Breeding for drought tolerance by integrative design: the case of common bean (*Phaseolus vulgaris* L.) in Ethiopia

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Abstract

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Drought stress is the most important limitation facing crops now and in the future. This makes improving adaptation to drought stress a major objective of crop breeding. Breeding efforts in common bean developed drought tolerant genotypes but the genetics and preferences of farmers for drought tolerance are largely un-studied. This study assessed genetic, physiological and social aspects of drought tolerance in common bean breeding. More specifically the study was envisioned as developing common bean varieties with increased levels of drought tolerance in farmers' preferred grain types for southern Ethiopia. Multiple approaches that combine laboratory, greenhouse and field level analysis with participatory experimentation deploying tools in the biological and social science arenas were used. The findings demonstrate that farmer use and management of common bean seed and varieties in southern Ethiopia are characterized by varying conditions, varying practices and the dynamics of a changing climate and market. Farmers recognize that climate is changing but only half of them have adapted some of the cropping practices. Marginality of production ecology related with climate change, market dynamics, cropping system and culinary preferences make common bean farming at farmer level a series of moving targets that are problematic for a drought breeding program to hit simultaneously. Moreover, exposure to new variety types influence farmers' preferences. In addition to this diversity in production environments and variation in farmers' preferences, the plant's response to drought stress is complex and diverse. Targeting specific plant responses to target areas is a difficult challenge for common bean breeders as shown by the quantitative trait loci (QTL) analysis. But, this study has generated information for specific qualitative and quantitative targets in the breeding design. These include: 1) farmers' preferences are not static nor are they consistent across gender, location, individual and co-evolve with exposure; 2) original differences in introduced germplasm from the primary centers of domestication were the base for East African common bean diversity and there are distinct germplasms at national or regional level; 3) the relevance of continued photosynthate accumulation as a trait for common bean drought tolerance; 4) root based QTL can be identified but may not be compatible with yield related traits although their selection can benefit from tightly linked markers; 5) only a few major QTLs with high QTL×E interaction were detected whereas a large proportion of genetic variance remained unexplained by the QTLs for traits related to drought avoidance, photosynthate accumulation and (re)mobilization, highlighting the difficulty in detecting QTL in drought studies in common bean; and 6) exposing farmers to new drought-tolerant variety types makes them aware of drought selection traits and creates new market niches for new products. Hence, more drought-tolerant common bean genotypes for a range of farmer conditions, markets and preferences can only be developed on the basis of an integrated understanding of farmers' production conditions, existing seed system practices and different physiological processes that regulate drought tolerance in the plant. In general, the results from the present work contribute to integrative breeding strategies that incorporate participatory, physiology and marker-aided selection to breed new varieties of drought-tolerant common bean that combine a range of mechanisms in farmers’ preferred grain types.

Keywords: Decentralized breeding, drought stress, farmer preference, genetic diversity, participatory variety selection, population structure, quantitative trait loci

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With love,
Asrat
September 2011, Wageningen, The Netherlands

Dedication

To my mother Azalech Dumalo, my son Henok Asrat, my daughter Betty Asrat and late Asamenech Kassa with love.

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CHAPTER 1

General introduction

Crop breeding and the smallholder farmers in harsh environments

Crop breeding is a key technology area of agricultural development. It has been the core element in agricultural development since the date of domestication of crop plants (Harlan 1992). Crop breeding encompasses intentional and/or unintentional creation, fixation and selection of variability based on the understandings of plants, environments and their interaction. In its primitive form, crop breeding started with selection, the art of discriminating among biological variations naturally present in a population to identify and pick desirable variants based solely on intuition, skill and judgment of the selector (Acquaah 2007). This form of selection is still an integral part of smallholder farming system in harsh environments where farmers save their seed from preferred plants for the next season sowing and share that with other farmers *via* social networks (Offei et al. 2010). However, there is a paradigm change in crop breeding from primitive natural variant based phenotype selection to the use of genotype to predict phenotype more precisely and efficiently (Borem et al. 2002). Furthermore with technological advancements, present day crop breeders are increasingly able to manipulate plant attributes beyond the natural barrier.

Crop breeding for smallholder farmers is problematic, especially in harsh environments. The performance and spread of varieties from modern crop breeding efforts in marginal stressful and low input environments have been small and sluggish. In contrast, progress of crop breeding has been incredible in favorable agricultural environments (Evans 1998; Evenson and Gollin 2003; Duvick 2005; Fisher and Edmeades 2010). Modern varieties from scientific breeding worked well in potential environments with high input and high-quality seed supply. However, the observed high yielding potential of these modern varieties is not realized in marginal low-input, risk-prone production ecologies of resource poor farmers (Scoones et al. 2005; Bellon 2006). The varieties developed by breeders either have not reached the smallholder farmers at all or did not fit to their specific agro-ecological and/or socio-economic conditions (Almekinders and Louwaars 1999; Tripp 2001). It can be argued that the poor adoption of modern varieties is at least partly caused by neglecting aspects of farmers' realities (or socio-technical context), perceptions and preferences.

In many aspects, smallholder farmer situations in harsh environment are different from the situation where many modern crop varieties from scientific breeding are developed. Farmers in harsh environments often practice their crop cultivation in micro-conditions of soils and climate regimes. Moreover, smallholder farmers in harsh environments are not only socio-culturally heterogeneous but also differ in their personal preferences for a particular type and form of plants that work for their individual conditions (Batterbury 2001; Offei et al. 2010). As a result, different breeding strategies have been sought to produce cultivars for different farmer conditions to facilitate crop production and optimize productivity in harsh environments. Participatory plant breeding (PPB) has been advocated as a complementary approach to centralized breeding, particularly suited to highly variable environments and variation in farmers' preference for variety types. PPB as an approach has been practiced in many crops to develop varieties that better suit smallholder farmers in harsh environments, largely by mimicking farmers’ conditions on station or by on-farm testing, seeking the farmers’ participation in one way or another. Remarkable results were registered in many crops in this regard in the last two decades around the globe (e.g. Almekinders and Hardon 2006).

Crop breeding is a constant pace in which breeders regularly seek new ways of cultivar development for increasing the productivity of farmers and meeting consumers’ needs and preferences. The phenomenon of global climate change is affecting the agricultural context by modifying crop production environments around the world (IPCC 2001, 2007). This means that new efforts are needed to breed cultivars of crops that adapt to the modified or new crop production environments but that are still appreciated by the smallholder farmers because these varieties appeal to their preferences and perceptions. The pursuit of new cultivars that better suit the smallholder farmers’ need and interest in harsh environments should be a multidisciplinary and integrated effort involving social science, plant breeding, physiology, genomics, simulation modeling, etc.

The challenges of common bean breeding for harsh environments

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for direct human consumption in the world, and is a traditional staple in many parts of eastern and southern Africa (Wortmann et al. 1998). Common bean is rich in protein, iron and other minerals (Broughton et al. 2003). Nearly 80% of total dry bean production occurs on high-poverty smallholder farms in developing countries (Hayman et al. 2008). In eastern and southern Africa, common bean is the second most important protein source and the third most important caloric source after cassava and maize (Pachico 1993). While traditionally a food security crop throughout the region, intra-regional trade of common bean is gaining in importance. In many parts of the developing world, common bean is evolving as an important source of foreign currency and cash income for smallholder farmers. It not only provides nutrients as human food and generates cash income but also provides fodder for livestock feed and improves soil fertility by its atmospheric nitrogen fixing capacity. Thus as a legume crop, it adds diversity to production systems on resource poor farmers’ fields and contributes to the farming system stability.

As compared to other lowland legumes like cowpea, common bean is less adapted to extreme conditions. The adaptation range and physiology of the cultivated genepool is influenced by ancestral traits associated with survival mechanisms of wild bean in its native habit which is mid-altitude with moderate temperature, organic soils, and seasonal abundant rainfall (Beebe et al. 2008). However, enormous variation in the genepools and races could give a wider window for manipulation of its adaptation through breeding. Cultivated common bean originated in Latin America from two recognized centers of domestication about 7000 to 8000 years ago (Gepts and Debouck 1991). The contrasting regions of domestication endowed the crop with a relatively high diversity. This diversity is broadly classified into two genepools:

the Mesoamerican and the Andean genepool (Gepts et al. 1986; Chacón et al. 2005). The two genepools are further sub-divided into races (Blair et al. 2009). So far the Mesoamerican race Durango from the semi-arid highlands of Mexico and race Mesoamerica native to the warm lowlands of Central America have been the most important sources of drought-adaptation genes in breeding programs (Singh et al. 1991; Rao 2001; Terán and Singh 2002; Miklas et al. 2006). On the other hand, among the races of Andean genepool which typically adapts best to cooler climates, race Nueva Granada is more heat tolerant than race Peru (Beebe et al. in press).

Abiotic stresses, either climatic or edaphic, are widespread, frequent in occurrence and often intense in magnitude, and thus pose a high total yield loss in common bean (Wortmann et al. 1998; Thung and Rao 1999). In common bean, drought stress is more serious than other abiotic stresses. Drought is a key challenge to the livelihood of vulnerable smallholder farmers in harsh environments causing significant harvest loss during production. Fortunately, drought is seldom a yearly event and its effect shows seasonal and spatial variation (Passioura 2007). At micro-regional level, drought is never uniformly distributed and its effect on common bean varies depending on the frequency, duration, and intensity of drought and the growth stage of the crop at which the stress occurs (Ramirez-Vallejo and Kelly 1998). The effects of drought stress on common bean have been well documented and include tissue specific plus whole plant effects (Acosta-Gallegos and Kohashi-Shibata 1989; Acosta-Gallegos and Adams 1991; Muñoz-Perea et al. 2006; Beebe et al. 2010). Drought stress can cause flower abortion, pod dropping and reduced seed filling (Masaya and White 1991). Overall biomass and seed yield, harvest index, number of pods and seeds, seed weight, and days to maturity are also affected (Nielsen and Nelson 1998; Ramĩrez-Vallejo and Kelly 1998). In addition, P uptake, N concentration and fixation are reduced under drought (Serraj and Sinclair 1998; Guida dos Santos et al. 2004).

Common bean is perceived by many farmers and development agencies as a food security crop because of its short life cycle in comparison to crops like maize. This short life cycle allows common bean to yield well with short rainy seasons compared to maize which often fails if rainfall is deficient in the middle or end of the season. However, even in so-called normal seasons, common bean can suffer moderate to severe water deficits since its production is mainly rain-fed and is affected by micro-climates which leave some areas with less rainfall than others at some time during the cropping season (Rao 2001; Amede et al. 2004).

Moreover, the drought problem is further escalated by rising temperatures linked to climate change, soil fertility decline, and land holding subdivision and shrinkage due to population pressure (Funk et al. 2005). In addition to this complexity of drought, the variation of farmers' perceptions of environmental constraints and farmers' preferences for grain types from region to region, combined with possible trade-offs represents a problem for the design of breeding programs. While culinary criteria for home consumption are demanding in terms of consumer traits, grain quality standards for market tend to be even more stringent (Beebe et al. 2010).

Therefore, a common bean breeding program not only should consider production constraints but also must respond to preference criteria that determine acceptability both at home use and in the market.

The state-of-art-of breeding common bean for drought tolerance

What traits to consider, how to measure a trait, when and where to measure and how to incorporate these traits in the preferred background and understanding their mechanism of inheritance have been central elements in common bean drought tolerance breeding. Research on drought tolerance in common bean traces back to the 1910s. Freeman (1912) and Currence (1928) reported drought tolerance in white pinto beans and tepary beans. Babb et al. (1941) studied drought tolerance on snap beans in the 1930s. The evolution of drought tolerance research at international level in CIAT (Centro Internacionale de Agricultura Tropical, Cali, Colombia) started in the late 1970s with the coincidence that lines selected for other traits expressed good adaptation to drought (White 1987). At that moment, breeding for drought tolerance was a wild idea and not acceptable for the scientists of that time (Jones et al. 2007). BAT 477, A195 and San Cristobal 83 were identified as drought tolerant lines in field screening from lines bred for other traits (White 1987). A systematic and organized international effort on drought breeding started in the 1980s with root traits and genetics studies. These studies identified deep rooting as mechanism of tolerance (Sponchiado et al. 1989) and additional new sources of drought tolerance from common bean race Durango (Rao 2001). The work continued in the 1990s on combination of sources for higher tolerance and looking for other mechanisms of tolerance. Rao (2001) reported greater (re)mobilization of photosynthates to seed under drought stress as additional mechanism of tolerance. These mechanisms are genetically determined although sensitive to local adaptation in common bean (Beebe 1998; Beebe et al. 2010). Drought tolerance in this study is generic term that combines all plant’s adaptation strategies to drought stress.

National Agricultural Research Institutes (NARI) and Universities in sub-Saharan Africa have been involved in some aspects of common bean drought research since the 1970s (PM Kimani, personal communication, 2007). For instance, studies on the effect of drought in common bean started in 1970 with the Bean-Cowpea Collaborative Research Support Program (CRSP) at the University of Nairobi. Since 1980, regional common bean networks engage in screening of genotypes for drought tolerance (Amede et al. 2004). In May 1999, a working group on drought named “Bean Improvement for Water Deficit in Africa” (BIWADA) was formed by regional common bean scientists in the ECABREN (East and Central Africa Bean Research Network) domain. BIWADA focused on germplasm screening and development of screening techniques. BIWADA activities were then implemented by NARIs in ECABREN member countries. Drought research in common bean in Ethiopia dates back to the late 1980s. Abebe et al. (1998) reported identification of drought tolerant lines from trials conducted at Melkasa from 1988 to 1992. Most recently breeding lines conferring drought tolerance from crossing local landraces with exotic sources were reported from the Awassa Agricultural Research Center (Asfaw et al. 2007).

Up to the end of the 1990s, many breeding efforts focused on looking for mechanisms of drought tolerance and broadening the germplasm base, neglecting other varietal traits of user preference (Singh et al. 2001). As of 2000, pyramiding of drought avoidance deep rooting traits and photosynthate (re)mobilization traits in commercially preferred seed types, QTL identification, gene tagging, and development of easy to use selection protocols draw the attention of breeders and physiologists at CIAT and the NARS (National Agricultural Research System) (SE Beebe and IM Rao, personal communication, 2008). But, the varietal development program for drought tolerance in common bean has progressed very slowly due to little understanding on the inheritance and physiological mechanism of drought tolerance, lack of high throughput drought phenotyping facilities and lack of reliable techniques for screening, the fact that the main focus in common bean breeding was on biotic stress, lack of staff with desired level of experience, low funding for drought research in the past and the negative image of drought research - generally considered a difficult area among scientists.

Nevertheless, an increase in yield potential under drought stress has been possible through breeding for sub-optimal rainfall conditions (Beebe et al. 2008). Furthermore, quantitative trait loci (QTL) analysis of drought tolerance traits in common bean has shown that some of the traits are heritable and initial studies in marker assisted selection of drought tolerance have been conducted (Schneider et al. 1997). Drought tolerance also affected yield potential under low soil fertility conditions indicating that common bean researchers need a better understanding of the genetic control of the traits that contribute to yield formation under drought stress conditions.

Rationale of the study

The potential advantage of drought tolerant varieties for vulnerable and risk-averse smallholder farmers in harsh environment is self-evident. Drought tolerance, as a trait to contribute to food security of many smallholder farmers in harsh environments, must be expressed in terms of increased grain yield under field conditions and combined with traits that correspond with the preferences and needs of farmers. But, drought tolerance is a genetically and physiologically complex trait for which plants have developed a range of strategies to balance the need for growth and reproduction at one hand and tolerance to adverse conditions on the other hand. Genetically, drought tolerance is a quantitatively inherited trait with a low heritability, showing limited genetic variation. In the harsh environments where the majority of smallholder farmers in developing countries grow their crops, mechanisms of drought tolerance are physiologically complex whereas drought is interacting with other stress factors, like high temperature, low soil fertility, soil acidity, salinity, diseases and pathogens. Therefore the expression of drought tolerance is affected by a high genotype-by-environment (G×E) interaction. As a result valuable drought tolerance can be masked by poor adaptation to a specific environment (Beebe 1998; Beebe et al. 2010).

This complexity in drought environments and the range of mechanisms that plants use for drought adaptation make it hard to identify strategies and selection criteria that contribute to high and stable yield under drought stress and non-stress conditions.

Breeding for drought tolerance is the main and most important goal for common bean programs in Ethiopia, the Pan-African Bean Research Alliance (PABRA) and the International Center for Tropical Agriculture (CIAT). The programs face challenges to fully understand the genetics and physiological mechanisms of drought tolerance and to incorporate traits related to drought tolerance in varieties that are attractive to bean farmers to be grown in their diverse environments. Preferred grain types grown by farmers vary in size, shape and color from region to region depending on culinary, market and cropping system requirements. The preferences in terms of culinary criteria for home consumption may be demanding in particular regions whereas grain quality standards for specific markets tend to be even more stringent (Beebe et al. 2010). This means that the complexity of drought tolerance as a trait and the diversity in environments and preferences, combined with possible trade-offs between drought tolerance and farmers' preferences, represent huge problems for the design of breeding programs. On the other hand, it is also essential to expose farmers with their specific ideas about quality to material that might be less suitable for consumption but much more yielding in their stressful environments thus creating awareness of the trade-offs and the consequences of the choices they either explicitly or implicitly make.

Understanding the mechanisms of drought tolerance, of the diversity of farmer's preferences and of their socio-economic and agro-ecological environments is an essential component in the development of a breeding strategy. Developing common bean varieties with increased levels of drought tolerance for such diverse users and conditions thereby represents an exemplary complex case in which an integrated plant breeding approach is needed. Such an approach should integrate physiological and genetic understanding of drought tolerance in the crop with identification of suitable germplasm, and decentralization and participation of farmers. But how to do so in practice is the challenge addressed in this thesis. The research in this thesis explores a range of methods to capture various aspects of the diversity in environments and farmer preferences, level of diversity in germplasm resources from harsh environments, detailed physiological and genetic understanding of the impact of drought stress on growth and reproduction in order to develop common bean varieties for smallholder farmers in southern Ethiopia.

Overall aim and objectives

The study explores the opportunities and potentials to integrate plant physiology, genomics and farming system perspectives in common bean breeding for variable environments of southern Ethiopia that are characterized by frequently occurring droughts, and where climate change is expected to have a major impact on the frequency and intensity of drought spells.

The specific objectives of this study were:

1. To describe variation in variety and seed management practices of farmers and changes that are related to drought stress and climate change.
2. To examine the genetic diversity and genetic relationships among and within landraces in the East African Highlands in relation to the Andean and Mesoamerican genepool using micro-satellite marker analysis combined with morphological evaluation.
3. To genetically analyze mechanisms of drought tolerance in common bean using a molecular breeding approach.
4. To explore a range of methods to capture farmer preferences and selection of advanced drought-tolerant genotypes.
5. To discuss integrating and emerging issues that contribute to the integrated plant breeding approach for drought tolerance in common bean.

Research design and process

Research design

The design of this research was a mixed-methods study ranging from laboratory to field level analysis and participatory experimentation. The study used a combination of quantitative and qualitative methods deploying both biological and social science tools for data collection, generation and analysis (Fig. 1). It used molecular marker tools to uncover genetic diversity in East African common bean germplasm and to dissect drought tolerance in common bean into component traits based on physiological and agronomic aspects of coping mechansisms. It used participatory tools to assess social aspects of drought tolerance in common bean farming systems and to adapt advanced drought lines to farmer conditions. The field study encompasses participation of farmers, breeders and extension agents to understand the implicit and explicit perferences of farmers via exposure to new diversity, interviews and observations.

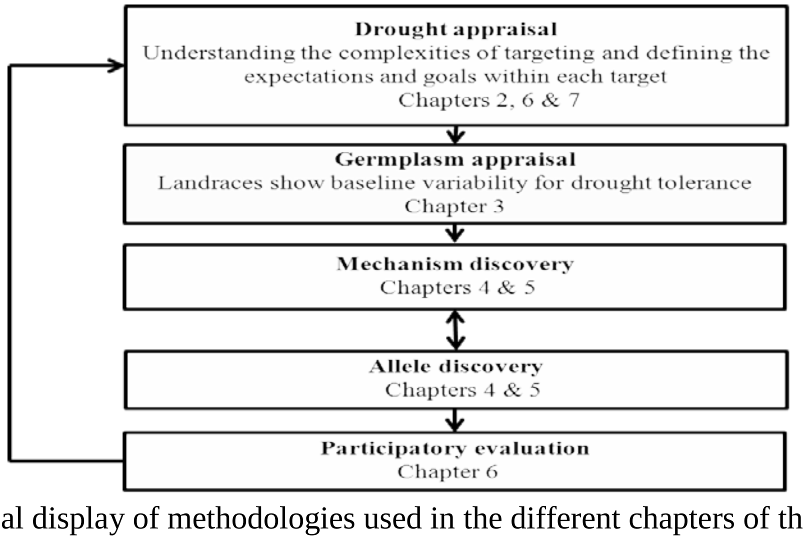

Fig. 1 Graphical display of methodologies used in the different chapters of the thesis

Description of field study sites

This study used different sites in two continents for field studies. CIAT Palimra, the Colombian site in Latin America, was used for greenhouse soil cylinder root study, morphological analysis of landraces and field drought phenotyping of recombinant inbred lines (RILs). Field site Kasinthula, Malawi in southern Africa and Awassa and Amaro, Ethiopian sites in East Africa, were used for field phenotyping of recombinant inbred lines (RILs) for quantitative trait loci analysis. More detailed field drought phenotyping of the RIL population was carried out at Palmira and Awassa sites. In addition, studies on farmer's varieties and seed management practices such as plant type selection, seed saving, and exchange practices, their associated knowledge and social relation, and participatory trials with advanced drought-tolerant genotypes were carried out in the southern region of Ethiopia.

Southern region (South Nation, Nationalities and Peoples' regional state) of Ethiopia is one of the major common bean production areas in Ethiopia. It is located in the southern and south-western part of Ethiopia between 4°43' – 8°58' N latitude and 34°88' – 39°14' E longitude. The region is a multi-ethnic society consisting of 56 ethnic groups with their own distinct geographical locations, languages, cultures, and social identities. The ethnic groups are categorized according to a language taxonomy as the Omotic, Cushetic, Nilo-Sahara and Semitic. Among them, the Omotic and Cushetic are the most populous and diversified with the largest area coverage (BoFED 2008). Based on ethnic and linguistic identities, the region is divided into 13 zones and 8 special districts. The 13 zones are subdivided into 126 districts which, in turn together with the 8 special districts, are subdivided into 3594 rural and 355 urban kebeles (the smallest administrative unit of the country).

The studies on farmer's variety and seed management practices and participatory variety trials were focused on seven rural communities in the region. Two rural communities were in Sidama zone (Awassa zuria and Boricha), one each in the Amaro and Konso special districts (woreda), one in the Gamo-Gofa zone (Gofa), one in Dawro zone (Loma) and one in the Gurage zone (Inseno) based on ecological marginality to drought and relative importance of common bean in terms of utilization and area planted, and ethnicity. Detailed descriptions of the study sites are presented in the respective chapters.

Diagnostic methods

Different diagnostic methods were employed for undertaking the study.

Focus group discussions. First, discussions with focus group composed of a mixture of people of different gender, ages, resources, and know-how on common bean were employed in four of the seven sites namely Amaro, Boricha, Konso and Loma in May/June 2008. At each of these four sites, three separate discussions were held to cover the different locations within the community. Each focus group discussion started with a minimum of 10 participants but in the process the number would increase, up to 30 in some cases, with curious volunteers from surrounding farms joining.

Contact farmer interview. The group discussion was backed up by detailed interview with contact farmers (two each at Amaro, Boricha and Konso), who were visited on a regular basis during the study period. Contact farmer interview assessed weather risk and adaptation strategies in common bean farming, details about varieties, seed selection and overall variety and seed management practices.

Individual household interviews. These were done at household level with purposively selected common bean farmers and captured the opinion of up to 375 farmers as data point for bean production, variety and seed management practices, valuation of bean variety traits, coping strategies to deal with drought stress on common bean and perceptions on genotype × environment interaction.

Genotyping

Different marker systems were used for genotyping. For molecular level diversity assessment, fluorescently labeled microsatellites representing cDNA-based and genomic markers with high polymorphism were used (Blair et al. 2009). In the molecular dissection of the mechanisms of drought tolerance the markers used included random amplified polymorphic DNA (RAPD) primers (Operon Technologies Inc., Alameda, CA), amplified fragment length polymorphisms (AFLP), and simple sequence repeats (SSRs) or microsatellites. Experimental procedures of genotyping for RAPD, and AFLP markers were as described in Blair et al. (2006) and Muñoz et al. (2004), respectively. The procedure for SSR markers was the same as described in Blair et al. (2003, 2008).

Phenotyping

Morphological trait measurements on landraces. Morphological variables were measured on plants raised in both the greenhouse and the field at CIAT headquarters in Palmira (1,000 m altitude, mean growing temperature 24^{o}C, Mollic soil), Colombia in 2007. In the greenhouse, plants were grown from August to October 2007 in plastic pots carefully packed with 5 kg of field soil from Palmira mixed with river sand in a 2:1 w/w (weight-by-weight) ratio. For field evaluation, plants were planted in November 2007. Morphological variables were assessed as per CIAT (1987) and Singh et al. (1991) protocols. Further details are provided in the respective chapters.

Greenhouse root cylinder dry-down experiment. For root and shoot traits phenotyping, a greenhouse study was conducted at CIAT (3°29’N and 76°21’W) located in Palmira using a natural Andisol from the common bean growing region of Darién, Colombia mixed with river sand (2:1 w/w). The plant material included 97 recombinant inbred lines (RILs) from a DOR364 × BAT477 cross along with 1 drought tolerant check (SEA 5) and 2 parents (DOR364 and BAT477). Pre-germinated uniform seedlings of each experimental genotype were planted in a closed-bottom transparent plastic cylinders each of which was inserted into PVC sleeve-tubes arranged in a row and column design and replicated three times. Plants were grown for 48 days in these plastic cylinders/PVC sleeve-tubes from October to

December 2007. Two contrasting water supply treatments were applied, namely 1) well-watered (WW) at 80% field capacity and 2) progressive water stress (WS) with no watering after 10 days of growth to simulate progressive water stress conditions similar to terminal drought stress conditions of some bean target environments in the tropics. Plants were harvested at 48 days after transplanting, equivalent to 38 days of withholding of water application in case of water stress treatment. Details on plant culture, data recording and analysis are given in the respective chapters.

Field managed drought stress experiment. Field experiments for phenotyping of drought tolerance were conducted at Palmira, Colombia in 2007, at Awassa Agricultural Research farms in Awassa and Amaro, Ethiopia and at Kasinthula, Malawi in 2009. The same experimental genotypes as those of the greenhouse root cylinder dry-down evaluation were used for drought tolerance phenotyping in the field. Drought stress was imposed by altering sowing dates (early versus late) during the main cropping season (‘*Belg*’ at Amaro and ‘*Meher*’ at Awassa) exposing the plants to higher rainfall with early planting and terminal drought with late planting. At Kasinthula, Malawi and Palmira, Colombia irrigated and rainfed experiments were carried out. A 10 × 10 triple lattice treatment design was used. Details on experimental procedure and data collection are given in the respective chapters.

Field experiments for participatory evaluation

Two types of participatory variety selection (PVS) trials were conducted, (i) researcher-managed on-station trials and (ii) farmer-managed on-farm trials. Researcher-managed on-station trials were planted in Amaro, Awassa, Gofa and Inseno, whereas farmer-managed on-farm trials were conducted in Awassa zuria, Boricha, Amaro and Inseno, southern Ethiopia. The PVS trials were carried out from June 2009 to December 2010. Germplasm used for selection included 38 diverse genotypes in 8 grain classes that included 34 advanced drought-tolerant lines of CIAT, 2 locally bred varieties from Awassa Research Center, 1 released variety from previous participatory plant breeding (PPB) activities and a farmer variety as local check. The PVS trials were selected and evaluated by farmers, breeders and extension agents. The on-farm PVS trials were hosted by farmers who were identified in consultation with extension agents, the peasant association (kebele = smallest administrative unit of the country) leaders and the researchers’ own contact developed through previous on-farm research. Also evaluator farmers were identified and invited in consultation with on-farm trial host farmers, extension agents and kebele leaders. All farmers hosting the on-farm PVS trials as well as farmer evaluators were experienced common bean producers in the locality. The evaluation characteristics of common bean varieties were some of the farmers’ selection traits previously identified in group discussions and individual interviews. The evaluation traits and procedures were explained to farmer-evaluators orally and with aid of plants in the field. Farmer-evaluators discussed each common bean variety trait in a freely interactive manner with the researcher and among each other until each evaluator fully understood how (s)he was going to rate each common bean genotype based on the scale. Apart from rating, evaluators were also asked to indicate whether each new drought tolerant line was worse, same or better compared with the local check in overall performance. The evaluators were also asked to select the common bean lines they would grow next season if they would get seed. Farmers used their own judgment to rate for the criteria and to select or reject the materials with no interference of the researcher. For on-station PVS evaluation and selection, interested farmer-evaluators were invited on-station. Likewise, in respective on-farm trials evaluator-farmers from surrounding farms were mobilized to evaluate and select the new bean types planted in their village. Those lines that were selected by trial farmers and evaluators were advanced to next season planting. The selections and evaluations were done when the crop was close to physiological maturity and also after threshing for four consecutive seasons. The grains of each line from previous year's harvest were present in transparent plastic bags at time of selection and evaluation at physiological maturity, to give the farmers options of selection and evaluation for seed characteristics. After threshing farmers gave scores for yield, seed characteristics and marketability and of course made their final refined selection decision. In addition grain yield (kg/ha) data were taken for each trial by the researcher. Details on experimental procedure and data analysis are given in the respective chapters.

Organisation of the thesis

This first chapter gives an introduction to main issues: crop breeding and the smallholder farmers in harsh environments, the challenges of common breeding for harsh environments, breeding concepts and methodologies used to improve drought tolerance in common bean, the need for drought tolerance as a trait and challenges to incorporate drought tolerance in common bean varieties grown by farmers, as well as problem definition, study objectives, research process and design. Chapter 2 provides the contextual analysis of farmers' varieties and seed management in common bean production in southern Ethiopia. It discusses social aspects of drought tolerance in the common bean farming system in the face of drought and climate change. Chapter 3 analysis population structure and diversity of common bean landraces from the East African Highlands using morphological phenotyping and microsatellite marker genotyping. This chapter answered questions related to whether variation in and between landraces exists in landraces from the East African Highlands and if it exists, whether the variation is the result of the original differences between the various introductions or whether it results from a continuous process of natural hybridization and selection by farmers and by the environment. Chapter 4 analyzes the molecular dissection of drought tolerance in common bean using root drought avoidance traits. Chapter 5 describes effects of drought stress on yield formation and traits contributing to yield formation under drought stress in common bean. It also identifies QTL for photosynthate acquisition, accumulation and (re)mobilization traits using recombinant inbred lines grown in eight different field environments across two continents. Chapter 6 deals with opportunities and possibilities of integrating scientist and farmer criteria for better targetting development of varieties with drought tolerance for smallholder farmers. Chapter 7 provides a synthesis on common bean breeding for drought and smallholder farmers.

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CHAPTER 2

Farmers’ variety and seed management and the challenges of drought and climate change in common bean farming in southern Ethiopia

This chapter is based on the article: Asfaw A, Almekinders CJM, Struik PC, Blair MW, Farmers’ variety and seed management and the challenges of drought and climate change in common bean farming in southern Ethiopia. Submitted *to Plant Genetic Resources.* The authors would like to thank the farmers of southern Ethiopia for their participation in this study and for sharing their knowledge on the different aspects of variety and seed management. We owe much gratitude to the field research assistants who helped to interview farmers and facilitated translation during group discussions. The authors acknowledge the support of NUFFIC fellowship to Asrat Asfaw and the Tropical Legume (TL1) project from the Bill and Melinda Gates Foundation. Thanks also go to Awassa Agricultural Research Center of Southern Agricultural Research Institute and the offices of agriculture at respective districts that helped during field work.

Abstract

This study reports the various elements and context that characterize the farmers' use and management of common bean seed and varieties in southern Ethiopia. The study used focus group discussions, contact-farmer interviews and surveys. The results demonstrate that farmers' cropping systems and preferences vary strongly. Moreover, the high level of environmental variation and the associated risks of crop failure have increased even more with the unpredictability of rains. While farmers are aware of climate change, only about half of them have adapted some of the cropping practices. At the same, the markets offer different opportunities and common bean production expands in areas at slightly higher elevations. In these conditions, common bean production is increasingly important for farmers. They currently manage only modest levels of common bean crop diversity. Farmers variety and seed management practices do not show a high level of specialization and at the same time the use of off-farm seed sources is relatively high. This situation provides opportunities for strategic development and introduction of common bean genetic diversity. Earlier maturing, more drought-tolerant common bean diversity for a range of conditions, markets and preferences can only be developed on an integrated understanding of farmers production conditions and existing seed system practices.

Introduction

Farmers’ variety and seed management practices are based on farmers’ experiences built over a lifetime. They have been the core elements of agricultural development since the domestication of crop plants and continue to be important for the future of agricultural crops (Harlan 1992). In highly variable and marginal environments, varieties tend not to be formally improved and seed is reproduced in the informal system. In such farming systems crop genetic diversity co-evolves with the social, economic and environmental context (Almekinders et al. 1994; McGuire 2007). There is growing interest among scientific communities and policy makers to understand how farmers adapt to such changing contexts in general and how the formal sector can support farmers to cope with the changing climate.

In general farmers are considered knowledgeable about their crops and their environment (Harlan 1992; Cleveland and Soleri 2007). Their variety and seed management is the result of a complex interaction between social-economical and agro-ecological factors in which new and old technologies are continuously assessed and appropriated (Almekinders et al. 1994; Dyer and Taylor 2008). Farmers’ selection practices in traditional farming are often effective in achieving goals of adaptation and genetic gain in terms of yield but also in terms of maintaining ritual, culinary and market traits (Cleveland and Soleri 2007). Farmers are often found effective in maintaining varietal ideotypes and changing or creating more preferred genotypes or variants in the informal seed system (Berthaud et al. 2001; Soleri and Cleveland 2001; vom Brocke et al. 2002; Nuijten et al. 2009). Specialized farmer-selectors are regarded as potential partners in participatory plant breeding (Almekinders and Elings 2001; Cleveland and Soleri 2007; Abay et al. 2008). However, it is not only the specialized farmers who make up an informal seed system: most farmers engage one way or another in seed production, seed diffusion and in design of new farming systems simultaneously (Offei et al. 2010). Hence, to design effective breeding strategies and understand opportunities to support farmers in mitigating the effect of climate change, the sum of all farmers’ actions matters in the farmers’ seed system (Dyer and Taylor 2008). Little, if any, is known when this comes to common bean farmers’ seed selection in southern Ethiopia.

East Africa in particular and Ethiopia especially are regions projected to be at risk from effects of climate change (Jones and Thornton 2003; Funk et al. 2005). Common bean is the principal food-security legume in this part of Africa, providing dietary protein and source of cash income for resource poor farmers (Wortmann et al. 1998; Broughton et al. 2003). The crop is believed to have been introduced together with maize via the east coast of Africa by Portuguese and Spanish traders in the 16th and 17th century (Greenway 1945; Gentry 1969). Since then common bean farming has been primarily shaped by farmers’ physical, climatic and social factors. These have resulted in a range of morphologically and genetically diverse landraces (Wortmann et al. 1998; Asfaw et al. 2009; Blair et al. 2010).

The formal common bean breeding program in Ethiopia has drought tolerance as a main and most important goal. The program faces the challenge to incorporate this trait in varieties that are adapted to be grown by common bean farmers in the multiple environments. Understanding the diversity of farmers and their socio-economic and agro-economic environments is therefore an essential component in the development of a breeding strategy.

This paper reports on farmers' variety and seed management practices such as plant type selection, seed saving, and exchange practices, their associated knowledge and social relation in southern Ethiopia. It places these practices in the context of farming systems that are diverse and prone to drought, and in which common bean (*Phaseolus vulgaris* L.) is the major food staple along with maize (*Zea mays* L.) and enset (*Ensete ventricosum* (Welw.) Cheesman). It looks in particular into aspects related to climate change: farmers' perception on drought, tendencies in the rainfall and practices to cope with the changes. It discusses the implications for breeding strategies.

MATERIAL AND METHODS

The study area

Southern region (South Nation, Nationalities and Peoples' Regional State) is one of the major common bean production areas in Ethiopia. It is located in the southern and southwestern part of Ethiopia between $4^{\circ}43'$ and $8^{\circ}58'$ N latitude and between $34^{\circ}88'$ and $39^{\circ}14'$ E longitude. The region forms a multi-ethnic society consisting of 56 ethnic groups with their own distinct geographical locations, languages, cultures, and social identities. The ethnic groups categorize according to a language taxonomy as the Omotic, Cushetic, Nilo-Sahara and Semitic super-language families. Among them, the Omotic and Cushetic are the most populous and diversified and cover the largest area (BoFED 2008). Based on ethnic and linguistic identities, the region is divided into 13 zones and 8 special districts (Fig. 1). The 13 zones are subdivided into 126 districts which, in turn together with the 8 special districts, are subdivided into 3594 rural and 355 urban kebeles (the smallest administrative unit of the country).

Subsistence-oriented rain-fed agriculture is the mainstay for about 90% of the region's population. The individual farm-land holding is very small and highly fragmented with 9% of the households having less than 0.1 ha, 46% having 0.1 - 0.5 ha, 26% having 0.51 - 1.0 ha, 15% having 1.1 - 2.0 ha and only 4% having more than 2.0 ha (BoFED 2008). Crop farming typically depends on hand or ox tillage and use of very few external inputs. The landscape is characterized by plain, mountainous and undulating terrain with an altitude ranging from 376 to 4207 m above sea level. These conditions endowed the region with diversity of agro-climate and large variety of crops. The diversity of people is therefore associated with a large diversity in the agro-ecological and social settings. People endure problems of recurring drought, low soil fertility, biotic stress, and continued shrinkage of landholding due to population pressure.

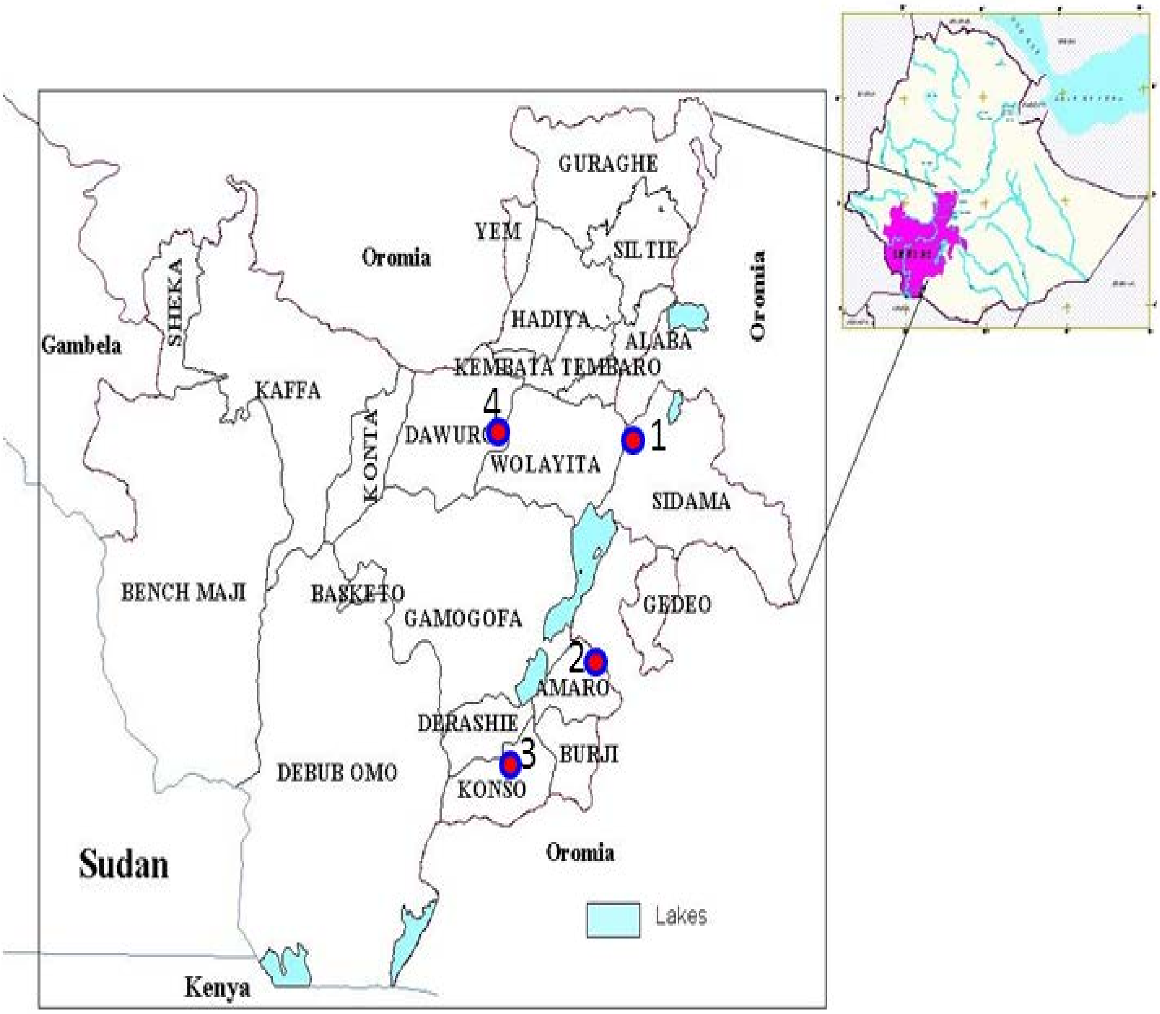

Fig. 1 The study sites in the South Nation, Nationalities and Peoples Regional State, Ethiopia. 1 Boricha, 2 Amaro, 3 Konso and 4 Loma

Common bean is grown in all 13 zones and 8 special districts in the region, in an altitude range of 1000-2200 m above sea level. Its production is concentrated in the Rift Valley where it grows from hot to warm sub-moist up to cool mid-elevation areas. This study focused on four sites in four districts of the region (Figure 1). The selection of the sites is based on the importance of common bean production and the marginality to drought throughout the area (Table 1). Boricha in Sidama zone is the most important bean production area in the Central Rift Valley part of the region. Boricha is a densely-populated mid-elevation plain with frequent intermittent droughts during the bean growing seasons (Amede et al. 2004). Amaro and Konso districts represent dry and semi-arid common bean growing ecologies constrained by terminal drought. Konso is characterized by degraded land with low soil fertility and terrace-based common bean farming. Amaro and Konso districts are located in the southern part of the Rift Valley and close to northern Kenya. Cross-border common bean trade and germplasm exchange with northern Kenya have existed for a long time (Asfaw et al. 2009). Loma district in the Dawro zone is a dry to humid mid-altitude area in the central-west part of the region where common bean is produced in a mountain and with sometimes hot conditions and intermittent droughts. The selected study sites have shown a long term downward trend in total rainfall per year and experienced a "green famine" in recent years (Funk et al. 2005).

Table 1 Physical and socio-economic characteristics of the study sites and survey farmers

Characteristics	Study sites			
	Boricha	Amaro	Konso	Loma
Area				
Altitude (m a.s.l.)	1650-1932	1305-1450	1200-2000	800-1900
Latitude (° north)	6.76-7.01	5.60-5.99	5.17-5.56	6.58-7.07
Longitude (° east)	38.07-38.39	37.54-38.01	37.01-37.69	36.93-37.43
Annual rainfall (mm)	963	927	617	1121
Total rainfall '*Belg*' (mm)	299	412	317	347
Total rainfall '*Meher*'(mm)	492	323	221	574
Major agro-ecology	Tepid to cool sub-humid	Hot to warm humid	Hot to warm humid, semiarid	Hot to warm sub-humid
Major soil type	Chromic luvisols, Eutric fluvisols	Eutric nitosols	Eutric regosols, Eutric nitosols	Eutric nitosols
Bean production system	Relay/sole	Intercrop/sole	Intercrop/sole	Relay/sole
Average bean area (ha) HH^{-1} †	0.30	0.28	0.41	0.73
Average bean yield (kg ha^{-1})	1550	750	690	1185
Importance of common bean	Cash, food	Food	Food	Cash, food
Ethnic group	Sidama, Wolayta	Koyra	Konso, Ale	Dawro
Survey farmers				
Number of respondents	104	110	90	71
Illiterate (%)	54	26	40	23
Female (%)	23	15	21	10
Average age (years)	44	40	41	44
Average family size (number)	8	7	9	8
Average land holding (ha)	0.81	1.06	1.94	1.58
Average land area share out (ha)	0.06	0.13	0.10	0.41
Average land area share in (ha)	0.18	0.16	0.04	0.43
Average land area rent out (ha)	0	0.02	0.03	0
Average land area rent in (ha)	0.04	0	0.06	0.01
% of HH land holding steep slope	9	7	21	13
% of HH land holding gentle slope	16	25	39	47
% of HH land holding flat slope	75	68	40	40

† HH household

Data collection

The study was carried out between May and December 2008. It used focus group discussions, interviews of and observations by contact-farmers throughout a season and surveys. The sampling unit in each interview session was the household.

Focus group discussions (FGD). Focus groups were composed of a mixture of people of different gender, ages, resources and know-how on common bean. Focus group discussions were conducted in May and June 2008. At each site, three separate discussions were held to cover the site’s geographical area. Each focus group discussion started with 10 or more participants who were identified and invited in consultation with extension agents and Kebele leaders. During the discussion the number would increase, up to 30 in some cases, with curious volunteers from surrounding farms. The discussions covered common bean cropping system, cropping calendar, inventory of common bean varieties grown or known in the locality, the preferred and non-preferred traits of the identified common bean varieties, perceived production constraints, perceptions on good and bad season for common bean farming and perceived changes of the characteristics of weather and common bean farming in the locality. Farmers were asked to rank common bean production constraints in order of importance using the proportional piling methodology (Mukherjee 1993). Each group received 100 common bean seeds to proportionally allocate to the constraints listed, based on their importance in causing yield loss. After calculating the rank for each group, severity of the constraints for the studies communities was determined using an index method (Smith et al. 2000). The constraints identified varied within and across study sites and resulted in ranking data of ordinal and different dimensionality. One group would identify four constraints and rank those four while another group could identify seven and rank those seven and so on. Accordingly the severity index value, s_j, for a constraint of rank r among a group of n constraints identified by group j is thus $s_j = 1+(r-1)/(n-1)$. This sets the most serious constraints across study sites ($r = 1$) to $s_j = 1.0$, the least serious constraint ($r = n>1$) to $s_j = 2.0$, and the remaining constraints assigned intermediate values between the two extremes.

Contact-farmer interviews. The group discussion was backed-up with information from contact-farmers (two each at Amaro, Boricha and Konso). Contact-farmers were farmers who stood out in the focus group discussions as most knowledgeable about the crop and the environment and who volunteered to share their knowledge. The contact-farmers were visited on a regular basis during the study period for follow-up interviews. The focus group discussion and contact-farmer interviews provided input for the farmer household surveys.

Household surveys. The surveys were held in at least three kebeles at each site and captured the opinion of 375 household heads, the majority being men (Table 1). Data were collected using a semi-structured interview on household composition, age and sex of household head, level of literacy, and a range of questions relating to the bean varieties grown and seed management practices. The survey also covered farmers’ perceptions on climate, their practices to cope with these changes and genotype × environment interaction.

Data analysis

The data collected were subjected to descriptive analysis using PASW statistics version 17, software. Sigmaplot version 10.0 (Systat Software, Inc, CA, USA) was used to develop graphs.

Results

Seasonality and cropping system in common bean farming

All study sites have a bimodal rainfall pattern that allows at least two cropping seasons in a calendar year, these being known as '*Belg*' (February – May) and '*Meher*' (June – October). The rainfall pattern is associated with a seasonal cycle of land preparation, weeding and harvesting (Fig. 2). The seasonal pattern in the region shows unpredictable and erratic rainfall variation (Funk et al. 2005). In many places, the first short rains in the '*Belg*' season are often less dependable than those of the second longer '*Meher*' season. On the other hand, in areas like Amaro and Konso with semi-arid ecology in the southern Rift Valley part of the country, total rainfall is higher during the '*Belg*' season than in the '*Meher*' (Table 1). Both cropping seasons are important for common bean: more than 85% of the surveyed farmers had planted common bean in both seasons of 2008. '*Belg'* is the major growing season at Amaro and Konso whereas in Boricha and Loma, '*Meher*' season is the more important common bean season. Farmers normally prepare the land at onset of rains.

Cropping practices in common bean production vary across study sites. In Amaro and Konso many farmers plant larger areas with common bean during '*Belg*' season when the rainfall is relatively more dependable than in the '*Meher*' season. In Boricha and Loma, many farmers plant larger common bean areas in the '*Meher*' rainy season because that is the more reliable rainy season there. During the '*Belg*' season, few farmers in Boricha and Loma plant small areas of common bean to combat hunger during May-June period when there is no other crop in the field to harvest, or to use this crop as seed source for the next planting.

In the group discussions farmers indicated that they normally grow cereal and pulse crops in sole, inter- or relay-cropping systems, and combine these with livestock rearing. Fifty-two percent of the survey farmers practiced more than one cropping system in the year 2008. Of the surveyed farmers 64, 50 and 38% practiced sole-, inter- and relay-cropping, respectively. In the '*Belg*' season, farmers usually practiced sole cropping of common bean or intercropping of common bean with maize or sorghum while in the '*Meher*' cropping season, common bean was often planted as a sole crop or as a relay crop with maize or sorghum. Common bean area under sole, intercrop and relay-cropping was 43, 26 and 31%, respectively, averaged over seasons and sites.

In the practice of a system with different crops, the sequence of sowing dates for each crop and variety is decided on the basis of the crops' growing period and labour availability. Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) are the major cereal crops which require longer growing seasons than common bean; consequently farmers prioritize planting maize and sorghum at onset of rains followed later by the sowing of common bean. This practice usually exposes common bean to water deficit during critical flowering and grain filling stage when the rains terminate prior to pod development. The water deficit problem is intensified when the May dry-spell is longer during '*Belg*' season in Boricha and Loma and rainfall is limited in the short ‘*Meher*' season in Amaro and Konso. Apparently farmers sacrifice the pulse crop for the more critical cereals which make up much of their diet. However, farmers in this study indicated that common bean can suffer moderate to severe water deficits, even in so-called normal seasons.

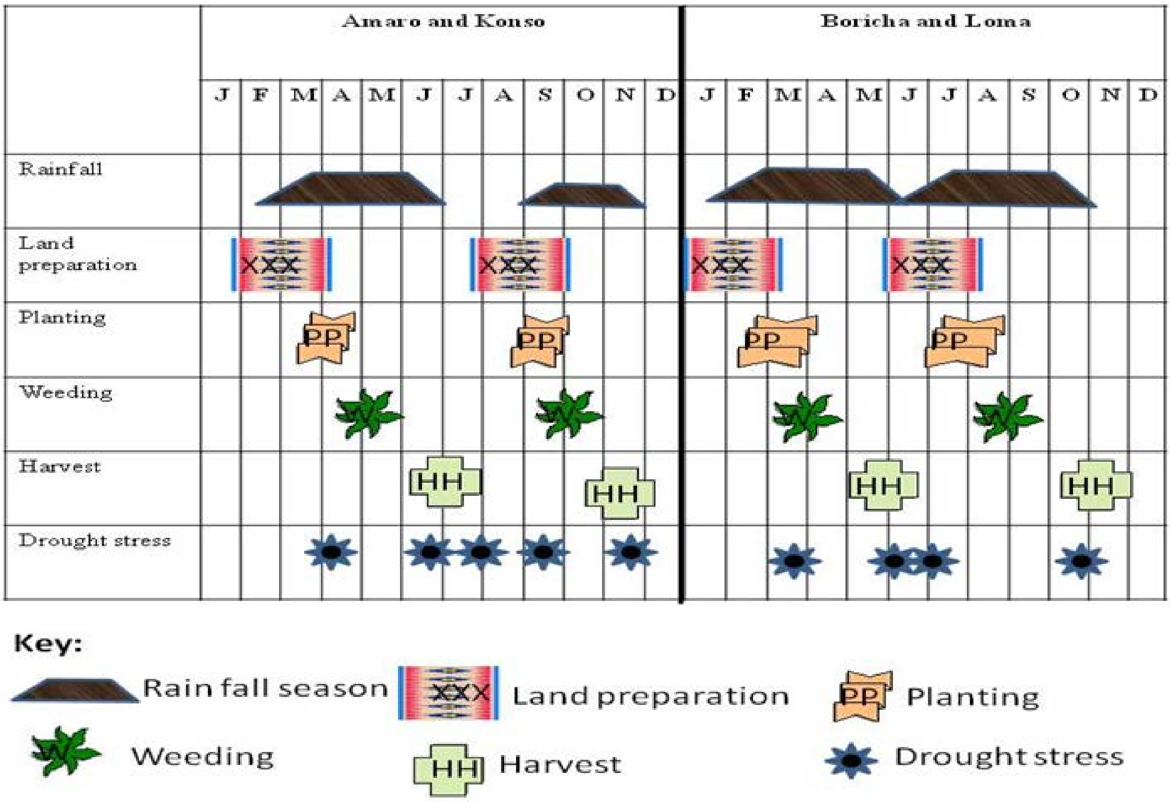

Fig. 2 Common bean cropping calendar in the southern region of Ethiopia according to farmer participants of focus group discussions

Varieties planted, their differentiation, distribution and origin

The survey indicated that 88% of the area planted with beans in 2008 was covered by farmer varieties whereas only 12 % was planted to improved varieties. Of all farmers, 96.2% was growing one variety (including variety mixtures), 3.3% was growing two varieties and only 0.5% had planted three varieties in 2008, which means an average of 1.04 varieties per farmer. Approximately 90% of survey farmers grew a pure bean variety while 10% was growing bean variety mixtures in the last five years (2003-2007). The practice of growing variety mixtures did not vary between the ‘*Belg*’ and ‘*Meher*’ seasons but farmers mostly practiced growing a pure variety under sole cropping and variety mixtures under inter- and relay-cropping systems. Varietal mixtures were grown by a considerable number of farmers at Amaro while few farmers were growing varietal mixtures in Konso and Loma. In variety mixtures, farmers mix 2 or 3 different grain types, like for example small-red and black or small-red and white. In Boricha farmers did not grow variety mixtures at all. Boricha farmers rather practiced planting more than one variety in separate plots as a sole or relay crop. Of the

improved varieties adopted by farmers in the region, 78.7% grew small-white, 19.7% small-red and 1.6% the large-seeded, red-mottled, Andean bean.
Farmers usually distinguish the common bean varieties by grain types. Although the study sites varied in ethnicity, ecological marginality, distance to market and to research center, there were no distinct differences in farmers' naming practice regarding their bean varieties. In all places farmers most often used seed color and seed size in naming common bean varieties although the words differed by language. During the survey season, seven grain types were grown with varying distribution across study sites (Table 2). Among these, farmers categorized varieties of small-red, small-white and black into sub-types based on seed shape and growth habit while the remaining ones were not. The varieties varied in their yielding potential, growth cycle and specific traits. Farmers described small-red as the most preferred variety for local-dish making and for selling at the market. Small-white varieties were preferred for 'kiki' (local stew) and uniform harvest maturity. Black-seeded varieties were not liked in many places, except Konso, because of its non-attractiveness for eating and the associated low marketability. Pinto beans (carioca-type) were preferred for their earliness and thereby the possibility to escape drought stress. However, women did not appreciate pinto beans because of their long cooking time. The red-mottled (calima-type), large cream and cream-mottled (sugar type) Andean common bean varieties were liked for their culinary quality: swelling in pot while cooking, good looking dish and flavorful. Farmers perceived large-seeded Andean and small-white varieties as performing well in sole cropping and on fertile land. Black and small-red type bean varieties were considered adapted to a wide range of production systems. Farmers stated that small-red seeded varieties prefer moist mid-altitudes whereas black and pinto beans perform better in the dry land. Farmers described Andean varieties as sensitive to sowing-time: they have a narrow window of planting.

The relative importance of bean varieties at different study sites varied (Fig. 3). Small-red was the primary class of beans cultivated by farmers in three of four study sites followed by small-white beans. Black beans were popular at Konso and Amaro where the growing area is very marginal due to drought. Only some farmers in Boricha and Amaro had planted the large seeded Andean type beans. Farmers identified three grain types in the Andean type, namely the red-mottled, cream-mottled and large cream beans of which red-mottled was dominant (data not shown). Medium-seeded pinto beans were exclusively grown by farmers in Amaro. The relative importance of bean varieties expressed as area planted showed large similarity to the importance expressed as percentage of farmers.

Farmers in Amaro and Konso prefer varieties with determinate growth habit for intercropping whereas in Boricha and Loma farmers like indeterminate varieties for relay-cropping. In the extremely dry and marginal environment of Konso, farmers planted black beans because of their moderate drought tolerance compared with other bean types. In areas receiving relatively better rainfall within Konso, farmers prefer growing small-red bean varieties for which there is a specialized seasonal grain market at Karate town in Konso. This market gets most of its

Table 2 Farmers' description of major bean varieties in relation to preferred and non-preferred traits elicited during focus group discussion

Type	Small-red	Small-white	Black	Pinto	Red-mottled	Large cream	Cream-mottled
Subtype	Light & dark red color	Round & flat seed shape	With & without tendrils	No	No	No	No
Description	Small-seeded with tendrils	Small-white	Small seed size and dull	Round shaped, medium sized, with stripes, growth habit with tendrils	Erect plant structure, thick stem, large seed size, calima type	Large seeded, long & slender seed shape, without tendrils	Large seed size, cream-mottled sugary type, with tendrils
Vernacular name	Dume (B), Engliz (L), Sora (A), Atima (K)	Wajo (B), Fillo (L), Boste (A), Atta (K)	Kolisho (B), Asimada (L), Manna (A), Abura (K)	Alga (A)	Logoma (B), Torgonia (L), Zorbo (A)	Sallo (A)	Logoma (B), Torgonia (L), Siroendo/ Zorbo (A)
Preferred trait	High market preference, preferred for local dishes, yields well, good for relay and sole cropping, well adapted to local conditions	Matures fast and uniformly, good for 'kiki' (local stew), good for sole cropping	Yields well, tolerates drought, disease & weevil, tastes good, adapted to all cropping systems	Matures fast, escapes drought, tastes good, gives less flatulence	Medium maturing, tastes like meat, fast to sell and good market price, good for fresh-grain consumption	Matures fast, tastes good, preferred for intercropping and fresh-grain consumption	Tastes good, cooks fast
Non-preferred traits	Does not tolerate drought, matures late, susceptible to disease and weevil, not suitable for intercropping, lacks uniform maturity, flatulence problem	Does not tolerate drought, susceptible to disease (rust) & weevil, low yield, requires fertilizer, shattering is a problem, not preferred in local dishes like '*nifro*'	Not attractive to eat, no market demand, late maturing, high flatulence	Low yield, not good for inter-cropping develops tendrils, lacks uniform maturity, does not cook fast	Low yield, does not tolerate drought and weed infestation, very susceptible to disease and weevil attack (seed storage is a big problem), prefers fertile soil	Does not tolerate drought, low yielding, susceptible to disease & weevil (seed saving is a big problem)	Very susceptible to drought, disease and weevil, matures late, yields are low
Growth cycle (days)	85-95	80-85	90-100	70	75-80	60-70	>90
Yield with good rain (kg/ha)¥	1267	896	929	844	865	600	683
Yield with bad rains (kg/ha) ¥	468	381	372	434	383	300	255

¥ farmer estimated yield, *A* Amaro, *B* Boricha, *K* Konso, *L* Loma supply from Gawada and relatively well-watered surrounding bean growing areas in Konso and is destined to northern Kenya via boarder town Moyale. Near Karate town in Konso farmers wished to grow small-red bean varieties for cash income but the marginal environment did not allow them to grow anything else but the black beans. In the Central Rift Valley near to the export market of Addis Ababa, farmers planted the small-white Ethiopian export class. In places like Boricha and Loma, where small-red beans were highly marketable, farmers' preference was mostly for this type while they preferred the large seeded Andean bean varieties for home consumption.

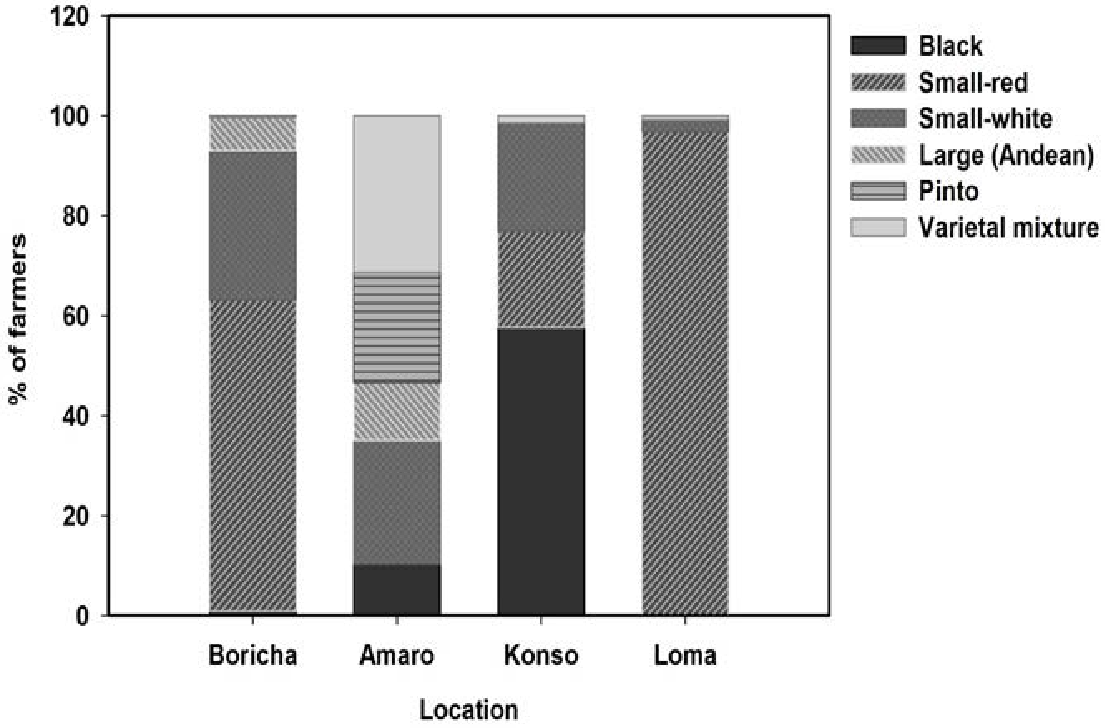

Fig. 3 The relative importance of common bean grain types by percentage of farmers growing the varieties in year 2008 in the region. Data of Andean varieties were pooled and areas planted in *Belg* and *Meher* are accumulated

In all study sites, farmers told that black beans were very old and probably the first introduced bean variety in the region. They said their forefathers used to grow black beans whereas the other grain types were introduced more recently. In six FGD in two of the four sites, there exists an anecdote of the rulers of the North to be served (black) beans at dinner on one of their tax collection missions. They liked the taste of new food they were exposed to and asked for the name. The local people gave the local name for the beans, but their guests replied that the common beans tasted like 'field butter' (***ye ehile kibe***). In the middle of the night the guest had stomach discomfort and became very much disappointed with the bad nature of the tasty food they had been served at dinner. Again they asked local people its name. The local people again replied "beans" in the local language. The guests replied that this crop was 'the most wild of all the crops' (***ye ehile awure***). Farmers say that this proves that beans were there before the northern rulers came and brought maize and teff (*Eragrostis tef* (Zucc.) Trotter) with them. Nor did the black beans come with them from other parts of the country like sorghum and cotton (*Gossypium hirsutum* L.) or the indigenous enset (*Ensete ventricosum* (Welw.) Cheesman). Rather, black beans seem to have been arrived via trading with neighboring tribes, probably from Northern Kenya.

It is likely that the introduction of black beans was soon followed by that of small-red beans. Small-red beans are known with the vernacular name '*Englize*' in Loma and in the neighboring Woylata where farmers consider it was adopted first, probably introduced by British from Kenya supporting Ethiopians in times of the Italian invasion (1936-1941). Pinto beans, specific to the Amaro area are said to have been introduced in the last decade or so from northern Kenya. Their local name '*Alga Hamare*' refers to the Alga or Burji ethnic group that is believed to have crossed the border into Kenya to visit relatives and in search for new seeds that would be adapted to the increasingly shorter growing period. The Andean cream-mottled bean varieties were probably introduced around the same time as the small-red beans. Because they are susceptible to drought, they are not widely grown and mostly as part of a variety mixture. White bean varieties are a relatively very recent introduction. They were brought during the Derge regime via extension programs related to the government export market strategy. White bean production in Ethiopia probably dates back to 1940s with the major grain and pulse export agreement between the government of Ethiopia and United Kingdom (Thodey 1969 cited in Assefa 1985). White beans are among the important export commodities of the country. White bean varieties are mainly improved varieties originating from the formal research system and exporters.

Other Andean grain types such as large cream and red-mottled are grown by a few farmers around Amaro and Boricha, respectively. According to farmers the lack of seed limits further diffusion. Large cream grain type in Amaro was a farmer introduction from the Central Rift Valley and may have been a local or formally improved variety. It is probably introduced due to its indeterminate growth habit and good fit into intercropping systems. Red-mottled beans were introduced through participatory varietal selection trials in early 2000 (Asfaw et al. 2006). Apart from the seven major grain types there are also minor grain types of varying different seed color and size that are the result of crossings within varietal mixtures. Few farmers grow this minor grain types in a variety mixtures and the off-types are not recognized or named as a variety.

Farmers' variety and seed selection practices

Farmer varieties like the small red, black and cream mottled are probably a mixture of lines. Some farmers recognize differences in seed color, seed size and growth habit within a seed lot, but they recognize it as a single variety because its components belong to a single grain type. Most surveyed farmers (71%) practiced some form of mass selection to save seed for next season. The selection intentionally or unintentionally may affect the variation of seed size, seed color and growth type of the seed lot. For example, when eliminating shriveled, under-developed or cracked seeds through winnowing, the farmers un-intentionally selected for genotypes in the seed lot with larger seed size. Among those practicing selection, 53.4% selected plants before harvest in the field, 15.3% at harvest just before threshing, and 6.9% separated seed from grain just after threshing while 17.6% practiced cleaning seed just before sowing. Nearly 7.3% of farmers combined practices of before harvest in-field plant selection and after-harvest seed sorting. Among those who practiced before harvest in-field plant selection, 29% selected from anywhere in the field, 38% selected from the best parts of the field including the border rows, 7% said they selected from any part of the field excluding border rows and 26% mentioned selecting from best part of the field excluding the border. In-field selection was mostly done by men while post-harvest seed sorting especially before planting was carried out by women and children. Seed storage was the responsibility of men in all study sites except in Konso where women managed seed storage.

When selecting seeds or plants to renew seed lots, farmers most often selected for well-filled grains, heavy pod load (large number of pods per plant), full seed color development, early maturity, absence of weevil damage (seeds not attacked by weevil), drought tolerance (plants survive drought stress and produce some seed), disease tolerance, seed size (mostly larger size preferred), clean pods (field resistance against insect pests), growth habit, immature and seed coats without cracks or other damage (Fig. 4). Primary criteria used for in-field selection were pod load and pod length, maturity, disease and insect pest resistance whereas screening seed for pest damage during storage, seed color development, grain fill, seed size, seed cracking and immature seeds was practiced at post-harvest seed selection. The use of the selection criteria was not the same across study sites. For instance, farmers practicing relay-cropping in Loma selected for indeterminate growth habit while those practicing intercropping in Amaro looked for determinate growth habit. No farmer used separate fields for producing seeds. However, some farmers at Boricha and Loma used '*Belg*' season plantings almost exclusively as a seed source for '*Meher'* plantings and seed lot renewal.

Although some farmers distinguished differences within a seed lot, farmers' knowledge on source of genetic variation was generally limited. More than half of the survey farmers (57%) reported observing off-type beans in their field sometimes. Those observing off-types considered different sources of the off-type seed: seed mechanical mixture (35%), natural occurrence (53%), or of unknown cause (12%). When observing off-types, 29% took no action and left off-types in the field, 65% would harvest them separately for sowing the following season and 6% rogued them out from the field. Those who harvested off-type seeds separately did not claim they were developing a new variety. They said to plant off-types separate in the next season and, depending on the performance, either eat it, mix the seeds with the mother seed stock again, or keep it as new variety if they see some future advantage for the line.

Some farmers at Amaro and Konso planted variety mixes to mitigate the effect of drought stress. Some farmers purchased seeds of different varieties, mixed them, and would re-select in seasons with drought stress those that survived for their variety mix next season. Other farmers bought seed lots that are mixtures. Most farmers considered early maturity as a drought escape, not as a real tolerance mechanism, but still preferred this trait under drought conditions in all the study sites. Farmers observed that early-maturing varieties were often more susceptible than late-maturing genotypes when drought came early.

Very few farmer-selectors considered strong root system and early vigour in their selection as a trait for drought tolerance. Farmers who used root pulling force resistance perceived deep rooting as allowing plants to extract moisture from deeper in the soil and to enhance plant survival during stress. Vigorous plants were considered as a sign of strength to survive harsh environments.

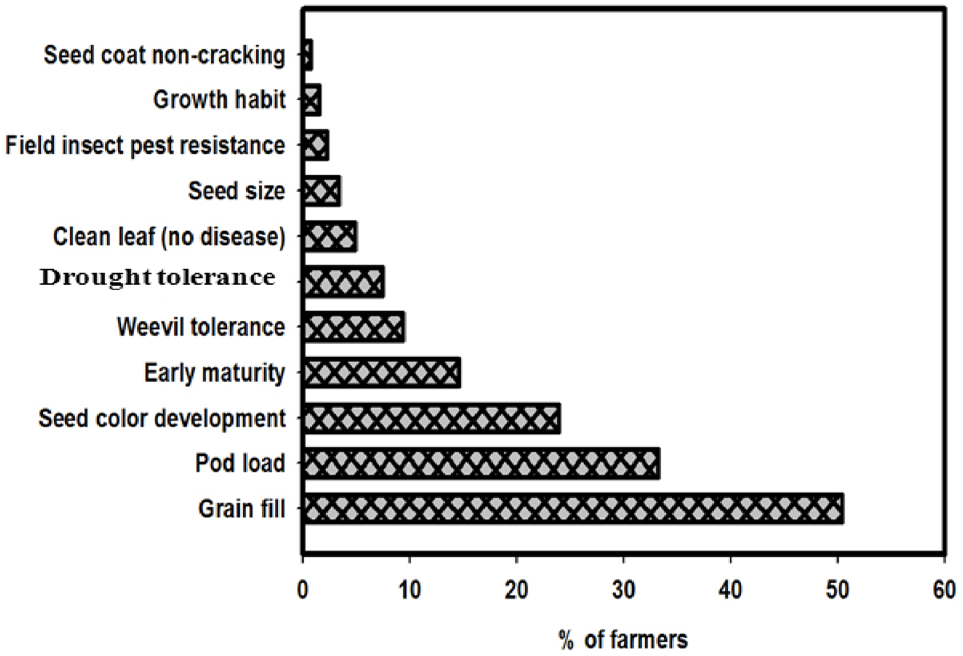

Fig. 4 Farmers' selection criteria for renewal the seed lot of their bean varieties. Traits and % of selector farmers using the traits for selection in farmer survey

Varietal change and seed replacement

Since about 40% of common bean seed used by survey farmers in 2008 originated from off-farm seed sources (Fig. 5), seed lot replacement rate is relatively high, given that common bean is self-pollinating and genetic degradation of varieties is therefore not very strong. Farmers replaced their seed lot when seed quality of particular variety had deteriorated for example when all the seed was shriveled, reduced in size, diseased or discolored, or when yields decreased. This could be the case after an extremely dry season, or when – as farmers considered – the seed was tired. Farmers also changed their seed lot when their seed was damaged by storage pest (weevil), after total crop failure and when all produce was eaten.

Farmers pointed out during the group discussions that refreshing seed stock every 3 to 4 years was good practice and that a younger seed stock was better than an older tired seed stock.

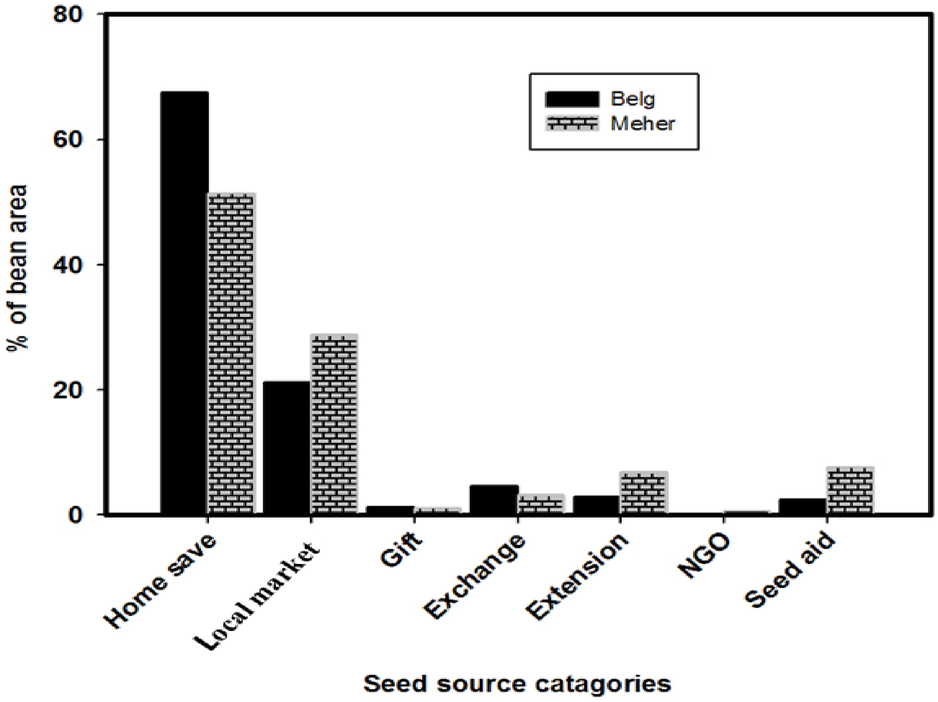

Fig. 5 Common bean seed source variation between seasons indicating off-farm seed procurement for renewal of varieties

Farmers grew the same variety without change for very long periods, on average for 10-11 years with low variety turnover (Table 3). However, the average age of a variety and seed lot turnover varied between varieties and across locations. Black and small-red varieties were grown for a longer time than pinto and cream-mottled varieties. Farmers in Boricha grew the same variety for shorter periods of time as compared to other sites. This site is relatively close to the research and extension site of SARI (Southern Agricultural Research Institute) and farmers pointed out that they changed their variety when new germplasm arrived to the farming system via the extension program and the visits of researchers or relatives or when they acquired new seed lots in trade exchanges with neighboring ethnic groups.

The seed-lot turnover highlighted in Table 3 refers to total seed lot replacement (usual after total loss). About 14% of survey farmers had fully refreshed their seed lot during the last five years with new, younger stock completely replacing the old lot; 11% had in the seed lot change also changed to a variety with a new grain type. More than 70% of survey farmers partially replaced their seed lot or mixed in new fractions of seed at least twice in the last five years (data not shown).

After total loss, 91% of the farmers replaced their seed stock from the local market, 5% from friends, 3% got a new seed stock as a gift from family, and 1% combining local market and friends. Of all survey farmers 48% had provided seed to other farmers in the last five years as a gift, of whom 55% were friends or families within the village, 14% were friends or families from outside the village and 32% were acquaintances from both inside and outside the village.

Table 3 Average 'age' (years) and the frequency of seed lot replacement for varieties in southern region, Ethiopia

	Boricha		Amaro		Konso		Loma	
Variety type	'Age' of variety (years)	Seed lot replacement frequency†	'Age' of variety (years)	Seed lot replacement frequency	'Age' of variety (years)	Seed lot replacement frequency	'Age' of variety (years)	Seed lot replacement frequency
Small-red	11	3	19	0	11	1	8	1
Small-white	6	2	9	1	3	1	1	0
Black			10	2	21	2	23	5
Red-mottled	6	2	12	1				
Pinto			6	1				
Cream-mottled (Sugar)			7	1				
Average	8	2	11	1	12	1	11	2

† Seed lot replacement frequency here refers to the number of times within a single age period farmers fully refresh seed lot of a variety

Farmers' perception on drought, rainfall and genotype × environment interaction in common bean farming

In the focus group discussions farmers came up with a list of factors that according to their experiences cause the greatest yield losses (Table 4). The constraints varied within and across study sites. However, farmers in all study sites except in one group at Loma considered drought as the most significant constraint, followed by low soil fertility and weevils (*Zabrotes subfasciatus* and *Acanthoscelides obtectus*). Other limiting factors included vertebrate pest, diseases (especially angular leaf spot (*Phaeoisariopsis griseola*), common bacterial blight (*Xanthomonas compestris pv. phaseoli*) and the rust (*Uromyces appendiculatus*)), weed infestation and field insect pests (especially bean stem maggot (*Ophiomyia spp*.)), worsening drought stress, pod borer (*Helicoverpa spp*.) and aphids (*Aphis fabae* and other aphids). Excess rainfall was not considered a very important constraint, but it was mentioned to occasionally reduce yields. Only drought and field insects were mentioned in all focus groups, the other factors were left out in at least one focus group. In Konso and Loma weed infestation was not considered a production challenge but rather a problem of laziness of the producer. At the mid-high altitude site in Loma farmers have slightly better rainfall and did not rank drought as the first constraint to common bean yield.

Table 4 Farmers' ranking of common bean productivity constraints using proportional piling methodology and severity index to rank the constraints across different groups. Data were collected in three separate focus group discussions (G1, G2 and G3) at four study sites

	Boricha			Amaro			Konso			Loma			
Constraints	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3	Severity index
Drought	1	1	1	1	1	1	1	1	1	1	1	2	1.02
Low soil fertility		2	3				2	2	2		2	3	1.31
Weevil	4		2	2	2	5	3	5	4	2	3	1	1.47
Vertebrate pest				4	3	2				3	5		1.49
Disease	2	4	5	6	5	5		4					1.67
Weed infestation	5		5	3	6	4							1.69
Field insect pest	3	5	4	7	6	3	4	3	3	4	4	4	1.71
Excess rain		3		5	4	7	5				6	5	1.81

From the group discussions, contact-farmer interviews and survey, it was clear that smallholder farmers observe changes in the climate that affect their bean production. In the survey, 58% of the farmers considered that common bean yields decrease while 35% perceived an increasing trend in the last 10 years. The majority of the farmers (93%) perceived that over the last ten years rainfall is increasingly inadequate and its distribution changing. It is increasingly common that rains start late and terminate early, while also dry-spells during growing period getting longer and more frequent. Moreover the number of rainfall showers reduces, their length becomes shorter and their drops smaller. They also observe that rainy clouds and fogginess decrease or even when it is cloudy or foggy there is no rain. Hail becomes less frequent or not seen at all and summers are considered hotter as compared to 30 or 40 years ago. Farmers shared the feeling that changing weather patterns shorten the growing season, resulting in a tendency to shift to early-maturing crops and varieties. This tendency seems stronger in the dry and lowland ecologies as compared to cooler mid- and highlands. Since common bean is faster maturing than cereals, farmers saw it as a crop that allows them to adapt to these changes. This explains why many farmers grew common bean in larger area as compared to 15 years. Due to the changing weather pattern, common bean farming is expanding and shifting to mid- and higher-altitude production ecologies where the crop was not used to be grown by farmers before. But farmers are increasingly concerned about the impact of climate change on common bean production. Farmers felt that frequent moisture stress events during recent cropping seasons had already resulted in a drop in common bean production per unit area but total production in their individual farms as well as their village increased in recent years due to a larger common bean area.

This trend is confirmed by the long-term common bean production statistics of the region. The regional statistics show an upward trend with strong variation from season-to-season while total rainfall of the main cropping season ('*Meher*') averaged over 15 meteorological stations in the same region shows counter-variation (own collected data, not shown). This inverse relation is explained by the decision of farmers to go for longer cycle crops whenever rainfall is adequate and using common bean in the seasons with delayed or scantier rains. Common bean has a shorter growing cycle which fits into the seasons where rains are short.

Farmers in group discussions and contact-farmer interviews talked about a ‘good’ or ‘bad’ season when evaluating common bean production. Rainfall deficit at any stage of common bean crop growth that reduced production or resulted in crop failure was considered a bad season for common beans. Farmers described how different rainfall phenomena presented themselves in good and bad season (Table 5). Farmers characterized the onset and end of the rain as increasingly unpredictable. Delayed start of the rains is the most common feature that has caused delayed planting and shortened growth periods. Farmers also indicated that it was not the total rainfall but rather the rainfall at the critical stages of crop growth that mattered. Exposure of common beans to dry spells towards the end of the season and early termination of rainfall can be critical for good flowering and pod filling. Two weeks without rain can in any stage of common bean growth significantly affect performance and ultimately reduce yield. Generally, farmers characterized the impact of drought on the common bean crop as caused by early season, mid-season (intermittent) drought or end of the season (terminal) drought. Yield losses were perceived as high when drought occurs before or during flowering in all study sites except Boricha. In Boricha, farmers perceived that higher yield losses could also occur due to drought stress during grain filling. In Boricha, farmers grew common bean primarily for cash (market) hence they attach yield loss due to drought not only to reduction in volume but also to loss of seed quality for marketability.

Farmers in all study sites shared the opinion that common bean is not drought tolerant but does not appreciate excessive rain either. Farmers said that varieties varied in their reaction to water availability: black beans were considered most drought tolerant whereas the large seeded Andean types were found very susceptible. On the other hand, varieties like ‘*Alga Hamare*’ (pinto) were perceived to escape terminal drought because of their early maturity. Farmers said that sometimes a farmer might lose a variety because of drought, but usually (s)he could still get the same variety from a local market, relatives or friends who had received better rainfall in the other parts of the village or outside the village. Farmers did not want to see any variety lost from their production system and indicated that more varieties would be useful in coming years to combat drought.

The survey showed that farmers perceived yield of the same bean variety as varying over locations and seasons/year. With regard to spatial yield variation, surveyed farmers perceived yield variability within field (69%), between fields (87%) and between villages (92%) whereas 87% had an understanding of time × genotype × environment interaction. Rainfall or weather variation and soil fertility differences were perceived as the main causes for spatial and temporal yield instability of their common bean crops (Table 6). Many farmers expressed an interest in varieties that show stable yields over time and space (77%), 12% wanted to have responsive varieties for a given year but some farmers (11%) reflected their quest for both. The reasons for having both stable and responsive varieties were to minimize risk or to fit the crop to the weather.

Table 5 Farmers' description of good and bad seasons in bean farming

Underlying factor	Description for good season	Description for bad season
Onset of rain	Starts normally at the usual time of the rains for the location. Starts in February for '*Belg*' season and June 20th onwards for '*Meher*' season for Boricha and Loma; Starts in March/April for '*Belg*' and August/early September for '*Meher*' in Amaro and Konso.	Starts later than the usual time causing delay in planting time, shortened growth period, pushing the crop into heat stress at the end of the season. Delay up to mid-April in '*Belg*' and delay up to July for Boricha and Loma and up to end of September for Amaro and Konso in '*Meher*' season.
End of rain	Ends normally in the season as in mid-May in '*Belg*' and October 15 for '*Meher*' season at Boricha and Loma whereas rain ends at end of May in '*Belg*' and end of November in '*Meher*' season for Amaro and Konso.	Ends earlier than usual. The rains stop when the crop is at flowering and grain filling, early termination of rain in the season for instance ends in beginning of May in '*Belg*' at all location and September for Boricha and Loma and October for Amaro and Konso in '*Meher*' season. Sometimes extended rain at end of the season causes harvest loss.
Mid-season drought	None, not a problem.	Very frequent mid-season drought, rain breaks for more than two weeks at flowering, main problem is exposure of plants to intermittent drought.
Rainfall distribution	Even rainfall distribution throughout the season, allows period of sun for farm operation, very normal, not windy, no hail, rain falls at reasonable intervals.	Excessive rain all the time without interruption, heavy rains all at once and no rain at other times in the season, very few showers in whole crop growth period in the season, high rainfall at flowering, low rainfall at flowering and grain filling stage of the crop, heavy and continued rain at physiological maturity.
Rainfall amount	Not too much not too little, soil moisture is good throughout the crop growth period.	Too much rain at some times causing erosion (loss of top soil and sometimes loss of crop) or in extreme cases water logging, very long rainy season not good for beans, or small amount of rains that does not allow crop development.

Table 6 Farmers’ perceived cause of genotype × environment interaction in common bean farming in southern Ethiopia

Causes	% of farmers mentioning the cause for G×E Spatial	 Temporal
Rainfall or weather variation	39.5	84.4
Soil fertility variation or decline	86.9	21.2
Disease and pest gradients	0.8	1.9
Slope and soil gradients	5.3	0.0
Seed bed preparation method variation	1.1	5.0
Sowing time variation	0.0	5.9
Weeding/hoeing/crop management difference	0.5	0.3
No idea	6.4	0.0

Source: farmer survey in this study, N = 375

Farmers’ adaptive strategies to reduce drought effect on common beans

About half of the surveyed farmers did not take any measure to reduce drought effect on common bean (Table 7). One third of the other half changed common bean production practices as a reaction to occurring drought effects. Two thirds of the other half changed practices proactively to reduce the vulnerability of common bean farming to drought. The use of early-maturing and drought-tolerant varieties was one of the popular adaptation strategies practiced by the other half of the farmers. This is apparent by recent introduction of the early-maturing pinto variety around Amaro and growing black beans in more drought marginal environments at Konso. The planting of varietal mixtures by some farmers is also meant to buffer drought risk on common bean yield in addition to diversity in varieties (see earlier description on bean farming).

Recently, in Loma and Boricha some farmers had turned to a repeated plowing of a part their land so as to be sure the land was ready for sowing at the first rains. When farmers experienced delayed starts in the rainy season, they turned to sowing earlier-maturing or drought-tolerant varieties. Also when first sowing was affected by a ‘false start’ of rain (i.e. early-season drought) then farmers either re-sowed common beans or shifted to earlier-maturing crops. Timely weeding was practiced to avoid competition for moisture. Dry planting and deep sowing were practiced to effectively use the earlier and often heavier rains. Conservation tillage mostly of the open furrow type was practiced to allow rainwater to infiltrate into the soil. Farmers adaptation strategies are unevenly distributed over individuals and study sites (data not shown).

Table 7 Farmers’ proactive and reactive changes in bean production practices to reduce drought effect on common bean

Reactive mechanisms	% of farmers	Proactive mechanisms	% of farmers
Use of supplementary irrigation	16.8	Use early-maturing varieties	33.3
Clean weeding of the field	12.5	Use drought-resistant varieties	22.7
Re-sowing	6.4	Conservation tillage / open ridges	14.9
Shift to another crop	4.5	Plant variety mixture	6.7
Reduce sowing area	3.7	Dry planting	5.3
Direct runoff into the field	0.5	Deep sowing	2.7
		Deep and repeated ploughing	0.5

Source: Farmer survey in this study, N = 375

Discussion

This study supports the generally observed diversity of agro-ecological and socio-cultural conditions for smallholder farmers in southern Ethiopia. Farmers in different areas have varying preferences for plant and grain types of common bean and most farmers traditionally grow a small-seeded red or black variety. The large majority of the farmers grows only one variety. Bean plantings comprised of a range of cropping patterns (sole, inter- and relay-cropping), with plantings in both the ‘*Belg*’ and the ‘*Meher*’ season, each with its specific features in the different regions. Farmers clearly notice that climate is changing. They observe increasing frequencies of delayed on-set of the rains, intermittent drought periods and early end of the rainy season. Together this increases the frequency of total crop failure and severe yield reduction. The study shows that farmer have their traditional agronomic practices to cope with the variation of climate within and between seasons, but so far only about half of the farmers have adapted their cropping practices in one form or another to the increasingly variation and shortening of the rainy seasons. Better weeding, deeper sowing and earlier land preparation are among the practices to better benefit the rains, but the use of drought resistant and earlier maturing varieties and crops probably represents the principal opportunities for farmers. This makes beans an increasingly important food security crop for farmers in South Ethiopia, as often the longer maturing maize or sorghum crop cannot complete its growing cycle when rains and sowing are delayed.

Crop genetic diversity is instrumental in the buffering of environmental variation. For farmers growing beans this means the use of several varieties in mixed or unmixed form, in either sole, inter- or relay cropping. However, given the relatively low level of common bean genetic diversity in individual farming systems and the study area as a whole, this buffering mechanism can be considered as highly underused.

The relative fast adoption of new early maturing pinto bean varieties that were introduced in Amaro may be indicative for the interest of farmers in bean diversity and points to the potential that crop genetic diversity has in supporting farmers to mitigate the effects of variable growing conditions.

Other factors also play a role in the demand for new diversity. For example, the expansion of common beans growing at the slightly higher elevations. Beans can now be grown in these slightly cooler environments because the increasingly shorter growing season now reduces the chance that late rains affect the harvest there. The market also proves to be an important driver of demand for new bean varieties. The small-white bean finds easy adoption since farmers in the Central Rift Valley experience that it fetches a good price when sold to bean exporters (Asfaw et al. forthcoming). Farmers are also interested in large-seeded Andean common bean types because they like it for consumption and they expect it to be easily marketable because of its superior culinary quality.

The study of farmers' seed saving practices does not show a high level of specialization. This does however not necessarily mean that farmers are not knowledgeable or that their knowledge cannot be developed. The high level of environmental variation and the associated risks of crop failure also imply that local crop development practices are less effective. This is underlined by the high frequency of seed stock replacement: keeping a seed stock of good quality apparently requires effort. The high turn-over of seed stocks also provides an opportunity to introduce new varieties. If farmers seek new seed for planting, the chances of them adopting new varieties increase. To make use of such opportunities, diversity needs to be available in the form of adapted germplasm and strategic introduction of adequate volumes of seeds.

Genetic diversity can be introduced in the form of more or less advanced lines. Work in Central America has indicated that local selection may result in more diversity and better adaptation (Humphries et al. 2005; Almekinders 2011) and also the selection work in the formal breeding program indicates that better adapted varieties can be acquired from local selection (Asfaw et al. forthcoming). Ideally, relatively small amounts of early generation seeds with promising backgrounds could be introduced to different environments from which varieties would be developed through local selection. However, farmers' involvement in selection of segregated materials requires not only expertise, but also investment and a high level of commitment. In highly variable environments like those of southern Ethiopia, participatory variety selection as a decentralized form of selection of advanced materials with farmers may be more feasible. Seasonal evaluation trials in which farmers select and keep the seeds of varieties they favors are less risky investments than on-farm selection processes of 2 to 4 generations. However, this means at the same time that the variety development and selection process is largely directed by the breeder. Knowledge of the local conditions, changes in agro-ecological and socio-economic conditions, and diversity caused by climate change and market dynamics are then crucial to define a breeding strategy that meets the need of the farmers. This study in particular shows that the farmers' needs cannot only be considered as an articulated existing demand. The demand is also partly an extrapolated demand for which it is essential to understand climate change and market trends and to know characteristics that the genepool can provide. Only on the basis of an integrated understanding of farmer production conditions and existing seed system practices a breeder can contribute to an overall package of mechanisms that harness and equip farmers to adapt to their dynamical context.

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CHAPTER 3

Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African Highlands

This chapter is based on the article: Asfaw A, Blair MW, Almekinders C (2009), Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African Highlands. *Theoretical and Applied Genetics* 120:1-12. The authors are grateful to the Genetic Resource Unit and Bean Project of CIAT as well as the Institute for Genomic Diversity at Cornell University for germplasm and assistance rendered during phenotyping and genotyping. The Generation Challenge Program (GCP) is gratefully acknowledged for funding associated with this study. We also thank the Cornell Computational Biology Service Unit for assistance in data analysis.

Abstract

The East African Highlands are a region of important common bean production and high varietal diversity for the crop. The objective of this study was to uncover the diversity and population structure of 192 landraces from Ethiopia and Kenya together with four genepool control genotypes using morphological phenotyping and microsatellite marker genotyping. The germplasm represented different common bean production ecologies and seed types common in these countries. The landraces showed considerable diversity that corresponded well to the two recognized genepools (Andean and Mesoamerican) with little introgression between these groups. Mesoamerican genotypes were predominant in Ethiopia while Andean genotypes were predominant in Kenya. Within each country, landraces from different collection sites were clustered together indicating potential gene flow between regions within Kenya or within Ethiopia. Across countries, landraces from the same country of origin tended to cluster together indicating distinct germplasm at the national level and limited gene flow between the two countries highlighting divided social networks within the regions and a weak trans-national bean seed exchange especially for landrace varieties. One exception to this may be the case of small red-seeded beans where informal cross-border grain trade occurs. We also observed that genetic divergence was slightly higher for the Ethiopian landraces compared to Kenyan landraces and that Mesoamerican genotypes were more diverse than the Andean genotypes. Common beans in eastern Africa are often cultivated in marginal, risk-prone farming systems and the observed landrace diversity should provide valuable alleles for adaptation to stressful environments in future breeding programs in the region.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the principal grain legumes of eastern and southern Africa, occupying more than 4 million ha annually and providing food for more than 100 million people (Wortmann et al. 1998). It is the second most important source of dietary protein and the third most important source of calories for lower income African households after cassava and maize (Broughton et al. 2003). Total production in sub-Saharan Africa is around 3.5 metric tons with 62% of production in East African countries of Burundi, DR Congo, Ethiopia, Kenya, Rwanda, Tanzania and Uganda making this the most important region for the crop within the continent. Although the crop is basically cultivated for home consumption in much of East Africa, it is also rapidly evolving into a cash crop in certain countries with Ethiopia earning about US$ 6.25 million (equivalent to 60 million ET Birr) from bean exports in 2005 (Teshale Assefa, personal communication 2007). Regional trade is significant for some trading partners, for example from Ethiopia, Tanzania and Uganda into Kenya.

Cultivated common beans originated in Latin America from two recognized centers of domestication about 7,000–8,000 years ago (Gepts and Debouck 1991). The multiple regions of domestication endowed the crop with relatively high diversity that is broadly classified into two genepools: Mesoamerican and Andean (Gepts et al. 1986; Singh et al. 1991a, b, c). The two genepools further differentiate into different races, such as Mesoamerica, Durango, Jalisco and Guatemala in the Mesoamerican genepool and Nueva Granada, Peru and Chile in the Andean genepool (Singh et al. 1991b; Beebe et al. 2000). The genepool and race differences have been validated using various marker systems including seed size, phaseolin (seed storage protein) patterns, plant morphology, isozymes, RFLP, RAPD, AFLP and microsatellite markers (Singh et al. 1991a, b, c; Becerra and Gepts 1994; Beebe et al. 2000, 2001; Islam et al. 2004; Blair et al. 2006, 2009).

Common beans are believed to have been introduced together with maize into the east coast of Africa by Portuguese and Spanish traders in the sixteenth and seventeenth century (Greenway 1945; Gentry 1969). Since then farmers have developed farming practices adapted to local conditions by preservation and exploitation of useful alleles which have resulted in a range of morphologically diverse landraces (Wortmann et al. 1998; Sperling 2001). Moreover, with recent efforts to improve on-farm level productivity by many national bean-breeding programs in Africa, new germplasm sources have been continually introduced to African farming systems from different parts of the world since the 1980s (CIAT 2005). The existence of both genepools (Andean and Mesoamerican) in Africa has furthermore been documented (Martin and Adams 1987) and probably is a result of original introductions and subsequent imports of novel germplasm. Given the wide range of landraces on the continent, Africa can be considered to be a secondary center of diversity for common beans (Allen and Edje 1990; Wortmann et al. 1998; Sperling 2001).

Despite recognition of the genetic diversity of common beans in eastern and southern Africa, it is not clear if the observed variation in and between landraces is the result of the original differences between the various introductions or whether they result from a continuous process of natural hybridization and selection by farmers and by the environment. Gene flow within and between genepools and races via spontaneous out-crossing in farmer's field or crossing programs in formal breeding could result in intermediate phenotypes that do not correspond well to any of the single race or genepool divisions (Beebe et al. 2001; Islam et al. 2004; Díaz and Blair 2006; Blair et al. 2007). Understanding the pattern of population-genetic structure and diversity of bean landraces and cultivars (hereafter accessions) and their relationships with the Andean and Mesoamerican genepools can therefore provide information on gene flow and be of great importance for future common bean breeding in the region. However, to date, the diversity assessment exercises in the region have mainly been limited to agro-morphological traits and no comprehensive marker evaluation of bean landraces has been conducted.

This study, therefore, aims to examine the genetic diversity and relationships among and within accessions from two East African countries (Ethiopia and Kenya) in relation to the Andean and Mesoamerican genepools using microsatellite marker analysis combined with morphological evaluation. Ethiopia and Kenya accessions were selected for analysis because these countries are among the ten largest common bean producers in sub-Saharan Africa (Hillocks et al. 2006) and bean production is very diverse in terms of agro-ecology, social settings and production systems (from monocrop to relay-cropping or intercropping) found in both countries. Throughout these countries, the crop is grown mainly by farmers with low external inputs and landraces remain the dominant source of seed for planting, although popular modern varieties have also been released in recent years, suggesting that landrace diversity may be under threat of being replaced by modern varieties in the near future, increasing the urgency of this germplasm characterization.

Material and Methods

Plant materials

A total of 192 accessions collected from a range of common bean production ecologies in Ethiopia and Kenya together with four control genotypes for the Andean and Mesoamerican genepools were grown in a greenhouse at CIAT for DNA extractions and analysis. The East African accessions were selected on the basis of their origin from the CIAT genetic resource unit collection (http://www.ciat.cgiar.org/urg/beans.htm) with 99 genotypes from Ethiopia and 89 genotypes from Kenya and the majority being landraces and only a few commercial cultivars (Supplementary table 1). The control genotypes were selected on the basis of their use in previous studies with microsatellite markers (Blair et al. 2006; Díaz and Blair 2006; Blair et al. 2007). These were: 'Calima' (G4494) a variety from Colombia, and 'Chauca Chuga'(G19833), a landrace from Peru as the Andean control genotypes; as well as DOR364 (or ‘Dorado’), a variety from CIAT/El Salvador, and ICA Pijao, a variety from Colombia as Mesoamerican controls. The seeds of these genotypes were also provided by the CIAT germplasm bank.

Morphological measurements

Morphological variables were measured on plants raised in both the greenhouse and the field at CIAT headquarters in Palmira (1,000 m altitude, mean growing temperature 24°C, Mollisol soil), Colombia in 2007. In the greenhouse, plants were grown from August to October 2007 in plastic pots carefully packed with 5 kg of field soil from Palmira mixed with river sand on a 2:1 w/w (weight-by-weight) ratio. A total of four plants were grown for each accession across two pots. For the field evaluation, 20 seeds of each accession were planted in November 2007 in a single 2-m long row with inter- and intra-row spacing of 60 and 10 cm, respectively. The plants were provided with optimum conditions for crop growth and development both under greenhouse and field conditions. The assessed morphological variables were bracteole size (small, medium or large), bracteole shape (cordate, ovate, lanceolate or triangular), outer base of the standard (banner petal), corolla type (smooth or striped), flower color (white, light purple or dark purple), growth habit (determinate bush, indeterminate bush, indeterminate prostrate or indeterminate climbing bean) and stem pigmentation (absent, light red or dark red). All variables were evaluated on four comparably aged plants per accession according to CIAT (1987) and Singh et al. (1991b). Additionally, primary and secondary seed colors and seed size were recorded after harvest for field grown seed.

Microsatellite marker evaluation

For molecular level diversity assessment, total genomic DNA for each accession was isolated from a bulked leaf tissue sample of 1-week old, paper-germinated plants from six randomly selected seeds using a CTAB extraction method as described by Afanador et al. (1993). Since these are CIAT genebank accessions that generally were purified from original landraces with each seed type within a landrace receiving a separate entry, we assumed that we were dealing with mainly single genotypes but that any heterozygosity would be captured within the six-plant sample. The DNA quality was evaluated on 1% agarose gels and quantified with QUANTITY ONE v. 4.0.3 software (Bio-Rad Lab., Hercules, CA). DNA was then diluted to 5 ng/µL for further use in the genotyping experiments. Microsatellite marker evaluation involved a total of 38 fluorescently labeled microsatellites selected to represent both cDNA based and genomic markers and for high polymorphism information content (Blair et al. 2006, 2009). The genomic microsatellites included BM139, 140, 141, 143, 151, 156, 165, 172, 175, 183, 187, 188A, 188B, 2001, 205; BMd12, 36, AG1 and GATs54, 91 (Gaitán et al. 2002). The gene based microsatellites included BMd1, 2, 8, 15, 16, 17, 18, 20, 45, 46, 47, 51 (Blair et al. 2003), and PV-ctt001a, PV-ctt001, PV-ag003, PV-ag001, PV-at001, PV-at003 (Yu et al. 2000). PCR amplification were carried out on a MJ Research Inc. PTC-100 thermo-cycler using 96-wall plates as described by Blair et al. (2006) with a 13 µL final reaction volume that included 3 µL of genomic DNA, 1.5 µL of each primer at concentrations of 0.16 µM, 0.78 µL of Mg buffer at a concentration of 1.5mM, 0.72 µL of 1 ×

PCR buffer (10mM pH 7.2 Tris-HCl, 50mM of KCl), 0.13 µL of dNTP at concentration of 0.2 mM and 0.15 µL of 1.0 unit *Taq* polymerase and 5.22 µL ddH_2O. The PCR products of different size and contrasting fluorescent labels were pooled and diluted with sterile deionized water to equalize signal strength. The DNA fragments from pooled PCR amplifications were then separated by capillary electrophoresis using an ABI3730 DNA analyzer (Applied Biosystems, Foster City, CA). The fragment analysis data from ABI3730 system were analyzed and allele sizes scored with GENEMAPPER version 3.7 software (Applied Biosystems). The observed allele size was then adjusted for the discrete allele size using AlleloBin software (http://test1.icrisat.org/gt-bt/download_allelobin.htm) and allele sizes for the control genotypes (Calima, G19833, DOR364 and ICA Pijao) were confirmed to be of the same sizes as in Blair et al. (2006, 2007) and Díaz and Blair (2006).

Genetic diversity analysis

The pattern of genetic diversity within and among accessions and across the countries of collection (Ethiopia vs. Kenya) was assessed for both morphological and molecular data using several software programs. Morphological marker data from greenhouse and field trial were averaged and subjected to frequency distribution analysis in SAS statistical package version 9.1.3 (SAS Institute 2003). The morphological traits were scored for each genotype based on presence and absence. These data were used to generate a binary matrix of presence and absence which was used for principal coordinate analysis (PCoA) and for creating the matrix of average taxonomic distance (i.e., DIST coefficient in the procedure) between accessions, respectively, in the SIMQUAL and SIMINT subprograms of NTSYS-pc, Version 2.10 (Rohlf 2002). Genetic relationships within and among accessions from the two East African countries based on genotypic data were assayed with a neighbor-joining method in DARWIN 5.0 software (Perrier et al. 2003; Perrier and Jacquemoud-Collet 2006). Genetic distance matrices were generated using the Peakall et al. (1995) method of calculating individual by individual genetic distances from co-dominant markers. Accordingly, for each SSR marker, with *i*-th, *j*-th, *k*-th and *l*-th different alleles, a set of squared distances was defined as $d^2(ii, ii) = 0$, $d^2(ij, ij) = 0$, $d^2(ii, ij) = 1$, $d^2(ij, ik) = 1$, $d^2(ij, kl) = 2$, $d^2(ii, jk) = 3$, and $d^2(ii, jj) = 4$. Genetic distance matrices for each locus were summed across loci assuming statistical independence. The genetic distance values were then subjected to PCoA as implemented by GENALEX version 6.1 software (Peakall and Smouse 2007). Patterns revealed by the first three coordinates of each accession were plotted using the Graph module and the G3D procedure of the software program SAS. Genetic diversity parameters such as number of alleles (N_A), number of effective alleles (N_E), Number of private alleles (N_{PA}), observed heterozygosity (H_O), standardized allelic richness (A_R), gene diversity (G_D), Shannon's information index (I), fixation index (F), percent polymorphic loci were estimated with FSTAT version 2.9.3 (Goudet 2001) for each pre-determined group based on origin of the accession of collection (by country) and genepool assignment as differentiated by neighbor-joining analysis and PCoA.

Molecular analysis of variance and population structure analysis

Partitioning of total genetic variation into within and among genepool diversity and country of origin was performed with a Molecular Analysis of Variance (AMOVA) procedure in GENALEX. To infer pattern of population structure, both population level and individual-based clustering approaches were employed. Global F_{ST} and pairwise F_{ST} were estimated using Weir and Cockerham's Q (Weir and Cockerham 1984). F_{ST} values and significance of estimates were calculated with FSTAT. Other parameters like gene flow, Nei's unbiased genetic distance and identity were computed to assess the degree of population differentiation using GENALEX. The Bayesian genotypic clustering method INSTRUCT (Gao et al. 2007) was used to validate population-based approaches and to infer population structure among the genotypes. INSTRUCT is an extended Bayesian clustering approach of STRUCTURE (Pritchard et al. 2000) that absorbs inbreeding or selfing rate for population inference. It quantifies the contribution of two forms of non-random mating: inbreeding (mating among relatives) and population substructure (limited dispersal of gametes) when determining the pattern of existing genetic variation (Gao et al. 2007). INSTRUCT was run for $K = 2$ to $K = 6$ in mode 2 for joint inference of population selfing rate and population sub-structure for five independent chains, each chain with 200,000 iteration steps, 100,000 burn-ins, and a thinning interval of 10 steps, assuming different starting points. Graphical representations of population assignments from INSTRUCT were produced from the program DISTRUCT (Rosenberg et al. 2002).

Results

Morphological diversity

Significant variation was observed for most morphological traits measured on the East African accessions with two to four character states found per trait as shown in Table 1. Significant differentiation of accessions from the two countries was observed for bracteole size, growth habit and seed size whereas for base of the standard, bracteole shape, flower color and stem anthocyanin pigmentation the difference was non-significant. In these cases, the majority of the accessions from both Ethiopia and Kenya had smooth outer base of the standard and no stem anthocyanin pigmentation.

A greater proportion of the accessions from Ethiopia had larger bracteole size, cordate or ovate bracteole shape, white flower color and smaller seed size, characteristics typical of the Mesoamerican genepool; and a larger proportion of accessions from Kenya had predominantly medium to large bracteole size, lanceolate or triangular bracteole shape and medium to larger seed size typical of the Andean genepool. The accessions from both countries were showing a range of growth habits and seed colors; however, type-III growth habit was prevalent in Ethiopian accessions and type I and II growth habit was prevalent in Kenyan accessions.

Table 1 Frequency distribution for morphological traits evaluated for East African accessions in relation to genepool control genotypes

Trait[a]	Origin			Controls (*N* = 4)[b]
	Ethiopian (*N* = 99)	Kenyan (*N* = 89)	χ^2-test	
Base of standard				
Smooth	72	67	0.1586n	2
Stripped	27	22		2
Bracteole size				
Small	20	17	18.96**	2
Medium	28	51		0
Large	51	21		2
Bracteole shape				
Cordate	27	13	5.128ns	1
Ovate	23	24		1
Lanceolate	33	38		0
Triangular	15	11		2
Flower color				
White	53	38	5.696ns	3
Light purple	23	35		0
Dark purple	23	16		1
Growth habit[a]				
I	15	29	12.95**	0
II	23	23		3
III	41	18		1
IV	20	19		0
Stem anthocyanin pigmentation				
Absent	74	75	3.870ns	2
Light red	15	11		1
Dark red	10	3		1
Seed size				
Small	56	20	27.10**	2
Medium	33	39		0
Large	10	30		2

Number (*N*) of each set of accessions is given when listing country of origin

ns non-significant; ** Highly significant ($P < 0.001$)

[a] Morphological traits evaluated in greenhouse and verified in field plantings at CIAT in Palmira, Colombia during summer 2007. Conventions are: I determinate bush, II indeterminate bush, III indeterminate prostrate, IV indeterminate climbing beans; seed size only measured from field trial

[b] Control genotypes were 'Calima' (G4494) a variety from Colombia and 'Chauca Chuga'(G19833) a landrace from Peru as Andean control genotypes and DOR364 (or 'Dorado') a variety from CIAT/El Salvador and ICA Pijao a variety from Colombia as Mesoamerican control

The dominant primary seed colors throughout the Ethiopian accessions were white, red and tan/brown whereas in Kenyan accessions purple, cream, yellow and red-seeded genotypes were common. A majority of the accessions in both countries were of a single primary color and had no secondary seed color; however, among those with secondary seed colors, red and cream mottled seed types were more prevalent in Ethiopia and Kenya, respectively.

Analysis of the morphological variables showed grouping of Andean and Mesoamerican genotypes combined with probable introgression between the genepools as shown by the PCoA in Fig. 1. In this graph, the first and second dimensions (Dim-1 and Dim-2) explained 21.0 and 10.6% of the total variation in the data set, respectively. Together, the first two-dimensions explained 31.69% of the total variation; and overall the PCoA analysis separated the Mesoamerican control genotypes from Andean control genotypes with concomitant clustering of some accessions into their respective gene pools. Many accessions, meanwhile, occupied intermediate positions between the two genepools and the control genotypes for the two genepools, probably due to introgression and/or shared morphological markers such as seed color and growth habit of the accessions in them.

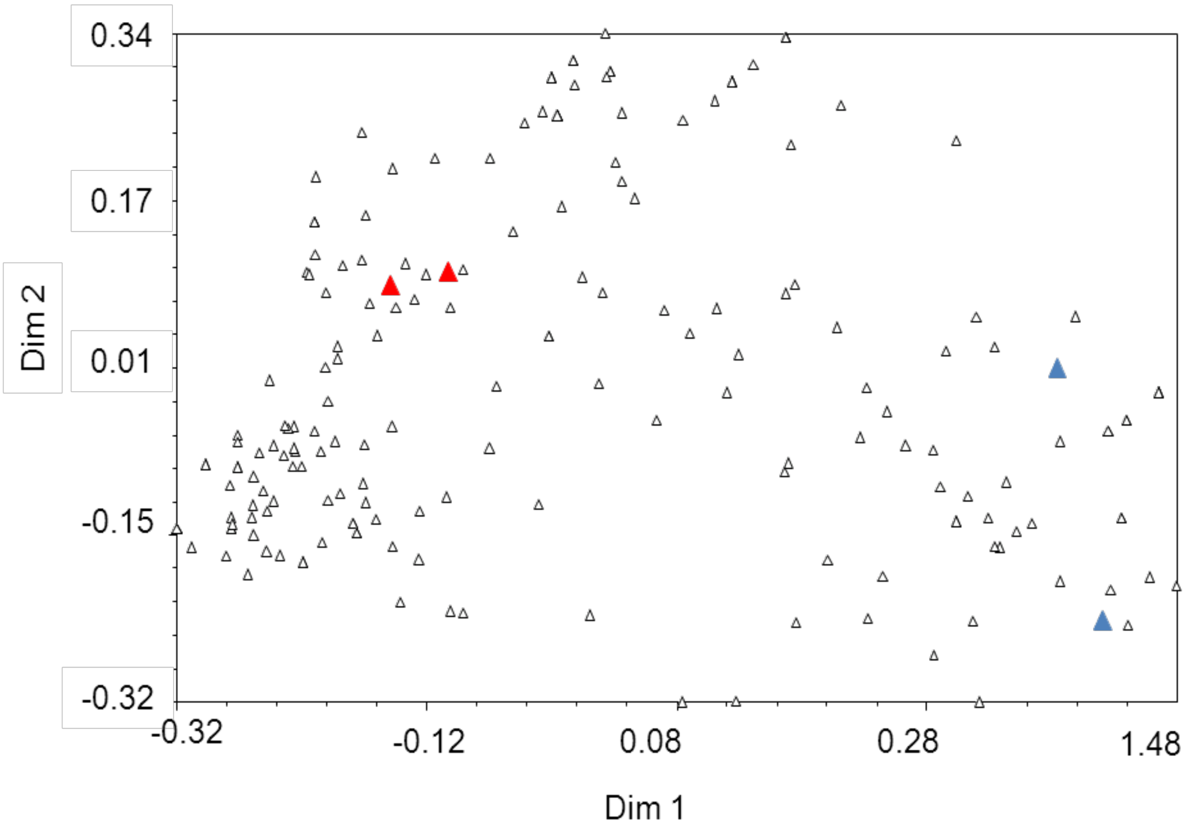

Fig. 1 Principal coordinates analysis of the 192 Ethiopian and Kenyan accessions based on nine morphological traits. *Filled triangles* to the *left* indicate placement of Andean control genotypes and *filled triangles* to the *right* indicate Mesoamerican control genotypes

Genetic associations among accessions

Genotyping results with the fluorescent microsatellite markers were also used to cluster the accessions, and genetic associations among accessions from Ethiopia and Kenya with respect to Andean and Mesoamerican control genotypes as shown in Figs. 2 and 3. In these graphs, distinct clusters were apparent with the SSR markers unambiguously assigning accessions to the Andean and Mesoamerican genepools both with neighbor-joining dendrograms (Fig. 2) and with the 3D plot of the PCoA based on pairwise genetic distances (Fig. 3). Within each country, accessions from the same collection site were often in different clusters and likewise accessions from different collection sites were clustered together (Fig. 2I, III) indicating the possibility of gene flow between sites and regions within Ethiopia and within Kenya. When comparing across countries in the overall analysis (Fig. 2II), accessions from the same country of origin tended to cluster together especially with the Andean genotypes indicating distinct germplasm at the national level and perhaps some cross-border gene flow between the countries.

Results of the PCoA were in agreement with those of the neighbor-joining dendrograms, with two major groups detected: one clearly representing the Andean genepool and the other the Mesoamerican genepool. The division of the accessions into two major groups showed that there was correspondence between the grouping of East African bean landraces and the respective genepools in the primary centers of diversity. However, the further differentiation into recognized bean races belonging to these genepools was not apparent, although some sub-grouping was observed in the analysis. The overall variation explained by the principle coordinate analysis was 79.7% with dimensions 1, 2 and 3 explaining 56.4, 12.0 and 11.3%, respectively.

Genetic diversity within and among accessions and country of collection

All of the microsatellite markers used in this study were polymorphic. The proportions of polymorphic loci were 89.5% in the control genotypes and 100% in both Ethiopian and Kenyan accessions (Table 2). A total of 389 alleles were detected among the 192 bean accessions with an average of 10.24 alleles per marker. The number of alleles per markers ranged from 2 in BMd46 to 35 in Pv-at001, with the mean number of effective alleles per locus not significantly different among the two East African collections but slightly lower in Kenyan (3.39) than in Ethiopian (3.72) accessions. Meanwhile, the mean number of private alleles per population was slightly higher for Kenyan (2.42) versus Ethiopian (1.25) accessions although allele richness was not significantly different between countries of origin and genepools.

AMOVA results showed that 66% of allelic diversity was attributed to individuals within genepool ($P < 0.001$) while only 34% was distributed among genepools. No significant variation for molecular diversity was observed between countries of collection denoting shared alleles among them. However, Ethiopian accessions had slightly higher level of gene diversity compared to Kenyan accessions. Within the country of origin and between the genepools, accessions within the Mesoamerican group of East African accessions had slightly higher gene diversity than those within the Andean group. Similarly, Shannon's information index was slightly higher for Ethiopian than for Kenyan accessions and for Mesoamerican genepool accessions compared to Andean genepool representatives. The observed heterozygosity and probable out-crossing values were low for all the study materials reflecting the inbreeding nature of the common bean crop. However, the heterozygosity and out-crossing values were slightly higher for the East African accessions (0.11–0.15) compared to the control genotypes (0.04) which might be explained by these genebank accessions resulting from the collection of varietal mixtures which are common in many farmer fields in the region. Higher observed heterozygosity was observed among Ethiopian accession than among Kenyan accessions overall and for the Mesoamerican genepool genotypes in Ethiopia and Andean genepool genotypes in Kenya.

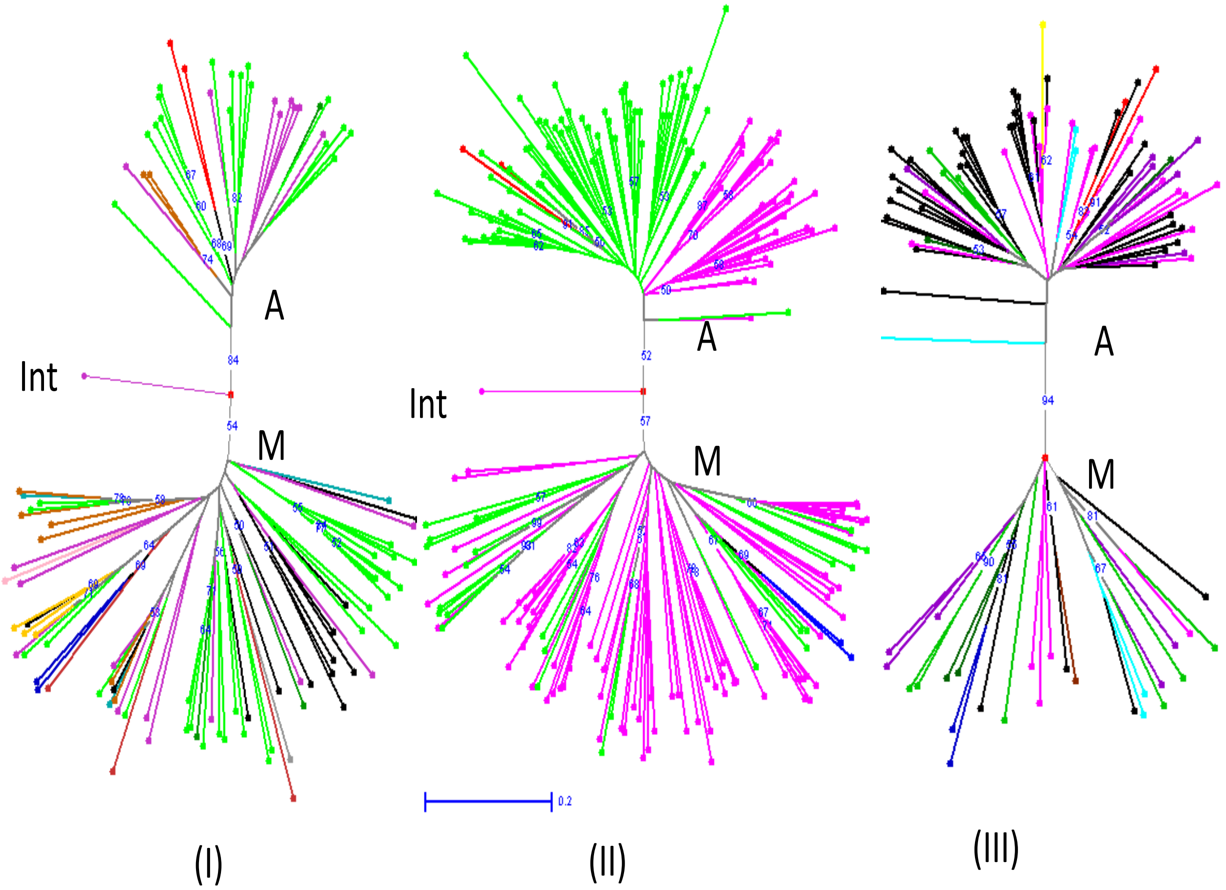

Fig. 2 Neighbor-joining dendrograms depicting genetic relationship between common bean accessions from Kenya and Ethiopia with respect to Andean and Mesoamerican control genotypes. **I** Ethiopian accessions, **II** global accessions (full set of the study materials) and **III** Kenyan accessions. *Different line shading* represent different collection sites within each of the countries (**I**, **III**) and country of origin (**II**). *Downward* facing *arrows* indicate Andean controls and *upward* facing *arrows* indicate Mesoamerican controls. *A* Andean, *M* Mesoamerican, *Int* introgression as explained in the text. *Numbers* along *branches* indicate bootstrap support (shown only for values greater than 50)

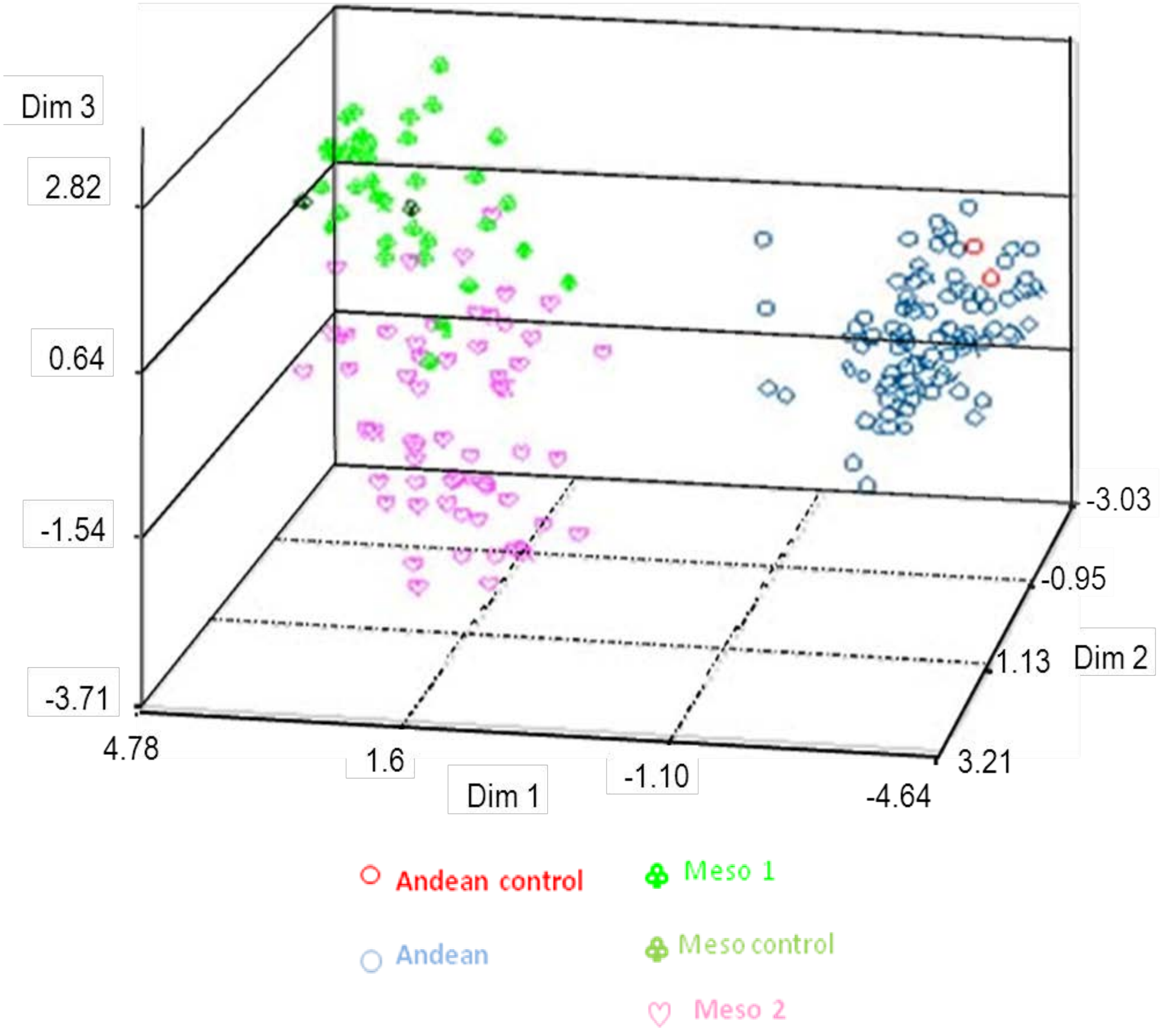

Fig. 3 Principal coordinate analysis based on microsatellite markers showing spatial distribution of Ethiopian and Kenyan accessions compared to Andean and Mesoamerican control genotypes. Each dimension explains 56.37% (Dim1), 12.01% (Dim2) and 11.30% (Dim3) of variation. The three dimensions together explained 79.68% of total variation present in the data set

Population differentiation and structure

Genetic differentiation in the East African bean landraces and cultivars was also analyzed with POPGENE (Yeh et al. 1997) and F_{ST} values among pairs of populations were found to range from 0.037 to 0.632 with an overall average of 0.273 (Table 3). Population differentiation was higher between genepools (F_{ST} = 0.189, $P < 0.001$) than between countries of origin (F_{ST} = 0.06, $P < 0.001$). However, for the comparison between the countries of origin, Andean genepool accessions were more highly differentiated (F_{ST} = 0.331, $P < 0.001$) than Mesoamerican genepool accessions (F_{ST} = 0.04, $P < 0.001$).

Correspondingly, some level of gene flow (Nm = 3.927) existed between the two neighboring East African countries, which was higher for Mesoamerican representatives (Nm = 6.421) than for Andean representatives (Nm = 3.940). Average Nei's unbiased genetic distance was high between genepools (0.665) but low between countries of origin (0.195). Within genepool, the Mesoamerican representatives presented lower genetic distances than the Andean genepool representatives in each country. Genetic identity was fairly high between the two countries (0.823); however, it was low between genepools (0.204–0.507) and intermediate within gene- pools (0.673–0.916).

Table 2 Mean SSR diversity for 38 microsatellite loci in Ethiopian, Kenyan and genepool control genotypes

	N	N_A	N_E	N_{PA}	A_R	G_D	H_O	I	F	t	P (%)
Total Andean	95	7.26	2.75	2.47	7.14	0.47	0.11	1.02	0.74	0.15	100.0
Total Mesoamerican	96	7.66	3.28	2.97	7.59	0.60	0.14	1.26	0.76	0.14	100.0
Ethiopian	99	7.68	3.72	1.95	7.51	0.64	0.14	1.36	0.79	0.12	100.0
Andean Eth.	28	3.95	2.23	0.26	3.79	0.44	0.11	0.82	0.76	0.14	86.8
Mesoamerican Eth.	70	6.45	3.09	1.05	5.15	0.59	0.15	1.18	0.73	0.16	100.0
Kenyan	89	8.16	3.39	2.42	8.09	0.59	0.12	1.28	0.81	0.11	100.0
Andean Kya.	65	5.97	2.56	1.29	4.70	0.45	0.12	0.95	0.71	0.17	94.7
Mesoamerican Kya.	24	5.40	3.28	0.58	5.28	0.59	0.13	1.17	0.77	0.13	94.7
Total Eth-Kenya	188	10.11	4.00	7.68	6.56	0.65	0.13	1.45	0.80	0.11	100.0
Andean-Meso checks	4	2.55	2.34	0.13	2.46	0.50	0.04	0.81	0.94	0.03	89.5
Grand total	192	10.24	4.03	-	10.20	0.65	0.13	1.45	0.81	0.11	100.0

N number of genotypes, N_A number of different alleles, N_E effective number of alleles, N_{PA} number of private alleles, A_R allele richness, G_D gene diversity according to Nei (1978), H_O observed heterozygosity, I Shannon's information index, F fixation index, $t = (1-F)/(1+F)$ out-crossing rate, P (%) percent polymorphic loci. *Eth* Ethiopian genotypes, *Kya* Kenyan genotypes

Population structure analysis with INSTRUCT confirmed the existence of the two genepools for the East African Highland common bean accessions (Fig. 4). The analysis for $K = 2$ populations showed individual genotypes from the two countries distributed between the two genepools which was congruent with neighbor-joining and PCoA that clearly separated the Mesoamerican and Andean genepools. At $K = 3$, the Mesoamerican genepool genotypes further separated into two sub-groups with a low level of admixture, while the Andean genepool genotypes did not show any separation. At $K = 4$, the Mesoamerican accessions further sub-divided into three groups but no meaningful interpretation of population structure could be made. At $K = 5$, the Andean group separated according to country of origin with very little admixture between Ethiopian and Kenyan Andeans, supporting earlier analysis that depicted distinct germplasm at this national level separation. At $K = 6$, the Andean groups further differentiated into three groups, principally in Kenya where two subgroups were highly admixed; while the Mesoamerican genepool maintained the same sub-grouping as observed at $K = 4$. INSTRUCT software predicated $K = 6$ as the optimum population structure in the study material, therefore no further population subdivisions were modeled. The morphological characteristics predominant in each sub-population at $K = 6$ are given in Table 4.

Table 3 Pairwise genetic differentiation, gene flow, unbiased Nei's genetic distance and identity among and between genepools and countries of origin in East African landraces and cultivars

Genepool [a]	F_{ST}						GD					
	AC	MC	AE	ME	AK	MK	AC	MC	AE	ME	AK	MK
Andean control (N = 2)	***	0.632	0.194	0.341	0.168	0.368	**	1.407	0.259	1.034	0.233	1.099
Mesoamerican control (N = 2)	0.141	****	0.478	0.217	0.472	0.205	0.245	**	1.590	0.398	1.453	0.309
Andean Ethiopia (N = 28)	1.040	0.274	****	0.202	0.060	0.229	0.772	0.204	**	0.666	0.108	0.811
Mesoamerican Ethiopia (N = 70)	0.483	0.902	0.985	****	0.208	0.037	0.355	0.672	0.514	**	0.699	0.088
Andean Kenya (N = 65)	1.235	0.279	3.940	0.954	***	0.217	0.792	0.234	0.898	0.497	**	0.724
Mesoamerican Kenya (N = 24)	0.430	0.972	0.842	6.421	0.902	****	0.333	0.734	0.444	0.916	0.485	**
	Nm						GI					

AC Andean control, *MC* Mesoamerican control, *AE* Andean from Ethiopia, *ME* Mesoamerican from Ethiopia, *AK* Andean from Kenya, *MK* Mesoamerican from Kenya. Genetic differentiation (F_{ST}) and unbiased Nsi's genetic distance (*GD*) in upper diagonals in right and left panels of the table. Gene flow (*Nm*) and Nei's unbiased genetic identity (*GI*) in lower diagonals in left and right panels, respectively

[a] Introgression was excluded and number (*N*) of accessions in each germplasm group was indicated

Discussion

The level of polymorphism in landraces and cultivars from Ethiopia and Kenya was found to be considerable, especially with microsatellite marker analysis. Our result identified common beans from this region as distinguishable into both Andean and Mesoamerican genepools as described by various authors (Gepts et al. 1986; Singh et al. 1991a, b, c; Becerra and Gepts 1994; Islam et al. 2002; Blair et al. 2006, 2009). The conservation of the genepool separation typical of the primary centers of diversity has been observed before for bean in southern Africa (Martin and Adams 1987) and is also a hallmark of bean diversity in other secondary centers of diversity outside of the Americas, such as Southwest Europe (Rodiño et al. 2006) and China (Zhang et al. 2008).

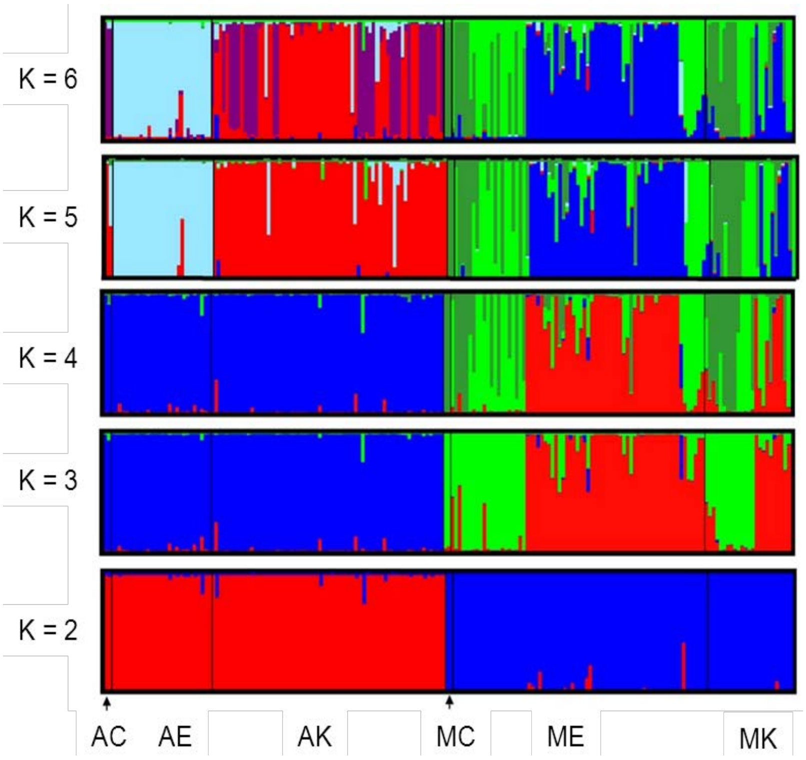

Fig. 4 Population structure for 192 common bean accessions from the East African Highlands compared to Andean and Mesoamerican control genotypes at $K = 2$ to $K = 6$. Predetermined group names indicated below figure are *AC* Andean control genotypes, *AE* Andean genotypes from Ethiopia, *AK* Andean genotypes from Kenya, *MC* Mesoamerican control genotypes, *ME* Mesoamerican genotypes from Ethiopia and *MK* Mesoamerican genotypes from Kenya

Table 4. Some characteristics of sub-populations identified at $K = 6$ population structure level for the East African common bean landraces

Population	Origin	N[a]	Growth habit [b]				Seed size			Bracteole size			Bracteole shape			
			I	II	III	IV	S	M	L	S	M	L	C	O	L	T
A1	Kenya	36	16	11	6	3	3	16	17	5	29	2	4	7	18	6
A2	Kenya	26	12	6	5	3		17	11	7	19			8	12	4
A3	Ethiopia	28	15	7	3	3	3	16	9	9	16	3	2	2	14	9
	Kenya	3	1	2				1	2	2	2				2	1
M1	Ethiopia	11		6	5		9	2				11	8	2	1	
	Kenya	10		3	5	2	9		1	2		8	3	5	2	
M2	Ethiopia	40		7	18	15	25	14	1	10	12	18	5	13	17	4
	Kenya	5			1	4	2	2	1	2	1	2	1	1	3	
M3	Ethiopia	19		3	14	2	17	2				19	12	5	1	1
	Kenya	9		1	1	7	6	3				9	5	3	1	

S small, *M* medium, *L* large, *C* cordate, *O* ovate, *L* lanceolate, *T* triangular

[a] Number (*N*) of genotypes in each sub-population with control genotypes and introgressed individuals not shown,

[b] Growth habit as described in Table 1

The separation of East African bean landraces into the two recognized genepools was stronger with SSR markers than with morphological markers indicating the success of this marker type in detecting genepools in common beans. Similar results were obtained in previous studies of accessions from primary and secondary centers of diversity analyzed with SSRs (Blair et al. 2006, 2009; Zhang et al. 2008). Despite the limitations of morphological analysis, the similarity distance matrices obtained using SSR markers was significantly correlated with that obtained with morphological markers ($r = 0.49731$, $P < 0.001$) based on the MXCOMP procedure of NTSYS-pc and testing using the normalized Mantel Z-statistics (Rohlf 2002). However, this positive and significant correlation might be misleading as distance matrices of morphological markers did not produce completely congruent patterns of population structure with that of SSR-based genetic distance matrices, which were of better resolution, indicating an under-estimate of genetic relationships with morphological markers. Discrepancy of clustering based on morphological and molecular markers has been attributed to hybridization or mutation that leads to divergent morphological or molecular profiles (Singh et al. 1991b). In addition, lower heritability or similarity of character states can also lead to poor separation based on morphological characteristics. Therefore, the use of informative molecular markers as a prior clustering criterion to improve the resolution power of morphological markers in common bean germplasm characterization is valid as was suggested by Singh et al. (1991a).

Microsatellite analysis showed generally low levels of introgression between the genepools compared to morphological analysis where character states were shared between the accessions belonging to each of the genepools. PCoA of morphological traits showed many intermediate genotypes while the same analysis for the SSR markers showed very few genotypes that were intermediate between the Andean and Mesoamerican clusters. Furthermore, in the neighbor-joining dendrograms only one genotype from Ethiopia (G18863) was intermediate between the genepools. In line with this, low gene flow and high genetic differentiation between genepools were observed in the present analysis. However, within genepool, gene flow was higher both between countries and within each country especially for Mesoamerican representatives.

These results suggest that the genetic divergence in East African bean landraces could be due to the original differences in introduced germplasm from the primary centers of origin combined with spontaneous out-crossing in farmer field and further farmer selection for adaptation to production niches and uses. For example, early flowering was common in many of the Andean accessions compared to the Mesoamerican accession in both greenhouse and field evaluation (data not shown). This lack of flowering synchronization could make inter-genepool hybridization a less likely phenomenon for the East African highland germplasm, even if varietal mixtures of both Andean and Mesoamerican phenotypes are a common farming practice in many parts of the region.

Another conclusion from the molecular analysis was that there was low gene flow between the two countries compared to gene flow within each country. This led to recognizably distinct germplasm at the national level especially for Andean genepool accessions. The molecular diversity was also reflected in the diversity for seed color, size and shape (data not shown) and the fact that the countries share very few seed types in the Andean genepool and only a few seed types in the Mesoamerican genepool. This might highlight different informal or formal institutional introductions and weak trans-national bean seed exchange or social and commercial networks especially for landrace varieties. One exception to this may be the case of small red-seeded beans in cross-border grain trade from southern Ethiopia to certain parts of Kenya. Apart from gene flow, an additional reason for the divergence in germplasm between Ethiopia and Kenya may be the existence of different farmers' selection preferences in each country in accordance with ecological adaptation, cooking value and market orientation. In fact, in Ethiopia, the small white and small red color classes are the preferred bean seed classes for export and local consumption, respectively, whereas in Kenya, large-seeded, red mottled seed types have high market preference (Wortmann et al. 1998).

Despite the distinct germplasm at the national level and at the genepool level, further race structure was not apparent in the East African common bean landraces. While all the population-genetic analysis employed in this study showed good congruence in the division of the landraces into two genepools, the number of sub-groupings varied depending on the analysis conducted. For example, INSTRUCT analysis suggested six sub-populations within the landraces with three of these corresponding to Andean genepool groupings and the other three to Mesoamerican genepool groupings; however, these six populations were not evident in the PCoA for the SSR markers where only three groupings, two in the Mesoamerican genepool and one in Andean genepool were found. Some of the genotypes clustered together in the neighbor-joining dendograms were assigned in different groups with INSTRUCT.

Andean diversity was found to be relatively high but difficult to subdivide with the Andean genepool control genotypes, Calima and G19833, representing Nueva Granada and Peru races, respectively (Blair et al. 2007) clustered together in both principal coordinate and population structure analysis. The closer placement of the two Andean control genotypes and concomitant overlap with other accessions from Ethiopia and Kenya might indicate the representation of the East African Andean genotypes, especially those from Kenya as part of a race Nueva Granada/ race Peru complex. This was evident in the distinction of the Andean groups in Table 4 where one group (A2) consisted of medium to large-seeded genotypes with small to medium sized bracteoles and a range of growth habits while another group (A1) was made up of only Kenyan genotypes with medium to large seeded and cylindrical or kidney seed shape genotypes having small to medium bracteole size, and ovate, lanceolate or triangular bracteole shape that corresponds to race Nueva Granada descriptors based on Singh et al. (1991b). The final Andean group (A3) consisted of Andean genotypes that were mostly from Ethiopia that had small to medium bracteole size, lanceolate or triangular bracteole shape, medium to large seed size, predominantly type I or II growth habit, and oval or rounded, cream spotted or tan seed shape.

Within the main Mesoamerican genepool grouping, most accessions clustered into three subgroups: M1 group included the Mesoamerican control genotypes DOR364 and ICA Pijao which are designated as race Mesoamerica in Díaz and Blair (2006) indicating the probable representation of East African Mesoamerican landraces by this race. This group possessed smaller seed size, larger cordate bracteole size and type II or III growth habit that corresponded to the race Mesoamerica description of Singh et al. (1991b). Another grouping under this genepool, M2, represented by 19 landraces from Ethiopia and nine landraces from Kenya had all large bracteole size and small to medium red, white or black seed with considerable admixture with the M1 populations suggesting that this represents another sub-grouping of race Mesoamerica. All small red-seeded genotypes including the dominant 'Red Wolayta' from Ethiopia were included under this group indicating that small red-seeded beans in Ethiopia have a narrow genetic background as compared to white and black beans that were distributed in the two other Mesoamerican sub-populations. Meanwhile, the majority of the small white-seeded genotypes from Ethiopia were represented in the third Mesoamerican sub-population, M3, which included 40 accessions from Ethiopia and five from Kenya almost all with small to medium seed size, indeterminate prostrate (III) or indeterminate climbing (IV) growth habit characteristic of the Durango–Jalisco race complex (Singh et al. 1991b; Díaz and Blair 2006). Hence, we suspect the apparent representation of this race complex in East Africa but further analysis would be needed to confirm this. A comparison of the East African beans to Latin American germplasm from the Caribbean, Central America, Mexico or Brazil as likely sources of germplasm sent to East Africa would also be valuable as would a comparison to European germplasm from the ex-Colonial countries that probably served as transit points for this diversity.

In this regard, many of the small red beans preferred in Ethiopia are typical of Central America (Singh et al. 1991a) and could have arrived through trade via Spain. Meanwhile, red mottled beans preferred in Kenya are typical of the Caribbean and could have followed the same route. Durán et al. (2005) characterized a large set of landraces of this seed type and found separation of the genepools based on morphological characteristics and RAPD markers with most of the large-seeded genotypes coming from the Eastern Caribbean. Rodiño et al. (2003, 2006) found small white beans in the Iberian Peninsula, and both studies observed inter-genepool introgression that may have produced new seed types.

In conclusion, our study found that population structure for the East African common bean landraces was based mainly on genepool origin and that introgression or gene flow was moderate. Given that beans in this region are often cultivated in marginal, risk-prone production ecologies (Wortmann et al. 1998), it will be interesting to correlate genetic diversity with drought tolerance and adaptation potential in future association mapping work. The results presented here also pave the way for rational use of East African germplasm and strategic crossing plans that could be used to identify transgressive segregation based on distinct germplasm at the national or regional level. In this regard, further phenotyping could identify the genotypes that would be the most valuable gene sources in future breeding programs in the region. Finally, the results also suggest that a considerable amount of common bean genetic diversity is present in East Africa motivating renewed conservation efforts for the region.

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CHAPTER 4

Quantitative trait loci for rooting pattern and shoot growth traits of common beans (*Phaseolus vulgaris* L.) grown under progressive water stress versus well-watered conditions

This chapter is based on the article: Asfaw A, Blair MW, Polanía JA, Rao IM, Quantitative trait loci for rooting pattern and shoot growth traits of common beans (*Phaseolus vulgaris* L.) grown under progressive water stress versus well-watered conditions. Accepted by *Molecular Breeding.* The authors thank CIAT bean breeding and physiology teams for assistance with lab analysis and greenhouse root phenotyping, respectively. Steve Beebe for population development. Conny Almekinders for study leave. This research was supported by funding from the Generation Challenge Program developing country scientists program, the Tropical legume (TL1) project from Bill and Melinda Gates foundation through Generation Challenge Program and a NUFFIC fellowship to Asrat Asfaw.

Abstract

Drought is the major abiotic constraint contributing to yield reduction in common beans (*Phaseolus vulgaris* L.) worldwide. An increasing scarcity of water in the future will make improving adaptation to drought stress a major objective of most crop breeding efforts. Drought avoidance by increased extraction of soil moisture from greater depth under drought conditions is an adaptive mechanism of common bean. A recombinant inbred line population of DOR364 × BAT477 was evaluated for rooting pattern traits in soil cylinder tubes under soil drying (progressive water stress) and non-stress (well-watered with 80% of field capacity) treatments in a greenhouse. One of the parents, BAT 477 is a deep rooting genotype while the other parent DOR 364 is a commercial cultivar in Central America. The recombinant inbred line population expressed quantitative variation and transgressive segregation for ten rooting pattern traits as well as five shoot traits of 48 days old plants. A mixed model QTL mapping analysis was carried out using a genetic map constructed with 165 genetic markers that covered 11 linkage groups of common bean genome. Genotype estimates were calculated from best design and spatial effects model for each trait. A total of 15 putative QTLs were identified for seven rooting pattern traits and four shoot traits. The QTLs detected were scattered over five of the 11 linkage groups. The QTLs detected for all the root traits except total root length and fine root length were main effect QTLs and did not interact with the level of water supply. The total root length and fine root length QTL with significant QTL × environment interaction only differed in magnitude of effect and interaction was of a non-cross over type. Other QTL for total root length, fine roots, thick roots, root volume and root biomass were co-localized and also explained relatively more genetic variance. This suggests the QTL affecting root traits in common beans are based on constitutive expression of genes and that drought avoidance based on deep rooting, longer root length, thicker roots, increasing root length distribution with depth, root volume and root biomass can be used in molecular breeding. The positive alleles for most of the QTLs detected in this study were derived from the paternal parent BAT477. The results from the present analysis highlighted the feasibility of marker-aided selection as an alternative to conventional labor-intensive, phenotypic screening of drought avoidance root traits.

Keywords: root cylinder, rooting depth, shoot:root ratio, specific root length

Abbreviations RD= Rooting depth; TRL = Total root length; FRL = Fine root length; TRL = Thicker root length; RV = Root volume; ARD = Average root diameter; SRL = Specific root length; RLD = Root length distribution with depth; Temp = Leaf temperature; SCMR = SPAD chlorophyll meter reading; LA = Leaf area; LTNC = Leaf total nonstructural carbohydrate; STNC = Stem TNC; SBDW = Shoot biomass dry weight; RBDW = Root biomass dry weight; R:S = Root : shoot ratio; RBMD = Root biomass distribution with depth; w/w = Weight by weight; v/v = Volume by volume

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most popular food legume of East and southern Africa and the Americas, providing proteins, vitamins and minerals to the diet of the poor (Broughton et al. 2003). About 60% of common bean produced around the globe are grown in regions subjected to either intermittent or terminal drought risk (Thung and Rao 1999), making drought the second largest contributor after disease to yield reduction (Rao 2001). In Africa, about 40% of bean production is under drought prone conditions and drought causes an estimated yield loss of more than 300,000 MT annually (Wortmann et al. 1998). In Latin America, up to 73% bean production is under drought risk. Meanwhile, only 7% of bean growing areas in the world are well-watered (Broughton et al. 2003). In some parts of the world, like sub-Saharan Africa and highland Mexico, drought episodes are frequent and complete crop failure is a common phenomenon (Rao 2001). Moreover, climate models predict that several regions where drought is already a problem such as the drought stressed areas of lowland and higher latitude East and southern Africa will become successively drier over the next decades (Jones and Thornton 2003).

Drought is the most limiting factor to plant survival with crop productivity affected in many agricultural regions (Boyer 1982). However, it is seldom a yearly event and its effect has seasonal and spatial variation (Passioura 2007). Drought tolerance is a prime target for molecular approaches to crop improvement (Salekdeh et al. 2009). The effects of drought stress on common beans have been well documented and include tissue specific plus whole plant effects (Acosta-Gallegos and Kohashi-Shibata 1989; Acosta-Gallegos and Adams 1991; Muñoz-Perea et al. 2006; Beebe et al. 2010). The effects are mostly dependent on the frequency, duration, and intensity of the stress and crop growth stage (Ramirez-Vallejo and Kelly 1998). Drought stress can cause flower abortion, pod dropping and reduced seed filling (Masaya and White 1991). Overall biomass and seed yield, harvest index, number of pods and seeds, seed weight, days to maturity are also affected (Nielsen and Nelson 1998; Ramĩrez-Vallejo and Kelly 1998). In addition, P uptake, N concentration and N fixation are reduced under drought (Serraj and Sinclair 1998; Guida dos Santos et al. 2004).

Genetic differences exist within common bean for adaptation to drought stress (Acosta-Gallegos and Kohashi-Shibata 1989; Acosta-Gallegos and Adams 1991; Ramirez-Vallejo and Kelly 1998; Muñoz-Perea et al. 2006) and have been exploited for breeding (Terán and Singh 2002; Singh et al. 2008; Beebe et al. 2008). Drought tolerance can come from either of the two recognized bean genepools (Mesoamerican and Andean). However, so far the Mesoamerican race Durango from dryland Mexico and race Mesoamerica native to the warm lowlands of Central America are the most important sources of valuable drought-adaptation genes (Singh et al. 1991; Terán and Singh 2002; Miklas et al. 2006). While the two races have been used as a consistent sources of improved drought adaptation for lowland tropical environments (Singh 1995; Terán and Singh 2002; Frahm et al. 2004; Singh et al. 2008; Beebe et al. 2008) little has been done for other races or for the Andean genepool (Beebe et al. 2010).

Plant mechanisms that improve drought adaptation in common beans include deep and balanced rooting system that increase extraction of soil moisture from lower soil depth (Sponchiado et al. 1989; White and Castillo 1992). Other adaptation strategies include maximizing water use for growth and seed yield through greater mobilization of photosynthates from vegetative structures to seed (Rao 2001). The plant adaptive strategies in common bean are mostly genetically determined although sometimes sensitive to local adaptation and environmental interaction (White 1987).

Efficient water uptake via enhanced root growth and development is an important factor for plants to adapt to drought stress environments (Turner 1979). This water uptake depends on root size (length or mass), activity and spatial distribution (Huang and Gao 2000). However, drought reduces root growth and development and can be a main cause of root death in the field (Smucker et al. 1991). A reduced root system under severe drought can aggravate plant susceptibility to insect pest (Muñoz-Perea et al. 2006) while enhanced healthy root system can help maintain plant water status, ultimately stabilizing yield under drought (Huang et al. 1997; Kamoshita et al. 2008). Genetic control of root characteristics determining drought adaptation of common beans is governed by the genotype of root system (White and Castillo 1989) and genetic variation exists for root traits under drought stress (Ho et al. 2005; Polanía et al. 2009) and other edaphic stresses (Lynch and Beebe 1995; Liao et al. 2004; Yan et al. 2004; Beebe et al. 2006). Even though the importance of root traits for adaptation to drought stress is well recognized in common bean breeding (Sponchiado et al. 1989; Rao 2001; Beebe et al. 2008), interaction with different soils makes root observation difficult to study. Hence selection for drought tolerance with QTL analysis would pave the way for application of marker assisted selection for root traits related to drought avoidance that are relatively more difficult and also time consuming to evaluate phenotypically.

Our objectives in this study, therefore, were (i) to evaluate variation for root traits among parents and recombinant inbred lines (RIL) population from the deep rooting genotype (BAT477) crossed with a small-red seeded genotype (DOR364) under contrasting water availability and (ii) to identify QTLs associated with drought avoidance root traits and a few shoot traits so as to provide fundamental genetic information for enhancing drought adaptation in this crop.

Material and methods

Plant material

A recombinant inbred line (RIL) population of 97 genotypes from the cross of DOR364 × BAT477 developed at CIAT (International Center for Tropical Agriculture) was used in this study. The population was developed through single seed descent to the F_5 generation followed by bulking for line development to create $F_{5:7}$ genotypes and is described further in Blair et al. (2010). DOR364 is a small-red seeded, high-yielding, commercial cultivar developed in Central America for resistance to *Bean golden yellow mosaic virus* and is acceptable in East Africa despite its dark red seed color. The line is an indeterminate upright short bush bean of type II growth habit (Schoonhoven and Pastor-Corrales 1987). It belongs to the race Mesoamerica of the Mesoamerican or the Middle American genepool as defined by Singh et al. (1991). It was developed under irrigated conditions and is less adapted to drought stress than BAT477, which is a cream-colored small-seeded breeding line with type II growth habit identified by CIAT as a drought tolerant genotype for lowland tropical environment and validated in Brazil and several other countries. This line combines a deep rooting ability with greater water uptake efficiency (White et al. 1994a, b) and like DOR364 also belongs to race Mesoamerica of the Mesoamerican genepool (Singh et al. 1991). All RILs plus both parents and drought tolerant SEA5 were used in the greenhouse experiment.

Experimental conditions

A greenhouse study was conducted at CIAT (3°29′N and 76°21′W) located in Palmira using a natural Andisol from the common bean growing region of Darién, Colombia mixed (2:1 w/w) with river sand. Soil cylinders were carefully packed with 4200 g of soil : sand mixture, with a final bulk density of 1.2 g cm^{-3}. Soil was fertilized with adequate level of nutrients (based on the rate of 80 kg/ha nitrogen, 50 kg/ha phosphorus, 100 kg/ha potassium, 101 kg/ha calcium, 29.4 kg/ha magnesium, 20 kg/ha sulfur, 2 kg/ha zinc, 2 kg/ha copper, 0.1 kg/ha boron and 0.1 kg/ha molybdenum) at planting by mixing with the soil. The seeds of the genotypes of each treatment were surface sterilized for 1 to 2 minutes in 10% (v/v) NaOCl, thoroughly rinsed with de-ionized water and germinated in rolled-up germinating paper placed upright in plastic tank. The seeds were germinated in darkness at room temperature for 72 hrs and uniform seedlings were selected for transplanting to closed-bottom transparent plastic cylinders (80 cm long with 7.5 cm diameter) each of which was inserted into PVC sleeve-tubes.

Plants were grown for 48 days in these plastic cylinders/PVC sleeve-tubes from October to December 2007 with an average maximum and minimum temperature of 39.7 ^{o}C and 20.5 ^{o}C (Fig. 1). The maximum photon flux density in the greenhouse at noon was 1100 µmol m^{-2} s^{-1} during the experiment. Two contrasting water supply treatments were applied namely 1) well-watered (WW) at 80% field capacity and 2) progressive water stress (WS) with no watering after 10 days of growth to simulate progressive water stress conditions similar to terminal drought stress conditions of some common bean target environments in the tropics.

The experimental genotypes were arranged in a row and column design and replicated three times within the greenhouse. The initial soil moisture for all the treatments was of 80% field capacity but the water-stressed plants suffered a reduction in soil moisture from 80% to 28% field capacity in average over the 38 days of the treatment period (Fig. 1) while the well-watered plants were maintained at 80% field capacity throughout the experimental period. The plants with well-watered treatment were maintained by weighing each cylinder every two days and applying water to the soil at the top of the cylinder to restore the appropriate moisture level of 80% field capacity. Plants with progressive water stress treatment received no water application and each cylinder was weighed at 2-day intervals for the determination of decrease in soil moisture content until the time of plant harvest.

Plants were harvested at 48 days after transplanting, in other words equivalent at 38 days of withholding of water application in the case of water stress treatment. Rooting depth was measured during the experiment at 2 day intervals using a ruler in cm scale, registering the total length reached by the visible roots at the edges of the plastic cylinder insert. Leaf temperature was measured in fully expanded leaf for each replication at 17 days after exposing the plants to water stress using an infrared thermometer (Telatemp model AG-42D, Telatemp CA, USA) held at 50 cm from the canopy surface in a 45° angle. Leaf chlorophyll content was measured with SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd, Japan). SPAD chlorophyll meter reading (SCMR) was recorded on a fully expanded young leaf for each replication at 17 and 34 days after exposing the plants to water stress and mean value of the two measurements was used for the analysis.

At harvest, leaf area (LICOR model LI-3000), shoot biomass distribution, and root distribution were measured. For root distribution traits, the cylinder was sliced into six layers (0-5, 5-10, 10-20, 20-40, 40-60 and 60-75 cm) and roots in each soil layer were washed free of soil and sand. The washed roots were preserved in a refrigerator before being scanned as images by a desk scanner (ScanJet Iic, Hewlett-Packard, USA). From the scanned images, total root length (m plant^{-1}), fine roots length (m plant^{-1}), thick roots length (m plant^{-1}), average root diameter (mm plant^{-1}), total root surface (cm^{3} plant^{-1}) were measured with an image analysis by WinRHIZO software (Regent Instruments INC, Quebec, Canada).

Root and shoot dry weight was determined after the roots and shoots were dried in an oven at 60 ^{o}C for 48 hours. Specific root length (SRL) was calculated as length per unit of root dry weight (m mg^{-1}). Leaf and stem TNC (total nonstructural carbohydrate content) was determined using NaOH as an extraction medium and anthrone reagent. Absorbance of the solution was measured with a spectrophotometer at 620 nm and TNC concentration was determined by comparison with glucose standards (Kang and Brink 1995). Differences in distribution of root length and root biomass within soil cylinder among genotypes were estimated by Gale and Grigal (1987) asymptotic equation: ($Y = 1\text{-}ß^{d}$) where Y = the fraction of root length/biomass accumulated from the soil surface to depth d (cm), and ß is a parameter that describes the shape of the cumulative distribution with depth. Higher ß values (closer to 1) indicate a greater proportion of root length/biomass deeper in the soil profile. Lower ß values (e.g. ß = 0·920) imply a greater proportion of root length/biomass nearer to the soil surface.

Data analysis

Analysis of variance of the variables in both drought-stressed and non-stressed treatments and Pearson’s correlations among the variables were carried out using the program Genstat version 12.1 (Payne et al. 2009). For analysis of variance the data were subjected to the Genstat mixed model analysis first for each single trait in each single environment and then for genotype × environment interaction (G×E) following Gilmour et al. (1997) using a Residual Maximum Likelihood (REML) procedure. Row and column effects were added to the model as random variable when significant for a trait. This was performed to remove the effect of cylinder tube position with respect to sunlight orientation in the greenhouse. Genotypes were considered as random in single environment data in order to estimate genetic or error variance parameter and generalized heritability values. Meanwhile genotypes were considered as fixed to get unshrunken means (best linear unbiased estimates = BLUEs) for QTL analysis in the best spatial model. Generalized heritability, as the proportion of total variance explained by genotypic component in best spatial model, was calculated as the formula $h^2_g = 1- [PEV/ 2\sigma^2_g]$ (Cullis et al. 2006; Oakey et al. 2006). PEV is the predicted error variance, or average variance of the difference as in VPREDICT statement in Genstat 12.1 edition) and σ^2_g is the genotypic variance. For multi-environment analysis in the same software, the BLUE mean from single environment was used. G×E interaction GEI pattern for the trait assessed using genotype plus genotype × environment interaction (GGE) biplot function was also implemented in the Genstat 12.1 software. Population distributions for the variables within each irrigation treatment were performed using graph program of Sigmaplot version 10.0 (Systat Software, Inc, CA, USA).

Molecular mapping of QTLs associated with root and shoot traits

The molecular markers used in the study included random amplified polymorphic DNA (RAPD) primers (Operon Technologies Inc., Alameda, CA), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) or microsatellites. Experimental procedures of genotyping for RAPD, and AFLP markers were as described in Blair et al. (2006) and Muñoz et al. (2004), respectively. The procedure for SSR markers was the same as described in Blair et al. (2003, 2008). The overall laboratory techniques for the three different marker analysis were presented in Blair et al. (2010). A new genetic linkage map was constructed using the software JoinMap® 4.0 for Windows (http://www.kyazma.nl/index.php/mc.JoinMap/) set to the Haldane 1 mapping function. To create grouping trees, mapping parameters were set to recombination frequency smaller than 0.15 and independence log of odds (LOD) score larger than 5.0. To order markers within a group, regression mapping algorithm was used first with start marker order of specific linkage group from previous bean genetic maps (Blair et al. 2003, 2008). The best marker order of the linkage groups was checked with best plausible positions in maximum likelihood mapping algorithm set to 1000 permutation. The mapping analysis was performed using marker data of 205 molecular markers segregating in the population of RILs. Naming of linkage groups was done by checking for each marker against known marker positions genetic maps constructed by Blair et al. (2003, 2008, 2010). For the final map to use in QTL analysis a subset of 162 markers was selected in such way that no two markers were located in the same position from resulting genetic map were used.

QTLs and their treatment or G×E interaction effects were mapped by mixed model based QTL mapping approach using a single trait and multi-environment option implemented in Genstat 12.1 (Payne et al. 2009). For QTL detection a three step mixed model analysis as described in Boer et al. (2007) was performed using a single interval mapping (SIM) procedure. In the SIM method, which operates based on Bayes theorem and Markov chain methodology (Lander and Green 1987), the QTL effects and positions were estimated based on flanking markers to accommodate uneven coverage of markers along the genome. Hence, additive genetic predictors (evaluation positions) based on marker phenotypes were estimated at maximum predictor distance of 10 cM which resulted in 197 positions to be tested in the genome. A genome-wide scan for significant QTL expression was performed using the SIM procedure taking each marker (between marker predicted position) fitted as fixed environment-specific QTL effect while maintaining the best variance-covariance structure based on Schwarz information criterion (SIC) (Schwarz 1978) set in Genstat GGE model previously determined in G×E interaction analysis.

The QTL threshold was based on peak value exceeding a value defined to control for multiple testing established by the Bonferonni correction (Lynch and Walsh 1998). The QTL effects were tested by a Wald test (Verbeke and Molenberghs 2000). The amount of variation explained by each QTL was calculated as per Mathews et al. (2008). The explained genetic variance (as percentage of the total genetic variance) was calculated as: % explained genetic variance by QTL = 100 × [1 – (genetic variance in the model with QTL/ genetic variance in model without QTL)].

Results

Variation of parents and derived lines for water uptake

The progressive water-stress treatment caused a reduction in soil water content (Fig. 1). The final soil moisture for the plants in progressive water stress treatment was on average 28% of field capacity value. The ability of parents and their derived lines varied in extracting water from the drying soil. The drought tolerant parent, BAT477, expressed better potential in extracting water from drying soil than the drought susceptible parent, DOR364. This difference was not clear during the initial soil drying period but the superiority of BAT477 in acquiring soil water from drying soil was expressed in critical stage of soil water depletion from the second week of drought stress onwards. This substantiated the ability of BAT477 for greater water uptake efficiency based on its deeper and thicker root system. Transgressive segregation was observed for water uptake efficiency from drying soil in the RILs developed from the two parents. This was apparent from better ability of RILs in extracting water from drying soil than the better parent BAT477. However, the advanced breeding line SEA5 which served as a drought-tolerant check expressed better water uptake efficiency in drying soil. This demonstrated the superior level of drought tolerance of SEA5 over BAT477, which is one of its predecessors in the pedigree of SEA5.

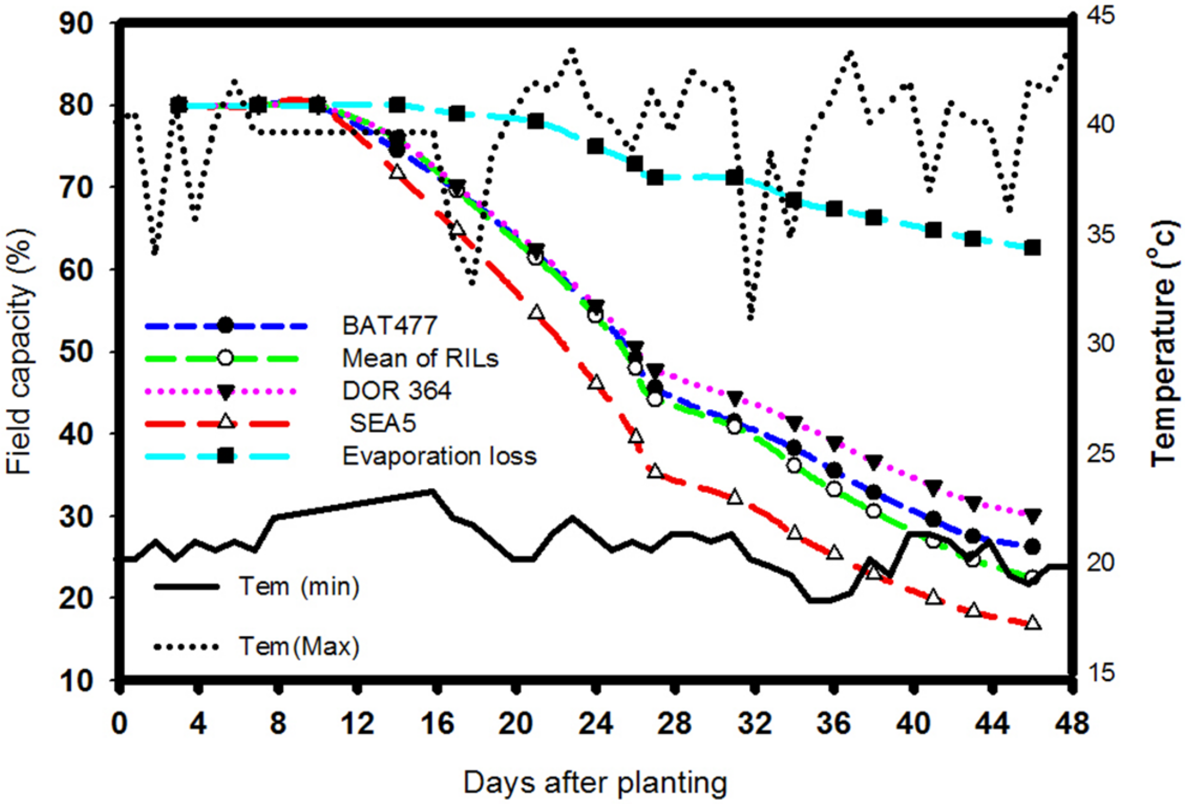

Fig. 1 Ability of genotypes including parents and their derived lines as well as the drought tolerant check SEA5 to extract water while growing on a drying soil in deep root cylinders in a greenhouse in Palmira Colombia (2007). Maximum and minimum temperatures (in ^{o}C) during soil drying are shown at the left y-axis, while the right y-axis shows % field capacity across days of growth and development of the plants

Variation of parents and the derived lines for root and shoot traits

The parents of the population were contrasting for root and also shoot traits in both well-watered and progressive water stress treatments (Table 1). The parental difference was significant for thick roots length, root volume, average root diameter, specific root length, leaf TNC and root biomass dry weight and non-significant for leaf temperature, SCMR, leaf area, stem TNC and shoot biomass dry weight in both well-watered and progressive water stress treatments. This was reflected by significantly larger root system (greater root volume and deeper rooting ability, larger and thick roots, wide average root diameter and higher root biomass) found in BAT477. For all the significantly different traits, BAT477 was better except for specific root length for which DOR364 was higher. The parental difference was non-significant in well-watered treatment for deep-rooting and root:shoot ratio while significant in the case of progressive water-stress treatment. For total root length and fine roots length, the parental variation was significant in well-watered treatments whereas this was non-significant under terminal drought treatment.

Table 1 Phenotypic variation and generalized heritability for rooting pattern and shoot traits in parents and recombinant inbred lines (RILs) of DOR364 × BAT477 cross in greenhouse soil tube well-watered and progressive water stress treatment

	Well-watered (WW)					Water-stressed (WS)				
	Parents			RILs mean	h^2_g	Parents			RILs mean	h^2_g
Trait	DOR364	BAT477	Diff			DOR364	BAT477	Diff		
Rooting depth (cm)	44.95	58.16	ns	56.03^{ns}	0.19	57.99	72.17	*	66.43*	0.31
Total root length (m plant^{-1})	37.25	67.80	**	60.20**	0.55	36.10	38.46	ns	41.39**	0.46
Fine root length (m plant^{-1})	34.07	60.92	**	53.97**	0.54	32.40	32.87	ns	36.48**	0.45
Thick root length (m plant^{-1})	2.66	5.73	**	5.14**	0.58	3.05	4.70	**	4.21**	0.51
Root volume (cm^3 plant^{-1})	3.29	6.27	**	5.53**	0.61	3.13	4.03	**	3.85**	0.49
Average root diameter (mm)	0.92	1.70	**	1.79**	0.71	1.76	2.23	**	2.02**	0.46
Specific root length (m g^{-1})	96.41	82.39	**	83.71**	0.40	91.42	80.09	**	83.28**	0.46
Root length distribution with depth (ß)	0.92	0.96	**	0.95**	0.64	0.94	0.96	*	0.96**	0.54
Root biomass distribution with depth(ß)	0.91	0.95	**	0.94**	0.53	0.94	0.96	*	0.95**	0.57
Leaf temperature (^{0}C)	25.20	25.23	ns	25.37^{ns}	0.10	27.91	26.70	ns	27.29^{ns}	0.05
SCMR	33.65	34.15	ns	35.87**	0.64	42.80	44.32	ns	42.99**	0.41
Leaf area (m^2 plant^{-1})	619.10	659.30	ns	634.10^{ns}	0.01	358.70	281.20	ns	310.40^{a}	0.21
Leaf TNC (mg g^{-1})	317.30	612.80	**	302.70**	0.57	108.90	297.40	**	207.90**	0.65
Stem TNC (mg g^{-1})	286.7	295.20	ns	359.30^{ns}	0.14	294.50	322.00	ns	295.30**	0.43
Shoot biomass DW (g plant^{-1})	3.83	5.66	ns	5.07^{ns}	0.08	2.14	2.39	ns	2.34^{ns}	0.05
Root biomass DW (g plant^{-1})	0.40	0.87	**	0.74**	0.56	0.41	0.49	*	0.50**	0.44
Root:shoot ratio	0.11	0.14	ns	0.15**	0.39	0.09	0.15	*	0.22**	0.35

[a], *,** Treatment differences significant at the 0.10, 0.05, and 0.01 probability level, respectively, ns is non-significant

† Diff difference between parents, TNC total non-structured carbohydrate, DW dry weight, Root length and biomass distribution with depth is extinction coefficient ß value from the equation described in Materials and Methods section

The RIL population distribution of root and shoot traits was quantitative and normally distributed except some skewing was observed for root length distribution with depth, average root diameter and leaf area in the progressive water stress treatment (Figs. 2, 3). Trangressive segregant RILs were observed for all the traits measured and this was true in both positive and negative directions in well-watered and progressive water-stress treatments. When these traits were subjected to analysis of variance, significant differences between the recombinant inbred lines were observed for all traits except leaf temperature and shoot biomass dry weight in both conditions. Differences for rooting depth, leaf area and stem TNC were non-significant in the well-watered treatment but significant in the progressive water stress treatment. Water stress significantly reduced root biomass and above ground shoot biomass for both parents and their derived lines. Rooting depth, average root diameter, leaf temperature, SCMR and root:shoot ratio however, were increased by the progressive water stress treatment. As expected, well-watered plants in general produced greater below ground and above ground biomass.

Correlation among root and shoot traits

Phenotypic correlations between root and shoot trait values of RILs in both well-watered and progressive water stress treatments are shown in Table 2. Positive and significant correlations were observed among rooting depth, total root length, fine roots length, thick roots length, root volume, average root diameter, root length distribution with depth, shoot biomass, root biomass, root:shoot ratio and root biomass distribution with depth in both well-watered and progressive water stress treatments. However, the correlations were positive and non-significant for root:shoot ratio with rooting depth and average root diameter in the progressive water stress treatment. Leaf area had a positive and in most cases significant correlation with root traits but was negatively associated with SCMR irrespective of water supply level. In this study deeper and thicker roots were strongly and positively associated with other root traits regardless of the degree of water supply. Specific root length was negatively associated with all other traits except with leaf TNC in water stress. The other shoot traits (leaf temperature, SCMR, leaf and stem TNC) were weakly or negatively correlated with root traits.

Heritability and QTL analysis with the DOR364 × BAT477 genetic map

The generalized heritability varied among different traits (Table 1) ranging up to 71% for average root diameter in well-watered treatment but being low for traits like leaf area, leaf temperature and shoot biomass in well-watered and progressive water-stressed treatments. Heritability values were intermediate for rooting depth, equaling 19 and 31% under well-watered and progressive water stress conditions, respectively, and root:shoot ratio, equaling 39 and 35%, respectively. However total root length, specific root length and component variables had high heritabilities in both treatments ranging from 45 to 61%. For stem TNC, heritability was higher in progressive water-stressed than in well-watered treatment. Finally heritability tended to be lower for traits that did not vary significantly between the parents.

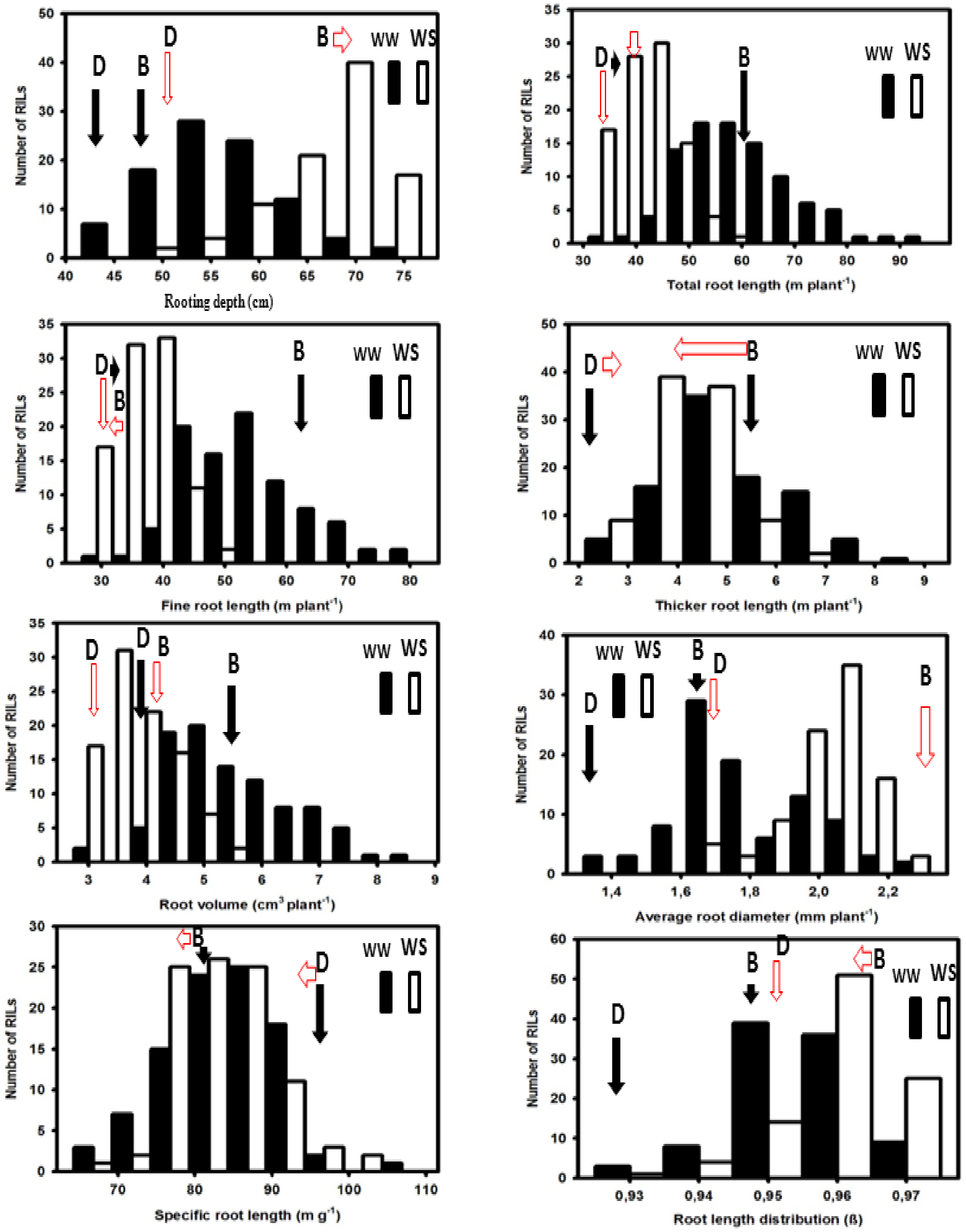

Fig. 2 Population distribution for rooting depth, total root length, fine roots length, thicker roots length, root volume, average root diameter, specific root length and root length distribution with depth among RILs of DOR364 × BAT477 cross under greenhouse well-watered and progressive water stress experiment. Maternal (DOR364 abbreviated as D) and paternal (BAT477 abbreviated as B) trait values indicated by arrows (dark arrow for well-watered (WW) and light arrow for water-stressed (WS) experiment). WW treatment is represented by black bar while white bar represents WS treatment

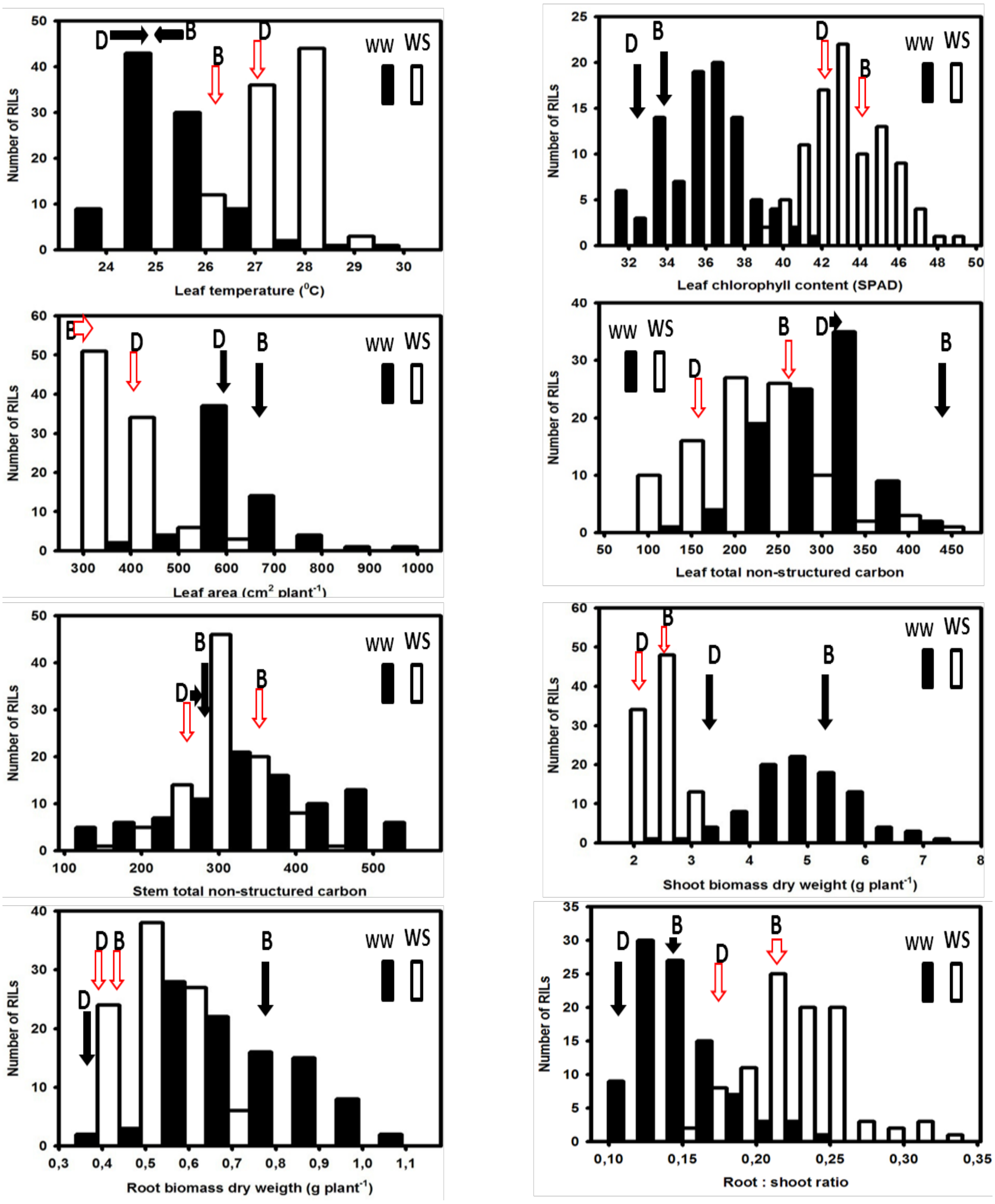

Fig. 3 Population distribution for leaf temperature, leaf chlorophyll content (SPAD), leaf area, leaf total non-structured carbon, stem total non-structured carbon, shoot biomass dry weight, root biomass dry weight and root:shoot ratio among RILs of DOR364 × BAT477 cross under greenhouse well watered and terminal drought experiment. Maternal (D) and paternal (B) trait values indicated by arrows (black arrow for well watered (WW) and light arrow for terminal drought (WS) experiments). Well watered (WW) treatment is represented by black bar while terminal drought (WS) in white bar

Table 2 Phenotypic correlations among root and shoot traits in greenhouse well-watered (WW) condition, above diagonal and water-stressed (WS) condition, below diagonal

	RD	TRL	FRL	TRL	RV	ARD	SRL	RLD	TEMP	SPAD	LA	LTNC	STNC	SBDW	RBDW	R:S	RBMD
RD	-	0.49**	0.48**	0.50**	0.55**	0.65**	-0.35**	0.64**	-0.10	0.09	0.43**	-0.09	-0.10	0.33**	0.59**	0.26*	0.68**
TRL	0.41**	-	0.99**	0.83**	0.95**	0.36**	-0.06	0.58**	0.02	-0.08	0.48**	0.04	0.13	0.45**	0.89**	0.49**	0.46**
FRL	0.37**	0.99**	-	0.79**	0.93**	0.34**	-0.03	0.57**	0.01	-0.10	0.47**	0.03	0.12	0.43**	0.87**	0.48**	0.45**
TRL	0.50**	0.77**	0.68**	-	0.94**	.48**	-0.33**	0.55**	0.10	0.05	0.41**	0.10	0.14	0.40**	0.87**	0.51**	0.50**
RV	0.47**	0.92**	0.86**	0.93**	-	.46**	-0.27**	0.62**	0.04	-0.01	0.50**	0.04	0.12	0.46**	0.95**	0.54**	0.51**
ARD	0.65**	0.32**	0.25*	0.57**	0.51**	-	-0.31**	0.52**	-0.02	0.21*	0.24*	-0.08	-0.004	0.21*	0.45**	0.26*	0.49**
SRL	-0.46**	-0.25*	-0.17	-0.54**	-0.41**	-0.43**	-	-0.30**	-0.02	-0.11	-0.43**	0.003	-0.14	-0.33**	-0.48**	-0.19	-0.24*
RLD	0.53**	0.37**	0.32**	0.53**	0.48**	0.52**	-0.41**	-	-0.02	-0.05	0.51**	-0.02	-0.003	0.38**	0.63**	0.29**	0.79**
TEMP	0.18	-0.04	-0.03	-0.03	-0.07	0.03	-0.09	0.17	-	0.09	-0.06	0.05	0.02	-0.08	0.03	0.11	-0.06
SCMR	0.16	0.27**	0.28**	0.15	0.22*	0.08	-0.03	0.14	0.07	-	-0.04	0.19	0.18	0.20	-0.03	-0.22*	-0.002
LA	0.34**	0.18	0.16	0.23*	0.23*	0.39**	-0.23*	0.35**	0.004	-0.14	-	0.12	0.18	0.58**	0.58**	0.05	0.34**
LTNC	-0.12	-0.01	-0.01	-0.05	-0.003	0.01	0.14	0.02	-0.19	0.23*	0.09	-	0.43**	0.34**	0.02	-0.31**	-0.01
STNC	0.06	0.22*	0.22*	0.13	0.17	-0.03	-0.03	0.10	-0.21*	0.16	0.07	0.47**	-	0.50**	0.15	-0.33**	-0.05
SBDW	0.53**	0.50**	0.50**	0.37**	0.45**	0.32**	-0.28**	0.39**	0.10	0.18	0.34**	0.20	0.19	-	0.50**	-0.43**	0.29**
RBDW	0.53**	0.91**	0.87**	0.86**	0.93**	0.45**	-0.61**	0.48**	-0.01	0.22*	0.22*	-0.07	0.16	0.51**	-	0.55**	0.49**
R:S	0.07	0.52**	0.48**	0.58**	0.58**	0.19	-0.40**	0.17	-0.09	0.08	-0.08	-0.27**	-0.01	-0.37**	0.61**	-	0.23*
RBMD	0.56**	0.52**	0.47**	0.62**	0.60**	0.50**	-0.43**	0.84**	0.23*	0.21*	0.27**	0.03	0.12	0.44**	0.60**	0.25*	-

*,** Correlation is significant at the 0.05 and 0.01 level (2-tailed)

RD rooting depth, *TRL* total root length, *FRL* fine roots length, *TRL* thicker roots length, *RV* root volume, *ARD* average root diameter, *SRL* specific root length, *RLD* root length distribution with depth, *TEMP* leaf temperature, *SCMR* SPAD chlorophyll meter reading, *LA* leaf area; *LTNC* leaf total nonstructural carbohydrate content, *STNC* stem nonstructural carbohydrate content, *SBDW* shoot biomass dry weight, *RBDW* root biomass dry weight, *R:S* root : shoot ratio, *RBMD* root biomass distribution with depth

The DNA polymorphism level for the DOR363 × BAT477 population with the molecular markers used in this study was low to moderate depending on the marker type as described in greater detail in Blair et al. (2010). As distinct from that study, the linkage map was constructed with a regression mapping algorithm using a total of 165 of the 205 polymorphic markers evaluated. This linkage map covered all 11 linkage groups of the bean genome with a total genetic distance of 798.6 cM which is in same range as reported by previous studies (Blair et al. 2006). Average length of the linkage group was 71.8 cM with average of 14.7 markers per linkage group. The advantage of this genetic map for QTL analysis was that mapping positions were represented by non-conflicting and non-overlapping markers such that a genome scan procedure was appropriate for all traits.

A multi-environment mixed model genome scan with the single interval mapping (SIM) procedure identified 15 regions which related to QTL presence for eight root and four shoot traits (Table 3; Fig. 4) at a genome-wide threshold significance level of $\alpha = 0.05$ based on the Bonferonni correction for the number of markers in the genetic map. Of the 15 putative QTL positions, six on b11 belonged to predicted between marker positions whereas the remaining nine corresponded to actual marker positions based on SIM procedure. The significant QTLs were scattered over 5 of the 11 linkage groups including b01, b08, b09, b10 and b11. The significant QTL were named by combining the first two or three letters of a trait with the linkage group and the order of QTL for the given trait on that linkage group (Table 3). The QTL for total root length, fine roots, thick roots, root volume and root biomass co-localized as predicted at overlapping loci on linkage group b11.

The co-localized QTL for thick roots, root volume and root biomass QTL on b11 was a main effect QTL that did not interact with water level and where the BAT 477 alleles was shown as red in subfigure 4.A2 contributed to an average increase in both well-watered and water-stressed treatments. The QTLs for total root length and fine roots length on b11, leaf TNC on b10, and shoot biomass on b08 and b09 showed significant QTL × environment interaction effects while other QTLs were not. Interestingly the QTL × environment interaction were of the non-crossover type and did not change direction with well-watered and progressive water stress treatments. The QTL × environment interaction effects in the study population were not attributed to the contrasting effects of the parental alleles between non-stress and stress environment but rather the differential expression of paternal alleles in different environment.

The positive QTL effects in Table 3 indicated that the BAT477 alleles generally contributed to an average increase of trait values whereas the negative effects were shown when alleles were derived from DOR364. The rooting depth QTL on b11 showed only main effect and as expected the positive alleles for QTL conferring deep rooting came from BAT477 in both well-watered and progressive water stress treatments (Subfig. 4.B2). The QTL on b01 for root length distribution with depth had only a main effect with the BAT477 allele providing for a deeper distribution. The QTL for SCMR was main effect QTL exclusively and was found on linkage group b09.

Table 3 Quantitative trait loci (QTL) of rooting pattern and shoot traits in multi-environment mixed model genome scan (SIM model) for recombinant inbred line (RILs) of DOR364 × BAT477 cross in greenhouse experiments†

Trait	QTL name	LG	Nearest marker	Marker interval	Wald	W_{porb}	QTL effect		% σ^2_g explained by full QTL model
							WW	WS	
Rooting depth (cm)	Rd11.1	11	P302	P302 - BMd43	7.90	0.006	1.45	1.45	20.5
Total root length (m plant^{-1})	Rl11.1	11	AB502	AB502 - P302	8.75	0.000	5.23	1.95	33.2
Fine roots length (m plant^{-1})	Frl11.1	11	AB502	AB502 - P302	7.74	0.001	4.54	1.42	29.6
Thicker roots length(m plant^{-1})	Trl11.1	11	AB502	P302 - AB502	18.86	0.000	0.43	0.43	29.6
Specific root length(m g^{-1})	Srl10.1	10	M902	AI1403 - P1501	9.50	0.003	1.75	1.75	17.4
Root volume (cm^3 plant^{-1})	Rv11.1	11	AB502	AB502 - P302	18.03	0.000	0.32	0.32	37.7
Root length distribution	Rld1.1	1	BM53	C1601 - Bma241	13.67	0.000	0.003	0.003	19.3
SCMR	Lch9.1	9	BMc255	Pv-ctt007 - F1002	9.89	0.002	0.56	0.56	14.8
Leaf TNC (mg g^{-1})	Ltc9.1	9	BMc255	Pv-ctt007 - F1002	18.17	0.000	19.81	19.81	27.0
	Ltc10.1	10	X903	U1403 - M1602	7.92	0.001	8.82	29.64	22.4
Stem TNC (mg g^{-1})	Stc10.1	10	BMa71	BMa76 - P401	8.38	0.004	14.36	14.36	5.7
Shoot biomass dry weight (g plant^{-1})	Sbm8.1	8	O1604	O1603 - BM211	5.64	0.005	0.323	0.014	10.7
	Sbm9.1	9	BMc255	Pv-ctt007 - F1002	9.27	0.003	0.314	0.08	9.8
Root biomass dry weight (g plant^{-1})	Rbm11.1	11	AB502	AB502 - P302	17.52	0.000	0.043	0.043	40.0
Root : shoot ratio	Rsr11.1	11	AB502	AB502 - P302	15.60	0.000	0.011	0.011	27.8

† LG Linkage group, WW non-stress well watered treatment, WS progressive water stress treatment, σ^2_g genetic variance, W_{Prob} Wald probability, SCMR SPAD chlorophyll meter reading, TNC total nonstructural carbohydrate content

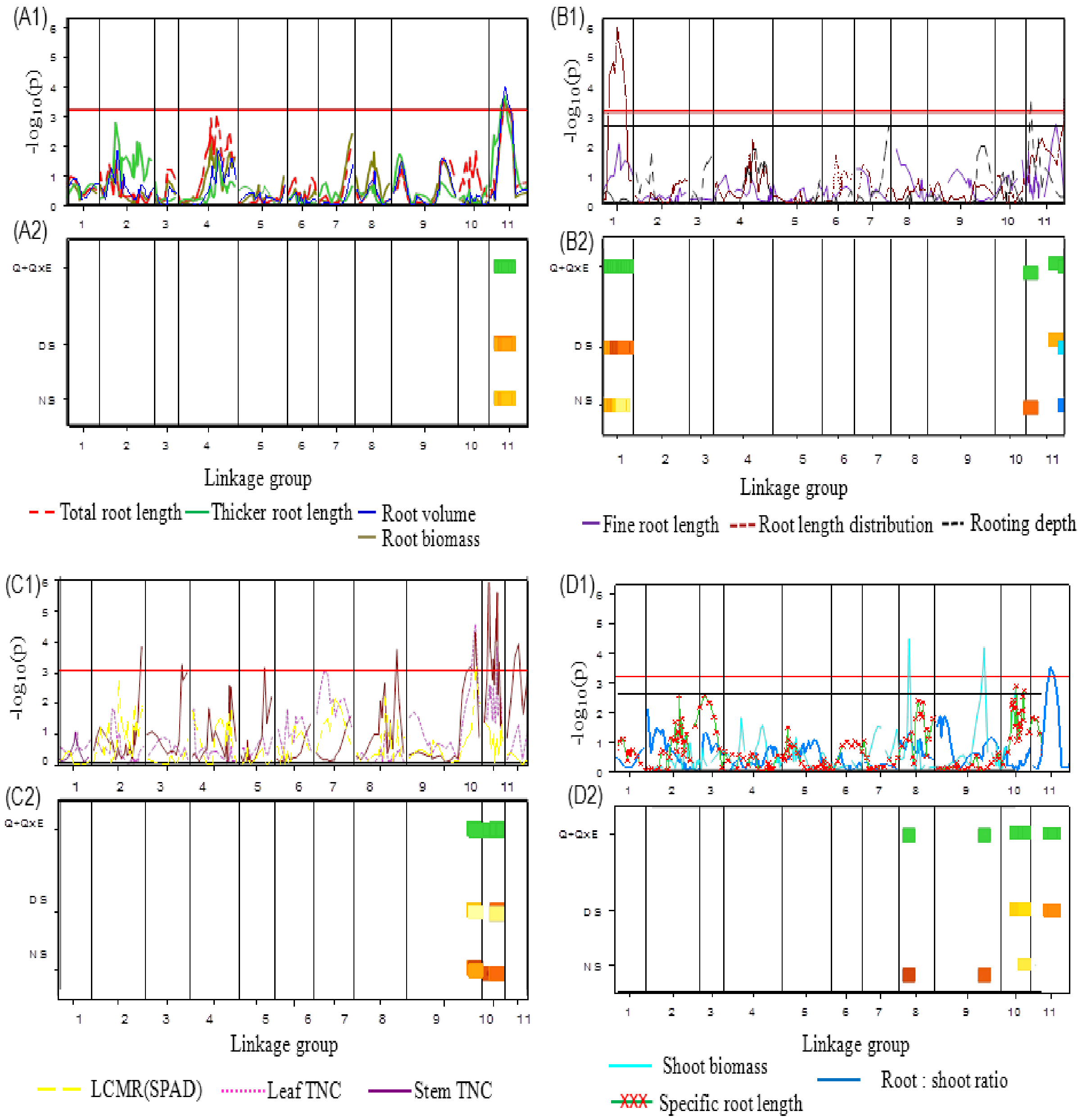

Fig. 4 Genome scan QTL traces for root and shoot drought related traits in the DOR364 × BAT477 recombinant inbred line mapping population in the greenhouse (Refer to Table 3 for QTL names and to Blair et al. (in press) for base genetic map). Co-localizing QTL for total root length, thicker roots length, root volume and root biomass are presented in LOD (–log10(P)) graph labeled subfigure A1. The QTL for fine roots length, root length distribution with depth and deep rooting are indicated in the subfigure B1, QTL for SPAD chlorophyll meter reading, leaf and stem TNC in subfigure C1 and QTL for shoot biomass, root : shoot ratio in subfigure D1. The *red horizontal line* in subfigures A1, B1, C1 and D1 represent α = 5% genome-wide significance threshold for all traits while the black *horizontal line* in subfigure B1 and D1 represents the –log10(P) value of 2.5 used as threshold for detection of rooting depth and specific root length QTL. The *vertical lines* represent the divisions between 11 linkage groups of the common bean genome. All heat maps use color in the top section to indicate the location of the QTL while the red and blue indicate environment-specific effect of the parental marker alleles. In this case, red represents drought tolerance from the paternal BAT477 marker allele and blue represents the drought tolerance from susceptible maternal DOR364 marker allele. The darker the color in these subfigures the larger the effect of the alleles in each case

The genetic variance explained by the final QTL models for each trait was as high as 40.0% for root biomass dry weight QTL on b11 and 37.7% for root volume QTL on b11. The QTL for rooting depth, specific root length and root length distribution over depth were intermediate in their contribution of genetic variance (17.4 to 20.5%) as were those for leaf TNC and SCMR. Total root length, fine roots, thicker roots and root:shoot ratio also had QTL in the range of 27.8 to 33.2% of variance explained. Meanwhile all the remaining QTL were of lower significance as measured by their effect on genetic variance. It was notable that all the QTL that expressed significant QTL × environment interaction had a lower proportion of genetic variance explained by the full QTL model as compared to QTL conferring main effects only. Thus, for those QTLs with significant QTL × environment interaction on average approximately 78.9% of the genetic variance was unexplained whereas for those with main effects on average approximately 24% of the genetic variance was explained, highlighting the difficulty of detecting QTL in drought studies for common beans even under controlled greenhouse conditions.

Discussion

Plants usually express differential adaptive strategies to drought stress. These include escape, avoidance or tolerance strategies (Levitt 1972), which may not be mutually exclusive but in practice are rarely combined within an agronomically superior genotype (Ludlow 1989). Instead a range of adaptive responses to drought and mechanisms of drought tolerance are found in plants. For example and most importantly for this study, an efficient water uptake via a deep and balanced root system is an important factor for plants to adapt to drought stress environments (Turner 1979; Huang et al. 1997; Kamoshita et al. 2008). However, soil makes selection for root traits related to drought adaptation a real challenge in crop improvement. This is because most of the methods used to evaluate roots are not only time consuming and laborious, but also destructive to the plant sample (Beebe et al. 2006) although some methods using tensiometers or dynamometers might resolve this.

Phenotypic selection for root traits therefore is a slow and labor-intensive process justifying the search for alternative strategies such as QTL tagging in controlled greenhouse test followed by indirect selection through the use of marker-aided breeding. The functional association of root traits and genetic markers via a QTL mapping approach in stable RIL population evaluated in replicated and properly designed experiments can contribute to better understanding of heritable variability of root traits of interest. This has been utilized in understanding the genetic control of root traits in improving drought tolerance in rice, *Oryza sativa* (Prince et al. 2000; Steel et al. 2006; Courtois et al. 2009), maize, *Zea mays* (Tuberosa et al. 2007; Ruta et al. 2010) and chickpea, *Cicer arietinum* (Gaur et al. 2008; Vadez et al. 2008).

In common beans, QTL analysis has been used to explore the importance of root hairs and rhizosphere acidification (Yan et al. 2004), basal root development and low phosphorus adaptation (Liao et al. 2004; Beebe et al. 2006; Ochoa et al. 2006) as well as root responsiveness to auxin (Remans et al. 2008). However, root QTL with effect on drought tolerance have not been reported to date in common beans as far as we know. In this study we used a mixed model methodology to dissect QTLs of root traits associated with contrasting water availability that would harness indirect selection for prolific root systems as a mechanism for adaptation to drought stress in common bean.

Considerable amount of quantitative variation was observed in the DOR364 × BAT477 RIL population used in this study for both root and shoot traits in conditions of contrasting water availability. Quantitative variation of root growth and development traits related to other edaphic stress has also been reported in other RIL population of common bean (Liao et al. 2004; Yan et al. 2004; Beebe et al. 2006). Our study showed progressive water stress significantly reduced root and above ground shoot growth and development in both parents and their derived lines except for deeper and thicker roots, leaf temperature, SCMR and root:shoot ratio that were enhanced under stress. This would probably indicate that plant growth and development in general and root length and mass in particular were strongly affected by water stress. The reduction in root:shoot ratio in well-watered treatment was probably in response to most favorable growing conditions and an increase in the root:shoot ratio, on the other hand, would indicate that the plant was probably growing under less favorable conditions (Harris 1992). Furthermore, plants developed deeper and thicker root systems with greater water uptake from drying soil in response to and most likely as an adaptation to decreasing water availability. Leaf SCMR values also increased with drought stress suggesting that the leaves became somewhat thicker.

In general, transgressive segregation in both directions was observed for all the traits including traits for which the parents did not show a significant variation. Larger transgressive segregation and relatively higher generalized heritability or repeatability in this greenhouse based experiment were observed in the well-watered treatment than progressive water stress treatment for the majority of the traits measured indicating the negative effect of drought stress on the expression of plant growth and development. However, the generalized heritability for deep rooting increased in water stress and expression of deeper and thicker root system in drought treatment suggested selection under drought stress would be more effective than selection under well-watered environment for genetic improvement or germplasm development program that targets improved drought adaptation in common bean.

The strong association of shoot biomass with many of the root traits regardless of the water supply treatments in this study suggests shoot biomass as a candidate trait to utilize in the indirect selection for drought tolerance because it is relatively easier to measure than root traits. However, its low heritability is a limitation in its application as a trait for selection of drought tolerance. The data supporting similar association of shoot biomass with basal root length have been reported (Liao et al. 2006). Leaf area has a positive and in most cases significant correlation with root traits but its low heritability as with shoot biomass might limit its application as a trait to measure in indirect selection of drought tolerance.

Furthermore it is interesting to see strong positive association of deeper and thicker roots with other root traits regardless of the contrasting water availability. This together with their relatively higher heritability could make these root traits useful for selection of drought tolerance, although confirmation would be needed to show that expression of the root length under greenhouse conditions mimics expression in the field.

The positive correlation of deep and thicker roots with root length and biomass distribution over soil horizons indicates genotypes with deep and thicker root system accumulate a greater proportion of root length and biomass deeper in the soil profile giving a better chance of extracting soil moisture from depth as a mechanism of adaptation to drought stress. Deeper and thicker roots might also be combined with greater photosynthate remobilization to grain (Rao 2001; Beebe et al. 2008). This is because deep rooting alone does not assure drought tolerance as root studies in Colombian field mollisols suggested deep rooting genotypes were not always the best yielding materials (Polanía et al. 2009). Hence for breeders to improve drought adaptation in common beans, pyramiding of various tolerance mechanisms might be needed and these would include improved photosynthate remobilization to grain under stress, thicker and deeper rooting that contributes to greater water uptake, and the ability to control stomatal opening (Beebe et al. 2010).

Our study provided better understanding of genetics of drought avoidance root traits and identified DNA markers linked to QTL for root traits using soil grown plants in cylinders with several notable observations made: Firstly, the root traits were all inherited quantitatively and showed moderate to somewhat high heritability. Heritability ranged up to 71% for average root diameter in well-watered treatment to between 54 and 58% and 45 and 51% for amount of fine and thicker roots or total roots in the two treatments, respectively. Total root volume also had high heritability of 61 and 49%, respectively. Meanwhile some shoot traits had low heritability (leaf area, leaf temperature and shoot biomass) probably because of little contrast between the parents which were both race Mesoamerican and type-II growth habit. Heritability tended to be lower for traits that did not vary significantly between the parents or derived lines and for water-stressed conditions compared to well-watered conditions. Root length distribution with depth and root biomass distribution with depth were traits with high heritability and which were stable in the two environments, actually being greater for water-stressed treatment than the well-watered treatment in the latter case.

A second observation was that the QTL detected for the all the root traits except total root length and fine roots length were main effect QTL and did not interact with the water regimes. Even the total root length and fine root length QTL with significant QTL × environment interaction showed less dependency on the water stress level and were QTL of the non-cross over type. The QTL × environment interaction effects in the study population were attributed to the differential expression of paternal BAT477 alleles in different environments.

The third observation was that the QTL for total root length, fine roots, thicker roots length, root volume and root biomass were often co-localized and also explained relatively greater genetic variance. This suggested that the QTL affected drought avoidance through a constitutive expression of genes for deep rooting, thicker roots, root length distribution with depth, root volume and root biomass and adaptive expression of genes for total root length and fine roots length. The positive alleles for most of the QTL detected in this study were derived from the paternal genotype BAT477 which is known to be deep-rooted, drought tolerant genotype in both greenhouse and field studies (Sponchiado et al. 1989; White et al. 1994a, b). This is in contrast to both parental contributions to the phenotypic expression of root traits for other edaphic stresses related to low phosphorus reported in other mapping population in common bean (Liao et al. 2004; Yan et al. 2004; Ochoa et al. 2006).

Finally many of the QTL for total root length, fine roots length, thicker roots length, root length distribution with depth, root biomass and root:shoot ratio were detected near each other on b11. The same linkage group has also been important in other QTL studies of root traits in other mapping population and loci on b11 have been shown to influence adventitious root length in the Mesoamerican by Andean cross G2333 × G19833 (Ochoa et al. 2006), root growth traits and P uptake in Andean × Andean cross G19833 × AND696 (Cichy et al. 2009) and root morphology traits for aluminum resistance in Mesoamerican × Andean cross DOR364 × G19833 (Lopez-Marin et al. 2009). Hence, this linkage group appears to possess genetic factors that may have an influence on many diverse root traits of interest for the inprovement of common bean. In this study the co-localization of QTL for these traits might be due to genetic linkage rather than pleiotropy as the genetic predictors were calculated at maximum distance of 10 cM. These traits also revealed strong correlation for their phenotypic expression in greenhouse root cylinders.

The yield QTL for grain per day or kg ha^{-1} detected for this population (Blair et al. 2010) did not co-localize with rooting depth QTL under either water stressed or non-stressed treatments. However this is not to say that rooting depth does not contribute to other parameters of plant adaptation under drought but warns us that careful genetic analysis will be needed to validate this trait for use in marker assisted breeding. The development of near isogenic lines for the root QTL is an obvious activity that can now be carried out given the markers in this narrow cross. Meanwhile the results from the greenhouse and the field may not be so different as QTL for leaf TNCs from the greenhouse were in the same position as seed weight QTL on linkage group b09 and one would expect both traits to be related physiologically due to translocation of carbohydrate from leaf to seed sink.

In conclusion, the present study represents the first efforts in common bean to identify QTL for drought avoidance root traits or to use the controlled condition of deep root cylinders to study the important effects of root and drought tolerance QTL. Using QTL dissection with molecular marker, we identified a prevailingly constitutive expression of genes underlying the QTL linked with drought avoidance root traits many on linkage groups b01 or b11 which explained up to 41% of genetic variance. Hence, the major constitutive QTL for root traits needs further detailed studies of chromosomal regions or candidate genes involved in drought tolerance. This knowledge is needed for legume breeding program to develop improved varieties that combine root-based drought tolerance with other shoot traits of interest using marker aided selection.

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CHAPTER 5

Multi-environment quantitative trait loci analysis for photosynthate acquisition, accumulation and remobilization traits in common bean under drought stress

This chapter is based on the article: Asfaw A, Blair MW, Geleano CH, Ricaure J, Chirwa R, Rao IM, Struik PC, Multi-environment quantitative trait loci analysis for photosynthate acquisition, accumulation and remobilization traits in common bean under drought stress. Submitted to *Genetics*. The authors are grateful for technical assistance in field phenotyping or laboratory analysis from Miguel Grajales and José Polonia in Colombia (CIAT), Fitsum Alemahayu in Ethiopia (SARI) as well as Macford Maseko and Lizzie Kalolokesya in Malawi (SABRN). We acknowledge germplasm development of Steve Beebe, Agobardo Hoyos and other previous CIAT staff as well as funding from the Tropical legume (TL1) project from the Bill and Melinda Gates foundation through Generation Challenge Program to Matthew W. Blair (Principal Investigator) and collaborators at the above institutions and a NUFFIC fellowship to Asrat Asfaw (PhD student and SARI researcher) through Wageningen University, the Netherlands

Abstract

Many of the world's common bean (*Phaseolus vulgaris* L.) growing regions are prone to either intermittent or terminal drought stress making drought the primary cause of yield loss under farmers' field conditions. Improved photosynthate acquisition, accumulation and then remobilization from leaves and stems to grains under drought stress has been observed as an important mechanism for adaptation to drought stress. The objective of this study was to tag quantitative trait loci (QTL) for photosynthate accumulation and remobilization to grain using a recombinant inbred line (RIL) population developed from the Mesoamerican intra-genepool cross of drought susceptible DOR364 and drought tolerant BAT477 grown under eight environments differing in drought stress across two continents, Africa and South America. The RIL population expressed quantitative variation and transgressive segregation for 11 traits associated with drought tolerance. QTL were detected by a mixed multi-environment model using a linkage map constructed with 165 genetic markers that covered 11 linkage groups of the common bean genome. A total of 9 consistent QTL were detected for 10 drought stress tolerance mechanism traits found on 6 of the 11 linkage groups. Significant QTL × environment interaction was observed for 6 of the 9 QTL. QTL × environment interaction was of the cross-over type for 3 of the 6 significant QTL with contrasting effect of the parental alleles across different environments. Among the 9 QTL the average genetic variance accounted for was 5.6% under drought stress and 11.2% in non-stress environments, highlighting the difficulty in detecting major QTL in drought studies for common bean under field conditions. Our results indicate the relevance of continued photosynthate accumulation as a trait for common bean drought tolerance.

Keywords: Biomass partitioning, Leaf area and chlorophyll content, QTL × environment interaction, Non-structural carbohydrates, Photosynthate remobilization and grain yield

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the major sources of dietary proteins, vitamins and minerals to millions of resource-poor farmers particularly in developing countries (Broughton et al. 2003). However, yield loss due to drought is a major problem for farmers who produce common bean under rainfed conditions. It is estimated that about 60% of common bean production around the globe is affected by drought in any given year including large areas of Latin America and Africa (Thung and Rao 1999). In Africa an estimated yield loss of 300,000 MT (metric tons) occurs annually (Wortmann et al. 1998) and the yield loss can be up to 80% when severe drought strikes the crop early in crop development (Rao 2001). Fortunately, drought is seldom a yearly event and its effect has both seasonal and spatial variation (Passioura 2007). At micro level, it can occur in different forms either throughout the season, early in the season, at mid-season or near the end of the season or life cycle of a crop. Thus, drought can have large effects on common bean growth either during early establishment, vegetative expansion, flowering or grain filling (Rao 2001). Furthermore, climate change will cause higher temperatures and greater evapotranspiration combined with erratic and lower rainfall which will intensify the problem for a smallholder crop like common bean, where opportunities for irrigation are limited (Beebe et al. in press).

Plants usually express differential adaptive strategies to drought stress. These include escape, avoidance, resistance and recovery strategies (Levitt 1972), which may not be mutually exclusive but in practice are rarely combined within a single agronomically-superior genotype (Ludlow 1989). Instead a range of adaptive responses to drought and mechanisms of drought tolerance are found in different genotypes. Mechanisms that provide drought adaptation in common bean include at a minimum 1) a deep rooting system with an appropriate architecture that increases extraction of soil moisture from a greater soil depth; 2) maximization of water use efficiency for photosynthesis, growth and development; and 3) higher photosynthate transport to seed under stress through efficient (re-)mobilization (Sponchiado et al. 1989; White et al. 1994a; Rao 2001; Beebe et al. 2008). Finally, phenological plasticity, involving early maturity, drought avoidance and recovery after drought, is an important mechanism when appropriate for the growing season (Acosta-Gallegos and White 1995).

These adaptive strategies for drought stress in common bean are known to be genetically determined for the most part (White 1987); however, full understanding of the underlying genes remains elusive. Plant traits for tolerance to drought have been identified in common bean lines of diverse backgrounds in both the Mesoamerican and Andean genepools (White 1987; Acosta-Gallegos and Kohashi-Shibata 1989, Acosta-Gallego and Adams 1991; Ramirez-Vallejo and Kelly 1998; Muñoz-Perea et al. 2006).

Breeding and selection for these traits have resulted in identification of remarkably tolerant parental lines such as BAT477, SEA5, SEA15 and a series of advanced lines in small red, cream stripped and black seeded commercial grain classes (Singh et al. 2001; Teran and

Singh 2002; Beebe et al. 2008). In any case, drought tolerance is a trait that can contribute to the food security of many resource-poor farmers who live in harsh, low-rainfall environments. Overall, however, drought tolerance is a genetically and physiologically complex trait which must be expressed in terms of increased grain yield under field conditions. In terms of inheritance, drought tolerance is a quantitative complex trait with low heritability for which appropriate selection criteria are largely absent (Schneider et al. 1997; Blair et al. 2010). In practical terms, selection for drought tolerance is difficult because the drought stress can present itself at different times in the growing season, and with different intensity, with its effect on crops being modified by soil type and fertility (Rao 2001). In the harsh environments where the majority of smallholder farmers in developing countries grow crops, mechanisms of drought tolerance are physiologically complex due to the interaction of drought with other stress factors, like high temperature, low soil fertility, soil acidity and salinity, pathogens and insect pests. It is therefore not surprising that drought tolerance is susceptible to genotype × environment (G×E) interaction. Valuable drought tolerance can be masked by poor adaptation to a specific environment (Beebe et al. in press).

The significant genotype × environment interaction, together with low heritability, polygenic control and epistatic gene action, hampers indirect trait-based selection as well as direct yield selection under drought stress conditions (Cattivelli et al. 2008). Moreover, genetic correlations between drought-adaptive traits are mostly low or negative presenting additional challenges for breeders (Doekiw et al. 2000). Nevertheless, an increase in yield potential under stress has been possible through breeding for sub-optimal rainfall conditions (Beebe et al. 2008). This drought tolerance also affected yield potential under low soil fertility conditions indicating that common bean researchers need a better understanding of the genetic control of traits that contribute to yield accumulation under stress conditions.

Molecular markers are powerful tools to analyze the genetic control of complex traits like drought tolerance (Blair et al. 2010). Segregation mapping has been used to evaluate quantitative trait loci (QTL) that control multi-genic traits such as biomass production and yield partitioning (Collins et al. 2008). So far few QTL analysis for drought tolerance have been reported in common bean and these have focused on yield components, phenology and rooting pattern traits (Schneider et al. 1997; Blair et al. 2010; Asfaw et al. submitted) instead of photosynthate remobilization traits.

The objective of this study, therefore, was to identify QTL associated with photosynthate acquisition, accumulation and remobilization traits such as canopy biomass dry weight, biomass partitioning indexes, stem and seed total nonstructural carbohydrate content, leaf area index and leaf chlorophyll content as well as final yield. This study measured these physiological traits under eight stress and non-stress environments in different countries and across two continents in a recombinant inbred line (RIL) population derived from a drought susceptible × drought tolerant cross.

Material and Methods

Plant material and drought trials under field conditions

A recombinant inbred line (RIL) population described in Blair et al. (2010) was used for this study. The population consisted of 97 F_5 derived advanced lines from single seed descent from the cross of DOR364 (drought susceptible) × BAT477 (drought tolerant). DOR364 is a small-red seeded, high-yielding, commercial cultivar developed in Central America for resistance to *Bean golden yellow mosaic virus* and is acceptable in East Africa despite its dark red seed color. The line is an indeterminate upright short bush bean of the type II growth habit (CIAT 1987). BAT477 is a cream-colored, small-seeded breeding line with type II growth habit identified by the International Center for Tropical Agriculture (CIAT) as drought tolerant and adapted to various tropical environments. BAT477 was derived from the cross (G3834 × G4493) × (G4792 × G5694) and combines deep rooting ability with greater water uptake efficiency (White et al. 1994a, b; Beebe et al. 2010). Both parents DOR364 belong to the race Mesoamerica of the Mesoamerican (a.k.a. Middle American) genepool as defined by Singh et al. (1991). All RILs plus both parents and one drought tolerant check SEA5 were used in the field experiments.

Field drought phenotyping experiments were conducted at two sites in Ethiopia, one site in Malawi and one site in Colombia. The trials were executed in Colombia in 2007 and in Ethiopia and Malawi in 2009 under the auspices of the Tropical Legumes I project. Drought stressed and non-stressed conditions were applied as separate experiments in each trial site which created eight environments for QTL analysis.

The field site in Colombia (CIAT-Palmira) and its soil and weather conditions are described in Blair et al. (2010) while the two field sites in Ethiopia were at Awassa and Amaro research farms of the Southern Agricultural Research Institute (SARI). The fourth set of experiments was in Malawi at the Kasinthula field station of the Department of Agricultural Research Service (DARS).

In Ethiopia, Awassa is located at 7°03′N latitude, 38°30′E longitude at an elevation of 1,700 m above sea level. The soil at this site is a well-drained sandy-loam (Flovisol, FAO classification) with pH 7.0. The yearly average maximum and minimum temperatures of the site are 26.9 °C and 12.4 °C, respectively, and annual rainfall is 959 mm on average. Rainfall at this site is divided into 296 mm and 444 mm, respectively, during the short ‘*Belg*’ rainy season (March - May) and the long ‘*Meher*’ rainy season (July - October).

The Amaro site is located at 5°50′N latitude, 37°55′E longitude at an elevation of 1,426 m above sea level. The soil at this site is a well-drained silt-clay-loam (Eurtic nitosols, FAO classification) with pH 6.5, with yearly average maximum and minimum temperatures of 27.6 °C and 15.2 °C, respectively, and annual rainfall of 927 mm (412 mm and 294 mm during the ‘*Belg*’ and ‘*Meher*’ growing seasons, respectively).

In summary for Ethiopia, the rainfall pattern is bimodal at both sites creating double common bean growing environments in a calendar year. With this in mind, the trials were planted in ‘*Meher*’ (from July – October) season at Awassa and in ‘*Belg*’ (March – June) season at Amaro.

The fourth field site was Kasinthula, Malawi, located in 16°0′S latitude, 34°5′E longitude and 70 m above sea level. The yearly average maximum and minimum temperatures of the site are 35.6 °C and 18.6 °C, respectively and annual rainfall is 800 mm on average. The soil surface horizon is sandy loam (UNDP 1970) while the subsurface horizon is sandy-clay-loam. Generally the soil is moderately drained with water table at 2.5 m below the ground surface. The average available water holding capacity is 100 mm m^{-1} of soil depth, and the pH is 7.4. The crop was planted off the rainy season, under irrigation in June, using two irrigation regimes: 1) no drought stress – where the crop was irrigated up to maturity stage, whenever the soil moisture field capacity was depleted by 30%, and 2) drought stress – where the crop was irrigated up to mid-pod filling stage, whenever the soil moisture field capacity was depleted by 70%, and thereafter the irrigation was cut off completely.

In all the experiments except those in Malawi, a 10 × 10 triple lattice treatment design was used. In Malawi results were for a subset of the lines as described in the results section. The plot sizes were 4 rows of 2 m length by 0.4 m width for Ethiopia sites, two row plots of 4 m length by 0.6 m width for the Colombia site and single row plots of 2 m length by 0.6 m with for the Malawi site. Recommended packages of agronomic practices were applied at each site.

Plant trait measurements

A list of the traits evaluated at the different trial sites is presented in Table 1. For quantifying physiological differences in drought tolerance, a number of plant attributes were measured through destructive sampling at mid-pod fill and at physiological maturity. For the plant attributes at mid-pod filling, a row length of 0.5 m (0.2 m^2) for each plot was selected and the plants were cut to the soil surface above the ground and put in a paper bag for processing in the laboratory. Plants were separated into leaves (without petioles), stems and the remaining (pods and reproductive structures) plant parts. The plant parts were put in separate paper bags and oven dried at 80 °C for 2 days. After drying of the samples, dry weight of each sample was measured to determine total dry matter production and dry matter distribution into different plant parts (leaf biomass, stem biomass and pod biomass). At harvest, plants within 0.5 m long row (0.2 m^2 area) were cut to the soil surface and oven dried at 80 °C for 2 days. The oven dried samples were then separated into plant parts: stem, pod wall and seeds and dry weight measurements were recorded. These data were collected at Awassa and Palmira but not in Amaro and Kasinthula. Physiological traits related with photosynthate accumulation and partitioning included canopy biomass dry weight at mid-pod filling, pod partitioning index, pod harvest index, stem biomass reduction and harvest index measured also only in Awassa and Palmira (Beebe et al. 2010). Canopy biomass dry weight at mid-pod fill was calculated as the sum of dry matter distribution into different plant parts (leaf biomass, stem biomass and pod biomass) at mid-pod and converted into kg ha^{-1}.

Table 1 Plant traits considered for QTL analysis at different sites in three countries (Colombia, Ethiopia and Malawi) for drought stress and non-stress trials

	Field site			
Traits considered	Awassa	Amaro	Kasinthula	Palmira
Grain yield (kg ha^{-1})	√	√	√	√
Canopy biomass dry weight (kg ha^{-1})	√			√
Pod harvest index (%)	√			√
Pod partitioning index (%)	√			√
Stem biomass reduction (%)	√			√
Harvest index (%)	√			√
Stem TNC (mg g^{-1})				√
Seed TNC (mg g^{-1})				√
Leaf are index (m^{2} m^{-2})				√
SPAD chlorophyll meter reading	√	√[1]	√	√
Canopy temperature depression (ºC)				√

[1] In drought stress environment only, *TNC* total nonstructural carbohydrate content

Pod partitioning index was determined as the ratio of dry weight of pods at harvest over dry weight of total biomass at mid-pod fill multiplied by 100. Similarly pod harvest index was calculated as the ratio of dry weight of seed over dry weight of pod at harvest multiplied by 100. Stem biomass reduction was calculated as the ratio of stem biomass at mid-pod filling minus stem biomass dry weight at harvest over stem biomass dry weight at mid-pod multiplied by 100.

In addition, other traits related with drought tolerance that were recorded at mid-pod filling included leaf chlorophyll content, leaf area index, canopy temperature depression and stem TNC (total nonstructural carbohydrate content) whereas seed TNC was determined at harvest. Data was also recorded at harvest for grain yield (in kg ha^{-1}) of all plots in all sites.

Leaf chlorophyll content was measured with a SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd, Japan). SPAD chlorophyll meter reading (SCMR) was recorded on a fully expanded young leaf of one plant for each replication. Leaf area was measured by leaf area meter (LICOR model LI-3000, Lincoln, Nebraska, USA) from plant parts separated as leaves during destructive mid-pod filling plant sampling and converted into leaf area index (m^{2} m^{-2}) for statistical analysis. Stem and seed TNC was determined using NaOH as an extraction medium and anthrone reagent. Absorbance of the solution was measured with a spectrophotometer at 620 nm and TNC concentration was determined by comparison with glucose standards (Kang and Brink 1995).

Canopy temperature depression (^{o}C) was measured as the difference in temperature between the leaf canopy and the surrounding air temperature using an infrared thermometer (Telatemp model AG-42D, Telatemp, Fullerton, CA, USA) held at 50 cm from the canopy surface in a 45^{o} angle. Leaf area index, canopy temperature depression, stem and seed total nonstructural carbohydrate were determined only at the Palmira site, while SCMR was evaluated at all four sites.

Phenotypic data analysis

Analysis of variance (ANOVA) of the variables in both drought-stress and non-stress environments, their genetic parameters such as genotypic and genotype × environment variance components and phenotypic correlations between grain yield and other variables were carried out using the program Genstat version 12.1 (Payne et al. 2009). For the ANOVAs a mixed model analysis was used first for each single trait in each single environment and then for genotype × environment interaction (G×E) following Gilmour et al. (1997) using a Residual Maximum Likelihood (REML) procedure. Block effects were added to the model as random variable when significant for a trait. This was performed to remove the spatial variation within the trial field. Genotypes were considered as fixed to get un-shrunken means (best linear unbiased estimates = BLUEs) for QTL analysis in the best spatial model.

To quantify the severity of drought stress on plant traits, the drought intensity index (DII) for each trait was calculated as DII = 1–Xds/Xns, where Xds and Xns are the mean experimental trait values of all genotypes grown under drought stress and non-stress, respectively (Fischer and Maurer 1978). For multi-environment analysis, BLUE mean from single environment was used.

G×E interactions (G×E) for the traits measured were assessed using genotype plus genotype × environment interaction (GGE) biplot function implemented in the Genstat software. Genetic correlations for grain yield between trial environments were calculated using factor analytic model of order k = 1 to model the genetic variance-covariance matrix. Broad sense heritability for each trait was estimated as the ratio of genetic variance over genetic variance plus genotype × environment variance obtained from the analysis of variance.

Molecular mapping and QTL detection

The molecular markers used in the study included random amplified polymorphic DNA (RAPD) primers (Operon Technologies Inc., Alameda, CA), amplified fragment length polymorphisms (AFLP), and simple sequence repeats (SSR) or microsatellites. Experimental procedures of genotyping for RAPD, and AFLP markers were as described in Blair et al. (2006) and Muñoz et al. (2004), respectively. The procedure for SSR markers was the same as described in Blair et al. (2003, 2008). The overall laboratory techniques for the three different marker analysis were presented in Blair et al. (2010).

A new genetic linkage map was constructed using the software JoinMap® 4.0 for Windows (http://www.kyazma.nl/index.php/mc.JoinMap/) set to the Haldane 1 mapping function. To create grouping trees, mapping parameters were set to a recombination frequency smaller than 0.15 and a log of odds (LOD) score larger than 5.0. Marker order within a group regression was determined with a mapping algorithm based on the marker order of specific linkage group from previous microsatellite-based common bean genetic maps (Blair et al. 2003, 2008). The best marker order of the linkage groups was checked with best plausible positions in maximum likelihood mapping algorithm set to 1000 permutation. The mapping analysis was performed using marker data of 205 molecular markers segregating in the population.

Naming of linkage groups was done by checking for each marker against known marker positions on genetic maps constructed by Blair et al. (2003, 2008, 2010). For the final map used in the QTL analysis a subset of 162 markers was selected in such a way that no two markers were located in the same position.

QTLs and their environmental interaction effects for all the traits were mapped by the mixed model based QTL mapping approach using a single trait and multi-environment option implemented in Genstat 12.1 (Payne et al. 2009). For QTL detection a three step mixed model analysis as described in Boer et al. (2007) was performed using a single interval mapping (SIM) procedure. In this method based on Bayes theorem and Markov chain methodology (Lander and Green 1987), the QTL effects and positions were estimated based on flanking markers to accommodate uneven coverage of markers along the genome. Hence, the additive genetic predictor or evaluation of positions was based on marker genotypes predicted at a maximum distance of 10 cM which resulted in 197 positions tested in the genome.

A genome-wide scan for significant QTL expression was performed using the single interval mapping (SIM) procedure and each predicted marker position fitted as fixed environment-specific QTL effects while maintaining the best variance-covariance structure based on Schwarz information criterion (SIC) (Schwarz 1978) set in the Genstat GGE model determined in G×E interaction analysis.

The QTL threshold was based on peak value exceeding a threshold defined for multiple testing by Bonferonni corrections (Lynch and Walsh 1998). The QTL effects were tested by a Wald test (Verbeke and Molenberghs 2000). The amount of variation explained by each QTL was calculated as per Mathews et al. (2008). The explained genetic variance (as percentage of the total genetic variance) was calculated as: % explained genetic variance by each QTL = 100 × [1 – (genetic variance in the model with QTL/ genetic variance in model without QTL)].

Results

Drought stress under field conditions

The optimum sowing time for common bean was determined for each site and based on this the optimum moment for planting so as to encounter drought stress. In Palmira, the dry season from June to September provided sufficient time for the contrasting irrigated and rainfed conditions while in Malawi the absence of rainfall in the winter season provided similar but colder conditions, the reason for which plantings were made at the lowland site and in the mid-season when temperatures were increasing.

In Ethiopia, the lack of an alternate season and reliable irrigation facilities led us to plant later in the long-rainy season to obtain terminal drought stress or near the beginning of the short-rainy season for intermittent and terminal drought stress. Near Awassa and for the '*Belg*' short rainy season the optimum planting date was at the onset of the rains in March. For the '*Meher*' long rainy season we planted in late June to the end of July for full rainfall and later in August for drought stress. Around Awassa, the '*Belg*' season is considered more minor for common bean production and farmers usually plant only a small area for seed production or to augment food production during the May-June when no other crop in the field to harvest. Optimum sowing time for common bean at Amaro for '*Belg*' season was at the onset of the first rainy season in April and for '*Meher*' season was at the end of August to early September. At this site, '*Belg*' is the main and relatively better season for common bean production as compared to '*Meher*' while the reverse holds true for the site at Awassa.

Figure 1 shows the weather conditions (rainfall, pan evaporation, maximum and minimum temperature) during the crop growing period at four of the locations in the different seasons. The rainfall and temperature data of Amaro, Ethiopia were not available for the full crop growing cycle; however, on the basis of the available data, the average maximum and minimum temperatures, respectively, were 20.2 °C and 16.0 °C in the drought non-stress trial (early planting) from 58 to 80 days after planting and 26.9 °C and 15.4 °C in the terminal drought stress trial (late planting) trial from 32 to 80 days after planting.

The average maximum and minimum temperatures for the other locations were 27.8 °C and 12.2 °C in non-stress (early planting) and 28.6 °C and 11.5 °C in terminal drought stress (late planting) at Awassa, Ethiopia, 30.9 °C and 19.4 °C at Kasinthula, Malawi and 30.6 °C and 18.6 °C at Palmira, Colombia, respectively. The total rainfall was 235 mm, 149 mm, 25 mm and 243 mm at Awassa early and late planting, Kasinthula and Palmira, respectively. The potential pan evaporation was 480 mm at Kasinthula and 431 mm at Palmira.

The weather parameters during the crop growth period indicated that the crop suffered terminal drought stress in late planting trials at Amaro and Awassa, Ethiopia and in the rainfed trial at Kasinthula, Malawi. By comparison drought stress was intermittent and mainly in early growth crop stages at Palmira, Colombia.

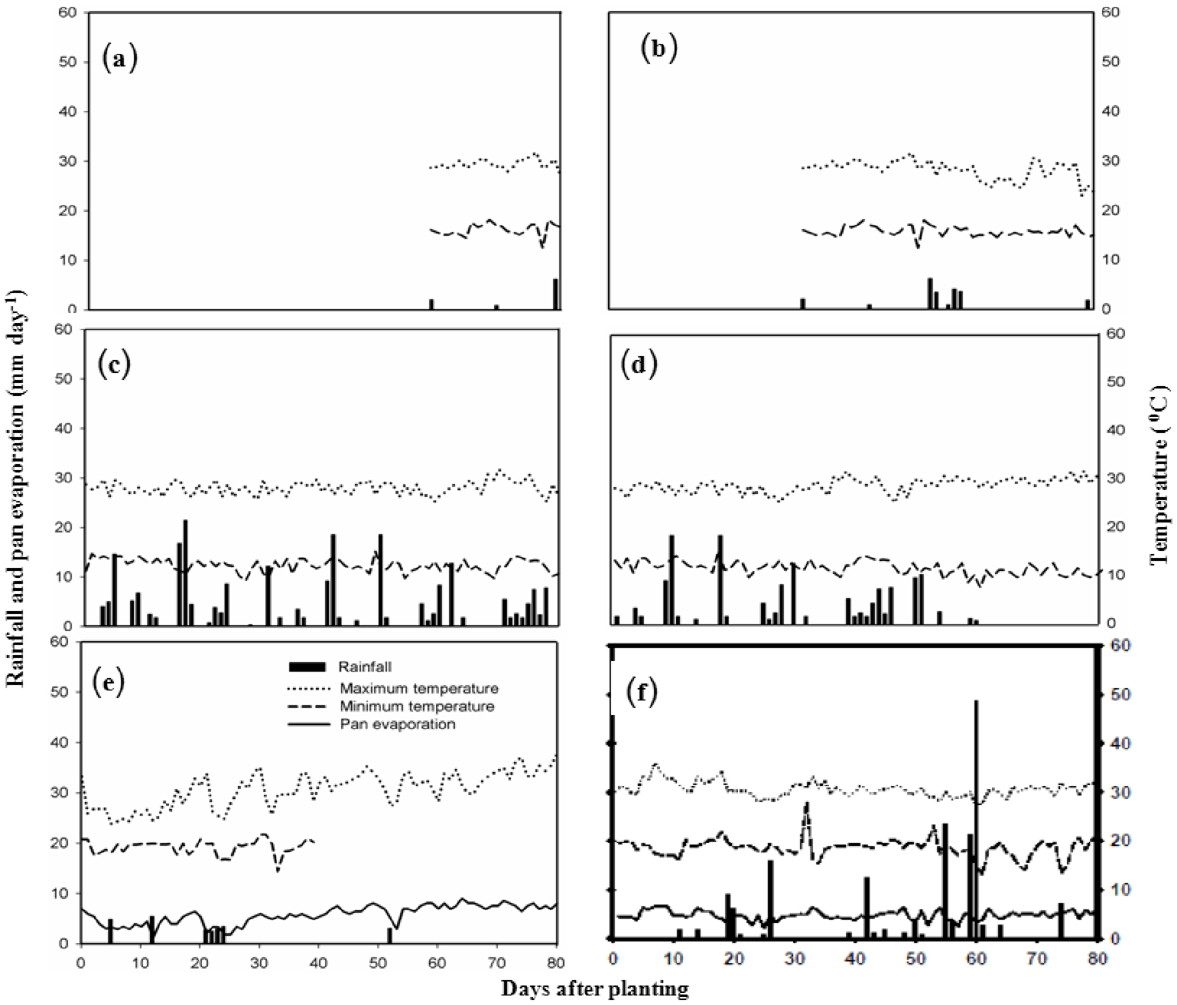

Fig. 1 Rainfall distribution, pan evaporation, and maximum and minimum temperature during the crop growth period at different trial locations in Colombia, Ethiopia and Malawi (for details see materials and methods). (**a**) Amaro early planting, (**b**) Amaro late planting, (**c**) Awassa early planting, (**d**) Awassa late planting, (**e**) Kasinthula and (**f**) Palmira

Yield effects and drought intensity indices

Mean grain yield over the four locations was 62% smaller in the stress environment than in the non-stress environments based on drought intensity index calculated from the mean yield of all genotypes under stress versus non-stress. Average grain yields of all genotypes including parents, RILs and drought tolerant check were 461, 510, 547, and 988 kg ha^{-1} in the drought stress environments in Amaro, Awassa, Kasinthula and Palmira, respectively. Meanwhile in the non-stress environments of the same sites they were and 1207, 2310, 1062, and 2024 kg ha^{-1}.

The mean grain yield showed 61.8%, 78.0%, 48.5%, and 51.2% reductions due to drought stress in the Amaro, Awassa, Kasinthula and Palmira sites, respectively. This showed that drought stress for grain yield was moderate to severe in stress created using early and late planting treatments of Amaro and Awassa compared to the irrigated and rainfed treatments of Kasinthula and Palmira. Drought stress also caused poor biomass accumulation and remobilization of photosynthate to grain.

Given this, the effect of drought stress was greater in biomass accumulation as compared to photosynthate remobilization. Mean canopy biomass dry weight at mid pod grain filling was reduced by 51% in the stress environments as compared to the non-stress environments. Mean reductions in traits related to photosynthate remobilization to grain were 2% for pod harvest index, 13% for pod partitioning index, 4% for stem biomass reduction, 12% for harvest index and 10% for stem total non-structural carbohydrate content. Furthermore, drought stress also caused a 29.5% average reduction in leaf area index and 18% average increase in canopy temperature in the stress environments as compared to the non-stress environments. On the other hand, the mean seed non-structural carbohydrate was 17% higher under the drought stress environments compared to the non-stress environments, and the SPAD chlorophyll meter readings increased by 4% on average in the same comparison.

Phenotypic variability of the parents and the derived lines

Significant differences were observed among RILs and between parents for majority of the traits measured in both drought stress and non-stress environments at the four locations and in the eight individual experiments (Table 2). The parental difference was significant for grain yield at all locations except in Kasinthula under non-stress conditions. The drought-tolerant paternal line, BAT477, out-yielded the drought-susceptible maternal line, DOR364, in the majority of the trials except at Amaro and Awassa in the drought stress environments. At Amaro and Awassa drought stress environments, BAT477 was affected by bean stem maggot that resulted in lower grain yield and lower overall performance. BAT477 was also better in canopy biomass accumulation compared to DOR364 except at Awassa under drought stress. For photosynthate remobilization traits like pod harvest index, pod partitioning index, stem biomass reduction and harvest index the parental differences were not significant except in the Awassa non-stress environment where DOR364 was better for pod harvest index, pod partitioning index and harvest index and where BAT477 was better for stem biomass reduction.

For stem non-structural carbohydrates, no differences were found both for the parents and the derived lines in any of the environments, while for seed non-structural carbohydrates BAT477 was better than DOR364 under drought stress in Palmira, the only site where these traits were measured. It would have been interesting to observe the results of these two traits in the other sites but the laboratory for measuring non-structural carbohydrates was specific to CIAT in Colombia and seed and stem shipments could not be arranged due to quarantine requirements.

Table 2 Mean values of traits measured in drought stressed (DS) and non-stressed (NS) conditions at four different locations (Palmira, Colombia in 2007 and Awassa and Amaro, Ethiopia and Kasinthula, Malawi in 2009) for parents, DOR364 and BAT477 and drought tolerant control check, SEA5 along with means and ranges of the recombinant inbred lines (RILs) from the population DOR364 × BAT477. Mean values are of three replications in each experiment. P value indicates level of significance for genotypic difference among RILs for each trait. Average standard error of the difference (AvSED) indicates the genotypic difference to declare significance among RILs, parents and the check genotype SEA5, advanced line from CIAT

Trait	Location	Env	Parents		RIL population				
			P1	P2	Mean	Range	P-value	SEA5	AvSED
Grain yield (kg ha^{-1})	Amaro (Eth)	DS	646	146	460	77-947	<0.001	1001	79.7
		NS	1849	2338	1202	192-2412	<0.001	1433	481.1
	Awassa (Eth)	DS	522	476	503	219-1003	<0.001	391	77.1
		NS	2118	2908	2361	1150-3622	<0.001	1852	261.0
	Kasinthula (ML)	DS	510	661	551	268-855	0.04	457	124.9
		NS	1344	1111	1068	517-1583	0.074	964	ns
	Palmira (Col)	DS	956	1126	986	709-1340	0.000	911	273
		NS	2075	2171	2029	1595-2556	0.013	2010	446
Canopy biomass (kg ha^{-1})	Awassa (Eth)	DS	3015	1958	2479	1062-4270	<0.001	2915	487
		NS	2851	4480	4243	2328-6783	<0.001	2895	546
	Palmira (Col)	DS	2163	2517	2223	1441-3269	0.008	2082	408
		NS	6180	6445	5362	4166-6930	0.273	5491	ns
Pod harvest index (%)	Awassa (Eth)	DS	72.9	72.5	72.2	57.0-81.8	<0.001	68.4	3.6
		NS	76.1	71.9	70.7	58.3-80.8	<0.001	65.8	3.5
	Palmira (Col)	DS	76.1	76.2	75.6	68.8-79.3	0.002	77.3	2.0
		NS	79.3	80.0	79.9	76.3-83.1	<0.001	80.1	1.1
Pod partitioning index (%)	Awassa (Eth)	DS	33.5	35.6	34.7	15.2-84.3	<0.001	37.0	8.6
		NS	97.4	74.7	66.8	29.7-97.7	<0.001	90.1	10.7
	Palmira (Col)	DS	56.7	54.7	67.5	37.4-87.7	0.335	71.1	ns
		NS	38.8	60.1	50.9	32.7-76.7	0.551	51.5	ns
Stem biomass reduction (%)	Awassa (Eth)	DS	31.9	26.5	31.5	3.82-60.4	0.125	20.9	ns
		NS	21.7	33.6	43.5	6.9-70.5	<0.001	11.4	15.1
	Palmira (Col)	DS	46.1	46.6	43.9	20.1-67.6	0.855	45.5	ns
		NS	34.5	31.1	34.9	11.0-57.1	0.447	19.2	ns
Harvest index (%)	Awassa (Eth)	DS	24.3	25.9	24.9	10.3-59.3	<0.001	25.4	6.5
		NS	73.9	53.6	47.5	18.0-73.6	<0.001	59.4	8.3
	Palmira (Col)	DS	43.6	42.2	52.5	28.7-94.4	0.396	56.9	ns
		NS	31.1	48.1	40.9	25.4-61.8	0.497	40.6	ns
Stem TNC (mg g^{-1})	Palmira (Col)	DS	227	131	199	125-278	0.756	206	ns
		NS	229	229	220	125-318	0.308	220	ns
Seed TNC (mg g^{-1})	Palmira (Col)	DS	261	332	358	272-451	0.101	501	61.9
		NS	333	261	310	244-398	0.120	320	ns
SPAD chlorophyll meter reading (SCMR)	Amaro (Eth)	DS	24.7	24.6	24.3	18.9-30.1	0.078	26.4	ns
	Awassa (Eth)	DS	24.3	24.0	22.2	16.1-28.7	<0.001	22.5	2.5
		NS	23.3	18.3	20.3	14.6-26.5	<0.001	20.8	2.7
	Kasinthula (ML)	DS	41.0	42.5	40.9	34.7-47.7	0.417	44.5	ns
		NS	37.9	37.3	39.3	32.5-45.9	0.690	46.6	3.3
	Palmira (Col)	DS	41.9	38.1	41.9	34.8-49.6	<0.001	39.5	3.0
		NS	31.6	34.6	32.5	20.5-46.2	<0.001	37.7	4.8
Leaf area index (m^{2}m^{-2})	Palmira (Col)	DS	1.63	1.83	1.70	1.10-2.41	<0.001	1.35	0.29
		NS	2.88	2.90	2.38	1.34-3.41	<0.001	1.98	0.42
Canopy temp. (^{o}C) depression (CTD)	Palmira (Col)	DS	3.54	4.14	3.35	1.11-6.36	0.005	4.70	1.12
		NS	3.82	4.60	4.11	1.62-6.26	0.347	4.94	ns

Env Environment, *DS* drought stress, *NS* non-stress, *AvSED* average standard error of difference, *Min* minimum, *Max* maximum, *Eth* Ethiopia, *Col* Colombia, *ML* Malawi, *TNC* total nonstructural carbohydrate content, *P1* DOR364, *P2* BAT477, *ns* non-significant, *temp* temperature

For other traits, namely the SPAD chlorophyll meter readings, the results were generally higher in drought stress environments than in non-stress environments for both parents and RILs whereas leaf area index was higher in non-stress environments than in drought stress environments. Using a temperature ‘gun’, BAT477 was found to be excellent in keeping the canopy temperature cooler as compared to DOR364 in both drought stress and non-stress environments. The drought tolerant control SEA5 was inconsistent in performance, sometimes being found to be better or worse than the parents for many of the traits measured across locations and stress levels.

The RIL population distributions were continuous for all traits suggesting quantitative inheritance in all cases and both in drought stress and non-stress environments (Suplementary Fig. 1). However, some skewing in distribution was observed for grain yield at Awassa in both drought stress and non-stress environments. Kurtosis was also significant for some partitioning traits (data not shown), mainly pod-partitioning index and harvest index at Awassa under drought stress. Some transgressive segregation was observed among the RILs for all the traits measured at each location and in each environment. This transgressive segregation was found both in positive and negative directions (Table 2). At each trial site and in each environment, several RILs were better or worse than the drought-tolerant paternal line BAT477 or drought-susceptible maternal line DOR364 for all the traits measured by this study.

Genotype × environment interaction, heritability and correlation

The genotypic (G) and genotype × environment (G×E) variance component and broad-sense heritability for all traits measured are presented in Table 3. The G×E variance was higher than genotypic variance for all the traits except leaf area index and SPAD chlorophyll meter reading confirming highly specific environmental effects on the expression of these drought tolerance traits. Genetic variance was equal to G×E variance for leaf area index while it was a little higher than the interaction effect for SPAD chlorophyll meter readings. This indicated the relatively high across environment repeatability of these two traits as compared to other traits measured in the study. The broad-sense heritability was low to medium in value for all the traits measured, being especially low for pod-partitioning index. The highest average heritabilities considering all sites were for leaf area index (0.51) followed by seed TNC (0.50), stem TNC (0.45), SPAD chlorophyll meter readings (0.43) and canopy temperature depression (0.42). Medium-low heritabilities (0.35 to 0.39) were observed for pod harvest index, grain yield, canopy biomass dry weight at mid-pod fill, stem biomass reduction and harvest index.

Figure 2 shows the patterns of G×E interaction in the experiment. Using a set of biplot displays, the lengths of the vectors connecting the environment to the origin corresponded to the amount of genetic variation expressed in that environment. Meanwhile, in the same biplots the cosine of the angle between environmental vectors approximated the correlation between environments with respect to the G×E interaction. Acute angles between two environments represented high positive correlations whereas wide obtuse angles (>90°) between two environments indicated their dissimilarity. G×E interaction effects for all traits measured were mainly caused by contrasting effects of each trial environment.

For example, for grain yield in the Palmira non-stress environment relatively little G×E interaction was observed compared to the drought stress environment, perhaps due the deep soils and high water table at this site. In contrast, strong environmental dissimilarities were observed between Palmira drought stress and non-stress environments compared to Awassa non-stress and Kasinthula drought stress environments. Similarly, non-stress environments were contrasting with the Awassa drought stress environment for grain yield; and finally, Amaro drought stress and non-stress environments tended to be closely correlated.

In general, low genetic correlations were observed both within African trial sites and between Africa and Colombia trial sites for grain yield performance (Table 4). The highest correlation was between environments at the Amaro site (r = 0.41, P<0.001); however correlations between drought stress and non-stress environments were not statistically significant at the other three sites (r = 0.01 to 0.09). Some significant correlations were observed between Awassa and Amaro for non-stress environments (r = 0.19, P<0.05) and drought (r = 0.20, P<0.05) environments. Interestingly grain yield in the Awassa non-stress environment was correlated with the Amaro drought stress environment (r = 0.26, P<0.01).

Table 3 Estimates of genotypic (G) and genotype × environment (G×E) variance components, broad sense heritability (h^2_b) and phenotypic correlation coefficients (r_p) of physiological traits with final grain yield under drought stress and non-stress trials in four locations across three countries (Colombia, Ethiopia and Malawi) for the DOR364 × BAT477 population

				r_p with grain yield under						
	Variance component			Drought stress				Non-stress		
Traits	G	G×E	h^2_b	PAL	AW	AM	KAS	PAL	AW	KAS
GY (kg ha^{-1})	2754	4669	0.37	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CBDW (kg ha^{-1})	20979	38058	0.36	0.41***	0.20*			0.25**	0.49***	
PHI (%)	0.515	0.792	0.39	0.25**	-0.21*			0.11	0.20*	
PPI (%)	2.61	12.0	0.18	0.15	0.30***			-0.02	-0.18*	
SBR (%)	6.5	11.9	0.35	0.03	0.08			0.06	0.06	
HI (%)	4.62	8.65	0.35	0.10	0.28**			0.00	-0.11	
STNC (mg g^{-1})	165	203	0.45	-0.02				-0.02		
SeTNC (mg g^{-1})	175	246	0.50	-0.09				0.03		
LAI (m^2 m^{-2})	0.0154	0.0154	0.51	0.46***				0.19*		
SCMR	0.550	0.539	0.43	0.20*	-0.02	0.13	-0.01	-0.21*	-0.37***	0.02
CTD (°C)	0.1046	0.1379	0.42	-0.05				0.11		

*, **, ***, significant at <0.05, <0.01 and <0.001 probability (one-tailed), respectively. *PAL* Palmira, *AW* Awassa, *AM* Amaro, *KAS* Kasinthula, *GY* grain yield, *CBDW* canopy biomass dry weight, *PHI* pod harvest index, *PPI* pod partitioning index, *SBR* stem biomass reduction, *HI* harvest index, *STNC* stem total nonstructural carbohydrate content, *SeTNC* seed total nonstructural carbohydrate content, *SCMR* SPAD chlorophyll meter reading, *CTD* canopy temperature depression

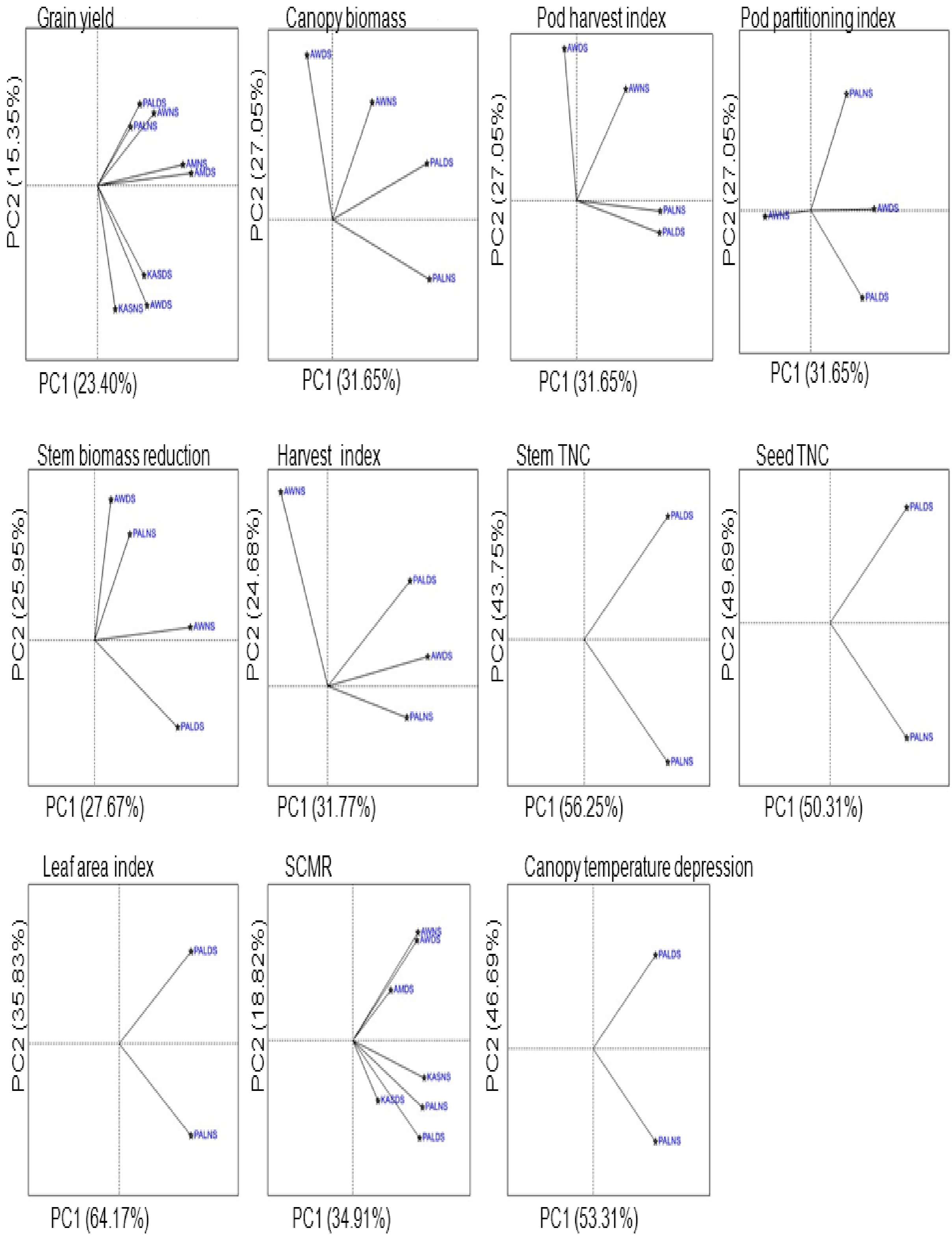

Fig. 2 Biplots indicating pattern of genotype × environment interaction for different traits measured in this study. Trait name indicated on top of respective biplot. *SCMR* SPAD chlorophyll meter reading, *TNC* total nonstructural carbohydrate content. Refer Table 4 for environment designation

The wide obtuse angles between Awassa drought stress and Palmira non-stress environments for canopy biomass dry weight at mid-pod fill, for pod harvest index and for harvest index indicated crossover type G×E interaction (Fig. 2). Patterns of G×E interaction for pod-portioning indices reflected the contrasting effects of drought stress and non-stress environments on this trait. For SPAD chlorophyll meter reading, Amaro and Kasinthula drought stress environments contributed low G×E interaction variance.

Table 4 Genetic correlations between drought stress (DS) and non-stress (NS) trial environments in four locations across three countries (Colombia, Ethiopia and Malawi) using a factor analytic model of order k=1 selected for modeling the residual genetic variance-covariance matrix for grain yield

Trial[a]	AMDS	AMNS	AWDS	AWNS	PALDS	PALNS	KASDS
AMNS	0.41***						
AWDS	0.20*	0.15					
AWNS	0.26**	0.19*	0.09				
PALDS	0.18*	0.13	0.06	0.08			
PALNS	0.12	0.09	0.04	0.05	0.04		
KASDS	0.19*	0.14	0.07	0.09	0.06	0.04	
KASNS	0.04	0.03	0.02	0.02	0.01	0.01	0.01

*, **, ***, Significant at <0.05, <0.01 and <0.001 probability (one-tailed), respectively
[a] *AMDS* Amaro drought stress, *AMNS* Amaro non-stress, *AWDS* Awassa drought stress, *AWNS* Awassa non-stress, *PALDS* Palmira drought stress, *PALNS* Palmira non-stress, *KASDS* Kasinthula drought stress, *KASNS* Kasinthula non-stress

The correlation values between traits and grain yield are shown in the last columns of Table 3 for both drought and non-stress environments. Positive and significant associations were observed between grain yield and biomass dry weight at mid-pod filling across locations and drought stress and non-stress environments. Under drought stress environments, the correlations with grain yield were positive (although not always significant) for the photosynthate partitioning traits pod harvest index, pod-partitioning index, stem biomass reduction and harvest index across locations, except for the negative and significant correlation for pod harvest index in Awassa. Pod harvest index and stem biomass reduction had positive correlations with grain yield under non-stress environments while they were negative or zero for pod partitioning index and harvest index under the same set of conditions.

The relationship between grain yield and stem or seed TNC were non-significant but between leaf area index it was positive and significant both under the drought stress and the non-stress environment (only assessed in Palmira). The relationship between grain yield and SPAD chlorophyll meter reading was inconsistent across locations and environments. It was negative under non-stress conditions in Awassa (r = -0.38, P<0.001) and Palmira (r = -0.21, P<0.05) while it was positive under drought stress conditions in Amaro (r = 0.13, P = 0.102) and

Palmira (r = 0.20, P<0.05). The correlation values between grain yield and SPAD chlorophyll meter reading were not significantly different from zero in Kasinthula both drought stress and non-stress environments and in Awassa drought stress. Grain yield was weakly correlated with stem and seed TNC, and canopy temperature-depression both under stress and non-stress environments.

QTL mapping

Three types of marker systems namely AFLP, RAPD and SSR/microsatellites were used to generate the linkage map for QTL detection. The DNA polymorphism level with the molecular markers used in this Mesoamerican intra-genepool cross was low to moderate depending on the marker type as described in greater detail in Blair et al. (2010). As distinct from that study, the linkage map presented here was constructed with a regression mapping algorithm using a total of 165 of the 205 polymorphic markers evaluated. This linkage map covered all 11 linkage groups of the common bean genome with a total genetic distance of 798.6 cM and had an average length *per* linkage group of 71.8 cM with an average distance between markers within a linkage group of 14.7 cM. The advantage of this genetic map for QTL analysis was that mapping positions were represented by non-conflicting and non-overlapping markers such that a genome scan procedure was appropriate for all traits.

With the single interval mapping (SIM) procedure in a multi-environment mixed model genome scan, a total of 9 significant QTL were identified. These were associated with yield, canopy biomass and photosynthate accumulation and partitioning related traits (Table 5). QTL were detected on 6 of the 11 linkage groups including b03, b05, b06, b08, b09 and b10 (Fig. 3). No significant QTL were detected for seed TNC and canopy temperature depression; however, QTL were identified for stem TNC and for SPAD chlorophyll meter readings.

The significant QTL were named by combining a three letter code for the trait with the linkage group and the order of the QTL for the given trait on each linkage group. The QTL for canopy biomass dry weight at mid-pod filling, pod harvest index, stem TNC and SPAD meter reading were significant with threshold LOD of 3.125 while QTL for grain yield, stem biomass reduction, harvest index and leaf area index were significant with a minimum LOD of 2.75. The QTL for grain yield on b08, canopy biomass dry weight at mid-pod fill on b03, for pod harvest index on b06, for pod partitioning harvest index on b03, for stem TNC on b05 and for SPAD chlorophyll meter reading on b06 showed inconsistent effects across environments while other QTL did not.

The QTL × environment interaction effects for grain yield, canopy biomass dry weight, and pod partitioning index were of crossover types whereas the interaction was non-crossover type for pod harvest index, stem TNC on b05 and SPAD chlorophyll meter reading. The QTL for stem TNC on b06 was consistent across environments. The crossover QTL × environment interaction effects in the study population were attributed to the contrasting effect of the parental alleles across different environments.

Table 5. Significant quantitative trait loci (QTL) and the percentage of genetic variance explained by the full QTL model for photosynthate acquisition, accumulation and partitioning traits in the DOR364 × BAT477 mapping population grown under drought stress and non-stress conditions at four different locations in three countries (Colombia, Ethiopia and Malawi) using multi-environment mixed model genome scan

							% of genetic variance [b]	
Trait	QTL[a]	LG	Marker	LOD	Wald	Pr_{wald}	Stress	Non-stress
Yield	*Yld*8.1	8	P103	2.83	3.03	0.003	19.45	14.45
Canopy biomass	*Cbm*3.1	3	AD1801	4.14	3.37	0.011	0.70	5.35
Pod harvest index	*Phi*6.1	6	Y501	3.30	4.92	0.001	0.00	6.45
Pod partitioning index	*Ppi*3.1	3	Q1701	3.14	3.28	0.013	0.95	10.00
Stem biomass reduction	*Sbr*9.1	9	Y1701	2.88	8.42	0.004	2.15	4.20
Harvest index	*Hri*3.1	3	Q1701	2.85	3.03	0.019	0.65	4.55
Stem TNC	*Stc*5.1	5	F601	4.77	11.72	0.000	1.20	17.60
	*Stc*6.1	6	M501	3.68	17.35	0.000	11.60	3.60
SCMR	*Scr*6.1	6	BMc238	10.79	11.39	0.000	19.68	42.23
Leaf area index	*Lai*10.1	10	N601	2.76	11.52	0.001	0.00	3.40

[a] QTL name based on association with yield (*Yld*), canopy biomass (*Cbm*), pod harvest index (*Phi*), pod partitioning index (*Ppi*), stem biomass reduction (*Sbr*), harvest index (*Hi*), stem total nonstructural carbohydrate (*Stc*), *SCMR* SPAD chlorophyll meter reading (*Scr*) and leaf area index (*Lai*). Decimal number represents linkage group and QTL order.

[b] The percentage of variance explained for each QTL under the full QTL model drought stress and non-stress conditions was calculated as average across the sites for the trait in respective drought stress and nons-tress environments. Pr_{wald} = Wald probability.

Figure 3 shows the results of the test for the specific effect of each QTL at each test environment. For grain yield the alleles from the drought resistant parent BAT477 had an increasing effect at Amaro drought stress, Awassa non-stress, Kasinthula drought stress, Palmira drought stress and non-stress conditions, but these BAT477 alleles had a decreasing effect at Amaro non-stress, Awassa drought stress and Kasinthula non-stress conditions.

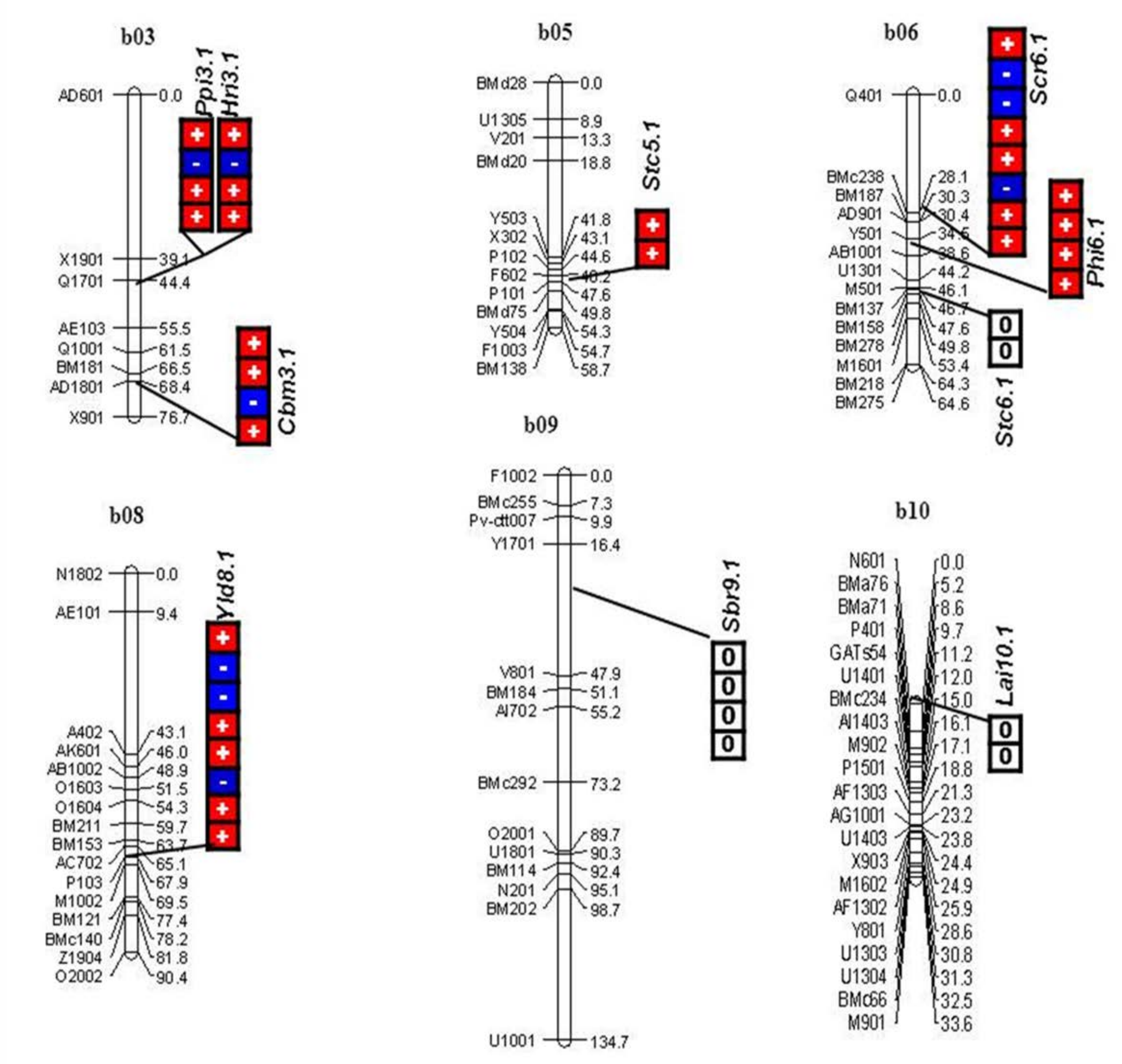

Fig. 3 Distribution of QTL for grain yield, canopy biomass dry weight at mid-pod filling, pod harvest index, pod partitioning index, stem biomass reduction, harvest index, stem total nonstructural carbohydrate, SPAD chlorophyll meter reading, and leaf area index on 6 of the 11 linkage groups of the DOR364 × BAT477 genetic map (refer to Table 5 for QTL names and to Blair et al. (2011) for base genetic map). Vertical bar with connectors to the corresponding positions on the linkage group represented each QTL. Co-localizing QTL indicated by connector pointing more than one trait. The blocks in vertical bar indicate the effect of the QTL in each environments (from top to bottom the environments are: AMDS, AMNS, AWDS, AWNS, KASDS, KASNS, PALDS and PALNS for grain yield, AMDS, AWDS, AWNS, KASDS, KASNS, PALDS and PALNS for SPAD chlorophyll meter reading, AWDS, AWNS, PALDS and PALNS for biomass accumulation and partitioning traits, PALDS and PALNS for stem total nonstructural carbohydrate and leaf area index (refer to Table 4 for environment abbreviations). The environment-specific effect of the parental marker alleles are indicated by either a '+' sign (red background) or a '-' sign (blue background) in the vertical bars. A '+' sign or red background represents the drought-tolerant paternal line BAT477 marker allele increasing the traits value whereas a '-' sign or blue background represents the drought tolerance from the susceptible maternal DOR364 marker allele. Main effects are indicated by '0' (white background).

For canopy biomass dry weight at mid-pod filling the alleles from BAT477 had an increasing effect at Awassa, both under drought stress and non-stress conditions and at Palmira under non-stress conditions and a decreasing effect at Palmira under drought stress. The co-localizing QTL for biomass partitioning traits namely pod partitioning and harvest index QTL on b03 showed a positive effect from the BAT477 alleles at Awassa drought stress and at Palmira under drought and non-stress conditions.

The QTL × environment interaction effects for pod harvest index and SPAD chlorophyll meter reading were attributed to differential expression of the BAT477 paternal alleles which increased the trait value of pod harvest index and decreased the trait value for SPAD chlorophyll meter reading across test environments. The QTL for stem biomass reduction, stem TNC on b06 and leaf area index were main effect QTL exclusively.

The genetic variance accounted for by the final QTL model for each trait was as high as 19.7% for the SPAD chlorophyll meter reading QTL on b06 (*Scr*6.1) and 19.5% for grain yield QTL on b08 (*Yld*8.1) both under drought stress environments. Meanwhile under non-stress conditions the SPAD chlorophyll meter reading QTL on b06 accounted for up to 42.2% of variance while the stem TNC QTL on b05 explained 17.6%.

QTL for stem TNC on b06 was intermediate in its contribution genetic variance (11.6%) while all remaining QTL were of low significance as measured by their effect on genetic variance under drought or non-stress environments. It was notable that all the QTL except that for grain yield and stem TNC on b06 had a lower proportion of genetic variance accounted for by the full QTL model under drought stress environments than under non-stress environments.

Discussion

Genotype × environment interactions for grain yield

Drought is a major constraint contributing to yield reduction in common bean production. In this study, drought stress treatments caused an average of 62% yield reduction relative to the non-stress environments. The effect varied from location to location but was always negative resulting in yield loss. The highest drought stress occurred during late planting environments of Awassa and Amaro as compared to Kasinthula and Palmira rainfed environments. This may have been also due to the combined effect of bean stem maggot and drought on plant establishment and yield. As a result, ranges in yield among RILs were large in Amaro and Awassa as compared to Kasinthula and Palmira (Table 2). A large yield variation among locations indicated that the drought stress environments used in this study were diverse and hence cross-environment selection would minimize selection efficiency while yield selection is a trait that must be evaluated on a *per* site basis.

All trial environments contributed considerably to the G×E variance of the traits considered in this study and one cannot pool the African sites versus the Latin American site for environmental effects (Fig. 2). The magnitude of G×E variances was larger than that of the genotypic variance for all traits except for that of leaf area index (Table 3) indicating there were sizeable differences in genotypic responses of RILs across environments for the vast majority of traits. Furthermore, large dissimilarities among test environments were observed to be the cause of high variance among genotypes (Table 4, Fig. 2). The genetic correlations for a given trait between trial environments, therefore, were generally low. This was especially the case for grain yield performance. Some exceptions were found for the same trait in the same location within African sites but overall the results indicated the lack of an ideal and representative test environment in discriminating the potential of test genotypes for reliable inference to be made on the performance across all other environments.

Breaking down grain yield into component traits

Understanding factors that account for larger differences in achieved yield compared with potential yield and the genetic enhancement for characters that contribute to yield formation are prime targets for physiology or molecular-aided approaches to crop improvement. Yield is a constant capacity system and a result of often inter-dependent traits (Yan and Wallace 1995). Two processes: namely, carbon assimilation rate and proportion of assimilates allocated to the storage organs, play an important role in determining achieved yield and yield potential of a crop or its varieties (Blum 1998). Carbon assimilation depends on sustained photosynthetic ability of the source while sink strength determines the ability of the storage organ to import and utilize the available assimilate. Meanwhile, photosynthate re-mobilization between source and sink is especially important for legumes which often remain green stemmed at the end of the season and which therefore are poor at removing carbohydrates from roots and stems to grain.

The present study accounts for genetic and environmental variation in grain yield of common bean by assessing the contribution of different traits to the three processes described above which can be summed up as: photosynthetic ability, photosynthate accumulation, and photosynthate partitioning. Traits assessed that contributed to plant photosynthetic ability included leaf area index, leaf chlorophyll content (assessed as SPAD reading) and canopy temperature depression. Canopy biomass production, stem and seed TNC were used to assess photosynthate accumulation while partitioning of photosynthates were assessed using pod harvest, pod partitioning, stem biomass reduction and harvest indices.

Impact of factors contributing to photosynthate acquisition and photosynthetic ability

Among the photosynthate acquisition, accumulation and partitioning traits, the proportion of variation in yield accounted for by variation in traits that could potentially contribute to plant photosynthetic ability under drought stress was variable. For example, leaf area index had a direct increasing effect on yield under both drought stress and non-stress environments (Table 3). Meanwhile other traits had no similar direct increasing effect on yield. The explanation for the importance of leaf area index could be in that leaf area reduction by inhibition of new leaf growth or via the earlier senescence of older leaves would lead to decreased transpirational area but also lower intercepted radiation throughout the growth season and ultimately to decreased biomass production (Pereira and Chaves 1993).

The positive and significant correlations of leaf area index with grain yield both under drought stress and non-stress environments in this study indicated that genotypes with maximum possible leaf area produced higher yield and *vice versa*. SPAD chlorophyll meter reading and canopy temperature depression showed slight increases in response to drought in both parents and RILs while that of leaf area index was decreased (Table 2). The genetic variance was about equal to the G×E variance for these traits indicating relatively high across environment repeatability (Table 4).

Despite this, SPAD chlorophyll meter reading showed inconsistent association with grain yield both under stress and non-stress environments while the correlations of grain yield with canopy temperature depression were not significantly different from zero. Slight increases in SPAD chlorophyll meter reading and canopy temperature depression during drought stress (Table 2) might suggest maintaining greener leaves and cooler canopy temperature would contribute to higher photosynthetic ability for sustained grain filling during stress or greater absorption and use of water during the growing period.

However, from our results it was difficult to make conclusions about the entire growth period as SPAD and canopy temperature readings were measured in single time points during this study. Therefore, the lack of well-structured correlations with grain yield may be as much a function of timing of measurements as physiological combinations of processes affecting both leaf chlorophyll and canopy temperature or water content during development.

Impact of factors contributing to photosynthate accumulation

Among the traits having to do with photosynthate accumulation, the proportion of variation in yield accounted for by variation in canopy biomass dry weight at mid-pod filling was significant. Canopy biomass dry weight at that stage had a direct positive effect on yield both under drought stress and non-stress environments across locations. Improved canopy biomass production, therefore might have contributed to drought tolerance. However, its utility as a trait for indirect selection for drought tolerance is questionable because the trait is not easy to measure and destructive, whereas there is also a large environmental effect on its expression.

Another study carried out by Ramirez-Vallejo and Kelly (1998) indicated that canopy biomass had a strong association with stem diameter which may be easier to measure and is certainly better in being non-destructive. But our personal experiences from field observations showed that genotypes with strong and thick stems were not always good yielding materials under drought stress.

Moreover, negative and non-significant correlation of stem TNC with grain yield indicated that the post-flowering drought stress in this study did not enhance stem photosynthate source remobilization to the seed in the RIL population. Alternatively stem TNC remobilization did not increase seed weight and per plant yield. This suggests the need to study the importance of stay green stems and delayed leaf senescence in common bean as an immediately-available source of photosynthates for remobilization to reproductive parts that might provide a longer window for better grain filling under drought stress. Blum (1998) practiced selection for stem reserves as potent trait to improve grain filling under drought stress in wheat.

On the other hand, stem and seed TNC had low or negative direct effect on grain yield both under drought and non-stress environments (Table 3). The effect of drought stress on biomass production was larger than that on grain yield while the effect was moderate on stem TNC (Table 2). Apart from these observations, slight increases in average seed TNC were observed across the RILs under drought stress. However, the correlation of seed TNC with grain yield was negative and low under the drought environments indicating that seed TNC increase is not a useful predictor of drought tolerance in common bean. Moreover, the genotype to G×E ratios for stem and seed TNC (0.81 and 0.71, respectively) and canopy biomass dry weight at mid-pod filling (0.55) were generally low indicating low across environment repeatability for these traits (Table 3).

Impact of factors contributing to photosynthate partitioning

Yield improvements via photosythate partitioning traits were also affected by drought stress but not as much as the photosynthetic ability and photosynthate accumulation related traits. In general, partitioning traits were positively associated with final grain yield under drought stress except for pod harvest index in the severe drought stress environment of Awassa.

Positive associations of partitioning indices with grain yield under drought stress suggest the importance of photosynthate remobilization from different vegetative structures of plant to contribute to increased seed weight. Data supporting similar relationships of yield with photosynthate partitioning indices under drought have been reported (Ramirez-Vallejo and Kelly 1998). However, negative correlation of pod harvest index with grain yield in the severe drought stress environment at Awassa indicates that this trait may not be useful as an indirect selection tool for all drought conditions.

Mobilization of photosynthates from pod wall reserve to final grain might be impaired by thicker pod wall formation under severe drought stress condition as compared to moderate drought stress conditions. Under moderate drought stress in Palmira, Colombia, pod harvest index had positive and significant correlation with grain yield but not in the more severe stress of Awassa, Ethiopia.

Finally, the partitioning traits particularly pod partitioning, stem biomass reduction and harvest indices had low associations with grain yield under non-stress environments. This could have been due to the indeterminate growth habit of the population, especially as some of the RILs continued to produce pods along with late rain showers even after the destructive sampling of mid-pod fill used to calculate these indices (Fig. 1). For the partitioning traits, even if no parental difference was observed, transgressive segregation among the RILs was important, making these traits of potential interest in further populations for drought improvement in common bean.

The need for pyramiding several genetically controlled traits related to drought tolerance

An understanding of the genetic control of each of the important traits that contribute to yield formation both under stress and non-stress conditions is imperative to breed cultivars that are fit to the target environment. For example, it appears that a deep and balanced rooting system under drought stress is needed for common beans to access to soil moisture from deeper in the soil profile during drought stress (Sponchiado et al. 1989; White et al. 1994a) and our results with canopy temperature depression may suggest this is true for some locations. However, deep rooting alone does not assure yield formation under drought stress as root studies in Colombian mollisols have shown that deep rooting genotypes are not always the best yield accumulators (Polanía et al. 2009).

Studies summarized by Rao (2001) further suggested remobilization of photosynthates from vegetative shoot structures to pod, and from pod wall to final grain as another important mechanism of drought tolerance in common bean with a carry-over effect to favorable environments (Beebe et al. 2008). However, in the face of drought stress, partitioning is altered to the detriment of grain filling and yield. Therefore, the accumulative effect of drought can result in poor remobilization of photosynthates to grain and at the same time to increased root growth, carbon accumulation in shoots, stay green stems, and late season re-flowering (Beebe et al. 2010).

Our results showed that improved remobilization of photosynthate to grain under drought condition may be an important mechanism to enhance yield formation under some conditions but also suggest that pyramiding of various tolerance mechanisms might be needed for breeders to improve drought adaptation in common bean. The best-bet mechanisms would be to pyramid deeper rooting that contribute to greater water uptake, improved photosynthetic ability via maximum possible leaf area for radiation interception and ability to control stomatal opening for better assimilate accumulation along with improved photosynthate remobilization to grain under drought stress.

Improving our genetic understanding of drought tolerance traits

Better understanding of the genetics of drought tolerance mechanisms is important to realize genetic gain in breeding programs. Studies of the association between traits related to drought tolerance and genetic markers using a QTL mapping approach in a stable RIL population evaluated in replicated multi-environment locations with properly designed experiments is useful in this regard. QTL analysis has been used to dissect the adaptation of common beans to low phosphorus but less so for drought.

For example, rooting pattern and drought avoidance (Asfaw et al. submitted), and yield components and phenology under drought stress (Blair et al. 2010) have been important components of drought tolerance in common bean. Meanwhile, root hairs and rhizosphere acidification (Yan et al. 2004), basal root development (Liao et al. 2004; Ochoa et al. 2006; Cichy et al. 2009), phosphorus use efficiency (Beebe et al. 2006) and root responsiveness to auxin (Remans et al. 2008) have been important for low phosphorus tolerance and adaptation. Some of the same traits involved in low phosphorus adaptation such as root hair density and root growth angle obviously also affect water uptake and therefore drought tolerance (Suriyagoda et al. 2010). Shallow roots while good for scavenging phosphorus from topsoil are obviously bad for water uptake in a receding water table. Despite this, some advantage of drought tolerant material in low phosphorus soils has been observed (Beebe et al. 2008) perhaps indicating a role for a larger rhizosphere that includes both shallow and deep roots together.

This study, amplified our understanding of the mechanisms and genetics of drought tolerance. Our principal achievement was to analyze three different traits related with the processes contributing to yield formation in common bean under drought, namely photosynthetic ability, photosynthate accumulation and photosynthate remobilization to grain. Specifically we identified the best DNA markers linked to these traits across different drought stress and non-stress environments. Harvest index which reflects the differences in photosynthate partitioning process was not easy to quantify in common bean because of leaf fall during pod filling and this was further analyzed through three indices, pod partitioning index, stem biomass reduction and pod harvest index. The stable QTL identified in this study, while few in number, would be the most useful to harness for indirect selection in breeding. For example the QTL for leaf area index and leaf chlorophyll content, canopy biomass, stem TNC, pod harvest, pod partitioning, stem biomass reduction and harvest index all appear to improve drought adaptation across various environments in common bean (Table 5).

Quantitative trait loci for drought tolerance

Multi-environment mixed model single interval mapping in the RIL population showed that QTL for the above mentioned traits were mostly distributed on linkage groups b03, b05, b06, b08, b09 and b10 (Table 5, Fig. 3). For the traits with significant QTL, mostly one QTL was detected highlighting the difficulty in detecting major QTL in drought studies for common bean under field managed conditions. A larger RIL population could identify more minor QTL but would be difficult to manage under the precision of replicated lattice design experiments used in our study. Notably, we found an important QTL for grain yield across various drought stress environments that was associated with the marker P103 on linkage group b08.

Previous studies with composite interval mapping in this and another recombinant inbred line population also found small numbers of QTL for yield under drought (Schneider et al. 1997; Blair et al. 2010). Yield QTL were not linked with or pleiotropic to biomass accumulation and photosynthate remobilization traits even though positive phenotypic correlations with some of these traits existed. This indicated that different sets of genes at different regions of the genome are activated in the yield accumulation process. This is due to the nature of yield as a complex trait determined by many physiological processes during photosynthesis, growth and development.

As remobilization of photosynthate from vegetative plant structures to pod wall and from pod wall to the final grain yield is an important mechanism in drought adaptation for common bean, it is interesting to see where QTL for these traits were located and to assess any possible functional relationship with other traits. In this regard, QTL for traits related with photosynthate accumulation and partitioning were distributed on linkage group b03, b05, b06 and b09 with genetic pleiotropy on b03 for pod partitioning and harvest index (Fig. 3). Meanwhile, the QTL for SPAD chlorophyll meter reading or SCMR was located on the same linkage group (bo6) with pod harvest index and both traits had similar pattern of association with grain yield particularly under stress. This indicated that the linkage group b06 may contain genes for increased plant photosynthetic ability under moderate drought stress and perhaps a larger number of chloroplasts per cell in leaf tissues and/or greater activation status of rubisco. Furthermore, these genes may result in a larger pool of photosynthates for remobilization to the pod wall and then to the grain, ultimately resulting in increased yield.

SPAD readings and QTL for this trait may be less useful under severe drought stress compared to moderate stress. This would be because very thick, small and dark green leaves under drought might be less photosynthetically active due to closed stomata despite high chlorophyll content. Leaves of common bean during severe drought became very dark green as compared to moderate stress or non-stress environments as reflected in higher SPAD meter reading (Table 2). Thick leaves would be expected to have more chloroplasts but construction and maintenance of thicker leaves is costly in terms of carbon (Lambers et al. 2008). Hence thicker leaves might have no advantage to productivity during severe drought stress in common bean but rather reflect a structural adjustment to the photosynthetic apparatus while conserving water as a survival strategy. Increased leaf thickness or reduced specific leaf area reflects a decreased cell expansion under drought stress.

Finally, the negative correlation of pod harvest index with final grain yield under severe drought stress indicated a major effect of severe drought on allocation of photosynthate from the pod wall to final grain production as reflected by seed weight. Differences in QTL location were observed for SPAD chlorophyll meter reading and stem TNC in pre-flowering, greenhouse-grown plants subject to terminal stress (Asfaw et al. submitted). Therefore, post-flowering measurements of stem TNC and SPAD reading may give different results from pre-flowering measurements of these traits. As a results we may postulate a different set of genes activating under drought stress in these different stages of plant growth as the plant moves from vegetative to reproductive phases.

QTL × environment interactions

The estimates of environment-specific QTL effects revealed that each site and season exerted a large effect on the expression of both yield and photosynthate remobilization or accumulation traits but less so on expression of photosynthate carbon assimilation traits. For example, it was notable that the QTL × environment interaction was of the cross-over type for grain yield and canopy biomass dry weight at mid-pod filling. Meanwhile, the co-localizing QTL for pod partitioning index and harvest index indicating greater relationship between these traits across environemnts. These traits also had low to medium genotype to genotype × environment interaction variance ratio confirming greater influence of the environment on trait expression.

The QTL × environment interactions indicate that site-specific mechanisms of drought tolerance are important. This makes sense from the perspective that no two drought events are the same and also that soil variability in terms of nutrients, porosity and structure strongly affect severity of drought effects. Weather variability in terms of night and day temperatures are also important characteristics of individual drought events.

Plants usually express differential adaptive strategies to drought stress, which may not be mutually exclusive but in practice are rarely combined within agronomically superior genotypes (Ludlow 1989). For example, deep rooting alone does not assure yield formation under drought stress as root studies in Colombia mollisols suggest deep rooting genotypes are not always the best yield accumulators (Polanía et al. 2009). Instead a range of adaptive responses to drought and mechanisms of drought tolerance that would not necessarily be liked genetically or related physiologically might be found in plants.

For examples, the QTL for grain yield and photosynthate remobilization traits did not overlap with any of the root depth or root pattern QTL detected for this population (Asfaw et al., submitted). This indicates that different sets of genes and physiological mechanisms determine activation of rooting depth traits that allow extraction of water from a greater depth compared to genes and mechanisms that affect photosynthate accumulation and its remobilization for yield improvement under drought stress.

Conclusions

There are three major insights to be noted from this study: 1) only a few major QTL were detected for traits measured in this study due to strict consideration of G×E interaction which is important for some low heritability traits; 2) high QTL × environment interaction for the significant QTLs was found except for the loci *Stc6.1*, *Sbr9.1* and *Lai10.1* (Fig. 3); and 3) low total genetic variance was explained by the QTL. The low QTL number, the high QTL×E interaction and the large proportion of genetic variance unexplained by the QTL highlight the difficulty in detecting QTL in drought studies under field stress.

Given the potential utility of improved photosynthate remobilization under drought stress as a mechanism for drought tolerance in common bean (Rao 2001; Beebe et al. 2010), the QTL tagged in this study for traits related with photosynthate remobilization will be useful in common bean breeding programs aimed at improving yield potential in stressful environments. Since phenotypic selection for such physiological traits is destructive and laborious, the discovery of QTL for traits such as stem TNC can pave the way for marker-aided selection to breed new varieties of drought tolerant common bean that combine a range of mechanisms in commercial grain types. Furthermore, the results from present work will permit future genetic studies to focus on certain parts of the genome and certain physiological processes that influence improved photosynthate acquisition, accumulation or remobilization for yield improvement under drought stress.

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CHAPTER 6

Participatory approach in common bean (*Phaseolus vulgaris* L.) breeding for drought tolerance for southern Ethiopia

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Abstract

Smallholder farmers in variable environments are characterized by varying variety preferences. This study targeted the identification of farmer preferences for common bean varieties in the drought-prone region of southern Ethiopia. To verify which traits breeders should consider and how these varied over sites, gender and wealth classes, various methods were employed and compared. The results showed considerable variation in farmers' variety preferences, but the most important variation consistently focused on a fairly consistent set of variety traits. Different methods indicated that farmers considered earliness as the most important trait. Culinary qualities and marketability showed to be at least as important as drought tolerance. Women clearly attached more importance to the culinary qualities than men, whereas the different methods pointed to men giving more importance to marketability. The local traditional varieties do not satisfy farmers in many of the traits they consider important and even marketability and culinary quality are sub-optimal. Some of the new drought tolerant genotypes yielded better than the local farmer varieties under drought-prone farmer field and on station conditions and farmers were attracted by the newly introduced grain types (large red-mottled, large cream mottled) as much as by the traditional grain types (small red, small white). The relatively high level of coincidence of farmer selections and selections made by breeders and extensionists, combined with the relative consistent set of important traits to consider suggest that diversity of farmers' demand is not the first concern. The challenge of the common bean breeding for southern Ethiopia therefore lies in combining different mechanisms of drought tolerance with culinary attractiveness and marketability. The market in the study region shows to be fairly dynamic and has as a consequence that farmer preferences for grain types and color are not static. Breeding that focuses on traditionally grown colours, shapes and sizes may therefore restrict farmers' access to novel attractive and adapted germplasm.

Keywords Decentralized breeding, farmer preferences, gender, grain types, marketability, participatory variety selection

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important food legume in Ethiopia. It is cultivated in a wide range of agro-ecologies and farming systems including well-watered and drought-stressed areas. Southern Ethiopia accounts for about 23% of the country's bean production area and 18% of its total production (CSA 2009). In this part of the country, bean production is carried out mainly by resource-poor smallholder farmers whose production conditions are diverse and harsh. Loss of common bean yield due to drought is a major problem to resource-poor farmers as its production is mainly rain-fed and some crop stages are particular sensitive to water supply (Amede et al. 2004). The problem of drought, whether intermittent or terminal, is becoming more serious over time in Ethiopia, both in frequency and in intensity. The drought problem is further escalated by rising temperatures linked to climate change, soil fertility decline, and landholding subdivision and shrinkage due to population pressure (Funk et al. 2005). Drought stress makes variety choice crucial, particularly in southern Ethiopia where agro-ecological and socio-cultural variation is large (Asfaw et al. forthcoming).

Many improved varieties with high on-station yield were developed in Ethiopia from local and international genepools (Asfaw 2008). However, in the drought-prone areas the majority of them are not adopted and common bean production continues to depend on a range of farmer varieties (Asfaw et al. 2009) and average regional yield is around 1 tonne per hectare (CSA 2009). In principle there are two breeding strategies to cope with drought-stress (Acosta-Gallegos and White 1995; Rao 2001; Beebe et al. 2010). One breeding strategy is to develop early-maturing varieties, to escape drought through early maturity. The other strategy is to develop drought-tolerant varieties, i.e. varieties that withstand or recover from drought stress during growth. Progress in breeding for drought tolerance has produced a pool of drought-tolerant genotypes that are potentially more interesting for farmers in these areas (Beebe et al. 2008). The genotypes are bred to combine high yield and drought tolerance, and include a range of different grain types. However, these genotypes have not yet been exposed to farmer preferences and drought conditions in southern Ethiopia.

Drought presents itself at different times in the growing season, and with different intensity, while its effects on crops are modified by soil type and fertility (Rao 2001). Different mechanisms play a role in drought tolerance and their importance depends on when the drought occurs (Beebe et al. 2010). Drought tolerance is therefore a genetically and physiologically complex trait for which there are no straightforward suitable selection criteria (Beebe 1998). Moreover, it shows low heritability and limited genetic variation (Blair et al. 2010). It is therefore not surprising that drought tolerance is susceptible to genotype by environment (G×E) interaction, for example where valuable drought tolerance can be masked by poor adaptation to a specific environment (Beebe 1998; Beebe et al. 2010). In addition to this complexity, the variation of farmers' preferences asks a diversity of visible traits to be combined with drought tolerance (Asfaw et al. forthcoming). The complexity of drought tolerance and the diversity in environments and preferences, combined with possible trade-offs, represent a problem for the design of breeding programs.

Ceccarelli and Grando (2005) argued for decentralized breeding programs but this does not *per se* respond to the needs of farmers for two reasons: (1) international breeding programs are often merely involved in the transfer of selection from one research station to another; (2) in national programs, the definitions of agro-ecological target environment do not include farmers' preference and needs. Hence, a breeding strategy that integrates selection criteria of farmers and other product-chain actors with the agro-ecological adaptation may be a more effective strategy to target the diverse environments and the user needs (Sperling et al. 2001; Witcombe et al. 2005b; Almekinders and Hardon 2006; Walker 2006; Ceccarelli and Grando 2009). Participatory Plant Breeding (PPB) as an alternative approach to breeding to overcome and even exploit the interaction of genotype, environment and socio-cultural or economic factors (G×E×S) is now widely advocated (Almekinders and Elings 2001; Nuijten 2005; Ceccarelli and Grando 2007; Dawson et al. 2008). Experiences around the globe revealed that farmers generally have the capacities to select the plant materials among and within crop varieties that fit their conditions and preferences (e.g. Sperling and Loevinsohn 1993; Ceccarelli et al. 2000; Witcombe et al. 2005a; Asfaw et al. 2006; Almekinders 2011). However, variation of farmer preferences and priorities, how to capture these, and the implications for the design of breeding programs have been less explicitly discussed.

Capturing and using farmers preferences and concerns in plant breeding has become more common practice over the last two decades (e.g. Ashby and Lilja 2004; Almekinders and Hardon 2006; Ceccarelli et al. 2007). Different types of methods have been developed, tested and applied in many different crops, including common bean. Most of these experiences have been very localized, working at community level with relatively small groups of farmers, using methodologies appropriate for the purpose (e.g., Ceccarelli et al. 2000; Bellon 2001; Christinck et al. 2005). Less attention has been given to methods to collect, analyze and exploit variation among farmers from the breeder point of view. Typically, a breeder in a developing country has to develop materials that serve a wide and varied agro-ecological area with large variation in farmer preferences and farmer agricultural practice. Several breeders and researchers used innovative methodologies to deal with this variation (Bellon and Reeves 2002; Virk and Witcombe 2008). This article contributes to designing approaches of decentralization and participation in common bean breeding by exploring different methods to capture farmers' preferences, understand the various sources of variation in farmers' preference and assess farmers' effectiveness in identifying among a pool of new genotypes that were developed for high yields in drought-prone conditions in southern Ethiopia and prevailing agricultural practices. It discusses the implications for decisions that breeders have to make in breeding programs.

Material and Methods

Study area description

This study was conducted in seven rural communities in the South Nation, Nationalities and People's Regional State of Ethiopia from 2008 to 2010 (Fig. 1): two rural communities in the Sidama zone (Awassa zuria and Boricha), one community each in the Amaro and Konso special districts (woreda), Gamo-Gofa zone (Gofa), Dawro zone (Loma) and the Gurage zone (Inseno). The study areas represented dry, warm and cool maize/sorghum based bean production ecologies with different soil types and with altitudes from 1305 to 2002 m above sea level. The seven communities differed in degree of drought stress, in the relative importance of common bean in terms of utilization and area planted, and in ethnicity (Table 1). At all sites common bean was grown twice per calendar year: in the '*Belg'* (February to May) and ‘*Meher*’ (June to October) seasons. '*Belg'* is the major growing season at Amaro and Konso whereas in the other areas '*Meher*' is the major season. In the '*Belg*' season, common bean is usually mono-cropped or intercropped with maize or sorghum. In the ‘*Meher*’ season, it is normally mono-cropped or relay cropped with maize or sorghum. Low moisture was the major limiting factor for common bean production in all communities and harvest was only possible when moisture was sufficient during the cropping season. The study areas had a long-term downward trend in total rainfall per year and experienced a “green famine” in recent years (Funk et al. 2005).

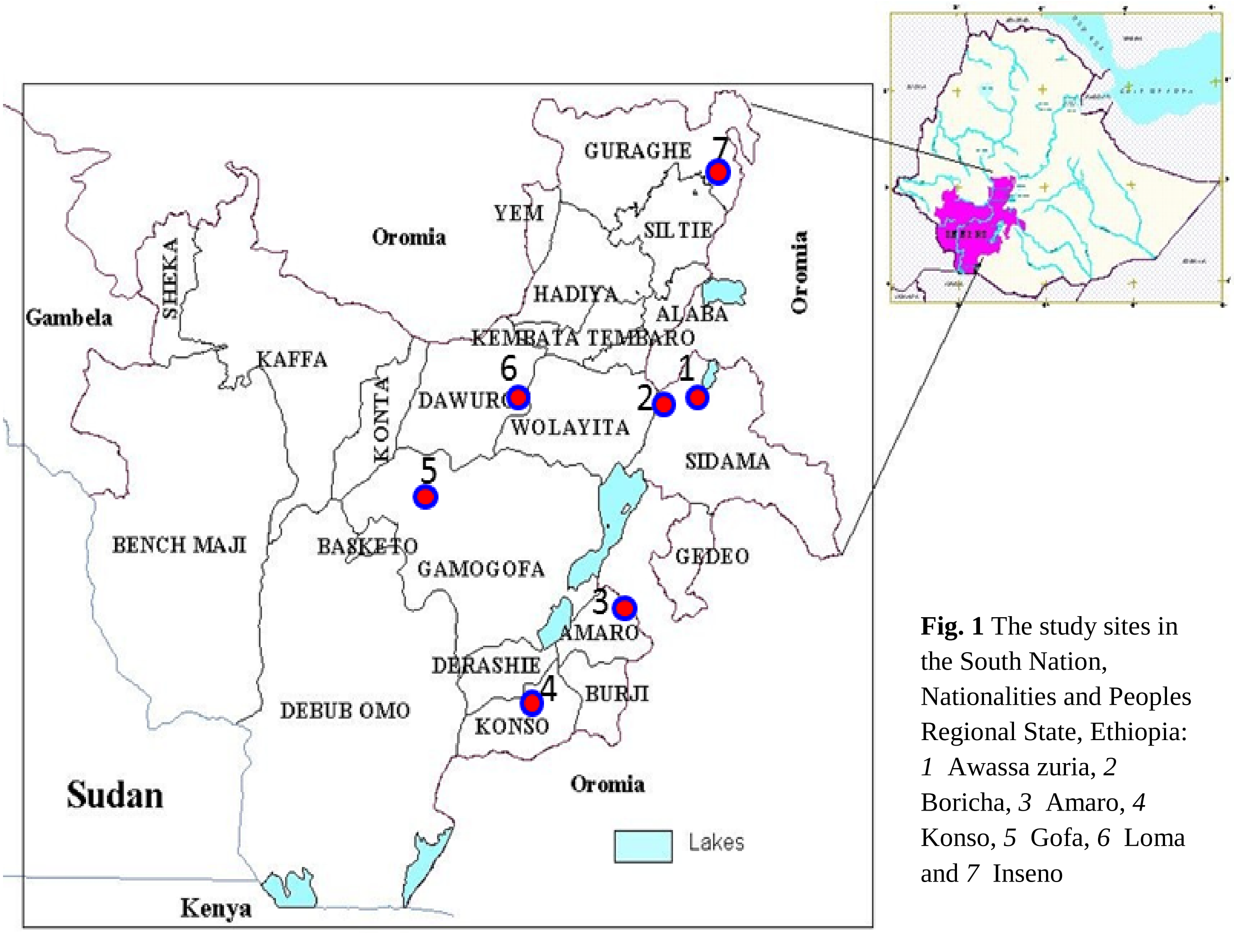


Fig. 1 The study sites in the South Nation, Nationalities and Peoples Regional State, Ethiopia: *1* Awassa zuria, *2* Boricha, *3* Amaro, *4* Konso, *5* Gofa, *6* Loma and *7* Inseno

Research design

This study analyzes farmers' evaluation of bean variety traits' in a set of currently used and new varieties. The first part uses different research methods to systematically compare and analyze the traits that common bean farmers value and the extent to which their current common bean varieties provide these traits. The second part reports on participatory on-station selection and the follow-up in on-farm selection and performance evaluation of the new drought tolerant bean genotypes.

Part I. Farmers' perceptions on choice of bean types

Focus group discussions. Focus group discussions (FGD) were held in May/June 2008 at four of the seven sites namely Amaro, Boricha, Konso and Loma (Table 1). At each of these four sites, three separate discussions were held to cover the different locations within the community. Each FGD was held in the open air and started with a minimum of 10 participants who varied in gender, age and wealth status. In the course of the process the number would increase, up to 30 in some cases, as curious volunteers from surrounding farms joined in. All FGD started with making an inventory of the common bean varieties grown and known in that locality, and of their traits. This inventory resulted in a portfolio of 32 common bean traits that farmers use to describe and recognize bean varieties.

First individual household interviews and stratification. In order to clarify the value of each of the 32 common bean traits, two methods were used. For the first method, a total of 178 households from the four sites were asked to classify the importance of each of the traits for describing and discussing common bean varieties as very important, somewhat important, or not important (see Table 3). From these data the percentage of people considering the trait as very important was calculated and an average importance of the trait (based on 1 = not important, 2 = somewhat important and 3 = very important) for men and women respondent groups was tested for statistically significant differences. Yield stability was added to this list of criteria, based on the experience that farmers considered stable yield over time and cropping seasons as important, increasing the number of traits to 33. For the second method, respondents were thereafter asked in the same interview to rank the six most important traits from the list of 33 bean traits and rank them in order of importance. In most households the man and wife were interviewed separately. In a few cases there was one interview per household because of absence of a man or a woman. The survey involved 178 households and captured the opinion of in total 324 individual farmers. To compare the variation in importance of these traits within and across the study sites, the answers of the respondents were stratified by gender, wealth classes and locations. Wealth-class stratification in poor, medium and better-off was made on the basis of the respondents' own perception (household position as compared with other ones in the village). For all traits a mean rank (MR) was calculated and the number of times (frequency) the trait was part of the famers' top six list. A rank index was calculated by dividing the mean rank by the number of times the trait appeared in the top six lists (frequency). A trait with lowest rank-index was ranked first and

Table 1 Physical and socio-economic characteristics of the study sites, and the data collection from each of them

Characteristics	Study sites						
	Awassa zuria	Boricha	Amaro	Konso	Gofa	Loma	Insono
Altitude (m a.s.l.)	1694-1964	1650-1932	1305-1450	1200-2000	1400	800-1900	1829-2002
Latitude (^{0} north)	6.49-7.15	6.76-7.01	5.60-5.99	5.17-5.56	6.19-6.67	6.58-7.07	7.86-8.11
Longitude (^{0} east)	38.19-38.43	38.07-38.39	37.54-38.01	37.01-37.69	36.83-37.19	36.93-37.43	38.43-38.63
Annual rainfall (mm)	959	963	927	617	1235	1121	1095
Total rainfall '*Belg*' (mm)	296	299	412	317	486	347	375
Total rainfall '*Meher*' (mm)	444	492	323	221	549	574	533
Major agro-ecology	Tepid to cool sub-humid	Tepid to cool sub-humid	Hot to warm humid	Hot to warm humid, semiarid	Hot to warm humid	Hot to warm sub-humid	Tepid to cool sub-humid
Major soil type	Eutric fluvisols	Chromic luvisols, Eutric fluvisols	Eutric nitosols	Eutric regosols, Eutric nitosols	Pellic vertisols	Eutric nitosols	Eutric fluvisols
Bean production system	Relay/sole	Relay/sole	Intercrop/sole	Intercrop/sole	Intercrop	Relay/sole	Sole
Average bean area (ha) per HH†	0.12	0.30	0.28	0.41	0.07	0.73	0.05
Average bean yield (kg/ha)	1490	1550	750	690	780	1185	1370
Major bean types grown in order of importance	Small-red, red-mottled, small-white	Small-red, red-mottled, small-white	Small-white, black, mixtures, pinto, small-red	Black, small-red, mixtures	Black, small-red	Small-red	Small-white, small-red, red-mottled
Importance of beans	Cash, food	Cash, food	Food	Food	Food	Cash, food	Cash
Ethnic group	Sidama	Sidama, Wolayta	Koyra	Konso, Gawada	Gofa	Dawro	Marako, Gurage
Data collection							
Group discussion		√	√	√		√	
Individual trait rating		√	√	√		√	
Variety profile rating		√	√	√		√	
Researcher managed OS-PVS	√		√		√		√
Farmer managed OF-PVS	√	√	√	failed		failed	√

† *HH* household, *kg* kilogram, *ha* hectare, *RF* rainfall, *OS-PVS* on-station participatory variety selection, *OF* on-farm participatory variety selection

the next lowest rank-index value was ranked second and so on. Accordingly, the six traits with the lowest rank index in consecutive order were considered top most important to survey farmers (see table 3).

Finally, the heads of each household (168 men and 10 women) were also asked to rate the 33 traits for the most common bean varieties they knew. The assessments poor, intermediate/acceptable and very good were assigned values of respectively 1, 2 and 3 and the average score per variety was calculated (see Table 6).

Second individual household interview. In the second individual interview, a hypothetical variety profile was used to elicit the preference of farmers for combining traits. From the six traits with the lowest rank index in the categories of men and women, respectively, two quantitative and two qualitative traits were selected to create the varietal profiles: yield, drought tolerance, culinary quality and marketability (see Table 2). Earliness was not included in the variety profile because it ranked first in importance by both men and women, hence it was assumed that there would be no variation in preference for this trait. Germination, which ranked high in importance for the male farmers, was excluded because it was more likely to be linked with seed quality and moisture during time of planting rather than with a variety trait. Specific adaptation was excluded because its meaning varied among farmers. A full factorial of four traits with two levels each generates 16 possible varietal profiles. Because this would be too difficult to evaluate in a meaningful way, only seven variety profiles were verbally presented to the farmers. Three hundred seventy five individual household members from the same four sites were asked to consider the seven profiles of the common bean varieties and to assign a rating to each profile using a five-point (1-5) preference scale: 5 for the most desirable variety profile(s) and 1 for the least desirable one(s). Most of the respondents in this second interview were the same as the ones from the first interview, but some new respondents replaced those who had moved or were engaged in other activities at the interview time. The relative importance score for each of the four traits was calculated by the formula described in Tano et al. (2003):

$$\psi a = [\max(V_{ga}) - \min(V_{ga})]/\Sigma\ \omega a,$$

where V_{ga} is the marginal value of the g^{th} level of the a^{th} trait; ψa represents the relative importance for the a^{th} trait; $\Sigma\ \omega a$ is the sum of the ranges, $[\max(V_{ga}) - \min(V_{ga})]$, across all traits. This ratio provides an indication of the traits the surveyed respondents valued most highly.

In the second interview we also asked farmers about the decision making of males and females in common bean farming. For decisions on plot selection, seed bed preparation, variety to plant, date to sow, amount of seeds to plant, seed source to use, date to harvest and share of harvest to keep for home use, selling and saving of seed, farmers gave the share of the influence of man and wife on the decision.

Statistical analysis. Non-parametric analysis were used to measure the extent to which farmers valued the common bean traits and the extent to which their current common bean varieties provided these traits (Table 3). Unpaired t test with Welch's correction was applied to test for statistical significant differences between ratings of male and female farmers for traits, using Graphpad Prism version 5.00 (Graphpad Software, San Diego California USA, www.graphpad.com). A Kruskal-Wallis one-way analysis of variance procedure was used to test for statistical differences in the ratings for the common bean variety traits among the three wealth groups and ethnic groups/communities and across the different farmer common bean types for the traits, using GenStat 12.1 (Payne et al. 2009) (Table 6).

Table 2 The varietal profile evaluated by farmers in southern Ethiopia

	Traits			
Profile	Grain yield[a]	Drought tolerance[b]	Culinary quality[c]	Marketability[d]
1	High	Tolerant	Preferred	High
2	High	Tolerant	Preferred	Less
3	High	Not tolerant	Preferred	Less
4	High	Not tolerant	Not preferred	High
5	Poor	Not tolerant	Preferred	High
6	Poor	Tolerant	Not preferred	Less
7	Poor	Tolerant	Preferred	High

[a] high yield means better than current farmer's yield and poor yield means lower than what farmer is achieving currently; [b] drought tolerance refers to the capacity of the plants to withstand or recover from drought stress in different parts of the growing season; [c] taste and attractiveness to eat; [d] easy to sell at local market

Part II. Participatory variety selection (PVS)

Decentralized participatory selection trials were used to assess how farmers' trait preferences elicited during the single trait evaluation and the hypothetical varietal profile exercise in the interviews compared with real selection in the field and to assess how farmers' preferences changed when they were exposed to new types of common beans. The trials were also used to assess how local adaptation – as expressed in yield - plays a role in breeding for drought adaptation in common beans.

Two types of PVS trials were conducted in the 2008-*Meher*, the 2009-*Belg* and *Meher* season and the 2010-*Belg* season: (i) researcher-managed on-station trials in Amaro, Awassa, Gofa and Inseno and (ii) farmer-managed on-farm trials in Awassa zuria, Boricha, Amaro and Inseno (Table 1). In total six on-station and seven farmer-managed trials were carried out using incomplete block designs replicated at least three times. In on-farm PVS farmers were considered as replications. PVS trials in Konso and Loma failed due to extreme droughts.

The first trials in the 2008-*Meher* season started with 38 genotypes from eight different grain classes (Supplementary Table 2). They included 34 advanced drought-tolerant genotypes of CIAT (International Center for Tropical Agriculture), two locally bred varieties from Awassa Research Center (Dume and ETAW-8-2A), one released variety from previous PPB activities (Ibado) and the most popular farmer variety as local check. The local checks varied among villages but for statistical analysis the local check was considered to be the same across villages. The breeding scheme for CIAT advanced drought genotypes was described in Beebe et al. (2008). In the participatory selection procedure if one farmer in one trial or site was of the opinion that the variety merited more evaluation, the variety was included in the next cycle of trials. Eventually this resulted in eight advanced CIAT materials and the one locally developed variety which were evaluated in all trials and compared with a local check. The data from these improved varieties and local checks were used to analyse yield performance, farmer selection and to compare them with breeder and extension agent selection (see Table 7, Figures 2, 3 and 4).

The on-farm trials were hosted by farmers who were identified in consultation with extension agents, the peasant association (Kebele = smallest administrative unit of the country) leaders and the researchers' own contact developed through previous on-farm research. Also evaluator farmers were identified and invited in consultation with on-farm trial host-farmer, extension agent and Kebele leaders. All farmers hosting the on-farm PVS trial as well as farmer-evaluators were experienced common bean producers in the locality. The selections and evaluations were done when the crop was close to physiological maturity and also after threshing for four consecutive seasons. The farmer-evaluators were asked to select the common bean genotypes they would grow next season if they were given the seed. Farmers used their own judgment to rate for the criteria and to select or reject the materials, without any interference from the researcher. The grains of each genotype from previous year's harvest were present in transparent plastic bags at the time of selection and evaluation at physiological maturity, to give the farmers options of selection and evaluation for seed traits. After threshing farmers gave scores for yield, seed traits and marketability and of course made their final refined selection decision. In addition grain yield (kg/ha) data were taken for each trial by the researcher.

To explore the correlation of seven important traits being valued as 'very good' by farmers and the chance of varieties to be selected, log odds ratio of farmers' assessments were calculated on the basis of logistical regression analysis. In all the six on-station and seven on-farm PVS trials, the farmer-evaluators (10 to 15 per site) were invited to evaluate the materials. The scale being used was: 1 = poor, 2 = intermediate/acceptable or 3 = very good. The evaluation traits of common bean varieties were discussed with the farmer-evaluators with verbal description and using plants in the field in a free, interactive manner until each evaluator fully understood how (s)he was going to rate each common bean genotype based on the scale. Apart from rating for the seven traits the evaluators were also asked to rate whether the new each drought tolerant genotype was ‘worse = 1’, ‘same = 2’ or ‘better = 3’ compared with the local check in overall performance. About 130 farmers participated in the PVS trials. Five bean breeders and three extension agents evaluated and selected in six on-station and seven on-farm trials using the same evaluation procedure.

Statistical analysis. For the analysis of the PVS trials we used ordinal logistic regression analysis for qualitative data and mixed model Restricted Maximum Likelihood (REML) analysis for quantitative grain yield data, as implemented in GenStat 12.1. The data from the PVS trials were highly non-orthogonal. For analysis of such unbalanced data, a mixed effect REML analysis for quantitative data and logistic regression analysis for qualitative data were used, as described in Coe (2002a, b) and Virk and Witcombe (2008). For logistic regression analysis, local farmer variety was used as reference point. In REML analysis genotype, trial type (on-farm or on-station) and village where PVS was conducted were fitted as fixed effect whereas years, seasons and farmers within village in on-farm trial and blocks in on-station trial were taken as random effect. To cope with the highly variable environments (i.e. sole, relay or inter-cropping situations, ‘*Belg*’ and ‘*Meher*’ plantings and different agro-ecologies) in the analysis of yield stability, we used the parametric cultivar superiority measure (Pi) of Lin and Binns (1988) and the non-parametric mean rank of Nassar and Huhn (1987) as measures of yield stability of the PVS genotypes across test environments. A smaller Pi estimate points to a more stable genotype. The lowest value of mean rank represents the highest stability. Graphs of figure 2, 3 and 4 were constructed with Sigmaplot version 10.0 (Systat Software, Inc, CA, USA). For figure 2, the selection frequencies of the new genotypes and the local variety by farmers, breeders and extension agents were used. For figures 3 and 4, the log odds ratios of the farmers’ qualitative assessment of the new genotypes were used. The genotype order in X-axis of figures 3 and 4 was based on random sorting of the log odds ratio of the genotypes for different traits.

Results

Inventory of farmers’ common bean varietal preferences

In the focus group discussions (FGD) in four of the seven research sites (Table 1) farmers used 33 different traits to describe and discuss bean varieties. These traits were differentiated into categories of agronomic field and storage performance, yield, market and use concerns (Table 3). In assessing the importance of these traits in describing and discussing varieties, the percentage of interviewed people considering the trait as ‘very important’ was analysed. The results show that the large majority of men and women found the traits very important; only three traits were considered ‘very important’ by 60% or fewer men and women: growth habit, shattering and seed shape. Yield stability, pod length and straw for feed scored intermediate, but for all other trait the mean score of men and women was higher than 2.0. In the statistical analysis of the mean rating of each of the 33 traits evaluated, 13 showed significant differences for men and women ratings ($p=0.001$). These traits mostly reflected culinary concerns to which women gave more weight and agronomic performances that men considered very important. However, the R^2 values related with the analysis of the mean ratings, which quantifies the fraction of all the variation in the samples that is accounted for by a difference between the two gender means, were extremely low. This indicates important variation in preference rating within the gender groups, i.e. across locations, wealth groups, or variation of idiosyncratic nature. Of the 33 traits male ratings varied significantly across locations for 18 traits while the females ratings differed for 26 traits (data not shown). However, the differences did not show a meaningful pattern. The results did not show clear differences between wealth classes either (data not shown).

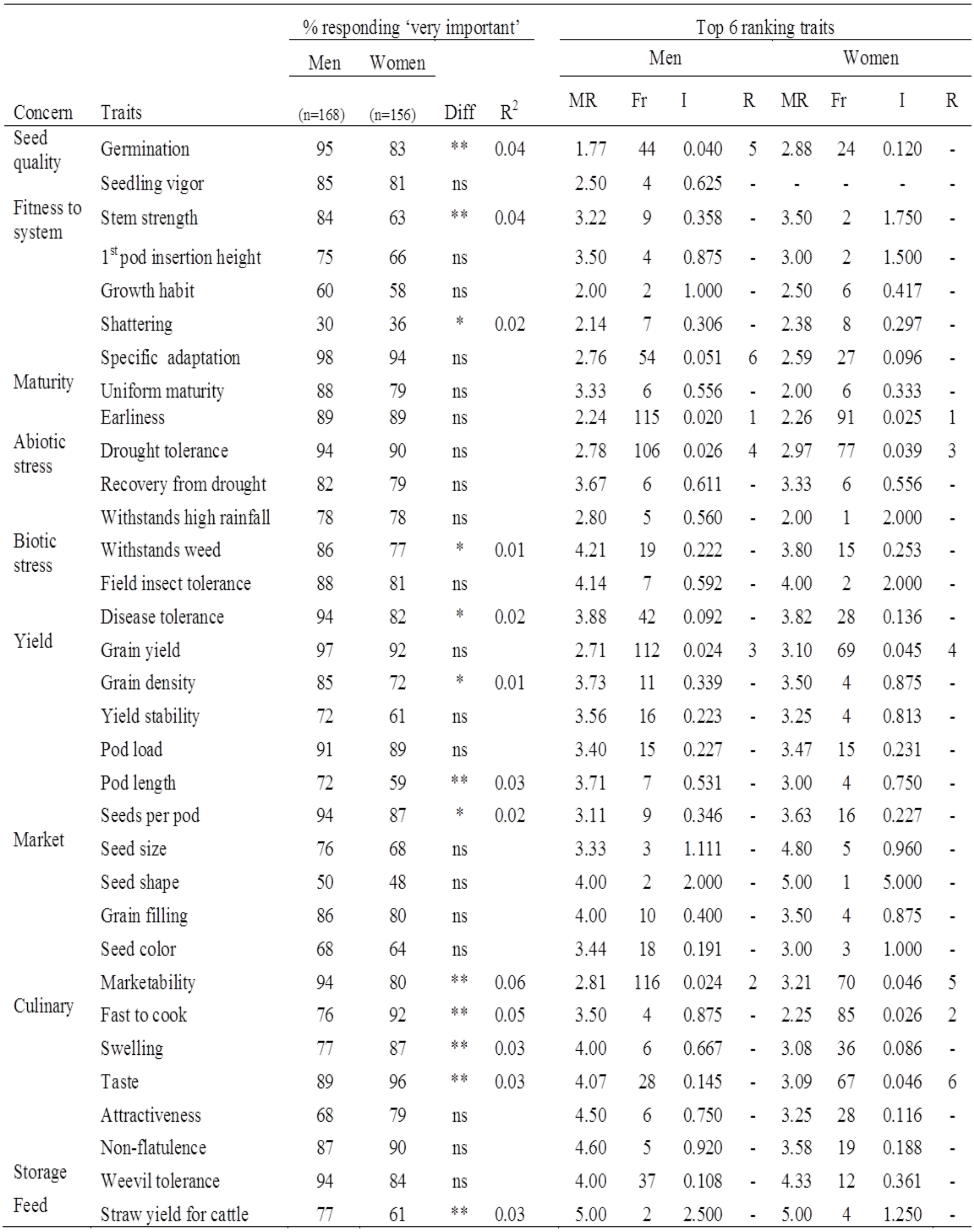
The information from the farmers' ranking of the six most important traits of a bean variety were used to calculate a mean ranking (MR), a frequency of being included in the ranking (Fr) and a rank index (I) (Table 3). This resulted in different identification of the most important traits as considered by farmers. MR considered order of importance of the trait in the top six lists but did not take into account the number of respondents mentioning it. Fr took into account the number of respondents mentioning the trait in the top six lists but did not give weight to the order of importance. Rank index weighed the traits based on order of importance and number of times the trait appeared in the top six most important list, hence provided a relatively good estimate of the top six most important traits for the survey farmers. When considering the six traits with the lowest rank indices, four were shared between the male and female groups, namely drought tolerance, marketability, earliness to maturity and grain yield (Table 3). For the men more specific adaptation and germination classified among the six most important traits, while for women fast-to-cook and good taste were most often included in the six most important traits. More generally all the listed traits except seedling vigor in the female group were considered most important at least for one farmer indicating individual preference variation for the bean traits in southern region of Ethiopia. Even yield stability which was added by the researcher was considered most important by some farmers.

Ranking of traits through the evaluation of the hypothetical variety profiles did not confirm the predominant importance of drought as found in the individual trait assessment and ranking methods. When farmers valued the hypothetical variety profiles, culinary quality/suitability for home consumption and marketability ranked more important than grain yield and drought tolerance in all locations (Table 4). There were no differences between ranking of men and women.

Intrahousehold decision making on bean farming and the linkages with preference

Table 5 presents gender involvement in common bean-farming decision making. The results show that men and women participated in the decisions related with common bean although their respective degrees varied across sites. The role of women was less prominent in different

Table 3 Common bean traits used by men and women to describe bean varieties, and their importance based on individual trait assessment in four communities using different methods



		% responding 'very important'				Top 6 ranking traits							
		Men	Women			Men				Women			
Concern	Traits	(n=168)	(n=156)	Diff	R^2	MR	Fr	I	R	MR	Fr	I	R
Seed quality	Germination	95	83	**	0.04	1.77	44	0.040	5	2.88	24	0.120	-
	Seedling vigor	85	81	ns		2.50	4	0.625	-	-	-	-	-
Fitness to system	Stem strength	84	63	**	0.04	3.22	9	0.358	-	3.50	2	1.750	-
	1st pod insertion height	75	66	ns		3.50	4	0.875	-	3.00	2	1.500	-
	Growth habit	60	58	ns		2.00	2	1.000	-	2.50	6	0.417	-
	Shattering	30	36	*	0.02	2.14	7	0.306	-	2.38	8	0.297	-
	Specific adaptation	98	94	ns		2.76	54	0.051	6	2.59	27	0.096	-
Maturity	Uniform maturity	88	79	ns		3.33	6	0.556	-	2.00	6	0.333	-
	Earliness	89	89	ns		2.24	115	0.020	1	2.26	91	0.025	1
Abiotic stress	Drought tolerance	94	90	ns		2.78	106	0.026	4	2.97	77	0.039	3
	Recovery from drought	82	79	ns		3.67	6	0.611	-	3.33	6	0.556	-
	Withstands high rainfall	78	78	ns		2.80	5	0.560	-	2.00	1	2.000	-
Biotic stress	Withstands weed	86	77	*	0.01	4.21	19	0.222	-	3.80	15	0.253	-
	Field insect tolerance	88	81	ns		4.14	7	0.592	-	4.00	2	2.000	-
	Disease tolerance	94	82	*	0.02	3.88	42	0.092	-	3.82	28	0.136	-
Yield	Grain yield	97	92	ns		2.71	112	0.024	3	3.10	69	0.045	4
	Grain density	85	72	*	0.01	3.73	11	0.339	-	3.50	4	0.875	-
	Yield stability	72	61	ns		3.56	16	0.223	-	3.25	4	0.813	-
	Pod load	91	89	ns		3.40	15	0.227	-	3.47	15	0.231	-
	Pod length	72	59	**	0.03	3.71	7	0.531	-	3.00	4	0.750	-
	Seeds per pod	94	87	*	0.02	3.11	9	0.346	-	3.63	16	0.227	-
Market	Seed size	76	68	ns		3.33	3	1.111	-	4.80	5	0.960	-
	Seed shape	50	48	ns		4.00	2	2.000	-	5.00	1	5.000	-
	Grain filling	86	80	ns		4.00	10	0.400	-	3.50	4	0.875	-
	Seed color	68	64	ns		3.44	18	0.191	-	3.00	3	1.000	-
	Marketability	94	80	**	0.06	2.81	116	0.024	2	3.21	70	0.046	5
Culinary	Fast to cook	76	92	**	0.05	3.50	4	0.875	-	2.25	85	0.026	2
	Swelling	77	87	**	0.03	4.00	6	0.667	-	3.08	36	0.086	-
	Taste	89	96	**	0.03	4.07	28	0.145	-	3.09	67	0.046	6
	Attractiveness	68	79	ns		4.50	6	0.750	-	3.25	28	0.116	-
	Non-flatulence	87	90	ns		4.60	5	0.920	-	3.58	19	0.188	-
Storage	Weevil tolerance	94	84	ns		4.00	37	0.108	-	4.33	12	0.361	-
Feed	Straw yield for cattle	77	61	**	0.03	5.00	2	2.500	-	5.00	4	1.250	-

Diff indicates significance of the difference for males and females rating using unpaired t test with Welch's correction, *ns* not significant. *, ** significant at 5 and 1 %, respectively. R^2 quantifies the fraction of all the variation in the samples that is accounted for by a difference between the gender means. *n* sample size of gender groups, *MR* mean rank (1 for first place, 6 for last place), *Fr* frequency of presence in top six list, *I* rank-index, *R* top six rank order for survey farmers

Table 4 Relative importance of the main common bean variety traits in four sites in southern Ethiopia based on varietal profile rating

	All respondents	Amaro	Boricha	Konso	Loma	All men from four sites	All women from four sites
Traits	N = 375	n = 110	n = 104	n = 90	n = 71	n = 309	n = 66
Grain yield	11.4(4)	17.3 (4)	8.7(4)	8.7(4)	9.2(4)	12.3(4)	13.7(4)
DT	22.8(3)	20.7(3)	21.9(3)	27.7(3)	20.6(3)	23.0(3)	23.1(3)
Marketability	30.9(2)	31.0(1)	27(2)	29.8(2)	36.5(1)	30.8(2)	28.8(2)
Culinary quality	34.9(1)	31.0(1)	42.4(1)	33.8(1)	33.7(2)	33.9(1)	34.4(1)

N total number of respondents in the survey, *n* sample size of respondents at respective site, *DT* drought tolerance. Number in parentheses indicates the rank of the trait based on the index value (see material and methods)

Table 5 Participation level of males and females in decision making in bean farming activities in southern region of Ethiopia†

	Participation level in %							
	Boricha		Amaro		Konso		Loma	
Farming tasks	M	F	M	F	M	F	M	F
Field selection	73	27	75	24	78	22	90	10
Seed bed preparation	79	20	78	20	78	22	86	12
Variety choice to plant	70	25	70	24	56	44	80	19
Sowing date	74	26	68	24	64	35	84	13
Seed amount to be planted	71	27	72	25	47	52	79	18
Seed source to use	69	31	67	27	57	44	68	32
Harvesting date	70	28	58	38	56	44	78	20
Harvest for home use	61	39	50	50	31	69	63	37
Harvest for sale	63	37	49	51	50	50	55	45
Harvest kept for seed	70	30	67	37	44	56	71	23

† Sum of male and female participation level of decision making for particular farming task per site is 100%. Sum less than 100 indicates influence of extension agents for the activities each bean farmer on average engaged in. *M* male, *F* female

aspects of agronomic activities but they were actively taking part in portioning the harvest for home use and sale. As compared with other sites, the involvement of females in common bean-farming decision making at Konso was higher. Particularly in relation to decisions on seed and variety use, the women in Konso seemed to be more influential than women in the other sites. This demonstrated that the importance attached to the trait is reflected in participation level of the decision making of men and women in the stages of common bean-farming process showing complementarity for their roles.

Farmers assessment of local varieties

Variation in preferences for different bean traits probably resulted in cultivation of diverse common bean types in the different parts of the region. Table 6 presents farmers' ratings for the performance of locally grown common bean varieties in the study sites, using the traits they considered to describe and discuss varieties. Farmers' assessment for the local varieties showed statistically significant differences for all traits identified. Farmer varieties were rated intermediate for most traits, indicating that currently grown farmer varieties are not offering what farmers most look for. This showed that an implicit demand for new germplasm existed and that new traits are needed that meet farmers' interest. Of the seven major grain types grown by farmers in the region, black beans rated relatively well for drought tolerance followed by pinto beans which according to farmers escaped rather than resisted drought stress due to their early maturity. The black beans were also perceived on average better in recovery from drought shock, straw yield for cattle, local specific adaptation, germination, seedling vigor, grain yield, pod load and seeds per pod as compared to other grain types. But black beans were generally considered bad in seed color, marketability and attractiveness to eat. The Andean types, such as large-cream and red-mottled rated average to high for seedling vigor, stem strength, seed size large, seed color, and attractiveness to eat whereas the cream-mottled (sugar) beans rated poor or intermediate for all the traits listed. The pinto bean rated intermediate to good for the majority of traits except for resistance to field insect pests and diseases or pod length, for which some farmers rated them as poor. Small-red seeded beans, a widely dominant grain type in the southern region of Ethiopia, rated intermediate for the majority of the traits and good for consumption, local adaptation, marketability, seedling traits and yield traits. The export classes of small-white beans were rated poor to intermediate for majority of the traits farmers valued.

Farmers' selection of new drought-tolerant genotypes

In 2008, a farmer in-field evaluation and selection process started with 38 diverse advanced genotypes and local test varieties representing different grain types. In the on-station and on-farm PVS trials during the 2008-*Meher* season, farmers rejected 25 of the advanced genotypes, and 13 were retained for further evaluation. In on-station and on-farm PVS trials in the following 2009-*Belg* season the farmers selected 9 from the 13 advanced genotypes.

Table 6 Average ratings of 33 identified common bean variety traits assessed as poor (= 1), intermediate (= 2) and very good (= 3) of seven farmer bean varieties by farmer household heads in southern Ethiopia

Traits	Black	Large-cream	Pinto	Red-mottled	Small-white	Small-red	Cream-mottled	P-value[a]
	n=157	n=43	n=43	n=70	n=178	n=178	n=87	
Germination	2.55	2.91	2.70	2.44	2.35	2.86	2.11	0.001
Seedling vigor	2.55	2.62	2.54	2.68	1.97	2.62	2.13	0.001
Stem strength	2.28	2.88	2.37	2.79	1.80	2.58	2.00	0.001
1st pod insertion height	1.99	2.12	2.26	2.39	2.37	2.23	2.07	0.001
Growth habit	2.25	2.70	2.54	2.23	2.28	2.55	2.18	0.001
Shattering	2.47	2.10	2.65	2.44	2.26	2.11	2.20	0.001
Adaptation	2.68	2.21	2.54	2.40	2.17	2.71	1.89	0.001
Uniform maturity	2.45	2.69	2.67	2.10	2.50	2.49	1.93	0.001
Earliness	2.14	2.86	2.88	1.91	2.58	2.43	1.71	0.001
Drought tolerance	2.64	1.90	2.48	1.86	1.91	2.43	1.87	0.001
Recovery from drought	2.50	2.01	2.14	2.11	2.07	2.31	1.92	0.001
Withstands HRF	2.21	2.56	2.40	2.44	1.94	2.31	1.92	0.001
Withstands weed	2.29	2.15	2.27	2.33	1.89	2.20	1.94	0.001
Field insect tolerance	2.21	1.60	1.79	2.13	1.81	2.18	1.81	0.001
Disease tolerance	2.23	1.78	1.73	2.09	1.96	2.23	1.70	0.001
Grain yield	2.58	1.86	2.29	2.23	2.22	2.68	2.06	0.001
Grain density	2.39	2.02	2.54	2.44	1.99	2.58	2.10	0.001
Yield stability	2.44	2.40	2.47	2.21	2.12	2.57	1.92	0.001
Pod load	2.62	1.93	2.17	2.30	2.15	2.49	1.91	0.001
Pod length	2.34	2.56	1.78	2.63	1.55	2.54	2.01	0.001
Seeds per pod	2.60	2.05	2.07	2.17	1.94	2.65	2.00	0.001
Seed size	1.96	2.51	2.52	2.67	1.70	2.48	2.21	0.001
Seed shape	2.14	2.43	2.65	2.30	2.47	2.35	2.09	0.001
Grain filling	2.42	2.54	2.77	2.44	2.15	2.65	2.00	0.001
Seed color	1.61	2.81	2.36	2.67	2.49	2.68	2.20	0.001
Marketability	1.40	2.42	2.86	2.50	2.27	2.75	1.81	0.001
Fast to cook	2.17	2.70	2.16	2.24	2.17	2.69	2.22	0.001
Swelling	2.25	2.48	2.72	2.53	1.89	2.66	2.42	0.001
Attractiveness	1.58	2.77	2.81	2.64	2.39	2.75	2.38	0.001
Taste	2.04	2.35	2.61	2.62	2.15	2.81	2.37	0.001
Non-flatulence	1.82	2.28	2.47	2.49	2.27	2.64	2.17	0.001
Weevil tolerance	2.08	1.63	2.34	2.00	2.02	2.30	1.95	0.001
Straw yield for cattle	2.67	2.49	2.55	2.20	2.42	2.80	2.37	0.001
Mean rating	2.26	2.34	2.43	2.35	2.13	2.52	2.05	

[a] P-value associated with a Kruskal Wallis one-way analysis of variance by ranks. *n* number of farmers assessed the variety traits

The rejected genotypes included all the black, cream and pinto (carioca) grain types. Some of the high-yielding genotypes in black and carioca grain types were rejected regardless of their drought tolerance. Farmers had however selected a kidney grain type (large-red) and a sugar (cream-mottled) grain type: both grain types are from the Andean genepool (large grain size) and not found among the traditionally grown local varieties. The other ones were two red-mottled grain types (Andean genepool), four small-red and one small-white one (Mesoamerican genepool).

Individual preferences were to keep two or three varieties for next season plantings in their own field, but the choice for genotypes varied between farmers within and across PVS sites. Generally farmers preferred early-maturing varieties at all study sites provided they were also suitable for their cropping system (intercropping or relay). Small-red seeded genotypes were selected at all sites except Inseno where the Ethiopian export class small-white grain type was selected for market preference. In Amaro, genotypes with indeterminate prostrate growth habit were perceived as not good for intercropping and hence not selected. On the other hand some farmers who practiced relay cropping in Boricha selected for indeterminate growth habit genotypes. In the evaluation of the genotypes farmers looked at pod load, pod length, seeds per pod, seed size and color, grain density and volume, early maturity, growth habit, pod clearance from base (pods not touching the ground), marketability, grain fill and full color development as indicators of drought tolerance, and uniform pod maturity as selection criterion in addition to their personal eye observation in many of the selection events.

There was variation for bean types between men and women evaluators. This was also observed during selection events: males often looked at the agronomic performance and market preference whereas females paid more attention to the culinary characters like attractiveness to eat, using color, seed size and seed hardness as indictors. This was apparent in all selection events and particularly reflected in the selection events in Amaro. In the 2008-*Meher* selection after harvest, the husband of one of the PVS-host farmers was overwhelmed by a good yield of black bean genotype and decided to keep this genotype for further evaluation on his farm considering it a good variety for drought. But his wife voiced the concern to her husband that she was not interested in cooking black beans since they were not attractive to eat. As a result the husband changed his selection to another grain type stating that *'it is difficult to grow a variety that women do not prefer'*.

Comparison of selection by farmers, breeders and extensionists

Figure 2 compares the selection made by farmers, breeders and extension agents. No single genotype was commonly selected by all farmers, breeders and extensionists. However, the Andean cream-mottled bean SAB626 was most often chosen by farmers, extension agents and breeders in many of the selection events. Extension agents and breeders in many of the selection events coincided with the farmers in rejecting the two genotypes SAB622 and SAB650. The two genotypes were rejected by extension agents and breeders because of their low yield performance as compared with other genotypes (Table 7) while some farmers still wanted to keep them compromising yield with other merits, mostly large seed size, and red-mottled and red-kidney seed color. Breeders rejected in all trials the local variety because of low yield and susceptibility to disease whereas in some of the selection events, farmers and extension agents were in favor of retaining the local variety. The reasons given by farmers and extension agents who selected the local variety were its high level of local specific adaptation and the marketability of the particular seed color. In general there was a fair coincidence among farmers, breeders and extensionists in the selection rest of the new genotypes. The sugar grain type SAB626, the small-red varieties Dume, SER16 and SER125 and the small-white variety SEC24 were the most widely preferred genotypes.

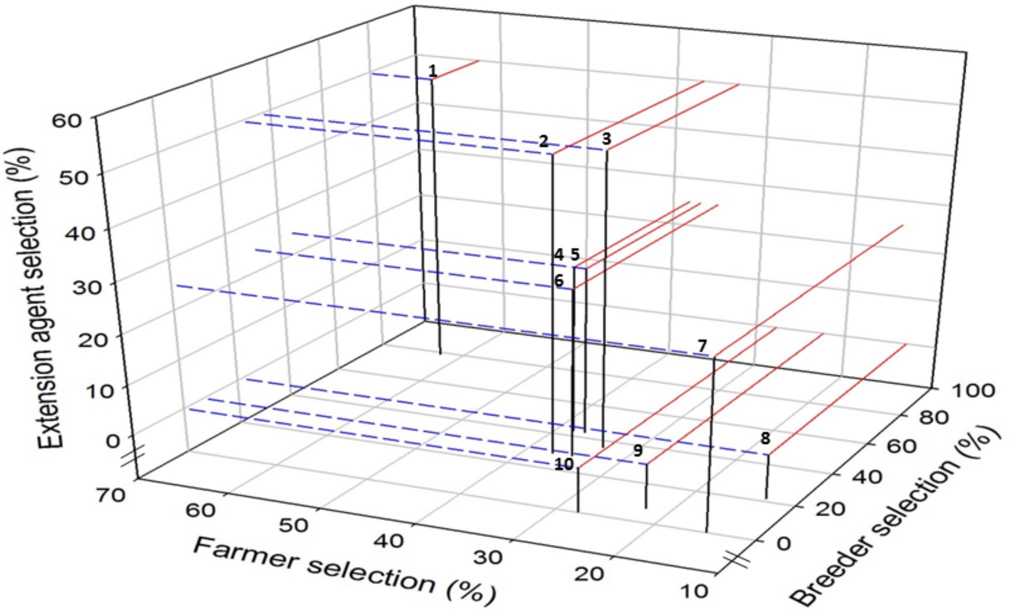


Fig. 2 Number of times (%) a genotype was selected by a farmer (N=149), extension agent (N=3) and breeder (N=5) during different selection events in the PVS. From a total of 103 farmers who participated in the selection events, 19 farmers participated twice. Genotypes are designated as *1* SAB626, *2* SER125, *3* SER16, *4* SEC24, *5* Dume, *6* Ibado, *7* Local, *8* SER48, *9* SAB650, *10* SAB622

Farmers assessment of the new drought-tolerant genotypes

For the same PVS trials, logistic regression analysis for farmers' evaluation of the new drought-tolerant genotypes for the common bean traits are presented in Figures 3 and 4. The log odds ratio in the two figures describe the chance of the genotype being rated 'very good' for the particular trait rather than being rated 'intermediate' or 'poor' – as compared to the baseline, being the local farmer variety. In other words, a higher log odds ratio for a genotype indicates that it is more likely to be rated as very good for the trait than another genotype.

Accordingly, the most often selected genotype SAB626 (Fig. 2) did best in grain fill, seed color, early maturity and drought tolerance whereas it did least well for marketability together with SAB-650 (Figs. 3 and 4).

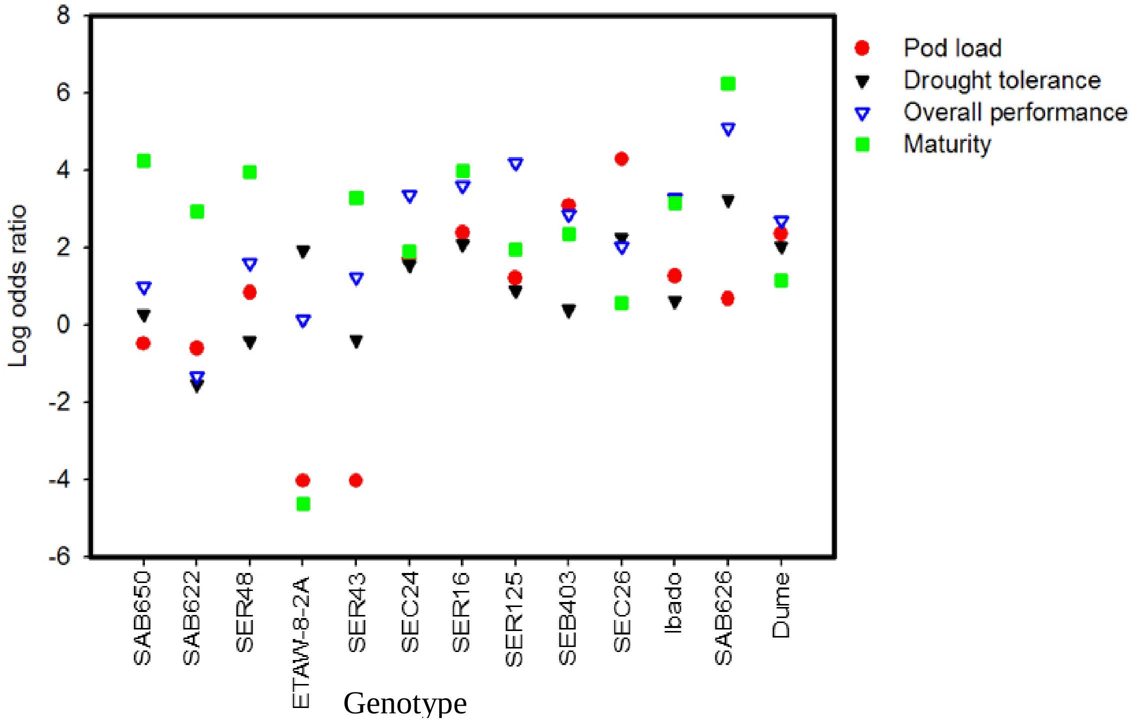


Fig. 3 Comparison of genotypes based of logistic regression analysis of farmers perceptions on pod load, drought tolerance, overall performance and maturity. Log odds ratio in y-axis indicates the chance of being in a high (very good in performance for that particular trait) or low (poor for the trait) response category. A higher log odds ratio indicates that the new genotype is most likely to be rated very good for the trait and vice versa as explained in the text

The farmers argued that the market is unfamiliar with cream-mottled grain type of SAB626s but they were thinking that over time it would gain acceptance. The other most preferred small-red grain types SER16 did well for grain filling. The unselected genotypes SEC26 and ETAW-8-2A performed well for marketability and grain filling. ETAW-8-2A was perceived as superior in pod length but was rejected due to its late maturity. SEC26 was rejected because of small seed size. The locally crossed and selected variety Dume was recognized as superior in grain yield as compared with the other CIAT-bred drought tolerant genotypes, but it was criticized for short pod length and dark-red seed color. In the southern region of Ethiopia bright-red seed color is most preferred. Six selected genotypes namely SAB626, SER125, SER16, SEC24, and varieties Dume and Ibado were assessed as better performing than the commonly grown local variety in overall performance (Fig. 4). Meanwhile SXB403 which did not qualify in the final selection was most likely rated better than the local variety in overall performance. This genotype was not favored by farmers rating for seed color and marketability (Fig. 3). On the other hand, SAB626 was considered to be good in overall performance but was rated poor for yield potential. This indicated how overall performance rating explains farmers' overestimation of yield. In general during in-field selection famers looked at many dimensions while making selection-decision.

Farmers' ratings for the quantitative trait drought tolerance, was little discriminative in assessing variation among genotypes. Farmers' ratings were discriminative for genotypic differences in the traits marketability, pod load and grain fill which are visible to their eyes to make judgment (Figs. 3 and 4). Variation among genotypes for seed color rating was narrow because the retained genotypes had seed colors that farmers favoured or believed to have potential. In discussions, farmers stated that drought tolerance *per se* was most difficult to rate or select for. Farmers indicated that they rather used proxy traits like grain fill, full color development, seeds per pod and pod length to assess for drought tolerance during in-field PVS. Overall performance is a similarly complex quantitative trait that is difficult for farmers to rate and select for.

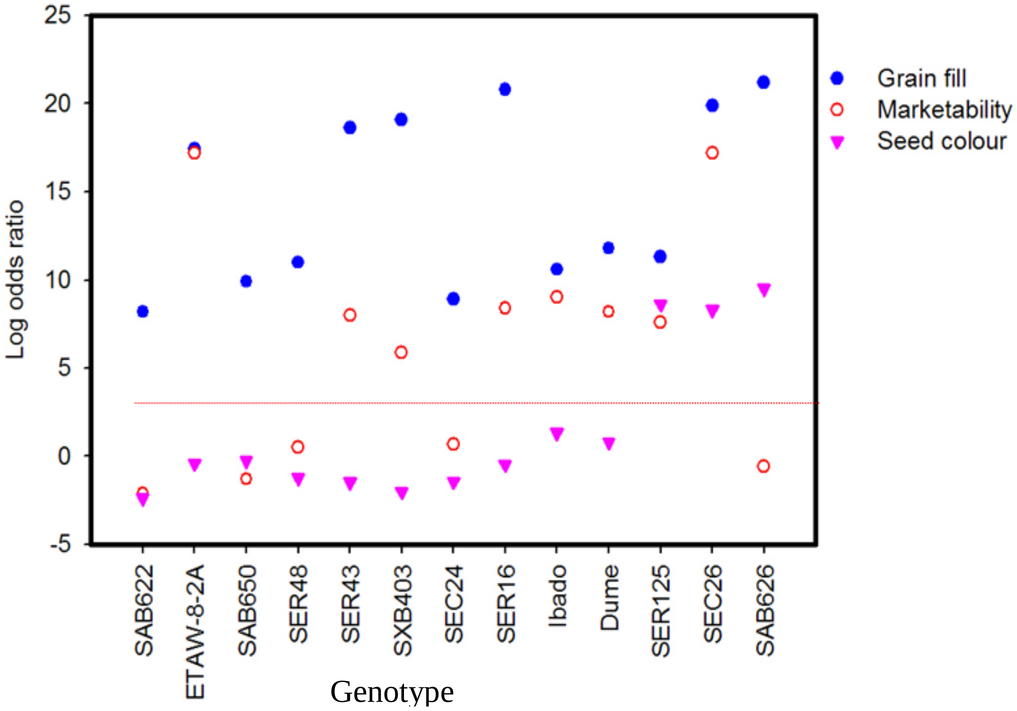


Fig. 4 Comparison of genotypes based of logistic regression analysis of farmers perceptions on grain fill, marketability and seed color. Log odds ratio in y-axis indicates the chance of being in a high (very good in performance for that particular trait) or low (poor for the trait) response category. A higher log odds ratio indicates that the new genotype is most likely to be rated very good for the trait and vice versa as explained in the text

Grain yield performance of the new farmer selected drought tolerant genotypes

Table 7 presents grain yield (kg ha^{-1}), a qualitative perception-based yield score and yield stability of 14 genotypes that were tested in on-station and on-farm PVS trials for more than one season. The mean grain yield ranged from 1318 (SAB622) to 2177 kg ha^{-1} (Dume). Based on the quantitative REML mean, seven genotypes namely Dume, SER16, SER43, SER125, SEC26, SER48 and SEC24 significantly out-yielded the local farmer variety. The qualitative yield scoring based on the farmer evaluations by which yield a genotype differed from the local check identified Dume, SXB403, SER125 and SER16 as superior to local varieties. The quantitative REML mean and qualitative farmer rating for yield (log odds ratio) showed significant correlation ($r = 0.54$, $P = 0.05$). This indicates that the farmers' perception based yield score might be a potential tool for breeders to consider in PVS trials in situations where quantitative yield measurement is not easy and economical, but not eventually for inclusion of the many concerns of farmers. This has been shown by high rating for SXB403 in yielding potential but this genotype eventually failed to qualify in the final selection decision because of its demerits for other farmers' preferences.

Table 7 Grain yield (kg/ha), log odds ratio for farmer perception-based yield score and stability of grain yield for genotypes selected by farmers in PVS. Stability parameters were calculated for 10 genotypes (local farmer variety and the nine new drought tolerant genotypes retained by farmers in their final selection)

	Grain yield		Stability parameters	
Genotypes	REML mean	Log odds ratio[a]	Pi	Mean rank
Dume	2177	3.53	40942	2.15
SER16	1988	1.65	103634	2.69
SER43	1900	0.14		
SER125	1810	2.04	218781	4.31
SEC26	1763	-0.02		
SER48	1752	-0.60	297830	5.53
SEC24	1622	1.33	369796	5.69
SAB650	1551	-3.16	661373	6.75
Ibado	1527	1.07	883615	6.33
SXB403	1527	2.10		
ETAW-8-2A	1484	-0.02		
SAB626	1479	0.86	625813	5.77
SAB622	1318	-1.48	843496	8.23
Local	1397		1157068	7
SED	158.8			

REML Restricted Maximum Likelihood, *Pi* genotypes superiority, *SED* standard error of difference. [a] Log odds ratio for farmers perception-based yield score, farmer variety was used as reference point as explained in the text

The analysis of the response pattern of the genotypes in the PVS trials identified the Awassa-bred variety Dume as superior yielder (Table 7). It combined least genotype superiority measure of Lin and Binns (1988) and low mean rank of Nassar and Huhn (1987) with high grain yield. Hence Dume, locally crossed and developed through decentralized selection scheme at target set of environments, is a more robust yielding genotype in the variable and drought-stressed southern region of Ethiopia than the other genotypes that were crossed and selected outside Ethiopia by the international bean program.

Discussion

Farmers' criteria in evaluating bean varieties

The FGD showed that common bean farmers in southern region of Ethiopia considered many traits in evaluating bean varieties. Some traits like pod load, pod length and seeds per pod can be seen as proxies that farmers use for estimating grain yield. Germination was often mentioned as a very important trait, especially by men. This was probably a consequence of farmers' negative experiences with seed provided by the extension service in 2006, a period prior to the survey, and might point to serious problems with seed quality in the region. Many of the other non-direct yield related traits that farmers consider important are rarely explicitly considered by common bean breeders: weed competitiveness, shattering, uniformity in pod maturing on the plant, grain filling, marketability, taste, attractiveness to eat and flatulence. Common bean breeders typically use in the range of 10 traits that relate to phenology, yield and yield components, physiological traits and disease resistance, of which some are not considered or overlooked by farmers in this study: yield stability, plant height and days to flowering. Furthermore, where farmers and breeders coincide, like in plant architecture, they may still do so differently: farmers look at plant architecture in the form of stem strength, growth habit and first pod-insertion height, whereas breeders consider plant height and distinguish four growth types (CIAT 1987). This means that farmers and breeders evaluate and select on the basis of different traits and also that there are traits they look at in different ways. This mismatch in the use of selection criteria might lead to valuable germplasm being discarded and less preferred varieties being developed by breeders.

Methods to capture the importance of farmers' criteria

In order to verify which traits breeders should consider to meet the demand and preferences of farmers, various methods were employed and compared. The method in which farmers were asked to indicate the importance of each the trait individually resulted little discriminative. On the basis of this method only two traits could be labeled as fairly unimportant given their relatively low percentage of farmers scoring it as very important: seed shattering and seed shape. All traits were considered very important by the majority of the farmers. Weevil tolerance, pod load, seed per pod, and non-flatulence appear among the most important while they do not surface among the most important with the use of the ranking-based methods. The ranking that was calculated on the average rating (1, 2 or 3) proved very similar as the ranking on the basis of the percentage of farmers scoring the traits as very important. The low discriminative value of this method can explain the lack of any pattern related to site, gender or wealth status and the low fractions of variation explained by the differences between men and women (R^2).

The methods based on mean ranking (MR) and frequency of being mentioned among the six most important traits (Fr) both indicate that seed shape and straw yield of common beans are traits with a low level of importance. However, although they are based on the same data set, there is variation in the traits that show up as most important. The MR method indicates shattering, growth habit and withstanding the rain among the most important traits, whereas they do not appear as important in the other methods. They are traits that have been mentioned by very few farmers, but nevertheless show up as important because of the way the average ranking was calculated (only when the trait was ranked among the six most important, it was counted). The Fr was much more discriminative, but does not differentiate well between the traits that are included by most farmers among the six most important traits. For the matter, we assume that the index I – which weighs the MR with the numbers of farmers who mentioned the trait – best reflects the importance of the various traits. The ranking of hypothetical variety profiles indicated that culinary traits and marketability are extremely important for farmers. In this method both men and women ranked them higher than drought tolerance and yield.

Although there is variation between the methods, overall there is a fair level of consistency in the traits surfacing as the most important ones. Earliness, drought tolerance and grain yield stood out as quite and similarly important to both men and women farmers. Marketability and germination were particularly valued by men, whereas culinary quality (fast to cook and taste) was more valued by women. These different rankings by men and women show the importance of gender differentiation. Had the ranking been done on the combined data, germination and none of the culinary traits would not have shown up among the six most important traits.

Farmers' preferences

Remarkably, farmers gave higher importance to earliness than to drought tolerance. Earliness allows escape from drought at the end of the season, whereas drought tolerance also refers to the capacity of the plants to withstand and recover from drought stress in different parts of the growing season. From this we may conclude that climate change impact on bean yields in these regions is currently more pronounced in the form of early ending of the growing seasons, but that other drought periods are importantly affecting bean production as well. For the common bean breeding this implies that a bean variety should preferably combine different mechanisms of drought tolerance.

Finally, seed color and size were less important traits than was expected on the basis of the generally assumed need to fit the locally preferred traditional color, size and shape of seeds

(Almekinders et al. 1994). On the other hand, farmers consider culinary quality and marketability among the most important variety traits. In the selection of hypothetical varieties marketability and culinary qualities even rank higher than drought tolerance and grain yield. This means that seed size and shape are important, but mostly because of their relation with marketability. In the justification of PPB and other farmer-oriented breeding approaches it is often claimed that taste, form and colour preferences of local people and markets vary, and for that matter are difficult to deal with in centralized formal breeding programs (e.g. Almekinders and Elings 2001). The results from this research confirm importance of culinary qualities and marketability, but at the same time indicate that farmers' preferences are not static and that local markets are dynamic. The local traditional varieties do not satisfy farmers in many of the traits they consider important and even marketability and culinary qualities remain sub-optimal. Especially the black bean has shortcomings, despite its high level of drought tolerance.

The dynamics of farmer preferences and markets also show in the selection of advanced bean genotypes from a pool of drought tolerant materials. Farmers were keen in trying out new bean types and especially the large-sized Andean grain type SAB 626 caught their attention because of its appreciated culinary traits: it scored low in marketability but was selected more often in the evaluation trials than the drought-tolerant, high- and stable-yielding Dume with the traditional small-red grain type. The fact that currently such grain types are not found in the market and farmers' confidence that a market for it would develop point to the potential of new materials that do not resemble the existing local traditional ones. Another example associated with the dynamics of marketability is the increasing commercial interest in the medium-sized bean. Export opportunities for this bean have also affected the market of the common bean farmers in southern Ethiopia. The importance of small-red grain types in the Konso market as compared to the locally adapted black bean further emphasizes that marketability is a condition for any new common bean variety to be adopted by farmers.

Conclusions

Although there was considerable variation in farmers' preferences and variety demand, the most important variation consistently focused on a fairly consistent set of traits that farmers felt as most important and that is relevant for a common breeding program: earliness, drought tolerance, culinary quality and marketability. Women considered culinary traits among the most important properties of a bean variety and if the results would not have been gender-differentiated, such information would have been lost. Other variation on the importance of the various variety traits across sites, gender and wealth groups showed no clear pattern in any of the methods used. Preference for particular grain types varied over the sites, but the results also clearly indicated the dynamics of markets and the non-static character of farmer preferences.

The relatively high level of coincidence of farmer selections and selections made by breeders and extensionists, combined with the relative consistent set of important traits to considers suggests that diversity of farmers demand is not the first concern. The challenge of the common bean breeding therefore lies more in combining different types of drought stress and escape with marketability and attractive culinary traits. In other words, a variety may perform excellent under varying types of drought stress, but it will not become a successful variety if it is not tasteful or has no market. The performance of the variety Dume strengthens the hypothesis that local selection of common bean varieties may result in more adapted better yielding varieties. In addressing the challenge, this work also showed that the conventional idea of developing varieties that have the same color, size and shape of grains as what farmers traditionally grow, may actually restrict the introduction and exposure of farmers to novel, attractive and adapted germplasm. Also for rural small scale producers preferences and markets are not static.

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CHAPTER 7

Synthesis: Breeding by integrative design

Introduction

This study addressed the challenges and complexity of breeding for drought stress in smallholder farmers conditions, taking common bean (*Phaseolus vulgaris* L.) and southern Ethiopia as the focus crop and target area, respectively. Drought stress is a major limiting factor to crop productivity worldwide and will only increase in importance in the face of climate change (IPCC 2001, 2007). Common bean is considered as a food security crop to smallholder farmers due to its short life cycle in many drought events. Smallholders farm common bean in harsh environments which is often characterized by variable conditions and preferences.

In this challenging scenario, breeders have a series of options to work with. Conventionally, breeding practices and indirect selection for physiological traits that improve yield under drought stress are used by breeders. In addition, genomics-based breeding can be used to combine different genes or sets of genes that adapt crop growth to drought stress conditions and improve yields (Tuberosa and Salvi 2006). Many different forms of farmer participation have been increasingly used over the last decades to identify adapted germplasm and farmers' preferences (Almekinders and Elings 2001; Ceccarelli et al. 2009). To effectively use and integrate the various options for common bean breeding and farmers in marginal areas in southern Ethiopia, this thesis explored aspects of plant physiology, genomics-based breeding and farmer involvement. In doing so, it brought together methods from plant physiology and genetics to identify traits related with drought tolerance and locate the region of genome that controls their inheritance (Chapters 4 and 5). The study also used molecular characterization (Chapter 3) and farmer evaluation (Chapter 2) for local genepool analysis, and used farmer criteria for identifying traits for specific (socio-economic and agro-ecological) adaptation and adapted genotypes (Chapters 2 and 6) (Fig. 1).

Hence the thesis capitalized on multiple approaches that combined laboratory, greenhouse and field level plant analysis with participatory experimentation deploying tools in the biological and social science arena. The various chapters thereby explored and approached improving drought adaptation in common bean using methods and knowledge from multiple disciplines. This chapter brings the findings of studying these aspects together and reflects on the implications for breeding.

In order to combine the different options to address the breeding challenges, the complexity of each of the above mentioned aspects needs to be understood. Drought as a phenomena is complex for how it presents itself, how crop plants respond to it and how it is genetically dealt with.

Drought stress seldom occurs as a single stress at farmer level; rather it presents itself at different stages of the crop growth with different intensity and its effect on crops being modified by farmer vulnerability, soil type and fertility, biotic stress, etc. (Rao 2001). At the micro-regional level, drought is not a yearly event and can occur in different forms either throughout the season, early in the season, at mid-season or near end of the season or life cycle of the crop (Rao 2001; Passioura 2007). The way plants respond to drought stress is complicated as well.

Plants usually utilize different physiological mechanisms in adaptation to drought stress. These include escape, avoidance, resistance and recovery strategies (Levitt 1972), which may not be mutually exclusive but in practice are rarely combined within an agronomically superior genotype (Ludlow 1989). Instead, a range of adaptive responses to drought stress and mechanisms of drought tolerance are found in different genotypes. In this study drought tolerance used as a generic term that combines all plant's adaptation strategies to drought stress. Drought tolerance in plant is genetically determined. Genetically, the complexity lies in the polygenic nature of the physiological traits related with drought tolerance which often show low heritability and high genotype × environment interaction (Blair et al. 2010a).

The challenge of breeding crop varieties for smallholder farmers means to find ways to cope with the variation of farmers' environments and preferences. Farmers face variation in climate, soils, and market in their specific conditions. On the other hand breeders face variation in target area, target farmers and complexity in physiological mechanism and inheritance of the drought tolerance traits to breed for.

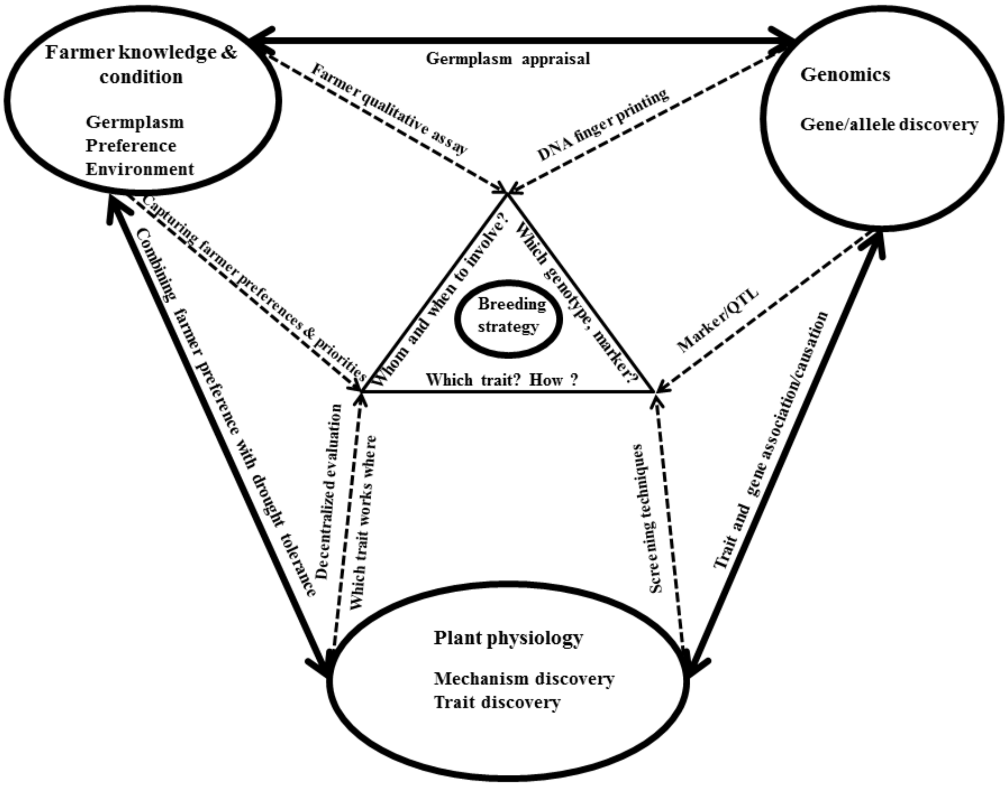

Fig. 1 Linkages between farmer knowledge, plant physiology and genomics and their integration into a breeding strategy targeting drought tolerance and farmers' preference in variable environments, using various methods and tools

In this chapter, I discuss the implications of the findings reported in earlier chapters for a breeding approach that takes the various challenges into account (see Fig. 1). I first discuss the implications of understanding the farmers' preferences: the germplasms/varieties farmers currently use, their desirable/undesirable traits and their relation with the socio-economic and agro-ecological environments. Through an inventory of varieties grown by farmers using participatory qualitative and quantitative methods, the level of visible diversity including which variety is grown by whom, where and why, and their respective desirable and undesirable traits are sorted for possible targeting in the breeding program. Then, the insights from molecular analysis of the local genepool are used to explore how breeding can use the germplasm variability for drought breeding. The insights from the mechanisms and alleles that regulate drought tolerance are used to define suitable traits and DNA markers linked to these traits in selection for drought tolerance. Finally, these will be used to discuss options for more effective, integrated common bean breeding for smallholder farmers in drought-prone areas of developing countries.

Diversity of environments and preferences as adoption challenge for drought tolerance

The study shows that the context of common bean production in southern Ethiopia is characterized by diverse and changing climatic conditions, varying soil types and farmers who use a range of different cropping practices (see Chapters 2 and 6). Farmer cropping practices encompass sole, inter- and relay-cropping in both the '*Belg*' and '*Meher*' seasons, each with its specific features in the different regions.

Within these diverse systems, drought stress is a major environmental determinant for farmer common bean production. At the micro-regional level, drought stress could be early in the season, intermittent or terminal in occurrence, but having large effects on common bean during vegetative expansion and flowering in farmers' fields (Chapter 2). Its effect is never uniformly distributed, but varied with farmers' field conditions and growth stages of the crop. In addition to the complex variation of drought, there was important variation in farmers' perceptions of which type of drought stress was most significantly affecting the performance of their bean crop.

Farmers were familiar with drought stress, particularly in some areas, and they also acknowledged that climate is changing and affecting their agricultural practices. More frequent occurrence of drought stress results in crop failure, decreased production. Farmers have also developed some strategies in their traditional agronomic practice to cope with the changing climate. About half of the farmers have adapted some of the cropping practices, but these adaptations are not uniform across regions and individuals. For example, farmers at Amaro and Konso grow mixture of several varieties whereas at Boricha farmers grow more than one variety in unmixed form.

Next to environmental variation and the associated perceptions of farmers, the research showed that farmers' preferences for specific variety types varied from region to region (Chapters 2 and 6). Farmers at the drier part of Konso rely on black bean varieties which is traditionally considered drought tolerant, whereas farmers at Loma use the susceptible small-red bean varieties in a relay cropping system and those in Amaro increasingly grow early maturing pinto beans.

Multiple cropping practices and stress factors faced by resource poor farmers in drought-affected ecologies resulted in a large number of traits that farmers consider in assessing varieties. A total of 32 traits were mentioned of which only three were considered less important (see Chapter 6). The importance of the preferred traits differed across gender and location, but showed no influence of farmer wealth class.

Most variation in importance of traits was non-structured variation. Nonetheless, a relatively consistent set of traits was considered important by farmers. The traits that farmers considered most important were earliness, drought tolerance, culinary quality and marketability. Earliness stood out as most important in all sites, whereas the seed color was mostly important indirectly because it influenced farmers perception of culinary quality and it determined marketability. Marketability resulted as important as drought tolerance although it depended on the method of assessment that was used to capture farmer perception. Marketability tended to be more important for men as compared to women – although this varied somewhat with the method that was used, whereas women emphasized the importance of culinary traits. This means that for common bean breeding to have an impact in southern Ethiopia this range of traits should be considered. It also implies that genotypes should be developed that possess the combination of these traits in order to be adapted to the varying environments and farmer preferences.

The relative consisency of set of traits that farmers consider does not mean that there is no variation in the farmer-preferred form, shape or color of the trait, as referred to before. In field selection, during focus group discussion and in individual interaction farmers frequently selected germplasm that did not match with their earlier mentioned preferences. This shows farmers' preference traits are not static but rather that they co-evolve with exposure.

At Amaro and Konso, where common beans are mostly produced for home consumption, farmers need drought tolerant, early-maturing varieties. Traditionally, the black grain type was the only germplasm sufficiently drought tolerant for their conditions. On the other hand, farmers at Amaro and Boricha still preferred to grow small red seeded varieties despite their relatively lower drought tolerance. Drought tolerance is relevant in their conditions, but culinary preference and marketability are considered more important. As a result farmers in these sites did not select any black and carioca grain types from the participatory variety selection (PVS) trials (Chapter 6). Furthermore, the experiences with the PVS trials showed that farmers' preference for new grain types like SAB626 (sugar bean type, i.e. cream-mottled) did develop through exposure. In addition, market dynamics and climate change affect farmers' preference and drive a shift from preferences for traditional seed colors and shapes to more commercial and better adapted germplasm. These experiences suggest that when breeding focuses on the traditionally grown seed colors, shapes and sizes only, the farmers' access to novel attractive and adapted germplasm may be restricted.

In general, the combination of climate change, market dynamics, and shifts in farmer preferences through exposure to new seed and variety types represent a constantly moving target for the common bean breeding program. Since common bean is a self-pollinating crop and because the local genepool in southern Ethiopia is only moderately diverse, the farmer based traditional system of bean variety development has had limited potential. The challenges of combining the large number of traits, the variation in which they occur and their constant modification in responses to the preferences and needs of the farmers therefore largely depend on the capacity of the breeding program. However, the relatively high level of use of off-farm seed sources provides opportunities for strategic introduction of new varieties. Hence more drought tolerant bean diversity for a range of farmer conditions, markets and preferences can only be developed with an integrated understanding of farmers production conditions and existing seed system practices. On the basis of this understanding a breeder can contribute to an overall package of mechanisms that harness and equip farmers to adapt to their dynamical context.

Landraces as baseline variability for bean breeders to approach drought tolerance

Farmers' qualitative assessment, morphological phenotyping and molecular genotyping are the range of available tools for a breeding program. Knowing which varieties are being grown where and why, capturing farmer preferences, their assessment of the varieties in use are insufficient information for a breeder to understand the level and structure of diversity in their local or potentially available germplasm base. Farmers' knowledge on local varieties and conditions is important, but it is constrained by the invisibility of genetic variation and their limited exposure.

Morphological phenotyping was used in this study to uncover diversity and population structure of bean landraces. Significant variation was observed for most morphological traits measured on East African landraces (Chapter 3). Analysis of the morphological variables showed grouping of Andean and Mesoamerican genotypes combined with probable introgression between the genepools (Fig. 1 in Chapter 3).

However, under-estimation of genetic relationships with morphological markers as compared to molecular markers was observed and discussed in Chapter 3. The results described in Chapter 3 demonstrated microsatellite/SSR marker types were of better resolution and successful in detecting baseline variability for breeders to approach drought tolerance in common bean. Microsatellite markers were also successful in detecting variability in common bean accession both from primary and secondary centers of diversity in other studies too (e.g. Blair et al. 2006; 2009; 2010b; Zhang et al. 2008).

The genetic analysis showed the existence of two distinguishable, distinct genepools in the East African Highlands: the Andean and Mesoamerican. The molecular analysis further indicated that Mesoamerican genotypes were more diverse than the Andean genotypes. Conservation of the genepool separation typical of the primary centers of diversity with little introgression between these groups indicated that the original differences in introduced germplasm from the primary center was the base for East African common bean diversity. This was also noticed in the analysis of farmer variety and seed management practices (Chapter 2). Farmers traditionally retire seed of a particular bean type or renew a different genotype through in-field plant selection as well as post-harvest seed sorting within and between grain types using multiple criteria when they feel that the varieties are no longer productive or do not meet their home use and market preference or when they find something better that catches their eye.

Given that common bean in East Africa is often cultivated in marginal, drought-prone production environments (Wortmann et al. 1998; Chapter 2), it will be interesting to correlate genetic diversity with drought tolerance and adaptation potential in future works. Preliminary evaluation of farmers varieties in another study at Awassa (my own observation) identified varieties from marginal ecologies were good in their adaptation to terminal drought stress. In general, the results presented here are an important ingredient for more rational use of germplasm in the East Africa. It allows for the design of a strategic crossing plan to mine transgressive segregants based on distinct germplasm at national or regional level.

Exploring mechanisms and alleles for breeding drought tolerant bean varieties

Since tolerance is not a single trait but rather is the overall manifestation of the sum of the different mechanisms in the plant (Rao 2001; Beebe et al. 2010, see also Chapters 4 and 5), plant breeders face the challenge to pyramid the different traits, mostly of which are also under complex polygenic control through which the drought tolerance is expressed, and to integrate these with grain yield and the other end-user preference like seed size, marketability etc. (Fig. 2).

The overall challenge lies on the proper mechanistic understanding of each of the traits related with drought tolerance and on having quick-easy, non-destructive, low cost and high throughput screening techniques for the traits responsible for drought tolerance.

The question is what are these desirable traits? How to choose a trait from a set of different traits? How to measure? When to measure and where to measure? Will the response to these questions lead to a paradigm shift in crop improvement from phenotypic selection to genotype selection? With current advancement in genomics, people have come to an understanding that phenotype is a modest predictor of genetic potential and that it is possible to locate and characterize genes controlling quantitative traits like drought tolerance (Collins et al. 2008). But this needs proper genotyping and high throughput phenotyping in order to use this knowledge of genomics and plant physiology at best.

In this study many traits related with drought tolerance in common bean were assessed in managed drought stresses environments in greenhouse, using soil cylinder tubes, and in the field (Chapters 4 and 5). Rooting depth, total root length, fine roots length, thicker roots length, root diameter, root volume, specific root length, root length distribution over depth, leaf area, canopy temperature depression, stem biomass reduction, pod harvest index, stem and seed total nonstructural carbohydrate content, leaf chlorophyll content, grain yield, etc. were the assessed traits.

The molecular dissection for the above mentioned traits using QTL mapping experiments identified a number of QTL, the mapping position of each identified QTL, the interaction of QTL with environment and the magnitude of QTL effect on explaining genetic variance. This provided better understanding of the genetics of drought avoidance root traits and photosynthate acquisition, accumulation and remobilization shoot traits in common bean. The traits studied were all inherited quantitatively. The QTL results clearly showed that the chromosomal regions determining variation in the traits studied that related with drought avoidance or photosynthate accumulation and (re)mobilization were scattered over the whole common bean genome.

For example, it was notable that the QTL for drought avoidance root traits like total root length, fine roots length, thicker roots length, root volume and root biomass were co-localized and also explained relatively better genetic variance. The result suggested that the QTL affected drought avoidance through a constitutive expression of gene for deep rooting, thicker roots, root length distribution with depth, root volume and root biomass and adaptive expression of genes for total root length and fine roots length (see Chapter 4). The QTL mapping analysis to dissect the inheritance of photosynthate acquisition, accumulation and (re)mobilization traits showed that their genetics is complex and that both heritability and variance are low (Chapter 5). The QTL for shoot traits related with photosynthate accumulation and partitioning were distributed on linkage group b03, b05, b06 and b09 with genetic pleiotropy on b03 for pod partitioning and pod harvest indices. The results furthermore indicated the relevance of continued photosynthate accumulation as a trait for common bean drought tolerance.

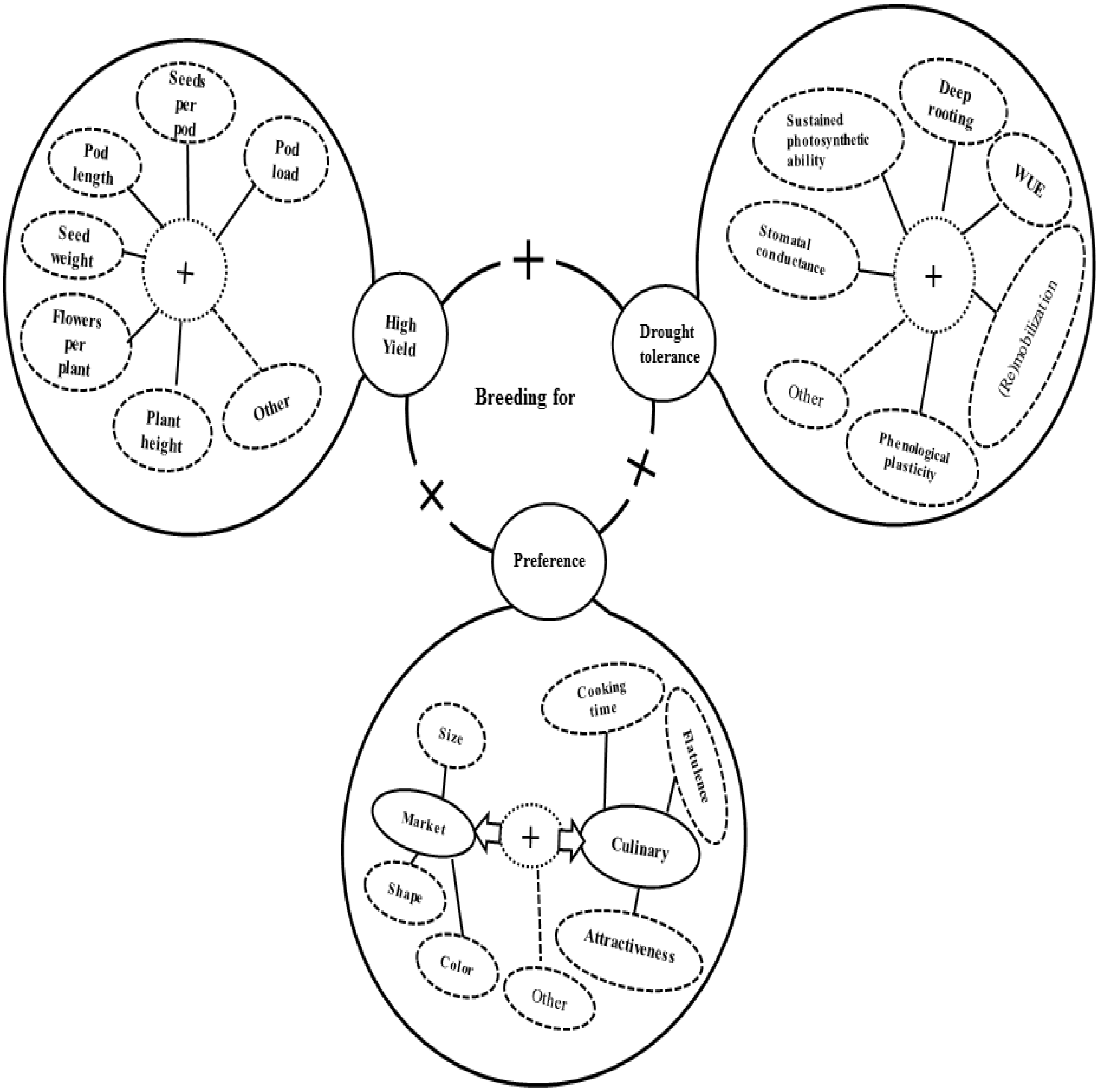

Fig. 2 The range of traits that are to be considered in a drought tolerance breeding program including farmers' preferred grain types for smallholder farmers in harsh environments

Functional relationship between the regions of genome that control the variation in the rooting depth, photosynthate accumulation and (re)mobilization traits and their effect on final yield under drought stress and non-stress conditions is the main focus for present and future research in common bean. The QTL for SPAD chlorophyll meter reading was located on the same linkage group with pod harvest index and both traits had a similar pattern of association with grain yield, particularly under stress. This might indicate that there is a region of genome that contains genes for increased plant photosynthetic carbon assimilation per unit leaf area under moderate drought stress and perhaps a larger number of chloroplasts per cell in leaf tissues and/or greater production of the protein Rubisco. Furthermore these genes may result in a larger pool of photosynthates for remobilization to the pod wall and then to the grain, ultimately resulting in increased yield.

In almost all the cases, yield QTL were not linked or pleiotropic to rooting depth or photosynthate acquisition, accumulation and (re)mobilization QTL in the RIL population studied even though positive phenotypic correlations with some of these traits existed (Chapter 5; Ramirez-Vallejo et al. 1998; Beebe et al. 2010). This however, is not to say that rooting depth or photosynthate acquistion, accumulation and (re)mobilization do not contribute to plant adaptation under drought stress. Rather this warns us that careful genetic analysis will be needed to validate these traits in marker assisted breeding. Furthermore the lack of co-localizing QTL for these traits and grain yield may imply that several QTL must be manipulated at the same time in order to obtain a significant impact in grain yield in common bean.

Yield is a complex trait determined by many physiological processes during growth and development (Yan and Wallace 1995). Earlier physiological work by CIAT and results of the present study suggest genotypes with deep and balanced root systems can utilize nutrients in their early growth stage and can tolerate drought when this comes at any stage of the crop growth. But, root studies in Colombian field mollisols suggested deep rooting genotypes were not always the best yielding materials (Polanía et al. 2009). Deep rooting therefore may reflect a survival response that coincides with suppressed reproductive development under drought stress and “waiting” for late rains for regrowth.

On the other hand, sustained photosynthate accumulation and improved translocation to grain under drought stress most likely reflects better source efficiency and sink strength (Chapter 5). The improved translocation efficiency under drought stress has also spillover effects in well-watered years (Beebe et al. 2008). This means genotypes with good photosynthetic ability and translocation efficiency perform well both under drought stress and non-stress conditions.

Strategies for drought-tolerance breeding in common beans

The findings of this study meant to contribute to an effective strategy for developing drought-tolerant varieties for smallholder farmers in highly diverse environments like those of southern Ethiopia. Considering the difficulties of such a breeding program, the study addressed the process of variety development by looking at the knowledge that was required as an input in this process: the knowledge on and of farmers and their environment, the germplasm they use, the knowledge of bean plant physiology and the molecular genetic analysis. This showed that important challenges are related to each of these aspects: the complexity of the environments, the variation of farmer preferences, the range of traits involved in drought tolerance and the available germplasm. While picturing each of the aspects was challenging, it moreover showed the need to integrate the challenges in order to obtain bean varieties that are adapted to the different environments. This implies that a breeding program needs to combine a great number of traits, and to consider the diverse and changing needs of smallholder farmers.

This study used a wide range of methods from different disciplines to identify relevant traits, i.e. those related to farmers' preferences and drought tolerance. Additional methods were used to evaluate and select germplasm, in the laboratory, and on experimental and farmer fields. The results showed the complexity of targeting: understanding of the environments, user preferences, germplasm structure and trait of interest that are needed to address important questions in the definition of a breeding program.

More specifically such questions would include: how diverse and dynamic are farmer environments and preferences and how to address that diversity? How does the baseline variability in germplasm look like and which germplasm to use? Which tolerance mechanisms do exist in available germplasm base? How and where to evaluate them and select for them? Which tolerance mechanism would farmers prefer to have in their varieties? Earliness, deep rooting, translocation efficiency or have farmers still other preference traits that they seek to have integrated with drought tolerance? If to involve farmers, how best to do so?

Diverse scientific disciplines are employed to study the mechanisms by which genetic and environmental variation modify grain yield and its composition at one hand (Wollenweber et al. 2005) and the ways of making better seeds for smallholder farmers in harsh environments at the other hand (Ceccarelli et al. 2009; Offei et al. 2010). There are notable advances within single disciplines in understanding these variations which could contribute to the increase in crop yield and variety adoption.

But what is increasingly needed is a more complete understanding of integrating multiple approaches to tackle such complex targets like drought stress linked with climate change for farmers who grow crops in harsh environments with dynamic markets. Hence, a breeding strategy should integrate knowledge in social science, plant breeding, genomics, plant physiology, soil science, agronomy, plant modeling techniques etc. Breeders should be able to interplay with all different disciplines in an integrative manner as presented in Fig. 1.

Capturing farmers' preferences and combining mechanisms of drought tolerance together with high grain yield in one final variety (Fig. 2) definitely requires an integrated breeding strategy that uses the knowledge of scientists as well as farmers (see Fig. 1 in Chapter 1). While many plant traits are involved in the adaptation to drought stress (Chapters 4 and 5), the heterogeneity in farmers' production environments (Chapter 2) and the variations in farmers' preference traits (Chapter 6) pose additional challenges for adoption of drought-tolerant varieties. Therefore manipulation of just one trait (aspect) is unlikely to provide major gains in drought tolerance particularly at smallholder farmers' environments. Knowledge of the local conditions, changes in agro-ecological and socio-economic conditions, and variation caused by climate change and market dynamics together with the mechanism and allele discovery at plant level are crucial to develop a variety that meets the needs for farmers.

The integration of the understanding on the different aspects leads to new strategic questions, like those related to the targeting. How essential is, for instance, decentralization of evaluation and selection to address the variation in farmers' preferences and conditions? In this study the variation in seed color preferences seemed a result of historically explained introductions, and actually farmers tended to converge around the traits that defined marketability and culinary quality.

Also, is it for instance realistic to aim for higher yield potentials when the average smallholder farmer in Ethiopia harvests on average 500 kg ha^{-1} under drought stress? And if we target for 1000 kg ha^{-1} under farmers conditions, what are the implications for the drought tolerance mechanisms to select for? Or are there trade-offs if we aim at improving realizable yields instead of potential yields? For instance, root traits seem important to improve drought tolerance. However, investment in roots might draw biomass away from the grain. In some cases extra investment in roots might be needed to get up to that goal of 1000 kg ha^{-1}. But roots take many forms, and targeting specific root phenotype to target areas is a difficult challenge that might be possible only with specific QTL selection. Roots and their balance with shoot growth and with reproductive development are topics that will require still more works across disciplines (breeders, physiologists, soil scientists). Other traits such as stomatal control also remain to be explored.

Fitting the right genotype in the right environment needs germplasm appraisal. Appropriate germplasm for target environments could be selected using criteria of various stakeholders but which trait is responsible for drought tolerance and how it is inherited required a detail genetic analysis. A breeding strategy is therefore to characterize the drought tolerance of various available common bean germplasms and identify the most important trait(s) related with that drought tolerance in the germplasm base.

Genomics is a powerful tool to dissect the complex traits like drought tolerance, but as pointed out in this study, there are many possible plant responses, and many target environments, so it is difficult to know where to target one's efforts in genomics. Since genomics requires intensive work and has substantial cost, and it will not be possible to target all possible drought responses, it is necessary to choose those traits that work across the greatest number of environments as a strategy. Grain filling or remobilization seems to be one such trait. Hence, it may be necessary to develop genetic maps and identify DNA markers linked to QTL to localize chromosomal regions or candidate genes involved in drought tolerance in common bean to design marker assisted selection.

Smart crossing to combine physiological traits with farmers' preferences and high grain yield is another core element of breeding by integrative strategy. A breeding strategy to confront diverse and dynamic preferences and complex mechanisms of tolerance should systematically and empirically combine contrasting parents to see what trait combination give a boost in yield under farmers conditions. The analysis of the germplasm in East African Highlands suggests potential for smart crosses between the distinct germplasm at national or regional level and with other potential donors. This may provide opportunity to create a series of lines with unique trait combination and see which combinations work in farmers conditions. Once there is proof of ‘what works where’, and what works across environments in improving drought tolerance to attain the “target yield”, then to bear down more in those traits or trait combinations in a breeding program.

Lastly, in the cyclic process of breeding, an integrative design should mainstream decentralized evaluation and selection together with farmer involvement in the process. This study explored farmer-based common bean improvement but does not show a high level of sophistication in farmers knowledge. Farmers’ knowledge may not be so well developed due to limited variation they are exposed to. This does not mean farmers’ knowledge cannot be enhanced or exploited. There are some weaknesses that need to be overcome and some strengths to build on. Common bean farmers in southern Ethiopia lack knowledge on creation of genetic variability in their germplasm resource. But they can select common bean types that are suitable to them during the PVS trials as discussed in Chapter 6. Farmers used plant introduction and varietal mixtures as source for variability to select upon as a smart strategy in the traditional system (Chapter 2). On the other hand, scientists have knowledge and the capacity to generate genetic variability. But, relatively little has been done in making available sufficient variability for farmers to choose from, given the dynamics of climate and market in the region.

Common bean farmers considered many traits in evaluating their bean variety to cope with the dynamism in their production system. Many of the traits considered by farmers such as taste, attractiveness to eat, flatulence, marketability etc. are not typically considered by breeders in their on-station data collection sheets (see Chapter 6 for detail). Moreover, findings indicated important gender differentiation for common bean preference traits. Women clearly attached more importance to the culinary qualities than men, whereas the different methods pointed to men giving more importance to marketability.

This means that breeders and farmers (men/women) evaluate or look at traits in different ways which might lead to valuable germplasm being discarded and less preferred varieties being developed by breeders. Hence, breeders should collaborate with farmers in developing drought tolerance bean varieties. Such collaboration should consider gender differentiation and should ensure their involvement at all stages of the variety evaluation process. Nonetheless, the findings from this study show there was a fair coincidence among farmers, breeders and extensionists in selecting genotypes (Chapter 6).

The findings also showed that farmers can be powerful partners in the drought work and their involvement in the breeding process provided them with some experience to recognize traits related to drought tolerance in common bean. Farmers' knowledge if combined with the scientists' point of view as well, would give an opportunity for interaction and complementary selection criteria often overlooked by formal drought breeding work at on-station. In such a scientist-farmer partnership, breeders can work closely with social scientists to assess what makes varieties attractive for farmers and design a strategy that catalyzes unsupervised variety dissemination. In participatory approaches to drought tolerance, farmers should participate in the beginning and end of the breeding process, scientists should help them by providing suitable variation and selection of suitable varieties should be done in farmers' fields.

Conclusions

This study has made an attempt to give an understanding of diversity of environments and preferences and mechanisms of drought tolerance using multiple approaches. There are six major insights to be gained from this study:

1) farmers' preferences are not static nor are they consistent across gender, location, individual and co-evolve with exposure;
2) original differences in introduced germplasm from the primary centers of domestication was the base for East African common bean diversity;
3) the relevance of continued photosynthate accumulation as a trait for common bean drought tolerance;
4) root based QTL can be identified but may not be compatible with yield related traits although their selection can benefit from tightly linked markers;
5) only a few major QTLs with high QTL×E interaction were detected whereas a large proportion of genetic variance remained unexplained by the QTLs for traits related to drought avoidance, photosynthate accumulation and (re)mobilization, highlighting the difficulty in detecting QTL in drought studies in common bean; and
6) exposing farmers to new drought-tolerant variety types makes them aware of drought selection traits and creates new market niches for new varieties.

The findings presented in this study are important ingredients for a breeding program that integrates participatory, physiology and genomics tools to breed new varieties of drought tolerant common bean that combine a range of mechanisms in farmers' preferred grain types. Involvement of farmers in all stages of the common bean variety evaluation process together with use of technological advancements in genomics and physiology is imperative.This would lead to better targeted common bean varieties suited in particular to the needs of resource-poor farmers in the harsh environments of Africa in general and southern Ethiopia in particular. Hence mainstreaming a decentralized participatory plant breeding approach as a novel component of integrated breeding for drought tolerance in common bean where different actors and their knowledge interact is suggested. As part of the integrated breeding, more molecular work on identification of genes for drought tolerance should be managed by an international common bean program and advanced laboratories whereas selection for the target environment should be transferred to national/local breeding programs in which breeders, extensionists, and farmers jointly select and recycle genotypes for their ultimate environment. In general, knowledge of the local conditions, changes in agro-ecological and socio-economic conditions, and diversity caused by climate change and market dynamics together with the mechanism and allele discovery at plant level are crucial to develop a variety that meets the needs of farmers. Only on the integrated understanding these various aspects, can a breeding program can develop a more drought tolerant common bean genotype(s) for a range of farmer conditions, markets and preference.

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Supplementary information

Supplementary table 1 Origin and description of bean accessions used in the study

Entry No	Accession	Country origin	Province/ state origin	Municipality	Growth Habit [a]	Seed Size[b]	Seed Color[c]	Inference[d]
1	A. melka[g]	Ethiopia	Melkasa	Melkasa	II	S	1	M1
2	Ayenew[g]	Ethiopia	Alemaya	Alemaya	III	M	2,4	M1
3	Calima[f]	Colombia	N/A	N/A	I	L	6,2	A2
4	DOR364[e]	El Savador	N/A	N/A	II	S	6	M1
5	G810	Ethiopia	Harerge	Harar	IV	S	2,6	M2
6	G1178	Ethiopia	Shewa	Addis Ababa	I	M	3	A3
7	G761	Ethiopia	Harerge	Dire Dawa	III	S	1	M3
8	G762	Ethiopia	Shewa	Addis Ababa	III	M	2,9	M3
9	G763	Ethiopia	Shewa	Addis Ababa	III	S	1	M3
10	G764	Ethiopia	Shewa	Addis Ababa	IV	L	2,6	A3
11	G765	Ethiopia	Shewa	Addis Ababa	IV	M	2,6	A3
12	G766	Ethiopia	Shewa	Bishoftu	IV	S	5,8	M3
13	G767	Ethiopia	Shewa	Bishoftu	III	S	2,8	M3
14	G768	Ethiopia	Shewa	Bishoftu	IV	M	8	M2
15	G768A	Ethiopia	Shewa	Bishoftu	IV	M	5	M3
16	G769	Ethiopia	Shewa	Bishoftu	III	S	1	M3
17	G1179	Ethiopia	Shewa	Addis Ababa	III	M	2,6	A3
18	G772	Ethiopia	Shewa	Addis Ababa	IV	M	1	M3
19	G773	Ethiopia	Shewa	Addis Ababa	II	S	1	M3
20	G774	Ethiopia	Shewa	Addis Ababa	III	M	2	M3
21	G775	Ethiopia	Shewa	Addis Ababa	IV	M	2,4	M3
22	G800	Ethiopia	Gojjam	N/A	IV	M	2,4	M3
23	G807	Ethiopia	Shewa	Mojo	IV	M	2,4	M3
24	G808	Ethiopia	Harerge	Harar	III	S	1	M2
25	G809	Ethiopia	Harerge	Harar	IV	S	8	M3
26	G1180	Ethiopia	Shewa	Addis Ababa	III	M	2,6	A3
27	G1181	Ethiopia	Shewa	Addis Ababa	III	S	4	M3
28	G1182	Ethiopia	Shewa	Addis Ababa	II	M	2,6	A3
29	G1183	Ethiopia	Shewa	Addis Ababa	II	S	8	M1
30	G1205	Ethiopia	Gojjam	N/A	II	M	2,6	A3
31	G1206	Ethiopia	Gojjam	N/A	IV	M	2	M3
32	G1211	Ethiopia	Shewa	Addis Ababa	II	M	2,5	A3
33	G1230	Ethiopia	Shewa	Mojo	I	L	3	A3
34	G1052	Ethiopia	Harerge	AUAREH	IV	S	1	M3
35	G1231	Ethiopia	Shewa	Mojo	I	L	2	A3
36	G1232	Ethiopia	Shewa	Mojo	I	M	2,6	A3
37	G1233	Ethiopia	Harerge	Harar	I	L	1,7	A3
38	G1405	Ethiopia	Shewa	Addis Ababa	II	S	1	M3
39	G1406	Ethiopia	Shewa	Addis Ababa	III	S	1	M3
40	G2576	Ethiopia	Harerge	Harar	II	M	2,6	A3

Supplementary table 1 continued........

Entry No	Accession	Country origin	Province/ state origin	Municipality	Growth Habit [a]	Seed Size[b]	Seed Color[c]	Inference[d]
41	G2928	Ethiopia	Harerge	Harar	III	S	6	M2
42	G5486	Ethiopia	N/A	N/A	III	M	6	M3
43	G6785	Ethiopia	Harerge	Harar	I	M	3	A3
44	G6980	Ethiopia	Harerge	Harar	I	L	2	A3
45	G1171	Ethiopia	Shewa	Addis Ababa	I	M	3	A3
46	G11481	Ethiopia	Harerge	Harar	I	L	1	A3
47	G11482	Ethiopia	Harerge	Harar	I	M	2	A3
48	G13071	Ethiopia	Harerge	Alemaya	III	S	6	M2
49	G13072	Ethiopia	Shewa	Shashamane	III	S	6	M2
50	G8130	Ethiopia	N/A	N/A	II	S	4,6	M1
51	G8242	Ethiopia	Shewa	A. Ababa	III	S	2,4	M3
52	G9319	Ethiopia	N/A	N/A	III	M	6	M2
53	G1172	Ethiopia	Shewa	A.Ababa	I	L	2	A3
54	G14177	Ethiopia	Harerge	Alemaya	IV	M	3	M3
55	G18750	Ethiopia	Harerge	Harar	III	S	2	M2
56	G18750A	Ethiopia	Harerge	Harar	III	S	2	M2
57	G18845	Ethiopia	N/A	N/A	II	S	6	M2
58	G18863	Ethiopia	Harerge	Harar	III	S	3	Introgression
59	G18863A	Ethiopia	Harerge	Harar	I	M	3	A3
60	G1173	Ethiopia	Shewa	A. Ababa	III	S	1	M3
61	G18865	Ethiopia	Harerge	Dire Dawa	III	S	2,6	A3
62	G19236A	Ethiopia	Gamo Gofa	Bako	II	S	8	M1
63	G19236B	Ethiopia	Gamo Gofa	Bako	III	S	2	M1
64	G19237	Ethiopia	Sidamo	Awassa	III	S	1	M3
65	G19237A	Ethiopia	Sidamo	Awassa	III	S	6	M2
66	G19256	Ethiopia	Gamo Gofa	Fasha	III	S	8	M1
67	G19257	Ethiopia	Harerge	Doketu	II	S	8	M1
68	G1174	Ethiopia	Shewa	A. Ababa	IV	S	1	M3
69	G20133	Ethiopia	Wolega	Nedjo	II	S	1	M3
70	G20134	Ethiopia	Wolega	Nedjo	III	S	4	M3
71	G20134A	Ethiopia	Wolega	Nedjo	II	S	3	A3
72	G20136	Ethiopia	Wolega	Nedjo	II	S	6	M2
73	G20137	Ethiopia	Wolega	Nedjo	II	S	1	M3
74	G20138	Ethiopia	Wolega	Nedjo	II	M	8	M3
75	G20139	Ethiopia	Wolega	Nedjo	I	M	3	A3
76	G20140	Ethiopia	Wolega	Gida	II	S	1	M2
77	G20141	Ethiopia	Ilubabor	Metu	III	S	8	M1
78	G1175	Ethiopia	Shewa	A. Ababa	III	S	1	M3
79	G20142	Ethiopia	Shewa	Shashamane	III	S	1	M3
80	G20143	Ethiopia	Shewa	Shashamane	III	S	8	M2

Supplementary table 1 continued........

Entry No	Accession	Country origin	Province/ state origin	Municipality	Growth Habit [a]	Seed Size[b]	Seed Color[c]	Inference[d]
81	G20144	Ethiopia	Gonder	Blue Nile FallS	II	S	1	M3
82	G20390	Ethiopia	N/A	N/A	III	S	1	M2
83	G20391	Ethiopia	N/A	N/A	III	S	1	M3
84	G20392	Ethiopia	N/A	N/A	III	S	1	M2
85	G20393	Ethiopia	N/A	N/A	IV	S	1	M3
86	G20394	Ethiopia	N/A	N/A	IV	M	1	M3
87	G20395	Ethiopia	Shewa	A. Ababa	IV	M	1	M3
88	G20396	Ethiopia	N/A	N/A	IV	L	1	M3
89	G1176	Ethiopia	Shewa	A. Ababa	I	S	3	A3
90	G20397	Ethiopia	N/A	N/A	III	S	1	M2
91	G20398	Ethiopia	N/A	N/A	III	S	1	M2
92	G1177	Ethiopia	Shewa	A. Ababa	I	M	3	A3
93	G754	Ethiopia	Shewa	A. Ababa	II	L	3	A3
94	G755	Ethiopia	Shewa	A. Ababa	III	S	1	M3
95	G756	Ethiopia	Shewa	A. Ababa	II	S	1	M3
96	G757	Ethiopia	Shewa	A. Ababa	IV	L	2,6	A3
97	G758	Ethiopia	Shewa	A. Ababa	II	M	2,6	A3
98	G759	Ethiopia	Shewa	A. Ababa	II	S	8	M1
99	G760	Ethiopia	Shewa	A. Ababa	III	S	1	M3
100	G19833[f]	Peru	Amazonas	N/A	III	L	3,6	A2
101	GLP x 92[g]	Kenya	KARI	N/A	III	L	2,6	M1
102	ICA Pijao[e]	Colombia	Valle Del Cauca	N/A	II	S	8	M1
103	G961	Kenya	Nairobi Area	Nairobi	IV	M	6	A1
104	G1370	Kenya	Nairobi Area	Nairobi	III	M	2,6	A2
105	G1371	Kenya	Nairobi Area	Nairobi	III	S	8	M2
106	G1372	Kenya	Nairobi Area	Nairobi	I	M	3	A2
107	G1373	Kenya	Nairobi Area	Nairobi	II	M	9	A2
108	G1374	Kenya	Nairobi Area	Nairobi	II	L	3	A2
109	G1375	Kenya	Nairobi Area	Nairobi	III	L	3,4	A1
110	G2888	Kenya	Kisumu	Kisumu	I	L	3	A2
111	G2889	Kenya	Nyanza Region	Chiganda	III	S	1,4	M1
112	G2889A	Kenya	Nyanza Region	Chiganda	II	S	1	M1
113	G962	Kenya	Nairobi Area	Nairobi	IV	S	3	M3
114	G2890	Kenya	Rift Valley	Hoey's Bridge	IV	S	6	M2
115	G2890A	Kenya	Rift Valley	Hoey's Bridge	IV	M	7,2	A2
116	G2890B	Kenya	Rift Valley	Hoey's Bridge	IV	M	5,7	A2
117	G2890C	Kenya	Rift Valley	Hoey's Bridge	I	L	6	A2
118	G2891	Kenya	Western Region	Kimilili	II	L	3	A2
119	G8045	Kenya	Nairobi Area	Nairobi	IV	M	7	A2
120	G11228	Kenya	N/A	N/A	II	M	3	A2

Supplementary table 1 continued........

Entry No	Accession	Country origin	Province/ state origin	Municipality	Growth Habit [a]	Seed Size[b]	Seed Color[c]	Inference[d]
121	G11242	Kenya	N/A	N/A	I	M	3	A1
122	G963	Kenya	Nairobi Area	Nairobi	IV	M	1	M3
123	G11243	Kenya	N/A	N/A	III	L	7,2	A1
124	G11246	Kenya	N/A	N/A	I	M	3	A2
125	G11251	Kenya	Nairobi Area	Nairobi	II	L	1,3	A1
126	G8046	Kenya	N/A	N/A	II	S	8	M1
127	G8047	Kenya	N/A	N/A	II	L	7,2	A1
128	G8048	Kenya	N/A	N/A	I	L	7,2	A1
129	G8049	Kenya	N/A	N/A	I	L	5,6	A2
130	G8052	Kenya	N/A	N/A	I	M	6,2	A2
131	G8053	Kenya	N/A	N/A	III	M	6	A2
132	G8054	Kenya	N/A	N/A	II	M	6	A2
133	G964	Kenya	Nairobi Area	Nairobi	III	L	3	A2
134	G8055	Kenya	N/A	N/A	IV	L	2,4	M3
135	G8056	Kenya	N/A	N/A	I	M	2,6	A2
136	G14499	Kenya	Nairobi Area	Nairobi	III	L	2,4	A1
137	G15950	Kenya	N/A	N/A	IV	S	1	M2
138	G19252	Kenya	Nairobi Area	Nairobi	II	M	2,6	A3
139	G19252A	Kenya	Nairobi Area	Nairobi	I	M	7,2	A1
140	G19252B	Kenya	Nairobi Area	Nairobi	III	M	7,2	A2
141	G20526	Kenya	N/A	N/A	I	L	6,2	A2
142	G20527	Kenya	N/A	N/A	IV	L	2,7	A1
143	G20528	Kenya	N/A	N/A	I	L	6	A1
144	G1365	Kenya	Nairobi Area	Nairobi	III	S	9	M3
145	G20529	Kenya	N/A	N/A	I	L	6	A1
146	G20531	Kenya	N/A	N/A	I	L	5,6	A1
147	G20536	Kenya	N/A	N/A	II	S	7	A1
148	G20537	Kenya	N/A	N/A	II	M	7	A1
149	G20538	Kenya	N/A	N/A	III	S	5,4	A1
150	G20539	Kenya	N/A	N/A	I	M	2,6	A1
151	G20540	Kenya	N/A	N/A	I	M	2,7	A1
152	G20541	Kenya	N/A	N/A	I	L	9,8	A1
153	G20541A	Kenya	N/A	N/A	I	L	9,8	A1
154	G20542	Kenya	N/A	N/A	III	L	9,8	A1
155	G1366	Kenya	Nairobi Area	Nairobi	I	L	7,2	A2
156	G20543	Kenya	N/A	N/A	I	L	9,8	A1
157	G20544	Kenya	N/A	N/A	I	L	7,2	A1
158	G20555	Kenya	N/A	N/A	II	S	2,6	A1
159	G20556	Kenya	N/A	N/A	III	M	7	A1
160	G20557	Kenya	N/A	N/A	II	M	2,7	A1

Supplementary table 1 continued........

Entry No	Accession	Country origin	Province/ state origin	Municipality	Growth Habit [a]	Seed Size[b]	Seed Color[c]	Inference[d]
161	G22748	Kenya	N/A	N/A	II	M	7	A1
162	G24070	Kenya	South Nyanza	Sare	IV	S	3	M2
163	G24070A	Kenya	South Nyanza	Sare	IV	S	3	M2
164	G24482	Kenya	Nyanza	Kisii	I	M	7,2	A1
165	G24483	Kenya	Western Region	Kakamega	II	M	2,6	A1
166	G1367	Kenya	Nairobi Area	Nairobi	I	L	7,2	A2
167	G24483A	Kenya	Western Region	Kakamega	I	L	7	A3
168	G24484	Kenya	South Nyanza	Migori	IV	M	2,8	M3
169	G24485	Kenya	Western Region	Kakamega	II	S	3	M2
170	G24486	Kenya	Western Region	Kakamega	IV	M	6	M2
171	G2456I	Kenya	N/A	N/A	IV	M	2,8	A1
172	G50117	Kenya	South Nyanza	Homa Bay	II	L	7,2	A3
173	G50118	Kenya	South Nyanza	Migori	I	M	7	A1
174	G50118A	Kenya	South Nyanza	Migori	II	M	6	A1
175	G50119	Kenya	Western Region	Kakamega	II	M	5,6	A1
176	G1368	Kenya	Nairobi Area	Nairobi	II	M	7	A2
177	G50121	Kenya	Western Region	Kakamega	I	M	3	A2
178	G50122	Kenya	Western Region	Kakamega	I	M	7,2	A2
179	G50123	Kenya	South Nyanza	Migori	IV	S	2,4	M1
180	G50125	Kenya	Nyanza	Kisumu	III	M	5,6	A2
181	G50126	Kenya	Western Region	Kakamega	II	S	9,8	M1
182	G50127	Kenya	Coast region	Kilifi Dist.	II	M	7,2	A1
183	G50541	Kenya	Rift Valley	Sotik	IV	M	5	M2
184	G50542	Kenya	South Nyanza	Homa Bay	IV	S	6	M1
185	G50543	Kenya	Western Region	Kakamega	III	S	8	M1
186	G1369	Kenya	Nairobi Area	Nairobi	I	L	7,2	A1
187	G50544	Kenya	Western Region	Kakamega	III	S	2,9	M1
188	G50545	Kenya	South Nyanza	Migori	III	S	6	M1
189	G50667	Kenya	N/A	N/A	IV	M	6	M2
190	G50808	Kenya	N/A	N/A	I	L	2,7	A1
191	Red Wolayta[g]	Ethiopia	EIAR	Wolayta	III	S	6	M2
192	Zebra[g]	Ethiopia	EIAR	Melkasa	III	M	2,4	M1

[a] Growth habit: I determinate bush, II Indeterminate bush, III Indeterminate prostrate, IV indeterminate climbing beans; [b] Seed size (g/100 seeds): *S* small (<25 g), *M* medium (25-40 g), *L* large (>40 g/100 seeds); [c] Seed color: primary and secondary color designations (separated by a comma) as *1* white, *2* cream, *3* yellow, *4* tan, *5* pink, *6* red, *7* purple, *8* black, *9* others; [d] population inference with INSTRUCT; [e] DOR364 (or 'Dorado') a variety from CIAT/El Salvador and ICA Pijao a variety from Colombia as Mesoamerican control; [f] 'Calima' (G4494) a variety from Colombia and 'Chauca Chuga'(G19833) a landrace from Peru as Andean control genotypes ; [g] released varieties from formal breeding programs.

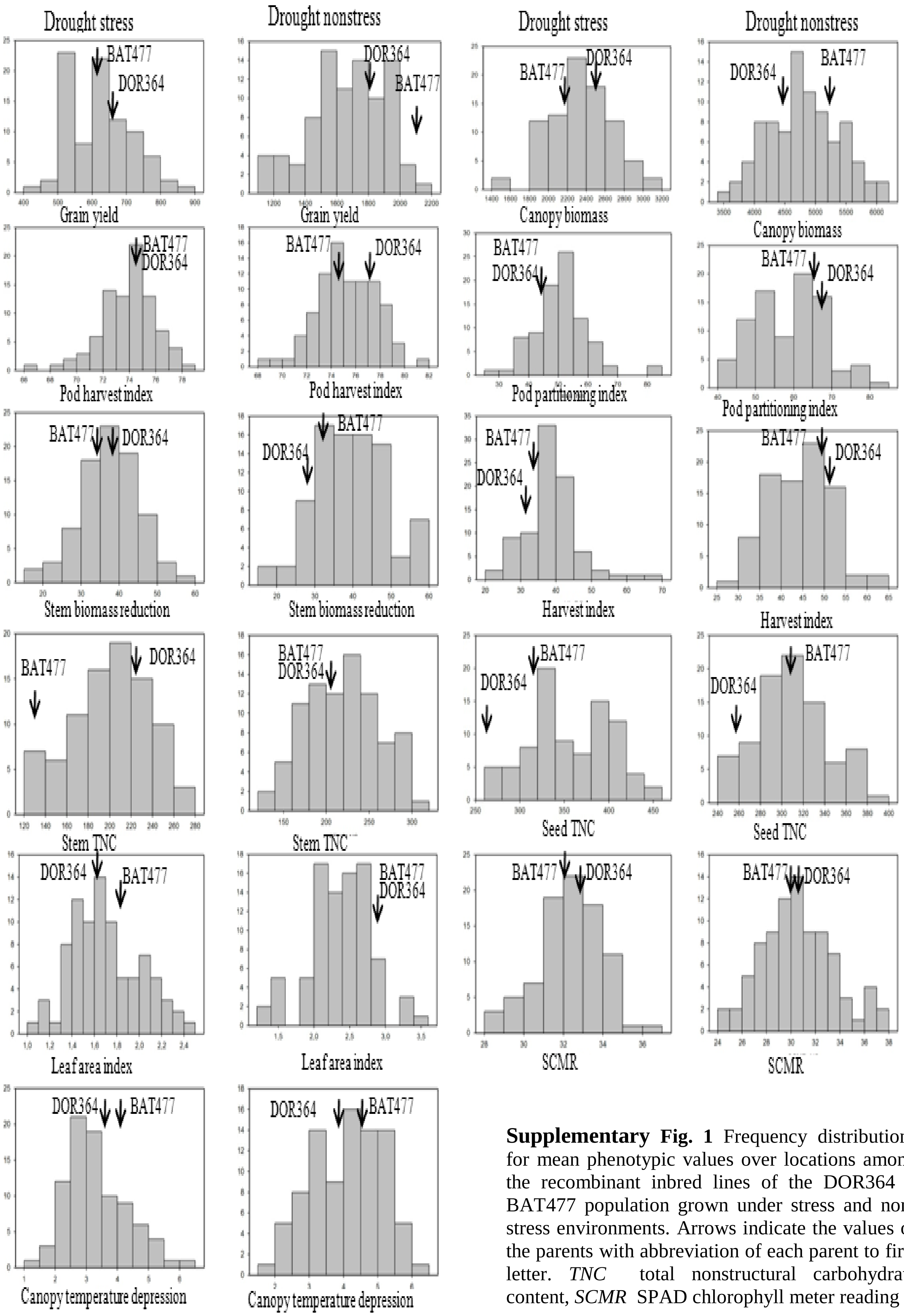
Abbreviations: *N/A* data not available, *A1-3* Andean subclasses, *M1-3* Mesoamerican subclasses

Supplementary table 2 Description of genotypes tested in PVS trials in southern Ethiopia from 2008-2010

				Test season and year‡			
Genotype	Grain type	Gene-pool	Status†	2008M	2009B	2009M	2010B
SAB-622	Red kidney	Andean	ADT from CIAT	√	√	√	√
SAB-676			ADT from CIAT	√			
SAB-679			ADT from CIAT	√			
SAB-680			ADT from CIAT	√			
SAB-626	Sugar		ADT from CIAT	√	√	√	√
SAB-629			ADT from CIAT	√			
SAB-691			ADT from CIAT	√			
SAB-692			ADT from CIAT	√			
SAB-646	Red-mottled		ADT from CIAT	√			
SAB-650			ADT from CIAT	√	√	√	√
SAB-651			ADT from CIAT	√			
SAB-659			ADT from CIAT	√			
Ibado			Released variety		√	√	√
SAB-710	Large White		ADT from CIAT	√			
SAB-713			ADT from CIAT	√			
SAB-735			ADT from CIAT	√			
ETAW 8-2A			Awassa line	√	√		
SEC-23	Small white	Meso	ADT from CIAT	√			
SEC-24			ADT from CIAT	√	√	√	√
SEC-25			ADT from CIAT	√			
SEC-26			ADT from CIAT	√	√		
SER-16	Small red		ADT from CIAT	√	√	√	√
SER-43			ADT from CIAT	√	√		
SER-48			ADT from CIAT	√	√	√	√
SER-125			ADT from CIAT	√	√	√	√
Dume			Released Awassa line	√	√	√	√
SXB-407	Carioca		ADT from CIAT	√			
SXB-409			ADT from CIAT	√			
SXB-410			ADT from CIAT	√			
SXB-415			ADT from CIAT	√			
SXB-403	Cream		ADT from CIAT	√	√		
SXB-405			ADT from CIAT	√			
SXB-412			ADT from CIAT	√			
VAX-1			CIAT line	√			
SEN-41	Black		ADT from CIAT	√			
SEN-56			ADT from CIAT	√			
NCB-226			ADT from CIAT	√			
NCB-280			ADT from CIAT	√			
Local[a]	Different		Farmers variety	√	√	√	√

† *ADT* Advanced drought lines from CIAT, Colombia. ‡ *M* *'Meher'* season, *B* *'Belg'* season

[a] Popular farmer variety grown in PVS village and its grain type changes from village to village



Supplementary Fig. 1 Frequency distributions for mean phenotypic values over locations among the recombinant inbred lines of the DOR364 × BAT477 population grown under stress and non-stress environments. Arrows indicate the values of the parents with abbreviation of each parent to first letter. *TNC* total nonstructural carbohydrate content, *SCMR* SPAD chlorophyll meter reading

Summary

Crop breeding for smallholder farmers is difficult, especially when they farm in harsh environments. Drought stress is a major limiting factor to crop productivity in many agricultural production systems. Improving crop performance in harsh environments where drought is limiting remains one of the most important and challenging issues for breeders, farmers, researchers, development agents and policy makers. In some regions the challenge will be even more daunting with an increasing scarcity of water in the future due to climate change. This will make improving adaptation to drought stress a major objective of crop breeding efforts. This thesis describes a process towards breeding by integrative design for developing common bean cultivars that improve productivity and meet farmers' needs and preferences in harsh environments, particularly in southern Ethiopia.

The introduction in Chapter 1 provides an overview of crop breeding efforts for smallholder farmers in harsh environments, the challenges of common bean breeding for such environments, and the state-of-art of common bean breeding for drought tolerance. It also describes the general research objectives and research design of the thesis project.

Chapter 2 explores various elements and contexts that characterize the farmers' use and management of common bean seed and varieties in drought-prone southern Ethiopia where the crop is an important source of food and cash income for the smallholder farmers. It analysis how farmer-based common bean improvement practice is used to cope with farmers' highly variable context within their food production and livelihood system. Discussions with focus groups, interviews with contact-farmers and surveys were the methodologies applied in the study. The results demonstrate that farmers' conditions and preferences for plant and grain types of common bean vary strongly. Moreover, the high level of environmental variation and the associated risks of crop failure have increased even more with the unpredictability of rains. Farmers recognize that common bean is not particularly drought tolerant, but it is attractive to them for its short life cycle and market opportunities. While farmers are aware of climate change, only half of them have adapted some of the cropping practices. They know different forms of drought stress and the response of their varieties to them but their variety and seed management practice do not show a high level of specialization. Farmers in the region carry out some level of in-field plant selection as well as post-harvest seed sorting using multiple criteria but their selection practice is a kind of mass selection, sometimes intentionally and sometimes un-intentionally. The selector-farmers focus on earliness, seed-color development, pod load and grain fill rather than on drought tolerance *per se*. However, farmer-based common bean improvement in the traditional system has had limited potential on providing choice for farmers due to self-pollinating nature of the crop. This implies that the local crop development practices are less effective for addressing diversity caused by climate change and market dynamics but at the same time with high potential because of relatively high off-farm seed source use. Farmers seek marketability, drought tolerance and culinary traits. This situation provides opportunities for strategic development and introduction of bean genetic diversity. Hence more drought tolerant bean diversity for a range

of farmer conditions, markets and preferences can only be developed on an integrated understanding of farmer’s production conditions and existing seed system practices. On the basis of this understanding a breeder can contribute to an overall package of mechanisms that harness and equip farmers to adapt to their dynamical context.

Chapter 3 uncovers the diversity and population structure of common bean landraces from East African Highlands using morphological phenotyping and microsatellite marker genotyping. The germplasm used in the study represented different common bean production ecologies and seed types common in two major bean producing countries, Ethiopia and Kenya in East Africa. The results demonstrated that the landraces showed considerable diversity, that their population structure was mainly based on genepool origin and that introgression or gene flow was moderate. With regard to distribution, Mesoamerican genotypes were predominant in Ethiopia while Andean genotypes were predominant in Kenya. The results also indicated that genetic divergence was slightly higher for the Ethiopian landraces compared to the Kenyan landraces and that Mesoamerican genotypes were more diverse than the Andean genotypes. Given that common bean in East Africa is often cultivated in marginal, risk-prone farming systems, the observed landrace diversity should provide valuable alleles for adaptation to stressful environments in future breeding programs in the region. The results presented in Chapter 3 would pave the way for rational use of East African germplasm and strategic crossing plans that could be used to identify transgressive segregation based on distinct germplasm at national or regional level.

Chapter 4 evaluates the mechanisms of drought tolerance and their inheritance with an analysis of quantitative trait loci (QTLs) for multiple traits related with drought avoidance by increased extraction of soil moisture from greater depth in a greenhouse root cylinder tubes drying-down experimental trials. A recombinant inbred line (RIL) population developed from the Mesoamerican intra-gene pool cross of drought susceptible DOR364 and drought-tolerant BAT477 was used for the study. The results showed that the RIL population expressed quantitative variation and transgressive segregation for ten rooting pattern traits as well as five shoot traits of 48 days old plants. Progressive water stress significantly affected root and above ground shoot growth and development in both parents and their derived lines. The study provided better understanding of the genetics of drought avoidance root traits and identified DNA markers linked to QTLs for root traits. The root traits showed moderate heritability. The molecular dissection of drought tolerance traits further suggested that the QTLs affecting root traits in common bean are based on constitutive expression of genes and that drought avoidance was based on deep rooting, longer root length, thicker roots, increasing root length distribution with depth, root volume and root biomass. The positive alleles for most of the QTLs detected in this study were derived from the paternal parent BAT477. The results in Chapter 4 represented the first effort in common bean to identify QTLs for drought avoidance root traits which highlighted the feasibility of marker-aided selection as an alternative to conventional labor-intensive, phenotypic screening of drought avoidance root traits.

Chapter 5 explores the mechanisms of drought tolerance and their inheritance with an analysis of quantitative trait loci for multiple traits having to do with photosynthate acquisition, accumulation and (re)mobilization to grains in field experimental trials. The study used the same experimental genotypes as those of the greenhouse root cylinder trials in Chapter 4. The experiments were executed under managed drought stress in the field in two continents. A mixed model QTL mapping analysis was executed to dissect the inheritance of photosynthate acquisition, accumulation and (re)mobilization traits under drought stress. The results showed that the genetics of drought tolerance traits studied in common bean are complex and that both heritability and variance are low. Furthermore, the results indicated the relevance of continued photosynthate accumulation as a trait for common bean drought tolerance.

Chapter 6 is a study of assessing methods to capture farmers' preference and farmers' selection of drought tolerant lines. By investigating farmers' perception on bean characteristics and the extent to which the current varieties and the advanced drought tolerant lines served farmers' needs in a participatory variety selection trial in seven sites in southern Ethiopia, we attempted to infer the multiple cropping practices, culinary and market requirements together with variable stress environments that farmers face in the region shaping farmers' preference in making choices about which bean genotype to grow. These criteria were not always static nor were they consistent across gender, location, individuals or other factors; rather they co-evolved through exposure. No single method effectively captured farmers' preference and predicted adoption; rather a combination of methods was needed for common bean drought tolerance breeding in southern Ethiopia. The results also indicated that none of the current farmers' varieties or any of the new drought tolerant genotypes was ideal for all farmers' preferences across all sites; however, some of the new drought tolerant lines yielded better than the local farmers' varieties under stress. Farmers who participated in the participatory variety selection (PVS) trial had the chance to observe broad genetic variability. They had gained some experience to recognize important traits related to drought tolerance in common bean. With this little effort, farmers could be really powerful partners in the drought work. Hence failing to recognize preference criteria and specific local adaptation in bean breeding would reduce the practical benefits of drought tolerant germplasm for small-holder farmers. The findings also showed that breeding focusing on varieties with traditional colours, shapes and sizes might therefore restrict farmers' access to novel attractive and adapted germplasm.

Finally, Chapter 7 synthesises the study results reported in the previous chapters and suggests a strategy for drought breeding in common bean using the approach of integrative design. The results in this study provided better understanding of diversity of environments, germplasms and preferences at one hand and the mechanisms of drought tolerance and their inheritance at the other hand. The chapter summarizes six major insights to be gained from the research in this thesis. These insights are: 1) farmers' preferences are not static nor are they consistent across gender, location, individual and co-evolve with exposure; 2) original differences in introduced germplasm from the primary centers of domestication was the base for East African common bean diversity; 3) the relevance of continued photosynthate accumulation as a trait for common bean drought tolerance; 4) root based QTL can be identified but may not be compatible with yield related traits although their selection can benefit from tightly linked markers; 5) only a few major QTLs with high QTL×E interaction were detected whereas a large proportion of genetic variance remained unexplained by the QTLs for traits related to drought avoidance, photosynthate accumulation and (re)mobilization, highlighting the difficulty in detecting QTL in drought studies in common bean; and 6) exposing farmers to new drought-tolerant variety types makes them aware of drought selection traits and creates new market niches for new varieties.

In general, the results from the present work are important ingredients for a breeding program that integrates participatory, physiology and genomics tools to breed new varieties of drought-tolerant common bean that combine a range of mechanisms in farmers’ preferred grain types.

Samenvatting

Plantenveredeling ten behoeve van kleine boeren is moeilijk, vooral wanneer ze onder moeilijke omstandigheden moeten boeren. Droogte-stress is in veel landbouwsystemen een belangrijke opbrengstbeperkende factor. Het verhogen van opbrengsten onder moeilijke omstandigheden waar droogte beperkend is, blijft één van de belangrijkste uitdagingen voor veredelaars, boeren, onderzoekers, ontwikkelingswerkers en politici. In sommige gebieden is de uitdaging zelfs nog groter vanwege een schaarste aan water die in de toekomst door klimaatsverandering alleen maar zal toenemen. Daarmee wordt het realiseren droogte-tolerantie een belangrijke opgave voor de veredelaar. Deze thesis beschrijft het proces om te komen tot een geïntegreerd ontwerp van een veredelingsprogramma om variëteiten van de gewone boon te ontwikkelen die een hogere productiviteit hebben en tegelijkertijd beantwoorden aan de behoeften en voorkeuren van boeren die deze gewassen onder moeilijke omstandigheden moeten verbouwen zoals in zuidelijk Ethiopië.

De inleiding in hoofdstuk 1 geeft een overzicht van plantenveredeling ten behoeve van kleine boeren die onder moeilijke omstandigheden moeten werken, de uitdagingen van de bonenveredeling voor dergelijke omstandigheden, en de huidige staat van kennis van de plantenveredeling voor droogte-tolerantie bij de gewone boon. Het beschrijft ook de algemene doelstellingen en het ontwerp van het onderzoeksprogramma waarop dit proefschrift is gebaseerd.

Hoofdstuk 2 onderzoekt verschillende aspecten van het gebruik en beheer van bonenzaad en – variëteiten in droogtegevoelig zuidelijk Ethiopië, waar het gewas een belangrijke bron van voedsel en inkomsten is. Hoofdstuk 2 analyseert hoe de boerenpraktijk van het verbeteren van de gewone boon wordt aangewend om hoofd te bieden aan de zeer gevarieerde omgeving waarin het voedsel moet worden geproduceerd en de zeer diverse manier van bestaan van de boeren. In de studie werd onder andere gebruik gemaakt van discussies met zogenaamde focus groups, interviews met sleutelfiguren onder de boeren en enquêtes. De resultaten lieten zien dat condities van boeren en voorkeuren voor plant- en korreltypes van bonen sterk varieerden. Bovendien zijn het hoge niveau van omgevingsvariatie en het daaraan gerelateerde risico van misoogst nog verder toegenomen met de onvoorspelbaarheid van de regens. Boeren erkennen dat de gewone boon niet specifiek droogte-tolerant is, maar het gewas is aantrekkelijk voor ze vanwege de korte groeiduur en de mogelijkheden om de bonen te vermarkten. Al zijn de boeren zich bewust van de klimaatsverandering, toch heeft slechts de helft van hen bepaalde teeltmaatregelen daadwerkelijk aangepast. Ze kennen de verschillende vormen van droogte-stress en de reacties van de variëteiten daarop, maar de manier waarop zij hun rassen en het zaad beheren getuigt niet van een hoog niveau van specialisatie. Boeren in de regio passen een zekere mate van selectie binnen het veld toe en sorteren ook het geoogste zaad op basis van verschillende criteria. Hun selectie is echter een vorm van massaselectie, waarbij soms met en soms zonder opzet wordt geselecteerd. De selecteur-boer let meer op vroegheid, ontwikkeling van zaadkleur, intensiteit van peulvorming en mate van korrelvulling dan op droogte-tolerantie *per se*. Echter, dit traditionele systeem van verbetering van de gewone bonen door de boeren zelf heeft de boeren slechts in beperkte mate in de gelegenheid gesteld te kiezen. Immers, de *Phaseolus* boon is een zelfbestuiver. Dit betekent dat lokale praktijken van gewasverbetering niet zo effectief zijn als antwoord op de diversiteit die veroorzaakt wordt door klimaatsverandering en de dynamiek van de markt. Tegelijkertijd hebben de lokale praktijken wel potentie omdat relatief veel gebruik wordt gemaakt van zaad van buiten de eigen boerderij. Boeren zoeken naar marktwaarde, droogte-tolerantie en culinaire eigenschappen. Deze situatie biedt mogelijkheden voor strategische ontwikkeling en introductie van genetische diversiteit van de boon. Teneinde voldoende diversiteit aan droogte-tolerante bonen te ontwikkelen die geschikt is voor een diversiteit aan omgevingen, markten en voorkeuren van boeren is het noodzakelijk te veredelen op basis van een geïntegreerd begrip van de productieomstandigheden van de boeren en hun bestaande zaaizaadsysteem. Op basis van dit begrip kan een veredelaar bijdragen aan een algemeen pakket van instrumenten die boeren kunnen benutten om zich aan te passen aan hun dynamische en veeleisende omgeving.

Hoofdstuk 3 legt de diversiteit en populatiestructuur van de landrassen van de gewone boon afkomstig uit de Oost-Afrikaanse hooglanden bloot, op basis van morfologische fenotypering en genotypering met gebruik van microsatellieten. Het kiemplasma dat gebruikt is in de studie is representatief voor verschillende productieomstandigheden en zaadtypes die algemeen voorkomen in twee belangrijke bonen-producerende landen in Oost Afrika, te weten Ethiopië en Kenia. De resultaten laten zien dat de landrassen aanmerkelijke diversiteit vertoonden, dat hun populatiestructuur hoofdzakelijk was gebaseerd op herkomst van de genenpool en dat introgressie of genenstroom beperkt was. Met betrekking tot de verdeling waren de Mesoamerikaanse genotypes dominant in Ethiopië terwijl de genotypes uit de Andes domineerden in Kenia. De resultaten gaven ook aan dat genetische divergentie iets hoger was voor de Ethiopische landrassen dan voor de Keniaanse landrassen en dat Mesoamerikaanse genotypes diverser waren dan de Andes genotypes. Gegeven het feit dat bonen in Oost-Afrika vaak verbouwd worden in marginale en risico-gevoelige teeltsystemen, zou de waargenomen diversiteit van landrassen waardevolle allelen moeten leveren voor aanpassing aan stressvolle omgevingen in toekomstige veredelingsprogramma's in de regio. De resultaten in hoofdstuk 3 bereiden de weg voor een rationeel gebruik van Oost-Afrikaans kiemplasma en strategische kruisingen die gebruikt zouden kunnen worden om transgressieve uitsplitsing te identificeren gebaseerd op verschillend kiemplasma op nationaal of regionaal niveau.

Hoofdstuk 4 evalueert de mechanismen van droogte-tolerantie en hun vererving met een analyse van loci van kwantitatieve eigenschappen (quantitative trait loci; QTLs) voor verschillende kenmerken die gerelateerd zijn aan het mijden van droogte door het meer onttrekken van vocht dieper in de bodem zoals onderzocht in uitdrogingsproeven op wortel-cylinder buizen in de kas. Voor deze studie werd een populatie van een recombinante inteeltlijnen (RILs) ontwikkeld van een kruising van de droogtegevoelige DOR364 en de droogte-tolerante BAT477 gebruikt, beide afkomstig uit dezelfde Mesoamerikaanse genenpool. De resultaten gaven aan dat de RIL populatie kwantitatieve variatie en transgressieve uitsplitsing tot uitdrukking bracht voor tien wortelpatroon-eigenschappen en vijf stengel-eigenschappen, gemeten aan planten van 48 –dagen oud. Progressieve droogtestress beïnvloedde de ontwikkeling van de wortels en van de bovengrondse stengel in beide ouders en de daarvan afgeleide lijnen significant. De studie leverde een beter begrip op van de genetica van worteleigenschappen die bijdragen aan mijding van droogte en identificeerde DNA merkers die gekoppeld waren aan QTLs voor worteleigenschappen. De worteleigenschappen vertoonden een beperkte erfelijkheidsgraad. De moleculaire analyse van eigenschapen die met droogte-tolerantie te maken hebben, suggereerde verder dat op de QTLs die coderen voor worteleigenschappen in de gewone boon continu getranscribeerde genen gelokaliseerd zijn; mijding van droogte hangt samen met diepere beworteling, langere wortellengte, dikkere wortels, een groter aandeel van de aanwezige wortellengte dieper in de bodem, wortelvolume en wortel-biomassa. De positieve allelen voor de meeste van de in deze studie aangetoonde QTLs waren afkomstig van de ouderlijn BAT477. Het onderzoek van hoofdstuk 4 is het eerste in zijn soort dat laat zien dat het mogelijk is QTLs te identificeren voor droogtemijdende worteleigenschappen, en dat het dus uitvoerbaar is merker-ondersteunde selectie toe te passen als alternatief voor de conventionele en arbeidsintensieve, fenotypische toetsing op droogtemijdende worteleigenschappen.

Hoofdstuk 5 beschrijft onderzoek naar de mechanismen van droogtetolerantie en de overervering daarvan. Het experimenteel veldonderzoek leverde een analyse op van de QTLs voor eigenschappen die gekoppeld zijn aan het aanmaken van fotosyntheseproducten, de ophoping van deze producten en de (re)mobilisatie ervan naar de korrels. In de studie werden dezelfde experimentele genotypes gebruikt als in het kasonderzoek met de wortelcylinders beschreven in hoofdstuk 4. De veldproeven werden uitgevoerd met droogtestress-regimes in twee continenten. Een zogenaamde “mixed model QTL mapping analysis” werd uitgevoerd teneinde de vererving te ontrafelen van de eigenschappen die gekoppeld zijn aan het produceren, accumuleren en (re)mobiliseren van assimilaten tijdens droogtestress. De genetica van eigenschappen die bijdragen aan droogtetolerantie van gewone boon is ingewikkeld; zowel de erfelijkheidsgraad als de genetische variatie is gering. Om droogte te kunnen weerstaan is het echter van belang dat de gewone boon fotosynthese blijft plegen.

Hoofdstuk 6 is een studie van methoden om voorkeuren van boeren vast te stellen en te bepalen hoe ze droogtetolerante lijnen selecteren. We hebben deze door middel van experimenten met participatieve selectie van variëteiten percepties van boeren ten aanzien van eigenschappen van bonen onderzocht, alsmede de mate waarin de huidige variëteiten en gevorderde lijnen met droogte-tolerantie in hun behoefte voorzagen. Dergelijke experimenten vonden plaats op zeven locaties in zuidelijk Ethiopia. Tevens hebben we geprobeerd af te leiden hoe de verschillende teeltmaatregelen, de culinaire en markteisen samen met de variabele stress-omgevingen waarmee boeren zich geconfronteerd zien de voorkeuren van boeren bij het nemen van de beslissingen over welk bonen-genotype te verbouwen, vormen. De criteria die boeren hanteerden waren niet altijd statisch en ook waren ze niet consistent over geslacht, locatie, individu of andere factoren; eerder ontwikkelden ze zich in samenhang met de ontwikkeling van het materiaal waaraan de boeren werden blootgesteld. Geen enkele methode was op zichzelf in staat de voorkeur van boeren te vangen of de adoptie te voorspellen; eerder was het een combinatie van methoden die nodig was voor het veredelen van bonen voor droogte-tolerantie in Ethiopië. De resultaten gaven ook aan dat geen van de huidige boeren-variëteiten noch enige van de nieuwe droogte-tolerante genotypen ideaal op alle locaties aan alle voorkeuren voldeed; echter, sommige van de nieuwe droogte-tolerante lijnen brachten meer op onder stresscondities dan de lokale boeren-variëteiten. Boeren die participeerden in de proeven met participatieve rassenselectie hadden de kans om brede genetische variabiliteit te observeren. Zij kregen enige ervaring om belangrijke eigenschappen te herkennen die gerelateerd zijn met droogte-tolerantie in bonen. Met een dergelijke geringe inspanning zouden boeren werkelijk sterke partners kunnen zijn in het droogte-werk. Daarom zou het niet kunnen onderkennen van de voorkeurscriteria en specifieke adaptatie in bonenveredeling de praktische voordelen van droogte-tolerant kiemplasma voor kleine boeren reduceren. De bevindingen lieten ook zien dat veredeling die zich richt op variëteiten met traditionele kleuren, vormen en groottes van de korrels de toegang van boeren tot nieuw attractief en aangepast kiemplasma zou kunnen beperken.

Hoofdstuk 7, ten slotte, synthetiseert de studieresultaten die gerapporteerd waren in de eerdere hoofdstukken en reikt een strategie aan voor droogte-veredeling in gewone boon die gebruik maakt van een integrerend ontwerp. De resultaten van deze studie leveren een beter begrip op van, aan de ene kant, diversiteit van omgevingen, kiemplasmas en voorkeuren en aan de andere kant, van de mechanismen van droogte-tolerantie en hun vererving. Het hoofdstuk vat de zes belangrijkste inzichten samen die uit deze studie volgen. Deze inzichten zijn: 1) voorkeuren van boeren zijn niet statisch en ook zijn ze niet consistent over geslacht, locatie, individu maar ontwikkelen zich in afhankelijkheid van de mate van blootstelling aan variatie; 2) oorspronkelijke verschillen in geïntroduceerd kiemplasma van de primaire centra van domesticatie waren de basis voor de Oost-Afrikaanse diversiteit in gewone boon; 3) het belang van continue ophoping van assimilaten als eigenschap voor droogte-tolerantie van de gewone boon; 4) QTLs voor worteleigenschappen kunnen weliswaar worden geïdentificeerd maar ze zijn misschien wel niet compatibel met opbrengst-gerelateerde eigenschappen, ook al kan goed op dergelijke eigenschapen geselecteerd worden dankzij gerelateerde merkers; 5) er werden slechts enkele belangrijke QTLs met een sterke QTL×milieu interactie ontdekt terwijl een groot deel van de genetische variantie onverklaard bleef voor de QTLs voor eigenschappen die gerelateerd zijn aan droogtemijding, ophoping en (re)mobilisering van assimilaten . Dit onderstreept hoe moeilijk het is QTLs in droogtestudies met de gewone boon te ontdekken; en 6) boeren blootstellen aan nieuw droogte-tolerante variëteits-types maakt hen bewust van eigenschappen van die met droogtetolerantie te maken hebben en creëert nieuwe markt-niches voor nieuwe variëteiten.

In het algemeen zijn de resultaten van dit werk belangrijke ingrediënten voor een veredelingsprogramma dat participatieve, fysiologische en genetische instrumenten integreert om nieuwe droogte-tolerante bonenvariëteiten te ontwikkelen die verschillende mechanismen combineren met zaadtypes die de boeren prefereren.

Biography

Asrat Asfaw was born on March 24, 1974 at Dawro, Ethiopia. He graduated with a BSc. degree in Plant Sciences (with distinction) in 1995 from Haramaya University (former Alemaya University of Agriculture), Ethiopia. He also holds an MSc. degree from CCS Haryana Agricultural University, Hisar, India, with a major in Plant Breeding and a minor in Genetics. He was awarded a gold medal for the highest overall grade point average in an MSc. study in the discipline of Plant Breeding completed in July, 2000. Asrat started his carrier as an agronomist in fostering agricultural development in rural areas in the Office of Agriculture at Dawro zone, southern Ethiopia, from 1995 to 1997. In 1998 he joined the Southern Agricultural Research Institute (SARI) as a researcher (plant breeder) at Awassa Agricultural Research Centre. His work at SARI involved improving agro-biodiversity and local seed systems by developing varieties adaptable to the environments and end users. He was also involved in management of breeding programs. During his stay at SARI, Asrat developed and released several improved varieties of different crops and produced several publications including journal articles, book chapters and newsletters. Currently Asrat is engaged in breeding for stress tolerance in common bean in collaboration with the International Centre for Tropical Agriculture. He has a strong interest in an integrated breeding program for impact in which molecular aspects, crop physiology and farmer involvement are combined.

Publications

Journal Articles

Asfaw A, Blair MW, Almekinders C (2009) Genetic diversity and population structure of common beans (*Phaseolus vulgaris* L.) landraces from the East Africa highlands. Theor Appl Genet 120:1-12

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PE&RC and WASS joint PhD Education Certificate: Asrat Asfaw Amele

With the educational activities listed below the PhD candidate has complied with the educational requirements set by C.T. De Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) and the Wageningen School of Social Sciences (WASS) which comprises of a minimum of total of 32 ECTs (= 22 weeks of activities).

Description	Department/Institute	Year	Credits
I. General			
CERES orientation program	CERES, Utrecht	2007	5,0
CERES presentation tutorials	CERES, Utrecht	2007	5,5
Literature review and proposal writing	Wageningen University	2007	6,0
II. Research Methods and Techniques			
Complexity in and between social and ecosystem	CERES, WUR	2007	3,0
Technography, researching Technology and development	Wageningen University	2008	3,0
In-service laboratory and greenhouse training for six months at International Center for Tropical Agriculture	CIAT, Colombia	2007/08	5,0
Plant breeding for drought tolerance	CSU, USA	2010	3,0
Marker-assisted breeding (MAB)	CIHEAM-IAMZ/GCP, Spain	2009	3,0
III Academic skills			
Scientific publishing	WGS, Wageningen University	2011	0,3
IV. Seminars and Workshop Presentations and Attendance			
Poster presentation at the international PAG conference	USA	2008	1,0
Oral presentation at International Drought Tolerance Symposium	CSU, Colorado	2010	1,0
Data management workshop organized by Generation Challenge Program (GCP)	Madrid, Spain	2010	1,0
Oral presentation at local seed business training organized by Wageningen International and Hawassa University	Hawassa University, Ethiopia	2008	1,0
Research seminars at TAD, CIAT, annual meeting of Tropical Legume Project		2007, 2010, 2011	2,0
Facilitation and presentation at advanced course in participatory plant breeding	Wageningen International, WUR	2007	2,0
Total			**41,8**