

## Estimating the Genetic Diversity of Ethiopian Noug (*Guizotia abyssinica* (L.f.) Cass.) Genotypes using SSR Markers

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### Abstract

Noug is an underutilized oilseed crop mainly cultivated in Ethiopia and India. Genetic diversity analyses among 100 noug genotypes were carried out using 14 simple sequence repeat markers to assess the diversity status among the genotypes. Analysis of molecular variance revealed that 70% of the total variation was observed among individuals within populations. The inbreeding coefficient within subpopulations value among the studied noug populations was recorded 0.04. The total number of alleles amplified was 135 with an average 9.6 alleles per locus, suggesting that simple sequence repeats are a powerful technique for assessment of genetic diversity. The mean value of major allele frequency and number of effective alleles was 0.31 and 3.33 respectively. Polymorphic information content ranged from 0.62 to 0.86 with a mean value of 0.78. The highest value was observed at the locus GA077. Similarly, the allelic diversity was very high with the mean value of observed and expected heterozygosity 0.02 and 0.63 respectively. The mean value of Shannon's information index and gene flow was 1.22 and 1.05 respectively. Accessions collected from Gojam showed the highest numbers of private alleles (1.14). The study identified Gonder, Shewa and Gojam as hot spot regions for genetic diversity of noug. Therefore, attention should be given to these areas in any noug improvement and conservation programs.

**Keywords:** Accessions; Analysis of molecular variance; Genetic diversity; Noug; Population; Simple sequence repeats

### Introduction

Noug is the most popular and indigenous oil seed crop of Ethiopia is grown for its edible oil and seed. It is characterized by creeping growth habit, yellow flowers, strictly out-crossing and self-incompatibility [1]. In Ethiopia it is mostly grown by small holder farmers. Central Statistics Agency reported over 0.95 million households cultivated on 0.29 million ha of land and it is the second widely grown oil seed next to sesame [2]. Ethiopian farmers prefer to grow noug because of the capability of the crop to grow and giving a good seed yield under low soil fertility, moister stress and poor management practices. In addition, noug tolerate crop pests and suitable for soil conservation and rehabilitation [3].

Despite the crop has originated in Ethiopia and the major oil seed widely growing in different parts of the country; it remained among the poor yielder crops. Its cultivation is plagued with the indeterminate growth habit, seed shattering, self-incompatibility, genetically low yielding characteristics, lodging, less or low response for management inputs, the difficulty of pollination behavior, disease, insect and parasitic weeds [4]. The availability of little genetic information and semi domesticated nature of the crop has also an obstacle for further improvement programs [5]. The information regarding the genetic diversity of the crop is still at infant stage compared to other staple crops. In order to combat production problems through breeding understanding the diversity states of the crop is crucial. Genetic variation within crop species is a fundamental resource, which has

been utilized in breeding programmes for the improvement of every crop. Exploration of genetic diversity in noug germplasms can open the door for improvement and provide information for the scientific community.

Characterization of germplasms is a prime concern to reduce the effort and expense spent on the identification of suitable parent lines and boost genetic improvement. Therefore, a better understanding of genetic variability within and between populations is important for further breeding and conservation programs [6]. To estimate the genetic diversity and relationship of genotypes a variety of molecular techniques has been developed. Different types of DNA-based molecular markers have been used for genetic analyses in noug, including RAPD [7,8], AFLP [7], ISSR [9,10] and SSR [5,11]. The SSRs are versatile and the marker of choice in molecular diversity study as they are mostly co-dominant markers, highly polymorphic, reproducible, greater distribution and abundance in the genomes, exchangeable between laboratories and are highly transferable between populations [12]. In noug a few hundred SSR markers have been developed and used for population genetic analyses [5,11,13]. Hence noug considering as orphan or neglected crop and only limited information is generated by the scientific community.

There are more than 1100 noug accessions at the Ethiopian Institute of Biodiversity which constitute a valuable resource in noug breeding. However, only few of these accessions have been properly characterized for their genetic diversity using modern molecular markers. The estimation of genetic diversity and characterization of noug accessions is usually done on the bases of phenotypic data; however, cannot provide sufficient information for more efficient exploitation of breeding material. To boost the economic benefit of the

farmers there is an urgent need to breed high yielding, high oil content and good agronomic performing varieties. Characterization of available gene pools is the primary step for developing resilient varieties. Hence, molecular characterization of germplasms collections ought to be a prerequisite to design suitable noug breeding program and conservation strategy. Therefore, the aim of the present study was to assess the genetic diversity among noug genotypes, which were collected from major growing regions of Ethiopia using SSR marker in order to generate information on the status of genetic variation for further improvement and conservation program.

## Materials and Methods

### Plant material and growth conditions

A total of 100 noug genotypes from Holeta Agricultural Research Center and Ethiopian Biodiversity Institute were used in this experiment. The DNA was extracted from 15-days old seedlings leaves using Edwards's protocol [14].

S No	SSR markers	Repeat motif	Forward primers	Revers primers	Expected size
1	GA013	CTT	GGTAATGGTAATGGAGGTTCTGG	CCTCATCAGAGTTCTTCGGGTTAT	424–455
2	GA018	AGC	GTTCCAGCCCATGAGTCATAAT	CTATCTCTATCTCGTGGGTTTTG	353–358
3	GA029	ATC and TC	CCATCATCAATGGCGTTACTC	GTCTCGTCTAGAAGCTTCATCCT	270–276
4	GA035	TGA	GATTTCTCAGGTGAAGGAGAAGAG	GCCCTCCCTACAACATACTTGATA	301–307
5	GA037	TA and GAA	GGTGTTTTGTGTAGTGGTCTGTC	GACTAGCCAGAAACCGAAGAATC	347–350
6	GA054	TA	AACGGTTTAGGAGACCTTGG	TCACCTGGCTCAGACTTGTTT	247–265
7	GA055	CT	CCTGAAACAAACCCCAACAA	CAGTACATCGCGGAGAGAGG	194–200
8	GA077	TC	TCAGCCAAACATTCCAAGC	AAACAACGCGCTAAAAACGA	487–490
9	GA081	TC	AATCTCGATTGGCTGAGTGG	AGGAAGTTGGGGCTTCGTAA	437–441
10	GA082	TC	TGTCCGTATGAAACCCATTGA	CAATGATCATGGGGACTGCT	197–197
11	GA117	CAC	CCCTTCATCCAATTCTAACGAC	AGGTCTAATCCCAGCCTCTCTAAT	336–339
12	GA127	CCT	CAATCTGCAACTACTGCCAATACC	CCAGTCAGAACCCTTGATCACTA	213–216
13	GA138	AAG	ATCAACTTCCCCATATACCTCTGG	CTTCTCTGTCACTTCTTTTGGAC	363–378
14	GA139	GAA	GTACATCCCAACTTTACCATCCAC	CTCTACAACCAACACCCTTTCC	223–241

**Table 1:** Description of the polymorphic microsatellite markers used in the analysis of noug genotypes.

The DNA quality and quantity were determined using the Nano Drop spectrophotometer (ND-8000 Thermo Fisher Scientific, USA) and visualized using 0.8% agarose gel and 1 × TAE buffer. Samples with high band intensity, lesser smear, purity with 1.8 to 2 were selected for further PCR analysis. A total of 14 EST derived SSR markers were used for genotyping the entire 100 noug genotypes [13] (Table 1).

### PCR Amplification

The PCR was performed with a total of 10 µl reaction volume containing 5 µl one Taq 2x Master Mix (M04821) with standard buffer, 1.5 µl genomic DNA with 20 ng/µl, 0.4 µl forward primer, 0.4 µl reverse primer and 2.7 µl nuclease-free water. The amplification was carried out in 96-well plates using Gene Amp<sup>®</sup> PCR system 9700 (Applied Biosystem, USA) with two touchdown PCR conditions. For primer GA018 and GA037 the touchdown PCR was programmed at 95°C initial denaturation for 2 minutes followed by 10 cycle at 94°C for 30 seconds, 60°C to 50°C for 45 seconds with 1°C decrease in every cycle and 72°C extensions for 90 seconds; followed by 26 cycles of 94°C for 45 seconds, 50°C annealing temperature for 60 seconds, 72°C for 90 seconds and a final extension for 20 minutes at 72°C.

For the rest 12 primers, the touchdown PCR amplification programmed at initial denaturation for 4 minutes at 94°C followed by 9 cycles at 94°C for 45 seconds, 60°C to 55°C for 60 seconds with 0.5°C decrease in each subsequent cycle at 72°C for 90 seconds. Then it was followed by 26 cycles of 94°C for 45 seconds, 55°C annealing temperature for 60 seconds, 72°C for 90 seconds and a final extension for 10 minutes at 72°C. The band separation was done by running the PCR products on 5% agarose gel for 3 hr at 90 constant voltages in 1% TBE along with BIONEER 25/100 base pair mixed DNA ladder. Amplified products were visualized using gel documentation 3UV trans-illuminator (Bio-Doc).

### Data scoring and analysis

The amplified products were scored based on fragment band size using PyElph gel images analyzer software V1.4 [15]. To estimate the population genetic structure and differentiation among and within genotypes based on their geographic origin the analysis of molecular variance (AMOVA) and genetic diversity parameters such as number of effective alleles per locus (Ne), Shannon information index (I), observed and expected heterozygosity, fixation index (F), gene flow (Nm) and percent polymorphism (% P) were analyzed using GenAlex

V6.502 software [16,17]. Percent polymorphism was computed based on the following formula:

$$\text{Percent polymorphism (\%)} = \left[ \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \right] \times 100$$

The polymorphic information content of each SSR marker was calculated as suggested by Anderson [18] using the Power Marker V3.25 software [19].

$$PIC = 1 - \sum_{i=1}^k p^2_{ij}$$

Where k is total number of alleles detected for an SSR marker, Pij is the frequency of j<sup>th</sup>SSR allele for the i<sup>th</sup>marker.

## Results

### Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance partitioned the total molecular variance within and among the accessions based on their source of origins and highly significant (P<0.001) variations were observed. The highest proportion 70% of the variation was attributed to genetic variability among individuals (AI) within populations, while smaller portion 5% of the total variation was observed among populations (AP) (Table 2).

Source of Variation	df	SS	MS	Variance		p	F-statistics
				Estimated	%		
Among Populations (AP)	9	128.7	14.3	0.3	5	>0.001	F <sub>st</sub> =0.04
Among Individuals (AI) with in population	90	848.8	9.4	4	70	>0.001	F <sub>is</sub> =0.74
Within Individuals (WI)	100	144	1.4	1.4	25	>0.001	F <sub>it</sub> =0.75
Total	199	1121.5	--	5.7	100	--	Nm =5.318

df=degree of freedom, SS=sum of squares and MS=mean squares, F<sub>st</sub>=inbreeding coefficient within subpopulations relative to the total, F<sub>is</sub>=inbreeding coefficient within individuals relative to the subpopulation, F<sub>it</sub>=inbreeding coefficient within individuals relative to the total NM=number of migrants (gene flow).

**Table 2:** Analysis of molecular variance (AMOVA) of 100 noug genotypes.

The population differentiation due to genetic structure were quantified using F-statistics (F<sub>it</sub>, F<sub>is</sub> and F<sub>st</sub>) also known as fixation indexes [20]. The F<sub>st</sub>, F<sub>it</sub> and F<sub>is</sub> value among the studied noug populations was 0.04, 0.75 and 0.74 respectively. The overall observed gene flow (Nm) or gene migration value was 5.318 which showed the

approximate number of individual's migration from one population to the other (Table 2). The pair-wise genetic differentiation among noug accessions within the source of origin ranged from 0.01 between Wollo and Gonder populations to 0.40 between Harerge and improved variety (Table 3).

	AR	GON	WLO	ILL	GOJ	HA	SHE	WEL	IV
AR	0	-	-	-	-	-	-	-	-
GON	0.08	0	-	-	-	-	-	-	-
WLO	0.08	0.01	0	-	-	-	-	-	-
ILL	0.18	0.13	0.13	0	-	-	-	-	-
GOJ	0.07	0.04	0.04	0.11	0	-	-	-	-
HA	0.27	0.26	0.24	0.39	0.23	0	-	-	-
SHE	0.08	0.05	0.04	0.12	0.03	0.21	0	-	-
WEL	0.09	0.05	0.05	0.13	0.03	0.24	0.04	0	-
IV	0.17	0.14	0.16	0.25	0.13	0.4	0.15	0.15	0

AR=Ararsi; GON=Gonder; WLO=Wollo; ILL=Illubabor; GOJ=Gojam; HA=Harerge; SHE=Shewa (central Ethiopia); WEL= Wellega; IV=Improved Variety

**Table 3:** Pairwise population matrix F<sub>st</sub> Values for assembled nine noug populations.

### Analysis of diversity parameters

The value of the studied diversity parameters was described in Table 4. The number of alleles ranged from seven for GA054 to 13 for GA077 giving an average of 9.6 alleles per locus. Highest and lowest numbers

of effective alleles were observed for marker GA054 (2.51) and GA081 (4.06). The gen flow ranged from 1.84 for marker GA054 to 0.65 for marker GA138. The lowest value of Shannon's information (I) index was recorded for marker GA054 and GA117 exhibited the highest

value. The average observed and expected heterozygosity across all the 14 SSR markers were 0.20 and 0.63 respectively. The observed heterozygosity ranged from 0.00 (GA029 and GA055) to 0.61 (GA035). The highest and lowest expected heterozygosity were recorded for

marker GA081 (0.70) and GA138 (0.54). The unbiased expected heterozygosity ranged from 0.58 (GA138) to 0.77 (GA081) with 0.68 mean value.

Markers	Major Allele freq.	Genetic parameters							
		Na	Ne	Nm	I	Ho	He	uHe	PIC
GA013	0.32	11	3.19	0.62	1.16	0.12	0.56	0.61	0.79
GA018	0.24	10	2.92	0.75	1.14	0.11	0.61	0.67	0.8
GA029	0.22	8	3.35	0.84	1.2	0	0.63	0.69	0.81
GA035	0.32	10	3.58	0.87	1.29	0.61	0.64	0.7	0.8
GA037	0.3	9	3.6	1.16	1.33	0.24	0.68	0.75	0.82
GA054	0.44	7	2.51	1.84	0.99	0.07	0.57	0.62	0.62
GA055	0.28	8	2.85	0.92	1.06	0	0.6	0.65	0.74
GA077	0.25	13	4	0.7	1.35	0.17	0.64	0.7	0.86
GA081	0.24	11	4.06	1.06	1.45	0.29	0.7	0.77	0.84
GA082	0.41	8	3.01	1.73	1.16	0.14	0.64	0.71	0.69
GA117	0.33	12	3.93	1.26	1.36	0.23	0.65	0.7	0.82
GA127	0.28	9	3.29	0.99	1.23	0.31	0.67	0.75	0.76
GA138	0.42	9	2.74	0.65	1.05	0.18	0.54	0.58	0.72
GA139	0.33	10	3.62	1.24	1.3	0.34	0.64	0.7	0.77
Mean	0.31	9.6	3.33	1.05	1.22	0.2	0.63	0.68	0.78

N<sub>a</sub>=Number of alleles; N<sub>e</sub>=Number of effective alleles; N<sub>m</sub>=Gene flow; I=Shannon's information index; H<sub>o</sub>=Observed heterozygosity; H<sub>e</sub>=Expected heterozygosity; uH<sub>e</sub>=Unbiased expected heterozygosity; PIC=Polymorphic information content.

**Table 4:** Summary of diversity parameters of 100 noug genotypes across 14 SSR markers.

The polymorphic information content (PIC) value ranges from 0.62 for marker GA054 to 0.86 for marker GA077 with a mean value of 0.78. This showed that all the markers used in the study were highly informative in expressing the genetic heterogeneity of the tasted noug accessions.

### Genetic relationship between populations

The mean value for numbers of observed alleles (Na) was higher for accessions collected from Gojam (7.29) followed by Shewa (6.29). Accessions collected from Gojam showed superior value for number of effective alleles and Shannon's information index.

Populations	Genetic parameters				
	Na	Ne	I	F	%P
Arsi	3.86	3.06	1.16	0.69	100
Gonder	5.86	4.14	1.54	0.65	100
Wollo	5.57	3.71	1.46	0.68	100
Illubabor	2.36	2.23	0.78	0.88	92.8
Gojam	7.29	4.34	1.64	0.73	100

Harerge	1.5	1.45	0.31	0.64	42.8
Shewa(Central Ethiopia)	6.29	4.16	1.54	0.78	100
Wellega	5.29	3.64	1.4	0.73	100
Improved Varieties	2.29	2.11	0.75	0.36	92.8
Mean	4.48	3.2	1.18	0.69	92.8
SE	0.2	0.12	0.05	0.18	6.24

Ave. Na =Average number of alleles; Ne=number of effective alleles; I=Shannon's information index; F=fixation index; %P= percent polymorphism; SE=standard error.

**Table 5:** Means of genetic parameters for nine noug populations studied under 14 SSR markers.

It appears that the degree of polymorphism between populations varied from 42.8% for accessions collected from Harerge to 100 % for the rest population (Table 5). The Harerge population showed poor mean value for all genetic parameters. The inbreeding coefficient (fixation index) value ranged from 0.36 for improved varieties to 0.88 for Illubabor accessions. On the other hand, accessions from Shewa

(Central Ethiopia) and Gojam populations had the highest number of alleles with frequencies  $\geq 5\%$  (excluding rare alleles) and number of effective alleles; however, Harerge populations had the lowest value. There is no any private allele observed from Wellega, Illubabor and

Harerge populations. While Shewa populations remarked the highest number of private alleles 0.43 followed by Gonder and Wollo populations 0.36 (Table 6).

Parameters	Populations								
	AR	GON	WLO	ILL	GO	HA	SHE	WEL	IV
Na Freq. $\geq 5\%$	3.86	4.57	4.57	2.36	5.14	1.5	5.5	4.64	2.29
No. Private Alleles	0.21	0.36	0.36	0	1.14	0	0.43	0	0.14
No. LComm Alleles ( $\leq 25\%$ )	0.29	0.5	0.5	0.14	0.57	0	0.79	0.14	0.07
No. LComm Alleles ( $\leq 50\%$ )	0.71	1.71	1.57	0.57	1.79	0.21	1.93	1.14	0.21
Shannon's Information Index (I)	1.16	1.54	1.46	0.78	1.64	0.31	1.54	1.4	0.75

Na=No. of Different Alleles; Na (Freq  $\geq 5\%$ )=No. of Different Alleles with a Frequency  $\geq 5\%$ ; Ne=No. of Effective Alleles; I=Shannon's Information Index; No. LComm Alleles ( $\leq 25\%$ )=No. of Locally Common Alleles (Freq.  $\geq 5\%$ ) Found in 25% or Fewer Populations; No. LComm Alleles ( $\leq 50\%$ )=No. of Locally Common Alleles (Freq.  $\geq 5\%$ ) Found in 50% or Fewer Populations.

**Table 6:** Mean of important allelic values recorded in nine noug population groups.

When we compare the number of locally common alleles (Freq.  $\geq 5\%$ ) which is found in 50% or fewer populations, Shewa, Gojam and Gonder populations showed the highest value. Similar scenario was observed regarding Shannon's index (I); Gonder, Shewa and Gojam ranked the top three values 1.64, 1.54 and 1.54 respectively. The result indicates that these populations may have the most important genetic diversity for noug improvement and conservation programs.

## Discussion

### Analysis of Molecular Variance (AMOVA)

The detection of genetic variation and diversity at the molecular level was performed by advanced molecular marker techniques. The information generated is useful for the development of new cultivars, germplasm classification and characterization [21]. According to the result highest genetic variability was observed among individuals within populations of noug accessions; because of the genetic material exchange by natural or human factors from the neighbor populations which can maximize the diversity of local germplasm but minimize the variations among populations. This leads to an increase in the distribution of alleles among different populations regardless of their geographical distance. In previous study 29 noug genotypes were genotyped with a set of 16 microsatellite markers and larger genetic variation was observed within population than among populations [13]. According to earlier report more genetic variation was observed within population than among populations [9,22]. Characterization of 35 India noug accession using 25 ISSR markers reported 95 % of variation with in the population but only 5% accounted among population [10]. The tested Ethiopian noug accessions using AFLP and RAPD markers reported 77% of genetic variation within populations [7]. This could be the result of the high degree of out crossing and strict self-incompatibility resulting in a relatively high gene flow among different noug populations of the country [1]. It is obvious for all out crossing species like noug; most of the genetic variability accounted among the individuals of a population than among the populations [9]. Genetic analysis of lettuce based on SSR marker reported 95% of variation among accessions within population than

among populations [23]. Similarly, analysis of molecular variance among open-pollinated oil palm genotypes showed that 99% of genetic variation was observed within populations rather than among populations [24]. The exchange of single gene, individuals and population on geographic scale (which is considered as gene flow) in conjugation with other evolutionary forces prevent differentiation between populations.

The threshold to determine the level of  $F_{st}$  value ranges from 0 to 0.05 considered as low, 0.05 to 0.15 moderate, 0.15 to 0.25 large and those greater than 0.25 mean very large genetic differentiations among populations [20]. According to the rule the level of  $F_{st}$  value the extent of genetic differentiation among the nine populations in terms of allele frequencies measured was small, this implied inter mating among individuals within similar populations was not significant. Previous studies also reported only a small proportion of genetic differentiation among noug populations based on RAPD and AFLP markers [7] and SSR marker [5]. Similar result was reported on sunflower [25] which strength the idea of low level of genetic differentiation implying high Nm value reveal large population sizes rather than gene exchange. It is obvious that geographic distribution, population size and opportunity for gene flow affect the genetic differentiation of a particular species [26].

Gene flow (Nm) values grouped into three categories: Nm  $> 1.00$  high, 0.25-0.99 intermediate and 0.000 – 0.249 low [27,28]. Therefore, the high Nm value 5.318 was observed in this study indicates high gene flow between populations which will agree with the AMOVA result showing low variation between populations. The low value of  $F_{st}$  implies that there is high frequency of identical alleles among accessions. This might be due to the geographic proximity of Wollo and Gonder resulting in admixture among the two populations because of seed exchange. The lowest  $F_{st}$  value observed between the two populations can be explained by high level of gene flow, which leads to genetic homogeneity of the populations. Harerge populations have larger  $F_{st}$  value between improved varieties. Low genetic material exchange and low distribution of improved varieties in Harerge area may maximize genetic heterogeneity between the populations. Lowest gene flow value between Harerge and Illubabor populations was

reported in previous study [9]. For proper estimation of population structure and divergence assessment of all relevant evolutionary forces such as gene flow, selection, genetic drift and mutation is important.

### Analysis of diversity parameters

The number of alleles detected in the present study was higher than the number of microsatellite alleles reported before [13,11]. The probable reason may be the higher number of genotypes used in the present study and the genotypic difference between noug collections along with their wider geographical distribution may create the variation. In addition, SSRs are co-dominant molecular markers that distinguish homozygote and heterozygote individuals and they produce a large number of alleles. EST derived SSR markers are also powerful in discriminating the genotypes based on their geographical origin [28]. As noug is a highly cross-pollinated crop therefore, a high number of alleles per locus could be a result of the natural out crossing among the landraces and also due to having a broad genetic base. Previous study reported lowest number of alleles per locus was reported in sunflower genotypes [29,30].

A total 208 alleles which are higher than the present study were reported on sunflower accessions and 0.5 average PIC with 0.51 expected heterozygosity [31]. The average values for number of alleles per locus, gene diversity, PIC and heterozygosity reported for safflower genotypes were lower than the present result [32] Another study on Brassica species using ten SSR markers also investigated a mean of 2.1 alleles [33]. In the present study all the markers showed distinct polymorphisms indicating the robust nature of microsatellites in revealing polymorphism. Even though the magnitude of PIC value was affected by population size, the genetic diversity in the selected genotypes, the genotypic method and locations of primers in the genome used for the study [34]; previous study reported high PIC value for the studied noug genotypes [16]. The strength of a marker can be determined by its PIC score. Greater the PIC value the stronger will be the marker for discrimination of genotypes. The use of markers with low PIC value should be avoided in genomic studies. SSR molecular markers have the highest PIC because of high mutation rate at microsatellites [35]. Therefore, the result confirmed the efficiency of the markers for further genetic diversity study of noug accessions in the future.

### Genetic relationship between populations

The wide range and high percentage value of polymorphism for populations indicated the existence of high genetic polymorphism in noug accessions. The high level of polymorphism found among the populations can be exploited in breeding programs to maximize the genetic diversity of the crop. The polymorphism detected in the present study was higher than the result reported using RAPD marker [7]. Previous study also reported highest level of polymorphism for noug accessions using ISSR markers [22]. This deviation might be due to the difference in discrimination power of the genetic marker used in the present study. Hence SSR markers are more powerful and informative for genetic diversity analysis than any other markers [20,36]. The lowest value of polymorphism observed for Harerge population might be because of the lowest number of genotypes incorporated in the study and the genotypic difference of the accessions. The inbreeding coefficient (fixation index) value closer to zero expected under random mating; therefore, improved varieties have high random mating than the other accessions. All populations

had positive fixation index which showed inbreeding is detected null alleles.

Populations showed an observable variation in loci carrying private alleles indicating the existence of a high genetic uniqueness among the genotypes. The detection of private alleles in the present study might be used as a source of important traits in the future noug breeding programs; because private alleles provide a unique genetic variability in certain loci. Moreover, the information gained from the presence of private alleles is quite fruitful to identify high diverse genotypes that can be integrated in breeding programs as parents to increase the allele richness in the gene banks [37]. For further investigation of functional traits if encoded by private or less common alleles, it may be possible to exploit the potentials of accessions in the populations in future noug improvement programs.

Previous result also reported private or rare alleles on sunflower genotypes [31]. Rare and private alleles are of high implication as they might be in linkage disequilibrium with genes underlying desirable traits. Such alleles are important for tagging core collection sites as they could be exclusive to a specific population and/or locality [38]. It should be noted that mutations within a population will most likely remain and hence screening more accessions with these markers may lead to identification of more rare and private alleles of significance for noug breeding. The result confirmed that Gonder, Shewa and Gojam populations had the highest genetic diversity measures. Therefore; these populations are premier in the future breeding programs to obtain noug accessions used as parents for crossing or as a source for population improvement.

### Conclusion

Genetic diversity is an important concept in any breeding program. For effective conservation and utilization of germplasms assessing the genetic diversity using molecular markers is important. The current study found the abundance of high genetic diversity in noug accessions collected from different parts of Ethiopia which are good for the introduction of new genes in the existing genotypes. The wide range of genetic variability in noug gene pool is beneficial for making a great progress in determining prime noug breeding and conservation strategies. The detection of private alleles in this study suggests the significant population differentiation at expressed portion of noug genome, which can be very useful from breeding point of view. Additionally, the markers are useful asset for assessing the large collections of noug accessions in Ethiopia that are yet to be characterized. SSR markers were exploited to provide an unbiased estimate of the diversity pattern in noug germplasms. The current study found the existence of high levels of diversity among 100 noug genotypes which are good for the introduction of new genes in the existing genotypes.

### Conflict of Interest

There is no potential conflict of interest.

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