Genet Resour Crop Evol
https://doi.org/10.1007/s10722-021-01302-5

RESEARCH ARTICLE

Genetic diversity and population structure analysis of Grass pea (*Lathyrus sativus* L.) accessions collected from North-Western Ethiopia using SSR markers

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Received: 21 March 2021 / Accepted: 7 November 2021

Abstract Grass pea (*Lathyrus sativus* L.) is a legume crop known to be an excellent source of protein, tolerant to drought, waterlogging, and salinity. The crop is used as an alternative source of protein to reduce malnutrition for resource-poor people and farmers leaving in marginal areas. However, due to the presence of a neurotoxin that causes lathyrism in the crop, it has been neglected and underutilized. As a necessary first step towards, therefore, this investigation was undertaken to assess the genetic diversity and population structure that existed within grass pea accessions collected from North-West Ethiopia using simple sequence repeat markers. Twenty-five grass pea accessions collected from the Ethiopian Biodiversity Institute were planted at the College of Agricultural Science, Ebonyi State University, Nigeria. The genomic DNA was extracted using Quick-DNA^{TM} ZR Plant/Seed Miniprep Kit and amplified in an ABI Veriti PCR machine with 10 pairs of SSR markers in IITA, Ibadan, Nigeria. Out of 10 SSR primers, only

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Published online: 22 January 2022

eight primers were polymorphic. A total of 41 alleles were detected with an average of 5.13. The Polymorphism Information Content and Gene Diversity values ranged from 0.074 to 0.944 with a mean of 0.474 and 0.536 respectively. The largest pairwise genetic distance (0.365) was detected between North Gondar and East Gojjam populations. The ΦPT (analog of FST test) estimated through AMOVA were 0.24, 0.20, and 0.05 for within accessions, among regions, and population within regions respectively. The highest genetic differentiation value (76%) resides within accessions followed by 20% among regions. Both the population structure and cluster analyses grouped the 25 grass pea accessions into two distinct subgroups. This grouping pattern indicates the presence of gene flow among geographic regions. In general, the findings of this study indicate that despite few numbers of SSR markers it was possible to detect genetic diversity among grass pea accessions indicating the power of the SSR marker in picking up the existing genetic diversity within the studied accessions.

Keywords Ethiopia · Grass pea · SSR · Gene diversity · Genetic structure · AMOVA

Introduction

Grass pea (*Lathyrus sativus* L.) is an essential legume crop in Ethiopia and is known to be rich in protein (28–32%), and micronutrients. Due to its high nutrition content and ability to adapt in marginalized areas, the crop is used for human consumption and animal feeds by subsistence farmers (Rafaat et al. 2021; Mahapatra et al. 2020; Urga et al. 2005). The *Lathyrus* exceeds 187 species among the species only *L. sativus* is widely cultivated as a food legume (Soren et al. 2020; Wang et al. 2015; Campbell 1997). The particular center of origin is unidentified, as different authors have reported different centers of diversity including South-West and Central Asia, Mediterranean, Iran-Turanian regions, Bangladesh, and East Africa mainly in Ethiopia (Dixit et al. 2016; Parihar et al. 2013,2015; Wang et al. 2015; Talukdar 2009). Currently, there are 435 *L. sativus* accessions, and 143 relative species a total of 578 *Lathyrus* accessions collections withheld in the Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia (https://www.ebi.gov.et/biodiversity/conservation/database-ms/).

Globally, 1.2 M t of *L. sativus* yields out of 1.5 M ha of land. Ethiopia is the third-major producer of the crop following Bangladesh and India (Gupta et al. 2018). It is the fifth major grain legume following faba bean, field pea, haricot bean, and chickpea regarding production, and area coverage (CSA 2020). It has various significant traits like drought, salinity, and waterlogging tolerance, and resistance to insect pests, which make the crop adaptable to mild to hot extreme environments (Tokarz et al. 2021; Wiraguna et al. 2020). It is resilience and penetrating root system allow the crop to grow in an extensive range of soil types including very poor soils and heavy clays and drought-stricken areas. It has been realized that grass pea can serve as a survival foodstuff in difficult situations making it a good food security crop (Asadova et al. 2020; Parihar et al. 2013; Girma and Korbu 2012).

The crop is exiled and ignored by governmental research institutes, non-governmental organizations, and donor groups in Ethiopia (Dixit et al. 2016). But, it is widely cultivated by farmers as a bonus crop in fallow lands because of its nitrogen-fixing ability. Besides, farmers also used it as an alternative crop between rice cultivation periods due to its drought-tolerant nature (Gupta et al. 2018). In Ethiopia, the seed of grass pea is used as a source of food and becoming valuable in replacing chickpea and field pea. The food is prepared in various forms such as sauce (shiro), boiled (nifro), and roasted (kolo) (Lambein et al. 2019; Urga et al. 2005). Currently, the price of this crop is comparable with other cereal crops in the local market attributable to its wide use as an alternative protein source and food security crop by resource-poor farmers who live in marginal lands in Ethiopia (Arslan et al. 2020).

Despite the possession of economically important traits, grass pea has the disadvantage of producing toxic and anti-nutritional compound referred to as β-N-oxalyl-L-α, β di-amino propionic acid (β-ODAP) also called β-N-oxalyl amino-L-alanine (BOAA), a neurotoxin that causes leg paralysis in humans including animals (Arslan et al. 2020; Lambein et al. 2019; Gupta et al. 2018; Hillocks and Maruthi 2012). To date, very limited research attention has been given thereto for improving this very essential legume plant. The fundamental reason for this omitting is the

presence of β-ODAP. Thus, it is among many African orphan crops with little scientific information available thereon, particularly at the molecular level (Tadesse and Bekele 2003a, b).

Crop characterization based on morphological features is a straightforward, affordable, and simpler method of knowing the potential of the crops under the study. Nevertheless, these features are extremely susceptible to the environment and cultural patterns. Hence, the use of molecular markers offers immense information and they are predominantly efficient and invulnerable to climate crisis unlike agro-morphological characters (Arslan et al. 2020). Thereby, to get a substantial advance in the breeding program and germplasm management of the crop, the use of molecular markers and genomic knowledge is a precondition (Akter et al. 2015; Tadesse and Bekele 2003a, b). Molecular markers that are previously been exploited efficiently for genomic studies in *L. sativus* comprises random amplification of polymorphic DNA (RAPD) (Barik et al. 2007; Croft et al. 1999), restriction fragment length polymorphism (RFLP) (Chtourou-Ghorbel et al. 2001), amplified fragment length polymorphism (AFLP) (Tavoletti and Iommarini, 2007), inter-simple sequence repeat (ISSR) (Uysal et al. 2018; Belaid et al. 2006). Recently, microsatellite markers /SSRs were mostly picked by breeders based on their polymorphic features and co-dominant aspect, along with several alleles per locus and plentiful in spreading across the genome (Gupta et al. 2018). Furthermore, it's valuable for genetic variation analysis (Arslan et al. 2020). However, there exist limited study on genetic diversity study of Ethiopian grass pea genotypes using SSR markers (Arslan et al. 2020; Soren et al. 2020, 2015; Wang et al. 2015; yang et al. 2014; Shiferaw et al. 2012; Lioi et al. 2011). For this study, we applied 10 polymorphic SSR markers selected from Wang et al. (2015) to evaluate the genetic diversity and population structure of 25 North-West Ethiopian-originated grass pea accessions.

Materials and methods

Plant materials and DNA extraction

The study was carried out using 25 grass pea accessions collected from the Ethiopian Biodiversity Institute (EBI), Ethiopia. These accessions mainly originated from four major grass pea growing administrative zones of the Amhara region; East Gojjam (seven accessions), South Gondar (six accessions), North Gondar (six accessions), and West Gojjam (six accessions). The detailed passport data of the accessions are presented in Table 1. Genomic DNA was isolated from the young leaves of three weeks old plants grown in plastic bags at Ebonyi State University, College of Agricultural Science (EBSU, CAS) campus, Abakaliki, Nigeria. The genomic DNA was extracted using Quick-DNA^{TM} ZR Plant/Seed Miniprep Kit (Zymo Research, California, USA) following the manufacturer's protocols. DNA quality and quantity were assessed using a nano-drop spectrophotometer (Thermo Fisher Scientific Inc, USA), and agarose gel electrophoresis.

SSR primers screening

Ten SSR primers were selected based on polymorphic information content (PIC) values ranging from 0.4107 to 0.7292. They were picked out from the list of SSR markers developed for *L. sativus* and related species by Yang et al (2014) and later used by Wang et al (2015). Details of the primers including primers name, sequence, annealing temperature, product sizes, number of alleles, and major allele frequencies are listed in Table 2.

Polymerase chain reaction (PCR)

PCR amplification was done in a total reaction volume of 25 µL comprising 2.5 µL of 10 × buffer, 1µL of 50 mM $MgCl_2$, 2 µL of 2.5 mM dNTP mix (Bioline, USA), 1 µL of 200 nM each of forward and reverse primers, 20 ng μL^{-1} of genomic DNA, 0.1 µL of five units of Taq DNA polymerase (Bioline), and made up to volume with nuclease-free water. The amplification was carried out in a Thermal Cycler machine (Applied Biosystem, ABI Veriti 96 well Thermal Cycler, Singapore) in IITA, Bioscience Lab, Ibadan, Nigeria; under the following reaction conditions: initial denaturation at 95 °C for 4 min followed by 35 cycles of 95 °C for 20 s, annealing at respective annealing temperatures (Ta) of each primer for 30 s, elongation at 72 °C for 30 s and final elongation at 72 °C for 5 min. The amplified products were resolved by gel electrophoresis using 2% SFR agarose gel and the

Table 1 Passport data of grass pea accessions used for the study

No.	Accessions	Zone	District	Latitude	Longitude	Altitude
1	238,935	South_Gondar	Fogera	11°-55′-24″-N	37°-54′-21″-E	2130.00
2	236,702	South_Gondar	Dera	-	-	1800.00
3	212,741	South_Gondar	Kemekem	12°-06′-00″-N	37°-42′-00″-E	2000.00
4	226,014	South_Gondar	Este	11°-27′-00″-N	37°-59″-00′-E	2645.00
5	238,931	South_Gondar	Fogera	11°-57′-45″-N	37°-43′-35″-E	1920.00
6	226,011	South_Gondar	Este	11°-03′-00″-N	38°-09′-00″-E	2520.00
7	226,017	North_Gondar	Dembia	12°-30′-00″-N	37°-24′-00″-E	1905.00
8	226,018	North_Gondar	Gondar zuria	12°-31′-00″-N	37°-20′-00″-E	1990.00
9	236,708	North_Gondar	Dabat	-	-	2730.00
10	238,928	North_Gondar	Gondar zuria	12°-24′-01″-N	37°-33′-09″-E	1990.00
11	242,216	North_Gondar	Gondar zuria	12°-30′-00″-N	37°-32′-00″-E	1975.00
12	46,107	North_Gondar	Gondar zuria	12°-37′-00″-N	37°-10′-00″-E	1950.00
13	236,697	West_Gojjam	Adet	-	-	2260.00
14	236,712	West_Gojjam	Jabi Tehnan	-	-	1820.00
15	238,947	West_Gojjam	Dega Damot	10°-38′-55″-N	37°-23′-58″-E	1900.00
16	238,920	West_Gojjam	Merawi	11°-25′-09″-N	37°-09′-54″-E	2050.00
17	238,942	West_Gojjam	Achefer	11°-44′-33″-N	36°-59′-06″-E	2050.00
18	238,945	West_Gojjam	Achefer	11°-48′-02″-N	36°-59′-59″-E	2030.00
19	24,812	East_Gojjam	Hulet Ej Enese	11°-05′-45″-N	37°-52′-28″-E	2452.00
20	30,357	East_Gojjam	Enarj Enawaga	10°-40′-57″-N	38°-11′-13″-E	2517.00
21	238,908	East_Gojjam	Dejen	09°-57′-28″-N	38°-18′-26″-E	2450.00
22	26,626	East_Gojjam	Debay Telatgen	10°-25′-26″-N	38°-07′-33″-E	2573.00
23	26,627	East_Gojjam	Baso Liben	10°-04′-44″-N	37°-44′-54″-E	2350.00
24	238,910	East_Gojjam	Enemay	10°-21′-29″-N	38°-09′-36″-E	2440.00
25	26,633	East_Gojjam	Awabel	10°-14′-14″-N	38°-03′-24″-E	2439.00

sizes of the amplicon were estimated with a 50 bp DNA ladder (Bioline). The gel photos were captured using a gel documentation system and the bands were scored as binary data ('0' for absence and '1' for the presence of specific bands).

Allelic diversity and population differentiation analysis

Genetic diversity was assessed using the allelic binary data set obtained from the eight polymorphic SSR markers. The values of gene diversity, polymorphic information content (PIC), expected heterozygosity and a major allele frequency per locus were estimated using Power Marker software v. 3.25 (Liu and Muse 2005). The Shannon information indices, the number of different alleles, and the number of effective alleles were determined with GenAlEx 6.5.1b2 software (Peakall and Smouse 2006). Population differentiation analysis was done using Analysis of Molecular Variance (AMOVA) and Principal Coordinate Analysis (PCoA) procedures with GenAlEx 6.5.1b2 software (Peakall and Smouse 2006). In this analysis, Gondar and Gojjam were considered as regions, whereas, zones were assigned as populations within regions.

Genetic structure and cluster analysis

To decipher the genetic structure of the grass pea populations, STRUCTURE software V. 2.3.4 Falush et al. (2003) and Pritchard et al. (2000) were used. The analysis was based on the ancestors model of 100,000 iterations and 300,000 Markov Chain Monte Carlo/MCMC/burn-in, using the admixture model and allele frequency correlated model with 10 independent runs (k = 1 to10). Evanno et al. (2005) technique was applied to identify the number of runs with the highest

Table 2 Details of the SSR primers used to determine genetic diversity in the North-West Ethiopia grass pea population with the annealing temperature (Ta), product sizes, number of alleles, and major allele frequencies

Primer name	Repeat Motif	Forward primer sequence(5′-3′)	Reverse primer sequence(3′-5′)	Ta^{0} C	Product size (bp)	No of Alleles	Major allele frequency
G9	(AAC)6	CAACCAGAGCAACCACAAGA	GGTTGCAAGAGGTTGCAGAT	55	170–300	2	0.68
G17	(AAT)5	CAGGTCCGGCTTATCTCTCA	TTGGTTTCAACCCACTCCTC	56	150–750	21	0.12
G68	(AC)9	GCACACAAGGGCACACTG	TGCGTCGTGTGTATGTGTTG	52	250	2	0.52
G157	(CAA)6	ACATCCAATCCCCACCATAA	AATGCATGGTTGTTGCTTGA	52	170–200	2	0.72
G245	(TG)6	CGTTGGTTGTTAGTCGGTCA	GAACGAAACAACGACGACAA	52	260–400	5	0.32
G15771	(TCG)5	AGTGCCTGATGGGAGTCAGT	CCGACGACGACGACTACTAA	56	200–300	2	0.64
G17922	(CCA)5	CACCACCATAACCACCTCCT	ATGCGATTGAAGGGATGAAC	55	140–370	5	0.44
G18109	(CGA)5	GACAGACACACGGCAAACAC	ACGTCGTCGTGTCGTTGTT	52	–	–	–
G19207	(AAG)5	ATCGTAAACCGTGAGGGTCA	AAGCTTGTGGTGGCTACTGC	56	200	2	0.96
G19337	(ACA)5	CGACAACACATACAGCAACAC	TGTTGTTCGTTGTTGTTAGTTAGTT	52	–	–	–

delta k values. For this, a web-based program, STRUCTURE HARVESTER ver 0.6.94 was applied to determine the ideal number of subgroups (Earl 2012). Afterward, the Clustering Markov Packager-CLUMPAK program was used to sum, graphically, and illustrate the results obtained by STRUCTURE software (Kopelman et al. 2015). For this function, formatted Q-matrices data and population data labels were used to separate individual accessions based on their membership coefficients generated from the 10 independent runs. The software assigned each accession to a particular subgroup based on its membership coefficients (Q). The membership coefficient threshold to assign grass pea individuals to a specific subgroup was 0.9. Thus, accessions with membership coefficients greater than 0.9 were allotted to particular subgroups, whereas, those with lower values than the limit were considered as genetically admixed accessions. Cluster analysis was computed with NTSYSpc Software v 2.02e (Rohlf, 1997) using the Jaccard's similarity matrix with the option of Sequential Agglomerative Hierarchic and Non-overlapping (SAHN) technique using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

Spatial genetic structure

The spatial autocorrelation investigation was conducted using genetic similarity matrix data and a geographic distance matrix generated from the coordinates of accessions. The investigation was done utilizing the "spatial" option in GenAlEx ver.6.51b2 software (Peakall and Smouse 2006). The significance of the spatial autocorrelation and heterogeneity was determined by a 95% confidence interval around the null hypothesis (r = 0). The estimation was executed with a preference of an even distance class of 20 km, and permutations of 9999, and a bootstrap of 1000.

Results

Genetic diversity estimated with SSR markers

Among the total of 10 SSR markers tested, eight amplified scorable bands with a fragment size ranging from 150 to 750 bp and PIC values from 0.074 to 0.941. These eight markers detected a total of 41 alleles in the 25 grass pea accessions ranging from two

alleles in marker G9 to 21 in G17 with an average of 5.13 alleles per locus. Gene diversity values varied from 0.077 in marker G19207 to 0.944 in G17 with an average value of 0.536 and the heterozygosity values ranged from 0.121 to 0.395 with an average of 0.326 per locus. The major allelic frequency ranged from 0.12 to 0.96 with an average of 0.55 per locus (Table 2 and Fig. 1).

Diversity among grass pea populations from different geographic zones of north-west Ethiopia

To measure diversity among the population from the regions, data of accessions collected from different geographic zones were pooled together (Fig. 2). The number of distinct alleles and effective alleles per locus ranged from 1.53 to 1.90 and 1.48 to 1.70 with an overall mean of 1.78 and 1.58, respectively. The smallest Shannon's diversity index was observed in East Gojjam (0.370), while the highest value was observed in West Gojjam (0.553) with an overall mean of 0.474. East and West Gojjam again followed the same pattern for expected heterozygosity with the lowest value of (0.259) in East Gojjam and highest (0.384) in West Gojjam and an overall mean value of 0.326. The largest percentage of polymorphism was observed in accessions from North Gondar and West Gojjam zones (89.47%), while the lowest percentage polymorphism was observed in accessions from East Gojjam (57.89%).

Genetic differentiation between population

Analysis of Molecular Variance (AMOVA) showed a highly significant difference ($P < 0.001$) among accessions between regions. The percentage of genetic variation among regions was 20% of the total genetic variation. However, contributions of zones within regions to the total variance were not significant (4%). Moreover, the largest genetic variance (76%) was detected among individuals within populations (Table 3). The obtained value of Phi-statistics (Φpt analogous to Fst) between regions was 0.20, and the value with the populations was 0.24. Whereas, the value of Φpt among populations within regions i.e. between adjacent zones (South and North Gondar) and between (East and West Gojjam) showed a low level of genetic differentiation (0.053) as indicated in Table 4. There was highly significant pairwise genetic differentiation ($P < 0.01$), the largest value was observed between populations of East Gojjam and North Gondar (0.365), followed by East Gojjam and South Gondar (0.231), and then North Gondar and West Gojjam (0.210) zones. While the lowest and non-significant pairwise genetic differentiation values were observed between populations of South Gondar and North Gondar zones (0.053) and between the population of West Gojjam and East Gojjam zones (0.072) (Table5).

Classification and principal coordinate analysis among the accessions

The genetic relationship among individual accessions according to geographical origin was inspected based on principal coordinate analysis using the genetic distance matrix. The two-dimensional biplot grouped all the accessions from East Gojjam together with only three out of six accessions from West Gojjam, while all accessions from North Gondar, 83.33% of accessions from South Gondar, and half of the accessions from West Gojjam were grouped. The contribution of the first and the second principal coordinates to the total genetic variation was 45.97% and 12.12, respectively (Fig. 3). The overall genetic variation explained by the three dimensions was 66.31%.

Population structure analysis

The genetic structure analysis of the 25 north-west Ethiopian grass pea accessions was determined by the highest ΔK values revealed at $K = 2$ (Fig. 4). The membership coefficient (≥ 0.9) was used to assign individuals to their respective subgroups. Based on this value, 52% of the accessions were assigned to subgroup one, while 24% of the accessions were assigned to subgroup two. The remaining 24% of the accessions (Acc 212,741, Acc 226,011, Acc 238,928, Acc 46,107, Acc 238,920, and Acc 238,942) were considered as admixed given the ordinarily shared ancestors with individuals allocated to the detected subgroups. The arrangement of every individual was represented by two different colors. The blue color indicates subgroup one, and the orange color indicates subgroup two (Figs. 5 and 6). Figure 6 clarifies the genetic assignment of each grass pea accession represented by a single line into two subgroups (blue

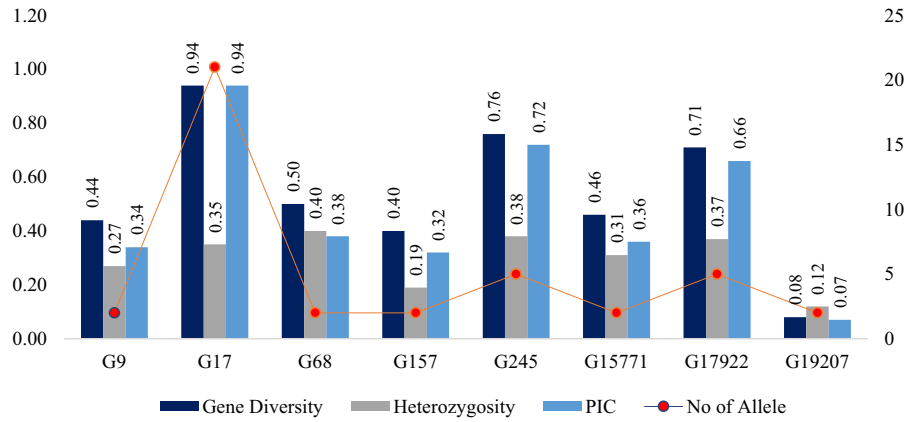

Fig. 1 Genetic diversity parameters were generated from 25 North-West Ethiopian grass pea accessions using eight SSR markers

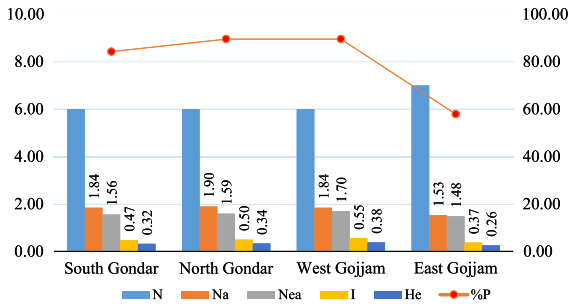

Fig. 2 The diversity of grass pea populations was obtained from the analysis of eight SSR loci based on geographic regions. Where: N = population size, Na = number of different alleles, N_{ea} = number of effective alleles, I = Shannon's information index, He = expected heterozygosity and % P = percentage of polymorphic loci in each population

and orange color) inferred from structure analysis at k = 2 based on SSR polymorphism.

Cluster analysis

Cluster analysis classified the accessions into two distinct groups using the Jaccard genetic similarity matrix. Cluster I and II consisted of 18 accessions and seven accessions, respectively (Fig. 7). The threshold value of the similarity coefficient determining the number of clusters was 70%. The clustering pattern similarly revealed that accessions from various geographic regions were grouped in the same cluster and vice-versa. Accessions from North Gondar and South Gondar showed more genetic diversity within zones, whereas accessions from East and West Gojjam showed less genetic diversity within zones.

Spatial autocorrelation analysis

The spatial autocorrelation result revealed a significant correlation between a geographic distance and genetic similarity matrices in North-Western Ethiopian grass pea accessions collected in the range of 30 km (Fig. 8). The grass pea accessions which were collected at a distance within 30 km were shown to have spatial autocorrelation. It was observed that as the geographic distance increased beyond 30 km, the associations between the genetic similarity and geographic distance began to diminish. Generally, the correlation between

Table 3 Summary of AMOVA partitioning of North-West Ethiopian grass pea populations based on regions

Source variation	df	SS	MS	Est. Var	%
Among regions	1	16.38	16.38	0.93	20
Among populations within regions	2	9.48	4.74	0.20	4
Within population	21	73.74	3.51	3.51	76
Total	24	99.60		4.64	100

df = degree of freedom, SS = sum of squares, MS = mean squares, Est. var. = estimate of variance, % = percentage of total variation based on 999 permutations

Table 4 Phi statistics values of grass pea accessions from different regions in North-West Ethiopia

Phi Statistic	Value	P rand ≥ data
PhiRT	0.20	0.001
PhiPR	0.05	0.099
PhiPT	0.24	0.001

the geographic distance and genetic similarity matrix increased positively until it reached (around 102.11 km) but a further increase in the sampling distance resulted in a decrease in the Mantel correlation coefficient. The result revealed the maximum geographic distance for close genetic similarity among the North-Western Ethiopian grass pea accessions as 30 km.

Discussion

Grass pea is the most widely cultivated legume by the resource-poor farmers living in marginal areas of Ethiopia. Despite the presence of neurotoxin β-ODAP content in its seeds and foliage that is known to cause leg paralysis in animals including humans, grass pea is becoming part of the main diet replacing other legumes like chickpea and field pea. The cultivation of the crop is favored in the drought-prone and less fertile areas due to the failure of the other cereals and pulses production under these conditions. This is attributed to the nature of the grass pea which is known to withstand and yield well under harsh environmental conditions. Furthermore, the rich protein content (28–32%), and the minerals add value to the crop that favors wide cultivation in Ethiopia. Due to this reason, the crop is considered as one of the alternative protein sources to reduce malnutrition and food insecurity of the ever-growing population of the country (Soren et al. 2015; Urga et al. 2005).

The impact of climate change is gradually affecting the existing crop production system and scientists are looking for climate-resilient crops. Grass pea is one of the crops that have the natural ability to maintain a

Table 5 Pairwise genetic differentiation (PhiPT) below diagonal values between geographic origins of North-West Ethiopian grass pea populations

	South Gondar	North Gondar	West Gojjam	East Gojjam
South Gondar	0.000			
North Gondar	0.033	0.000		
West Gojjam	0.137*	0.210**	0.000	
East Gojjam	0.231**	0.365**	0.072	0.000

**Indicates high significance at ($P < 0.01$) and *indicates significance at ($P < 0.05$) P-value based on 999 permutations

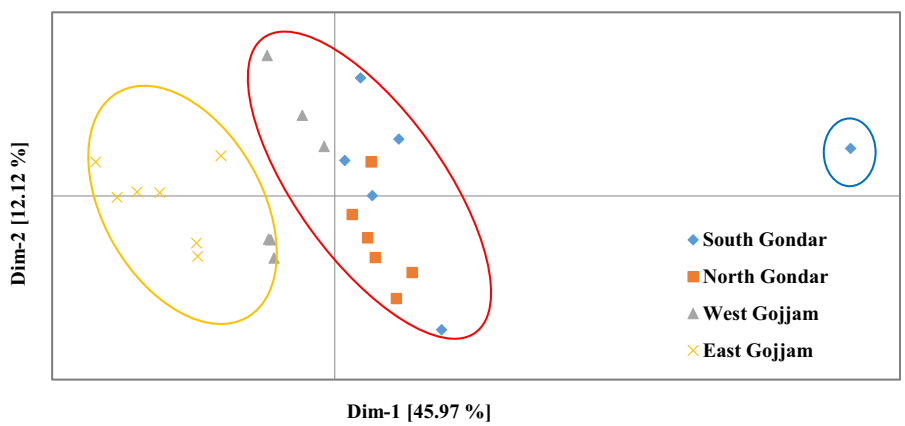

Fig. 3 Two-dimensional principal coordinate analysis [PCoA] of North-West Ethiopian grass pea population-based on SSR polymorphism

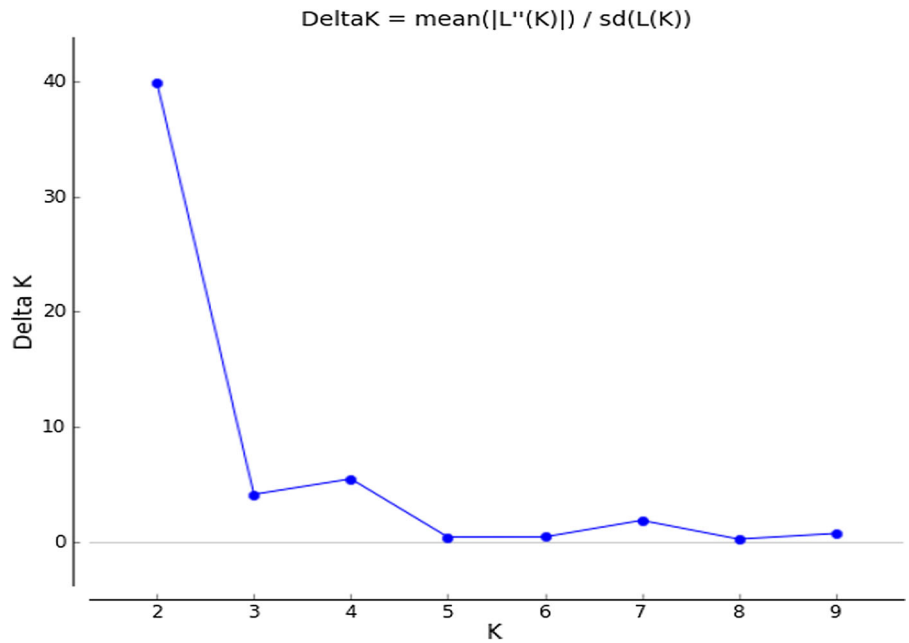

Fig. 4 Population genetic structure estimation of North-West Ethiopian grass pea accessions, the number of subgroups determined by the highest delta K value at k ranging from 1 to 10 using SSR markers data

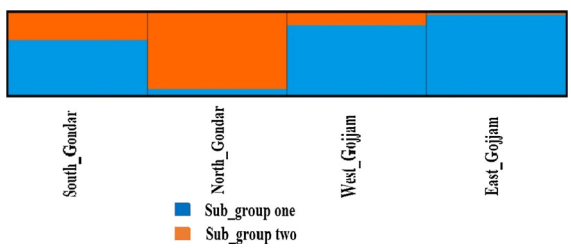

Fig. 5 Genetic assignment of North-West Ethiopian grass pea populations inferred from structure analysis at k = 2 based on SSR polymorphism. The analysis assigned the populations into two subgroups (1st = blue color and 2nd = orange color)

high yield under harsh environmental conditions and as result, its production is increasing. However, there is still a need to improve the crop for better yield and increased tolerance to biotic and abiotic stresses in the face of increasing climate change. To achieve this, understanding the genetic variations and relationships within and among grass pea populations in Ethiopia is a prerequisite. As a result, we applied previously developed SSR markers for *L. sativus* and its relatives were used to assess the diversity and population structure analysis of the North-Western part of Ethiopian grass pea populations (Yang et al. 2014; Wang et al. 2015).

The average number of alleles per locus among the studied accessions was 5.13. This is lower than the result obtained by Wang et al. (2015) who reported an average number of alleles per locus of 8.6. However, these authors used more markers (30 polymorphic SSR markers) unlike the eight SSR markers used in this study. In addition, the authors combined different species of *Lathyrus* presumably introducing more polymorphic alleles than the single *L. sativus* species we have studied.

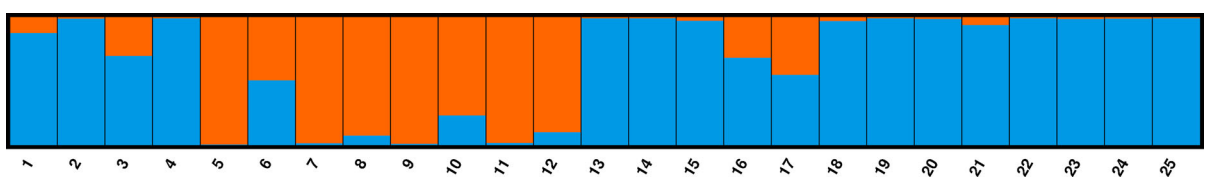

Fig. 6 Genetic assignment of each grass pea accession represented by a single line into two subgroups (blue and orange color) as inferred from structure analysis at k = 2 based on SSR polymorphism

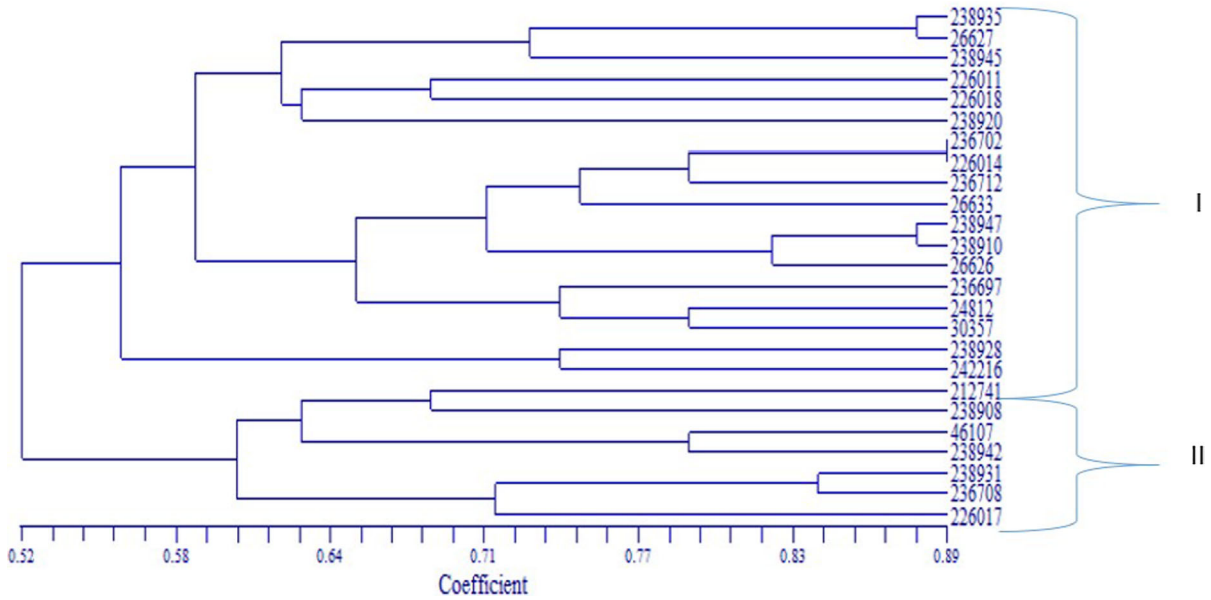

Fig. 7 A dendrogram of North-West Ethiopian grass pea accessions constructed by the UPGMA method based on SSR polymorphism using the Jaccard genetic similarity matrix data. The dendrogram clustered the 25 grass pea accessions in two major groups

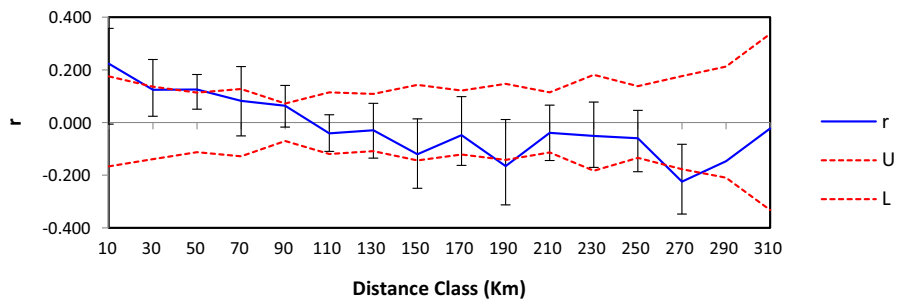

Fig. 8 Spatial correlation between geographic distance and genetic similarity matrices for North-West Ethiopian grass pea accessions. The broken lines indicate the 95% confidence interval for the Null hypothesis of random distribution, the solid line in the center indicates the correlation coefficient (Mantel r), and the whiskers indicating the magnitude of error after bootstrapping 1000

The polymorphism among the studied accessions based on the eight SSR loci yielded major allele frequencies ranging from 0.12 to 0.96 with a mean value of 0.55. A related result has been reported by Shiferaw et al. (2012) who studied the genetic diversity of the Ethiopian grass pea based on 11 EST-SSR markers and detected major allele frequency ranging from 0.29 to 0.88 with a mean of 0.62. Moreover, close results have been reported by Soren et al. (2015) who observed major allele frequency in the range of 0.39 to 0.97 with an average of 0.63 from 176 Indian grass pea accessions using 19 EST-SSR analysis markers.

In the present study, gene diversity, and PIC values ranged from 0.074 to 0.944 with a mean gene diversity of 0.536 and a mean PIC value of 0.474. A similar result has been described by Wang et al. (2015) who evaluated the genetic diversity of grass pea and its related species using 30 SSR markers and as high as 266 accessions from Europe, Asia, and African including 17 related species. These authors reported gene diversity and PIC values in the range of 0.0688–0.8505 with a mean gene diversity of 0.534 and a mean PIC value of 0.4817. Furthermore, our result also agreed with the work of Soren et al. (2015) and Shiferaw et al. (2012) on gene diversity values.

These authors respectively applied 19 EST-SSR and 11 EST-SSR markers on grass pea accessions and detected gene diversity values ranging from 0.04 to 0.73 with a mean of 0.45 and 0.205 to 0.805 with a mean of 0.477.

Our study revealed expected heterozygosity values that ranged from 0.259 to 0.384 with a mean of 0.326. These values are low compared to the result (0.23–0.50 and a mean of 0.42) obtained by Soren et al. (2015) using 19 polymorphic EST-SSR primers. The result was also slightly lower than the values obtained by Shiferaw et al. (2012) who have investigated 11 polymorphic EST-SSR primers to evaluate the expected heterozygosity of 20 Ethiopian grass pea accessions and obtained 0.354 to 0.478 with a mean of 0.430. These observed differences may be attributed to the used of grass pea accessions from close geographical regions, different genotype, different primers type and number of accissions we used.

Shannon's information index detected in the present study ranged from 0.370 to 0.553 with a mean value of 0.474. The detected values are close to the values (0.42–0.69 with a mean of 0.61) reported by Soren et al. (2015) with 19 EST-SSR primers in the population of 176 Indian grass pea accessions. However, the result was very low as compared to the values (0.595–0.855 with a mean of 0.760) reported by Shiferaw et al. (2012) who evaluated 20 grass pea accessions from seven regions of Ethiopia (Shewa, Wollo, Gojjam, Gondar, Tigray, Arsi, and Hararge) using 11 EST-SSR primers. The wider geographical location covered in their study and the type of primer may have contributed to the observed variation in the reported Shannon's information index between the current and the above reports.

The present study was relatively in a close population that would be important in germplasm management and breeding program. Piloting a close study on a particular population could be desirable when conducting genetic improvement in grass pea. The study revealed the existence of a moderate level of genetic diversity among the North-Western Ethiopian grass pea populations. The highest level of genetic differentiation was observed among the grass pea accessions within populations, suggesting that geographic regions were not the major contributor of the observed genetic variations in North-West Ethiopian grass pea accessions. A report based on phenotypic characterization of Ethiopian grass pea showed the genetic variation was distributed across regions (Taddese and Bekele 2003a). Shiferaw et al. (2012) who worked on the genetic diversity of Ethiopian grass pea using EST-SSR markers showed an equivalent level of genetic variation among their studied regions but their study area covered more distant regions than the present study. This finding and those of the above two reports indicated geneflow among grass pea accessions across the regions and zones. The presence of low genetic differentiation among adjacent zones implies high seed exchange among farmers as the towns and villages in zones are closer than the two regions. However, gene flow from where to where is still unknown identifying origin of the gene flow needs further studies. In this study, the Analysis of Molecular Variance (AMOVA) revealed a high significant genetic difference among North-Western Ethiopian grass pea accessions between regions, among populations, and within populations. This finding is comparable to an earlier report published by (Shiferaw et al. 2012). A similar pattern of genetic variations based on AMOVA has been reported by Wang et al. (2015) for a larger population of 266 grass pea accessions, and 17 relative species from a very wide geographical origin including Europe, Asia, and Africa, using as many as 30 SSR markers.

Based on Phi statistic (Φpt) values the detected levels of genetic differentiation among individual accessions (0.24) and between the two regions (0.20) were found to be moderate according to Wright (1978) categorization. Shiferaw et al. (2012) have earlier reported a closely related value (0.15) which also falls within the moderate level of genetic differentiation. The study also revealed low-level genetic differentiation (Φpt) (0.05) among the population within regions. Therefore, when the geographic distance between zones decreases, the genetic differentiation becomes lower. Similarly, the Pairwise Genetic Differentiation (PhiPT) analysis result also supports that increase in geographic distant increases the level of genetic differentiation among the grass pea accessions while a decrease in geographic distance correspondingly decreases genetic differentiation.

The discrimination of the 25 North-Western Ethiopian grass pea accessions based on Principal Coordinate Analysis to great extent reflected the influence of geographic proximity on the genetic pattern. In the biplot graph grass pea accessions were collected from four geographic zones grouped into

two distinct clusters leaving one singleton. Similarly, the presence of high genetic differentiation between these two groups indicated there is significant gene flow among adjacent zones due to their geographic zones. The level of genetic variation explained by the first principal coordinate was 45.95% of the variation. This is close to the values reported by Wang et al. (2015) for 266 grass pea accessions collected from Europe, Asia, and Africa and characterized with 30 SSR primers. The level of genetic variation explained by the first principal coordinate according to this author was 43.42. The related results reported by Shiferaw et al. (2012) who have studied the genetic diversity through 11 polymorphic EST-SSR markers for 20 grass pea accessions originated in Ethiopia from seven regions of the study areas. It was grouped into three clusters so that, Cluster I consists of accessions from Tigray, Gojjam Gondar, and Wollo. Cluster II contained accessions from all regions while cluster III was mainly composed of Shewa and Gojjam and some accessions were categorized in this cluster from Gondar and Wollo. This showed that a gene flow took place across all regions. Tadesse and Bekele (2003a, b) have similarly reported clustering 50 Ethiopian grass pea accessions from different regions (Gondar and Gojjam), and (Tigray and Wollega) into the same subgroup suggesting possible gene flow in the Ethiopian grass pea populations across regions.

Understanding the distribution of genetic variation among geographic regions would be important for the grass pea improvement program and planning germplasm collection and conservation. Genetic structure analysis using molecular markers plays a crucial role in choosing raw materials for breeding experiments, germplasm maintenance, and provision. The identification of genetically structured groups provides distinct, stable, and accurate parental and breeding lines through membership coefficients. In this study, population genetic structure analysis revealed two distinct subgroups. Based on the membership coefficient ($Q \geq 0.9$) 76% of the accessions were assigned to distinct subgroups. This indicates that there is a level of high gene diversity between the two subgroups. These grouping could be vital for future grass pea breeding experiments when crossing accessions from subgroup one with subgroup two which might give a heterotic effect. Soren et al. (2020) successfully assorted 50 diverse geographic grass pea accessions using 60 polymorphic SSR markers data set. Additionally, Aci et al. (2020) successfully distinguished a mixture of *L. sativus* and its relatives (*L. cicera* and *L. ochrus*) based on genetic structure analysis using five polymorphic SSR markers data set. Whereas, structure result in this finding, demonstrated that both subgroups contained accessions collected from all the zones in different proportions indicating the absence of region-based genetic structuring of the grass pea population in the study areas. Some of the accessions were admixed indicating 24% of the accession's ancestors comes from both subgroups and they share some part of the genome. Thus, the population genetic structure analysis and clustering pattern in the present study indicated the presence of gene flow between adjacent zones due to the exchange of seeds among farmers allowing each zone to show an equivalent level of variations. The germplasm exchange among farmers is a basis for increasing the diversity of the local population which might affect the spreading of alleles among various populations regardless of their geographical distance (Louette et al. 1997).

The spatial autocorrelation analysis revealed how far the North-Western Ethiopian grass pea accessions are separated regarding the geographical distance. The result showed that beyond 30 km the genetic similarity associated with geographical distance begins to drop. This indicates that the farmers presumably go as far as 30 km to fetch seeds from the market or through germplasm exchange with neighboring villages. This result could be used as a potential indicator of the distance perimeter to cover during germplasm collection in the study areas. This might help to avoid the risk of collecting genetically similar accessions within a short distance.

Conclusion

This study confirmed that the microsatellite/SSR markers previously developed by Yang et al. (2014) and evaluated by Wang et al. (2015) for *L. sativus* and related species are vital tools for genetic diversity study in grass pea. The result showed the existence of a moderate level of genetic diversity among the North-Western part of Ethiopian grass pea populations which largely resides among the accessions. It also revealed the occurrence of gene flow among grass pea accessions across the geographical location of North-

Western Ethiopia. Although the present investigation evaluated a limited number of grass pea accessions with few SSR primers, the finding can provide valuable information on how geographic regions shape genetic diversity and how to exploit this information for future conservation, in situ maintenance, and breeding programs for grass pea.

Acknowledgements Special thanks to Ebonyi State University (EBSU), Faculty of Science and Department of Biotechnology, Abakaliki, Nigeria, and GENEs Intra-Africa Mobility Project EACEA/2017/2552 with Ref number EBSU/2018/GENES/MSc/ SCH-M/03 for hosting and sponsoring this research work. I appreciate Ethiopian Biodiversity Institute (EBI) for providing grass pea accessions. My superior acknowledgments go to Dr. Melaku Gedil of IITA Bioscience Lab, for his technical assistance, and for affording consumable chemicals used for the molecular part of the study throughout the Lab work.

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