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GENETIC DIVERSITY AND POPULATION STRUCTURE OF *LUPINUS ALBUS* (L.) FROM THE AMHARA REGION OF ETHIOPIA USING SEED STORAGE PROTEIN MARKERS

Selamawit Kelemu[1,3], Eleni Shiferaw[2*] and Faris Hailu[3]

[1]Department of Biology, Raya University, P.O.Box 42, Maichew, Ethiopia
[2]Ethiopian Biodiversity Institute, P.O.Box 30726, Addis Ababa, Ethiopia
[3]Department of Biology, Wollo University, P.O.Box 1145, Dessie, Ethiopia

Abstract: The genetic diversity in 48 lupin (*Lupinus albus* (L.) accessions collected from the Amhara region, Ethiopia, was assessed using seed storage protein markers (SDS-PAGE). A total of 30 different protein bands with sizes ranging from 11 to 100 kDa were detected. The average number of protein bands, the percentage of polymorphism, and gene diversity in the accessions were 16.96, 20.35, and 0.072, respectively. Genetic diversity estimates showed that West Gojam and Bahir Dar areas could be the most important sources for lupin genetic resources. The pair-wise comparison of genetic distances (GDs) among the accessions ranged from 0.011 to 0.378. The most distantly related accessions were accession 6, collected from the West Gojam zone, and accession 28 from the Bahir Dar area. Principal coordinate analysis (PCoA) showed the absence of a distinct group, and most of the accessions were intermixed. Population structure analysis revealed that the 48 lupin accessions could be assigned to three clusters. Similar to PCoA, no defined grouping based on geographic origin was observed. Accessions from different geographic origins being grouped together could be attributed to a common origin for the various accessions in the different zones, or it could be the result of seed-mediated gene flow among different lupin growing areas of the country.

Key words: diversity, white lupin, SDS-PAGE.

Introduction

Lupinus is a large and diverse genus of the legume family Fabaceae, containing both annual and perennial herbaceous species and some shrubby and tree types (Ainouche and Bayre, 1999). *Lupinus albus* (L.), commonly known as white lupin or lupin, is one of the cultivated plants in the genus believed to have originated in the Balkan Peninsula (Kurlovich and Kartuzova, 2002), and it has

*Corresponding author: e-mail: teleni08@gmail.com

been cultivated in the Mediterranean region, North Africa and the Nile valley (Westphal, 1974). Lupin can grow under environmental and edaphic conditions that are not tolerated by other crops (Hill, 1977). As reviewed by Nigussie (2012), lupin is used for many purposes, which include pasture improvement, ornamentation, erosion control, soil stabilization as green manure, and pest control. It has a high protein and fiber content (Erbas et al., 2005; Tizazu and Emire, 2010). It also has positive roles in combating obesity, diabetes, and cardiovascular disease (Magni et al., 2004; Belski et al., 2010; Duranti and Morazzoni, 2011). However, the extensive use of lupin for food or feed is hindered by its alkaloid content (Yeheyis et al., 2010). In Ethiopia, it is grown mainly by subsistence farmers, and it covers 1.2% of the total pulse growing area, of which 99.2% of the produce came from the Amhara region (CSA, 2018). Compared to other legume crops grown in the country, lupin could be considered a neglected and underutilized crop.

Studying the genetic diversity of crops provides information that can be used to identify germplasms with valuable traits. Genetic diversity analysis in lupin has been carried out using agro-morphological traits (Atnaf et al., 2015), SSR markers (Atnaf et al., 2017), DArT markers (Raman et al., 2014), and ISSR markers (Oumer et al., 2015). Seed storage proteins are relatively inexpensive and informative markers and could show variation both within and between species (Shewry et al., 1995). In lupin, seed protein markers have been used to distinguish between genotypes and differentiate cultivars (Pollard et al., 1996; Vaz et al., 2004). No prior study employing seed proteins was conducted to assess the genetic diversity of Ethiopian lupin germplasm. Hence, this study was initiated to assess the utility of seed proteins for diversity assessment, assess the level of genetic variation among accessions, and assess the genetic structure of lupin collected from the Amhara region, Ethiopia.

Material and Methods

Plant materials and SDS-PAGE electrophoresis

Forty-eight lupin accessions obtained from the Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia, were used in this study (Table 1). To capture the intra-accession diversity, each accession was represented by 17 seeds. Individual seeds were ground to a fine powder with mortar and pestle. Extraction buffer (200 µl of 0.002M sodium borate) was added to 0.02 g of each sample, mixed by vortexing, and the homogenate was centrifuged at 10,000 rpm (Hermel Z233 M-2) for 5 min. The extracted crude protein was recovered as supernatant. The denaturing agent (0.02 M Tris-base (pH 8.6), 0.03 M sodium dodecyl sulphate (SDS), 8.3% glycerol, 2% β-mercaptoethanol and bromophenol blue) was added to

the supernatant protein sample in a 1:1 ratio. Samples were denatured for 5 min at 90°C before electrophoresis.

Gel solution prepared from 30% acrylamide and N, N-methylbisacrylamide in a 29:1 ratio was used for electrophoresis. Proteins were separated using 5% stacking and 10% resolving gel in the Tris-glycine buffer (pH 8.3). The denatured sample (25 µl) was loaded on the gel and run at 100 volts until the tracking dye (bromophenol blue) reached the bottom of the gel following the discontinuous method of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). A standard protein marker (NEB P7712s) was included with each run. After electrophoresis, gels were stained overnight using a staining solution (0.25 g Coomassie blue diluted in 100 ml ethanol and 100 ml distilled water). For distaining, a mixture of ethanol and distilled water (1:1) was used.

Table 1. The list of accessions used in this study with accession code (Acc. code), accession number (Acc. no) and collection zone.

Acc. code	Acc. no.	Collection zone	Acc. code	Acc. no.	Collection zone
1	105007	East Gojam	25	239003	Agew Awi
2	105003	Bahir Dar area	26	208464	Agew Awi
3	239014	North Gondar	27	105006	West Gojam
4	239059	East Gojam	28	239020	Bahir Dar area
5	239047	West Gojam	29	239026	Agew Awi
6	259046	West Gojam	30	239057	East Gojam
7	239036	West Gojam	31	239019	West Gojam
8	239009	West Gojam	32	212754	South Gondar
9	239010	West Gojam	33	239016	West Gojam
10	238997	West Gojam	34	105001	West Gojam
11	239024	Bahir Dar area	35	105005	Agew Awi
12	239007	Agew Awi	36	238998	West Gojam
13	239018	West Gojam	37	238999	West Gojam
14	238993	Bahir Dar area	38	239000	West Gojam
15	239017	South Gondar	39	239004	Agew Awi
16	239025	Bahir Dar area	40	239001	West Gojam
17	238994	Bahir Dar area	41	239006	West Gojam
18	242265	West Gojam	42	239011	Bahir Dar area
19	216014	East Gojam	43	239060	North Gondar
20	239021	Bahir Dar area	44	239054	West Gojam
21	216016	East Gojam	45	239051	West Gojam
22	239034	West Gojam	46	239015	West Gojam
23	239005	Agew Awi	47	239012	North Gondar
24	216015	East Gojam	48	238996	Bahir Dar area

The accession number is a unique identifier number at the Ethiopian Biodiversity Institute.

Data analysis

The presence (1) or absence (0) of each band was scored using the standard protein marker as a reference. The resulting binary data matrix for the 48 accessions (816 individual seeds) was used to perform diversity analysis within and between the accessions. GenAlEx version 6.5 (Peakall and Smouse, 2012) was used to compute the percentage of polymorphic bands (PPB), gene diversity (He), the pair-wise comparison of genetic distances (GDs) among accessions and Principal coordinate analysis (PCoA). The genetic structure was analyzed using STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003). To determine the most likely number of populations (K), a burn-in period and value of MCMC (Markov Chain Monte Carlo) were set to 100,000 replications. Assumed K values (1 to10) were checked, and to assure the consistency of the results between runs with the same K, ten replicates were run for each assumed K value. The most probable K-value was determined by following the simulation method of Evanno et al. (2005) using the web-based software STRUCTURE HARVESTER (Earl and VonHoldt, 2012).

Results and Discussion

Based on the relative mobility of seed proteins on the gel, a total of 30 protein bands with sizes ranging between 11 and 100 kDa were detected (Figure 1). The number of bands per accession ranged from 12 (accession 26) to 22 (accession 16).

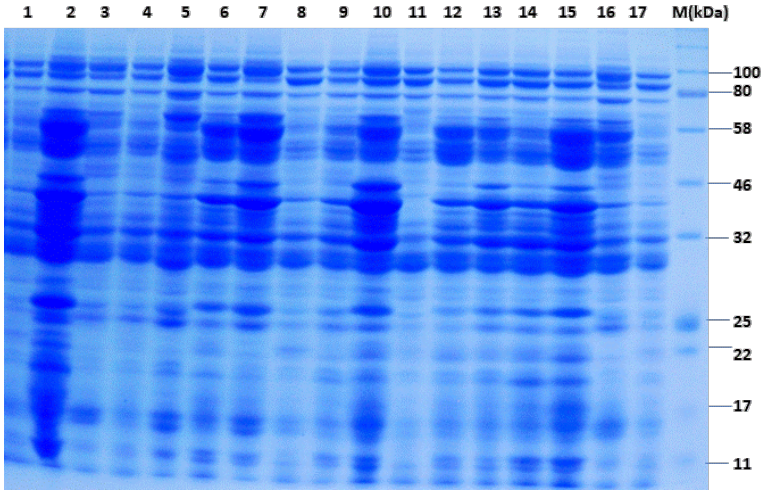

Figure 1. The representative SDS-PAGE image showing the intra-accession variability in accession 9 (acc. no 239010); numbers 1–17 indicate individual seed samples; M – Protein weight marker.

The percentage of polymorphic bands ranged from 0% to 43.33%, with an average value of 20.35%. High percentages of polymorphic loci were observed within accession 16, which was collected from the Bahir Dar area, followed by accessions 13 and 37 from the West Gojam zone (Table 2). The least band polymorphism (0.00%) was found within accessions 3, 4 and 26, which were collected from North Gondar, East Gojam, and Agew Awi zones, respectively. The highest gene diversity estimate was shown by accession 16 (He = 0.166), which was collected from the Bahir Dar area, followed by accession 13 from West Gojam (He = 0.158). Oumer et al. (2015) and Atnaf et al. (2017) reported higher values of gene diversity estimates for lupin collection from the Amhara region using ISSR and SSR markers, respectively. This showed the limited potential of seed proteins in revealing variations within accessions. Likewise, limited intra-species variations using seed proteins were also reported in legumes such as chickpea (Ghafoor et al., 2003) and groundnut (Javid et al., 2004).

Table 2. The summary of genetic diversity measures of the 48 white lupin accessions.

Accession code	N	PPB	He	Accession code	N	PPB	He
1	19	26.67	0.098	25	17	16.67	0.053
2	17	30.00	0.099	26	12	0.00	0.00
3	13	0.00	0.00	27	18	10.00	0.038
4	15	0.00	0.00	28	15	23.33	0.053
5	16	20.00	0.073	29	17	23.33	0.104
6	17	10.00	0.049	30	18	30.00	0.107
7	19	30.00	0.117	31	18	30.00	0.105
8	21	33.33	0.069	32	17	20.00	0.074
9	18	20.00	0.068	33	14	13.33	0.051
10	17	20.00	0.076	34	14	10.00	0.032
11	17	23.33	0.081	35	18	20.00	0.077
12	17	23.33	0.103	36	16	13.33	0.066
13	21	40.00	0.158	37	21	36.67	0.106
14	17	26.67	0.084	38	18	23.33	0.080
15	20	30.00	0.079	39	16	16.67	0.078
16	22	43.33	0.166	40	17	23.33	0.077
17	14	26.67	0.112	41	16	23.33	0.076
18	18	20.00	0.097	42	16	13.33	0.064
19	17	13.33	0.035	43	16	6.67	0.028
20	17	30.00	0.100	44	17	20.00	0.062
21	15	20.00	0.075	45	13	3.33	0.007
22	17	33.33	0.116	46	19	33.33	0.121
23	17	16.67	0.055	47	17	6.67	0.031
24	15	10.00	0.034	48	18	13.33	0.049
Mean					16.96	20.35	0.072

N (number of bands); PPB (percentage of polymorphic bands) and He (expected heterozygosity/gene diversity); Mean (mean values of N, PPB and He for the 48 accessions).

Based on the high number of different protein bands, the percentage of polymorphic bands and gene diversity estimates, the West Gojam and Bahir Dar areas could be the most important sources for lupin genetic resources. High gene diversity for accessions from West Gojam was also reported by earlier studies (Atnaf et al., 2015; Atnaf et al., 2017). The Bahir Dar area is another location that showed a higher level of diversity using the seed storage protein, which was not reported in the earlier studies.

The seed protein profile transformed into a binary matrix was used to calculate genetic distance. Genetic distances (GDs) between all pair-wise combinations among the 48 accessions ranged from 0.011 to 0.378 (data not shown). The most distantly related accessions were accession 6 collected from the West Gojam zone and accession 28 from the Bahir Dar area (GD = 0.378), followed by accessions 6 and 12 from West Gojam and Agew Awi (GD = 0.363) zones, respectively. The least distances were recorded between accession 47 from North Gondar and accession 27 from West Gojam (GD = 0.011) and between accession 43 from North Gondar and accession 4 from East Gojam (GD = 0.013). Close distance observed among accessions collected from different areas indicated the presence of genetic similarity among them. The principal coordinate analysis (PCoA) also revealed no distinct grouping based on geographic origin (Figure 2). The first three axes explained a cumulative variation of 44.66%. This could be due to the presence of a shared protein profile as a result of seed exchange among farmers or common origin. The existence of a dominant informal seed system might have contributed to the presence of similar genetic backgrounds for accessions collected from the different geographical areas (Forsberg et al., 2015). Similar observations were made for other legume species from Ethiopia (Shiferaw & Porceddu, 2018; Ayelign et al., 2020).

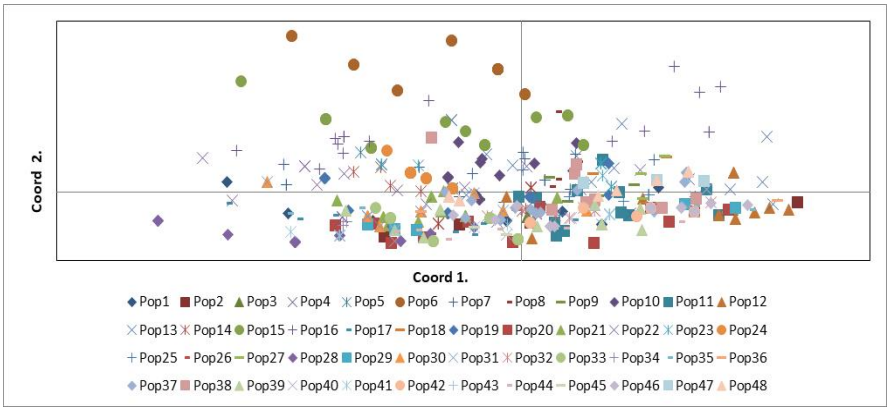

Figure 2. The principal coordinate analysis (PCoA) bi-plot showing the clustering pattern of the 816 individual samples representing the 48 lupin accessions.

The Bayesian approach-based clustering method allows to define the population structure, assign individuals to populations, and identify admixed individuals (Pritchard et al., 2000). The assignment of the 48 accessions, represented by 816 genotypes, to different populations and the determination of their population structure revealed K = 3 (three groups) to be the most likely number of clusters.

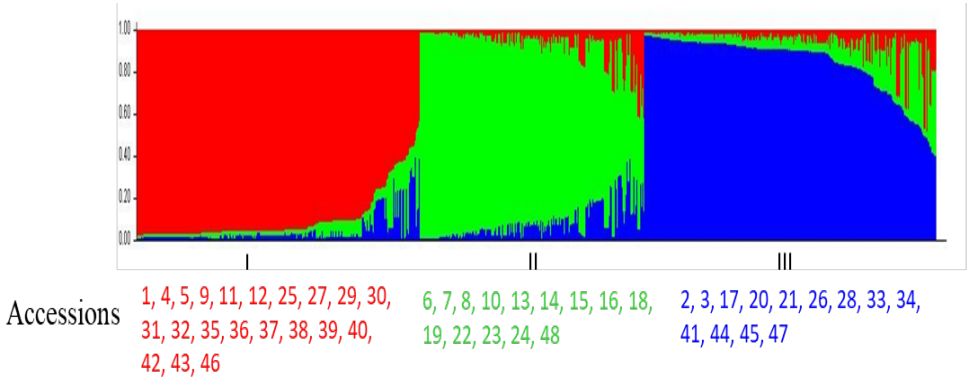

Figure 3. The model-based clustering of lupin accessions indicating the grouping of the 48 accessions into three clusters.

The first cluster (I) contained 21 accessions, while clusters II and III contained 14 and 13 accessions, respectively (Figure 3). Both PCoA and the model-based structure analysis showed substantial admixtures of lupin collections. The majority of the accessions from the Agew Awi zone were grouped in cluster I, while the rest of the accessions from different zones were grouped within the same cluster. This contrasts with the finding by Atnaf et al. (2015), where accessions from Agew Awi were distributed over different clusters. This difference could be attributed to the different types of markers used in the two studies. No defined grouping based on geographic origin was observed. The structure analysis result is largely consistent with PCoA since accessions grouped in the similar quadrat in the PCoA were also grouped in the same cluster in the structure analysis. The absence of a clear relationship between geographic origin and diversity pattern was also reported in other legume species from Ethiopia (Negisho et al., 2017; Tekalign et al., 2019). Accessions from different geographic origins being grouped together may indicate the existence of similar genetic backgrounds or a common origin for the various accessions in the different zones, or it could be the result of seed-mediated gene flow among different lupin growing areas of the country.

Conclusion

This study has analyzed the utility of seed storage proteins in detecting genetic variability in some collections of *L. albus* from the Amhara region, Ethiopia, the major growing region of the crop. Accessions from the West Gojam zone showed a higher level of diversity. The present study also indicated the Bahir Dar area as an important site for lupin diversity. The clustering of the accessions did not follow geographical origin. This could be due to the seed-mediated gene flow among different geographic zones or because of a common germplasm source. An extensive study on specific traits of germplasms from the West Gojam zone is recommended to fully realize the potential benefits of this genetic resource in breeding programs and improve the crop.

Acknowledgments

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GENETSKA RAZNOVRSNOST I STRUKTURA POPULACIJE *LUPINUS ALBUS* (L.) IZ REGIONA AMHARA U ETIOPIJI KORIŠĆENJEM PROTEINSKIH MARKERA IZ SEMENA

Selamawit Kelemu[1,3], Eleni Shiferaw[2*] i Faris Hailu[3]

[1]Odsek za biologiju, Univerzitet Raya, P.O.Box 42, Majčev, Etiopija
[2]Etiopijski institut za biodiverzitet, P.O.Box 30726, Adis Abeba, Etiopija
[3]Odsek za biologiju, Univerzitet Wollo, P.O.Box 1145, Dese, Etiopija

R e z i m e

Genetska raznovrsnost kod 48 genotipova lupine (*Lupius albus* (L.) prikupljenih iz regiona Amhara u Etiopiji, procenjena je korišćenjem proteinskih markera skladištenih u semenu. Detektovano je ukupno 30 proteinskih traka veličina u rasponu od 11 do 100 kDa. Prosečan broj proteinskih traka, procenat polimorfizma i raznovrsnost gena u domaćim populacijama iznosio je 16, 96, 20, 35 odnosno 0,072. Procene genetičke raznovrsnosti pokazale su da oblasti Zapadnog Godžama i Bahir Dara mogu biti najvažniji izvor genetskih resursa lupine. Poređenje parova uzoraka ukazalo je da se genetska rastojanja među njima kreću od 0,011 do 0,378. Najmanje srodni bili su uzorak 6, iz oblasti Zapadni Godžam, i uzorak 28 iz oblasti Bahir Dar. Primenom analize glavnih komponenata (PCA) nisu dobijene posebne grupe, većina uzoraka je bila pomešana. Primenom populacione strukturne analize 48 uzoraka lupine podeljena su u tri klastera. Slično kao kod PCA nije primećeno grupisanje uzoraka na osnovu geografskog porekla. Zajednička grupisanost uzoraka različitog geografskog porekla može se pripisati njihovom zajedničkom poreklu, ili bi to mogao biti rezultat protoka gena posredstvom semena između različitih oblasti uzgajanja lupine u zemlji.

Ključne reči: raznovrsnost, bela lupina, SDS-PAGE.

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[*]Autor za kontakt: e-mail: teleni08@gmail.com