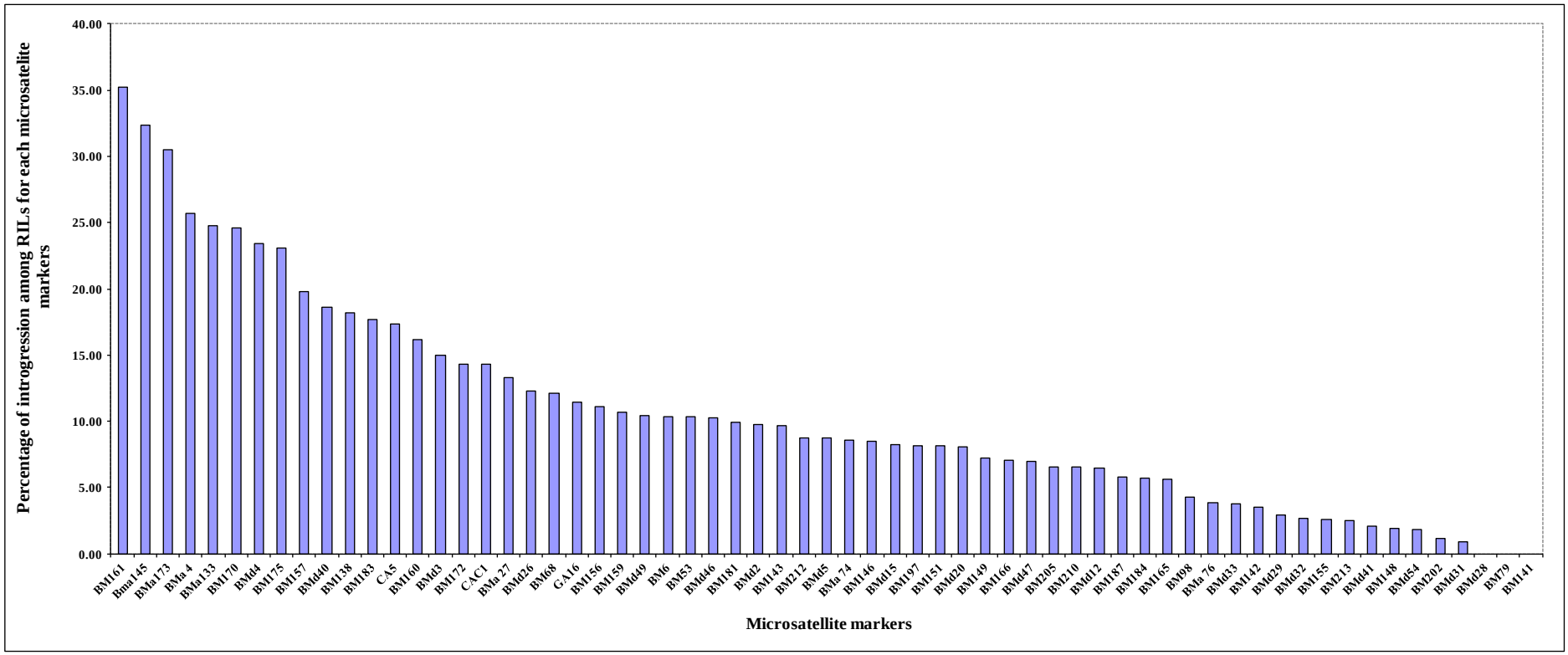

Figure 35: Percentage of introgression of genes from the two parents (SER16 and G35346-3Q) among RILs population for each SSR marker

IV.6.3. Genetic mapping of root characteristics (SRL, VRD, MRD, and FRP under HAl treatment, and FRP and RDW in WW treatment) of RILs in soil tube study

The map was created based on linkage of 62 of the microsatellites, with several of the markers remaining unlinked. The map has a total length of 154.91 cM distributed across 10 linkage groups. The number of markers varied from 2 on b10 to 16 on the linkage group b01. The average linkage distance between pairs of markers in all linkage groups was 12.5 cM. Beebe et al. (2006) in their study on QTL for root architecture traits correlated with phosphorus acquisition in common bean find an average distance between markers of 7.2 cM. The maximum distance (15.3 cM) separating two markers occurred in linkage group b05, and the minimum distance between two markers was 1.18 cM in linkage group b10. Six quantitative trait loci (QTLs) were identified for root traits over different environments (High Al and irrigated field). For either greenhouse nutrient solution or soil tube trials, they were mapped to only five linkage groups (b01, b02, b03, b04 and b05) (Fig. 36). Only QTLs for MRD_High_Al and RDW_WW were located on the same linkage group (b04). Parameters associated with these QTL appear in Table 27, indicating the parent which contributed the positive effect of the QTL and its magnitude.

The use of moleculer markers will improve the understanding of genetic factors conditioning these traits. QTLs were identified for only 6 traits in this study on root characteristics mapping, and only one QTL was identified per trait. A total of 19 QTLs associated with root hair, acid exudation and P-uptake traits were detected on 8 linkage groups by Yan et al. (2004). In this study, the six QTLs were identified for 4 different traits under high Al stress including fine root proportion, root diameter, root depth at 34 days in soil tube and specific root length. The large genetic distance (>20 cM) in some regions of the linkage map and size of the population in this study may have prevented the detection of QTL with small effects.

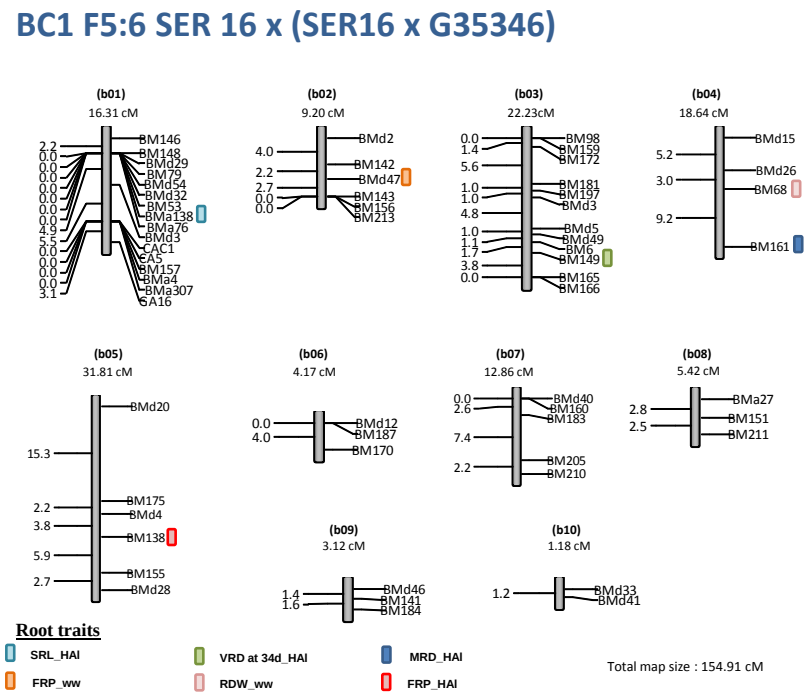

Figure 36: Root trait characteristics (SRL, VRD, MRD, FRP, RDW) over different environments (High Al and well watered soil tube) and the QTLs are located on different linkage groups

Root trait characteristics over different environments:

- FRP_HAl = Fine root proportion high Al -toxic acid soil tube (%)
- MRD_HAl = Root diameter high Al -toxic acid soil tube (mm)
- VRD at 34d_High_Al = Rooting depth at 34 days high Al -toxic acid soil tube (cm)
- SRL_High_Al = Specific root length high Al -toxic acid soil tube (m/g)
- FRP_WW = Fine root proportion-well under watered soil tube (%)
- RDW_WW = root dry biomass weight under well watered soil tube (g)

IV.6.4. Genetic mapping for yield (drought stress and irrigated) and yield components (DTF on irrigated and Al-toxic soil field, DTM on irrigated field, PWBP and PHI on drought field) among RILs

To cope with climate change under stress conditions, common bean population from interspecific crosses are thought to be more promising for selection of high yield potential than those from intraspecific crosses. This study was conducted to identify genetic loci associated with quantitative traits responsible for seed yield and yield components under stress (drought and aluminum toxicity) in common bean. Eight QTLs associated with phenology, yield and yield components were identified; and were located on only two linkage groups (b01 and b05) (Fig. 37). Table 28 presents these QTL and their magnitude.

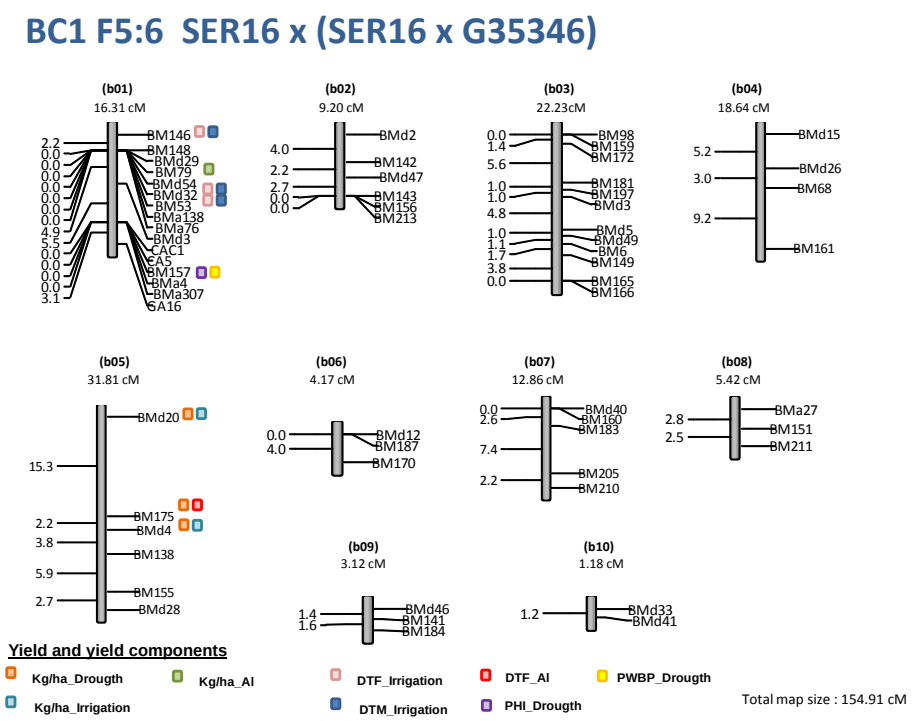

Figure 37: Genetic map for yield and yield components among RILs over different environments (Drought, High Al and irrigated field) and where QTLs are located on different linkage groups

Yield and yield components over different environments:

- Kg/ha_Drougt = Seed yield in drought-stressed field
- Kg/ha_Irrigation = Seed yield in irrigated field
- Kg/ha_Al = Seed yield Al stressed field
- DTF_Irrigation = Days to flower in irrigated field
- DTM_Irrigation = Days to maturity in irrigated field
- DTF_Al = Days to flower in Al stressed field
- PHI_Drougth = Pod harvest index in drought-stressed field
- PWBP_Drougth = Pod wall biomass in drought-stressed field

The number of QTL per trait ranged from one to three. Only one QTL was introgressed separately from other traits, and was identified on BM79 for seed yield in the high Al stressed field. Blair et al. (2006) found a total of 13 QTL for plant height, yield and yield components along with a single QTL for seed size showed positive alleles from a wild bean parent while the remaining QTL showed positive alleles from the cultivated parent. Many QTLs for yield and related traits derived from different wild accessions or species were mapped to identical chromosomal regions (Mallikarjuna and Sarla, 2007). All other QTLs from our study were inherited per pairs including days to flower with days to maturity in the irrigated control planting (BM146, BMd32, BM53), Pod harvest index with Pod wall biomass in drought-stressed field, seed yield in drought-stressed

treatment with seed yield under irrigated field (BMd20, BMd4), and seed yield in drought-stressed treatment with days to flower in the Al stressed field. The contributions of QTL for yield under drought, irrigated and Al-toxic soil were respectively 10.47-13.16 % ($P<0.001$), 11.52-12.02 % ($P<0.0001$), and 10.92% ($P<0.01$). For yield components QTL (DTF_irrigation, DTF_Al, DTM_irrigation, PHI_drought, and PWBP_drought), the corresponding contribution ranged from 21.98 to 29.2 % at $P<0.001$. Blair et al. (2012) studying the crop improvement for drought tolerance in the Middle American genepool found that similar traits were inherited quantitatively with the greatest number for seed weight followed by yield per day, yield per se, days to flowering and days to maturity.

Table 28: QTL identified for yield and phenology traits in a population of recombinant inbred lines of common bean with *Phaseolus coccineus*.

Trait	Test m	Chrom	Marker	Position	LOD Score	Additive	Source	R2	TR2	S	ID-Marker
g100s_Drought	1	2	1	0	3.4979	-2.855	G35346-3Q	0	0	0	BMd2
g100s_Drought	2	5	2	18.3	4.2374	2.06	SER16	0	0	0	BM175
kgha_Drought	1	5	2	18.3	2.7277	201.858	SER16	0	0	0	BM175
Kgday_Drought	1	5	2	18.3	3.1722	3.11	SER16	0	0	0	BM175
DTF_Irrigated	1	1	9	2.8	0.4977	-2.58	G35346-3Q	0	0	0	BMa76
DTF_Irrigated	2	5	2	18.3	3.0096	-1.157	G35346-3Q	0	0	0	BM175
DMF_Irrigated	1	1	1	0	5.3755	-3.207	G35346-3Q	0	0	0	BM146
g100s_Irrigated	1	5	2	18.3	3.7963	1.984	SER16	0	0	0	BM175
kgha_Irrigated	1	5	1	0	2.8374	364.084	SER16	0	0	0	#N/A
Kgday_Irrigated	1	5	1	0	2.8586	5.696	SER16	0	0	0	#N/A
DTF_Alum	1	1	9	2.8	0.2797	-2.718	G35346-3Q	0	0	0	BMa76
DTF_Alum	2	5	2	18.3	4.7985	-2.089	G35346-3Q	0	0	0	BM175
DTF_Alum	3	5	4	24.8	3.5112	-1.936	G35346-3Q	0	0	0	#N/A
DTM_Alum	1	1	9	2.8	0.0341	-3.212	G35346-3Q	0	0	0	BMa76
DTM_Alum	2	5	2	18.3	3.5331	-1.874	G35346-3Q	0	0	0	BM175
PHI_Drought	1	1	15	12.4	0.4984	1.567	SER16	0	0	0	BMa307
PWBP_Drought	1	1	15	12.4	0.4988	-1.568	G35346-3Q	0	0	0	BMa307

- Empirical LOD thresholds based on 1000 permutations used fro QTL detection as recommended by Churchill and Doerge (1994),
- Determination coefficient based on marker (R2) and model including background markers (TR2),
- g100s (100 seed weight), kgha (seed yield), Kgday (seed yield per day).

Summary statement:

Quantitative Trait Loci (QTL) associated with Aluminum and drought tolerance in common beans

A backcross to the recurrent common bean parent was pursued to create recombinant inbred lines. The mapping population has been phenotypically evaluated both under greenhouse and field conditions. Polymorphisms between the mapping populations were determined on parental survey gels. Alleles of the progenies were scored based on the amplified parental bands. Total map length for the base map was 156.91 cM, and the average linkage distance between pairs of markers in all linkage groups was 12.5 cM. The percentage of introgressed lines per marker varied from 0 to 35.2 % with an average performance of introgression of about 10.5 %. Six QTL for root architecture traits for common bean were identified for two environements. QTLs associated with tolerance to Al-toxic acid soil were four for 4 different root traits: SRL (BMa138 at b01), VRD at 34d (Bm149 at b03), MRD (BM161 at b04), and FRP (BM138 at b05). The two other QTLs were associated with roots under well watered conditions were mapped at b02 with BMd47 marker (as for FRP) and at b04 with BM68 marker (as for RDW). Eight QTLs associated with phenology, yield and yield components were identified. They were all located on only two linkage groups (b01 and b05). Only one QTL was introgressed separately from others and was on BM79 for seed yield under high Al stressed field. All other QTLs from this group were inherited per pairs including days to flowering and days to maturity under irrigated field conditions (BM146, BMd32, BM53), Pod harvest index and Pod wall biomass in the drought-stressed field, seed yield in drought stress and seed yield under irrigated conditions. Among these QTLs, three were associated to phenology including days to flowering (DTF-irr.) under irrigated field conditions and under Al-toxic acid soil (DTF-Al), and day to maturity (DTM-irr.) under irrigated field conditions. Three other QTLs were identified for drought stress tolerance, for irrigated yield, and tolerance to Al-toxic acid field conditions. Only two QTLs for performance under drought were identified for yield component traits including pod harvest index (PHI) and pod wall biomass proportion (PWBP).

CHAPTER V. CONCLUSIONS AND PERSPECTIVES

The objective of this study was to explore the diversity found in common beans and its wild relatives to develop abiotic (Aluminium toxicity and drought) and biotic (*Fusarium* root rot) stresses resistance bean genotypes, and to identify QTLs for resistance to these stresses.

While screening potentiel parents for resistance to abiotic (Aluminium toxicity and drought), we found that root phenotyping for Al resistance indicated that total root length (TRL) and tap root elongation rate (TRER) could be considered as the most important root characteristics when identifying Al resistant bean genotypes using hydroponics, while total root length (TRL) and visual root depth (VRD) are the most useful root traits to be considered for evaluating tolerance to Al-toxic acid soil. These root traits had shown the sensitivity of genotypes to toxic-Al stress through root growth inhibition. They predicted how roots of resistant genotypes continue to branch under Al stress and maintained their exploratory capacity in soil, and contributed to remobilization of photosynthates to the reproductive plant parts.

The results from hydroponics and soil tube studies indicated that these two methods of evaluation were effective in screening for resistance to individual and combined stress factors of Al and drought. The greater level of Al-resistance found in *P. coccineus* genotypes (G35346-2Q and G35464-5Q) offers the opportunity to obtain much higher resistance in common bean through interspecific crosses. Another *P. coccineus* accession G35346-3Q showed ability to tolerate combined stress factors of Al and drought. The use of this genotype in common bean improvement for resistance to these two stress factors is likely to be more productive for combined stresses than considering resistance to them separetly. Populations created from multiple stress resistance donors could be more stable and capable to produce grain under stress in the face of climate change. In conclusion, our data reaffirm that the runner bean genotype, G35346-3Q, is an Al resistant material and could be a very good source for improving acid soil tolerance in common bean.

Regarding the phenotypic evaluation of Interspecific recombinant inbred lines for their tolerance to individual and/or combined stress of Al and drought, a number of root and shoot traits evaluated using soil tube system, several root parameters expressed well in the best lines for shoot development and may contribute to shoot biomass accumulation of those lines. Higher values of total root length and rooting depth found with ALB91, ALB88, ALB77 and ALB 89 contributed to greater shoot development. The use of both systems could contribute to evaluate breeding materials to identify genotypes that combine Al resistance with acid soil tolerance. However, results on Al

resistance obtained from hydroponic system were not correlated well with the data on acid soil tolerance from soil tube system indicating that use of either system alone can eliminate some useful genotypes. Two genotypes (ALB88 and ALB91) emerged as lines with multiple traits for resistance to Al-stress. Different concentrations of Al in hydroponic system and different levels of Al saturation in soil system could be tested to further test the relationship of root traits with shoot traits. This knowledge will be useful to understand the physiological basis of differences in ranking of genotypes under acid soil field conditions across seasons and years. The results from this work will be useful for identification of molecular markers for Al resistance in *Phaseolus* species and to improve acid soil adaptation in common bean.

11 genotypes including G35346-3Q, ICA-Quimbaya, ALB28, ALB43, Tio canela 75, ALB49, ALB122, ALB125, ALB118, ALB60, and ALB20 maintened high root surfsce area under drought, active uptake and may maximize nutrient diffusion to the root surface. Under combined stress of Al and drought, ALB 91 emerged as superior genotype in developing high root density, and may be a potential candidate for further improving other trait of the plant.

For the greenhouse screening for resistance to resistance to *Fusarium* root rot, high inheritance of was observed. The diversity was very high within the recombinent inbred lines (RILs) as result of a combination of a large number genes for that trait. One of the specific objectives of this study was to transfer *Fusarium* root rot resistance from *P. coccineus* into small seeded Mesoamerican red bean SER16 was met. More than five lines scored as resistant as the coccineus parent. The low *Fusarium* symptom score and root growth inhibition for resistance to *Fusarium* was effectively transferred while success for root dry biomass weight (RDW) was limited. Resistant RILs (ALB45, ALB41, ALB126, ALB84, ALB49, ALB34, ALB88, and ALB85) were selected, and are under evaluation in fields with a history of *Fusarium solani* in Nyamagabe (Rwanda) for the identification of candidates lines for realise.

Field evaluation of phenology and seed yield of the recombinant inbred lines (RILs) for resistance to abiotic stresses (Al-stress and drought) has allow to quantify biomass accumulation combined with remobilization of photosynthate to grain (or harvest index). Greater yield compared to SER 16 was observed in some lines under Al toxicity, and under intermittent drought in 2008. However, in a combined analysis of terminal drought and irrigated conditions in 2009, no line yielded significantly more than SER 16. It appears that in these environments remobilization in the derived lines was inadequate to take advantage of the biomass derived from runner bean. In that season the

lines may have expressed some sensitivity to high temperatures that was inherited from runner bean. In any case, augmenting biomass accumulation may induce more vegetative development in the crop, with a concomitant reduction in sink strength and remobilization. Maintaining good remobilization while increasing biomass is a particular challenge for breeders. Two elite recombinant inbred lines (ALB6 and ALB36) were identified as drought resistant linnes.
Improvement for aluminum resistance was possible due to the availability of the resistance in coccineus roots, and it was possible to transfer it to Al susceptible line SER16. This resistance results in greater shoot biomass. Maintaining good remobilization while increasing biomass is a particular challenge, further research is needed to design optimal efficiency of roots to support higher yield in specific environments. Greater rooting alone does not necessary result in greater yield. Under high Al saturation soil in Quilichao, RILs (ALB21, ALB1, ALB63, ALB25, and ALB5) were identified as less Al-toxic acid soil sensitive lines, and are now under screening in many country in Eastern Africa.

In this study we identified 14 QTLs including six for root architectural traits, and evaluated their relationship with QTLs for Aluminum toxicity and drought tolerance in common beans. We also identified eight QTLs associated with seed yield and yield components under drought-stressed and Al toxic acid soil stressed field.

It is difficult to predict the potential of crosses for bean line developments and increase the efficiency of the breeding programmes. Interspecific genetic improvement for common bean has shown its potential for increasing productivity on drought and aluminum toxic soil. In general, RILs identified in the current work would be further evaluated in multi-location trial in Rwanda prior to their official release to farmers for cultivation.

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