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# Genetic Divergence Analysis of Ethiopian White Lupine (Lupinus albus L.) Accessions in Bale Zone, South East, Ethiopia

**Tadesse Adere [1] and Temesgen Bedassa Gudeta[2]**

[1]Department of Biology,Gasera Preparatory School, P.O. Box 37, Robe, Ethiopia.
[2]Department of Biology, Madda Walabu University, P.O. Box 247, Robe, Ethiopia.
*Corresponding Author: E-mail:tasgabifenet@gmail.com alternative E-mail:singitentem@gmail.com
Tele: +251911785364

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**White lupin (Lupinu albus L.) is a promising leguminous crop for human consumption, green manure, forage and has been traditionally cultivated for several years in Mediterranean region along the Nile valley where it ~~is~~was originated. In Ethiopia, it has been exclusively produced by smallholder farmers; mainly for its food grain and maintenance of soil fertility. The objectives of the study were to cluster the genotypes into genetically different groups and to quantify the genetic distance among the clusters. Field experiments were conducted on 36 Ethiopian white lupin accessions at two locations namely Madda Walabu University Research Site and Gasera farmers' farm land during the main cropping season of the year 2017. Randomized Complete Block Design with three replications was used and eighteen morphological quantitative traits were studied at both locations. The collected data were adjusted to mean values and the mean values were standardized to mean of zero and unity variance in order to minimize biases due to differences in scales of measurement and then subjected to analysis of variance using appropriate SAS computer software. The combined analysis of variance showed very highly significant ($p \leq 0.0001$) and highly significant ($p \leq 0.01$) differences among the studied genotypes for all characters, except for the traits number of primary branches and pod thickness. Although all the tested 36 white lupin landraces were originally collected from diverse agro-ecologies of Ethiopia, in this study they were grouped in to only five clusters based on the studied genetic traits. The maximum inter-cluster distance ($D^2$=641.87) was observed between cluster three and five; the lowest value ($D^2$=28.70) was observed between cluster two and four. Maximum intra-cluster value ($D^2$= 3.07) was exhibited by cluster four followed by cluster one and cluster two. Cluster three and five each held solitary accession, hence, their intra-cluster, $D^2$, value was zero. Therefore, crossing of accessions from cluster three and five will give rise to maximum genetic segregation. The selection of parents on the basis of divergence analysis would be more promising for breeding program.**

**Key Words:** Bale Zone,Cluster analysis, Gasera, Genetic divergence, Madda Walabu, White lupin.

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## INTRODUCTION

White lupin (*Lupinus albus* L.) originated in the Balkin region of the northeast Mediterranean; it is now distributed throughout the Mediterranean region and from the Azores Islands across North Africa to Ethiopia and Kenya (Vipin *et al.,* 2013). In Ethiopia, white lupin is locally known as *Gibto;* Zerihun (2012) reviewed different vernacular names given to white lupin, such as *Gibto* in Ethiopia; *Thermus* in Greece; *Tumus* in Arabian countries and *Termiyeor Acibakla* in Turkey. The author explained that the reason why the local community in North-western Ethiopia gave the name *Gibto* is due to the legend that the crop was introduced to Ethiopia from Egypt, where the Amharic /local/ language name given to Egypt is '*Gibts*'. White lupin is a predominant species produced by small scale farmers mainly in the Amhara region (Gojjam and Gondar). The crop is also produced in other parts of the country, such as in Benshangul, S.N.N.P.R, Oromyia, and Regional States of Ethiopia in decreasing order of area coverage and total production (CSA, 2014).

White lupin is one of the four lupins (*Lupinus albus*, *Lupinusangustifolius*, *Lupinus luteu* L., *Lupinus mutablis*) widely known commercially and agriculturally important, large seeded annual legume crop for human consumption and animal feed in some countries. The composition of the seed and especially the high protein content makes white lupin highly suitable for livestock diets. Its adaptation to poor soil makes it economically feasible (OECD, 2008; Wheeler & McCormack, 2010).The presence of quinolizidine alkaloids and some anti-nutritional factors results in characteristically bitter taste making the crop unacceptable for food/feed (Santiago *et al* 2010; Erbas, 2010). Among the methods, soaking after roasting, boiling, germination, fermentation and alkaline treatments can be mentioned. It needs appropriate processing methods which can reduce the alkaloid content and thereby enhance its utilization as food and feed. Chemical treatment of lupin grain is the most common processing method suggested to reduce alkaloid contents of the crop (Yeheyis et *al.,* 2012).The genetic diversity of white lupine and other species of *Lupinus* have been characterized using morphological and agronomical attributes (Gonzalez et *al.,* 2007).Ramana *et al*. (2008) explained that *Lupinus albus* has 2n=50.

Moreover, research based information on the agro-morphological and genetic characteristics of the crop are not available to design future breeding strategies and promote the crop production and utilization. Such research gaps contributed to the prevailing major problems such as lack of early maturing, high-yielding, low/free alkaloid content varieties, and disease resistant cultivars. 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, 2002; 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*., 2015b; 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.,* 2012). 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. In Ethiopia, about 250 accessions are conserved (Atnaf *et al.,* 2017; EBC, 2017); however; information is inadequate on the extent of its genetic diversity, and related genetic aspects. *Lupinus albus* and its uses are not well known and adapted particularly in Bale zone. Before any hybridization work, genetic diversity of the existing genotypes needs to be known. Moreover, evaluation of genetic diversity is important to know the source of genes for particular trait within the available germplasms. Therefore, the current study aimed to analyze genetic divergence among 36 Ethiopian white lupin genotypes.

## MATERIAL AND METHOD

### Description of the Study Site

The experiment was conducted during the *Genna* season (from March to June, 2017) at two locations namely: Research site of Madda Walabu University main campus (RSMWU), Robe and farmer's farm land of Gasera village in Bale Zone. As Bale Zone is known by its bimodal rainy seasons, there are two cropping seasons in the region *Ganna (Kiremt)* which ranges from March to June and *Bona (Bega)*ranges from July to December; crops which are planted on *Kiremt* season are collected in *Bega* season which is the dry period of the area.The study area, Bale zone, is geographically located between 5.360N-8.120N and 39.210E-42.230E in the South Eastern parts of Ethiopia. The region extends over 18 districts. Based on traditional agro-climatic classification which is mainly depends on altitude and mean temperature the study area is classified in to Highland (Dega), Weina Dega (midland) and Quolla (lowland) (Bekele *et al*., 2017). Madda Walabu University, one of the research sites for this study, is located in Robe town 430 km away from the capital city, Addis Ababa, to the South-east direction. The geographic location of RSMWU is $07^0$ 08′ 45″N and $40^0$ 00′ 13″ E with an elevation of 2460 m. a. s. l. The average annual mean temperature of the research site is 18 $^0$C & 22 $^0$C, night and day respectively. Gasera is one of the villages in Bale Zone,

at the distance of 575km away from Addis Ababa to South East. Geographically, Gasera is located at $07^0$ 10′ 33″N and $40^0$ 04′ 11″ Ewith an elevation of 2340 m. a. s. l. The average annual rainfall of 823-1567mm.The two locations represent high lands region of Bale zone. The dominant soil type is pellic, vertisol and slightly acidic (PH=6.2) Jin *et al.,* 2015.

**Experimental Materials**

Thirty six (36) white lupin accessions were used. The accessions were collected by Ethiopian Biodiversity Conservation (EBC) from diverse agro ecological locations of the country varying in altitude, rainfall, Latitude, Longtude and collection dates (accession passport), Table 1. The present study evaluated each stated white lupin germplasm collections in terms of some of their morphological traits. As it could be understood from the bellow Table 1 showing geographic origin and administrative units (Zone, District and locality) of Ethiopian white lupin accessions, the plant is growing in northern part of Ethiopia only; particularly in Amhara National Regional state. White lupin has been under production in different administrative regions of Ethiopia (CSA, 2014). However, the CSA reports indicated that the Amhara National Regional state is the major producer and contributor to the national total production.

**Experimental Design and Procedures**

Randomized Complete Block Design (RCBD) with three replications was used at both locations. Each replication contained 36 plots. The distance between plots was 50cm. Each genotype was sown in $1.20m^2$ (1.20m length x 1m width) plots containing four rows with inter-row spacing of 30 cm. Drilling mechanism was applied to provide seed hole along the row. Six seed holes per row with 20cm gap hence were prepared. The layout and randomization was taken place as per the standard procedure set by Cochran and Cox (1957). For each genotype, two seeds per hole were thoroughly sown in the row, but later, ten days after germination, at true leaf stage, the plants in each hole of the row on the plots were thinned out as it should have about 20cm gap from plant to plant. 24 plants per plot were maintained. However, the two middle rows alone were used for data collection for the parameters that were recorded as per plant to avoid border effect. Planting was carried out in the first week of March, 2017 for both locations. Three weeding activities and two hoeing practices were carried out and no fertilizer and chemicals were applied. All pertinent crop management practices were implemented with strict close supervision as per the recommendations adopted for the respective site.

**Data Collection**

The following data were collected during the experimental time both from the whole plot and from the sample plants that were randomly selected from the middle two rows of each plot.

**Data on the whole plot basis**

**Days to emergence (DE)**: This was carried out by taking the total number of days from date of planting to when 50% of the seedlings in each plot appeared above the ground level.

**Days to flowering initiation (DFI)**: This was determined by counting the number of days from date of sowing to date of some plants in each plot starts to bloom.

**Days to flowering (DF)**: The actual count of number of days from the date of planting to the date on which about 50% of the plants in each plot produce flower.

**Daysto maturity (DM)**: Days to 90% physiological maturity was determined as the number of days from sowing to the date when the peduncles turned to yellow straw color. It was recorded when no green color remained on chaff and Peduncles of the plants.

**Seed yield per plot (SYPL)**: 7% moisture adjusted seed (dry seed yield) from the possible total harvestable rows of each experimental plot was recorded in grams.

**Seed yield per hectare (SYH)**: It was the value of seed yield per plot converted to kg/ha.

**Hundred Seeds weight (HSW):** It was recorded as the weight in grams of 100randomlytakenand7%moisture-adjusted seeds from each experimental plot.

**Biomass yield per plot (BYPL)**: The above ground biomass yield at the time of harvesting was determined in grams from each experimental plot *viz.* from the net plot size.

**Data on plant (sample) basis**

Data on plant basis were recorded for the following characters on five randomly taken plants from possibly harvestable rows of experimental unit (plot). These data were expressed as average of randomly taken five plants in each experimental plot *viz*. mean values of these measured samples were utilized to estimate the performance of each germplasm collection for the traits under consideration. Detailed accounts of each data type and collection methods are discussed here under.

**Table 1:** Passport data (geographic origin and administrative units) of Ethiopian white lupin accessions

| No | Acc.# | Genus | Species | Region | Zone | District | Locality | Latitude* | Longitude | Altitude | C. Date |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | AC.24850 | *Lupinus* | *Albus* | Amara | Misrak | goncha | NA | 10-57-11- | 38-04-46- | 2496 | 25/12/2014 |
| 2 | AC.26634 | *Lupinus* | *Albus* | Amara | Misrak | Gozamen | Layamba | 10-28-28- | 37-51-06- | 2883 | 15/02/2015 |
| 3 | AC.26635 | *Lupinus* | *Albus* | Amara | Misrak | machakal | mahasar | 10-36-25- | 37-41-51- | 2793 | 03/02/2015 |
| 4 | AC.26636 | *Lupinus* | *Albus* | Amara | Misrak | Senan | Yatad | 10-03-54- | 37-46-41- | 2975 | 03/05/2015 |
| 5 | AC.26637 | *Lupinus* | *Albus* | Amara | Misrak | dabre | Zagab | 10-22-23- | 37-23-31- | 2163 | 03/09/2015 |
| 6 | AC.26638 | *Lupinus* | *albus* | Amara | Misrak | dabre | Atkaram | 10-18-44- | 37-29-37 | 2225 | 09/09/2015 |
| 7 | AC.26639 | *Lupinus* | *albus* | Amara | Misrak | Baso | Wagaj | 10-09-78- | 37-39-56- | 2281 | 15/03/2014 |
| 8 | AC.26640 | *Lupinus* | *albus* | Amara | Misrak | Baso | Gutto | 10-09-53- | 37-43-13- | 2301 | 15/03/2014 |
| 9 | AC.26641 | *Lupinus* | *albus* | Amara | Misrak | yalamlam | wagaj | 10-09-46- | 37-43-27- | 2318 | 15/03/2014 |
| 10 | AC.29054 | *Lupinus* | *albus* | Amara | Agew | Dengla | Gerar | 11-19-03- | 36-44-43- | 2215 | 07/06/2008 |
| 11 | AC.29055 | *Lupinus* | *albus* | Amara | Agew | Dengla | woleta | 11-19-21- | 36-43-35- | 2122 | 07/06/2008 |
| 12 | AC.29056 | *Lupinus* | *albus* | Amara | Agew | Dangila | mehal | 11-21-40- | 36-46-06- | 2201 | 07/06/2008 |
| 13 | AC.29057 | *Lupinus* | *albus* | Amara | Agew | Dangila | IayAfat is | 11-20-38- | 36-45-26- | 2254 | 07/06/2008 |
| 14 | AC.29058 | *Lupinus* | *albus* | Amara | Agew | Dangila | IayAfat is | 11-20-12- | 36-46-04- | 2112 | 07/06/2008 |
| 15 | AC.105001 | *Lupinus* | *Spp.* | Amara | Mirab | Jabi | Woinma, | 10-45-00- | 37-06-00- | 2280 | 02/01/1978 |
| 16 | AC.105002 | *Lupinus* | *Spp.* | Amara | Debub | Este | Gudie | 11-37-00- | 38-01-00- | 2420 | 08/01/1979 |
| 17 | AC.105006 | *Lupinus* | *Spp.* | Amara | Mirab | Dembech | Debremek | 10-40-00- | 37-34-00- | 2430 | 09/01/1980 |
| 18 | AC.105007 | *Lupinus* | *Spp.* | Amara | Misrak | Guzamn | about | 10-18-00- | 37-47-00- | 2430 | 15/03/1980 |
| 19 | AC.216014 | *Lupinus* | *albus* | Amara | Misrak | Baso | Aba | 10-09-00- | 37-40-00- | 2320 | 05/01/1986 |
| 20 | AC.216015 | *Lupinus* | *albus* | Amara | Misrak | Machake | Debre | 10-16-00- | 37-27-00- | 2280 | 05/01/1986 |
| 21 | AC.216016 | *Lupinus* | *albus* | Amara | Misrak | Machake | Genet abo | 10-23-00- | 37-27-00- | 2240 | 05/01/1986 |
| 22 | AC.225802 | *Lupinu* | *Spp.* | SNNP | Semen | Dita | 5km | 06-15-00- | 37-32-00- | 2800 | 07/01/1988 |
| 23 | AC.239003 | *Lupinu* | *albus* | Amara | Agew | Dangela | Smalta/Ge | 11-14-18- | 36-50-93- | 2190 | 10/01/1997 |
| 24 | AC.239004 | *Lupinu* | *albus* | Amara | Agew | Dangela | Zelesa/Ge | 11-30-29- | 36-51-58- | 2220 | 10/01/1997 |
| 25 | AC.239005 | *Lupinu* | *albus* | Amara | Agew | Dangela | Shangana | 11-09-82- | 36-52-10- | 2360 | 10/01/1997 |
| 26 | AC.239006 | *Lupinu* | *albus* | Amara | Agew | Dangela | Ashewa | 11-09-02- | 36-51-90- | 2400 | 10/01/1997 |
| 27 | AC.239007 | *Lupinu* | *albus* | Amara | Agew | Dangela | Ziguda/kid | 11-16-76- | 36-52-81- | 2190 | 10/01/1997 |
| 28 | AC.239017 | *Lupinu* | *albus* | Amara | Debub | Dera | Amora | 11-55-24- | 37-54-21- | 2130 | 12/01/1997 |
| 29 | AC.239046 | *Lupinu* | *Spp.* | Amara | Mirab | Bure | Tilil | 10-50-56- | 37-01-88- | 2520 | 15/01/1997 |
| 30 | AC.239047 | *Lupinu* | *albus* | Amara | Mirab | Bure | Kurb | 10-49-65- | 37-02-45- | 2660 | 15/01/1997 |
| 31 | AC.239048 | *Lupinu* | *albus* | Amara | Mirab | Bure | Bradi | 10-47-88- | 37-03-24- | 2600 | 15/01/1997 |
| 32 | AC.239051 | *Lupinu* | *Spp.* | Amara | Mirab | Bure | 157km | 10-42-45- | 37-07-33- | 2120 | 15/01/1997 |
| 33 | AC.239054 | *Lupinu* | *albus* | Amara | Mirab | Dembech | Mekelabo | 10-34-29- | 37-28-24- | 2210 | 15/01/1997 |
| 34 | AC.239055 | *Lupinu* | *albus* | Amara | Mirab | Dembech | 213km | 10-32-86- | 37-30-61- | 2160 | 15/01/1997 |
| 35 | AC.239057 | *Lupinu* | *albus* | Amara | Misrak | Machake | Yewla | 10-25-07- | 37-33-93- | 2380 | 15/01/1997 |
| 36 | AC.239059 | *Lupinu* | *albus* | Amara | Misrak | Guzamn | 3km | 10-18-35- | 37-44-07- | 2420 | 16/01/1997 |

*Source: EBC,* 2017; Acc# = Accession number, NA= Not Available, * = above sea level (m.a.s.l), C.Date= Collection Date

**Plant height at flowering (PHF):** Height of five randomly selected plants during flowering period from each experimental unit was measured in centimeter from the ground to top of the plant and the average height was recorded as plant height at flowering.

**Number of primary branches (NPB):** Number of productive branches extending from the main stem was recorded on five randomly selected plants of each experimental plot and the means were recorded as number of primary branches per plant. Counting was done at the time when flowering was completely over and fruits were still green but old enough to judge that they would give seeds.

**Number of secondary branches (NSB):** Number of branches extending from the primary branches was recorded on the same plants used to determine the number of primary branches from each plot and the means was recorded as number of secondary branches/plant.

**Number of pods per node of the plant (NPN):** The total number of health pods was taken at physiological maturity from each node of five randomly selected plants, the average was used.

**Number of pods per plant (NPP)**: the total number of health pods was taken at physiological maturity from five randomly selected plants

**Pod length (PL):** the average length of pod expressed in cm at physiological maturity from five randomly selected plants

**Pod thickness (PT):** the average thickness of pod expressed in cm at physiological maturity from five randomly selected plants

**Seed number per pods (SNPP)**: The number of seed was counted per pods from five randomly selected plants

**Number of seeds per plant(NSP):** This was recorded as average total number of seed of five randomly taken plants from each experimental plot and the means were recorded as number of seed per plant.

**Seed yield per plant(SYP)**: The average weight in grams of seeds adjusted 7% moisture content was obtained from five randomly selected plants on each plot.

**Biomass yield per plant (BYP)**

The above ground biomass yield at the time of harvesting was determined in grams from five randomly taken plants and their mean value was taken as biomass yield per plant. This value again converted to kg /ha

**Harvest index (HI):** It was recorded as the ratio of the moisture-adjusted seed yield per plant to the above ground biomass yield per plant in percentage as given by the formula;

$$\mathbf{HarvestIndex(HI)} = \frac{Moistere\ adjusted\ seed\ yieldin\ gram\ per\ plant}{Biomass \text{ in gram } perplant}$$

## RESULTS AND DISCUSSION

### Clustering and Genetic Divergence Analysis

### Cluster analysis

To undergo effective breeding program, parents to be crossed should belong to different genetic cluster. The more distant the parents within over all limits of fitness the greater the chances of obtaining higher amount of heterotic expression in first filial generation, F1 and broad spectrum of variability in segregating populations Atnaf *et al.,* 2017. However, crossing of genotypes belonging to the same genetic cluster would not be expected to yield desirable recombinants. Before any hybridization process takes place, genetic diversity of the existing genotypes needs to be known. The study of divergence analysis is the indication of either presence or absence of genetic diversity among genotypes or the cluster containing the genotypes based on the existing genetic traits. Moreover, evaluation of genetic diversity is important to know the source of genes for particular trait within the available germplasms (Atnaf *et al.,* 2015b). Genetic diversity, in turn, provides hint to produce greater heterosis between diverse genotypes or groups. On the other hand, clustering analysis may show the way in which genotypes with similar genetic characters come in to similar cluster or group, so hybridization between these similar genotypes is less effective to produce more hybrid vigor offspring. Therefore, selection by crossing accessions from different clusters through hybridization can bring about improvement.

In this particular study, to avoid the effect due to differences in scale (measurement), the means of each character were standardized prior to analysis. The distance among the accessions for clustering was estimated using the Euclidean distance method for the mean values of the traits studied at both locations using SAS software. The number of clusters was determined using the cubic clustering criteria (CCC) as described by Mohammad and Trasanna (2003). Figure 1 shows the Dendrogram of genotypes based on distance matrix (among the accessions) and their respective cluster

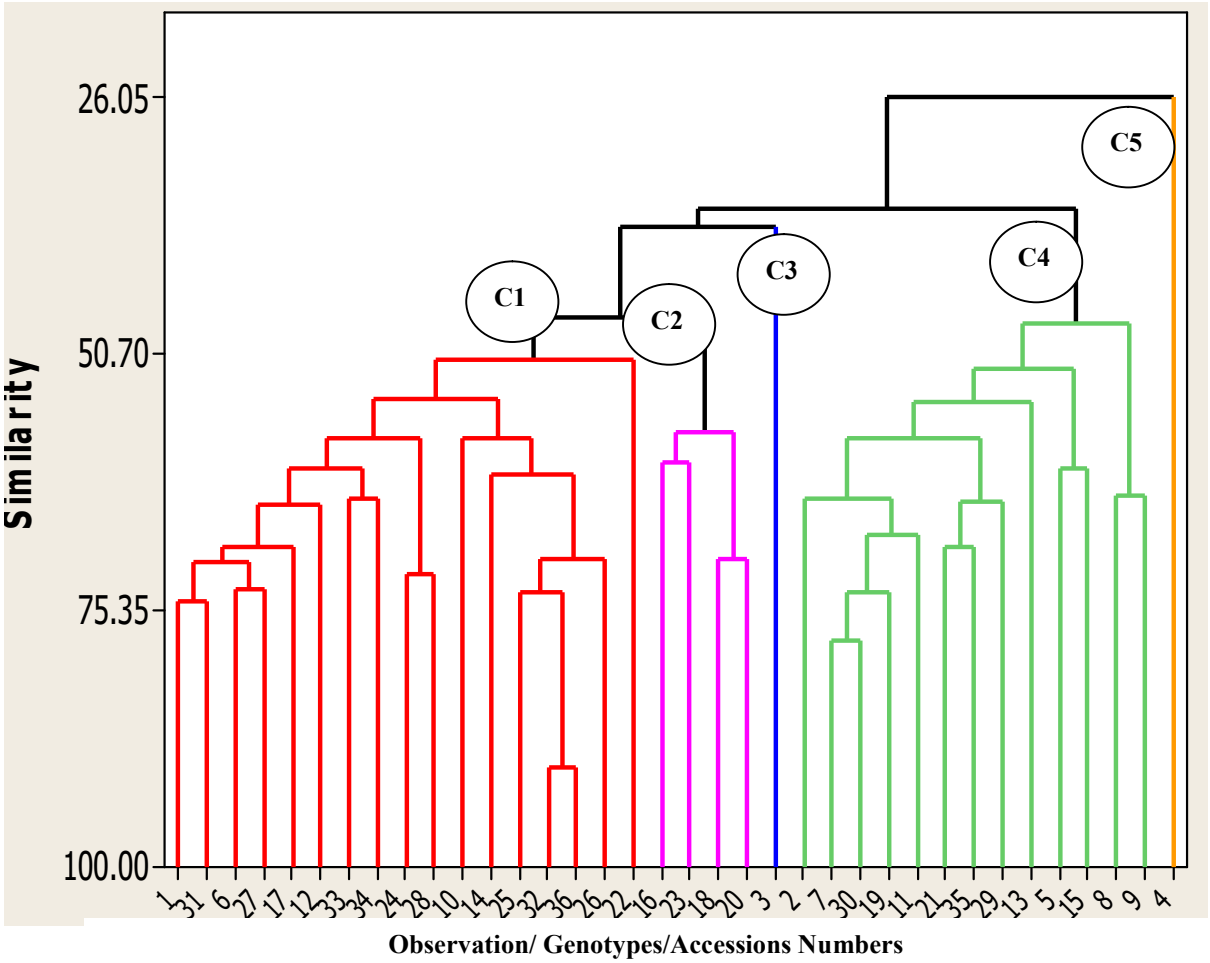


Figure 1: Dendrogram with average linkage and Euclidean Distance of 36 white lupin *Lupinus albus* L. accessions based on average linkage cluster analysis.C1= Cluster 1, C2= Cluster 2, C3= Cluster 3, C4= Cluster 4, C5= Cluster 5

groups. Each extension of the dendrogram and the numbers at the base of the dendrogram represented a genotype, an accession. All the studied accessions were grouped in to five different clusters in which different members within a cluster being assumed to be more closely related in terms of the traits under study with each other than those members in different clusters. Similarly, members in clusters with non-significant distance were assumed to have more close association with each other than those had significant distance clusters. This can mean that members in clusters with significant distance tend to be genetically more divergent than those had non-significant distance. Out of the total thirty six tudied white lupin landraces, 17 of them were grouped in Cluster1 (C1), whereas accessions [16], [23], [18] and [20] were included in Cluster 2 (C2), Cluster 3 (C3) includes a solitary accession [3] which was originally collected from Machakal, East Amhara region, Cluster 4 (C4) holds 13 accessions whereas the last cluster (C5) include a solitary accession [4].

**Cluster 1(C1):** This consisted of seventeen white lupin landraces originally collected from different areas of the country namely Amhara (Dangila, Debre, Dembecha, Dera,Gozamen) and SNNP (Dita), Table 1 and 7. This cluster encompasses 47.22% of the total studied accessions. Based on the overall combined mean performance this cluster includes accessions with the characteristics of early flowering, had much more number of primary branches and longer pods. However, they were with less values of seed yield per plant, hundred seed weight, seed yield per hectare and low harvest index, so they are less productive when compared to the overall mean performance (X) of the accessions in terms of the studied traits.

**Cluster 2 (C2):** This cluster included four accessions; which covers 11.11% of the total studied genotypes. They were formerly collected from Este, Dangila, Gozamen and Machakel districts of the country, Ethiopia, Table 2. The accessions under this cluster exhibited high numbers of both primary and secondary branches, high

**Table 2:** Grouping 36Ethiopian white lupin *(Lupinus albus*L.*)* accessions in to different clusters and collection regions.

| Clusters | Total No of accessions in the cluster | Lists of accessions in the Cluster | Collection regions |
|---|---|---|---|
| C1 | 17 (47.22%) | AC.24850 [1], AC.239048 [31], AC.26638 [6], AC.239007 [27], AC.105006 [17], AC.29056 [12], AC.239054 [33], AC.239055 [34], AC.239004 [24], AC.239017 [28], AC.29054 [10], AC.29058 [14], AC.239005, [25] AC.239051 [32], AC.239059 [36], AC.239006 [26] and AC.225802 [22], | Amhara (Dangila, Debre, Dembecha, Dera,Gozamen) and SNNP (Dita) |
| C2 | 4 (11.11%) | AC.105002 [16], AC.239003 [23], AC.105007 [18], AC.216015 [20] | Amhara (Este, Dangila, Gozamen and Machakel) |
| C3 | 1(2.78%) | AC.26635 [3] | Machakal (East Amhara region) |
| C4 | 13 (36.11%) | AC.26634 [2], AC.26639 [7], AC.239047 [30], AC.216014 [19] AC.29055 [11], AC.216016 [21], AC.239057 [35], AC.239046 [29], AC.29057 [13], AC.26637 [5], AC.105001 [15], AC.26640 [8] and AC.26641[9] | Amhara (Gozamen, Baso, Bure, Dangila, Debre, Jabi,and yalamlam) |
| C5 | 1(2.78%) | AC.26636 [4] | West Amhara region (Senan) |

Numbers in the bracket represent accession numbers at the base of the Dendrogram (Figure 1).

in seed yield per plant and harvest index, with higher number of seeds per plant and per plot than the overall mean value. They required longer duration of time to start flowering and longer period for 50% flowering and maturation. On the other hand, they had intermediate hundred seed weight and were shorter in their height at flowering. They are less productive, even, the maximum

**Cluster 3:** This cluster held only one (solitary), AC.26635 [3], genotype which was originally collected from Machakal district of Amhara regional state of the country, Figure 1 and Table 2. It was relatively very lately emerging accession (it took above 14 days) and also required relatively longer days to start flowering, to reach at 50% flowering and for maturation than the overall average days. The accession was shorter in height at flowering but contained much less number of primary branches than the overall mean value. It was also characterized by having intermediate seed yield per plant, yield per hectare and harvest index. Nevertheless, it possessed relatively low biomass yield per plant with less hundred seed weight.

**Cluster 4:** This cluster consisted of thirteen different germplasm collections which hold 36.11% of the total studied accessions. The white lupin accessions included in this cluster were collected from seven different administrative districts namely: Gozamen, Baso, Bure, Dangila, Debre, Jabi,and yalamlam in Amhara regional state of Ethiopia, Table 1 and 2. They require intermediate period to start flowering, but longer maturation. They were extremely high in length at flowering and possessed intermediate numbers of primary branches, less secondary branches. When compared to the overall mean performance of the whole studied accessions in yield per hectare (3982.33 $Kgha^{-1}$), the thirteen accessions under this cluster exhibited extremely high yield per hectare (6968.28 $Kgha^{-1}$). This shows that the most productive landraces are found under C4. Therefore, one can select best performing germplasm here, from C4, out of the studied accessions in terms yield.

performance of these four accessions (AC.105002 [16], AC.239003 [23], AC.105007 [18], AC.216015 [20]) in terms of their plot yield converted to hectare (1476.41 $Kgha^{-1}$) is very much less than the overall mean performance value 3982.3 $Kgha^{-1}$ Table 5.

**Cluster 5:** This cluster consisted of only one accession (AC.26636 [4]) which was collected from particular area of West Amhara region of the country called Senan. It was relatively very early emerging accession but required relatively longer days to start flowering, to reach at 50% flowering and for maturation than the overall average days. It was short at flowering in height and had intermediate numbers of both primary branches. The mean values of eleven traits (61%) of the accessions namely (DFI, DF, NPB, NPP, NSP, DM, SYP, SYPL, SYH, BYPL, and HI) in this cluster highly exceeded their overall mean values, Table 3.

Generally, to identify genetic divergence and useful variability among genotypes due to certain genetic traits is by grouping or clustering of genetic stocks based on suitable scale is quite imperative (Temesgen *et al.,*

**Table 3:** Range and Mean of quantitative traits for the five clusters of white lupin accessions tested at both RSWWU and Gasera farmer's farm land during the main cropping season of the 2017.

| Studied traits | Cluster1( C1) | | | Cluster2 ( C2) | | | Cluster3 ( C3) | | | Cluster4 ( C4) | | | Cluster5 ( C5) | | | X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | **Mean** | Max | Min | **Mean** | Max | Min | **Mean** | Max | Min | **Mean** | Max | Min | **Mean** | Max | |
| DFI | 57 | **66.12** | 87 | 71.3 | **89.72** | 108.00 | - | **98.20** | - | 57 | **70.34** | 97.7 | - | **93.00** | - | **71.83** |
| DF | 66 | **76.39** | 121.5 | 80 | **99.42** | 116.00 | - | **121.5** | - | 68.3 | **81.27** | 117.3 | - | **112.83** | - | **82.97** |
| PHF | 56 | **66.63** | 85.5 | 62.67 | **74.16** | 99.50 | - | **59.83** | - | 80.3 | **106.39** | 137.8 | - | **76.5** | - | **82.22** |
| NPB | 4 | **6.13** | 9 | 7 | **8.25** | 10.00 | - | **4.05** | - | 5.33 | **7.59** | 9.67 | - | **4.33** | - | **4.23** |
| NSB | 6 | **10.54** | 16 | 14 | **17.25** | 20.00 | - | **4.83** | - | 8.83 | **14.59** | 23.17 | - | **8.03** | - | **12.53** |
| PL | 8 | **11.36** | 13 | 8 | **9.25** | 12.00 | - | **6.67** | - | 6 | **9.40** | 12.2 | - | **8.17** | - | **10.23** |
| NPP | 19 | **33.57** | 68 | 26.7 | **39.41** | 51.00 | - | **36.00** | - | 40 | **84.38** | 103.3 | - | **87.67** | - | **54.13** |
| SNPP | 3 | **5.37** | 7 | 3 | **4.5** | 6.00 | - | **6.33** | - | 3 | **3.81** | 5.5 | - | **4.33** | - | **4.70** |
| NSP | 71 | **143.20** | 262 | 80 | **111.75** | 149 | - | **173.83** | - | 154 | **288.79** | 393.7 | - | **218** | - | **195.21** |
| DM | 207 | **189.71** | 170.33 | 192.3 | **210.83** | 231.3 | - | **179.83** | - | 180.7 | **197.02** | 222 | - | **205.33** | - | **194.84** |
| SYP | 29.23 | **69.27** | 222.87 | 41.18 | **46.85** | 51.03 | - | **68.68** | - | 91.44 | **149.87** | 198.7 | - | **101.67** | - | **96.77** |
| SYPL | 109.1 | **268.86** | 729.6 | 122.63 | **145.88** | 170.52 | - | **467.31** | - | 102.9 | **855.07** | 1194.8 | - | **791.84** | - | **486.92** |
| SYH | 787.6 | **2197.64** | 6168.8 | 1035.5 | **1301.10** | 1476.4 | - | **3827.3** | - | 3169 | **6968.28** | 9503.3 | - | **6382.4** | - | **3982.3** |
| HSW | 9.35 | **25.68** | 44.35 | 34.4 | **40.30** | 46.25 | - | **15.72** | - | 18.3 | **35.53** | 43.35 | - | **22.63** | - | **30.48** |
| BYPL | 1.03 | **1.55** | 2.20 | 1.6 | **1.77** | 2 | - | **1.26** | - | 1.2 | **2.51** | 3.45 | - | **2.12** | - | **1.93** |
| HI | 26.26 | **57.66** | 87.36 | 25.66 | **36.84** | 47.29 | - | **61.59** | - | 66.66 | **82.41** | 91.8 | - | **84.28** | - | **65.13** |

2013b). Similarly, choice of genetically divergent parents for hybridization under transgressive breeding program is also dependent upon categorization of breeding materials on the basis of appropriate criteria.According to Sharma (1998), quantitative classification that serves as a sound basis of grouping any two or more genotypes based on minimum divergence or resemblance between them offers a quantified degree of divergence among genotypes or populations. The nature of distribution of the accessions within each cluster on the dendrogram indicated relationships among the groups.

**Genetic Divergence analysis**

The genetic distances ($D^2$) among the accessions indicated the presence of a wide diversity where the minimum and maximum distances were 17.23 and 129.26, respectively, with average distance of 63.21. The studied genetic divergence of the 36 Ethiopian white lupin genotypes was grouped into five clusters using Mahalanobis $D^2$ statistics. This analysis was carried out to know the extent of divergence in the genotypes to identify the superior genotypes for further utilization in hybridization program and to find out the contribution of different characters towards genetic divergence among the studied white lupin accessions.

Nevertheless, the dendrogram did not indicate any clear divisions among the white lupin accessions based on their geographical locations. 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 most divergent clusters, in this study, were cluster three and five ($D^2$=641.87). Each of the two clusters consists of only one accession. Cluster three constitutes an accession from Machakal district, while cluster five constitutes a single accession from West Amhara region of the country called Senan Table 2. The second maximum inter-cluster distance was found between cluster one and five ($D^2$= 529.28). The third most divergent clusters were cluster two and five ($D^2$=507.96).Cluster two holds four accessions collected from Este, Dangila, Gozamen and Machakel districts of the country. The forth most divergent clusters in terms of inter-cluster distance were between cluster four and five ($D^2$=439.85), cluster four was constituted from a thirteen accessions collected from Gozamen, Baso, Bure, Dangila, Debre, Jabi and Yalamlam in

**Table 4:** Pairwise, average intra-cluster (the bold diagonal) and inter-cluster (off diagonal) distance ($D^2$) among the five clusters of Ethiopian *Lupin albus* L. accessions tested at two research sites of Bale zone during the main cropping season of the year 2017.

| Clusters | C1 | C2 | C3 | C4 | C5 |
|---|---|---|---|---|---|
| C1 | **2.89** | | | | |
| C2 | 61.94* | **1.84** | | | |
| C3 | 87.02* | 227.57** | **0** | | |
| C4 | 28.70* | 82.18* | 79.61* | **3.07** | |
| C5 | 529.28** | 507.96** | 641.87** | 439.85** | **0** |

*= significant and **= highly significant at P = 0.05, P = 0.01 respectively.

Amhara regional state of Ethiopia, Table 1 and 2. In the study carried out on Molecular genetic diversity and population structure of 212 Ethiopian white lupin landraces by Atinaf *et al.,* 2017 grouped it in to thirteen different clusters based on their studied quantitative traits.

On the other hand, intra-cluster genetic distance (the bold diagonal values in Table 4), was analyzed and estimated for the five formed clusters. This can indicate that genotypes that were grouped into the same cluster would most likely be diverged slightly from one another as the combined characters are measured. Accordingly, among the five clusters formed, cluster four (**C4**) showed the maximum intra-cluster value ($D^2$=3.07) followed by cluster one (**C1)** ($D^2$= 2.89). Information on the genetic distance between parents is necessary to assist transgressive segregation, since the higher the genetic distance between parents, the higher the heterosis in progeny can be observed. According to Hussain *et al*., 2014 diverse parents from various clusters are helpful in planning the breeding program by planning the crosses and increased use of heterosis and genetic diversity. Bhanupriya *et al*. (2014) reviewed the work of Joshi *et al.* (2004) and Anand and Murrty (1968) and then remarked that one of the important approaches to breeding is hybridization and subsequent selection where the parents' choice is the first step in plant breeding program through hybridization.

Generally, in 2017, cluster four consisted of 36.11% of the accessions with higher yield (6968.28Kgha$^{-1}$) along with higher values for most of the yield components. Therefore, crossing of accessions from cluster two to either cluster one or with cluster three with the traits of interest of other traits may be advantageous to improve yield. In this connection, noted that when parents are selected based on $D^2$ statistics, three points should be taken into consideration. These are:

(1) The relative contribution of each character;
(2) The choice of clusters with maximum statistical distance; and
(3) The selection of one or two genotypes from such clusters. Other characters, like disease resistance, earliness and quality, should be considered.

## CONCLUSION

In conclusion, despite the diverse favorable agroecologies, germplasm availability and released opportunities, research attention given to this crop was very low till recent time. The present investigation indicated that there is wide range of genetic diversity in the tested germplasm for most of the characters studied. Hybridization among accessions from different clusters identified in this study could lead to considerable genetic improvement by following appropriate selection strategies in the segregating generations. However, it would be worthwhile to study more available germplasm over years and locations to identify more diverse accessions as well as to confirm the importance of the traits identified as predictors of seed yield and/or oil content. Hybridization among accessions from different clusters identified in this study could lead to considerable genetic improvement by following appropriate selection strategies in the segregating generations. In general, maximum genetic segregation and genetic recombination is expected from crosses that involve parents from highly significant distant clusters. In the present investigation, therefore, crossing of accessions from cluster three and five will give rise to maximum genetic segregation. The selection of parents which are identified on the basis of divergence analysis would be more promising for a hybridization program.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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