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Evaluation of Genetic Variation in Ethiopian Malt Barley Cultivars Using Malt Quality-Linked Markers and Phenotypic Traits

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Abstract

Germplasm conservation and breeding programs can be enhanced by understanding the genetic diversity at molecular level. In Ethiopian malt barley cultivar, the pattern of genetic diversity at DNA level is poorly characterized. This study is aimed to evaluate the genetic diversity of 22 Ethiopian malt barley cultivars using malt quality linked markers, brewing and agro morphological traits. Twenty one SSR markers were used to analyze all accessions and total of 244 alleles were found with a mean of 8 alleles per locus. The analysis indicated the existence of high polymorphic information content ranging from 0.82 to 0.92 and the mean Shannon diversity index (I) was 1.95, indicating that they are highly informative markers. The result of molecular variance analysis pointed out that the highest variation was found among the cultivars (94%), indicating its importance to develop appropriate conservation strategy and parental selection for new competing barley variety development. The highest β-glucan content was recorded for IBon174/04(706±54), while the lowest β-glucan content was recorded for the cultivar Focus (231±54.5). The value of extract content is the highest for the cultivar Focus (81.8±0.8), while the low extract content was recorded for HB-1533(75.8±0.8). Agromorphological analysis also indicated the existence of phenotypic variation for dwarf plant development in Explorer (0.58±0.09m) and high seeds per spike for yield in Beka (31±1.3), Suba (29.7±1.3), and HB-52(30±1.3). Therefore, Ethiopian malt barley breeding and conservation can be enhanced and speedup by utilizing the information generated from the study.

Introduction

Barley grains are one of the raw materials to produce malt, which in turn processed to produce beer. It is one of the first cereal grains that humans have ever grown and is the fifth largest food source in the world. Barley is the fourth most widely cultivated cereal in the world, after rice, maize, and wheat. Central and Northern Europe and Russia are the main producers of barley. Barley is produced in Ethiopia at altitudes ranging from 1500 to 3500 meters above sea level (a.s.l), but it is primarily planted between 2000 and 3000 meters a.s.l. It ranks sixth in area and production among Ethiopia's grains. Barley is Ethiopia's most significant staple food crop and is grown primarily for human use. In addition, barley straw is an excellent source of animal feed, particularly during the dry season. It's also a good material for thatching roofs and mattresses.

Increase in brewery industries that utilize malt barley result an increase in global malt barley demand. Recent economic growth and urbanization in the last two decades resulted in a rise of beer and malt industries in Ethiopia. In addition to industrial demand, malt barley is also used for human food, animal and poultry feed. Locally malt barley is eaten as Kollo, enjera, tella, genfo and besso, which also create extra demand for malt barley.

Despite grain production increase, barley malting quality traits are highly desirable to produce premium quality beer.Some of the key paramteres used to determine barley malt quality are Diastatic Power (DP), Viscosity (VIS), β-glucan content (BG), Malt Extract (ME) and Alpha-Amylase (AA)(Cu et al., 2016; Gao et al., 2004)

Barley varieties with high malt extract is desirable to brewing industries as it serves as soluble elements of malt such as carbohydrates, proteins, fermentable sugars and enzymes for hydrolysis of starch. Barley varieties withy high betaglucan content is desirable in food industries as it serve as dietary fiber. However, breweries require barley cultivars of lower betaglucan content to reduce haze formation, filtration problems and viscosity during brewing process (McClear et al., 1985). Therefore, enhancing malt barley production and quality is crucial to satisfy malt barley demand and increase farmer’s income.

Many of the malt quality traits such as malt extract and beta glucan content are controlled by quantitative trait loci (QTL). The concentration of the loci controlling the traits varies from cultivar to cultivar and localized in all barley chromosomes (Fang et al., 2019). However, Chromosome 2 and 4 are known as the major chromosomes harboring malting quality QTLs. accurate identification of genotypic and phenotypic variations are critical for effective exploitation of genetic resources. Evaluation of genetic diversity is important for effective selection of germplasm for barley malt quality breeding research in Ethiopia. To facilitate effective utilization of genetic resources for better malt quality traits and number of effectors have been made to develop molecular markers and utilize them. For instance, SNPs, Dart , RAPD and other PCR based markers were used to select barley cultivars with better malt quality(Zewodu et al., 2024, Tiegist et al., 2023; Tiegist & Abebe, 2010; Cu et al., 2016, Ovesna et al., 2001; Coventry et al., 2003; Murioz-Amatriain et al., 2010; Emebiri et al., 2009; fechter et al., 2010). There have been some studies on barley germplasm at morphological and molecular levels

In the present study, we made an effort to characterize Ethiopia malt barley cultivars using malt quality linked markers and phenotypic traits. These genetic data can be used to associate the markers with the malt quality traits. In addition, it offers opportunity to detect diversity in cultivars and genes related with malt quality, and plan breeding and conservation for malt quality traits.

Materials and Methods

Plant materials and Study area

Twenty two malt barley cultivars evaluated in this study were collected from Holeta Agricultural research center. These cultivars includes Holker, Bekoji-I, M-21, EH-1847, Bahati, HB-1963, HB-1964, Sabin, HB-1533, Beka, Grace, Ibon 174/03, HB-120, HB-52, Planet, Suba, Explorer, Proctor, Fatima, Focus, Henerike, and Traveller .The cultivars were planted on the experimental field of Holeta Agricultural research center(HARC).

DNA Extraction and PCR Amplification

Total genomic DNA was extracted from young barley leaves using the CTAB protocol. , PCR amplification was carried out in a final volume of 10 µL µL containing 10×PCR buffer, MgCl2 (25 mM), dNTP mixture (10 mM), 0.5 µL primer(10 pmole) (Table 1), Taq DNA polymerase (1 U/µL), and template DNA (80 ng/µL). The thermocycler is set up to run one cycle of denaturation at 94 degrees Celsius for 3 minutes, then 35 cycles of 94 degrees Celsius for 1 minute, 55–60 C for 30 sec s, and 72 degrees Celsius for 1 minute, before finishing with a 10-minute extension step at 72 ^{O}C. The resulting PCR products were resolved in 3% agarose gel. The SSR marker were scored as codominat markers using PyElph softwares and verified manually.

Agronomic trait analysis

Each variety was sowed in five 2.5-meter rows and 2.5 meters long, with a spacing of 10 and 20 centimeters between plants and rows, respectively. The experiment was set up using a complete randomized block design (CRBD) with three replications. Every agronomic technique, fertilizer application, weeds control, and so forth have been implemented by the agricultural sector's standard for barley as reported by Zhang and Li, (2010). Only the two middle rows of each plot have been used for data collection and subsequent analyses to prevent boundary effects. For every genotype, the following agronomic characteristics were measured: plant height, panicle length, spike length, number of seeds per spike, number of effective tillers, days to heading, days to flowering, thousand grain weight, days to maturity, and grain yield.

Table 1 Malt quality linked SSR marker used for genotypic the cultivars.

Malt quality traits analysis.

NIRSS Machine was used to evaluate friability, protein content, extract content, and beta-glucan contents using spectral characteristics. The near infrared reflectance spectroscopy (NIRSS machine) then correlates the spectral data obtained from these samples with their corresponding quality parameters as reported by (Zhang and Chen, 2013). This is typically done by shining near-infrared light onto the sample and measuring the reflected light. The machine collects spectral data across a range of wavelengths. The barley grains were scanned for its spectral information by means of FT_NIR spectrometer, using 600ml sample cuvette 32 mount of scans and 16/cm resolution and rotating mode. The spectral data was collected across a range of wave length such as 867 and 2535nm (3944 –11528/cm) as reported by Abeshu (2021).

Statistical analysis

Different statistical software packages were used to research the genetic diversity and population structure of the malt barley germplasm. Locus-based diversity parameters including number of observed alleles (Na), effective number of alleles (Ne), Shannon's information statistic (I), Nei's genetic diversity (h), total genetic diversity (HT), genetic differentiation (HS), genetic differentiation statistics by locus (Gst), estimate of the number of migrants (gene flow) (Nm) and percent polymorphic loci (PPL) was analyzed using GenAIEx6.51b2. Agromorphological and malt quality traits were analyzed using standard procedures. This was investigated statistically using mean and standard deviations and analysis of variance (ANOVA) using R version 4.3.1. A Tukey test at a significant level of 5% was used to determine significant differences for the tested traits

Results

Analysis of Molecular Variance

Two components contributed to individual cultivars variance, one was due to within individuals and, the other was due to among individuals. An AMOVA analysis showed that 94% of the variance in allele frequencies was among individuals, and 5%of the variance was observed within individuals (Table 2). Only 1% allele frequency variation was observed among Ethiopian and exotic malt barley cultivars.

Table 2 Summary AMOVAshowing partitioning of genetic variations within and among individuals and populations.

Summary AMOVA Table					
Source	df	SS	MS	Est. Var.	%Variation
Among Pops	1	20.576	20.576	0.078	1%
Among Indiv	20	378.265	18.913	9.218	94%
Within Indiv	22	10.500	0.477	0.477	5%
Total	43	409.341	39.966	9.773	100%

df degree of freedom, SS sum of squares, MS mean square, Est. Var. estimated variation, PV percent of variation

Number of amplified alleles and PIC values

The number of amplified bands (number of different alleles (Na) varied from 5 to 12 bands (Table 3). Loci gave 6-10 amplified bands that were common. Marker HVALAAT gave the highest amplified bands (about 12 amplified bands), meanwhile, BMAG877 gave the lowest (about 5 amplified bands) (Table 3). High PIC values were observed in the whole cultivar for each marker tested (above 0.8). The level of polymorphism (PIC) for each SSR locus across the 22 barley cultivars ranged from 0.82 to 0.92. The higher number of amplified bands and the higher PIC values demonstrated that observed genotypes were diverse.

Malt quality evaluation

Six malt quality traits were investigated for the twenty two malt barley cultivars. Significantly high beta-glucan content was observed for cultivar Ibon174/04, while low β-glucan content was observed for cultivar Focus. The extract content of barley cultivar Focus was highest of all. The seed moisture content of the cultivar M-21 is lowest of all (Table 4). However, the mean hectoliters for all of the cultivars were not significantly different except HB-120.

Agromorphological trait evaluation

Nine agronomic traits, including days to heading, days to maturity, plant height, pedicels length, number of effective tillers, grain width, spike length, number of seeds per spike , thousand grain weight and yield per plot was examined on 22 Ethiopian malt barley cultivars. The result in the **Table 5** below shows that the cultivars were not significantly different for panicle length, and grain width. However, highest plant length was observed for cultivar Suba (1.44m), and the lowest for cultivar Explorer (0.58m). Significantly high seeds per spike were observed for cultivar Beka which is also comparable with Suba and HB-52.

Table 3 Summary of genetic diversity indices for the 21 malt quality linked marker.

S/No	Markers name	Chrs Pos	Na	Ne	Ho	He	uHe	I	Gst	F	Fis	Fit	Fst	PIC
1	AWBMS80	1HS	7.0	5.95	0.00	0.81	0.85	1.84	0.012	1.00	1.00	1.00	0.06	0.87
2	BMAC90	1HS	9.0	8.95	0.00	0.89	0.93	2.22	-0.012	1.00	1.00	1.00	0.04	0.92
3	EBMAC501	1HS	6.0	5.84	0.00	0.83	0.87	1.81	0.005	1.00	1.00	1.00	0.05	0.87
4	HVM2	1HS	8.0	7.06	0.00	0.86	0.90	2.02	-0.018	1.00	1.00	1.00	0.03	0.88
5	HVALAAT	1HS	12.0	9.63	0.58	0.88	0.93	2.36	0.001	0.34	0.34	0.36	0.03	0.83
6	HVM20	1HS	9.0	7.68	0.05	0.87	0.91	2.11	-0.003	0.93	0.94	0.94	0.04	0.92
7	BMAG692	2HL	7.0	5.84	0.00	0.83	0.87	1.85	0.000	1.00	1.00	1.00	0.05	0.87
8	HVM36	2HS	10.0	9.07	0.09	0.88	0.92	2.24	0.011	0.89	0.89	0.89	0.06	0.93
9	BMAC134	2HS	8.0	6.72	0.00	0.84	0.88	1.97	-0.005	1.00	1.00	1.00	0.04	0.89
10	HVM54	2HL	10.0	9.61	0.00	0.88	0.92	2.23	0.005	1.00	1.00	1.00	0.05	0.93
11	EBMAC415	2HL	9.0	7.68	0.06	0.87	0.91	2.11	0.009	0.93	0.94	0.94	0.06	0.92
12	EBMAC541	3HL	10.0	7.75	0.19	0.87	0.91	2.19	0.014	0.78	0.78	0.79	0.06	0.91
13	BMAG877	3HL	5.0	4.09	0.00	0.75	0.79	1.54	0.032	1.00	1.00	1.00	0.08	0.82
14	HVM67	4HL	6.0	5.04	0.00	0.79	0.84	1.72	-0.009	1.00	1.00	1.00	0.04	0.83
15	HVM6	5HL	7.0	6.05	0.00	0.83	0.87	1.89	0.006	1.00	1.00	1.00	0.05	0.88
16	GMS1	5HL	6.0	5.11	0.00	0.80	0.84	1.74	-0.013	1.00	1.00	1.00	0.04	0.83
17	HVAMY2	7HS	8.0	7.56	0.00	0.86	0.91	2.07	0.002	1.00	1.00	1.00	0.05	0.91
18	HVBTAI3	4HL	7.0	6.06	0.00	0.83	0.87	1.84	0.006	1.00	1.00	1.00	0.05	0.88
19	HDAMYB	4HL	7.0	6.24	0.00	0.84	0.88	1.89	-0.007	1.00	1.00	1.00	0.04	0.87
20	HVBAMY	4HL	7	6.374	0.000	0.839	0.881	1.917	-0.016	1.000	1.000	1.000	0.033	0.87
21	HVCMA	7HL	5	4.607	0.000	0.722	0.757	1.478	0.073	1.000	1.000	1.000	0.122	0.84
Minimum			5	4.09	0	0.722	0.757	1.48	-0.018	0.34	0.339	0.62	0.032	0.82
Maximum			12	9.634	0.585	0.888	0.932	2.36	0.073	1	1	1	0.122	0.92
Average			8	6.807	0.047	0.837	0.878	1.95	0.004	0.947	0.947	0.949	0.052	0.880

Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), Ho = Observed Heterozygosity = No. of Hets / N, He = Expected Heterozygosity = 1 - Sum pi^2 , uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He, F = Fixation Index = (He - Ho) / He = 1 - (Ho / He), Fis = (Mean He - Mean Ho) / Mean He, Fit = (Ht - Mean Ho) / Ht, Fst = (Ht - Mean He) / Ht, Nm = [(1 / Fst) - 1] / 4

Table 4 Malt quality traits performance of Ethiopian malt barley cultivars.

Cultivars	B-glucan content (Mean ±SE)	Extract content (Mean ±SE)	Friability (Mean ±SE)	Hectoliter (kg/l). Weight(g)	Moisture content (%)	Protein content (%)
Focus	231 ±54.5[1]	81.8± 0.8[4]	73.8±6.72[45]	68.6±1.66[12]	11.7±0.172[234]	8.89±0.669[1]
Explorer	280 ±54.5[12]	81.1± 0.8[34]	81.9±6.72[5]	65.8±1.66[12]	11.7±0.172[234]	10.13±0.669[123]
Henerike	292 ±54.5[123]	78.9± 0.8[1234]	57.3±6.72[12345]	66.7±1.66[12]	10.9±0.172[12]	10.94±0.669[1234]
Planet	343 ±54.5[1234]	80.5± 0.8[234]	65.1±6.72[2345]	66.7±1.66[12]	11.7±0.172[234]	9.45±0.669[12]
Fatima	378 ±54.5[12345]	81.6± 0.8[34]	67.5±6.72[2345]	66.4±1.66[12]	11.8±0.172[234]	9.47±0.669[12]
Traveller	419 ±54.5[123456]	81.6± 0.8[34]	71.4±6.72[345]	68.1±1.66[12]	11.7±0.172[234]	10.12±0.669[123]
HB-52	441 ±54.5[123456]	77.8± 0.8[1234]	25.1±6.72[1]	71.0±1.66[12]	12.1 ±0.172[4]	11.96±0.669[234]
Proctor	445 ±54.5[123456]	78.5± 0.8[1234]	49.8±6.72[12345]	69.5±1.66[12]	11.5±0.172[1234]	10.49±0.669[123]
Sabin	480 ±54.5[123456]	80.6± 0.8[234]	71±6.72[345]	69.3±1.66[12]	11.7±0.172[234]	10.15±0.669[123]
Grace	488 ±54.5[123456]	81± 0.8[34]	72.4±6.72[345]	66.0±1.66[12]	11.9±0.172[234]	10.39±0.669[123]
EH-1847	547 ±54.5[23456]	78.3± 0.8[1234]	46.7±6.72[1234]	67.9±1.66[12]	11.3±0.172[1234]	12.77±0.669[34]
HB-1963	551 ±54.5[23456]	78.5± 0.8[1234]	48±6.72[1234]	71.9±1.66[2]	12.0±0.172[34]	10.78±0.669[1234]
Beka	559 ±54.5[23456]	79.1± 0.8[1234]	48.5±6.72[12345]	70.5±1.66[12]	11.8±0.172[234]	12.42±0.669[34]
HB-1533	565 ±54.5[23456]	75.8± 0.8[1]	34.6±6.72[12]	66.3±1.66[12]	11.1±0.172[123]	13.18±0.669[4]
Bekoji-I	571 ±54.5[23456]	77.8± 0.8[1234]	34.7±6.72[12]	70.5±1.66[12]	11.9±0.172[234]	11.54±0.669[1234]
HB-120	578 ±54.5 [3456]	77.4± 0.8[123]	44.6±6.72[1234]	62.2±1.66[1]	11.8±0.172[234]	11.85±0.669[234]
Bahati	585 ±54.5[3456]	78.7± 0.8[1234]	47.9±6.72[1234]	67.4±1.66[12]	11.6±0.172[1234]	12.2±0.669[34]
Holker	594 ±54.5[456]	78.2± 0.8[1234]	53±6.72[12345]	65.7±1.66[12]	11.2±0.172[123]	11.57±0.669[234]
Suba	597 ±54.5[456]	78.8± 0.8[1234]	40±6.72[123]	69.6±1.66[12]	11.6±0.172[234]	11.83±0.669[234]
HB-1964	669 ±52.6[56]	78.8± 0.8[1234]	43.5±6.83[1234]	62.8±1.72[12]	11.0±0.178[12]	11.28±0.675[1234]
M-21	677 ±54.5[6]	76.2± 0.8[12]	39.5±6.72[123]	64.5±1.66[12]	10.7 ±0.172[1]	12.01±0.669[234]
Ibon 174/03	706 ±54.5[6]	77.6± 0.8[1234]	39.6±6.72[123]	67.7±1.66[12]	11.4 ±0.172[1234]	11.81±0.669[234]

Same numbers (within the column) indicates no significant difference in data expressed as means ±SD at $p<0.05$ according to Tukey test.

Table 5 Agromorphological traits performance of Ethiopian malt barley cultivars

Cultivar	PH (m)	PL(cm)	NET	MD	HD	GW (cm)	SPS	1000.gw	YPP (g)
Focus	0.63±0.09^{123}	0.31±0.13^{1}	5±0.01^{123}	173±0^{c}	101±0^{g}	2.76 ±0.07^{1}	23.7±1.3^{12}	45.7± 3.9^{12}	489 ±110^{12}
Explorer	0.58±0.09^{1}	0.33±0.13^{1}	8±0.01^{234}	168±0^{b}	95±0^{c}	2.62±0.07^{1}	25.7±1.3^{123}	41.6± 3.97^{12}	276±110^{12}
Henerike	0.59±0.09^{12}	0.07±0.13^{1}	3±0.01^{12}	183±0^{f}	97±0^{d}	2.75±0.07^{1}	21.0±1.3^{1}	57.6± 3.97^{2}	216±110^{1}
Planet	0.67±0.09^{1234}	0.07±0.13^{1}	3±0.01^{1}	168±0^{b}	100±0^{f}	2.69±0.07^{1}	27.3±1.3^{123}	46.8± 3.97^{12}	492±110^{12}
Fatima	0.59±0.09^{12}	0.10±0.13^{1}	2±0.01^{1}	165±0^{a}	95±0^{c}	2.59±0.07^{1}	26.3±1.3^{123}	44.7± 3.97^{12}	317±110^{12}
Traveller	0.63±0.09^{123}	0.32±0.13^{1}	10±0.01^{456}	168±0^{b}	92±0^{b}	2.65±0.07^{1}	25.0±1.3^{123}	50.3± 3.97^{12}	403±110^{12}
HB-52	1.06±0.09^{34567}	0.12±0.13^{1}	15±0.01^{789}	178±0^{d}	87±0^{0}	2.62±0.07^{1}	30.0±1.3^{23}	47.7± 3.97^{12}	714±110^{2}
Proctor	0.75±0.09^{12345}	0.36±0.13^{1}	8±0.01^{34}	168±0^{b}	102±0^{h}	2.68±0.07^{1}	28.7±1.3^{23}	34.0± 3.97^{1}	268±110^{12}
Sabin	0.8±0.09^{123456}	0.08 ±0.13^{1}	5±0.01^{123}	112±0^{2}	69±0^{2}	2.62±0.07^{1}	25.3±1.3^{123}	44.7± 3.97^{12}	272±110^{12}
Grace	0.73±0.09^{12345}	0.08 ±0.13^{1}	10±0.01^{456}	168±0^{b}	83±0^{8}	2.52±0.07^{1}	28.0±1.3^{123}	52.3± 3.97^{12}	587±110^{12}
EH-1847	1.04±0.09^{23456}	0.08±0.13^{1}	9±0.01^{345}	150±0^{7}	107±0^{i}	2.66±0.07^{1}	24.7±1.3^{123}	46.9± 3.97^{12}	490±110^{12}
HB-1963	1.09±0.09^{4567}	0.39±0.13^{1}	16±0.01^{890}	138±0^{6}	86±0^{9}	2.63±0.07^{1}	27.0±1.3^{123}	58.3± 3.97^{2}	719±110^{2}
Beka	1.21±0.09^{67}	0.09±0.13^{1}	35±0.01^{a}	181±0^{e}	98±0^{e}	2.65±0.07^{1}	31.0±1.3^{3}	53.7± 3.97^{12}	676±110^{12}
HB-1533	1.02±0.09^{1234567}	0.08 ±0.13^{1}	10±0.01^{456}	133±0^{5}	74±0^{4}	2.53±0.07^{1}	24.7±1.3^{123}	46.8± 3.97^{12}	486±110^{12}
Bekoji-I	1.17±0.09^{567}	0.07±0.13^{1}	19 ±0.01^{90}	153±0^{9}	82±0^{7}	2.68±0.07^{1}	25.7±1.3^{123}	57.3± 3.97^{2}	532±110^{12}
HB-120	1.14±0.09^{567}	0.10±0.13^{1}	20±0.01^{0}	163±0^{0}	90±0^{a}	2.60±0.07^{1}	25.7±1.3^{123}	55.9± 3.97^{2}	608±110^{12}
Bahati	1.14±0.09^{567}	0.07±0.13^{1}	15±0.01^{789}	131±0^{4}	77±0^{6}	2.53±0.07^{1}	28.3±1.3^{23}	57.3± 3.97^{2}	531±110^{12}
Holker	1.11±0.09^{4567}	0.07±0.13^{1}	8±0.01^{34}	178±0^{d}	114±0^{j}	2.52±0.07^{1}	27.3±1.3^{123}	52.7± 3.97^{12}	352±110^{12}
Suba	1.44±0.09^{7}	0.10±0.13^{1}	31±0.01^{a}	151±0^{8}	76±0^{5}	2.62±0.07^{1}	29.7±1.3^{23}	50.7± 3.97^{12}	661±110^{12}
HB-1964	0.99±0.09^{123456}	0.10±0.13^{1}	13±0.01^{5678}	122±0^{3}	72±0^{3}	2.62±0.07^{1}	25.7±1.3^{123}	58.4± 3.97^{2}	520±110^{12}
M-21	0.93±0.09^{123456}	0.08±0.13^{1}	11±0.01^{4567}	173±0^{c}	82±0^{7}	2.47±0.07^{1}	23.7±1.3^{12}	55.7± 3.97^{2}	447±110^{12}
Ibon174/03	0.82±0.09^{123456}	0.33±0.13^{1}	14±0.01^{678}	107±0^{1}	61±0^{1}	2.51±0.07^{1}	25.7±1.3^{123}	54.7± 3.97^{12}	738±110^{2}

Same lowercase letters or numbers (within the column) indicates no significant difference in data expressed as means ±SD at p<0.05 according to Tukey test.

Principal coordinate analysis

The Principal Coordinate Analysis (PCoA) conducted on 22 Ethiopian malt barley cultivars resulted in the population being divided into three main groups (Figure 2). This analysis accounted for 60.9% of the total variation in the data. The first principal coordinate axis accounted for 50.3% of the variation, the second coordinate axis for 10.6%, and the third for 39.1%. The cultivars were organized into three distinct subgroups with notable genetic admixture: First Group: Suba and HB-1963 were clustered together. Second Group: Grace was isolated in this group. Third Group: This group contained nineteen cultivars, which were further subdivided as follows: Bahati ,HB-120 ,Ibon 174/03, Fatima Bekoji-I Traveller, HB-1533 ,EH-1847, Planet, Beka, Henerike, Holker, Proctor, Focus, HB-52, HB-1964, M-21, Sabin, Explore .

Hierarchical clustering

The cluster was conducted for 22 Ethiopian malt barley cultivars resulted in the population being divided into three main groups (Figure 3). The cultivars were organized into three distinct subgroups (the Clusters C1, C2, and C3) with notable genetic admixture: First Group: Suba and HB-1963 were clustered together .Genotype's grouped in cluster C1 had medium to the highest thousand grain weight 50.7± 3.97^{12} and 58.3± 3.972 and yield per plot area 661±11012 and 719±110^{2} while with the optimum range of protein content respectively. **Second Group:** Grace was isolated in this group. This second cluster (C2) consists of only one. This genotype group in the cluster is characterized by medium β-glucan content. Third Group: This group contained nineteen cultivars, which were further subdivided as follows: These include. Another cluster (C3) had 10 cultivars and was considered as the largest cluster with two subgroups. The cultivars grouped in this cluster include Bahati ,HB-120 ,Ibon 174/03, Fatima Bekoji-I Traveller, HB-1533 ,EH-1847, Planet, Beka, Henerike, Holker, Proctor, Focus, HB-52, HB-1964, M-21, Sabin, Explore (Fig. 3). Cultivars grouped in cluster III had the highest β-glucan content, high thousand grain weight and yield compared with other clusters (Table 4 &5).

Discussions

Crop successful genetic improvement program depends on the genetic diversity study. Such genetic diversity analysis was essential for plant breeders to perform strategic integration and target selection while preserving important economic traits of individual crops (Yirgu et al., 2023). In present study, we found 21 of the malt quality linked markers studied were polymorphic. Therefore, each of the primers was capable of differentiating the malt barley cultivars, so could be routinely used for assessing diversity, spectrum and malt quality trait introgression in Ethiopian malt barley cultivars.

The result of AMOVA indicated that variation among individual cultivars was higher than the variation with in individuals. The low level of variation within individuals indicates that the cultivars possess common alleles. Therefore variation among individuals is more important than with in individuals. Similar result was also reported by other researchers (Dido et al., 2022, Dido et al., 2022). This variance could be also explained by mutation rate and selection effect on the cultivars (Skot et al., 2005).

The average polymorphic information content (PIC) in the study is 0.88, with a range from 0.82- 0.94, with an overall mean of 0.88. Higher PIC of 0.78 and 0.73 was reported from ICARDA and Ethiopia, respectively (Dido et al., 2022, Jilal et al., 2008). In general, a PIC value greater than 0.5 is useful in genetic studies since it can distinguish a marker's polymorphism. Most of the markers in the current study had a score above 0.5, indicating that they were highly informative SSR markers that could be utilized in genetic diversity studies (Botstein et al., 1980) and breeding programs for malt quality trait introgressions. The high PIC observed in all of the SSR primers could be attributed to the diverse nature of the barley cultivars. In addition, high PIC values of the markers also indicate their multi-allelic nature of markers. These markers were highly informative and indicating the diversity of these cultivars and its potential as a source of novel alleles for malt barley breeding. Especially the marker Marker HVALAAT was highly polymorphic and can be used for breeding purpose. Therefore, all of the PCR products derived from these SSR markers are sufficient to monitor malt quality trait introgression in breeding materials.

The loci such as HVALAAT showed high observed heterozygosity than expected heterozygosity. This indicated that the presence of inbreeding depression or high mutation associated with the loci. However, high fixation index was recorded for the marker HVCMA, which indicates high level of inbreeding associated with the loci.

The ideal moisture content of malt barley cultivars may vary from 10-12% depending on their genetic make-up and local environment (Deme et al., 2020). The tested barley cultivars have moisture content level of 10-12%, which was in agreement with the international standard. The extract content for malt barley is in the range of 80% to 84% on a dry basis. However, low extract content was recorded for HB- 1533 has (75.8± 0.81%). Factors like enzyme starch content in the seed is known to be key players for variation in extract content (Kumar et al., 2013). Friability of 80% and above is preferred for brewing. Very low friability was recorded for the cultivar HB-52 (25.1±6.72^{1} %). Similar studies reported friability below 80% for barley genotyoes (Edney and Mather, 2004).The standard protein content for malt barley range from 8-12% on dry basis. Most of the tested cultivars were in 8-12% range, which is in agreement is the standard. However, the protein content of HB -1533 was the highest (13.18±0.669%), indicating its low suitability for brewing.

The hectoliter weight is an important measure of malt barley quality, reflecting the density and overall quality of the grain. For malt quality barley, the optimum hectoliter weight is generally considered to be around 63-71 kg/hl (kilograms per hectoliter), which is equivalent to 630-710 grams per liter. Higher hectoliter weights usually indicate plumper, well-filled kernels, which are preferred for malting because they tend to produce higher extract yields and better overall malt quality(Mathre, 1997; Kunze, 2004).All most all of the tested cultivars were in optimum range except HB-120 (62.2±1.66 kg/l). Studies indicated that heavier kernels are typically plumper, which is beneficial for water absorption during steeping. Therefore, higher hectoliter weights correlate with higher starch content, leading to higher extract yields, which is a critical factor for brewing efficiency.

The optimal heading and maturity dates for the best malt barley cultivar depend on several factors, including the specific growing region, local climate, and desired phenotypic and malt quality traits (Briggs, 1998; Mathre, 1997; Kunze, 2004). The tested malt barley cultivars were not significantly different for grain width, and spike length. However, highest plant length was observed for cultivar suba (1.44m), and the lowest for cultivar Explorer (0.58m). Significantly high seeds per spike were observed for cultivar Beka which is also comparable with Suba and HB-52. Therefore, the cultivar Beka can be used for developing high yielding malt barley cultivars in future breeding programs.

Conclusion

In conclusion, understanding the genetic variation of barely cultivars will provide useful information and abundant source genes for malt barley breeding program in Ethiopia to make new cultivars adapting to a wide range of ecological regions. The study of genetic variations among Ethiopian malt barley cultivars has demonstrated significant diversity in malt quality traits, as well as in the genetic markers associated with these traits. Therefore, the SSR marker liked to brewing traits could be used to monitor traits, evaluate genetic diversity and determine the geographic origin of the barley cultivars.

Declaration

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Author Contributions: MN performed the experiment and prepared the manuscript. GH conceived the project, analysed data, reviewed the manuscript. All authors read and approved the final manuscript.

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Ethical approval: Not applicable

Competing interests: The authors have no conflicts of interest to declare.

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Table

Table 1 is available in the supplementary files section

Figures

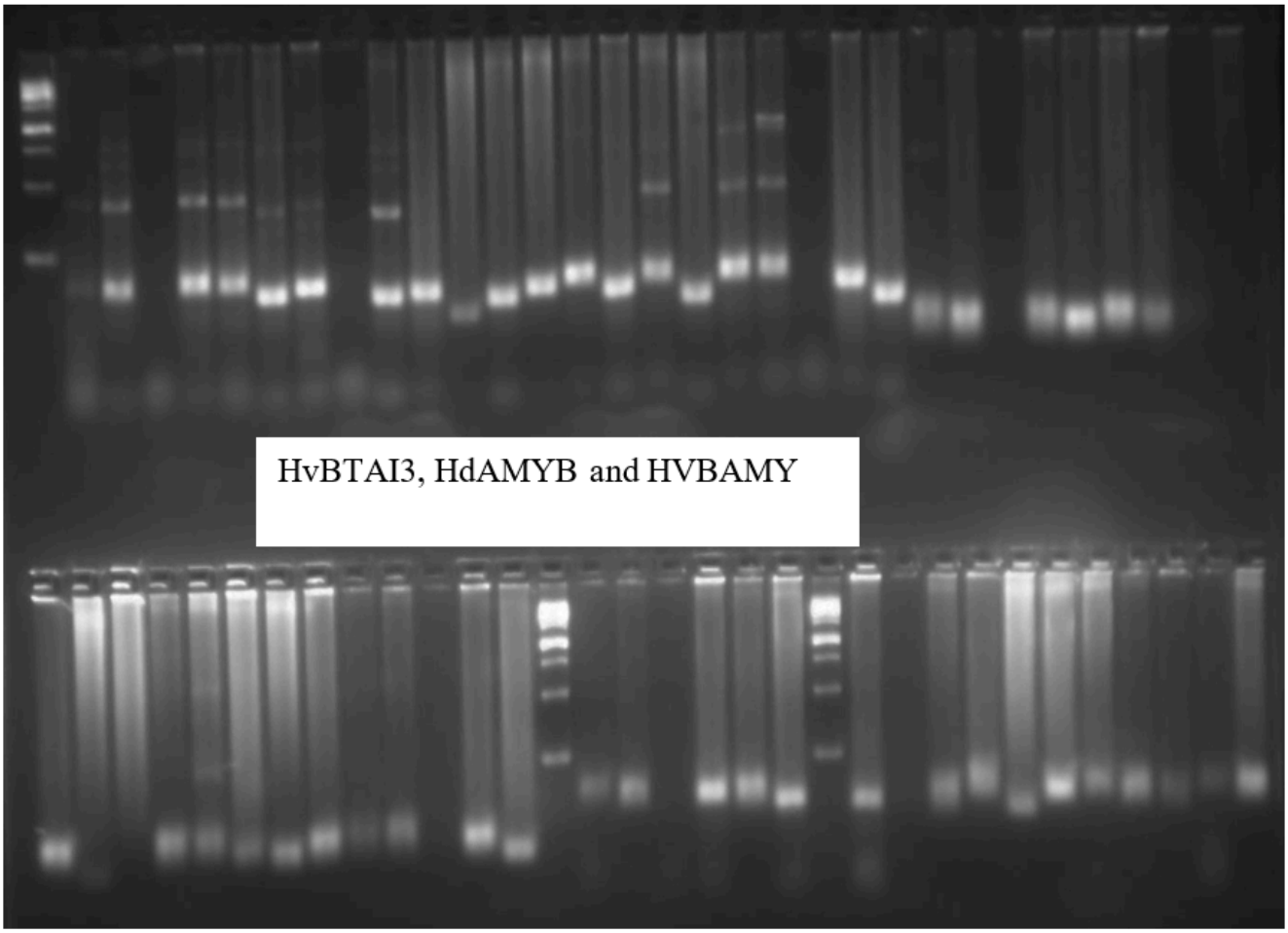


Figure 1

SSR markers profile of the 22 malt barley cultivars for the malt quality linked markers

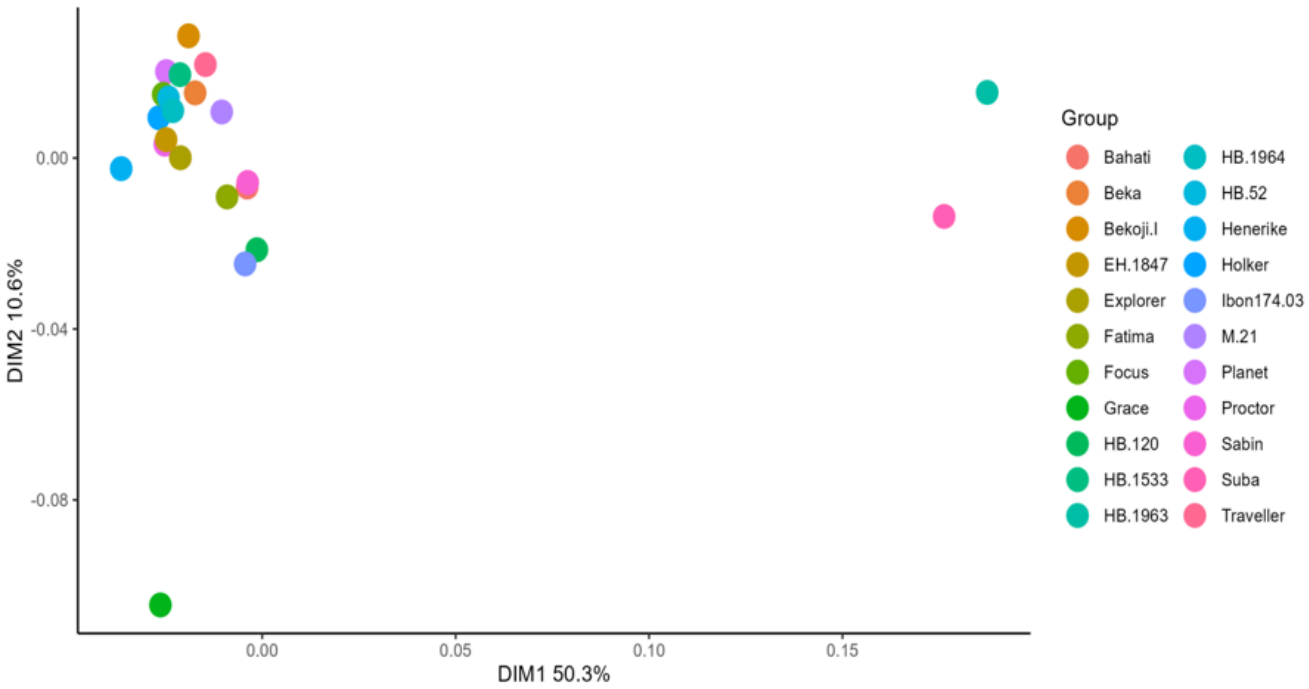


Figure 2

The PCoA plot shows the genetic relationships between the genotypes. Genotypes closer together are more genetically similar. The first two axes explain a large portion of the variance, indicating a good representation of the genetic diversity. Therefore PCoA of 22 malt barley cultivars using malt quality linked SSR marker

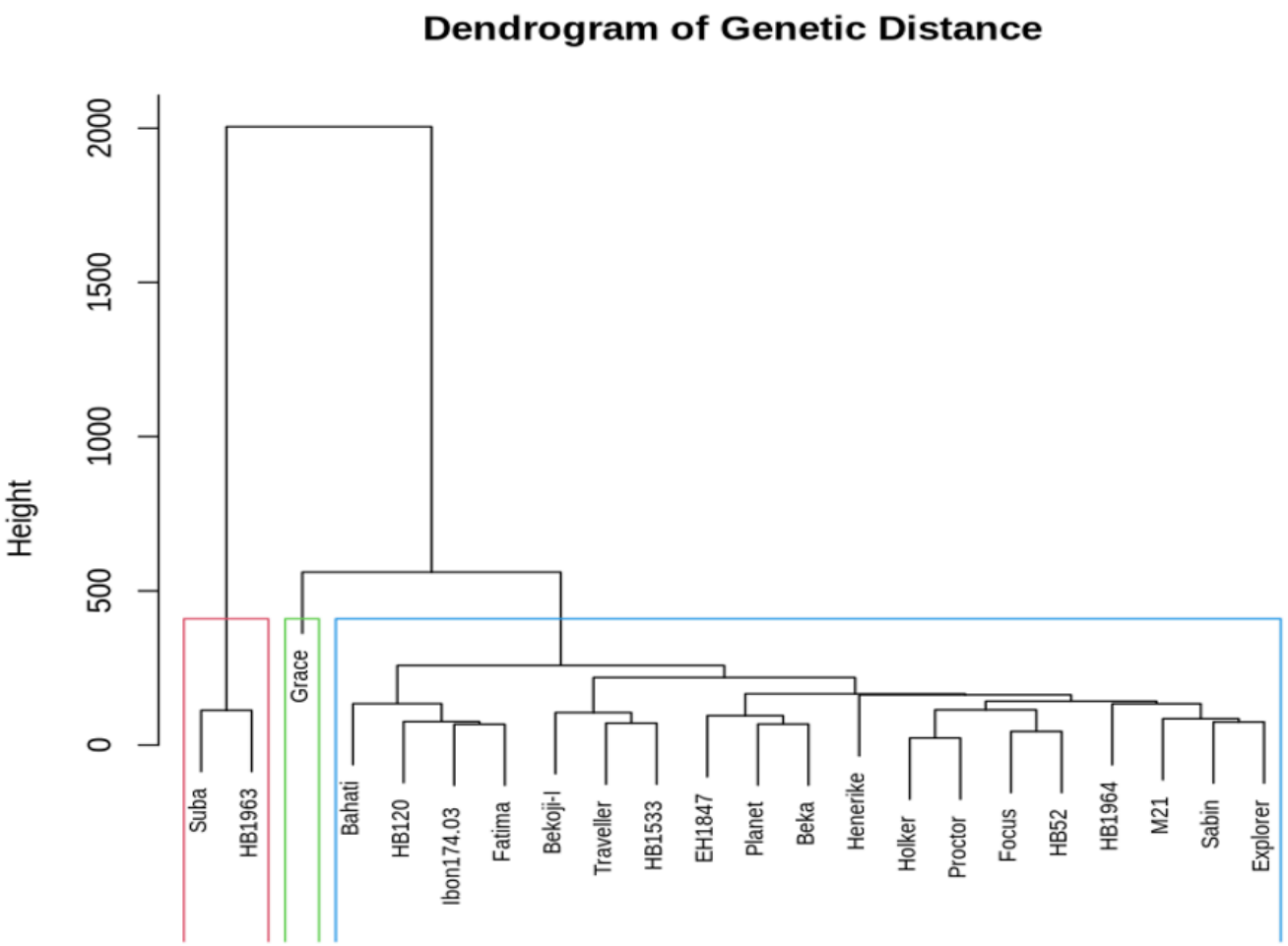


Figure 3

hierarchical clustering showing genetic relationship among 22 Ethiopian malt barley cultivars using malt quality linked SSR markers by using the genetic distance matrix and the dendrogram was generated using R4.3.1 software

Supplementary Files

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- MogesArticlesubmissionTable.docx
- Table1MaltqualitylinkedSSRmarkerusedforgenotypicthecultivars.docx