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Agro-morphological genetic diversity assessment of Amaranthus genotypes from Ethiopia based on qualitative traits

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Abstract

Amaranths are a promising plant in the family of Amaranthaceae because of their nutritional and functional properties, such as their high antioxidant content and dietary fiber content. However, it's being disregarded for several reasons, such as ignorance, a lack of in-depth research, and the plant's long-term genetic growth in Ethiopia, among other factors. In the current work, we described the genetic diversity of 120 amaranth genotypes using qualitative criteria. The experiment, which was configured with an alpha lattice design and duplicated twice, was run for two seasons in 2020 and 2021. Twenty qualitative descriptors were looked for in the gathered data. Among the 20 qualitative variables that were assessed, the chi-square test result indicated the presence of prevailing phenotypic variation. The results of the agro-morphological characterization also revealed a significant amount of variance. The overall mean of the Shannon diversity indices (H') was 0.61. The indices for germination rate, leaf margin, prominence of leaf veins, and the existence of auxiliary inflorescence varied from 0.12 to 0.99. The estimated diversity indices showed more intra-regional diversity (0.66) than inter-regional diversity (0.34), demonstrating the existence of gene flow between growing regions. Shannon–Weaver Diversity Index, ranged from 0.00 for auxiliary inflorescence to 1.94 for leaf coloration, with an overall mean of 19 characters (95%) that were found to have high diversity (> 0.76) while auxiliary inflorescence was invariant. Except for auxiliary inflorescence, all qualitative features showed a lot of variation. Additionally, *Amaranthus hybridus L. subsp. cruentus* (L.) Theil recorded the greatest Shannon diversity index (0.47) while Amaranthus spinosus L. recorded the lowest (0.00). The hierarchical clustering grouped all the genotypes into three clusters. The first cluster included the most genotypes (58), followed by the second (47), and the third cluster contained the fewest (15). Principal component analysis showed that the first six principal components with eigenvalues greater than one contributed 72% of the variability among genotypes. The study unequivocally demonstrated that, even when the genotypes were grouped into a small number of clusters, there was still enough divergence within the clusters to demonstrate the genotypes of amaranth to have a high genetic diversity. These results indicate that there is substantial genetic diversity among Ethiopian amaranth genotypes, which should be safeguarded and may be utilized in breeding in the future.

Keywords Agro-morphological, Descriptor, Diversity, Genotype

Introduction

Plant biodiversity is the primary source of food, feed, shelter, medicine, and a variety of other products and services that make life feasible and enjoyable on Earth (Padulosi et al. 2002). The number of plant species utilized by humans around the world accounts for only

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one-third of the total number of species used by generations of people from various cultures (Heywood and Watson 1995). Incorporating alternative crops with a good nutrient profile could promote dietary diversity and be a crucial part of raising food quality to battle unmet hunger and boost biodiversity. Amaranth is one of the few highly nutritive crops that serve multiple purposes, including those of vegetable, cereal, medicinal plant, dye plant, pasture, fuel, and ornamental (Sheikh and Singh 2013; Mlakar et al. 2009). Research and development on amaranths could result in a simple and affordable method of eradicating malnutrition, boosting people's health, and ensuring food security.

The name *Amaranthus* comes from the Greek word amarantos, which also means "never fading," "immortal," "everlasting," "non-wilting," or "one that never perishes."(Anjali et al. 2013) because its flowers last for a long time (Anjali et al. 2013; Rastogi and Shukla 2013). High genetic variability is prevalent in *Amaranthus*, which signifies variation in plant form (erect to prostrate), seed color, seed yield, protein content, resistance to pests and diseases, and adaptation to soil type, pH, climate, rainfall, and day length (Assad et al. 2017; Tejaswini et al. 2017). *Amaranthus* is a genus comprising 87 species (Jacobsen et al. 2003). Due to a lack of systematic investigations into amaranth, the number of its species is unclear (Costea and DeMason 2001). *Amaranthus* is a versatile crop that is grown all over the world for use as a vegetable, cereal, medicinal plant, dye plant, pasture, fuel, ornamental, and weedy plant (Wu et al. 2000; Sheikh and Singh 2013). Amaranth, a long-standing part of traditional African agriculture, is semi-domesticated in Ethiopia and other East African countries, primarily as a vegetable (Alemayehu et al. 2015). Due to the widespread usage of local names and the large genetic variation present in Ethiopia, the country has been suggested as a hub of amaranth diversity. Eleven species have been identified in the flora regions of Ethiopia and Eritrea (Demissew 2010).

Amaranthus was named a promising economically valuable underutilized plant by the National Academy of Sciences and it is regarded as the most important raw resource for food and nutrition, as well as a powerful medicinal plant (Shodiev et al. 2021). According to Ishimoto and Monteiro (2010), these plants contain each attribute of a functional food. Moreover, because amaranth flour is gluten-free, it might be a suitable source of nutrition for people who are intolerant to gluten. Amaranth leaves are anti-cancerous; they prevent the erratic division of cancer cells in the breast, colon, and liver, making them suitable for cancer patients as well (Li et al. 2015). As a result, including these grains in one's diet has the potential to enhance one's diet while also providing health advantages. Moreover, amaranth grains have a greater protein content than other typical cereals (Gamel et al. 2004). Pseudo cereals' nutritional value is primarily determined by their protein content. Amaranth grains are an excellent source of proteins with a well-balanced amino acid profile, with a high concentration of lysine and sulfur-containing amino acids (Haros and Schoenlechner 2017). Amaranth usually has more protein than quinoa or buckwheat (Haros and Schoenlechner 2017). The high concentration of lysine, which is required by the human body but cannot be produced by it, is a significant benefit of amaranth. Amaranth is unusually high in this essential amino acid, which is found in low or limited amounts in other grains and plant sources. Amaranth is equivalent to other animal proteins in terms of lysine and tryptophan levels (Correa et al. 1986).

Morphological features are helpful for early evaluation since they are quick, easy, and may be utilized as a generic technique for evaluating genetic diversity across morphologically different genotypes. Regarding the morphological features investigated, the current study showed significant variance among the germplasm. *Amaranthus* has a remarkable morphological diversity and can adapt to a wide range of ecological and geographical environments (Lee et al. 2008). Despite the vast phenotypic diversity across and within amaranthus species, the genus has just a few taxonomic characteristics that are unique to it (Sammour et al. 1993; Juan et al. 2007). Genetic diversity is mostly determined by the agro-morphological characterization of genotypes and it is essential for biodiversity conservation and crop improvement efforts to be successful (Shah et al. 2018). The assessment of a crop species' genetic diversity is an excellent place to start when it comes to crop improvement since it provides a framework and a roadmap for choosing parental lines and designing a breeding program. It's crucial to the creation of new crop varieties with desirable characteristics. As a result, determining the genetic diversity of current genotypes is a prerequisite for crop improvement. Unfortunately, genetic research of the genus amaranth is very limited in Ethiopia due to lack of extensive research, discrimination, and lack of knowledge, the improvement of this plant is limited. Furthermore, researchers are less interested in this "orphan crop," as it is known (Jonah et al. 2012). The study on amaranth has significantly aided in improving amaranth growth by building a value chain for the manufacturing of value-added products based on amaranth that may aid in food security and nutritional quality. Even though several countries have reported on the distribution of amaranth species used like cereal grains and the production of ancient grain products, Ethiopia has yet to conduct similar research.

The amaranth genus reportedly exhibits high levels of morphological variety (Molin and Nandula 2017; Srivastava and Mahavidyalaya 2015; Oyetunde et al. 2021). For describing species genotypes, the qualitative features have been considered advantageous (Gerrano et al. 2015). Amaranth genotypes have not yet been the subject of any specialized research in Ethiopia. The objective of the current study is to define the genetic diversity in Ethiopian amaranth genotypes by including qualitative agro-morphological features. Therefore, study on genetic varieties is essential for giving information for efforts pertaining to domestication, breeding, and propagation, as well as for the preservation of the genetic resources of plant species. Consequently, the results are intended to promote future genetic research aiming at strengthening this unusually nutrient-rich and climate-resilient crop species by employing qualitative agro-morphological features.

Materials and methods

Experimental site

The experiment was conducted at the Hawassa University Farm and Research Center in the years 2020 and 2021. Geographically, it is situated at 7° 2′ 54.7503" N and 38° 30′ 17.1608" E, at a height of 1709 m above sea level, in Ethiopia's Sidama area, about 275 km from Addis Abeba Fig. 1. The pH range in the experimental region was between 6 and 6.5, and the soil texture class was clay loam. The region's average lowest and highest monthly temperatures are 14.10 °C and 27.9 °C, respectively. The experimental farm had an average rainfall of 1379.16 mm throughout two growing seasons).

Plant material and experimental procedures

A total of 120 amaranth genotypes from seven different species— *A. hybridus* L. *subsp. hybridus, A. hybridus* L. *subsp. cruentus (*L.*)* Theil.*, A. caudatus* L.*, A. viridis* L*, A. spinosus* L.*, A. palmeri* S. Wats.*, and A. species*—were used for the qualitative genetic diversity study, with 34 coming from the Ethiopian Biodiversity Institute (EBI), 2 from the Melkasa research center, 15 from the Werere research center (Afar region), and the remaining 69 coming from three different regions of Ethiopia in 2019 (Oromia, Tigray, Amhara, and Sidama, as well as the Southern Nations Nationalities and Peoples' Region). While the remaining two genotypes do not and are referred to as released variations, one hundred eighteen genotypes include passport information (Table 1).

Experimental layout and crop management

The experiment was planted on April 15th, 2020, and 2021 of the two growing seasons, the seeds were sown at the same location by using an alpha lattice design with two replications under rain-fed conditions. The two growing seasons were considered as two environments.

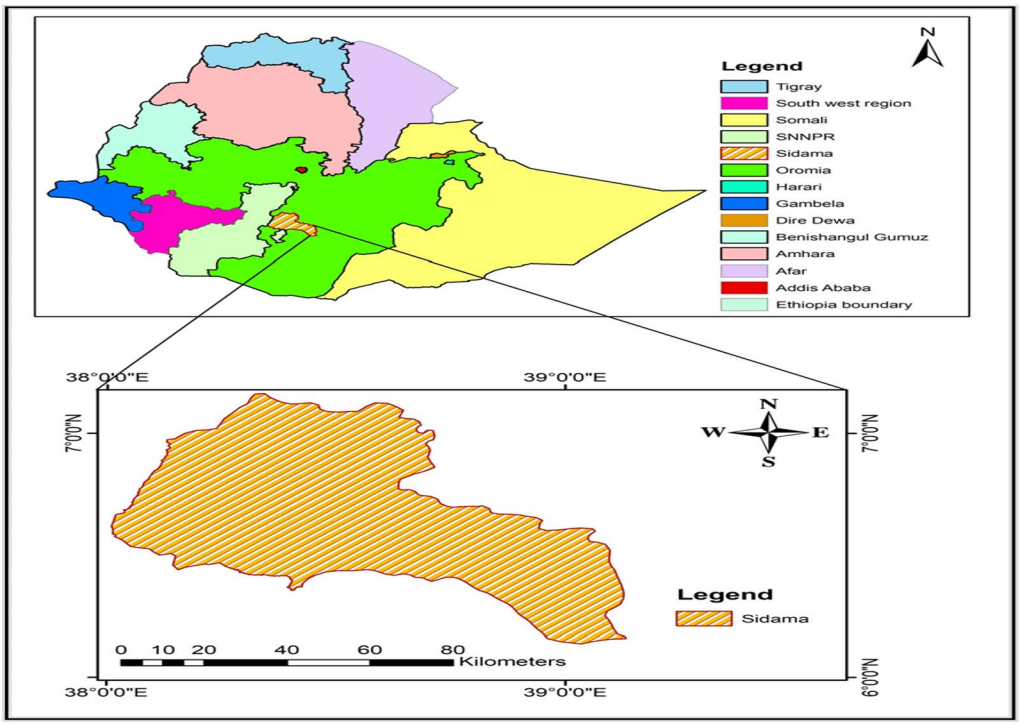

Fig. 1 Map of the study area

Table 1 List and location of plant materials included in the study

Genotype Name	Easting	Northing	Altitude
KEN-065	321813	583339	1756
YRC-048	411783	675273	1995
KAZ-077	327268	599608	1202
KEN-067	321745	585148	1760
211457	326440.36	589244.33	1560
242917	812954	793136	2200
KEN-011	299762	603070	1739
241764	306077	591230	1520
HA-003	636126	1046188	726
KAZ-076	327267	599336	1202
208683	758052	1043629	2270
KEN-016	322071	584756	1745
MEK-084	552161	1490423	2155
HAL-039	407697	806621	1834
212892	315406	622936	2200
211455	328390	645017	1150
HAW-041	444076	776871	1750
KAZ-055	326719	609804	1213
DRA-053	324631	614748	1290
ALE-023	299964	597729	1370
KEN-015	320119	586057	1819
ALE-069	299971	597724	1376
DIL-050	421120	709519	1459
KAZ-057	326722	609738	1214
KEN-022	321811	585162	1751
SHIA-007	627807	1036846	735
240815	354053.54	754795.3	1950
209056	312304.71	699421.8	1660
ABL-004	369796	734542	1367
ALE-034	295826	597501	1316
ALE-024	299885	597965	1368
SAA-004	636126	1046188	726
212582	572790	1234490	1840
ALE-068	299970	597736	1384
212581	581861	1245570	2920
KAZ-007	327352	599054	1187
204645	327492.26	590316.19	1600
225717	348942	740795	1440
KEN-019	321816	585168	1754
91003	233325	835289	2040
242533	665945	1167179	1250
KEN-013	319707	586409	1772
BNT-026	247228	615060	1618
AC-NL	Released variety	Released variety	1550–2400
Madiira II	Released variety	Released variety	1550–2400
BKD-027	232336	637249	1427
204644	334751.47	653902.46	1200
225718	348942	740795	1440
ALE-070	299981	597743	1329
OTI-044	431913	721434	1893
KAZ-008	326435	604708	1204
MEK-081	552793	1489902	2143
KAZ-056	326684	609708	1214
KEN-018	321785	585144	1761
BA-001	628326	1026376	743
ALE-074	299762	603000	1655
209057	341220.34	740810	1580
KAZ-059	327268	599608	1202
KEN-010	319325	585745	1729
KEN-012	319703	586470	1760
215567	372960	768376	2100
ALE-025	300048	597628	1349
SHD-001	434789	761187	1874
ALE-073	301337	604237	1693
DAL-043	429799	738442	1790
SA-001/07	624955	1028024	736
SRA-002	632177	1031734	742
219284	358268	783157	1750
HA-008	634804	1044976	730
ALE-071	299973	597729	1372
240814	322866.04	764187.65	1250
KAZ-060	319338	585751	1734
MEK-083	552491	1490761	2151
BKD-031	234460	634949	1400
WON-049	418632	699405	1743
KAZ-058	327268	599608	1202
KAZ-006	327308	598524	1188
BKD-029	234471	634953	1409
WA-003	628204	1033787	741
YRB-002	424075	769481	1857
KEN-021	321818	585166	1752
KAZ-079	326433	604711	1204
KEN-064	321816	585168	1762
SHL-040	433633	805425	1662
KEN-017	322084	584791	1762
242532	665945	1167179	1250
SA-008/07	624844	1026573	740
240813	323825.31	589809.06	2750
208764	698483.15	943746.8	1850
MEK-080	552552	1490147	2162
BA-016	629359	1027955	740
WA-001	627878	1033651	740
SA-025/07	625039	1026746	735
DMG-038	380667	777365	1881
KAZ-078	327269	599337	1200
242531	195529	1185259	1250
DA-006	630310	1034519	735
211456	314087.91	599272.76	1570

Table 1 (continued)

Genotype Name	Easting	Northing	Altitude
CHU-045	431835	721533	1910
DUF-003	394497	770836	2113
ADZ-037	389243	797508	1912
212583	570917	1258445	1640
208025	300580	1354876	2100
225713	311940	696674	1600
KEN-014	319660	586850	1690
91001	315299	584231	1890
212893	324653	628440	1380
ALE-033	282131	591864	589.5
KEN-020	321817	585166	1753
K3A-005	631670	1035541	738
242530	665945	1167179	1250
BKD-028	234471	634953	1409
SA-005/07	624847	1026721	737
KAZ-009	325782	604450	1213
242534	665945	1167179	1250
212890	382230	795996	2180
DAL-042	431596	753041	1780
MEK-082	552972	1490517	2140
225715	258450	696873	1780
ALE-075	299919	603075	1640

Seeds of a genotype were consistently sown in two rows with a spacing of 0.75 m between the rows. Since the seeds are quite tiny, they were combined with sand in 1:4 ratios and were sown on seedbeds and covered with finely powdered farmyard manure. At 14 and 22 days after sowing (DAS), seedlings were thinned down to 6 plants per row with a spacing of 30 cm between plants. According to Grubben and Van Sloten (1981a) and Shukla et al. (2006), the experiment followed standard cultural practices. Weeding was done by hand-hoeing at 2-week intervals following germination and whenever necessary.

Agro-morphological qualitative traits and data collection

To characterize the genotypes under study, observations were made on various morphological qualitative traits at distinct phenological stages. As suggested by the International Board for Plant Genetic Resources based on taxonomic keys (Grubben and Van Sloten 1981b), data for amaranth descriptors were recorded. Data also were collected on some characters that were not shown in the list of standard descriptors since these were considered necessary for the characterization. In this investigation, a total of 20 qualitative characters were considered for characterization. Visual observation was used to evaluate all of the qualitative traits. The colors of the different plant parts were determined using the RHS color chart (Voss 2002). Vegetative, inflorescence, and seed data were among the 20 characteristics evaluated. The list of qualitative agro-morphological traits along with their phenotype code is shown in Table 2.

Data analysis

Frequency and Shannon diversity index

The frequency distribution of qualitative traits and basic statistical parameters (number of genotypes, percentage of genotype, Shannon diversity index) based on qualitative morphological traits were analyzed using Minitab 19.1 (Minitab 2019). The phenotypic frequency distribution of the phenotypic markers was calculated for each genotype. The Shannon Weaver Diversity Index was constructed using frequency data. The Shannon–Weaver diversity index (H') was used to assess and interpret the phenotypic diversity for each of the markers using phenotypic frequencies. This index, according to (Perry and McIntosh 1991), is given as:

$$H' = -\sum_{(i=1)}^{n} piInpi; Hmax = lnS$$

Where n is the number of phenotypic classes in a given categorical character, and pi is the relative proportion of the total number of entries (N) in the Ith class I (1, 2... n). S is the number of traits category in a qualitative trait of Ethiopia genotypes, H' is the Shannon–Weaver diversity index of a given character, which is lastly standardized to H' as a ratio of H' by ln (n) to normalize and keep the value between 0 and 1, by pooling various characters across the locations (regions), the additive properties of *H*' were used to evaluate the diversity of locations and characters within the population. For a monomorphic population, the index has a minimum value of zero. The index value rises as polymorphism grows and reaches its maximum value when all phenotypic classes have equal frequencies (Yang et al. 1991).

Based on their collecting region and released variety, the genotypes were divided into eight groups for data analysis: Afar, Amhara, Benishangul and Gamuz, Unknown, Oromia, Sidama, Southern Nations Nationalities and Peoples Regions in Ethiopia, and Tigray. The phenotypic diversity of the overall sample and the sample group for each collecting region were both measured using Shannon and Weaver (1949a).

The Wachira et al. (1995), techniques were used to divide the phenotypic diversity across regions of collection $= \frac{(H'sp - H'cr)}{H'sp}$ and within collections $= \frac{(H'cr)}{H'sp}$

Where: H_{cr} and H_{sp}, are Shannon–Weaver diversity index across collection regions and species, respectively.

Table 2 List of qualitative agro-morphological traits used along with their phenotype code, Description and phenotype scores

Qualitative marker	Phenotypic Code	Descriptor and code
Germination rate	GR	1 = Rapid, (< 2 days), 2 = Slow (2–7 days), 3 = Very slow (> 7 days),4 = Irregular
Growth habit	GH	1 = Erect, 2 = Prostrate
Branching index (score if erect)	BI	1 = No branches, 2 = Few branches (all near the base of the stem), 3 = Many branches (all near the base of the stem), 4 = Branches all along the stem
Stem pubescence	SP	0 = None, 3 = Low, 7 = Conspicuous
Stem pigmentation	SPG	1 = Green, 2 = Purple or pink, 3 = White, X = Mixture
Spines in leaf axils	SLA	1 = Absent, 2 = Present, X = Mixture
Leaf pubescence	LP	0 = None, 3 = Low, 7 = Conspicuous
Leaf pigmentation	LPG	1 = Entire lamina purple or pink, 2 = Basal area pigmented, 3 = Central spot, 4 = Two stripes (V-shaped),5 = One stripe (V-shaped), 6 = Margin and vein pigmented, 7 = One pale green or chlorotic stripe on normal green, 8 = Normal green, 9 = Dark green, 10 = other (specify), X = Mixture
Leaf shape	LS	1 = Lanceolate, 2 = Elliptical, 3 = Cuneate, 4 = Obovate, 5 = Ovatainate, 6 = Rhombic, 7 = Oval,8 = other (specify), X = Mixture
Leaf margin	LM	1 = Entire, 2 = Crenate, 3 = Undulate, 4 = other (specify), X = Mixture
The prominence of leaf veins	PLV	1 = Smooth, 2 = Rugose (veins prominent)
Petiole pigmentation	PP	1 = Green, 2 = Dark green, 3 = Amaranth, 4 = Dark purple, 5 = White, X = Mixture
Terminal inflorescence shape	TIS	1 = Spike (dense),2 = Panicle with short branches, 3 = Panicle with long branches, 4 = Club-shaped at tips, 5 = Thyrse, 6 = Raceme, X = Mixture
Terminal inflorescence Attitude	TIA	1 = Erect, 2 = Drooping
Inflorescence density index	IDI	3 = Lax, 5 = Intermediate, 7 = Dense
Inflorescence color	IC	1 = Yellow, 2 = Green, 3 = Pink, 4 = Red, 5 = Amaranth, 6 = Golden, 7 = White, X = Mixture
Seed shattering	SSH	1 = Low (< 10%), 2 = Intermediate (10–50%), 3 = High (> 50%),
Seed color	SC	1 = Pale yellow, 2 = Pink, 3 = Red, 4 = Brown, 5 = Black
Presence of axillary inflorescence	PAI	1 = Absent 2 = Present
Sex type	SX	1 = Monoecious, 2 = Dioecious, 3 = Polygamous

Cluster-analysis

To group the 120 genotypes and to bring out the patterns of similarity and dissimilarity based on 20 qualitative morphological characters, the Past version (4.0.9) and different branches of the trees were differently colored using Fig Tree v1.4.3 (Rambaut 2014) were used to perform cluster-analysis based on ward hierarchical grouping using Euclidian distance. The optimum number of clusters in a data set was determined by using the average silhouette method for the dendrogram with various packages of R software (Maechler 2019).

Genetic distance analysis

By utilizing Mahalanobis distance (D^2) statistics to calculate the genetic distance between the clusters, the connection between them was evaluated (Mahalanobis 2018). Using the PROC procedures methods of SAS version 9.4 (Allison 2012), extended Mahalanobis' D^2 statistics were used to quantify the genetic divergence within and across clusters.

Principal component

Principal component analysis (PCA) was used to ascertain which attributes were most responsible for the overall variation. To have a significant genotype dispersion, the PCA components with cumulative variability must account for at least 75% of the overall variability (Govindaraj et al. 2020).To identify the number of principal components (PCs), eigenvalues larger than unity were employed as a criterion (Iezzoni and Pritts 1991). The eigenvalues frequently decline gradually as the number of PCs rises, and their sum is equal to the number of features employed in the study. The features with the greatest absolute values of eigenvectors for each PC were identified by comparing the eigenvectors of the PCs, and these traits were then taken into consideration for selection because of their significant contributions to yield improvement (Dhakar et al. 2019).Characteristics with higher coefficients, typically 0.6 or more, on the PC axis are generally considered more important (DeLacy and Cooper 1990; Jolliffe 2002). The positive and negative loading indicates the presence of positive and negative

correlation trends between the components and the variables. Therefore, the above-mentioned traits that load high either positively or negatively contributed more to the diversity, and they were the ones that most distinguished the clusters.

With the help of the statistical program FactoMineR and factoextra packages using R studio, the eigenvalues, and eigenvectors of the PCs were calculated using FactoMineR and factoextra packages using R studio. Additionally, the first two PCs' scree plots were generated using the FactoMineR and factoextra packages using R studio.

Results

Qualitative traits variation

For various qualitative traits, several morph types were observed in the current study. Nineteen of the twenty qualitative traits were found to be variable (discriminated 120 amaranth genotypes) and one was determined to be invariant (did not discriminate the genotypes). The genotypes could be distinguished by their germination rate, branching index, stem pubescence, stem pigmentation, spines in leaf axils, leaf pubescence, leaf pigmentation, leaf shape, leaf margin, prominence of leaf veins, petiole pigmentation, terminal inflorescence shape, terminal inflorescence attitude, inflorescence density index, seed shattering, seed color, and sex type (Table 3). Visually collected qualitative data is presented in the form of a table and figure, revealing the frequencies and relative percentages of each phenotype occurring in amaranth genotypes in combined forms, as well as the percentage of genotypes possessing a specific phenotypic trait (Table 3). In amaranth genotypes' several qualitative traits were observed, examined, and displayed in a histogram Fig. 2.

The genotype's predominantly (75%) germination rate was slow (2–7 days) and the rest of the germination rate was very slow (>7 days). The growth habit of most of the genotypes was erect (86.67%) and 13.33% of the genotypes were prostrate. The branching index of genotypes having an erect type growth habit showed branches all along the stem were 85.83% and many branches all near the base of the stem were 4.17%. Stem pubescence for a majority of the stem was low (79.17%), 19.17% of the genotypes were absent, and the remaining 1.67% of the genotypes had conspicuous stem pubescence. Stem pigmentation showed significant color variation, with approximately 41.67% of genotypes being mixtures, 40.83% being green, white, and pale colors, each having 7.5%, and the remaining 2.53% being red (Fig. 3a). The terminal inflorescence shape was largely (39.5%) panicle with long branches, followed by a mixture (32.5%), thyrsi (14.17%), club-shaped at tips (8.33%), and raceme (4.17%), and the least common was panicle with short branches and spike (dense) (83%). The majority of genotypes had terminal inflorescence attitudes erect (87.5%), whereas the rest showed drooping (12.5%). Inflorescence density indexes of lax, intermediate, and dense types were observed in the present study. Among them, 46 genotypes (38.33%) had a lax inflorescence density index, 42 genotypes (35%) had an intermediate inflorescence density index, and 32 (26.67%) had a dense inflorescence density index. Six types of inflorescence color were observed in the present study of which 59 genotypes (49.17%) had amaranth color inflorescence, 8 genotypes (6.67%) had pink color inflorescence, 34 genotypes (28.33%) had green color inflorescences, 9 genotypes (7.5%) had golden and white inflorescence colors each, and the remaining 1 genotype (0.83) had red color inflorescence (Fig. 3b). The majority of genotypes (75.83%) had black seed colors, followed by white (15%), brown (6.67%), pale yellow (1.7%), and red (0.83%) (Fig. 3c). The leaf pigmentation presented a marked variation, 27.5% were normal green, 15% dark green, 17.5% margin and vein pigmented, 11.6% one pale green or chlorotic stripe on the normal green, 9.17% one stripe (V-shaped), 8.33% two stripes (V-shaped), 8.33% the entire lamina purple or pink, 1.67% mixture, and 0.83% had the central spot (Fig. 3d). A majority (86.67%) of the genotypes were devoid of spines in the leaf axis, and 13.33% of the genotypes had spines in the leaf axis. The predominant leaf-shape genotypes were lanceolate (57.5%), elliptic (25.83%), and ovatinate (10.83%), whereas the rest were rhombic (5.83%) in shape. With regards to the distribution frequency of leaf margin, significant variation was observed among the genotypes (98.33%) that had undulated leaf margin, followed by crenate (1.67%). Similarly, it was observed that 98.33% of the genotypes had the prominence of leaf veins that were rugose (veins were prominent), while 1.67% showed smooth leaf veins. Four types of petiole pigmentation were noticed in the present study, among which were green (40.00%), white (32.5%), mixture (25%), and amaranth (2.5%). In general, the distribution frequency of the phenotypes revealed considerable variances, with certain morph types being uncommon while others being frequent and proportionately dispersed over the genotypes (Table 3).

Estimation of (%) of frequency distribution, and Chi-square

Table 3 shows the Estimation of (%) of frequency distribution, and chi-square. The chi-square test value showed the existence of dominant phenotypic variation among the evaluated 20 qualitative traits. The observed value was higher than the expected phenotypic value. However, all scored qualitative expressive traits showed a very highly significant difference except for the inflorescence density index which is not significant. This indicates the recorded data showed dominant phenotypic classes for seed color (171.5) followed by a terminal inflorescence

Table 3 Specific qualitative morphological traits, their scores, equivalent phenetic characters, (%) contribution to variation, and Chi-square for 20 morphological qualitative traits during the analysis of 120 Genotypes from Ethiopia

Qualitative marker	Character state	Proportion	Chi-square	% Contribution
Germination rate	Slow (2–7 days)	90.00	112.1***	75.00
	Very slow (> 7 days)	30.00		25.00
Growth habit	Erect	104.00	64.5***	86.67
	Prostrate	16.00		13.33
Branching index	Many branches (all near the base of the stem)	17.00	61.63***	14.17
	Branches all along the stem	103.00		85.83
Stem pubescence	None	23.00	118.95***	19.17
	Low	95.00		79.17
	Conspicuous	2.00		1.67
Stem pigmentation	Green	49.00	61.7***	40.83
	Pale(golden)	9.00		7.50
	Red	3.00		2.53
	White	9.00		7.50
	Mixture	50.00		41.67
Spines in leaf axils	Absent	104.00	64.5***	86.67
	Present	16.00		13.33
Leaf pubescence	None	40.00	13.3***	33.33
	Low	80.00		66.67
Leaf pigmentation	Mixture	2.00	58.2***	1.67
	Entire lamina purple or pink	10.00		8.33
	Central spot	1.00		0.83
	Two stripes (V-shaped)	10.00		8.33
	One stripe (V-shaped)	11.00		9.17
	Margin and vein-pigmented	21.00		17.50
	One pale green or chlorotic stripe on normal green	14.00		11.67
	Normal green	33.00		27.50
	Dark green	18.00		15.00
Leaf shape	Lanceolate	69.00	78***	57.50
	Elliptical	31.00		25.83
	Ovatainate	13.00		10.83
	Rhombic	7.00		5.83
Leaf margin	Crenate	2.00	112***	1.67
	Undulate	118.00		98.33
The prominence of leaf veins	Smooth	2.00	112.1***	1.67
	Rugose (veins prominent)	118.00		98.33
Petiole pigmentation	Green	48.00	37.8**	40.00
	Amaranth	3.00		2.50
	White	39.00		32.50
	Mixture	30.00		25.00
Terminal inflorescence shape	Spike (dense)	1.00	121.9***	0.83
	Panicle with short branches	1.00		0.83
	Panicle with long branches	47.00		39.17
	Club-shaped at tips	10.00		8.33
	Thyrse	17.00		14.17
	Raceme	5.00		4.17
	Mixture	39.00		32.50
Terminal inflorescence attitude	Erect	105.00	67.5***	87.50
	Drooping	15.00		12.50

Table 3 (continued)

Qualitative marker	Character state	Proportion	Chi-square	% Contribution
Inflorescence density index	Lax	46.00	2.6^{ns}	38.33
	Intermediate	42.00		35.00
	Dense	32.00		26.67
Inflorescence Color	Green	34.00	42.05***	28.33
	Pink	8.00		6.67
	Red	1.00		0.83
	Amaranth	59.00		49.17
	Golden	9.00		7.50
	White	9.00		7.50
Seed shattering	None	20.00	16.1***	16.67
	Low (< 10%)	19.00		15.83
	Intermediate (10–50%)	45.00		37.50
	High (> 50%)	36.00		30.00
Seed color	White	18.00	171.5***	15.00
	Pale yellow	2.00		1.70
	Red	1.00		0.83
	Brown	8.00		6.67
	Black	91.00		75.83
The presence of auxiliary inflorescence	Present	120.00	–	100.00
Sex type	Monoecious	106.00	70.5***	88.33
	Dioecious	14.00		11.67

ns non- significant

** significant at $p \leq 0.01$

*** significant at $p \leq 0.001$

shape (121.9), stem pubescence (118.95), germination rate (112.1), the prominence of leaf veins (112.1), leaf margin (112), leaf shape (78), sex type (70.5), growth habit (64.5), spines in leaf axils (64.5), branching index (61.63), stem pigmentation (61.70), and leaf pigmentation (58.2) showed dominant than inflorescence density index (2.6), leaf pubescence (13.3), seed shattering (16.1), petiole pigmentation (37.8), and inflorescence color (42.05).

Estimates of phenotypic diversity

Tables 4, 5 show the Shannon–Weaver diversity index (H') estimated across all genotypes, across collection regions, and within collection regions, and the maximum Shannon's Weaver diversity index for all 20 qualitative morphological traits examined across all genotypes. Individual morphological qualitative Shannon diversity indices (H') ranged from 0.12 for germination rate, leaf margin, the prominence of leaf veins, and the presence of auxiliary inflorescence to 0.99 for inflorescence density index, with an overall mean of 0.61. Low genetic diversity (0.12) and monomorphic were found in the phenotypic markers auxiliary inflorescence, germination rate, leaf vein prominence, and leaf margin. In contrast, intermediate genetic diversity (0.52–0.60) and polymorphism were found in the phenotypic markers growth habit, sex type, branching index, stem pubescence, spines in leaf axils, and terminal inflorescence attitude. Stem pigmentation, terminal inflorescence shape, seed color, leaf shape, petiole pigmentation, seed shattering, leaf pigmentation, leaf pubescence, inflorescence color, and inflorescence density index all had a high variety index (0.69–0.99).

The average diversity index (H') of individual characters across collection regions ranged from 0.01 (for germination rate) to 0.95 (for leaf pigmentation) with an overall mean value of 0.43 (Table 5). Four pigmentation features—leaf pigmentation, petiole pigmentation, inflorescence color, seed color, and one seed trait—seed shattering—were determined to be the most diversified traits across all collecting regions with H' varied from 0.68 to 0.95. However, with H' < 0.3, there was less variation across all areas in germination rate, sex type, spines in leaf axils, growth habit, the prominence of leaf veins, inflorescence density index, branching index, leaf margin, presence of auxiliary inflorescence and terminal inflorescence attitude.

Whenever the phenotypic diversity was split within $\frac{(H'cr)}{H'sp}$ and between collection regions $\frac{(H'sp-H'cr)}{H'sp}$. It was found that there was more intra-regional diversity (0.66)

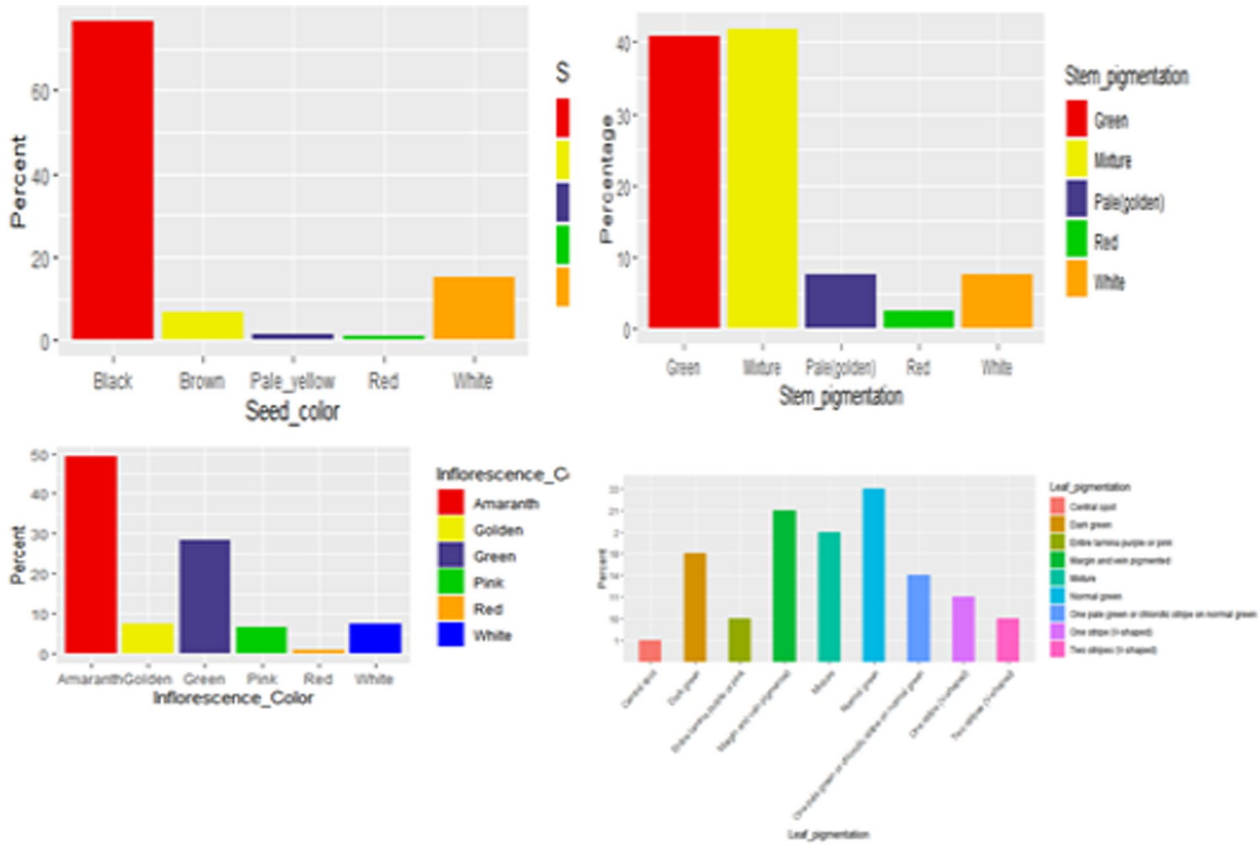

Fig. 2 Various qualitative characteristics of amaranth genotypes

than inter-regional diversity (0.34), demonstrating the existence of gene flow between growing regions. The studied qualitative traits with an H' value greater than 0.83 included stem pubescence, stem pigmentation, leaf pigmentation, leaf shape, leaf margin, petiole pigmentation, terminal inflorescence shape, inflorescence color, and seed shattering, seed color were the main contributors to heterogeneity within the collection region. Even while there was a greater degree of phenotypic variety found within collection regions (0.66), there was also a sizable level of diversity found between regions (0.34) (Table 4). The regional diversity was greatly influenced by the branching index (0.97), germination rate (0.92), growth habit (0.88), spines in leaf axils (0.88), and sex type (0.88). Although characters from various regions were pooled together, the mean value of H' varied from 0.20 for Tigray to 0.63 for Sidama (Table 5). Except for Unknown, Tigray, and Afar, which had lower diversity indices (H' = 0.20, 0.25, and 0.33, respectively), the majority of the collection regions had H' values above 0.40. In each collecting location, specific characteristics showed various degrees of variability. Overall, Sidama, Oromia, Benishangul &Gumuz, Amhara, and SNNPR genotypes showed higher diversity indices for most of the evaluated characters.

Table 6 displays the Shannon diversity indices for each of the seven *Amaranth species* that were the subject of the investigation. *A. hybridus* L.*subsp. cruentus* (L.) Theil had a mean value of 0.47 while *A. spinosus* L. had a mean value of 0.00. The greatest Shannon diversity index was recorded by *Amaranthus hybridus L. subsp. cruentus* (L.) Theil (0.47) is followed by *A. hybridus* L. *subsp. hybridus* (0.41), *Amaranthus caudatus* L. *(0.38), Amaranthus viridis* L. *(0.36), Amaranthus palmeri* S. Watson *(0.35), and Amaranthus spp. (0.21),* while *Amaranthus spinosus* L. had the lowest (0.00). On the other hand, all of the studied species displayed monomorphism, sex, growth habit, and spines in the leaf axils. *Amaranthus hybridus* L. *subsp. cruentus* (L) Theil, *A. hybridus L. subsp. hybridus, A. caudatus* L., and *A. viridis* L., genotypes exhibit monoecious character and upright growth. However, *Amaranthus palmeri* S. Watson is primarily prostrate and dioecious. On the other hand, *A. hybridus* L. *subsp. cruentus* (L) Theil was found to have the highest values (which are highly polymorphic) in seed color, terminal inflorescence shape, petiole pigmentation, inflorescence

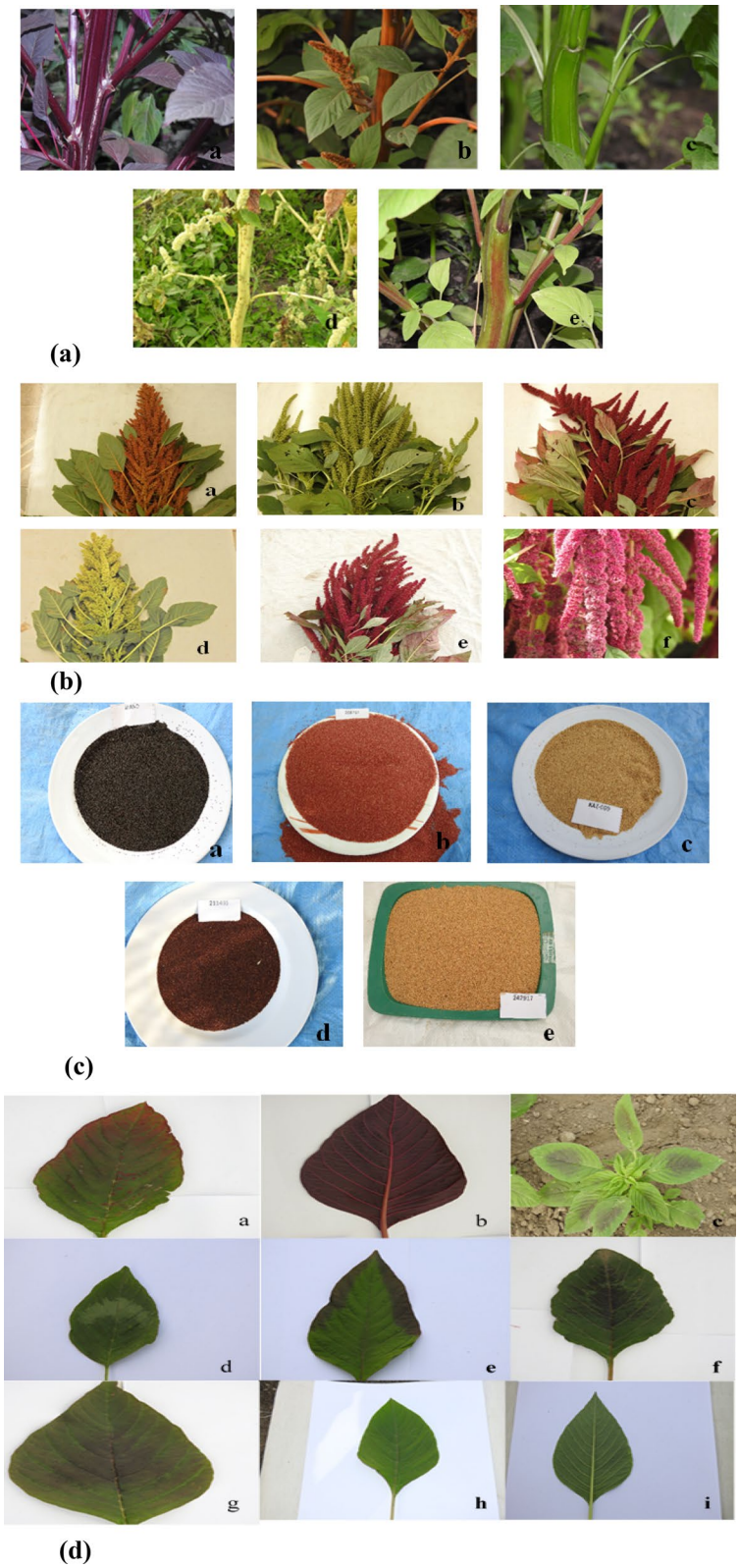

Fig. 3 **a** *Stem* color of amaranth (**a**) Red color (**b**) Golden (pale) color (**c**) Green color (**d**) White color (**e**) Mixed color. **b** *Inflorescence color* (**a**) Golden color (**b**) Green color (**c**) Red color (**d**) White color (**e**) Amaranth color (**f**) Pink color. **c** *Seed color of amaranth* (**a**) Black color (**b**) Red color (**c**) White color (**d**) Brown color (**e**) Pale yellow color. **d** *Leaf pigmentation* (**a**) Mixture (**b**) Entire lamina purple or pink (**c**) Two stripes (V-shaped) (**d**) One stripe (V-shaped) (**e**) Margin and vein pigmented (**f**) Central spot (**g**) One pale green or chlorotic stripe on normal green (**h**) Normal green (**i**) Dark green

Table 4 Qualitative morphological traits, their Shannon–Weaver diversity index, across genotypes, within and among collection regions, and maximum Shannon's Weaver diversity index for 20 morphological qualitative traits during the analysis of 120 Genotypes from Ethiopia

Qualitative marker	$H'_{sp.}$	H'_{cr}	$H'_{cr}/H'_{sp.}$	$(H'_{sp}-H'_{cr})/H'_{sp}$	H_{max}
Germination rate	0.12	0.01	0.08	0.92	0.69
Growth habit	0.57	0.07	0.12	0.88	0.69
Branching index	0.59	0.02	0.03	0.97	0.69
Stem pubescence	0.52	0.44	0.85	0.15	1.1
Stem pigmentation	0.69	0.55	0.80	0.20	1.61
Spines in leaf axils	0.57	0.07	0.12	0.88	0.69
Leaf pubescence	0.92	0.52	0.57	0.43	0.69
Leaf pigmentation	0.88	0.95	1.00	−0.08	2.2
Leaf shape	0.78	0.84	1.00	−0.08	1.39
Leaf margin	0.12	0.14	1.17	−0.17	0.69
The prominence of leaf veins	0.12	0.07	0.58	0.42	0.69
Petiole pigmentation	0.84	0.80	0.95	0.05	1.39
Terminal inflorescence shape	0.70	0.88	1.26	−0.26	1.95
Terminal inflorescence attitude	0.55	0.25	0.45	0.55	0.69
Inflorescence density index	0.99	0.59	0.60	0.40	0.69
Inflorescence color	0.95	0.80	0.84	0.16	1.79
Seed shattering	0.87	0.75	0.86	0.14	1.39
Seed color	0.76	0.68	0.89	0.11	1.61
The presence of auxiliary inflorescence	0.12	0.1	0.83	0.17	0.69
Sex type	0.60	0.07	0.12	0.88	0.69
Mean	*0.61*	*0.43*	*0.66*	*0.34*	*1.10*

H' sp, the diversity index for each character calculated from the entire data set; H'cr, the average diversity index of each character pooled over the seven collection regions and one unknown; H'_{cr}/H'_{sp}, the proportion of diversity within collection regions; $(H'_{sp} - H'_{cr})/H'_{sp}$, the proportion of diversity between collection regions, H_{max} = maximum Shannon's weaver diversity indexed

density index, stem pigmentation, and stem and leaf pubescence. Whereas *A. hybridus L. subsp. hybridus* had the highest values in petiole pigmentation, inflorescence color, seed color, and leaf pigmentation (which is highly polymorphic). In contrast, *Amaranthus palmeri* S. Watson has the highest values for leaf shape, petiole pigmentation, leaf margin, and the prominence of leaf veins. The presence of axillary inflorescence was highest for *A. spp.*, and petiole pigmentation was highest for *Amaranthus caudatus* L.; *Amaranthus viridis* L. showed the highest values in terminal inflorescence shape, inflorescence color, and seed shattering. The lowest values for all the studied features were revealed in *A. spinosus* L, but only for one phenotypic class (monomorphic) (Table 6).

Cluster analysis of 20 qualitative traits

Based on qualitative data of 20 traits, 120 amaranth genotypes were typically grouped into three basic clusters (Fig. 4). Three distinctive genotype clusters were observed based on the diversity among several qualitative morphological traits (Fig. 5). Cluster I comprised (58) genotypes collected from Tigray, Amahara, Benishangul & Gumuz, Oromia, Southern Nations Nationalities and Peoples' Region, and Sidama, followed by cluster II, which had 47 genotypes collected from Amhara, Southern Nations Nationalities and Peoples' Region, Benishangul & Gumuz, Tigray, Sidama, Oromia, and released variety. Cluster III had the fewest genotypes (15) collected from the Afar region (Table 7).

Cluster I was mainly distinguished by high seed shattering (54.83%), intermediate seed shattering (43.55%), and low seed shattering (1.61%), all of which were monoecious sex types. Out of the total 80.65% had mixture stem pigmentation, 9.68% had white stem pigmentation, and 8.06% had green stem pigmentation. In terms of branching index, 96.77% had all branches along the stem and the remaining had many branches (all at the base of the stem), also distinguished by the absence of spines in the leaf axils. Cluster II was mainly characterized by monoecious sex types with intermediate and low seed shattering (each accounting for (40.47%), no seed shattering (11.90%), and strong seed shattering (7.14%). Likewise, genotypes in cluster II lacked spines in the leaf axils, and inflorescence color varied, with 35.71% having green inflorescence color, (23.80%) having white inflorescence color, 14.29%

Table 5 Estimates of the Shannon–Weaver diversity index across the seven collection regions and one released variety (Unknown) for each qualitative character

Qualitative Traits	Regions							
	Afar	Amhara	Benishangul & Gumuz	Unknown	Oromia	Sidama	SNNPR	Tigray
GR	0.00	0.00	0.00	0.00	0.00	0.00	0.1	0.00
GH	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.00
BI	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00
SP	0.57	1.00	0.00	0.00	0.81	0.81	0.31	0.00
SPG	0.00	1.00	0.96	0.00	0.81	0.89	0.74	0.00
SLA	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.00
LP	0.00	0.81	0.97	0.00	0.81	0.81	0.74	0.00
LPG	0.84	1.00	0.96	1.00	1.00	0.93	0.89	0.97
LS	0.93	0.81	0.67	1.00	0.81	0.95	0.60	0.97
LM	0.57	0.00	0.00	0.00	0.00	0.55	0.00	0.00
PLV	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PPG	0.93	0.81	0.96	0.00	0.95	0.96	0.81	0.96
TIS	0.85	0.99	0.96	1.00	0.81	0.99	0.70	0.73
TIA	0.00	0.00	0.00	0.00	0.81	0.55	0.66	0.00
PAI	0.00	0.00	0.72	0.00	0.00	0.00	0.10	0.00
IDI	0.00	1.00	0.97	0.00	1.00	0.81	0.95	0.00
IC	0.72	0.95	0.95	0.00	1.00	0.93	0.91	0.96
SS	0.00	0.81	0.97	1.00	1.00	0.77	0.72	0.73
SC	0.57	0.00	0.97	1.00	1.00	0.96	0.91	0.00
SX	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.00
Mean	*0.33*	*0.46*	*0.50*	*0.25*	*0.54*	*0.63*	*0.43*	*0.20*

SNNPR (Southern Nations, Nationalities, and Peoples' Region).; Refer to abbreviated code Table 2

having pink inflorescence color, 9.52% having purple inflorescence color, and 4.76% having a mixture of inflorescence color. Cluster III genotypes are characterized mainly by dioecious sex types, no seed shattering, having spines in the leaf axis, and a high inflorescence density index.

Intercluster distance (D^2) analysis on qualitative traits

Table 8 presents intra- and inter-cluster distances. There were marked variations in intra-cluster distances, which ranged from 1.32 to 4.03. The highest intra-cluster distance was recorded in cluster III (4.03), which contained fifteen genotypes, followed by cluster II (2.10), which contained forty-seven genotypes. The lowest intra-cluster distance was observed in cluster I (1.32) with fifty-eight genotypes, and cluster II showed the second lowest intra-cluster distance (2.10) with the highest (47) number of genotypes. The highest inter-cluster distance was observed between clusters I and III (253.23), followed by clusters II and III (250.20). The lowest inter-cluster distance was observed between clusters I and II (29.63).

Diversity based on qualitative traits

Six main components were preserved based on eigenvalues and scree plots in the principal component analysis (PCA) of 19 qualitative traits in 120 amaranth genotypes, accounting for almost 72% of the overall variance (Table 9). The scree plot also showed that the first four PCs were important in exploiting the variation in amaranth genotypes (Fig. 6). According to the qualitative traits, the first six PCs explained 32%, 13%, 10%, 6%, 6%, and 5% of the total variation, respectively. For PC1, growth habit, branching index, spines in leaf axils (SLA), and seed shattering (SSH) were the main causes of the relationship between variables and components. PC2 explained 13% of the total variation, with the highest weights assigned to stem pigmentation (SPG), leaf pigmentation (LPG), petiole pigmentation (PP), inflorescence density index (IDI), and seed shattering (SS) (SSH). PC3, which accounted for 10% of the overall variance, was mostly associated with seed color (SC), leaf shape (LS), and terminal inflorescence shape (TIS). The terminal inflorescence attitude (TIA), prominence of leaf veins (PLV), and leaf pubescence (LP) account for 6% of the variation in the PC4. Similarly, only leaf pubescence (LP),

Table 6 Estimates of the Shannon–Weaver diversity index across the seven *Amaranthus species* for each qualitative character

Traits	*A. hybridus L. subsp. cruentus*	*A. hybridus L. subsp. hybridus*	*A. caudatus L*	*A. palmeri S. Wats*	*A.spp*	*A. viridis L*	*A. spinosus L*
GR	0.00	0.14	0.00	0.00	0.00	0.00	0.00
GH	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BI	0.20	0.14	0.00	0.00	0.00	0.00	0.00
SP	0.60	0.41	0.00	0.55	0.00	0.00	0.00
SPG	0.70	0.52	0.70	0.00	0.00	0.00	0.00
SLA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LP	0.95	0.69	0.35	0.57	0.81	0.00	0.00
LPG	0.89	0.83	0.77	0.84	0.00	0.81	0.00
LS	0.88	0.76	0.78	0.92	0.81	0.81	0.00
LM	0.00	0.00	0.35	0.57	0.00	0.00	0.00
PLV	0.00	0.00	0.00	0.57	0.00	0.00	0.00
PPG	0.80	0.94	1.00	0.92	0.00	0.81	0.00
TIS	0.83	0.66	0.57	0.84	0.00	0.95	0.00
TIA	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PAI	0.00	0.00	0.00	0.00	1.00	0.00	0.00
IDI	0.92	0.69	0.80	0.00	0.00	1.00	0.00
IC	0.95	0.86	0.72	0.72	0.81	0.95	0.00
SS	0.64	0.67	0.97	0.00	0.81	1.00	0.00
SC	0.94	0.81	0.59	0.57	0.00	0.81	0.00
SX	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	*0.47*	*0.41*	*0.38*	*0.35*	*0.21*	*0.36*	*0.00*

Refer to abbreviated code Table 2

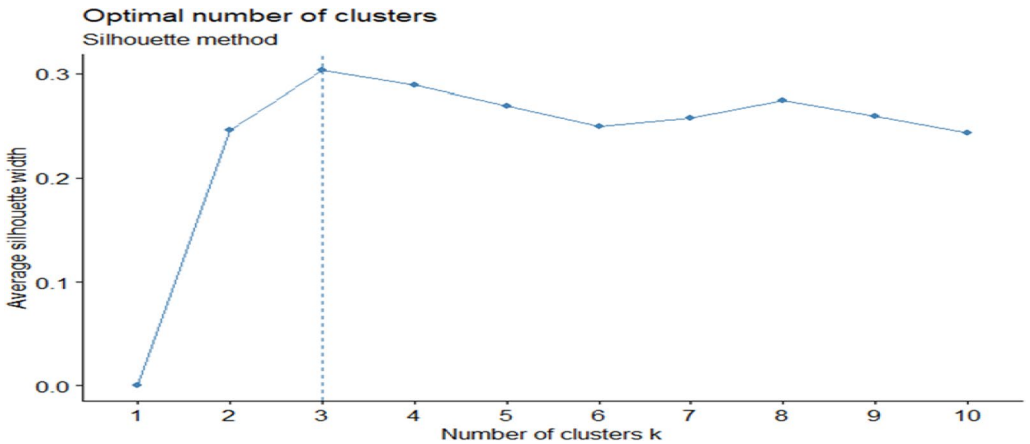

Fig. 4 Silhouette widths to determine the number of clusters (K) for qualitative data

terminal inflorescence shape (TIS), and germination rate (GR) scored heavily for PC5, which accounted for 6% of the overall morphological variance in these genotypes. Leaf margin (LM), prominence of leaf veins (PLV), and terminal inflorescence attitude (TIA) weighted significantly in PC6, which explained 5% of the overall variance.

Dim. 1 (32.4%) is defined by nine variables, seven of which are strongly positively correlated, including namely: spines in leaf axils (SLA; 98%), growth habit (GH; 96%), sex (SX; 93%), branching index (BI; -91%), inflorescence density index (IDI; 61%), inflorescence color (IC; 59), seed, and two of which are negatively correlated, such as shattering (SSH; -75%), stem pubescence (SP; -70%), and leaf pubescence (LP; -62%). Dimension 2 (12.4%) is defined by five variables, two of which are strongly positively correlated: petiole pigmentation (PP;

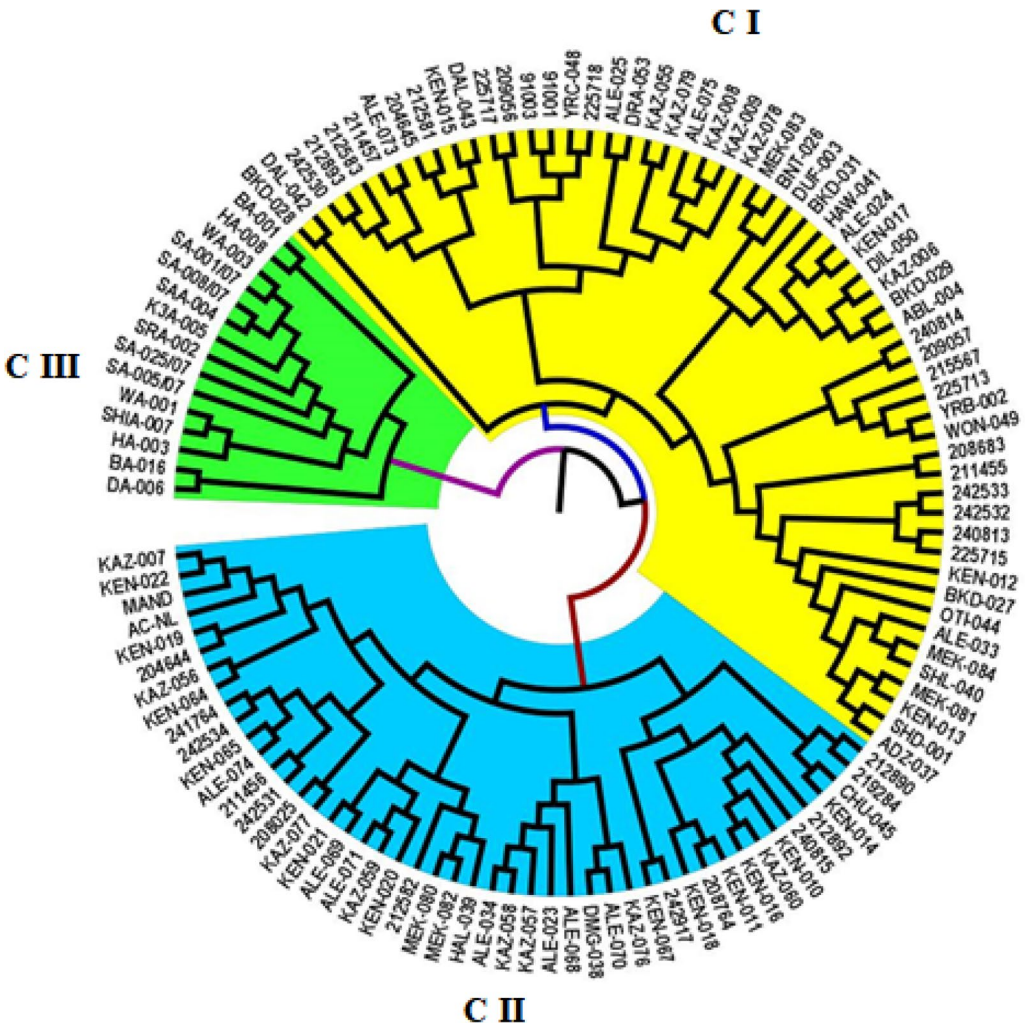

Fig. 5 Cluster analyses of the 120 amaranths genotypes based on 19 qualitative traits. (Codes represent the genotypes. See Table 1 for the details

83%), stem pigmentation (SPG; 77%), and three of which are negatively correlated: leaf pigmentation (LPG; -65%), inflorescence density index (IDI; -50%), and seed shattering (SSH; -46%) (Fig. 7).

Discussion

Plant breeders can only succeed in any crop improvement program if they understand the genetic heterogeneity that exists across a species' genotypes (Roy et al. 2012; Mavengahama et al. 2013). Modern plant breeding would be difficult to imagine, let alone being successful, without a constant supply of genetically varied plants (Duvick 2007). To offer information for plant breeding initiatives, an agro-morphological assessment of available germplasm is essential (Lin 1991), and they can use the information to evaluate genotypes to include in a breeding program. Due to the extensive phenotypic variety, inter-specific relations of the genus *Amaranthus* are poorly recognized. Trait variation assists in the selection of the best lines for improvement in trait-assisted selection (Fasahat et al. 2016; Moradi et al. 2019; Dewi et al. 2020). Moreover, extensive genetic variability among the genotypes offers several opportunities for breeding to select desired features (Laxmi et al. 2020).

It has been thought that qualitative characteristics are useful for describing species germplasm (Abe and VegeTable 2015). For instance, most Africans prefer to include green-leaved varieties in their diet, whereas the Chinese prefer red-leaved varieties. These have an impact on consumer preferences, the socioeconomic environment, and natural selection as they determine the specific traits that can be found in society (Akaneme and Ani 2013).

Table 7 Clustering of 120 *Amaranths species* of genotypes into 3 clusters using the mean of 20 agro-morphological qualitative characters

Cluster No	Genotypes	Regions	No of Genotypes
I	MEK-081,BKD-031,KAZ-078,OTI-044,208,683,KEN 012, 204,645,WON-049,215,567,209,056,240,814,242,533,ALE-033,KAZ-055, 240,813, 209,057, DIL-050, KAZ-006, BKD-027, BKD-029, KAZ-008, 225,715, KAZ-009, 225,718, 211,457, ALE-024, ALE 073, KEN-015, 212,581, 91,001, 242,530, DAL-043, 225,717, HAW-041, 211,455, KAZ-079, 242,532, SHD-001, BKD-028, ALE-025, DRA-053, MEK-083, 212,583, KEN-017, DAL-042, 91,003, 212,893, MEK-084, SHL-040, ABL-004, KEN-013, YRC-048, DUF-003, ALE-075, 225,713, ADZ-037, YRB-002, BNT-026	Tigray, Amahara, Benishangul and Gumuz, Oromia, Southern Nations, Nationalities, and Peoples' Region and Sidama	58
II	ALE-074, 212,890, KAZ-057, KEN-020, CHU-045, KAZ-076, 242,534, 211,456, 241,764, ALE-068, MEK-082, 242,531, KEN-065, KAZ-059, 219,284, 242,917, ALE-023, 208,764, KAZ-077, KAZ-058, KEN-064, KEN-021, KEN-014, AC-NL, KAZ-056, ALE-034, 204,644, 212,892, HAL-039, 208,025,MEK-080, KEN-022, MAND, KEN-067, 240,815, ALE-071, KEN-010, ALE-070,KAZ-007, KAZ-060, KEN-011, KEN-016, KEN-019, 212,582, DMG-038, ALE-069, KEN-018	Amhara, Southern Nations, Nationalities, and Peoples' Region, Benishangul& Gumuz, Tigray, Oromia, and Sidama, Released a variety	47
III	SAA-004, HA-003, DA-006, BA-016, SRA-002, K3A-005, SA025/07, SA-008/07, SA-001/07, SHIA-007, SA-005/07, HA-008, BA-001, WA-001, WA-003	Afar	15

Although qualitative features are often unaffected by environmental variables, they may be used to accurately identify genotypes and perhaps aid in the removal of duplicates and closely similar materials (Merrick et al. 2023; Esayas et al. 2016). Furthermore, when the range of quantitative features is constrained, they help classify varieties (Ghafoor and Ahmad 2003). In this study, the results showed the existence of a significant morphological variation of genotypes in qualitative characteristics such as germination rate, branching index, stem pubescence, stem pigmentation, spines in leaf axils, leaf pubescence, leaf pigmentation, leaf shape, leaf margins, vein prominence, petiole pigment, inflorescence terminal shape, inflorescence terminal attitude, inflorescence density index, seed burst, and seed color. Similar reports for the existence of large variability have been made by different researchers (Juan et al. (2007), Delgado et al. 2022, Nyasulu et al. 2021, Gherase et al. 2020, Gerrano et al. 2013, Gueco et al. 2016, Khanam et al. 2012, Sarker and Oba 2021, Paredes-Lopez 2018, Lakshmidevamma et al. 2022). Nyasulu et al. (2021), and Gherase et al. (2020) reported a slightly larger range of variances for qualitative features such as leaf color, inflorescence, and seed color. Furthermore, the majority of genotypes had an erect growth habit; erect plant types might be used for breeding genotypes in irrigation-dependent areas, whereas prostrate plant types could be used for creating elite genotypes for rain-fed places that will aid in moisture conservation. Similarly, the consistent result was also reported by Iqbal et al. (2003) for cowpea germplasm.

Table 8 Intra (bold) and inter-cluster distances (D^2) for 20 qualitative traits of Amaranth Genotypes from Ethiopia

Generalized Squared Distance to clusters				Mean distance
Cluster	I	II	III	
I	**1.32***	29.63^{NS}	253.23***	141.43
II		**2.10^{NS}**	250.20***	139.92
III			**4.03^{NS}**	251.72
Overall mean				**177.69**

* significant at P < 0.05, **highly significant at P < 0.01, and ***very highly significant at P < 0.001; NS = non-significant at P > 0.05. Chi-Square (χ^2) 20 DF = 31.41; at 37.57 at P < 0.01; 45.32 at P < 0.001; Inter-cluster is off-diagonal; and intra-cluster is diagonal bold

The predominance of genotypes with lanceolate leaf shapes in the current study contributes to the adaptation of the genotypes used for hybridizing the introduced rainfed variety and their capacity to tolerate the drought. A similar result was reported by Iqbal et al. (2003). Genotypes with ovatinate and rhombic leaf forms are anticipated to be the best for absorbing moisture and producing dietary proteins. These findings coincide with those made available by (Dwivedi et al. (2001), Iqbal et al. 2003). As a result, the observed discrepancy in leaf phenotype may be exploited as a key marker for selection in the breeding of better genotypes for different purposes.

High diversity within cultivated crops may be caused by human or natural selection, exchange of seeds, genetic drift, and natural variances (Yeshitila et al. 2023; Joshi

Table 9 Eigenvectors and eigenvalues of the first six principal components (PCs) for 19 qualitative characters of 120 genotypes

Traits	Principal Components					
	PC1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	6.15	2.42	1.82	1.20	1.11	1.03
Proportion	0.32	0.13	0.10	0.06	0.06	0.05
Cumulative	0.32	0.45	0.55	0.61	0.67	
			Eigenvectors			
Growth habit [GH]	0.96	0.08	−0.08	0.08	0.10	0.03
Branching index[BI]	−0.91	−0.09	0.16	−0.11	0.08	−0.03
Stem pubescence[SP]	−0.70	−0.03	−0.10	0.31	−0.08	−0.08
Stem pigmentation [SPG]	0.23	0.77	0.06	0.22	0.03	0.08
Spines in leaf axils [SLA]	0.96	0.08	−0.08	0.08	0.10	0.03
Leaf pubescence [LP]	−0.62	0.16	−0.10	0.45	−0.09	0.00
Leaf pigmentation [LPG]	−0.10	−0.65	0.18	0.23	0.04	0.15
Leaf shape [LS]	−0.10	−0.10	0.67	0.10	0.38	0.20
Leaf margin [LM]	−0.39	−0.10	0.03	0.17	0.29	−0.51
Prominence of leaf veins [PLV]	−0.35	0.09	0.18	−0.60	−0.07	0.45
Petiole pigmentation [PP]	−0.02	0.83	0.03	−0.05	−0.04	0.09
Terminal inflorescence shape [TIS]	0.16	0.15	0.63	−0.09	−0.31	−0.08
Terminal, inflorescence attitude [TIA]	−0.24	−0.12	−0.31	0.36	0.24	0.68
Inflorescence density index [IDI]	0.61	−0.50	0.24	0.07	0.01	0.06
Inflorescence color [IC]	0.59	0.28	0.32	0.27	0.03	−0.03
Seed shattering [SSH]	−0.75	0.46	−0.04	0.15	0.00	0.06
Seed color [SC]	0.14	−0.08	−0.76	−0.22	0.03	−0.04
Germination rate [GR]	0.06	0.23	0.01	−0.24	0.81	−0.11
Sex [SX]	0.93	0.03	−0.11	0.16	−0.08	0.05

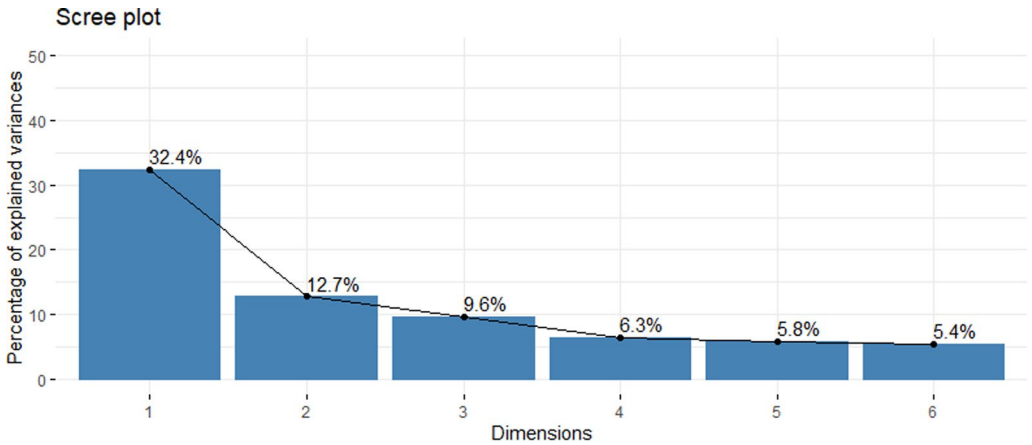

Fig. 6 Scree plot showing eigenvalues in response to some principal components for 19 agro-morphological traits of 120 amaranth genotypes

and Baniya 2006). Wider inflorescence trait variabilities seen in the genotypes under study demonstrate the divergence of the genotypes and might be useful in selection and enhancement procedures. Large inflorescence variability, which has been cited as a key characteristic in identifying amaranth genotypes, has also been demonstrated in earlier investigations (Yadav et al. 2014).

The current studies reveal that amaranths exhibit a variety of pigmentation in many phenotypic markers, including the coloring of the seed, stem, leaves, petioles, and inflorescence. This is a result of the variety of betacyanin pigmentation patterns (Paredes-Lopez 2018; Das 2016). Amaranth can therefore be used for several utilitarian and aesthetic uses due to the

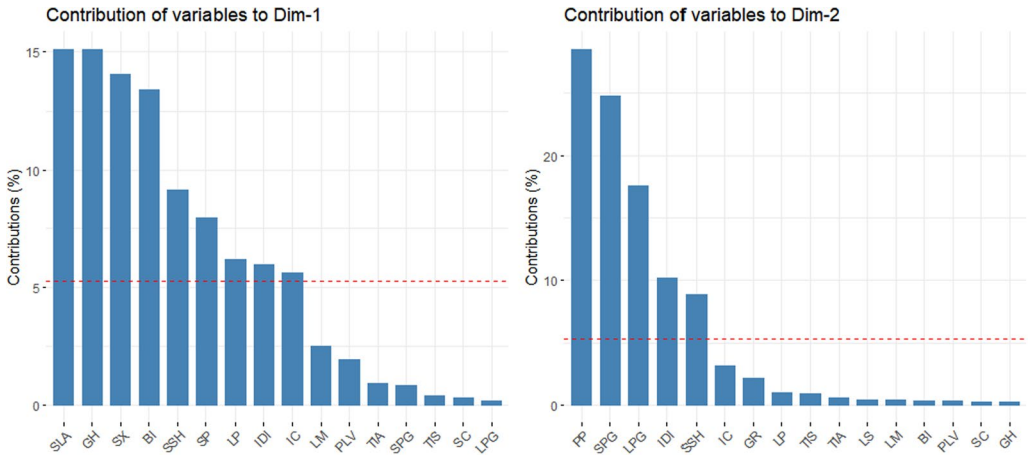

Fig. 7 Contribution of the variables to the formation of the two principal components

pigmentation's apparent variance. They serve as beneficial genetic markers for managing breeding hybrids, evaluating natural outcrossing, identifying varieties, and mapping the genome. Moreover, according to Wu et al. (2000), Khanam et al. (2012), and Sarker and Oba (2021) different expressions of natural pigments such as betacyanins, including amaranthine, iso-amaranthine, betanin, iso-betanin, anthocyanin's, carotenoids, and chlorophylls, cause broad-spectrum coloration in *Amaranth species*.

As compared to other leafy vegetables, *Amaranthus* leaves offer a unique source of antioxidant pigments like betalain, β-xanthin, and β-cyanin. They are also a great source of other antioxidant pigments like anthocyanins, carotenoids, and chlorophylls (Sarker et al. 2018b), as well as naturally occurring antioxidant phytochemicals like vitamin C, phenolic acids, and flavonoids (Sarker et al. 2018a). These naturally occurring antioxidant molecules are important for the food business because they act as natural preservatives in food items in addition to their health-promoting properties (Repo-Carrasco-Valencia et al. 2010). Vegetables' inherent antioxidants have drawn the attention of scientists and consumers lately. These organic antioxidants guard against several diseases such as retinopathy, arthritis, emphysema, cancer, heart disease, cataracts, and neurological conditions (Isabelle et al. 2010; Steffensen et al. 2011). It is resistant to abiotic circumstances like droughts with a range of (Sarker and Oba 2018b)and salinities (Sarker and Oba 2018a). More than six key genes have been identified to govern pigment expression patterns (Wu et al. 2000; Paredes-Lopez 2018). This suggested that the genotypes based on these data had several important genes that expressed a wide range of changes in the color of the stem, inflorescence, leaves, and seeds. The taste, color, and flavor of food are the most important characteristics that influence its popularity. As a result, people all across the world are becoming more and more interested in colorful foods. Because of consumer interest in the aesthetic, nutritional, and safety aspects of food, colored food items have increased the need for natural pigments like betacyanins, along with amaranthine, iso-amaranthine, betanin, iso-betanin, anthocyanins, carotenoids, and chlorophyll (Sarker and Oba 2021).

The red coloration of the stem/leaves is caused by anthocyanin. Anthocyanin is associated with enhanced resistance to abiotic stressors such as drought (Dabravolski and Isayenkov 2023), heavy metals(Kgang 2010) (opposition to herbivores and pathogens (Coley and Aide 1989). It acts as a sunscreen against damaging UV -B radiation, and as an antioxidant, which protects the plant under stress. *Amaranthus* leafy vegetables are known to have a greater concentration of antioxidant components than beet, cabbage, leafy lettuce, and carrots (Kgang 2010; Amin et al. 2006; Hunter and Fletcher 2002).

The results of the chi-square test indicated that among the 20 qualitative markers examined, dominant phenotypic variation existed. The observed value exceeded the phenotypic value that was predicted. However, there was a highly significant difference in all evaluated qualitative expressive traits except the inflorescence density index. It implies that the dominating phenotypic classes were apparent in the data compiled. Abaynesh (2019), reported a similar result in *Coffea arabica* L.

The standardized Shannon diversity index Shannon and Weaver (1949b) was classified as low (0–0.33), intermediate (0.34–0.66), and high (0.67–1). Auxiliary inflorescence, germination rate, prominence of leaf veins, and leaf margin had a low genetic diversity index and

were monomorphic, whereas growth habit, branching index, stem pubescence, spines in leaf axils, and terminal inflorescence attitude had an intermediate diversity index. Seed color, stem pigmentation, leaf pigmentation, leaf shape, petiole pigmentation, terminal inflorescence shape, inflorescence density index, inflorescence color, and seed shattering all had a high genetic diversity index and a wide range of polymorphisms. The genetic diversity found using qualitative traits in this study suggests that the tested genotype differs in genetic variants. Morphological diversity index (H') estimates for individual traits range from 0.12 for auxiliary inflorescence, germination rate, leaf vein prominence, and leaf margin to 0.99 for inflorescence density index, with an overall Shannon Weaver index (H′) of 0.61. The high H' in this study was largely due to stem pigmentation, terminal inflorescence shape, seed color, leaf shape, petiole pigmentation, seed shattering, leaf pigmentation, leaf pubescence, inflorescence color, and inflorescence density index all had a high variety index, indicating that these traits contributed to most of the genetic variability among the genotypes. Similar results were also obtained in studies reported by Adhikari et al. (2022) in grain amaranth and Zavinon et al. (2019) in pigeon peas. Therefore, wide genetic variation among genotypes offers a larger genetic pool, providing several possibilities for breeding to select desired features. In amaranths, Gerrano et al. (2017) found comparative results in terminal inflorescence. The Shannon index (HI) increased with both genotype abundance and evenness. Gueco et al. (2016), reported a high diversity index (0.67) in 18 amaranth germplasm collections in the Philippines.

It was additionally pointed out that other crops in Ethiopia and other countries have higher levels of variability for several qualitative traits within collecting locations (Nsabiyera et al. 2013; Aklilu et al. 2016; Zigene et al. 2022; Awol 2018). All growing regions might be a source of notable characteristics since there is a higher amount of within-region variability, and all regions should be given a comparable weight for future initiatives including the collection, characterization, and conservation of amaranth crops. For amaranth and other crops, comparable results were also reported (Gerrano et al. 2013, Nyasulu et al. 2021, Adhikari et al. 2022).

Low Shannon's diversity index signifies a lack of variety and imbalanced frequency classes for a particular trait. The results of H' between 0 and 1 range from the lowest value of 0.12 to the highest value of 0.99 revealing the existence of variability among tested traits of overall amaranths genotypes of Ethiopia. A similar result was also indicated in most studied traits by Lakshmidevamma et al. (2022).

All of the amaranth species were examined for various qualitative morphological traits, both individually and collectively. Morphological markers were shared by a variety of species because frequent outcrossing and hybridization widened the *Amaranthus* gene pool and produced a large number of morphotypes (Sauer 2017). In addition, from a taxonomic standpoint, *Amaranthus* is regarded as a difficult genus because there aren't many characteristics that set its species apart from one another, the diagnostic features are small and hard to see, the genus has a wide geographic distribution, and there are lots of hybrid forms. These factors make taxonomy difficult and contribute to the genus' general reputation as a challenging genus among systematists (Das 2016; Costea and DeMason 2001). As a result, it was difficult to classify genotypes into morphotypes or relate traits to species. According to the study's findings, however, amaranth species classified as grains (such as *Amaranthus hybridus L. subsp. cruentus* (L.) Theil, *Amaranthus hybridus L. subsp. hybridus,* and *Amaranthus caudatus* L.), exhibit more variation in morphological traits like stem pigmentation and seed color than the weedy types (*A. viridis* L.*, A. spinosus* L., and *A. palmeri* S. Wats). *Amaranthus* species differ from one another in several characteristics, including the kind of reproduction (monoecious vs. dioecious). Similar results were reported by Stetter and Schmid (2017)

Samples were classified according to how similar or close together they were (Tahir et al. 2013). There is a lot of genetic variation among the amaranth genotypes, as seen by the distribution pattern of all the genotypes into three groups. Additionally, Ward's method was employed since it is widely acknowledged to be the most effective method for conducting hierarchical cluster analysis (Murty and Arunachalam 1966; Shankar et al. 2012). Clustering is a valuable tool for examining links between closely linked cultivars or genotypes by grouping units based on their similarity in particular features or response patterns (Hair et al. 1995). Moreover, clustering is a useful tool for studying relationships between closely related genotypes by categorizing units based on their similarity in certain characteristics or response patterns. Crop species' genetic relationships are important because they enable a particular set of breeding populations as well as the collection of data on genetic diversity (Aghaee et al. 2010).

Although genetic diversity is typically linked to geographic variety, it is not always directly connected to geographic distribution (Rahman and Munsur 2009). When the amaranth genotypes were grouped in this study using the three clustering techniques, it became clear that two of the clusters had no discernible pattern (cluster I and II) whereas the third did (cluster III). While some

genotypes from the same collecting site were grouped, others from various sites were clustered. This suggests that there is no relationship between the population and its place of origin. Furthermore, cluster analysis was used to demonstrate the spatial grouping of genotypes and the co-occurrence of species in a single cluster. As a result, cluster III was established by all of the genotypes from the Afar region. However, despite the genotypes obtained from Afar, genotypes from Southern Nations, Nationalities, and Peoples' Region, Amhara, Tigray, Sidama, Oromia, and Benishangul & Gumuz were grouped into clusters I and II, where the genotypes differed substantially from those in cluster III, demonstrating similarity in adaptability. Nevertheless, the lack of a geographic location correlation in this study shows that the populations of various regions share genetic similarity and may have derived from the same breeding materials (Tahir et al. 2013) or the random grouping of populations from different geographic regions into different clusters suggests that factors other than geographic influence, such as breeding material exchange, genetic drift, and natural and artificial selects, are responsible for diversification (Murty and Arunachalam 1966). Similar results were found in Indian accessions of amaranth by (Shankar et al. (2012)). In other words, cluster analysis revealed that the amaranth genotypes obtained from several Ethiopian regions had a high degree of genetic diversity. As a result, crossing these genetically distinct parents could produce desirable recombinants and transgressive segregants, which in turn could produce varieties that perform better than those that have already been released.

Lower heterotic F1 offspring are produced when clusters with short distances between them are crossed. Crossing of best genotypes from distant clusters is expected to produce transgressive segregants that exceed both parents (Mussa et al. 2003). According to Darkwa et al. (2016), maximum genetic recombination is expected from the hybridization of the parents selected from divergent cluster groups. However, crossings involving parents chosen from clusters I and III, followed by clusters II and III, are likely to have the highest levels of genetic recombination and variation in the next generation of offspring. The highest inter-cluster distance showed that the genotypes in cluster III have diverged from those of cluster I. Nevertheless, depending on the specific objectives of the hybridization, it is also important to take into account the distinctive advantages of each genotype and cluster (Chahal and Gosal 2002).

Clusters I and II had the lowest inter-cluster divergence, demonstrating the genetic closeness of these clusters' genotypes. Therefore, based on our findings, it is expected that choosing a parent for hybridization from cluster III may result in the desired heterosis for heterotic amaranth hybrids. In contrast, all of the clusters' inter-cluster distances were greater than their intra-cluster distances, suggesting more genetic diversity among the genotypes of various groupings. The results were in agreement with (Rahman and Munsur 2009; Kumar 2019).

The results of PCA revealed that the genotypes of amaranth exhibit significant genetic variety. The first two PCs had a bigger contribution than the other PCs, even though the individual PCs contributed to the overall variance in different ways. In this study, the top two PCs explained 45.1% of the total variation, although the third (9.6%), fourth (6.3%), fifth (5.8%), and sixth (5.4%) PCs made less contribution to the overall variation. Additionally, according to Chahal and Gosal (2002), the first few PCs often contributed more to the overall variance. SLA, GH, SX, BI, SSH, SP, LP, IDI, and IC were the key contributors to the observed variance in the first PC, whereas PP, SPG, LPG, IDI, and SSH had a significant impact on the overall variation in the second PC. Contrarily, it was widely thought that the characteristics inside the first PC with substantially bigger absolute values of the eigenvector that are closer to unity had a greater effect on clustering than those with smaller absolute values that are closer to zero (Chahal and Gosal 2002). However, in this study, each of the examined variables alone contributed less (0.02 to 0.96) to the overall variance in the first PC; as a result, the clustering of the genotypes into various groups was more influenced by the traits' additive effects. The value of the eigenvector denotes the relationship between characteristics and PCs. For a particular characteristic, a negative eigenvector denotes a negative correlation between that trait and the given PC, as well as the opposite.

Conclusion

The diversity patterns among the 120 genotypes that make up our amaranth diversity array are examined in this study. This study lays the groundwork for Ethiopian amaranth genetic advancement since crop improvement and selection may make use of the variety that was identified. The large genetic diversity among genotypes revealed by the morphological qualitative features utilized in the current study will help with the classification, improvement, and preservation of genetic resources.The majority of the investigated characters showed similarity across collection regions. Still, some phenotypic traits, including germination rate, inflorescence density index, growth habit, spines in leaf axils, sex type, branching index, and inflorescence color, were found to be variable and contributed to regional differentiation. To distinguish genotypes in various growing regions, these traits might be utilized as important markers. Future actions

for collection, conservation, and development should be based on real diversity and give equal weight to the growing areas since the genotype variability within the expanding areas is larger, making all regions a potential source of valuable genes. Additionally, growing genotypes with distinct traits, a broader genetic basis, and unique identification may be achieved by selecting for the diversity and distinctive qualities found. Likewise, some qualitative characteristics such as amaranthus leaves provide a special source of antioxidant pigments such as β-cyanin, β-xanthin, and betalain. The development of amaranth varieties can use anthocyanins, carotenoids, and chlorophylls as well as naturally occurring antioxidant phytochemicals like vitamin C, phenolic acids, and flavonoids, among others. This is significant for the food industry because these phytochemicals promote health and act as natural preservatives in food items. Furthermore, the existence of genetic variation was shown by the Shannon-Waver diversity index (H') in many qualitative traits. Therefore, it can be declared that a higher degree of divergence was detected among tested genotypes based on H'. Clusters I and III, then II and III, had the greatest distance between them. As a result of this, heterozygous offspring can be produced. The first two main components contributed a significant portion of the variance in amaranth genotypes. A substantial amount of the variance in amaranth genotypes was contributed by the characteristics SLA, GH, SX, BI, SSH, SP, LP, IDI, and IC. Moreover, the findings also have breeding implications for improved grain or vegetable amaranth variants, particularly for leaf and blossom color uniqueness while selecting for economically relevant seed color features. Therefore, the selection of these traits could be effective for the improvement of amaranth genotypes. This finding indicates that broad collection from a range of altitudes and origin locations is far more valuable than amaranth collection from particular agroecology, indicating that it catches significant allele variation from varied growth situations. Because of this, this material might be improved by conventional breeding (direct selection) or by using modern methods to investigate the genetic underpinnings of the phenotypic variations of these features. With enough variability to allow for the selection of genotypes for desirable characteristics in Ethiopian breeding programs for amaranth, these genotypes may offer a starting point for the production of amaranth of Ethiopian genotypes. To further enhance the crop, future molecular marker diversity studies combined with estimates of phenotypic variety will provide a comprehensive picture of the extent and distribution of variability. Also, to map QTLs and validate candidate genes for distinct characteristics, molecular characterization of the genotypes that have been realized must be conducted.

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Author contributions
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Availability of data and materials
On a reasonable request, the corresponding authors will provide you with all the data and materials used in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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