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Full Length Research Paper

Assessment of genetic diversity among released and elite Ethiopian barley genotypes using simple sequence repeat (SSR) markers

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Barley is a major cereal grown widely and used in several food products, beverage production and animal feed. Being the fourth most important cereal crop in the world and the fifth rank in Ethiopia, it is a cash crop and used as a source of malt by the brewery industries, as food for human and feed for animals. Genetic diversity assessment is a key component in breeding programs. High level of polymorphism, codominant and multi allelic nature of simple sequence repeats (SSRs) markers make them preferable for diversity analysis in plant species. In this study, 22 SSRs markers were used to characterize the genetic diversity of 39 released and elite barley varieties collected from barley breeding program in Ethiopia. The amplification of SSRs loci were obtained for 35 primer pairs and only 22 of them showed clear polymorphic patterns which produced a total of 73 alleles with an average of 5 alleles per locus. The data generated by these informative primers were sufficient to discriminate the analysed barley genotypes. Based on the dissimilarity matrices ranging from 0.11 to 0.58, the genotypes were grouped into three major groups. The calculated polymorphism information content (PIC) values ranges from 0.17 to 0.60 with an average of 0.47 which shows the importance of the markers for future diversity analysis of barley. Locus HVACL1 and HVM36 shows higher PIC and locus HVBDHN7 shows lower PIC in this characterized barley genotype. This result will be useful for barley germplasm management and improvement in terms of biodiversity protection and design of new crosses for future breeding purpose.

Key words: Barley, elite, polymorphism information content (PIC), released, similarity, simple sequence repeats (SSRs), varieties.

INTRODUCTION

Barley (*Hordeum vulgare* L.), being the fourth most important cereal crop in the world, and ranks fifth in Ethiopia (CSA, 2012), is a cash crop and used for brewing malts, animal feed and human consumption (Hayes et al., 2002). The Ethiopian landrace barleys have been known to the botanical communities, notably

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from Vavilov's extensive collections and study. As cited by Abebe and Bjornstad (1997) Scheiemann stated that Ethiopia is considered as secondary center of diversity or center of origin for barley, which belongs to Poaceae. The diversity of barley in Ethiopia is quite high for an extended history of cultivation and variant agro-ecosystems (Eticha et al., 2010). Environmental factors such as varied soil types, altitudinal variation and climatic factors contribute to the diversity of barley manifested in Ethiopia. The entire cultivated barley of Ethiopia is a farmer variety or landrace (Hadado et al., 2010). The morphological characterizations of landraces of barley were studied (Eticha et al., 2010). The development of molecular markers makes it easy to assess genetic diversity in crops at DNA level (Reif et al., 2003). Molecular markers such as RAPD (Fernández et al., 2002; Meszaros et al., 2007), AFLP (Zhang and Ding, 2007a), ISSR (Fernández et al., 2002), STS (Meszaros et al., 2007) and SSR (Turuspekov et al., 2001; Matus and Hayes, 2002; Feng et al., 2006; Meszaros et al., 2007; Zhang et al., 2007b) can be used to estimate genetic diversity. Ramsay et al. (2000) developed SSR markers for molecular characterization and linkage mapping of barley. Molecular diversity of *H. vulgare* L. was studied using SSR markers (Wang et al., 2010; Hadado et al., 2010) and the primers were designed by Ramsay et al. (2000). Chaabane et al. (2009) also characterized barley collections of Tunisia, Syria and Denmark by SSR markers. But, the barley collections were not from Ethiopia.

SSRs are codominant, abundant, informative and their detection is very simple (Matus and Hayes, 2002). This makes them an excellent molecular marker system for analysis of genetic diversity. In this study, the authors used a set of 35 SSRs from seven linkage groups (five per each) of barley genome of which 22 were polymorphic to characterize 39 released and elite verities of barley obtained from barley breeding program of Holeta Agricultural Research Center. The objectives of this study were to assess genetic diversity and relationship of released and elite barley varieties for use in improvement and germplasm management.

MATERIALS AND METHODS

Plant materials

A total of 39 released and elite barley varieties were used in this study (Table 1). These barley varieties were provided by barley breeding program units of Holeta Agricultural Research Center.

Genomic DNA extraction

Five seeds of each genotype were sown in plastic pot of dimension (6.8 x 6.8 x 7.8 cm) and allowed to grow in greenhouse compartment in 2016, at National Agricultural Biotechnology Research Center. The soil mixture was red ash, frost soil and animal dung in the ratio of 1:1:1 and sterilized at a temperature of 150°C for 3 h. Two weeks later, the seedlings ranging from five to seven leaves were targeted and approximately, 100 mg young leaves tissues of each genotype were used for DNA extraction. DNA was extracted from each fresh and dried leaf following modified CTAB method (Doyle and Doyle, 1990).

The presence and absence of gDNA was checked in agarose gel electrophoresis (0.8% Agarose in 100 ml of 1XTAE, 5 µL of gDNA+1.5 µL of 1X Loading dye) run for 30 min at 100 V (Figure 1). DNA quality and concentration was estimated using Nano Drop Spectrophotometer (ND-8000, Thermoscitnific). DNA samples were then diluted to a concentration of 20 ng/µL using ddH_2O and stored at -20, -80 and -196°C (Yuanzheng and Angell, 2005).

Acquisitions of SSR markers and PCR amplification

SSR markers acquisition

Literature based search was done to find appropriate SSR markers for barley. Accordingly, thirty five SSR markers were found from Wang et al. (2010). All of them were screened for amplification and usefulness and 22 of them were found to be polymorphic (Table 2). The consistency of the band profiles SSR markers was assessed across the DNA samples by repeating amplifications and only the repetitive PCR products were scored.

PCR optimization, primer screening and PAGE

Polymerase chain reaction was optimized starting from the reaction set up described in Wang et al. (2010). Accordingly, PCR was carried out in a 25-µL final volume containing 2 µL of 20 ng/µL genomic DNA templates, 2.5 µL of 1X PCR buffer containing 15 mM Mg^{2+}, 0.5 µL of 15 mM dNTP mixture (2.5 mM of each), 1.25 µL of 5 u/µL of Taq DNA polymerase, and 0.25 µL of 10 µM forward and reverse primers and 1.6 ng/µL of gDNA (20 ng/µL of stock) for amplification. Depending on the primer pair used, DNA amplification was performed using master cycler (Pros, eppendorf), with a thermo cycler program of 1 cycle 4 min at 94°C of initial denaturation, followed by 35 cycles 30 s denaturation at 94°C, 30 s annealing (specific for each primer) (Table 2) and 30 s of extension at 72°C. The final extension was for 10 min at 72°C with final holding at 4°C. For primer Bmac0032, gradient PCR between 45-65°C was applied to get an optimum annealing temperature (Table 2). The success of the PCR and the associated yield was assessed in 2% agarose gel (2 g agarose in 50 ml of 1xTAE, 5 µL of gDNA+1.5 µL of 1X Loading dye with gel red (mix (1000:1)) and run for 30 min at 100 V. Once the optimization is over, the same PCR setup (as described above) was applied for amplification of SSRs with all the 22 primers across the entire barley genotypes studied. Microsatellite allele separation was carried out using polyacrylamid gel electrophoresis also called native DNA PAGE with a dual vertical electrophoresis apparatus (Cleaver, CS500 volt). The recipes used were polyacrylamide gel (5 µL of 10x TBE, 22 ml of 40% (29:1, acrylamid: bis acrylamid solution), 66 µL of TEMED, 80 µL of Nuclease free water) and 5 µL of PCR product +3µL of 1X Loading dye) run at 150 V for 1:30 h. PAGE picture (Figure 2) was captured using gel documentation system (3uv bench top, M-20 transilluminator).

Data analysis

The number of alleles detected by each SSR marker was estimated for each genotype and all SSR marker loci were scored as described by Struss and Plieske (1998). Data obtained from SSR analysis were scored as presence (1) or absence (0) of fragments for each barley genotype. Polymorphism information content (PIC), number of allele, allele frequency and gene diversity were

Table 1. List of released and elite barley varieties.

S/N	Varieties	Type	Row number	Maturity category	Seed source/pedigree*
1	HB-120	Malt	2	late High land potential	BSM2012
2	HB 52	Malt	2	High land potential	BSM2012
3	HB1533	Malt	2	High land potential	BSM2012
4	Holker	Malt	2	High land potential	BSM2012
5	Beka	Malt	2	High land potential	BSM2012
6	M-12	Malt	-	- ---	BSM2012
7	EH1847	Malt	2	Late High land	BSM2012
8	IBON174/03	Malt	2	Mid high land	BSM2012
9	Bekoji1	Malt	2	Late high land	BSM2012
10	Sabini	Malt	2	Mid high land	BSM2012
11	Bahati	Malt	2	Late high land	BSM2012
12	Ferie Gebes	Malt	2	Late high land	NMBADT 2012 P#2,15
13	HB1307	Food	6	Medium to late	Breeder seed
14	Shegie	Food	6	Medium to late	Breeder seed
15	HB42	Food	6	Medium to late	Breeder seed
16	Ardu1260B	Food	6	Medium to late	Breeder seed
17	Dimtu	Food	1r	Medium to late	Breeder seed
18	Cross 41/98	Food	6	Medium to late	Breeder seed
19	EH1493	Food	-	Medium to late	Breeder seed
20	Yedogit	Food	6	Medium to late	FBADT2012,-LS P#10
21	Estayish	Food	6	Medium to late	FBADT2012-LS P#5
22	Tiret	Food	6	Medium to late	FBADT2012-LS P#12
23	Shedeho	Food	6	Medium to late	FBADT2012-LS P#15
24	Hardu	Food	6	Medium to late	FBADT2012-LS P#6
25	Agegnehu	Food	6	Medium to late	FBADT2012-LS P#9
26	Tolose	Food	-	Medium to late	Seed Stock
27	Abdane	Food	-	Medium to late	FBADT2012-LS P#7
28	Baleme	Food	-	Medium to late	Seed stock
29	Dribie	Food	6	Early	FBADT2012-LS P#4
30	Tila	Food	6	Early	FBADT2012-LS P#5
31	Abay	Food	6	Early	FBADT2012-LS P#8
32	Biftu	Food	6	Early	FBADT2012-LS P#1
33	Dafo	Food	6	Early	FBADT2012-LS P#6
34	Dinsho	Food	2	Early	FBADT2012-LS P#12
35	Mulu	Food	ir	Early	FBADT2012-LS P#9
36	Setegne	Food	6	Early	FBADT2012-LS P#11
37	Misrach	Food	6	Early	FBADT2012-LS P#7
38	Basso	Food	6	Early	FBADT2012-LS P#2
39	Mezezo	Food	6	Early	FBADT2012-LS P#10

*BSM: Breeder seed maintenance; NMBADT: National Malt Barley Adaptation Trial; FBADT: food barley adaptation trial.

calculated using Power Marker V3.25 (Liu and Muse, 2005) (Table 3). Dendrogram was constructed using Darwin 6.0 software (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006) based on the dissimilarity matrices and neighbour joining (NJ) clustering method.

RESULTS

PIC statistics and SSR analysis

The PIC values of markers can provide an estimate of discrimination power in a set of accessions by taking not only the number of alleles, but also the relative frequencies of each allele (Smith et al., 2000). Based on this, the PIC value of this study was calculated using Power marker v3.25 and found to range from 0.17 to 0.60 and provide an estimate of discrimination power in a set of released and elite barley accessions. Similarly, number of allele, allele frequency and gene diversity was calculated using power marker v3.25, and resulted in minimum and maximum values of 2, 4 for number of

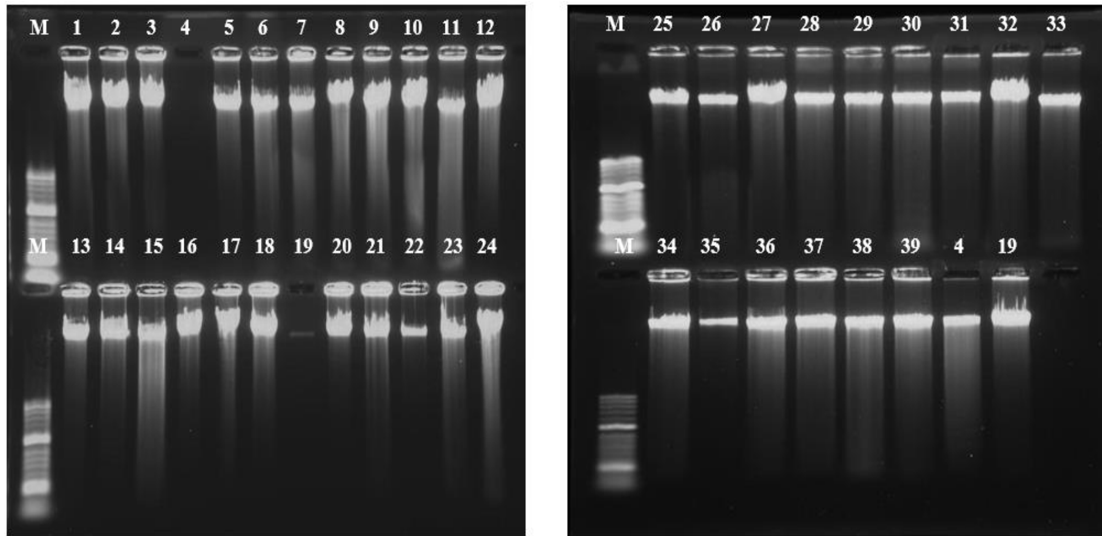

Figure 1. Genomic DNA extracted from the 39 barley cultivar (last two are repetitions) following CTAB method and loaded in 0.8% Agarose Gel concentration; M is size marker.

Table 2. Primer sequences, fragment sizes and repeat types of 22 barley SSR markers.

Locus	Chromosome	Repeat motif	Primer sequence (5′→3′)	Ta (°C)	Expected size (bp)
HVBDG	5(1H)	(CT)6	GAGAGAGAAAGAGAATGGCAGG AAAAAACTGCACCCAATCACTT	60	145
HVDHN7	7(5H)	(AAC)5	TTAGGGCTACGGTTCAGATGTT ACGTTGTTCTTCGCTGCTG	58	177
HVRCABG	4(4H)	(GA)6	TTTAAAAGAAAAGTGAATGGC TAATGAAGAATGAGGAGAAGC	55	123
HVM40	4(4H)	$(GA)_6(GT)_4(GA)_7$	CGATTCCCCTTTTCCCAC ATTCTCCGCCGTCCACTC	55	160
HVACL1	7(5H)	$(AT)_7$	TTTGGAATTATTCTGTGGGACC GGGATTCAATCAAGTATTCGGA	60	150
HVM36	2(2H)	$(GA)_{13}$	TCCAGCCGACAATTTCTTG AGTACTCCGACACCACGTCC	55	114
HVLEU	7(5H)	$(ATTT)_4$	TTGGAAGTGTACAGCAATGGAG TGAAAGGCCCCACAAGATAG	60	166
Bmag0006	3(3H)	$(AG)_{17}$	TTAAACCCCCCCCCTCTAG TGCAGTTACTATCGCTGATTTAGC	58	174
Bmag0217	1(7H)	$(AG)_{19}$	AATGCTCAAATATCTATCATGAA GGGGCTGTCACAAGTATATAG	58	196
Bmag0853	3(3H)	$(GA)_{15}$	ACAAGTATCCTGCAAACCTAA CGACCTTCTTAATGGTTAGTG	55	183
Bmag0905	3(3H)	$(TC)_{14}$	TTTATCTCCCCCTAGATAGAAG TCTCCGTATATTTAGGAAACG	55	177
Bmag0508A	3(3H)	$(AG)_{14}$	TCTCCGTATATTTAGGAAACG TATCTCCCCCTAGATAGAAGG	55	175
Bmag0807	6(6H)	$(TC)_{18}$	GGATATAAGGGTCCATAGCA AATTACATCAAATAGGCTCCA	55	111
Bmag0375	4(4H)	$(AG)_{19}$	CCCTAGCCTTCCTTGAAG TTACTCAGCAATGGCACTAG	58	135
Ebmag0793	2(2H)	$(GT)_{13}(AG)_{36}$	ATATATCAGCTCGGTCTCTCA AACATAGTAGAGGCGTAGGTG	55	177
HVBKASI	2(2H)	$(C)_{10}(A)_{11}$	ATTGGCGTGACCGATATTTATGTTCA CAAAACTGCAGCTAAGCAGGGGAACA	60	197

Table 2. Contd.

Bmac0032*	5(1H)	$(AC)_7T(CA)_{15}(AT)_9$	CCATCAAAGTCCGGCTAG GTCGGGCCTCATACTGAC	53.4	215
Bmac0209	3(3H)	$(AC)_{13}$	CTAGCAACTTCCCAACCGAC ATGCCTGTGTGTGGACCAT	58	176
Bmac0216	2(2H)	$(AC)_5$	GTACTATTCTTTGCTTGGGC ATACACATGTGCAAAACCATA	55	190
EBmac0501	5(1H)	$(AC)_{13}$	ACTTAAGTGCCATGCAAAG AGGGACAAAAATGGCTAAG	58	151
EBmac0679	4(4H)	$(AC)_{22}$	ATTGGAGCGGATTAGGAT CCCTATGTCATGTAGGAGATG	55	148
Bmac0577	4(4H)	$(AC)_{12}$	TCATACAGAAGCCCACACAG TGCATGTTCATTCTAGACAGG	53	146

Source; Wang et al. (2010); *For that primer, annealing temperature is the result of optimization in this study.

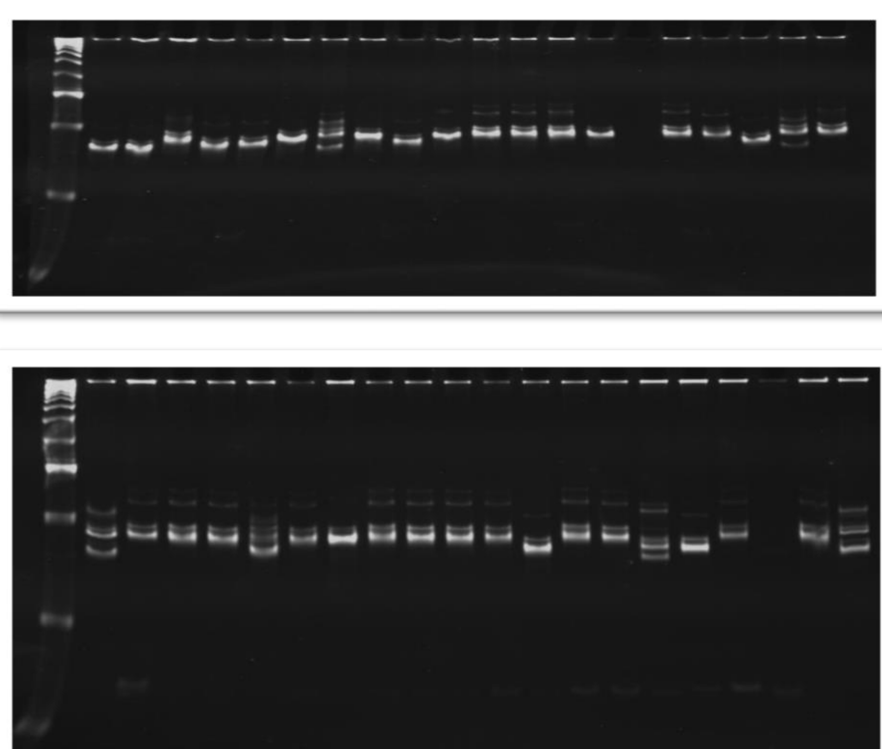

Figure 2. An example of marker profile of barley cultivars with the SSR marker HVM40. Where the first lane in both A and B is ladder size marker and the rest of the lanes represent the 39 barley genotypes except the 15th lane in A which is a gap.

allele, 0.38, 0.90 for allele frequency and 0.18, 0.67 for gene diversity, respectively (Table 3).

Dendrogram obtained with SSR markers

Dendrogram obtained from application of Darwin 6.0 using the dissimilarity matrices (Table 4), grouped the genotypes into three major groups. Cultivar Misrach and Mezezo showed greater genetic distance as compared to Cultivar Dafo, and HB120 with HB52 which showed lower genetic distance (Table 4).

DISCUSSION

SSR markers in barley genetic diversity analysis

In this study, 22 SSR markers were chosen for 39 released and elite barley genetic diversity analysis and they were from Chr. 1, Chr. 2, Ch. 3, Chr. 4, Chr. 5, Chr. 6 and Ch7 (Table 2). As far as genome coverage is

Table 3. PIC, gene diversity, major allele frequency and number of alleles generated from 22SSR markers across the genome of 39 released and elite barley cultivars.

S/N	Marker	Major allele frequency	Allele no.	Gene diversity	PIC
1	Bmac0032	0.54	4	0.62	0.57
2	HVBDG	0.64	2	0.46	0.35
3	HVBDHN7	0.90	2	0.18	0.17
4	HVRCABG	0.54	3	0.52	0.41
5	HVM 40	0.62	4	0.54	0.47
6	HVACL1	0.49	4	0.65	0.60
7	HVM36	0.38	4	0.67	0.60
8	Bmac0209	0.62	4	0.51	0.42
9	Bmac0216	0.77	3	0.37	0.31
10	Bmag0006	0.51	3	0.60	0.53
11	EBmac0501	0.46	4	0.62	0.55
12	EBmac0679	0.46	3	0.59	0.50
13	Bmag0217	0.62	4	0.52	0.45
14	Bmag0853	0.49	3	0.63	0.56
15	HVLEU	0.56	2	0.49	0.37
16	Bmag0905	0.59	3	0.52	0.43
17	Bmag-0508	0.49	4	0.57	0.48
18	HABKASI	0.74	3	0.39	0.33
19	EBmag0793	0.72	4	0.45	0.42
20	Bmac0577	0.49	3	0.55	0.44
21	Bmag0807	0.62	4	0.52	0.45
22	Bmag0375	0.74	3	0.39	0.33
	Mean	0.57	5	0.54	0.47

concerned, it may be arguable that the number of selected markers is low for barley genetic diversity study. However, we still obtained many alleles, and most of them were polymorphic. Although, some barley germplasms were not discriminated by the cluster analysis (Figure 3), the general classification was informative. It indicated that the genetic structure of barley germplasms in the study was high, which mainly attributed to difference in the genetic background of the studied barley cultivar. Therefore, it is inferred that the SSR marker used were relatively of high efficiency for barley genetic analysis and could reveal the genetic differences of barley germplasms as described in previous studies (Maroof et al., 1994; Struss and Plieske, 1998; Turuspekov et al., 2001; Matus and Hayes, 2002; Feng et al., 2006; Zhang et al., 2007b; Mikel and Kolb, 2008).

Genetic diversity for barley germplasm protection and barley breeding

With the cluster analysis, we were to identify relatively fewer number of genotype group (Figure 3) instead of total discerning of the whole genotypes in many possible groups. Two possible reasons may be attributed to the obtained result. One of the reasons could be some of the studied materials were duplications of the others or might have been developed from very closely related sister lines. In each case, the obtained result is justified. The other could be the smaller number of SSR markers used leading to smaller genome coverage which otherwise could be a good source of additional discrimination power used. In either case, we optimistically consider the SSR markers appear to provide an optimal platform to identify duplicated materials in the barley germplasm collection (Struss and Plieske, 1998), and they are helpful in managing the barley collections for subsequent barley improvement programs.

However, the result suggests that a more comprehensive result could also be obtained if more representations of germplasms and number of good genome coverage are considered future barley diversity study.

SSR markers in new variety protection

As a general knowledge and fact, molecular fingerprinting is an effective and accurate way to identify crop varieties (Nandakumar et al., 2004). In this study, it was found that

Table 4. Dissimilarity matrices for 39 studied barley varieties.

Units	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
2	0.15																																				
3	0.33	0.31																																			
4	0.33	0.31	0.24																																		
5	0.26	0.18	0.21	0.26																																	
6	0.38	0.32	0.31	0.35	0.23																																
7	0.24	0.26	0.24	0.29	0.26	0.31																															
8	0.31	0.33	0.22	0.32	0.29	0.29	0.16																														
9	0.42	0.35	0.34	0.34	0.31	0.31	0.43	0.37																													
10	0.30	0.36	0.26	0.35	0.37	0.40	0.31	0.24	0.39																												
11	0.36	0.33	0.37	0.32	0.24	0.33	0.37	0.44	0.32	0.42																											
12	0.41	0.34	0.38	0.24	0.35	0.38	0.38	0.36	0.38	0.38	0.31																										
13	0.45	0.38	0.38	0.33	0.31	0.34	0.42	0.41	0.38	0.38	0.26	0.28																									
14	0.37	0.34	0.33	0.43	0.35	0.43	0.33	0.36	0.47	0.39	0.41	0.37	0.32																								
15	0.36	0.38	0.37	0.46	0.43	0.46	0.32	0.25	0.50	0.24	0.53	0.45	0.41	0.31																							
16	0.49	0.42	0.37	0.46	0.39	0.33	0.46	0.35	0.46	0.38	0.44	0.41	0.31	0.36	0.40																						
17	0.54	0.47	0.38	0.42	0.39	0.33	0.47	0.41	0.38	0.47	0.35	0.46	0.41	0.47	0.54	0.45																					
18	0.29	0.21	0.34	0.29	0.31	0.44	0.39	0.42	0.34	0.35	0.27	0.33	0.33	0.33	0.46	0.42	0.38																				
19	0.34	0.27	0.35	0.31	0.32	0.36	0.35	0.33	0.31	0.32	0.33	0.30	0.25	0.29	0.42	0.38	0.33	0.15																			
20	0.41	0.34	0.38	0.29	0.35	0.30	0.38	0.36	0.33	0.38	0.41	0.18	0.41	0.42	0.49	0.41	0.41	0.38	0.30																		
21	0.38	0.32	0.31	0.31	0.23	0.27	0.31	0.42	0.39	0.40	0.24	0.30	0.20	0.34	0.46	0.33	0.43	0.35	0.32	0.34																	
22	0.38	0.31	0.25	0.34	0.22	0.26	0.34	0.37	0.34	0.31	0.37	0.33	0.29	0.33	0.37	0.32	0.42	0.38	0.31	0.29	0.22																
23	0.44	0.37	0.26	0.31	0.29	0.28	0.36	0.43	0.41	0.37	0.29	0.35	0.31	0.35	0.43	0.34	0.34	0.36	0.37	0.31	0.18	0.12															
24	0.47	0.45	0.44	0.49	0.42	0.45	0.44	0.47	0.49	0.49	0.43	0.43	0.47	0.53	0.43	0.51	0.57	0.49	0.53	0.47	0.45	0.39	0.42														
25	0.43	0.32	0.31	0.35	0.32	0.32	0.35	0.38	0.35	0.40	0.33	0.30	0.25	0.24	0.38	0.33	0.43	0.31	0.27	0.34	0.32	0.22	0.28	0.41													
26	0.46	0.43	0.38	0.33	0.40	0.29	0.33	0.41	0.47	0.43	0.46	0.50	0.50	0.47	0.50	0.50	0.47	0.47	0.47	0.42	0.43	0.38	0.35	0.49	0.39												
27	0.41	0.34	0.33	0.33	0.31	0.20	0.38	0.36	0.33	0.43	0.36	0.37	0.32	0.46	0.45	0.41	0.41	0.42	0.38	0.37	0.34	0.38	0.39	0.39	0.30	0.37											
28	0.46	0.36	0.44	0.39	0.41	0.32	0.44	0.46	0.44	0.40	0.42	0.38	0.38	0.47	0.42	0.42	0.47	0.35	0.32	0.38	0.32	0.31	0.32	0.36	0.32	0.43	0.30										
29	0.38	0.26	0.30	0.34	0.32	0.26	0.30	0.37	0.38	0.31	0.37	0.33	0.38	0.38	0.32	0.37	0.46	0.34	0.35	0.29	0.26	0.16	0.17	0.31	0.22	0.33	0.29	0.17									
30	0.46	0.40	0.35	0.39	0.41	0.32	0.44	0.46	0.44	0.36	0.38	0.43	0.38	0.47	0.42	0.42	0.43	0.39	0.40	0.43	0.36	0.26	0.23	0.36	0.27	0.43	0.30	0.17	0.17								
31	0.41	0.34	0.42	0.38	0.39	0.34	0.38	0.41	0.33	0.34	0.36	0.37	0.41	0.50	0.45	0.45	0.46	0.38	0.34	0.32	0.38	0.29	0.35	0.39	0.30	0.42	0.32	0.25	0.19	0.25							
32	0.45	0.39	0.34	0.43	0.40	0.26	0.38	0.41	0.43	0.43	0.45	0.38	0.41	0.42	0.49	0.41	0.46	0.46	0.35	0.29	0.35	0.26	0.32	0.39	0.26	0.38	0.29	0.31	0.26	0.26	0.33						
33	0.51	0.49	0.35	0.40	0.46	0.41	0.49	0.51	0.44	0.49	0.55	0.47	0.55	0.53	0.55	0.51	0.48	0.49	0.45	0.34	0.45	0.35	0.37	0.58	0.45	0.44	0.47	0.45	0.39	0.41	0.51	0.35					
34	0.34	0.36	0.30	0.40	0.32	0.31	0.30	0.33	0.40	0.31	0.33	0.47	0.43	0.34	0.38	0.43	0.44	0.40	0.45	0.43	0.41	0.39	0.37	0.46	0.31	0.34	0.29	0.45	0.31	0.36	0.34	0.44	0.54				
35	0.28	0.25	0.24	0.24	0.26	0.34	0.29	0.36	0.38	0.34	0.26	0.28	0.32	0.32	0.41	0.49	0.46	0.24	0.25	0.37	0.30	0.29	0.31	0.39	0.20	0.42	0.32	0.30	0.24	0.30	0.37	0.33	0.43	0.34			
36	0.42	0.39	0.34	0.34	0.41	0.44	0.43	0.46	0.43	0.39	0.46	0.42	0.50	0.43	0.50	0.50	0.47	0.34	0.31	0.38	0.48	0.30	0.36	0.40	0.31	0.38	0.46	0.35	0.30	0.35	0.38	0.34	0.35	0.44	0.24		
37	0.53	0.46	0.46	0.54	0.47	0.42	0.50	0.44	0.46	0.46	0.49	0.49	0.49	0.50	0.49	0.39	0.45	0.46	0.37	0.40	0.50	0.41	0.47	0.42	0.32	0.54	0.40	0.37	0.36	0.42	0.40	0.36	0.56	0.47	0.40	0.41	
38	0.47	0.45	0.40	0.44	0.42	0.41	0.40	0.38	0.44	0.36	0.43	0.43	0.43	0.39	0.43	0.47	0.39	0.40	0.26	0.39	0.41	0.35	0.42	0.46	0.31	0.49	0.39	0.26	0.31	0.36	0.34	0.35	0.50	0.41	0.29	0.30	0.21
39	0.38	0.35	0.39	0.39	0.36	0.39	0.29	0.42	0.43	0.39	0.37	0.38	0.46	0.47	0.54	0.54	0.47	0.43	0.35	0.38	0.35	0.38	0.45	0.44	0.35	0.47	0.38	0.35	0.34	0.39	0.29	0.34	0.56	0.40	0.29	0.39	0.36	0.25

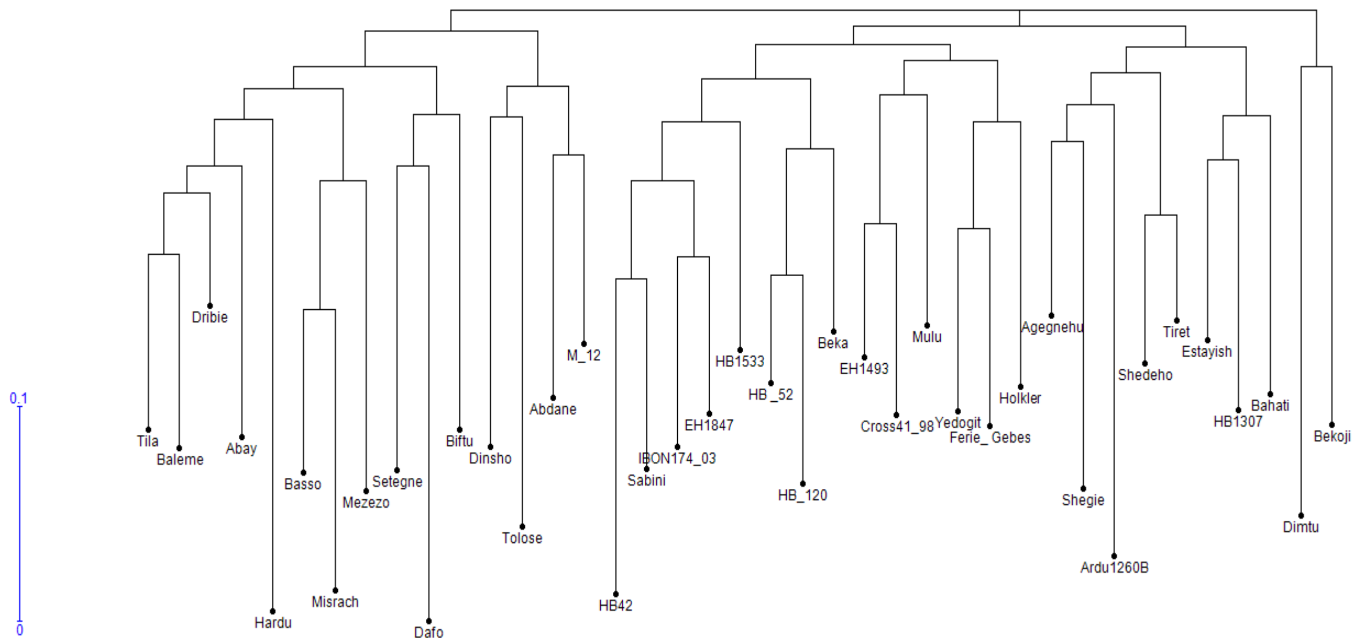

Figure 3. Dendrogram constructed using 22 SSR markers across 39 barley cultivars.

the SSR marker have at least distinguished the released barley cultivars from other elite barley cultivars. This suggests that we can protect the breeder rights of the released barley cultivars by using SSR markers. Similarly, we can identify all barley landraces by SSR fingerprinting which intern allows us to find out whether there are varietal duplications or mistakes in a given germplasm collection.

Conclusions

In conclusion, genetic variation is a raw material for plant breeding and assessments of existing similarities or differences in any crop germplasm pool. It plays a great role in a predictable area to improve agricultural production and productivity, to solve food insecurity in developing world. This study was conducted to determine the levels of genetic variation in released and elite Ethiopian barley materials. The good information content of the markers used, estimated extent of diversity and limited clustering among the studied barley materials are basic outcomes of this study upon which a more comprehensive study can be built. Relatively speaking however, the results can still be used for the consumption of barley breeding programs where breeders should think of the distinctness of the varieties already released and their future plans to release new ones. Finally, diversity study such as this is useful for the establishment of genetic relatedness and molecular characterization of barley germplasm. This in turn benefits barley breeding programs to make choice of the genotypes to be used in crosses and will facilitate the germplasm management.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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