Genetic Diversity and Population Structure, Trait Interrelationships, Yield Stability and Socioeconomic Importance of White Lupin (*Lupinus albus* L.) Landraces in Ethiopia

A Thesis submitted

TO

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BY

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In Partial Fulfillment of the Requirements for the

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Dedication

This thesis is dedicated to the everlasting love and memories of my hero and beloved father, Atnaf Tiruneh, who, to me, never died but simply departed; and to my ever beloved mother, Ayalnesh Bezie, for her unconditional and true love and dedication in all times.

DECLARATION

I, the undersigned, declare that the thesis hereby submitted for the Degree of Doctor of Philosophy (PhD) in Biology (Applied Genetics) to the Department of Microbial, Cellular, and Molecular Biology, Addis Ababa University is my own work and has not previously been submitted at another university. The materials obtained from other sources have been duly acknowledged in the thesis.

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Acronyms and Abbreviations

AEC	Average tester coordinator
AEC	Average environment coordination
AMMI	Additive main effects & multiplicative interactions
AEA	Average environment axis
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BecA	Biosciences east and central Africa
CASCAPE	Capacity building for scaling up of evidence-based best practices in agricultural production in Ethiopia
ddH_2O	Double distilled water
EBI	Ethiopian Biodiversity Institute
ECSA	Ethiopian Central Statistical Authority
EIAR	Ethiopian Institute of Agricultural Research
EPCC	Ethiopian Population Census Commission
FAO	Food and Agriculture Organization of United Nations
FAOSTAT	FAO Statistics
GAM	Genetic advance as percent of mean
GCV	Genetic coefficient of variation
G x E	Genotype by environment interaction

GGE	Genotype plus genotype by environment interaction
GT	Genotype by Trait
Ha	Hectare
ILRI	International Livestock Research Institute
IPGRI	International Plant Genetic Resources Institute
MFLP	Microsatellite-anchored fragment length polymorphisms
PARC	Pawe Agricultural Research Center
PAs	Peasant associations
PCA	Principal component analysis
PCV	Phenotypic coefficient of variation
PIC	Polymorphic information content
QTL	Quantitative trait loci
RCBD	Randomized complete block design
SAS	Statistical Analysis System
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for Social Sciences
SSR	Simple sequence repeats/microsatellites
TLU	Tropical Livestock Unit

Genetic Diversity and Population Structure, Trait Interrelationships, Yield Stability and Socioeconomic Importance of White Lupin (*Lupinus albus* L.) Landraces in Ethiopia, Mulugeta Atnaf (2017), Addis Ababa University.

Abstract

White lupin (*Lupinus albus* L.*)* is one of four economically important species of the *Lupinus* genus, and has been traditionally cultivated for several thousand years along the Nile valley, including Ethiopia. Lupins are known to perform multifaceted functions, such as for human food and beverage, livestock feed, ecological importance, pharmaceutical values and social contributions. In Ethiopia, white lupin has been sustaining quite long in the farming system, and is produced exclusively by smallholder subsistence farmers. However, despite it has long been produced in the country, the crop received little attention by different development actors and have several undesirable characteristics. To address these, setting up practical lupin breeding program targeting the aforementioned major lupin production constraints is quite essential and necessary. Hence, the present study attempted to avail various pertinent socioeconomic and genetic and/or breeding information which are fundamental to realize lupin improvement in the country.

Detailed baseline survey involving 303 households sampled from white lupin production areas of north western Ethiopia was conducted, to ascertain the extent of lupin production constraints and document farmers experiences and practices on lupin cropping, processing and utilization and marketing. Study results identified the major production practices and

constraints to lupin production in the areas. The results would form a useful guide for the development of well-tailored breeding objectives for the improvement of white lupin for Ethiopian farmers and consumers. This approach is useful not only to document farmers experiences and practices, but also ensures participation of farmers to develop demand led lupin technologies.

A phenotyping experiment aiming at characterizing the landraces using agronomic and phenological traits which comprises 143 landrace accessions was under taken at Merawi, Ethiopia. Further characterization of genetic diversity and population structure of 212 landraces using 15 polymorphic SSR markers were done. Another experiment with objectives to evaluate the performance and stability of white lupin landraces in different locations; and characterize white lupin growing environments in Ethiopia was conducted at six different locations in north western Ethiopia. The phenotyping and over-locations experiments were considered to understand the relationships among traits, and to document trait profile of white lupin landraces.

Phenotypic characterization revealed that Ethiopian white lupin landraces were significantly different for most of the traits studied, and a significant number of local accessions performed as high as 5 metric tonnes per hectare of grain yield. Cluster analysis showed that landraces were grouped into 17 clusters of different sizes, of which five were singletons. Some landraces were grouped together regardless of their geographic origin. On the other hand, landraces from Awi, South Gondar and West Gojam were distributed over many clusters. Genetic distances between many pairs of clusters were significant, justifying crosses between parents from them would be desirable genetic recombinations.

Molecular characterization further revealed the genetic diversity vested on the landraces. The SSR markers revealed 98 from 212 landraces, with an average of 6.5 alleles per locus. The average gene diversity was 0.31. Twenty eight landraces harbored one or more private alleles from the total of 28 private alleles identified in the 212 white lupin accessions. Seventy-seven rare alleles with a frequency of less than 5% were identified and accounted for 78.6% of the total allele detected. Analysis of molecular variance (AMOVA) showed that 92% of allelic diversity was attributed to individual accessions within populations while only 8% was distributed among populations. At 70% similarity level, the UPGMA dendrogram resulted in the formation of 13 clusters comprised of 2 to 136 landraces, with the two control genotypes and five landraces remaining distinct and ungrouped. Population differentiation and genetic distance were relatively high between Gondar and populations collected by Australians. High level of gene flow (Nm), ranging from 10.60 to 31.46, was detected between the four major populations namely West Gojam, Awi, East Gojam and Gondar. A model-based population structure analysis divided the white lupin landraces into two populations. All Ethiopian white lupin landrace populations, except most of the landraces collected by Australians and some from Awi, were grouped together with significant admixtures. The study also suggested that 34, as core collections, were sufficient to retain 100% of SSR diversity.

Higher heritability and genetic advance as percent of mean was observed for grain yield, indicating the possibility of improving this trait through selection. Different patterns of associations and accession by trait interactions were observed in different environments. However, genotype by trait biplots consistently indicated that grain yield had positive

associations with most of the traits; especially, with number of pods per plant, plant height and seeds per pod. The study identified some accessions with desirable performances as good for specific trait and/or trait groups that could be considered as sources of genes for the traits they have best performed.

The genotype by environment interaction study depicted that the white lupin landraces studied had differential performances at different test locations implying the presence of crossover interaction. The first two principal components (PC1=41.6% and PC2=21.8%) of the genotype plus genotype by environment interaction (GGE) explained 63.4% of the GGE sum of squares. Two white lupin growing mega-environments were identified in north western Ethiopia. All test locations were found to be representative with different degrees of reliability whereby Fenote Selam and Dibate were found to be most representative. In addition, all test locations, except Mandura and Injibara had generally similar and good discriminating power. Fenote Selam and Dibate were found to be the most representative and discriminating environments and are characterized as most desirable test locations for white lupin improvement in north western Ethiopia. Genotype 2 (G2) was found to be the highest yielding and most stable landrace across the test environments, and hence identified as most desirable genotype for production.

Key words: Discriminating power, Ethiopian farming system, Farmers' experiences, Genotype by location interaction, Genotype by Trait, Landrace populations, Mega-environment, Representativeness, White lupin.

Chapter 1

General Introduction

Taxonomy

Lupinus is a large genus in family Fabaceae. Lupin is a common name used in Europe and Australia for both wild native and domesticated species, while lupine is commonly used for native *Lupinus* in North America. However, both lupin and lupine are being used in Africa. Taxonomically, lupins are classified in order Fabales, family Fabaceae, tribe Genisteae and genus *Lupinus* (Clements *et al*., 2005). The number of species belonging to this genus is not well defined and different workers mentioned range of numbers from 200 to over 1700 (Kurlovich, 2002b; Dunn and Gillett, 1966; Hondelmann, 1984). The genus constitutes both annual and perennial herbaceous species, and some shrubby and tree types (Ainouche and Bayer, 1999). Based on origin and distribution regions, species in the genus can be grouped in to Old World and New World species. Most species of the genus occur in the New World, with two main centers of species diversity in western North America and the Andes. In contrast, only 12-15 species are found in the Old World, predominantly around the Mediterranean, and northern and eastern Africa (Eastwood *et al*., 2008; Pascual, 2004; Święcicki *et al*., 2015).

The Old World species have been divided into two distinct groups, Malacospermae and Scabrispermae, primarily based on seed coat texture: the smooth seeded and the rough seeded species, respectively (Gladstones, 1984). Three of the Old World lupin species, *L. albus*, *L. angustifolius* and *L. luteus* which have got agricultural importance since the 20th century, are characterized by smooth seeds, and belongs to Malacospermae group. They exhibit variable

chromosome numbers ranging from 2n = 40 to 52 (Naganowska *et al*., 2003; Wink *et al*., 1999). *Lupinus albus*, commonly named as white lupin has a chromosome number of 2n=50.

Origin and distribution

Lupinus is a diverse and widespread genus found in both lowland and mountain regions (Cardoso *et al*., 2013; Drummond *et al*., 2012). There are two geographically isolated groups within the genus: the Old World and New World lupins (Cowling, 1999). The genus *Lupinus* originate in the Old World and subsequently dispersed to the New World (Aïnouche *et al*., 2004; Drummond *et al*., 2012). Three different centers of diversity have been proposed for this diverse *Lupinus* genus. These include, the Mediterranean and northern and eastern African regions, North America and South America (Hondelmann, 1984; Kurlovich, 2002b). Some advancements from molecular evolution studies suggest that the Old World species including *L. albus* originated in the Mediterranean and northern and eastern African regions, while two lineages lead to the New World species in North America and South America (Wink *et al*., 1999; Wolko *et al*., 2011). Furthermore, within the major Old World species groupings, there are more local regions considered to be the species centers of diversity. For example, *Lupinus albus* L., the semi-domesticated large seeded forms (var. *albus*) have long been cultivated around the Mediterranean and in the Nile valley including in Egypt, Ethiopia and Sudan. Majority of lupin species are distributed in temperate and subtropical zones of North and South America. The remaining species, especially Old World species are distributed in the Mediterranean region and Africa (Gladstones, 1998; Wolko *et al*., 2011). Domestication of lupins occurred first in the Mediterranean region and the American continent, however real breakthrough that made lupin

a modern agricultural crop occurred in Europe and Australia (Kurlovich, 2002a). In their wild form, lupins are characterized with seeds which are water impermeable, pods easily shattering and high level of alkaloids that causes lupin seeds toxic for human and animal consumption. Domesticated lupin species have been grown as a cultivated crop in many countries on five continents. Among the four agriculturally important lupin species, viz., *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis*, the first three are Old World species that is due to their larger seed size and well formed embryo.

Production and importance

Lupin production in the world has been highly oscillating. Its cultivation expanded across northern Europe and in Australia quite dramatically through the 20th Century but has recently declined almost as dramatically in these same countries. During the last 15 years (2000-2014) total world production range from 0.76 million tonnes in the year 2006 to 1.63 million tonnes in 2005 (Fig. 1a), whereas lupin area harvested somehow consistently decreased from 1.3 million hectare in the year 2000 to 0.65 million hectare in 2013 as the lowest during the period. These trends in production and area harvested are more similar in Australia. This could be due to the fact that Australia is producing the lion share of lupin production in the world that could dictate world trend. In Africa, both lupin production and area harvested has shown similar decreasing trend during the mentioned period (FAOSTAT, 2016). Similarly, in Ethiopia the trend is mainly decreasing with some exceptions in some years where there was increments both in production and area coverage (Atnaf *et al*., 2015a; ECSA, 2013; ECSA, 2014). Considering the same period, Oceania account for 75% of lupin production in the world followed by Europe which account for

about 19% of production (Fig. 1b). The remaining small proportions are contributed by Americas (5%) and Africa (2%).

Major factors that contribute to the declining trend in production and area harvested have been documented by different researchers and found to be variable in different regions and/or countries. For example, in Western Australia, lupin production has declined mostly due to a large reduction in the area sown. Producers assessed lupins to have lower profitability relative to wheat, especially in seasons with late sowing rains and in the 2000s such seasons were more frequent. There has been a steady decline in lupin production in Europe since the 1980s for two main reasons: widespread of anthracnose which devastates the crop, and free imports of soybean by some European countries have seen greater use of soybean meal for feed (Sweetingham and Kingwell, 2008). In Ethiopia, different assessments pointed out that lack of attention by different actors especially the research system which effected unavailability of improved varieties profiled with high productivity, low alkaloids content, resistant to major lupin diseases and early maturing that eventually caused decrease in production and area coverage (Atnaf *et al*., 2015b; Yeheyis *et al*., 2010).

Lupins are known to perform multifaceted functions, such as for human food and beverage, livestock feed, ecological importance, pharmaceutical values and social contributions (Hall *et al*., 2005a; Lqari *et al*., 2005; Johnson *et al*., 2006). The composition of the lupin grain is quite unique, being high in protein and soluble fiber, with no starch and very low fat (Petterson *et al.,* 1997). Thus, it has a very low glycemic index which has significant implications for human health especially for modern societies with an increasing incidence of obesity and associated

risk of diabetes and cardiovascular disease (Hall *et al*., 2005b). In nutritional terms, lupin seed is an attractive alternative to soybean for human consumption. Food laboratory studies have shown that the protein and fiber components have excellent functional properties (Lqari *et al*., 2005). Lupin ingredients have been included in a range of highly palatable breads and other baked goods, meat products and beverages (Hall *et al*., 2005a). Studies have also indicated the substantial health attributes of lupin such as diets supplemented with lupin grain may play an important role in treating type 2 diabetes, particularly in overweight and obese people, beneficially influence satiety (appetite suppression) and energy balance, improve blood lipids, lower hypertension, and improve bowel health (Archer *et al*., 2005; Hall *et al*., 2005b; Johnson *et al*., 2006). Lupins also lack anti-nutritional factors such as trypsin inhibitors and saponins (Sweetingham and Kingwell, 2008).

The nutritive value of lupins in the diets of monogastric and ruminant animals has been thoroughly determined and reviewed by Australian and European researchers as a protein and energy source (Petterson, 2000). The absence of starch makes lupin attractive to ruminants (Edwards and van Barneveld, 1998). In Ethiopia, the use of lupin as livestock feed is limited due to the fact that farmers are cultivating local white lupin cultivars which are bitter due to high level of alkaloids (Yeheyis *et al*., 2010). Hence, debittering lupin grain is necessary to feed to animals as pointed out by same authors, although some animals especially sheep and goat are fed the local lupin which is bitter. Of course, these animals are feeding not on the seed, rather different parts of lupin crop especially finely threshed plant parts mainly pod walls left after threshing. Meanwhile, researchers reported the importance of lupin not only for maintenance

but also for optimum performance of ruminants in the form of concentrate supplement feeds (Tefera *et al*., 2015; Yeheyis *et al*., 2012a).

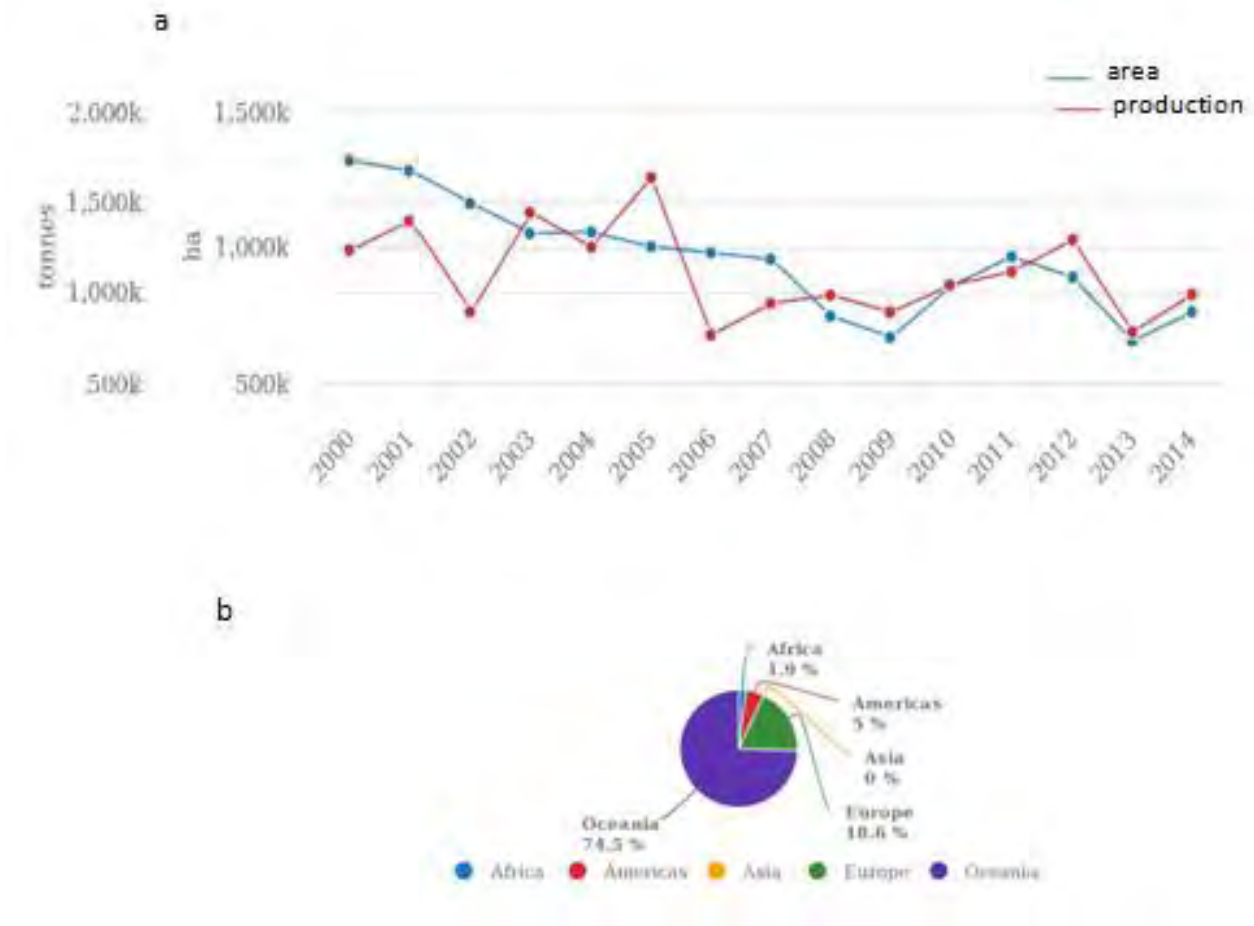

Figure 1: (a). Trends in world lupin production during the last 15 years (2000 - 2014); (b). Share of lupin production by region during the same period (Source: FAOSTAT, 2016).

Lupins are a valuable component of different farming systems and play key role in crop rotations; important in nitrogen fixation and hence significantly reduce the fertilizer use of the

succeeding crop, and good for disease and insect pest management. Lupin can potentially fix and accumulate a total of 150 to 400 kg/ha per year nitrogen (Jansen, 2006; Takunov, 1999; Reeves *et al*., 1990). Thus, it significantly contributes to yield increment and/or profit margins of the succeeding grain crop usually cereals. Apart from these direct values of lupin as rotational component in the cropping system, its role further implicate in the ecosystem/environment, because the use of lupin as legume in cropping systems could reduce the use of fossil fuel which otherwise is utilized in the manufacture of synthetic fertilizer, thereby releasing CO_2, and the transport and spreading of organic and synthetic nitrogen fertilizers consumes additional fuel. Further, nitrates from fertilizers and soil nitrogen reserves may also leach through the soil column into groundwater, and the denitrification of nitrates from synthetic or organic sources is the primary source of nitrous oxide (N_2O), a powerful greenhouse gas, from agricultural soils (Philippot *et al*., 2011). Hence maintaining the reactive nitrogen within the plant, as happens in a symbiotic legume in the growing season, avoids some potential environmental damage. On the other hand, previous scientific research reported that lupin could offer considerable human food in marginal lands with limited water availability (Small, 2012).

Genetic Resources and Improvement

The main aim of germplasm collections and gene banks is to maintain the genetic variation of crop plants and their wild relatives. The diverse and widespread nature of genus *Lupinus* that resulted from differentiated regions of origin and secondary distribution, and also the environmental requirements of the four agricultural important lupin crops have led to a broad

interest in these genetic resources as a material for basic research and applied breeding. The first collection mission for the genus *Lupinus* was the Vavilov's expedition to the Mediterranean basin in 1926; and the collections have been maintained at the Vavilov's Institute at St. Petersburg, Russia. In addition, other institutions with substantial importance either as lupin genetic resource repositories or as lead institutions for description, characterization and evaluation of collections include, the International Lupin Association, the Biodiversity/ International Plant Genetic Resources Institute, and the lupin project of the Department of Western Australia/University of Western Australia at Perth (Święcicki *et al*., 2015).

Current total holdings of *Lupinus* germplasm accessions based on IPGRI Directory of Germplasm Collections Database around the world, are estimated to be close to 40,000; and Wolko *et al*. (2011) reported list of world countries which have maintained more than 100 lupin accessions in their national gene banks. However, Ethiopia has not been listed while the Ethiopian Biodiversity Institute has conserved more than 275 *albus* lupin accessions. Significant number of the world's gene bank accessions represent the three major Old World, domesticated, smooth-seeded species, viz., *L. albus*, *L. angustifolius*, and *L. luteus*; and one New World species, *L. mutabilis*. Although a relatively large number of accessions of these species are conserved, information on their passport, collection site, and preliminary evaluation requires further enrichment especially for *L. mutabilis* collections, because the usefulness of germplasms highly relies on its accurate characterization and evaluation (Xiao *et al*., 2004).

Several diversity studies have been conducted on lupin germplasm collections, which have provided useful knowledge for crop improvement programs. To this effect, several workers

evaluated different gene pools of lupin landraces including *albus* lupin for their variability under different growing environments and have found out appreciable variability for the various lupin traits such as lupin grain yield and other agronomic and adaptive traits (Annicchiarico *et al*., 2010; Annicchiarico and Carroni, 2009; Christiansen *et al*., 2000; Gonzalez-Andres *et al*., 2007; Kurlovich, 2002a; Lagunes-Espinoza *et al*., 2000; Rubio *et al*., 2004; Noffsinger and van Santen, 2005). Various activities have been carried out in screening germplasms for resistance/tolerance of major lupin diseases, including anthracnose in *L. albus* and *L. angustifolius* (Talhinhas *et al*., 2000; Thomas and Sweetingham, 2004), Pleiochaeta brown spot and root rot in *L. angustifolius* (Cowling *et al*., 1997) and root rot in *Lupinus albus* (Luckett *et al*., 2008) and phomopsis in lupin species (Shankar *et al*., 1999).

Lupinus albus is predominantly self pollinating, however, out-crossing rates of 8–10% were reported (Green *et al*., 1980). Like other crops, lupins can be improved through classical breeding typically through pedigree selection of pure lines from natural germplasm resources. However, with the phylogenetic distance between the Old World and New World lupin species, interspecific crossing attempts between these groups have been difficult. Fertile segregating populations and/or hybrids under natural conditions from inter-specific crossing among smooth seeded Old World species populations including between *L. albus* and *L. angustifolius*, were not so far reported except fertile F1 and F2 plants (Kurlovich and Kartuzova, 2002; Clements *et al*., 2008). This is mainly because, Old World lupin species experience very variable number of chromosomes that range from 2n=40 for *L. angustifolius* to 2n=50 for *L. albus* and 2n=52 for L.

micranthus, *L. luteus*, and *L. hispanicus* and heterogeneity among these species (Naganowska *et al*., 2006; Naganowska *et al*., 2003).

Nevertheless, intraspecific hybridizations have been successful in improving various important traits mainly in the four agriculturally important *Lupinus* species including white lupin (*Lupinus albus*). Sources of genetic variation are mutations and recombinations. The natural variation of lupins is not too broad for some important traits from a breeding point of view. Therefore, in the event of a lack of desirable characteristics, a useful tool is mutation induction. In white lupin breeding so far, achievements that could worthily be mentioned include, decrease in alkaloid content in seeds (<0.02%), improvement of harvest index (vegetative growth decreased), shortening of vegetation period, selection of restricted branching and finally introducing these characters to cultivars (Święcicki, 1986; Boersma *et al*., 2007). Cultivars bred to profile the above mentioned improvements are suitable for modern farming, and such cultivars have been released and under production in many European countries and in Australia since long time (Clements *et al*., 2005; Cowling *et al*., 1998a; Gladstones, 1970; Hondelmann, 1984).

Meanwhile, knowing much incompatibility of interspecific hybridization between lupin species which limits production of viable seeds (Święcicki *et al*., 2000) and to facilitate efficiency and effectiveness of conventional lupin breeding programs, application of biotechnological methods and tools has demonstrated an important role in lupin improvement. Employment of biotechnology in contemporary lupin breeding programs allows broadening the available genetic variability, development of new germplasm, development of markers for marker

assisted breeding and direct transformation of agronomically important genes into the genome of interest. Several successful transformation protocols including for root culture of *L. albus*, herbicide resistance gene and sulfur rich sunflower seed albumin gene to *L. angustifolius*, have been published for lupins as reviewed by Święcicki *et al*. (2015), however transgenic lupins have never been yet produced and commercialized.

Marker-assisted selection (MAS) is a very promising tool to improve efficiency and accelerate selection in breeding programs as field-based selection for agricultural traits is often time-consuming and less efficient. MAS ensures selection in early generations and parallel screening of multiple traits based on simple molecular marker genotype data (Holland, 2004). However, compared to the other three *Lupinus* species especially *L. angustifolius*, the research towards marker development for practical MAS is much less advanced in white lupin. Currently, four microsatellite-anchored fragment length polymorphisms (MFLP)-derived simple PCR-based markers closely linked to agricultural traits have been reported. These are marker "PauperM1" linked to the low alkaloid gene pauper (Lin *et al*., 2009), three markers (WANR1, WANR2, and WANR3) linked to QTLs conferring anthracnose resistance (Yang *et al*., 2010), and some QTLs which are not converted to simple PCR- based markers for practical MAS were identified for other important traits including for flowering time (Phan *et al*., 2007), phomopsis blight and pleiochaeta root rot (Cowling *et al*., 2014; Raman *et al*., 2014a).

Generally, given the increased interest in lupins for healthy food, expanding livestock and aquaculture feeds from plant derived proteins, and with the need for sustainable production

through reduced fertilizer input and nutrient leaching into subsoil, lupins can offer a viable option for world agriculture in the future.

Rationale and Relevance of this study

White lupin (*L. albus*) is produced in Ethiopia exclusively by smallholder subsistence farmers, mainly for its food grain and soil fertility maintenance values (Atnaf *et al*., 2015a; Yeheyis *et al*., 2010). White lupin sustained quite long time in the Ethiopian farming system. It is mainly due to its ability, more than any other legume, to strive well in degraded land and at the same time maintain and bring back the land to production suitable for other crops in the typical mixed crop - livestock farming system of north western Ethiopia. Traditionally, it has been thought and used to cure some disorders and diseases including high blood pressure. Meanwhile, the crop has social value, being generally consumed in bad crop harvest year and by challenged wealth community. Moreover, marketing of its processed products essentially the two branded local products from white lupin, viz., lupin snack and the high sprit local alcohol drink locally called 'Gebto arekie', and sometimes the grain itself is mainly operated by women and youth (Atnaf *et al*., 2015a).

However, obtaining food and beverage from white lupin involves quite long and laborious steps to get rid of a bitter test due to the presence of high level of alkaloids in Ethiopian white lupin local cultivars. On top of this, the local varieties being used by farmers have several undesirable characteristics, such as low yield potential, susceptibility to major lupin diseases and late maturing. Nevertheless, despite the importance of lupin in improving the fertility of degraded

farm land and providing cheap sources of protein for poor family, only very limited research and development effort has been made to improve its productivity and quality in Ethiopia. Consequently, there is no single improved food variety of white lupin developed and delivered to the farmers until this time (Atnaf *et al*., 2015a; Yeheyis *et al*., 2010). Therefore, there is a need to develop well adapted white lupin varieties profiled with preferred qualities including high grain yield, low alkaloids level, early maturing and resistant to major lupin diseases and insect pests.

Supporting Ethiopian farmers' long time engagement of lupin farming with provision of improved varieties is quite important (Yeheyis *et al*., 2010). It needs a practical lupin breeding program targeting major production constraints in the typical crop-livestock mixed farming system/ cropping system of north west Ethiopia where lupin is a key rotational component. To setting up lupin improvement program, it is advisable to assess, generate and document baseline information that would serve as a platform: assess and document the vast experiences and practices of Ethiopian lupin farmers acquired from their long time engagement on lupin production and utilization; characterize the landrace genetic resources that is maintained in the Ethiopian Biodiversity Institute (EBI) in terms of genetic diversity and relationship; and generate genetic information including heritability and genotype by environment interaction on key agronomic traits, trait associations /interrelationships of objective traits and trait profiles of the Ethiopian white lupin landraces. To this effect, there has been few works carried out and limited information documented. In terms of assessing and documenting farmers indigenous practices and experiences, Yeheyis *et al.* (2010) attempted to assess and document some of the

practices and constraints although it was from livestock feed and nutrition perspective and with small number of farmers.

With regard to Ethiopian landrace accessions, the Ethiopian Biodiversity Institute (EBI) conserved more than 275 white lupin landrace accessions (Personal communication to EBI officers). The Ethiopian white lupin landraces represent a unique gene pool (Raman *et al.,* 2014b). It has been reported that it is sources of genes for worldwide *L. albus* breeding, particularly resistance to the wide-spread and devastating fungal disease anthracnose (Adhikari *et al*., 2009); sources of traits for climate resilience including drought resistance, and proteoid/cluster roots development for phosphorus scavenging (Massonneau *et al*., 2001). However, the landrace accessions had never been systematically characterized using molecular markers and agronomic traits. On the other hand, research results by several researchers (Annicchiarico *et al*., 2010; Gonzalez-Andres *et al*., 2007; Rubio *et al*., 2004) indicated that the relationships among white lupin traits are consistently influenced by the unpredictable conditions of the growing environments. Thus, genotype selection should be based on multiple trait data in variable environments within the target regions. However, little is known about trait relations and trait profiles of white lupin genotypes in tropical growing conditions including in Ethiopia.

In addition, several workers evaluated different gene pools of white lupin landraces under different growing environments and have found out that white lupin grain yield and other agronomic and adaptive traits are responsive to different growing conditions, implying the presence of genotype by environment interactions (Christiansen *et al*., 2000; Lagunes-Espinoza

et al., 2000; Kurlovich, 2002a; Rubio *et al*., 2004; Noffsinger and van Santen, 2005; Gonzalez-Andres *et al*., 2007; Annicchiarico *et al*., 2010; Annicchiarico and Carroni, 2009). Yeheyis *et al.* (2012b) also reported the importance of genotype by environment interaction (G X E) on the performance of grain yield and other traits in sweet and bitter cultivars of different lupin species evaluated in three different lupin growing locations of Ethiopia. However, no comprehensive multi-environment evaluation of Ethiopian white lupin has been undertaken so far.

The present study would bring a useful guiding platform for the development of well-tailored breeding objectives for the improvement of white lupin for Ethiopian farmers and consumers: Ethiopian white lupin germplasm accessions that can serve as genetic resources for traits which they profiled with and; high yielding and stable landraces would be identified; production environments would be characterized. In addition, genetic diversity and relationship would be determined, and core collection would be established.

Overall aim and objectives

The overall aim of the study was to generate pertinent genetic and socio-economic information which would be useful to the development of improved technologies which in turn enhance lupin production and productivity in Ethiopia for poverty alleviation; and food and nutrition security with the following general and specific objectives.

The general objective of this study was:

To characterise white lupin landrace populations using molecular markers and agronomic traits, study trait associations and yield stability; and access farmers' practice and experiences on socio-economic importance of white lupin in Ethiopia.

The specific objectives were:

1. To document farmers' experience and practices, and constraints, on white lupin production, product processing and utilization; and to asses importance and role of lupin in the farming/cropping system
2. To characterize the genetic diversity of Ethiopian white lupin landrace accessions using agronomic traits and SSR markers
3. To cluster the Ethiopian white lupin accessions into similarity groups and assess pattern of diversity of the accessions
4. To propose a core collection of Ethiopian white lupin landraces using SSR markers data
5. To understand the relationships /association among traits in Ethiopian white lupin landraces; and asses trait profile of Ethiopian white lupin landraces
6. To evaluate the performance and stability of white lupin landraces in different growing environments; and to characterize white lupin growing environments in Ethiopia

Outline of the thesis

This thesis is based on combinations of household survey, across location field experiments and molecular laboratory activities. There are seven chapters including this general introduction

(Chapter 1), five experimental chapters (Chapters 2- 6), and a summary, conclusion and recommendations (Chapter 7).

In chapter 2, we ascertain the major production practices and constraints of lupin production in northwestern part of Ethiopia through household survey; and document farmers experiences and practices on lupin cropping, processing and utilization, product development and marketing. The survey encompasses four major lupin production zones in the country, namely, West Gojam, East Gojam, Awi and South Gondar. The results would form a useful guide for the development of well-tailored breeding objectives for the improvement of white lupin for Ethiopian farmers and consumers.

Chapters 3 and 4 describe genetic diversity, population structure and core collection establishment of Ethiopian white lupin landrace accessions. The characterization was based on phenotyping of 143 Ethiopian white lupin landrace accessions plus one genotype introduced from Germany for agronomical and phenological traits, and SSR marker genotyping of 212 Ethiopian white lupin landraces plus two control out-groups against 15 polymorphic markers. These marker systems, morphological and molecular, provided sufficient and complementarily description and characterization of the Ethiopian white lupin landrace accessions. It uncovered the genetic diversity vested within the population and among the populations of Ethiopian white lupin landraces; determined the relationship and population structure, and established core collections of white lupin landrace accessions. The results of these chapters provide information useful for setting up practical improvement programs of white lupin; and would suggest how national collection missions on white lupin would be executed in the future.

Chapter 5 deals with trait relationships/ associations and profiles of white lupin landraces. This study was based on two sets of experiments. The first comprehensive experiment that consisted of 143 accessions collected from major lupin growing areas of Ethiopia was evaluated at Merawi and the second experiment that consisted 12 selected accessions was evaluated across six different lupin growing locations in northwestern Ethiopia. The study revealed that trait associations and profiles of white lupin landraces have been found different for different environments due to genotype by location interactions; and a number of landraces were identified as good for specific attributes and/or attribute groups that could be considered as sources of genes for the attributes they have best performed.

Chapter 6 analyzes genotype by environment interaction (G X E) and grain yield stability of Ethiopian white lupin landraces. It also characterizes white lupin growing environments in Ethiopia. The experiment involved 12 white lupin landraces selected from the comprehensive phenotyping experiment based on desirable performance for grain yield and resistance to major lupin diseases such as lupin rust, pleiochaeta root rot, brown leaf spot and phomopsis. Test locations within mega-environment were evaluated for their representativeness and discriminating power. Fenote Selam and Dibate were found to be the most representative and discriminating locations and are characterized as most desirable test locations for white lupin improvement in north western Ethiopia. One genotype (G2) was found to be the highest yielding and most stable landrace across the test environments, and hence identified as most desirable genotype for production.

Chapter 7 summarizes and synthesizes some aspects of genetics and breeding information of white lupin under Ethiopian context that are generated in the experimental chapters of this thesis; and social, environmental and ecological aspects and implications of white lupin in Ethiopia. It further articulates recommendations and future works based on outputs of the present study.

Chapter 2

Farmers' Appraisals on Production and Utilization Practices and Constraints of White Lupin in Ethiopia: Platform for lupin Improvement

Submitted

Farmers' Appraisals on Production and Utilization Practices and Constraints of White Lupin in Ethiopia: Platform for lupin Improvement

Abstract

White lupin (*Lupinus albus* L.) is an important grain legume in the Ethiopian farming system. However, research interventions on the crop have been limited in the country. Consequently, varieties cultivated by farmers contain undesirable characters: they contain high alkaloids level, are susceptible to diseases and insect pests and are low yielders. In order to ascertain the extent of these constraints with the farmers and document their experiences and practices on lupin cropping, processing and utilization and marketing, detailed baseline survey was conducted in white lupin producing areas of north western Ethiopia; namely, West Gojam, East Gojam, Awi and South Gondar, during 2013/14 cropping season. Questionnaires were administered among local farmers. Data were collected for different parameters such as cropping systems, cultural practices, yield, constraints to production and utilization. The data were summarized into averages, percentages or ranges. The study showed that farmers in the study areas have ample experiences of lupin farming and have similar purposes of growing lupin and is targeted for food, fertility restoring value, cash, medicinal value and as feed as their order of importance. Furthermore, the study results identified the major production practices and constraints of lupin production and processing in the areas. In line with this, the study indicated that majority of lupin farmers perform minimum crop management practices to grow lupin. However, there are significant variations of farmers' agronomic practices. Prevalence of different lupin diseases, infestation of insect pests and lack of improved varieties were among the top production constrains repeatedly complained by individual farmers and at the group discussion. On top of this, all farmers agree that bitterness in white lupin is the most persistent and outstanding constraint that limits lupin utilization. Generally, the results identified from this study would form a useful guide/platform for the development of well-tailored breeding objectives for the improvement of white lupin for Ethiopian farmers and consumers.

Key words: Ethiopian farming system, White lupin, Farmers' experiences

Introduction

Ethiopia has diverse agro-ecology that permits different farming systems. Crops, livestock and trees are major components of the farming systems in the country (ADBG, 2008). Food legumes are among the various crops produced in all the regions in different amounts across the country next to cereals both in area coverage and volume of production (ECSA, 2015). More than 12 legume species are grown in different parts of the country. Of these, faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), grass pea (*Lathyrus sativus* L.), and white lupin (*Lupinus albus L.*) are among the legumes adapted and grown by farmers in the north western part of the country (ECSA, 2015). Mixed crop-livestock production is the typical farming system in north western parts of Ethiopia.

White lupin has been traditionally cultivated for several thousands of years in the Mediterranean region where it originated, and along the Nile valley (including Ethiopia) (Wolko *et al*., 2011). It is a promising leguminous crop for human consumption, green manuring, forage and has substantial importance in human nutrition and health (Hall *et al*., 2005a; Lqari *et al*., 2005; Johnson *et al*., 2006). White lupin is produced in Ethiopia exclusively by smallholder subsistence farmers, mainly for its food grain and soil fertility maintenance values (Atnaf *et al*., 2015a; Yeheyis *et al*., 2010), and it has sustained quite long time in the farming system. Traditionally, it has been thought and used to cure some human disorders and diseases including high blood pressure. Moreover, the crop has social value, being generally consumed in bad crop harvest year and by challenged wealth community. Furthermore, marketing of its processed products and sometimes the grain itself is mainly done by women and youth (Atnaf

et al., 2015a). However, food and beverage produced from white lupin involves quite long and laborious steps to get rid of the bitter test due to the presence of high level of alkaloids in Ethiopian white lupin local cultivars. The local cultivars being used by farmers have several undesirable characteristics, such as low yielding potential, susceptibility to major diseases (Atnaf *et al*., 2015b) and high contents of alkaloids (Yeheyis *et al*., 2011).

Despite lupin's importance in improving the fertility of degraded farm land and providing cheap sources of protein for poor family, only very limited research and development efforts have been made to improve its productivity and quality in Ethiopia. Therefore, there is a need to develop well adapted white lupin cultivars with farmers' preferred traits including high grain yield, low alkaloids level and resistant to major lupin diseases and insect pests. Nevertheless, any national improvement and conservation endeavor should consider the vast experiences and practices of Ethiopian lupin farmers acquired from their long time engagement in lupin production and utilization. Ascertaining lupin constraints along the value chain and documenting farmers' experiences is important not only to use it as a baseline information to setting up national lupin improvement programs, but also it ensures farmers participation to develop demand led lupin technologies.

Hence, it is quite important to ascertain these constraints with the farmers and document farmer's experience about different strategies to alleviate these constraints. Farmer's long time indigenous practices in lupin production, product processing and utilization; knowledge and experiences on value and role of lupin in cropping systems, and knowledge and belief on health and nutritional value of the crop, are all worthy to be documented. Yeheyis *et al.* (2010)

attempted to assess and document some of the practices and constraints considering only 112 households and two districts in a single zone in Ethiopia mainly from feed and/or nutrition perspectives. Nevertheless, there is no compressive assessment and documentation of these practices and constraints of lupin farmers in Ethiopia especially from the perspective and prospects of white lupin improvement in the country. Hence, this study attempted to fill this gap and coin some research questions for breeders and provide important recommendations for policy makers regarding lupin production and processing. Meanwhile, assuming that there might be some complementation between the two independent studies, those two districts which were considered by Yeheyis *et al.* (2010) were systematically excluded, and four other districts from each four different agro-ecological zones of Ethiopia considered, in which these four zones together contribute more than 95% of lupin production in the country. The purposes of this research were, therefore, to understand and document farmers' experience and practices, and constraints, on white lupin production, product processing and utilization; and ecological importance and role of the crop in the Ethiopian farming system.

Materials and Methods

Description of study area

The study was undertaken from July - November 2013 in four different agro-ecological zones of northwestern Ethiopia, namely; West Gojam, Awi, East Gojam and South Gondar. In each zone, the study covered one district; South Achefer from West Gojam, Fagta Lekoma from Awi,

Machakel from East Gojam and Dera from South Gondar. Districts were selected purposively based on their lupin production potential and representativeness in agro-ecology. The four zones approximately produce 95% of lupin production in the country (ECSA, 2015). The districts are known for high lupin production in Ethiopia since long time. Regarding soil types, South Achefer and Dera districts are mainly characterized by nitosols, while Fagta Lekoma and Machakel are dominated by acrisols and luvisols, respectively (Adet research center, unpublished data). Map of the study areas is provided in Figure 1.

Mixed crop-livestock production is the typical farming system in all the study districts. Crop production is dominant in agriculture as well as in the farming system and hence main means of livelihood in all the study districts. Livestock is also an important component and plays key role in the farming system. Crop and livestock production are highly integrated where livestock provides draft power and manure for crop production while crop production provides feeds and crop residues for the livestock sub-system.

Survey and sample design

In the study, a cross-sectional survey was designed to lead the data collection and analysis. A combination of probability random sampling and non-probability sampling methods were used. A multistage sampling was employed. Stage I- selection of districts: Districts were purposively chosen and stratified based on lupin production potential (high potential districts) and farming system. Stage II – selection of peasant associations (PAs): PAs were selected based on lupin production area in each district into two with respect to production area, above and below the

average of the district. The sample PAs were selected only from the above district average group. Stage III – selection of villages: Within each of the selected PA, villages were picked randomly.

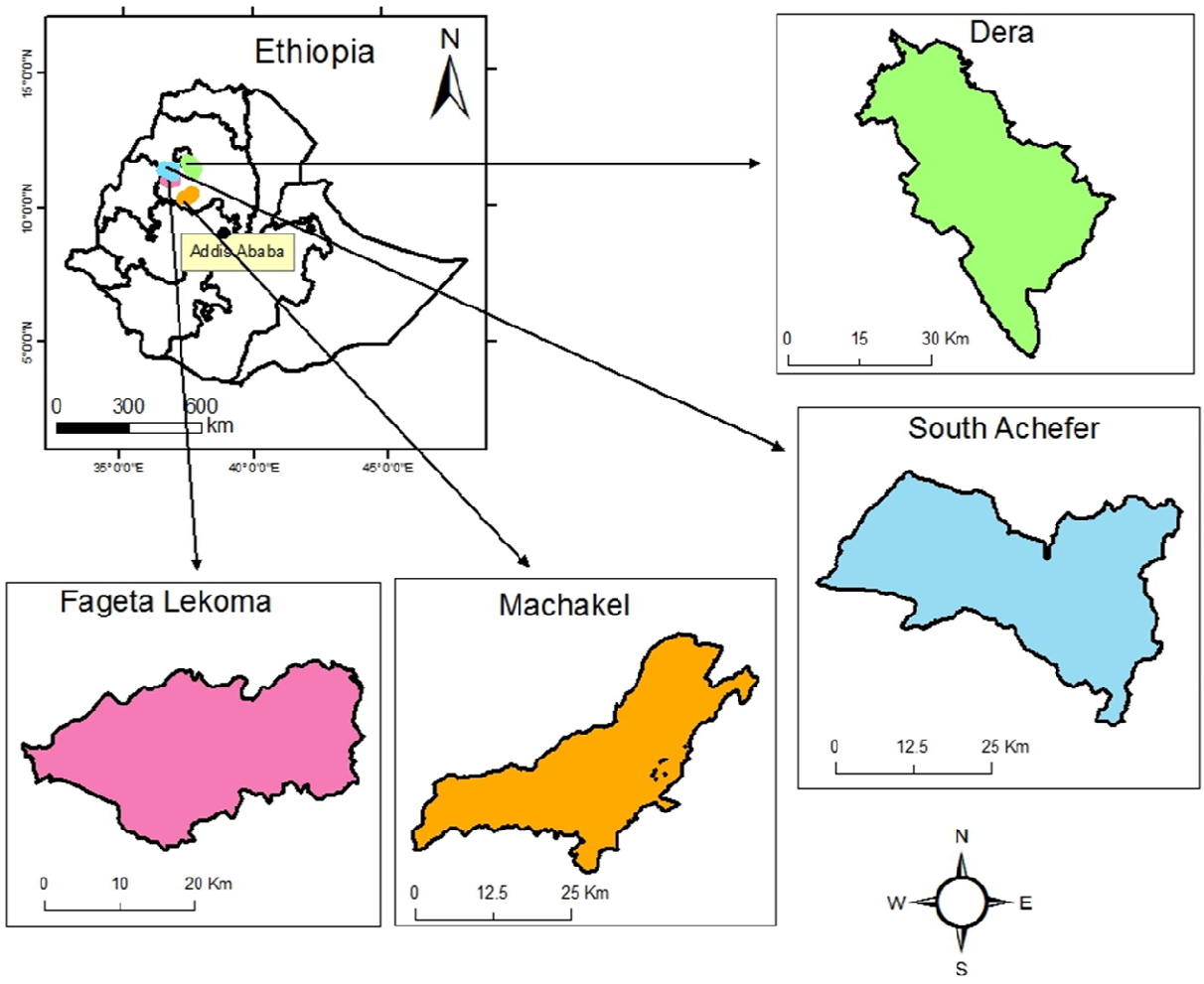

Figure 1: Map of study areas in northwestern Ethiopia.

Stage IV – selection of households: Using simple random sampling (https://www.random.org/), respondents were selected randomly from the already selected villages. The sample household size in each village was assumed to be proportional to the total household size of all selected villages. Female household heads were included proportionally in the sample. The sampling frame used to get the right sample size and type was an up-to-date list of households in the target areas. Ideally, the initial number of sample households in each village were slightly above the required number of sample households to easily replace some households who were not around or not willing to participate. Sample size was determined based on households population number and resource availability and hence 303 sample households were selected for this study across the four study districts.

Data collection and analysis

Data were collected using different techniques. First, secondary data were collected to get initial understanding of the farming system of selected areas through secondary sources, such as Central Statistics Authority, regional, zonal and district agricultural offices reports and consultation; and focused group discussions. For the formal survey, structured questionnaires were prepared, pre-tested and administered in randomly selected 303 household heads. Data on household and farmland characteristics; farmers' practices and constraints on lupin production, processing, utilization and marketing; cropping system experiences; and institutional support services including extension and marketing, were collected using a structured questionnaire. The questionnaire was administered by group of researchers selected from Pawe Agricultural Research Center of the Ethiopian Institute of Agricultural Research.

Trainings were given to the selected data enumerators to ensure that everyone understands each issue of the questionnaire in the same way.

The data collected from primary sources were coded and entered into a computer software, Statistical Package for Social Sciences (SPSS), version 20. The data were checked for consistence and completeness and analyzed using the same software. We employed descriptive statistics such as frequencies or percentages and ranges, cross-tabulations, means and ratios to analyze, summarize and present the data. Analysis was conducted by disaggregating the data by district to capture differences among the study districts. Comparisons were made using F-test (one-way ANOVA), and non-parametric tests including K-independent samples and Chi-square depending on the type of variables.

Results and Discussion

House hold and farm characteristics

This survey covered a total of 303 households, whereby the four study districts, Dera, South Achefer, Fagta and Machakel constitute 24%, 30%, 17% and 29% of the total households, respectively. The survey shows that 96% of the households were male headed and the rest were female headed. The proportion of female headed households (4%) found in this study is much lower than the national average, which is 17.6%. Heads of the household had an average of 41 years of age in both Dera and Machakel districts, 42 years in South Achefer and 43 years in Fagta Lekoma, the youngest head of household is 20 years and oldest head of household

being 82 years across all districts. However, the mean ages of the household heads are not significantly different among the districts ($P<0.05$). Slightly aged (mean of 47) farming populations reported in central part of the country (Asfaw *et al*., 2010). Household average size did not show significant difference among districts and the mean value ranges from 5.49 in Dera to 6.5 in South Achefer. These figures are higher than the regional (4.3) and national (5.2) averages (EPCC, 2008; Asfaw *et al*., 2010). On average, proportion of male and female household members are equal across all districts. This is in line with the national average sex proportion (EPCC, 2008). However, unequal proportions of male to female ratio reported in different parts of the country (Asfaw *et al*., 2010).

All respondents are exclusively Orthodox Christians (100%), and Amharic speakers except farmers from Fagta Lekoma who speaks Awigna in addition to Amharic. Result shows majority (95%) of the respondents are married with single wife, while few remains single, divorced and widowed. This situation is similar with a situation reported in central part of Ethiopia (Asfaw *et al*., 2010). Educational level of head of the household was significantly different among the districts, whereby 71% respondents of Dera, 68% of Machakel, 52% of Fagta and 37% of South Achefer did not attend any formal education, i.e. they are either illiterate or have basic adult education. On the other hand, 57% of South Achefer households, 42% of Fagta, 28% of Machakel and 25% of Dera, attended primary school education. Only 5% of the households, on average across the four districts, went to secondary school education. These illiteracy level is quite high especially in Machakel and Fagta that would adversely affect informed agriculture.

Survey result shows that majority of respondent households are primarily engaged in farming (99%) without significant difference among the districts, few are agricultural paid laborers, carpenter and petty traders. On average, four out of six family members of the households are involved in family farming either in full time or as part time which makes two third of the family members productive while only one third remains dependent. Farmers participation in agricultural group activities are significantly different between districts, whereby 96% respondents of Fagta, 88% of South Achefer, 85% of Machakel and 77% of Dera, are involved in agricultural group activities. Time needed to get agricultural related services significantly vary among the districts, in which farmers in Machakel need to walk for 38 minutes to get the service whereas farmers in Fagta and Dera took 29 minutes of walk. Similarly, access to farmers cooperative services varies among districts in terms of minutes of walking. Across the study area, farmers in Dera need to walk more (for about 53 minutes) to get cooperative services, whereas South Achefer farmers have got the service after 38 minutes of walk on average.

The survey result indicate that there are different forms of land tenure arrangements in the study districts where own farmland, shared-in and rented-in farmlands are the dominant ones in the farming system. Similar land tenures were reported in central parts of the country (Asfaw *et al*., 2010) and north western highlands (Teshome *et al*., 2014). Households own farmland vary between districts and ranges from 1.03 ha in Fagta to 1.6 ha in Machakel, whereas households owe 1.13 ha in Dera and 1.26 ha in South Achefer. These figures are smaller (average, 0.35 hectare) in the central part of Ethiopia (Asfaw *et al*., 2010). Crop farming is by far the dominant activity and means of livelihood across the study areas. Maize, tef and finger

millet are the major cereals produced in South Achefer, while wheat, tef, finger millet, barley and maize are common in Dera. Oat (*Avena* species) is very common in addition to wheat, tef and barley in Machakel, whereas potato is common next to tef in Fagta. Food legumes are key components of farming in the study areas. Faba bean, field pea and lupin are the major food legumes across the study areas though with varying scale of production, for example, lupin is very common in Fagta. Other food legumes, such as chick pea and grass pea are also important in the farming system.

Livestock on the other hand, are an integral part of farming and asset of the household. Common livestock species being rearing across the study areas are, cattle, sheep, donkey and poultry. In addition, horses in Fagta and mule in South Achefer are important. Nevertheless, total livestock holding on average by households which was quantified by Tropical Livestock Unit (TLU) vary among districts and range from 4.25 TLU in Dera to 6.52 TLU in Machakel, whereas households own 5.3 TLU at Fagta and 5.83 TLU at South Achefer. Yeheyis *et al.* (2010) reported similar figures at Mecha (5.62 TLU) and Sekela (4.25 TLU), north west Ethiopia. However, the livestock holdings are higher in Lume-Ejere (8.3 TLU) and Gimbichu (7.9 TLU) districts in central Ethiopia (Asfaw *et al*., 2010).

Lupin cropping experiences and practices

Lupin farming experience of farmers did not vary significantly among study districts and they have on average 16 years of experience as a household (Table 1a), otherwise experience of lupin farming in the area trace back to their fathers and fore-fathers. Average land allotted to

lupin is higher at Machakel (0.32ha) and Fagta (0.3ha) which are high altitude agro-ecologies in the study areas, while it is relatively small in mid-altitude agro-ecologies, Dera (0.23) and South Achefer (0.25). However, the proportion of land allotted to lupin compared to the household own land are similar, which is about 20% in the study districts except Fagta where it goes up to 29%. A study about white lupin in north west Ethiopia indicated relatively high proportion of land allocation to lupin at high altitude areas which are characterized as low fertile and degraded crop land (Yeheyis *et al*., 2010). The high proportion of allotted land to lupin in high altitude agro-ecologies might be associated with the understanding and experience of farmers about the two fold potential of lupin, viz., lupin strives well in these infertile and degraded crop lands better than any other crop and at the same time it has the potential to bring back the degraded land suitable for other crops production which otherwise couldn't be grown (Small, 2012; Schulze *et al*., 2006). Further, Small (2012) pointed out that given the growing need to produce human food in marginal lands with limited water availability and limited energy expenditure, lupin offers considerable promise.

The study result indicates that farmers across the study area have similar purposes of growing lupin, whereby substantial number of them (54.5%) explained that their lupin farming is targeted for food, fertility restoring value and to fetch cash from transaction of lupin grain and its processed products (Table 1a). The next, reasonably good number of farmers (34%) justify their lupin farming is not only for the three aforementioned lupin values, but also for livestock feed and medicinal value. The remaining respondents, growing lupin for food and fertility restoration (8.3%), fertility value (1.7%) and for food only (1%). Only two households (<1%)

explained that they are farming lupin for cash. The implication is that farmers are conscious about the multifaceted uses of lupin, from farm to kitchen and even its cash and medicinal values, while deciding lupin growing. Several researchers reported that legumes, including lupin, could offer multiple functions as mentioned above (Atnaf *et al*., 2015a, 2015b; Kassie, 2011; Yeheyis *et al*., 2010).

Frequency of growing lupin by households significantly vary between study districts (Table 1b). At Dera, large proportion of the respondents (78%) exercising lupin farming every year, and only the remaining 12% and 4% are doing lupin farming every other year and every third year, respectively. On the other end, at South Achefer, 32% , 42%, and 17% of the households are growing lupin, every year, every other year and every third year, in that order. About half (49%) in Fagta, and two third (64%) in Machakel, of the respondents are practicing lupin farming every year, whereas 39% and 21% of the households, in Fagta and Machakel, respectively, are growing lupin every other year. Only small proportion of the households in both Fagta (12%) and Machakel (15%) are doing lupin farming every third year. Generally, more than 85% of the farmers across the study districts had lupin farming activity either every year or every other year.

Farmers were interviewed about lupin cultivar they are using, and invariably 97% replied that they are using a local white lupin cultivar which they received mainly from their elderly, local market and fellow farmer (Table 1a). Only few (5 from Dera, 4 from South Achefer and 1 from Machakel) farmers explained that they are using two different types of lupin cultivars, viz., local white lupin cultivar and sweet lupin which is not white lupin, i.e., narrow leaved/blue lupin. The

present result is in contrary with the one reported by Jansen (2006) who claimed the presence of two types of cultivated white lupin (large seeded as produced in Egypt and Sudan, and small seeded type with small leaves) in Ethiopia. Report of sweet lupin cultivar by few farmers in Dera and South Achefer corresponds to the intervention report of introducing sweet blue lupin to these areas by CASCAPE project (Abebe *et al*., 2015). Farmers usually save their next seed from the present harvest. In the present study, it is revealed that about 98% of the respondent farmers save their own seed from their lupin grain production. The amount of seed they save vary both within the district and among districts. The average amount of saved seed range from 10 kg at South Achefer to 24 kg at Machakel, while the amount is 16 kg at both Dera and Fagta.

Farmers land preparation practice for lupin planting significantly vary among the study districts (Table 1b). The experience and practice in South Achefer is totally different, where 64% and 31.5% of the households, ploughing their land, four and three times, respectively for lupin planting. Only small portion of respondents (4.35%) ploughing land twice for lupin planting. On the other extreme, however, substantial number of households in Machakel (39%) and Dera (23%) do not plough their land even at once to sow lupin; and about 43% of the households in both districts ploughing their land for lupin planting only once. Nearly quarter (24.3%) of the respondents in Dera and only 9.2% in Machakel are practicing two times ploughing. The remaining 7% in both districts are ploughing 3-4 times. At Fagta, more than half (56%), and 34% of the households are doing one and two times ploughing, respectively. Only small number of the respondents, 8% and 2%, are practicing 3 and 4 times ploughing, respectively. According to farmers, the main reason, if not the sole, that farmers in South Achefer do more frequent

ploughing for lupin planting is not purposely for the advantage of the lupin to be planted, rather it accounts for the advantage of the succeeding cereal crop. Similar reasoning by farmers was reported in Mecha, a neighboring district to South Achefer, by Yeheyis *et al.* (2010).

Farmers experience and practice in lupin planting time have been found to be different both within the district and among the districts. About half of the respondents in Machakel (55%) and Dera (47%) explained that they are planting their lupin field early in the main growing season, i.e., in June. The next good number of farmers in both districts (37% in Machakel and 43% in Dera) are sowing their lupin seed in July. Only small number of farmers in both districts do planting in August, whereas 15% of farmers in Dera practicing late planting in September. Similarly, on late planting, 21% of South Achefer farmers are doing September lupin planting. Significant amount of lupin field in South Achefer are planted in July (50%) and August (49%). There is also some (23%) early planting in June in this district. In Fagta, however, majority of lupin planting (64%) are doing in August, while 24% and 14% do planting in July and June, respectively. Apart from the noted climatic difference among the study districts which directly dictate planting time, farmers perception about lupin and their engagement to other priority crops mainly cereals impact lupin planting time in a particular season. Two distinct planting times, main planting from June-August and late planting in September, are apparent in mid-altitude agro-ecologies, i.e., in South Achefer and Dera. Their purposes are a bit different where the late planting mainly targets for fertility restoring value for the next cereal crop, whereas the main planting targets for grain production could be for food and/or cash, and fertility maintenance.

The survey result revealed that farmers within the district and among study districts are using variable amount of lupin seeding rate for planting (Table 1b). In Dera, for example, the amount of seed used for planting per "timad" (common land unit by farmers, and is equivalent to a quarter of a hectare) ranges from 5-30 kg, while more than half (53.42%) and about 38% of the farmers, are using 15-20 kg/"timad" (60-80 kg/hectare) and 5-10 kg/"timad" (20-40 kg/hectare), respectively. The seeding rate varies (3 kg/"timad" - 50 kg/"timad") more in both Fagta and Machakel, where 40% of the households in Fagta and 31% in Machakel are using 5-10 kg/"timad" (20-40 kg/hectare), and 34% in Fagta and 25.3% in Machakel are exercising 15-20 kg/"timad" (60-80 kg/hectare). The remaining 22% of farmers in Fagta and 29.9% in Machakel are using 25-30 kg/"timad" (100-120 kg/hectare) seeding rate. At South Achefer, the trend is more close to farmers at Fagta, where 40%, 28% and 20% of the farmers are sowing 5-10 kg/"timad" (20-40 kg/hectare), 15-20 kg/"timad" (60-80 kg/hectare) and 25-30 kg/"timad" (100-120 kg/hectare) lupin seed, in that order. This result didn't clearly show association of agro-ecologies with amount of seeding rate which is in contrary with a previous report that showed farmers in higher altitudes use high amount of seeding rate of lupin (Yeheyis *et al*., 2010).

The majority (98.3%) of farmers across the study districts explained that they are broadcasting their lupin seed while sowing their lupin field. Only few (5 famers, 1.7%) are row-planting their lupin field. All of the farmers interviewed in all districts mentioned that they are not applying any chemical fertilizer to their lupin field. While they were asked why they are not applying chemical fertilizers, majority (93.6%) explained, they believe that fertilizer is not needed for lupin rather lupin recover the fertility of their degraded land. Yeheyis *et al.* (2010) reported

similar result where all interviewed farmers are not applying chemical fertilizers and broadcasting the seed while sowing lupin. This result is in agreement with previously documented scientific reports by several researchers who showed the potential of lupin that striving well in low fertile and degraded soils (Small, 2012; Schulze *et al*., 2006; Baer, 2005).

Farmers in all the study districts except South Achefer had similar experience of weed management of their lupin farm (Table 1b). More than 93% of farmers in the three (Dera, Fagta and Machakel) study districts do not weed their lupin field. Only few of the farmers (about 5-7%) are weeding lupin field in the three of study districts. However, more than half (56.5%) of South Achefer farmers weed lupin farm. These farmers who are weeding their lupin farm were asked about how often they do weeding, and majority (91%) mentioned that they are doing once, while the remaining 9% are weeding two times. On the other hand, the farmers who do not weed their lupin farm gave significantly different reasons between study districts. Majority (92.3% out of those who are not weeding) of South Achefer and about half (53.1%) of Machakel farmers mentioned that lupin suppresses weeds (as their reason of not to weed the lupin farm), while only about 30% at both Dera and Fagta mentioned the same reason. More than half (58.3%), about 42% and 32% of the farmers, in Fagta, Dera and Machakel, in that order, think that the weeds in the lupin field can be used as feed source. However, none of the farmers mentioned this reason in South Achefer. Few farmers in all the study districts had some other reasoning, including traditional thinking, and weeding lupin field does not pay.

Lupin harvesting time differed among study districts. This is directly associated with planting time differences and, climatic and edaphic differences of the study districts. The majority of

farmers (89%) in Dera and about 69.5% in South Achefer harvest their lupin crop in January, while, in Fagta and Machakel, both are high altitude agro-ecologies, harvesting is in February and March. Farmers use different maturity indicators to make sure that the lupin crop is ready for harvesting, viz., while leaves shade down, pods and stubble drying and becomes yellow, whole plant drying, "kua kuwu" sound while touching the plant. All farmers are using either big stick and animal power separately or a combination of both to thresh their lupin harvest. They have also similar practice and experience with the kind of material they are using to store the lupin grain. Majority are using local store called "gota" made of mud, and some use grain sacks.

Lupin cropping constraints

Farmers were interviewed and focused group discussions made about lupin cropping constraints, and ranges of production constraints were mentioned. Prevalence of different lupin diseases, infestation of insect pests and lack of improved varieties were among the top production constrains repeatedly mentioned by individual farmers and at the group discussion. According to farmers, "michi" or "wag" is the common disease to all study districts and is mainly characterized as drying of part or whole plant. This type of symptoms might be associated with common lupin fungal diseases, such as pleiochaeta root rot, brown leaf spot and phomopsis. Occurrence of these diseases in Ethiopia was reported by Atnaf *et al.* (2015b). However, further diagnosis and identification should be done to further characterize these diseases. Farmers in Machakel mentioned frequent infestations of their lupin crop by insect pest called "green-black- gray" worm. It mainly occurs at podding stage. This insect pest might be associated with Australian native budworm which was reported in Ethiopia (Atnaf *et al*., 2015b).

Table 1: Farmers' experience on lupin cropping explained as percentage or mean value depending on the type of measurement employed

(a). These variables were not significantly different among study districts and are analyzed and presented across study districts

Parameter	values/measurement	percentage	mean	range	Sig.
Experience of lupin farming	number of years		15.90	0 - 60	0.45
Purpose of growing lupin	food only	0.99			0.09
	fertility restoring only	1.65			
	cash	0.66			
	food & fertility restoring	8.25			
	food, fertility & cash	54.50			
	food, fertility, cash, medicinal & feed	34.00			
Status of growing different lupin cultivars	yes	3.30			0.11
	no	96.70			
Seed saved to next planting	yes	98.00			
	no	2.00			
Method of sowing	broadcasting	98.35			0.84
	row planting	1.65			
Use of fertilizer	yes	0.66			0.20
	no	99.34			
Reason for not applying	not needed	93.65			0.30
	not worthy	3.68			
	others	1.67			
Proportion of land allocated to lupin	ratio of land allocated for lupin to own land (%)		22.33	19-29	

(b). Agronomic variables that are significantly different among study districts and are presented separately for each study district

Agronomic parameters	Unit of measurement	Study districts					Sig.
		Dera	Fagta	Machakel	SA	Mean	
Seed rate	5-10kg/timad	38.36	76.71	31.03	43.96	38.21	0.002
	15-20kg/timad	53.42	34.00	25.29	30.77	35.22	
	25-30kg/timad	8.22	22.00	29.89	21.98	20.93	
	30-40kg/timad	0.00	4.00	13.79	3.30	5.65	
Weeding culture	yes	6.76	6.00	6.90	56.52	21.78	0.000
	no	93.24	94.00	93.10	43.48	78.22	
Land holding	in hectares	1.13	1.03	1.60	1.26	1.26	0.002
Land allotted to lupin	in hectares	0.23	0.30	0.32	0.25	0.28	0.003
Frequency of ploughing lupin	not at all	22.97	0.00	39.08	0.00	16.83	0.000
	once	43.24	56.00	43.68	0.00	32.34	
	twice	24.32	34.00	9.20	4.35	15.51	
	three times	8.11	8.00	3.45	31.52	13.86	
	four-times	1.35	2.00	4.60	64.13	21.45	
Frequency of growing lupin	every year	78.38	47.92	64.37	32.22	55.67	0.000
	every other year	16.22	39.58	20.69	42.22	29.00	
	every two years	5.41	12.50	14.94	16.67	12.67	
	every three years	0.00	0.00	0.00	5.56	1.67	
	every four years	0.00	0.00	0.00	3.33	1.00	

Note: Sig. = Significant test and was performed based on Independent Samples Kruskal Wallis one way ANOVA test; SA = South Achefer.

All farmers explain that the local cultivar they are using took on average about 180 days to mature and it is too long. Atnaf *et al.* (2015b) reported similar result that farmers cultivars as well as Ethiopian white lupin germplasm accessions in general took longer time to reach physiological maturity, 179 days on average. Moreover, early sown lupin usually being affected by diseases. In addition, the local cultivar has low uniformity in maturity and shattering problems which affect harvesting and would have negative consequences on grain yield. Some farmers were mentioning pod thorniness as constraint for lupin production especially it makes hand harvesting harder. Other production constraints mentioned by farmers include, threshing consumes more labor, frost, animal damage as lupin is usually late and left alone in the field and the areas are characterized by free grazing especially after crop harvest, hail storm, traditional belief that seeds/grain stored for two or more years could not be used for seed, land shortage and lack of awareness on lupin production packages. Yeheyis *et al.* (2012b) pointed out the importance of early maturing lupin cultivars compared to farmers late lupin cultivars in areas experiencing free grazing problems.

Farmers were requested to comment on what should urgently be done towards alleviating production constraints in lupin. Lack of improved varieties, and diseases and insect pests constraints were put as top production constraints that should be shortly targeted in all study districts. Farmers in Machakel gave more weight to diseases and insect pests whereas farmers in Dera gave more emphases to the lack of improved varieties. According to farmers, an improved variety should have a profile of high productivity, low alkaloids level, resistant to major lupin diseases and common insect pests and relatively early maturing. Farmers in all

study districts, except in Fagta, appreciated agronomical constraints, such as lack of optimum research recommendations for spacing/plant population, row planting and tillage practices.

Lupin in the cropping system

Farmers are usually wise and put different criteria to decide which legume crop should get into the rotation scheme. Among others, nitrogen fixing capacity, stubble (residue) yield and its utilization, extent of input requirement, drought adaptation/resilience, disease and insect pest resistance, tolerance to weed infestation and grain yield productivity, were considered by the farmers to comparatively choose among the legumes adapted and grown in the study areas. Faba bean, field pea and lupin are common legumes in the study districts, while chick pea and grass pea are also produced. Hence, comparison was made among the common ones. The comparison was based on the available production technologies of respective legumes, and in fact technological interventions have not been similar among these legumes. Ordinal measures (poor, average/fair, good and very good) of the mentioned criteria were used for the comparison.

According to farmers experience and judgment, majority of them gave good (by 48% of the respondents) to very good (46%) value of nitrogen fixing capacity for lupin, whereas faba bean and field pea have got average (44%) to good (42% faba bean, 37% field pea) nitrogen fixation capacity (Fig. 2). This farmers judgment and experience is in line with scientific results documented earlier where lupin can potentially fix and accumulate a total of 150 to 400 kg/ha per year nitrogen (Jansen, 2006; Takunov, 1999; Reeves *et al*., 1990), while faba bean up to 200

kg/hectare per year. Farmers evaluation gave priority to lupin (49% scored as good and very good) followed by field pea (42%) and faba bean (36%) for stubble yield. However, field pea (59% of respondents evaluated as good) followed by faba bean (48%) found to be better than lupin (26%) in residue utilization. Nevertheless, nearly all farmers fairly consider residues of three of the legumes as important for different purposes. More than 85% of respondent farmers judged lupin require minimal inputs, and about half of the respondents mentioned fair/average input requirement of field pea, whereas 60% of contacted farmers explained that faba bean uses more inputs than lupin and even field pea (Fig. 2).

Majority of participated farmers (80.5%) evaluated that lupin had good drought adaptation, whereas about 65% of the respondents disfavoring faba bean for drought adaptation. On the other hand, about 50% of the farmers gave fair/average for field pea drought adaptation capacity, while the other 50% judged as poor. This result is in agreement with previous scientific results reported where lupin could offer considerable human food in marginal lands with limited water availability (Small, 2012). The three legumes considered had comparable resistance/tolerance to diseases, where almost 50% of the respondents favor these legumes for resistance/tolerance to their respective diseases. However, still the other 50% of the respondents speak out that these legumes have disease problems. Farmers assessment on insect pest resistance of these legumes indicate that the legumes are susceptible, as confirmed by almost 65-70% of respondents. However, these legumes are found to be different in their weed infestation tolerance, where lupin is by far superior than both faba bean and field pea. About 97% of the respondents agree on lupin weed tolerance potential, whereas 70% and 40%

disfavoring the potential of faba bean and field pea to tolerate weed infestations, respectively. Conversely, about 60% and 30% of the respondents appreciating weed infestation tolerance of field pea and faba bean, respectively.

Among the respondents, 77% appreciate lupin had good grain productivity as compared to faba bean and field pea, where majority of farmers put both faba bean (51% of the respondents) and field pea (63%) as fair/average in their grain productivity. The productivity judgment by farmers in this study is a bit different than that documented by the Central Statistics Authority (ECSA, 2012; ECSA, 2013; ECSA, 2014; ECSA, 2015) where faba bean (1.8 tonnes per hectare) and field pea (1.4 tonnes per hectare) had slightly higher national as well as regional average productivity than lupin (1.1 tonnes per hectare). This could be attributed to the difference in research interventions on these legumes where quite a number of improved varieties of both faba bean and field pea have been developed and delivered to the farmers unlike lupin in which case there is no single improved variety available and delivered to farmers (Atnaf *et al*., 2015a).

Cognizant to the above analyses and comparisons, most farmers consider lupin as key rotational legume crop in the cropping system across all the study agro-ecologies. Thus, knowledge, experience and practices of farmers on value and role of lupin in farming and/or cropping systems were assessed. The frequency of putting lupin into the rotation greatly varies among the study districts, however, they all plant cereal following lupin.

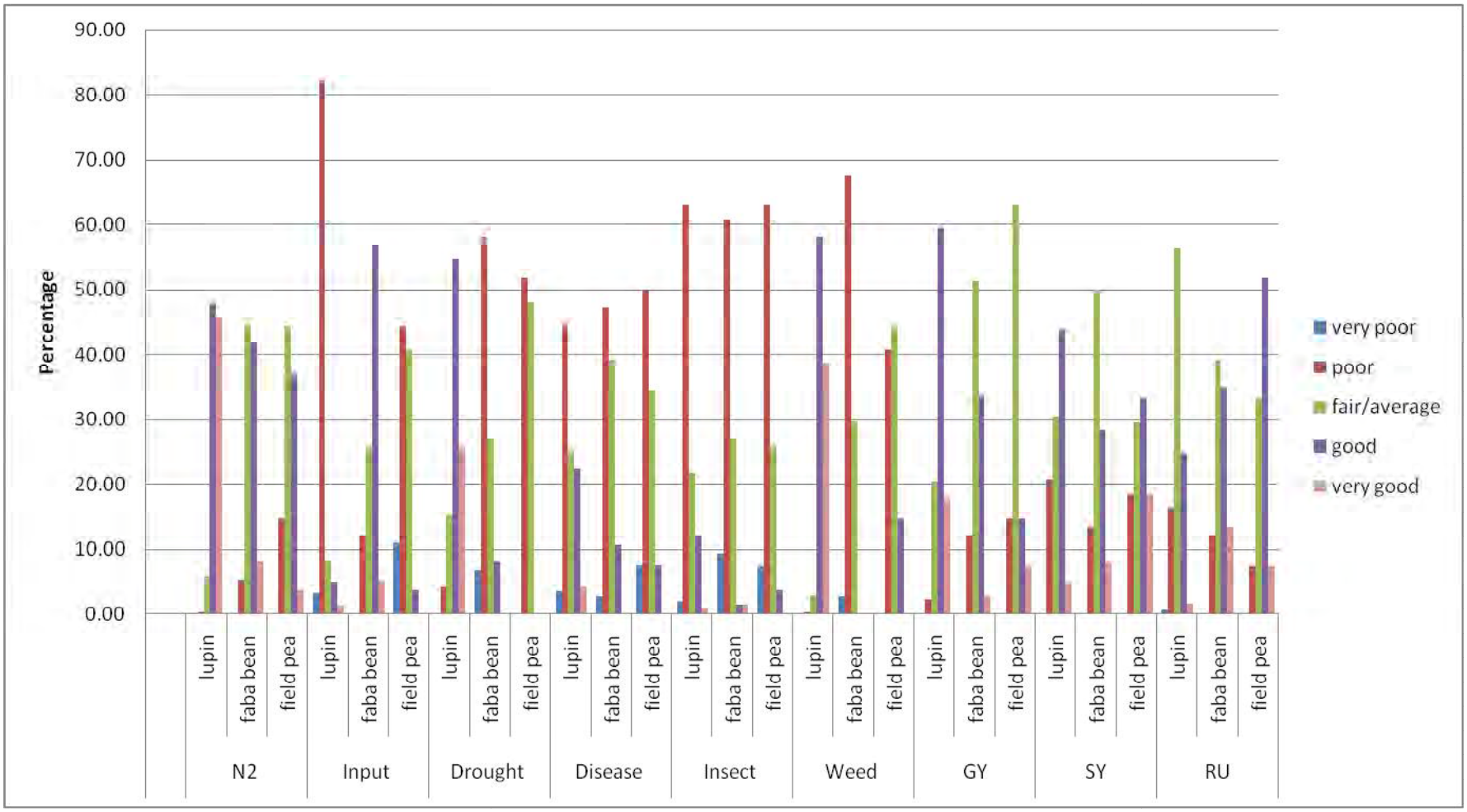

Figure 2: Comparisons of common legumes grown in the study area usually done by the farmers to choose among the legumes to put into the rotation scheme. Parameters considered are: N2= Nitrogen fixation capacity; Input = Input requirement; Drought= Drought adaptation/resilience; Disease= Disease resistance; Insect= Insect pest resistance; Weed= Weed tolerance; GY= Grain yield; SY= Stubble yield; and RU= Residue utilization.

At Fagta, more than half (56%) of respondent farmers put lupin every other year in the rotation and nearly 50% of the respondents at Machakel put lupin in the rotation in third and fourth year, whereas significant number of farmers both in Dera and South Achefer bring lupin back to the rotation in the fourth and fifth year. This difference in frequency of putting lupin into the rotation between highland agro-ecologies (Fagta and Machakel) and midland-agro-ecologies (Dera and South Achefer) might be due to the fertility status difference between these agro-ecologies (farmers' experience).

Farmers perceptions about the role of lupin in crop rotation were assessed, and the majority of the respondent farmers, 90% of Fagta, 81.6% Machakel, 79.7% Dera and 72.8% South Achefer, explain that lupin has fertility restoring value in the rotation (Fig. 3). About 14% at each district, except Fagta, where it is 8%, respondents mention that they put lupin in the rotation for both fertility restoring value and weed control; while, about 13% South Achefer, 7% Dera and 5% Machakel respondent farmers justify that lupin in the rotation play a role for fertility maintenance, weed control, and disease and insect pest build up/cycle break down. Moreover, farmers practice about lupin residue management was assessed, and about 37% of the respondents put the residue into the soil in different forms and ways. These farmers' perceptions about the role lupin plays in the rotation agree with previous reports documented by Yildiz (2011).

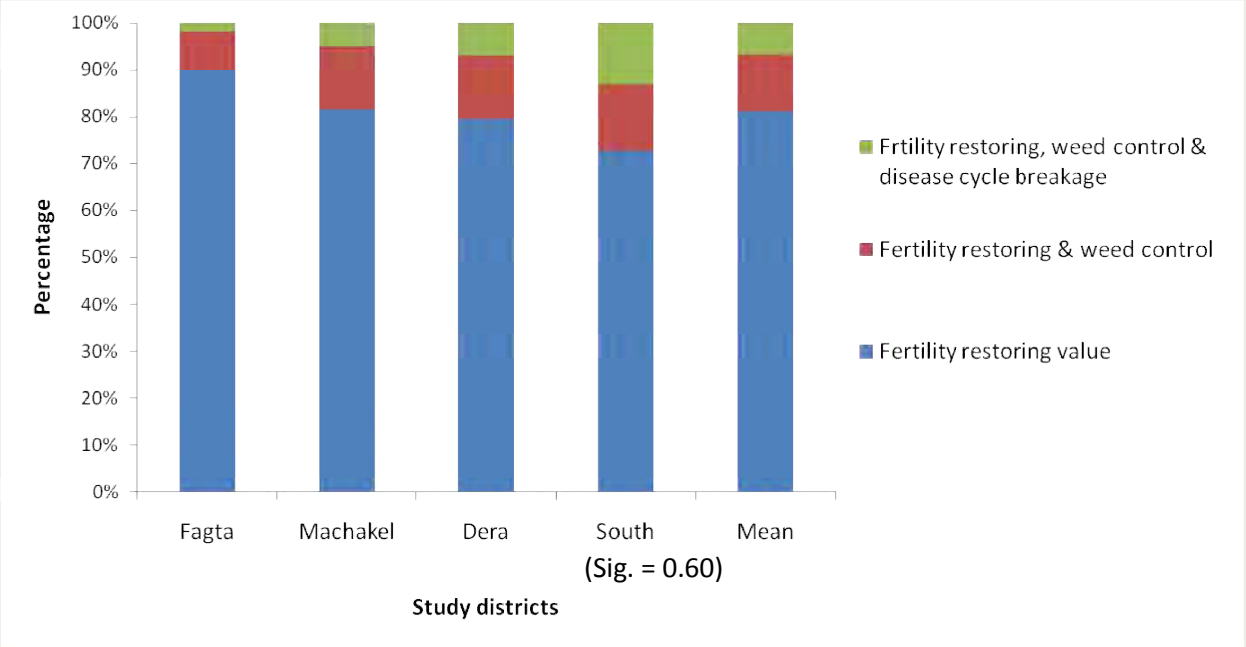

Figure 3: Farmers' perceptions on the role of lupin in crop rotation in north western Ethiopia. South= South Achefer.

In this study, farmers revealed that the rotation sequence varies among study districts and hence we provided rotation sequences of five years starting from lupin at each study district as follows, and the order among the crops within a year is based on frequencies of the crop being produced by farmers, i.e., the first being most produced and the last least grown by farmers in that particular year. Dera: Lupin -- wheat/millet/tef/barley -- tef/maize/millet/potato -- tef/lupin/millet/noug/linseed/maize/barley -- lupin/tef/wheat/maize/millet/barley. South Achefer: lupin -- maize/tef/millet -- millet/maize/tef -- maize/tef/millet/noug/lupin -- lupin/maize/millet/tef. Fagta: Lupin -- tef -- lupin/potato -- tef/maize/lupin -- lupin/potato/tef. Machakel: Lupin -- wheat/tef/barley -- oat/barley/lupin/tef/noug -- lupin/tef/wheat/oat/noug/barley -- lupin/tef/wheat/oat.

Lupin Intercropping practice by farmers varies among study districts, where more than half (58%) of the respondent farmers in Dera are exercising lupin intercropping whereas only 18% are practicing in Fagta. About 28% of the respondent farmers of both Machakel and South Achefer are experiencing lupin intercropping in the farming system. The crops with which farmers intercrop lupin differs among study districts. At Dera, they usually intercrop with noug (*Guizotia abyssinica*), maize, linseed, potato and sometimes with barley and finger millet. Oat (*Avena* species) and noug are the crops in which lupin intercropped with at Machakel, while maize, noug and finger millet at South Achefer. Noug is consistently companion crop with lupin in the intercropping practice of majority of the farmers. The present result is not in full agreement with a study conducted by Yeheyis *et al.* (2010) in two districts of north Western Ethiopia and reported very less practice (only 8% of the respondents) of intercropping lupin with other crops. Farmers associate the purpose of intercropping with risk minimization that would have happen due to natural disasters, i.e., hail, drought and pest outbreak, etc.

Lupin utilization and product development experiences and constraints

Almost all respondent farmers explain that their lupin farming is targeted for multiple purposes including for food, fertility maintenance and as cash. Moreover, some households consider the importance of lupin as medicinal and as feed for livestock. According to farmers, lupin is usually consumed in the form of snack, and used for making local alcoholic drink "Gebto arekie" as medicinal beverage. These products are very popular among the producers themselves and other consumers. The snack is commonly consumed by wealth challenged groups, and sometimes considered as food usually during food shortage times due to bad harvest in the

year. Most farmers agree that the lupin snack is among the foods usually consumed when a family faces food deficit. Moreover, snack preparation is highly associated with water availability, and hence relatively easy preparation and more consumption is usually common in summer where most rivers in the study districts get enough volume from rain water. Mainly on consumers side, however, the snack is becoming more popular in local drink "tella" houses and beer groceries, where the demand is becoming year round. Moreover, some women farmers mention their experience of using lupin grain for preparation of a local sauce called "shiro".

The local alcohol "Gebto arekie" is mainly demanded for its medicinal value especially to manage hypertension. The alcohol is getting popular and has got a brand name called "dembecha" from its nationwide users. The name came from the place where it is widely processed and marketed. It is produced by distilling a fermented brew prepared in the same way like other cereal-based alcoholic beverages, except that in this case flour of white lupin seeds are used as one of the major ingredients (Ambaye *et al*., 2002). In fact, seeds of lupin should go through debittering process before it is utilized for the alcohol preparation (personal communication, women processors).

All farmers repeatedly complain that bitterness in white lupin is the most persistent and outstanding constraint that limits lupin utilization and make processing and product development laborious and time taking. Yeheyis *et al.* (2011) reported high level of alkaloids in Ethiopian landraces. Farmers added, debittering is necessary rather mandatory to develop any product from lupin. For example, snack preparation involves roasting seeds on metal plate, soaking in water for about 5-8 days with repeated (1-3 times) washing (Yeheyis *et al*., 2010;

farmers' experience). Usually the processed seeds are spiced and made ready for eating. In addition to being laborious and time taking, farmers indicate additional challenges in the processing and product development value chain of lupin. These include, availability and accessibility of river water to soak the seeds, theft and take away by runoff while soaking in river water, lack of uniformity of the bitterness while debittering that might be due to non-uniform seed size which affects uniformity while roasting, and traditional belief that lupin will have negative effect if touched by hand while roasting for processing. Furthermore, farmers raise, lack of training to prepare different recipe and products, and lack of effective debittering options that do not require much labor and longer time. Yeheyis *et al.* (2010) pointed out the importance of effective debittering options especially to consider lupin as livestock feed. On the contrary, however, lupin plants employ alkaloids and other secondary products as defensive weapons against herbivores and microbial infections (Wink, 2011) as well as anti-bacterial, anti-fungal and anti-viral purposes for the lupin plant (Wink, 2008). Moreover, lupin alkaloids are important in pharmaceutical industry (Yildiz, 2011) and cosmetics products.

Farmers' experience about lupin as feed was assessed considering some parameters, such as which livestock species prefer to fed lupin, feeding potential of lupin, palatability and lupin residue as feed (Table 2). More than half of the respondent farmers (56%) replied that lupin is poorly palatable to livestock, whereas only 34% gave fair/average evaluation. Similarly, about two third of the respondents even do not believe on the potential of lupin as livestock feed. A previous study done by Yeheyis *et al.* (2010) documented similar farmers' perceptions about palatability and potential of lupin as livestock feed. Nevertheless, in this study about 38% of the

Table 2: Farmers' experience and evaluation of lupin as feed expressed as percentage

Parameters	Unit	district				Sig.
		Dera	Fagta	Machakel	SA	
Which animals prefer lupin	cattle	4.05	4.00	5.81	9.89	0.66
	sheep	13.51	10.00	6.98	8.79	
	goats	14.86	4.00	27.91	19.78	
	equines	2.70	42.00	4.65	0.00	
	not preferred by animals	17.57	4.00	16.28	26.37	
	sheep & goat	47.30	30.00	37.21	35.16	
	cattle and equines	0.00	6.00	1.16	0.00	
Lupin feeding potential	very poor	14.86	18.00	27.91	23.91	0.01
	poor	39.19	32.00	48.84	36.96	
	fair/average	36.49	38.00	23.26	33.70	
	good	9.46	12.00	0.00	5.43	
	very good	0.00	0.00	0.00	0.00	
Lupin palatability	very poor	8.11	0.00	18.39	14.13	0.001
	poor	31.08	30.00	54.02	53.26	
	fair/average	50.00	46.00	24.14	25.00	
	good	9.46	24.00	3.45	7.61	
	very good	1.35	0.00	0.00	0.00	
Lupin crop residue utilization	very poor	1.35	0.00	0.00	1.09	0.03
	poor	14.86	6.00	17.24	22.83	
	fair/average	60.81	54.00	55.17	55.43	
	good	18.92	40.00	26.44	19.57	
	very good	4.05	0.00	1.15	1.09	

Note: Sig. = Significant test and was performed based on Independent Samples Kruskal Wallis one way ANOVA test.

farmers agree on the feeding potential of lupin. Relative preference of lupin feed by livestock species was assessed, and small ruminants (sheep and goat) relatively prefer more than any livestock species. This result is in line with a previous scientific result documented by Urbaniak (1996) where he showed that sheep exhibit a higher tolerance to lupin alkaloids than monogastric animals. According to farmers, equines especially horses are the next species that prefer lupin feed. The parts of the lupin crop currently preferred by these animals are the finely threshed plant parts, mainly pod walls left after threshing.

Information and marketing

Agricultural information sources of farmers, access to lupin marketing and their lupin marketing activities were assessed. Majority of the farmers (92.5%) reveal that government extension and fellow farmers, mostly in combination and sometimes the former alone, are the main sources of agricultural information (including crop production, soil fertility and about crop protection measures) for the farmers, while some relies on radio (9.6%), extension materials (5%), and news paper and television (2%) as sources of the agricultural information (Table 3). These figures are significantly different among study districts. About 97% of the farmers mention that they had interactions with the government extension workers about farming during the last twelve months since execution of the survey work; and 89% of them visit the extension agents once per month to every week, while the remaining do interact once and twice a year. Farmers rely more on other fellow farmers (37%), followed by traders (30%) as source of information for grain market price including lupin than any other sources. Other sources mentioned were cooperatives (19%), television and/or radio (7%), and government extension (6%). Whereas,

mainly cooperatives (70%) and government extension (27%) for fertilizer price, and government extension (87%) for pesticide price, are sources of information that farmers are contacting while buying these inputs. Asfaw *et al.* (2010) documented somehow different results with the present study where they reported government extension and neighbor/fellow farmers are the major sources of (input) market information in three districts in central Ethiopia, whereas the present study showed government extension and farmers cooperatives are the major sources of (input) market information mainly for fertilizer and pesticides. The same authors reported neighbor/fellow farmer and government extension are the major sources of (output) market information in the order of importance, whereas the present study revealed that farmers rely more on other fellow farmers and traders in the order of importance for output market mainly for grain market information.

Results of this study revealed that farmers access to village and district market, and transport cost to the main market significantly vary among districts (Table 3). Availability of village mark*et al*so varies among districts, where 85% of the households at Dera, 55% at Machakel, 49% at South Achefer and 36% at Fagta had village markets. In fact unavailability of village market does not necessarily show difficulty to market access, because it might be due proximal availability of district market. This is evidenced by relatively less amount of time, which is 56 minutes at South Achefer and 78 minutes at Fagta, that farmers need to walk to get the district market, in contrary to much amount of time needed at Dera, 128 minutes. Farmers in three of the study districts (Dera, Fagta and Machakel) need to walk more than an hour (63 -66 minutes) to access the nearby village market, whereas those at South Achefer walk about 39 minutes for the

market access. For farmers having transport access to the district market, the transport cost range from 4.4 ETB at Fagta to 10 ETB in Machakel, while it costs 9 ETB and 5.4 ETB, in Dera and South Achefer, respectively. One of the key constraints in successful market participation by smallholders is the high transaction cost resulting from proximity to markets. Those smallholders residing at a far distance from the market tend to experience high transaction cost, which in turn reduces the incentives for market participation (Asfaw *et al*., 2010).

Interviewed farmers were divided into two almost equal groups with regard to availability of reliable lupin market, where 43% agree on availability whereas more than half (57%) mention that there is no available reliable market for lupin. One way ANOVA showed price of lupin grain varied significantly between study districts and mean price ranges from 2.51 ETB (Ethiopian birr) per kg at Dera to 3.42 ETB per kg at Machakel (Table 3) in 2013 cropping year. Individual price variations within districts were also appreciable, where it is higher at Machakel (range, 1.4 - 7.0) and squeeze down at Dera (range, 1.0 - 4.0). About 69% of the farmers believe that the current overall price of lupin is low and not fair, especially while they compare with other legumes they are producing; while, 31% of them considers the current price as fair and good. Pack animals (61.8%) followed by cart pulled by mule (25.7%) are the common means of transport used by the farmers to transport lupin grain to the market across the study areas except at South Achefer where significant (69%) number of the farmers use cart. Some farmers in all districts are also using head carry (10.6%) as means of transporting the grain to the market. Most farmers (77.6%) sell their lupin grain in the local market, while 15% of them do for outside trader. Some farmers consider other market places including assembler (5.3%).

Table 3: Sources and access of agricultural and market information for farmers, expressed as percentage or mean value depending on the type of measurement employed

Parameter	Unit	district				Combined	Sig.
		Dera	Fagta	Mkl	SA		
Main sources of agricultural information	Radio	1.35	6.00	6.90	4.40	4.64	0.002
	Gov't	39.19	40.00	56.32	42.86	45.36	
	TV	0.00	0.00	2.30	0.00	0.66	
	Ext. material	2.70	6.00	8.05	3.30	4.97	
	News paper	0.00	2.00	3.45	0.00	1.32	
	Fellow farmer	2.70	0.00	0.00	1.10	0.99	
	Gov't & farmer	52.70	44.00	20.69	36.26	37.09	
	Gov't & radio	1.35	2.00	2.30	12.09	4.97	
Interaction*	yes	94.59	100.00	94.25	98.91	96.70	0.124
	no	5.41	0.00	5.75	1.09	3.30	
Frequency of advice from government extension in the past 12 months	once / year	2.82	0.00	4.65	2.17	2.68	0.001
	twice / year	11.27	10.00	5.81	7.61	8.36	
	once / month	36.62	14.00	24.42	22.83	25.08	
	several times	39.44	36.00	33.72	46.74	39.46	
	every 15 days	1.41	6.00	3.49	5.43	4.01	
	every week	8.45	34.00	27.91	15.22	20.40	
Sources of output market (price of lupin grain)	Gov't	9.46	12.00	1.16	6.53	6.60	0.476
	Traders	31.08	24.00	33.72	29.35	30.13	
	Farmers	40.54	32.00	32.56	41.30	37.09	
	Radio/TV	1.35	8.00	12.79	5.43	6.95	
	Others	17.57	24.00	19.77	17.39	19.21	
Sources of input market (price of fertilizer)	Gov't	48.65	28.00	18.39	18.48	27.39	0.000
	Traders	1.35	0.00	0.00	0.00	0.33	
	Farmers	2.70	0.00	0.00	1.09	0.99	
	Radio/TV	0.00	0.00	0.00	3.26	0.99	

	Others	47.30	72.00	81.61	77.17	70.30	
Sources of input market (price of pesticides)	Gov't	75.68	95.83	90.80	85.87	86.38	0.004
	Traders	8.11	2.08	8.05	6.52	6.64	
	Farmers	2.70	0.00	0.00	1.09	1.00	
	Others	13.51	2.08	1.15	6.52	5.98	
Place where lupin were sold	Local market	77.08	71.43	81.36	79.17	77.63	0.466
	Assembler	8.33	0.00	6.78	5.56	5.26	
	outside trader	8.33	24.49	11.86	15.28	14.91	
	whole sealer	6.25	0.00	0.00	0.00	1.32	
	farmers coop.	0.00	4.08	0.00	0.00	0.88	
Availability of reliable market	yes	53.42	34.00	32.56	50.00	43.19	0.015
	no	46.58	66.00	67.44	50.00	56.81	
Common means of transport of goods to market	vehicle/car	0.00	4.00	0.00	0.00	0.66	0.000
	pack animal	79.45	60.00	87.36	24.18	61.79	
	cart	0.00	18.00	6.90	69.23	25.91	
	head carry	19.18	14.00	5.75	6.59	10.63	
	at farm gate	0.00	2.00	0.00	0.00	0.33	
	others	1.37	2.00	0.00	0.00	0.66	
Lupin market value	low	45.95	20.00	28.74	33.70	33.00	0.445
	not fair	16.22	56.00	51.72	26.09	35.97	
	fair	35.14	20.00	16.09	33.70	26.73	
	good	2.70	4.00	3.45	6.52	4.29	
Walking time*	minutes	63.57	65.83	66.25	39.33	58.28	0.006
Walking time**	minutes	128.77	78.67	86.09	56.30	86.13	0.000
Price of lupin	ETB per kg	2.5115	2.5918	3.4220	2.8542	2.8726	0.000

Mkl= Machakel; SA= South Achefer; Gov't= Government; Ext. material= Extension material; Interaction*= Interaction with an extension agent in the past 12 months; walking time*= Minutes of walking time to the nearest village market; Walking time**= Minutes of walking time to district/main market; and Sig. = Significant test and was performed based on Independent Samples Kruskal Wallis one way ANOVA test.

Gender aspects, and societal implications of lupin, Ethiopian context

It is noted above that on average, four out of six family members participate in family farming activities. Their participation in lupin farming value chain (production-utilization-marketing and revenue management) considering gender were assessed (Table 4). It was found out that there is division of labor for lupin land preparation, sowing, processing, and lupin sales revenue management, whereas both male and female are almost equally involving in harvesting and marketing of lupin grain, i.e., no division of labor for the two activities. The division of labor did not vary across study districts for the mentioned activities except for land preparation in which it greatly varies with districts.

In all districts, land preparation is mainly done by male, otherwise, by both male and female; it is not left for female alone. The participation of female family members in lupin land preparation, though together with their male counterparts, is higher (42%) at South Achefer and lower (18%) at Fagta. Sowing is mainly operational by male members (98%), while females are involving together with the males in some cases. About 93% of the participated farmers agree that there is no division of labor to harvest lupin, i.e., both male and female members do participate while harvesting lupin. Debittering and product development activities in lupin are highly skewed to female members, while involvement of males are also appreciable in soaking and washing activities in the process.

Marketing of lupin grain involves both male and female household members, however, marketing of end products from lupin essentially the snack and "Gebto arekie", are totally

operated by young female members of a household as witnessed by individual farmers and group discussions with processor female farmers. The income from sales are managed mainly by both the husband and wife (75%), while some do by wife alone (18%) and few (7%) say by the husband. Somehow similar result was reported by Yeheyis *et al.* (2010) on white lupin in north west Ethiopia where there is division of labor in lupin production and utilization activities. However, it disagrees with the present study in such a way that the present study revealed that there is no division of labor for harvesting and marketing of lupin.

It was clearly mentioned during focused group discussions in all study districts that white lupin are strongly associated with small scale farmers especially with the resource poor ones. Among the justifications indicated by farmers, the following were the major ones, all agree that lupin growing requires low input and hence it is grown by resource poor farmers. Lupin is among the few crops that are usually consumed when a family faces food deficit in the study areas. Marketing of the two most important end products from lupin, the snack and "Gebto arekie", are exclusively operated by female youths.

Table 4: Gender differentiations in white lupin value chain in Ethiopia expressed as percentage

		district					
Parameter	Unit	Dera	Fagta	Machakel	SA	Average	Sig.
Land preparation	male	72.22	81.63	70.59	56.52	70.24	0.016
	female	0.00	0.00	0.00	1.09	0.27	
	both	27.78	18.37	29.41	42.39	29.49	
Sowing	male	71.23	55.10	70.93	57.61	63.72	0.083
	female	1.37	0.00	0.00	2.17	0.89	
	both	27.40	44.90	29.07	40.22	35.40	
Harvesting	male	6.85	2.04	5.81	5.43	5.03	0.559
	female	0.00	0.00	2.33	2.17	1.12	
	both	93.15	97.96	91.86	92.39	93.84	
Processing	male	0.00	0.00	1.16	0.00	0.29	0.131
	female	69.86	67.35	56.98	53.26	61.86	
	both	30.14	32.65	41.86	46.74	37.85	
Marketing	male	8.22	2.04	3.49	3.30	4.26	0.977
	female	9.59	16.33	12.79	15.38	13.52	
	both	82.19	81.63	83.72	81.32	82.22	
Sales money handling	male	13.70	6.12	6.98	3.26	7.51	0.427
	female	17.81	18.37	16.28	19.57	18.00	
	both	68.49	75.51	76.74	77.17	74.48	

SA= South Achefer; Sig.= Significance

Chapter 3

Extent and Pattern of Genetic Diversity in Ethiopian White Lupin Landraces for Agronomical and Phenological Traits

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Extent and pattern of genetic diversity in Ethiopian white lupin landraces for agronomical and phenological traits

Abstract

White lupin (*Lupinus albus* L.*)* is one of the four economically important species of the *Lupinus* genus, and has been traditionally cultivated for several thousand years along the Nile valley, including in Ethiopia. An experiment comprising 143 Ethiopian white lupin landraces and one genotype from Germany was undertaken at Merawi in Ethiopia. The objective of the study was to cluster the Ethiopian white lupin accessions into similarity groups and assess the extent and pattern of diversity of the accessions. Data on 10 quantitative agronomic traits were recorded. Landraces were significantly different for most of the traits studied, and a significant number of local accessions performed as high as 5 metric tonnes per hectare of grain yield. Cluster analysis showed that landraces were grouped into seventeen clusters of different sizes, of which five were singletons. Some landraces were grouped together regardless of their geographic origin. On the other hand, landraces from Awi, South Gondar and West Gojam in Ethiopia were distributed over many clusters. Hence, the result did not support definite relationship between geographic diversity and genetic diversity. Genetic distances between many pairs of clusters were significant, justifying crosses between parents from them to be desirable genetic recombinations and, hence, transgressive segregants.

Keywords: Ethiopia, landrace populations, *Lupinus albus*

Introduction

White lupin (*Lupinus albus* L., Fabaceae) is one of the four economically important species of the *Lupinus* genus*,* which consists of over 300 annual species (Hondelmann, 1984). The other three agriculturally important species of the genus are: *Lupinus angustifolius, Lupinus luteus and Lupinus mutabilis.* Molecular evolution studies suggest that three of the four economic species originate from the Mediterranean, eastern and northern Africa regions; while the fourth important species, *Lupinus mutabilis* originate from the New World (Wolko *et al*., 2011). Lupins have an ancient history in agriculture that trace back to more than 4000 years (Kurlovich, 2002a). Though, its domestication first occurred in the Mediterranean and eastern Africa, a real breakthrough that made lupin a modern agricultural crop occurred in Australia and Europe (Clements *et al*., 2005). White lupin (2n=50) is widely known, commercially important, large seeded, annual lupin species in the world. It is a promising annual legume crop for human consumption, green manuring and forage. It has also substantial human nutrition and health importance (Hall *et al*., 2005a; Lqari *et al*., 2005; Johnson *et al*., 2006).

White lupin has been traditionally cultivated for several thousand years along the Nile valley, including in Ethiopia. It is locally known in Ethiopia as '*Gebto'*, and is mainly produced by small holder subsistence farmers around Lake Tana, the origin of the Blue Nile. Its production by small holder farmers in the area is targeted for grain and soil fertility maintenance values. According to the Ethiopian Central Statistical Agency (ECSA) (2013) report, 107,379 farmers cultivate lupin on a total area of 33,170.03 hectare; producing 36,880.77 metric tonnes of grain

in 2013 main cropping season. However, farmers production has not yet been supported by research and/or technology interventions (Yeheyis *et al*., 2010; Atnaf *et al*., 2015a).

Understanding of the genetic variation between and within populations is an important step for every management strategy directed towards the improvement and conservation of these populations (Xiao *et al*., 2004). More than 275 white lupin landrace accessions have been collected mainly from north western Ethiopia, including Gojam and Gondar, and have been conserved in the Ethiopian Biodiversity Institute. With the exception of some passport data, these accessions have never been phenotyped and/or characterized for important agronomic and phenological traits including grain yield.

Multivariate analyses are useful approaches to characterizing populations such as for white lupin, as it considers several agronomic parameters or traits simultaneously. Clustering takes a set of units into account and classify them into groups based on their observed characteristics. Principal components analysis is aimed at reducing the dimensionality. That is, it aims to find a smaller number of dimensions (usually 2 or 3) that exhibit most of the variation present in the data. This can help to identify the relative importance of individual traits. The objective of this study was to cluster the Ethiopian white lupin accessions into similarity groups and assess the extent and pattern of diversity of the accessions.

Materials and Methods

Germplasm

One hundred forty three Ethiopian white lupin landraces received from Ethiopian Biodiversity Institute (EBI), plus one sweet genotype from Germany, were used in this study. The landraces considered represent almost 50% of the total collections at EBI, Ethiopia. The collections were mainly from north western part of Ethiopia, including Gojam and Gondar (Fig. 1). There were few landrace accessions from south and north Ethiopia. Detail description of the landraces; and map of collection area of the accessions are provided in Appendix table 1 and Figure 1, respectively. The landraces were phenotyped at Merawi (11^{o}42'N, 37^{o}17'E) during 2013/2014 cropping season, with supplemental irrigation. Merawi, with an altitude of 1960 meters above sea level, receives 1576.55 mm mean annual rain fall and its soil is characterized by nitosols with pH range of 4.8 - 5.5 (Yihenew, 2002).

Experimental design, field management and data collection

The trial was laid down in a 12 x 12 simple lattice design. A plot consisted of two rows, 2.5 meter long, with a spacing of 75 cm between rows and 25 cm between plants was used. No fertilizer was applied to the crop. Agronomic and plant protection practices were applied uniformly across plots for the duration of the experiment. Data were collected either on plot basis or plant basis. Grain yield was collected in a plot and later converted to metric tonnes per hectare, 100 seed weight, number of days from emergence to 50% flowering and 75% physiological maturity were determined on plot basis; whereas, number of pods per plant and

seeds per pod, plant height, pod length and diameter, and number of branches on main axis were recorded on plant basis. Plant data were assessed on five plants randomly taken from each plot.

Data analysis

The data were checked for outliers and normality of residuals using Breeding View of Breeding Management System, before proceeding to analysis (BMS, 2015). Adjusted mean values (best linear unbiased estimators/ BLUE) for all the traits for further analyses were also generated using the same software. Mean trait data were standardized to mean zero and unity variance in order to minimize biases due to differences in scales of measurement. Multivariate analyses such as Cluster Analysis and Principal Component Analysis, were used. The Principal Components Analyses were meant to identify large contributing traits to the total variation among the populations. Nonhierarchal, and hierarchal clustering of accessions based on the Average Linkage Method were performed using SAS (SAS, 2004), and GenStat software (GenStat, 2013), respectively. Statistics, pseudo F statistic and pseudo t^2 statistic generated by SAS were examined to decide the number of optimum clusters.

Genetic distances between clusters as standardized Mahalanobis's D^2 statistics were calculated as:

$$D^2ij= (X_i - X_j)\, s^{-1}\, (X_i - X_j)$$

Where D^2_{ij} is the distance between cases i and j; x_i and x_j are the vectors of the values of the variables for cases i and j; and s^{-1} is the pooled within groups variance-covariance matrix.

The D^2 values obtained for pairs of clusters were considered as the calculated values of Chi-square ($\chi 2$) and were tested for significance at (1% and 5%) probability levels against the tabulated value of $\chi 2$ for 'P' degree of freedom, where P is the number of parameters considered (Singh and Chaudhary, 1985).

Principal components based on correlation matrix, and Euclidian distances were calculated using GenStat software. One of the major reasons that analyses of principal components shall be based on correlation matrix was to standardize each variate (by subtracting its mean and dividing by its standard deviation), which is very useful as the parameters considered in this study did not share a common scale of measurement. Principal components having Eigen value greater than one was considered as significant and presented in the result.

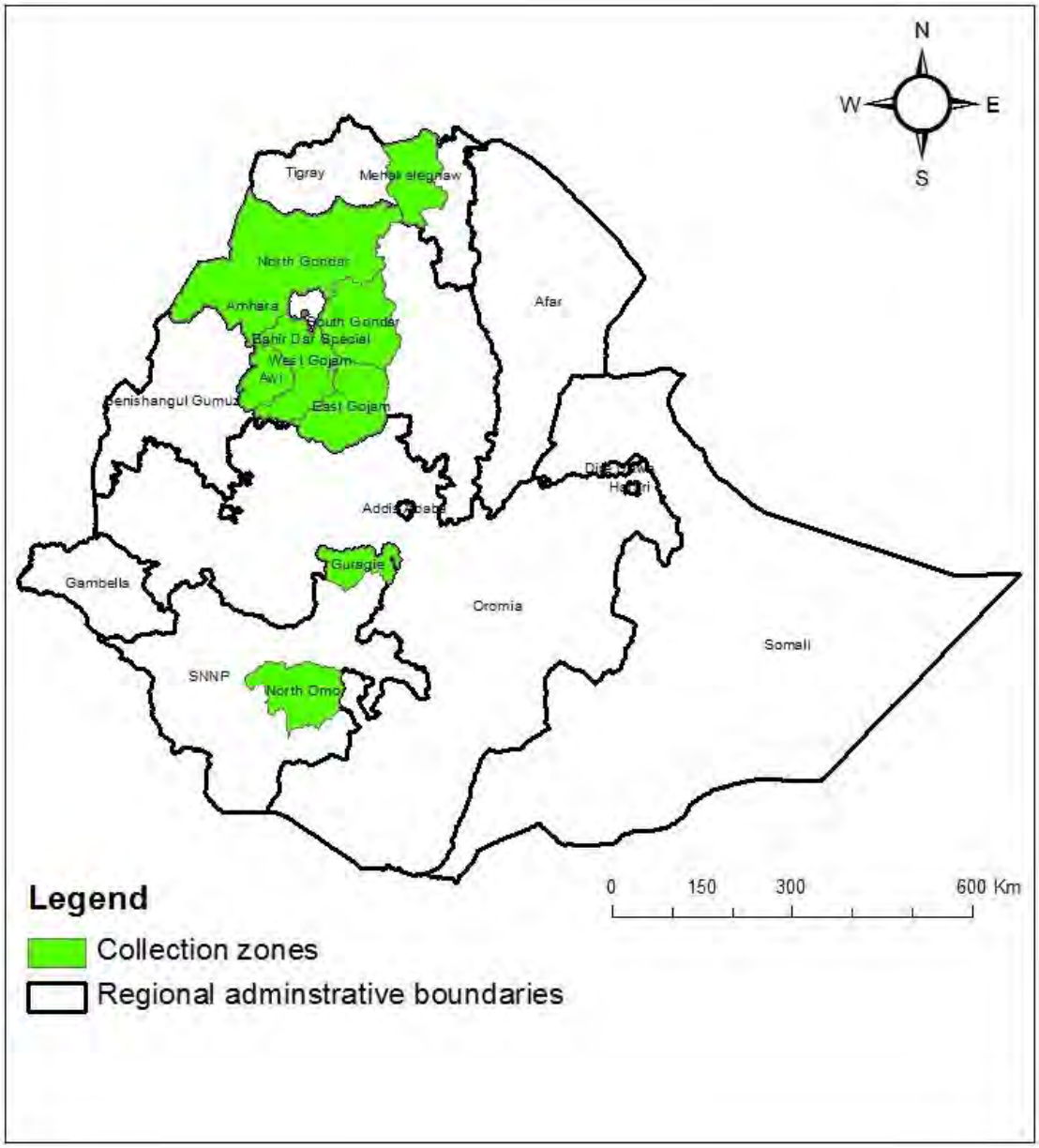

Figure 1: Map of collection areas of white lupin accessions which are provided in Appendix table 1.

Results

Landraces were significantly different among themselves for most of the traits studied at genotypic and phenotypic levels (variances not shown). The performance of the landrace accessions phenotyped showed that there was a significant number of white lupin landraces which performed as high as 5 metric tonnes per hectare grain yield (Appendix table 2). A sweet narrow leafed lupin (*Lupinus angustifolius*) genotype, introduced from Germany, called Sanabor (Acc144) performed the least (1.01 tonnes per hectare) for grain yield; worse than the least performed local landrace, Acc57 (2.66 tonnes per hectare). The landraces in general were late maturing, i.e. took a mean of 179 days to maturity. The earliest local accession (Acc12) took 168 days to mature, which is still long time. However, Sanabor (Acc144) was the earliest and took 131 days to maturity.

Different lupin diseases such as lupin rust, pleiochaeta root rot , brown leaf spot and phomopsis occurred at different pathogenic level on the local accessions. The severity of rust was scored using 1-9 scaling, and some level of variability in resistance/tolerance of the local accessions were observed (data not shown). In general, however, the local accessions showed moderate resistance to lupin rust. Australian native budworm was observed in the present study at podding stage on the local accessions.

Cluster analysis

The landraces were grouped into 17 clusters (Table 1; Fig. 2). Twelve of the seventeen clusters comprised of more than one landrace accessions; whereas five clusters were singletons (each

containing single accession). The first three clusters contained 100 (70%) accessions out of the total landraces considered. Cluster I contained 78 accessions (54%) out of 144. It was followed by clusters III, V, and II containing 12, 11, and 10 accessions, in that order. Clusters XIII, XIV, XV, XVI, and XVII contained one accession each; whereas the other 8 clusters consisted of accessions ranging from 2 – 7.

Cluster I was mainly (about 90%) constituted by accessions from three bordering zones of Gojam namely: West Gojam, Awi and Bahir Dar Zuria. Among these, more than 56% were from West Gojam (Table 2). Cluster VII consisted of accessions from a single origin, East Gojam. Similarly, cluster X was made of mainly accessions from South Gondar. The four accessions that did not group and form four separate clusters were Acc10 (Cluster XIII), Acc3 (cluster XIV), Acc116 (cluster XV), and Acc20 (XVI). Three of these accessions (Acc10, Acc3, and Acc20) were collected from the same origin, Awi. The genotype from Germany (Acc144), Sanabor, did not group with any local accessions and was put in a separate cluster, XVII.

Table 1: Grouping of the 143 Ethiopian white lupin landraces into different diversity clusters

Cluster number	Number & % of accessions	Accessions grouped	Origins
I	78(54.17 %)	Acc141, Acc80, Acc119, Acc99, Acc2, Acc5, Acc112, Acc24, Acc25, Acc61, Acc66, Acc36, Acc69, Acc48, Acc127, Acc60, Acc114, Acc7, Acc19, Acc63, Acc29, Acc41, Acc101, Acc137, Acc21, Acc67, Acc109, Acc87, Acc102, Acc42, Acc65, Acc64, Acc82, Acc140, Acc38, Acc76, Acc97, Acc143, Acc50, Acc85, Acc68, Acc74, Acc79, Acc104, Acc26, Acc35, Acc4, Acc34, Acc78, Acc37, Acc71, Acc95, Acc47, Acc92, Acc88, Acc44, Acc81, Acc108, Acc40, Acc94, Acc96, Acc28, Acc9, Acc100, Acc17, Acc51, Acc75, Acc12, Acc27, Acc39, Acc70, Acc86, Acc14, Acc62, Acc90, Acc11, Acc72, Acc131	West Gojam, East Gojam, Awi zone, Bahirdar zuria, South Gondar, North Gondar & Mehakelegnaw
II	10(6.94 %)	Acc23, Acc59, Acc133, Acc142, Acc111, Acc16, Acc135, Acc93, Acc6, Acc132	West Gojam, East Gojam, Awi zone, South Gondar, North Gondar & North Omo
III	11(7.64 %)	Acc22, Acc31, Acc126, Acc33, Acc113, Acc128, Acc125, Acc46, Acc91, Acc32, Acc103	West Gojam, East Gojam, Awi zone & South Gondar
IV	7(4.86 %)	Acc117, Acc118, Acc115, Acc54, Acc110, Acc56, Acc122	East Gojam & South Gondar
V	11(7.64 %)	Acc134, Acc58, Acc52, Acc98, Acc106, Acc15, Acc49, Acc138, Acc139, Acc83, Acc8	West Gojam, East Gojam, Awi zone, South Gondar & Gurage
VI	3(2.08 %)	Acc13, Acc89, Acc84	West Gojam & Awi zone
VII	3(2.08 %)	Acc123, Acc124, Acc107	East Gojam
VIII	2(1.39 %)	Acc130, Acc136	North Gondar & North Omo
IX	3(2.08 %)	Acc120, Acc121, Acc18	West Gojam & Awi zone
X	5(3.47 %)	Acc43, Acc57, Acc55, Acc45, Acc73	South Gondar & West Gojam
XI	4(2.78 %)	Acc129, Acc30, Acc1, Acc53	East Gojam, Awi zone & South Gondar
XII	2(1.39 %)	Acc105, Acc77	West Gojam & East Gojam
XIII	1(0.69 %)	Acc10	Awi Zone
XIV	1(0.69 %)	Acc3	Awi Zone
XV	1(0.69 %)	Acc116	East Gojam
XVI	1(0.69 %)	Acc20	Awi Zone
XVII	1(0.69 %)	Acc144	Germany

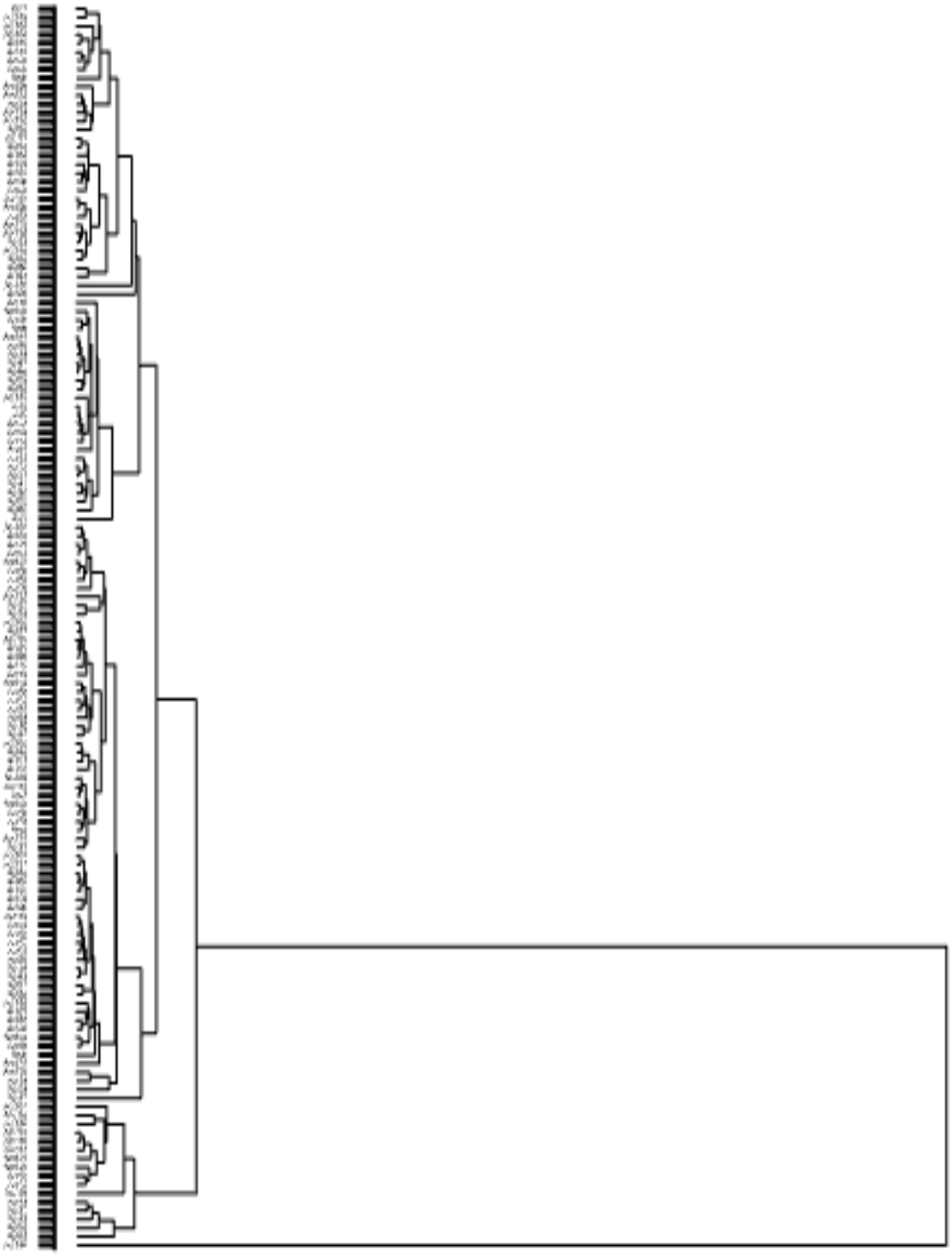

Figure 2: Dendrogram of 143 Ethiopian white lupin accessions and one genotype from Germany based on average linkage hierarchical cluster analysis between groups (Table 1).

Table 2: Clustering pattern of Ethiopian white lupin landraces from different origins over 17 clusters

Origin	Number of Accessions	Number of accessions in each clusters																
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
West Gojam	56	44	1	2	-	3	2	-	-	2	1	-	1	-	-	-	-	-
East Gojam	21	3	1	5	5	1	-	3	-	-	-	1	1	-	-	1	-	-
Awi zone	31	16	3	2	-	3	1	-	-	1	-	2	-	1	1	-	1	-
Bahir Dar Zuria	10	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
South Gondar	17	3	2	2	2	3	-	-	-	-	4	1	-	-	-	-	-	-
North Gondar	3	1	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
North Omo	2	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Gurage	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Mehakelegnaw	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Germany	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total	144	78	10	11	7	11	3	3	2	3	5	4	2	1	1	1	1	1

Cluster trait performance is presented in Table 3. Clusters I, II, and III were not significantly divergent. These clusters accessions characterized as good yielders, relatively early to flower and mature, large seeded, large number of pods per plant; medium plant height, pod length and diameter, and number of branches on the main axis. Among the singleton clusters, cluster XIV contained an accession (Acc3) with the highest yield and relatively short plant height, large seeded, and large number of pods per plant. On the contrary, cluster XV having Acc116 was characterized by lowest yielder and small seeded. The other singleton cluster XVI contained one accession with the highest number of pods per plant, large seeded and average in grain yield.

Pair-wise generalized squared distances (D^2) among the seventeen clusters are presented in Table 4. There were 136 possible pair-wise genetic distances between any two clusters. Among these, only 9 genetic distances (between clusters I and II, I and III, I and IX, II and III, III and V, III and VI, IV and XI, V and XI, and X and XI) were not significant ($p>0.05$). The remaining genetic distances were significant ($p<0.05$), to highly significant ($p<0.01$). The maximum distance was found between clusters XVI and XVII ($D^2 = 1697$), and the distances between any cluster and cluster XVII (the cluster containing Sanabor) were maximum and very highly significant ($p<0.01$).

Table 3: Cluster mean for 10 characters in Ethiopian white lupin landraces

Par	Cluster																	
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	GM
DF	78.76	80.12	80.10	86.05	84.77	80.52	91.30	86.46	80.00	92.17	89.74	86.99	85.18	82.56	87.49	92.64	62.95	81.2
DM	176.3	180.9	180.5	188.6	182.0	177.8	189.3	190.3	175.9	187.3	183.8	176.5	181.3	173.0	192.4	177.1	131.5	178.9
PH	133.0	124.5	133.8	129.0	137.6	123.8	126.8	146.9	129.9	124.7	132.8	119.7	129.7	123.6	128.3	136.3	48.26	131.3
BR	7.79	7.34	8.55	8.76	8.84	9.47	8.42	8.59	7.93	10.23	9.13	9.37	7.79	9.69	10.37	7.71	10.37	8.2
PL	8.82	8.90	8.45	8.22	8.79	8.86	8.18	8.60	8.67	8.55	8.70	9.02	8.66	9.01	7.31	9.31	4.91	8.7
PD	6.56	6.75	6.27	6.31	6.50	6.61	6.18	6.60	6.79	6.51	6.33	6.84	7.15	6.79	5.83	7.18	**	6.53
PN	83.27	84.90	97.51	84.85	88.53	83.58	89.56	91.92	65.23	83.64	86.56	74.75	99.47	92.20	86.20	102.8	25.80	84.75
SN	5.44	5.26	5.25	5.14	5.30	5.07	5.00	5.60	5.23	5.08	5.40	5.25	5.80	5.70	5.20	5.40	4.30	5.35
SW	32.26	31.08	30.54	28.31	31.50	32.97	25.94	29.86	35.89	30.99	29.50	35.93	32.87	33.27	24.44	34.40	17.93	31.53
GY	4.88	4.26	4.64	3.62	4.23	3.98	3.20	3.73	4.47	2.96	3.65	3.96	4.87	5.79	2.71	4.53	1.01	4.48

Par=Parameter; DF=Days to 50 % flowering; DM=days to 75 % physiological maturity; PH=Plant height in centimeter; BR=Number of branches on the main axis; PL=Pod length in mil meter; PD=Pod diameter in mill meter; PN= Number of pods per plant; SN= Number of seeds per pod; SW=100 seed weight in gram; GY=Grain yield in tones per hectare; GM=Grand mean of a given trait/parameter **=missed value.

Table 4: Intra (bolded diagonals) and Inter cluster distance between Ethiopian white lupin landraces categorized in to 17 clusters

CL	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
I	**1.23**																
II	13.56	**5.33**															
III	11.67	13.60	**5.14**														
IV	61.47**	35.32**	35.04**	**6.05**													
V	25.22**	22.93*	17.11	20.46*	**5.14**												
VI	22.18*	21.10*	16.04	42.48**	20.34*	**7.74**											
VII	128.6**	83.36**	87.34**	20.20*	54.16**	97.84**	**7.74**										
VIII	67.95**	53.28**	52.90**	21.32*	21.32*	66.56**	46.96**	**8.55**									
IX	12.86	26.47**	29.06**	62.63**	25.82**	21.70*	128.3**	68.53**	**7.74**								
X	129.5**	97.41**	96.24**	31.07**	45.95**	76.02**	25.46**	46.44**	107.7**	**6.72**							
XI	70.19**	53.34**	52.13**	17.13	14.17	50.53**	24.41**	19.48*	65.07**	15.75	**7.17**						
XII	51.86**	49.06**	52.61**	51.46**	22.01*	26.88**	83.05**	62.75**	30.86**	42.79**	28.50**	**8.55**					
XIII	31.73**	28.27**	30.43**	43.48**	22.79*	51.66**	81.80**	36.74**	42.95**	85.66**	40.00**	45.31**	**0.00**				
XIV	31.80**	60.73**	44.39**	102.5**	54.83**	48.57**	174.1**	116.8**	47.46**	151.0**	95.20**	59.18**	48.63**	**0.00**			
XV	153.5**	120.7**	102.3**	33.69**	88.17**	107.5**	49.35**	65.43**	152.9**	56.28**	65.22**	129.2**	126.4**	194.2**	**0.00**		
XVI	120.9**	108.4**	113.5**	105.0**	62.66**	121.2**	89.37**	87.83**	114.4**	83.35**	53.70**	61.96**	64.70**	138.6**	217.2**	**0.00**	
XVII	1252**	1278**	1213**	1341**	1364**	1151**	1437**	1563**	1270**	1438**	1420**	1324**	1494**	1302**	1269**	1697**	**0.00**

CL=Clusters; * & ** = Significant at 0.5 and 0.1 alpha levels, respectively.

Principal component analysis (PCA)

The first three principal components were found to be significant (Eigen value greater than 1) and accounted for about 75% of the total variation (Table 5).

Table 5: Eigen vectors, explained variance, and Eigen values of the first significant 3 Principal components for 10 parameters of 143 Ethiopian white lupin landraces

	Eigen Vectors		
Parameter	PCA1	PCA2	PCA3
Number of branches on the main axis	-0.249	0.342	0.111
Days to flowering	-0.051	0.547	0.196
Days to maturity	0.094	0.544	-0.030
Grain Yield	0.405	-0.264	-0.196
Pod diameter	0.395	0.167	0.379
Plant height	0.370	0.216	-0.281
Pod length	0.424	0.055	0.269
Number of pods per plant	0.228	0.339	-0.480
100 seed weight	0.350	-0.129	0.523
Number of seeds per pod	0.339	-0.097	-0.334
Eigen value	3.823	2.599	1.046
Explained variance %	38.230	25.990	10.460
Cumulative variance	38.230	64.220	74.680

PCA=Principal component analysis

The first PCA component explained 38% of the total variance, and the first and second PCA components accounted for 64% of the variation. Parameters that contributed relatively more with an Eigen vector value (0.424 - 0.339) for the first PCA, were grain yield, pod length, pod diameter, plant height, 100 seed weight, and number of seeds per pod. Thus, this PCA was associated with yield and architectural traits of lupin. Most of the variations accounted to the second PCA were contributed by two phenological traits, days to flowering and maturity; and hence, this PCA was associated with growth duration of the accessions. The third PCA explained about 10.5% of the variation, and yield component traits such as 100 seed weight and number of pods per plant contributed much of its variation.

Discussion

Presence of significant genetic variation among Ethiopian white lupin landraces and performance of significant number of landraces as high as 5 metric tonnes per hectare grain yield indicates a huge available genetic potency in terms of grain yield that could easily be exploited through breeding and selection. A similar result was reported by Christiansen *et al*. (2000), from Egypt, where they showed the importance of landraces for breeding. Another report by Gonzalez-Andres *et al.* (2007) showed variability of Spanish white lupin local accessions for grain yield and its components. Some level of variability in resistance/tolerance of the local accessions were observed for lupin rust (data not shown). In general, however, the local accessions showed moderate resistance to lupin rust. Occurrences and importance of

those white lupin diseases, and resistance breeding achievements have been reported in different parts of the world including Australia (Thomas, 2003; Thomas *et al*., 2008; Luckett *et al*., 2009).

The performance of Sanabor (The genotype introduced from Germany) was the least (1.01 tonnes per hectare). However, a completely contradictory grain yield performance was reported by Yeheyis *et al.* (2012b) where Sanabor performed better than our landraces. Nevertheless, the authors considered a single local accession that could not represent the available huge diversity in the country. In any case, however, Sanabor could not perform comparably for grain yield with those local accessions performed as high as 5 tonnes per hectare.

Presence of highly significant variation in maturity between Sanabor and the Ethiopian landraces suggests that the former could be used as a source of genes for earliness to improve late maturing Ethiopian landraces. However, fertile segregating populations and/or hybrids under natural conditions from inter-specific crossing between the two populations (landraces from *Lupinus albus* and Sanabor from *Lupinus angustifolius*) were not so far reported except fertile F1 and F2 plants (Kurlovich and Kartuzova, 2002; Clements *et al*., 2008).

In this study, landraces were grouped into 17 diverse clusters containing significantly different numbers of landraces, which ranged from 1 to 78. About 90% of the landraces constituted the largest cluster (Cluster I) were from three bordering zones of Gojam namely: West Gojam, Awi, and Bahir Dar Zuria. This result indicates that there might have been exchange of seeds, and

seed trade between farmers, and gene flow across boundaries of those areas (Forsberg *et al*., 2015). On the other hand, accessions from non-bordering origins were entirely constituted by a particular cluster such as landraces from South Gondar and East Gojam constituent cluster IV, and those from North Gondar and North Omo form cluster VIII. Gemechu *et al*. (2005) reported similar result on local field pea and faba bean accessions in Ethiopia. One possible reason among others could be, the landraces might have been introduced from a similar source.

Landraces from the same origin were not all grouped into the same cluster, except accessions from Bahir Dar Zuria in which all the 10 accessions from this zone grouped into Cluster I. This result is in agreement with that of a Moroccan lupin local accessions. The local accessions were clustered regardless of their geographic origin (Sbabou *et al*., 2010). Distribution of accessions of similar origin into different significantly divergent clusters, might indicate the diversity of accessions within the origin. The distributions of accessions from Awi, East Gojam, and West Gojam, over different clusters, were quite high covering 10, 9, and 8 clusters, respectively. Moreover, the three singleton clusters each contained accessions from Awi. This might indicate that accessions from Awi were more diverse than others. The distribution and pattern of accessions, over significantly different clusters from these three major geographic origins, would suggest future collections of local accessions in those geographic regions with particular emphasis to Awi, followed by East Gojam, and West Gojam in that order of importance, for future national collection mission in white lupin. Supportive research result, that Ethiopian accessions formed a very distinct and separate grouping/gene pool than others, was reported from Australia (Raman *et al.,* 2014b).

The maximum distance among Ethiopian accessions lies between Clusters XV and XVI (D^2= 217.23); followed by distances between clusters XIV and XV (D^2=194.18), and clusters VII and XIV (D^2=174.11). Those cluster pairs exhibited the first two maximum genetic distances were all singletons. Maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances. Thus, it could maximize opportunities for transgressive segregation, as there is a higher probability that unrelated accessions would contribute unique desirable alleles at different loci. Genetic distance, as good indicator of transgression and heterosis, has been reported by several authors on many crops (Atnaf *et al*., 2013a; Pickup *et al*., 2013). Hence, the attempt to cluster Ethiopian white lupin accessions using multivariate analyses; in the present study, is of practical importance to start a white lupin breeding program. However, the selection of parents for a particular cross should also consider the special advantages of each cluster and each accession within a cluster, depending on specific objectives of hybridization programs. Members within a cluster are assumed to be more closely related, in terms of the trait under consideration, with each other than to members in different clusters (Saeed, 2008; Million, 2012; Habtamu and Million, 2013).

Principal components analyses in this study showed that the first three PCAs explained about 75% of the variation. The amount of explained variance by the first PCA and parameters that contributed relatively more clearly indicated that grain yield and architectural traits of lupin are important traits that could be considered for lupin breeding and selection. Two important

phenological traits (Days to flowering and maturity) accounted most of the variations explained by the second PCA. A similar finding on common bean was reported by Hirpa *et al.* (2013).

Chapter 4

Molecular Genetic Diversity and Population Structure of Ethiopian White Lupin Landraces: Implications for Breeding and Conservation

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Molecular Genetic Diversity and Population Structure of Ethiopian White Lupin Landraces: Implications for Breeding and Conservation

Abstract

White lupin is one of the four economically important species of the *Lupinus* genus and is an important grain legume in the Ethiopian farming system. However, there was limited research effort to characterize the Ethiopian white lupin landraces. Fifteen polymorphic simple sequence repeat (SSR) markers were used to assess the genetic diversity and population structure of 212 Ethiopian white lupin (*Lupinus albus*) landraces and two genotypes were used as out-groups. The SSR markers revealed 108 different alleles, 98 of them from 212 landraces and 10 from the two control genotypes, with an average of 6.5 alleles per locus. The average gene diversity was 0.31. Twenty eight landraces harbored one or more private alleles from the total of 28 private alleles identified in the 212 white lupin accessions. Seventy-seven rare alleles with a frequency of less than 5% were identified and accounted for 78.6% of the total allele detected. Analysis of molecular variance (AMOVA) showed that 92% of allelic diversity was attributed to individual accessions within populations while only 8% was distributed among populations. At 70% similarity level, the UPGMA dendrogram resulted in the formation of 13 clusters comprised of 2 to 136 landraces, with the two control genotypes and five landraces remaining distinct and ungrouped. Population differentiation and genetic distance were relatively high between Gondar and Ethiopian white lupin populations collected by Australians. High level of gene flow (Nm), ranging from 10.60 to 31.46, was detected between the four major populations or collection areas namely West Gojam, Awi, East Gojam and Gondar. A model-based population structure analysis divided the white lupin landraces into two populations. All Ethiopian white lupin landrace populations, except most of the landraces collected by Australians and from Awi, were grouped together with significant admixtures. The study also suggested that 34 accessions, as core collections, were sufficient to retain 100% of SSR diversity.

Keywords: Genetic diversity, landraces, *Lupinus albus*, SSR markers, white lupin

Introduction

White lupin (*Lupinus albus*; 2n=50) has been traditionally cultivated for several thousand years in the Mediterranean region where it originated, and along the Nile valley (including Ethiopia) (Wolko *et al*., 2011). It is one of the four economically important species of the *Lupinus* genus*,* which consists of over 300 annual species (Hondelmann, 1984). White lupin is widely known, commercially important, large seeded, annual lupin species in the world; and it is a promising annual legume crop for human consumption, green manuring and forage. White Lupin has also substantial human nutrition and health importance by providing cheap protein as well as functional food products (Johnson *et al*., 2006; Arnoldi, 2005; Hall *et al*., 2005a; Lqari *et al*., 2005).

White lupin production in Ethiopia is exclusively done by smallholder subsistence farmers, and is targeted for its grain and soil fertility maintenance values (Yeheyis *et al*., 2010). Traditionally, it has been thought and used to cure some disorders and diseases including high blood pressure. Moreover, the crop has social value, being generally consumed in bad crop harvest year and by the poor. Marketing of its processed products and sometimes the grain itself is mainly done by women and youth (Atnaf *et al*., 2015a). However, preparing food and beverage from white lupin involves quite long and laborious steps to get rid of a bitter test due to the presence of high level of alkaloids in Ethiopian white lupin local cultivars. Moreover, there was limited research intervention from the Ethiopian research system to support farmers' production and hence there is no single improved food variety delivered to the farmers until now (Atnaf *et al*., 2015a; Yeheyis *et al*., 2010).

Major collections of important crop plants are held in gene banks around the world. These collections serve as repositories of the biodiversity available for each species and thus are valuable source for useful genes to plant breeders. For any improvement and conservation management or intervention strategy of any populations, understanding the genetic variation between and within populations is an important step (Xiao *et al.*, 2004). The efficient maintenance and use of germplasm are commonly restricted due to the lack of genetic information on the large number of accessions in these collections (Virk *et al.*, 1995).

Despite its importance to improve the fertility of degraded farm land and as cheap sources of protein for poor family, only very limited research and development efforts have been made to improve lupin productivity and quality in Ethiopia. The Ethiopian Biodiversity Institute (EBI) conserved more than 275 white lupin landrace accessions. However, except the passport data and some phenotypic characterization, the landrace accessions have never been systematically characterized using molecular markers (Atnaf *et al*., 2015b). The utility of the germplasm in gene bank collections for breeding purposes depends largely on the accuracy of the evaluation data (Gilbert *et al*., 1999). The genetic diversity of white lupin and other species of lupin conserved in different parts of the world has been characterized using morphological and agronomical attributes (Gonzalez-Andres *et al*., 2007), isozymes/proteins (Vaz *et al*., 2004) and molecular markers including random amplified DNA polymorphism, amplified fragment length polymorphism and inter simple sequence repeats (Talhinhas *et al*., 2003).

Assessment of genetic diversity on the basis of morphological traits is not very reliable, since it may be influenced by the environment coupled with a small number of traits with known

inheritance. Molecular markers have the distinct advantages of being independent of climatic variables, and are very numerous. Microsatellites have been used and found to be good choice in genetic diversity, population structure, and gene mapping studies of different legumes including soybean (Kuroda *et al*., 2009), mung bean (Sangiri *et al*., 2007), lentil (Jin *et al*., 2008), field pea (Xiao-Xu *et al*., 2008), faba bean (Ya-ming *et al*., 2011), chickpea (Sefera *et al*., 2011) and white lupin (Raman *et al*., 2014b). The use of molecular markers can also help to select materials for establishing a core collection, *i.e.*, a group of accessions from an existing germplasm collection that is chosen to represent the genetic spectrum of the entire collection (Hao *et al.*, 2006). The core collection idea has been applied in different crops such as dry beans (Paredes *et al*., 2010; Gepts, 2000; Beebe *et al*., 2001; Rodino *et al*., 2003) and rice (Yan *et al*., 2007a).

Thus, the objectives of this study were: to characterize the genetic diversity of Ethiopian white lupin accessions using SSR markers, to determine the intra-and inter-population genetic diversity and to propose a core collection of white lupin.

Materials and Methods

Plant materials and DNA extraction

The 212 white lupin landrace accessions and one of the two control genotypes used in this study were obtained from the Ethiopian Biodiversity Institute (EBI), whereas one of the control genotype was kindly provided by Dr. Alemayehu Assefa (Researcher at Adet research center,

Ethiopia). The landraces were mainly collected from northwestern Ethiopia including Gojam and Gondar as revealed from the passport data. The number of sampled landraces represents 75% of the Ethiopian white lupin landrace collections; and it was purposively sampled from all lupin producing zones of the country. Twenty (20) white lupin accessions, believed to have been collected by Australians in Ethiopia and donated to the Ethiopian Biodiversity Institute, and two genotypes each from different species (*Lupinus angustifolius* and *Lupinus mutabilis*) used as out-group, were included in this study. Full descriptions; and map of collection areas of these accessions are provided in Appendix table 1 and Figure 1 in chapter 3.

A total of 178 accessions were grown in Ethiopia and sampled leaves were dried using silica gel and transported to BecA-ILRI hub, Nairobi. The remaining 36 accessions were planted at BecA screen house, for DNA extraction. Approximately equal amount of leaves from five different plants per accession were bulked to represent the accession (Gilbert *et al*., 1999). Genomic DNA was extracted from bulked leaves of 15 days to one month old seedlings from each accession, using QIAGEN kit at the BecA-ILRI hub. Genomic DNA quality was checked through 1% agarose gel electrophoresis and Thermo Scientific NanoDrop Spectrophotometer 2000c. The total amount of DNA was quantified with a NanoDrop Spectrophotometer and normalized to 10ng/μl for polymerase chain reaction (PCR) and genotyping.

PCR and SSR markers assay

For SSR assays, a total of 16 polymorphic SSR markers based on reports by Raman *et al*. (2014b) and Phan *et al*. (2007) were selected (Appendix table 3). PCR amplification reactions were carried out on GeneAmp PCR system 9700 thermo cycler in a total reaction volume of 10 μl

lyophilized negative dye BIONEER 96 well plate, 1.5 - 2.5 μL of genomic DNA template, 0.4μL of each primer at concentrations of 0.2 μM, 0.2μL Mg buffer at a concentration of 0.5 mM to top-up the one present in the BIONEER, and 6.5 - 7.5 μL ddH_2O. PCR amplification was carried out at 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, 55°C -65°C (depending on the particular primer) for 30s to 1min and 72°C for 2 min; and a final extension at 72°C for 15 minutes. Annealing temperatures of each primer were determined by primer digital software (primerdigital.com). PCR products of contrasting florescent labels were pooled based on dye color and band intensities to have uniform signal strength. The pooled PCR amplicons were denatured with Hi-Di formimide at 95°C for 3min and then separated by capillary electrophoresis using an ABI3730 DNA genetic analyzer (Applied Biosystems, Foster City, CA) using GeneScan- 500 Internal LIZ and 1200 Internal LIZ Size Standards based on size of amplicons. The fragment analysis data from ABI3730 system were analyzed and allele sizes scored with GENEMAPPER V 4.1 software (Applied Biosystems).

Genetic diversity analysis

The genotypic data were subjected to various within and among groups genetic diversity measures. Basic statistics were calculated using the PowerMarker genetic analysis package (version 3.25) (Liu and Muse, 2005) to measure the diversity at each SSR locus, including the total number of alleles (NA), major allele frequency (MAF), accession-specific alleles, observed heterozygosity (Ho), number of genotypes (NG), polymorphism information content (PIC) and expected heterozygosity (He). The numbers of rare alleles (RA), common alleles (CA) and abundant alleles (AA), partitioning of total genetic variation into within and among pre-grouped

populations through molecular analysis of variance (AMOVA), pair-wise *Fst* and gene flow, were computed using GenAlEx version 6.5 (Peakall and Smouse, 2012).

Genetic relationships analysis

Genetic distances between each pair of accessions and between pre-grouped populations were measured based on both shared allele frequencies and Nei's genetic distance (Nei *et al*., 1983) using PowerMarker (Liu and Muse, 2005). Genetic distance matrices for each locus were summed across loci assuming statistical independence. Pair-wise genetic frequency based dissimilarity or distance matrix between individuals were calculated according to Nei *et al*. (1983) as implemented in PowerMarker. The resulting dissimilarity matrix was subjected to tree construction using the un-weighted pair group method with arithmetic mean (UPGMA) analysis using the same software.

Construction of core collections

Core collections are a group of accessions from an existing germplasm collection that is chosen to represent the genetic spectrum of the entire collection (Hao *et al.*, 2006). Core collections formed for the purposes of maximizing the representativeness of genetic diversity as suggested by Marita *et al*. (2000) ensuring that all accessions in the entire collection are maximally represented, and this core collections provide the best option for obtaining a single ‘‘multipurpose’’ or generalist core collections compared to any type of core collection (Odong *et al*., 2013). To construct the genetic core collections we used allelic data to calculate the dissimilarity matrix as implemented in DARwin v 6.0.10 software (Perrier and Jacquemoud-

Collect, 2010) following random sampling method. The efficiency of the strategy was assessed by comparing the total number of alleles captured for each run using the same software.

Population structure analysis

The model-based software Structure 2.3.4 (Pritchard *et al*., 2000; Falush *et al*., 2003) was used to infer the population structure for the sampled landrace set using a burn-in of 10,000, a run length of 100,000, and a model allowing for admixture and correlated allele frequencies. At least five runs of Structure were conducted by setting the number of populations (K) from 1 to 20. The model choice criterion to detect the most probable value of K was, both the LnP(D) value for each given K and ΔK, an *ad hoc* quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by Structure (Evanno *et al*., 2005). Once the best K was found, the analysis was re-run in the same software using a burn-in of 10,000, a run length of 500,000 with the same aforementioned model. CLUMPAK: "a program for identifying clustering modes and packaging population structure inferences across K" (CLUMPAK server) was used (Kopelman *et al*., accepted to *Molecular Ecology Resources*).

Result

Genetic diversity

Two hundred twelve Ethiopian white lupin landrace accessions and the two out-groups were amplified using 16 SSR markers, which successfully amplified across all accessions. However, one marker (Lup146) produced very large fragment size, which could not be sized with the

available largest GeneScan- 1200 LIZ Size Standard at the Biosciences east and central Africa (BecA) and hence was eliminated from genotyping. Moreover, the remaining 15 SSR markers were checked for medium to high polymorphism before embarking for further analysis. The 15 SSR markers revealed 108 alleles, of which 98 alleles were from the 212 white lupin landrace accessions and 10 alleles from the two out-groups belonging to two different species other than *Lupinus albus*. SSR locus diversity data are summarized and provided in Table 1. The number of allele (NA) per locus varied among the markers, ranging from 3 (SSR55 & GLNA) to 12 (Lup197 & Lup257), with an average of 6.53 alleles. The major allele frequency (MAF) per locus varied from 0.48 (CHS9) to 0.95 (Lup125), with an average of 0.80 per marker. Among the 15 SSR markers, the overall Polymorphism information content (PIC) values ranged from 0.10 (Lup125) to 0.63 (Lup197), with an average of 0.28. The observed heterozygosity (Ho) values ranged from zero (Lup125 and DSI) to 0.96 (CHS9), with an average of 0.18, and the expected heterozygosity (He) values ranged from 0.1 (Lup125) to 0.67 (Lup197), with an average of 0.31.

Twenty eight landraces harbored one or more private alleles (Table 2) from the total of 28 private alleles identified in the 212 white lupin accessions (Table 1). Thirteen of the 15 SSR loci contained one or more of the private alleles identified (Table 1). The RA (rare allele) ranged from 1 (GLNA) to 10 (Lup257 and Lup197) with a total of 77, with each allele having a frequency less than 5%. It accounted for 78.6% of the total allele detected (Table 2). Eight of the SSR loci (SSR55, PT1-1, PT1-2, SSR26a, EST01, Lup125, CHS9 and Lup197) did not contain common alleles, whereas two loci (SSR9a and SSR9b) possessed two common alleles each, with a frequency of 5–50%.

Table 1: Statistics of genetic diversity across 15 SSR loci in the 212 White lupin landrace accessions and the two control genotypes

Marker	MAF	NG	NA	NPA	Ne	I	He/GD	Ho	PIC
SSR55	0.91	4	3	1	1.26	0.32	0.16	0.15	0.14
PT1-1	0.87	10	6	2	1.43	0.50	0.24	0.19	0.22
SSR26a	0.80	5	4	2	1.50	0.47	0.33	0.32	0.28
PT1-2	0.89	9	5	1	1.35	0.41	0.20	0.15	0.19
LSSR14	0.91	6	5	0	1.21	0.36	0.17	0.17	0.16
EST01	0.90	10	6	1	1.36	0.52	0.19	0.11	0.19
Lup125	0.95	4	4	2	1.16	0.26	0.10	0.00	0.10
AnM13	0.85	6	5	1	1.54	0.56	0.26	0.11	0.25
GLNA	0.89	4	3	0	1.41	0.47	0.21	0.08	0.20
DSI	0.91	4	4	1	1.21	0.32	0.17	0.00	0.16
CHS9	0.48	11	8	1	2.29	0.93	0.56	0.96	0.46
SSR9a	0.75	13	11	6	1.18	0.80	0.42	0.12	0.40
Lup257	0.72	23	12	1	1.98	1.03	0.46	0.09	0.44
SSR9b	0.73	13	10	5	1.85	0.88	0.45	0.15	0.42
Lup197	0.51	16	12	4	2.91	1.31	0.67	0.09	0.63
Total		138	98	28					
Mean	0.80	9.20	6.53	1.9	1.58	0.61	0.31	0.18	0.28

MAF, major allele frequency; NG, number of genotypes; NA, number of alleles; NPA, number of private allele; Ne, number of effective alleles; I, Shannon's information index; He, expected heterozygosity; Ho, observed heterozygosity; PIC, polymorphic information content.

The analysis of molecular variance showed that 92% of allelic diversity was attributed to individual accessions within populations while only 8% was distributed among populations (Table 3). No significant variation for molecular diversity was observed among populations based on areas of collection depicting shared alleles among them. However, accessions collected by Australians followed by those from Awi had fairly higher level of gene diversity and Shannon's diversity index.

Genetic relationships

Frequency based genetic distances between all pair-wise combinations among the 212 white lupin accessions and the two control genotypes were calculated as per Nei *et al*. (1983) distance method implemented in PowerMarker software. Pair wise genetic distance ranged from 0 (accessions are more similar) to 1.0 (between some Ethiopian accessions with the out-groups) indicating the presence of genetic diversity among accessions.

The resulting distance matrix was used to construct an UPGMA tree. The generated dendrogram revealed a complex accession distribution pattern with no clear grouping based on geographic origin. At 70% similarity level, the UPGMA dendrogram resulted in the formation of 13 clusters comprised of 2 to 136 landraces, with the two control genotypes and five landraces remaining distinct and ungrouped (Fig. 1; Table 4). The largest cluster, cluster I is constituted of 136 landrace accessions with the following proportions from the different collection areas in the country: West Gojam (63); Gondar (26); East Gojam (23); Awi (17); and Australia donated (7).

Table 2: Detail of allele types identified and landraces containing private alleles as revealed by 15 SSR loci among the 212 white lupin landrace accessions

Marker	R_A	C_A	A_A	Total	Accessions containing one or more private alleles
SSR55	2	0	1	3	
PT1-1	6	0	0	6	
SSR26a	4	0	0	4	
PT1-2	5	0	0	5	
LSSR14	3	1	1	5	
EST01	5	0	1	6	
Lup125	3	0	1	4	Aus14, Aus19, Aus17, Aus9, Acc30, Acc10, Acc9, Acc158, Acc12, Acc24, Acc118, Acc107, Acc122, Acc164, Acc110, Acc114, Acc131, Acc44, Acc39, Acc143, Acc41, Acc70, Acc102, Acc100, Acc101, Acc177, Acc170, Acc96
AnM13	3	1	1	5	
GLNA	1	1	1	3	
DSI	2	1	1	4	
CHS9	7	0	1	8	
SSR9a	9	2	0	11	
Lup257	10	1	1	12	
SSR9b	7	2	1	10	
Lup197	10	0	2	12	
Total	77	9	12	98	28

R_A, number of rare (<5%) alleles; C_A, number of common (5–50%) alleles; A_A, number of abundant (>50%) alleles.

Table 3: Analysis of molecular variance (AMOVA) in Ethiopian white lupin landrace populations

Source of variations	Degree freedom	Sum square	Mean square	Estimated variances	Proportion of explained variance	Statistics	value	P value
Among Populations	4	132.41	33.1	0.66	8%			
Within Populations	207	1491.63	7.21	7.21	92%	PhiPT	0.084	0.001
Total	211	1624.03		7.87	100%			

The second largest cluster comprised 22 landraces the majority of which are from West Gojam (11) and Awi (7), and two each from East Gojam and Gondar. The third largest cluster is cluster X that harbored 15 landrace accessions from all the collection areas. The dendrogram did not show any clear divisions among the white lupin accessions based on their geographical locations except that West Gojam and Gondar which are neighboring areas shared most accessions and clustered together as clearly shown in cluster I.

For the different areas of collection, genetic distances between populations from different parts of Ethiopia and the control genotypes were greater than any other combinations of paired population. Gondar population is the most similar to population of West Gojam (0.001), whereas populations collected by Australians showed the greatest genetic distance with populations of Gondar (0.154). An UPGMA tree of the six collection areas including the control populations was constructed based on Nei's genetic distances and resulted in the formation of

three groups with regard to areas of collection or origin (Fig. 2). The first group contained Gondar, West Gojam and East Gojam. Awi and populations collected by Australians in Ethiopia were grouped into the second and third groups, respectively. The control genotypes isolated apart and clustered in a separate and different group. The dendrogram revealed patterns of genetic relationship among proximity areas of collection.

Figure 1: UPGMA dendrogram showing the genetic relationships among the 212 Ethiopian white lupin landrace accessions and the two control genotypes.

Table 4: Clustering patterns of Ethiopian white lupin landraces from different origins over the clusters

Areas of collection/Origin	No of accessions	Number of landraces in each cluster													
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	Singletons
West Gojam	87	63	11	2	1	1	-	1	1	1	5	-	-	1	-
East Gojam	35	23	2	-	1	2	1	1	-	-	2	-	-	1	2
Awi	38	17	7	-	-	1	1	-	3	1	4	2	1	-	1
Gondar	32	26	2	2	-	-	-	-	-	-	2	-	-	-	-
Australia donation	20	7	-	-	1	-	-	-	-	-	2	-	8	-	2
Control genotypes	2	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Total	214	136	22	4	3	4	2	2	4	2	15	2	9	2	7

-, Nil

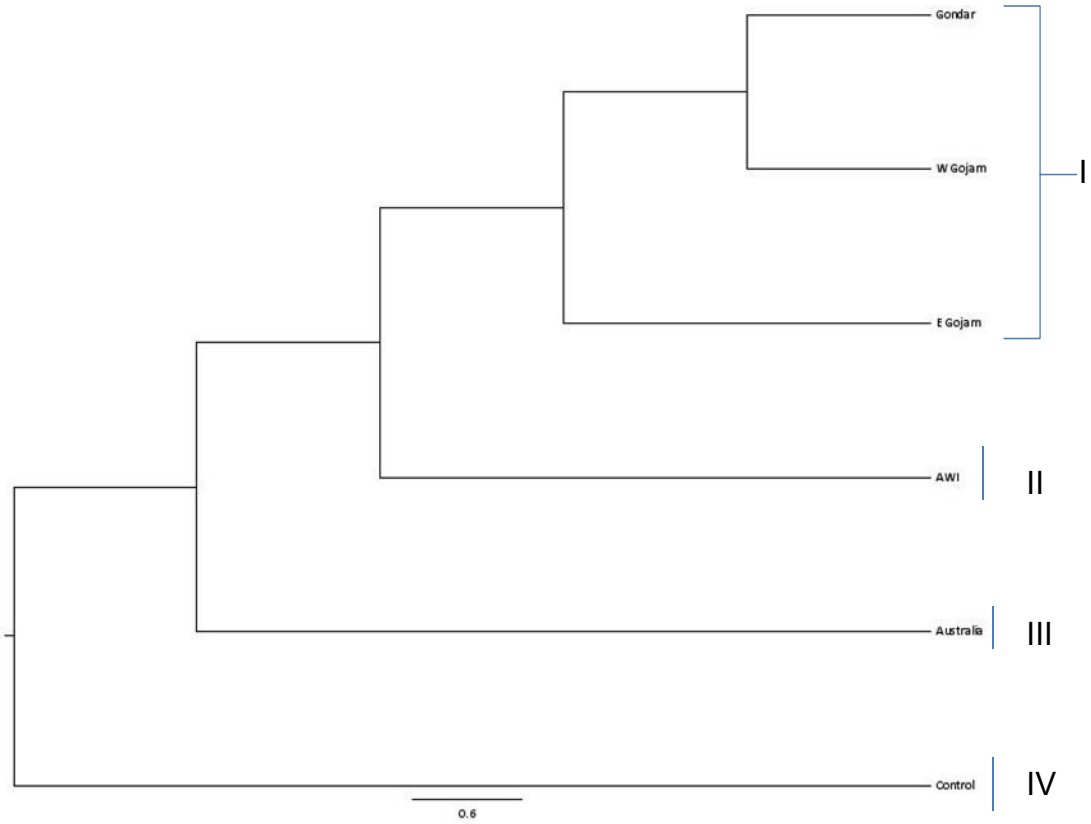


Figure 2: UPGMA dendrogram showing the genetic relationships among the Ethiopian white lupin landrace populations collection areas.

Construction of core collection

The purpose of developing genetic core collections is to provide a restricted set of accessions, feasible to handle, and representing the genetic variability among individuals in a large source of germplasm. Genetic core collections were constructed to maximize the allelic diversity among Ethiopian white lupin landrace accessions based on 15 polymorphic SSR markers. Based on random method, thirty four accessions (core G-34) were sufficient to retain 100% of SSR

diversity, i.e. captured all the 98 alleles which were detected from the 212 Ethiopian white lupin landraces. Detail description of the thirty four white lupin landraces which is proposed to constituent national core collection for Ethiopian white lupin landrace accessions are provided in Table 5. These accessions (core G-34) represent 16% of the whole 212 Ethiopian white lupin accessions considered in this study and populations from West Gojam, Awi and Australian collections contributed more accessions to the core collection than others (Fig. 3). This result shows that only a small number of accessions are needed to retain the whole allelic diversity of Ethiopian white lupin landrace populations.

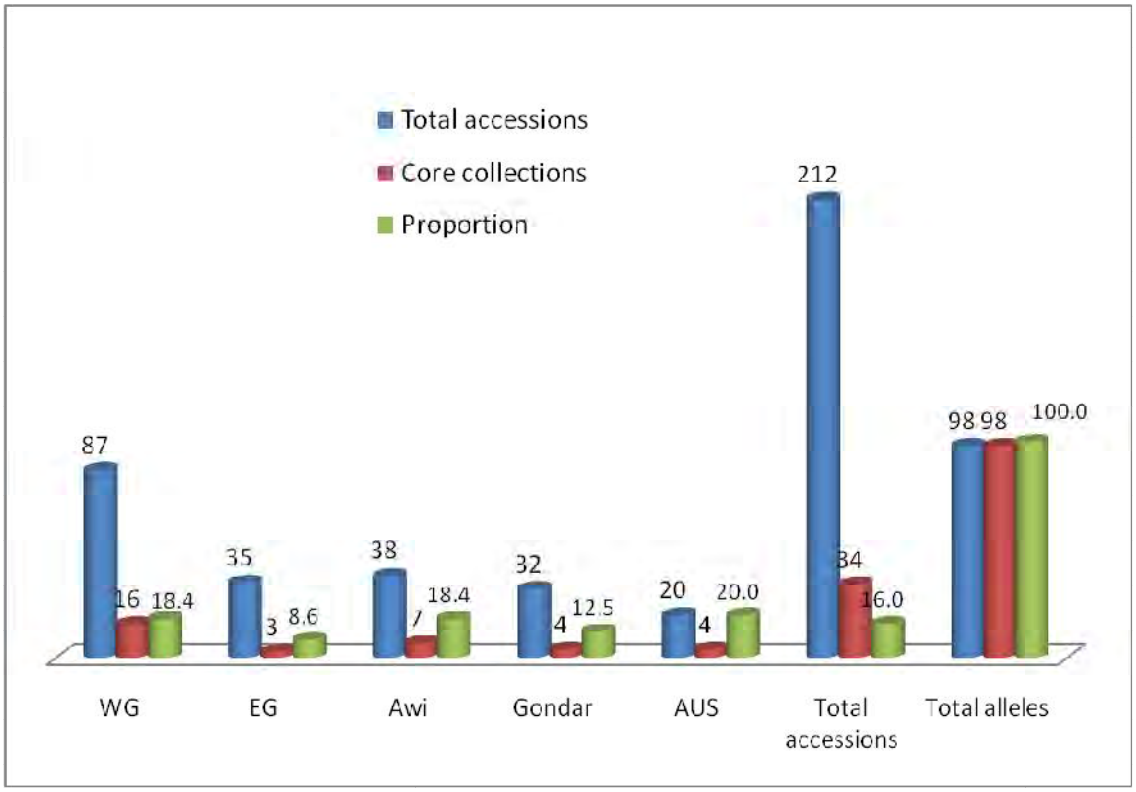


Figure 3: Proportion of the core collection to the total accessions and contributions of pre-defined populations (WG, West Gojam; EG, East Gojam; Awi; Gondar; and AUS, Australian collections and donations) to the core collection.

Table 5: Description of Ethiopian white lupin landrace accessions proposed to constituent national core collection for Ethiopian white lupin accessions

Acc No	EBI code	Zone	District	Altitude	Acc No	EBI code	Zone	District	Altitude
AEG122	105015	EG	Machakel		AWG102	238999	WG	Mecha	2050
AEG129	242260	EG	Machakel	2400	AWG103	239001	WG	Mecha	2050
AEG164	216015	EG	Machakel	2280	AWG162	239031	WG	Achefer	2010
AGR131	239012	NG	G zuria	1930	AWG169	239036	WG	Achefer	2000
AGR46	242313	SG	Dera	1960	AWG170	239037	WG	Achefer	2000
Aus14					AWG177	239048	WG	Bure W	2600
Aus17					AWG179	239050	WG	Bure W	2300
Aus7		Australia collections and donations			AWG39	239011	BD Sp	Bahir Dar	2090
Aus9					AWG41	239022	BD Sp	Bahir Dar	1930
AW15	242276	AWI	Banja	2590	AWG66	242297	WG	Achefer	2010
AW158	242289	AWI	Fagta	2375	AWG74	239030	WG	Achefer	2010
AW18_s		AWI			AWG79	242310	WG	Bd Z	1880
AW21	242292	AWI	Dangila	2060	AWG83	239046	WG	Bure w	2520
AW24	236617	AWI	Dangila	2040	AWG92	242270	WG	Dembecha	2050
AW30	242287	AWI	Fagta	2550	AWG96	242305	WG	Mecha	2000
AW9	242283	AWI	Banja	2160	GVar03				
AWG101	238997	WG	Mecha	2060	GVar04	Variants identified while phenotyping the EBI collections			

Acc No, Accessions number given in the study; EBI, Institute of Biodiversity Conservation; WG, West Gojam; EG, East Gojam; Bd Z, Bahir Dar zuria; Bure W, Bure Womberema; BD SP, Bahir Dar Special; NG, North Gondar; SG, South Gondar; G zuria, Gondar Zuria; Fagta, Fagta Lekoma

Population differentiation and structure

Different population genetics parameters including genetic differentiation (*Fst*), gene flow (Nm), Nei's unbiased genetic distance and Nei's genetic identity were analyzed using GenAlEx 6.5 (Peakall and Smouse , 2012). *F*st values among pairs of populations ranged from 0.008 (between West Gojam and Gondar) to 0.472 (between Gondar and the control) with an overall average of 0.179 (Table 6). Population differentiation was higher between the control and any of the five populations than any pair combination of the five populations. High level of gene flow (Nm), ranging from 10.60 - 31.46, was detected among the four major collection areas namely West Gojam, Awi, East Gojam and Gondar. Nei's unbiased genetic distance was high between the control and any of the five populations than any pair combination of the five populations. Among the five landrace populations, relatively high genetic distance was exhibited between Gondar and populations collected by Australians. Genetic identity was very high among the five populations (0.858 - 0.999); and very low between the control and any of the five populations (0.345 - 0.358).

A total of 15 SSR markers were used to analyse the population structure of the panel of 212 white lupin landrace accessions and the two control genotypes using a model-based approach in Structure, giving 1 to 20 possible clusters (K). The results were then permuted for each K value using the Structure software; and the results were collected through structure harvester (Earl and vonHoldt, 2012). The LnP(D) value for each given K increased with the increase in K, but as there was no clear change in the LnP(D) value, the probable K value could not be inferred

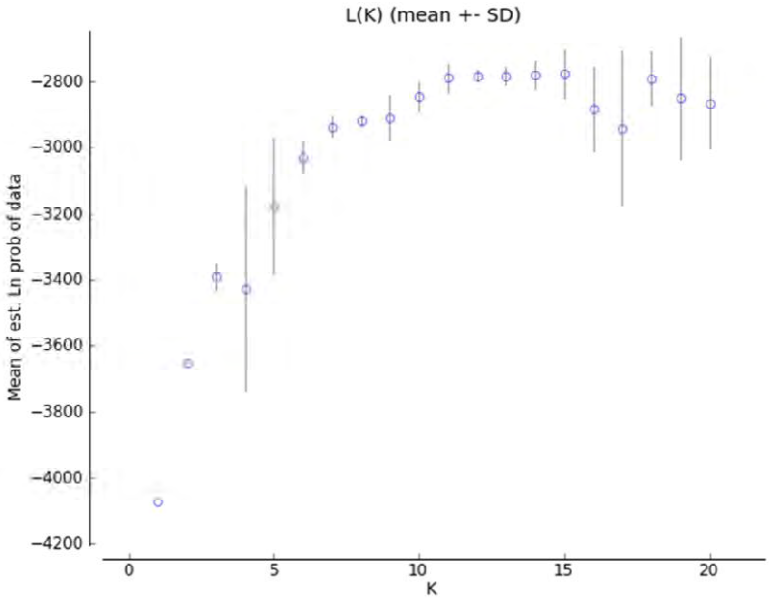
(Fig. 4a). However, on applying the second-order statistics (ΔK) developed by Evanno *et al*. (2005), a sharp peak in ΔK at K = 2 was observed, suggesting the presence of two major populations (Fig. 4b). The analysis for *K* = 2 populations showed individual landrace accessions from the five collection areas distributed between the two populations (Fig. 5; Table 7). However, significant proportion of the populations, 74% from West Gojam, 78% Gondar, 70% East Gojam and 56% Awi grouped together in population one with significant admixtures; whereas majority (more than 75%) of those collected by Australians and 44% of Awi grouped to a second population (Table 7). Generally, this model based grouping was somehow congruent with UPGMA.

Table 6: Pair-wise genetic differentiation, gene flow, unbiased Nei's genetic distance and identity among populations based on areas of collection in Ethiopian white lupin landraces

Populations	Nm						GD					
	Au col	AWI	Con	EG	GR	WG	Au col	AWI	Con	EG	GR	WG
Au col (N=20)		2.99	0.42	2.47	1.75	2.02		0.11	1.04	0.11	0.15	0.13
AWI (N=38)	0.08		0.35	11.16	11.17	18.48	0.89		1.03	0.02	0.01	0.01
Control (N=2)	0.37	0.42		0.31	0.28	0.29	0.35	0.36		1.05	1.06	1.07
E Gojam (N=35)	0.09	0.02	0.45		10.60	17.75	0.89	0.98	0.35		0.01	0.01
Gondar (N=32)	0.13	0.02	0.47	0.02		31.15	0.86	0.99	0.35	0.99		0.00
W Gojam (N=87)	0.11	0.01	0.47	0.01	0.01		0.88	1.00	0.35	1.00	1.00	
	Fst						GI					

Au col, Australian collections; Con, Control genotypes; EG, East Gojam, GR, Gondar; WG, West Gojam. Gene flow (*Nm*) and unbiased Nei's genetic distance (*GD*) in upper diagonals in right and left of the table; Genetic differentiation (*F*st) and Nei's unbiased genetic identity (*GI*) in lower diagonals in left and right of the table

a



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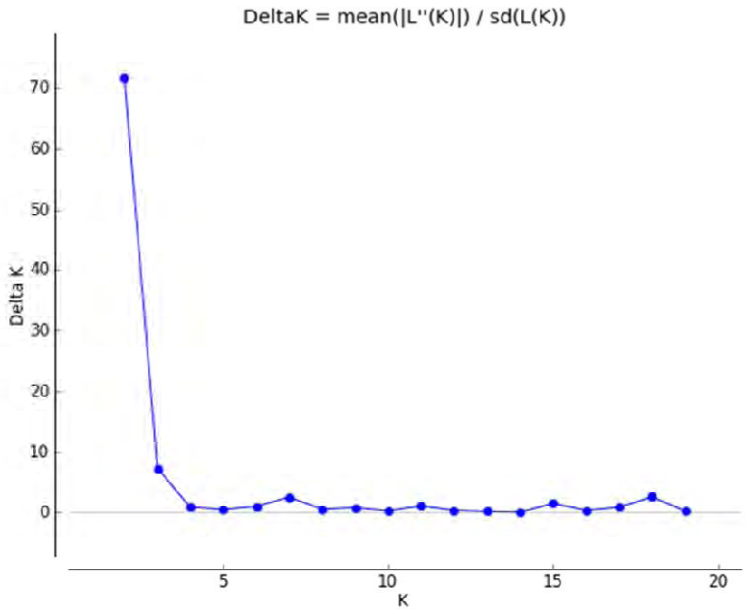

Figure 4: Inferred k from first and second order statistics: (a) LnP(D) value for K values 1 -20; (b). ΔK from structure analysis of 214 lupin accessions.

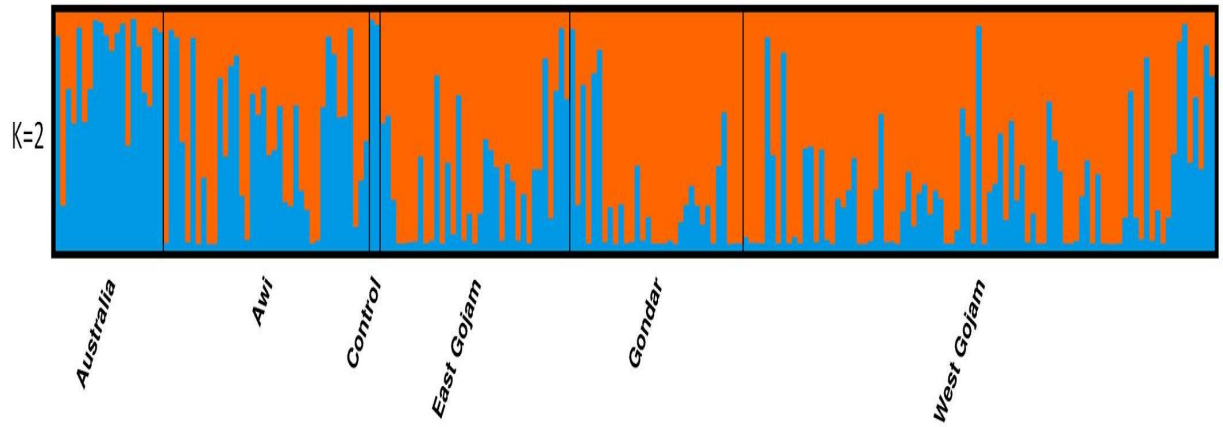

Figure 5: Population structure of 212 Ethiopian white lupin landraces based on 15 SSR panels as obtained from Structure 2.3.4 software.

Table 7: Proportion of membership of each predefined population, in each of the clusters obtained at the best k, i.e., k=2

Pre-defined Populations	Number of accessions	Cluster I		Cluster II	
		Proportion	number	Proportion	number
Australia	20	22.90	5	77.10	15
Awi	38	55.90	21	44.10	17
Control group	2	4.10	0	95.90	2
East Gojam	35	70.40	25	29.60	10
Gondar	32	78.60	25	21.40	7
West Gojam	87	74.10	64	25.90	23
Total accessions	214		140		74
Average distance*		0.10		0.58	

Average distance* = Average distance (expected heterozygosity) between individuals in the same cluster

Discussion

Genetic diversity and SSR allelic distribution

In this study, SSR polymorphism was high as revealed by the high allelic richness per locus, which varied widely among the markers from 3 to 12 alleles (average 6.5 alleles). Relatively moderate to high PIC, Ho and He values were also observed in most markers as well as high number of private alleles, indicating a high level of genetic diversity in the Ethiopian lupin landraces studied. Our results are supported by previous studies based on morphological and agronomic traits, ISSR, AFLP, SSR and DArT marker systems analyses, which reported high genetic diversity in white lupin landraces in Ethiopia (Atnaf *et al*., 2015b; Abdie *et al*., 2015; Raman *et al*., 2014b) and in other parts of the world including Egypt (Christiansen *et al*., 2000), Morocco (Sbabou *et al*., 2010) and Spain (Gonzalez-Andres *et al*., 2007; Rubio *et al*., 2004). Meanwhile, twenty-eight Ethiopian white lupin landraces harbored one or more private alleles of the total of 28 private alleles identified in the 212 white lupin accessions. Moreover, we identified a total of 77 rare alleles accounting for 78.6% of the total alleles discovered in this study with each allele having a frequency of less than 5%. A similar result was reported by Gwag *et al.* (2010) who obtained high frequency of rare alleles (34 alleles; 51.5%) among mung bean accessions, and also indicated that these private alleles contributed greatly to the overall genetic diversity of the collection in other crops (Roussel *et al*., 2004; Yifru *et al*., 2006). Hence, it is important to include rare alleles to maximize the genetic variation in gene bank collections and to utilize them in a breeding program (Yifru *et al*., 2006).

Our results also showed that 92% of allelic diversity was attributed to individuals within populations while only 8% was distributed among populations. No significant variation for molecular diversity was observed among populations based on areas of collection depicting shared alleles among them. This result is supported by previous studies on white lupin (Abdie *et al*., 2015) using ISSR markers though their sample size was too small (only 39); on common bean (Asfaw *et al*., 2009a) using SSR markers. However, relatively high molecular variance (59%) among populations was reported on chickpea (Saeed *et al*., 2011) from diversified populations including wild relatives. The relatively higher variance obtained among populations of chickpea could be attributed to the presence of wild relatives included in that study. Some reports indicate that the level of polymorphism depends on the type of germplasm (He *et al*., 2011), floral biology, marker used (Baraket *et al*., 2011; Sharma *et al*., 2011), primers selected (Kong *et al*., 2011; Sharma *et al*., 2011) and the sampling strategy (Kong *et al*., 2011).

Genetic relationships and patterns of clustering

The dendrogram based on UPGMA revealed two major clades with a complex accession distribution pattern. Nevertheless, at about 70% similarity, the UPGMA dendrogram revealed 13 clusters comprised of 2 to 136 landraces while the two control genotypes and five landraces remaining distinct and ungrouped. Similar relationship and clustering pattern were reported for Ethiopian white lupin landraces based on agronomical and phenological traits (Atnaf *et al*., 2015b). Raman *et al*. (2014b) also found comparable clustering patterns of white lupin accessions originated from different countries including Ethiopia. The same study also claims that Ethiopian white lupin landraces formed a separate and distinct group from others.

In this study, sixty four percent of the landraces (136) were grouped in cluster I. This cluster was constituted by 81% from Gondar, 72% of West Gojam and 65% of East Gojam. This is not surprising since these areas are bordering on one another. Previous phenotypic characterization of Ethiopian white lupin landraces showed similar results (Atnaf *et al*., 2015b). This result may indicate seed exchange, and/or trade between farmers, leading to gene flow across boundaries within those areas (Forsberg *et al*., 2015). Nevertheless, the dendrogram did not indicate any clear divisions among the white lupin accessions based on their geographical locations. On the other hand, accessions from non-bordering regions grouped together in particular clusters as similarly observed through a study on local field pea and faba bean accessions in Ethiopia (Gemechu *et al*., 2005). One possible reason of explanation could be that the landraces might have been introduced from a similar source. A supportive result is documented by Sbabou *et al*. (2010) who found that white lupin local accessions in Morocco clustered regardless of their geographic origin.

Distribution of accessions of similar origin into different clusters might indicate the existence of accession diversity within the populations of origin. The distributions of accessions from West Gojam, Awi and East Gojam, over different clusters, were high covering 10, 9, and 9 clusters, respectively. Moreover, three out of the five singleton landraces were from these areas. This might indicate that accessions from these areas are more diverse than others. The distribution and pattern of accessions, over clusters from these three major geographic origins, would suggest future collections of local accessions in those geographic regions. This result is consistently in agreement with the agronomic and phenologic characterization of sub set of

these landraces (Atnaf *et al*., 2015b); and it is further supported by Raman *et al*. (2014b) who showed that Ethiopian accessions formed a very distinct and separate grouping/gene pool than others.

Population differentiation among populations of West Gojam and Gondar was very low and very high among Gondar and the control. Consequently, high level of gene flow (Nm, ranging from 10.60 - 31.46) was detected between the four major collection areas namely West Gojam, Awi, East Gojam and Gondar. Since these four major collection areas are bordering on one another, there might have been exchange of seeds and seed trading between farmers across those areas (Forsberg *et al*., 2015). Moreover, this result is clearly reflected on the model based structure analysis findings showing significant admixtures of gene pool across populations. Among the five landrace populations, relatively high genetic distance exhibited between populations of Gondar and populations collected by Australians.

Cluster analysis of populations based on areas of collection showed that population grouping did not follow strictly geographical proximity. Therefore, we evaluated them to understand whether alternative clustering methods such as model based clustering would resolve similar patterning. From the relationship analysis, it was observed that there were two major populations corresponding to the major collection areas on one hand and populations collected by Australians on the other. By the structure analysis, these two major populations were resolved but with significant admixtures. The cluster analysis also provided some evidence for gene flow between the subpopulations. Generally, there was good correspondence between

the population pattern observed in the dendrogram and the population structure identified using structure.

Construction of core collections

The sampling percentage of a core collection has long been under debate. Brown (1989) suggested a sampling percentage of 5% - 10%. Yonezawa *et al*. (1995) thought 20% - 30% of the sampling percentage was needed to well conserve the genetic diversity of the whole germplasm collection. The present study resulted in 16% proportion of core collections to the base collections. Slightly higher sampling percentage than the present study was reported by previous researchers, for example, 22% in dry beans (Bascur and Tay, 2005) and 27% in faba bean (Liu and Hoa, 2010). In general, most core collection sizes are 10% - 30% of the initial collection (Coimbra *et al*., 2009; Upadhyaya *et al*., 2009; Zhang *et al*., 2010). However, a perfect ratio or fixed size for all core collections does not exist, and different plant or different constructing goals need different sampling percentage (Wang *et al*., 2014).

Our results demonstrated the great potential of using molecular data to construct a core collection and thus improve the management and utilization of the Ethiopian white lupin landrace germplasm collections. Hu *et al*. (2000) showed that core collection based on genotypic values retained larger genetic variability and had superior representatives than those based on phenotypic values. Nevertheless, because we used a relatively small number of genic and genomic SSR markers for the genetic analysis, the data presented here should not be used alone when deciding on which accessions from the germplasm collection should be discarded

or maintained. Additional molecular markers, including more SSRs and single nucleotide polymorphisms (SNPs), should be used to provide better coverage of the genome. Moreover, this information should be coupled with structural, agronomic and morphological data to make a final decision on the accessions to be maintained.

Chapter 5

Genotype by Trait biplot Analysis to Study Associations and Profiles of Ethiopian White Lupin (*Lupinus albus* L.) Landraces

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Genotype by trait biplot analysis to study associations and profiles of Ethiopian white lupin (*Lupinus albus* L.) landraces

Abstract

Limited information is available on trait relations and profiles of white lupin landraces in tropical growing conditions including Ethiopia. The objectives of this study were to understand the relationships among traits, and to document trait profile of white lupin landraces. As a component of this study, two sets of experiments were conducted. The first comprehensive experiment that consisted of 143 accessions collected from major lupin growing areas of Ethiopia was evaluated at Merawi and the second experiment that consisted 12 selected accessions was evaluated across six locations. In both experiments, significant variations were observed among the accessions for most of the studied traits. Higher heritability and genetic advance as percent of mean was observed for grain yield. Genotype by trait biplots captured 55% - 66% of the variations due to genotype by trait interactions. Trait association and trait profile biplots were constructed for highland, mid-altitude and lowland environments, and across all locations. Different patterns of associations and genotype (accession) by trait interactions were observed in different environments. However, genotype by trait biplots consistently indicated that grain yield had positive associations with most of the traits; especially, with number of pods per plant, plant height and seeds per pod. The study identified some accession with desirable performances as good for specific trait and/or trait groups that could be considered as sources of genes for the traits they have best performed. G8 consistently showed higher grain yield, G2 had higher number of branches and higher number of seeds per plant, G7 had more number of pods per plant and larger seed size and G4 produced longer pods. The accessions used in this study were found to be useful sources for genetic variability for future breeding that targets to improve grain yield and other agronomic traits of white lupin in Ethiopia.

Keywords: Genotype by location interaction; Mega-environment; Selection; Trait inheritance.

Introduction

Most challenges in plant breeding can be summarized into two main categories. One is genotype-by-environment interaction on key traits, and the other is undesirable associations among desired traits (Yan, 2014a). Genotype evaluations on the basis of multi-environment trials and multiple traits are important components in plant breeding (Yan *et al*., 2000; Yan and Rajcan, 2002). Effective interpretation and utilization of the multi-environment trial data based on multi-variate considerations are very crucial at all stages of plant breeding. Yet, accurate estimation, interpretation, and decision making of such data remains a major challenge to breeders (Yan and Rajcan, 2002). Interrelationships among specific breeding objectives mostly impact choice of selection and breeding methods. If all breeding target traits were either positively correlated, or independently inherited, selection would not be much more difficult than selecting for a single trait. However, strong negative correlations between key traits often exist, which make breeding and selection very challenging (Lewis, 2006).

Understanding of the genetic variations between and within populations is key for any breeding strategy (Xiao *et al*., 2004), and it is important to ascertain the variations available in terms of agronomic and plant structure traits (Rubio *et al*., 2004). Heritability of a trait is important in estimating its response to selection, and genetic improvement of quantitative traits requires reliable estimates of heritability. It estimates the relative magnitude of genotypic and phenotypic variance for the trait and gives an idea of the total variation accounted to genotypic effects (Allard, 1960).

Numerous methods have been used to understand trait relations and overall genotype profile of different crops. Recently, a genotype by trait (GT) biplot, which is an application of the GGE biplot technique, is becoming an effective tool for exploring multi-trait data (Yan and Kang, 2003). It graphically displays the genotype by trait table as a biplot, and allows the visualization of the associations among traits across the genotypes (Yan and Rajcan, 2002), and trait profile of the genotypes (Ma *et al*., 2004; Rubio *et al*., 2004; Yan and Frégeau-Reid, 2008; Yan and Kang, 2003). It also provides information on the usefulness of cultivars for production as well as generates information that helps to detect less important (redundant) traits, and identify those that are appropriate for indirect selection for a target trait. The GT biplot has been utilized to study trait relations and genotype evaluation in several crop species including soybean (Yan and Rajcan, 2002), white lupin (Rubio *et al*., 2004), common bean (Hirpa *et al*., 2013; González *et al*., 2006), cowpea (Oladejo *et al*., 2011), durum and bread wheat (Akcura, 2011; Reza *et al*., 2011), and aromatic peppers (Abu *et al*., 2011).

White lupin is a promising leguminous crop for human consumption, green manuring, forage and has substantial importance in human nutrition and health (Hall *et al*., 2005a; Lqari *et al*., 2005; Johnson *et al*., 2006). It has been traditionally cultivated for several thousands of years in the Mediterranean region, and along the Nile valley where it has been originated (Kurlovich, 2002a; Wolko *et al*., 2011). It is produced in Ethiopia exclusively by smallholder subsistence farmers, mainly for its food grain and soil fertility maintenance values (Atnaf *et al*., 2015a; Yeheyis *et al*., 2010). The local varieties being used by farmers have several undesirable characteristics, such as low yield potential, susceptibility to major diseases (Atnaf *et al*., 2015b)

and high contents of alkaloids (Yeheyis *et al*., 2012c). Therefore, there is a need to develop well adapted white lupin varieties with farmers' preferred traits including high grain yield, low alkaloids level and resistant to major lupin diseases.

Several researchers studied the relationships among agronomic traits in white lupin (Annicchiarico *et al*., 2010; Gonzalez-Andres *et al*., 2007; Rubio *et al*., 2004). The results of these studies indicated that the relationships among white lupin traits are consistently influenced by the unpredictable conditions of the growing environments. Thus, genotype selection should be based on multiple trait data in variable environments within the target regions. However, little is known about trait relations and trait profiles of white lupin genotypes in tropical growing conditions including Ethiopia. The objectives of this study were two folds:1) to understand the relationships among traits in Ethiopian white lupin landraces, and (2) to assess trait profile of Ethiopian white lupin landraces. The information generated would be used to design improvement strategies of white lupin in Ethiopia that focus on grain yield and other important agronomic traits.

Materials and Methods

Germplasm

Two independent experiments were considered under this study. The first one, which is a comprehensive experiment, consisted of 143 Ethiopian landrace white lupin accessions and described in chapter 3. Summary of the accessions and from where they were collected are

provided in Table 1, and detail descriptions and passport data; and map of collection area of the accessions are provided in Appendix table 1 and Figure 1 in chapter 3. The second experiment focused on 12 white lupin landrace accessions (Table 2) selected from the first experiment based on desirable performance for grain yield and resistance to major lupin diseases such as lupin rust, pleiochaeta root rot, brown leaf spot and phomopsis (Atnaf *et al*., 2015b).

Table 1: Summary of Ethiopian white lupin landraces used for the comprehensive experiment

		Altitude (masl)*	
Zone	Number of Accessions	Minimum	Maximum
Awi	31	1740	2600
Bahir Dar Zuria	10	1790	2090
East Gojam	22	2060	2500
Gurage	1	NA	NA
Mehakelegnaw	1	NA	NA
North Gondar	3	1820	1930
North Omo	2	2800	2800
South Gondar	17	1860	2850
West Gojam	55	1880	2520
Unknown	1	NA	NA
Germany	1	NA	NA
Total	144	NA	NA

masl = meters above sea level; NA = Not applicable/available

Table 2: Description of white lupin landraces used for the focused experiment

Genotype		Source	
Code	EBI designation	Zone	Country
G1	242281	Awi	Ethiopia
G2	238996	Bahir Dar special	
G3	238999	West Gojam	
G4	236615	West Gojam	
G5	239029	West Gojam	
G6	239007	Awi	
G7	242306	West Gojam	
G8	239003	Awi	
G9	239045	Awi	
G10	239032	West Gojam	
G11	207912	Mehakelegnaw	
G12	Local	West Gojam	

EBI=Ethiopian Biodiversity Institute; G1-G11 are codes representing respective local landrace accessions; Zone means an administrative boundary from where the white lupin landrace accessions were collected

Experimental design and field management

Two types of experiments, comprehensive and focused were carried out under this study. The comprehensive experiment was executed at Merawi, north western Ethiopia during 2013/2014 season with supplemental irrigation. Detail description of Merawi experimental location is given in Table 3. The experiment was laid out in a 12 x 12 simple lattice design. Each plot

consisted of two rows of 2.5 m length with spacing of 75 cm and 25 cm between rows and between plants, respectively. The seeds were hand planted in rows. As there is no research recommended fertilizer rate for white lupin growing in Ethiopia, fertilizer was not used for this experiment. Supplementary irrigation was applied as necessary. Other agronomic management and crop protection practices were applied uniformly to all the plots during the period of experimentation.

Table 3: Description of the study locations for the focused experiment

Location	Altitude (masl*)	Latitude	Long	Annual RF(mm**)	M min T (0^c)	M max T (0^c)	Soil Type	Agro-ecology
Debre	2630	$11^0$89'N	$38^0$09'E	1500	11.8	23	Luvisol	Highland
Injibara	2560	$10^0$57'N	$36^0$56'E	2300	8.0	22	Acrisol	Highland
Merawi	1960	$11^0$42'N	$37^0$17'E	1577	7.8	25.8	Nitosol	Mid-alt
Fenote	1917	$10^0$42'N	$37^0$16'E	1344	12.9	29.4	Nitosol	Mid-alt
Dibate	1615	$10^0$77'N	$37^0$26'E	1000.0	15	29	Nitosol	Lowland
Mandura	1400	$10^0$95'N	$37^0$43'E	1300.0	15	35	Nitosol	Lowland

Debre = Debre Tabor; Fenote = Fenote Selam; *= Meters above sea level; Long = Longitude; ** = Rain fall in millimeter; M min T = Mean minimum and M max T = mean maximum temperature, in degree Celsius; Agro-ecology is mainly based on altitudes; Mid-alt = Mid-altitude. [Source: Yihenew (2002)]

The focused experiment was conducted in the main season of 2014/15 over six locations; namely, Debre Tabor, Injibara, Merawi, Fenote Selam, Dibate and Mandura. These locations represent major lupin growing areas of Ethiopia. Detail descriptions of the test locations are presented in Table 3. Completely randomized block design with four replications was used for

this experiment. Each experimental unit consisted an area of 15 m^2 (5m x 3m) with similar intra-row and inter-row spacing with the comprehensive experiment. Planting, fertilization, agronomic management and crop protection practices used were similar to that of the comprehensive experiment.

Data collection

For both experiments, data were recorded for grain yield and agronomic traits. Grain yield was recorded on plot basis in grams, and adjusted to 14% moisture content and expressed as tons per hectare (tha^{-1}). Hundred seed weight (gm) was determined using random seeds from each experimental unit after grain moisture content was adjustment to 14%. Number of days from emergence to 50% flowering and 75% physiological maturity were recorded as days to flowering and days to maturity, respectively. Number of pods per plant, number of branches on the main axis and plant height (cm) were recorded as the average of five randomly selected plants per plot. Five randomly selected pods from each of these plants were used to record number of seeds per pod, pod length (mm) and pod diameter (mm).

Data analysis

All the data collected were checked for outliers and normality of residuals using Breeding View of Breeding Management System before proceeding to any subsequent analysis (BMS, 2015). Consequently, an outlier accession (Acc144), which was found to significantly affect the result was excluded from the subsequent analyses of the comprehensive experiment. The same

software was used to generate adjusted mean values for all the traits using best linear unbiased estimators (BLUE).

Phenotypic and genotypic coefficients of variations, genetic advance as percent of mean, and heritability in broad sense were estimated using the formula suggested by Singh and Chaudhary (1985) as follows:

$$PCV = \frac{\sqrt{\delta^2 p}}{\bar{X}} X\ 100 \qquad [1]$$

$$GCV = \frac{\sqrt{\delta^2 g}}{\bar{X}} X\ 100 \qquad [2]$$

$$\delta p = \sqrt{\delta^2 p} \qquad [3]$$

$$H = \frac{\delta^2 g}{\delta^2 p} \qquad [4]$$

$$GAM = \frac{HK\delta_p}{\bar{X}} X\ 100 \qquad [5]$$

where, δ^2_p and δ^2_g are phenotypic and genotypic variances, respectively; PCV and GCV are phenotypic and genotypic coefficients of variation, respectively; $\bar{X}$ is the grand mean; H is the broad sense heritability; GAM is genetic advance as percent of mean; δ_p is phenotypic standard deviation; and K is selection differential at 5% selection intensity (2.06).

For the interpretation of genetic parameters, heritability values were categorized as suggested by Robinson *et al*. (1949): 0 -30% as low, 30 -60% as moderate, and >60% as high. The values of GAM were categorized as recommended by Johnson *et al*. (1955): 0 - 10% as low, 10 -20% as moderate, and >20% as high.

Adjusted mean values of the traits were used for the analysis of genotype by trait and trait associations. The adjusted mean values were standardized to mean zero and unity variance in order to minimize biases due to differences in scales of measurement. To display the genotype by trait two-way data in a biplot, the formula suggested by Yan and Rajcan (2002) was used:

$$\frac{T_{ij}-\bar{T}_j}{sj} = \lambda_1 \zeta_{i1} \boldsymbol{\tau}_{j1} + \lambda_2 \zeta_{i2} \boldsymbol{\tau}_{j2} + \varepsilon ij \qquad [6]$$

where, T_{ij} is the average value of genotype *i* for trait *j*, T_j is the average value of trait j over all genotypes, s_j is the standard deviation of trait *j* among the genotype averages; ζi_1 and ζi_2 are the first principal component (PC1) and the second principal component (PC2) scores, respectively, for genotype *i*,$\boldsymbol{\tau}_{J1}$and $\boldsymbol{\tau}_{J2}$ are the PC1 and PC2 scores, respectively, for trait *j*, and ε_{ij} is the residual of the model associated with the genotype *i* and trait *j*.

Equation [6] is a principal component analysis of standardized data with two principal components. Because different traits use different units, the standardization is necessary to remove the units.

PC1 and PC2 must be scaled so that the one value is symmetrically distributed between the genotype scores and the trait scores. A GT biplot is constructed by plotting the PC1 scores against the PC2 scores for each genotype and each trait. To display the PC1 and PC2 in a biplot, the λ values in Equation [6] above are absorbed into the genotype and trait scores so that the equation is written as:

$$\frac{T_{ij}-\bar{T}_j}{sj} = \zeta_{i1}\boldsymbol{\tau}_{j1} + \zeta_{i2}\boldsymbol{\tau}_{j2} + \varepsilon ij \qquad [7]$$

In addition, Pearson correlation coefficients were computed using GGE software to augment the trait relationships visualized in the GT biplot. Moreover, GT analysis was done following mega-environments classification approach. A mega-environment is a sub-region of a crop species' growing region within which the same or similar genotypes perform best (Gauch and Zobel, 1997). Understanding the mega-environment constitution of the target region for a given crop is a prerequisite for determining proper strategies of genotype evaluation and cultivar recommendation.

Results and Discussion

Significant differences were observed among the Ethiopian white lupin landraces for the 10 agronomic and phenological traits studied, except for pod diameter, in the comprehensive experiment. Details of diversity analysis and mean performances of these landraces were previously presented in chapter 3 and published as Atnaf *et al*. (2015b). Hence, this chapter mainly focuses on the study of trait relationships, and trait profiles, inheritance and gain from selection among Ethiopian white lupin landraces evaluated under two sets of experiments.

Estimates of variance components, heritability and genetic advance

Phenotypic coefficients of variations (PCV) were higher than genotypic coefficient of variation (GCV) for all traits studied in both experiments (Table 4 a & b). The differences between PCV

and GCV were accounted to the environmental conditions under which the genotypes were grown. The extents of the difference between PCV and GCV indicate degree of environmental influences on the traits. The difference ranged from 0.61 in days to maturity to 8.42 in number of pods per plant which indicate significant environmental influence on the expression of number of pods per plant; whereas it was negligible for days to maturity. Very similar results were observed in the focused experiment executed across six different locations in Ethiopia (Table 4b). For the comprehensive experiment, phenotypic variability ranged from 3.87 for days to maturity to 17.66 for grain yield while it ranged from 0.86 to 34.61 in the focused experiment for the same traits. Genotypic variability ranged from zero for pod diameter to 13.05 for grain yield in the comprehensive experiment and from 0.68 for physiological maturity to 20.6 for grain yield in the focused experiment.

Estimates of broad sense heritability ranged from near zero for pod diameter to 85% for days to flowering for the comprehensive experiment (Table 4a). For the focused experiment, it ranged from 35% for pod diameter to 77% for days to flowering (Table 4b). Of the studied traits, days to flowering and maturity, and grain yield consistently exhibited high heritability in both experiments. Annicchiarico *et al*. (2010) reported high heritability for flowering time. High heritability indicates high proportion of genetic variance that could be inherited, and would be exploited by breeders to select superior genotypes based on phenotypic performance (Peter *et al*., 2008; Tazeen *et al*., 2009). Plant height showed moderate to high heritability for both experiments. Number of branches on the main axis, hundred seed weight, number of seeds per pod and pods per plant, and pod length showed moderate heritability, which depicts

considerable influence of environment on the expressions of these traits. Contrary to the current findings, Annicchiarico *et al*. (2010) reported high broad sense heritability for seed weight in European white lupin genotypes. Heritability of pod diameter was zero for the comprehensive experiment, and low for the focused experiment, indicating the predominance of environmental effects more than that of genetic effects for the trait.

Estimates of genetic advance as percent of mean were high only for grain yield in both experiments; moderate to high for pods per plant (Table 4 a & b). Days to flowering, plant height, hundred seed weight and number of branches on the main axis had moderate (>10 %) genetic advance for the comprehensive experiment, but low for the focused experiment; whereas all the other traits had low level of genetic advance in both experiments. Since high heritability does not always guarantee high genetic gain from selection, heritability should be considered combined with genetic advance in predicting selection for superior genotypes (Ali *et al*., 2002; Singh, 2000). In the present study, high estimate of GCV, heritability and genetic advance as percent of mean were observed only for grain yield while moderate heritability and genetic advance as percent of mean were observed for hundred seed weight, plant height and pods per plant. High heritability but low genetic advance as percent of mean were observed for days to flowering and physiological maturity; whereas moderate heritability and low genetic advance were realized for number of seeds per plant, pod length and number of branches on the main axis.

Table 4: Estimates of parameters of variability for different traits in white lupin landraces

(a). Variability parameters in 143 white lupin landrace accessions at Merawi (Comprehensive experiment)

Traits	Minimum	Mean	Maximum	Variability		Heritability (%)	GAM
				PCV	GCV		
DF	65.74	81.20	92.05	5.97	5.14	84.92	10.45
DM	140.77	178.86	190.78	3.87	3.26	81.12	6.47
BR	7.27	8.20	10.05	12.83	8.08	55.70	14.72
PH	75.99	131.29	142.64	8.39	6.05	66.08	11.43
PD	6.53	6.53	6.53	8.69	0.00	0.00	0
PL	71.22	87.06	89.97	6.21	3.36	43.20	5.52
PP	66.60	84.75	92.19	15.19	6.77	31.67	9.91
SP	4.99	5.35	5.54	5.65	2.55	33.98	3.95
SW	24.28	31.52	35.09	9.47	5.84	53.58	10.45
GY	2.05	4.48	5.38	17.66	13.05	69.31	25.21

(b). Variability parameters in 12 white lupin accessions evaluated across six locations in Ethiopia (Focused experiment)

Traits	Minimum	Mean	Maximum	Variability		Heritability	GAM
				PCV	GCV		
DF	57.50	60.32	64.25	2.93	2.29	77.12	4.65
DM	164.25	167.10	169.25	0.86	0.68	72.60	1.29
BR	3.71	4.87	5.86	9.85	4.99	39.40	7.99
PH	105.85	128.00	146.90	7.30	4.05	48.17	7.25
PD	6.05	7.31	8.47	7.11	4.33	35.49	5.20
PL	66.85	75.20	83.10	4.72	2.63	62.87	6.12
PP	31.45	64.26	93.55	21.29	13.31	60.70	26.62
SP	3.58	4.91	5.74	9.37	5.31	45.34	8.76
SW	17.25	22.81	27.90	10.65	6.89	44.35	9.73
GY	1.25	2.63	4.39	34.61	20.60	75.50	53.83

DF=Days to 50 % flowering; DM= Days to 95 % maturity; BR= Number of primary branches on the main axis; PH=Plant height in centimeter; PD= Pod diameter in millimeter; PL= Pod length in millimeter; PP=Pods per plant; SP=Seeds per pod; SW= 100 seed weight in gram; GY= Grain yield in ton per hectare; PCV= Phenotypic coefficient of variation; GCV= Genotypic coefficient of variation; GAM= Genetic advance as percent of mean.

Panse (1957) stated that high heritability coupled with high genetic advance indicates the additive gene effects while high heritability coupled with low genetic advance indicates the

non-additive gene effects for the control of a particular character. Accordingly, the presence of high estimates of GCV, heritability and genetic advance as percent of mean for grain yield in this study indicate the preponderance of additive gene action in governing the expression of the trait and consequently high expected genetic gain through selection. High estimates of heritability and low genetic advance observed for some of the traits suggest the presence of non-additive gene action and/or significant genotype by environment interaction in the expression of the traits that will make selection difficult for the improvement of these traits. Several researchers reported similar results to the present finding on different legume and other crop species, such as mungbean (Payasi, 2015), soybean (Malek *et al*., 2014), cowpea (Ajayi *et al*., 2014), ricebean (Geeta *et al*., 2015), and barley (Dyulgerova and Valcheva, 2014).

Trait relationships and trait profiles in white lupin landraces

Different trends of trait associations and trait profiles were observed under different environments due to genotype by location interaction effects, as depicted by the GT biplot for different locations in this study (Fig. 1a -e). Annicchiarico *et al*. (2010) reported similar finding in white lupin accessions evaluated in Italy and France. The important question here is, which of the biplots should be chosen? The biplot in Figure 1e, for example, may be regarded as an integration of the GT patterns from all trials conducted across location, but it is not 100% true to any of the single location trial. These conflicting patterns were caused by, and were an indication of, genotype by location interactions. In such cases, GT data analysis by mega-environment could be a solution (Yan, 2014a). Thus, the GT biplots that reveal trait

relationships and trait profiles of white lupin landraces used in this study are presented and discussed following altitude based mega-environments.

GT biplots in this study captured 55% - 66% of the variations due to genotype and genotype by trait interactions. As a previous report by Yan and Rajcan (2002), this result reflects the complexity of the interrelationships among the measured traits. Nevertheless, the fundamental patterns among the traits could still be captured by the biplots (Kroonenberg, 1995). Similar results were reported by Rubio *et al*. (2004) for white lupin accessions evaluated in Spain. In the biplot, the cosine of the angle between two traits approximates the correlation between the traits; and hence associations among traits could easily be visualized from the biplot. Two traits are positively correlated if the angle between the vectors is acute (<90°); negatively correlated if the angle is obtuse (>90°); and not correlated if the angle is right angle (Yan and Tinker, 2006). These associations could be confirmed from Pearson correlation coefficients between any two traits (Table 5). However, some discrepancies might be expected as the biplots explained only 55 - 65 % of the variations attributed to the genotype and genotype by trait interactions.

In higher altitude locations (Injibara and Debre Tabor), grain yield had strong and positive association only with plant height; positive relationship with seeds per pod, pods per plant and pod length; and positive but weak associations with pod diameter and days to flowering as revealed by the acute angles between the vectors of these traits (Fig. 1a). Results of Pearson correlation coefficients showed similar relationship among these traits (Table 5a). On the other hand, grain yield had significantly negative correlation with days to maturity and hundred seed weight. In agreement with the current study, Rubio *et al*. (2004) reported positive associations

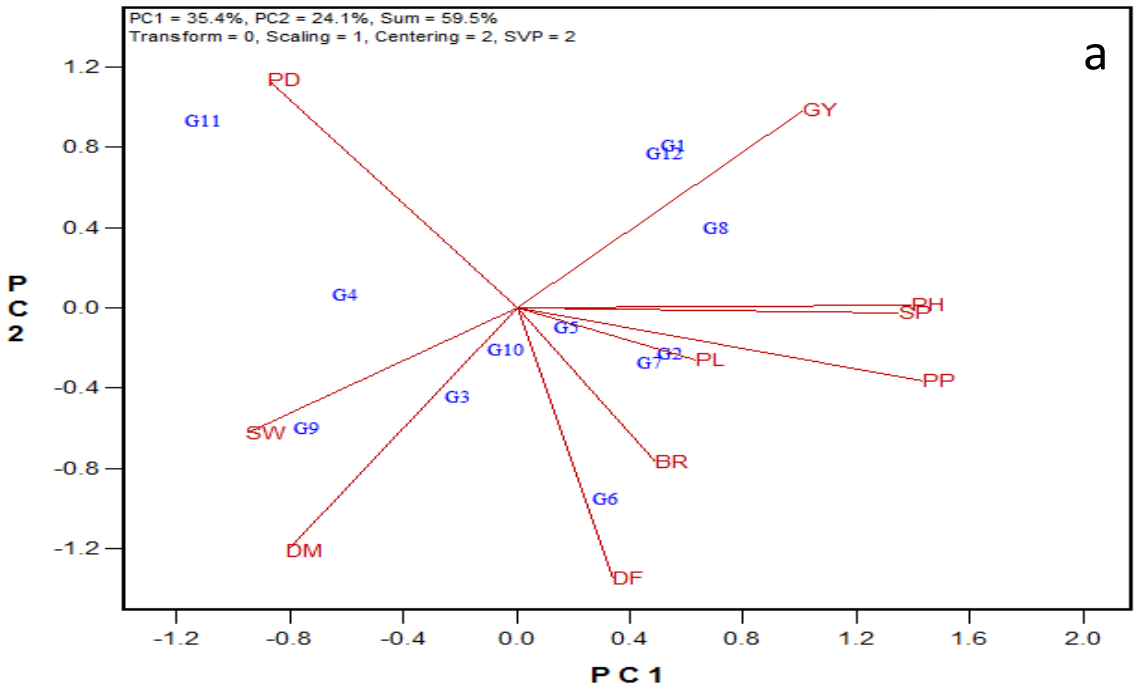
between grain yield and plant height in white lupin genotypes evaluated in Spain. Traits that had strong positive associations have a tendency to discriminate accessions in similar fashions, whereas traits with negative associations have a tendency to discriminate accessions in opposite direction.

At mid-altitude environments (Fenote Selam and Merawi), however, grain yield had positive associations with all measured traits, with relatively stronger relationship with hundred seed weight, pods per plant, branch number and plant height (Fig. 1b; Table 5b). In the comprehensive experiment, similar associations were observed between grain yield and most other traits (Fig 1c; Table 5b). However, grain yield had negative relationships with phenological traits (days to flowering and days to maturity) and branch number on the main axis. This could be due to an excessive and extended rainy period occurred at Merawi during the experimentation, which significantly delayed the flowering and maturity period. Grain yield had strong and positive relationships with plant height, pods per plant and branch number on the main axis in the low altitude environments (Dibate and Mandura). On the other hand, grain yield was negatively associated with hundred seed weight, days to flowering and pod diameter (Fig. 1d; Table 5a). Strong positive relationship between grain yield and pods per plant was previously reported in white lupin genotypes (Huyghe *et al*., 1990).

Trait relationships based on data averaged over six locations for the focused experiment revealed that grain yield had positive relationship with all traits measured except with days to maturity, pod diameter and hundred seed weight (Fig. 1e; Table 5c). Although the GT biplot captured most important relationships, it did not entail all the associations that have been

apparent between traits in the respective mega-environments. Hence, it is advisable to consider trait associations under the context of mega-environments as presented in this paper. The inconsistencies in associations of some of the traits over different environments might be due to environmental effects and genotype by environment interactions.

The length of the trait vector projected from the origin shows the ability of the traits in discriminating among accessions. Traits with longer vectors do have high discrimination power and vice versa. Thus, grain yield and most agronomic traits had longer vectors, and hence high discrimination power. Whilst pod length and number of branches on the main axis consistently showed shorter projection of vector in all environments; and days to maturity & flowering, 100 weight and seeds per pod had shorter vectors in some of the environments. This indicates the inability of these traits to discriminate among accessions in the respective environments.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G11
G1
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PH
SP
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SW
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DM
DF
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0.4
0.0
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-0.8
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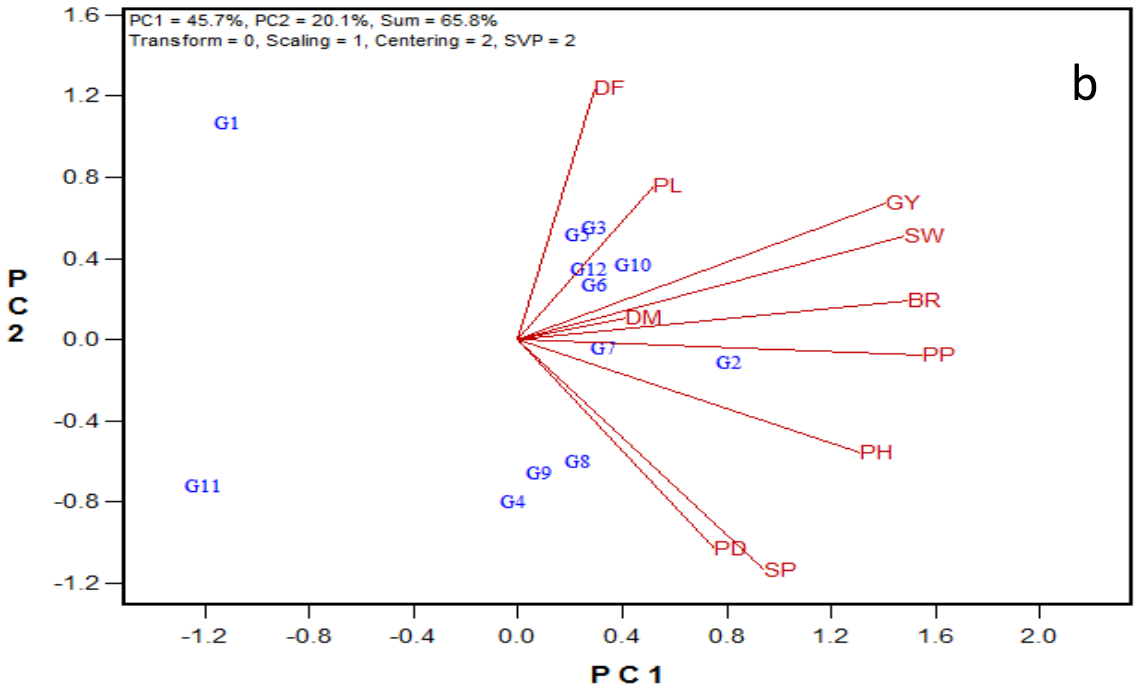
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G6
BR
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G11
G4
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SP
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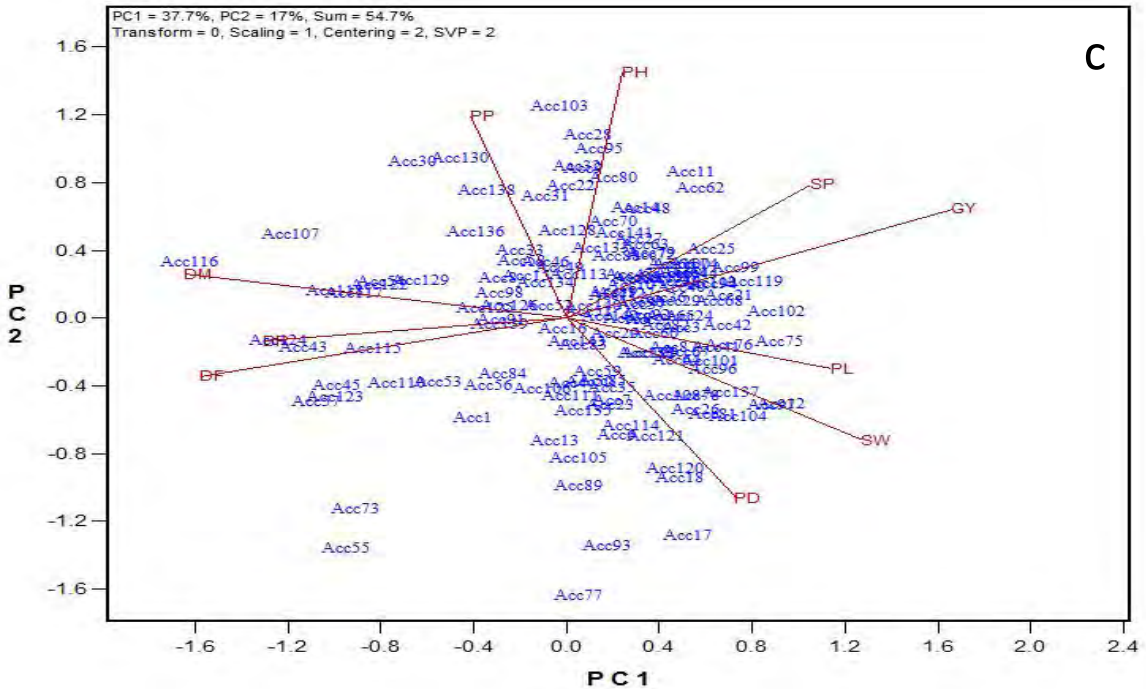
P C 1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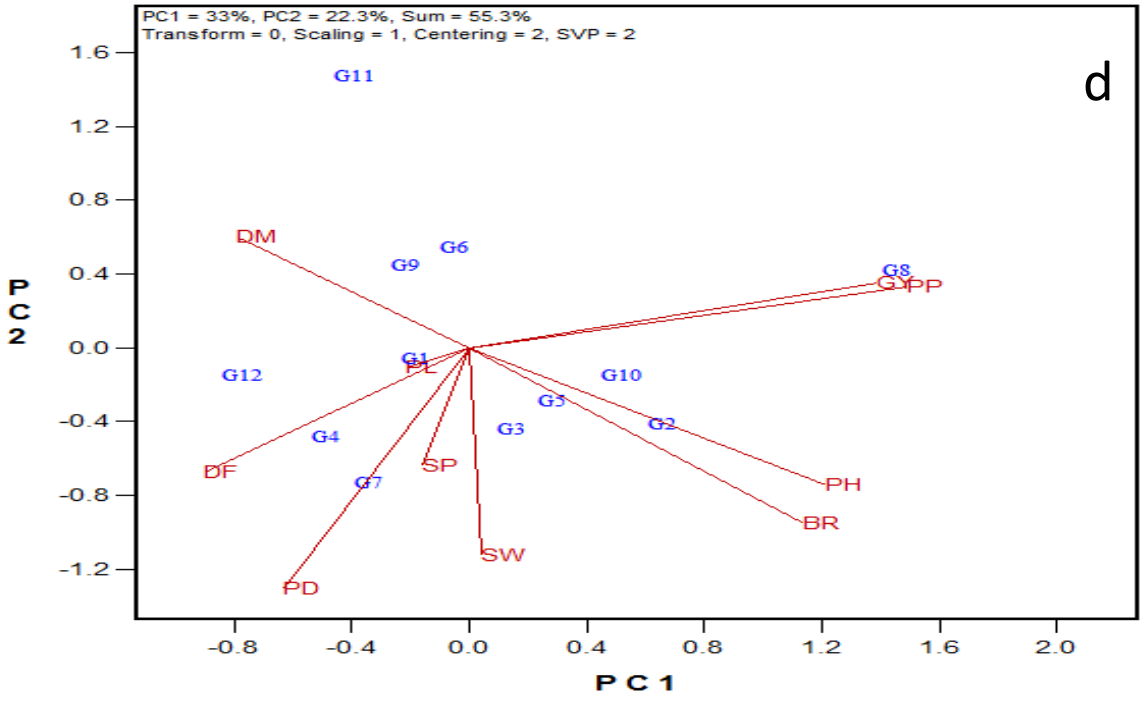
PC 1
PC 2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G3
G2
DF
SP
G7
PH
BR
SW
PD
PC 1
PC 2

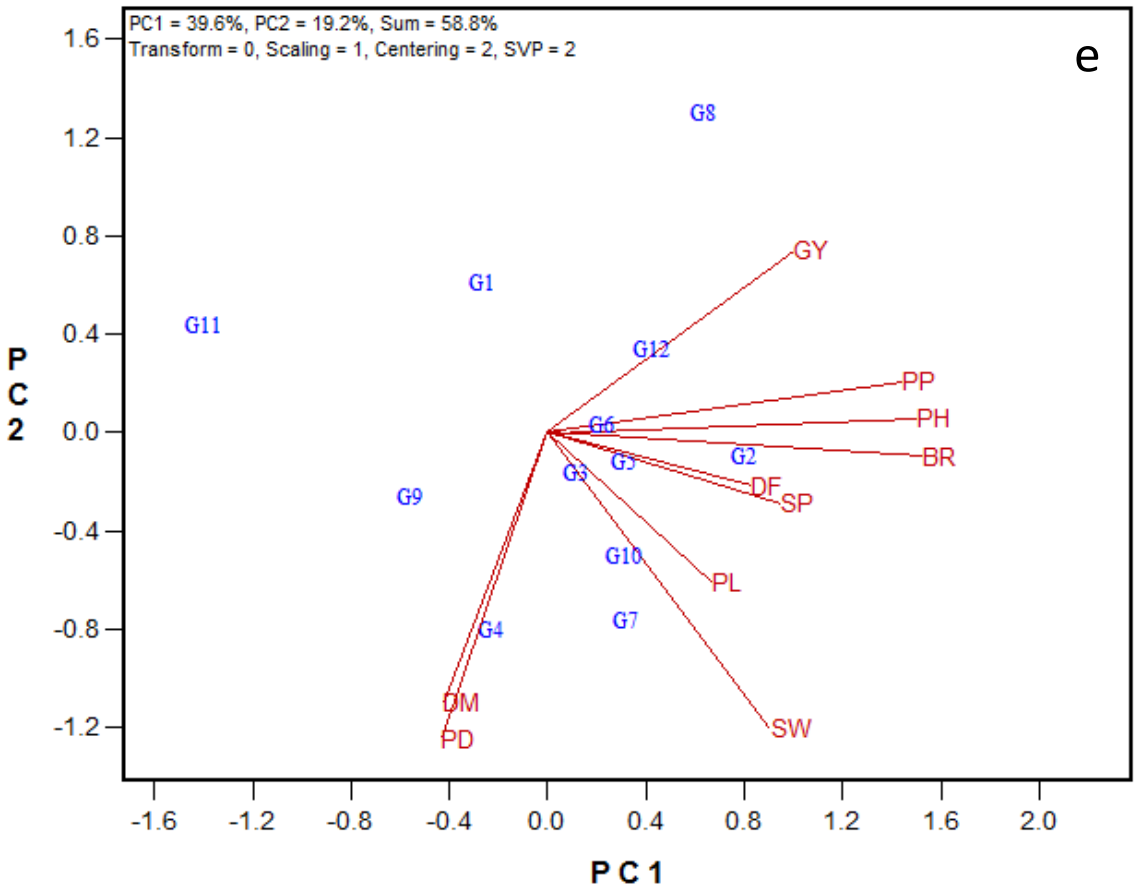


Figure 1: Biplots showing the interrelationships among different traits for white lupin landraces evaluated in different scenarios. (a) In high altitude environments (Debre Tabor and Injibara); (b) In mid-altitudes (Fenote Selam and Merawi); (c) At Merawi which evaluated 143 landrace accessions; (d) In low altitude environments (Dibate and Mandura); and (e) Across six testing sites representing highland, mid altitude and lowland ecologies. G1 - G12 are codes for white lupin landraces evaluated in all the six environments; whereas Acc1 - Acc144 in Figure 'c' are codes to represent accessions phenotyped at Merawi, Ethiopia. GY = Grain yield; PP = Pods per plant; SP = Seeds per pod; SW = 100 seed weight; PH = Plant height; BR = Number of branches on the main axis; PL = Pod length; PD = Pod diameter; DF = Days to flowering; and DM = Days to physiological maturity.

Table 5: Pearson correlations coefficients among grain yield and related traits in Ethiopian white lupin landraces based on genotype by trait data generated from different agro-ecologies of Ethiopia

(a). Twelve accessions evaluated at two highland (above diagonal) and two lowland (below diagonal) environments

Traits	DF	DM	BR	PH	PD	PL	PP	SP	SW	GY	
DF		0.627	0.398	0.105	-0.522	0.388	0.188	0.081	-0.085	-0.127	DF
DM	0.044		0.002	-0.378	-0.126	0.066	-0.307	-0.379	0.32	-0.636	DM
BR	0.002	-0.419		0.158	-0.423	-0.033	0.254	0.127	0.031	0.028	BR
PH	-0.123	-0.415	0.821		-0.429	0.275	0.672	0.643	-0.27	0.493	PH
PD	0.322	-0.132	0.046	0.067		-0.025	-0.689	-0.351	-0.047	0.089	PD
PL	-0.033	-0.166	-0.02	-0.182	0.027		0.234	0.308	-0.211	0.194	PL
PP	-0.626	-0.346	0.471	0.564	-0.402	-0.261		0.74	-0.332	0.21	PP
SP	0.127	-0.283	0.127	-0.095	0.415	0.759	-0.124		-0.332	0.244	SP
SW	0.068	0.017	0.334	0.31	0.579	-0.305	-0.129	-0.075		-0.751	SW
GY	-0.533	-0.323	0.445	0.407	-0.547	0.095	0.762	0.089	-0.081		GY

(b). Twelve accessions evaluated at two mid-altitude environments (above diagonal) and 143 white lupin landraces evaluated at Merawi (below diagonal)

Traits	DF	DM	BR	PH	PD	PL	PP	SP	SW	GY	
DF		-0.245	0.282	-0.099	-0.174	0.075	0.017	-0.357	0.451	0.426	DF
DM	0.649		-0.061	0.017	0.321	0.317	0.214	-0.134	0.346	0.342	DM
BR	0.611	0.462		0.726	0.207	0.29	0.875	0.485	0.78	0.733	BR
PH	-0.153	0.073	-0.061		0.504	0.258	0.663	0.698	0.532	0.449	PH
PD	-0.077	-0.287	-0.157	-0.222		-0.396	0.318	0.522	0.391	0.191	PD
PL	-0.276	-0.4	-0.218	0.052	0.371		0.294	-0.002	0.24	0.304	PL
PP	0.143	0.277	0.244	0.278	-0.096	-0.04		0.629	0.787	0.795	PP
SP	-0.296	-0.402	-0.307	0.234	0.07	0.266	0.107		0.211	0.157	SP
SW	-0.383	-0.508	-0.183	-0.032	0.416	0.443	-0.264	0.162		0.933	SW
GY	-0.744	-0.667	-0.517	0.284	0.119	0.38	0.071	0.482	0.458		GY

(c). Twelve white lupin accessions evaluated across six locations

Traits	DF	DM	BR	PH	PD	PL	PP	SP	SW	GY
DF		-0.153	0.354	0.299	0.084	0.302	0.196	0.121	0.366	0.507
DM	-0.153		-0.156	-0.255	0.168	0.294	-0.183	-0.313	0.433	-0.449
BR	0.354	-0.156		0.826	-0.338	0.26	0.877	0.37	0.604	0.427
PH	0.299	-0.255	0.826		-0.27	0.319	0.774	0.527	0.448	0.48
PD	0.084	0.168	-0.338	-0.27		0.038	-0.447	0.318	0.273	-0.273
PL	0.302	0.294	0.26	0.319	0.038		0.173	0.269	0.365	0.167
PP	0.196	-0.183	0.877	0.774	-0.447	0.173		0.459	0.478	0.35
SP	0.121	-0.313	0.37	0.527	0.318	0.269	0.459		0.219	0.318
SW	0.366	0.433	0.604	0.448	0.273	0.365	0.478	0.219		-0.057
GY	0.507	-0.449	0.427	0.48	-0.273	0.167	0.35	0.318	-0.057	

DF = Days to 50% flowering; DM = Days to 95%maturity; BR = Number of primary branches on the main axis; PH = Plant height in centimeter; PD = Pod diameter in millimeter; PL= Pod length in millimeter; PP = Pods per plant; SP = Seeds per pod; SW = 100 seed weight in gram; GY = Grain yield in tons per hectare

A genotype may be regarded as a package of traits; genotype evaluation must be based on multiple traits that are considered as breeding objectives (Yan, 2014a). In the focused experiment, the genotypes were evaluated for multiple traits across locations and a polygon view of the which -wins-where biplots of GT were constructed based on mean values of the traits (Fig. 2a-e). The biplots identified the best accession (s) for specific trait or group of traits.

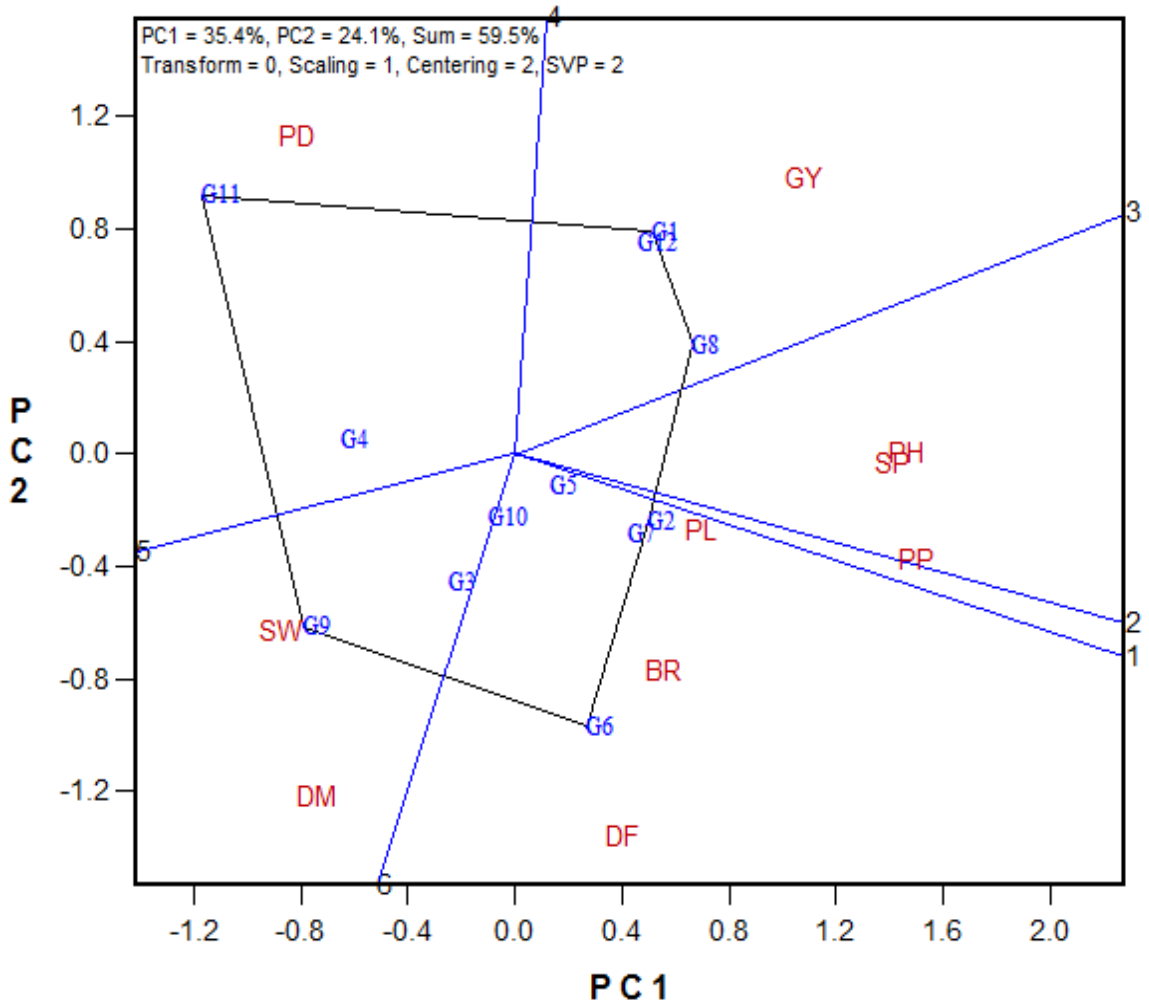
Accordingly, landraces G1, G2, G6, G8, G9 and G11 were vertex genotypes; and hence, were the most responsive in high altitude environments (Fig. 2a). Vertex accessions show higher values for the traits that fall within the same sector in the biplot (Yan *et al.*, 2007b). Hence, in the highland ecology, G1 and G8 had the higher grain yield; G2 had the highest number of pods per plant and longest pods; G6 had the highest number of branches and was the latest in flowering; G9 had the heaviest seed weight and was the latest in maturity; and G11 had wider pod diameter than the other accessions. However, GT relationships observed in the highland

ecologies were not consistent with the relationships observed in other ecologies. For example, in the mid-altitude ecology (Fig. 2b), G2 had the highest mean values for seven out of the 10 measured traits, except for days to maturity, pod length and diameter. In contrary, G11 had the lowest performance for all the traits considered. In the low altitude environments, G2 and G8 showed desirable performances for grain yield, pods per plant, plant height and branch number whereas G7 had higher number of seeds per pod, larger seeds, longer pods but flowered late. G2 showed taller plant height, higher number of branches, higher number of seeds per plant but delayed in flowering. G8 had the highest grain yield and number of pods per plant. G7 had more number of pods per plant and larger seed and G4 produced longer pods and matured later whereas G11 had lower values for all the traits measured. The polygon view of the genotype by trait biplot for the data combined across locations (Figure 2e) showed that accessions G8 and G2 had higher grain yield, whereas G11 followed by G9 and G4 had lower grain yield. G8 also showed better grain yield in the highland (Figure 2a) and lowland (Figure 2d) ecologies, indicating the inherent higher yield potential of this accessions. Hence, G8 could be recommended for further exploitation in breeding programs.

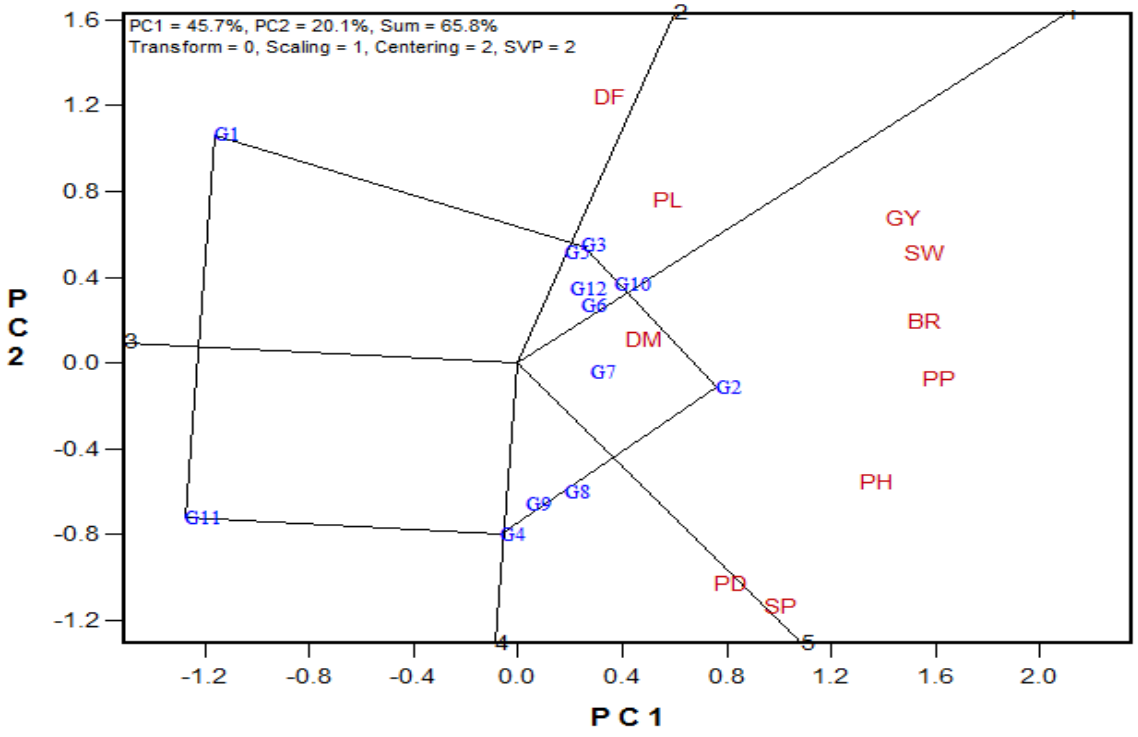
Figure 2c presents trait profiles of 143 white lupin landrace accessions evaluated at Merawi. The biplot gave more opportunity to assess which accessions were good for which trait (s) that would help as an excellent baseline information for white lupin improvement in Ethiopia and elsewhere. This biplot is divided into 10 sectors; out of which three sectors are without traits. Sectors without traits indicate that the accessions falling in these sectors including the vertex accessions, for instance Acc55, Acc102 and Acc30, had low values for all the traits studied. On

the other hand, sectors in which both accessions and traits fell could clearly and visually depict the best accession for each trait. For instance, accession (Acc119) is the vertex accession in the grain yield (GY) sector and hence the accession was the highest yielder, and Acc103 had more pods per plant (PP) and the tallest plant height (PH) in that particular sector.

a



b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PL
GY
SW
BR
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PP
PH
PD
SP
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G3
G5
G12
G10
G6
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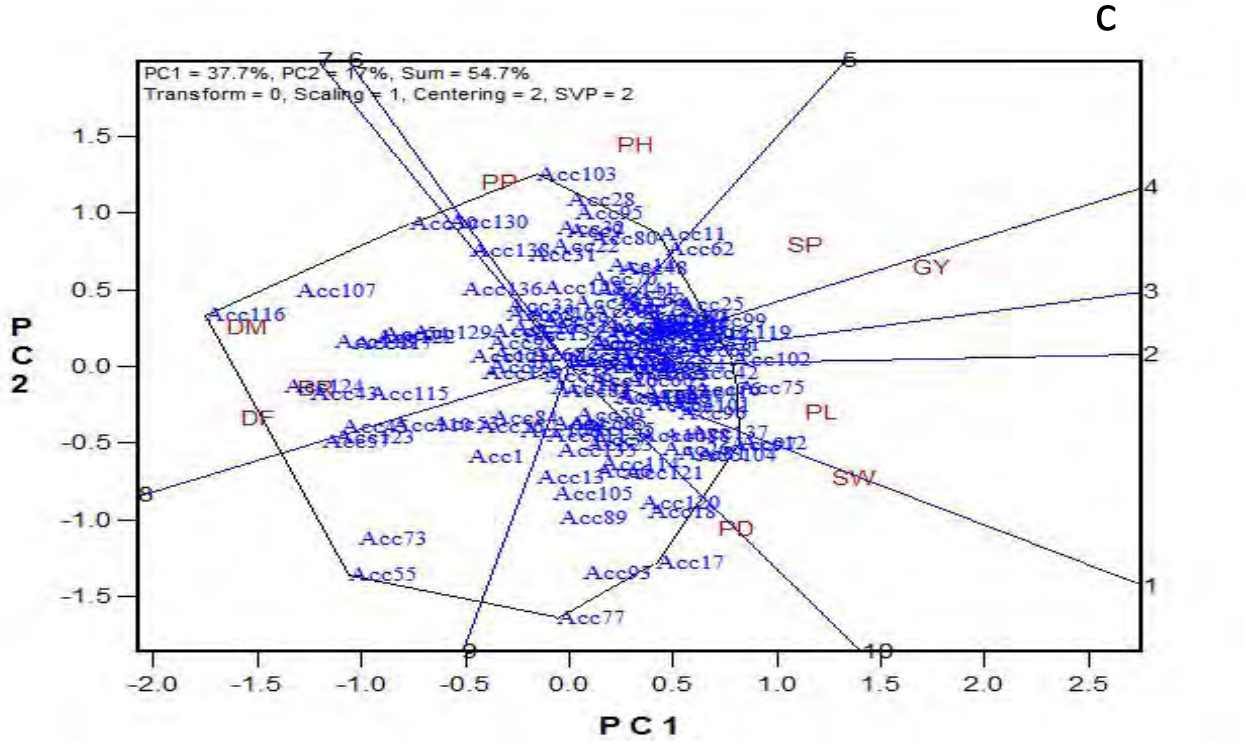
G2
G8
G9
G4
G11
1
2
3
4
5
PC 2
PC 1

c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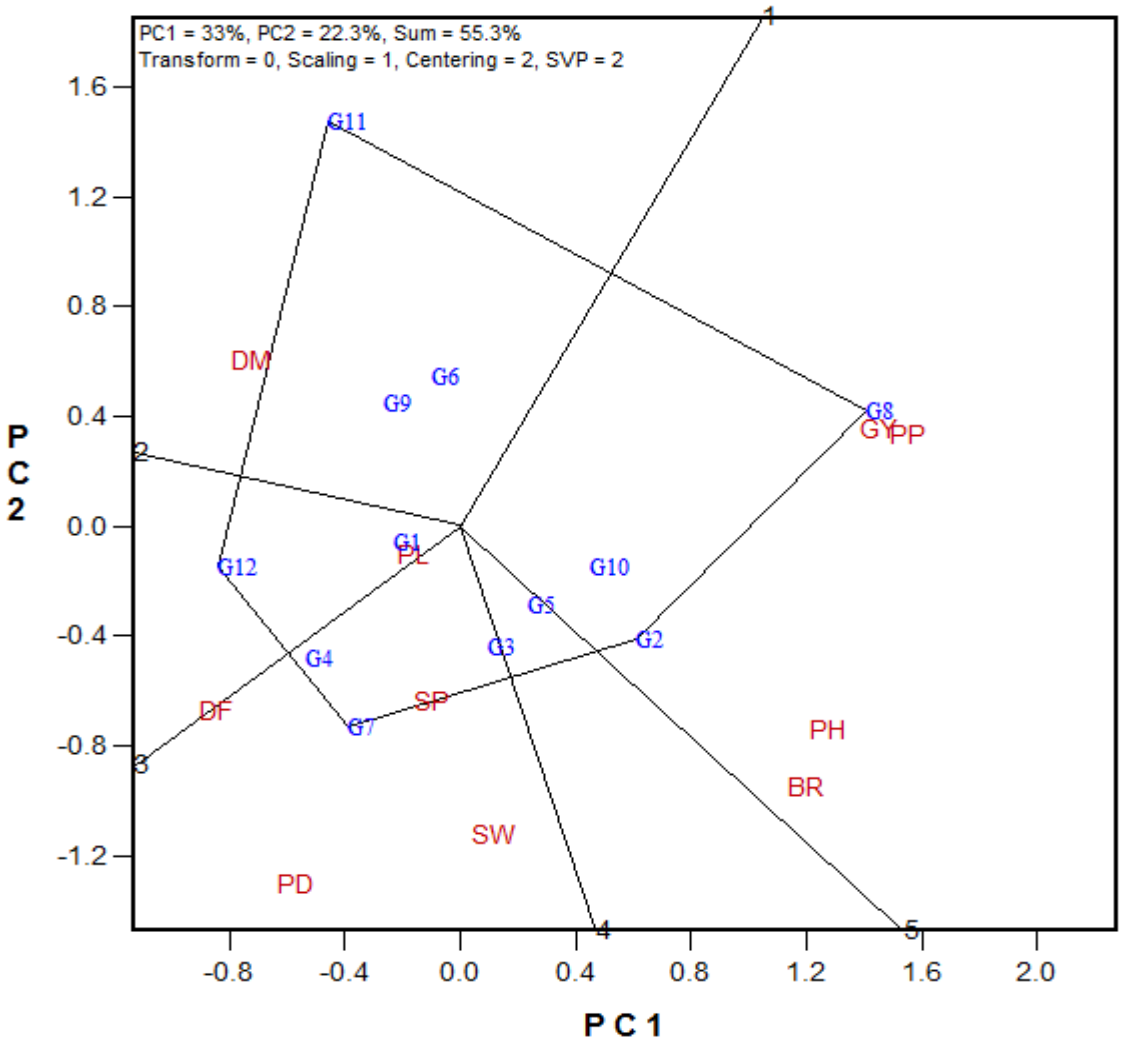
PH
PP
SP
GY
DM
DF
PL
SW
PD
Acc103
Acc107
Acc116
Acc115
Acc1
Acc73
Acc55
Acc77
Acc89
Acc105
Acc17
Acc93
1
2
3
4
5
6
7
8
9
10
PC 2
PC 1

d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1.6
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
PC 2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
PC 1
1
2
3
4
5
G11
G6
G9
G8
G1
G12
G10
G5
G2
G4
G3
G7
DM
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DF
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PH
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SW
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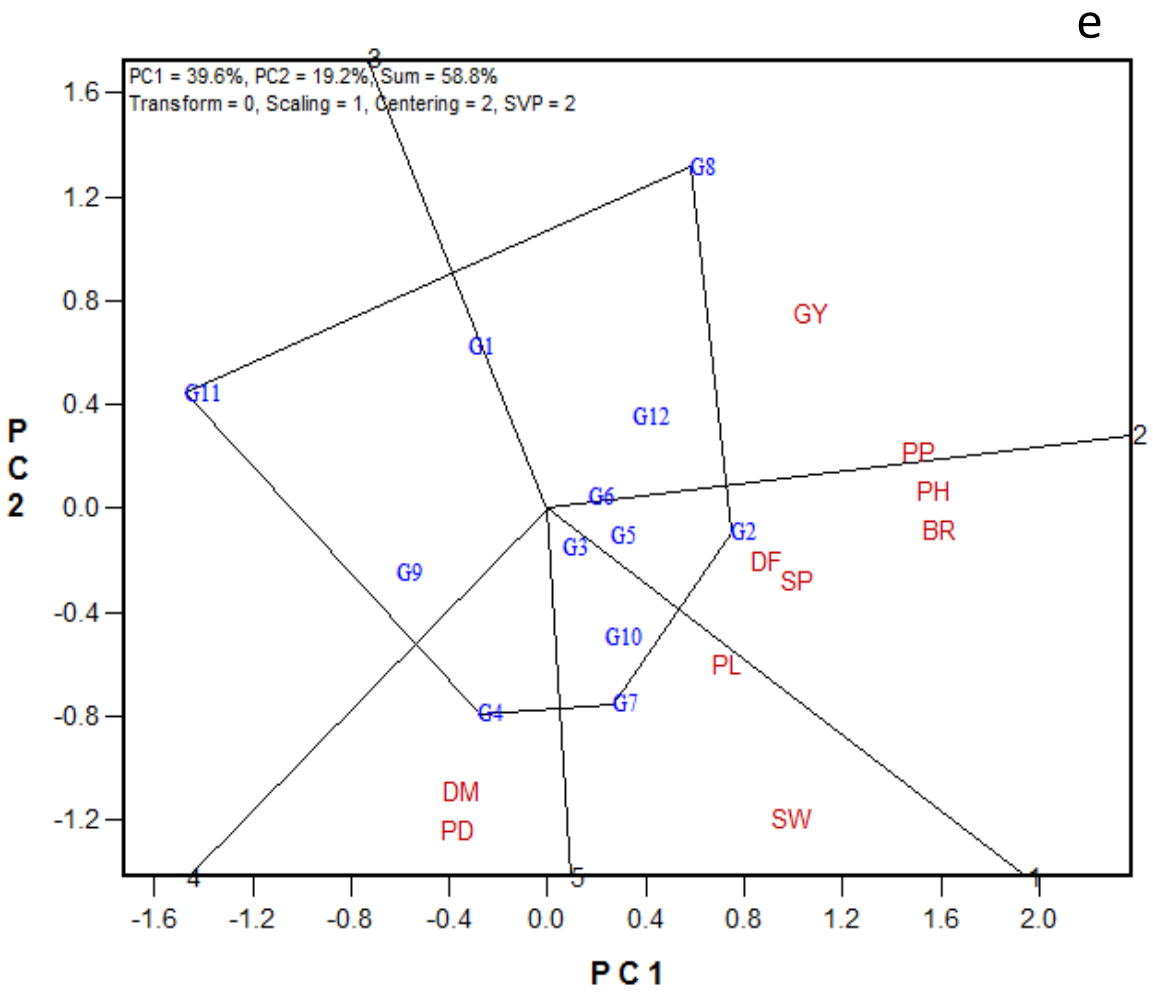

Figure 2: Polygon view of the genotype by trait biplot for different environments in Ethiopia, demonstrating white lupin landrace comparison on the basis of a GT biplot. (a) High altitude (Injibara and Debre Tabor) environments; (b) Mid-altitude (Fenote Selam and Merawi) environments; (c) Merawi which evaluated 143 landrace accessions; (d) Low altitude (Dibate and Mandura) environments; and (e) Across six testing sites representing highland, mid altitude and lowland ecologies. G1 - G12 are codes for white lupin landraces evaluated in all the six environments; whereas Acc1- Acc144 in Figure 'c' are codes to represent accessions phenotyped at Merawi, Ethiopia. GY = Grain yield; PP = Pods per plant; SP = Seeds per pod; SW = 100 seed weight; PH = Plant height; BR = Number of branches on the main axis; PL = Pod length; PD = Pod diameter; DF = Days to 50% flowering; and DM = Days to 95% physiological maturity.

Chapter 6

Genotype by Environment Interaction and Grain Yield Stability of Ethiopian White Lupin (*Lupinus albus* L.) Landraces

Under review (Experimental Agriculture, Cambridge University)

Genotype by Environment Interaction and Grain Yield Stability of Ethiopian White Lupin (*Lupinus albus* L.) Landraces

Abstract

Genotype by environment interaction is a common phenomenon in crop production and remains an important issue in genotype evaluation and recommendation. However, no comprehensive multi-environment evaluation of Ethiopian white lupin has been undertaken so far. This study was undertaken with the objectives to evaluate the performance and stability of white lupin landraces in different locations; and characterize white lupin growing environments in Ethiopia. Twelve Ethiopian white lupin landraces were evaluated across six different locations in Ethiopia during the 2014/15 main growing season using randomized complete block design with four replications. The genotype main effect plus genotype by environment interaction (GGE) biplots analysis was used to visualize the patterns of the interaction components. The results depicted that the white lupin landraces studied had differential performances at different test environments implying the presence of crossover interaction. The first two principal components (PC1=41.6% and PC2=21.8%) of the GGE explained 63.4% of the GGE sum of squares. Two white lupin growing mega-environments were identified in north western Ethiopia. All test locations were found to be representative with different degrees of reliability whereby Fenote Selam and Dibate were found to be most representative. In addition, all test locations, except Mandura and Injibara, had generally similar and good discriminating power. Fenote Selam and Dibate were found to be the most representative and discriminating environments and are characterized as most desirable test locations for white lupin improvement in north western Ethiopia. G2 was found to be the highest yielding and most stable landrace across the test environments, and hence identified as most desirable genotype recommended for production.

Key words: Genotype x Environment; Genotype plus Genotype x Environment; Lupin; Mega-environment; Representativeness; discriminating power

Introduction

Genotype by environment interaction (G x E) is a common phenomenon in crop production; and remains an important issue in genotype evaluation and recommendation that suits for specific or wider production environments. G x E is understood as the differential ranking of genotypic performances among locations or years. Basically, there are two different approaches towards G x E. The first approach is development of high yielding genotypes with low G x E interaction; that is, widely adapted genotypes across mega-environments. The second approach is exploitation of G x E by breeding genotypes for maximum yield and stability within mega-environments. Plant breeders conduct multi-environment trials primarily to identify superior cultivars for a target region and to determine mega-environments (Yan *et al*., 2000). Targeting of cultivars to specific locations becomes difficult when G x E is present, because yield is less predictable and cannot be interpreted based only on genotypic and environmental means (Ebdon and Gauch, 2002).

Several statistical methodologies have been proposed and employed to analyze and visualize the nature and magnitude of G x E for multi-environment trail data. More recently, however, additive main effect and multiplicative interaction (AMMI) (Gauch, 2006; Zobel *et al*., 1988) and genotype plus genotype by environment interaction (GGE) proposed by Yan *et al*. (2000) models are becoming popular for multi-environment trial data analysis. Furthermore, GGE is found to be the best fit for mega-environment analysis ('which-won-where' pattern), genotype evaluation (mean vs. stability), and test environment evaluation (discriminating power vs. representativeness) (Amira *et al*., 2013; Asfaw *et al*., 2009b; Yan *et al*., 2007c; Samonte *et al*.,

2005). Several researchers recognized GGE as the most useful method to analyze and visualize the pattern of G x E in multi environment cultivar evaluation of different crop species, including wheat (Yan *et al*., 2000), maize (Fan *et al*., 2007), soybean (Atnaf *et al*., 2013b; Jandong *et al*., 2011; Asfaw *et al*., 2009b), common beans (González *et al*., 2006; Kang *et al*., 2006), oilseeds (Brar *et al*., 2010) and kenaf (Atnaf *et al*., 2014).

White lupin (*Lupinus albus* L.) is an annual legume crop that is traditionally cultivated around the Mediterranean and along the Nile valley where it is used for human consumption, green manuring and as forage. It is adapted to wide ecological conditions from Alaska to Argentina (Cowling *et al*., 1998b) characterized by low pH (Huyghe, 1997), low nutrients availability (Marschner *et al*., 1987) and high salinity, excess nitrate (Hernández *et al*., 1999). Several workers evaluated different gene pools of white lupin landraces under different growing environments and have found out that white lupin grain yield and other agronomic and adaptive traits are responsive to different growing conditions, implying the presence of G x E (Christiansen *et al*., 2000; Lagunes-Espinoza *et al*., 2000; Kurlovich, 2002a; Rubio *et al*., 2004; Noffsinger and van Santen, 2005; Gonzalez-Andres *et al*., 2007; Annicchiarico *et al*., 2010; Annicchiarico and Carroni, 2009).

Similarly, region-specific top-yielding (as high as 5 tha^{-1}) white lupin landraces with useful adaptive traits were reported by several investigators in different parts of the world, such as, in Western Europe (Julier *et al*., 1993), Southern Europe (Rubio *et al*., 2004; Annicchiarico and Carroni, 2009; Annicchiarico *et al*., 2010) and east and north African countries, including Ethiopia, Egypt and Morocco (Atnaf *et al*., 2015b; Sbabou *et al*., 2010; Christiansen *et al*., 2000).

This indicates specificity in performance and adaptation of the white lupin germplasms considered in those studies. Yeheyis *et al.* (2012b) also reported the importance of G x E on the performance of grain yield and other traits in sweet and bitter cultivars of different lupin species evaluated in three different lupin growing locations of Ethiopia. However, no comprehensive multi-environment evaluation of Ethiopian white lupin has been undertaken so far. The objectives of this work, therefore, were to (1) evaluate the performance and stability of white lupin landraces in different growing environments, and (2) characterize white lupin growing environments in Ethiopia.

Materials and Methods

The experiment was conducted at six different locations in Ethiopia; namely, Debre Tabor, Injibara, Merawi, Fenote Selam, Dibate and Mandura during the 2014/15 main growing season. The locations represent most lupin growing environments that range from low to high altitude agro-ecologies (Table 1). Twelve white lupin accessions were used for the study (Table 4); of which, eleven were collected by the Ethiopian Biodiversity Institute (EBI) from different localities of northwestern Ethiopia and one accession (G12) is a local cultivar commonly grown by farmers in West-Gojam zone.

The experiment was laid out in randomized complete block design with four replicates, except at Merawi where two replications were used. A plot with an area of 15 meter square (5m x 3m) was used. Spacing between plants and rows were 25 cm and 75 cm, respectively. The plots

were hand planted in rows; and fertilizer was not applied at any of the locations during the cropping period. Agronomic and plant protection management practices were applied uniformly across the plots for the duration of the experiment.

Grain yield data was recorded in grams on plot basis from four harvestable rows and later expressed as tons per hectare (tha^{-1}) after adjusting moisture content to 14 percent. The data were subjected to combined analysis of variance employed in the GGE software (Yan, 2001). The existence of significant G x E variance for grain yield justified further partitioning of the G x E variance into principal components. Partitioning of the G x E was performed using the GGE model (Yan, 2001). The GGE refers to the genotype main effect and the G x E, which are the two most important sources of variation for cultivar evaluation in multi-environment trials (Yan *et al*., 2007c). A GGE biplot displays the genotypic main effect and G x E of a genotype by environment data set (Yan *et al*., 2000). This biplot is specially and perfectly used for mega-environment analysis based on genetic correlation between environment and the which-won-where pattern; test environment evaluation based on their discriminating ability and representativeness; and genotype evaluation based on their mean performance and instability within a mega-environment provided that a given data set has a high correlation between PC1 and genotype main effects (Crossa *et al*., 2002). This correlation was calculated using the GGE software.

The GGE bi-plot was constructed using the first two principal components (PC1 and PC2) derived from subjecting environment centered yield data (Yan *et al*., 2000). The GGE model used was:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} \quad [1]$$

Where Y_{ij} is measured mean of genotype *i (=1,2,….,n)* in environment *j (=1,2…,m),* μ is the grand mean, β_j is the main effect of environment *j,* $\mu + \beta_j$ being the mean yield across all genotypes in environment *j,* λ_1 and λ_2 are the singular values (SV) for the first and second principal component (PC1 and PC2), respectively. ξ_{i1} and ξ_{i2} are eigenvectors of genotype i for PC1 and PC2, respectively. η_{1j} and η_{2j} are eigenvectors of environment *j* for PC1 and PC2, respectively. ε_{ij} is the residual associated with genotype *i* in environment *j*.

PC1 and PC2 eigenvectors cannot be plotted directly to construct a meaningful biplot before the singular values are partitioned in to the genotype and environment eigenvectors. Singular value partitioning was implemented by

$$g_{i1} = \lambda_1^{f_1} \xi_{i1} \; and \; e_{ij} = \lambda_1^{1-f_1} \eta_{ij} \quad [2]$$

Where f_1 is the portion factor for PC_1. The f_1 can range between 0 and 1. To visualize relationship among genotypes, the GGE biplot based on genotype metric (that is, f=1; S.V.P=1) and environment metric (f=0; S.V.P=2) are appropriate. GGE biplot is important to visualize relationships among environments. So the following formula from equation [1] was formulated to generate the GGE biplot:

$$Y_{ij} - \mu - \beta_j = g_{i1} e_{1j} + g_{12} e_{2j} + \varepsilon_{ij} \quad [3]$$

If the data were environment standardized, the common formula to generate the GGE biplot was as follows:

$$\frac{Y_{ij} - \mu - \beta_j}{s_j} = \sum_{i=1}^{k} g_{i1} e_{1j} + \varepsilon_{ij} \qquad [4]$$

Where s_j is the standard deviation in environment *j, i=1,2,….,k,* g_{i1} and e_{1j} are PC_1 scores for genotype *i* and environment *j*, respectively. In the present study we used environment standardized model, equation [4].

Table 1: Description of test locations used for the experiment

Location	Altitude (masl*)	Latitude	Longitude	Annual RF(mm)**	M min T (0^c)	M max T (0^c)	Soil Type
Debre Tabor	2630	$11^0$89'N	$38^0$09'E	1500.0	11.8	23	Luvisol
Injibara	2560	$10^0$57'N	$36^0$56'E	2300.0	8.0	22	Acrisol
Merawi	1960	$11^0$42'N	$37^0$17'E	1576.6	7.8	25.8	Nitosol
Fenote Selam	1917	$10^0$42'N	$37^0$16'E	1343.6	12.9	29.4	Nitosol
Dibate	1615	$10^0$77'N	$37^0$26'E	1000.0	15	29	Nitosol
Mandura	1400	$10^0$95'N	$37^0$43'E	1300.0	15	35	Nitosol

*= Meters above sea level; ** = Rain fall in millimeter; M min T and M max T= Mean minimum and mean maximum temperature in degree Celsius, respectively

Results and Discussion

Analysis of variance and mean values

Statistically significant variations were observed among the white lupin genotypes for grain yield at individual locations (Table 2). However, mean performances of the genotypes greatly varied from location to location (Table 4), which might attribute to the environmental suitability of the test locations for white lupin production. Among the six locations, the highest mean grain yield of 3.72 tha^{-1} was recorded at Debre Tabor while the lowest (0.40 tha^{-1}) was observed at Mandura. Combined analysis of variance for grain yield over locations exhibited highly significant ($p<0.001$) mean squares due to genotype, environment, and G x E among the Ethiopian white lupin landraces (Table 3). Significant variations among the genotypes indicates the differences in the inherent genetic potential of the landraces that makes selection possible, whereas differences among the environments showed the variability in potential suitability of the test locations for white lupin production.

Before conducting GGE analysis, a basic analysis of variance is useful to get an idea about the relative magnitudes of the various sources of variation. According to Yan *et al*. (2014b), GGE biplot analysis is suitable whenever G and/or G x E are statistically significant as observed in the current study (Table 4). Annicchiarico *et al*. (2010) and Annicchiarico and Carroni (2009) reported the importance of genotype and genotype by environment interaction variances in white lupin grain yield in Italy and France. Across locations, G2 (2.44 tha^{-1}) and G12 (2.22 tha^{-1}) showed higher grain yield, whereas G9 (1.60 t ha^{-1}) and G4 (1.61 tha^{-1}) had lower grain yield.

Environment effect explained about 86.87% of the total sum of the square, indicating that most of the experimental variations were attributed to the environment. This indicates that the locations were diverse. Of most relevance is the G/(G+G x E) ratio, which is 0.26, indicating that G x E was relatively large in the dataset. This indicates that G x E must be considered in genotype evaluation and that GGE biplot analysis would be essential to reach meaningful conclusions about the genotypes (Yan, 2014b).

Table 2: Summary statistics for grain yield (t ha^{-1}) for individual test locations

Environment	Mean	Max	SE	LSD5%	SD	H
Fenote Selam	2.09	2.70	0.31	0.41	0.47	0.90
Mandura	0.40	0.56	0.12	0.17	0.07	0.26
Dibate	0.75	1.01	0.13	0.18	0.13	0.73
Debre Tabor	3.72	4.91	0.83	1.13	0.69	0.64
Injibara	3.43	4.58	1.22	1.65	0.81	0.43
Merawi	1.27	2.03	0.08	0.16	0.59	0.99

Landraces = Number of landraces; Max = Maximum; SE = Standard error; LSD5% = Least significant difference at 5% probability level; SD = Standard deviation; and H = Heritability in the broad sense

Table 3: Analysis of variance for grain yield of 12 white lupin landraces grown at six locations in Ethiopia

Source	DF	SS	MS	F	Probability	% SS
Genotype (G)	11	17.54	1.59	3.7	<0.001	3.38
Environment (E)	5	451.13	90.23	208	<0.001	86.87
G X E	55	50.67	0.92	2.1	<0.001	9.76
Rep (E)	16	1.98	0.12	0.3	1	
Error	176	76.33	0.43			
TOTAL	263	597.64				
G+E+G x E	71	519.33				
G/GGE (G + G x E)				0.26		

G x E = Genotype by environment interaction; Rep (E) = Replication nested in each location; G/GGE = Ratio of genotype to (genotype plus genotype by environment interaction); DF = Degree of freedom; SS = Sum squares; MS = Mean squares; F = F value; and % SS = Percent sum squares explained

Larger G x E effect in this study suggests the possible presence of different mega-environments with different winner genotypes (Yan and Kang, 2003). Previous researches indicated the importance of G x E effect in white lupin in Western Europe (Annicchiarico and Carroni, 2009); in narrow leafed lupin in Western Australia and Western Europe (Annicchiarico and Carroni, 2009; Bob and Mario, 2003). The present experiment also depicted that the performance of

white lupin landraces were different at different testing environments (different winners at different locations) due to the existence of large G x E. The existence of different winner genotypes in different mega-environments complicates the selection process and cultivar recommendation in breeding programs (Comstock and Moll, 1963).

GGE analysis

The present data set showed 0.971 correlations between the primary effects and the genotype main effects which justifies the use of GGE biplot (Crossa *et al*., 2002; Yan *et al*., 2000). Analysis of principal components showed that the first three PCs had information ratio (IR)> 1.0. However, still the first two principal components usually (the 2-D biplot) display the most important patterns (Yan, 2014b). Thus, in this study, the first two principal components (PC1 and PC2) of the GGE explained 61.8% (PC1 = 34.3% and PC2 = 27.5%) of the GGE sum of squares using environment standardized model.

White lupin mega-environment analysis in Ethiopia

Two forms of the GGE biplot were used in mega-environment analysis; the location vector form (Fig. 1) and the which-won-where form (Fig. 2). The location vector view of the GGE biplot (Fig. 1) facilitates visualization of the genetic correlations between test locations in ranking genotypes based on yield. Lines that connect the biplot origin with environment markers are known as environment vectors and the angle between the vectors of two environments is related to the correlation coefficient between the environments which is approximated by the cosine of that angle (Yan, 2002). Acute angles indicate a positive correlation, obtuse and right

angles show negative and no correlation, respectively (Yan and Kang, 2003). Five of the six locations used for the current study appeared to be positively correlated. The two high altitude locations, Injibara and Debre Tabor, apparently grouped very close to each other. Two low-altitude locations (Dibate and Mandura) and one mid-altitude location, Fenote Selam, were positively correlated. The pattern of relationship among locations generally followed altitudinal and agro-ecological based similarities. The angles between test locations are an indication of the relative magnitude of G versus G x E (Yan, 2014b).

On the other hand, the study showed larger G x E between some locations, for example, strong negative correlation between Merawi and Injibara. The presence of negative correlations among locations suggests that the target region may consists of different mega-environments. The grouping pattern observed in this study suggests that there are two white lupin mega-environments in north western Ethiopia (Fig. 1). The first one was high altitude mega-environment represented by Injibara and Debre Tabor; and the second one was mid to low altitude locations that includes Fenote Selam, Mandura, Dibate and Merawi. However, to confirm patterns observed in the current study, there should be a multi-years data to see the repeatability of the pattern over the years.

Mega-environments are often defined by the which-won-where patterns (Yan *et al*., 2000; Gauch and Zobel , 1997). Visualization of the 'which-won-where' pattern in the polygon view is helpful to estimate possible existence of different mega-environments in the target environment (Yan and Rajcan, 2002; Yan *et al*., 2000; Yan and Tinker, 2006).

Table 4: Mean grain yield (tha^{-1}) of 12 white lupin landraces tested at six different locations in Ethiopia

Landrace	EBI	Source	Fenote	Merawi	Debre	Injibara	Dibate	Mandura	Mean
G1	242281	Awi	1.98	0.33	4.91	3.14	0.69	0.41	1.91
G2	238996	BD	2.70	1.71	4.23	4.58	1.01	0.42	2.44
G3	238999	WG	2.69	1.03	3.29	2.50	0.75	0.34	1.77
G4	236615	WG	1.47	1.42	2.88	2.96	0.62	0.32	1.61
G5	239029	WG	2.15	2.03	3.98	3.11	0.84	0.33	2.07
G6	239007	Awi	2.40	0.80	3.17	3.90	0.66	0.44	1.90
G7	242306	WG	1.90	1.81	3.37	3.17	0.72	0.36	1.89
G8	239003	Awi	1.58	1.56	4.17	4.04	0.82	0.56	2.12
G9	239045	Awi	1.71	2.02	2.74	2.08	0.69	0.37	1.60
G10	239032	WG	2.44	1.25	4.06	2.88	0.78	0.49	1.98
G11	207912	MK	1.47	0.38	3.29	4.36	0.84	0.39	1.79
G12	Local	WG	2.62	0.84	4.55	4.41	0.53	0.40	2.22
Mean			2.09	1.27	3.72	3.43	0.75	0.40	1.94

EBI= Ethiopian Biodiversity Institute; G1-G12 are codes representing white lupin accessions; Fenote = Fenote Selam; Debre = Debre Tabor; BD = Bahir Dar special; WG = West Gojam; MK = Mahakelegnaw

The polygon was drawn on genotypes placed away from the biplot origin so that all genotypes are contained in the polygon. The perpendicular lines radiating from the origin of the biplot

divide the biplot area as well as the test locations into sectors. Figure 2 presents a polygon view of twelve white lupin landraces tested at six different locations in Ethiopia. In the biplot, the six locations were fell into three distinct sectors. The first sector was marked by radiate lines 1 and 2, where the two high altitude locations (Injibara and Debre Tabor) fell in, landrace (G12), a local cultivar obtained from West-Gojam zone, was the vertex genotype in this sector and hence the nominal winner genotype in these locations. This is consistent with mean performance of the cultivar in these locations (Table 4); G12 showed higher grain yield at Injibara and Debre Tabor. The cultivar generally took longer to mature (data not shown) and hence showed better performance in the highland locations with longer growing season. It is generally agreed among the crop expert that earlier maturing genotype, owing to its shorter life cycle, is predisposed to lower yields than a later maturing genotype which has the opportunity to draw nutrients and photosynthesize over a longer period. Several workers documented that white lupin germplasm pools with taller stature, more leaves and branches on the main axis and late flowering and maturity tended to give more yield in environments offering longer growing durations (Annicchiarico *et al*., 2010; Annicchiarico and Carroni, 2009; Rubio *et al*., 2004; Kurlovich, 2002a), which exactly agrees with the present finding. The second sector was defined by the radiate lines 1 and 6. Low to mid altitude locations (Mandura, Dibate and Fenote Selam) fell in it, and the landrace (G2) was on the vertex for this sector, suggesting that G2 was the nominal winner at these locations. The third sector which was delineated by radiate 5 and 6 contained only a single location, Merawi; but no genotype fell in the sector.

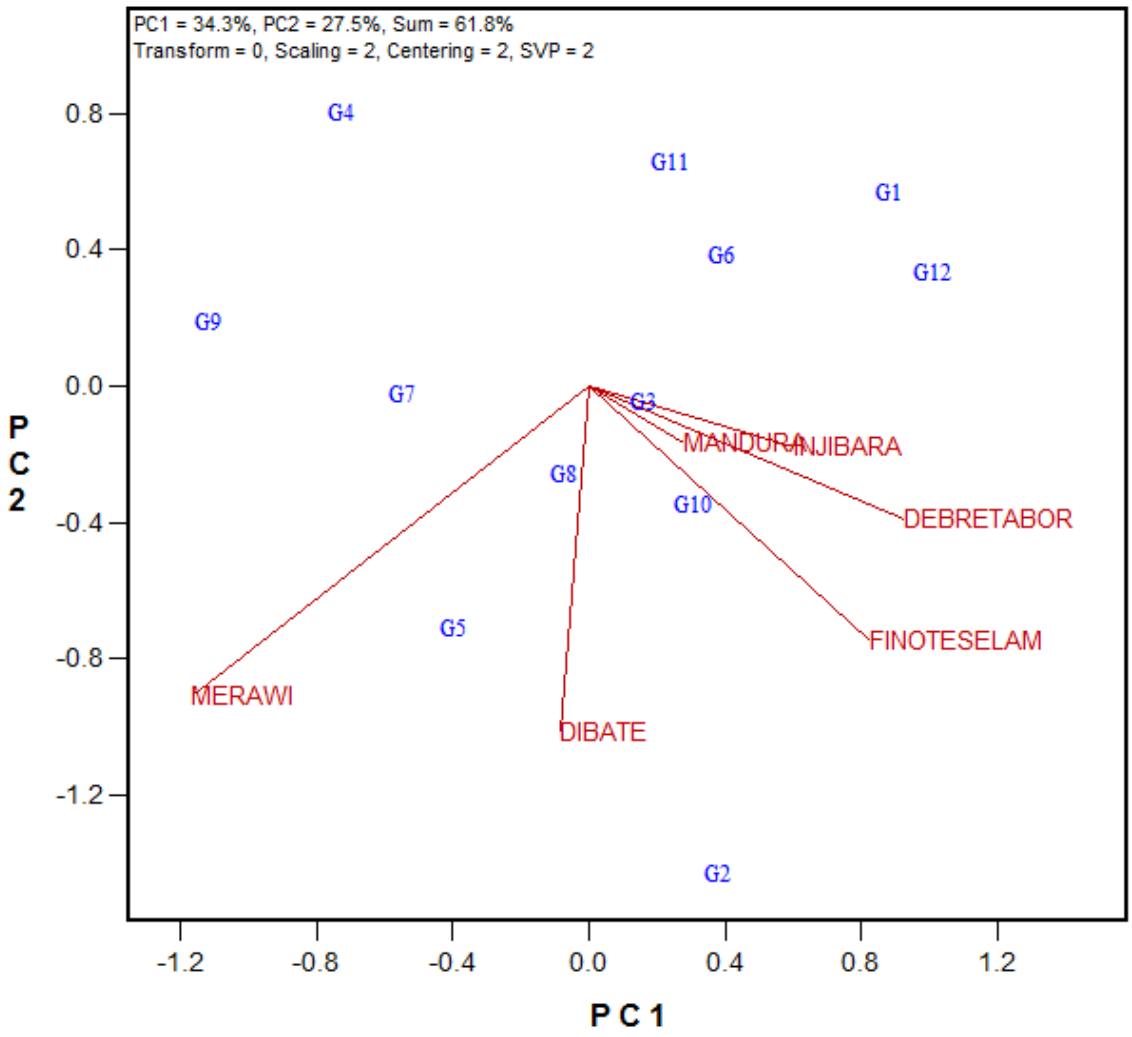

Figure 1: Location vector view of the GGE biplot for grain yield of 12 white lupin landraces evaluated across locations in Ethiopia. G1- G12 are codes representing white lupin landraces as described in Table 4.

Results of this study depicted the existence of three white lupin mega-environments in north western Ethiopia. However, the third sector in which Merawi fell in (without any genotype) is not distinctively different from the second sector, which contains locations with low to mid

altitude. Lack of clear mega-environment classification might be attributed to the fact that the bi-plots of the present data explained only 63% of the variation. Furthermore, the polygon view of GGE biplot is not consistent with the results of the location vector which suggested two mega-environments. Above all, it is important to note that conclusions from mega-environment analysis have a long-term effect on breeding and cultivar recommendation and must be based on multiyear data (Yan, 2014b); and both the location vector view and the which-won-where forms of the GGE biplot (Figures 1 and 2) for mega-environment delineations are useful and should be used complementarily.

Mean and stability of white lupin landraces

To identify widely adapted landraces, the Mean vs. Instability form of the GGE biplot containing all test locations were used (Fig. 3). In the biplot, the average environment is indicated by the small circle, which is a virtual environment defined by the average coordinates of all the environments (locations) to represent the target environment. The line passing through the biplot origin is called the average environment axis (AEA) (Yan and Kang, 2003). The double arrow line which is perpendicular to AEA and passes through the origin is termed as average environment coordination (AEC) in which the arrows point to higher instability for the genotypes (i.e., greater contribution to G x E) regardless of the direction. In the present data set, all locations were placed on the same side of the AEC in Figure 3, indicating that the G/G x E is sizable and that the AEA is meaningful for genotype evaluation.

Thus, the landrace G2 has the longest positive projection, indicating that it had the highest mean yield across locations; whereas G4 has the longest negative projection onto AEA,

indicating that it had the lowest mean yield across locations. All other landraces were ordered between these two extremes. The landraces G12 and G3, for example, have projections near the origin on the AEA, had mean yields close to the grand mean of the trial. With regard to stability, length of a line drawn from each genotype onto the AEC indicates instability of the genotype or its contribution to G x E. Hence, G2 contributed little to G x E and, therefore, was stable, whereas landraces like G12, G1 and G9 contributed more to G x E and were unstable. Annicchiarico and Carroni (2009) reported both specific and wide adapted white lupin cultivars tested in climatically contrasting environments in Italy. Similarly, Annicchiarico *et al*. (2010) reported that germplasm pools from Madeira and Canaries tended to adapt to wider environments whereas others from Europe, East Africa, West Asia, and the Mediterranean adapted to specific growing environments in Italy and France.

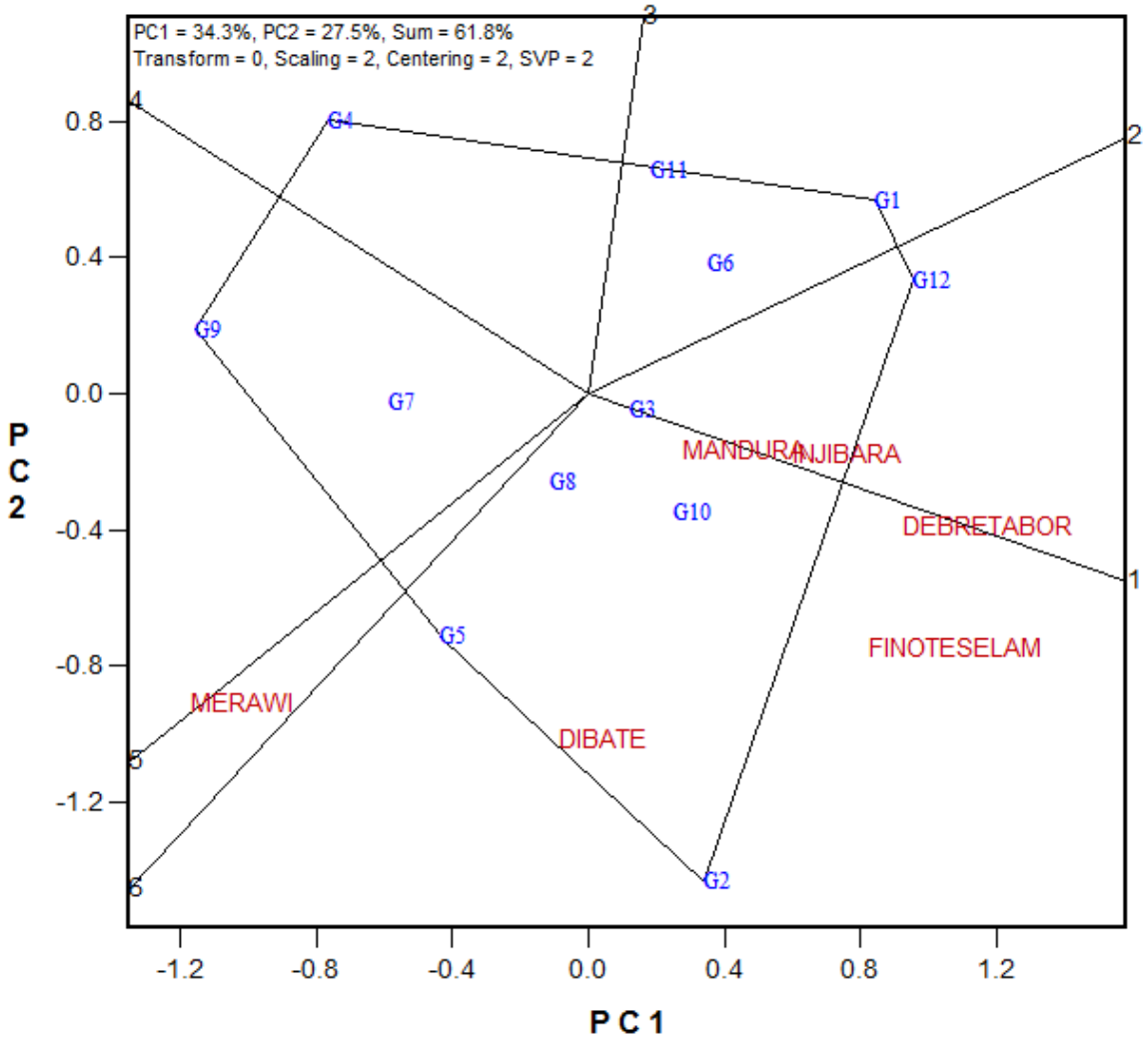

Figure 2: Polygon view of the GGE biplot to show which white lupin landrace wins where. G1–G12 are codes for white lupin landraces.

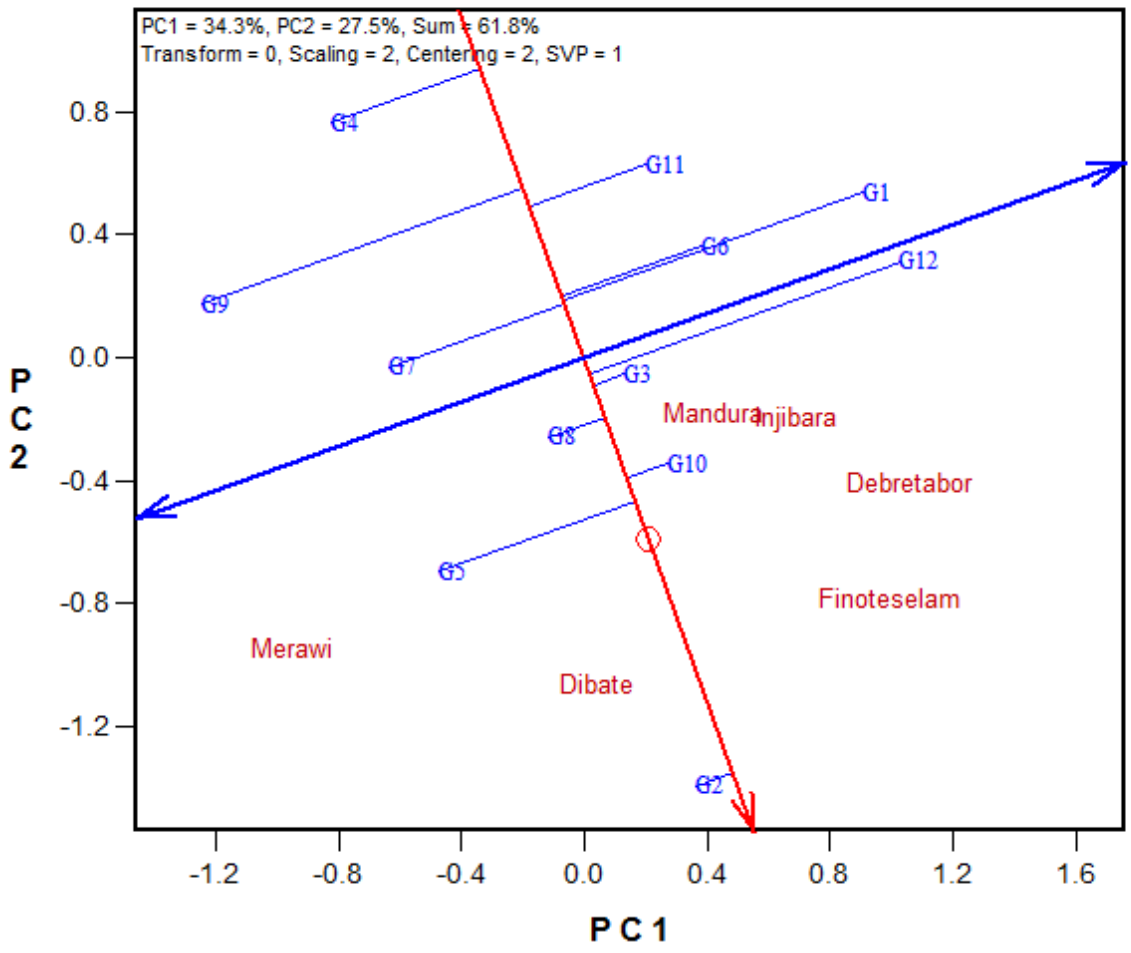

Figure 3: The Mean vs. Instability view of the GGE biplot for the white lupin grain yield evaluated across locations in Ethiopia.

An ideal genotype should have the highest possible mean performance and be absolutely stable (i.e., contributes zero to the G x E) (Yan and Kang, 2003). This ideal genotype is defined by the small circle in Figure 4. The desirability of the genotypes is judged by their closeness to this "ideal" genotype. Thus, G2 was the most desirable whereas G4 was the least desirable

genotypes in this study. Other landraces based on distance from the ideal genotype were ranked as G10>G5>G8>G3>G12>G7>G6>G1>G11>G9, indicating that those ranked last were less desirable as they were further from the ideal genotype. The distances of genotypes from the ideal genotype (GGE distances) as well as their ranks relative to the ideal genotype are presented in Table 5. The smaller the distance of a genotype from the ideal genotype, the more desirable it is.

Table 5: Instability values, distance to ideal genotype and ranks of 12 white lupin landraces based on GGE analysis

Landrace	Instability	Rank*	Distance to Ideal
G2	0.10	1	0.80
G10	0.30	2	1.80
G5	-0.10	3	1.80
G8	0.10	4	2.00
G3	0.00	5	2.10
G12	-0.50	6	2.40
G7	0.30	7	2.50
G6	-0.50	8	2.50
G1	-0.20	9	2.60
G11	-0.20	10	2.80
G9	0.50	11	3.00
G4	0.20	12	3.20

G1 -G12 are codes for white lupin landraces; Rank* = Rank on closeness to Ideal

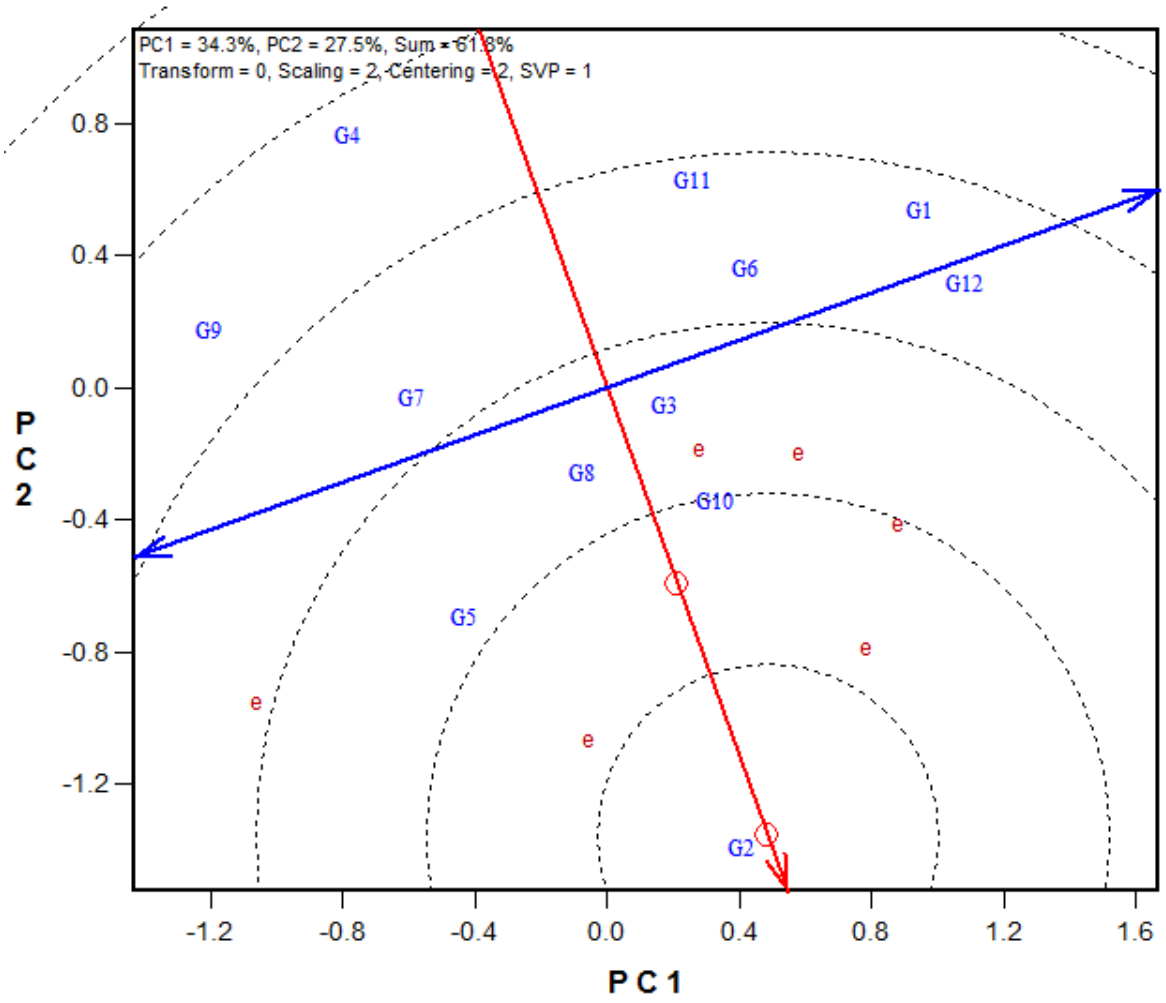

Figure 4: Ranking landraces based on both mean and instability.

White lupin test locations evaluation in Ethiopia

Figure 5 presents the representativeness and discriminative power of white lupin test locations in Ethiopia based on standard deviation (SD)-scaled and heritability (*h)*-weighted data which is an appropriate biplot for test location evaluation (Yan and Holland, 2010). In the biplot (Fig. 5; Table 6), it was appeared that all locations showed positive correlations with the average environment axis (AEA), and hence all locations were representative. However, the degree of representativeness differs in that Fenote Selam and Dibate were most representative white lupin growing locations in north western Ethiopia whereas Merawi was the least representative.

The length of the location vectors approximates the *h* of the locations, which is a measure of the discriminating power of the locations. All locations except Mandura and Injibara had generally similar and good discriminating power (Fig. 5; Table 6). The circle at the center of the concentric circles in Figure 5 represents the "ideal test location." It is a virtual location defined to have the longest vector of all locations and to be absolutely representative (i.e., it has zero contribution to G x E and therefore is located on the AEA). The closer a location to this ideal location, the more desirable it is as test location. The concentric circles help visualize this distance. In the biplot, locations Fenote Selam and Dibate were closest to the ideal location. Meanwhile, it was observed that locations were not redundant and hence provided different information about the performance of white lupin landraces. Nevertheless, test locations evaluation should be concluded based on multi-years data (Yan, 2014b). An "ideal" test

environment should be both discriminating of the genotypes and representative of the mega-environment (Yan *et al.*, 2007c).

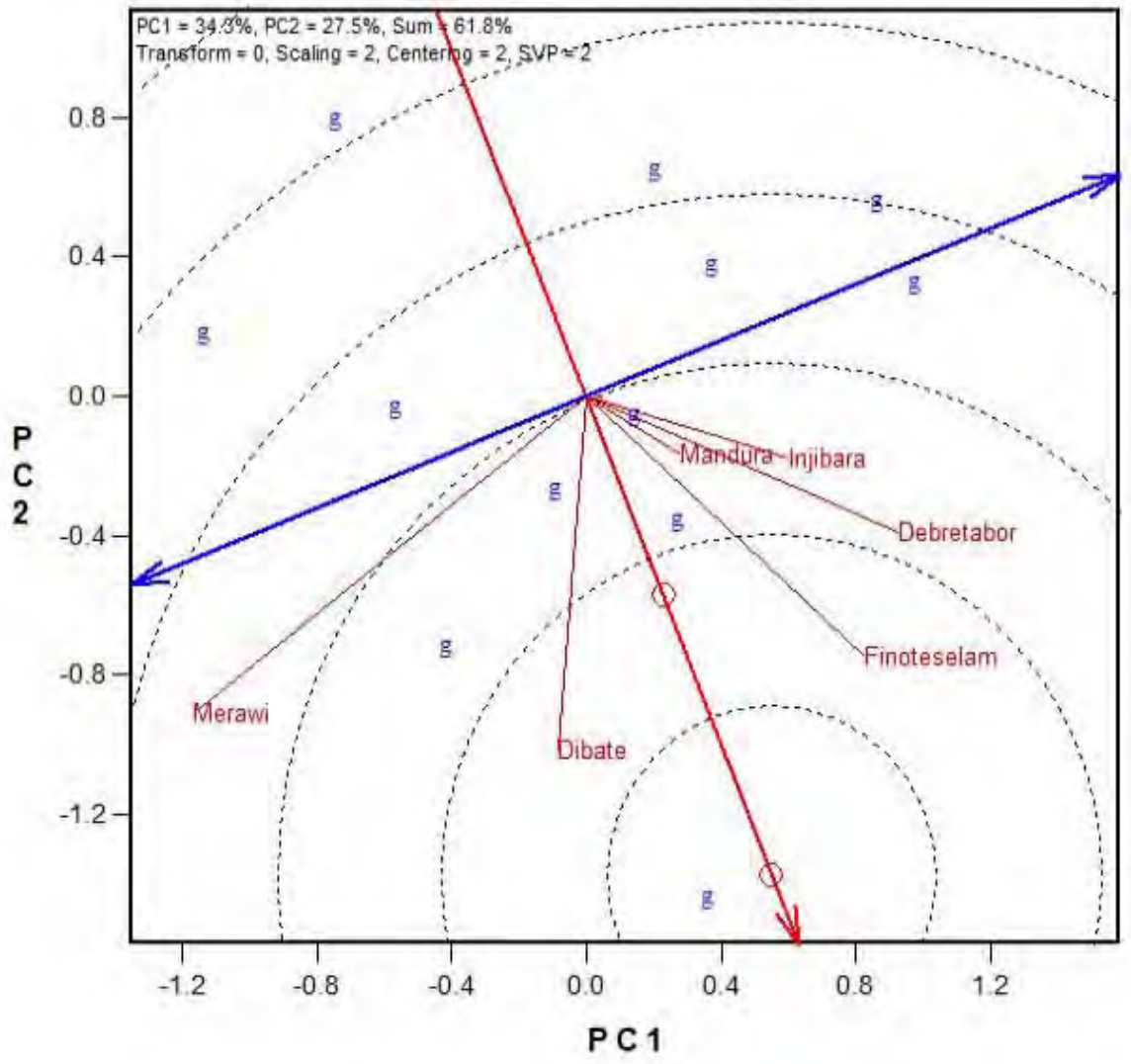

Figure 5: Representativeness and discriminative power of white lupin test locations in Ethiopia.

Table 6: White lupin test location evaluations (Vector length, Correlation with AEA and distance to the ideal location) in Ethiopia

Location	Vector Length	Correlation With AEA	Distance to Ideal location
Fenote Selam	1.12	0.90	1.36
Mandura	0.33	0.81	2.01
Dibate	1.03	0.90	1.42
Debre Tabor	1.01	0.71	1.72
Injibara	0.63	0.63	1.94
Merawi	1.48	0.28	2.33

AEA = Average environment axis

Chapter 7

Summary, Conclusion and Recommendations

Summary and Conclusion

Lupins are known to perform multifaceted utilities, such as for human food and beverage, livestock feed, ecological importance, pharmaceutical values and social contributions. The composition of the lupin grain is quite unique, being high in protein and soluble fiber, with no starch and very low fat. Despite little attention given by different actors, white lupin (*Lupinus albus*) has been sustaining for quite long time in the Ethiopian farming system, and it is produced exclusively by smallholder subsistence farmers, mainly for its food grain and soil fertility maintenance values (Atnaf *et al*., 2015a; Yeheyis *et al*., 2010). However, farmers' local varieties have several undesirable characteristics, such as low yield potential, high level of alkaloids, susceptibility to major lupin diseases and late maturing. Therefore, there is a need to develop well adapted white lupin varieties profiled with preferred qualities including high grain yield, low alkaloids level, early maturing and resistant to major lupin diseases and insect pests. To this effect, setting up practical lupin breeding program targeting major production constraints is quite essential and necessary. The present study attempted to avail various pertinent socioeconomic and genetic and/or breeding ingredients which are fundamental to realize lupin improvement in the country.

Given the long standing lupin farming experience of Ethiopian farmers, any national improvement and conservation endeavor should consider the vast experiences and practices of lupin farmers acquired from their long time engagement in lupin production and processing. Hence, ascertaining lupin constraints along the value chain and documenting farmers' experiences is important not only to use it as a baseline information to setting up national lupin

improvement programs, but also it ensures farmers participation to develop demand led lupin technologies. The research findings would be used as platform to setting up breeding programs aiming at lupin improvement. It further inform development practitioners and other stakeholders in the lupin value chain.

Farmers in the study areas have ample experiences of lupin farming and have similar purposes of growing lupin and is targeted for food, fertility restoring value, cash, medicinal value and as feed as their order of importance. This would imply that farmers are conscious about the multifaceted uses of lupin, from farm to kitchen and even its cash and medicinal values, while deciding lupin growing. However, frequency of growing lupin by farmers significantly vary among study districts that is mainly dictated by the fertility status of their farmland, i.e., farmers owing low fertile lands such as those in Fagta and Machakel growing lupin more frequent than farmers in South Achefer. Nevertheless, more than 85% of the farmers across the study districts had lupin farming activity either every year or every other year that would indicate the importance of the crop in that particular farming system.

The study indicated that the majority of lupin farmers perform minimum crop management practices to grow lupin. However, there are significant variations of farmers' agronomic practices (plot preparation, planting time, mode of planting, seeding rate, fertilizer application, weed management, harvesting time and harvesting indicators) among the study districts and within a study district. For example, in South Achefer more than 95% of the farmers are ploughing their land three-four times in preparation for lupin planting unlike substantial number of farmers in Machakel and Dera who do not plough their land even once to sow lupin.

Similarly, weeding lupin field is not common in three of the four study districts (Dera, Fagta and Machakel), but more farmers in South Achefer weed their lupin farm.

According to farmers, lack of improved varieties profiled with low alkaloids level, resistance/tolerance to major lupin diseases and insect pests, and early maturing are top priority lupin production constraints that should be shortly targeted. All farmers repeatedly complain that bitterness, which is caused by high level of alkaloids, in white lupin is the most persistent and outstanding constraint that limits lupin utilization, and processing and product development laborious and time taking. On the contrary, however, lupin plants employ these alkaloids and other secondary products as defensive weapons against herbivores and microbial infections as well as anti-pathogenic purposes for the lupin plant. It seems there is a tradeoff between the bitterness (which is caused by high level of alkaloids) of lupin seed for food and feed, and its importance as biological weapon for the lupin plant against disease causing pathogens and insect pests. However, researchers are working on various aspects of these lupin alkaloids including their biosynthesis and transportation; and they confirmed that the major sink of lupin alkaloids is on the seed and it is transported through phloem. This would bring some clues of possibility of developing varieties in which the transport of alkaloids from the phloem into the seeds is interrupted; such plants would maintain their defense against pathogens and insect pests in their aerial parts, but would produce alkaloid-poor sweet seeds (Wink, 2011).

In an effort to characterize the Ethiopian white lupin landrace accessions, both the molecular and agronomic and/or phenological markers confirmed that there exists genetic diversity in the

Ethiopian white lupin landrace accessions with several rare alleles. However, the results of both marker systems also claim much redundancy in the landrace collections as more than 64% of them clustered together. Moreover, it was confirmed from the molecular experiment that only 34 accessions are sufficient to retain 100% SSR diversity i.e. captured all the 98 alleles which were appeared to present in the whole accessions considered for this study. The extent and pattern of the existing genetic diversity does not strictly follow the geographic origin or areas of collection. The molecular study also showed that genotyping combined with clustering and population structure analysis is a powerful method for characterizing germplasm. It was also able to access and evaluate some polymorphic SSR loci which can be effectively used in future genetic analysis studies and molecular breeding programs.

Based on the agronomic and phenological performance measure, most of the genetic distances between clusters are significant, suggesting desirable genetic recombination and variation in subsequent generation from crosses that involve parents from those clusters characterized by maximum distances. Thus, this could maximize opportunities for transgressive segregation as there is a higher probability that unrelated accessions would contribute unique desirable alleles at different loci. From phenotyping of the agronomic and phenological traits of the landraces, it was revealed that significant number of landrace accessions perform as high as 5 metric tonnes per hectare of grain yield which is a huge performance and could be exploitable.

Generally, the results suggest, future breeding programs could exploit the available genetic diversity harbored in the Ethiopian white lupin landraces; to design and implement appropriate conservation including core collection establishment; and future national collection mission in

white lupin. Hence the attempt to characterize Ethiopian white lupin landraces using multivariate analyses and molecular markers with the present study is of practical importance to start a white lupin breeding program. On the other hand, the distribution and pattern of landrace accessions over significantly different clusters from the three major geographic origins would suggest future collections of local accessions in those geographic regions with particular emphasis to East Gojam, Awi and West Gojam for future national collection mission in white lupin.

Significant differences were observed for most traits among the white lupin accessions used in the current study, indicating the presence of considerable amount of genetic variations among Ethiopian white lupin local accessions. The study revealed high estimates of GCV, heritability and genetic advance as percent of mean for grain yield which indicates the possibility of improving this most important trait through selection. Trait associations and trait profiles were found to be different for different environments due to genotype by location interactions. Analysis of trait relationships and trait profiles based on averaged data over all locations captured most important relationships and trait profiles, though it did not entail all the associations and profiles apparent in the respective mega-environments as the biplots explained 55% - 66% of the variations. Nevertheless, the current results indicated the need to consider trait associations and trait profiles under the context of different mega-environments. GT biplots were found to be effective to reveal and visualize important relationships among attributes and trait profiles of Ethiopian white lupin landraces for ease multi-variate selection.

G x E is an important factor in genotype evaluation and recommendation of varieties that are suitable for specific or wider production environments. The genotype main effect plus G x E (GGE) biplots are effective enough to analyze and visualize pattern of G x E. Results of the present study depicted differential performance of white lupin landraces at different test environments implying crossover interactions. The results suggested two white lupin mega-environments in north western Ethiopia. Fenote Selam and Dibate were most representative locations; and all locations except Mandura and Injibara had generally similar and good discriminating power for white lupin landraces. Fenote Selam and Dibate combined both representativeness and discriminating power and characterized as desirable test locations for white lupin improvement in northwestern Ethiopia. However, mega-environment delineation and test location evaluation should be concluded based on multi-years data over environments. G2 was found to be the highest yielding and most stable landrace across the test environments. Hence, it is the most desirable genotype to be directly recommended for farmers' use and/or to be used as a source material for future white lupin breeding that targets high yielding and stable genotypes. Findings of this study provide useful information for white lupin breeding and commercialization in Ethiopia.

Recommendations and future works

Based on farmers responses and requirements, and out puts of molecular and agronomic field experiments included in the present study, the following recommendations and implicated future works are suggested as below:

- All farmers repeatedly complain that high level of alkaloids is one of the major and persistent constraints in lupin production and utilization in Ethiopia. Alkaloids quantification and profiling of the landraces, and hence screening landraces for low alkaloids are suggested to be done, before seeking other options, like introducing sweet lupin from a different growing environment which might have high yield penalties as it has been witnessed by the performance of Sanabor (in this study), a sweet blue lupin introduced from Germany.
- Prevalence of different diseases were among the top production constraints as indicated by farmers. They call it "mich" or "wag" is the common disease across all study areas, and is mainly characterized as drying of part or whole plant. The symptoms might be associated with common lupin fungal diseases, such as pleiochaeta root rot, brown leaf spot and phomopsis; and the occurrences of these diseases in Ethiopia were reported. However, further diagnosis and identification should be done to further characterize these diseases; and evaluate and screen the landraces against these prevalence.
- The present study demonstrated the great potential of using SSR marker data to construct a core collection and thus improve the management and utilization of the Ethiopian white lupin germplasm collections. Nevertheless, the core collection construction result presented in this study is limited to relatively small number of SSR markers used in this study. Hence, additional molecular markers, including more SSRs and SNPs, should be used to provide better coverage of the genome.

- Genotype by environment interaction analysis of grain yield in the present study suggested two white lupin mega-environments in north western Ethiopia; and identified desirable testing locations based on representativeness and discriminative power. However, mega-environment delineation and test location evaluation should be concluded based on multi-years data over environments. Hence, it is highly suggested to generate white lupin multi-years over environments data to conclude mega-environment delineation and test location establishments of white lupin in Ethiopia.

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Appendices

Appendix table 1: Description of the Ethiopian white lupin landrace accessions and the control genotypes used for the study.

ACC no	EBI code	Zone	District	Altitude	ACC no	EBI code	Zone	District	Altitude
Acc1	242279	Awi	Ankesha	2310	Acc37	238993	BD Sp	Bahir Dar	1990
Acc2	242280	Awi	Ankesha	2185	Acc38	238994	BD Sp	Bahir Dar	2020
Acc3	242281	Awi	Ankesha	2310	Acc39	239011	BD Sp	Bahir Dar	2090
Acc4	242282	Awi	Ankesha	2410	Acc40	239020	BD Sp	Bahir Dar	1940
Acc5	242266	WG	Dembecha	2110	Acc41	239022	BD Sp	Bahir Dar	1930
Acc6	239044	Awi	Banja	2600	Acc42	239023	BD Sp	Bahir Dar	1930
Acc7	242277	Awi	Banja	2560	Acc43	228519	SG	Dera	
Acc8	242278	Awi	Banja	2560	Acc44	242311	SG	Dera	1860
Acc9	242283	Awi	Banja	2160	Acc45	242312	SG	Dera	1960
Acc10	242284	Awi	Banja	1960	Acc46	242313	SG	Dera	1960
Acc11	236619	Awi	Banja	2570	Acc47	242314	SG	Dera	2160
Acc12	239045	Awi	Banja	2600	Acc48	242315	SG	Dera	2380
Acc13	242273	Awi	Banja	2490	Acc49	242316	SG	Dera	2460
Acc14	242274	Awi	Banja	2450	Acc50	242268	WG	Dembecha	2010
Acc15	242276	Awi	Banja	2590	Acc51	239018	WG	BD Z	1950
Acc16	105018		Unknown		Acc52	242319	SG	Dera	2510
Acc17	105005	Awi	Dangila	1940	Acc53	105002	SG	Este	2420
Acc18	228520	Awi	Dangila		Acc54	226034	SG	Este	2560
Acc19	242290	Awi	Dangila	2240	Acc55	242321	SG	Este	2630
Acc20	242291	Awi	Dangila	2160	Acc56	242219	SG	Farta	2280
Acc21	242292	Awi	Dangila	2060	Acc57	242322	SG	Farta	2850
Acc22	242293	Awi	Dangila	2100	Acc58	242323	SG	Farta	2760
Acc23	242294	Awi	Dangila	2060	Acc59	212754	SG	Fogera	1950
Acc24	236617	Awi	Dangila	2040	Acc60	239008	WG	Achefer	2070
Acc25	239003	Awi	Dangila	2190	Acc61	239029	WG	Achefer	2030
Acc26	239004	Awi	Dangila	2220	Acc62	239033	WG	Achefer	2000
Acc27	239005	Awi	Dangila	2360	Acc63	239038	WG	Achefer	2150
Acc28	242253	EG	Machakel	2140	Acc64	242295	WG	Achefer	2050
Acc29	239007	Awi	Dangila	2190	Acc65	242296	WG	Achefer	1975
Acc30	242287	Awi	Fagta	2550	Acc66	242297	WG	Achefer	2010
Acc31	242288	Awi	Fagta	2425	Acc67	242298	WG	Achefer	1990
Acc32	239017	SG	Dera	2130	Acc68	242299	WG	Achefer	2000
Acc33	242254	EG	Machakel	2150	Acc69	242300	WG	Achefer	2060
Acc34	242286	Awi	Guangua	1740	Acc70	242301	WG	Achefer	2090
Acc35	105003	BD Sp	Bahir Dar	1790	Acc71	242302	WG	Achefer	2000
Acc36	239021	BD Sp	Bahir Dar	1940	Acc72	239009	WG	Achefer	2000

Appendix 1: Continued....

Acc no	EBI code	Zone	District	Altitude	Acc no	EBI code	Zone	District	Altitude
Acc73	239027	WG	Achefer	2060	Acc109	242272	WG	Bure W	2500
Acc74	239030	WG	Achefer	2010	Acc110	105007	EG	Guzamn	2430
Acc75	239032	WG	Achefer	2000	Acc111	216013	EG	Guzamn	2500
Acc76	239034	WG	Achefer	2020	Acc112	239028	EG	Achefer	2060
Acc77	242308	WG	Bd z	1975	Acc113	242248	EG	Guzamn	2450
Acc78	242309	WG	Bd z	2000	Acc114	242252	EG	Guzamn	2350
Acc79	242310	WG	Bd z	1880	Acc115	105008	EG	Machakel	
Acc80	239015	WG	Bd z	1910	Acc116	105009	EG	Machakel	
Acc81	239016	WG	Bd z	1920	Acc117	105010	EG	Machakel	
Acc82	239019	WG	Bd z	2000	Acc118	105011	EG	Machakel	
Acc83	239046	WG	Bure w	2520	Acc119	238996	BD S	Bahir Dar	2050
Acc84	239051	WG	Bure w	2120	Acc120	239035	WG	Achefer	2050
Acc85	236620	WG	Damot	2110	Acc121	239002	WG	Merawi	2070
Acc86	105006	WG	Dembecha	2430	Acc122	105015	EG	Machakel	
Acc87	242263	WG	Dembecha	2380	Acc123	105016	EG	Machakel	
Acc88	242264	WG	Dembecha	2430	Acc124	105017	EG	Machakel	
Acc89	242265	WG	Dembecha	2450	Acc125	242255	EG	Machakel	2200
Acc90	242267	WG	Dembecha	2060	Acc126	242256	EG	Machakel	2200
Acc91	242269	WG	Dembecha	2010	Acc127	242257	EG	Machakel	2120
Acc92	242270	WG	Dembecha	2050	Acc128	242258	EG	Machakel	2300
Acc93	105001	WG	Jabi	2280	Acc129	242260	EG	Machakel	2400
Acc94	242303	WG	Mecha	1950	Acc130	105004	NG	Belesa	1820
Acc95	242304	WG	Mecha	1950	Acc131	239012	NG	G zuria	1930
Acc96	242305	WG	Mecha	2000	Acc132	208464	Awi	Dangela	2100
Acc97	242306	WG	Mecha	2010	Acc133	239060	NG	G zuria	1900
Acc98	242307	WG	Mecha	2010	Acc134	208365	GUR	Gumer	
Acc99	236615	WG	Mecha	2000	Acc135	225802	N O	Dermalo	2800
Acc100	236616	WG	Mecha	2060	Acc136	242320	N O	Dermalo	2800
Acc101	238997	WG	Mecha	2060	Acc137	207912	Mk	Adwa	
Acc102	238999	WG	Mecha	2050	Acc138	Local	WG	Dembecha	
Acc103	239001	WG	Mecha	2050	Acc139	Local	Awi	Fagta	
Acc104	239010	WG	Mecha	2050	Acc140	Local	WG	Achefer	
Acc105	242249	EG	Baso	2300	Acc141	Local	WG	Mecha	
Acc106	242250	EG	Baso	2310	Acc142	Local	SG	Dera	
Acc107	242251	EG	Baso	2300	Acc143	Local	BD SP	Bd z	
Acc108	242271	WG	Bure w	2450	Acc144	Sweet	Gm	Gm	

Appendix table 1: Continued....

ACC no	EBI code	Zone	District	Altitude	ACC no	EBI code	Zone	District	Altitude
ACC145	228520	Awi	Dangila		Acc180	239052	WG	Dembecha	2130
Acc146	239000	WG	Mecha	2050	Acc181	239053	WG	Dembecha	2150
ACC147	242275	Awi	Banja	2550	Acc182	239054	WG	Dembecha	2210
ACC148	239014	NG	G zuria	1920	Acc183	239055	WG	Dembecha	2160
ACC149	242318	SG	Dera	2400	Acc184	239056	EG	Machakel	2240
ACC150	105012	EG	Machakel		Acc185	239057	EG	Machakel	2380
ACC151	105013	EG	Machakel		Acc186_1	239058	EG	Machakel	2390
ACC152	105014	EG	Machakel		Aus1	239741			
ACC153	229805	Awi	Banja		Aus2	239742			
ACC154	239059	EG	Gozamn	2420	Aus3	239743			
ACC155	242317	SG	Dera	2400	Aus4	239744			
ACC156	238998	WG	Mecha	2060	Aus5	239745			
ACC157	242312	SG	Dera	1960	Aus6	239746			
ACC158	242289	Awi	Fagta	2375	Aus7	239747			
ACC159	242285	Awi	Guangua	1790	Aus8	239748			
ACC160	238995	BD SP	Bahir Dar	2020	Aus9	239749			
ACC161	236618	Awi	Dangila	2100	Aus10	239750	Australia collections and donation		
ACC162	239031	WG	Achefer	2010	Aus11	239751			
ACC163	216014	EG	Baso	2320	Aus12	239752			
ACC164	216015	EG	Machakel	2280	Aus13	239753			
ACC165	216016	EG	Machakel	2240	Aus14	239754			
ACC166	239024	BD SP	Bahir Dar	1900	Aus15	239755			
ACC167	239025	BD SP	Bahir Dar	1900	Aus16	239756			
ACC168	239026	BD SP	Bahir Dar	1900	Aus17	239757			
ACC169	239036	WG	Achefer	2000	Aus18	239758			
ACC170	239037	WG	Achefer	2000	Aus19	239759			
ACC171	239039	WG	Achefer	2080	Aus20	239761			
ACC172	239040	WG	Achefer	2130	Var01				
ACC173	239041	WG	Achefer	2150	Var02				
ACC174	239042	WG	Achefer	2120	Var03	Variants identified while phenotyping the EBI collections			
ACC175	239043	WG	Achefer	2080	Var04				
ACC176	239047	WG	Bure W	2660	Var54_1				
ACC177	239048	WG	Bure W	2600	Var54_2				
ACC178	239049	WG	Bure W	2480	Acc186_2	Variant from accession 186			
ACC179	239050	WG	Bure W	2300	Am	Control genotype*			

Acc no, Accession number; EBI, Ethiopian Biodiversity Institute; WG, West Gojam; EG, East Gojam; BD SP, Bahir Dar Special; SG, South Gondar; Fagta, Fagta Lekoma; Bd Z, Bahir Dar zuria; Jabi, Jabi Tehnan; Baso, Baso Liben; Bure W, Bure Womberema; NG, North Gondar; NO, North Omo; GUR, Gurage; MK, Mehakelegnaw; Gm, Germany; G Zur, Gondar Zuria; Control genotype*, identified while phenotyping white lupin landraces from EBI and found to be different species (*Lupinus mutabilis*).

Appendix table 2: Mean performance of the 143 Ethiopian white lupin accessions at Merawi, Ethiopia

Accessions	Pod	Seed	PH	BR	PD	PL	DF	DM	SW	GY
Acc001	72.65	5.30	135.60	9.23	7.07	8.72	89.04	182.75	31.03	3.54
Acc002	81.43	5.60	128.18	7.74	6.52	8.57	80.02	175.25	31.68	4.70
Acc003	92.20	5.70	123.60	9.69	6.79	9.01	82.56	173.02	33.27	5.79
Acc004	81.69	5.30	121.75	8.16	6.49	8.47	82.67	180.41	32.35	4.75
Acc005	87.94	5.70	132.73	7.75	6.55	8.60	79.09	174.06	32.75	4.63
Acc006	67.80	5.20	123.78	6.60	6.37	8.90	83.66	176.15	31.47	4.31
Acc007	84.61	5.50	124.81	8.46	7.17	8.71	81.56	178.67	32.71	4.45
Acc008	83.54	5.10	143.85	10.16	6.10	9.07	83.20	185.88	33.17	4.44
Acc009	92.65	5.50	134.69	7.46	5.91	8.37	77.69	180.43	28.99	4.93
Acc010	99.47	5.80	129.74	7.79	7.15	8.66	85.18	181.28	32.87	4.87
Acc011	92.80	5.70	143.30	7.09	6.59	8.77	78.03	181.69	32.04	5.27
Acc012	83.75	5.40	122.99	7.16	6.74	9.18	78.69	167.93	35.03	5.29
Acc013	77.33	5.20	126.95	9.32	6.87	8.81	81.56	178.88	32.72	3.92
Acc014	100.59	5.60	132.62	8.79	6.37	8.83	80.08	172.97	29.91	5.13
Acc015	97.00	5.30	133.50	9.19	6.37	8.63	82.53	180.19	31.94	4.27
Acc016	91.00	5.30	124.90	7.99	6.29	9.12	81.06	179.02	29.70	4.04
Acc017	68.44	5.30	118.50	7.36	7.56	8.92	79.20	172.38	31.76	4.24
Acc018	63.98	5.10	133.01	7.22	7.00	8.44	80.21	178.97	38.26	4.61
Acc019	71.35	5.50	130.30	7.13	5.98	8.35	78.54	176.25	29.64	4.48
Acc020	102.77	5.40	136.26	7.71	7.18	9.31	92.64	177.15	34.40	4.53
Acc021	84.30	5.70	131.38	7.14	6.71	8.90	78.23	175.20	32.63	5.08
Acc022	95.70	5.60	137.08	8.94	6.08	8.58	80.23	178.70	30.52	4.64
Acc023	81.40	5.20	126.10	7.69	6.74	9.21	79.56	184.02	32.76	4.30
Acc024	76.04	5.40	137.70	7.16	6.95	8.61	77.70	174.88	31.04	4.91
Acc025	85.14	5.60	132.98	7.33	6.23	8.50	76.93	174.98	33.48	5.38
Acc026	76.81	5.40	128.63	7.58	7.30	9.03	78.43	170.25	29.94	4.44
Acc027	87.24	5.20	130.68	7.73	5.80	9.25	77.43	181.98	30.06	5.68
Acc028	97.04	5.60	130.67	7.74	5.09	8.49	78.94	177.43	30.46	4.93
Acc029	85.66	5.20	139.65	8.16	6.78	8.65	79.53	175.12	33.98	5.40
Acc030	99.45	5.60	138.54	9.23	5.85	8.60	89.41	183.24	27.24	3.74
Acc031	100.87	5.60	136.06	8.97	6.28	8.33	81.51	181.38	31.97	4.54
Acc032	108.95	5.20	137.04	7.83	6.31	8.36	78.41	179.24	30.22	5.12
Acc033	92.04	5.20	127.87	8.64	5.66	8.53	79.94	181.93	30.70	4.71
Acc034	81.96	5.60	133.45	7.76	6.77	8.34	80.53	175.12	29.81	4.98
Acc035	80.65	5.20	128.54	7.93	6.64	8.57	79.91	179.24	34.12	4.49

Pod=Number of pods per plant; Seed=Number of seeds per pod; PH=Plant height in centimeter; BR=Number of branches on the main axis; PD, PL= Pod diameter pod length in millimeter, respectively; DF=Days to flowering; DM=Days to maturity; SW=100 seed weight in grams; and, GY=Grain yield in tonnes per hectare

Appendix table 2: Continued...

Accessions	Pod	Seed	PH	BR	PD	PL	DF	DM	SW	GY
Acc036	87.70	5.40	132.67	7.07	6.59	8.68	78.95	176.49	32.34	4.74
Acc037	80.58	5.30	131.79	8.09	6.15	8.95	79.50	175.31	31.32	4.98
Acc038	76.25	5.10	135.72	7.83	6.16	9.01	77.97	179.26	31.86	4.81
Acc039	84.34	5.20	136.39	6.93	6.66	8.97	76.49	180.22	30.78	5.27
Acc040	76.67	5.20	136.71	7.27	6.36	8.31	77.01	172.88	31.82	5.42
Acc041	89.06	5.30	136.68	7.94	7.45	8.74	78.93	175.20	34.59	5.36
Acc042	87.51	5.70	130.63	7.82	7.19	8.76	79.50	171.30	32.39	5.10
Acc043	88.76	5.10	133.33	9.93	6.11	8.60	93.96	191.52	31.43	2.93
Acc044	84.58	5.40	134.29	6.49	6.29	8.76	77.00	173.81	31.62	4.71
Acc045	90.17	5.10	130.71	11.57	6.69	8.83	91.51	184.38	29.26	3.29
Acc046	92.51	5.10	133.93	7.98	6.09	8.60	79.93	177.25	29.69	4.30
Acc047	87.11	5.70	131.59	8.44	6.38	8.95	82.94	178.01	31.79	4.85
Acc048	90.66	5.50	143.02	7.65	6.56	8.88	79.99	179.47	31.16	4.95
Acc049	87.23	5.40	141.09	8.01	6.60	8.79	84.00	184.57	31.93	4.33
Acc050	81.96	5.30	128.89	8.12	7.07	8.35	79.98	176.35	31.08	4.21
Acc051	78.33	5.50	128.97	8.82	5.93	8.73	80.96	176.29	31.77	4.43
Acc052	81.86	5.20	136.02	7.95	6.03	9.18	86.49	182.97	31.85	4.43
Acc053	72.03	5.40	124.28	8.22	6.07	8.70	89.94	183.83	28.56	3.46
Acc054	78.90	5.30	137.71	8.73	6.10	8.32	88.89	189.72	28.85	3.48
Acc055	71.57	4.90	117.86	9.82	6.33	8.26	93.42	184.35	34.27	2.89
Acc056	84.60	5.20	127.71	8.13	6.95	8.40	85.39	183.22	30.15	3.87
Acc057	77.88	5.30	125.84	9.95	6.22	8.22	91.94	186.53	29.76	2.66
Acc058	96.03	5.40	137.68	8.62	6.65	8.68	86.44	179.83	30.28	4.19
Acc059	82.15	5.30	125.22	7.09	6.65	8.99	80.42	183.49	30.97	4.28
Acc060	85.64	5.40	133.76	9.15	6.88	8.71	79.54	176.47	34.16	5.11
Acc061	86.14	5.40	137.93	7.69	6.56	8.82	78.43	176.94	33.17	5.15
Acc062	85.44	5.90	139.26	8.45	6.19	9.12	78.04	176.47	31.21	5.18
Acc063	71.50	5.60	136.01	7.43	5.64	8.54	77.89	174.72	31.08	4.54
Acc064	75.67	5.40	134.69	7.74	6.52	9.09	78.38	177.72	34.61	4.35
Acc065	71.99	5.40	137.47	7.50	6.45	8.73	77.03	175.89	32.01	4.59
Acc066	87.47	5.40	141.06	7.72	6.71	8.67	79.42	173.85	32.39	4.87
Acc067	71.34	5.60	130.93	7.49	6.87	8.81	78.93	175.44	30.62	4.80
Acc068	87.86	5.60	134.55	8.26	6.84	8.98	76.45	174.34	33.77	4.92
Acc069	82.53	5.30	138.52	7.02	6.41	8.58	78.46	174.29	32.50	5.00
Acc070	76.19	5.30	141.67	7.86	5.46	8.41	75.96	175.84	32.51	4.45
Acc071	77.27	5.30	130.29	7.74	5.91	8.26	80.38	176.72	32.41	5.18
Acc072	97.79	5.60	131.80	8.66	6.73	9.37	79.49	182.25	31.40	5.19
Acc073	89.80	5.00	115.89	9.89	7.21	8.82	89.99	189.70	30.25	3.03

Appendix table 2: Continued...

Accessions	Pod	Seed	PH	BR	PD	PL	DF	DM	SW	GY
Acc074	89.99	5.40	137.41	8.03	6.57	9.25	77.00	179.90	33.18	5.28
Acc075	88.30	5.60	132.69	7.19	7.00	9.41	77.99	175.70	35.86	5.04
Acc076	77.99	5.50	129.00	7.96	6.47	9.15	78.49	168.75	31.92	5.08
Acc077	62.37	5.20	117.68	9.32	6.80	8.85	85.51	176.58	37.34	3.64
Acc078	68.67	5.40	126.30	7.33	6.35	8.99	78.47	176.95	33.79	4.87
Acc079	92.54	5.40	138.29	8.14	6.71	9.06	76.04	178.03	30.74	4.66
Acc080	95.01	5.50	140.64	8.19	6.12	8.82	78.99	179.11	30.72	4.83
Acc081	72.87	5.50	127.33	8.22	6.67	9.08	76.51	176.08	35.66	4.56
Acc082	86.15	5.40	131.23	7.68	6.34	9.06	77.51	174.22	33.04	4.19
Acc083	89.86	5.30	135.54	8.56	7.02	9.48	84.51	178.87	30.32	4.10
Acc084	90.44	4.90	127.49	9.64	6.26	8.58	79.04	178.53	32.96	3.90
Acc085	80.30	5.10	129.04	9.19	6.59	8.91	79.99	175.70	31.86	4.84
Acc086	79.59	5.50	141.21	7.53	6.54	8.84	78.00	181.40	32.34	4.20
Acc087	84.30	5.70	122.74	7.89	6.80	8.54	78.49	176.70	32.78	4.89
Acc088	86.46	5.20	143.61	8.62	6.37	8.76	78.07	177.57	32.77	4.67
Acc089	82.98	5.10	116.90	9.45	6.71	9.18	80.95	175.97	33.21	4.11
Acc090	88.54	5.00	140.28	8.80	6.55	9.04	78.47	182.48	35.47	5.42
Acc091	89.52	4.80	134.42	8.72	6.45	8.41	78.02	179.80	30.00	4.14
Acc092	90.19	5.60	127.11	8.03	6.73	9.15	80.00	177.40	30.59	4.58
Acc093	79.08	5.10	109.35	7.25	7.08	8.83	77.04	178.76	32.83	3.60
Acc094	71.94	5.60	142.94	7.58	6.66	9.20	79.02	177.17	31.84	4.94
Acc095	94.23	5.50	141.06	8.06	5.77	9.16	78.48	180.24	28.97	4.65
Acc096	74.47	5.40	133.94	6.74	7.02	9.21	77.99	178.95	32.28	4.73
Acc097	68.07	5.70	126.23	8.06	6.71	9.31	79.99	169.80	33.57	5.26
Acc098	88.45	5.20	138.81	8.27	6.47	9.09	87.50	183.70	29.85	4.02
Acc099	88.64	5.70	132.36	7.63	6.43	8.76	77.54	174.76	34.95	5.28
Acc100	91.61	5.40	143.25	8.44	6.86	9.03	80.52	179.86	34.86	5.26
Acc101	84.49	5.40	124.30	6.81	6.51	8.76	76.44	175.53	34.26	4.73
Acc102	89.53	5.70	131.32	7.22	7.06	8.98	79.47	173.44	34.38	5.49
Acc103	102.72	5.60	141.02	8.24	5.99	8.63	79.03	187.07	29.76	4.94
Acc104	83.85	5.50	125.91	7.57	7.29	9.32	78.00	172.70	33.08	4.53
Acc105	87.13	5.30	121.82	9.42	6.88	9.20	88.47	176.44	34.52	4.28
Acc106	90.76	5.20	128.53	8.96	7.00	8.54	83.94	179.01	32.88	4.08
Acc107	106.97	5.00	136.54	8.54	6.29	8.22	90.99	190.95	27.15	2.97
Acc108	75.90	5.20	126.15	7.63	6.80	8.19	77.51	174.84	33.69	5.20
Acc109	85.71	5.40	130.29	8.47	6.68	8.93	79.47	176.79	33.82	4.53
Acc110	77.86	4.80	131.64	8.66	6.55	8.32	83.50	186.25	27.80	3.40

Appendix table 2: Continued...

Accessions	Pod	Seed	PH	BR	PD	PL	DF	DM	SW	GY
Acc111	96.42	5.30	120.42	7.70	7.07	9.16	83.07	182.07	30.53	3.99
Acc112	80.76	5.50	137.54	7.16	6.70	9.02	78.50	174.75	30.69	4.95
Acc113	101.98	5.20	133.31	8.60	6.83	8.38	80.04	179.66	32.33	4.85
Acc114	85.23	5.40	126.22	8.12	7.40	9.06	83.47	179.94	32.90	4.41
Acc115	88.06	5.00	124.34	8.45	6.35	8.17	86.56	188.49	27.80	3.66
Acc116	86.20	5.20	128.34	10.37	5.83	7.31	87.49	192.38	24.44	2.71
Acc117	88.76	5.30	122.24	8.95	5.98	8.04	85.56	193.99	28.17	3.84
Acc118	82.23	5.10	130.99	8.70	6.00	8.04	87.01	194.07	27.84	3.66
Acc119	82.86	5.60	136.82	7.57	6.68	8.82	77.46	173.47	34.07	5.39
Acc120	61.64	5.20	132.69	8.01	6.75	9.08	79.90	175.80	35.17	4.20
Acc121	70.07	5.40	124.06	8.56	6.63	8.51	79.90	172.82	34.25	4.59
Acc122	93.54	5.30	128.48	9.72	6.22	8.27	85.41	184.76	27.58	3.40
Acc123	79.88	5.20	116.74	8.49	6.26	8.19	91.49	187.28	25.78	3.38
Acc124	81.84	4.80	127.28	8.22	5.97	8.13	91.41	189.76	24.91	3.24
Acc125	96.01	5.10	125.05	8.58	6.47	8.23	82.01	180.68	28.81	4.40
Acc126	92.81	5.10	127.85	8.98	6.28	8.41	79.51	181.68	30.68	4.39
Acc127	84.94	5.40	134.09	8.91	6.42	8.79	79.90	176.30	32.02	4.90
Acc128	99.47	5.20	138.53	8.55	6.52	8.46	82.44	178.45	31.26	4.97
Acc129	102.08	5.30	132.71	9.85	6.34	8.79	90.55	185.49	31.16	3.87
Acc130	89.49	5.70	149.58	8.10	6.37	8.39	85.98	190.86	29.42	3.51
Acc131	77.47	5.50	133.43	6.72	6.63	8.57	77.96	172.80	27.23	4.71
Acc132	96.47	5.30	128.14	7.32	7.19	8.78	77.00	185.09	34.49	4.54
Acc133	92.92	5.60	133.09	7.42	6.70	8.74	81.97	179.12	28.63	4.45
Acc134	90.69	5.50	133.18	9.30	6.49	8.64	84.98	176.36	30.24	4.35
Acc135	76.67	5.00	124.44	7.52	6.69	8.49	77.45	179.85	30.67	4.36
Acc136	94.35	5.50	144.29	9.09	6.82	8.82	86.94	189.73	30.29	3.95
Acc137	76.89	5.50	124.95	6.92	6.80	8.58	78.42	174.57	34.75	5.03
Acc138	94.87	5.40	144.23	9.15	6.35	8.21	84.44	184.95	31.09	4.41
Acc139	73.55	5.30	140.79	9.09	6.39	8.39	84.44	185.23	32.91	3.93
Acc140	88.08	5.00	137.44	8.29	6.59	9.04	78.99	180.28	30.89	4.67
Acc141	88.63	5.40	137.13	8.10	6.23	8.67	78.53	178.40	31.29	4.92
Acc142	85.10	5.30	128.10	6.87	6.70	8.80	79.95	181.36	28.75	4.73
Acc143	79.11	5.30	126.05	8.08	6.37	8.40	81.01	175.68	29.53	4.57
Acc144	25.80	4.30	48.26	10.37	**	4.91	62.95	131.49	17.93	1.01
Mean	84.75	5.35	131.29	8.20	6.53	8.70	81.2	178.86	31.53	4.48
Mean SED	12.05	0.27	8.20	0.84	0.58	0.48	2.49	4.00	2.42	0.55

**= missed value

Appendix table 3: List of SSR markers used for the study

Number	Marker Name	Forward sequence	Reverse sequence
1	Lup125	TGGTCCTATGTTGAAAGACC	CTGTACAACCAAATTACACCAG
2	Lup146	ATTCCTCTTACCTAGTGCTTA	CCCTTGGGATTGATTGGTC
3	Lup197	CTGGTTCTGTTCTTCAACTGTA	CCTATATGCCTTGGTCCAT
4	PT1-1	CAAATGGAAAATCTTTGGAAGAG	CTAACATAAGACCTAAACATATGGAACAG
5	AnMtS13	GCGACGTGCCCTCCAAGTCC	AGGACCCATGGAATCATTACCTCC
6	GLNA	GAATGGTGCTGGTGCTCACACA	TGGTGGTGTCTGCAATCATGGAAG
7	CHS9	ATCCAGCCAACTGTGTTGAACAAAGCA	GATTTTGGTTGGCCCCATTCTTTTATAG
8	LSSR14	GGTGACCCTCACCAGAACAT	GGTCCTTTGATGATGGTGCT
9	LSSR55	CGAGGAAAGAGCAGTTTCAAG	CCTGGTAGTCCTTGGGTTCA
10	LSSR9a	CCCCTGCTCCGAATATATGA	AAGCCCAATGATTGTTCTGG
11	LSSR9b	CAAAGGATGGTTTCTTGTTAGGTC	CCTCGCACATTTCCTACCCA
12	LSSR26a	TGCTCATGTTGCCAAGACTC	CGCAACAATCAGCTATAAGCC
13	La1-EST01	GCGCTAAATTTCTATCTTTTAACACT	CCCAACCATATTTTCCACCAAC
14	PT1-2	TTGTTGTCCCTGCAGAGATTTT	CAAACATAAGACCTAAACATATGGAACAG
15	DSI	GAAGCCAAAAAGTATGAAGGGCCACGCAC	CATGGTGCATAAAACTCAACCAAGACATC
16	Lup257	GCTGGAGCAGATTATTGTGTA	CCTGGCAGACAGTTGCTT

Appendix table 4: The Evanno table output.

K	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	-4072.3000	0.1225	—	—	—
2	-3649.0200	2.3004	423.2800	164.8200	71.6473
3	-3390.5600	40.7329	258.4600	294.8400	7.2384
4	-3426.9400	310.6000	-36.3800	287.2800	0.9249
5	-3176.0400	205.4422	250.9000	105.0800	0.5115
6	-3030.2200	49.3836	145.8200	51.1600	1.0360
7	-2935.5600	29.7394	94.6600	77.3600	2.6013
8	-2918.2600	15.8659	17.3000	9.0400	0.5698
9	-2910.0000	71.0800	8.2600	58.7800	0.8270
10	-2842.9600	44.8498	67.0400	13.3000	0.2965
11	-2789.2200	42.4041	53.7400	46.6400	1.0999
12	-2782.1200	19.0902	7.1000	7.2400	0.3793
13	-2782.2600	26.4415	-0.1400	4.7000	0.1778
14	-2777.7000	44.1208	4.5600	2.2600	0.0512
15	-2775.4000	70.9565	2.3000	108.3600	1.5271
16	-2881.4600	128.1073	-106.0600	47.6800	0.3722
17	-2939.8400	234.4353	-58.3800	208.1800	0.8880
18	-2790.0400	78.8900	149.8000	209.0400	2.6498
19	-2849.2800	183.8866	-59.2400	44.0000	0.2393
20	-2864.5200	136.9635	-15.2400	—	—

Biography of the author

Mulugeta Atnaf Tiruneh was born on February 2, 1980 in Gojam, Ethiopia. He graduated with a BSc. degree in Plant Sciences in 2001 from the then Debub University, now Hawassa University, Ethiopia. He also obtained an MSc. degree from Haramaya University, Ethiopia specialized in plant breeding in 2006. Mulugeta resumed his work career in December 2001 as a junior researcher in the same Institute he is still working, the Ethiopian Institute of Agricultural Research. He has stationed in one of the Institute's research center called Pawe which is located in Northwestern part of the country. Since then, Mulugeta has served as researcher and administrator with different positions and capabilities. His research mainly involved improvement of food legumes such as soybean, common bean and chick pea; and managed the release of a number of improved varieties of these crops for the farmers. Hence, Mulugeta, together with his teammates have been awarded for such an outstanding accomplishment in various levels up to the national level. He also authored several publications in journal articles, book chapters, conference proceedings and newsletters. He also coordinated National soybean research from April 2011 - January 2012. During the periods 2008 - 2012, Mulugeta served as director of Pawe research center. He moved the center forward and transformed it in delivering

and scaling up best-bet technologies to the surrounding farming community. Such exemplary achievements were recognized at national, regional and institute levels and as a result the center has awarded two consecutive national awards from the then prime minster of Ethiopia. Moreover, Mulugeta gratified by the Institute and obtained a certificate of good governance for his committed and rigorous governance he has shown in his directorship responsibility. Currently, Mulugeta is working on lupin breeding and socioeconomics in Ethiopia as his PhD thesis project.

Publications

Peer reviewed Journal articles emanated from this thesis

Mulugeta Atnaf, Kassahun Tesfaye, Kifle Dagne, Dagne Wegary (2016). Genotype by Trait analysis to study Trait Associations and Profiles of Ethiopian White Lupin (*Lupinus albus*) Landraces. Australian Journal of Crop Science 11(1): 55-62.

Mulugeta Atnaf, Dagne Wegary, Kifle Dagne, Kassahun Tesfaye (2016). Genotype by Environment Interaction and Grain Yield Stability of Ethiopian White Lupin (*Lupinus albus L.*) Landraces. Experimental Agriculture.

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Other Journal articles

Mulugeta Atnaf, Kassahun Tesfaye, Kifle Dagne (2015). The Importance of Legumes in the Ethiopian Farming System and Overall Economy: An Overview. American Journal of Experimental Agriculture 7(6):347-358.

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