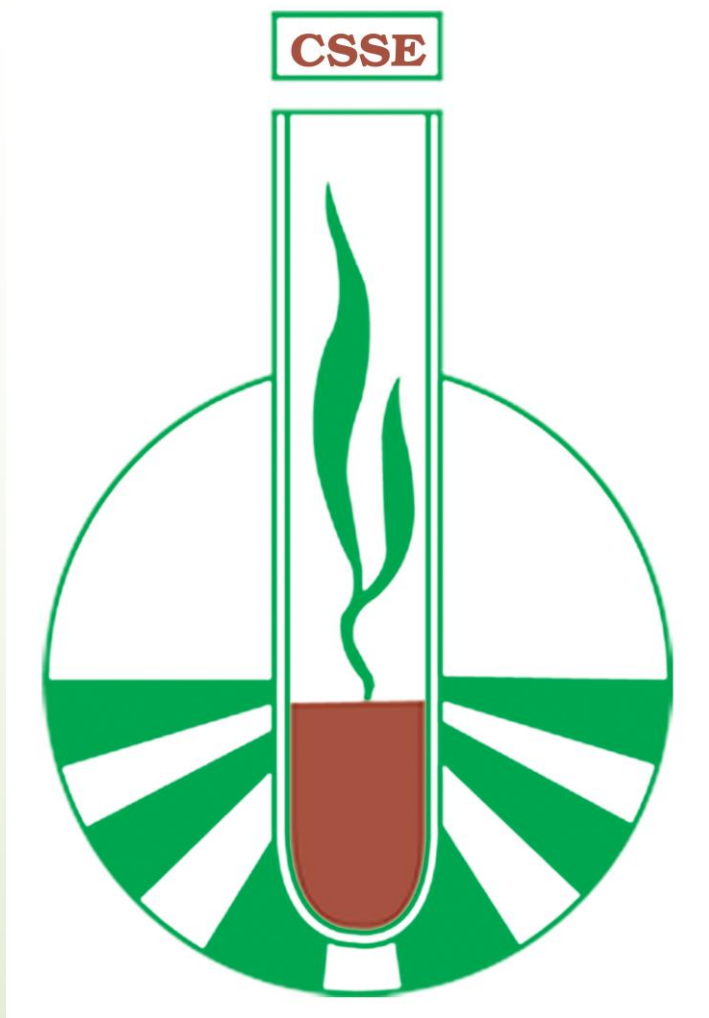


Ethiopian Journal of Crop Science

Volume 6 (Special volume)

Number 2 (2018)

ISSN: 2072-8506



An Official Journal of the Crop Science
Society of Ethiopia (CSSE)
Addis Ababa, Ethiopia



Ethiopian Journal of Crop Science

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Ethiopian Journal of Crop Science

Published by the Crop Science Society of Ethiopia (CSSE)

Vol. 6 (Special volume)

Number 2 (2018)

ISSN 2072-8506

Editor: Dagnachew Lule (PhD)

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Ethiopian Journal of Crop Science (EJCS)

Volume 6 (Special Volume), Number 2, 2018

ISSN 2072-8506

Addis Ababa, Ethiopia

Ethiopian Journal of Crop Science

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Innovative Partnership in Chickpea Seed Production and Technology Dissemination: a Decade of Lessons in Ethiopia

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Abstract

Innovative partnership in chickpea seed production and technology dissemination was assessed in increasing chickpea productivity through facilitated access to quality seed. The study was designed to evaluate the benefits gained from partnership implemented for the last 10 years in chickpea technology by seed system of crop production. Farmers' participatory variety selection (FPVS)' has been implemented as epicenter of the new innovation. The varieties selected by the farmers were ultimately demonstrated and visited by large number of farmers and other stakeholders. This was complemented with the establishment and technical backstopping of farmers' seed producer associations to produce and market quality seed to fill the gap by the formal sector. Intervention with chickpea technologies were initiated right after training partners in chickpea seed and grain value chain. From 2008 to 2015, over 350 FPVS trials were conducted concurrently with demonstrations. The farmers' seed producer associations cover the lion's share of the seed supply and became an innovative approach that enhanced the chickpea seed system. The production of certified and quality declared seed increased from 632.7t in 2008 to 3290 tons in 2014. The drastic increase in productivity and production of chickpea during the intervention period were largely attributed to the interventions made. The national chickpea productivity has doubled in a decade's time (2006-2015) and attained peak of 1.9ton ha⁻¹ in 2014. Similarly production showed drastic increase during the intervention period where the production in 2015 was 124% more compared to the production in 2006. Lessons were drawn from the approach that many development parties and the government policy recognized the approach as key player for enhancing the chickpea sector.

Keywords: Chickpea, demonstration, participatory variety selection, seed, technology

Introduction

Ethiopian agriculture accounts for 85 percent of employment (Dercon *et al.*, 2012; Spielman *et al.*, 2010). The country's population of 92 million is expected to grow to 160 million by 2050 (Josephson *et al.*, 2014). As a result, farm sizes would be rapidly declining, increasing the need for agricultural intensification (Headey *et al.*, 2014). Accordingly, increasing the productivity of smallholders through improved technology has become a policy priority for development agencies and the Ethiopian government (Abebaw and Haile, 2013).

Chickpea (*Cicer arietinum* L.) is an important food legume in Ethiopia. The country is considered as a secondary center of genetic diversity for chickpea and the wild relative of cultivated chickpea, *Cicer cuneatum*, which is found in Tigray region of the country (Anbesa and Bejiga, 2002; Kanouni *et al.*, 2011). Chickpea is largely grown in central, northern and northwest highlands of Ethiopia. According to FAOSTAT (2014), Ethiopia (3.2%) is among the chickpea producing countries of the world next to India (69%), Australia (5.7%), and Pakistan (5.3%) and Myanmar (3.4%). In 2015/16, the total area covered by chickpea is about 0.26 million hectares, with a total production of 0.47 million ton and productivity of 1.83 tons ha⁻¹ (CSA, 2016).

Increasing the productivity and production of pulse crops in general and chickpea in particular is critical to satisfy the national demand for increased food and nutrition security. The national chickpea improvement program has made significant research to increase the productivity and released 27 improved varieties since 1974 at both federal and regional levels (MoANR, 2015). The yield advantage of improved chickpea varieties is up to four folds more than farmer's local varieties (Fikre, 2014). Some of these improved varieties meet local and export market standards owing to their quality. The adoption of improved varieties and technologies found high in recent years, which led to an increase in the productivity and production (Fikre, 2014). This might be attributed to the confidence and adoption by the farmers improved chickpea technologies as a result of their participation in selecting technologies of their preference at the early stage of the popularization.

The wide scale adoption of chickpea technologies in Ethiopia created huge demand for seeds of improved varieties, as obviously we have seen significant imbalance between demand and supply annually. In the present condition, if one calculates the ideal seed demand on area basis of 258 000ha it is about 27.7 thousand tones, however, with the assumption that Ethiopia being center of secondary diversity for the crop, it appear convincing to keep some 25% of production for land races /traditional

cultivars/ to keep the diversity mainstreamed. Hence, with 75% coverage it is about 20.8 thousand tones; and if we further divide this in to four cycle of replacement, then it would be like 5 thousand tons. This being the case, on the contrary, the annual approximate chickpea seed supply does not surpass 2 to 3 thousand tons, a half below the demand. This could highly be attributed to low interest of the few formal public seed enterprises and private seed companies to produce seed of pulse crops (Atilaw and Korbu, 2016) but rather are predominantly engaged in production of seed of wheat, tef, barely and hybrid maize (IFPRI, 2010). Bishaw *et al.* (2008) reported that the formal sector supplies about 2% seed requirements of the major cool season food legumes such as chickpea.

Unlike the commercialized hybrids, the informal seed sector is the major player of the seed system in chickpea (Fikre *et al.*, 2012). Cognizant of these facts, development of reliable and sustainable seed multiplication and supply system was found to be critical, particularly for crops where the private sector interest is weak. Therefore, efforts were made to tackle this bottleneck in a pluralistic approach where community based seed production was considered as alternative approach by the tropical legumes (TL) project and found to be effective in addressing the shortage of seeds of improved chickpea varieties.

Hence to enhance the uptake of improved chickpea technologies the research and development interventions have generally aimed at (1) equipping the farmers with knowledge of improved chickpea grain and seed production technologies, (2) building farmers' confidence on improved technologies through participatory variety selection, (3) wide scale demonstration of varieties selected by farmers and (4) improving availability of improved seed through community based seed production. This study, therefore, provides highlights of the innovative approaches followed in the Ethiopian chickpea as enhancer of change for seed multiplication and dissemination of improved chickpea technologies.

Methodology

Interventions on multiplication and dissemination of improved chickpea technologies have been made in the main rainy seasons since 2007. Over 67 districts in 18 zones of the four major chickpea growing regions; namely, the Oromia, Amhara, Southern Nations Nationalities and Peoples' (SNNP) and Tigray regional states of Ethiopia were covered (Figure 1). To address chickpea farmers in these regions, interventions were made by eight regional and federal agricultural research centers in collaboration with zonal and district agricultural offices under the coordination of Debre Zeit Agricultural Research Center hosting the Tropical Legumes (TL I, II and III) project.

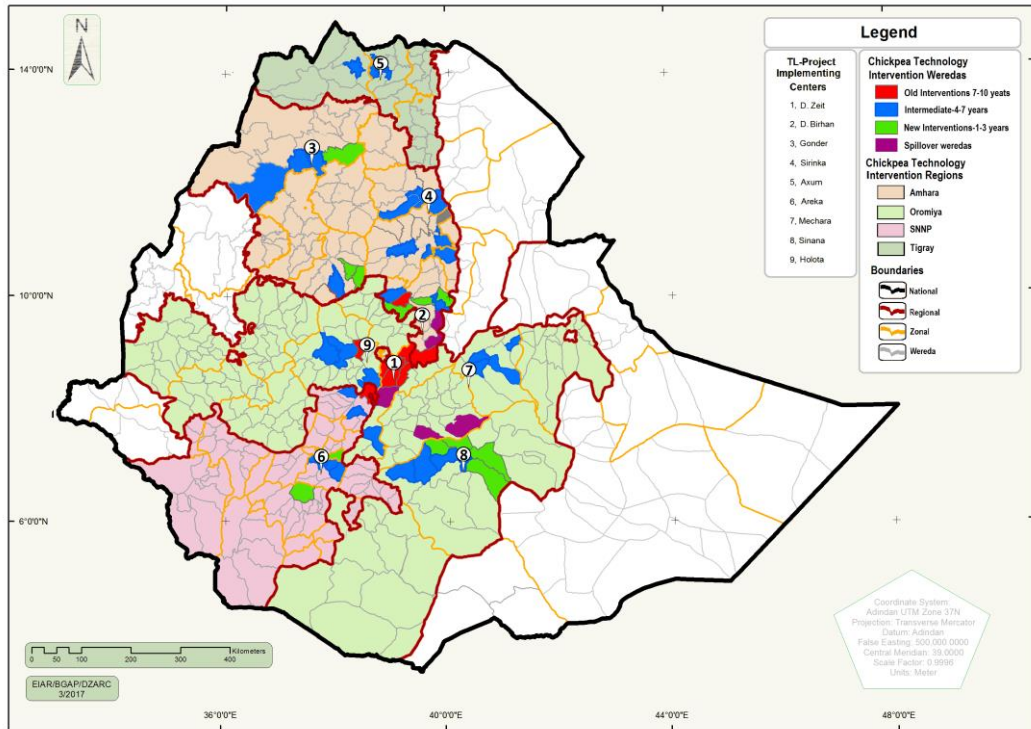


Figure 1. Chickpea technology intervention districts under the TL III project in Ethiopia

The assessment was derived from the decadal interventions experience through participatory variety selection followed by demonstration and seed business incubation. Perceptions reflected by implementing farmers and domain community have been synthesized in the discussion. The approach has segments of technology adaptation and seed incubation as feeding system of sustainable wide scope technology scaling. Hence, as approach, segments of key points, which are integral components, were elaborated.

Capacity and Partnership Building

Interventions in technology popularization and improving

chickpea seed system has been started with building the right attitude of actors. This mainly was about awareness creation and technical training that subsequently subordinate to commended input. The trainings on principles and techniques of chickpea grain and seed production were given directly to farmers and seed producers as well as development agents and agricultural experts who further train other farmers.

To catalyze the uptake of improved technologies, an innovative platform of relevant stakeholders has been established. The multitasked platform provided support in identifying technology demanded, assessing product specialty options, updating market signals, evaluation of

interventions and feedbacks. The national and regional chickpea stakeholders' innovation platform met annually and share responsibilities on actions to be taken in improving technology multiplication and dissemination.

Farmers' Participation in Variety Selection

Farmers' participatory variety selection (FPVS) (Cherinet and Tazebachew, 2014) approach has been used by the Tropical Legumes project to build farmers' confidence on improved chickpea technologies (Mekasha *et al.*, 2016; Mekasha *et al.*, 2017). Farmers implementing FPVS trials were selected by development agents and trained on chickpea production and trial management. The trials were composed of a set of 6-8 improved varieties that could vary with locations. A local variety commonly grown in the area was included as a check. The trails are planted on 10 m x 10 m plots in three replications. The selected farmers grew and managed the trials under close supervision of researchers and development experts. The trials were then evaluated at the vegetative and maturity stage by groups of 10-15 farmers using their own criteria related to yield and yield components. Based on the ratings of the criteria, varieties of their preference were identified for further popularization.

Demonstration of Identified Technology

Based on the above premises, the varieties selected through FPVS were

demonstrated on a quarter of a hectare (Kebebew *et al.*, 2011). The demonstration plots were designed for wider visits to grab the attention of farmers located in proximity to roads, meeting places, administration, villages, etc. The demonstrations were handled with recommended management practices under close supervision of researchers and agricultural development experts.

The demonstration plots were visited by large number of farmers invited from adjacent villages on formally organized field days, in addition to the small group visits frequently initiated by development agents. The farmers, who implemented the demonstrations and/or earlier participated in FPVSs share their observations and experiences on the technologies. Briefings were also given by researchers on the merits of the technologies such as better yield, quality and disease resistance and major management practice to create awareness to the visiting farmers. Discussions were made on these occasions where queries raised were entertained by researchers, development experts and farmers. Finally, the interest of the farmers on the demonstrated technologies was considered for scaling up decisions.

Decentralized Seed Production and Sourcing

In order to satisfy the demand for seeds of improved varieties, the informal seed system was strengthened through establishment and technical backstopping of farmers' seed producer associations. The seed

producers often were farmers who are organized and with better technical skill to undertake seed production. Such a decentralized seed sourcing (DSS) approach was based on premises of access by geography, volume and low transaction cost and affordability.

Data Analysis

Data on FPVS trials and demonstrations were used to express interventions made and data from FAOSTAT and the Ethiopian central statistical agency (CSA) were used to assess impacts of the interventions. Non-parametric statistics was applied to elaborate progresses, differences and inferences. Simple regression equations were generated using Excel software to assess trends over years.

Results and Discussion

Building the Right Aptitude for Technology Promotion

The first step of intervention was meant to start from the basics and move to the innovative concept. Lead and elite, risk-taker farmers are assumed to be critical community groups to start with. Intervention in chickpea development started with awareness creation and inclusive training in building the technical capacity enhancing the seed system and dissemination of improved technologies. Between 2006 and 2016, technical trainings on management of improved chickpea technologies were delivered to over 3,000 agricultural experts and development agents as training of trainers, who further trained over 14,500 farmers. Actors engaged in the seed system were equipped with knowledge and skills in seed production. Table 1 summarizes the number of trained manpower from 2006 to 2016.

Table 1. Numbers of different stakeholders trained on chickpea technology

Year	Number of trainees						Total
	Researchers and technicians	Agricultural experts and development agents	Farmers	Farmers' Cooperative unions	Community Seed producers	Others	
2006	30	155	250	5	-	35	475
2007	50	210	470	10	2	63	805
2008	57	243	603	15	6	79	1,003
2009	72	399	934	28	10	153	1,596
2010	85	463	1,785	20	12	263	2,628
2011	100	563	2,175	13	13	200	3,064
2012	78	475	2,000	12	14	185	2,764
2013	81	402	3,024	13	12	215	3,747
2014	45	89	1,321	-	-	3	1,458
2015	-	135	964	-	-	-	1,099
2016	-	36	1,328	-	-	5	1,369
Total	598	3,170	14,854	116	69	1,201	20,008

Evaluating the impact of the training, overall perception changes towards improved technology was created effectively, like from nearly zero to as high as three to four folds. Because some two decades back, chickpea technology was applied by research to farmers' fields and rejection of the technology was very common; while in a decade and half down the line, farmers load technologies from research sources with big ambition. Another indicator of change is that more than 10 seed associations have been able to sale their seed with premium, showing perception towards improved technology is growing well.

Seed producer farmer practiced the knowledge they acquired and managed to produce quality seed and became major suppliers to bridge the gap. This is in agreement with the report by Atilaw and Korbu (2016) that states the informal sector remains the dominant and almost the sole supplier of improved chickpea seeds. Livelihood changes are evident with

some well adapted farmers and they have been featured as story tellers.

Farmers' Confidence on Improved Technologies Hastened their Promotion

Most farmers are often reluctant of new things to test at their own risk. Hence, it has been customary to just start from simple to complex and small to big. In 2008/09, farmers' participatory variety selection (FPVS) approach was started in three districts, namely; Gimbichu, Lume and Minjar-Shenkora, by Debre Zeit Agricultural Research Center with the support of TL projects. A lot of lessons were driven to improve the approach for more effect. Since then the scope of intervention has expanded and covered over 67 districts in 18 zones of the major chickpea growing regions; namely, Oromia, Amhara, Southern Nations, Nationalities and Peoples' (SNNP) and Tigray regional states (Figure 1). This rapid progress was made possible by progressive inclusion of eight research centers that

collaborated in implementing the project in major chickpea producing areas and integrated efforts of various institutions related to the chickpea development that resulted in increased awareness of farmers' perception of improved varieties.

In this approach, farmers (male and female) from the respective locations had evaluated the varieties in the FPVS trials in groups of 10 to 15 individuals at the vegetative stages and maturity by setting their own selection criteria. The common criteria used by

the farmers for selection of varieties of their preference were plant height, plant stand, branching habit, disease resistance, earliness, yield, pod number, seed size and seed color. From 2008/09 to 2016/17 over 350 FPVS were conducted where 16 improved chickpea varieties were evaluated under different agro ecologies over years (Figure 2). Among these 14 improved varieties were selected by the farmers in various localities for wider scale production and dissemination.

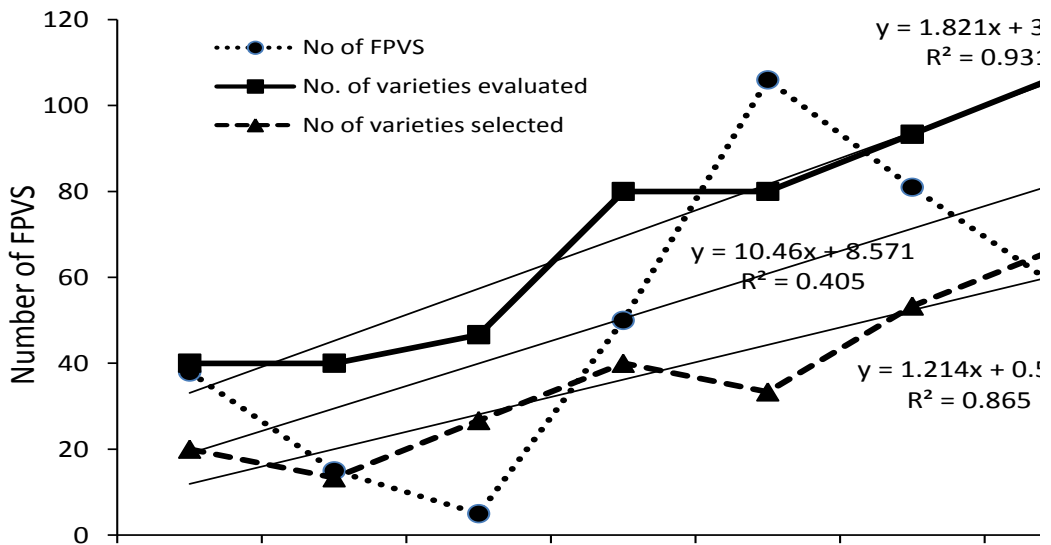


Figure 2. Chickpea varieties evaluated and selected by farmers' participatory variety selection (FPVS) in Ethiopia

Technology development for boosting agricultural productivity is worth investments, if and only if, it reaches the farmers and changes their livelihoods. A collaborative arrangement that brings together several organizations working towards technical and social change or organizations that are involved in

generating, diffusing and adapting new knowledge in agriculture is a way out to improve and build the capacity of the conventional extension system (Aliy, 2016). Different platforms were used at different times to enhance research-extension-farmer linkages. Earlier efforts made to popularize improved chickpea

technologies through demonstration helped in promote the technologies to the farmers with some limitations in acceptance and adoption. Aliy (2016) suggested that a paradigm shift is needed to employ farmers' participatory research approach, that relies on farmers' experimentation and farmers' interaction with important market opinion and backstopping. The briefings made on group and field day visits by the farmers involved in FPVS and demonstration built confidence of the large group of farmers on the technologies. It is natural that farmers can easily learn from their fellow farmers other than any one else. Therefore, such participatory technology

popularization approach was used as smooth awareness creation and learning ground that created significant demand by the farmers. Knowledge empowerment of farmers through electronic and print media, organizing field days, farmers' fairs, and conducting training programs, demonstrations, and farmer-participatory varietal selection trials (FPVS) will help strengthen the local seed systems and enhance the adaption of new improved cultivars (Laxmipathi *et al*, 2016). As a result of this approach recent interventions enhanced technology dissemination and fastened their uptake, as the varieties demonstrated are those selected by the farmers' participation.

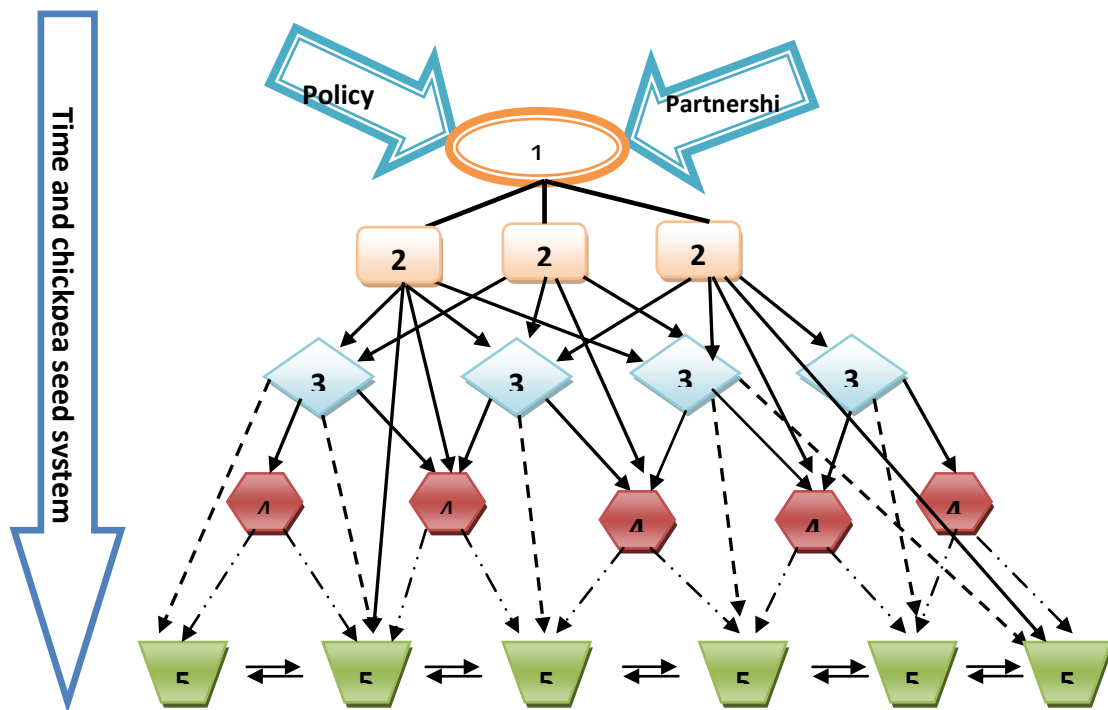


Figure 3. Schematic representation of the current chickpea seed system in Ethiopia

1 = National improvement program, 2 = Research Centers, 3 = Public seed enterprises, 4 = CBOs

(Unions, Farmers' seed producer associations), 5 = Private seed producers, 6 = Farmers

→ = EGS, -- → = Certified, - . . → = QDs, == → = Farmer to farmer seed exchange

Chickpea Seed System Developmental Scheme in the Innovation

Describing the developments of the complex in the seed system, one can find out a narrative scheme as indicated in Figure 3. It is interesting that as in the technology adaptation there are interactivity in the seed system. This could mainly be due to its stage of development, from whole farmers based (own saved) to the community segments (intermediary) and heading to the destiny of private system.

Farmer to farmer seed exchange, either in kind or sales is believed to cover significant scale of the seed transaction. Atilaw and Korbu (2016) reported that self-saved or farmer to farmer seed exchange, accounting for over 95% of the seed used by smallholder farmers, is playing indispensable role in the supply and expansion of chickpea technologies.

The intermediate sector obtains basic seed either from the research system or the public seed enterprises and sells the quality seed produced to farmers for grain production. Due to technical support from the research and agricultural development system their capacity of the intermediate sector has been progressively built. As a result, seed laboratories under the Ministry of Agriculture have recently started evaluating the status of community seed producers and provide them with competency certificate in recognition of their capacity to produce certified

seed, taking their infrastructural, financial and technical capacity into consideration. We can witness among the 18 seed organizations established during the decade, some 75 have got evaluation and pass through certificate of competence for their produce. There are also few seed organizations who try to promote to private limited. There are partner institutions and projects who were parts of the success in the due course

The formal seed sector in general is not a major supplier, as far as pulse crops are concerned. They devote much effort in the production of cereals primarily hybrid maize and wheat and less on legume seed (Bishaw *et al.*, 2008; Aliy, 2016; Emmanuel *et al.*, 2016). According to Bishaw *et al.* (2008) the formal sector supplies about 2% seed requirements of the major cool season food legumes such as chickpea taking into account the area under production and the seed supply from the public or private sector. Emmanuel *et al.* (2016) reported TL-II baseline studies in Ethiopia, Malawi and Tanzania indicated that there was very limited awareness about improved legume varieties, and that neither public- nor private-sector interventions to produce and market legume seeds had a successful track record in these countries.

Even though the contribution of the formal sector is not significant, the public seed enterprises (Figure 3) obtain early generation seed from the

research and produce pre basic, basic and certified seed of chickpea. They sell basic seed to private seed companies and community based organizations such as farmer seed producer association and unions to produce certified seed which is marketed to chickpea farmers.

The birth of Intermediate Seed Sectors and its Impact

The participatory popularization efforts resulted in a growing demand for improved technologies as discussed above. This demand, however, could not be satisfied because the formal legume seed system in general and chickpea is weak. This led to the birth of innovative seed system, called community based seed production (CBSP) synonym with farmers seed

producer association, in filling the critical gaps.

The chickpea improvement program, with the support of the TL projects, worked on development of CBSP system by encouraging active farmers to engage in seed production. These farmers were rendered with special support by building their knowledge on basic techniques of seed production, providing technical supports and early generation starter seed. This resulted in gradual establishment of 20 legal farmers' seed producer associations to date (Table 2). The associations became strong covering the major share of chickpea seed supply and became effective chickpea seed corridors of the country.

Table 2. Farmers' seed producer associations working on chickpea seed production

No	Name of Seed Producer Association (SPA)	Number of members		Location	
		Male	Female	Region/State	District
1	Hundaf Hate'u	74	6	Oromia	Ada'a
2	Megertu Denkaka	56	6	Oromia	Ada'a
3	Giche-garebabu Kolbe	66	8	Oromia	Ada'a
4	Ude	67	7	Oromia	Ada'a
5	Biftu	121	27	Oromia	Lume
6	Guchi	59	9	Oromia	Ada'a
7	Memihir Ager	53	3	Amhara	Minjar
8	Diquana	?	?	Amhara	West Belesa
9	Kalay	110	13	Amhara	West Belesa
10	Geta Amba	197	6	Amhara	Siyadebirna Wayu
11	Timret	167	6	Amhara	Siyadebirna Wayu
12	Teseffa	150	15	Amhara	Ensaro
13	Weyra Amba	46	3	Amhara	Moretina Jiru
14	Hawi Boru	137	23	Oromia	Chefe Donsa
15	Lemlem Chefe	113	13	Oromia	Chefe Donsa
16	Chala	55	2	Oromia	Lume
17	Utuba Jiregna	?	?	Oromia	Adaa
18	Gudina	100	8	Oromia	Lume
19	Burka	?	?	Oromia	Lume
20	Birbisa	19	7	Oromia	Lume

This is in agreement with the report by Aliy (2016) that state that the huge gap as well as uneven access to scarcely available improved chickpea seed has created the development of an alternative and innovative seed production and delivery systems that responds to the immediate demand of the resource poor farmers. The author indicated that community-based seed production, which operates neither purely as commercial seed business nor as farmer managed ones, has been playing an important role in seed production and supply. Bishaw and Sushil (2016) also emphasized on the importance of mobilizing and engaging farmers in seed production and marketing and promoting farmer based smallscale enterprises due to the inefficiency of the formal sector in pulse crops seed production. For sustaining seed production, informal seed systems, community based as well as individual farmer based are instrumental to meet seed

requirements and to disseminate new varieties (Laxmipathi *et al*, 2016).

Due to the innovative technology multiplication and dissemination approaches, chickpea seed production of various classes have increased considerably (Figure 4). Breeder seed production showed an increase of 113% over the 6 years (Figure 4a). It increased from 3.8 tons at the start of the project (2008) to 8.1 ton in 2014, which means an annual increment of 0.72 tons. On the other hand pre-basic and basic seed production showed 246% increment over the years with an average annual increment of 16.1 tons (Figure 4b). There has also been 420% increment in the production of certified and QDS seed in 2014 compared to that of 2008 (Figure 4c). The production of certified and QDS seed in 2014 (3290 t) was more than five- fold over that was produced at the start of the project (632.7t) in 2008.

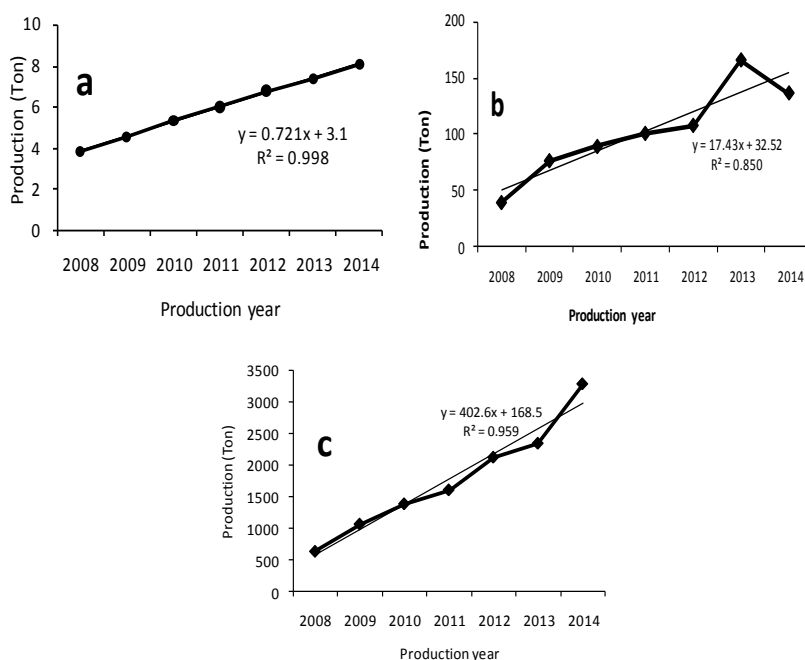


Figure 4. Chickpea seed production in Ethiopia (2008-15); (a) breeder seed, (b) pre-basic and basic seed and (c) certified/QDS seed.

Considering the area under chickpea, however, the amount of seed produced is far below the demand. For instance, the area under chickpea production in 2015/16 was 258,486.29 ha. To cover 75% of this area with improved varieties (leaving the 25% for landrace maintenance) 23,264 tons of seed is required at an average seeding rate of 0.12t/ha. Hence the highest amount of certified and QDS seed produced in 2014 (3290 t) was only 14% of the required amount. Much effort is, therefore, needed to strengthen the seed system to satisfy the demand for the development of the chickpea sector.

Impact of Research and Development Intervention

The national chickpea productivity and production data of the central

statistical authority of Ethiopia shows low tonnage of chickpea production in the early years (Figure 5). Stimulated by the innovative interventions chickpea production increased drastically during the decade and attained 124% increase in 2015 over that of the production in 2006. The 96.5% yearly increment in chickpea production ($R^2=0.965$) in the period could be largely attributed to the significant improvement in the productivity (90.5% yearly) of the crop, without undermining the increment in the production area that had 78% annual increment. The national productivity of the crop was one ton per hectare in 2006. It nearly doubled in decade's time and attained peak of 1.9 ton ha⁻¹ in 2014, which is among the highest record globally. This significant improvement in the

national productivity and production of chickpea was the result of the innovative intervention in the multiplication and dissemination of improved technologies. Asfaw *et al.*

(2012) and Simone *et al* (2016) also reported that dramatic increase in improved chickpea adoption had a strong positive effect on household welfare.

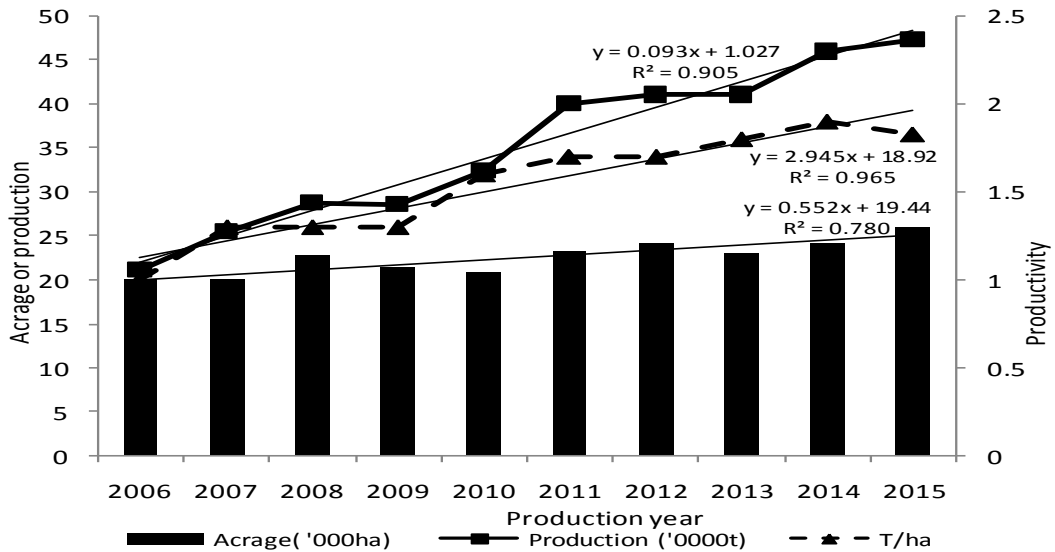


Figure 5. National area coverage, production and productivity of chickpea in Ethiopia (2006-2015). Source: Compiled from CSA (2006-2015).

By the end of the Growth and Transformation Plan of the country for chickpea productivity was set 3.9 tons per ha by 2012, hence there is too high to go looking the ambitious plan. The two pulling factors farmers would be encouraged with are: 1) productivity gain per unit area, which is comparable to some high input cereals, 2) market for seed and grain for chickpea which is among the highest. Chickpea production using improved varieties is profitable, as reported by Fikre (2014), that it fetches about USD 2,000 per hectare. The low production cost of the crop is among the contributing factors. This time around, in layman's estimate, chickpea grain price hit USD 3,600 per hectare (3,000

ETB/ha x 1.2 USD/kg). This could even be complimented to 15% seed premium values. The growing demand in both the domestic and export markets that provides a source of cash for smallholder producers as reported by Abera (2010) and Shiferaw and Teklewold (2007) would make chickpea production an important business.

Conclusions and Recommendation

Intervention in the development of chickpea sector started with awareness creation and training of relevant actors, as building their capacity helps

in enhancing the seed system and dissemination of improved technologies. An innovative approach of farmers' participatory variety selection (FPVS) built confidence of farmers on new varieties and enhanced uptake of technologies in addition to the efforts on popularizations.

The legume seed system in general and of chickpea in particular has limited involvement of the formal seed sector. To overcome the chickpea seed shortage the Tropical Legumes project took an innovative approach of bringing CBSP or FSPA into picture. The program worked on development of CBSP system by encouraging active farmers to engage in seed production through building their technical capacity in seed production, providing advisory supports and starter seed that resulted in the establishment of 'seed producer associations' in the country. The associations got the strength of covering the lion's share of the chickpea seed supply and became an effective national seed corridor. Due to the innovative technology popularization and seed multiplication approaches chickpea seed production of various classes have increased considerably over years in response to the increasing demand. Currently few of the associations are engaged in certified seed production as a result of the recognition they got due to their improved infrastructural, financial and technical capacity. The increased availability of seed in turn resulted in the drastic increment in the national

chickpea productivity and production and thereby income of the farmers.

In spite of the improvements made in increasing the availability of seed, the amount of certified and QDS seed being produced is still too low to satisfy the demand. Hence, much effort is needed to strengthen the seed system for the development of the chickpea sector. It is, therefore, critical to strengthen the informal seed system. To this effect, establishing more farmers' seed producer associations in other chickpea growing regions is necessary to avail seed in the vicinity of the farmers. More over the capacity of such association need to be built so that they would be able to produce quality seed that could be certified under the proper regulation system. Efforts should also be made in popularizing improved technologies to unaddressed potential chickpea production areas to boost the national production.

Acknowledgement

The authors are grateful to the Bill and Melinda Gate Foundation for supporting the chickpea improvement program through Tropical Legumes projects that made significant contribution to the R&D success.

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Stability Analysis in Chickpea Genotype Sets as Tool for Breeding Germplasm Structuring Strategy and Adaptability Scoping

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Abstract

Chickpea research program has come across realizing the importance of restructuring the working germplasm pool in Ethiopia where we have 39 divergent agroecological zones (AEZ). Though chickpea is not suit to all, it adapts in more than 30% of the agroecologies having different scale of responses. Hence, as show case we have tried to scan the agroecologies discrimination power based on crop using three sets of bred-crop responses. Evidently enough, germplasms in all the sets have revealed differential responses for economical yield and associated traits, from the three set of 57 entries put under 47 environments. The AMMI stability value and stability index have been able to discriminate genotypes with designated position; and supposed the breeding program would signify values by attempting both environment and genetics still as key considerable factors.

Keywords: Adaptability, Agro-ecology, Breeding, Genotype, stability

Introduction

Chickpea (*Cicer arietinum* L) in Ethiopia is crop on a high moment of development and transformed from simple precursor to principal crop of the producing households. Ethiopia as secondary center of diversity (van-der-Maesen, 1987) is making advantage in developing the chickpea industry faster and bolder by time. Unlike the altitudinal range (1400-2800 m asl) suggested by Anbessa and Bejiga (2002), the crop adaptation range has

verified to expand further to thermal zones where the altitude goes down to 600m asl with reasonable yield in Afar (Semera and Werer) and South Ethiopia (Woito) demonstrating high yield of more than 2 tons/ha (Shimelis, 2017). This would at least double the command area of suitability of the crop in magnitude of multimillion hectares.

During the 2014/2015 cropping year, 1.08 million smallholder Ethiopian farmers produced 458,682 tons of

chickpea on 239,755 ha of land with an average productivity of 1.913 tons ha⁻¹ (CSA, 2015). This makes Ethiopia one of the high productive geography as it demonstrated doubling value of the global average. However, the yield potential of chickpea in Ethiopia still is verified to hit 6t/ha (Asnake, 2016) of course under optimal genetics x management x environment combination.

Classical breeding programs extensively employ the effect of genetics and environment as drivers of genotypic responses and eventually determines their outcome of characterization and evaluation. Response traits could be varying in the germplasm resources, that breeding programs employ structuring depending on the objectives of the study. Genotype evaluation for traits of interest using multiple locations and years often pose complication of selection as the interaction of G x E could lead to unpredicted outcome (Farshadfar *et al.*, 2012). The yield stability evaluation based on genotype by year or genotype by location by year interaction (G x E x Y) is a good selection parameter (Annicchiarico, 1997); and would lead to stratification of the genotypes on narrow base adaptability or wider adaptability. In Ethiopian chickpea some 25% of the released varieties have been developed for specific regional adaptation, as they are released by the RARIs. However, even with this refinement, the level of interaction can remain high, because breeding area does not reduce the interaction of genotypes

with location on years (Eberhart and Russell, 1966; Tai, 1979). The other commonest strategy for reducing GxE interaction involves selecting genotypes with better stability across a wide range of environments in order to better predict their behavior (Farshadfar *et al.*, 2011). GxE analysis is important to identify superior varieties and their adaptation to and stability in diverse agroecologies (Kanouni *et al.*, 2015).

Differential performance of chickpea genotypes under diverse environmental conditions decreases yield stability (Padi, 2007). Inefficiency in the GxE analysis of variance may result in wrong selection of genotypes for yield. There are many models for managing GxE interaction in which its applicability depends on the experimental data, the number of environments, and the accuracy of collected data and environmental information. In this study, we used The Additive Main effects and Multiplicative Interaction (AMMI) a widely applicable model that combines analysis of variance (AOV) to partition the genotype main effects, environment and their interaction in yield stability analysis as its reliability recently been forwarded (Hongyu and Garc, 2014). Moreover, AMMI provides an initial diagnosis of the model and is well-suited for data analysis with many environmental influences. It also allows greater unfolding of the G×E interaction and summarizes the patterns and relationships between genotypes and environments, and improves the

accuracy of trait estimates (Gauch, 1988; Zobel et al., 1988; Crossa et al., 1990). Using AMMI stability value and mean yield, YSI incorporates both mean yield and stability in a single criterion. Low value of this parameter shows desirable genotypes with high mean yield and stability. The objectives of this study were (i) to identify genotypes in the three sets that have both high mean yield and stable yield performance across different environments, (ii) to study the relationships, similarities and dissimilarities among yield–stability statistics that is implicated on genotypes structuring

Materials and Methods

Description of eco-location and genotypes

A study was undertaken by the national chickpea research program using germplasms of different genetic background to determine their level of

stability and consistency in their biological yield responses. Fifty-seven advanced breeding genotypes in three sets (Table 2) together with appropriate checks were evaluated each over three seasons between 2008 and 2010 at eight diverse elevations (1500's m asl to 2400's m asl) eco-locations (Table 1). In the three years a total of 47 environments, however, some locations shared among the three sets have been considered. The experiments were put under three independent sets (desi types set (A), kabuli type early set (B), late set (C)) using Randomized Complete Block Design (RCBD) with independent and combined analysis for each set of genotypes. Each genotype was planted in 30cm by 10cm inter and intra row spacing in three replications. Production was all under rain fed condition. The eco-climatic characteristics of the research locations are presented in Table 1.

Table 1: Characteristic features of test eco-locations

Research Location	Altitude /m asl/	Rainfall mean /mm/	Temp. mean /°C/ min and max	Soil
Debre Zeit /DZ/	1900	851	8.9-28.3	Vertisol
Alem Tena /AT/	1575	728	12.9-29.8	Sandy-loam/light
Chefe Donsa /CD/	2450	750-1200	7-26	vertisol
Sinana /Sin/	2400	1150	9-21	Nitosol
Minjar/Min/	1810	600-1000	10-28	Light vertisol
Akaki/Ak/	2200	1025	7-26	vertisol
Arsi Negele /AN/	1913	915	17.7	Sandy-loam
Dhera/Dh/	1650	680	14-27.8	Silty loam/andosol/
Arsi Robe /AR/	2420	890	6-22.1	Heavy clay/vertisol/
Adet /Ad/	2240	1270.5	8-25	Nitosol
Ambo /Am/	2175	1018.29	10.02-26.89	vertisol
Hawassa /Haw/	1700	1141	13.1-27.1	Light soil

Table 2. Over location performance of set genotypes over test year1, year 2 and year 3 of 2008, 2009 and 2010 respectively [eco-locations suffix 1, 2, 3 indicates the test year]

	Over location yield performance test Desi late= Set A [DZ1-AK1-CD1-Sin1-DZ2- AK2-CD2-AR2-Ad2-DZ3- AK3-CD3-AM3]	Over location yield performance test kabuli early=Set B [Min1-AT1-DZ1-AN1-DH1-Min2- AT2-DZ2-Haw2-DZ3-Min3-AN3]	Over location yield performance test kabuli =Set C [AK1-CD1-DZ1-Min1-Sin1-AK2- CD2-DZ2-AN2-Ad2-AM2-AR2- HaW2-AK3-CD3-DZ3-AN3-Min3]
1	Akaki	Chefe	Chefe
2	ICC-3195	DZ-10-4	DZ-10-4
3	ICCV-00104	FLIP 00-60C	FLIP 01-12C
4	ICCV-00110	FLIP 00-73C	FLIP 01-21C
5	ICCV-00202	FLIP 01-16C	FLIP 01-40C
6	ICCV-03103	FLIP 01-29C	FLIP 01-45C
7	ICCV-03107	FLIP 01-2C	FLIP 01-46C
8	ICCV-03203	FLIP 01-56C	FLIP 01-52C
9	ICCV-04111	FLIP 02-25C	FLIP 01-57C
10	ICCV-92219	FLIP 02-39C	FLIP 01-58C
11	ICCV-95138	FLIP 02-46C	FLIP 01-60C
12	ICCV-97030	Teji	FLIP 01-7C
13	ICCX-910121-5	X96TH-52-14/2000	FLIP 01-8C
14	ICCX-910144-4	X98TH-51-1-3	FLIP 02-02C
15	ICCX-940002-F5-242P-1-1-1	X98TH-81-2	FLIP 02-11C
16	ICCX-940002-F5-294P-1-1-1	X98TH-82-4	FLIP 02-22C
17	ICCX-940002-F5-335P-1-1-1	X98TH-82-7	FLIP 98-218C
18	ICCX-940002-F5-6P-1-1-1		Habru
19	ICCX-940002-F5-88P-1-1-1		ICCV-04305
20			ICCV-04307

Statistical analysis

AMMI analysis

The grain yield data were subjected to combined analysis of variance and AMMI analysis which is a combination of analysis of variance and multiplication effect analysis. AMMI analysis of variance was used to partition total sum of squares into its components: genotype and environment main effects, GxE interaction and the residual term. Subsequently, multiplication effect analysis is used to partition GxE deviations into different interaction principal component axes (IPCA),

which can be tested for statistical significance through ANOVA.

The AMMI model equation for i^{th} genotype in j^{th} environment in r blocks (replication) formulated by Gauch, (1992) was used to analyze G x E interactions as;

$$Y_{ijr} = \mu + G_i + E_j + B_r (E_j) + \sum_{k=1}^n \lambda_k Y_{jk} \alpha_{en} + P_{ij} + \varepsilon_{ij}$$

Where Y_{ijr} is the yield of genotype (i) in environment (j) for replicate (r), μ is the total yield mean, G_i is the main effect of genotype or the genotype (i) mean deviation (genotype mean minus

total yield mean), Ej is the main effect of environment or the environment (j) mean deviation, $Br(Ej)$ is the effect of the block r within the environment j, r is the number of blocks, k is the singular value for IPCA axis k (k is the number of remain IPCA axis in AMMI model) γ_{jk} and j_k are the genotype (i) environment (j) Eigen vector value (i.e. the left and right singular vectors) for IPCA axis k, P_{ij} is the residual containing all multiplicative terms not included in the model, n is the number of axes or principal components (IPCA) retained by the model, and ϵ_{ij} is the experimental error, assumed independent with identical distribution

$$ASV = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}} (IPCA1_{score}) \right]^2 + (IPCA2_{score})^2}$$

Where $\frac{SS_{IPCA1}}{SS_{IPCA2}}$ is the weight given to the IPCA1-value by dividing the IPCA1 sum of squares by the IPCA2 sum of squares, $IPCA1_i$ = IPCA1 score of the i^{th} genotype and $IPCA2_i$ = IPCA2 score of the i^{th} genotype. The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller ASV scores indicate a more stable genotype across environments.

Another component of stability in AMMI model is yield stability index (YSI) and was calculated as: $YSI =$

We considered stability parameters based on Farshadfar *et al.* (2011), which affirmed that Yield stability index (YSI) which incorporate AMMI stability value (ASV) and mean grain yield in a single non-parametric index, and Rank sum as explained by sum of rank mean (R) plus standard deviation of ranks (SDR), were found the most desirable indices for discriminating the most stable genotypes with high grain yield.

Calculations were performed using R software version 3.1.3 using the full data (including all replicates data) for AMMI model. The AMMI stability value (ASV) as described by Purchase (2000) was calculated as follows:

$rASV + rY$ where, $rASV$ is the rank of AMMI stability value and rY is the rank of mean grain yield of genotypes (rY) across environments. YSI incorporates both mean yield and stability in a single criterion. Low values of this parameter show desirable genotypes with high mean yield and stability

AMMI 2 biplot for demonstrating the magnitude of G×E

Genotypes and environments, in the three sets, were overlaid on the biplot and their responsiveness was drawn

based on their distance from the reference origin on the AMMI 2 biplot. Genotypes which are close to the origin are considered non-sensitive to environmental interaction.

The relationships among and between environments and genotypes on the graph of AMMI 2 biplot help to predict relative performance of a given genotype in a given environment by drawing connecting segments (blue line) between all the genotypes located at the outer side and then creating lines from the origin (0, 0) that cut these segments perpendicularly (i.e. the red dotted line is perpendicular to the green line). If any environment point lies on the red dotted line, genotypes found at the two ends of the segment will produce equal yields in that environment. On the other hand, if an environment point lies on one side of the red line, the closer genotype will produce a higher yield in that environment (Yan et al., 2000; Yan and Kang, 2002; Yan and Kang, 2003). Thus, genotypes in the current study were judged based on their discriminative environments for adaptation or suitability.

Results and Discussion

Adaptive response of yield by environment

Three analytical evidences ANOVA, AMMI biplot ASV (AMMI Stability Value) and YSI (Yield Stability Index) have been done across the three experimental sets and used as support of the discussion. Combined analysis of variance (Table 3) of environment (year + location) by genotypes resulted in highly significant differences ($P \leq 0.01$) in their interaction for the three sets. The significant interactions of genotypes $\times$ environments (locations and years) suggest that grain yield of genotypes varied across environments. Similarly, there are differential responses of the genetic constituents. Significant differences for genotypes, environments and GE interaction indicated the effect of environments in the GE interaction, genetic variability among the entries and the possibility of selection for narrow base or wider based adaptability genotypes. It was reported that GE interaction in multi-locations within a year is more important than GE interaction with year (Chandra et al., 1974). As GE interaction was significant, therefore we can further proceed and estimate phenotypic stability (Farshadfar and Sutka, 2006).

Table 3: ANOVA Table for National Variety Trial of Desi, early Kabuli and late Kabuli chickpea sets evaluated across diverse agro ecologies.

Set A (D)	Df	Sum Sq	Mean Sq value	F	Pr(>F)	Remark
ENV	17	2548388156	149905186	121.8507	< 2.2e-16 ***	Coeff var: 17.5 Mean YLD: 2595.2
REP(ENV)	53	65202523	1230236	5.9941	< 2.2e-16 ***	
GEN	19	62856597	3308242	16.1187	< 2.2e-16 ***	
ENV:GEN	323	322211444	997559	4.8604	< 2.2e-16 ***	
Residuals	1647	338035059	205243			
Set B (KE)						
ENV	11	956889433	86989948	194.9231	< 2.2e-16 ***	Coeff var: 19.4 Mean YLD: 2054.0
REP(ENV)	24	10710677	446278	2.8248	7.816e-06 ***	
GEN	16	39774570	2485911	15.7349	< 2.2e-16 ***	
ENV:GEN	176	209204374	1188661	7.5238	< 2.2e-16 ***	
Residuals	996	157355455	157987			
Set C (KL)						
ENV	12	1076408410	89700701	68.0685	< 2.2e-16 ***	Coeff var: 14.8 Mean YLD: 2830.1
REP(ENV)	39	51394199	1317800	7.5436	< 2.2e-16 ***	
GEN	19	109856180	5781904	33.0979	< 2.2e-16 ***	
ENV:GEN	228	227028264	995738	5.7000	< 2.2e-16 ***	
Residuals	1461	255223890	174691			

Key: ENV= environment, rep= replication, gen= genotypes, df= degree of freedom, pr= probability

Mean grain yield of the three sets of genotypes ranged from 1327 kg/ha in set B to 3057kg/ha in Set C (Table 4) clearly demonstrating the high productivity differences in the Sets for yield scale. Genotypes of annual crops evaluated for grain yield on a multi-locational, multi-year basis frequently show GE interaction that complicates the selection or recommendation of materials. Coping with genotype-year or genotype-location-year interaction effects is possible only by selection for yield stability across environments defined as location-year combinations (Annicchiarico, 1997). If the deriving interest is to get low G x E, interaction as is the case with many programs, the two possibility are either to follow homogeneous environmental cluster, which is difficult to realize (Tai, 1979), or to go for reducing GxE

interaction by selecting genotypes with a better stability across a wide range of environments in order to better predict behavior (Yaghotipoor and Farshadfar, 2007), which is the dominant practice of breeding programs. Calculating the account of the total Sum of Squares/TSS/, in all the three sets A, B and C, environment was dominant factor accounting for 76%, 70% and 63%, respectively and followed by the interaction effect as the next high for all, but Set B. This would suggest the existence of the different environment groups conditioned by complex and easily unpredictable parameters. From this parallel analysis of the three sets, interestingly enough, environment is universally dominating factor that highly influences the system with its unpredictability outcomes.

Table 4. AMMI stability value based rankings of genotypes with average yield performances in the three sets.

	Set A (D)					Set B (KE)					Set C (KL)						
	ASV	YSI	rASV	rYSI	means		ASV	YSI	rASV	rYSI	means		ASV	YSI	rASV	rYSI	means
G15	9.7	4.0	3.0	1.0	2715.6	G1	19.8	6.0	5.0	1.0	2256.1	G18	8.54	8.00	7.00	1.00	3057.4
G20	27.8	19.0	17.0	2.0	2625.4	G13	17.5	5.0	3.0	2.0	2110.5	G1	7.28	5.00	3.00	2.00	2909.5
G13	26.3	19.0	16.0	3.0	2618.2	G10	41.0	19.0	16.0	3.0	2066.7	G17	13.98	16.00	13.00	3.00	2888.8
G8	22.9	18.0	14.0	4.0	2597.5	G12	25.4	13.0	9.0	4.0	2045.4	G3	3.11	5.00	1.00	4.00	2836.4
G16	21.2	18.0	13.0	5.0	2563.7	G8	18.8	9.0	4.0	5.0	2037.2	G13	6.14	7.00	2.00	5.00	2812.0
G18	3.9	7.0	1.0	6.0	2523.5	G14	31.6	18.0	12.0	6.0	2003.9	G10	15.14	20.00	14.00	6.00	2767.7
G17	12.4	11.0	4.0	7.0	2482.1	G17	23.2	14.0	7.0	7.0	1986.7	G9	16.50	22.00	15.00	7.00	2757.3
G19	20.1	19.0	11.0	8.0	2477.4	G7	24.5	16.0	8.0	8.0	1983.6	G8	11.57	18.00	10.00	8.00	2745.4
G10	20.5	21.0	12.0	9.0	2450.1	G16	38.6	24.0	15.0	9.0	1972.4	G7	10.04	18.00	9.00	9.00	2733.3
G5	16.2	18.0	8.0	10.0	2449.5	G15	28.3	21.0	11.0	10.0	1909.4	G5	7.32	14.00	4.00	10.00	2691.3
G11	9.5	13.0	2.0	11.0	2419.4	G6	22.2	17.0	6.0	11.0	1908.5	G6	12.00	22.00	11.00	11.00	2647.9
G3	18.5	21.0	9.0	12.0	2353.4	G3	5.6	13.0	1.0	12.0	1882.8	G12	27.03	30.00	18.00	12.00	2638.5
G14	23.8	28.0	15.0	13.0	2351.7	G11	25.8	23.0	10.0	13.0	1850.7	G11	8.34	19.00	6.00	13.00	2608.0
G1	15.8	21.0	7.0	14.0	2331.4	G4	33.1	27.0	13.0	14.0	1833.1	G15	13.04	26.00	12.00	14.00	2583.2
G12	29.2	33.0	18.0	15.0	2303.9	G5	17.5	17.0	2.0	15.0	1787.8	G4	21.61	31.00	16.00	15.00	2558.6
G2	19.8	26.0	10.0	16.0	2303.0	G9	37.7	30.0	14.0	16.0	1766.9	G14	9.88	24.00	8.00	16.00	2557.1
G9	14.7	23.0	6.0	17.0	2297.1	G2	48.9	34.0	17.0	17.0	1327.0	G16	7.47	22.00	5.00	17.00	2517.2
G6	30.7	38.0	20.0	18.0	2290.3							G19	41.03	37.00	19.00	18.00	2480.7
G4	14.6	24.0	5.0	19.0	2245.6							G20	26.85	36.00	17.00	19.00	2470.8
G7	30.0	39.0	19.0	20.0	2202.8							G2	42.56	40.00	20.00	20.00	1941.6
												G2	42.56	40.00	20.00	20.00	1941.6

Key: ASV= AMMY stability value, YSI= yield stability index, r= rank

AMMI stability values (ASV) and bi-plot analysis

ASV measure was proposed by Purchase *et al.* (2000) in looking provision for a quantitative stability measure, such a measure is essential in order to quantify and rank genotypes according to their yield stability. In fact, ASV is the distance from zero in a two-dimensional scatter-gram of IPCA1 (interaction principal component analysis axis 1) scores against IPCA2 scores. The distance from zero is then determined using the theorem of Pythagoras (Purchase *et al.*, 2000). In ASV method, a genotype with least ASV score is the most stable, accordingly, G18 followed by G11 of set A i.e., Desi type, G3 followed by G5 of set B i.e., kabuli early, and G3 followed by G5 of set C i.e., Kabuli late, were the most stable genotypes. In general the importance of AMMI model is in reduction of noise even if principal components do not cover much of the GE sum square (Gauch, 1992; Gauch and Zobel, 1996).

G18, G3, G3, despite being the most stable genotypes of set A, set B and set

C, respectively, their respective yield compromise of 7.6%, 19.7%, 7.8% compared with the first rank of YSI, is of particular interest. It follows that, stability alone cannot be the basis for screening and selection of genotypes for release since some genotypes are stable for poor yields across environments (Yan and Kang, 2003) and selecting them would lead to development of poorly competent variety.

As far as stability is concerned G18>G11> G15 from experiment set A (Figure 1 and Table 4 set A); genotypes G1, G13 and G10 from experiment set B (Figure 2 and Table 4 set B); genotypes G3, G1 and G13 from experiment set C (Figure 3 and Table 4 set C) were the first three genotypes that were located to the right side of AMMI 1 biplot in decreasing order of the stability value. However, in terms of yield stability index, with some alteration in stability order genotype G15>G18> G17 from set A; from experiment set B genotype G13> G1> G8; and also genotypes from experiment set C G3>G1>G13 had the most desirable trait of high yielding and stability as well.

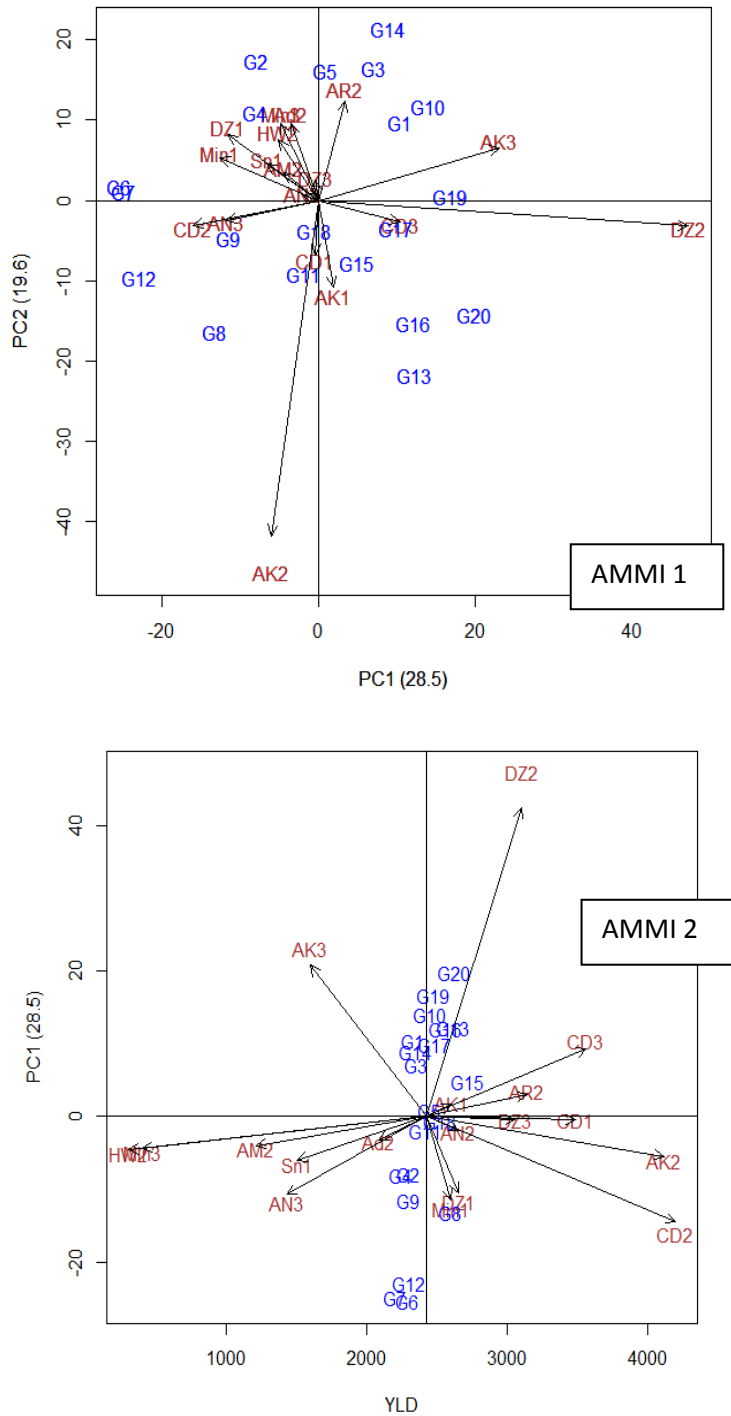


Figure 1. AMMI Biplot for desi chickpea genotypes

Graphical representation of the AMMI 1 biplot for additive and interaction effects

The adaptation habit for most of genotypes of the experiment set A (Desi type late) in figure 1 of AMMI 1 biplot, could be categorized mainly into two with exclusion of the three stable genotypes. The first genotype category comprises of nine genotypes which had positive IPCA1 scores namely G3, G14, G1, G17, G16, G13, G10, G19 and G20 according to increasing order of their interactive nature with environment. The second genotype category consisted of seven genotypes having negative IPCA1 score. Except G8 which had higher yield performance, the remaining six genotypes (G2, G4, G9, G12, G7 and G6) had low yield performance and hence had nothing to be retained in the breeding scheme. Similarly, environments had shown variation in main effect as well as in interaction effect (Figure 1).

According to Duarte and Vencovsky (1999), stability is evaluated in the y-axis (IPCA1) by AMMI1, whereas AMMI2 analysis revealed stable environments and genotypes located near the origin, with low scores for the two axes of the interaction (IPCA1 and IPCA2). On this basis of their effect on yield performance of genotypes; environments were grouped into two groups as high and low yielding. Environments Akaki 2008, Arsi Negelle in year 2009, Minjar in year 2008, Debre Zeit in year 2008, Debre Zeit in year 2010, Debre Zeit in year

2009, Arsi Robe in year 2009, Chefe Donsa in year 2008, Chefe Donsa in year 2010, Akaki in year 2009 and Chefe Donsa in year 2009 were identified as high yielder out of which Akaki in year 2008, Arsi Negelle in year 2009, Debre Zeit in year 2010 and Chefe Donsa in year 2008 were found to be stable environments. Those environments were closely located on the x-axis indicating that they were stable. The second environment group was those environments located on the left side of the Y-axis having low yield performance. Environments Hawassa in year 2009 and Minjar in year 2010 were locations identified with poor yield performance of the genotypes.

On biplot of AMMI 2 (Figure 1) genotypes in experiment set A (Desi type) G6, G7, G14, G20, G13, G8 and G12 and environments Debre Zeit in year 2010, Akaki in year 2009, Chefe Donsa in year 2009, Debre Zeit in year 2008, Arsi Robe in year 2009 and Akaki in year 2010 were identified as the most responsive/interactive since they were located far from the origin (0, 0) (Purchase, 1997). On the other hand, genotypes G8, G11 and G15 which were located close to the origin and hence were considered non-sensitive to environmental interaction (Figure 1). The distribution of environments on AMMI 2 biplot were concentrated on quadrants II forming a cluster of environments which most likely influence the performance of genotypes distributed in quadrant II and quadrant IV. It is worth to note that extending vectors formed by

environments to their opposite directions help see the relationships among and between environments and genotypes (Mcdermott & Centre, 2012) . Accordingly, genotypes in quadrant IV (G13, G16, G17 and G20) and also genotypes in quadrant II (G2, G4, G6, G7) were influenced by environments AN3, DZ3, AM2, Sn1, Min1, DZ1, HW2, Min2 and Ad2.

AMMI1 biplot presentation (Figure 2) of seventeen chickpea genotype in an experiment set B revealed that two of the genotypes (G5 and G3) were identified as stable lines, though were poor in yield performance. There were only three genotypes (G1, G13 and G10) that performed higher yield over grand mean (2054kg/ha) of all observation. G1 (2256 kg/ha) with the highest yield performance was released variety named Chefe confirming that breeders' capability in developing cultivars with better yield trait, and since none of the genotypes could surpass the check the breeding program need to see into more vigor of

genetic combination and recombination. Similarly, yield of the genotypes (in experiment set B) in most of environments (seven out of twelve) was also found to be low (Figure 2).

In contrary to this, environments Debre Zeit in year 2009, Debre Zeit in year 2008, Minjar in year 2009, Debre Zeit in year 2010, and Minjar in year 2008 were high yielding environments among which Debre Zeit in year 2010 was found to be most stable environment. It is important to notice the seasonal influence on physically same location to behave as distinct environment. It is also worth to note that the definition of environment in the current study was referred to as combination of physical attributes of a location and the climatic and other attributes of a specific season (i.e. soil type, fertility, topography, temperature, rainfall, pest/disease challenge) that affect the plant growth (Mcdermott & Centre, 2012).

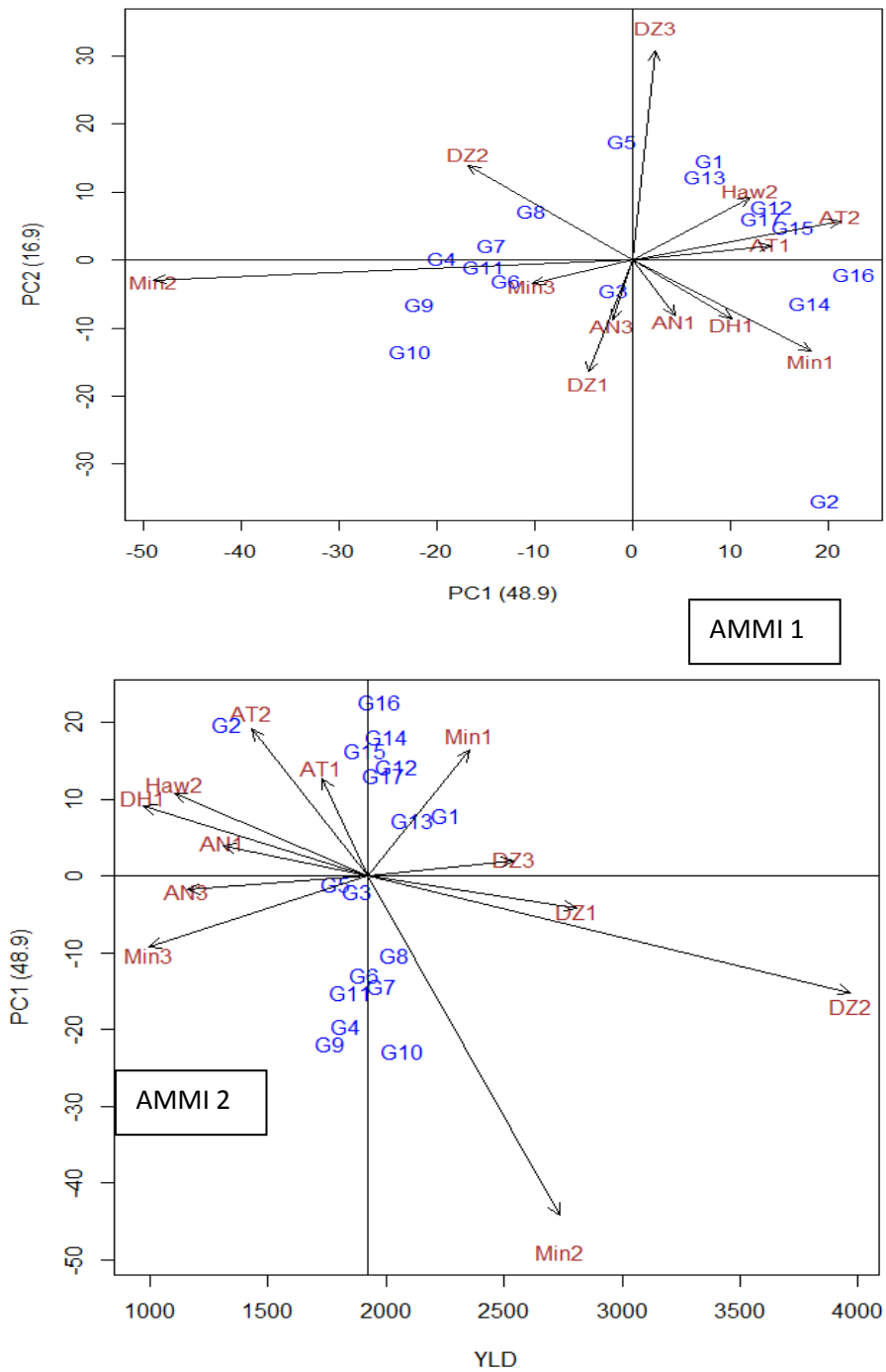


Figure 2. AMMI Bi-plot for early maturity kabuli chickpea genotypes

Discriminative nature of the environments that would be exerted on genotypes can be determined from the magnitude of IPCA scores (Figure 2). Environments with large IPCA score had more discriminative of genotypes, while environments with IPCA scores near zero exhibit little interaction across genotypes and less discrimination among genotypes (Thangavel *et al.* 2011; Funga *et al.*, 2017). Accordingly, Debre Zeit and Minjar in year 2009 were most discriminative environments as indicated by long distance from the origin of the biplot graph (Figure 2). Genotypes with positive IPCA1 scores respond positively (adaptable) to the environments that have positive IPCA1 scores (i.e. their interaction is positive). Those that respond negatively to the environments (less adapted) have negative IPCA1 scores as stated elsewhere (Samonte *et al.*, 2005).

The AMMI1 biplot (figure 2) revealed that genotypes G1, G13, G17, G12, G15, G14, G16 and, G2 with positive IPCA1 scores responded positively to the environments distributed on the first and second quadrant of AMMI1 biplot regardless of the merit of genotypes. Similarly, genotypes distributed over the third and fourth quadrants of AMMI1 biplot has negative IPCA-1 scores and hence respond positively to the environments located in the same quadrants. For instance, genotypes G8, G6, G7, G11, G4, G9 and G10 respond positively to environments Arsi Negelle in year 2010, Minjar in year 2010, Minjar in

year 2009, Debre Zeit in year 2009 and Debre Zeit in year 2008.

Predicting the relative performance of a given genotype in a given environment for this study had no use since there were no new genotypes performing better than the already registered chickpea variety Chefe (G1) (Figure 1 and Figure 2), however, it gives clues on breeding genotypes development and competence. It could also be a case where the genetic composition of the commercial varieties are well constructed, that new approaches need be sought.

There were ten genotypes in experiment set C (Kabuli late type) such as G18, G1, G17, G3, G13, G10, G7, G8, G9 and G5 that performed higher yield than grand mean (2830.1kg/ha) with different stability habit (Figure 3). According to the value of IPCA1 score and ASV score G3 was the most stable line followed by G13. However, the mean yield performance of these two genotypes were at par amount to grand mean (2830kg/ha) which expelled us to find other high yielding genotype using yield stability index (YSI). Accordingly, Genotypes G18, G1, G17 and G3 were identified as high yielding lines in a decreasing order. As a matter of chance, the first two high yielding genotypes (G18 and G1) are released varieties and registered in the Official Varieties Catalogue of Ethiopia as 'Habru' and 'Chefe'. As a result of this, it is not important discussing on the remaining genotypes

because of their poor yield performance instability nature.

However, it is possible to predict the relative performance of a given genotype in a given environment according to the procedure given by Yan and Kang, (2003), Yan and Kang (2002) and Yan et al. (2000). Hence, genotypes in the current study were assigned to their adaptive environments as follow. On segment formed by G2 and G12 on AMMI 2 biplot (Figure 3) the two genotypes (G2, G12) at the vertex had a capacity to give equal yield at Ambo in year 2010 and the other genotypes performed better at environments close

in the either side of the dotted line. Similarity, on segment formed by G12 and G19 seven genotypes such as G7, G13, G1, G14, G6, G10 and G12 won at Akaki in year 2009, while G5, G18, G16, G17 and G20 performed better at Debre Zeit in year 2008, Akaki and Debre Zeit in year 2010. On third segment formed by G19 and G2, genotypes G19 and G2 perform at equal magnitude at Akaki and Chefe Donsa in year 2008 since both environments were located on red dotted line. Likewise, G3, G15, and G4 showed specific adaptation at Adet in year 2009, Sinana in year 2008 and Arsi Robe in year 2009 (Figure 3)

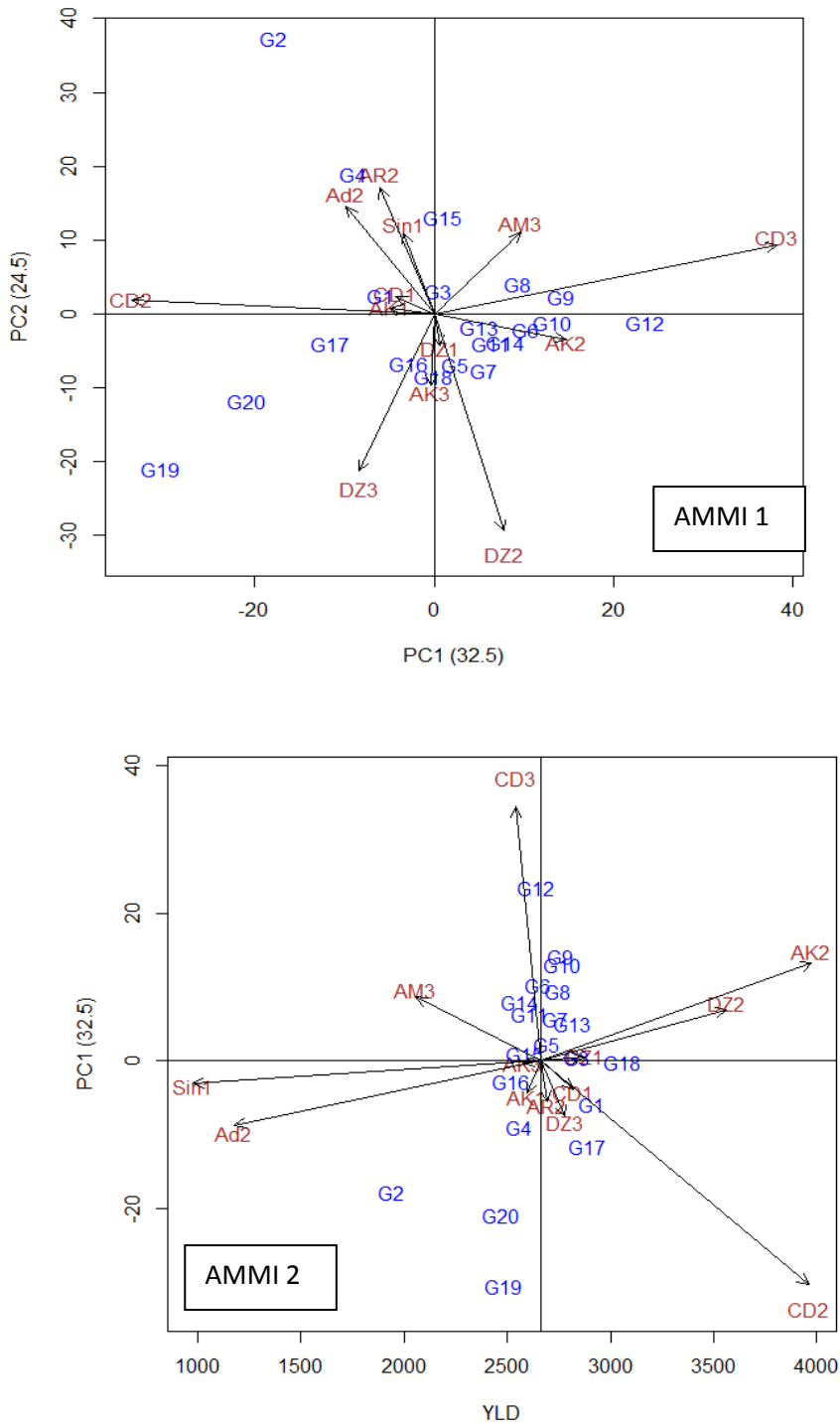


Figure 3. AMMI bi-plot for kabuli late maturing genotypes of chickpea

Conclusions and Implications

In the current study with exclusion of the released variety Chefe seven new test genotypes were identified from the three sets of experiments as stable genotype with comparable yield performance. Genotype G15(ICCX-940002-F5-242P-1-1-1), G18 (ICCX-940002-F5-6P-1-1-1)> G17 (ICCX-940002-F5-335P-1-1-1) from set A; genotype G13(X96TH-52-14/2000), G8 (FLIP 01-56C) from experiment set B and genotypes G3 (FLIP 01-12C), and G13(FLIP 01-8C) from experiment set C were genotypes identified having both high mean yield and stable yield performance across different environments.

From computing the ASV and presentation of AMMI 1 biplot, genotype with low IPCA 1 and AMMI stability value associated parameters, witness that decision making could be supported for the breeding and evaluation programs before getting into the final steps. Evidently, the analysis has demonstrated that the breeding genotypes in test seem to bounce with highest point, where we had non-surpassing the standard check. This could come from two perspectives; 1/ the germplasm enhancement program need be improved with innovative approach 2/ the right environment of potential expression is not well matched. Agro-eco-environments have different capacity set ups in discriminating

genotypes which critical processes of the breeding. In dealing with the three sets, the tool of analysis clearly demonstrated scenario of breeding considerations like stability, high yield, seed market quality (not considered here) or a combination of them to make the forward decision.

It is important to underline such type of studies are key informants of both in forward and back ward germplasm management in the breeding program. It is also important this analysis is subject to time bound (periodic functionality) and changes in time as environmental changes are experiencing in due course.

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Screening of chickpea for resistance to Ascochyta blight (*Didymella rabies*) under Field Conditions

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Abstract

Ascochyta blight caused by Ascochyta rabiei is serious foliar disease reducing yield potential of chickpea in Ethiopia and previously released desi type of varieties gradually lost their resistance. The present study was conducted to identify the sources of resistance to ascochyta blight in desi type of chickpea during 2013 to 2014 main cropping season. Eighteen chickpea genotypes and two released chickpea varieties were screened in Randomized Complete Block Design with three replications in AlemTena and Dhera sub-centers, hotspot areas for Ascochyta blight. Analysis of variance revealed that there were highly significant differences ($P \leq 0.001$) among the tested genotypes against ascochyta blight disease. From the overall observations, the resistance of the tested genotypes to ascochyta blight, when mapped against year by location, showed eight accessions were susceptible, six moderately susceptible, two moderately resistant and two resistant genotypes. This indicated that high variation among the tested genotypes in level of resistance against ascochyta blight and clear indication of environmental factors to ascochyta blight level of aggressiveness. One genotype (ICCV-96836) and one improved variety (Arerti) were identified as resistant, and their resistance levels were consistent over locations and years. Further study on inheritance of resistance, existing physiological race through Marker Assisted Selection (MAS) for their level of aggressiveness and intensive screening of the genotypes against ascochyta blight on additional testing location is suggested.

Keywords: Ascochyta blight, inheritance, resistance, screening

Introduction

Chickpea (*Cicer arietinum* L.) is cultivated globally on 13.5 million hectares of land from which 13.1 million tons of grain is produced (FAO, 2014). The total area covered by chickpea in Ethiopia is estimated at 258,886.29 ha and from this a

corresponding mean annual production of 472,611.40 tons of chickpea grain is produced (CSA, 2016). It is an important source of human food and animal feed in many parts of the world and it contains protein (28.9%), oil (3%) and carbohydrate (60%) (Ali *et al.*, 2013). Chickpea fixes a significant amount of nitrogen, adds organic matter and breaks the disease cycles of

important cereal pathogen (Pande *et al.*, 2011) and for this main reason is commonly used in rotation with cereals like tef and wheat on heavy soils (Geletu and Anbesa, 1994).

The average chickpea yield in Ethiopia is usually below 2 tons ha⁻¹, although its potential yield is more than 5 t/ha (Melese, 2005). The biotic and abiotic factors, drought, water logging, poor cultural practices and low or no protection against weeds, diseases and insect pests, are responsible for the low productivity of the crop. Although more than 70 pathogens have been reported on chickpea from different parts of the world so far, only a few of them are currently recognized as significantly important pathogens to chickpea production (Pande *et al.*, 2010). Of several diseases affecting chickpea, ascochyta blight caused by *Ascochyta rabiei* [teleomorph: *Didymella rabiei* (Kovachski) von Arx] is the most serious foliar disease of chickpea during wet, cool and humid seasons (Benzohra *et al.*, 2012). It is known to occur in almost all countries where chickpea is grown (Nene, 1982). In Ethiopia ascochyta blight is believed to be an introduced disease, with seeds of the cultivars Punjab7, C 217/3, C 410 (Bejiga, 1984).

Ascochyta blight can cause up to 100% crop loss under favorable environmental conditions where the relative humidity is greater than 60% and temperature range of 10-25 °C (Alwawi *et al.*, 2009; Jamil *et al.*, 2010). The use of resistant cultivars, which have high degree of variation

in resistance, is the most efficient and effective means of controlling ascochyta blight (Pande *et al.*, 2005). The existence of a range of different resistance sources indicates the need for characterization of potential resistance genes that may be pyramided by breeding programs in developing durable resistance (Tekeoglu *et al.*, 2000). There are also a growing number of reports indicating that resistance declines as the plant matures. For example, it has been reported that the resistance to ascochyta blight is age related which tends to decrease as time goes on, and with different stages in the life cycle of the host plant and the vegetative stage do not maintain resistance up to reproductive stage (Chongo and Gossen, 2001). One of the emerging challenges in breeding scheme to breakdown ascochyta blight disease/lack of durable resistance within the released varieties, high variability of pathogen population in terms of their virulence, quantitative nature of inheritance and presence of minor gene in commercial chickpea cultivars. Cultivars available in ICARDA lack complete resistance to *Ascochyta rabiei*. Partial resistance in cultivars adapted to the western Iran tends to break down after the onset of flowering. Partially resistant cultivars contribute to the development of new pathotypes of the disease by imposing selection pressure, possibly resulting in increased virulence or aggressiveness within the pathogen population (Singh *et al.*, 1992). With a genetically diverse population of *Ascochyta rabiei*, it is important not

only to develop cultivars with durable forms of resistance, but also to monitor changes in the population structure to anticipate resistance breakdown in existing cultivars. Nevertheless, the chickpea national program has been searching for sources of resistance to ascochyta blight in local landraces. However, those landraces were found to be totally susceptible to ascochyta blight. Therefore, germplasms have been introduced from international chickpea improvement centers and screened under Ethiopian condition. From these introductions, ascochyta blight resistant kabuli type chickpeas were obtained and released for large scale production. However, desi type of chickpea resistant to ascochyta blight has not been found from both landrace and introductions, which necessitate to continue the screening work. The present study was therefore, to identify desi type of chickpea genotypes resistant to ascochyta blight disease under field conditions.

Materials and Methods

Field experiments were conducted during 2013 and 2014 main cropping seasons at AlemTena (8°18'24.4"N, 38°57'05.3"E and 1610 m.a.s.l) and Dhera (08°19'10"N, 38°19'13"E and 1650 m.a.s.l.) which are the sub centers of Debre zeit Agricultural Research Center. At Alem Tena, the average annual rainfall is about 728

mm and the maximum and minimum annual mean temperatures are 29.8°C and 12.9°C, respectively, and the relative humidity ranges between 67% and 83%. At Dhera, the average annual rainfall is about 620 mm and the maximum and minimum annual mean temperature are 27.8 and 14°C, respectively, relative humidity ranges between 75% and 85%. The soil type at both location is light silty-loam (DZARC, 2013).

A total of 18 desi type of chickpea genotypes were used. Arerti (resistant) and Mariye (susceptible) varieties were used as check. The testing genotypes were obtained from International Crops Research Institute for Semi-Arid Tropics. The experiments were laid out as a randomized complete block design (RCBD) with three replications. The plot sizes were 4.0 m and 1.20 m. Spacing was kept at 30 cm and 10 cm between rows and seeds, respectively (DZARC, 2005).

Ascochyta blight severity assessment was started immediately after disease onset was visible as lesion on upper leaf and wilting of leaf tips was observed. Severity was recorded on plot base and assessed three times every seven to ten days' intervals using 1-9 point disease ratings scale (Pande *et al.*, 2011) the last score data was used since it is diseases severity that is progress from seedling to podding stage. The disease severity scoring scale (1-9) description as

follow: 1=no visible symptoms; 2=minute lesions prominent on the apical stem; 3=lesions up to 5 mm in size and slight drooping of apical stem; 4=lesions obvious on all plant parts and clear drooping of apical stem; 5=lesions on all plants parts, defoliation initiated, breaking and drying of branches slight to moderate; 6=lesions as in 5, defoliation, broken, dry branches common, some plants died; 7=lesions as in 5, defoliation, broken, dry branches very common, up to 25% of plants died; 8=symptoms as in 7 but up to 50% of the plants died and 9= symptoms as in 7 but up to 100% of the plants died.

Test genotypes were further categorized for their reaction to AB infection on the basis of Gowen *et al.* (1989) scale, according to this scale; 1-<2= Highly resistant(HR); 2<4= resistant (R); 4-<6=moderately resistant (MR); 6<7= moderately susceptible (MS); 7-<9= susceptible (S); and 9-10=highly susceptible (HS).

Data Analysis

Data on disease severity were subjected to analysis of variance (ANOVA) using R statistics using General Linear Model. Mean separation was based on the LSD at the 5% probability level. Since the disease severity over locations and years were tested using combination testing method that the ratio of larger error mean square to lower mean square values less 5 acceptable and homogenous, the results were analyzed in combined way.

Results and Discussion

The reaction of the 18 chickpea genotypes to ascochyta blight disease is presented in Tables 1 and 2. There was a significant ($P<0.05$) difference among the genotypes to ascochyta blight disease reaction response. At Dhera nine genotypes were susceptible, six genotypes were moderately susceptible, one genotype was moderately resistant, but only two genotypes showed resistant reaction (Table 1).

The wide range of variations among the tested genotypes and across the experimental station could be depends on differences in the amount of inoculums in the soil, extent of rain fall distribution, duration of leaf wetness, variations in the genetic diversity within population of *Ascochyta rabiei* and aggressiveness of pathotype difference. These all variation comparison differences studies also reported (Ahmed *et al.*, 2006; Jamil *et al.*, 2010).

At AlemTena in 2014 main cropping season, ten genotypes were found susceptible, two genotypes were moderately susceptible, three genotypes were showed moderately resistant, two genotypes were resistant and one genotype displayed highly susceptible. At Dhera, twelve genotypes were susceptible, two genotypes were moderately susceptible, one genotype found to be moderately resistant, one genotype was recorded as resistant and two

genotypes were found to be highly susceptible to ascochyta blight diseases (Table 2). Our results revealed that there was a definite variation among desi type of chickpea genotypes in terms of their susceptibility to aschochyta blight. This could be attributed to the intensive high rainfall at both locations, which could potentially create conducive environmental conditions for ascochyta blight

epidemics. Similar were reported by Reddy and Singh (1984). Overall, ten genotypes were moderately resistant, two were exhibited susceptible, four were found moderately susceptible, but one pipeline genotypes and one improved variety (Arerti) were resistant over different locations and seasons with consistent reactions to ascochyta blight disease under field conditions.

Table 1. Mean of ascochyta blight severity score on 18 chickpea genotypes in 2013 at Alem Tena and Dhera sub center.

Genotypes	Pedigree	(Alem Tena) Mean $\pm$ SD	Reaction	(Dhera) Mean $\pm$ SD	Reaction
ICCV-10509	WACAPE2099 x ICC 14917	7.00 ^b $\pm$ 1.00	S	7.66 ^{abc} $\pm$ 0.57	S
ICCV-10502	ICCV 98503 x ICCV 96836	5.33 ^d $\pm$ 0.57	MR	8.00 ^{ab} $\pm$ 0.00	S
ICCV-10503	Sona x PBG 5	6.66 ^b $\pm$ 0.57	MS	8.00 ^{ab} $\pm$ 1.00	S
ICCV-10516	ICCV 98503 x ICCV 96836	7.00 ^b $\pm$ 0.00	S	7.66 ^{abc} $\pm$ 0.57	S
ICCV-10510	WACAPE2099 x ICC 14917	5.66 ^{cd} $\pm$ 0.57	MR	7.00 ^{bcd} $\pm$ 1.00	S
ICCV-10512	WACAPE2099 x ICC 14917	5.66 ^{cd} $\pm$ 0.57	MR	5.00 ^d $\pm$ 0.00	MR
ICCV-10505	Moti x PBG 5	6.33 ^{cd} $\pm$ 0.57	MS	6.00 ^{de} $\pm$ 0.00	MS
ICCV-10511	WACAPE2099 x ICC 14917	5.33 ^d $\pm$ 0.57	MR	6.33 ^{cde} $\pm$ 0.57	MS
ICCV-10517	ICCV 98503 x WACAPE 2098	5.66 ^{cd} $\pm$ 0.57	MR	7.66 ^{abc} $\pm$ 2.30	S
ICCV-10508	ICC 37 x PBG 5	5.00 ^d $\pm$ 0.00	MR	6.33 ^{cde} $\pm$ 1.15	MS
ICCV-10506	ICC 37 x PBG 5	6.66 ^b $\pm$ 0.57	MS	7.00 ^{bcd} $\pm$ 1.73	S
ICCV-10501	ICCV 98503 x ICCV 96836	5.66 ^{cd} $\pm$ 0.57	MR	6.33 ^{cde} $\pm$ 1.15	MS
ICCV-10504	Sona x PBG 5	5.00 ^d $\pm$ 0.00	MR	6.33 ^{cde} $\pm$ 1.15	MS
ICCV-10515	Moti x ICCV 04516	3.66 ^e $\pm$ 0.57	R	3.66 ^{fg} $\pm$ 0.57	R
ICCV-10514	ICCV 96970 x ICCV 04516	6.33 ^{bc} $\pm$ 0.57	MS	7.00 ^{bcd} $\pm$ 0.00	S
ICCV-10507	ICC 37 x PBG 5	5.33 ^d $\pm$ 0.57	MR	7.00 ^{bcd} $\pm$ 0.00	S
ICCV-10513	WACAPE2099 x ICC 14917	5.33 ^d $\pm$ 0.57	MR	6.00 ^{de} $\pm$ 1.00	MS
ICCV-96836	(BDN 9-3 x K 1184) x ICP 87440	3.33 ^e $\pm$ 0.57	R	3.33 ^g $\pm$ 0.57	R
Arerti		3.33 ^e $\pm$ 0.57	R	3.33 ^g $\pm$ 0.57	R
Mariye		8.33 ^a $\pm$ 0.57	S	9.00 ^a $\pm$ 0.00	HS
Grand mean		5.63		6.43	
CV (%)		10.02		14.22	
Error		0.319		0.836	

Key: Means sharing similar letters do not differ significantly at $P < 0.05$. Whereas HS= highly susceptible, S= susceptible, MS= moderately susceptible, MR= moderately resistant and R= resistant.

During the 2014 cropping season, almost all chickpea genotypes showed a high degree of susceptibility to ascochyta blight at both sites. This will in turn increase relative humidity that could potentially aggravated the severity. Both testing sites are hotspot for ascochyta blight disease, and specific race is not identified so far. As a reflection of this, there were high variations in disease pressure and the

nature of disease as it is polycyclic type that implies that variability in pathogen population among *Ascochyta rabiei*. More recently, pathotype/race in Dhera were highly aggressive pathotypes/races than the ones found at Alem Tena (from our observation). Similarly, based on the evaluation of the resistance level of desi type of chickpea, it was observed that maximum number of varieties were susceptible under high humidity and moist conditions as well as cooler temperature (Bokhari *et al.*, 2011). To date the different varieties of chickpea at different research institutes are mostly susceptible to present races of *Ascochyta rabiei* (Ghazanfar *et al.*, 2010).

Table 2. Mean of ascochyta blight severity score on 18 chickpea genotypes in 2014 at Alem Tena and Dhera.

Genotypes	Pedigree	(Alem Tena) Mean $\pm$ SD	Reaction	Dhera Mean $\pm$ SD	Reaction
ICCV-10509	WACAPE2099 x ICC 14917	8.66ab $\pm$ 0.57	S	8.66ab $\pm$ 0.57	S
ICCV-10502	ICCV 98503 x ICCV 96836	8.66ab $\pm$ 0.57	S	8.66ab $\pm$ 0.00	S
ICCV-10503	Sona x PBG 5	7.66bc $\pm$ 1.15	S	7.66bcde $\pm$ 1.15	S
ICCV-10516	ICCV 98503 x ICCV 96836	7.66bc $\pm$ 0.57	S	8.00abcd $\pm$ 0.00	S
ICCV-10510	WACAPE2099 x ICC 14917	7.00cd $\pm$ 1.00	S	7.33cde $\pm$ 1.15	S
ICCV-10512	WACAPE2099 x ICC 14917	5.33e $\pm$ 0.57	MR	7.33cde $\pm$ 0.57	S
ICCV-10505	Moti x PBG 5	8.00abc $\pm$ 0.00	S	8.00abcd $\pm$ 0.00	S
ICCV-10511	WACAPE2099 x ICC 14917	7.66bc $\pm$ 1.15	S	8.00abcd $\pm$ 1.00	S
ICCV-10517	ICCV 98503 x WACAPE 2098	8.33ab $\pm$ 0.57	S	8.33abcd $\pm$ 0.57	S
ICCV-10508	ICC 37 x PBG 5	7.00cd $\pm$ 0.00	S	6.33cde $\pm$ 1.15	MS
ICCV-10506	ICC 37 x PBG 5	6.33de $\pm$ 0.57	MS	6.66e $\pm$ 0.57	MS
ICCV-10501	ICCV 98503 x ICCV 96836	9.00a $\pm$ 0.00	HS	8.66ab $\pm$ 0.57	S
ICCV-10504	Sona x PBG 5	6.33de $\pm$ 1.15	MS	9.00a $\pm$ 0.00	HS
ICCV-10515	Moti x ICCV 04516	3.33f $\pm$ 0.57	R	4.00f $\pm$ 0.00	MR
ICCV-10514	ICCV 96970 x ICCV 04516	7.66bc $\pm$ 0.57	S	9.00a $\pm$ 0.00	HS
ICCV-10507	ICC 37 x PBG 5	5.66e $\pm$ 0.57	MR	8.00abcd $\pm$ 1.00	S
ICCV-10513	WACAPE2099 x ICC 14917	5.33e $\pm$ 0.57	MR	7.00de $\pm$ 0.00	S
ICCV-96836	(BDN 9-3 x K 1184) x ICP 87440	3.33f $\pm$ 0.57	R	3.33f $\pm$ 0.57	R
Arerti		3.66f $\pm$ 0.57	R	3.33f $\pm$ 0.57	R
Mariye		8.66ab $\pm$ 0.57	S	9.00a $\pm$ 0.00	HS
Grand mean		6.76		7.35	
CV (%)		10.19		8.34	
Error		0.476		0.376	

Key: Means sharing similar letters do not differ significantly at $P < 0.05$. Whereas HS= highly susceptible, S= susceptible, MS= moderately susceptible, MR= moderately resistant and R= resistant.

Results generated from a two-way interaction effects, comprising genotypes by locations is presented in (Table 3). Accordingly, eight genotypes were susceptible, six genotypes were moderately susceptible, two were displayed resistant and two genotypes were moderately resistant. This result is in contrast to the findings of others

reported (Ilyas *et al.*, 1991; Reddy and Singh, 1990; Reddy and Singh, 1993). The information on the resistance chickpea genotypes to ascochyta blight detected in the present study provided a clear clue that there are a few genetic variations in desi type of chickpea for the resistant gene that can be exploited for disease control through resistance breeding. It was reported through

genetic studies that resistance of chickpea against ascochyta blight disease is due to either a single dominant gene or recessive gene (Singh and Reddy, 1990; Singh and Reddy, 1991).

Though the information available in the current literatures are inconclusive, the number of genes governing the resistance in those genotypes and genetic variability of pathogen and the environmental conditions are the possible reasons for the observed differences. Therefore, it is yet to be

resolved by conducting more experiments on mode of inheritance resistance using molecular techniques for introgression with other high yielding genotypes. Nevertheless, the presence of resistant genotypes in our collection suggested that those genotypes could be regarded as potential sources of resistant against ascochyta blight, which is in agreement with reports of other similar works (Collard *et al.*, 2003; Flandez-Galvez *et al.*, 2003).

Table 3. Combined mean of ascochyta blight severity on chickpea genotype over locations.

Genotypes	Pedigree	Mean $\pm$ SD	Reaction
ICCV-10509	WACAPE2099 x ICC 14917	8.00b $\pm$ 0.51	S
ICCV-10502	ICCV 98503 x ICCV 96836	7.66bc $\pm$ 1.49	S
ICCV-10503	Sona x PBG 5	7.50bcd $\pm$ 1.00	S
ICCV-10516	ICCV 98503 x ICCV 96836	7.58bcd $\pm$ 0.51	S
ICCV-10510	WACAPE2099 x ICC 14917	6.75ef $\pm$ 1.05	MS
ICCV-10512	WACAPE2099 x ICC 14917	5.83g $\pm$ 1.02	MR
ICCV-10505	Moti x PBG 5	7.08de $\pm$ 0.99	S
ICCV-10511	WACAPE2099 x ICC 14917	6.83ef $\pm$ 1.33	MS
ICCV-10517	ICCV 98503 x WACAPE 2098	7.50bcd $\pm$ 1.56	S
ICCV-10508	ICC 37 x PBG 5	6.33fg $\pm$ 0.98	MS
ICCV-10506	ICC 37 x PBG 5	6.66ef $\pm$ 0.88	MS
ICCV-10501	ICCV 98503 x ICCV 96836	7.41cd $\pm$ 1.62	S
ICCV-10504	Sona x PBG 5	6.66ef $\pm$ 1.66	MS
ICCV-10515	Moti x ICCV 04516	3.66h $\pm$ 0.49	R
ICCV-10514	ICCV 96970 x ICCV 04516	7.50bcd $\pm$ 1.08	S
ICCV-10507	ICC 37 x PBG 5	6.50f $\pm$ 1.24	MS
ICCV-10513	WACAPE2099 x ICC 14917	5.91g $\pm$ 0.90	MR
ICCV-96836	(BDN 9-3 x K 1184) x ICP 87440	3.33h $\pm$ 0.49	R
Arerti		3.41h $\pm$ 0.51	R
Mariye		8.75a $\pm$ 0.45	S
Genotype	**		
Location	**		
Year	*		
Genotype X location	**		
Grand mean	6.545		
CV (%)	10.9		
Error	0.509		

Key: Means sharing similar letters do not differ significantly at $P < 0.05$. Whereas HS= highly susceptible, S= susceptible, MS= moderately susceptible, MR= moderately resistant and R= resistant.

Conclusion

The present study result showed that most of the genotypes displayed susceptible to moderately susceptible reactions and only two promising genotypes were identified as resistant to ascochyta blight. There was high disease pressure and distribution in each location and season. Sources of resistance identified during this study can further be exploited in breeding program in the development of disease resistant varieties after determining their genetics through marker assisted selection. Therefore, intensive work is necessary on identification of source of resistance continually for stable as well as for the study of inheritance of ascochyta blight and mechanism of resistance. Molecular techniques for pathogen variability studies need more focus because polycyclic nature of disease as it breaks source of resistance with climate change attributions. Identification of major races and pathotypes aggressiveness and evaluation of inheritance resistance studies at different crop growth stage will also be crucial to determine their level of resistance.

Acknowledgement

We thank Debre zeit Agricultural Research Center for financial support by Chickpea and Lentil national program. We also acknowledge Eresi Megersa, Tayu Shewansagad, Wancha Bejiga and Beley Bekele for their help during data collection and trial management. We thank ICRISAT for

providing genotypes and facilitating the preparation of manuscripts.

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Assessing the Competitiveness of Smallholders Chickpea Production in the Central Highlands of Ethiopia

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Abstract

Chickpea is an important food legume in Ethiopia which provides sources of livelihood for millions of smallholder farmers. Currently, it has become an important high value crop that promotes commercialization. This study attempted to assess the competitiveness of smallholders' chickpea production in the central highlands of Ethiopia. To this end, the domestic resource cost coefficient (DRC) analysis was employed using farm household surveys, price and trade data. Current evidences showed that there has been a significant upswing in the trends of local chickpea prices both in nominal and real terms since the last eight years. As a result, chickpea has become an important cash crop in high potential major chickpea growing areas where farmers dispose the largest proportion of their chickpea production (82%) for marketing purpose. One of the major challenges of Ethiopian chickpea export is related to the bulk of the chickpea trade (84%) being absorbed by the local markets. Moreover, the current Ethiopia's export market share has remained at 4% while the largest proportion (94%) is destined to low value export markets. Despite all those limitations, Ethiopian chickpea export is highly competitive in major export markets as evidenced by the DRC value which was found to be 0.20. Therefore, the competitiveness of Ethiopian chickpea in high value markets could be improved by focusing on quality, volume, and supply continuity.

Key words: chickpea, domestic resource cost coefficient

Introduction

Chickpea (*Cicer arietinum*) is an important food legume mainly produced by smallholder farmers under rainfed condition in Ethiopia. Chickpea provides a multi-functionality to smallholder farmers in terms of sources of nutrition, cash income, animal feed, and soil fertility maintenance (Geletu et al., 1996;

Verkaart et al., 2017). The crop is grown by about over one million households covering 240,000 ha of land while it is the second major food legume next to faba bean by accounting for 17% of the total food legume production in Ethiopia (CSA, 2015a).

Chickpea is widely grown across the vertisol-dominated highlands with

altitude ranging from 1400 to 2300 meters above sea level and mean annual rainfall of 700-2000 mm (Geletu *et al.*, 1996). The major chickpea growing regions include Amhara (Gondar, Gojam, Wello, and North Shewa Zones) and Oromia (East, West, and Northwest Shewa, and Arsi Zones) which account for 49% and 47% of the total chickpea production, respectively, while the contribution of Tigray and SNNP is about 2% (CSA, 2015a).

The current agricultural development strategy of Ethiopia gives a high priority on the commercialization of smallholder farmers by promoting the production of high value crops that are competitive in local and export markets (MoFED, 2016). Chickpea is one of the newly emerging export commodities being promoted for expansion in Ethiopia. Currently, chickpea has become the second pulse export commodity next to common bean in generating foreign exchange revenues (ERCA, 2015; Shiferaw and Hailemariam, 2007).

However, the local market absorbs the largest proportion of the total chickpea trade volume (Shiferaw *et al.*, 2007). Moreover, the integration of smallholder farmers to the market economy still remains very limited. About 56% of the total chickpea production is consumed at farm level while only 25% is sold for cash incomes (CSA, 2015b). The low level of adoption of improved chickpea technologies has been cited as the major factor contributing to high yield

gaps and subsequent limited marketable surplus (Shiferaw *et al.*, 2007, Shiferaw and Teklewold 2007). The current average chickpea productivity (1.9 t/ha) still remains below the attainable yield (3-4 t/ha) that could be achieved through adoption of improved chickpea production technologies (Leges *et al.*, 2005; Chilot *et al.*, 2010).

Given the high value of the commodity and its significance for poverty alleviation, several research for development interventions have been made in major chickpea growing areas of the country over the past decades to improve the productivity and profitability of smallholder chickpea production. With this context, the objective of this study was to assess the competitiveness of chickpea production in the central highlands of Ethiopia.

Methodology

Description of the study areas

The study was conducted in East Shewa Zone of Oromia National Regional State. It is bordering Addis Ababa in the East where its main town, Adama, is situated in the Rift Valley at 98 km east of the capital on the highway to Djibouti. The total population of the zone was 1.36 million where 75% of them are living in the rural areas (PCC, 2008). The total zonal area coverage is estimated to be over 526,000 ha where cultivated land accounted for 90% of the total land mass of the zone (CSA, 2014). The

average cultivated land per household is 1.7 ha higher than the national average land holding of 0.95 ha (ibid.). The farming system is generally characterized by mixed crop-livestock production system where livestock provides sources of draught power, cash income, food, transport, and fuel. The livestock population includes 1.15 million cattle, 949.33 thousand sheep and goats, 353.20 thousand equines, and over 3000 camels (CSA, 2015c). East Shewa Zone is normally considered as one of the high potential agricultural areas in the region with suitable agro-ecology for the production of different cereal and food legume crops. Chickpea is the major crop which accounts for 32% of the total food legume production in the zone (CSA, 2015a).

In terms of infrastructure, the zone is situated relatively in a better accessible parts of the country due to its proximity to Addis Ababa. The main highway linking the capital to the East, Southeast and Southern parts of the country crosses the zone while it has also a recently built express highway linking Addis Ababa to its main town, Adama. Despite the availability of some intra-zone all-weather roads connecting different districts, rural inaccessibility still remains a major development challenge in rural Ethiopia.

Data Sources and Collection Methods

Primary data were generated from Tropical Legumes II (TL-II) farm household surveys updated with rapid market assessment survey. The TL-II farm household surveys (baseline and adoption) were conducted in three project intervention districts, namely Gimbichu, Lume-Ejere and Minjar-Shenkora involving 700 randomly selected sample farmers in three rounds (2008, 2010, and 2014) to establish a panel dataset. To supplement and substantiate the farm household data, a rapid market assessment survey was also conducted in November 2016 in the Zone with additional terminal markets in Addis Ababa and Adama cities. The rapid market assessment survey involved Focus Group Discussion (FGD) and Key Informant Surveys using semi-structured questionnaires to generate data on chickpea production (input and technology use), marketing and trade. Farmers, cooperatives, agricultural experts, traders, and exporters were interviewed during the survey. In addition, weekly chickpea prices which were collected at Bishoftu and Arerti markets during 2009-2013 were also used for price analysis.

The secondary data collected were monthly chickpea price data for Addis Ababa and Adama (2012-2015) from Ethiopian Grain Trade Enterprise (EGTE) while chickpea trade data were obtained from the Ethiopian Revenue and Customs Authority

(ERCA). Other published and web-based data sources were used in this study.

Data Analysis

The Domestic Resource Cost ratio (DRC) analysis was employed for assessing the competitiveness of chickpea production in export markets using the Policy Analysis Matrix (PAM) framework. The analytical framework involves the estimation of chickpea enterprise budget in terms of private and social (economic) values for computing the DRC values which measure chickpea competitiveness. The application of the DRC methodology is widely documented in the literature (Morris, 1990; Masters & Winter-Nelson, 1995) while an elaborative explanation of the analysis using PAM is given in Monke and Pearson (1989).

The PAM structure has four columns (Table 1). The first column is for revenue, the second and third are for costs and the last is for profitability. The two cost columns are consisting of tradable inputs and domestic factors. Furthermore, PAM has three rows. The first two rows represent the two different versions of the profit equation where the first row (D) is evaluated using actual (market) prices while the other (H) is evaluated at shadow (social prices). The third row measures the effect of government policy (market failure) where each entry is simply the difference between its value in the first and second rows, i.e. as the difference between observed parameters and parameters that would exist if the distortions were removed.

Table 1. General structure of Policy Analysis Matrix (PAM)

	Revenues	Costs		Profits
		Tradables	Domestic Factors	
Private Price	A	B	C	D = (A-B-C)
Social Price	E	F	G	H = (E-F-G)
Divergences	I = (A-E)	J = (B-F)	K = (C-G)	L = (D-H) = (I-J-K)

Source: Monke & Pearson (1989)

The DRC value was computed using the returns to land and management obtained from chickpea enterprise budgets in both private and social

prices. The DRC for chickpea was calculated by dividing the costs of domestic factors by value added at social prices:

$$DRC = \frac{(\text{labor cost} + \text{capital cost}) \text{ in social prices}}{(\text{revenues} - \text{cost of tradable inputs}) \text{ in social prices}}$$

Here, if the DRC value exceeds one, then it indicates that the country does not have a comparative advantage in

chickpea production. However, for values less than one, the local chickpea production is considered to

be competitive in export markets, i.e. the value added measured in social prices exceeds the costs of domestic resources in social prices used in chickpea production.

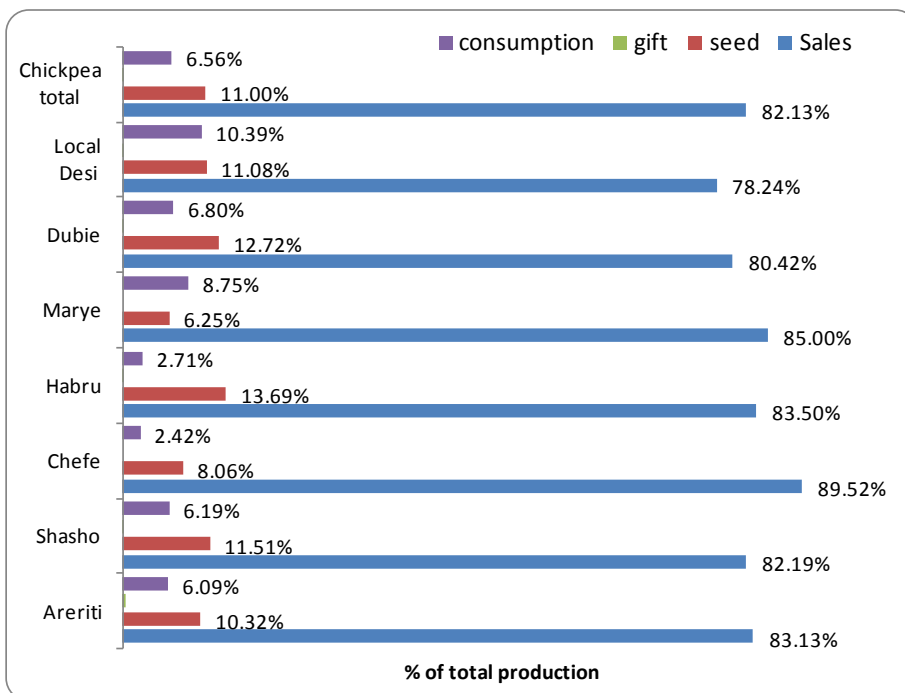
Results and Discussion

Chickpea Market Participation

The integration of Ethiopian smallholder farmers to the market economy still remains very limited and at subsistence level. Evidences have showed that pulses have been increasingly becoming an important source of cash income to farmers for the high demand from local and export markets. It is estimated that nearly

25% of the total chickpea production in Ethiopia is delivered to the market while the remaining 75% is consumed at farm level (CSA, 2015b).

However, farmers who have better access to the market were observed to have better productivity and market linkage where significant proportion of their chickpea production is delivered to the market. TL-II project intervention areas were relatively located in high potential areas with better access to the market. As a result, farmers, on average, sold 82% of their total chickpea production while household chickpea consumption is limited to 7% (Figure 1).



Source: TL-II Adoption Survey, 2014

Figure 1. Utilization of chickpea, 2013/14 cropping season

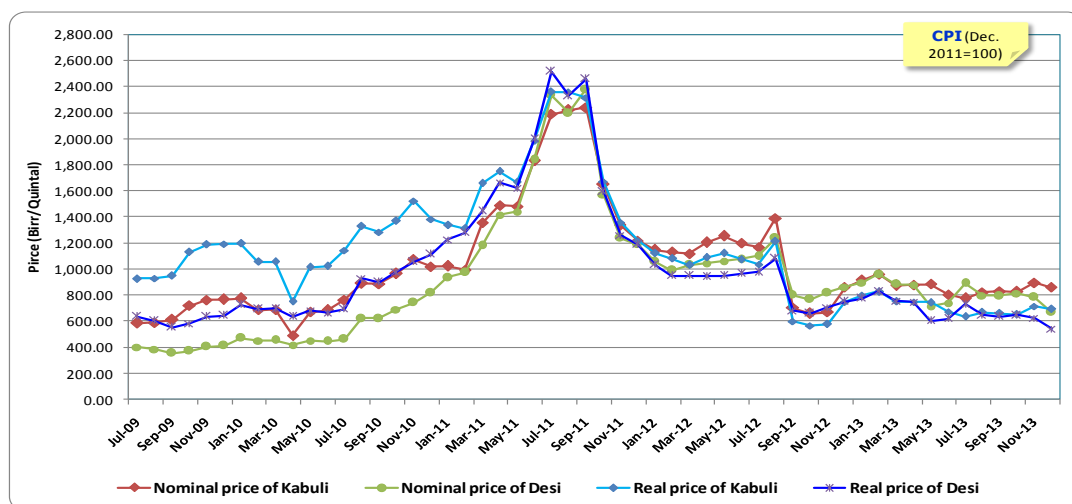
Our rapid assessment survey conducted in December 2016 showed similar results where about 85% of the total chickpea production was destined for market and the rest for home consumption.

In Ethiopia, there is a high and growing demand for pulses in local markets which is closely associated with the deep-rooted values of these crops in the diets of the population. Chickpea is an important staple food legume used for the preparation of various traditional foods in Ethiopia. Currently, there has been a growing trend in the processing and marketing of chickpea in local markets by medium and small-scale processors which are locally called *Baltina*. They are engaged in the processing and marketing of chickpea in the form of husked and split chickpea (*Kik*) or dhal, chickpea flour for sauce (*Shiro*), and roasted (*kollo*). The desi types are highly preferred for *Kik* and *Shiro* while the Kabulis are for roasted chickpea (*Kollo*). Chickpea is also used for the preparation of unleavened bread.

Domestic chickpea price trends

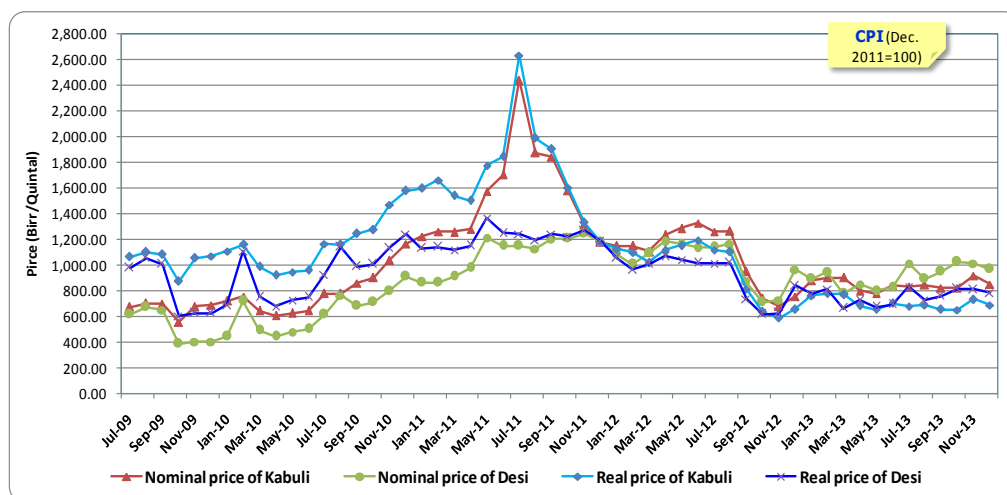
Although the local prices of most food grains have generally increased since 2006, there has been a significant upswing in the trends of local chickpea prices both in nominal and real terms since late 2009 (Figures 2&3). Nominal and real chickpea price trends were calculated based on a weekly price data collected by DZARC from Bishoftu (Ada'a district) and Arerti markets (Minjar-Shenkora district). In general, the nominal chickpea prices increased significantly during the period from September 2010 to September 2012. Chickpea hits a historical high price level during the year 2011 where the nominal chickpea (both Kabuli & Desi types) prices exceeded Birr 2000 per 100 kg during July-August 2011. This price increment was equivalent to 189% for Kabuli chickpea and 431% for Desi chickpea in December 2009.

The real prices of chickpea also showed a similar trend indicating that much of the price increment was not closely associated with the high inflation rate in the country (Figures 2 & 3).



Source: Socioeconomics DZARC & CSA Monthly CPI Report (2009-2013).

Figure 2. Nominal and real price trend of chickpea, Bishoftu (DebreZeit) Market, Ada'a District (July 2009-December 2013).



Source: Socioeconomics DZARC & CSA Monthly CPI Report (2009-2013).

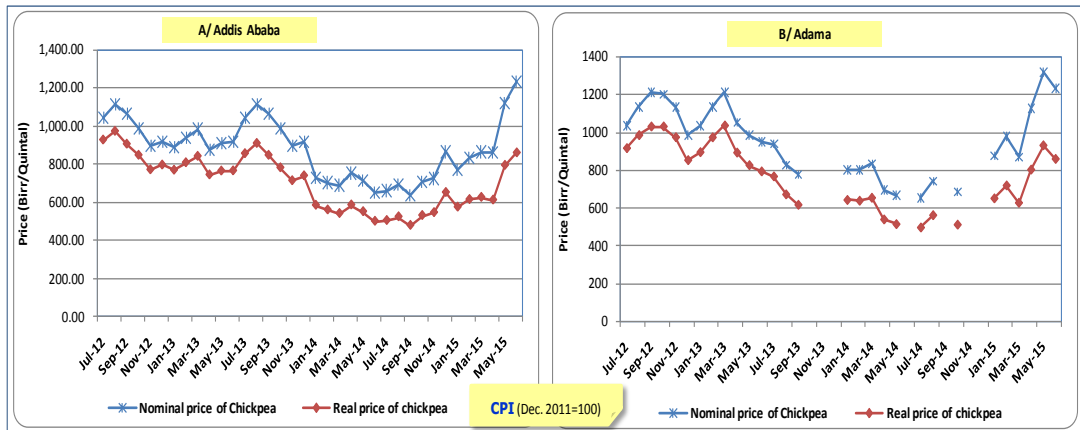
Figure 3. Nominal and real price of chickpea, Arerti Market, Menjar-Shenkora District (July 2009-December 2013).

Chickpea prices started sharply declining since the last quarter of 2011 while sustaining a declining trend until the third quarter of 2014 (Figure 4). The price percentage change showed that the average prices in December 2013 have been much lower than their values in the same month since 2010

and even less than their average values in the first month of the last quarter in the same year. For instance, the average prices of Kabuli and Desi chickpeas in December 2013 were 29% and 44% lower than their values in the same month in 2011, respectively. The declining trend was

much worse for Desi than Kabuli types. And yet, chickpea price started

showing an increasing trend since the first quarter of 2014.



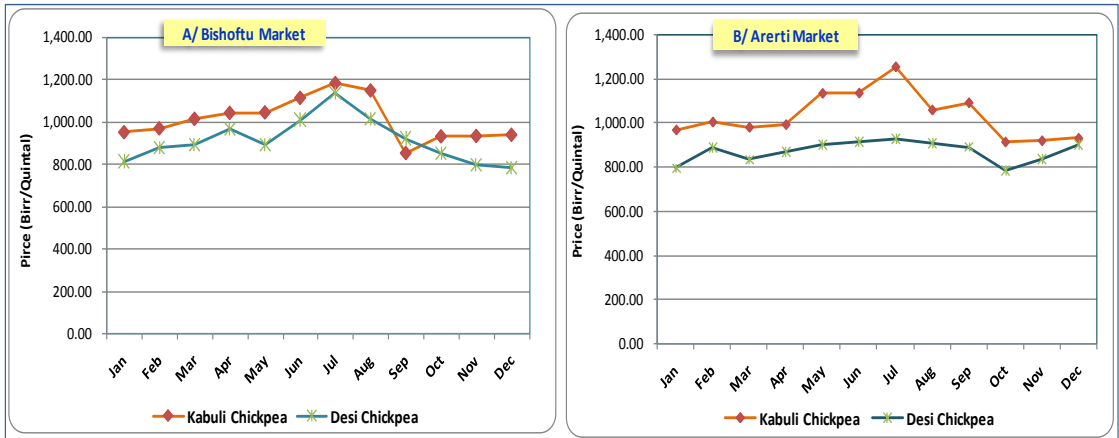
Source: EGTE & CSA Monthly CPI Report (2012-2015).

Figure 4. Nominal and real prices of chickpea in Addis Ababa and Adama (2012-2015).

The sharply increasing local chickpea price trend observed during 2010-2011 was closely associated with the increasing global chickpea price trends. Studies showed that the dramatic rise in local pulse prices since the past few years have been largely driven by persistent growth in export demand mainly from Sudan, Pakistan, United Arab Emirates, India and Turkey (Shiferaw and Teklewold, 2007; Shiferaw *et al.*, 2007; FAO/WFP, 2012; USAID, 2013).

Similar to other food grains, chickpea markets are also characterized by high

seasonal price fluctuations due to the very nature of Ethiopian rainfed farming system. Normally, chickpea prices are getting high during the planting season (June-August) when grain supply is limited in the market while prices reach at their low level during the harvesting season (December-May) when there is high inflow of grain supply in the market (Figure 5). It was found that the prices of Kabuli and Desi chickpeas increased by 16% and 21% during the cropping season, respectively.



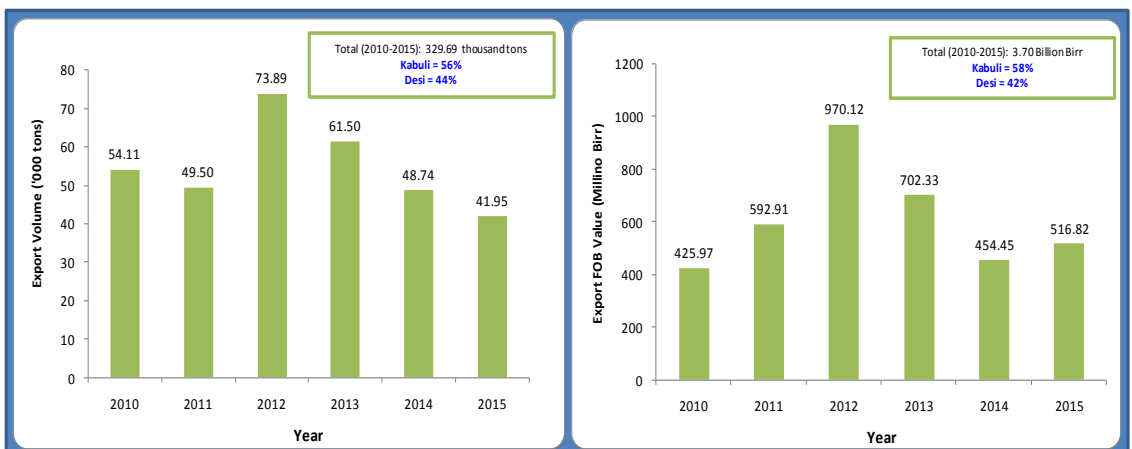
Source: Socioeconomics, DZARC (2009-2013).

Figure 5. Seasonal Chickpea nominal price patterns (average 2009/2013).

In general, kabuli chickpea types are marketed at higher prices as compared to the desi types. The price of Kabuli types was, on average, 15% higher than that of the Desi types. Similar finding reported 36% high price of Kabuli over Desi type chickpea (Shiferaw and Teklewold, 2007).

Ethiopian Chickpea Trade

The total chickpea trade volume in Ethiopia is estimated at 339 thousand tons where 84% is supplied to the local market while the export market absorbs 16%. Chickpea is the second major export commodity among pulse crops by generating nearly 25% of the total pulse export earnings. Ethiopia has exported a total of 329.70 thousand tons chickpea during the period 2010-2015 with a total income of 3.70 Billion Birr (Figure 6).

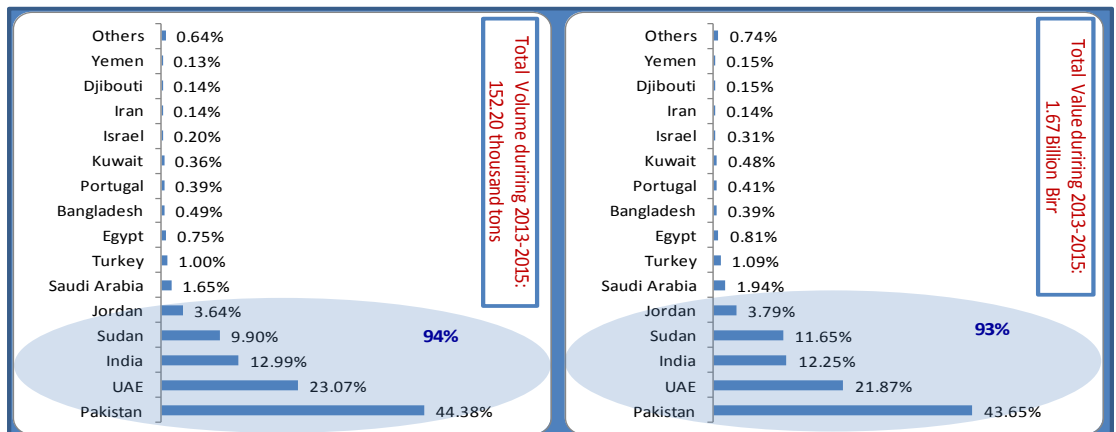


Source: Computed from ERCA Data, 2015).

Figure 6. Ethiopian Chickpea Export Trade

Kabuli types chickpeas accounted for 56% of the total export volume while the remaining was Desi types. One of the major limitations of Ethiopian chickpea export arises from the fact that the largest proportion has been sent to low value chickpea export destination markets (Figure 7). Evidences showed that about 94% of the total Ethiopian chickpea export

during 2013-2015 was sent to Pakistan (44.4%), UAE (23.10%), India (13%), Sudan (10%), and Jordan (3.64%). Moreover, yearly export fluctuation has been an apparent feature where export performance is highly influenced by weather condition in South Asia and Australia and demands in South Asian markets.



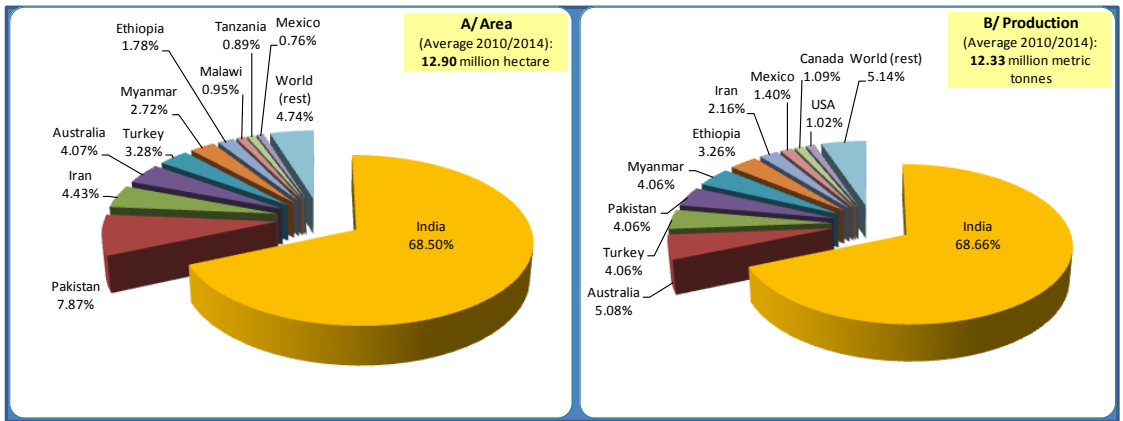
Source: Computed from ERCA Data, 2015).

Figure 7. Major Ethiopian Chickpea Export Destinations Markets (2013/2015).

Major competitors in Global Chickpea Markets

Chickpea is the second major pulse crop in the world with a total production of 12.33 million tons from 12.90 million ha of land (FAOSTAT, 2017). While there are no precise data showing global chickpea production disaggregated into desi and kabuli types, some estimates showed that Kabuli types account for 15 to 30% of world chickpea production while the desi type accounts for the remaining proportion (Knights *et al.*, 2007;

Reddy *et al.*, 2007). Globally, India is the leading chickpea producer contributing 68.7% of world chickpea production followed by Australia (5.10%), Pakistan (4.1%), Turkey (4.1%), and Myanmar (4.1%) (Figure 8). Here, the top-10 producing countries all together covered 95% of the world chickpea production. Ethiopia stands sixth among the major chickpea producing countries by accounting for 3.26% of global chickpea production.



Source: Computed from FAOSATA.

Figure 8. Major Chickpea Producing Countries in the world (average 2010/2014)

Unlike production, the scenarios for global chickpea export trade are completely different. For instance, the average global chickpea export trade during 2010/13 was estimated at 1.8 million tons per annum worth of 1.12 Billion USD where Australia was the leading chickpea exporter contributing 41.53% of global chickpea export volume (Figure 9). The other major chickpea exporting countries are India, Russia, Mexico, Argentina, Ethiopia, USA, Canada, Myanmar and Tanzania. The top 10

chickpea exporting countries alone accounted for 94% of the world chickpea export market share. Russia has been emerging as the new strong competitor next to Australia in terms of price and volume of chickpea export and it is important to note that although Ethiopia's chickpea export market share still remains very limited, it has been improving and reached 4% of global chickpea export market share which is much better than early 2000s below 2% export share.

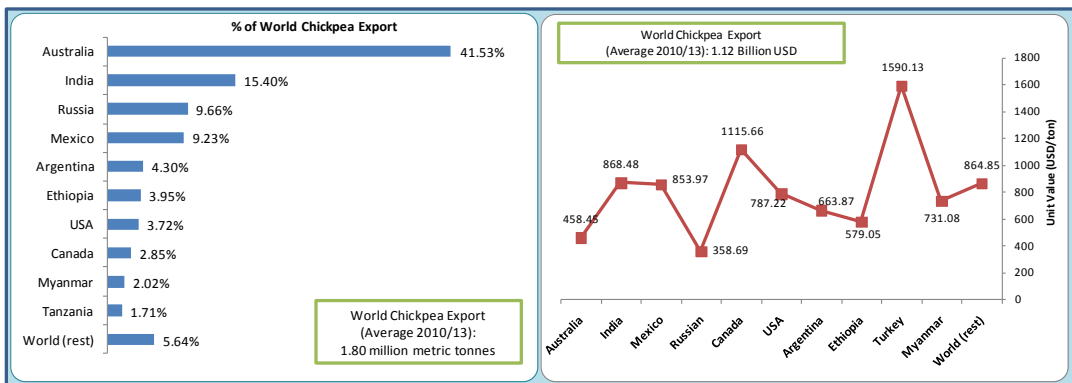
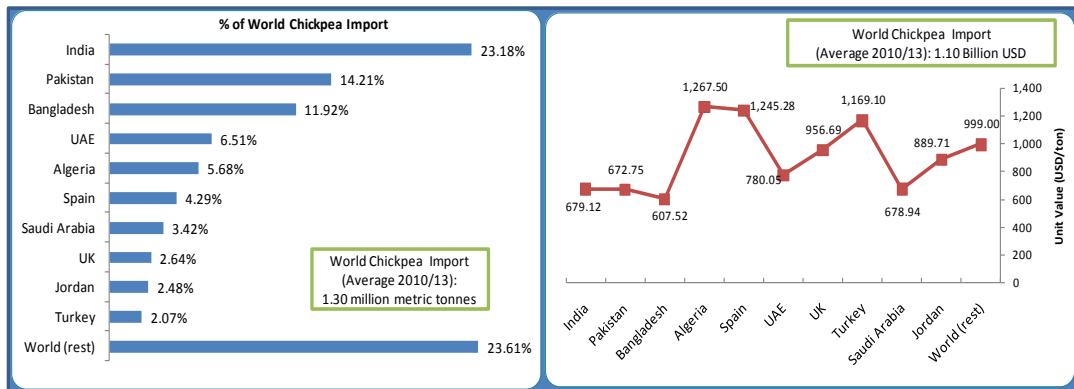


Figure 9. Global Chickpea Export Trade (2010-2013). (Source: Computed from FAOSATA)

The largest global chickpea destination markets are India, Pakistan and Bangladesh accounting for 49.31% of the world chickpea import (Figure 10). Other major chickpea importing countries are in Middle East, North Africa and some European countries.

The largest chickpea destination market, the Indian sub-continent, pays lower unit value (between 600 and 700 USD ton⁻¹) as compared to those high value markets in North Africa and Europe (Figure 10).



Source: Computed from FAOSATA

Figure 10. Global Chickpea Import Trade (2010-2013).

Competitiveness of Ethiopian Chickpea Export

While Ethiopia is listed to the top-10 chickpea producing countries, its export market share has been very low (<4%). So, it is important to understand whether Ethiopian chickpea production is competitive in export markets or not. Accordingly, the DRC value (0.20) indicated that Ethiopian chickpea is highly competitive in major chickpea export markets (Table 2). This further implies

that Ethiopia's resource endowments in terms of soil, agro-ecology and labor enable the country to produce chickpea at comparatively lower costs than other countries. Therefore, Ethiopian chickpea is highly competitive in external markets. This is in conformity with the largest proportion of Ethiopian chickpea export trade to low value export markets in Asia and the Middle East.

Table 2. Policy Analysis Matrix for chickpea, 2015 (values in Birr/ha)*

Price	Tradables		Domestic Resources		Profits
	Output	Inputs	Labor	Capital	
Private Price	21,500.00	2,855.50	7,670.00	380.81	10,593.69
Social Price	32,750.00	3,059.89	5,752.50	45.84	23,891.77
Divergences	-11,250.00	-204.39	1,917.50	334.97	-13,298.08

*The DRC value was computed using the returns to land and management obtained from chickpea enterprise budgets in both private and social prices

Conclusion

Chickpea is a strategic commodity that could greatly contribute to the commercialization of smallholder farmers and generation of foreign exchange revenues. There has been a significant upswing in the trends of local chickpea prices both in nominal and real terms since the last eight years. As a result, chickpea has become an important cash crop in high potential major chickpea growing areas where farmers dispose the largest proportion of their chickpea production for marketing purpose. And yet, price fluctuation is also an apparent problem which is closely associated with the export market influenced by the production situation in major chickpea growing countries. Since Ethiopia is a price taker in export market, high export demand usually causes to increase the local price and vice versa. One of the major challenges of the chickpea sector is related to the bulk of the chickpea trade being supplied to the local markets. Moreover, while the trend of chickpea export has been on the increase, the largest proportion was sent to low value chickpea export markets in the Indian subcontinent and Sudan. Yearly fluctuation has also been an apparent feature of Ethiopian chickpea export where export performance is highly influenced by external factors from major chickpea

export markets. Despite with all those challenges, Ethiopian chickpea export is highly competitive in international markets. However, concerted efforts should be made to improve the competitiveness of the chickpea value chain in terms of quality, volume, and continuity.

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Agronomic and Economic Evaluation of Wheat-Chickpea Double Cropping on the Vertisol of Takusa, North Western Ethiopia

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Abstract

Takusa district in north Gondar zone has a high potential for double cropping per one growing season. Farmers in the area, however, do not practice double cropping so far, a reflection of lack of research outputs that addressed its feasibility. The objective of this study was, therefore, formulated around the need to evaluate the technical and economic feasibility of double cropping in the area using wheat-chickpea. Experiments were established at Takusa district during the 2015 main cropping season. The trials were laid down in factorial arrangement of randomized complete block design (RCBD) with three replications. Three bread wheat varieties (Senkegna, Tay and Dinknesh) and two chickpea varieties (Habru and Natoli) were used. The combined data showed that, wheat variety Dinknesh took the shortest days to mature (81 days) compared to Senkegna and Tay (97 days each) varieties. The highest thousand seed weight and grain yield was observed on variety Denknesh and has significance difference at $P < 0.05$ with the other two varieties. Sole planting of Natoli (2926 kg/ha) and Habru (2103kg/ha) chickpea varieties gave relatively higher yield when compared with their respective double cropping combination. The Marginal rate of return (MRR) result showed that double cropping Natoli chickpea variety with Denekinesh wheat variety had 104% MRR. The land equivalent ration demonstrated double cropping rewards to a maximum of 1.99, implying the yield and benefit maximization per unit area per season. The highest grain yield in the double cropping system was obtained with Dinknesh wheat variety (2709kg/ha) double cropped with Natoli chickpea variety (2562 Kg/ha) and this combination could be recommended for similar agroecologies.

Keywords: Chickpea, Double cropping, Wheat

Introduction

Chickpea (*Cicer arietinum* L.) is an annual grain legume or “pulse crop” that is used extensively for human consumption, market and fertility

restoration. According to central statistics agency (CSA, 2015), chickpea production in Ethiopia covers 239,711 ha of land and the productivity was 1.91t/ha. Hence the Amhara region took a share of 124,854 ha, of which 41,787 ha of

chickpea production is located in north Gondar zone. The production of chickpea is dominantly distributed in the mid altitude of the zone. The production in the area is mainly done in sole cropping system. Its production takes place with residual moisture planted at the end of August or beginning of September. Both chickpea and wheat share common agroecology on the vertisol of North Western Ethiopia.

Bread wheat is one of the most staple food crops in the world and is one of the most important cereal crop cultivated in Ethiopia. It is also one of the most important crops in Amhara region and has a high potential for the expansion (Alelign, 1988). Bread wheat production is practiced recently in the mid altitude of North Gondar zone and mainly produced entirely as sole cropping.

Double cropping could maximize benefit from same area and season. It is a key to look for best combination and compatibility of crops to exhaust the opportunity from the system. It was reported that double cropping have many advantages, such as it reduces the risk of field loss due to drought, insect and disease, obtain a better use of vertical space and time in limited farmland (Beuerlein, 2001). Legumes are able to fix and incorporate nitrogen into the system and improved soil structure, avoiding the formation of hardpan and promote better aeration. In one of the studies, it was reported that double-cropped

wheat and soybean used 54-70% of the annual rainfall; while only 40% of the incident PAR (photosynthetically active radiation) was utilized (Caviglia *et al.*, 2004). From the producers' point of view, double cropping systems increase the value and income of agricultural production. There is no solid evidence documented on the double cropping between wheat and chickpea in Ethiopia. However, as a synonym double cropping of chickpea with wheat has been found to be quite remunerative. For example, it was clearly shown that 50:50 chickpea-wheat mixed cropping gave the highest land-equivalent ratio (Asaduzzamam *et al.*, 1989). Furthermore, a high net return from wheat-chickpea mixed cropping at a ratio of 5:1 was reported (Sharma *et al.*, 1987). Even though evidences and promotions are not well advancing, there has been some practices in central parts of Ethiopia where cropping of principal crops (cereals) with precursor (chickpea) using the main cropping season. According to Muluneh *et al.*, (2014) concluded that double-cropping of early-maturing, improved forage crops and residual soil moisture-based planting of chickpea and grass pea could improve feed availability, and labor and land productivity.

Mid altitude of North Gondar zone including Dembia, G/zuria and Takusa have a high potential to produce two crops in one cropping season as a double cropping. The rain fall distribution combined with the high retention of vertisol, enables the area to support two

crops of high compatibility viz., wheat under full rain fall and chickpea suit for partial phenological residual moisture. The soil in the area has vertic nature with high water holding capacity. However, farmers are still practicing sole cropping because there is neither verified research outputs not awareness created and promotion that support for the practicing of double cropping in the area. It is thus vital and timely to evaluate the feasibility of double cropping in the area, in terms of technical and economic feasibility concept. Therefore, the objective of the experiment was to evaluate the economic and technical feasibility of wheat-chickpea in double cropping combination for improved production system.

Materials and Methods

The wheat-chickpea sequenced experiment was done in two sites (research sites and on farm) at Takusa district during the 2015 cropping season. The trials were laid down in factorial arrangement of randomized complete block design (RCBD) with three replications. Three bread wheat (Senkegna, Tay and Dinknesh) and two chickpea varieties (Habru and Natoli) were used. The plot size of the experiment was 1.8m x 3m (=5.4m²). Each experimental plot had 9 rows for bread wheat and six rows for chickpea. Planting was done by hand drilling at seed rate of 150 kg ha⁻¹ for bread wheat and 130 kg ha⁻¹ for chickpea. Fertilizer was applied at the rate of 41/46 kg/ha N and P₂O₅, respectively for bread wheat. All DAP fertilizer was applied once at planting. Urea fertilizer, however, was applied in split

at planting and tillering stages for bread wheat.

Common agronomic parameters including; Heading days, Maturity days, Plant height, thousand seed weight of wheat, pod per plant, seed per pod, Hundred Seed Weight of chickpea and grain yield were measured based on representative sample of each treatment from both species.

Partial budget analysis was done to compare the financial feasibility of each treatment. Partial budget analysis is the way of calculating the total cost that varies the net benefits of each treatment in an on farm expert (CIMMYT, 1988). The partial budget analysis includes the average yield for each treatment, adjusted yield and gross benefit. All costs that show variation due to the treatment effects were recorded. The major cost items that vary across treatments were seed, fertilizer, labour and draft power. Other cost of production other than cost that varies was similar for all treatments. To calculate the net benefit gained from each treatment, the farm gate grain price of chickpea and wheat, average yield and adjusted yield for each treatment were taken. The adjusted yield is the yield adjusted downward by certain percentage to reflect the difference between the experimental yield and the yield farmers could expect from the same treatment. Then dominance analysis was carried out to compare the increase in terms of cost that varies

with its respective benefits. Those treatments which have lower net benefits but have higher or the same cost that vary were dominated and rejected from the analysis.

Rainfall distribution

The planting date of the experiment was on June 20, 2015, and all the three bread wheat varieties were planted at the same time. During this time the soil was at field capacity and there was enough moisture for the germination and growth of the wheat crop (see Fig 1, for rainfall distribution).

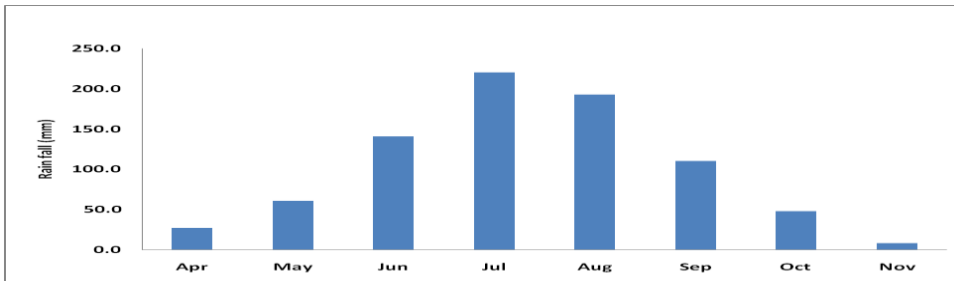


Figure 1. Rainfall in 2015 at Takusa, met station

Results and Discussion

The analysis of variance showed that in all the agronomic parameters such as heading days, maturity days, spike length, number of tiller per plant, thousand seed weight and grain yield; there were significance ($p < 0.05$) difference among treatments.

From the three bread wheat varieties tested, variety Dinknesh (with heading days of 53) was found to be much earlier than the other two tested wheat varieties (Table 1). Variety Senkegna and Tay mature lately and both took 97 days to mature, whereas the early maturing bread wheat variety Dinknesh took 81 days. This

parameter (maturity date) have significance effect on effective utilization of natural resources especially water in the double cropping farming system. Hence the subsequent chickpea crop planted immediately after the harvest of the wheat appears to get enough moisture for the growth. Planting time is critical in double-cropping systems as maturity times and dates have greatly affected productivity (Sanford et al., 1973). However, the other two bread wheat varieties mature on October 4-6, 2016, which have weaker compatibility in double cropping concept and affected the productivity of the subsequent chickpea crop.

Table 1. HD, MD, PH, Tiller per plant, Spike length, TSW and Grain yield (Delgi Station) of wheat crop.

Treatment	HD	MD	PH	No.of till	Spike length (cm)	TSW	Yield (kg/ha)
Senkegna	63.3 ^b	96.3 ^a	71.1 ^{bcd}	5.7 ^{abc}	7.8 ^{ab}	25.1 ^e	1211 ^c
Tay	65.6 ^a	96.6 ^a	73.4 ^{abcd}	3.9 ^{bc}	8.9 ^a	27.1 ^{cde}	1153 ^c
Dinknesh	53.0 ^c	82.3 ^b	75.8 ^{abc}	4.4 ^{abc}	7.4 ^b	32.7 ^{ab}	2511 ^a
Senkegn +Habru	64.6 ^{ab}	97.6 ^a	72.4 ^{abcd}	6.0 ^{ab}	7.3 ^b	29.5 ^{bcd}	1815 ^{abc}
Tay + Habru	65.6 ^a	98.0 ^a	74.2 ^{abcd}	6.3 ^a	7.9 ^{ab}	30.0 ^{abcd}	1438 ^{bc}
Dinknesh + Habru	52.6 ^c	82.0 ^b	80.6 ^a	3.60 ^c	7.0 ^b	34.5 ^a	2595 ^a
Senkegna + Natoli	64.6 ^{ab}	98.0 ^a	69.8 ^{cd}	6.2 ^a	7.2 ^b	27.9 ^{cde}	1610 ^{bc}
Tay + Natoli	65.6 ^a	98.0 ^a	66.0 ^d	5.2 ^{abc}	7.0 ^b	25.3 ^{de}	1189 ^c
Dinknesh + Natoli	53.0 ^c	82.3 ^b	78.6 ^{ab}	5.0 ^{abc}	7.0 ^b	30.8 ^{abc}	2123 ^{ab}
LSD%	1.43	3.40	8.29	2.31	1.26	4.7	844.5
CV%	1.36	2.12	6.50	25.8	9.68	9.42	28.0
LS	**	**	*	*	*	*	*

Key: *, ** indicate significance at 0.05 and 0.01 probability levels, respectively. LS, Level of Significance, HD, Heading days, MD, Maturity days, PH, Plant height, TSW- thousand seed weight)

In the tested three bread wheat varieties, variety Dinknesh gave the tallest plant height (78cm), followed by Tay and Senkegna (Table 1). From the yield determining parameters, variety Senkegna gave high number of tiller per plant compared to other varieties (Table 1). Variety Tay has long spike length (7-8.9 cm) followed by Senkegna (7.2-7.8 cm) and Dinknesh (7-7.4 cm) varieties (Table 1), but variety Dinknesh has higher thousand seed weight (31-34gm) than the other varieties and gave relatively good grain yield ranged between 2.1and 2.59 t/ha.

Similarly variety Dinknesh planted at farm site took shortest days to heading and to mature, when compared with other bread wheat varieties. At on farm Takusa site, there was significance difference between treatments on spike length and thousand seed weight as well. Varieties Tay and Senekegna gave relatively longer spike length than Denknesh. However, Denknesh has high thousand seed weight than the two bread wheat varieties and thus gave highest grain yield in this site (Table 2 and Figure 2).

Table 2. HD, MD, PH, Tiller per plant, Spike length, TSW and Grain yield (Delgi On-farm) of wheat crop.

Treatment	HD	MD	PH	No.of till	Panicle length	TSW	Yield (kg/ha)
Senkegna	61.3 ^b	95.6 ^a	90.5 ^{ab}	5.9 ^{ab}	8.4 ^{a-c}	29.6 ^b	2195 ^b
Tay	61.3 ^b	96.0 ^a	88.2 ^{a-c}	4.9 ^b	8.2 ^{a-c}	29.2 ^b	2129 ^b
Dinknesh	49.3 ^c	81.0 ^b	80.6 ^{bc}	5.8 ^{ab}	6.8 ^c	36.2 ^a	3337 ^a
Senkegn + Habru	61.3 ^b	97.3 ^a	89.2 ^{ab}	5.5 ^{ab}	8.1 ^{a-c}	31.0 ^b	2585 ^{ab}
Tay + Habru	63.0 ^{ab}	96.0 ^a	91.6 ^a	6.5 ^{ab}	8.6 ^{ab}	30.2 ^b	2337 ^b
Dinknesh + Habru	49.0 ^c	81.3 ^b	84.5 ^{abc}	6.8 ^a	6.9 ^{bc}	36.2 ^a	3264 ^a
Senkegna + Natoli	61.6 ^b	96.6 ^a	86.5 ^{abc}	6.2 ^{ab}	7.8 ^{a-c}	30.5 ^b	2505 ^{ab}
Tay + Natoli	66.0 ^a	96.6 ^a	91.5 ^a	6.4 ^{ab}	8.9 ^a	30.8 ^b	2086 ^b
Dinknesh + Natoli	49.6 ^c	81.3 ^b	78.4 ^c	5.3 ^{ab}	6.7 ^c	35.5 ^a	3294 ^a
LSD%	3.84	1.82	10.4	1.75	1.74	3.3	888
CV%	3.82	1.15	6.93	16.9	12.8	5.93	19.4
LS	**	**	*	*	*	*	*

Key: *, **= indicate significance at 0.05 and 0.01 probability levels, respectively. LS, Level of Significance, HD, Heading days, MD, Maturity days, PH, Plant height, TSW- thousand seed weight

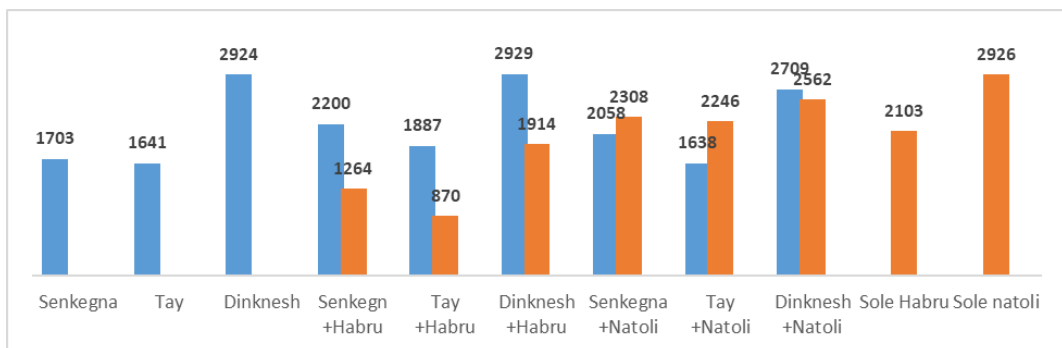


Figure 2. Yield responses (kg/ha) of wheat and chickpea under double cropping pattern

Even if it might be a inappropriate tool for this type of cropping, but for mixed or intercropping, as indices of efficiency we have tried to calculate LER of double crop over monocrop counterparts. LER compares the yields from growing two or more crops together with yields from growing the same crops in monocultures or pure stand. LER calculated as the sum of the fractions of the intercropped yields divided by the sole-crop yield. Based on this LER has fallen between 0.53 and 1.20 for wheat and between 0.41 and 0.91 for chickpea; with total LER value

ranging between 0.76 and 1.99. This means a concept in the moisture, radiation and nutrient use efficiency for output maximization, just as high as doubling than sole system counterparts. However, there is time extension for two crops in tandem than sole cropping despite the best compatibility design implemented. A proper efficiency analysis of a cropping system should take the use of the field time into consideration, because increasing the number of component crops or harvests could allow the use of field time, soil and

aerial resources more efficiently (Worku, 2014). In another study it was observed that intercrops increased water resource capture compared to the sole counter parts as a result of extended duration of the intercropping system (Coll *et al.*, 2012). Furthermore, in a study aimed to compare two-, three- and four-component intercrops grown together, it was reported that more crops in a mixture are likely to increase the chance for yield advantage and weed suppression (Nelson *et al.*, 2012). Growing two pulse components sequentially under intercropping instead of one could permit to fully utilize the growing season.

The combined analysis of variance showed that, except plant height, all recorded parameter showed

significance difference between treatments. Treatment with site interaction did not have significant effect on heading days, maturity days, number of tiller per plant, spike length thousand seed weight and grain yield. There are inconsistencies on scenarios, as many factors interact to affect the recorded parameters at production site. Hence in some cases, it is possible that yield in double cropping could be comparable to sole. In our observation, planting of chickpea immediately after wheat allow the chickpea plant to get sufficient moisture (a more yield determining factor), which might support the above assertion. Combined analysis for parameters in wheat showed common significance values for treatments and sites (Table 3).

Table 3. Combined Analysis of variance of HD, MD, PH, Tiller per plant, Spike length, TSW and Grain yield (at 2 sites) of wheat crop.

Source of variation	HD	MD	PH	No.of till	Spike length	TSW	Yield (kg/ha)
Rep	Ns	Ns	*	Ns	Ns	ns	Ns
Treatment (T)	**	**	Ns	*	*	**	**
Site (S)	**	*	**	*	Ns	**	**
T*S	Ns	Ns	**	Ns	Ns	Ns	Ns

Key: *, **= indicate significance at 0.05 and 0.01 probability levels, respectively. HD, Heading days, MD, Maturity days, PH, Plant height, TSW- thousand seed weight)

The combined data also showed that, variety Dinknesh took short days to mature (81 days) when compared to Senkegna (97 days) and Tay (97 days) varieties (Table 4). The shortest spike length was observed on variety Denknesh (8.86cm) whereas the

longest spike length obtained on variety Tay (8.58cm). The highest thousand seed weight and grain yield was observed on variety Denknesh and has significant difference at $P < 0.01$ with the other varieties (Table 4).

Table 4. Mean grain yield and other agronomic traits of wheat crop

Treatment	HD	MD	PH	No.of till	Panicle length	TSW	Yield (kg/ha)
Senkegna	62.3 ^c	96.0 ^a	80.8	5.85 ^a	8.1 ^{abc}	27.3 ^c	1703 ^c
Tay	63.5 ^{bc}	96.3 ^a	80.9	4.4 ^b	8.58 ^a	28.2 ^{bc}	1641 ^c
Dinknesh	51.2 ^d	81.7 ^b	78.2	5.2 ^{ab}	7.16 ^{cde}	34.5 ^a	2924 ^a
Senkegn + Habru	63 ^{bc}	97.5 ^a	80.8	5.7 ^a	7.3 ^{abcde}	33 ^b	2200 ^{bc}
Tay + Habru	64.8 ^{ab}	97.0 ^a	82.9	6.4 ^a	8.30 ^{ab}	30.1 ^c	1887 ^c
Dinknesh + Habru	50.8 ^d	81.7 ^b	82.6	5.2 ^{ab}	6.96 ^{ed}	35.4 ^a	2929 ^a
Senkegna + Natoli	63.2 ^{bc}	97.3 ^a	78.2	6.2 ^a	7.50 ^{bcde}	29.2 ^{bc}	2058 ^{bc}
Tay + Natoli	65.8 ^a	97.3 ^a	78.8	5.8 ^a	7.96 ^{abcd}	28.1 ^{bc}	1638 ^c
Dinknesh + Natoli	51.3 ^d	81.8 ^b	78.5	5.2 ^{ab}	6.86 ^e	33.2 ^a	2709 ^{ab}
LSD%	1.93	1.88	6.38	1.36	1.007	2.84	661
CV%	2.77	1.75	6.78	20.84	11.16	7.88	25.7

Key: *, **= indicate significance at 0.05 and 0.01 probability levels, respectively, HD, Heading days, MD, Maturity days, PH, Plant height, TSW- thousand seed weight

The two chickpea varieties namely Habru and Natoli used for our experiment, responded differently for double cropping farming system. The analysis of variance in Table 5 showed that there was significant difference in most recorded parameters except plant height.

Table 5. Analysis of variance of Chickpea during the 2015 cropping season.

Source of variation	MD	PH	Pod per plant	Seed per pod	HSW	Yield (kg/ha)
Treatment	**	NS	**	*	*	**
Rep	NS	NS	NS	NS	NS	NS
CV	2.2	10.3	22.1	15.8	4.2	19

Key: *, **= indicate significance at 0.05 and 0.01 probability levels respectively. NS, Non Significance. HD, heading days, MD, Maturity days. HSW- Hundred Seed Weight)

Sole cropping of chickpea crop took longer maturity days (~ 110 days) than the wheat-chickpea combination, which took nearly 90 days (Table 6). One of the possible reasons for maturity difference is the moisture regime, which triggers the crop to mature fast or to remain vegetative, as chickpea is known with its indeterminate growth habit. Sole planting of Natoli (2926 kg/ha) and Habru (2103kg/ha) chickpea varieties gave relatively higher yield (about

double), when compared with their respective double cropping combination. In contrast there is clear distinction trend between sole and double cropping responses in wheat, which is difficult to define as wheat is not expected to suffer from any competition.

Table 6. Analysis of variance of for major traits of Chickpea varieties

Treatment	MD	PH	Pod per plant	Seed per pod	HSW	Yield (kg/ha)
Sole Habru	111 ^a	50.1	52.2 ^{abc}	1.26 ^{ab}	28.08 ^{ab}	2103 ^b
Senkegn +Habru	90 ^b	44.8	42.1 ^c	0.9 ^b	28.64 ^a	1264 ^{cd}
Tay + Habru	89 ^b	44.2	51.5 ^{abc}	0.9 ^b	27.3 ^{abc}	870 ^d
Dinknesh + Habru	110 ^a	51.1	54.9 ^{abc}	1.33 ^a	29.1 ^a	1914 ^{bc}
Sole Natoli	112 ^a	48.2	44.4 ^{bc}	1.26 ^{ab}	25.8 ^c	2926 ^a
Senkegna +Natoli	91 ^b	43.4	62.2 ^{ab}	1.53 ^a	27.08 ^{abc}	2308 ^{ab}
Tay +Natoli	91 ^b	43.6	70.8 ^a	1.2 ^{ab}	26.2 ^{bc}	2246 ^b
Dinknesh +Natoli	111 ^a	45.1	48.3 ^{bc}	1.4 ^a	26.58 ^{bc}	2562 ^{ab}
LSD%	3.89	8.42	20.7	0.34	2.05	674
CV%	2.2	10.3	22.1	15.8	4.2	19

The highest grain yield in wheat-chickpea double cropping farming system was obtained from Dinknesh wheat variety (2709kg/ha) with Natoli chickpea variety (2562 Kg/ha) and this combination is recommended for areas having similar agro ecology as Takusa district has for double cropping farming system.

Financial analysis

Partial budget analysis was done to compare the financial feasibility of each treatment. The result of partial budget analysis in Table 7 indicates that lowest cost and benefit was obtained from sole *Habru* chickpea

variety while the highest cost and benefit was recorded when *Natoli* chickpea variety was double cropped with *Denekinesh* wheat variety. To do the dominance analysis, the total cost that varies in each treatment with its net benefit was listed in ascending order. Except treatments which contain sole *Natoli* chickpea variety double cropped with *Denekinesh* wheat variety, all other treatments were dominated and rejected from the analysis since the total cost that varies was higher but had relatively lower net benefit.

Table 7. Marginal rate of return of wheat chickpea double cropping system

Treatment	Sole Habru	Sole Natoli	Sole Senkegna	Sole Tay	Sole Dinknesh	Senkegn + Habru	Tay + Habru	Dinknesh + Habru	Senkegna +Natoli	Tay + Natoli	Dinknesh + Natoli
wheat Yield kg/ha	0	0	1703	1641	2924	2200	1887	2929	2058	1638	2709
Adj. yield wheat (kg/ha)	0	0	1532.7	1476.9	2631.6	1980	1698.3	2636.1	1852.2	1474.2	2438.1
Chickpea yield kg/ha	2103	2926	0	0	0	1264	870	1914	2308	2246	2562
Adj. yield chickpea (kg/ha)	1892.7	2633.4	0	0	0	1137.6	783	1722.6	2077.2	2021.4	2305.8
Gross filed benefit	26497.8	36867.6	15327	14769	26316	35726.4	27945	50477.4	47602.8	43041.6	566620.2
wheat production cost birr/ha	0	0	10140	10140	10140	10140	10140	10140	10140	10140	10140
Chickpea production cost birr/ha	4670	4670	0	0	0	4190	4190	4190	4190	4190	4190
TCV (ETB/ha)	4670	4670	10140	10140	10140	14330	14330	14330	14330	14330	14330
NB (ETB/ha)	21827.8	32197.6	5187	4629	16176	213960.4	13615	36147.4	33272.8	287110.6	423320.2
Dominance analysis	D		D	D	D	D	D	D	D	D	
MC (ETB/ha)											9660
MNB (ETB/ha)											101340.6
MRR (%)											104.913

Therefore, marginal rate of return (MRR) was calculated for the two treatments only. The MRR result showed that double cropping of *Natoli* chickpea variety with *Dinknesh* wheat variety had 104% MRR. This means that one birr spending on the treatment (double cropping *Natoli* chickpea variety with *Dinknesh* wheat variety) over sole *Natoli* chickpea variety can cover the cost and have a return of birr 1.04. Therefore, there is a great concern to integrate double cropping as feasible system in the community of agri-practitioners. It is also important to note that, in the era of continuous land fragmentation, cropping system that intensify yield vertically are of great options, to be included in the package for promotion.

Conclusion and Recommendation

Maturity days of both crop varieties have significance effect on the total grain yield. Wheat variety *Dinknesh* took relatively shortest days to mature, whereas, *Senkegna* and *Tay* variety took long days to mature, but variety *Dinknesh* has short spike length when compared to others. The highest grain yield in wheat-chickpea double cropping farming system was obtained from *Dinknesh* wheat variety (2709kg/ha) with *Natoli* chickpea variety (2562 Kg/ha) and this combination is recommended for areas having similar agro ecology with *Takusa* district for double cropping farming system. This association has the highest biological efficiency,

largest total productivity and the optimal monetary return. Thus, the combination will be useful to address both the food requirement and cash needs of farmers. It is also important to note that, in the era of continuous land fragmentation, cropping system that intensify yield vertically are of great options, to be in the technology package and promotion.

Acknowledgements

This research received the full financial and logistical support of ICRISAT and Gondar Agricultural Research Center. Mr Amare Tsehay was highly involved in executing the experiment in the field where sticking in water logging and muddy soil conditions was common experience.

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Genetic Variability and Heritability in Ethiopian Grasspea (*Lathyrus sativus* L.) Accessions

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Abstract

Comprehensive information on genetic variability and selection parameters is very crucial to design breeding strategies. However, very limited information is available in Ethiopian grasspea germplasm. Therefore, the present study was conducted to estimate genetic variability, broad sense heritability and genetic advance; and determine scope of selection for 18 quantitative traits using 426 Ethiopian grasspea accessions. The analysis of variance showed highly significant ($P < 0.0001$) differences for most of the traits demonstrating the presence of high genetic diversity among the studied grasspea accessions. Higher differences between PCV and GCV estimates were observed for number of pods, effective pods and seed yield signifying the importance of environmental factors influence. High heritability coupled with high genetic advance was observed for many of agronomically important traits indicating that the high heritability is due to additive gene effects and therefore, selection can be effective for the improvement of grasspea for those traits. On the contrary, low heritability coupled with low genetic advance was recorded for number of seeds per pod indicating greater contribution of non-additive gene effects on the expression of this trait.

Keywords: Additive effect, Coefficient of variation, Genetic advance

Introduction

Grasspea (*Lathyrus sativus* L., $2n = 2x = 14$) is a grain legume cultivated for food, feed and fodder in many countries. It has very important economic and ecological significance in developing countries like Ethiopia, India, Bangladesh, Pakistan and Nepal

(Campbell, 1997; Dixit *et al.*, 2016). In Ethiopia, grasspea is one of the principal legumes cultivated by smallholder farmers for its drought tolerance, resistance to diseases and pests; and for its ability to restore soil fertility (Dejene and Lijalem, 2012). The annual total area of cultivation and production of the country was reported to be 159,105.70 ha and

287,674.40 tons, respectively (CSA, 2016). The crop is becoming more important than previous times in agricultural systems; and thus, its area of cultivation is expected to increase in the future (Dejene and Lijalem, 2012). It is also highly adapted to adverse edaphic conditions (Polignano *et al.*, 2005; Vaz-Patto *et al.*, 2006). For these reasons, grasspea is considered as a promising crop for climate change adaptation, diversification of cropping systems and sustainable agriculture (Vaz-Patto *et al.*, 2006; Hillocks and Maruthi, 2012). Despite these advantages, grasspea is one of the most inadequately studied and exploited crops (Polignano *et al.*, 2005; Kumar *et al.*, 2011).

Genetic variability plays the fundamental role in any plant breeding program. Quantifying genetic diversity present in crop species is of greatest importance as it provides the basis of selection for traits of interest (Singh, 2002). Additionally, reliable estimates of genetic and environmental variations are helpful in estimating the heritability and predicted genetic gain from selection (Singh and Narayanan, 1997; Holland *et al.*, 2003; Visscher *et al.*, 2008). Overall, comprehensive knowledge on genetic variability, heritability and genetic advance allows geneticists and breeders to design breeding strategies for the improvement of crop productivity and quality (Johnson *et al.*, 1955).

Heritability and genetic advance are important selection parameters (Singh and Narayanan, 1997). Heritability

estimates can be grouped as broad sense heritability or narrow sense heritability (Holland *et al.*, 2003). Broad sense heritability provides information on the relative magnitude of genetic and environmental variation in specific population (Singh, 2002; Holland *et al.*, 2003; Visscher *et al.*, 2008). Genetic advance is the measure of genetic gain under selection and depends on genetic variability, heritability and selection intensity. Genetic advance also indicates the mode of gene action in the expression of traits and helps in choosing breeding methods (Singh and Narayanan, 1997). Thus, heritability estimates coupled with genetic advance are more reliable and helpful in predicting the gain under selection than individual consideration of the parameters (Johnson *et al.*, 1955).

In grasspea, genetic variability studies had been conducted by many researchers (Wuletaw and Endashaw, 2003; Turk *et al.*, 2007; Sedehi *et al.*, 2008; Talukdar and Biswas, 2008; Rahman *et al.*, 2010; Hobdari *et al.*, 2012; Parihar *et al.*, 2015) using quantitative traits and proved the presence of high genetic variation among studied genotypes. Heritability and genetic advance had also been estimated for yield and related traits by several authors (Wuletaw and Endashaw, 2003; Turk *et al.*, 2007; Parihar *et al.*, 2015). However, very limited research has been done on variability, heritability and genetic advance in Ethiopian grasspea accessions. In addition, previous study considered only small number (50) of

accessions compared to the total accessions conserved in Ethiopian Biodiversity institute. Moreover, the accessions were evaluated for small number (9) of quantitative traits. As a result, very limited information is available on these parameters in Ethiopian grasspea accessions. Therefore, the present study was conducted to estimate genetic variability, heritability and genetic advance of yield and related traits and to determine scope of selection in Ethiopian grasspea accessions.

Materials and Methods

Experimental Site, Materials and Design

The experiment was conducted at Debre Zeit Agricultural Research Center (DZARC) in Central Ethiopia during 2013/14 main cropping season. The mean annual rainfall of the center during the cropping season was 850 mm. The minimum and maximum temperatures were 8°C and 26.1°C, respectively, with a mean annual temperature of 19.32°C. The soil of the testing site is characterized by dark

clay vertisols with pH of 7.45 (Niguse, 2014). The study was carried out using 426 grasspea accessions obtained from Ethiopian Biodiversity Institute, originated from different regions of Ethiopia (392), Eritrea (24) and Australia (10) (Figure 1). All the available accessions were included in the study purposively to generate comprehensive information. The experiment was laid down using randomized complete block design with two replications. The blocks were folded into six sub blocks to reduce variations within the block and each sub block was planted with 71 accessions. Accessions were assigned randomly to the rows within the blocks. Each accession was grown on a 3 m long single row with spacing of 30 cm between rows and 10 cm between plants within a row. Therefore, the area of each block was 21.3 m (71 accessions x 30 cm of space between rows) length by 20.5 m (6 blocks x 3 m long row + 2.5 m of total space between sub blocks) width. The distance between the two blocks was 1m.

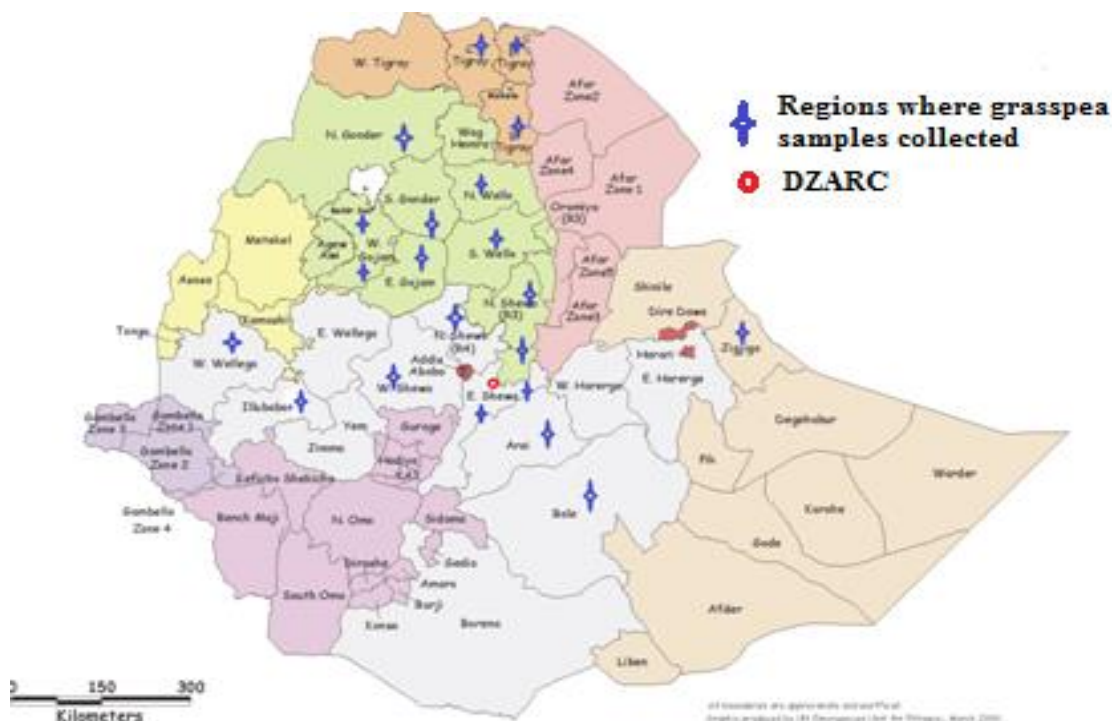


Fig. 1. Map of Ethiopia indicating different regions where 392 grasspea accessions collected for *ex situ* conservation and DZARC-the test site

Data Collection

Data collection was started from seed emergence and date of planting was used as a basis for all data recording purposes. Data were collected both on plant and plot bases from a total of 23 quantitative traits using descriptors for *Lathyrus* species (IPGRI, 2000). On plant basis, data were collected for plant height (cm), number of primary branches, length of primary branch (cm), leaflet length (cm), leaflet width (cm), leaf petiole length (cm), number of pods per plant, number of effective pods, pod length (cm), pod width (cm), number of seeds per pod and seed yield per plant (g) from six randomly selected and tagged plants of each accession. On plot basis, data were recorded on date of emergence,

days to 50% flowering, days to maturity, 100-seed weight (g), biomass (g), grain yield per plot (g) and harvest index (%).

Data Analyses

Genotypic and phenotypic variability

Mean, range, standard error of the mean and coefficient of variation were computed for all recorded traits. All quantitative data were subjected to the analysis of variance (ANOVA) as suggested by Gomez and Gomez (1984) using SAS Software (SAS Institute, 2004) to evaluate the extent of genetic variability among accessions. The means were compared using Duncan's Multiple Range (DMRT) at $\alpha = 0.05$ probability.

Genotypic and phenotypic variances and the respective coefficients of variation were estimated following the procedure given by Burton and DeVane (1953) for traits showing significant variation among accessions. Thus,

Genotypic variance (σ_g^2) = (MSg-MSe)/r, and

Phenotypic variance (σ_p^2) = $\sigma_g^2 + \sigma_e^2$

Where, MSg = mean square for genotypes

MSe (σ_e^2) = mean square for error (environmental variance); and

r = number of replications.

Genotypic Coefficient of Variation (GCV) = $[\sqrt{(\sigma_g^2) / (\bar{X})}] \times 100$

Phenotypic Coefficient of Variation (PCV) = $[\sqrt{(\sigma_p^2) / (\bar{X})}] \times 100$

Where, $\bar{X}$ is grand mean

Heritability

Heritability (H^2) in broad sense of traits was estimated as the ratio of the genotypic variance (σ_g^2) to the phenotypic variance (σ_p^2) (Burton and DeVane, 1953): $H^2 = [\sigma_g^2 / \sigma_p^2] \times 100$ where; $\sigma_g^2 = (MSg-MSe)/r$; $\sigma_p^2 = \sigma_g^2 + MSe$; MSg, MSe and r = mean squares for genotypes, mean square for error and the number of replications, respectively.

Genetic advance

Genetic advance (GA) of traits was computed using the formula suggested by Johnson et al. (1955) as follows:

$GA = (k) (\sigma_p) (H^2)$; and GA (as % of the mean) = $[GA / (\bar{X})] \times 100$

Where, k= selection differential (k=2.06 at 5% selection intensity)

σ_p = phenotypic standard deviation

H^2 = heritability (broad sense); and

$\bar{X}$ = Grand mean.

Results and Discussion

Analysis of Variance

The mean squares from the analysis of variance revealed highly significant ($P \leq 0.0001$) differences among grasspea accessions for the traits like plant height, number of primary -branches, number of pod per plant, number of seeds per pod, days to flowering, days

to maturity, 100 seeds weight, seed yield per plant, biomass, and harvest index (Table 1). These results indicated the presence of genetic diversity among grasspea accessions and the possibility of improving these traits through strong selection. The existence of high variability for different traits among grasspea accessions had been reported by

previous authors (Wuletaw and Endashaw, 2002; 2003; Turk *et al.*, 2007; Talukdar and Biswas, 2008; Grela *et al.*, 2010; Parihar *et al.*, 2015). Most of these authors reported the presence of highly significant differences for plant height, number of primary branches, number of pod per plant, number of seeds per pod, days to flowering, days to maturity, 100 seeds weight, seed yield per plant, biomass, and harvest index. Furthermore, in other studies of similar nature, it was found that there was highly significant differences for plant height, number of primary branches, number of pod per plant, number of seeds per pod, days to flowering, days to maturity, 100 seeds weight, and seed yield per plant in filed pea, cowpea, soybean, faba bean and

chickpea genotypes, respectively (Singh *et al.*, 2011; Kumar *et al.*, 2013; Diriba *et al.*, 2014; Ammar *et al.*, 2015; Getachew *et al.*, 2015). Therefore, these findings showed the importance of these agronomic traits for diversity studies and crop improvement in legumes. However, on contrary to the present result, non-significant difference for plant height among 50 Ethiopian grasspea accessions were also reported (Wuletaw and Endashaw, 2003). This difference may be due to differences in accessions and environmental conditions of the research sites used by the researchers. In the present study, many more accessions were included than accessions used by the previous investigators.

Table 1. Mean squares and simple statistics for 18 quantitative traits of 426 grasspea accessions

Traits	MSg (425)	MSe (425)	Mean	Range	S.E	CV (%)
PH	184.39***	39.17	68.72	42.32 - 100.23	0.36	9.11
NPB	1.19***	0.38	4.26	2.00 - 7.17	0.03	14.50
LPB	171.01***	42.15	66.99	41.00 - 95.33	0.35	9.69
LL	0.27 ^{ns}	0.26	4.24	2.92 - 12.23	0.02	11.91
LW	0.015***	0.004	0.58	0.23 - 1.13	0.00	11.05
PtL	0.122 ^{ns}	0.11	2.08	1.02 - 5.40	0.01	15.84
ONPP	1406.57***	293.8	102.94	43.00 - 262.00	1.00	16.65
EPP	1084.82***	230.11	94.45	39.00 - 232.00	0.88	16.06
PL	0.055 ^{ns}	0.06	2.78	2.20 - 6.38	0.01	8.40
PW	0.0045 ^{ns}	0.004	0.87	0.60 - 1.33	0.00	7.53
NSP	0.13***	0.074	3.59	2.50 - 4.50	0.01	7.57
DF	55.02***	2.8	46.02	36.00 - 64.00	0.18	3.64
DM	127.53***	9.74	106.65	86.00 - 129.00	0.28	2.93
SW	0.82***	0.32	8.40	5.50 - 12.00	0.03	6.72
SYP	23.59***	6.72	14.30	3.45 - 30.79	0.13	18.12
GYP	0.8***	0.28	2.76	1.16 - 4.6	0.37	18.34
BY	4.91***	0.78	5.96	2.07 - 11.67	0.62	14.80
HI	0.0007***	0.002	0.47	0.32 - 0.81	0.03	9.72

Note: PH = plant height (cm), NPB = Number of primary branches per plant, LPB = Length of primary branch (cm), LL = Leaflet length (cm), LW = Leaflet width (cm), PtL = Petiole length (cm), NPP = Number of pod per plant, EPP = Effective pods per plant, PL = Pod length (cm), PW = Pod width (cm), NSP = Number of seeds per pod, DF = Days to flowering, DM = Days to maturity, SW = 100 seed weight (g), SYP = Seed yield per plant (g), GYP = Grain yield per hectare (t/ha), BY = Biomass (t/ha), and HI = Harvest index; ns, and *** indicate insignificance and very highly significance at 5% and 1%, respectively).

Simple Measure of Variability

The mean values, ranges and coefficients of variation for 18 quantitative traits showed considerable variation among grasspea accessions (Table 1). The wide ranges especially for plant height, days to 50 % flowering, number of pods per plant, days to maturity, seed yield per plant, biomass and grain yield per hectare indicated the diversity among the grasspea accessions and suggest the possibility of improving these traits through selection, and hybridization. The existence of variability among grasspea accessions for these parameters had been reported previously by several authors (Wuletaw and Endashaw, 2002; 2003; Rahman *et al.*, 2010; Dikshit *et al.*, 2014; Ahmadi *et al.*, 2015; Parihar *et*

al., 2015). However, the results reported elsewhere revealed low mean performance with comparable ranges and coefficient of variation compared to the present results for most of traits considered (Rahman *et al.*, 2010; Ahmadi *et al.*, 2015). Similarly, lower mean values with comparable ranges but very high coefficient of variation were reported for many of the traits evaluated (Parihar *et al.*, 2015). These differences in mean performance might come from differences in genetic factors or environment conditions under which genotypes were evaluated.

Table 2. Estimates of coefficients of variation, heritability and genetic advance for 14 agronomic traits in 426 grasspea accessions

Traits	σ^2_e	σ^2_g	σ^2_p	GCV (%)	PCV (%)	H ² (%)	GA (5%)	GAM (5%)
PH	39.17	72.61	111.78	12.40	15.39	64.96	14.15	20.59
NPB	0.38	0.41	0.79	14.94	20.80	51.59	0.94	22.10
LPB	42.15	64.43	106.58	11.98	15.41	60.45	12.86	19.19
LW	0.004	0.01	0.014	12.79	16.80	57.89	0.12	20.04
NPP	293.80	556.39	850.19	22.91	28.33	65.44	39.31	38.19
EPP	230.11	427.36	657.47	21.89	27.15	65.00	34.33	36.35
NSP	0.07	0.03	0.10	4.66	8.90	27.45	0.18	5.03
DF	2.80	26.11	28.91	11.10	11.68	90.31	10.00	21.74
DM	9.74	58.90	68.64	7.20	7.77	85.81	14.64	13.73
SW	0.32	0.25	0.57	5.95	8.99	43.86	0.68	8.12
SYP	6.72	8.44	15.16	20.31	27.22	55.66	4.46	31.21
GYP	0.28	0.26	0.54	18.47	26.62	48.15	0.73	26.41
BY	0.78	2.07	2.85	24.11	28.30	72.58	2.52	42.32
HI	0.002	0.003	0.005	10.64	14.27	55.56	0.08	16.33

Key: PH = plant height, NPB = Number of primary branches per plant, LPB = Length of primary branch, LW = Leaflet width, NPP = Number of pod per plant, EPP = Effective pods per plant, NSP = Number of seeds per pod, DF = days to flowering, DM = days to maturity, SW = 100 seed weight, SYP = seed yield per plant, GYP = Grain yield per plot, BY = Biomass, HI = Harvest index).

In the present study, the mean performance of grain yield per hectare varied from 1.16 to 4.6 t with the mean value of 2.76 t ha⁻¹ and coefficient of variation of 18.34%. The highest grain yield per hectare (4.6 t) was recorded from A60 followed by A385 (4.38 t) and A105 (4.33 t); while the lowest yield per hectare was obtained from A96 (1.16 t). Biomass per hectare also varied from 2.07 t to 11.67 t with the mean value of 5.96 t and coefficient of variation of 14.8%. The highest biomass per hectare (11.67 t) was recorded from A385 followed by 126 (10.72 t) and A60 (10.39 t); while the lowest biomass per hectare was obtained from A71 (2.07 t). Days to maturity ranged from 86.00 to 129.00 days with the mean value of 106.65 days and coefficient of variation of 2.93%, showing a wide range of variation among accessions. The earliest accession was A90, which took 87.00 days to mature, followed by A76 and A71 with 89.00 and 89.50 days to mature, respectively. The late maturing accession was A323, which took 126.00 days to mature. However, unlike to the present results, higher mean values and high coefficient of variation were reported by previous studies (Wuletaw and Endashaw, 2002; Wuletaw and Endashaw, 2003) for many of the traits considered in Ethiopian grasspea accessions. These differences in mean performance and coefficient of variation might be due to differences in environmental conditions of the two research sites. Grela *et al.* (2010) also reported that grain yield, plant height, biomass and number of pods per plant are highly

affected by rainfall amount and other environmental factors.

Estimates of variances and genetic parameters

Estimates of variances

Estimates of genotypic (σ^2_g), phenotypic (σ^2_p) and environmental (σ^2_e) variances were estimated for the studied traits (Table 2). Phenotypic variance was relatively high for the traits like number of primary branches, number of seeds per pod, 100-seed weight, seed yield per plant and harvest index. This indicated that the phenotypic expression of these traits was greatly influenced by environmental factors; and selection on phenotypic bases of these traits may not be effective for genetic improvement unless the environmental conditions are optimized. Similarly, in another studies, relatively higher phenotypic variance for number of plant height, primary branches, number of pods per plant and seed yield per plant were reported (Wuletaw and Endashaw, 2003). On the contrary, degree of difference between phenotypic variance and genotypic variance was relatively low for leaflet width, days to 50% flowering, days to maturity and biomass. This shows that the phenotypic expression of these traits was relatively less affected by environmental factors; and selection on phenotypic bases of these traits will be effective. Wuletaw and Endashaw (2003) also indicated lower degree of difference between phenotypic and genotypic variances

for days to 50% flowering and biomass. However, the same authors reported relatively high and low degree of differences between phenotypic and genotypic variances for days to maturity and harvest index, respectively. This variation of phenotypic expression of the two traits between studies might be mainly due to differences in environmental conditions of the two research sites.

Coefficients of variation

Genetic (GCV) and phenotypic (PCV) coefficients of variability values for 14 traits varied from 4.66% for number of seeds per pod to 25.2% for biomass per hectare and 7.77% for days to maturity to 29.11% for biomass per hectare, respectively (Table 2). Estimates of GCV and PCV had been reported for the same traits of grass pea by previous investigators (Kumar and Dubey, 2001; Wuletaw and Endashaw, 2002; Wuletaw and Endashaw, 2003; Ahmadi *et al.*, 2015; Parihar *et al.*, 2015). It has been reported that GCV and PCV values, > 20%, 10-20% and < 10% are regarded as high, moderate and low, respectively (Deshmukh *et al.*, 1986).

High GCV value was obtained for traits like number of pods per plant, effective pods per plant, seed yield per plant, grain yield per hectare and biomass (Table 2). This indicated the existence of considerable genotypic variability among grasspea accessions for these traits and greater influence of genetic factors for the expression of

these traits. High GCV estimates for number of pods and seed yield per plant were reported by several authors (Kumar and Dubey, 2001; Talukdar and Biswas, 2008; Parihar *et al.*, 2015). Similarly, Wuletaw and Endashaw (2003) reported high GCV for number of pods per plant and biomass. In the present study, moderate GCV estimate was revealed for traits like plant height, length of primary branches, number of primary branches, leaflet width, days to 50% flowering and harvest index; and low GCV estimate was observed for days to maturity, number of seeds per pod and 100-seed weight. These results indicated greater influence of environmental factors for the expression of these traits compared to traits revealed high GCV estimates.

In line with the present results, moderate GCV was reported by Kumar and Dubey (2001) for number of branches per plant; by Wuletaw and Endashaw (2003) for days to 50% flowering; and by Ahmadi *et al.* (2015) and Parihar *et al.* (2015) for harvest index. Similarly, low GCV estimates were reported by Wuletaw and Endashaw (2003) and Ahmadi *et al.* (2015) for days to maturity and 100-seed weight; and Parihar *et al.* (2015) for days to maturity and number of seeds per pod. However, on the contrary, low GCV estimate was reported by Wuletaw and Endashaw (2003) for number of primary branches; and by Ahmadi *et al.* (2015) for biomass. These differences might be due to differences between sets of accessions used for the studies or

environmental conditions of research sites where accessions were grown for characterization.

High PCV was revealed for traits like number of primary branches, number of pods per plant, effective pods per plant, seed yield per plant, grain yield per hectare and biomass (Table 2). These results reflected the presence of considerable phenotypic variation among grasspea accessions for these traits. The high PCV estimates for number of pods and seed yield per plant and biomass per hectare were in harmony with the previous reports by several authors (Kumar and Dubey, 2001; Wuletaw and Endashaw, 2003; Talukdar and Biswas, 2008). Similarly, Parihar *et al.* (2015) reported high PCV estimates for number of pods per plant, seed yield per plant, biomass and grain yield per hectare.

In the present study, moderate PCV values were exhibited for traits like plant height, length of primary branches, leaflet width, days to 50 % flowering and harvest index. On the contrary, low PCV was observed for days to maturity, number of seeds per pod and 100-seed weight, indicating existence of lesser phenotypic variability among grasspea accessions that might be due to greater influence of environmental factors for the expression of the traits. Similar results were reported by Wuletaw and Endashaw (2003) for days to 50% flowering and harvest index. Ahmadi *et al.* (2015) also reported moderate PCV estimate for harvest index. In

agreement with the present result, low PCV estimate was reported by Wuletaw and Endashaw (2003), Ahmadi *et al.* (2015) and Parihar *et al.* (2015) for days to maturity. However, in contrast to the present study, low PCV estimate was reported by Wuletaw and Endashaw (2003) for traits like number of primary branches and plant height; and by Ahmadi *et al.* (2015) for plant height. These differences might be due to differences in genetic bases of the studied materials for these traits or higher influence of environmental factors for their expression.

Higher differences between PCV and GCV estimates were observed for number of pods, effective pods and seed yield per plant, and grain yield per hectare (Table 2) indicating the complexity of these traits and the importance of environmental factors in influencing the expression of these traits. High differences between PCV and GCV were also reported in grasspea by previous authors (Wuletaw and Endashaw, 2003; Parihar *et al.*, 2015) for number of pods and seed yield per plant and biomass per hectare. Similar results were reported by Talukdar and Biswas (2008) for number of pods per plant and seed yield per plant. However, difference between PCV and GCV estimates was relatively very slight in the case of days to maturity and days to 50% flowering, signifying minimal influence of environment and a reasonable effect of genotypic factors on the expression of these traits. Similar results were reported

previously in grasspea by several authors (Wuletaw and Endashaw, 2003; Ahmadi *et al.*, 2015; Parihar *et al.*, 2015) for days to maturity and days to 50% flowering. In general, coefficients of genotypic and phenotypic variation suggest that there is good scope for improvement through selection for number of pods per plant, effective pods per plant, seed yield per plant, grain yield per hectare and biomass per hectare. Similar results were reported for traits like number of pods per plant, seed yield per plant and biomass per hectare in grasspea accessions (Wuletaw and Endashaw, 2003; Parihar *et al.*, 2015).

Broad sense heritability

Heritability estimates for traits under study varied from 27.45% for number of seeds per pod to 90.3% for days to 50% flowering (Table 2). According to Johnson *et al.* (1955), these heritability estimates can be classified as low (< 30%), moderate (30-60%) and high (> 60%) levels. Hence, high heritability estimate was recorded for days to 50% flowering, days to maturity, followed by biomass per hectare, number of pods per plant, effective pods per plant, plant height and length of primary branches. These results indicated that expression of these traits was least influenced by the environmental factors, signifying a close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to phenotypic expression. However, selection may not be useful for these traits, because broad sense heritability is based on total genetic variance which includes both fixable

(additive) and non-fixable (dominance and epistatic) variances (Singh and Narayanan, 1997). Similarly, Wuletaw and Endashaw (2003) reported high heritability estimates for days to 50% flowering, number of pods per plant and biomass per hectare. High heritability had been also reported in grasspea for days to 50% flowering, days to maturity and plant height (Ahmadi *et al.* (2015); Parihar *et al.*, 2015).

Likewise, Kumar and Dubey (2001) revealed high heritability estimates for plant height in grasspea. Furthermore, similar to the present findings, high heritability estimates were reported (Kumar *et al.*, 2013; Diriba *et al.*, 2014; Getachew *et al.*, 2015) for days to 50% flowering and days to maturity in field pea, cowpea, mung bean and chickpea, respectively. However, on the contrary to the present result, low heritability was reported for plant height and biomass per hectare by Wuletaw and Endashaw (2003) and Parihar *et al.* (2015), respectively.

Traits like seed yield per plant, number of primary branches, harvest index, grain yield per hectare, leaflet width and 100 seeds weight revealed moderate level of heritability. For such traits, phenotypic expression is influenced by environmental factors and the non-additive gene effects; and hence, genetic improvement through selection is difficult due to masking effects of the environment on the genotypic effects (Johnson *et al.*, 1955). Further, the lowest heritability estimate was recorded for number of seeds per pod (27.45%); this indicated that a small proportion of the

phenotypic variation is caused by variation in genotypes, signifying that the phenotypic expression of this trait was highly influenced by environmental factors with less contribution of genetic factors. In agreement with the present results, Wuletaw and Endashaw (2003) and Parihar *et al.* (2015) reported moderate level of heritability for number of primary branches, 100 seeds weight, seed yield per plant and grain yield per hectare.

Genetic advance

In present study, genetic advance as a percent mean (GAM) ranged from 5.03% for number of seeds per pod to 45.24% for biological yield per hectare (Table 2). These results indicated that selecting the top 5% of the accessions could result in an advance of 5.03% to 45.24% over the respective population mean. As suggested by Johnson *et al.* (1955), estimates of genetic advance can be classified as low (< 10%), moderate (10-20%) and high (> 20%).

High GAM was recorded for traits like biomass per hectare, number of pods per plant, effective pods per plant, seed yield per plant, grain yield per hectare, number of primary branches, days to 50% flowering, plant height and leaflet width. These results indicated that the expression of these traits is mainly governed by additive gene effects; and therefore, improvement of such traits can be achieved through selection. In harmony to the present findings, Wuletaw and Endashaw (2003) reported high GAM for days to 50%

flowering, number of pods per plant and biomass per hectare. Similarly, Parihar *et al.* (2015) reported high GAM for plant height, number of primary branches and pods per plant and seed yield per plant.

On the contrary, length of primary branches, harvest index and days to maturity revealed moderate level of GAM. In addition, low genetic advance was recorded for traits like number of seeds per pod and 100 seeds weight. This indicated that expression of these traits is governed by non-additive gene effects; and hence, heterosis breeding may be useful for the improvement of these traits than selection. Similarly, Parihar *et al.* (2015) reported low GAM for number of seeds per pod in grasspea accessions. However, in contrast to the present results, Wuletaw and Endashaw (2003) reported moderate level of GAM for 100 seeds weight and seed yield per plant; and low level of GAM for days to maturity, number of primary branches and plant height. Additionally, Parihar *et al.* (2015) reported moderate level of GAM for days to maturity and biomass per hectare. These differences might be due to difference in magnitude of the different gene effects or the influence of environmental factors.

Scope of selection

In the present study, high heritability coupled with high GAM was observed for plant height, number of pods per plant, effective pods per plant, days to 50% flowering and biomass per hectare (Table 3), indicating greater

contribution of additive gene action for the expression of these traits; and therefore, improvement can be achieved through selection in these traits. Similarly, Wuletaw and Endashaw (2003) reported high heritability coupled with high GAM for days to 50% flowering, number of pods per plant and biomass per hectare. In addition, high heritability coupled with high GAM had been reported in grasspea accessions by Ahmadi *et al.* (2015) for biomass per hectare. However, it is not necessary for a trait showing high heritability to exhibit high GAM or the vice-versa (Johnson *et al.*, 1955). In the present study, moderate heritability and high GAM estimates were revealed for length of primary branches, number of primary branches, leaflet width, seed yield per plant and grain yield per hectare, indicating that these traits are governed by additive gene effects, however the exhibited low heritability might be due to high environmental influences. Therefore, selection for these traits may be effective in early generations of grasspea accessions with larger possibility of delay for further generations.

On the contrary, high heritability coupled with moderate level GAM was recorded for days to maturity. In addition, moderate heritability coupled with moderate genetic advance was recorded for harvest index. These results indicated the existence of intermediate expression in these traits for both additive and dominance gene effect. Similar results were reported for days to maturity (Ahmadi *et al.*,

2015; Parihar *et al.*, 2015). Furthermore, moderate heritability coupled with low GAM was recorded for 100 seeds weight, suggesting that the expression of seed weight is governed by non-additive gene action. However, the exhibited moderate heritability might be mostly due to favorable influence of the environment rather than the genetic factors. Additionally, low heritability and low GAM were recorded for number of seeds per pod indicating that the expression of this trait is governed by non-additive gene effects; and influenced negatively by environmental effects. In general, these results indicate the predominance of non-additive gene action in the inheritance of seeds per pod and seed weight, suggesting that selection may not be effective for the improvement of these traits, and rather heterosis breeding may be useful. Similar suggestion was given for the traits exhibiting non-additive gene action (Singh and Narayanan, 1997).

Conclusions

In conclusion, the analysis of variance showed the presence of high genetic diversity among the studied grasspea accessions. Traits like pods per plant, seed yield per plant and grain yields per hectare were highly influenced by the environment factors compared to other traits. The role of additive gene action was high for the expression of plant height, pods per plant, days to 50% flowering and biomass yield; and therefore, selection can do

improvement on these traits. High heritability coupled with high GAM was observed for plant height, number of pods per plant, effective pods per plant, days to 50% flowering and biomass per hectare showing that the high heritability is most likely due to additive gene effects; and the importance of selection for the improvement of grasspea for these traits. On the contrary, the role of additive gene effects was low for seeds per pod indicating limited scope of selection for improvement for this trait; rather heterosis breeding may be useful.

Acknowledgements

The authors wish to appreciate Ethiopian Biodiversity Institute and Debre-Zeit Agricultural Research Center for the provision of accessions; and experimental land and labor support, respectively, for execution this study.

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Heat Tolerance Responses of Chickpea (*Cicer arietinum* L.) Genotypes in the Thermal Zone of Ethiopia, a Case of Werer Station

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Abstract

Chickpea (*Cicer arietinum* L.) is an important cool season food legumes with indeterminate growth habit. The crop is valued for its nutritive seeds and use as animal feed in many developing countries. The productivity of the crop is constrained by several abiotic stresses, among which high temperature is one of the key determinants of crop. The present study was conducted to screen, evaluate and select Chickpea genotypes possessing high yield potential under heat stress condition at Werer Agricultural Research Center. The experiment was laid in RCBD of three replications using eighteen early maturing chickpea genotypes of ICRISAT in 2015. Two times planting (i. e. 23 Jan and 24 February) was done each on 4.8m² plot with 30cm and 10 cm spacing, and data was determined on the two central rows. Growth period maximum temperature of $\geq 35^{\circ}\text{C}$, considered threshold for heat assessment, was sufficiently interfaced in both planting days. Combined analysis of variance revealed existence of highly significant differences among the tested genotypes for most of the agronomic traits. The top 3 best performing lines with extra early phenology were ICCV 09309 (1187 kg/ha), ICCV 10103 (1035 kg/ha) and ICCV 10108 (1014 kg/ha). Delayed planting posed more stress on the crop and yield, possibly the increasingly progressing temperature interfered beyond physiological adjustment of the crop. Heat tolerance indices like STI, TOL, SSI, MP and GMP calculated on the basis of grain yield, and genotypes ICCV-10102, ICCV-09309, DZ-2012-CK-0034 and DZ-2012-CK-0041 showed lower TOL and higher STI values indicating as tolerant genotypes relative to others

Keywords: Chickpea, heat stress, tolerance, phenology, Yield

Introduction

Chickpea (*Cicer arietinum* L.) is annual crop belongs to family leguminaceae, subfamily papilionaceae

and genus *cicer* (Van der Maesen, 1987). Among the global pulse crops, chickpea has consistently maintained a much more significant status, ranking second in area of production (15.3%) after common bean and third in

production (14.6%) after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) (Knights *et al.*, 2007; FAO, 2008; Gaur *et al.*, 2010). The crop is one of the first grain legumes domesticated in old world.

The crop faces various abiotic stresses among which heat stress is progressively posing major production constraint in warmer short-season environments. High temperature often occurs in combination with high solar irradiance, drought, and strong wind, all of which can aggravate plant injury even in well watered plants (Hall, 1992). For example, exposure of chickpea plants to high temperature 30–35°C at flowering stage can cause substantial yield loss (Summerfield and Wein, 1980; Saxena *et al.*, 1988) as it interferes with reproductive biology of the crop. High temperature during the grain filling period can reduce the individual seed size as it might interfere with assimilate translocation period to sink at maturity which may lower grain yield per plant (Ong 1983). Grain yield was reduced by 53-330 kg/ha for every 1°C seasonal temperature rise in India (Kalra *et al.* 2008). In spring sown crops, the mean grain yield decreased compared with autumn sown materials due to seasonal temperature fluctuations (26-38°C) during the reproductive stage (Ozdemir and Karadavut 2003). In Bangladesh, a six week delay in sowing from the optimum period was observed to reduce the grain yield by 40% and flowering and maturity was also accelerated (Ahmed *et al.* 2011) as it

coincides with increasingly aggressive thermal condition.

Heat stress is a function of plant genotype, high temperature, and water status and soil type. The occurrence and severity of heat stress varies in different regions from year to year. Depending on timing, duration and interaction, observed heat stress can be grouped into chronic and acute, each of which involve different coping mechanisms, adaptation strategies and ultimately, breeding techniques (Blum, 1988; Wery *et al.*, 1993). A simple but effective field screening technique for heat tolerance at the reproductive stage in chickpea has been developed at ICRISAT (Gaur *et al.*, 2013, 2014). It involves advancing sowing date to synchronize the reproductive phase of the crop with the occurrence of higher temperatures ($\geq 35^{\circ}\text{C}$). This method was effective in identifying heat tolerant germplasm at ICRISAT and several other locations in the world (Gaur *et al.*, 2013, 2014). So far no effective screening technique has been developed to tackle this problem in Ethiopia. Heat tolerance is therefore important under Ethiopian condition where temperature is high.

Previous chickpea improvement efforts by centers under Ethiopian Institute of Agricultural Research (EIAR) focused on developing high yielding and drought tolerant varieties. As a result, about twenty-two improved chickpea varieties for increased yield and drought tolerance were released and some of which are currently under cultivation (Asnake,

2014). However, no variety has been released so far in Ethiopia for high temperature (heat) stress tolerance, despite global warming is on alert and some signals are evidenced in Ethiopia, at national level, and neither population thematized effort being attempted by the national improvement program. Therefore, the objective of this experiment is to assess variability among genotypes for heat tolerant and high potential yield under thermal zone of Ethiopia by screening in hot spot location.

Materials and Methods

Description of the study area

The study was conducted at Werer Agricultural Research Center, found in Afar National Regional State. The center is located at 9°20'31" N latitude and 40°10'11" E longitude in the Middle Awash Rift valley 280 km far from Addis Ababa. The station is delimited at 740 masl. The climate is semi-arid with a bimodal rainfall of 533mm annually. The long rainy season occurring from July to September accounts for 264 mm rainfall and the short rainy season from February to April accounts 156 mm. The minimum/maximum annual temperatures are 18.9°C/38°C, while

the average annual temperature is 28.4°C. The area receives the average daily sunshine of 8.5 hours with an average solar radiation of 536 calories per square centimeter day⁻¹ (cal/cm²/day) (Girma Menkir and Awulachew Sileshi 2007). The soils of the study area is predominantly fluvisol followed by Vertisols (Wondimagegne and Abere, 2012). The Fluvisol soils are coarser in texture than Vertisols and their textural classes range between clay and silt loams. The soils are brown in color and turn to dark brown when moist. The pH of the soil is slightly alkaline and ranges from 7.2 to 8.5.

Experimental Procedure

Field evaluation of 18 early maturing chickpea (*Cicer arietinum* L.) germplasms (Table 1) including both kabuli and desi types was conducted during 2015 off season adapting Gaur et al. (2013 and 2014), using two planting dates of 23 January and 24th February. It involves advancing sowing date to synchronize the reproductive phase of the crop with the occurrence of higher temperatures ($\geq 35^{\circ}\text{C}$). This method was employed to optimize planting date and to effectively identify heat tolerant germplasm.

Table 1. List of plant materials (genotypes) used in experiment. K=Kabuli, D=Desi, €=indicating standard check

ICCV-10409 (K)	ICCV-07313 (K)	ICCV-10307 (K)
ICCV-09315 (K)	ICCV-09311 (K)	DZ-10-11 (D)€
DZ-2012-CK-0034 (D)	ICCV-10102 (K)	ICCV-09304 (K)
ICCV-09309 (D)	ARERTI (K)€	DZ-2012-CK-0044 (D)
ICCV-10107 (D)	DZ-10-4 (K)€	ICCV-10311 (K)
ICCV-10404 (K)	ICCV-09301 (K)	ICCV-09307 (K)
ICCV-10108 (D)	ICCV-10103 (D)	DZ-2012-CK-0041 (D)

The treatments were laid in RCBD with three replication. Both dates of planting of the 18 chickpea genotypes including the standard checks were sown when temperature rises above 30 °C. The gross plot size was 4.8m² (4m x 1.2m) accommodating 4 rows of 4m length. Spacing of 30cm between rows and 10cm between plants were used and harvesting was done from two central rows of each plot (2.4m²).

Measurements of growth and yield parameters

This study used measure of plant growth, yield traits and temperature prediction at different developmental stages of chickpea as tools for heat tolerance screening. Days to first flowering (DFF), days to 50% flowering (D50%F), days to first podding (DFP), days to end of podding (DEP) and days to maturity (DM) were recorded for each genotype. At physiological maturity, five plants were randomly selected and plant height (cm) and first pod height (cm) was determined. Grain yield was collected from two central rows of each plot (2.4m²) and the aerial parts of the plants from 2 central rows were air dried at 38°C for 48 h to determine shoot dry weight. At harvest, five plants were randomly collected and yield components (pod number per

plant, seed number per plant and hundred seed weight) were recorded. Harvest Index (%) was calculated as (grain yield/total shoot dry weight) x 100.

The plant growing days at different developmental stages were also calculated following the procedure of Vargas (1998). Vegetative period (VP) was defined as the number of days from sowing to one day before flowering date was recorded for the plot. The days from first flower to first pod was considered the flowering period (FP). The grain filling period (GFP) was defined as the number of days from first pod to maturity and, Maturity period (MP) was defined as the number of days from first flowering to maturity. Then, the average maximum and minimum temperatures were calculated at different developmental stages (VMax; VMin; FMax; FMin; GMax ; GMin; Mmax; Mmin). Grain yield was considered as the dependent variable and the influence of temperature was determined from different developmental stages (Vargas *et al.* 1998). All measured parameters (plant phenology, growth parameters, yield and yield components) were subjected to analysis of variance (ANOVA) using

PROC GLM of SAS software version 9.1 (Anonymous, 2002) and mean separation was done using LSD (0.05).

Estimation of Heat Indices

Heat indices including stress susceptibility index (SSI) $\left(\frac{1-(Y_p/Y_s)}{1-(\bar{Y}_s/\bar{Y}_p)}\right)$ (Fischer and Maurer, 1978), stress tolerance (TOL) $(Y_p - Y_s)$ (Rosielle and Hamblin, 1981), mean productivity (MP) $(Y_s + Y_p)/2$ (Rosielle and Hamblin, 1981) stress tolerance index (STI) $= \frac{(Y_p)(Y_s)}{(\bar{Y}_p)^2}$ (Fernandez, 1992) and geometric mean productivity (GMP) $(\sqrt{Y_s \times Y_p})$ (Fernandez, 1992) were calculated using the formula indicated in their respective references, where “ Y_s ” is the yield of genotype under stress, “ Y_p ” is the yield of genotype under irrigated conditions, “ $\bar{Y}_s$ ” and “ $\bar{Y}_p$ ” are the mean yields of all genotypes under stressed and non-stressed conditions, respectively, and “ $1 - (\bar{Y}_s/\bar{Y}_p)$ ” is the stress intensity.

Result and Discussion

Variation in atmospheric temperature

The maximum/minimum sowing air temperatures during the first planting was 31°C/9.3°C and maximum temperature reached the threshold level of 35°C at 12 days after sowing. For the second planting, Maximum/minimum sowing temperatures were 33.5°C/ 12.5°C and maximum temperature raised to 36 °C at 2 days after sowing (Fig 1). This is an indication for the rapid increment of temperature during the chickpea growing period, which might have posed greater factor of yield reduction, possibly from physiological interference beyond adjustment, in the second date sown chickpea. In this study, the average grain yield of chickpea genotypes reduced by half during the second planting (430 kg/ha) as compared to first planting (860 kg/ha) (Table 5). Similar report by Singh *et al.* (1982) indicated that, peak photosynthetic rate was observed at 22°C in chickpea, but the net photosynthetic rate showed to be reduced at 28°C.

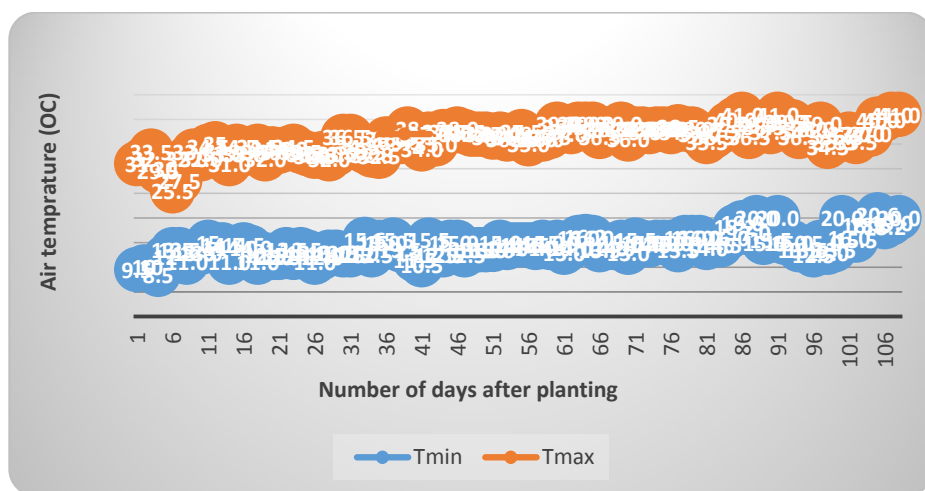


Fig 1. Maximum and minimum air temperatures at WARC during the chickpea growing season

Thus, observations in the present study suggested that, the experimental location is suitable site for screening heat stress tolerance of chickpea and the performance of genotypes to the existing temperature would result in screening and identification of genotypes tolerant to heat stress.

Effect of atmospheric temperature on developmental stages of chickpea

The result of this study indicated that high temperature significantly reduced the vegetative, flowering, grain filling and maturity periods of chickpea as

planting delayed from Jan 23 to Feb 24 (Table 2). The overall vegetative period (VP), flowering period (FP), grain filling period (GFP) and maturity period (MP) was reduced from 33 to 31, 16 to 13, 33 to 30 and 49 to 43 days at late sown chickpea (Table 2).

The calculated average maximum and minimum temperature at different developmental stage V_{max} , V_{min} ; F_{max} , F_{min} ; GF_{max} , GF_{min} and M_{max} , M_{min} were considerably higher during the second planting as compared to the first planting (Table 3).

Table 2. Mean number of days for different chickpea developmental stages during P1 and P2.

Planting dates	Chickpea developmental stages			
	VP	FP	GFP	MP
P1	33	16.1	33.2	49.2
LSD(0.05)	2.0	4.0	8.9	9.1
P2	31.3	13.2	30.3	43
LSD(0.05)	1.8	2.89	6.4	8.8

Key: VP = one day before first flower – sowing date; FP= days to first pod – days to first flower; GFP= maturity date – one day after pod formation; MP=maturity date-days to first flower

Table 3. The average maximum and minimum temperatures for each developmental stages.

Planting	Average maximum and minimum temperature of chickpea developmental stages					
	V _{max}	V _{min}	F _{max}	F _{min}	GF _{max}	GF _{min}
P1	32.8	12.5	36.4	13.6	37.5	14.7
P2	36.7	14.2	37.8	14.5	38.1	16.4

Key: V_{max} = Average maximum temperature during vegetative period, V_{min} = Average minimum temperature during vegetative period, F_{max} = Average maximum temperature during flowering period, F_{min} = Average minimum temperature during flowering period, GF_{max} = Average maximum temperature during grain filling period, GF_{min} = Average minimum temperature during grain filling period

The VP exposed to max/min temperature of 39⁰C/8.5⁰C and 41⁰C/10.5⁰C during first and second planting, respectively. The FP experienced max/min temperature of 40⁰C/10.5⁰C and 40⁰C/11.5⁰C during first and second planting, respectively. Whereas, the GF exposed to max/min temperature of 39⁰C/11⁰C and 41/12⁰C of during first and second planting, respectively. This indicates late sowing exposed the chickpea plant to high temperature and reduced the length of vegetative growth stage causing flowering earlier. This might be the reason for yield reduction during second planting as compared to the first (Table 5).

The current result is supported by old reports of Saxena *et al.*, (1988), indicated that exposure of chickpea plants to high temperature of 30⁰C-35⁰C at flowering and grain filling stage caused substantial yield loss.

Combined Analysis of Variance

Results from combined analysis of variance revealed that there were significant genotypic differences among the tested chickpea genotypes for most of the traits considered in this study (Table 4). Except for days to maturity (DM), highly significant difference ($p \leq 0.01$) for all the studied phenological & growth parameters, such as days to first flowering (DFF), days to 50% flowering (DFFF), days to first podding (DFP) and number of primary branches (NBR) were observed among the tested chickpea genotypes under heat stressed environment (Table 4).

Table 5. Analysis of Variance for sum of squares of chickpea traits grown under heat stress at Werer for both planting date.

Characters	Planting date 1						Planting date2				
	Source of variation						Source of variation				
	Varieties (20)*	Replication (2)	Error (40)	mean	CV	R2	Varieties 20)	Replication (2)	Error (40)	mean	CV (%)
DFF	71.09**	3.48	1.48	33.52	3.62	0.96	65.74**	1.33	1.38	32.38	3.63
DFFF	44.02**	6.78*	2.04	43.46	3.29	0.92	71.07**	0.33	2.70	39.33	4.18
DFP	47.3**	46.9*	5.9	49.4	5.09	0.68	30.2**	7.04	3.4	46.0	4.00
DM	33.02 ^{ns}	104.64*	24.60	82.54	6.01	0.47	24.75 ^{ns}	63.54	28.46	75.84	7.03
FPH	6.49**	4.12	2.17	17.86	8.25	0.61	33.48**	5.19	5.83	16.62	14.53
PPP	1574.21**	757.48	408.1	50.10	40.3	0.67	897.52**	251.44	194.31	36.68	38.00
SPP	2480.84**	1824.91*	482.5	60.57	36.2	0.73	1391.21**	277.48	291.11	43.24	39.46
HSW	368.46**	1.42	12.21	31.24	11.2	0.94	343.08**	4.01	5.55	29.00	8.12
YLD	225729.73**	66068.59	7706	860.0	32.3	0.60	207198.96**	30847.83	34399.89	430.92	43.04
			20758								
BMY	557821.18**	151265.21	4	1982	22.99	0.58	575546.07**	230512.00	112863.12	1243.33	27.02
HI	236.33**	87.01	87.61	42.65	21.9	0.58	556.40**	78.05	83.43	32.60	28.02

Key: *, **, ns indicate significance at 0.05 and 0.01 probability levels, and non-significant, respectively. ¥ =figures in parenthesis refer to degrees of freedom, CV coefficient of variation. DFF=Days to first flowering, DFFF=Days to fifty percent flowering, DFFP=Days to fifty percent podding, DEP=Days to end of podding, DM=Days to maturity, FPH=First pod height, PPP=Number of pods per plant, SPP=Number of seeds per plant, HSW=Hundred seed weight, YLD=Yield, BMY=Biomass yield, HI=Harvest index.

Table 4. Combined ANOVA for studied traits of chickpea genotypes under high temperature during the dry season of 2015 at Werer.

Sources of Variation							
S/No	CHARACTERS	Varieties (20)*	Replication (2)	Planting Date (1)	Planting Date*Varieties (20)	Error	CV (%)
1	DFF	134.47**	3.88	41.143**	2.36	1.42	3.61
2	DFFF	109.74**	4.79	536.51**	5.34**	2.37	3.72
4	DFP	73.10**	44.9	346.7**	3.7	4.6	4.53
5	DM	40.02 ^{ns}	101.17	1413.37**	17.75	27.52	6.62
6	FPH	25.07 ^{ns}	8.16	48.91**	14.89	3.93	11.50
7	PPP	2334.91**	227.39	5666.87**	136.82	312.95	40.77
8	SPP	3617.21 ^{ns}	474.50	9464.00**	254.83	417.06	39.35
9	HSW	701.61**	0.34	158.01**	9.93	8.79	9.84
10	YLD	408721.67**	4051.52	5800294.89**	24207.02	56637.02	36.87
11	BMV	898336.69**	53624.41	17192467.06**	235030.56	164319.42	25.14
12	HI (%)	711.46**	86.47	3183.13**	81.27	85.35	24.55

Key: *, **, ns indicate significance at 0.05 and 0.01 probability levels, and non-significant, respectively. * = figures in parenthesis refers to degrees of freedom, CV = coefficient of variation. DFF = Days to first flowering, DFFF = Days to fifty percent flowering, DFFP = Days to fifty percent podding, DEP = Days to end of podding, DM = Days to maturity, FPH = First pod height, PPP = Number of pods per plant, SPP = Number of seeds per plant, HSW = Hundred seed weight, YLD = Yield, BMV = Biomass yield, HI = Harvest index

Similarly, yield and yield related traits such as grain yield, biomass yield, number of pods plant⁻¹, hundred seed weight and harvest index showed highly significant variation ($p \leq 0.001$) among the tested genotypes, whereas, difference in number of seeds plant⁻¹ among chickpea genotypes remained statistically insignificant (Table 4), though the Cv was high due possibly uneven surface irrigation management. The present result is in agreement with report by Singh *et al.* (1990), who observed variations for grain yield, biomass weight, harvest index, and other traits such as number of pods plant⁻¹ and hundred seed weight in chickpeas. Generally, out of the 12 traits studied, most traits exhibited highly significant differences under heat stress, indicating the presence of variability in responses of chickpea genotypes to high temperature that

underlines the utility of the materials for applied breeding programme and these sources of heat tolerance can be used for physiological and genetic studies in heat tolerance breeding

Highly significant difference ($p \leq 0.01$) was the case due to planting date influence days to first flowering (DFF), days to 50% flowering (DFFF), days to first podding (DFP), days to maturity (DM) and first pod height (FPH) (Table 4). Number of pods plant⁻¹ (NPP), number of seeds plant⁻¹ (SPP), hundred seeds weight (HSW), grain yield (YLD), biomass yield (BMV) and harvest index (HI) varied highly significantly as planting delayed from Jan 23 to Feb 24, implying the impact of heat stress on late sown crops (Table 4). With regard to interaction between planting dates and tested genotypes, significant

interaction effect was recorded on number of fifty percent flowering. This is an evidence for the influence of planting date (increased temperature) on flowering and pod setting potential of chickpea genotypes under heat stressed condition. (Table 2 & 4).

Performance of Chickpea

Genotypes

Crop phenology and growth parameters

Significant difference in crop phenology was observed among the chickpea genotypes for both planting dates (p1 and p2). The ANOVA showed that there were highly significant differences in flowering times (days to 1st flowering and days to 50% flowering) and days to 1st podding among germplasms for both p1 and p2 (Table 5 & 6). However all genotypes tended to mature more or less close to each other irrespective of their differences in pre-maturity phenology (Table 5 & 6).

Over all mean values of germplasms revealed that all of the test genotypes took lesser mean number of days ranging between 28-36 and 37-47 days for 1st flowering and 50% flowering compared to the standard checks Arerti, DZ 10-4 and DZ 10-11 (40-47 days). There were about 3 weeks gap between the earliest DZ-2012-CK-0034 (28 days) followed by ICCV 10307 (29 days) and the latest (Arerti) germplasms which took 48 days to show the first flower. On the other hand, the standard check Arert (57 days) and DZ 10-4 (53 days) were among the latest genotype to produce the first pod and 50% podding, while ICCV 09301 (43days) and ICCV 10307 (44days) were the earliest (43-44 days) to produce the first pod and 50% podding (Table 6). Though there was no statistically marked difference among the genotypes in their number of days to attain maturity, almost all the tested genotypes matured little earlier (76-81 days) than the standard checks Arerti (82-84 days) (Table 6,8,9).

Table 6. Mean values of phenological traits and growth parameters of chickpea genotypes under heat stress at Werer, 2015.

S/No	Treatment /Entry	DFF	DFFF	DFP	DEP	DM	PLHT	FPH
1	ICCV-10409 (K)	30.3hij	40.2def	44.8gh	62.3e-j	79.8abcd	27.9cde	15.2b-f
2	ICCV-09315 (K)	30.8ghi	39.3ef	44.5gh	61.0e-j	78.2bcde	28.0cde	13.6hi
3	DZ-CK-0034 (D)	28.0k	38.8fg	48.3def	60.0hij	77.8cde	31.9abc	14.0e-h
4	ICCV-09309 (D)	31.2fgh	39.3ef	48.0def	63.2d-i	80.0abcd	28.6cde	15.2a-e
5	ICCV-10107 (D)	29.7ij	38.8fg	46.7efg	62.0e-j	79.2a-e	28.1cde	13.0i
6	ICCV-10404 (K)	31.3efgh	39.8ef	44.3gh	62.5e-j	79.2a-e	28.7cde	13.6hi
7	ICCV-10108 (D)	32.3e	38.7fg	50.8c	65.5cde	81.7abcd	29.6bcde	14.0d-h
8	ICCV-07313 (K)	31.7efgh	39.0fg	46.5efg	58.8ij	76.7cde	27.5e	14.3c-h
9	ICCV-09311 (K)	31.0fghi	40.2def	44.8gh	60.0hij	79.3a-e	28.1cde	13.7fghi
10	ICCV-10102 (K)	32.7e	40.8cde	45.2gh	60.2ghij	80.5abcd	28.2cde	15.1b-g
11	ARERTI (K)	47.5a	55.2a	57.0a	72.7a	84.7a	27.7de	15.8ab
12	DZ-10-4 (K)	42.3b	46.8b	53.2b	71.2ab	82.3abc	32.9ab	13.7ghi
13	ICCV-09301 (K)	31.2fgh	39.2ef	43.2h	64.0d-h	78.8a-e	28.9bcde	13.8e-h
14	ICCV-10103 (D)	31.5efgh	39.2ef	51.2bc	64.8def	81.0abcd	34.6a	14.7b-h
15	ICCV-10307 (K)	29.0jk	37.3g	44.3gh	58.5j	73.8e	26.7e	13.5hi
16	DZ-10-11 (D)	40.0c	47.7b	49.8cd	69.5abc	77.3cde	29.8bcde	13.1i
17	ICCV-09304 (K)	32.7e	41.7cd	46.3fg	61.3e-j	76.0de	31.8abcd	15.5abc
18	DZ-CK-0044 (D)	30.5hi	39.0fg	49.3cd	64.7defg	78.0bcde	28.9bcde	15.5abcd
19	ICCV-10311 (K)	30.7ghi	39.3ef	44.3gh	60.3f-j	77.8cde	30.1bcde	14.6b-h
20	ICCV-09307 (K)	32.0efg	46.8b	48.3cde	67.5bcd	83.8ab	26.8e	16.7a
21	DZ-CK-0041(D)	35.7d	42.2c	50.3cd	63.3d-i	77.0cde	30.3bcde	14.3c-h
Grand mean		33	41.4	47.7	63.5	79.2	29.3	14.4
LSD (0.05)		1.4	1.8	2.5	4.5	5.9	4.1	1.5
CV (%)		3.6	3.7	4.5	6.2	6.5	12.2	9

Key: DFF=Days to first flowering, DFFF=Days to fifty percent flowering, DFP=Days to first podding, DEP=Days to end of podding, DM=Days to maturity, PLHT=Plant height, FPH=First pod height

The result of the study probably illustrate plasticity response of the crop, whereby plants grown under high temperature and low rain fall condition make adjustment of the environment through alteration of their normal physiological growth and development process. The decline in number of days to flower and maturity in the tested genotypes shorten their vegetative period so that their transformation in to reproductive phase assures their seed production under the existing high temperature. It has been reported that early phenology (time to flowering, podding and maturity) plays critical role in adaptation of chickpea cultivars to different environments (Berger et al. 2004, 2006) and Early phenology is a key trait for adaptation of chickpea to short season environments as it helps crop to escape from end season stresses (drought, temperature extremities). Hence the tested chickpea genotypes identified with early phenological traits in this study can make progress in genetic studies and breeding for early phenology in targeted high temperature areas. Highly significant variation among chickpea genotypes was observed in their plant height and 1st pod height. The highest plant height (34.6cm) and 1st pod height (16.7) measure was taken from ICCV 10103 and ICCV 099307, respectively. ICCV 10307 (26cm) and ICCV 10107 (13cm) were found to be the shortest of all in their height and 1st pod height (Table 6).

Yield and yield components

Highly significant inherent variation among the chickpea genotypes was observed for number of pods/plant⁻¹, hundred seeds weight, grain yield, biomass yield and harvest index for both planting dates whereas, number of seeds plant⁻¹ exhibited non-significant differences (Table 7). The highest number of pods per plant (93) and seeds per plant (106) was found for desi types ICCV- 10103, followed by ICCV 10108 (92), while the lowest number of pods per plant (19) and seeds per plant (22) was observed in the kabuli type ICCV 09304 (Table 7).

Maximum hundred seed weight was observed in the kabuli types ICCV- 10404 (45g) and ICCV 09311 (43g), while the lowest hundred seed weight was recorded from the standard checks DZ 10-4 (10g), DZ 10-11 (11g) and Arerti (20g). The result of this study also showed there were highly significant differences ($P \leq 0.01$) in biomass yield and grain yield and harvest index among chickpea genotypes for both planting dates (P1 and P2) (Table 7). The highest grain yield was obtained from ICCV 09309 (1187 kg/ha), ICCV 10307 (1035 kg/ha) and ICCV 10108 (1014 kg/ha), while the lowest grain yield was obtained from the standard check Arerti (308 kg/ha) followed by ICCV 09307 (328 kg/ha) and DZ – 10-11 (338 kg/ha). The possible explanation for this is that the standard check has been developed under high potential ecology and miss more of stress response genes to respond or adapt. 13 lines achieved significantly higher

yield level than the best yielding standard check DZ 10-4 (399 kg/ha) with marked yield advantage. Results further revealed that the high yielding genotypes produced high biomass yield as compared to low yielding ones. The highest biomass yield was obtained from ICCV-09309 (2352 kg/ha) followed by ICCV-10108 (2224 kg/ha), which were also the highest seed yielder (Table 7). According to the report by Asnake, (2014), released cultivars gave average grain yield up to 3350 kg ha⁻¹ based on on-farm evaluation and 2600-5000kg ha⁻¹ on

research stations all under potential chickpea agroecology. Reduction of yield due to increased temperature has been reported by Karla et al., 2008, who observed chickpea grain yield decreased by 53-301kg ha⁻¹ in 1⁰C increase of temperature. In this study we roughly calculated the reduction in seed size and yield for Arerti, and it was like exposed to 10C⁰ more temperature than its normal adaptation, and reduced yield 10 fold, and seed size by 1/3rd.

Table 7. Mean values of yield and yield components of chickpea genotypes under heat stress at Werer, 2015.

S/NO	Treatment	PPP	SPP	HSW	BMV	YLD	HI
1	ICCV-10409 (K)	29.5fghi	32.8fghi	39.2bc	1625.2cde	585.5defg	33.2def
2	ICCV-09315 (K)	36.7d-i	44.0d-h	39.6bc	1765.8bcde	810.2bcd	46.0ab
3	DZ-CK-0034 (D)	50.7cde	55.7cde	29.3e	1929.8abcd	963.0abc	49.8a
4	ICCV-09309 (D)	77.0ab	87.7ab	22.0fgh	2351.7a	1188.8a	50.8a
5	ICCV-10107 (D)	50.5cde	60.7cd	25.4f	1085.7h	520.7efgh	43.0abcd
6	ICCV-10404 (K)	33.5d-i	36.3e-i	45.9a	1585.0c-g	615.7def	36.6bcde
7	ICCV-10108 (D)	78.2a	89.3ab	21.0gh	2224.0ab	1013.5ab	44.8abc
8	ICCV-07313 (K)	34.5d-i	41.8d-i	39.9bc	1921.5abcd	912.0bc	48.4a
9	ICCV-09311 (K)	27.7ghi	31.2ghi	42.7ab	1360.3efgh	589.7defg	42.8abcd
10	ICCV-10102 (K)	43.0c-g	54.5cdef	38.6cd	1785.8bcde	799.0bcd	42.5abcd
11	ARERTI (K)	31.3e-i	39.0d-i	20.1gh	1946.2abc	308.0h	16.1g
12	DZ-10-4 (K)	57.2bc	92.5a	10.4i	1509.2c-h	398.5fgh	26.0fg
13	ICCV-09301 (K)	22.2hi	19.7i	35.7d	1609.0cdef	480.3efgh	24.7fg
14	ICCV-10103 (D)	93.0a	106.0a	20.3gh	2115.2ab	1034.8ab	48.3a
15	ICCV-10307 (K)	22.3hi	26.7ghi	38.5cd	1087.7h	480.3efgh	40.2abcd
16	DZ-10-11 (D)	48.2cdef	67.2bc	10.9i	1077.8h	337.7gh	26.8ef
17	ICCV-09304 (K)	19.0i	22.8hi	41.5bc	1141.3h	382.2fgh	27.7ef
18	DZ-CK-0044 (D)	51.5cd	58.3cde	19.1h	1346.5efgh	639.0def	45.1abc
19	ICCV-10311 (K)	38.8c-i	45.0c-h	38.3cd	1475.5d-h	711.0cde	46.1ab
20	ICCV-09307 (K)	27.0hi	29.8ghi	31.2e	1769.8bcde	326.8gh	15.9g
21	DZ-CK-0041(D)	39.5c-h	49.0c-g	23.1fg	1154.2fgh	458.3efgh	35.2cdef
Grand mean		43.4	51.9	30.1	1612.7	645.5	37.6
LSD (0.05)		19.9	22.6	3.4	459.9	271.2	10.6
CV (%)		40	37.9	9.9	24.8	36.6	24.6

Key: PPP=Number of pods per plant, SPP=Number of seeds per plant, HSW=Hundred seed weight, YLD=Yield, BMV=Biomass yield, HI=Harvest index

The result of this study showed, 13 lines out of 18, achieved significantly higher yield level than the best yielding standard check DZ 10-4 (399 kg/ha). This significant grain yield increment among the test genotypes is due to their comparatively higher heat tolerance and therefore we can use them as source of heat tolerance in further breeding activities. It was also indicated the top 3 high yielding chickpea genotypes are desi types which achieved significantly higher yield level than kabuli type's chickpea genotypes (Table 7). Study by Tibebu (2011), confirmed that desi chickpea types were high yielder, better in biomass rate and harvest index over kabuli types of chickpeas, which could come from inherent variability in the two types.

Effect of planting date on chickpea growth

Planting date caused significant difference in crop phenology (DFF, D50%F, DFP, DEP and DM) and among the studied genotypes (Table 8). Mean values of Number of days to 1st flowering, number of days for 50%

flowering, number of days for 50% podding and number of days to reach maturity decreased significantly as planting date delayed from Jan 23 to Feb 24 (Table 2). There were 2-4 days difference in flowering times among genotypes and variation in crop maturity was 14 days during P1 and 11 days during P2 (Table 8). The result indicated the overall crop cycle further shortened under late sowing condition and this was associated with high temperature during the second planting. The result further revealed that chickpea phenology had negatively significant association with maximum temperature. Plant height and 1st pod height reduced from 31cm to 28cm and 18cm to 11cm, as planting date delayed from Jan 23 to Feb 24. The relatively taller chickpea plants may be attributed to the longer growing period and vigorous growth associated with earlier planting. In contrast, there was no statistically significant difference among germplasms in their number of primary branches (Table 8).

Table 8. Mean performances for phenological traits and growth parameter of chickpea genotypes grown under heat stress at Werer, 2015

S.N	Treatment /Entry	PD	DFF	DFFF	DFP	DEP	DM	PLHT	FPH
1	ICCV-10409 (K)	1	30.33hij	43.33c	48.33g	62.67def	82.67a-d	18.60b-f	18.60b-f
		2	30.33f-i	37.00de	49.33fgh	62.00c-f	77.00abc	16.60cde	16.60cde
2	ICCV-09315 (K)	1	31.00f-i	42.67cde	48.67fg	62.67def	81.33a-d	17.60b-h	17.60b-h
		2	30.67e-i	36.00de	48.33gh	59.33def	75.00abc	15.40d-g	15.40d-g
3	DZ-2012-CK-0034 (D)	1	29.00ij	41.67c-f	53.67bc	64.00cde	83.66abc	16.87e-h	16.87e-h
		2	27.00j	36.00de	51.67e-h	56.00f	72.00bc	19.87bc	19.87bc
4	ICCV-09309 (D)	1	32.66efg	41.67cdef	54.33bc	64.67cde	86.33ab	19.33a-d	19.33a-d
		2	29.67hi	37.00de	53.33d-g	61.67c-f	73.67abc	16.00c-f	16.00c-f
5	ICCV-10107 (D)	1	29.67ij	42.00c-f	52.67bcd	63.00def	83.66abc	16.27fgh	16.27fgh
		2	29.67hi	35.67de	48.67gh	61.00c-f	74.67abc	12.00g	12.00g
6	ICCV-10404 (K)	1	31.00f-i	42.33c_f	49.33efg	63.67de	82.67a-d	17.20c-h	17.20c-h
		2	31.67efg	37.33de	48.67gh	61.33c-f	75.67abc	16.13cde	16.13cde
7	ICCV-10108 (D)	1	33.67e	40.33ef	52.67bcd	64.33cde	84.33abc	17.67b-h	17.67b-h
		2	31.00e-i	37.00de	56.33a-e	66.67a-d	79.00ab	14.27d-g	14.27d-g
8	ICCV-07313 (K)	1	32.00e-h	42.33c-f	52.00cde	61.67def	80.67bcd	18.27b-f	18.27b-f
		2	31.33e-h	35.67de	48.67gh	56.00f	72.67abc	16.00c-f	16.00c-f
9	ICCV-09311 (K)	1	32.00e-h	42.67cde	48.67fg	58.00f	82.67a-d	17.93b-g	17.93b-g
		2	30.00ghi	37.67d	49.00fgh	62.00c-f	76.00abc	14.60d-g	14.60d-g
10	ICCV-10102 (K)	1	33.67e	43.67c	49.67d-g	61.67def	83.66abc	19.60abc	19.60abc
		2	31.67efg	38.00d	48.67gh	58.67ef	77.33abc	17.20cde	17.20cde
11	ARERTI (K)	1	48.33a	56.67a	62.00a	74.33a	89.33a	18.27b-f	18.27b-f
		2	46.67a	53.67a	59.67ab	71.00a	80.00ab	22.40b	22.40b
12	DZ-10-4 (K)	1	42.67b	47.67b	55.33b	70.33ab	83.66abc	16.20fgh	16.20fgh
		2	42.00b	46.00b	61.00a	72.00a	81.00a	18.00cd	18.00cd
13	ICCV-09301 (K)	1	32.00e-h	41.33c-f	48.00g	65.00b-e	82.00a-d	17.47c-h	17.47c-h
		2	30.33f-i	37.00de	48.00h	63.00b-f	75.67abc	16.80cde	16.80cde
14	ICCV-10103 (D)	1	32.66efg	40.67def	53.33bc	66.33b-e	84.67abc	18.87a-e	18.87a-e
		2	30.33f-i	37.67d	56.00a-e	63.33b-f	77.33abc	15.13d-g	15.13d-g
15	ICCV-10307 (K)	1	28.66j	40.0f	48.67fg	61.00ef	75.00d	15.33h	15.33h
		2	29.33i	34.67e	48.00h	68.00abc	72.67abc	16.60cde	16.60cde
16	DZ-10-11 (D)	1	40.33c	48.33b	54.66bc	69.33abc	84.67abc	15.73gh	15.73gh

17	ICCV-09304 (K)	2	39.67c	47.00b	53.00d-h	69.67ab	70.00c	14.00efg	14.00efg
		1	33.00ef	42.33c-f	48.67fg	61.67def	77.33cd	19.00a-e	19.00a-e
18	DZ-2012-CK-0044 (D)	2	32.33de	41.00c	54.00c-e	61.00c-f	74.67abc	16.30cde	16.30cde
		1	31.00f-i	40.33ef	51.67c-f	64.33cde	81.00bcd	20.00ab	20.00ab
19	ICCV-10311 (K)	2	30.00ghi	37.67d	55.00b-e	65.00a-e	75.00abc	12.13fg	12.13fg
		1	30.67f-j	42.00c-f	48.00g	61.00ef	80.00bcd	16.73e-h	16.73e-h
20	ICCV-09307 (K)	2	30.67e-i	36.67de	48.67gh	59.67def	75.67abc	17.10cde	17.10cde
		1	32.00e-h	47.67b	55.67b	67.00bcd	86.67ab	21.10a	21.10a
21	DZ-2012-CK-0041(D)	2	32.00def	46.00b	59.00abc	56.00f	81.00a	27.20a	27.20a
		1	37.67d	43.00cd	54.66bc	63.67de	77.33cd		17.07d-h
Grand mean		2	33.67d	41.33c-f	57.33a-d	63.00b-f	76.67abc		15.20d-g
		1	33.52	43.46	51.94	64.30	82.54		17.86
LSD		2	32.38	39.33	52.49	62.68	75.84		16.62
		1	2.01	2.36	3.29	5.40	8.19		2.43
		2	1.94	2.71	5.17	7.42	8.80		3.98

Key: DFF=Days to first flowering, DFFF=Days to fifty percent flowering, DFP=Days to first podding, DEP=Days to end of podding, DM=Days to maturity, PLHT=Plant height, FPH=First pod height

Planting date also caused significantly affected number of pods plant⁻¹, number of seeds plant⁻¹, hundred seeds weight, grain yield, biomass yield and harvest index (Table 9). However the interaction effect between genotypes and planting date remained statistically insignificant. Average number of pods per plant and seeds per plant were decreased from 50 to 37 and from 60 to 43 as planting date was delayed from Jan 23 to Feb 24 (Table 9). This might be due to increased temperature during late planting which associated with loss of stigma receptivity, poor pollen germination and failure of pollen fertilization and pollen formation and it might lead to embryo abortion of small endosperms as reported earlier (Egli 2005). Delayed

planting significantly reduced average hundred seed weight from 31g to 29g (Table 9).

This might be due to prevalence of higher temperature in delayed planting (Fig 1 and 2) at the reproductive stage which caused reduced remobilization of photosynthate to grain yield. Planting date also negatively affected in grain yield of chickpea genotypes to reduce by half during the second planting (430 kg/ha) as compared to first planting (860 kg/ha)(Table 9). This might be linked with reduced pollen viability and stigma receptivity through oxidative stress in the leaves which causes failure of fertilization (Kumar, 2012).

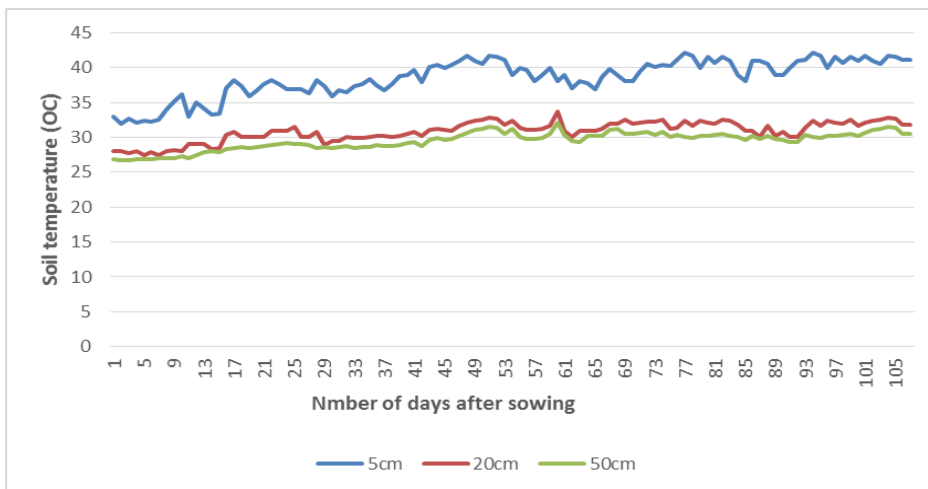


Fig 2. Maximum and minimum soil temperatures at WARC during the chickpea growing season for considered soil depths

Table 9. Mean performances for yield and yield components of chickpea genotypes grown under heat stress at Werer, 2015.

S.N	Treatment /Entry	PD	PPP	SPP	HSW	YLD	BMV	HI
1	ICCV-10409 (K)	1	36.00de	40.67ef	41.97bcd	852.7b-f	2164.7a-e	39.66b-e
		2	23.00e-h	25.00fgh	36.47cde	318.3f-i	1085.7c-f	26.74fgh
2	ICCV-09315 (K)	1	40.33de	49.00def	40.10bcd	1003.0a-e	2132.0a-e	48.14a-d
		2	33.00c-h	39.00d-h	39.07a-d	617.3b-e	1399.7bcd	43.89a-d
3	DZ-2012-CK-0034(D)	1	55.33cde	58.33def	30.43fg	1186.0abc	2378.7abc	49.89abc
		2	46.00b-e	53.00b-e	28.17f	740.0abc	1481.0bcd	49.81ab
4	ICCV-09309 (D)	1	87.00abc	99.67abc	23.10hi	1416.7a	2836.3a	49.89abc
		2	67.00ab	75.67ab	20.90gh	961.0a	1867.0ab	51.81a
5	ICCV-10107 (D)	1	51.33de	56.67def	26.73gh	822.3b-f	1616.7def	49.45abc
		2	49.67bc	64.67a-d	23.97g	219.0hi	554.7f	36.58b-f
6	ICCV-10404 (K)	1	36.67de	40.67ef	48.97a	867.3b-f	2153.7a-e	39.93b-e
		2	30.33c-h	32.00e-h	42.73a	364e-i	1016.3def	33.30c-f
7	ICCV-10108 (D)	1	92.00ab	104.67ab	22.50hi	1340.0a	2818.0a	47.54a-d
		2	64.33ab	74.00ab	19.53h	687.0a-d	1630.0abc	42.06a-e
8	ICCV-07313 (K)	1	32.00e	41.67ef	38.23cde	1051.0a-d	1935.0b-f	56.31a
		2	37.00c-g	42.00d-g	41.57ab	773.0abc	1908.0ab	40.44a-f
9	ICCV-09311 (K)	1	33.67e	37.67ef	43.50abc	797.7c-f	1758.0c-f	45.85a-e
		2	21.67fgh	24.67gh	41.80ab	381.7d-i	962.7def	39.83e-f
10	ICCV-10102 (K)	1	50.67de	64.33cde	41.00bcd	1059.3a-d	2215.0a-d	47.88a-d
		2	35.33c-g	44.67c-g	36.10de	538.7b-g	1356.7b-e	37.08a-f
11	ARERTI (K)	1	37.00de	44.33def	20.43i	489.3f	1821.3c-f	26.02f
		2	25.67e-h	33.67e-h	19.77h	126.7i	2071.0a	6.12i
12	DZ-10-4 (K)	1	67.67bcd	113.00ab	10.77j	508.0f	1591.3def	31.60ef
		2	46.67bcd	72.00abc	10.00i	289.0ghi	1427.0bcd	20.30ghi
13	ICCV-09301 (K)	1	24.33e	25.67f	36.43de	725.7def	2129.0a-e	32.05ef
		2	20.00fgh	13.67h	34.87e	235.0ghi	1089.0c-f	17.38hi
14	ICCV-10103 (D)	1	111.33a	128.33a	21.17hi	1259.0ab	2597.0ab	48.47a-d
		2	74.67a	83.67a	19.47h	810.7ab	1633.3abc	48.11abc
15	ICCV-10307 (K)	1	30.00e	34.67ef	36.90de	719.0def	1370.7f	50.64abc
		2	14.67gh	18.67gh	40.07abc	241.7ghi	804.7ef	29.84e-h
16	DZ-10-11 (D)	1	56.3cde3	78.67bcd	12.43j	521.7f	1459.0ef	33.91def
		2	40.00c-f	55.67a-e	9.40i	153.7i	696.7f	19.61ghi
17	ICCV-09304 (K)	1	28.00e	33.67ef	44.87ab	636.0def	1679.0c-f	35.54c-e
		2	10.00h	12.00h	38.13b-e	128.3i	603.7f	19.96ghi
18	DZ-2012-CK-0044(D)	1	55.33cde	62.67de	19.27i	805.0b-f	1623.0def	52.28ab

19	ICCV-10311 (K)	2	47.67bcd	54.00b-d	19.00h	473.0c-g	1070.0def	38.01a-f
		1	43.00de	53.33def	39.20bcd	756.3c-f	1572.0def	43.61a-e
20	ICCV-09307 (K)	2	34.67c-g	36.67d-h	37.47cde	665.7a-e	1379.0bcd	48.59ab
		1	31.00e	36.67ef	33.43ef	565.7ef	2182.7a-e	25.33f
21	DZ-2012-CK-0041(D)	2	23.00e-h	23.00gh	29.00f	88.0i	1357.0b-e	6.49i
		1	53.00de	67.67cde	24.67hi	679.0def	1591.3def	41.74a-e
		2	26.00d-h	30.33e-h	21.60gh	237.7ghi	717.0f	28.69e-f
Grand mean		1	50.10	60.57	31.24	860.03	1982.11	42.65
		2	36.68	43.24	29.00	430.92	1243.33	32.60
LSD		1	33.34	36.25	5.77	458.10	751.85	15.45
		2	23.00	28.16	3.89	2.028	554.39	15.07

Key: DFF=Days to first flowering, DFFF=Days to fifty percent flowering, DFP=Days to first podding, DEP=Days to end of podding, DM=Days to maturity, PLHT=Plant height, FPH=First pod height

Estimation of Heat Tolerance indices

For better evaluation the genotypes for heat tolerance, some selection indices, including STI, TOL, SSI, MP and GMP were used. Tolerance indices were calculated on the basis of grain yield. The greater the TOL value, the larger yield reduction under heat stress conditions and the higher heat sensitivity. A selection based on minimum yield reduction under stress conditions in comparison with no stress conditions (TOL) failed to identify the most tolerant genotypes (Farshadfar *et al.*, 2014). Rosielle and Hamblin (1981) reported that selection based on the tolerance index often leads to selecting cultivars which have low yields under no stress conditions. The greater SSI and TOL values, the greater sensitivity to stress, thus a

smaller value of these indices is favored.

Therefore, according to the result obtained from this study (Table 10), the genotypes showed the greater TOL values were ARERTI, ICCV-10404 and ICCV-09311. In contrast, the genotypes ICCV-10102, ICCV-09309, DZ-2012-CK-0034 and DZ-2012-CK-0041 showed lower TOL and higher STI values, and that means these materials were found heat tolerant genotypes relative to others, but may be with poor yield potential. Hence, the heat tolerant materials can be used in the breeding program either for direct advancement based on their agronomic merits or could be used in the crossing program for further manipulation.

Table 10. Grain yield (Kg/ha) and heat tolerance related characters influenced by different genotypes.

Treatment /Entry	Grain Yield							
	Potential	Stress	% yield reduction	SSI (%)	MP	TOL	STI	GMP
ICCV-10409 (K)	2450.79	585.50	76.11	1.08	1518.15	1865.29	0.24	1197.89
ICCV-09315 (K)	2548.12	810.17	68.21	0.97	1679.14	1737.96	0.32	1436.80
DZ-2012-CK-0034 (D)	1847.03	963.00	47.86	0.68	1405.01	884.03	0.52	1333.67
ICCV-09309 (D)	2029.55	1188.83	41.42	0.59	1609.19	840.71	0.59	1553.32
ICCV-10107 (D)	1556.69	520.67	66.55	0.95	1038.68	1036.02	0.33	900.29
ICCV-10404 (K)	3369.64	615.67	81.73	1.17	1992.65	2753.98	0.18	1440.34
ICCV-10108 (D)	2104.31	1013.50	51.84	0.74	1558.90	1090.81	0.48	1460.38
ICCV-07313 (K)	2461.23	912.00	62.95	0.90	1686.62	1549.23	0.37	1498.21
ICCV-09311 (K)	2820.48	589.67	79.09	1.13	1705.07	2230.82	0.21	1289.63
ICCV-10102 (K)	1417.89	799.00	43.65	0.62	1108.44	618.89	0.56	1064.37
ARERTI (K)	3519.45	308.00	91.25	1.30	1913.73	3211.45	0.09	1041.15
DZ-10-4 (K)	1633.55	398.50	75.61	1.08	1016.03	1235.05	0.24	806.83
ICCV-09301 (K)	1860.23	480.33	74.18	1.06	1170.28	1379.89	0.26	945.27
ICCV-10103 (D)	2540.62	1034.83	59.27	0.85	1787.73	1505.79	0.41	1621.46
ICCV-10307 (K)	2004.17	480.33	76.03	1.09	1242.25	1523.83	0.24	981.16
DZ-10-11 (D)	1702.43	337.67	80.17	1.15	1020.05	1364.77	0.20	758.19
ICCV-09304 (K)	1849.54	382.17	79.34	1.13	1115.85	1467.37	0.21	840.73
DZ-2012-CK-0044 (D)	1666.67	639.00	61.66	0.88	1152.83	1027.67	0.38	1031.99
ICCV-10311 (K)	2250.00	711.00	68.40	0.98	1480.50	1539.00	0.32	1264.81
ICCV-09307 (K)	2509.81	326.83	86.98	1.24	1418.32	2182.98	0.13	905.70
DZ-2012-CK-0041 (D)	1384.27	458.33	66.89	0.96	921.30	925.94	0.33	796.53

K=Kabuli, D=Desi. €=indicating standard check chickpea varieties

Conclusion

The Combined analysis of variance showed there were highly significant differences among the tested genotypes for most of the traits considered, except for DM, FPH and SPP, indicating the existence of variability among tested genotypes and the potential for selection under heat stress environments. The overall mean values of germplasms revealed that all of the test genotypes took lesser mean number of days ranging between 28-36 and 37-47 days to show 1st flowering and 50% flowering than the standard checks Arerti, DZ 10-4 and DZ 10-11 (40-47 days). Though there

was no statistically marked difference among the genotypes in their number of days to attain maturity, almost all the tested genotypes matured earlier (76-81 days). Since, ICRISAT had classified chickpea varieties matured in < 85 days as extra early, 85- 115 as early and > 115 days as late maturing varieties, all of the test genotypes used in this study can be regrouped as extra early maturing and can make progress in breeding for early phenology in targeted high temperature areas. Highly significant variation among the chickpea genotypes was observed for number of podsplant⁻¹, hundred seeds weight, grain yield, and biomass yield

and harvest index for both planting dates. Based on the observation on yield performance, most of the test genotypes gave significantly higher biomass yield and grain yield than all of the standard checks under heat stress condition of $\geq 35^{\circ}\text{C}$. The top 3 best responding genotypes under heat stressed environment were ICCV 09309 (1187 kg/ha), ICCV 10103 (1035 kg/ha) and ICCV 10108 (1014 kg/ha). These heat tolerant chickpea materials can further be taken to breeding advance as source parents. It was also indicated the top 3 high yielding chickpea genotypes are desi types which achieved significantly higher yield level than kabuli types chickpea. This might suggest that, desi types constituency of heat combating genes better compared to kabuli types.

Planting date between last week of January and February significantly affected plant parameters as the thermal condition was increasingly differed. The result indicated that high temperature stress significantly reduced the mean values of DFF, D50%F, DFP, DEP, DM, VP, FP, GFP, MP, PLHT, FPH, NBR, PPP, HSW, YLD and BMY with delayed sowing. It may therefore be concluded that the heat screening planting protocol can be conducted somewhere between mid-January to beginning of February, with the analogy that in mutagenesis treatment about 50% deformation is assumed right dose to likely impose the expected change.

For better decision making heat indices including STI, TOL, SSI, MP

and GMP could be calculated on the basis of grain yield. Accordingly, the tested chickpea genotypes showed the greater TOL values were ARERTI, ICCV-10404 and ICCV-09311, indicating their poor tolerance whereas genotypes ICCV-10102, ICCV-09309, DZ-2012-CK-0034 and DZ-2012-CK-0041 showed lower TOL and higher STI values, and they are found relatively better heat tolerant genotypes. This study is in its early state of emergence, and further optimization of protocols, facilities and analytical approaches should be coming down the course.

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Phenotypic Characteristics and Preliminary Symbiotic Effectiveness of Rhizobia Associated with Haricot Bean Growing in Diverse Locations of Southern Ethiopia

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Abstract

Phenotypic characteristics of one hundred thirteen rhizobia nodulating haricot bean (*Phaseolus vulgaris* L.) growing on soils of nine different locations from southern Ethiopia were studied. Their tolerance to varying temperature, salinity, soil pH, heavy metals, antibiotics, their symbiotic effectiveness and cultural characteristics were determined. Eight reference species belonging to four different genera were also included in the analyses. The analyses allowed the description of a wide physiological diversity among tested isolates. Numerical analysis, based on numerical taxonomic approach, of the phenotypic characteristics, using Unweighted Pair Group Method with Average algorithm as implemented in NTSYspc21 software package, showed that, the tested isolates fell into five major diversity groups (designated as group I-V), when 82% level of relative similarity were used as cut-off point. Four strains were found to occupy a separate branch, thus designated as U (unclustered) group. While strains belonging to groups 1-IV were found to associate with recognized species belonging to *Rhizobium*, *Ensifer* and *Mesorhizobium* genus, the remaining test strains in cluster V and U were found to occupy distinct branches of their own on the dendrogram. Under laboratory condition, they were able to grow at pH ranging from 4 to 10.5; majority tolerated salt concentration (0.5-1%) and grew at a maximum temperature between 35 and 40 °C. The isolates were able to utilize a wide range of carbon and amino acid source and tolerated range of antibiotics and heavy metals. Based on the symbiotic effectiveness test, a number of potential isolates have been identified for inoculation trials.

Keywords: Haricot bean, numerical taxonomy, rhizobia, Southern Ethiopia

Introduction

Common bean (*Phaseolus vulgaris* L.) is a leguminous plant of worldwide with social and economic values, and is one of Africa's most essential pulses, cultivated on greater than 4 million ha (Broughton et al., 2003). It provides protein (20-25%) and carbohydrate in Ethiopia, common bean is one of the major pulse crops widely cultivated by smallholders, either as a sole crop or intercropped with sorghum, maize and other cereals (Ohlander, 1977), where in all cases soil fertility is depleted. Despite its high economic importance, the average yield of bean obtained by smallholder farmers in Ethiopia is low (1.5 tons/ha) (CSA, 2014), which is far below the potential yield from research managed experimental plots (4.6 tons/ha) (Assefa and Teshale, 2007), a reflection of, among other things, low soil fertility (Beyene and Tsigie, 1986).

The yield gap and soil N fertility issues could partly be alleviated by using soil bacteria generally known as rhizobia involved in biological nitrogen fixation (BNF) process with legumes including common bean. BNF is a key process for the conversion of nitrogen gas (N_2) into ammonia (NH_3) performed by bacteria belonging to the different genera within the family rhizobiaceae. Common bean, in most cases, substantially make use of the BNF process, thus able to fix up to

50 kg N ha⁻¹ (Bliss, 1993). With the respect to its symbiotic adaptability, haricot bean is generally regarded as promiscuous, non-selective host that is nodulated by several groups of root nodule bacteria (Hardarson et al., 1993) belonging to different genera including *Rhizobium* (Amarger et al., 1997; Martínez-Romero et al., 1991; Ramírez-Bahena et al., 2008; Segovia et al., 1993), *Ensifer* (Andrade et al., 2002) and *Mesorhizobium* (Grange and Hungria, 2004). As a reflection of its promiscuous nature, common bean is considered as a poor nitrogen fixing plant in comparison to other grain legumes. Furthermore, because of its inherent behavior as being nodulated with inefficient population of native common bean nodulating rhizobia in soil (Brockwell and Bottomley, 1995; Rodriguez-Navarro et al., 2000), it generally respond poorly to rhizobial inoculation in the field condition (Buttery et al., 1987; Graham, 1981; Hardarson et al., 1993). There are several reports, however, that indicated nitrogen fixation and the yield in common bean can be increased through inoculation of the crop with highly efficient rhizobial strains (Giller and Cadisch, 1995). For example, elite *Rhizobium* isolates were shown to improve the productivity of common bean (Hungria et al., 2003; Mostasso et al., 2002). In inoculation trials in Ethiopia, using *Rhizobium* species (strain HB 429, currently recognized as being national commercial inoculant) and eight *P. vulgaris*

cultivars, it was clearly demonstrated that rhizobial inoculation increased the total yield of the crop pod by 18% (Beshir et al., 2015). On the other hand, it was shown that effectiveness of the indigenous rhizobia that nodulate common bean vary depending upon location and host genotypes. This necessitates isolation and evaluation of more common bean nodulating rhizobia with regard to their diversity and effectiveness to fully realize the benefit of biological nitrogen fixation in this crop. Accordingly, many isolating works have been performed using soil from different sites in Ethiopia (Aserse et al., 2012; Ashenafi, 2017; Beyene et al., 2004; Workalemahu and Assefa, 2007). Therefore, this study was conducted with the objectives to isolate and characterize rhizobia from root nodules of common bean (*Phaseolus vulgaris* L.) plants grown at different locations in Southern Ethiopia, thus to determine their diversity on the

basis of morphological, physiological and biochemical characteristics in reference to known species. Furthermore, preliminary screening of their symbiotic effectiveness on haricot bean was also assessed with the objective to identify the most efficient nitrogen fixing isolates for further field trials.

Materials and Methods

Collection of nodules

The nodules were collected from nine different locations in Southern Nation Nationalities and Peoples Regional State (SNNPRS) in Ethiopia (Table 1), where common bean has been grown with no previous history of inoculation with rhizobia. Characterization and symbiotic effectiveness of the test strains were conducted at Hawassa University, College of Agriculture in the soil microbiology laboratory.

Table 1. Geographical locations, and other associated parameters from which the test strains were isolated

Site	pH of the soil	Max and min Mean annual T(°C)
Humbo	7.2	17.6-25
Areka	7.4	17.6-25
Bodity	6.6	17.6-22.5
Goffa	6.8	17-29
Hawassa	7.8	12-27
Badiwacho	7.1	17.6-22.5
Kachabira	6.7	15.1-22.5
Jinka	6.4	17-27.5
Durame	5.9	17.6-25

Source: SNNPRS finance and economy Bureau, metrological data, 2007

Rhizobial isolation

Nodules stored on silica gel were rehydrated in water and surface sterilized following the procedures as described elsewhere (Somasegaran and Hoben, 1994b). To verify whether the nodules were surface-sterilized or not, the last rinse of the nodules were streaked on a Yeast Extract Mannitol Agar (YMA) plates, incubated and observed for the growth of any microorganisms. The sterilized root nodules were then crushed aseptically in sterile petri-dish placed in laminar air flow hood by sterile crushing glass rods using a drop of sterilized normal saline (0.85% NaCl) solution. Loop-full of the suspensions of the crushed root nodules were streaked with sterile inoculating loop on Yeast Extract Mannitol Agar (YMA, pH=7) plates containing 0.025% (w/v) Congo Red and incubated at 28°C for 4 days (Vincent, 1970). After 4 days, a single colony from each isolate was selected and re-streaked on new YMA plates for further purification and characterization

Presumptive tests of the isolates

After isolation on YMA, the purity of the isolates were determined based on different morphological characteristics of colonies and absorption of Congo Red (Somasegaran and Hoben, 1994b). Gram staining and the growth of colonies on Peptone Glucose Agar

(PGA) medium were also determined following procedures described elsewhere (Lupwayi and Haque, 1994).

Authentication and symbiotic effectiveness of the test isolates

All test isolates were evaluated for their ability to produce nodules on the homologous host species (*Phaseolus vulgaris* L.). Seedlings were grown in modified Leonard jars filled with washed and sterilized river sand. Four surface-sterilized and pre-germinated seedlings were planted aseptically into each of three replicate pots. The seedlings were later thinned to one by snipping on the top, as described previously (Chen et al., 2008). A single colony of each isolate was picked from yeast extract-mannitol agar (YMA) plates and multiplied in yeast extract-mannitol broth (YMB). Each seedling was inoculated with 1 ml bacterial culture at exponential growth phase (Somasegaran and Hoben, 1994a). Non-inoculated seedlings of the plant species, either supplied with mineral nitrogen (as 0.1% KNO₃ in nutrient solution) or grown without nitrogen, served respectively as positive and negative controls. Seedlings were grown under natural sunlight and temperature and were fertilized with one-quarter-strength modified Jensen's N-free medium (Somasegaran and Hoben, 1994a). Nodule assessment and harvesting

took place 5 weeks after inoculation (Maâtallah *et al.*, 2002; Somasegaran and Hoben, 1994a). At harvest, plants were scored for nodulation as effective, moderately effective and ineffective.

Morphological characteristics

Individual colonies were characterized based on their colony color, size, shape, capacity to produce extracellular polysaccharide (EPS) gum and colony texture following procedures described elsewhere (Somasegaran and Hoben, 1994a).

Acid and base production

The isolates were grown in YEMB and loop full of suspension 10^8 /ml were streaked on to YEMA contain 0.5% BTB. The color change (yellow) was observed after 3 days for fast growing and blue remains for slow grower (Jordan, 1984).

Carbon source use (CSU), tolerance to salinity and temperature (TST) and pH assays

Assays for CSU, TST and pH were performed for all the test isolates and the reference strains. For CSU, the following carbon sources including L-arabinose, D-fructose, D-galactose, D-glucose, sucrose, maltose, D-mannose, raffinose, L-rhamnose, D-sorbitol, xylitol, trehalose, xylose, dulcitol, arabinose, myoinositol, inulin, ribose, starch, citric acid, and

malonic acid were tested following methods described before (Somasegaran and Hoben, 1994a). Similarly the test isolates were assessed for TST and pH on YMA culture medium with NaCl concentration of (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 9.5, 10, 10.5% (W/V), temperature of 35, 40, and 45°C following methods detailed earlier (Maâtallah *et al.*, 2002), and pH of 4, 4.5, 5, 5.5, 6, 7, 8, 9, 9.5, 10 and 10.5 (Hungria *et al.*, 2000).

Nitrogen sources utilization

Sixteen different amino acids utilization namely: D-serine, L-histidine, L-pyroglutamic acid, ureidine, thymidine, inosine, L-arginine, L-alanine, L-asparagine, L-asparatic acid, L-glutamic acid, L-proline, L-theronine, L-leucine, L-phenylalanine, and Glycine, were investigated following methods as described before (Amarger *et al.*, 1997).

Intrinsic antibiotic resistance

The intrinsic antibiotic resistance test was performed by using YMA (Rodriguez-Navarro *et al.*, 2000), to which filter sterilized (0.2µm) antibiotics (µg/ml) was added; streptomycin 10; kanamycin sulphate salt 5, 15; novobiocin 0.5, 1.5; spectinomycin dihydrochloride pentahydrate 2.5, 5; neomycin trisulphate salt 5, 20; chloramphenicol 5, 15; erthromycin

10, 20; lincomycin hydrochloride 100; and trimethoprim 200.

Intrinsic heavy metal resistance

The resistances of strains to heavy metals were determined on solid Tryptone Yeast Extract Agar following methods detailed elsewhere (Zhang *et al.*, 1991).

Phosphate solubilizing ability

This was determined by inoculating and incubating the isolates on Pikovskaya Agar medium. This ability was detected based on growth and presence of clear zone around the colonies (Nautiyal, 1999).

Numerical taxonomy

Traits were coded 1 for positive and 0 for negative. A cluster analysis of 92 phenotypic variables for a total of 121 strains (113 new isolates and eight reference strains including *Azorhizobium caulinodans*, *Ensifer meliloti*, *Mesorhizobium mediterraneanum*, *Rhizobium etli*, *R. gallicum*, *R. giardinii*, *R. leguminosarum* and *R. tropici*) were carried out using similarity coefficient and dendrogram was constructed by the unweighted pair group method with arithmetic mean (UPGMA) clustering method using NTSYS-pc version 2.1.

Results

In this study, a total of 113 isolates were obtained from the root nodules

of haricot bean (*Phaseolus vulgaris* L.) that were grown in soils from nine localities in southern Ethiopia including Jinka (12 isolates), Bodity (19 isolates), Gofa (19 isolates), Hawassa (11 isolates), Badiwacho (4 isolates), Durame (24 isolates), Kachabira (10 isolates), Humbo (3 isolates) and Areka (11 isolates) were isolated and characterized. All isolates were Gram negative and rod shaped bacteria with no ability to absorb Congo red, when grown on YEMA-CR medium. Furthermore, none of them grew on peptone glucose medium supplemented with bromocresol purple, indicating that all isolates were presumed to be rhizobia. Up on authentication, (i.e when re-inoculated into the seedlings of variety, Red Wolaita), all the isolates were shown to induce nodulation on the host (data not shown), confirming that they were root nodule bacteria.

Cultural and morphological characteristics

The result for cultural and morphological characteristics is presented on Table 2. Accordingly, the isolates showed different cultural characteristics indicating the existence of diversity among rhizobia nodulating haricot bean in the region. In this study 91% of the isolates showed colony diameter of ≥ 2 mm, when incubated for three days. Likewise the isolates displayed various colony textures of white translucent (42%), watery translucent

(36%), milky (19%) and dull glistening color (3%). Seventy seven percent of the isolates also showed convex shape; whereas 21% of the isolates were found to be flat. Fifty four percent of the isolates have produced copious amount EPS (Extracellular polysaccharide) while 19% produced medium and 27% were low in EPS production. The production of exopolysaccharides (EPS) by the isolates could be an adaptive feature in providing protection to bacteria against environmental stresses including

temperature, salinity, and pH fluctuations in the soil. Rhizobia with wider elasticity of withstanding environmental stresses could be suitable for the development of commercial inoculants. All of the test isolates changed the YEMA-BTB media in to yellow. Turning of YEMA-BTB media into moderately yellow to deep yellow color is a characteristic feature of acid producing and fast growing rhizobia (Somasegaran and Hoben, 1994b).

Table 2. Summarized cultural and morphological characteristics of the isolates.

Properties	Traits	No of isolates	Proportion (%)
Colony color	WHT	48	42
	WT	41	36
	MK	21	19
	DG	3	3
	Total	113	100
Colony size(diameter)	> 5mm	2	2
	4.00-5.00mm	19	17
	3.00-3.99mm	34	30
	2.00-2.99mm	48	42
	< 2.00mm	10	9
	Total	113	100
Colony shape	C	87	77
	F	26	23
	Total	113	100
Colony texture	E	49	43
	B	64	57
	Total	113	100
EPS	CO	61	54
	M	21	19
	L	31	27
	Total	113	100
YMA-BTB reaction	Ac	113	100
	Ba	0	0
	Total	113	100

Key: WT= watery translucent, WTH= white translucent, MK= milky, DG= dull glistening, F= flat, C= convex, L= low, M= medium, CO= copious amount, E=Elastic, B= buttery, Ac= acid, Ba= base

Physiological and biochemical characteristics

Biochemical and physiological characterization of isolates revealed the existence of versatile and tolerant haricot bean nodulating rhizobial isolates in our collection. The test isolates exhibited a wide range of variation in their NaCl tolerance (data not shown). More than 42% of the isolates grew at 1% NaCl while only 3% of the isolates continued to grow at up to 9% NaCl. The result indicated that most isolates were very sensitive to salt concentrations. However, strains from Gofa, Jinka and Hawassa were comparatively salt tolerant than other sites. Isolates HB19a and HB19b, both from Gofa were found to be the most salt tolerant that grew on the medium containing up to 10% NaCl. On the contrary, isolates from Durame (pH of the soils 5.9), Humbo and Badiwacho (pH of the soils 7.1) were highly salt sensitive, thus they were unable to grow at 1% NaCl.

With the respect to their pH profile, all the test isolates were able to grow on a wide pH values ranging between 6 and 9 while only 53% of the isolates were able to grow acidic pH (pH=4). On the other hand, 91% of the isolates were able to grow at pH value of 10. All isolates from Jinka and Kachabira were able to grow at pH value of 5. In addition, Kachabira isolates were able to grow in a wide range of pH values (5-10.5). With the respect to temperature requirement, fifty four of the isolates were able to grow at 40°C, whereas only 12% of the isolates were able to grow at 45°C (Table 3). At higher temperature extremes, the proportion of tolerant isolates decreased. Yet there is no critical boundary line to be drawn that discriminated between tolerance and intolerance, be it for thermal or pH regime. It might also be important whether the area of adaptation has role on tolerance level association mapping.

Table 3. pH and temperature tolerance of the rhizobia isolated from different locations. Numbers indicate the number of isolates that were able to grow

Site	strains from each site	pH levels								Temperature (°C)		
		4.0	4.5	5	5.5	6-9	9.5	10	10.5	35	40	45
Jinka	12	9	10	12	12	12	11	10	10	12	8	1
Gofa	19	14	14	16	19	19	17	17	13	19	7	4
Bodity	19	9	10	14	17	19	17	16	10	19	12	4
Hawassa	11	8	8	8	9	11	10	10	9	11	8	1
Badiwacho	4	2	3	4	4	4	4	4	3	4	1	0
Durame	24	5	10	12	18	24	24	24	14	24	13	3
Kachabira	10	7	9	10	10	10	10	10	10	10	1	0
Humbo	3	2	2	3	3	3	3	3	3	3	1	0
Areka	11	4	5	7	8	11	11	9	6	11	9	0
Total	113	60	71	86	100	113	107	103	78	113	61	13

%	53	63	76	88	100	95	91	69	100	54	12
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With the respect to carbon source utilization, many of the isolates were versatile in utilization of different carbon and nitrogen sources. Of the carbon sources tested, 12 sources (mannitol, glucose, xylitol, trehalose, sucrose, raffinose, maltose, arabinose, sorbitol, myoinositol, ribose, rhaminose) were assimilated by all (113) isolates, whereas D-fructose, mannose, D-xylose, dextrin, inulin were assimilated by 96%-99% of the isolates. Dulcitol, galactose and starch were utilized by 86-89% of the isolates. Taken together, our results clearly revealed that monosaccharide and disaccharide supported maximum growth, followed by polysaccharides and none of strains were able to assimilate organic acids. On the other hand, of the total of 16 nitrogen sources tested, all isolates were able

to metabolize 13 of them (data not shown). D-serine and L-proline were utilized by 84% and 99% of the isolates, respectively.

Intrinsic antibiotic resistance (IAR) and heavy metal resistance

The result of intrinsic antibiotic resistance of the isolates is summarized in Table 4. The data showed that most of the isolates were tolerant to novobiocin and lincomycin and sensitive to Streptomycin. The result showed that the strains exhibited a wide range of differences in their antibiotic resistance. The results in this experiment showed that at least 86% of the isolates resist heavy metals.

Table 4. Antibiotic and heavy metal resistance of the isolates.

Antibiotics & Metals	Concentration (µg/ml)	No of resistant strains	Proportion (%)
Neomycin	5	111	98
	20	91	81
Kanamycin	5	104	92
	15	93	82
Novobiocin	0.5	111	98
	15	111	98
Spectinomycin	2.5	101	89
	5	94	83
Trimethoprim	200	109	96
Chloramphenicol	5	113	100
I	15	111	98
Erythromycin	10	104	92
	20	104	92
Lincomycin	100	111	98
Streptomycin	10	89	79
Cu	100	113	100
Al, Fe	500	113	100
Pb	500	108	96
Zn	100	97	86

Numerical taxonomy

In total 92 traits were examined, based on which the isolates and reference strains were characterized for cluster analysis. Using 82% similarity level as a cut point, a total of 113 isolates and eight reference strains of recognized species, were grouped into five major clusters (designated as I, II, III, IV, and V) and four un-clustered positions on the dendrogram (Figure 1). Out of the five clusters, four of them (I-IV) contained reference strains while one cluster (V) comprised only the test isolates. Of the five clusters, cluster I and II contained 87% of the studied isolates.

Cluster I included 55% of the isolates and clustered with the reference

strain *Rhizobium leguminosarum* at 82% relative similarity. At 87% level of similarity this major cluster I further subdivided into four sub-clusters (designated IA, IB, IC and ID). Sub-cluster 1D contained *R. leguminosarum*, the other sub-clusters were loosely linked to this reference strain. Sub-cluster ID comprised the isolates from four specific locations; Kachabira (4), Durame (1), Humbo (2), and Bodity (1) while sub-cluster IC included isolates only from Areka and Durame. However, sub-cluster IA and IB contained the isolates from nine different locations.

Cluster II comprised 36 new isolates and reference strain, *Ensifer meliloti* at 84% similarity cut point, which

further divided into three distinct sub-clusters, designated IIA, IIB and IIC. Sub-cluster IIB closely related to reference strain *Ensifer meliloti*. Sub-cluster IIC contained strains from Bodity, Durame and Hawassa. None of the isolates from Humbo and Badiwacho were clustered with sub-cluster IIA and IIB.

Cluster III consisted two strains from Durame (HB 109, HB 108), one strain from Kachabira (HB 110) and one from Bodity (HB 92b). These isolates formed cluster with *Mesorhizobium mediterraneum* at 85% similarity cut point in the dendrogram. Cluster IV contains four isolates: HB 114 from Areka, HB 113 from Kachabira, HB 39b and HB 20c from Gofa which were clustered along with *Rhizobium tropici* and *Rhizobium etli* at about 84% and 89% relative similarity, respectively. The main distinguishing characteristics of these isolates were that all of them could not tolerated Zn concentration and grew on glycine. Cluster V consists of only three isolates (HB 19a, HB19b from Gofa and HB 62 from Hawassa), for which the dendrogram suggested distant relatedness to the rest of the isolates found in other clusters and reference strains. Generally, these isolates showed the lowest relatedness (64% similarity) with the rest of the test strains and to the reference strains used in this study. However, the strains should be tested in field conditions and their

phylogenetic positions should be further explored. The test isolates which occupied unclustered positions include HB 20b from Gofa, HB 2a from Jinka, HB 57b from Hawassa, and HB 119 from Durame and were more divergent from the isolates that were grouped in each of the main cluster (I–V). None of these isolates were related to each other and other isolates including the reference strains included in this study.

Nodulation capacity and effectiveness of the isolates

Isolates of haricot bean nodulating rhizobia indigenous to Southern Ethiopia were evaluated for their symbiotic potential in the greenhouse, and the results of which are presented in Table 5. In this study haricot bean rhizobia exhibited a high degree of heterogeneity concerning their symbiotic performance on sand culture. Of the isolates tested 69.9% formed deep green leaf, pink/red nodule and strong stem and, therefore they were effective. All isolates from Bodity and Hawassa were effective indicating that these soils harbour effective strains as compared to other sites.

About 16% of the tested isolate were found to be moderately effective. All isolates from Humbo were moderately effective. Only 2.65% isolates formed white nodules, thus were ineffective (from Jinka), however, the rest isolates from

different locations formed pink and green nodules. It is to be noted that the pattern of nodulation also varied widely between isolates. Ineffective isolates (for example HB27) formed many small white nodules, while

most of the effective isolates (for example HB 1a, HB 1b and HB 139) formed nodules which were mainly pink/dark pink in color.

Table 5. Summarized nodulation and symbiotic performance of rhizobia nodulating haricot bean isolated from different locations in southern Ethiopia.

Geographic location	strain number	Effective*	Moderately effective*	Ineffective*	Nodule size (mm)	Nodule No	Nodule dry Weight(gm)
Jinka	12	75	0	25	1.4-4.5	18 – 204	0.01-0.09
Bodity	19	100	0	0	2.0-4.5	39 – 150	0.04-0.11
Gofa	19	84	16	0	2.0-5.0	38 – 203	0.06-0.12
Hawassa	11	100	0	0	2.0-4.0	34 – 162	0.04-0.13
Badiwacho	4	75	25	0	2.5-3.0	71 – 103	0.05-0.09
Durame	24	70	30	0	1.75-4.5	40 – 109	0.05-0.10
Kachabira	10	80	20	0	2.5-3.7	47 – 132	0.04-0.10
Humbo	3	0	100	0	2.9-3.7	63 – 78	0.06
Areka	11	64	36	0	2.5-4.0	22 – 107	0.04-0.09
Total	113	80	18	2	-	-	-
%	100	70	16	3	-	-	-

* Percent of nodules scored as effective, moderately effective and ineffective qualitatively, Effective = Deep green leaf, strong stem and pink nodule, moderately effective = Pale green leaf, medium stem strength, Ineffective = Yellow leaf, weak stem, white nodule.

Discussion

In earlier studies on rhizobia from common bean in Ethiopia, it was clearly demonstrated that indigenous rhizobia nodulating common bean were diverse (Abebe, 1987; Beyene *et al.*, 2004; Mitiku; Workalemahu and Assefa, 2007). Similarly, this study showed that rhizobia capable of eliciting nodules on haricot bean (*Phaseolus vulgaris*) were present at all the locations investigated in Southern Ethiopia. The colony diameter ($\geq 2\text{mm}$) determined in 91% of the test isolates within 3 days of incubation clearly signifies the fast growing nature of common bean

rhizobia, which is also the case in other studies of similar nature (Aguilar *et al.*, 2004; Amarger *et al.*, 1997; Andrade *et al.*, 2002; Beyene *et al.*, 2004; Küçük *et al.*, 2006; Workalemahu and Assefa, 2007). In addition, the acid production behavior of the test isolates corroborates earlier findings on common bean rhizobia (Aguilar *et al.*, 2004).

Rhizobium phaseoli is one of the most halotolerant rhizobia and several isolates have been reported to grow at high salt concentrations (4-5%) (Hungria *et al.*, 2000). Similarly, two isolates of common bean from Southern Ethiopia tolerated 2%

concentration of salt (Workalemahu and Assefa, 2007). Isolates that were tolerant of up to 9% NaCl were also found to be highly resistant to alkaline pH of 10 except HB 19a. This result agreed with the results of other works from Egypt (Shamseldin and Werner, 2005). The isolates which were tolerant to high salt concentration and alkaline pH could be a candidate for field trials where salinity and alkalinity domain is increasingly a constraint, in lowland valleys and western hemisphere. The presence of isolates that could resist higher NaCl concentration offers a possibility for choosing inoculant for production sites where salinity is a problem (Mensah *et al.*, 2006). The same author concluded that the population count of rhizobia was inversely proportional to the salt concentration; with most growing at lower concentrations while only a few are able to grow at higher concentrations. In earlier investigations rhizobia of common bean tolerant to varying NaCl concentration levels have been reported, including 2% (Jordan, 1984), 3% (Odee *et al.*, 1997), 5% (Maâtallah *et al.*, 2002), 10% (Zahran, 1999) and 14% (Abdelmoumen *et al.*, 1999).

With the respect to their pH profile, all isolates were able to grow on a pH range from 6 – 9 while 53% of the isolates were able to grow at pH 4. The results indicated that 100% of the isolates were tolerant to pH 9

which is similar to the study conducted in Ethiopia (Workalemahu and Assefa, 2007). On the contrary the same authors reported that almost all their haricot bean isolates from Southern Ethiopia were unable to tolerate pH lower than 5. It can be suggested that isolates, which were able to grow at acidic pH in the lab, could be taken further to the field condition (with acidic soil pH) for field trials to develop them into inoculant. Currently, it is estimated that about 40% of the total arable land of Ethiopia is affected by soil acidity (Taye, 2007). In earlier studies, it was found and reported that all strains of *R. tropici* were able to grow at pH 4, with few representatives of *R. giardinii* and *R. leguminosarum* bv. *phaseoli* (Amarger *et al.*, 1997). Similarly, *R. tropici* affiliated rhizobia from common bean, and that tolerated pH of 4.2 were identified (Graham *et al.*, 1994). On the other hand, 91% of the isolates were able to grow at pH value of 10 and in agreement with other findings on common bean isolates identified elsewhere (Küçük *et al.*, 2006). All isolates from Jinka and Kachabira were able to grow at pH value 5. In addition, Kachabira isolates were able to grow in a wide range of pH value; from pH value 5-10.5. Strains grew in wide pH range can be taken as a promising alternatives for inoculant production in areas where acidity and alkalinity are problem for haricot bean production.

With the respect to the temperature profile, and in agreement to our findings, several research identified that rhizobia from common bean were found to resist an incubation temperature of 40°C (Hungria *et al.*, 2000), 42°C (Küçük *et al.*, 2006) and 44°C (Diouf *et al.*, 2000). Similar response of *R. tropici* to incubation of high temperature was recorded, tolerating 40°C on different growing media (Martínez-Romero *et al.*, 1991). Furthermore, rhizobia capable of growing at temperature values ranging between 32 and 47°C (Hungria and Vargas, 2000) were identified, although tolerance varies among species. In our study, thus, it can generally be concluded that, most of the isolates can overcome high temperatures (35°C and 40°C) which are one of the major problems for biological nitrogen fixation and might be used as haricot bean inoculants in areas where high temperature is a problem for bean production especially in rift valley areas in Ethiopia. However, further work is needed to verify their performance in the field condition.

Regarding the carbon source utilization, most bean nodulating bacteria could utilize dulcitol except *R. tropici*. Similar finding was also shown by (Andrade *et al.*, 2002) that the *R. tropici* strains were unable to grow on dulcitol. Fast growing rhizobia were reported to utilize a large variety of carbon sources than slow growing counterparts (Gao *et*

al., 1994; Kalita and Małek, 2004). Our results also revealed that monosaccharide and disaccharide, support maximum growth, followed by polysaccharides and none of strains were able to assimilate organic acids. Similar findings have been reported (Kumari *et al.*, 2009; Küçük *et al.*, 2006). Nitrogen source utilization by the test isolates revealed similar pattern as that pattern generated from carbon source utilization, and this is supported by other studies. For example, (Amarger *et al.*, 1997) and (Andrade *et al.*, 2002) found that most of the bean isolates could utilize wide range of amino acids. The most resistant amino acid was glycine; where only 61% of the tested isolates were unable to utilize it. This is similar to glycine utilization of Brazilian isolates (Amarger *et al.*, 1997), Ethiopian isolates (Workalemahu and Assefa, 2007) and fast growing rhizobia (Jordan, 1984). The ability to utilize a wide range of carbon and amino acid sources have significance for rhizobia strain classification and provide an ecological advantage to the rhizobia in colonizing the soil and competing with other microorganisms around the rhizosphere, where carbon and nitrogen sources are the limiting factors.

Conclusion

The combined results (from cultural, physiological and symbiotic

characteristics) of this study have revealed that there are indigenous rhizobia capable of eliciting nodules on the host legume, *Phaseolus vulgaris*, growing at different locations in Southern Ethiopia. The diversity of the isolates that were collected from different sites might be the result of the promiscuous nature of the host or the presence of compatible rhizobial population in soils. Characterization of 113 isolates based on cultural, physiological and biochemical characteristics indicates that there is a wide diversity in rhizobial strains nodulating haricot

bean in Ethiopia. The ability to utilize a wide range of carbon and amino acid sources and ability to tolerate a wide range of antibiotics and heavy metals may give competitive advantage for the test isolates under field condition where such factors affect their performance during colonization of the rhizosphere. Isolates that tolerated high pH, low pH and high NaCl concentration could be best candidates for inoculum production for haricot bean in locations where salinity and acidity is a problem.

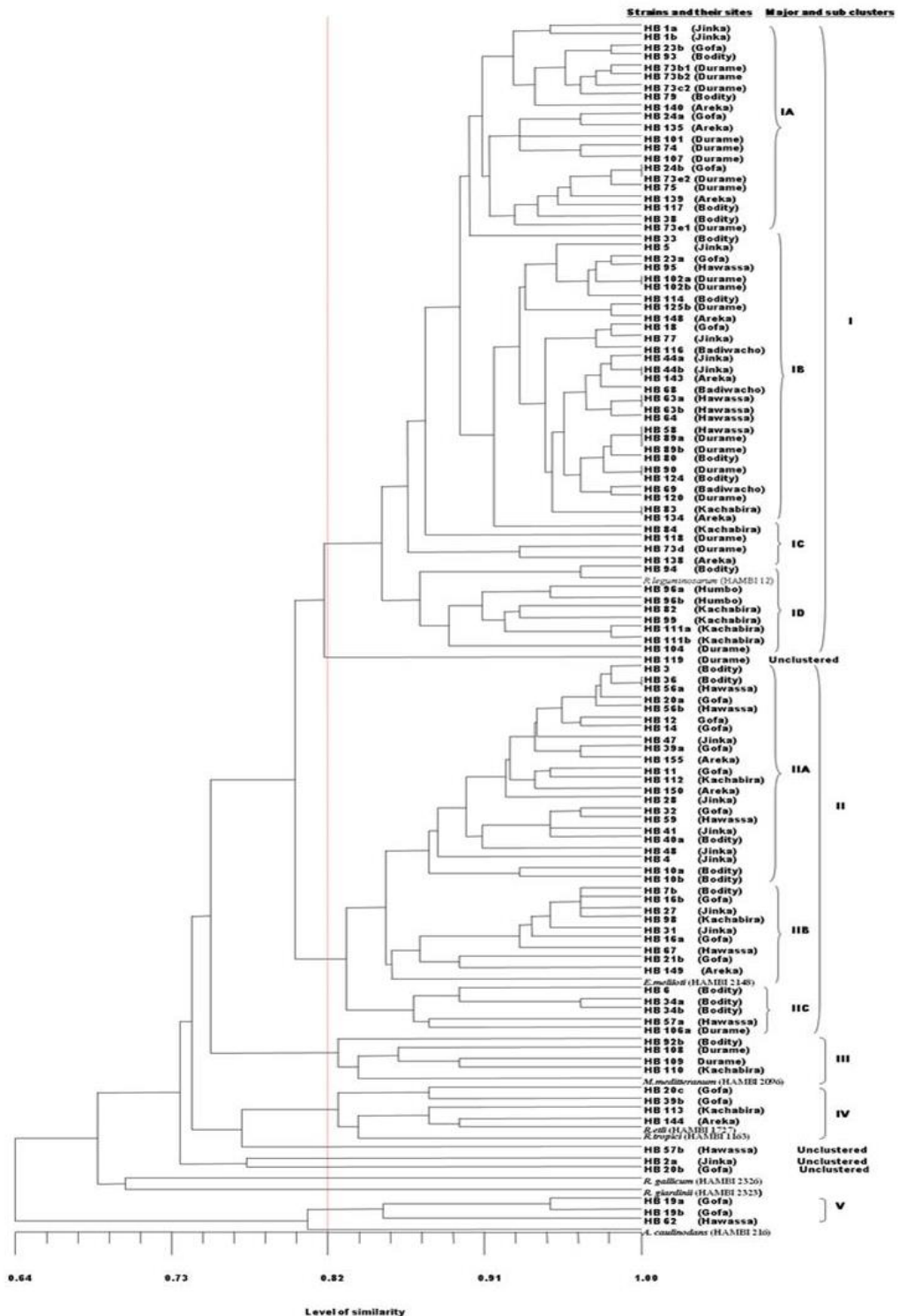


Fig. 1. Dendrogram showing the grouping of the total study isolates and eight reference strains

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Carbon Isotope Discrimination as A Selection Tool for High Water-Use Efficiency and Yield of Groundnut (*Arachis hypogaea* L.) Genotypes Grown Under Rain-Fed Conditions in Dewachefa District, North-East Ethiopia

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Abstract

Water availability is increasingly becoming scarce and limiting agricultural productivity in North-eastern part of Ethiopia. Identifying crop varieties with better water-use efficiency coupled with improved plant growth, and greater grain yields can significantly increase crop production, food and nutritional security in areas of low rainfall. Field experiment was conducted at Gerbi in Dewachefa District during 2012 and 2013 to evaluate plant growth, grain yield and water-use efficiency of 16 groundnut varieties using the ^{13}C natural abundance techniques. The analysis result showed significant differences ($p \leq 0.05$) in plant growth, grain yield and water-use efficiency among the varieties both in 2012 and 2013. The average grain yield markedly varied among varieties, ranging from 604 kg ha⁻¹ for Manupinter to 2018 kg ha⁻¹ for Tole-1. Improved varieties such as Fetene, Manupinter, Sedi, Bulki, Nc-343, Tole-2 and Werer-962 were greater in water-use efficiency although the grain yield was low. But Tole-1 and Shulamith were better in water-use efficiency coupled by high grain yield. The significant difference between the varieties for those traits indicated the possibility of further breeding for enhanced plant growth, high grain yield and better water-use efficiency. Tole-1 and Shulamith are relatively better in water use efficiency and grain yield, and thus potentially drought tolerant varieties for use by farmers.

Key words: Plant growth, grain yield, $\delta^{13}\text{C}$, water-use efficiency

Introduction

Low crop productivity and fast population growth is the main problem facing in sub-Saharan Africa (Gillbert *et al.*, 2014). Ethiopia is one of the countries where the population is growing at a faster rate (2.53 %) and

has resulted in practically most cultivable lands were being ploughed, eliminating expansion of cropped area as the main driver of agricultural growth, which had been the case in 1990s (World Bank, 2007). The rapid population growth in sub-Saharan African country greatly increases

human misery and demands an increased proportion of agricultural products. On the other hand, growth in food production is not in equal footings with population pressure (FAO, 2009). Population pressure on agriculture will have devastating effects on smallholder farmer's ability to feed themselves and their families (Josephson *et al.*, 2014). Therefore, countries in sub-Saharan Africa must seriously giveaway in which theses effects could be mitigated and meet future food needs (FAO, 2009). Strengthening food production capability of the region by wisely exploiting its existing human and natural resources could possibly be critical option to avert the existing situation (Msangi, 2014).

Drought in African agriculture is currently the main constraint to increased crop productivity and food security (Bekele *et al.*, 2014). Availability of water to agricultural crops is essential, whether supplied through irrigation or rainfall, and is one of the most important inputs essential for plant growth, tissue expansion and several biological processes (Farooq *et al.*, 2009). In most parts of the African continent, water availability is the single most important factor that limits agricultural production (Madhusudhan and Sudhakar, 2014). Drought is known to hinder plant growth by negatively affecting photosynthesis, respiration, absorption, translocation and utilization of mineral nutrients, and cell division (Kumar *et al.*, 2012). In certain drought-tolerant crop species,

morphological and metabolic changes occur in response to drought, which enables the plants to adapt to such environments (Chaves *et al.*, 2003). Identifying crop species/genotypes that are greater in water-use efficiency, coupled with improved plant growth, and greater grain yields is important and can significantly increase crop production, food and nutritional security in areas with low annual rainfall.

Groundnut is a multipurpose high-value grain legume that is predominantly grown in countries of Asia and Africa, but with over 70% of the world total production coming from India, China, and the United States of America (Prasad *et al.*, 2009). In Africa the major groundnut-producing countries include Nigeria, Sudan, Senegal, Niger, Chad, Ghana, and Congo (FAO, 2014). Groundnut can serve as a cheap source of high quality protein for both human consumption and for use as animal feed (Ennin *et al.*, 2004; Ibañez *et al.*, 2008). The groundnut grain is rich in oil (48 - 50%) and protein (25 - 28%), and also serves as a source of vitamins, minerals, antioxidants, biologically-active polyphenols and flavonoids (Janila *et al.*, 2013). The oil is also being tested for possible use as bio-diesel (Reddy *et al.*, 2003). Processed groundnut paste is spread on bread, biscuits, sandwiches and candies as food (Benton, 2003). In Ethiopia, it is also used as a substitute for milk in the preparation of "maciyato" during fasting periods (Alemayehu *et al.*, 2014).

Although groundnut is considered drought-tolerant, its production is mostly under rain-fed conditions in the arid and semi-arid tropics, where the rainfall is insufficient and unpredictable (Songsri *et al.*, 2013). In groundnut, drought is reported to delay flowering, decrease chlorophyll content, reduce N₂ fixation, biomass accumulation, pod and grain yield (Reddy and Anbumozhi, 2003; Pimratch *et al.*, 2008; Khan *et al.*, 2012; Madhusudhan and Sudhakar, 2014). Therefore, identifying groundnut genotypes that exhibit improved plant growth and greater water-use efficiency under conditions of soil water deficit could potentially increase groundnut production in low-rainfall environments. The ¹³C isotope technique can therefore be employed as a useful tool for studying water-use efficiency in breeding programs.

Isotopic discrimination of ¹³C during photosynthesis in C₃ plants has been studied intensively, and the differences in the level of discrimination have been correlated with differences in water-use efficiency (Farquhar *et al.*, 1982; Farquhar and Richards, 1984). The significant variation in water-use efficiency (measured as ¹³C in plant tissue) among plant species and genotypes allow the selection of material with greater in water-use efficiency under water deficit conditions (Moghaddam *et al.*, 2013). Rowland *et al.* (2012) have in fact found the $\delta^{13}\text{C}$ values of groundnut genotypes to correlate with their water-use efficiency, thus making the

technique a potential tool for selecting drought tolerance in crops such as groundnut. The aim of this study was to identify groundnut genotypes that have improved plant growth, greater grain yield, and higher water-use efficiency under field conditions of Dewacheffa District, North-east Ethiopia.

Materials and Methods

Site description, experimental design and treatments

The study was conducted at Gerbi in Dewacheffa District located in North-east Ethiopia, during the 2012 and 2013 cropping seasons. Dewacheffa District is located at latitude 10°35' North and longitude 39°41' East, at an altitude range of 1000 - 2500 m above sea level and an annual daily temperature of 12 - 33 °C. The area experiences a bimodal rainfall with the short rainy season beginning in January and ending in March, and the long rainy season starting in June and ending in Mid-October. The mean annual rainfall was 924.2 and 1078 mm for the 2012 and 2013 cropping seasons, respectively. The experiment was laid out in a randomized complete block design with four replications. Each plot measured 3 m x 2 m (6 m²) with five rows. Spacing between blocks, plots, rows and plants were 1.5 m, 1 m, 60 cm and 10 cm, respectively. Sixteen groundnut varieties namely; Bulki, Fayo, Fetene, Lote, Manupinter, Nc-343, Nc-4x, Roba, Sedi, Shulamith, Tole-1, Tole-2, Werer-961, Werer-962, Werer-963 and

Werer-964 were used. Seeds were planted during the main cropping season in July 2012 and 2013.

Source of groundnut seed

The seed of groundnut varieties used for this study were obtained from Werer Agricultural Research Centre, Ethiopian Institute of Agricultural Research, where these varieties were evaluated and reported to have a yield potential of up to 3.3 t ha⁻¹ under rain-fed conditions, and 44 - 53% oil content.

Plant sampling and processing

Plant sampling for dry matter yield and ¹³C isotopic analysis was done at

flowering to early pod-filling stage. Five plants were randomly dug up from the inner three rows of each plot, and separated into shoots, roots and nodules. The shoots were oven-dried (60°C), weighed and ground to fine powder (0.85 mm) for storage prior to ¹³C analysis.

¹³C/¹²C isotopic analysis

To analyze for ¹³C/¹²C, about 2.0 mg of finely ground groundnut shoot samples were weighed into aluminium capsules, and run on a mass spectrometer. The ¹³C natural abundance or δ¹³C (‰), was calculated as Farquhar et al. (1989):

$$\delta^{13}\text{C} (\text{‰}) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1000$$

Where ¹³C/¹²C_{sample} is the isotopic ratio of the sample, and ¹³C/¹²C_{standard} is the isotopic ratio of PDB, a universally accepted standard from the Belemnite Pee Dee limestone formation Craig (1957). Shoot C content was calculated as the product of %C (obtained from the mass Spectrometer) and shoot DM.

Statistical analysis

All data were subjected to a normal distribution test before being subjected to statistical analysis using SAS (System Analysis Software) version 9.0. Analysis of variance (ANOVA) was done to test for treatment

differences and where such differences occurred, the Duncan's multiple range test was used to separate the means. Pearson correlation and linear regression analyses were done to determine the relationships between the measured parameters.

Results

Shoot dry matter and grain yield

There were highly significant (p≤0.001) differences in plant growth (measured as dry matter yield) between and among the 16 groundnut

varieties planted in 2012 and 2013 (Figure 1A and 1B). In 2012, shoot DM yield ranged from 32.2 g plant⁻¹ in Werer-961 to 52.9 g plant⁻¹ in Tole-1 (Figure 1A). The variety Tole-1 accumulated the largest shoot DM, followed by Tole-2, Sedi, Manupinter and Werer-962. In contrast, varieties Werer-961, followed by Nc-4X, Lote and Werer-963 produced the lowest shoot DM yield (Figure 1A). Although plant growth differed significantly between and among the 16 groundnut genotypes in 2013, most genotypes showed similar growth in that year (Figure 1B). The genotype Tole-1, followed by Tole-2, Sedi and Werer-962 produced, relatively the largest biomass in 2012 and also recorded the greatest amount of shoot DM in 2013 (Figure 1B).

The data for grain yield revealed significant difference between and among the 16 groundnut genotypes in 2012, which ranged from 598 kg ha⁻¹ of grain for Manupinter to 2069 kg ha⁻¹ for Tole-1 (Figure 1B). Most of the varieties produced grain yield above 1000 kg ha⁻¹, with Tole-1 producing well above 2000 kg ha⁻¹. Only six varieties (namely, Manupinter, Tole-2, Werer-962, Nc-343, Sedi and Bulki) produced grain yield below 1000 kg ha⁻¹ (Figure 1B). The grain yield in 2013 (Figure 2B) was similar to that of 2012, with Tole-1 producing the highest grain yield (2147 kg ha⁻¹), followed by Fayo, Shulamith and Werer-961. In contrast, varieties Manupinter, Sedi, Werer-962 and Nc-343 produced yields below 1000 kg ha⁻¹ (Figure 2B).

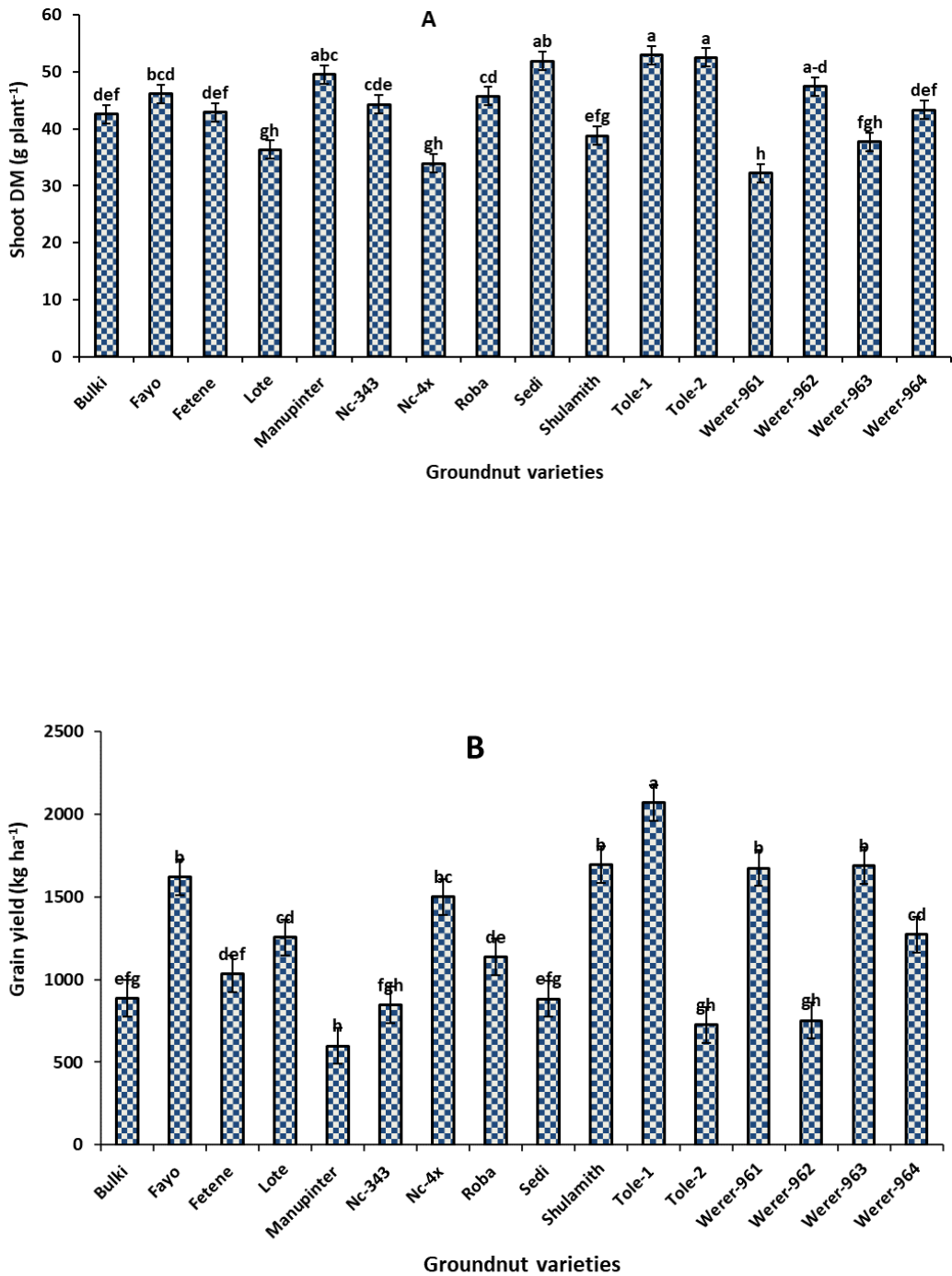


Figure 1: Plant growth (A) and grain yield (B) of sixteen groundnut genotypes grown at Gerbi in 2012. Vertical lines on bars represent Standard Error (S.E)

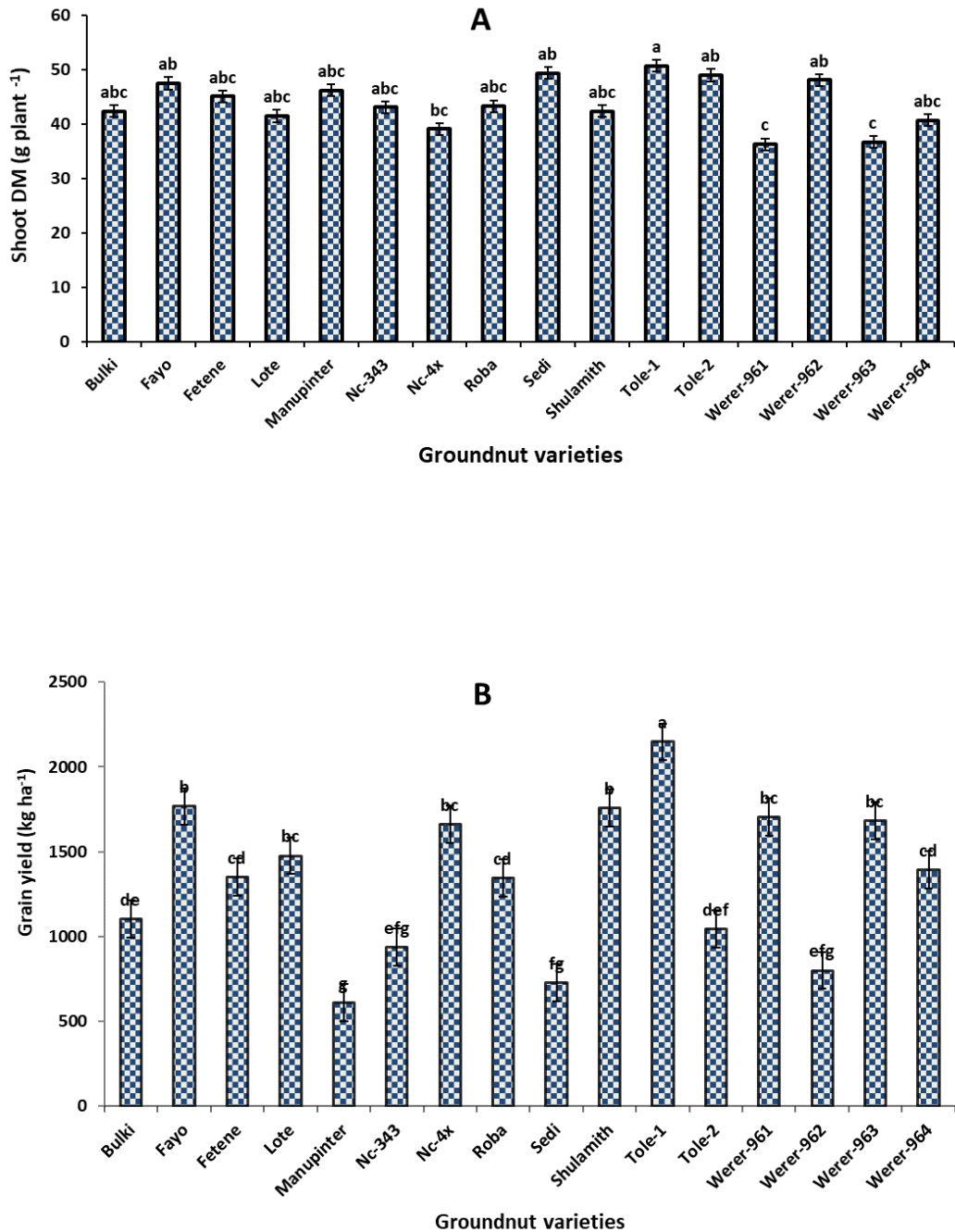


Figure 2: Plant growth (A) and grain yield (B) of sixteen groundnut genotypes grown at Gerbi in 2013. Vertical lines on bars represent S.E

Carbon concentration and content of groundnut

Shoot C concentration and content differed among the 16 groundnut varieties in 2012 and 2013 (Table 1 and 2). The variety Fetene recorded much greater shoot C concentration, followed by Werer-964 and Werer-963, while Fayo, Shulamith and Nc-343 showed the least shoot C concentration in 2012 (Table 1). But, the shoot C concentration in 2013 ranged from 41.0% for Werer-964 to 42.6% in Werer-961. Shoot C concentration in 2013 followed the same trend as in 2012 (Table 2). Shoot C content in 2012 was much higher in Tole-1, Sedi, Tole-2, Manupinter and Werer-962 due to their greater biomass (Table 1). However, varieties Werer-961, Nc-4x and Lote also showed less C concentration due to their lower shoot DM. In 2013, the shoot C content was higher in genotype Tole-1, followed by Sedi, Tole-2 and Werer-962, while the lowest C content was recorded in Werer-961 and Werer-963 (Table 2).

C/N ratio

The photosynthetic N-use efficiency (expressed as C/N ratio) of the groundnut shoots differed significantly among the 16 varieties tested during 2012 and 2013 cropping seasons (Table 1 and 2). The C/N values of groundnut shoots in 2012 was greater for variety Sedi (14.9 g g⁻¹), followed by Fetene and Werer-961, due to their lower N. Varieties Fayo, Nc-4x, Roba and Tole-2 showed the lowest C/N values (Table 1). The C/N ratio ranged

from 9.3 g g⁻¹ for Lote to 12.4 g g⁻¹ for Fetene in 2013, and these values were relatively lower when compared to those of 2012 (Table 2).

$\delta^{13}\text{C}$ as measure of water-use efficiency

Shoot $\delta^{13}\text{C}$ values of groundnut varieties were used to estimate water-use efficiency in this study. Here, higher shoot $\delta^{13}\text{C}$ (i.e. lower discrimination) means greater water-use efficiency and conversely lower $\delta^{13}\text{C}$ implies lower water-use efficiency (i.e. higher discrimination). The shoot $\delta^{13}\text{C}$ values differed significantly between and among the 16 groundnut varieties in 2012 and 2013 (Table 1 and 2). Eleven varieties exhibited greater $\delta^{13}\text{C}$ values, ranging from -26.9‰ to -26.6‰, with Werer-963, Werer-964, Fayo, Nc-4x and Roba showing the lowest $\delta^{13}\text{C}$ values (Table 1). The same pattern was observed in 2013 where most of the varieties recording similarly high $\delta^{13}\text{C}$ values, but Werer-964 and Sedi had much lower $\delta^{13}\text{C}$ values (Table 2).

Correlation and regression analysis

From the correlation and regression analysis, C content was significantly correlated with shoot DM ($r = 0.99^{***}$). Similarly, $\delta^{13}\text{C}$ was significantly correlated with C/N ratio at ($r = 0.38^{**}$). Furthermore, C/N ratio was significantly correlated with C concentration at ($r = 0.50^{***}$) (Figure 3).

Table 1. Shoot C concentration, C content, C/N-fixed and $\delta^{13}\text{C}$ of sixteen groundnut varieties grown at Gerbi in 2012

	C	C content	C/N-fixed	$\delta^{13}\text{C}$
Treatment	%	g plant ⁻¹	g g ⁻¹	‰
Genotype				
Bulki	41.6de	17.7cd	11.9def	-26.6a
Fayo	40.8f	18.8bc	10.9g	-27.2cd
Fetene	43.3a	18.6bc	13.6b	-26.7ab
Lote	41.6de	15.1ef	12.5cd	-26.9abc
Manupinter	42.0cde	20.8ab	12.1def	-26.7ab
Nc-343	41.3ef	18.3cd	11.7d-g	-26.6a
Nc-4x	42.0cde	14.3ef	11.3fg	-27.1cd
Roba	41.9cde	19.2bc	11.4efg	-27.0bcd
Sedi	42.3bcd	22.0a	14.9a	-26.7ab
Shulamith	41.3ef	16.0de	11.7d-g	-26.9abc
Tole-1	41.6de	22.0a	12.1def	-26.9abc
Tole-2	41.8cde	21.9a	11.4efg	-26.6a
Werer-961	41.7cde	13.4f	13.3bc	-26.7ab
Werer-962	41.7cde	19.7abc	12.2de	-26.8abc
Werer-963	42.5abc	16.0de	11.9def	-27.3d
Werer-964	42.8ab	18.5bc	12.2de	-27.3d
F-Statistics	4.91***	9.76***	10.10***	2.84**
CV (%)	1.33	9.50	5.23	-1.12

Values (Means $\pm$ SE) with dissimilar letters in a column are significant at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and ns=not significant

Table 2. Shoot C concentration, C content, C/N-fixed and $\delta^{13}\text{C}$ of sixteen groundnut varieties grown at Gerbi in 2013

	C	C content	C/N-fixed	$\delta^{13}\text{C}$
Treatments	%	g plant ⁻¹	g g ⁻¹	‰
Genotype				
Bulki	42.4abc	18.0a-d	10.9bc	-26.9a
Fayo	41.6b-f	19.8a-d	10.4bcd	-27.2abc
Fetene	42.6a	19.2a-d	12.4a	-26.8a
Lote	41.2def	17.1a-d	9.3d	-27.4abc
Manupinter	41.6b-f	19.2a-d	9.9cd	-26.8a
Nc-343	41.1ef	17.7a-d	10.1cd	-27.0ab
Nc-4x	41.5c-f	16.2cd	10.0cd	-27.2abc
Roba	42.5abc	18.4a-d	11.6ab	-27.3abc
Sedi	42.1a-e	20.8ab	11.4ab	-27.5bc
Shulamith	42.3abc	18.0a-d	10.5bcd	-27.3abc
Tole-1	42.5abc	21.5a	10.5bcd	-27.1ab
Tole-2	42.3abc	20.7abc	9.9cd	-27.2abc
Werer-961	42.6a	15.5d	11.0bc	-27.2abc
Werer-962	41.8a-f	20.1abc	10.0cd	-27.1ab
Werer-963	42.2a-d	15.5d	10.6bc	-26.9a
Werer-964	41.0f	16.7bcd	10.2cd	-27.8c
F-Statistics	3.08**	2.00*	4.31***	2.04*
CV (%)	1.47	14.63	6.97	-1.33

Values (Means $\pm$ SE) with dissimilar letters in a column are significant at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and ns=not significant

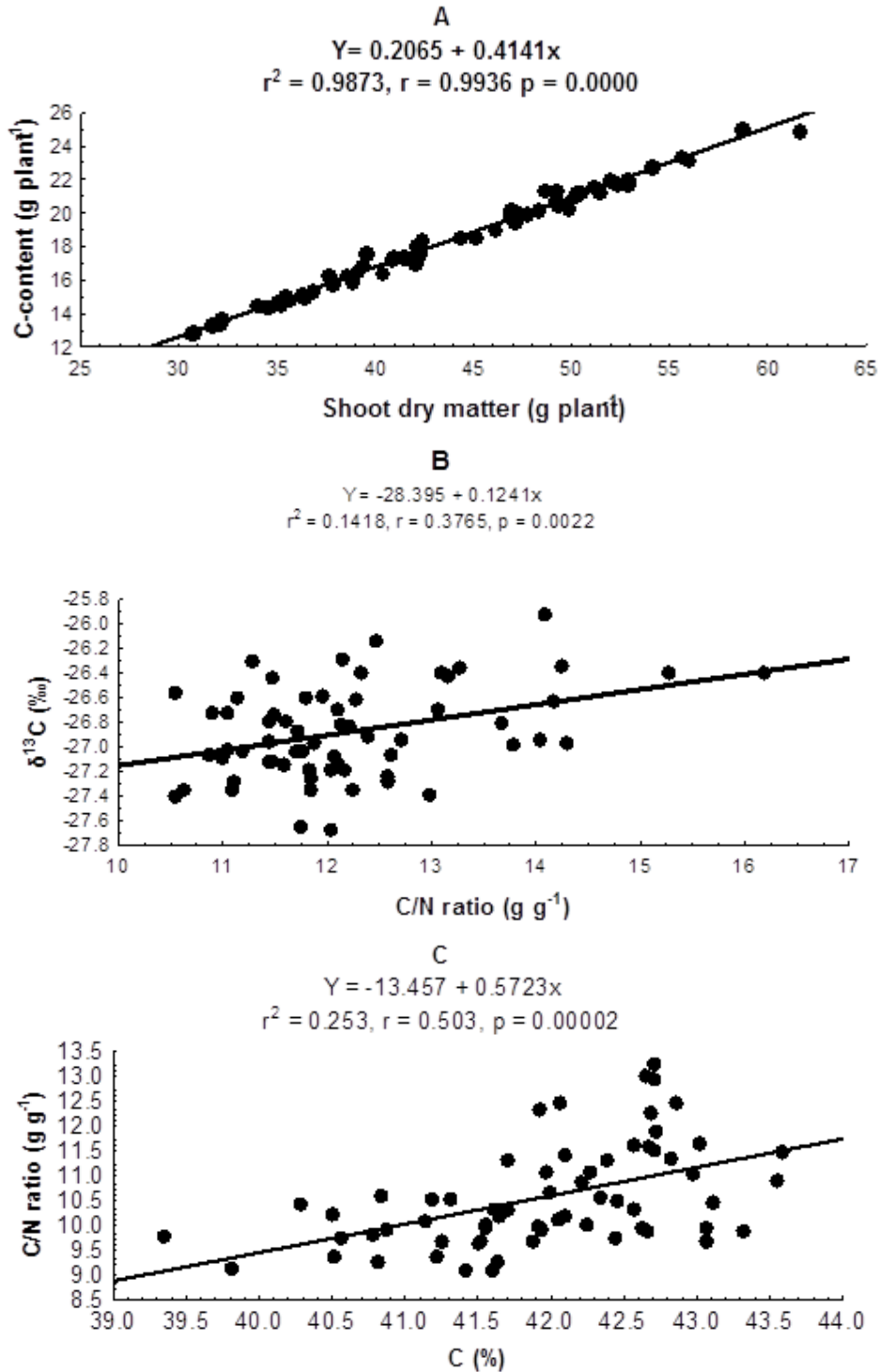


Figure 3: Correlation and regression analysis between shoot C content and shoot DM (A), $\delta^{13}\text{C}$ and C/N ratio (B) and C/N ratio and %C (C) for groundnut varieties planted at Gerbi in North-east Ethiopia.

Discussion

In this study, sixteen groundnut varieties were evaluated under rain-fed conditions at Gerbi, North-east Ethiopia, in 2012 and 2013 for their plant growth, grain yield and water-use efficiency. The evaluated 16 groundnut varieties revealed marked differences in both plant growth, grain yield and their water-use efficiency. As shown in Figures 1 and 2, varieties such as Tole-1, Tole-2, Werer-962, Sedi, Roba, and Fayo produced huge shoot biomass during 2012 and 2013, which directly contribute to greater N contribution for the N economy of the cropping system. Interestingly, varieties Tole-1 and Fayo which produced higher biomass, also recorded greater grain yield. Furthermore, it is important to note that varieties Fayo, Nc-4X, Shulamith, Werer-961 and Werer-963 also produced grain yield greater than 1500 kg ha⁻¹ regardless of the year of experimentation, with Tole-1 producing grain yield above 2000 kg ha⁻¹ during both 2012 and 2013 cropping seasons. In general, 10 of the 16 groundnut varieties were promising, and after further field testing could be used by farmers in Ethiopia as they produced grain yield above 1000 kg ha⁻¹. But it is crucial to identify the factors underling the low grain yield of varieties such as Manupinter and Nc-343 for increased groundnut production in the study area.

Carbon accumulation, C/N ratio and $\delta^{13}\text{C}$ of sixteen groundnut varieties were evaluated in a two-year field experiment in North-east Ethiopia. The data revealed marked variation in shoot C concentration, C content, C/N ratio and $\delta^{13}\text{C}$ among the 16 groundnut varieties tested during the 2012 and 2013 cropping seasons (Tables 1 and 2). Although, the shoot C concentrations of the 16 varieties were very close to each other, they were statistically significant, and ranged from 40.8 to 43.3% in 2012 and 41.0 to 42.6% in 2013, figures equivalent to those reported for groundnut Rowland *et al.* (2012) and Bambara groundnut Mohale *et al.* (2014). The variation in shoot C content was more related to plant growth than to differences in concentration, thus varieties with greater shoot biomass (Tole-1, Tole-2, Sedi, Manupinter and Werer-962) showed higher C content (Table 1 and 2). There were, in fact genotypes with high C concentration that showed low C content due to reduced shoot biomass. The positive relationship observed between C content and shoot dry matter yield ($r = 0.99^{***}$) (Figure 3A) clearly indicates the direct relationship between shoot photosynthesis and plant biomass accumulation Makoi *et al.* (2010).

The rate of photosynthetic CO₂ fixation is regulated by N nutrition, as greater amounts of plant N is expected to increase chlorophyll and Rubisco biosynthesis, as well as stimulate, the rates of photosynthesis, and hence photosynthate production to support increased N₂ fixation Kaschuk *et al.*

(2009). The C/N ratio is therefore a good measure of N nutrition in plants, and indicates the C-to-N relationship at the metabolic level Mohale *et al.* (2014). As results of the reduction of N_2 to NH_3 by bacteroids inside root nodules, the C/N ratio of symbiotic legumes is usually low, less than 24 g g^{-1} when compared to non-legumes Hobbie *et al.* (1998). The C/N ratios in this study ranged from 10.9 to 14.9 g g^{-1} in 2012 and 9.3 to 12.4 g g^{-1} in 2013, which is below 24 g g^{-1} and comparable to those of other symbiotic legumes Mohale *et al.* (2014).

Plants with C_3 photosynthesis tend to discriminate against the heavier ^{13}C isotope during CO_2 reduction by Rubisco due to the slower diffusion of $^{13}\text{CO}_2$ than $^{12}\text{CO}_2$ across the stomatal aperture and the heavier weight of ^{13}C . As a result, the ratio of ^{13}C to ^{12}C in C_3 plant tissues becomes lower than the atmosphere Farquhar *et al.* (1982). However, decreased stomatal aperture during soil moisture deficit often results in lower ^{13}C discrimination with less leaf water lost via transpiration, thus increasing the $^{13}\text{C}/^{12}\text{C}$ isotopic ratio in plant tissue Farquhar *et al.* (1989). The $\delta^{13}\text{C}$ of C_3 plants have therefore been used as a measure of water-use efficiency (WUE). The $\delta^{13}\text{C}$ values of groundnut varieties in this study ranged from -27.3 to -26.6‰ in 2012 and -27.8 to -26.8‰ in 2013. Of the 16 groundnut varieties evaluated, only five (namely, Werer-963, Werer-964, Fayo, Nc-4x and Roba) in 2012 and two (namely, Werer-964 and Sedi) in 2013 showed lower water-use efficiency, the rest

exhibited tolerance of greater water-deficit (Tables 1 and 2).

Although varieties Fetene, Manupinter, Sedi, Bulki, Nc-343, Tole-2 and Werer-962 showed greater WUE in 2012, they recorded lower grain yield (Figure 1). In contrast, varieties Fayo, Werer-963 and Nc-4x recorded lower water-use efficiency but relatively greater grain yield (Figure 1). Plants can adjust leaf stomatal conductance to balance CO_2 intake for photosynthesis and water conservation via transpiration Ma *et al.* (2012). Greater WUE is largely accomplished either through high net photosynthetic rates, or low transpiration, both of which are regulated by stomatal conductance (Cowan 1982; Nobel 1991). An increase in stomatal conductance can therefore cause a decrease in $\delta^{13}\text{C}$ while a decrease in leaf stomatal conductance can increase shoot $\delta^{13}\text{C}$, with the later leading to increased water-use efficiency (Farquhar *et al.* 1982; Liu *et al.* 2005). Therefore, the varieties with greater $\delta^{13}\text{C}$ and lower plant growth identified in this study probably had low photosynthesis rates as a result of lower stomatal conductance Condon *et al.* (2004). The greater water-use efficiency with lower grain yield production is consistent with data from other studies Hubick *et al.* (1986); Condon *et al.* (2002). Taken together, this study has identified groundnut varieties with greater WUE and higher grain yield (Tole-1, and Shulamith), suggesting that these varieties probably have an efficient use of water without affecting

photosynthetic rates, that is greater photosynthetic capacity with higher transpiration efficiency Wright *et al.* (1994). The significant variation among the 16 groundnut varieties in water-use efficiency measured as shoot ^{13}C permitted the identification of groundnut varieties with greater WUE under field conditions. The technique represents a useful tool for selecting water-use efficient groundnut material in breeding programs for meeting the negative effects of climate change.

Acknowledgments

The author is very grateful to the Bill and Melinda Gates Foundation, Ethiopian Institute of Agricultural Research, the South African Research Chair in Agrochemurgy and Plant Symbioses, and Tshwane University of Technology.

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Ethiopian Journal of Crop Science (EJCS)

Guidelines for Contributors

1. Objectives of the journal

- To establish communication among crop science professionals within and outside the country
- To enhance orderly dissemination of crop research information to the farming community through extension and development agencies
- To promote the application of crop research findings and development experiences in the teaching process of all fields of crop science
- To foster exchange of scientific knowledge and information and its application in increasing crop productivity

2. Scope of the journal

The journal publishes peer-reviewed original research articles in various disciplines of crop science and related fields. It also publishes review articles, short communications and crop technology (variety) registrations.

2.1. Research Articles: Treated under this category are both basic and applied original research findings related to crop research Ethiopia. Articles from regional and international sources covering tropical and sub-tropical crop research findings could also be considered for publication.

2.2. Review Articles: Included here are scientific papers covering advances and current topics in various fields of crop research and development. Normally review articles will be prepared by experts in selected fields, volunteered or through invitation by the Editorial Board of the journal.

2.3. Short Communications: The column is intended for brief scientific notes including preliminary results, scientific observations, experimental techniques and recent technological advances in crop science.

2.4. Technology registration: New crop related technologies that have got

official letter of registration can be published in the journal. Crop variety or technology registration requires the following: 1) a letter of confirmation from the technology host institute indicating that the technology or variety has been developed by the author (s); 2) an official letter of approval by the national organization responsible for technology/variety release and registration; and 3) that the technology/variety for registration is not more than two years old since its acceptance/approval. The contents should include a title preferably containing the official name of the technology, author(s) and their affiliations, and description (morphologic, agronomic, genetic, adaptation agro-ecology, performance, etc.) of the technology/variety.

3. General format and style - General Instructions

· The Ethiopian Journal of Crop Science publishes original manuscripts of high standard dealing with crop research and development. Reviews on different subjects of crop research and development appropriate to tropical and sub-tropical regions will also be considered.

At least one of the authors must be a member of The Crop Science Society of Ethiopia for papers to be published in the Ethiopian Journal of Crop Science free of charge. Subsidized publication charges are assessed for papers submitted from non-member authors but no publication charges are assessed for invited review articles.

- Manuscripts should be written in standard grammatical English and should not have been published in other professional journals or simultaneously submitted to another such journals. Manuscripts in the form of thesis, workshop/conference proceedings, and research and development project reports are legible to publication on the EJCS. Papers based on thesis materials should be presented with the thesis advisor as co-author and indicate the institutions and years the work was done. Except in unusual cases, articles should be limited to 3,500 words.
- Manuscripts must be typed, double-spaced, only on one side of an A4 (21 × 30 cm) size paper. Leave margins of 2.5 cm at the top, bottom and sides of each page. Number all pages. Main text headings should be centered and typed in capitals for only the first letter of each word in the heading. Sub-headings are typed in small letters starting from the left- hand margin and italicized.
- Papers submitted following the guidelines will be sent to at least two reviewers (within and/or outside the country) selected by the Editorial

Board for comments and critical review to improve the technical and editorial standard of the paper. Manuscripts may be returned to the corresponding author for further improvement or as not being acceptable for publication. For accepted articles authors are requested to send both soft and hard copies of the revised versions giving details of the word processing software used.

- Formatted manuscript will be returned to the author for proof reading. Proofs should be read carefully; corrections of only typographical errors should be made and returned to the Editorial Office within one month of receipt. One issue of the journal will be provided to the corresponding author of a published article free of charge.

4. General outline for research articles (Full length paper) – Specific instructions

The format of the submitted manuscripts should conform to an acceptable standard editorial style that contains title, author(s) and institutions(s), abstract, key words, introduction, materials and methods, results and discussion, acknowledgement (if any), references, and tables and figures as major components.

4.1 Title Page

The title page must contain the following

- 1) **Title:** The title should be selected carefully. It should be concise, specific and descriptive enough to contain key words or phrases indicating the contents of the article.
- 2) **Author(s) and Institution(s):** The name(s) of the author(s) and the institution(s) they are affiliated to along with the addresses should be provided.
- 3) **Running title:** The author(s) should also suggest a short running title of less than 35 characters, including spaces.
- 4) **Corresponding author:** Name of the author to whom correspondence should be sent

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The following shall appear on the abstract page

- 1) **Full title:** Do not include name of authors and their affiliations
- 2) **Abstract text:** An abstract reflecting the content of the paper is required. The abstract should not exceed 250 words for research and review papers and 100 words for short communications. It must include reasons for the study objectives, methods used, results and conclusions. Specific rather than general statements must be used in the abstract. For example, it is more informative to state that 40 and 60 kg of N were studied instead of two rates of N. The scientific names of crops or other organisms used in the study and full chemical names must be included in the abstract when the common names are first mentioned. Abstract need not be divided into paragraphs.
- 3) **Keywords:** Five to seven index words may be important to the journal editor and the audience for information storage, retrieval and to identify the article from among several other contributions. Important keywords may include phrases and nouns that characterize the study but the use of phrases longer than three words may not be recommended. Together with the title and abstract, keywords comprise most of the content of the article that will be available to people looking for it. Keywords must not repeat the title but they must amplify it.

4.3 Introduction: This part of the paper should state the justification or underlying hypothesis for doing the work, the major objectives of the research or investigation and provide a brief review of literature, which should be limited to information essential to orient the reader.

4.4 Materials and Methods: Under this heading a brief and concise description of the conditions under which the investigation was carried out and the procedures, techniques, and experimental designs used should be stated.

4.5 Results and Discussion: The major findings and assessment of the significance of the results of the investigation and the possible practical implications may be discussed with reference to the problems indicated in the introduction or stated as objective. Conclusions can also be presented as part of this section.

4.6 Acknowledgments: Acknowledgements, if any, may be given in a separate section immediately following the results and discussion.

4.7 References: The references must include only publications cited in the text.

Limit the number of references to those that are most pertinent and up-to-date to economize space. It is the responsibility of authors to check on the accuracy of references as reviewers and editors are not expected to do so.

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(a) Examples of Journal Articles

Collaku, A., Harrison, S.A., Finney, P.L. and D.A.van Sanford. 2002. Clustering of environments of southern soft red winter wheat region for milling and baking quality attributes. *Crop Sci.* 42: 58–63.

Gemechu Keneni, Belay Asmamaw and Mussa Jarso. 2001. Efficiency of drained selection environment for improving grain yield in faba bean under undrained target environments on Vertisol. *Euphytica* 122: 279–285.

(b) Example of a Book

Falconer, D.S. 1989. Introduction to quantitative genetics. 3rd ed. Longman, London, England.

(c) Paper in Proceedings

Edmeades, G.O., J. Bolanos and S.C. Chapman. 1997. Value of secondary traits in selecting for drought tolerance in tropical maize. pp. 222-234. *In*: Edmeades G.O., M. Banzinger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Developing drought and low N-tolerant Maize. Proceedings of a symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico. Mexico D.F.,

CIMMYT.

Unpublished and other source materials not readily available in libraries should be mentioned in parentheses in the text or as a footnote. Abbreviate periodical names according to accepted standards.

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4.10 Standard samples: It is suggested that authors consult with previous issues of the journal. It is also suggested that it would be of help for authors to consult with the *Publications Handbook and Style Manual* for ASA, CSSA, and SSA publications or with the *CBE Style Manual* regarding specific points of manuscript preparation

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6. Frequency Publication

Twice/year (January/July)

7. Submission of manuscript

Authors are required to submit manuscript in three identical and standard hard copies and a soft copy preferably as e-mail attachment. Use standard word processing software (such as WORD 2003, 2007 and above versions) for text manuscript processing for submission. Publications can be sent to:

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Ethiopian Journal of Crop Science

P.O. Box 2003, Addis Ababa

Ethiopia

Ethiopian Journal of Crop Science

Special Issue

Vol. 6 (Special volume)

Number 2

2018

ISSN 2072-8506

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