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Selection for drought and bruchid resistance of common bean populations

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Indice

Riassunto

Il fagiolo (*Phaseolus vulgaris* L.) è una leguminosa tra le più coltivate al mondo per il consumo umano, su una superficie di più di 14 milioni di ettari, ma fortemente limitata dalla siccità. Un altro dei fattori limitanti per il fagiolo è rappresentato dagli insetti che attaccano i semi, appartenenti ai coleotteri bruchidi. La comprensione dei meccanismi di resistenza alla siccità e agli insetti è utile per la selezione di varietà superiori. Inoltre la partecipazione dei coltivatori al processo di selezione è importante al fine di identificare le caratteristiche migliori di piante e semi. Gli obiettivi di questo studio sono: (i) condurre una valutazione fenotipica di 81 genotipi in relazione alla resistenza alla siccità; (ii) selezionare i genotipi migliori per resistenza alla siccità, produzione e caratteristiche commerciali; (iii) selezionare linee resistenti ai bruchidi anche mediante marcatori genetici associati al gene dell'arcelina; (iv) valutare i genotipi mediante la partecipazione dei coltivatori in Etiopia.

Nel primo studio sono state impiegate 78 linee, due parentali e un controllo (Awash melka) in condizioni di stress idrico e di irrigazione presso il centro etiope di Melkassa nel 2008 e nel 2009, secondo un disegno di blocchi randomizzati con tre repliche. Sono state analizzate le seguenti variabili: resa in seme, semi per baccello, peso di 100 semi, biomassa della pianta, LAI (leaf area index) e PHI (pod harvest index). I valori di resa in seme sono variati da 404 a 1580 kg/ha, con differenze significative tra i genotipi. I genotipi G80, G13, G19, G40, G87, G6, G28, G21,G24, G70, G22, G78, G60, G100 e G14 hanno dato risultati migliori in condizioni di stress idrico. I genotipi G78, G80, G6 e G19 e hanno dato buoni risultati anche con l'irrigazione, mostrando differenze significative anche per la biomassa della pianta. La resa in seme in condizioni di stress è correlata positivamente con il numero di semi per m^2, il numero di baccelli per m^2, il peso di 100 semi, la biomassa e il PHI.

Nel secondo studio sono state utilizzate in laboratorio 40 linee avanzate per la resistenza ai bruchidi, con 4 repliche di 30 semi ciascuna. Ogni gruppo di semi è stato infestato con 6 coppie del bruchide Zabrotes subfasciatus provenienti dall'allevamento presso il CIAT di Cali, Colombia. Due marcatori genetici microsatelliti sono stati utilizzati per la caratterizzazione delle linee in merito alla presenza del gene per l'arcelina, unitamente all'analisi della proteina stessa. Le stesse linee sono state infine utilizzate in una prova di campo condotta in Etiopia. I dati raccolti hanno riguardato variabili relative alla performance degli insetti e alla resa delle linee in campo. Le linee RAZ 4, RAZ 101, RAZ 173, RAZ 44 e RAZ 174 hanno mostrato una resistenza elevata per tutte le variabili considerate. Nel complesso la resa in campo è stata moderatamente più elevata per le linee suscettibili (2.11 t/ha, SE = 0.05) rispetto alle resistenti (1.8 t/ha, SE = 0.02). La resistenza è stata sempre associata alla presenza di una proteina da 35 kDa che rappresenta l'arcelina 1. I marcatori microsatellite BMy 11 e Pvatct 001 hanno confermato l'associazione con il gene per l'arcelina.

Nel terzo studio sono state condotte indagini con coltivatori etiopi nel 2008 (16 coltivatori) e nel 2009 (20 coltivatori) provenienti dalle aree di Boffa e Siredodota. Sono state utilizzate le prove del primo studio presso il centro di Melkassa, valutando sia le piante sia il prodotto. I semi sono stati inoltre mostrati a esportatori e commercianti per la valutazione di qualità. Nel 2008 tale processo ha portato alla selezione di 25 genotipi superiori, di cui 4 apprezzati commercialmente. Nel 2009 è stato individuato un sottogruppo di 12 genotipi, all'interno dei quali è stato possibile elencare i 5 migliori (G60,

G53, G40, G80 e G5) in relazione a misura, colore e forma del seme. I criteri di selezione sono variati tra coltivatori maschi e femmine. La valutazione commerciale, basata su caratteri in parte simili, ha portato alla selezione di tre linee (G40, G60, G80).

Summary

Common bean (*Phaseolus vulgaris* L.) is world's most important grain legume for human consumption and the crop is grown annually on more than 14 million hectares. Drought stress limits common bean production worldwide. Understanding drought resistance mechanisms and identifying key plant traits may help to select the superior performers of crop under drought stress. Storage insect attacks on stored beans are also known to be substantial all over the world. Understanding the resistance mechanisms to bruchid weevils and identifying resistant genes can help to develop resistant varieties. Participatory variety selection also helps to select genotypes that possess farmers preferred plant and grain traits. The main objectives of the study were (i) to conduct phenotypic evaluation of a set of 81 genotypes along with two parents for drought resistance and identifying key plant traits related to superior performance under drought stress; (ii) to select the most promising genotypes that combine drought resistance with seed yield and market potential;(iii) to select bruchid-resistant advanced lines and apply marker-assisted selection useful for the identification of arcelin gene; (iv) to evaluate bean genotypes using participatory variety selection.

In the first study, a total of 78 lines, two parents and one standard check (Awash melka) were evaluated under drought stress and irrigated (control) conditions at Melkassa research center (39^0 12'N and 8^0 24'E and 1550 meters above sea level) over two season (2008 and 2009) in Ethiopia. A 9x9 lattice experimental design with three replications (two rows of 3m long with 0.4m wide) was used. The seeds were planted at plant to plant distance of 10 cm. Data were taken on seed yield, seed number and pod number per plant, 100 seed weight, Shoot biomass, leaf area index (LAI) and pod harvest index (PHI). Data were analyzed using SAS 2002. Pearson correlation test and principal component analysis were used to determine the relation between and among measured variables. Significant ($P< 0.05$) genotypic differences were recorded in drought and irrigated conditions for grain yield, seeds per plant, pods per plant and 100 seed weight. The mean values of yield for the 81 lines ranged from 404 to 1580 kg/ha grown under moisture stress, while in the irrigated conditions, yield ranged from 1560 to 3985 kg/ha. Genotypes G80,G13, G19, G40, G87, G6, G28, G21,G24, G70, G22, G78, G60, G100 and G14 performed better under drought stress, and they also showed higher values for seeds per plant and pods per plant. Genotypes G78, G80, G6 and G19 were found to be responsive to irrigated conditions. Significant differences among genotypes for their LAI and PHI values were found under drought condition but a significance difference for canopy biomass was only found under irrigated conditions. Canopy biomass under drought conditions was higher with genotypes such as G80, G6, G87, G76 and G58 compared with the poor lines G16, G35 and G101. Genotype G103, G70, G2, G105, G74, G69, and G49 had significantly better LAI value than the standard check (Awash melka) and SxB 405 under drought conditions. There were also higher PHI recorded for G24, G78, G19, G14, G72, G60, G13, G100 and G87. Grain yield under drought conditions was positively correlated with seed number per m^2, pod number per m^2, 100 seed weight, canopy biomass and PHI. Genotypes such as G14, G21, G28, G60, G22, G24, G19, G78, G40 and G6 had positive association with grain yield, seed number, pod number, 100 seed weight and PHI.

In the second study, a set of 40 advanced lines of RAZ (resistance against zabrotes) and susceptible commercial varieties were tested for bruchid resistance using four

replicates of 30 seeds. Each replicate of advanced lines and commercial varieties at 10% seed moisture was infested with 6 pairs of newly emerged Mexican bean weevil (*Zabrotes subfasciatus)* from the stock rearing of CIAT Colombia. Two microsatellite markers analysis were used for the marker assisted selection scheme and protein analysis was done for presence or absence of arcelin. A field trial was also conducted in Ethiopia. Data were collected on number of eggs at 15 days, number of emerged adults, percentage emergence, adult dry weight and yield. RAZ 4, RAZ 101, RAZ 173, RAZ 44 and RAZ 174 showed consistently high resistance for all the parameters measured. The average yield of susceptible varieties (2.11 t/ha, SE = 0.05) was moderately higher than that of the resistant lines (1.8 t/ha, SE = 0.02). Arcelin protein analysis of 21 highly resistant advanced lines and 5 susceptible varieties together with the controls also showed a high level of accuracy. Resistance was associated entirely with the presence of the heavy 35KDa band representing Arcelin 1. The molecular markers BMy 11 and Pvatct 001 confirmed that they are more tightly associated with the arcelin gene and they produced bands that were 208 and 192 bp long for resistance lines.

In the third study, a total of 16 farmers were invited in the 2008 season and 20 farmers in the 2009 season from Boffa and Siredodota areas to Melkassa research farm in Ethiopia to evaluate the 80 genotypes of common beans at podding and maturity growth stages. Seeds of selected genotypes were exposed to exporters and traders for quality assessment. A total of 25 genotypes were selected in 2008, both individually and in a group by farmers. Four genotypes were selected by exporters and traders. In 2009, a total of 12 genotypes from a total of 25 were selected by farmers from the two sites. Farmers from Boffa as well as from Siredodota conducted a last group selection of the genotypes under field conditions and ranked the top five genotypes (G60, G53, G40, G80 and G5) in terms of seed size, contrasting color and contrasting shape. The main selection criteria used by male farmers from both Boffa and Siredodota were grain yield, drought resistance, earliness, pod load, vegetative vigor, pod filling, marketability and color (brilliance). Female farmers also used their own selection criteria, grain yield, drought, earliness, pod load, color (brilliance) and suitability for stew. Exporters and traders evaluated and selected G40, G60, and G80. Exporters' and traders' selection criteria were seed size, color, shape, split seed, slightly stained (anthracnose) and moisture content of the seed. The study conducted over two years implied that there is a need to combine the classical breeding with participatory variety selection for effective and efficient selection of bean genotypes under drought conditions. Insect bioassay should also be supported by marker assisted selection for identification of better resistant genotypes to bruchids.

Irrigated field at Melkassa Station, Ethiopia

Drought stressed field at Melkassa Station, Ethiopia

Preparing plants for DNA (left) and drought (right) experiment at CIAT Colombia

Bean sampling at mid-pod filling at Melkassa Station, Ethiopia

Dr Rao (CIAT) (left) and Prof Battisti (right) visiting Melkassa station, Ethiopia

Samples for biomass assessment

Leaf area index measuring and harvesting

Soil sampling and soil moisture measurement

Chapter I

Introduction

General Introduction

Common bean (*Phaseolus vulgaris* L.) is world's most important food legume for human consumption with annual production value of over U.S $ 10 billion (Rao, 2001). Production is principally in the Latin America and eastern and southern Africa, where three quarters of 12 million metric tons of the world production is grown annually (Beebe et al., 2009). Dry bean is grown annually on more than 14 million hectares (Singh, 1999). It provides an inexpensive source of protein for low income consumers. The consumption of dry bean is high in east, central and southern Africa (Rao, 2001). Although less important as source of calorie than cereals, beans supply important carbohydrates as well (Graham et al., 2007). It complements cereals and other carbohydrate rich foods in providing complete nutrition to people of all ages (Singh, 1999). Beans are grown under different cropping systems ranging from modern mechanized, irrigated and intensive production of monoculture as well as under different relay, strip, and intercropping with maize, other cereals, sugar cane and coffee throughout the world (Rao, 2001; Singh, 1999).

Mechanism and traits related to drought resistance in bean

Drought stress limits common bean (*Phaseolus vulgaris* L.) production worldwide (Wortmann et al., 1998). Intermittent and terminal droughts are the major kinds of drought and are endemic to some parts of Africa and Latin America where beans are commonly produced as major crop for local consumption. As much as 60 % of the common bean production areas in the developing countries are estimated to suffer from moderate to severe drought, and this makes the drought stress as the second most important constraint next to diseases to seed yield (Rao, 2001). The level of yield reduction is determined by the type, intensity and duration of drought stress (Thung and Rao, 1999). In some semi arid area of Eastern and Southern Africa, drought is the major problem reducing the mean seed yield by 50 % or more (Wortmann, 1998). As a result, the average world yields of common beans is less than 900 kg/ha (Singh 2001).

Genotypic differences among varieties based on the seed yield under drought stress conditions have been reported for common bean (White et al., 1994; Teran and Singh, 2002; Beebe et al., 2008). Drought stress is known to significantly reduce the individual yield components (number of pods per plant, number of seeds per plant, seeds per pod and seed weight), resulting thereby in reduction of common bean seed yield. These traits are mostly affected when drought stress is imposed during post flowering stage. The yield component mostly affected during the drought stress period is the pods per plant with about 63.3 % reduction followed by seeds per pod (28.9 %) and seed weight (22.3 %). Under drought stress conditions, differences among bean genotypes have been observed for shoot biomass accumulation (Ramirez-Vallejo and Kelly, 1998; Rosales-Serna et al., 2004; Rao et al., 2009). Positive associations have been confirmed between canopy biomass and seed yield under drought conditions (White et al., 1994; Ramirez-Vallejo and Kelly, 1998; Polania et al., 2008; Rao et al., 2009). According to Rao et al. (2009) and Beebe et al. (2009) in addition to shoot biomass accumulation, leaf area index (LAI) and pod harvest index (PHI) have strong positive correlation with seed yield under drought conditions. Pod harvest index is considered as one of the key partitioning indices that measure the remobilization of photosynthates to seed. According to Beebe et al. (2009), PHI reflects plant efficiency in partitioning of photosynthates from vegetative shoot structures to pods and from pod wall to grain, which varies with the genotypes and is affected by drought. Strong positive associations have been reported between PHI and grain yield under drought stress and non stress conditions (Polani et al., 2008; Beebe et al., 2009; Rao et al., 2009).

Bruchids resistance mechanisms and application of microsatellite markers

Economical losses due to insect attacks on stored beans are known to be substantial all over the world. Losses are higher in third world countries where good storage facilities are not usually available (Schoonhoven and Cardona, 1986). Economically important bruchid species affecting beans are Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) and the bean bruchid, *Acanthoscelides obtectus* (Say) (Schoonhoven and Cardona, 1982). *Z. subfasciatus* is a pest confined to warmer areas and is a storage pest, while *A. obtectus* is found in colder areas (higher altitude or latitude) infesting beans in the field and storage. Together these insect pests are estimated to cause an average of 13% grain loss to bean crops grown in developing countries (Cardona and Kornegay, 1999). *Z. subfasciatus* is the dominant species of storage insect in Eastern and Central Africa (Nchimbi and Misangu, 2002). The damage caused by *Z. subfasciatus* in farm storage was reported to be 38.1%, varying between 3.2% up to 80% at some locations, causing serious problem to most farmers in Ethiopia (Ferede, 1994). Arcelin, a lectin-like protein, present in wild bean accessions is the factor responsible for resistance to the Mexican bean weevil (Osborn et al., 1988). To date, seven variants of Arcelin have been discovered and these variants are all highly similar but provide different levels of resistance (Osborne et al., 1988, Lioi and Bollini, 1989 and Acosta-Gallegos et al., 1998). Arcelin is inherited as a monogenic dominant trait that provides the highest level of resistance to bruchids when it is in the homozygous state while with heterozygous Arc^{+}/Arc^{-} state individual seed is less resistant than with Arc^{+}/Arc^{+} (Kornegay et.al., 1993). Researchers at CIAT (International Centre for Tropical Agriculture, Bean Programme based at Cali, Colombia) have used the Arc1 variant widely in their breeding program to create resistant breeding lines known as RAZ (Resistant against Zabrotes) through back crossing (Cardona et al., 1990). In addition to bioassay for bruchids resistance identification there are additional methods such as using protein analysis and DNA based markers. The advantage of using the microsatellites over the time-consuming protein based selection is that it is rapid and has high precision.

Participatory variety selection in common bean

Participatory variety selection is thought to have the potential to identify more crop varieties that are better adapted to farmers' local area or quality requirement with in shorter time and has also been addressing the needs of diversified range of users, widen farmers' knowledge to speed up the selection in short time, accelerating dissemination and enhancing cultivar diversity through farmer involvement (Mekbibe, 1997). It can also minimize the number of unacceptable varieties and maximize the number of options available to farmers (Habtu et al., 2006). Exporters and traders deal with different seed quality and their involvement at varietal selection before release is crucial since they are the one who bridge the consumers and producers. The level of farmers and exporters/ traders involvement for participatory variety selection is somehow different but it should be at their own and personal efforts. Therefore, including information on farmers' and traders' perspective of plant and seed grain character preferences to these criteria will be beneficial to the variety selection process. In this regard, cost can be minimized and adoption rates increased if the farmers are exposed fully to participate in variety testing and selection To make participatory research effective, the plant breeders, farmers and traders should work together to test the range of new bean varieties that can fit farmers and consumers preferences.

The main objectives of this study were (i) to conduct phenotypic evaluation of a set of 81 common bean genotypes along with two parents for drought resistance and identifying key plant traits related to superior performance under drought stress; (ii) to select the most promising genotypes that combine drought resistance with seed yield and market potential; (iii) to select bruchid-resistant advanced lines and apply marker-assisted selection useful for the identification of arcelin gene; and (iv) to evaluate bean genotypes using farmer participation.

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Chapter II

Improving drought resistance in white pea bean (*Phaseolus vulgaris* L.) with market potential

Introduction

Drought stress limits common bean (*Phaseolus vulgaris* L.) production worldwide (Wortmann et al., 1998). Intermittent and terminal droughts are the major kinds of drought and are endemic to some parts of Africa and Latin America where beans are commonly produced as major crop for local consumption. As much as 60 % of the common bean production area in the developing countries is estimated to suffer from moderate to severe drought, and this makes the drought stress as the second most important constarint next to diseases to seed yield (Rao, 2001). The level of yield reduction is determined by the type, intensity and duration of drought stress (Thung and Rao, 1999). In some semi arid area of Eastern and Southern Africa, drought is the major problem reducing the mean seed yield by 50 % or more (Wortmann, 1998). As a result, the average world yields of common beans is less than 900 kg/ha (Thung and Rao, 1999).

Early work on moisture stress tolerance in common beans, mainly focused on grain yield for identifying drought resistant genotypes from evaluation using both drought and non stress conditions (White and Singh, 1991; Thung and Rao, 1999; Teran and Singh, 2002). There is a strong argument that combination of yield components with shoot attributes can help to identify better performing genotypes under drought conditions. In addition to yield components and plant attributes, the efficiency of partitioning of photosynthates to grain yield has also been the reliable approach to identify drought resistant genotypes under moisture stress conditions (Beebe et al., 2008; Rao et al., 2009; Beebe et al., 2009). Identification of of plant attributes that contributes to greater seed yield under drought stress conditions can contribute to genetic improvement of drought resistance in common bean. However, progress in breeding using physiological traits or mechanisms for improving drought resistace has been slow for most field crops (Rao, 2001). Nevertheless, some reports on mobilization of photosyhtnthates (from leaves and stem to pods, and from pod walls to grain) (Beebe et al., 2008, Rao et al., 2009; Beebe et al., 2009), biomass accumulation and distribution under drought stress (Rosales-Serna et al., 2004) have proven the merit of this approach. In common beans, better understanding of the key adaptive morphological and physiological traits and mechanisms that are associated with growth, biomass partitioning and yield under drought stress conditions can contribute to development of rapid and reliable selection criteria that are needed to identify drought resistant genotypes.

Genotypic differences among varieties based on the seed yield under drought stress conditions have been reported for common bean (White et al., 1994; Teran and Singh, 2002; Beebe et al., 2008). Drought stress is known to significantly reduce the individual yield components (number of pods per plant, number of seeds per plant, seeds per pod and seed weight), there by resulting in reduction of common bean seed yield. These traits are mostly affected when drought stress is imposed during post flowering stage. The yield component mostly affected during the drought stress period is the pods per plant with about 63.3 % reduction followed by seeds per pod (28.9 %) and seed weight (22.3 %). This indicates the relative importance of each component on seed yield depending on timing (crop growth stage), intensity and duration of drought stress (Barrios et al., 2005).

Deep rooting ability identified as akey mechanism for improving drought resistance in a number of field crops including common beans (Sponchiado et al., 1989; Rao, 2001). Shoot biomass accumulation is also considered as one of the plant traits useful to identify drought resistant genotypes (White et al., 1994; Rosales-Serna et al., 2004; Rao et al., 2009). Under drought stress conditions, differences among bean genotypes have been observed for shoot biomass accumulation (Ramirez-Vallejo and Kelly, 1998; Rosales-Serna et al., 2004; Rao et al., 2009). Positive associations have been confirmed between canopy biomass and seed yield under drought conditions (White et al., 1994; Ramirez-Vallejo and Kelly, 1998; Polania et al., 2008; Rao et al., 2009). Recently, another plant attribute was found to be useful to identify drought resistant genotypes was efficient biomass partitioning to from vegetative structures to pod and from pod wall to seed (Polania et al., 2008; Beebe et al., 2009; Rao et al., 2009). According to Rao et al. (2009) and Beebe et al. (2009) in addition to shoot biomass accumulation, leaf area index and pod harvest index have strong positive correlation with seed yield under drought conditions. This may be because of some genotypes producing greater leaf area to generate more photosynthates and partition a greater proportion of these assimilates to grain under drought conditions (Polania et al., 2008; Beebe et al., 2009; Rao et al., 2009). Pod harvest index is considered as one of the key partitioning indices that measure the remobilization of photosythates to seed. According to Beebe et al. (2009), pod harvest index (PHI) reflects plant efficiency in partitioning of photosynthates from vegetative shoot structures to pods and from pod wall to grain, which varies with the genotypes and is affected by drought. Strong positive association have been reported between PHI and grain yield under drought stress and non stress conditions ((Polani et al., 2008; Beebe et al., 2009; Rao et al., 2009).

Although there is significant progress in improving drought resistance of small seeded Mesoamerican beans, the progress with large seeded Andean is limited (Beebe et al., 2008; Beebe et al., 2009). Like other bean market classes, the white pea bean has also produced in drought affected part of Africa and central America; it is mainly produced for canning process.

The objectives of this study were: (i) to conduct phenotypic evaluation of a set of 81 genotypes along with two parents for drought resistance; (ii) to identify key plant traits related to superior performance under drought stress; (iii) to select the most promising genotypes that combine drought resistance with seed yield and market potential.

Materials and Methods

Location

A field experiment was conducted at Melkassa research farm, Ethiopian Institute of Agricultural Research, (39^0 12'N and 8^0 24'E and 1550 meters above sea level), with average annual rainfall of 750mm maximum and minimum temperature of 28 and 14^0C respectively. The soil texture of the field site was sandy loam with the pH 7.6

Plant materials and experimental design

One white pea bean genotype, ICA Bunsi was crossed with SXB 405 to generate a population segregating for drought resistance and possessing commercial quality white pea bean seed. SXB 405 from the International Center for Tropical Agriculture (CIAT) breeding program combines drought resistance with resistance to diseases, especially common bacterial blight (CBB) and possesses a type II growth habit. ICA Bunsi was developed by the Instituto Colombiano Agropecuario (Colombian Agricultural Institute) for canning and was released as Awash-1 in Ethiopia. Additional traits such as, high yielding potential, commercial seed type and disease resistance were considered in the selection of parents to improve the value for the East and Central Africa region of any beneficial small white pea bean genotypes resulting from this work.

The original cross made between ICA Bunsi and SXB 405 in 2007 at CIAT Palmira, Colombia. The crosses were advanced by Single Seed Descent (SSD). The final single plant selection was made in the F4 generation and the seeds from each F4:6 genotypes were harvested in bulk. The F4:6 seed was shipped to Melkassa, Ethiopia in 2008 and was immediately planted to increase the quantity of seed without selection. A total of 78 lines (coded as G1 to G105), two parents and one standard check (Awash melka) were selected and produced for testing (Appendix 1). The advanced yield tests of a selected group of lines, parents and standard check were evaluated under moisture stress and non stress (control) at Melkassa research center in 2008 and 2009. The experiments were hand planted on 27 September 2008 for first season and on 22 March 2009 for second season. A 9x9 experimental design lattice with three replications of two rows plots of 3m long with 0.4m between rows was used. The seeds were planted at an intra row spacing of 10 cm. Seedlings were thinned to one per hill ten days after emergence. Di-ammonium phosphate (DAP) fertilizer was applied during planting at the rate of 100kg/ha. Gramineus weeds and broad leaf weeds that emerged after sowing were controlled by hand weeding.

Data collection

Biomass and seed yield

A 0.5 m segment from each plot was sampled to measure dry weights of leaf biomass, stem biomass and pods and reproductive structures at mid pod filling. Biomass was oven dried at 70^{0}C for 48 hour (Rao et al., 2007). Dry weight of stem biomass, pod biomass, pod wall and seed biomass were determined by harvesting a 0.5m of row at maturity and then oven dried it at 70 ^{0}C for 48 hours. Number of seeds per plant and pods per plant were counted on 4 randomly selected plants within the sampling area at the harvest. One hundred seeds were randomly selected from those harvested from each plot and these were used to determine 100 seed weight. Grain yield was determined for each plot, and later corrected to 10% moisture content. Pod harvest index (dry weight of seed/ dry weight of pod at harvest x 100), canopy biomass (leaves + stem + pods + seeds), leaf area index (leaf area per unit ground surface) and seed number per area (seed/m^2) were computed using the CIAT protocol (Rao et al., 2007)

Irrigation schedule and management

In both years, a preirrigation of approximately 38.5 mm was applied to every row in each plot, three days before seeding both for drought and irrigated experiment. A second irrigation was applied, also to every row, to encourage a good plant stand after emergence. Irrigations were supplemented by furrow using flume; flow measuring device for open channel where water travels through a restriction and the flow rate is determined using the water height on a staff gauge. Water balance for each application was calculated based on excess or deficit water in the bean root zone relative to field capacity. Rain fall was minimal in both years (Table 1). Soil moisture measurements made at depths of 0-30, 31-60 and 61-90 cm at 10 days interval between planting and physiological maturity. Irrigation was applied when the root zone water deficit equaled the maximum allowable depletion of the available soil water. The soil moisture was monitored gravimetrically by oven drying the soil sample at 105 ^{0}C for 24 hours to obtain the dry weight for each sampled plot. The soil moisture content was calculated as percent by weight using the formula, ((Wet weight-dry weight)/dry weight) x 100. For drought treatment a total of 3 irrigations were applied (each 38.5 mm) and supplemental irrigation was suspended after 80 % of each plot flowered until the crop was physiologically mature. But the control experiment was kept irrigated until physiological maturity, and a total of 6 irrigations were given (each 38.5 mm).

Data Analysis

All data collected and derived were statistically analyzed using SAS program. Effects of treatments were estimated using analysis of variance (ANOVA) and means were computed using the least significant Difference (LSD) at P=0.05. The relationships between selected parameters were investigated using the pearson's correlation test (level of probability at 5% and 1%). The biplot display of principal component analysis was used to determine the relationship between more than two variables.

Table 1: weather and soil data during the growing period

year		Temperature (^{0}C)		Panevaporation (mm)	Relative humidity (%)	Sunshine hours (h/day)	Soil temperature (^{0}C). at depth(cm)				Soil class	pH
	Rainfall(mm)	Max	Min				5	10	50	100	sandy loam	7.6
2008	112.2	27.3	11.4	200.2	52.8	8.9	23.5	22.7	23.8	24.3		
2009	89.7	31.1	17.8	268.6	41.5	8.8	28.0	27.2	27.6	27.1		
Mean	100.9	29.2	14.6	234.4	47.1	8.8	25.8	24.9	25.7	25.7		

Results

Total Rainfall, Supplemental irrigation and Temperature

During the crop growing season, average maximum and minimum air temperatures in 2008 were 27.3 and 11.4 ^{0}C and in 2009 were 31.1 and 17.8 ^{0}C respectively. Total rainfall during the crop growth period was 112.2 mm in 2008 and 89.7mm in 2009. This occurred as sporadic rains at planting and during the vegetative stage and served as residual moisture for the bean crop. The pan evaporation was 200.2 mm in 2008 and 268.9 in 2009(Table 1). The plots under irrigated environment received a total 329.5 mm including both rainfall and supplemental irrigation (6 irrigations) where as the plots under drought environment received 215.1 mm with 3 irrigations. Drought stress influenced the grain yield and several other shoot traits of each line (Tables 2 and 3).

Yield and yield components

Significant ($P < 0.05$) genotypic differences were recorded in drought and irrigated conditions for grain yield, seeds per plant, pods per plant and 100 seed weight (Table 2). The mean values of yield for the 81 lines ranged from 404 to 1580 kg/ha grown under moisture stress, while in the irrigated conditions, yield ranged from 1560 to 3985 kg/ha (Table 2). Seeds per plant for the same 81 genotypes ranged from 38 to 110.2 under drought and from 66.1 to 150.1 under irrigated conditions. Mean values for pods per plant ranged from 9.4 to 21.8 under drought and 14.9 to 32.1 under irrigated conditions. Similarly mean values of 100 seed weight ranged from 15.9 to 24.2 under drought and from 18.5 to 27.3 under irrigated conditions. Genotypes G80,G13, G19, G40, G87, G6, G28, G21,G24, G70 and G22 performed better under drought stress with seed yields ranging from 1436 to 1580 kg/ha compared with the standard check(Awash melka) with 1072 kg/ha, and they also showed higher values for seeds per plant and pods per plant (Table 2). These lines had mean values of 100 seed weight ranging from 19.4 to 21.5 g under drought conditions; they are therefore classified as small seeded types. The less adapted genotypes such as G103, G101 and G10 ranked below both the trial mean and the standard check for the grain yield, seeds per plant and pods per plant under drought conditions. Genotypes G78, G80, G6 and G19 were also responsive under irrigated conditions

Table 2: Combined mean values over two years of 81 genotypes for grain yield (GY), seeds/plant, pods/plant and 100 seed wt (g) under drought and irrigated conditions in 2008 and 2009 at Melkassa, Ethiopia.

		Irrigated				**Drought**		
Genotype	**GY(kg/ha)**	**Seeds/plant**	**Pods/plant**	**100seed Wt(g)**	**GY(kg/ha)**	**seeds/plant**	**Pods/plant**	**100 seed wt(g)**
G1	3019	93.3	23.9	18.9	1100	47.9	12.4	15.9
G2	3628	83.2	18.4	19.6	1189	47.4	10.8	19.8
G3	3427	89.2	16.2	24.5	1149	64.4	12.1	20.5
G4	2776	98.3	19.2	24.1	1055	110.2	19.4	20.6
G5	2857	104.9	19.9	22.8	1366	72.7	16.1	21.9
G6	3468	128.9	26.9	22.0	1495	89.5	21.8	20.4
G7	2429	89.6	17.8	22.1	1117	58.1	13.9	18.7
G8	3155	76.5	20.6	22.2	1093	88.0	14.9	19.7
G9	2511	108.2	22.8	26.1	1112	59.6	15.1	22.8
G10	2931	117.5	25.7	22.2	932	53.3	12.1	19.6
G11	2947	146.6	24.9	23.9	979	44.7	10.9	19.2
G12	3312	91.1	19.5	20.3	1166	62.1	13.2	19.0

	Irrigated				Drought			
Genotype	**GY(kg/ha)**	**Seeds/plant**	**Pods/plant**	**100seed Wt(g)**	**GY(kg/ha)**	**seeds/plant**	**Pods/plant**	**100 seed wt(g)**
G13	3236	107.4	23.9	21.6	1555	86.7	20.2	19.4
G14	3470	112.3	23.3	21.0	1292	91.2	18.3	19.8
G15	3301	103.4	22.6	21.8	1120	60.2	16.1	19.9
G16	2911	95.7	22.5	20.6	848	66.3	15.7	19.0
G17	2685	71.1	15.3	22.9	1107	54.9	13.9	20.6
G18	3180	86.4	19.0	23.8	1211	63.6	13.1	20.1
G19	3455	115.7	23.2	22.9	1535	78.8	18.6	19.8
G21	3203	95.2	20.5	22.3	1477	88.4	20.3	20.5
G22	2879	66.3	16.6	23.2	1436	90.5	17.5	20.7
G23	3365	104.7	22.8	19.8	1028	55.5	12.8	18.0
G24	2871	109.6	25.8	22.2	1445	88.5	20.1	20.5
G26	2491	96.4	23.2	21.5	1238	46.1	13.6	19.7
G27	2869	118.3	25.0	19.2	1393	73.5	15.3	18.7
G28	3244	97.5	23.1	21.4	1491	84.6	19.8	20.0
G30	3915	83.7	17.5	23.4	1263	55.6	13.0	21.6
G31	2896	128.6	31.4	22.0	1320	81.4	18.6	20.4
G32	3397	66.1	18.6	23.1	1228	54.9	12.3	21.7
G34	3685	72.4	18.0	23.5	1255	53.0	12.8	19.5
G35	2668	67.5	16.3	22.6	1024	49.1	18.9	21.7
G36	3092	118.2	20.5	20.5	1173	59.7	13.3	18.5
G37	3545	106.6	20.8	21.8	1207	49.4	9.4	18.5
G38	2031	150.1	24.9	21.9	609	70.4	15.2	17.8
G39	2089	77.0	22.6	20.5	998	65.5	13.8	17.8
G40	3094	89.0	22.0	25.1	1524	74.5	18.1	21.3
G41	3711	103.7	24.3	21.8	1102	67.3	16.0	17.1
G43	3692	106.5	20.5	21.0	1150	43.4	11.9	18.3
G44	1710	120.9	25.3	20.1	672	78.5	17.0	18.2
G45	3073	69.2	16.3	21.6	1130	61.9	15.2	18.6
G46	3362	80.8	16.8	26.9	1202	60.8	13.1	24.2
G48	3525	102.6	19.3	23.9	1229	59.5	13.9	22.1
G49	3323	115.4	23.2	22.2	1400	65.4	14.5	18.8
G50	3846	130.1	28.1	25.5	1039	59.4	16.0	22.1
G51	3369	83.2	22.4	25.2	1200	54.0	13.5	22.4
G52	3340	126.4	25.2	20.2	966	47.3	11.8	18.6
G53	3767	75.3	17.4	21.8	1283	61.2	13.8	19.8
G54	3624	71.5	15.0	22.9	1236	52.5	11.8	20.2

	Irrigated				Drought			
Genotype	GY(kg/ha)	Seeds/plant	Pods/plant	100seed Wt(g)	GY(kg/ha)	seeds/plant	Pods/plant	100 seed wt(g)
G55	3442	95.6	20.7	20.9	1166	63.3	13.0	19.1
G56	2891	86.5	21.0	21.3	1248	62.2	16.2	19.0
G58	3206	123.3	24.7	21.4	1200	87.7	20.2	18.0
G60	3647	102.9	23.2	22.8	1380	82.5	20.1	20.9
G62	3049	115.7	25.9	21.4	1087	52.8	15.1	19.3
G69	3966	96.3	18.7	22.0	1182	51.9	12.9	20.5
G70	3142	110.9	24.3	24.8	1443	64.1	13.6	21.5
G72	3389	93.3	20.7	21.4	1342	77.6	17.9	20.2
G74	3366	75.1	21.7	22.3	976	79.8	20.3	20.1
G75	3985	98.6	26.0	20.2	1290	67.4	15.4	17.7
G76	2981	85.5	20.7	22.4	1281	78.3	16.8	20.6
G77	3305	82.5	19.3	24.1	1209	77.4	15.1	19.8
G78	3500	102.7	21.6	22.7	1391	83.5	19.2	19.9
G79	3671	94.2	18.9	20.8	1212	71.5	15.3	17.9
G80	3483	95.9	22.7	21.1	1580	84.6	19.7	20.2
G85	2859	75.2	18.0	22.6	1007	75.2	18.4	20.3
G86	3259	121.7	24.5	19.2	1063	66.6	19.9	16.7
G87	3300	102.0	22.5	22.6	1502	77.3	18.6	20.1
G88	3610	90.4	17.8	20.9	1099	77.5	14.9	18.0
G90	3621	125.8	32.1	20.6	1362	69.0	16.1	18.3
G92	3044	67.9	14.9	26.7	877	58.8	13.5	22.9
G95	3160	115.2	22.4	21.1	1074	67.7	16.3	18.7
G96	3548	104.7	24.0	22.6	1268	79.2	19.1	20.4
G97	3899	94.2	21.4	24.1	1337	74.4	18.4	20.9
G99	3319	110.4	24.3	22.3	1221	68.1	15.2	20.5
G100	3443	131.4	29.1	22.5	1295	75.0	18.3	20.2
G101	3325	73.3	18.6	21.6	912	38.0	13.7	18.1
G103	1560	110.7	20.4	20.2	404	57.4	15.3	17.4
G104	3082	92.0	16.7	19.7	993	51.2	12.9	17.7
G105	3251	116.8	22.0	20.0	1141	41.7	12.6	16.9
SXB 405	3289	87.1	17.0	27.3	1242	57.1	11.9	23.9
Am(check)	3330	74.7	23.3	18.5	1072	64.3	15.5	16.8
ICA Bunsi	3209	120.2	21.2	20.1	999	72.7	16.4	16.8
Mean	**3204**	**97.5**	**21.6**	**22.1**	**1185**	**66.7**	**15.5**	**19.7**
LSD(0.05)	**703**	**26.2**	**4.5**	**1.3**	**339**	**20.3**	**4.1**	**1.4**
CV	**23**	**24.3**	**19.4**	**5.3**	**18**	**24.8**	**21.7**	**6.3**

Plant attributes

Significant differences among genotypes for their leaf area index (LAI) and pod harvest index (PHI) were found under drought condition but a significance difference for canopy biomass was only found under irrigated condition (Table 3). The mean values of canopy biomass ranged from 2475 to 4756 kg/ha under drought while in irrigated condition, canopy biomass ranged from 3352 to 8154 kg/ha. The LAI of the same 81 genotypes ranged from 0.351 to 1.153 under drought and from 0.378 to 2.605 under irrigated conditions. Mean values for PHI ranged from 59.1 to 80.6 under drought and from 66.2 to 77.1 under irrigated condition (Table 3). Canopy biomass was higher in genotypes such as G80, G6, G87, G76 and G58 compared with the poor lines G16, G35 and G101 under drought condition. There was also high PHI recorded by G24, G78, G19, G14, G72, G60, G13, G100 and G87 where as G105, G36, and G101 were below average under drought condition. Genotype G103, G70, G2, G105, G74, G69, and G49 had significantly higher LAI than the standard check (Awash melka) and SXB 405 under drought condition. Similarly, genotypes G87and G19 had comparable LAI and better grain yield under drought conditions compared with standard check (Table 3). Despite low LAI for genotypes such as G13 and G80, their yield performance was better than the standard check. Genotype G56 and G4 recorded low LAI under drought conditions compared with the standard check.

Table 3: Mean values over two years of 81 genotypes for grain yield (GY), Leaf area index (LAI), canopy biomass (CB) and pod harvest index (PHI) under drought and irrigated in 2008 and 2009 at melkassa, Ethiopia

	Irrigated				**Drought**			
Treat	**GY**	**LAI**	**CB**	**PHI**	**GY**	**LAI**	**CB**	**PHI**
G1	3019	0.378	4979	71.4	1100	0.739	3429	71.0
G2	3628	1.280	3579	71.4	1189	1.057	3663	69.7
G3	3427	1.072	3893	72.6	1149	0.947	3546	69.2
G4	2776	0.894	5080	71.7	1055	0.354	3159	66.7
G5	2857	1.654	3743	73.6	1366	0.424	3513	72.3
G6	3468	1.192	5268	72.8	1495	0.387	4531	71.4
G7	2429	2.314	4391	72.9	1117	0.914	3098	68.4
G8	3155	0.945	7296	76.0	1093	0.392	3482	71.6

	Irrigated				Drought			
Treat	**GY**	**LAI**	**CB**	**PHI**	**GY**	**LAI**	**CB**	**PHI**
G9	2511	2.225	4392	68.7	1112	0.601	3251	65.0
G10	2931	0.566	5422	68.8	932	0.758	3561	65.3
G11	2947	1.366	4790	72.4	979	0.635	3738	74.9
G12	3312	0.852	4594	66.3	1166	0.915	3140	70.6
G13	3236	2.605	4423	71.1	1555	0.450	2788	77.3
G14	3470	2.428	6766	74.1	1292	0.759	3305	79.2
G15	3301	0.918	5503	73.1	1120	0.554	3418	62.5
G16	2911	1.145	4729	72.6	848	0.423	2475	65.4
G17	2685	0.698	6213	70.2	1107	0.701	3308	65.8
G18	3180	0.876	5918	71.6	1211	0.509	3151	65.3
G19	3455	0.969	6699	68.8	1535	0.862	3058	79.3
G21	3203	0.935	6747	67.6	1477	0.553	3345	69.3
G22	2879	0.598	3958	69.8	1436	0.599	3311	69.4
G23	3365	0.898	5974	74.5	1028	0.498	2979	74.7
G24	2871	1.273	5733	73.0	1445	0.590	3403	80.6
G26	2491	1.098	4180	69.5	1238	0.714	3009	63.4
G27	2869	1.356	4423	71.4	1393	0.697	3480	65.7
G28	3244	0.941	5618	67.8	1491	0.422	3178	70.4
G30	3915	1.065	8154	71.3	1263	0.613	3628	71.1
G31	2896	1.323	5561	69.8	1320	0.647	3020	72.8
G32	3397	1.275	4995	71.7	1228	0.826	3727	71.3
G34	3685	1.204	5383	75.2	1255	0.866	3036	70.4
G35	2668	1.024	7281	70.7	1024	0.560	2495	67.6
G36	3092	0.753	5307	69.4	1173	0.522	2734	61.0
G37	3545	1.350	5154	75.4	1207	0.886	2810	72.9
G38	2031	1.260	4178	70.7	609	0.732	3149	68.9
G39	2089	1.266	3855	66.2	998	0.609	2640	65.0
G40	3094	0.878	4445	73.8	1524	0.546	3596	74.6
G41	3711	0.975	4243	74.6	1102	0.666	3338	67.8
G43	3692	0.875	5884	73.8	1150	0.498	2833	70.2
G44	1710	1.044	3703	70.7	672	0.561	3238	71.5
G45	3073	1.057	5115	70.4	1130	0.583	2648	66.0
G46	3362	1.400	5047	73.4	1202	0.644	3130	73.0
G48	3525	0.900	4091	74.2	1229	0.505	3619	73.7
G49	3323	1.603	4916	69.2	1400	1.000	3159	67.2
G50	3846	0.846	4650	71.8	1039	0.521	2889	73.6
G51	3369	0.793	3612	74.6	1200	0.496	2890	70.1
G52	3340	1.110	4955	71.6	966	0.591	2914	69.3
G53	3767	1.170	4395	76.3	1283	0.636	2723	71.1
G54	3624	0.690	7674	72.1	1236	0.436	2902	66.8

	Irrigated				Drought			
Treat	**GY**	**LAI**	**CB**	**PHI**	**GY**	**LAI**	**CB**	**PHI**
G55	3442	0.694	4022	69.5	1166	0.455	3022	73.7
G56	2891	0.574	6400	70.6	1248	0.351	2725	71.3
G58	3206	1.667	4442	72.4	1200	0.956	3787	66.8
G60	3647	1.415	3984	75.6	1380	0.736	3251	77.5
G62	3049	1.234	3852	71.1	1087	0.752	3222	67.1
G69	3966	1.634	6369	73.0	1182	1.008	3296	70.5
G70	3142	1.485	5587	74.1	1443	1.068	3703	72.0
G72	3389	1.302	4676	72.7	1342	0.885	2902	77.9
G74	3366	1.796	6181	71.1	976	1.041	2970	64.7
G75	3985	1.255	5771	71.3	1290	0.656	3035	65.7
G76	2981	1.212	6312	72.2	1281	0.785	4199	71.6
G77	3305	1.201	3645	72.2	1209	0.687	3762	69.8
G78	3500	1.320	5249	70.5	1391	0.808	3437	80.4
G79	3671	1.415	5029	72.3	1212	0.720	3040	71.3
G80	3483	0.870	6087	73.1	1580	0.426	4756	72.8
G85	2859	0.858	5827	72.1	1007	0.497	2691	66.5
G86	3259	1.180	5237	74.8	1063	0.690	3786	63.9
G87	3300	1.693	4798	72.0	1502	0.941	4278	75.4
G88	3610	1.479	4538	75.8	1099	0.772	3573	67.4
G90	3621	0.809	6334	74.4	1362	0.517	2829	70.0
G92	3044	1.577	3352	73.0	877	0.857	2843	64.7
G95	3160	1.394	4937	73.2	1074	0.711	3154	69.2
G96	3548	1.162	4903	73.4	1268	0.616	3218	73.5
G97	3899	1.696	5090	73.7	1337	0.946	3010	73.9
G99	3319	1.093	4015	69.4	1221	0.766	2965	65.6
G100	3443	1.016	4894	75.0	1295	0.684	3398	75.5
G101	3325	1.406	6024	70.5	912	0.911	2568	61.6
G103	1560	2.063	5407	69.2	404	1.153	3320	69.3
G104	3082	1.162	7488	77.1	993	0.739	3109	68.2
G105	3251	1.620	4852	72.1	1141	1.049	3062	59.1
SXB405	3289	1.428	4854	73.9	1242	0.805	2975	73.3
AM(check)	3330	1.052	3895	75.0	1072	0.568	2979	68.7
ICA Bunsi	3209	1.099	4055	74.8	999	0.573	2603	68.0
Mean	**3204**	**1.212**	**5124**	**72.1**	**1185**	**0.686**	**3221**	**70.0**
LSD(0.05)	**703**	**0.239**	**1271**	**NS**	**339**	**0.109**	**NS**	**5.4**
CV	**23**	**10.100**	**19**	**7.3**	**18**	**9.100**	**19**	**6.1**

Relationships between Drought seed yield and Irrigated seed yield

The mean grain yield of 81 genotypes under drought conditions was 1185 kg/ha compared with mean irrigated yield of 3204 kg/ha (Figure 1).The difference in drought versus irrigated yield was also wide for the two parents and the commercial check, and all the three were close to the respective treatment average. Among the genotypes (advanced lines) tested, the lines G6, G13, G19, G21, G28,G70, G80 and G87 performed better in drought conditions and they had significantly higher yield than Commercial ICA Bunsi parent and standard check (Awash melka). These lines were also responsive to irrigation (Figure 1). The lines G103, G38, G44, G16, G92, and G10 were poor performers under both drought and irrigated conditions (Figure 1). Among 78 advanced lines 29 of them performed marginally better than the donor parent (SXB 405) under drought condition. The performance of the standard check (Awash melka) under drought was poorer than normally expected.

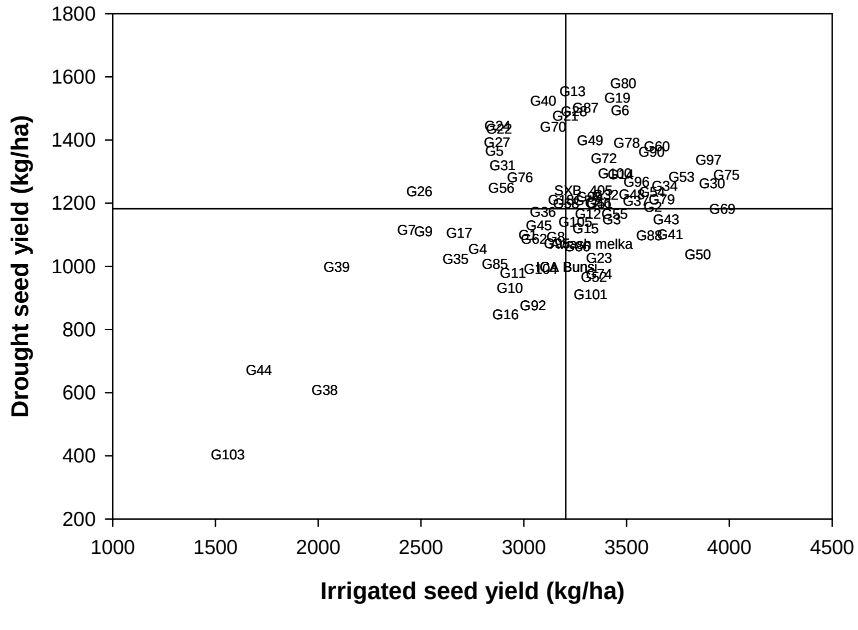

Figure 1: Yield (kg/ha) of genotypes under conditions of drought and irrigation in a sandy loam soil at Melkassa, Ethiopia. Superior yielding genotypes under both drought and irrigated conditions appear in the upper, right hand quadrant

Relationship between Drought seed yield and Drought canopy biomass

Genotypic differences were observed under drought conditions in canopy biomass (leaves + stem + pods + seeds) production at- mid pod filling growth stage (Figure 2). The mean canopy biomass value under drought condition was 3221 kg/ha. Among the tested genotypes of common beans, G80, G6, G87, G76 G40, G28, G21, G24, G22, G27, G60, G100 and G14 had relatively good biomass and grain yield compared with standard check (Awash melka) and parent (SXB 405) under drought conditions (Figure 2). About 69 % of the total 81 genotypes gave better biomass production than the standard check (Awash melka) ranging from 3009 (G26) to 4756 (G80) kg/ha. Compared with the other lines, G16, G35 and G101 had lower canopy biomass production and grain yield under drought condition (Figure 2). Relative to irrigated conditions, drought stress caused significant reduction in above ground biomass production. Likewise, drought stress imposed at early pod filling stage of the crop significantly reduced canopy biomass of the 81 genotypes (Table 3). It is also important to note that the genotype G13 and G19 with much lower value of canopy biomass yielded similar to the genotype G80.

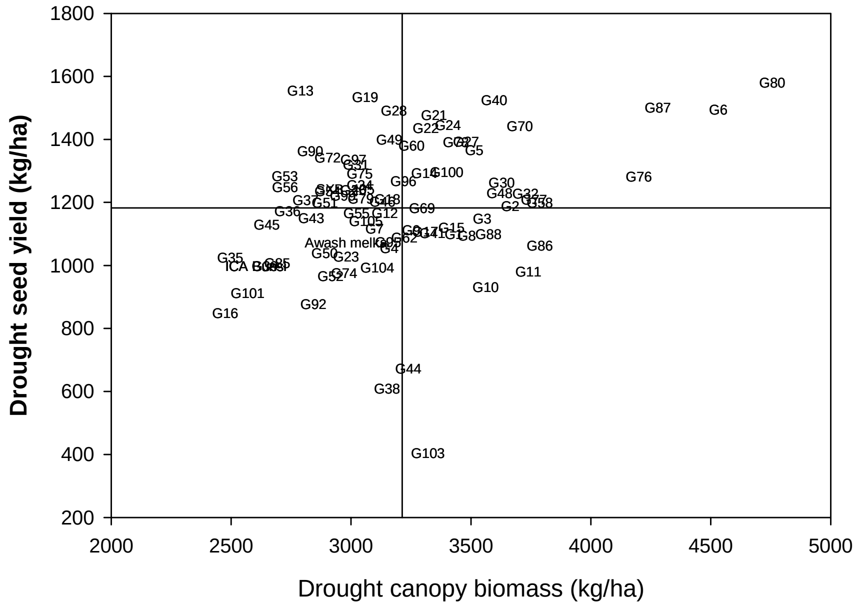

Figure 2: Relationship between seed yield and canopy biomass (vigor) under drought conditions in a sandy loam soil at Melkassa, Ethiopia. Genotypes with greater vigor and seed yield appear in the upper, right quadrant.

Relationship between drought seed yield and drought leaf area index

There was significant (P <0.05) genotypic differences recorded both in LAI and grain yield of 81 genotypes under drought conditions (ANOVA not shown). The relation of LAI and grain yield showed that G70, G49, G87, G97, G72, G19, G78, G60 and G14 had relatively better LAI with good grain yield under drought condition compared with standard check (Awash melka) and SXB 405(Figure 3). Comparatively, lower values of LAI were recorded for G80, G13, G6, G28, G40, G21, G24 and G22. It is interesting that these lines achieved greater seed yield with low LAI. This shows that they were able to assimilate photosynthates and remobilize them to grain under low LAI. Genotype G44 and G16 performed poorly both in grain yield and LAI.

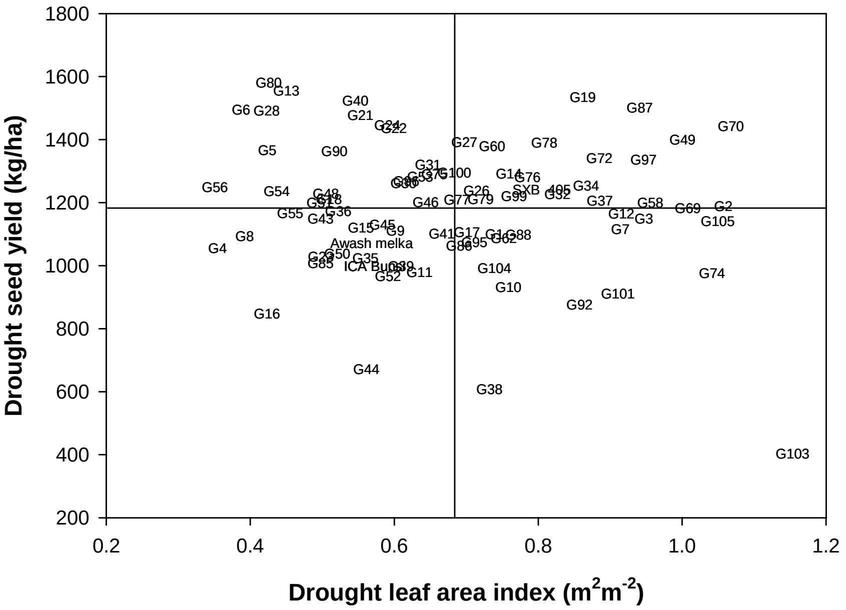

Figure 3: The relationship between seed yield and leaf area index under drought stress in a sandy loam soil at Melkassa, Ethiopia. Genotypes with higher leaf area index and seed yield appear in the upper; right hand quadrant.

Relationship between drought seed yield and drought pod harvest index

Pod harvest index (PHI) is the measure of mobilization of photosynthates from pod wall to seed formation (CIAT, 2008). The relationship between drought PHI and drought grain yield showed that G19, G13, G24, G78, G14, G72, G60, G100, G87, G40, G80, G70, G6 and G28 were superior in maintaining greater values of PHI under drought stress conditions. Similarly genotype G21 and G22 were better in reallocating photosynthates to seeds compared with the standard check (Figure 4). About 25 % of the 81 genotypes showed greater values of PHI and seed yield under drought stress conditions. Six genotypes (G105, G101, G92, G16, G38 and G103) were markedly lower than the other genotypes in their ability to mobilize photosynthates from pod walls to seeds. These lines performed poorly in their PHI compared to the standard check (Awash melka) and SXB 405 under drought conditions.

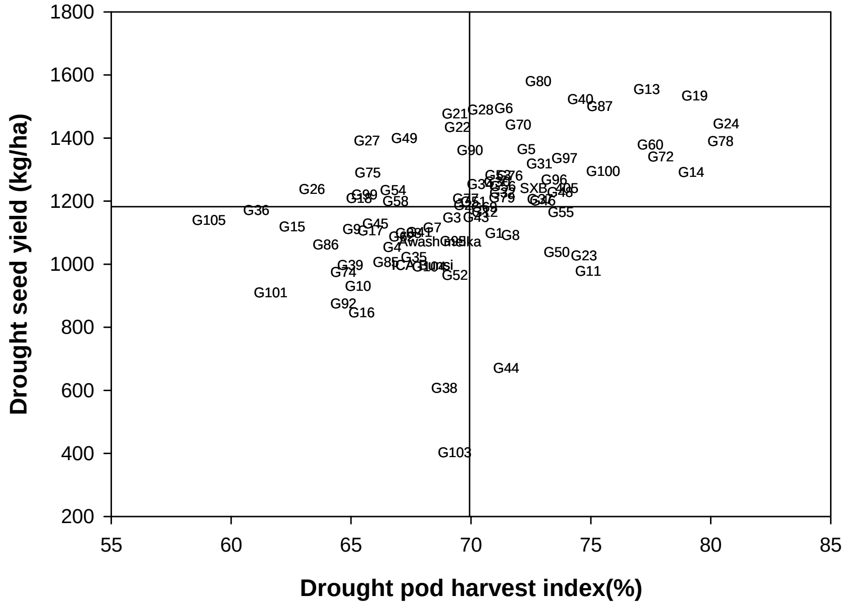

Figure 4: Relationship between pod harvest index and seed yield under drought conditions in a sandy soil loam at Melkassa, Ethiopia. Genotypes with greater pod harvest index and grain yield appear in the upper; right hand quadrant

Relationship between drought seed yield and drought seed number per area

Under drought stress conditions, the relationship between drought seed number per area and drought grain yield indicated that 13 genotypes (G6, G21, G28, G24, G22, G13, G60, G80, G72, G78, G19, G40 and G87) were outstanding in their seed number per area (Figure 5). They had also better performance than the standard check and SXB 405. Similarly G49, G27 and G100 had also better seed per area. 37 % of the 81 genotypes showed better adaptability for their number of seed per area compared with the standard check and SXB 405. Three advanced lines, such as G105, G101 and G103 produced lower number of seed per area.

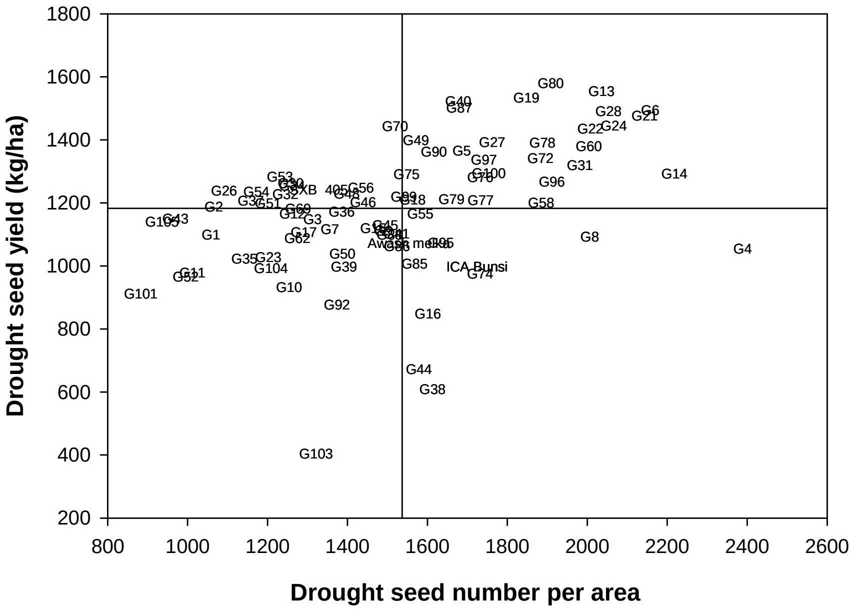

Figure 5: Relationship of seed number per area (seeds/m^2) with seed yield under drought conditions in a sandy loam soil at Melkassa, Ethiopia. Genotypes with higher grain yield and superior seed number per area (seeds/m^2) appear found in the upper; right quadrant

Correlation coefficients

Grain yield under drought conditions was positively correlated with seed number per m^2 (0.444**), pod number per area (0.368**), 100 seed weight (0.327**), canopy biomass (0.328**) and PHI (0.438**) (Table 4). PHI was the only trait positively correlated with the grain yield under irrigated conditions (0.409**) (Table 5). Seed number per area was strongly associated with pod number per area (0.822**), canopy biomass (0.268**) and PHI (0.395**) under drought conditions (Table 4). In addition, seed number was more strongly associated with pod number per area (0.747**) but negatively associated with 100 seed weight (-0.222*) under irrigated conditions (Table 5). Similarly, pod number per area under drought conditions was positively associated PHI (0.292**), but negatively correlated with 100 seed weight (-0.303**) under irrigated conditions. PHI was positively correlated with 100 seed weight (0.280*) and canopy biomass (0.232*) under drought conditions. LAI showed negative association with seed number per area (-0.265*).

Table 4: Correlation coefficient among leaf area index (LAI), seed number per area(SN), pod number per area (PN), 100 seed weight (100 SW), canopy biomass (CB), grain yield (GY) and pod harvest index (PHI) for 81 common bean genotypes evaluated at Melkassa, Ethiopia in 2008 and 2009 under drought conditions

Traits	LAI	SN	PN	100 SW	CB	GY	PHI
LAI							
SN	-0.265*						
PN	-0.208	0.822**					
100W	-0.098	0.149	0.092				
CB	0.108	0.268**	0.186	0.137			
GY	-0.126	0.444**	0.368**	0.327**	0.328**		
PHI	-0.051	0.395**	0.292**	0.280*	0.232*	0.438**	

* indicates significant at p<0.05

** indicates significant at P< 0.01

Table 5: Correlation coefficient among leaf area index (LAI), seed number per area (SN), pod number per area (PN), 100 seed weight (100 SW), canopy biomass (CB), grain yield (GY) and pod harvest index (PHI) for 81 common bean genotypes evaluated at Melkassa, Ethiopia in 2008 and 2009 under irrigated conditions

	LAI	SN	PN	100 SW	CB	GY	PHI
LAI							
SN	0.142						
PN	0.022	0.747**					
100W	0.088	-0.221*	-0.303**				
CB	-0.137	-0.152	-0.135	-0.073			
GY	-0.105	-0.102	-0.042	0.095	0.182		
PHI	0.075	-0.020	-0.054	0.066	0.020	0.409**	

* indicates significant at $P<0.05$

**indicates significant at $P<0.01$.

Principal component analysis

Principal component of plant traits under drought revealed that the first PCA explained 0.61 of the variation with grain yield, seed number, pod number, 100 seed weight and PHI demonstrating that all these traits had a positive relationship. Thus the first dimension showed the yield potential and drought resistance. Considering the yield dimension and positive value of this biplot, genotypes plotted in same direction had better yield under drought conditions (Figure 6). The second PCA explained 0.29% of the total variability and had positive correlation with LAI. Selection of genotypes that have high PCA1 and low PCA2 were considered as good yielders under drought conditions. Genotypes such as G14, G21, G28, G60, G22, G24, G19, G78, G40 and G6 had positive association with grain yield, seed number, pod number, 100 seed weight and PHI while genotypes G11, G105, G103, G101 and G10 were associated with less adaptation under drought conditions (Figure 6). Similarly, LAI negatively correlated with grain yield and was located in the opposite direction (Figure 6). Meanwhile, canopy biomass was somewhat associated with grain yield since drought resistance genotypes had better biomass production than drought susceptible genotypes. Biplot analysis under irrigated conditions showed that the grain yield, PHI and 100 seed weight had positive direction to the first PCA (PCA1) with that explained 0.74 of variability (Figure 7). These three traits showed highly positive correlation among each others. LAI, seed number and pod number per area showed positive association with each other and positive dimension toward second PCA (PCA2) with variability of 0.14 but negatively correlated with grain yield. Those genotypes found along the yield direction quadrant showed better yield than those found in opposite direction. Genotypes such as G34, G75, G69, G37 and G43 had good yield where as genotypes G44, G38, G103, G39, G11 and G7 performed poorly under irrigated conditions.

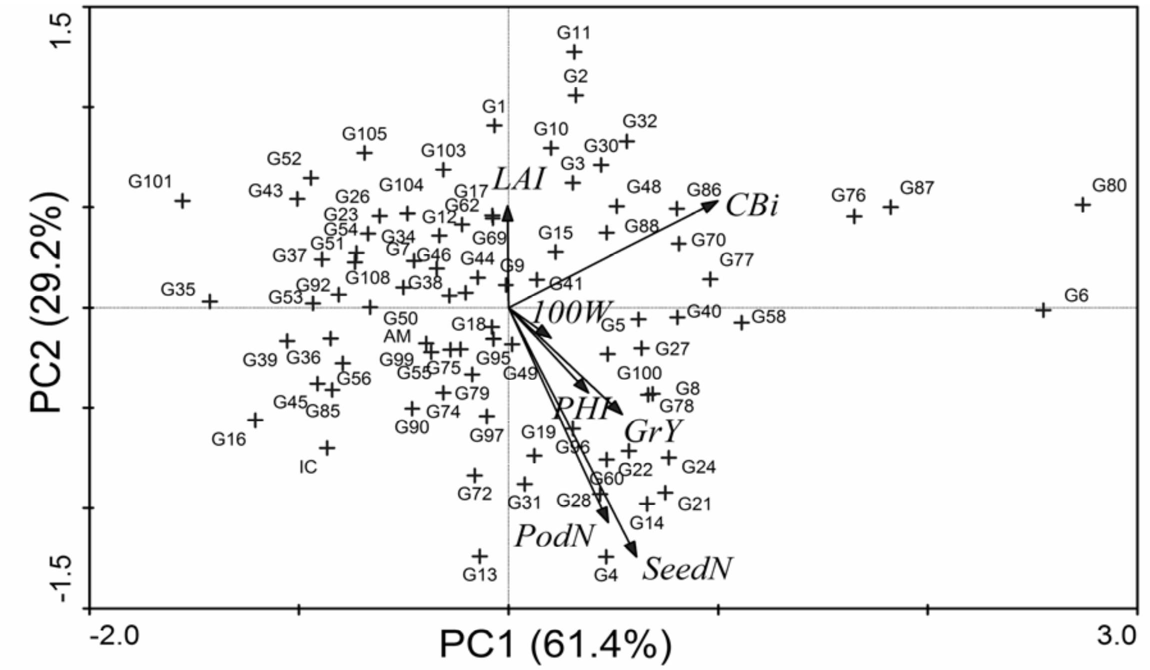

Figure 6: Principal component analysis (PCA) for grain yield (GrY), leaf area index (LAI),canopy biomass (CBi), 100 seed weight (100W), seed number per m^2(SeedN), pod number per m^2(PodN) and pod harvest index (PHI) under drought conditions over two years at Melkassa, Ethiopia

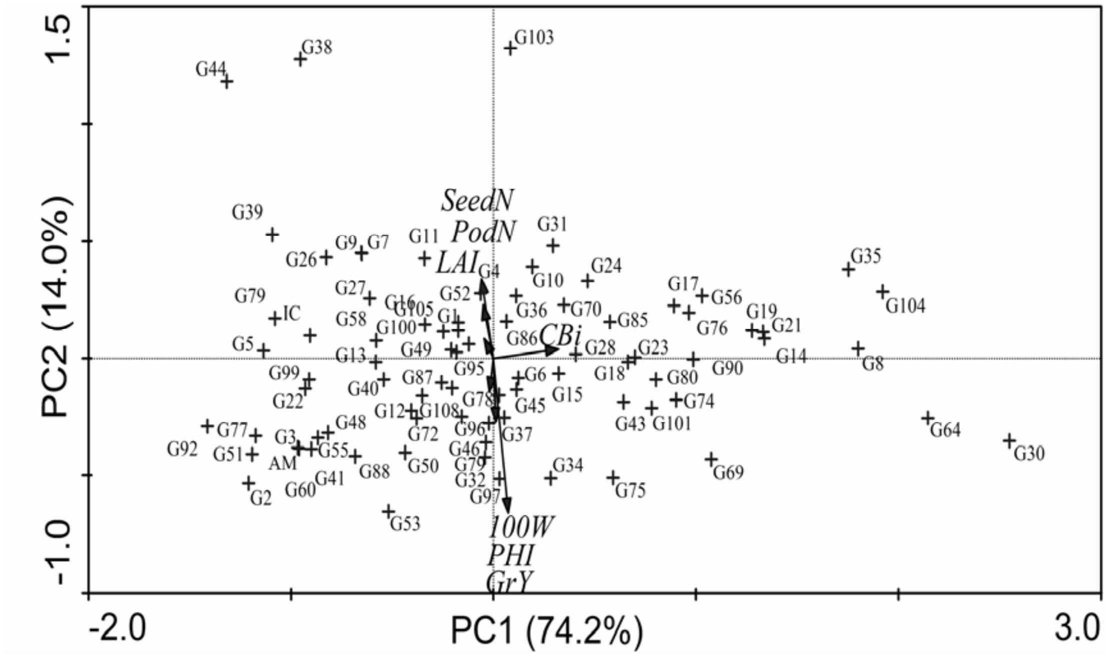

Figure 7: Principal component analysis (PCA) for grain yield (GrY), leaf area index (LAI),canopy biomass (CBi), 100 seed weight (100W), seed number per m^2(SeedN), pod number per m^2(PodN) and pod harvest index (PHI) under irrigated conditions over two years at Melkassa, Ethiopia

DISCUSSION

Seed Yield and Yield Components

The results from this two years study at Melkassa farm demonstrate the potential adaptation of common bean to semiarid environments in the central rift valley of Ethiopia where drought is the major constraint to bean production. The bean genotypes tested in this study showed marked genotypic variability in seed yield and yield components. Drought stress decreased the seed yield and affected yield components in all tested genotypes. Genotypic differences were observed in seed yield, seed number per plant, pod number per plant and 100 seed weight under both drought stress and irrigated conditions. Genotypes G78, G80, G6 and G19 had consistently higher values of grain yield, seed per plant and pod per plant both under drought stress and irrigated conditions. This indicates that the genotypes with greater sink strength (high values of pod and seed number per plant) responded better to drought stress and also yielded well under non stress conditions (Table 2). Conversely, G103, G38, G101 and G10 were confirmed as drought sensitive at Melkassa based on lower grain yield, seeds per plant and pods per plant. Similar results have been reported by Beebe et al. (2008) that the selected F6:8 genotypes gave two to three times better yield than the best check cultivar (Tio Canela) under terminal drought stress conditions. As expected, drought stress reduced mean values of the seed yield and these results are consistent with previous reports (White et al., 1994b; Teran and Singh, 2002b; Frahm et al., 2004). The reduction in pods per plant, number of seeds per pod and 100 seed weight due to drought contributed marked decrease in average grain yield (Acosta and Kohashi, 1989; Acosta and White, 1995; Barrios et al., 2005). Acosta and Kohashi (1989) found 42 and 50 % grain yield reduction for genotypes 'Bayo Cal-era and Ojo de Cabra' when drought was induced from vegetative stage to physiological maturity. A good level of consistency was observed for grain yield by the genotypes as indicated by the significant and positive association found between drought stress and irrigated growth conditions confirming previous reports of Frahm et al. (2004) and Beebe et al. (2008). Thus, if optimum performance and adaptation under drought are to be achieved then the genotypes should have greater yield potential under non stress condition.

Pod number per plant

Pod number per plant is an important trait of seed yield and the most affected yield component under stress conditions (Table 2). The reduction in pod number per plant observed in tested genotypes under drought conditions was due to adverse effect of drought at reproductive stage. Floral and pod development limitations due to drought stress in genotypes tested under drought condition resulted in lower pod and seed number per plant compared with the same genotypes that were tested under irrigated conditions , and this contributed to lower seed yield (Table 2). In line with the suggestions of Castaneda-Saucedo et al. (2009), the greater moisture stress during the reproductive stage exposed the plant to floral abortion and resulted in low seed yield. Maintenance of pod number under drought stress conditions in common bean is an important yield component that determines seed yield. Genotypes such as G6, G74, G21, G58, G13, and G24 were less affected by drought compared to standard check, SXB 405 and the other genotypes that were tested under drought conditions. These genotypes were also responsive to irrigated conditions (Table 2). A similar result reported by Martinez et al. (2007) that a genotype Orfeo was relatively less affected by drought for its number of pods per plant and seed yield compared with the cultivar Arroza Tuscola under the same water regimes. In our study, reduction in pod number per plant was observed in genotypes G37, G2 and G11. Drought stress imposed during flowering and pod setting causes flower and pod abortion (Barrios et al., 2005). Castaneda-Saucedo et al. (2009) also reported that the reduction in number of pods per plant during pod formation caused a loss of 40% in seed yield. Similarly, Martinez et al. (2007) indicated in his report that the reduction in number of pods per plant was observed in cultivars Arroza Tuscola and Barbucho. Studies by Singh (1995) and Sponchiado et al. (1989) also showed that moisture stress imposed at reproductive stage had an effect on an individual yield component. Drought susceptible genotypes during the 2008 and 2009 trails showed a slow and weak development of pod and seed setting as drought stress continued toward physiological maturity. This resulted in poor plant growth and failure to maintain good grain production during drought stress (Szilagyi, 2003). In line with these Barrios et al., 2005 reported that abortion and slow development of pods were common in drought susceptible genotypes when drought was imposed at pod setting stage. The total number of flowers in some susceptible varieties may be reduced up to 47% under drought conditions influencing the number of pods per plant (Lopez et al., 1996). However, pod setting itself may also vary among different common bean varieties in response to drought. In general, pod number per plant is the main yield component that is most affected by water deficit during flowering stage and can reduce seed yield up to 70% depending on the duration and severity of the drought period (Andriani et al., 1991).

Seed number per plant

The number of seeds per plant was affected by drought (Table 2). The two year combined analysis for seeds per plant showed significant differences among genotypes tested both under drought and irrigated conditions. A significant reduction of seeds per plant was found under drought conditions indicating that drought had an adverse effect on seed formation and filling of the bean genotypes. G4, G14, G22, G6, G24, G21, G8, G58, G13, G28, G80 and G60 had relatively more number of seeds per plant compared with standard check (Awash melka) and SXB 405. These genotypes with relatively better number of seeds per plant remobilized more assimilate from vegetative structures and pod walls to the seeds. A similar work reported by Beebe et al. (2009) and Rao et al.(2009) indicates that a high number of seeds/ha were produced and this could be due to remobilization of photosynthates from vegetative parts to pod wall and to seeds. Conversely, G101, G105, G43 and G11 produced the lowest relative seeds per plant (i.e. they were the most drought sensitive genotypes). This may be due to weak sink strength, which was largely responsible for low seed yield under drought conditions. Other studies have also shown that drought imposed during the reproductive development of faba bean can minimize the number of flowers, pods and number of seeds per pod (Xia, 1997; Loss and Siddique, 1997). Similarly, failure to tolerate abortion of flowers and pods due to drought was associated with reduced number of seed per plant in soybean (Andriani et al., 1991).

100 seed weight

Drought affected the seed size of the bean genotypes (Table 2) and this observation was in agreement with the previous reports on seed filling and seed quality of the check (Tio Canela) compared with the elite lines under drought stress(Beebe et al. 2008). Similarly, Teran and Singh (2002) reported that drought stress, on the average reduced common bean 100 seed weight by 13%. Seed yield reduction of up to 60 % observed in common beans under drought stress was attributed to losses of 63.3% in pods per plant, 28.9% in seed per pod and 22.3% in seed weight (Barrios et al., 2005).

Compared to the better seed size in drought resistant genotypes (ranged from 19.4 to 20.9 g 100 seed^{-1}), the standard check(Awash melka) and commercial ICA-Bunsi had lower seed size (16.8 g 100^{-1}) (Table 2). This is an advantage of drought resistance genotypes for commercial value of the harvested seed that can meet either domestic or international market requirements besides contributing to the yield and quality of the beans. Reduction caused by drought in 100 seed weight was also reported by White and Izquierdo (1991) and Acosta- Gallegos and Shebata (1989). Differences observed in seed size and quality in elite lines under drought stress in the present study (Table 2) supports the findings by Beebe et al. (2008) that the genotypes that maintain seed filling and grain quality are the drought resistant ones.

Response of shoot traits to drought stress

Canopy biomass

Shoot biomass accumulation is considered an important trait to attain good seed yield in grain legumes. Severe drought has an influence on biomass production of genotypes. Reduced biomass was seen in drought stressed genotypes of common beans but there were genotypes that produced relatively better biomass under drought stress conditions (Table 3; Figure 2). Significant and positive correlations between yield and shoot biomass were recorded under drought conditions over two years evaluations (Table 4). A few genotypes G80, G6, G87 and G76 showed the competitive advantage over sensitive genotypes (G16, G35 and G101) and the standard check (Awash melka) in maintaining relatively better yield during the same period under drought conditions (Table 3; Figure 2). This mainly due to their greater vigor as reflected by higher values of canopy biomass. These genotypes were contrasting to genotypes such as G13, G19 and G28 that yielded at similar level with much lower values of canopy biomass indicating the significance of remobilization of photosynthates stored in vegetative structures to reproductive parts. Therefore it appears that genotypes such as G80, G6, G87 and G76 have the needed vigor under drought stress but lack the greater ability to mobilize the photosynthates to grain. Our results agree with the reports on cowpea species in which higher biomass reduction was found for fast growing species than for slow growing counterparts when exposed to drought stress at early growth stage (Abayomic and Abidoye, 2009). According to CIAT (2007) and CIAT (2008), reduced shoot biomass due to moisture stress was observed in almost all genotypes of common beans. However, some genotypes showed better shoot biomass production than others. Korir et al. (2006) indicated that shoot biomass was significantly reduced by moisture stress after 28 and 42 days of emergence in all genotypes tested. They also reported that shoot biomass accumulation was lower at low moisture level and they concluded that the biomass was highly affected with level of water supplied to the bean plants. Biomass distribution/diversion to plant parts during early growth stage is considered as an adaptation response to drought stress of resistant genotypes (Specht et al., 2001). In addition to reduced growth of stems and branches of common beans, there was also leaf area reduction, decreased rate of new leaf production and decline in photosynthetic rate operating simultaneously and contributing to reduced growth and biomass accumulation (Jaleel et al., 2009; Rosales-Serna et al., 2004; Korir et al., 2006). In line with this and other published reports (CIAT, 2007; Beebe et al., 2009; Ramirez-Vallejo and Kelly, 1998; Rosales-Serna et al., 2004; Lei et al., 2009), we found that there are differences in biomass accumulation and allocations among bean cultivars under drought stress and those genotypes with efficient biomass partitioning to reproductive structures are primarily the better drought adapted than the genotypes with biomass accumulation ability *per se*. Our data, therefore, support that a strong association occurs between shoot biomass and grain yield in dry beans when grown under drought conditions with a few genotypes showing the greater ability to mobilize photosynthates to grain (CIAT, 2007; Rao et al. 2007; Beebe et al., 2008; Rao et al., 2009).

As it is shown in Table 4, a strong correlation was found between canopy biomass and seed number per area (seed/m^2) under drought stress conditions. Data supporting similar relationships of shoot biomass with grain yield under drought have been reported (CIAT, 2007; Beebe et al., 2009; Rao et al., 2009). Similarly, Rosales-Serna et al. (2004) indicated that there was positive and highly significant relationship between shoot biomass and seed yield under drought stress conditions. Ramirez-Vallejo and Kelly (1998) also indicated that total shoot biomass was significantly associated with grain yield and seed number in irrigated conditions but in our findings opposite results were obtained (Table 5).

Leaf area index

Leaf area index (LAI) is the leaf area per unit ground surface. This is basic to any analysis of stand growth or light interception, and especially to the performance of net photosynthesis at canopy level. In our experiment drought decreased the shoot biomass and the individual plant leaf area compared with irrigated thus the LAI in drought stress is lower in all genotypes (Table 3). Analysis of variance showed that the LAI was significantly different among genotypes tested both under drought stress and irrigated conditions. Among 81 genotypes tested G103, G70, G2, G105, G74, G69 and G49, these had significantly better LAI values than the standard check (Awash melka) and SXB 405, and except for G70 and G49, the rest had lower grain yield under drought conditions(Table 3 and Figure 3). This may be due to limited assimilate supply as well as more biomass accumulation ability rather than partitioning to reproductive structures (CIAT, 2007). A similar result was reported by CIAT (2002) indicating that ICA Pijao and SEA 17 had higher leaf area values but low yield than the drought resistant *P. acutifius* genotypes such as G 40068 and G 40159 indicating that mobilization of photosynthates to grain was limiting seed yield. In our findings, moderate reduction in LAI but good grain yield was recorded in genotypes such as G87, G19, G14, G78, G60 and G100 under drought stress conditions (Table 3 and Figure 3). This could be due to a remarkable increase in assimilating remobilization from vegetative parts of the plant to reproductive structures (Foster et al., 1995; CIAT, 2007; CIAT 2008; Rao et al., 2009). This is consistent with several reports that moderate to high values of LAI for some elite lines are indicative of superior adaptation to drought conditions (Rao et al., 2007; Rao et al., 2009).

The leaf area was higher at vegetative stage before drought was imposed on plants and as the available soil water was depleted from early pod filling to physiological maturity. Other researchers also observed a progressive reduction in LAI of common bean plants under drought stress (CIAT, 2007; Rao et al., 2009). Our results further indicate that genotypes G13 and G80 had lower values of LAI compared with the standard check (Awash melka) and SXB 405 but produced higher yield under drought conditions (Table 3 and Figure 3). The reason most likely is the strong assimilate export from vegetative parts of the plant to reproductive structures. Similarly Kenneth and Anthony (1980) reported that in spite of less leaf area due to severe drought during the podding stage, there are genotypes that mobilized reserves to reproductive structures. A similar report by Kenneth and Anthony (1980) indicates that increasing levels of drought resulted in a decreased leaf area, number of leaflets and average leaflet area. Reduced number and size of leaf or inhibited expansion of developing foliage under drought resulted in loss of leaf area, all these accounted for grain yield reduction (Rao, 2001)

In contrast to better performing genotypes in relationship to LAI and seed yield under drought conditions, G44 and G16 produced low grain yield and exhibited less LAI (Figure

3). This may be due to the greater sensitivity of leaf expansion process to drought stress that could result in reduction of biomass and grain yield under drought conditions (Rao et al., 2007). Three genotypes, DOR 390, Tio Canela 75' and BKI 11 adapted poorly among the 24 genotypes tested under drought stress conditions (CIAT, 2004). However resistant lines reduced their plant size, leaf area and LAI for moderating and reducing injury under drought stress. This is consistent with the work done by Barrios et al. (2005) showed that that there was significant differences observed among genotypes under moisture stress and non stress conditions. A similar report by Rao et al (2007) on common beans shows that greater value of LAI for some elite lines indicating adaptation to drought conditions. Similarly, George (2001) observed that with scarcity of water, the plants lack the raw materials for the synthesis of an extensive leaf system and LAI remains insufficient.

The results of this study shows that substantial improvement of leaf area occurred in advanced lines (represented by G70, G49, G87, G97, G19, G7 and G100) selected for adaptation to drought stress conditions compared with the poor adapted genotypes and standard check (Awash melka) (Table 3; Figure 3). Relatively better values of LAI under both water supply regimes for the advanced lines was associated with grain yield while a few lines such as G13, G19 and G28 took advantage of moderate LAI values to produce higher grain yield under drought stress. It is very likely that these three genotypes used less water to produce greater amounts of grain and therefore could be considered as efficient in water use (Blum, 2005). Further research work is needed to test the water use efficiency of these genotypes.

Pod harvest index

Pod harvest index (PHI) is one of the key partitioning indices that measure the remobilization of photosynthates to seeds (Beebe et al., 2009; Rao et al., 2009). Genotypic differences observed under drought conditions were related to the variation found in partitioning of photosynthates to seed or reproductive structures (Table 3 and Table 4). Following the approach used by Rao et al. (2007) and Beebe et al. (2009), the PHI was computed from dry seed yield/dry pod biomass X 100. PHI was significantly higher for G24, G78, G19 and G14 compared with drought parent (SXB 405) under drought conditions. During the same period the PHI for standard check (Awash melka) was lower than the drought parent (SXB 405) and some other potential genotypes (G60, G13, G100 and G87). The higher PHI of the four genotypes (G24, G78, G19 and G14) was accompanied by a remarkable increase in partitioning of photosynthates to seed (Table 3; Figure 4). On the other hand, the lower PHI under drought conditions resulted for less partitioning of photosynthates to seed for G105, G36 and G101. These results indicate that the drought susceptible genotypes (G105, G36 and G101) have genetically lower efficiency in partitioning of photosynthates to reproductive structure than the superior genotypes (G24, G78, G19 and G14), i.e. higher efficiency in remobilizing of photosynthates under drought stress conditions occurring during pod and seed filling coincide with important gains in PHI for drought resistant genotypes of common beans (Beebe et al., 2009; Rao et al, 2007; CIAT, 2008; Polania et al., 2008).

According to Beebe et al. (2009), PHI reflects plant efficiency in partitioning of photosynthates from vegetative shoot structures to pods and from pod wall to grain, which varies with the genotypes and is also affected by drought. In line with this, the mechanism that contributes to differences between resistant and sensitive genotypes is mainly associated to the selection made for efficient remobilizing of photosynthates to seed (Foster et al., 1995). Our research results support the conclusion that a strong association exist between PHI and the grain yield when genotypes grown under drought stress conditions (CIAT, 2008; Barrios et al., 2005; Rao et al., 2006; Rao et al., 2009).

As indicated from the relationship between PHI and grain yield under drought in Fig 3, the extent of yield reduction for drought sensitive genotypes was more marked than for the drought resistant ones. The lower yield for drought sensitive genotypes is due to restriction of efficient partitioning of photosynthates to pod and seed, therefore, drought resistant varieties can be more efficient in photoassimilate production and remobilization to grain (Samper and Adams, 1985). Similarly, grain yield under soil moisture stress is highly associated with the mechanism of dry matter partitioning and temporal biomass distribution (Kage et al., 2004). The bean genotype Pinto villa exhibited the highest average harvest index values, 0.36 in Texcoco and 0.67 in Cotaxtla, this is a marked difference interms of capability of the cultivar to redistribute stored assimilates to the seed(Rosales et al., 2004). The decreases in PHI (Table 3; Figure 5) observed for the tested genotypes (G105, G36 and G101) under moisture stress is within the range of that reported for advanced lines of common beans (CIAT, 2008). According to George (2001), high common bean yields were determined by high total dry matter production as well as high harvest index under drought conditions. Similar reports suggesting that improving harvest index is a viable strategy for crop improvement and also maintaining high heritability for harvest index is the selection for increased harvest index in common beans is high (Ludlow and Muchow, 1990; Ramirez-Vallejo and Kelly, 1998; Beebe et al., 2009).

Higher correlation of PHI with grain yield, seed number per area and pod number per area (Table 4) implied that the genotypes tested under drought conditions attained their higher yield mainly due to partitioning of photosynthates from vegetative part of the plant to seed. In addition, canopy biomass and 100 seed weight were associated with PHI under drought conditions. Data supporting similar relationship of PHI with grain yield, pod number per area, seed number per area, canopy biomass and 100 seed weight under drought have been reported (CIAT, 2007; CIAT 2008; Ramirez-Vallejo and Kelly, 1998). In our data, PHI was also positively correlated with grain yield under irrigated conditions indicating that the PHI is a key trait to quantify genotypic variation in mobilization of photosynthates to seed (Rao et al, 2007; Beebe et al., 2009; Rao et al.; 2009).

Selection based on combination of grain yield with yield components and other shoot attributes could contribute for progress in improving drought resistance of common beans (Beebe et al., 2009; Rao, 2001; Ramirez-Vallejo and Kelly, 1998). Principal component analysis was also useful to assess the relationship among different plant attributes at once and their comparison in each moisture regime for identifying the superior genotypes (Figure 6 & Figure 7). As is shown in Fig 6, the first principal component analysis indicated that grain yield, seed number per area, pod number area, 100 seed weight and PHI positive association with the seed yield. This indicates that the combination of these attributes and components toward the same and positive direction helps us to identify genotypes with high yield under drought stress (Figure 6) (Golabadi et al., 2006; Talebi et al., 2008). Unlike the yield dimension, LAI shows toward the second PCA and negatively correlated with grain yield and was located in opposite direction. This implies that the second dimension designated as drought sensitive dimension (Talebi et al., 2008). Therefore, genotypes in the same sector of the graph as LAI vector were inferior in their performance under drought stress. Consistent with our findings, Kaya et al. (2002) reported that the genotypes with larger first PCA and lower second PCA scores have better yields than those with lower first PCA and higher second PCA.

CONCLUSIONS

Increasing drought stress in the post flowering period of the bean crop significantly reduced the seed yield, pod number per plant and seed number per plant. However, the genotypes that performed better under drought conditions maintained better seed yield, pod number per plant and seed number per plant than the standard check (Awash melka) and donor parent (SXB 405). Compared with the drought susceptible genotypes, drought resistant genotypes maintain better sink strength (better number of pods and seeds per plant), which was mainly responsible for higher seed yield under drought stress conditions. Drought resistant genotypes also showed better 100 seed weight than the drought susceptible genotypes under drought conditions. These genotypes were also responsive to increased water availability under irrigated conditions. This feature confirms that drought resistant genotypes also have better advantage over drought sensitive genotypes under favorable conditions (like irrigation). Generally yield gain was recorded for resistant genotypes with better pods per plant, seeds per plant and 100 seed weight under drought conditions.

Drought stress decreased shoot biomass of the bean genotypes in the post flowering period, this resulted in a difference between drought resistance and drought susceptible genotypes. Drought resistant genotypes maintained better biomass accumulation and partitioning to reproductive organs than the drought sensitive genotypes under drought stress conditions as it was correlated to seed yield. Generally, there were differences found in biomass accumulation and allocations among bean genotypes tested under drought conditions and those genotypes with efficient biomass partitioning to reproductive structures were better adapted to drought stress. Thus strong partitioning of shoot biomass to reproductive structures contributed to increase seed in yield in dry beans.

LAI was lower in drought stressed conditions. In most drought resistant genotypes moderate reduction of LAI was found but better seed yield was recorded. This is because of the increase in efficiency of remobilizing assimilates from vegetative parts to plant reproductive structures. Thus drought resistant bean genotypes reduced their plant size; leaf area and LAI for moderating and reducing injury under sever moisture stress conditions. In addition to canopy biomass and LAI, PHI could also make substantial contribution for resistance of bean genotypes under drought conditions. Drought also affected the partitioning of assimilate from pod to seed but the resistant genotypes performed well under drought conditions. Drought resistant genotypes exhibited high PHI values with efficient partitioning of photosynthates from vegetative shoot structures to pod wall to seed under drought conditions.

The field evaluation of bean genotypes at Melkassa resulted in identification of 13 genotypes (G6, G78, G87, G100, G19, G13, G21, G22, G24, G28, G40, G79, and G80) that were better in their adaptation to drought stress conditions compared with standard check (Awash melka) and donor parent (SXB 405). The good performance of the genotypes under drought conditions was associated with grain yield, pod number per plant, seed number per plant, 100 seed weight, shoot biomass, LAI and PHI. G101 and G105 were poorly adapted under drought conditions. In the present study SXB 405 was considered as donor parent for drought trait. This is a line developed for Brazil with intention of combining resistance to drought and diseases (Steve Beebe personal communication). From our data we confirmed that most of the traits that contributed for drought resistance, remobilization of assimilates to grain and better sink strength to genotypes were from SXB 405 where as the other donor parent was ICA Bunsi (small white pea bean), which contributed traits like white color, small seed size and round shape. In summary, this work has confirmed that canopy biomass, LAI, and PHI are reliable plant traits for selection of drought resistant genotypes under drought conditions. Over two years, a strong correlation between seed yield, yield components and plant attributes have been observed in our drought research work. It has also demonstrated that simple and easily measured plant traits such as shoot biomass, LAI and PHI could be successfully used to select better performing genotypes under drought stress in common bean breeding program. The approach outlined in this research work shows that common bean breeders should be able to select drought resistant genotypes. Other traits might be studied for their possible relation and contribution to selection of drought resistance, for instance chlorophyll content, canopy temperature depression, soil moisture content and daily climate data might allow the bean breeder to more fully understand the selection process of drought resistant genotypes.

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Chapter III

Framers and traders participation in common bean genotype evaluation under drought conditions

Introduction

Selection of varieties in beans has usually been mainly based on seed yield. Different breeding lines have been developed and their performances evaluated at different environmental conditions over years to identify the best varieties for final release. The evaluation and selection activity which is mainly dominated by breeders takes more time to identify a given variety from nurseries to final yield trial. Nevertheless, the speed up of the variety releases for final use and their dissemination is slow. In addition, classical breeding programs are mainly designed for specific needs of different kinds of farmers and environments since a small farmer deals with a variable environment and multiple objectives that will affect his or her choice of varieties (Sperling and Loevinsohn’ 1996). Conversely, conventional breeder effectively performs in developing varieties that can be used fairly homogenous and stable environment, but less effective under more complex and drought prone environment.

Participatory variety selection is thought to have the potential to identify more number of crop varieties better adapted to farmers’ local area or quality requirement with in shorter time and has also been addressing the needs of diversified range of users, widen farmers’ knowledge to speed up the selection in short time, accelerating dissemination and enhancing cultivar diversity through farmer involvement. (Mekbibe, 1997). It can also minimize the number of unacceptable varieties and maximize the number of options available to farmers (Habtu et al., 2006).

Exporters and traders deal with different seed quality and their involvement at varietal selection before release is crucial since they are the one who bridge the consumers and producers. The level of farmers and exporters/ traders involvement for participatory variety selection is somehow different but it should be at their own and personal efforts. Therefore, including information on farmers’ perspective of plant and seed grain character preferences to these criteria will be beneficial to the variety selection process. In this regard, cost can be minimized and adoption rates increased if the farmers are exposed fully to participate in variety testing and selection (David and Sperling, 1999).

To make participatory research effective, the plant breeders, farmers and other concerned bodies should work together to test the range of new bean varieties that can fit farmers preferences and suit their socioeconomic situations. Therefore, the objective of the present research was to select diverse and productive common bean lines adapted to drought conditions and accepted by farmers', traders' and consumers' at large using farmers' indigenous knowledge and breeding scientific approach.

Materials and Methods

Site, Design and farmer selection

The standard experimental breeding plots were used for Participatory Variety Selection (PVS) in 2008 and independent plots were used in 2009 at Melkassa research farm, Ethiopia. Eight genotypes (advanced lines) were sown on a single replication plot using 9X9 lattice design under drought condition. Each genotype was planted in a plot of two rows and 3 m length. In November 2008, participant farmers were selected based on their indigenous knowledge of bean production and their willingness to participate in the PVS process. The farmers were from Boffa (17 km away from the research station) and Siredodota (10 km away), and have had a good collaboration with Ethiopian Institute of Agricultural research (EIAR) via a Japan International Cooperation agency (JICA) –funded project which catalyzes the formation of farmer research groups (FRGs) in the central rift valley. Farmer selection was also done with the help of district level extension workers who were more familiar with farmers.

Participatory variety selection

Participatory variety selection (PVS) was implemented in this study to select different white bean genotypes that possess farmer's plant and grain traits. Farmers were first asked to select among 80 genotypes(advanced lines). In the 2008 season 8 men and 2 women from Boffa and 6 men and 2 women from Siredodota(Table 1) were invited to Melkassa research farm to evaluate the 80 genotypes of common beans at two different crop growth stages (Appendix 1 description of bean genotypes) .

1. Podding stage: Farmers were advised to select by themselves and then in group to evaluate 78 advanced lines, a commercial parent (ICA Bunsi) and the standard check (Awash melka). At the beginning of the evaluation farmers gathered to discuss what they thought were important criteria for selecting a given line at a particular development stage. These criteria were ranked and the top ones, of prime importance to farmers, were used in a more detailed evaluation, line by line. These specific criteria used were: pod filling potential, pod load, biomass and disease reaction.

2. Physiological maturity: Farmers followed the same procedure as at podding stage, but with some criteria added, relevant to the stage of development of the plant: earliness, yield potential, drought resistance and uniform maturity. Informal interviews were made immediately after the field evaluation with three male and one female farmer from each site.

In 2009, the 25 genotypes selected by farmers in the previous season were planted separately from the breeding experiment. The planting was done in a plot of two rows and 3 m length per genotype and was treated under drought conditions. A total of 20 participant farmers (16 men and 4 women), including two new male farmers from Siredodota (Table 1), were invited to the station and they were asked to select among 25 genotypes. The evaluation was done at podding and at physiological maturity stage, evaluation was also done individually and in a group. A week after participant farmers made the selection, 4 non-participating farmers who were not grouped under bean FRG, were invited from Boffa and asked to give another independent evaluation (after having been explained of the PVS objectives). These farmers evaluated the same 25 genotypes at physiological maturity, using the same criteria as determined by participant farmers. Also in 2009, after harvesting the seed samples of selected genotypes were taken to farmers' village to display for final seed quality assessment.

Exporters and traders selection

Among 35 private companies involved in exporting beans, we selected Agricultural Commodity Supply (**ACOS)** and Omar Baobed, based on their high volume export capacity and experience at international market. Three traders (wholesalers and assemblers) were selected in addition to exporters. The wholesalers and assemblers were many in number: among these, we selected two wholesalers from Boffa (Yosef Reta and Mohamed

Beshir) and another two from Siredodota (Mohamedo Anowar and Kedir Chebsa). We also included one assembler from Boffa (Mokonnen w/Amanuel) and one from Siredodota (Bezabeh Tarekegn). Following harvesting and threshing, seed samples of the 25 genotypes in 2008 and 6 genotypes in 2009, which were selected by farmers under drought conditions, were taken to Agricultural Commodity Supply (ACOS) and Umer Baobed (bean exporters), to quality experts in Nazareth, and to wholesalers and assemblers at Boffa, and Siredodota. At the beginning in 2008, we explained that the 25 genotypes had been selected by farmers at different growth stages and under field conditions and they were also informed about the range of different white color groups included for the evaluations. The exporters and traders were asked their perceptions of the quality in terms of seed size, color, shape and overall appearance, based on standard requirements on the international market and specific requirements by importers and canners. At the end, samples of seeds were displayed at their own places of business. The same methods were followed for evaluating 6 genotypes in 2009.

Table 1: Number of farmer selector and non selector evaluators during the selection and evaluation stages at Melkassa, rift valley region, Ethiopia

Season	Boffa		Siredodota	
	Male	Female		
2008	8	2	6	2
2009	12	2	8	2

Results and Discussion

The farmers who participated and evaluated the genotypes generally had long experience in bean production. The farmers were most interested parameters like pod load, vigor/biomass, yield, drought resistance, earliness, and then the overall performance. In the 2008 season, 78 advanced lines, commercial ICA Bunsi and standard check (Awash melka) were considered for evaluation.

During the 2008 season the farmers evaluated both at pod and physiological maturity stage at Melkassa. Farmers responded positively to the new genotypes they evaluated. The selection by each farmer varied from a minimum of three to up to five genotypes and a total of 25 genotypes were selected in 2008, both individually and in a group (Table 2). Seven genotypes were more preferred among selected genotypes by Boffa and five genotypes were preferred by Siredodota. Genotypes evaluated and selected by farmers gave a yield range of (0.197 to 347 g/plot). Male farmers from Boffa showed more preference for vigour/biomass as animal feed was more important there than at Siredodota. Earliness was given more emphasis by Siredodota farmers than Boffa. Yields, resistance to drought and pod load were common for both groups. Disease evaluation was not given much emphasis since there was not economically important disease incidence in the dry season.

Table 2: Lists of genotypes selected by farmers and their characteristics

Genotype	**Seed color**	**Growth habit**	**Seed size**
G5	White(brilliant)	Type II	Small
G6	White (brilliant)	Type II	small
G14	White(brilliant)	Type II	Small
G18	White(dull)	Type II	small
G19	White(dull)	Type II	Small
G21	White(brilliant)	Type II	small
G22	White(brilliant)	Type II	Small
G24	White(dull)	Type II	small
G28	White(brilliant)	Type II	Small
G31	White(brilliant)	Type II	small
G32	White(brilliant)	Type II	Small
G40	White(brilliant)	Type II	Small
G53	White(dull)	Type II	small
G58	White(dull)	Type II	Small
G60	White(brilliant)	Type II	small
G74	White(brilliant)	Type II	Small

Genotype	Seed color	Growth habit	Seed size
G76	White(brilliant)	Type II	small
G79	White(dull)	Type II	Small
G80	White(brilliant)	Type II	small
G87	White(brilliant)	Type II	Small
G90	White(brilliant)	Type II	small
G97	White(brilliant)	Type II	Small
G100	White(brilliant)	Type II	small
G105	White(brilliant)	Type II	Small
Awash melka	White(dull)	Type II	small

In 2009, a total of 12 genotypes were selected among 25 by farmers from the two sites. In the same year 4 non participating farmers from Boffa were invited to station and evaluated the bean genotypes at physiological maturity and they selected 7 genotypes in common. These lines were G28, G60, G97, G40, G80, G5 and G87. These genotypes were also selected by participant farmers. Surprisingly, genotypes selected by female farmers were similar to those genotypes selected by male farmers from the two areas (Boffa and Siredodota) both in 2008 and 2009. But more preference was given for G6, which was preferred by 95 % of female farmers from both areas followed by G40. Qualitative discussions showed that both groups of female farmers gave priority to pod load and earliness, as these features are important for consumption and beans are the first food to become available after the annual "hungry gap". Women in the central rift valley usually mix and boil immature bean seed with maize (Nifro) and thus provide for their family home consumption.

Farmer selection for seed quality

In 2008, farmers were not given seeds of the selected genotypes for seed quality evaluation since there was an overall shortage of seed in the season. In 2009, after the farmers selected their best genotypes under field conditions, seeds of 12 genotypes were taken to one village where both groups of farmers came together for group evaluation of seed quality after beans had been harvested. In the same year, farmers from Boffa as well as from Siredodota made a last group selection and ranked the top five genotypes interms of seed size, contrasting color and contrasting shape among the genotypes selected under field conditions (Table 3). In terms of quality assessment, the role and contribution of women farmers was more important than that of male farmers. Half a kilogram of each genotype selected for seed quality was given to Boffa and Siredodota female farmer groups for further evaluation in food preparation, for *wasa* **(sauce)** or *wot* (local stew). Female farmers from the two areas found all genotypes to be acceptable for stew making but ranked G40), G5, G80, G53 and G60 in that order. Based on their evaluation of Shiro (flour made from white beans for making stew) and Kike (split beans), female farmers decided to use the beans for home consumption especially for preparation of stew (wot), as is the custom in Boffa and Siredodota areas for white pea beans. In 2009, farmers also requested seed of their preferred genotypes for testing under their local environment, so seeds of two genotypes per farmer were randomly given from 12 genotypes selected at the end of the 2009 since there was a shortage of seeds in the season. Five hundred (500) seeds were prepared from each line and given to all 20 farmers who participated in the evaluation process.

Table 3: Rating of genotypes selected by participant male and female farmers groups during seed quality assessment

Genotype	Seed size	Contrasting color	Contrasting shape	Total score
G60	1	2.1	1.7	1.0
G53	2.5	1.7	2.1	1.2
G40	1.7	3.3	1	1.3
G80	2.4	2.6	2.2	1.5
G5	1.3	3.2	2.5	1.8
G6	3.8	1.5	4.6	2.3
G87	4.2	2.5	3	2.8
G79	5	3.8	1.3	3.2
G97	1.3	4.6	3	3.5
G100	4.6	1.3	4.2	3.8
G28	2.1	4.2	3.3	4.2
G14	4.2	4.2	3.3	4.5

Score: (1-5) scale; 1= very good, 2=Good, 3=average, 4=poor, 5=very poor

Exporters and traders selection

In 2008, G14, G28, G60 and G100 were selected by exporters and traders, based on visual assessment (seed size, color/brilliance and shape) from among the genotypes displayed. In 2009, we followed the same procedure; six genotypes, including previously selected ones were evaluated. The two additional genotypes (G40 and G80) were initially rejected in 2008 due to their low moisture content but, in 2009, exporters and traders requested them to be evaluated together with the previously selected genotype from the 2008 season. G40, G60 and G80 were finally selected by exporters and traders.

Farmers selection criteria

Participatory variety selection (PVS) method contributed much to facilitate variety selection based on farmers' preference, and also to highlight key criteria. Based on the combined selections over two years, including field and seed quality evaluations, the main selection criteria used by male farmers from both Boffa and Siredodota were grain yield, drought resistance, earliness, pod load, vegetative vigor, pod filling, marketability and color(brilliance). Female farmers also used their own selection criteria, grain yield, drought, earliness, pod load, color (brilliance) and suitability for stew. Table 4 shows the frequency of use of different criteria for selection: four in particular proved important for both male and female farmers. Surprisingly, male farmers were much more concerned with vegetative vigor as compared with female farmers, especially those farmers from Boffa area. Meanwhile female farmers showed a preferred criterion for suitability for stew (50% citing as an important). Note that this last criterion is not taken in to account by formal breeder.

Table 4: Frequency (%) of selection criteria applied by male and female farmers at Melkassa, Ethiopia

Rank	Criteria	Male(16)	Female(4)	Total score
1	Yield	100	100	100
2	Drought	100	94	93
3	earliness	81	96	82
4	Pod load	87	75	78
5	Vegetative vigor	70	20	43
6	Pod filling	50	25	35
7	marketability	50	20	33
9	Color/brilliance	10	16	11
8	Suitability for stew	10	50	28

Exporters and traders selection criteria

The participant exporters, wholesalers, assemblers, and the non-participating exporters involved in the evaluation of the genotypes, used similar selection criteria across sites. Seed size, color, shape and overall appearance were the top criteria for grading the beans. They gave equal value for each criterion for the domestic market even though there is some acceptable limit for each criterion at international level. Exporters indicated that they have additional criteria when they purchase the product in bulk from wholesalers, big private and government organizations. These criteria focused on whether the beans were broken and split, uniformity (size and color), contrasting shape, staining (due to anthracnose) and moisture content (Table 5). However, these criteria are not fully applied when they purchase the bean from the small scale farmers. Instead they deduct the price of rejects (unwanted size, discolored and broken) by 5-20% from the normal price set on that day. They also further sort out the unwanted materials. At the domestic level, within Ethiopian households, the mechanisms for exchanging white beans based on quality standards are not well developed. The exchanges are done based mainly on visual detection only. However, when exported to the international markets, there is a grade set by the quality standard authority of Ethiopia, and the standards set by importing countries. These standards are S1 (first grade), S2 (second grade) and S3 (third grade). We were also told that first grade (S1) is mostly exported to Europe where as second (S2) and third grade (S3) are exported to Asian and African countries. Generally, the exporters ranked first and top priority both for broken/split seed and slightly stained seed by anthracnose and followed by seed size and color.

Table 5: Frequency (%) of selection criteria applied by exporters during bulk purchase

Description/criteria	Tolerance in %	score	Rank
Broken &split seed	Max,1	100	1
Seed size	460-550seeds/100gm	80	2
Seed color	Good white/brilliance		
Contrasting shape	Max, 1	70	3
Slightly stained seed/anthracnose	0.1	100	1
Moisture content	13-17	50	4

Conclusion

This research was initiated to evaluate genotypes using participatory variety selection(PVS) method in bean improvement under moisture stress conditions. As it has been demonstrated by farmers new and promising genotypes were identified using PVS methodology and this method contributed much to facilitate variety selection based on farmers' preference. PVS methodology clearly showed that farmers were capable of evaluating and identifying a number of drought resistant genotypes using more than 9 different selection criteria over two years. Most important farmers' selection criteria were identified under drought conditions. Farmers rated and selected genotypes such as G60, G53, G40, G80 and G5 not only for their drought resistance but also for seed and food quality (color, shape, size and stew making)

The exporters and traders in seed quality assessment demonstrated that they were able to make significant contribution to selecting market demanded genotypes. They also identified the bean genotypes by variety, and not by areas of adaptation. Among exporters and traders, there was common understanding about seed quality of beans with regard to seed quality demand in domestic and international market. Like farmers, exporters and traders also identified their selection criteria when they evaluated the white pea beans. In addition to seed size, color and shape, they use broken and split seed, slightly stained/ anthracnose and moisture content of the seed as selection criteria. Using their selection criteria, exporters and traders evaluated and selected genotypes such as G40, G60, and G80. In summary, the experience from this study implied that there is a need to combine the PVS with conventional breeding approach and it also demonstrates the potential for bringing together in an integrated fashion, the selection indices of farmers, exporters/traders and breeders.

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Chapter IV

Selection of bruchid resistance and yield testing of advanced breeding lines of common bean (*Phaseolus vulgaris* L.) in Ethiopia

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Selection of Bruchid Resistance and Yield Testing of Advanced Breeding Lines of Common bean (*Phaseolus vulgaris* L.) in Ethiopia

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Abstract

The study was carried out to select bruchid-resistant advanced lines of common bean crossed at International Centre for Tropical Agriculture (CIAT) and to evaluate yield performance under field conditions in Ethiopia. Forty advanced lines and commercial varieties were tested for resistance to *Z.subfasciatus* using four replicates of 30 seeds were infested with 6 pairs of newly emerged Mexican bean weevil (*Z.subfasciatus)* from the stock rearing of CIAT Colombia. Data were taken on number of eggs, number of emerged adults, and days to adult emergence (DAE) and adult dry weight. The insect bioassay and protein analysis were done at CIAT (Colombia) and the field evaluation was assessed in Ethiopia**.** The resistant lines containing Arcelin 1 were characterized for resistance to the Mexican bean weevil. RAZ 4, RAZ 101, RAZ 173, RAZ 44 and RAZ 174 showed consistently high resistance for all the parameters measured**.** The average yield of susceptible varieties (2.11t/ha, SE = 0.05) was moderately higher than that of the resistant lines (1.8 t/ha, SE = 0.02). The number of days to maturity varied between 78 and 96, without any significant difference between susceptible commercial varieties and RAZ lines. Using arcelin protein analysis with 21 highly resistant advanced lines and 5 susceptible varieties plus controls also showed a high level of accuracy. Resistance was associated entirely with the presence of the heavy 35KDa band representing Arcelin 1.

Key words: Dry bean, Bruchids, *Zabrotes subfasciatus*, resistance, arcelin

Introduction

Common beans (*Phaseolus vulgaris* L.) are an important crop for food, cash and agro-ecosystem improvement in the central, southern, eastern and western Ethiopia. According to the 2005 CSA (Central Statistic Agency of Ethiopia) data, this crop is grown by nearly 2.5 million small scale farmers on about 250,000-300,000 hectares with annual production near to 234,900 metric tonnes. It is often cultivated by smallholder farmers in small plots of land in association with other crops or as sole crop with low external input. It is rapidly progressing as an important export earning of the country in recent years. The crop also is playing significant role in smallholder economy being an important source of proteins and incomes as cash.

Economical losses due to insect attacks on stored beans are known to be substantial all over the world. Losses are higher in third world countries where good storage facilities are not usually available (Schoonhoven and Cardona, 1986). Economically important bruchid species affecting beans are Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) and the bean bruchid, *Acanthoscelides obtectus* (Say) (Schoonhoven and Cardona, 1982). *Z. subfasciatus* is a pest confined to warmer areas and is a storage pest, while *A. obtectus* is found in colder areas (higher altitude or latitude) infesting beans in the field and storage. Together these insect pests are estimated to cause an average of 13% grain loss to bean crops grown in developing countries (Cardona and Kornegay, 1999). *Z. subfasciatus* is the dominant species of storage insect in Eastern and Central Africa (Nchimbi and Misangu, 2002). The damage caused by *Z. subfasciatus* in farm storage was reported to be 38.1%, varying between 3.2% up to 80% at some locations, causing serious problem to most farmers in Ethiopia (Ferede, 1994).

It has been reported by many authors that insecticides applied as dusts for admixture of seeds or sprays, are effective for the control of bruchids (Tsedeke, 1995). Most of the pesticides used were effective but not applicable at farm level because the farmers' storage structures are not air tight for the use of fumigation and in general, it is not easy to mix insecticide with food grains for protection against insect. Under such circumstances, alternative methods like use of resistance varieties that could be easily utilized by farmers need to be considered.

Arcelin, a lectin-like protein, present in wild bean accessions is the factor responsible for resistance to the Mexican bean weevil (Osborn *et al.*, 1988). To date, seven variants of Arcelin have been discovered and these variants are all highly similar but provide different levels of resistance (Osborne *et al*., 1988, Lioi and Bollini, 1989 and Acosta-Gallegos *et al.*, 1998). Within the allelic series the level of resistance is progressively lower in the variants Arc5 > Arc4 > Arc1 > Arc2 > Arc6 > Arc3 when the background is the wild progenitor. However, in the cultivated background the alleles that provide the highest resistance are Arc1 > Arc2 > Arc5 > Arc3 > Arc4 (Cardona and Kornegay, 1999) with differences in resistance level thought to be due to protein differences or carbohydrate content (Harmsen *et al.*, 1987).

Arcelin is inherited as a monogenic dominant trait that provides the highest level of resistance to bruchids when in the homogenous state with heterozygous Arc^{+}/Arc^{-} individual seed less resistance than Arc^{+}/Arc^{+}(Kornegay *et.al.*,1993). The precise mode of action of arcelin is not known, but the arcelin negatively affects the digestion process in the larval gut, resulting in lower emergence rate and lighter weight of surviving adults (Minney *et al.*, 1990). Researchers at CIAT (International Centre for Tropical Agriculture, Bean Programme based at Cali, Colombia) have used the Arc1 variant widely in their breeding program to create resistant breeding lines known as RAZ (Resistant against Zabrotes) through back crossing (Cardona *et al*., 1990). The objective of this study was selection of bruchid-resistant advanced lines of common bean crossed at CIAT and their yield performance under field condition in Ethiopia.

Materials and Methods

Plant materials and Bruchid screening

A set of 40 advanced lines of RAZ and susceptible commercial varieties were used in the trial. Of these, 30 were RAZ Mesoamerican and Andeans genotypes crossed at CIAT; 10 were also Mesoamerican and Andean susceptible commercial varieties of different colour (table 1). Insects for this experiment were reared on the variety Calima and drawn from a mass culture maintained at CIAT in a controlled environment chamber (27^{0} and 70% RH). The 40 advanced lines and commercial varieties were tested for bruchid resistance using four replicates of 30 seeds. Each replicate of advanced lines and commercial varieties at 10% seed moisture was infested with 6 pairs of newly emerged Mexican bean weevil (*Z.subfasciatus)* from the stock rearing of CIAT Colombia. Beans and weevils were put into small mesh covered clear plastic vials whose walls were covered with sandpaper (No. 150, rough side of sand paper facing inwards), to avoid egg laying on the plastic surface rather than on the bean seed coat. Vials were incubated at 27°C and 70% relative humidity in a dark room. Data were collected on number of eggs at 15 days after infestation at which point initial insect parents were removed, number of emerged adults, percentage emergence and adult dry weight. In addition, to facilitate comparisons of resistance levels among genotypes, data on progeny per female and days to adult emergence were used to calculate Dobie's (1974) Index of susceptibility (IS).

$$IS = \frac{\log \text{progeny/female}}{\text{Days to adult emergence}} \times 100$$

Table 1: Resistant lines and susceptible varieties used in the experiment and their market classes.

Genotype	**Reaction to bruchids**	**SOURCE**	**COLOR**	**SIZE**
Amelka	S	Ethiopia	White	Small
Bat 41	S	Africa	Colored	Medium
Cal 96	S	Africa	Colored	Large
Cal 143	S	Africa	Colored	Large
Carioca	S	CIAT	Colored	Small
Emp 250	S	CIAT	Colored	Medium
Erico23	S	Ethiopia	White	Small
Ibunsi	S	CIAT	White	Small
Ipijao	S	CIAT	colored	Small
Pc 50	S	CIAT	Colored	Large
Raz 11	R	CIAT	White	Small
Raz 17	R	CIAT	Colored	Small
Raz 19	R	CIAT	White	Small
Raz 2	R	CIAT	White	Small
Raz 20	R	CIAT	Colored	Small
Raz 22	R	CIAT	White	Small
Raz 26	R	CIAT	White	Medium
Raz 34	R	CIAT	White	Small
Raz 36	R	CIAT	White	Small

Genotype	Reaction to bruchids	SOURCE	COLOR	SIZE
Raz 37	R	CIAT	White	Small
Raz 4	R	CIAT	Cream	Small
Raz 40	R	CIAT	White	Small
Raz 42	R	CIAT	White	Small
Raz 44	R	CIAT	White	Small
Raz 49	R	CIAT	Colored	Small
Raz 54	R	CIAT	Colored	Small
Raz 7	R	CIAT	White	Small
Raz 101	R	CIAT	Colored	Small
Raz 105	R	CIAT	Colored	Large
Raz 11-1	R	CIAT	White	Small
Raz 111	R	CIAT	White	Small
Raz 114	R	CIAT	White	Small
Raz 119	R	CIAT	White	Small
Raz 120	R	CIAT	White	Small
Raz 136	R	CIAT	White	Small
Raz 138	R	CIAT	White	Small
Raz 151	R	CIAT	Colored	Small
Raz 173	R	CIAT	White	Medium
Raz 174	R	CIAT	White	Medium
Raz 24-2	R	CIAT	colored	Large

CIAT: International Center for Tropical Agriculture. R: Resistance. S: Susceptible. Seed size is expressed as weight in grams of 100 randomly chosen seeds. Small: <25gm, medium: 25-40gm and large: >40gm.

Yield assessment and Statistical Analysis

The same advanced lines and commercial varieties used for the laboratory study were grown for yield evaluation in Ethiopia during 2008 cropping season at Melkassa Research Center (39^0 12'N and 8^0 24'E and 1550 meter above sea level). The test lines and varieties were laid in a randomized block design with four replications. The plot size was two rows of 3m length with 40 and 10cm between and within row spacing, respectively. Data were recorded for yield (t/ha), physiological maturity and moisture content at 10% adjustment. The analysis of variance (ANOVA) was used Statistica computer software with arcsin transformation of percentage data whenever necessary.

Protein extraction and Arcelin determination

The presence of the arcelin protein and the arcelin alleles were determined according to the methods described by Ma & Bliss (1978) where a 0.75g of bean flour was dissolved in 250µl of extraction buffer, vortexed and centrifuged at 14,000 for 15 minutes. The supernatant was transferred and mixed with 50µl cracking buffer, which was vortexed, boiled for 5 minutes, allowed to cool and centrifuged before loading 5µl on to a stacking polyacralymide gel. Samples were run at a constant 150 volts until the samples passed in to running gel where a constant 25 mA was maintained. Protein gels were stained for 4 to 5 hours in 120ml of 0.025% Coomassie Blue R-250, 45.4% methanol, 9.2% acetic acid, and 45.4% distilled water then transfer to distaining solutions (I: 10% acetic acid, 50% methanol and II: 7% acetic acid, 50% methanol) for approximately 4 to 5 hours.

Results

Bioassay and yield

The number of eggs laid on beans of each line differed significantly among genotypes ($F_{(39,120)} = 6.5$, $p < 0.01$), varying from 62.2 (SE 7.9) for RAZ 42 to 221.5 (SE 26.7) for RAZ 174(Figure 1). Days to adult emergence (DAE) also differed significantly among lines ($F_{(39,120)} = 23.5$ $p<0.01$), with susceptible commercial varieties clearly showed low number of days ranging from 31 to 34 whereas the resistant lines did prolong DAE (Figure 1). The percentage of adult emerged showed significant difference among genotypes tested ($F_{(39,120)} = 229.0$). Percentage of unemerged adults was higher on RAZ 4, RAZ 120, RAZ 42, RAZ 101, RAZ 173, RAZ 119, RAZ 44, RAZ 174, and RAZ 151 and a considerable variation in emergence rate was observed also among resistant lines, with values ranging from 33.4% to 0.7% (Figure 2). A significantly higher emergence of adults was also recorded for varieties Exrico-23, pc-50, Icapijao, Icabunsi and Carioca (Figure 2). The dry weight of the adults obtained from the bioassay varied significantly among genotypes ($F_{(39,120)} = 6.4$ $p<0.01$). The heaviest adults were found in susceptible ones (figure 2) and the difference in dry weight of the adults from the heavies (Cal 96) and that of the lightest (RAZ 44) was about 66%. The index of susceptibility of RAZ 4, RAZ 101, RAZ 173, RAZ 44, RAZ 174, RAZ 36, RAZ 2 and RAZ 20 showed resistance with lower value ranging from 1 to 3 whereas all commercial varieties had significantly higher susceptibility index ranging from 8.6 to 9.7 while the remaining lines were intermediate resistance with susceptibility index ranging from 3.1 to 5.0(Figure 3). The seed yield had also significant difference among genotypes ($F_{(39,120)} = 6.3$ $p<0.01$). The average yield of susceptible varieties (2.11t/ha, SE = 0.05) was significantly higher than that of the resistant lines (1.8 t/ha, SE = 0.02). The number of days to maturity varied between 78 and 96, without any difference between susceptible commercial varieties and RAZ lines ($F_{(39,120)} = 0.74$, $p = 0.78$)

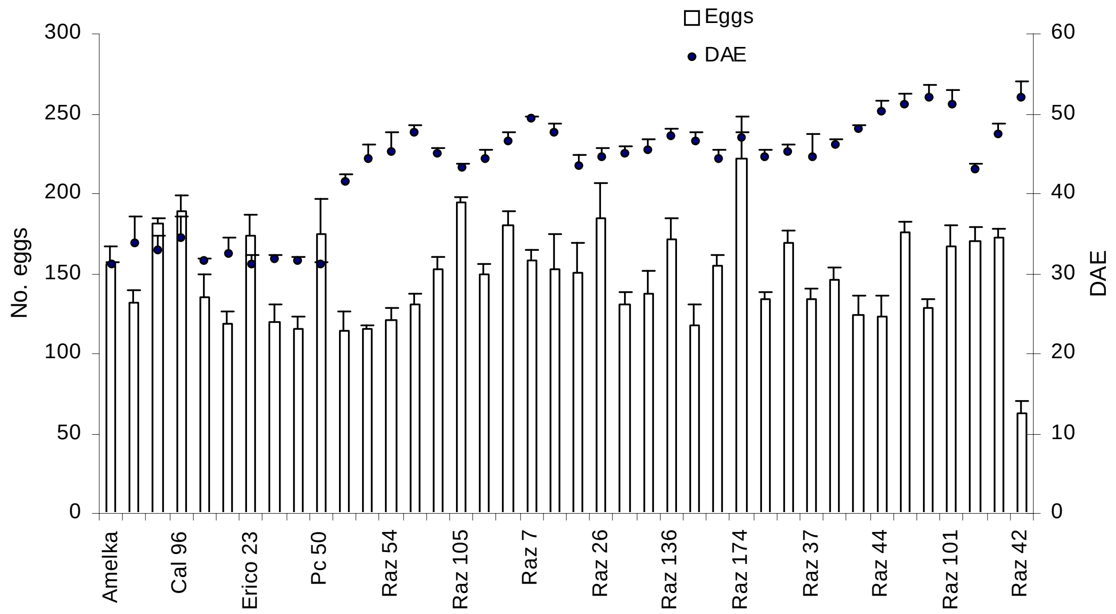

Figure 1: Number of eggs (± SE) and days to adult emergence (± SE) of mexican bean weevil in bioassay with 10 susceptible and 30 resistant (RAZ) lines, ordered by increasing days to adult emergence(DAE)

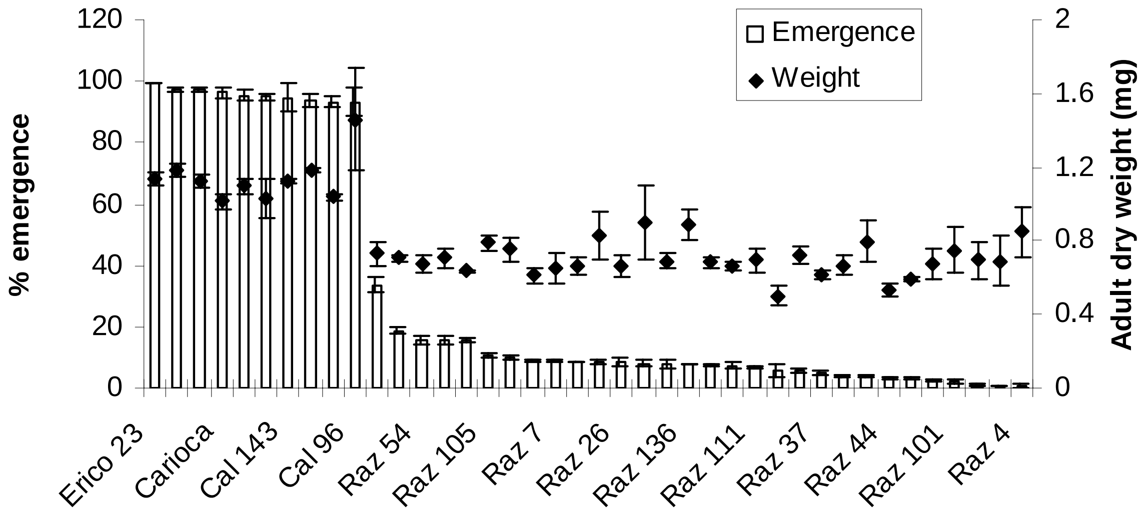

Figure 2: Percentage of emergence (± SE) and dry weight of adult Mexican bean weevil (± SE) in bioassay with 10 susceptible and 30 resistant (RAZ) lines, ordered by decreasing values of emergence.

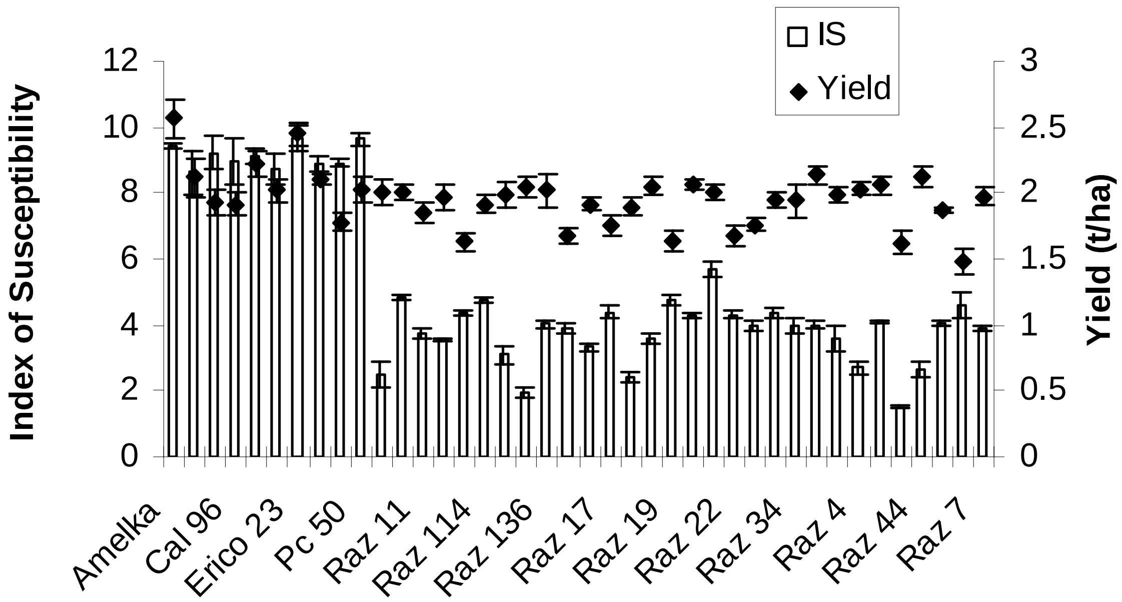

Figure 3: Index of susceptibility (± SE) and yield of (± SE) of bean lines tested in bioassay with 10 susceptible and 30 resistant (Raz) and under field conditions in Ethiopia, ordered by decreasing index of susceptibility.

Protein extraction and Arcelin determination

Electrophoretic patterns for advanced lines with high reconfirmed levels of resistance to Mexican bean weevil are shown in Fig.4 and 5. Lanes 1, 3,5,7,9 and 11 correspond to advanced RAZ lines whereas 2, 4, 6, 8 and 10 were susceptible varieties. Lanes 12 and 13 were standard checks for resistance and susceptible, respectively. The advanced resistance lines (except RAZ 101) were identical in terms of seed size, colour and growth habit. Using Arcelin protein analysis with 21 highly resistance advanced lines and 5 susceptible commercial varieties plus controls also showed a high level of accuracy. Resistance was associated entirely with the presence of the heavy 35KDa band that represents Arcelin 1, the seed protein extract.

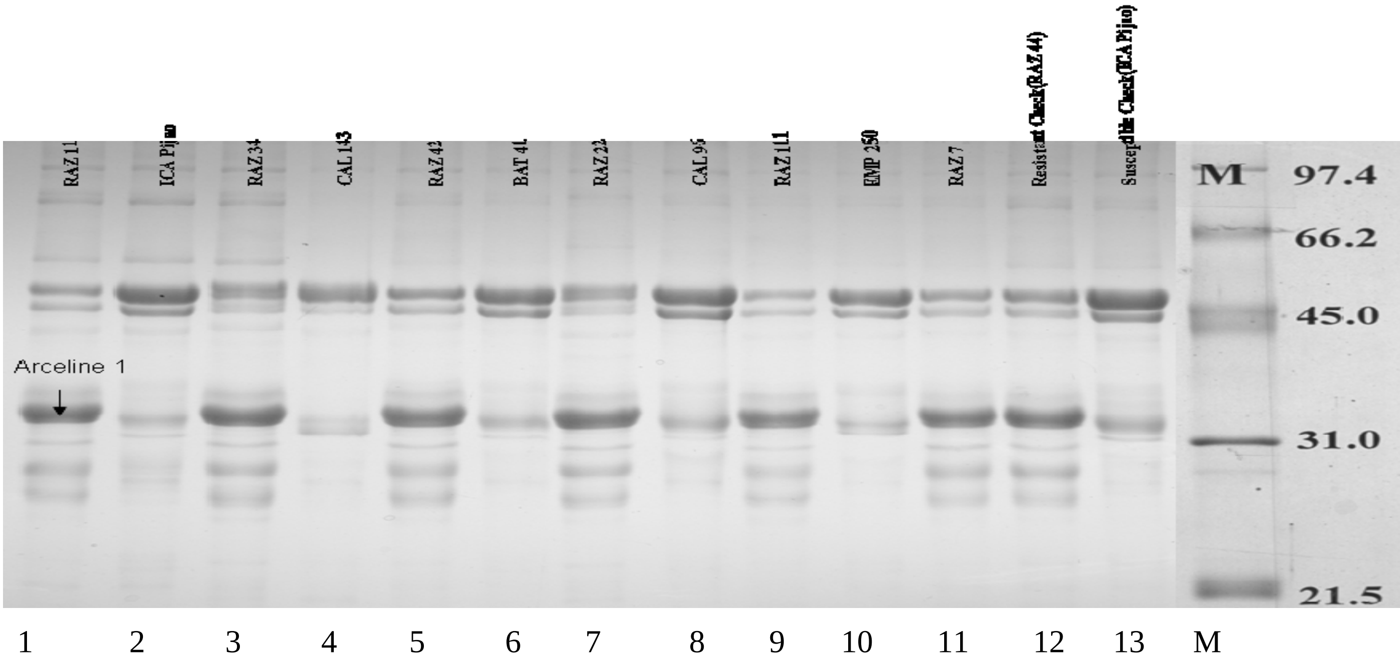

Figure 4: Electrophoretic patterns of resistance lines and susceptible varieties with reconfirmed 35kDa for presence of Arcelin 1. Lane 1: RAZ 11;3:RAZ 34;5:RAZ 42; 7:RAZ 22; 9:RAZ111; 11:RAZ 7;12:Resistance check(RAZ 44) & 13: Susceptible check (Icapijao). 2, 4, 6,8,10 are susceptible varieties (Icapijao, Cal.143, Bat41, Cal96 & EMP 250). M: standard molecular marker. Arrow point to Arcelin 1 bands.

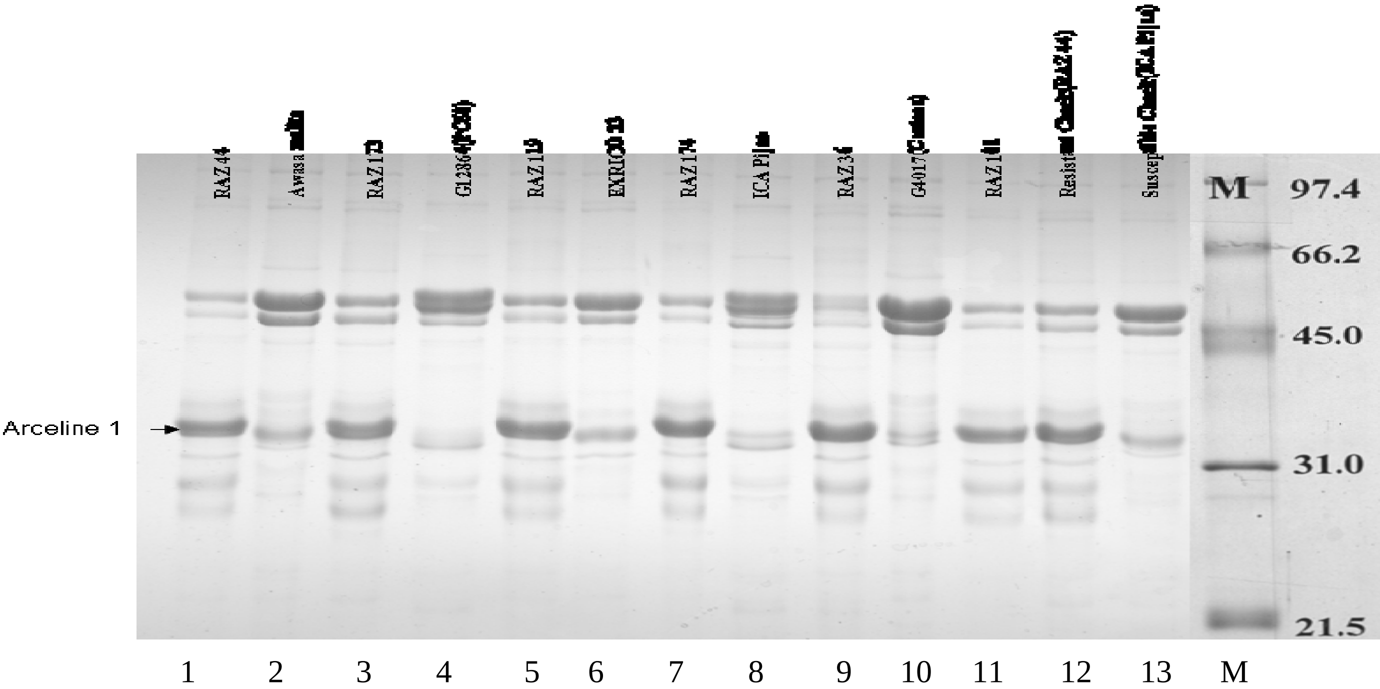

Figure 5: Electropheretic patterns of resistance lines and susceptible varieties with reconfirmed 35 kDa for presence of Arcelin 1. Lane 1: RAZ 44; 3: RAZ 173; 5: RAZ 119; 7: RAZ 174; 9: RAZ 36; 11: RAZ 101; 12:Resistance check(RAZ 44) & 13: Susceptible check(Icapijao). 2,4,6,8 & 10 are susceptible varieties (Awashmelka, PC 50, Exr-23, Icapijao & Carioca). M: Standard Molecular markers. Arrow point on Arcelin 1 bands.

Discussion

The highest level of antibiosis resistance expressed as reduced emergence, prolonged life cycle and reduced progeny weight were observed in all advanced lines (RAZ) regardless of their level of resistance. These indicators of resistance to bruchids have been studied by several workers. Redden and McGuire(1983) indicated mean emerging day or developmental time from egg to adult, cumulative adult emergence as percentage of number of eggs at 45 and 56 days as best separation of resistance and susceptible lines. Resistance was expressed as reduced oviposition, a prolonged larval development period and reduced progeny weight (Schoonhoven *et al.*, 1983). According to Mueke (1984), number of eggs oviposited, days to adult emergence, weight of adult and index of susceptibility as reliable measures of resistance and susceptibility. This experiment measured most of the above mentioned criteria as an indicator of resistance and susceptibility and the result indicated that the advanced lines containing arcelin 1 variant had good levels of resistance to *Z.subfacsiatus*. RAZ 4, RAZ 120, RAZ 42, RAZ 101, RAZ 173, RAZ 119, RAZ 44, RAZ 174, and RAZ 15**1** exhibited a higher degree of resistance to *Z.subfasciatus* for percent emergence, days to adult emergence and adult dry weight therefore, the back cross lines containing Arcelin 1 variant have been similarly ranked in past studies for resistance to *Z.subfasciatus* (Harmsen, 1989; Cardona *et al.*, 1990).

Ranking of the advanced lines as resistance factors was facilitated by comparison on Index of susceptibility (IS). It is linearly correlated with the intrinsic rate of increase and thus with the logarithm of the numbers of insects that will be produced in a given period of time, providing a reliable estimate of resistance levels. Under our experimental conditions, IS values for susceptible varieties and checks usually range from 8.6 to 9.7. Genotypes with low IS values are rated as highly resistant and those with high values as susceptible ones (Cardona *et al.,* 1989). Consequently, RAZ 4, RAZ 101, RAZ 173, RAZ 44, RAZ 174, RAZ 36, RAZ 2 and RAZ 20 confer higher level of resistance to Mexican bean weevil. These results agree with those reported by Cardona *et al* (1990) and Cardona *et al.* (1989). In general, there was a marked difference in a susceptible index among the 40 varieties tested ranging from 1.5 for RAZ 173 and over 9.5 for the varieties Cal 96 and Cal 143. This indicated that there was a remarkable variation in this value for the varieties tested.

Electrophoretic patterns for advanced lines with high reconfirmed levels of resistance to Mexican bean weevil was associated entirely with the presence of the heavy molecular weight 35KDa band that represents arcelin 1 in seed (Romero Andreas *et al.,* 1986). By using serological techniques for identification of resistant progeny, two to three generation can be evaluated each year.

In summary most of the advanced lines crossed at CIAT Colombia showed high to intermediate level of resistance in all the parameters tested as compared to commercial varieties. The advanced lines RAZ 4, RAZ 101, RAZ 173, RAZ 44 and RAZ 174 were consistently resistant to all parameters measured and can therefore be used as source of resistance in a breeding program especially some of which were derived from Awash-1(commercial variety of Ethiopia). As varieties resistant to *Z. subfasciatus* are currently unavailable in the country, it is suggested the national bean research program tests the promising lines for their agronomic excellence and commercial values and then releases the best performing ones for production.

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Chapter V

Marker assisted selection of arcelin1 in common beans *(Phseolus vulgaris* L.*)*

Abstract

Arcelin is a seed protein which has been extensively studied but not widely used by bean breeding program for commercial purpose. The objective of this study was developing marker assisted selection procedure useful for identification of arcelin 1 gene controlling bruchid resistance. Two microsatellite markers Bmy11 and Pvatct001 were used for the marker assisted selection scheme on 40 advanced lines and commercial varieties to verify the association between these markers and the arcelin1 gene. The SSR markers BMy-11 and Pvatct-001 confirmed they are tightly associated with the arcelin1 gene and they produced bands that were 208 and 192 bp long for resistant lines. They were more accurate in eliminating susceptible plants than in distinguishing the levels of resistance.

Key words: Dry bean, Bruchids, *Zabrotes subfasciatus*, resistance, arcelin

Introduction

Economical losses due to insect attacks on stored beans are known to be substantial on the entire world. Losses are higher in third world countries where good storage facilities are not usually available (Schoonhoven and Cardona, 1986). Economically important bruchid species affecting beans are Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) and the bean bruchid, *Acanthoscelides obtectus* (Say) (Schoonhoven and Cardona,1982). *Z. subfasciatus* is a pest confined to warmer areas and is a storage pest, while *A. obtectus* is found in colder areas (higher altitude or latitude) infesting beans in the field and storage. Together these insect pests are estimated to cause an average 13% grain loss to bean crops grown in developing countries (Cardona & Kornegay, 1999). *Z. subfasciatus* is the dominant species of storage insect in Eastern and Central Africa (Nchimbi and Misangu, 2002). The damage caused by *Z. subfasciatus* during farm storage was 38.1%, varying between 3.2% up to 80% at some locations, causing serious problem to most farmers in Ethiopia (Ferede, 1994).

Arcelin is inherited as a dominant trait that provides the highest level of resistance to Zabrotes subfasciatus. It is a monogenic dominant trait that provides the highest level of resistance to bruchids when in the homozgous state, with heterozygous Arc^{+}/Arc^{-} individual seeds showing incomplete resistance compared to Arc^{+}/Arc^{+} (Kornegay et.al., 1993). So far the exact mode of action of arcelin on bean bruchids is not known. Minney et al. (1990) reported that there is a probability that the protein may not be digestible to insect. The bean entomologist in CIAT (International Centre for Tropical Agriculture) selected Arc1 allele among the seven Arc variants for breeding program to develop bruchid resistant advanced lines known as RAZ (Resistant against Zabrotes) using back crossing breeding methods (Cardona et al., 1990).

The objective of this study was to verify the possibility to develop a marker-assisted selection procedure, useful for the identification of arcelin 1 gene in our materials based on the association between this gene and some SSR common bean markers developed at CIAT. For this purpose two SSR loci were assayed in 40 advanced breeding lines of common bean to be evaluated for bruchid resistance.

Materials and Methods

Plant material: Thirty resistant advanced lines and 10 susceptible commercial varieties were used for microsatellite markers analysis (Table 1).

DNA extraction

The DNA extraction technique was a standard organic solvent (phenol/chloroform) based "microprep" used in CIAT molecular laboratory and based on method of Afandor et al. (1998). Briefly, 200µl of extraction buffer was added to 0.5g of young trifoliate leaf tissue that was ground with a plastic pestil. A further 600µl of extraction buffer was added and the mixture was incubated at 65^{0}C for 45 minutes before adding chloroform: octanol (24:1) mix, shaking for 30 minutes and precipitating the supernatant with 500µl of isopropanol at -20^{0}C and 125µl of sodium acetate in a new eppendorf tube. The DNA was pelleted by centrifuging at 12,000 rpm for 10 minutes and cleaned with 500µl of 70% ethanol. The DNA pellets were then dried and resuspended in 150µl of ddH_2O to a concentration of 50-

100ng/µl. DNA was then diluted to 10ng/µl for use as template for the amplification of microsatellites.

Microsatellite markers

Two microsatellite markers, Bmy11 and Pvatct001 were used to assay their association with the arc 1 gene and develop marker assisted selection scheme. These microsatellite markers were selected from eight previously identified in common bean by CIAT. PCR conditions consisted of initial denaturation at 94^0C for 5 minutes followed by 30 cycles 94^0C for one minute, 47^0C for one minute and 72^0C for 2 minutes, ending with a final extension period of 72^0C for 5 minutes. The microsatellites were run in 4% polyacrilamide (29:1 acrylamide:bisacramide) gels at 150 constant watts(1800-2000 volts) and 45^0C constant temperature in Biorad Sequi-Gen GT vertical gel rings for 1.5 hrs. Gels were silver-stained with recirculating tank system as described in Blaire et al. (2003).

Table 1: Resistant lines and susceptible varieties used for Marker Assisted Selection and their market classes.

NAME	SUSCEPTIBILE/ RESISTANCE	SOURCE	COLOR	SIZE
Amelka	S	Ethiopia	White	Small
Bat 41	S	Africa	Coloured	Medium
Cal 96	S	Africa	Coloured	Large
Cal 143	S	Africa	Coloured	Large
Carioca	S	CIAT	Coloured	Small
Emp 250	S	CIAT	Coloured	Medium
Erico23	S	Ethiopia	White	Small
Ibunsi	S	CIAT	White	Small
Ipijao	S	CIAT	Colored	Small
Pc 50	S	CIAT	Coloured	Large
Raz 11	R	CIAT	White	Small
Raz 17	R	CIAT	Coloured	Small
Raz 19	R	CIAT	White	Small
Raz 2	R	CIAT	White	Small
Raz 20	R	CIAT	Coloured	Small
Raz 22	R	CIAT	White	Small

NAME	SUSCEPTIBILE/ RESISTANCE	SOURCE	COLOR	SIZE
Raz 26	R	CIAT	White	Medium
Raz 34	R	CIAT	White	Small
Raz 36	R	CIAT	White	Small
Raz 37	R	CIAT	White	Small
Raz 4	R	CIAT	Cream	Small
Raz 40	R	CIAT	White	Small
Raz 42	R	CIAT	White	Small
Raz 44	R	CIAT	White	Small
Raz 49	R	CIAT	Coloured	Small
Raz 54	R	CIAT	Coloured	Small
Raz 7	R	CIAT	White	Small
Raz 101	R	CIAT	Colored	Small
Raz 105	R	CIAT	Coloured	Large
Raz 11-1	R	CIAT	White	Small
Raz 111	R	CIAT	White	Small
Raz 114	R	CIAT	White	Small
Raz 119	R	CIAT	White	Small
Raz 120	R	CIAT	White	Small
Raz 136	R	CIAT	White	Small
Raz 138	R	CIAT	White	Small
Raz 151	R	CIAT	Coloured	Small
Raz 173	R	CIAT	White	Medium
Raz 174	R	CIAT	White	Medium
Raz 24-2	R	CIAT	coloured	Large

CIAT: International Center for Tropical Agriculture. R: Resistance. S: Susceptible

Results and Discussion

The two microsatellites were found to be clearly associated with arcelin-1 gene our advanced breeding lines: of these, microsatelite BMy-11 was known to be nearer to arcelin-1 gene than pvatct-001. These microsatellite markers BMy 11 and pvatct 001 produced bands that were 208 and 192 bp long in resistant lines, whereas susceptible ones had bands of 240 and 200 respectively. These two markers confirmed that they were associated Arc 1 locus, as previously observed in a different market classes by CIAT, (2006). BMy-11 and pvatct-001 also showed that the percentage of precisely identifying susceptible advanced lines was higher (95.4 and 94.9%, respectively) than the percentage of correctly identified highly resistant or resistant genotypes (60.2 and 62.0%, respectively). It is interesting to note that the markers were more precise in discriminating susceptible lines than in distinguishing levels of resistance (highly resistance, resistance or intermediate) among genotypes containing arceline 1 allele. Similar results were reported on Andean bean genotypes (CIAT, 2006).

Primer BMY 11

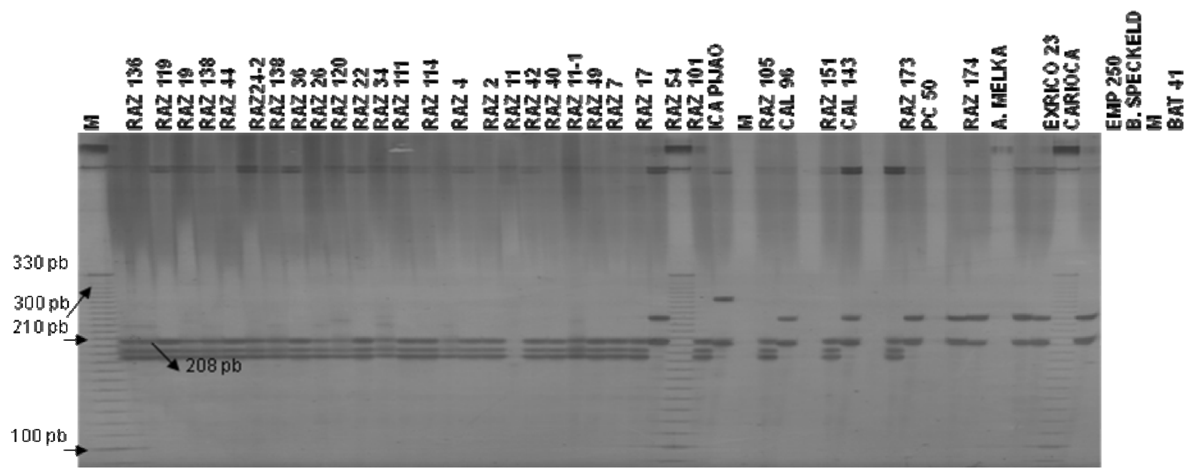

Figure 1: SSR marker BMY11: bruchid resistant lines share a 208 pb band, susceptible lines show a 240 pb band.

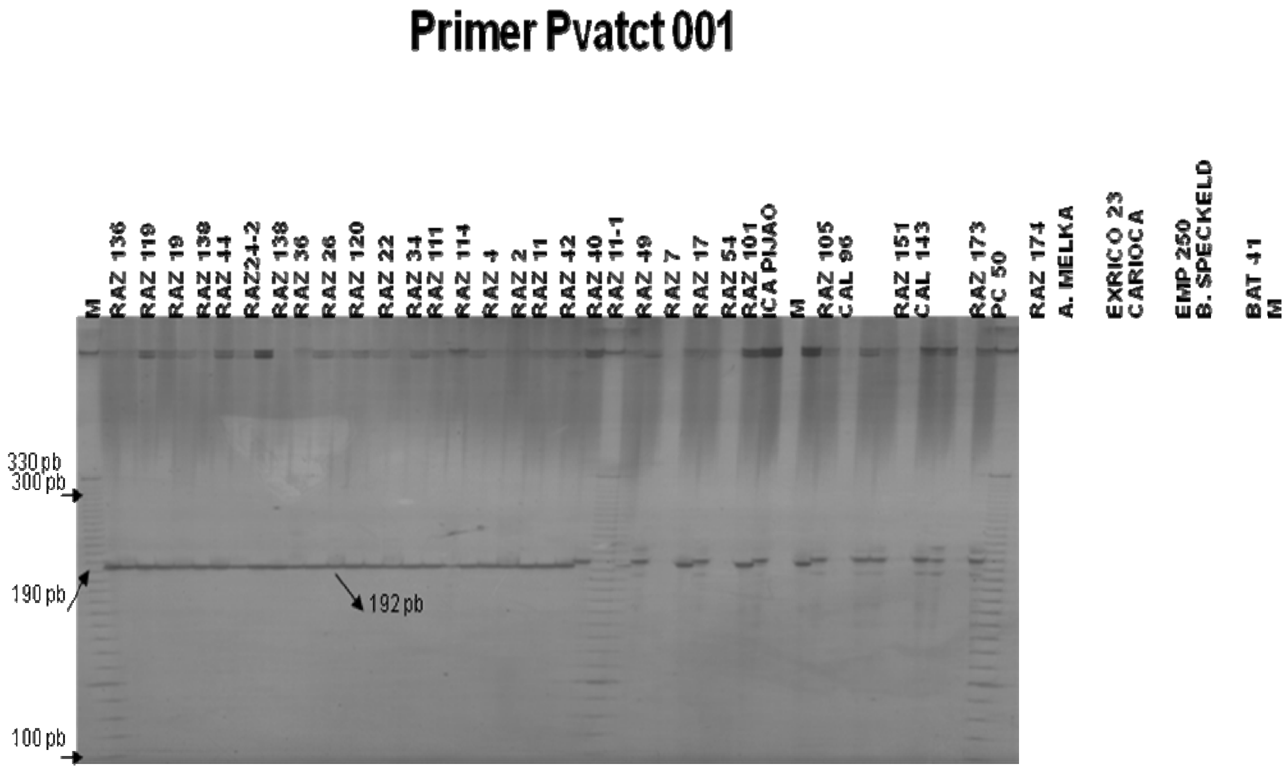

Figure 2: SSR marker Pvatct001: bruchid resistant lines share a 192 pb band, susceptible lines show a 200 pb band.

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Chapter VI

General conclusion

Earlier studies have shown genotypic variation in common bean for drought and bruchid resistance (Beebe et al., 2008; Cardona et al., 1990). Evaluation and selection of genotypes under field conditions based on yield components and other plant attributes have not been attempted before in common bean. for industrial canning nor have farmers been incorporated in the process of selection of drought resistance. Breeding methods for drought have not been validated in condition of east Africa. Furthermore, dry bean is sensitive to bruchid (storage pest) and hence the cumulative effects of drought during post flowering in the field and attack of bruchid in storage need to be determined. It was hypothesized that common bean genotypes selected under drought conditions with the participation of farmers exhibit significant advantage over those genotypes that area sensitive to drought. Differential responses in number of emerged adults, percent emergence and adult dry weight of the bean genotypes also contribute for differences in bruchid resistance.

A number of common bean genotypes with high seed yield, pod number per plant, seed number per plant and 100 seed weight have been identified and these genotypes also showed greater values of shoot biomass, leaf area index and pod harvest index than the standard check (Awash melka) and donor parent (SXB 405) under drought conditions. Over two years, a strong correlation between seed yield, yield components and plant attributes have been observed under drought conditions. It has clearly demonstrated that simple and easily measured plant traits such as shoot biomass, leaf area index (LAI) and pod harvest index (PHI) could be successfully used to select better performing genotypes under drought stress in a common bean breeding program. This study has also confirmed that farmers and traders were capable of making significant contributions to identification of superior genotypes under drought conditions, having selected some of the highest yielding genotypes under drought within the population. Thus, agronomic criteria of drought resistance were totally compatible with traits of marketability and commercial value.

The study has also identified a significant number of genotypes that showed high to intermediate level of resistance to bruchids compared to commercial varieties using parameters such as number of emerged adults, percent emergence and adult dry weight. Arcelin protein analysis for resistant and susceptible lines also showed a high level of accuracy for presence or absence of arcelin 1 gene in a given cultivar. Resistance was entirely associated with the presence of the heavy 35kDa band. In addition, two molecular markers (BM 11 and pvatct 001) were found to be clearly associated with arcelin 1 gene.

The approach outlined in this research work shows that common bean breeders should be able to select drought resistant genotypes based on field evaluation. There is need for further research work on other physiological traits for their possible relation and contribution to selection of drought resistance, for example chlorophyll content, photosynthetic efficiency and canopy temperature depression. Combining the physiological analysis with measurement on soil moisture content and daily climate data might allow the bean breeder to more fully understand the selection process of drought resistant bean genotypes. So far there is not any commercial variety that is resistant to bean bruchid and further research on bruchids would provide much needed focus on a combined approach such as marker assisted selection, insect feeding screening in laboratory and subsequent field evaluation of the bean genotypes. Most of the time the bean bruchid resistant genotypes are low yielders under field conditions, hence bean breeders and entomologists should work together to develop dry bean genotypes that could be resistant to bruchids and high yielding under field conditions.

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Appendix 1: List of 81 bean genotypes including the two parents (ICA Bunsi and SXB 405) and standard check (Awash melka)

Entry	G code	Seed size	Seed color	Line code
1	G1	Small	White	NAE 1
2	G2	Small	White	NAE 2
3	G3	Small	White	NAE 3
4	G4	Small	White	NAE 4
5	G5	Small	White	NAE 5
6	G6	Small	White	NAE 6
7	G7	Small	white	NAE 7
8	G8	Small	White	NAE 8
9	G9	Small	White	NAE 9
10	G10	Small	White	NAE 10
11	G11	Small	White	NAE 11
12	G12	Small	White	NAE 12
13	G13	Small	White	NAE 13
14	G14	Small	white	NAE 14
15	G15	Small	White	NAE 15
16	G16	Small	White	NAE 16
17	G17	Small	White	NAE 17
18	G18	Small	White	NAE 18
19	G19	Small	White	NAE 19
21	G21	Small	white	NAE 21
22	G22	Small	White	NAE 22
23	G23	Small	White	NAE 23
24	G24	Small	White	NAE 24
26	G26	Small	White	NAE 26
27	G27	Small	White	NAE 27
28	G28	Small	white	NAE 28
30	G30	Small	White	NAE 30

Entry	G code	Seed size	Seed color	Line code
31	G31	Small	White	NAE 31
32	G32	Small	White	NAE 32
34	G34	Small	White	NAE 34
35	G35	Small	white	NAE 35
36	G36	Small	White	NAE 36
37	G37	Small	White	NAE 37
38	G38	Small	White	NAE 38
39	G39	Small	White	NAE 39
40	G40	Small	White	NAE 40
41	G41	Small	White	NAE 41
43	G43	Small	White	NAE 43
44	G44	Small	White	NAE 44
45	G45	Small	White	NAE 45
46	G46	Small	White	NAE 46
48	G48	Small	White	NAE 48
49	G49	Small	white	NAE 49
50	G50	Small	White	NAE 50
51	G51	Small	White	NAE 51
52	G52	Small	White	NAE 52
53	G53	Small	White	NAE 53
54	G54	Small	White	NAE 54
55	G55	Small	White	NAE 55
56	G56	Small	white	NAE 56
58	G58	Small	White	NAE 58
60	G60	Small	White	NAE 60
62	G62	Small	White	NAE 62
69	G69	Small	White	NAE 69
70	G70	Small	white	NAE 70
72	G72	Small	White	NAE 72
74	G74	Small	White	NAE 74

Entry	G code	Seed size	Seed color	Line code
75	G75	Small	White	NAE 75
76	G76	Small	White	NAE 76
77	G77	Small	white	NAE 77
78	G78	Small	White	NAE 78
79	G79	Small	White	NAE 79
80	G80	Small	White	NAE 80
85	G85	Small	White	NAE 85
86	G86	Small	White	NAE 86
87	G87	Small	White	NAE 87
88	G88	Small	White	NAE 88
90	G90	Small	White	NAE 90
92	G92	Small	White	NAE 92
95	G95	Small	White	NAE 95
96	G96	Small	White	NAE 96
97	G97	Small	White	NAE 97
99	G99	Small	White	NAE 99
100	G100	Small	White	NAE 100
101	G101	Small	White	NAE 101
103	G103	Small	White	NAE 103
104	G104	Small	White	NAE 104
105	G105	Small	white	NAE 105
108	SXB 405	medium	Cream	CAE 108
109	Awash melka(check)	Small	White	NAE 110
110	ICA Bunsi	Small	White	NAE 111