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Exploring Genetic Diversity, Population Structure, and Interrelationships Among Ethiopian Barley (Hordeum vulgare L.) Landraces Through SSR Marker Analysis

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Abstract:

Characterization of genetic resources held in both in situ and ex situ GenBanks have important implications for future applications in association mapping studies, genetic selection, breeding, and conservation efforts. This study aimed to evaluate the genetic diversity, population structure, and relationships among 384 Ethiopian barley genotypes collected from various barley-growing regions of Ethiopia using 49 simple sequence repeat (SSR) markers. Analysis of these markers revealed a total of 478 alleles, with an average of 9.755 alleles per locus, of which 97.07% were found to be polymorphic. The Nei's genetic diversity index (h) was 0.654, and the Shannon diversity index (I) was 0.647, indicating moderate-high genetic diversity among the studied barley genotypes. At the population level, the mean percentage of polymorphic loci (PPL) was 98.37%, with h = 0.388 and I = 0.568. The highest genetic diversity was observed in the Arsi population (PPL = 100%, h = 0.439, I = 0.624), while the lowest was in the population from Jimma (PPL = 75.51%, h = 0.291, I = 0.430). Analysis of molecular variance (AMOVA) demonstrated significant genetic differentiation within and between populations ($P < 0.001$), with 84.21% and 15.79% of the variation occurring within and among populations, respectively. Additionally, the study revealed a coefficient of gene differentiation of 0.053 and a gene flow value of 4.467 among populations. The 384 barley genotypes were grouped into seven genetic clusters based on STRUCTURE, neighbour-joining tree, and principal coordinate analysis, which were significantly correlated with their geographic distribution. These results highlight significant variations within and among populations, providing valuable insights for the design of conservation strategies, including both in situ and ex situ conservation approaches.

Keywords: genetic diversity; landraces; population structure; simple sequence repeat; Hordeum vulgare

Introduction:

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Barley (Hordeum vulgare L.) is one of the earliest domesticated annual cereal crops and is a member of the grass family Poaceae [1], which includes various cytotypes with different chromosome numbers [2]. This versatile crop is cultivated under diverse environmental conditions and requires minimal production inputs [3]. In Ethiopia, barley is an important food crop, ranking fifth among cereal crops after teff, maize, sorghum, and wheat. It is grown twice a year during the main rainy season (Meher)[4] from June to September and the short rainy season (Belg) from March to May [5]. Barley cultivation constitutes approximately 18.0% of the country's total cereal cultivation, with landraces accounting for over 90.0% of the barley varieties grown in Ethiopia [6].

Materials and methods:

Plant Material:

A total of 384 barley (H. vulgare L.) accessions were used in this study, consisting of 376 landraces and eight cultivars [7]. The eight cultivars, including Abdanie, Guta, Dafo, HB-1964, HB-1966, HB-42, Ardu-12-60B, and Aruso (six-rowed barley), were obtained from Holetta and Sinana Agricultural Research Centres [8]. The landraces were obtained from the Ethiopian Biodiversity Institute (EBI) along with their passport data [9]. For data analysis, only the improved cultivars were considered to study the relationships within and among barley genotypes [10]. In cases where the sample size of landraces from a particular region was less than five, they were included in an adjacent region to minimize experimental errors due to small sample size [11]. This reduced the original 42 agro-ecological zones to 15 zones for analysis [12]. The 384 accessions were distributed across different regions in Ethiopia as follows: 188 landraces from Oromia, 88 from Amhara, 57 from Tigray, 42 from SNNP, and nine from Benishangul Gumuz [13]. The major barley-growing highland regions, Oromia, and Amhara, were well represented with more samples [14]. All the landraces were of the spring growth type, with 176 being two-rowed and 200 being six-rowed barley types [15].

Collection Sites:

The collection sites of the barley landraces are shown in Figure 1, created using QGIS-v. 3.8.0 software and GPS coordinates of the collection sites [16].

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Population Structure:

All sets of barley accessions, both landraces and cultivars, were considered as a population, while each grouping based on administrative zones [17], kernel row number, and breeding status (landraces and improved cultivars) was treated as a subpopulation [18].

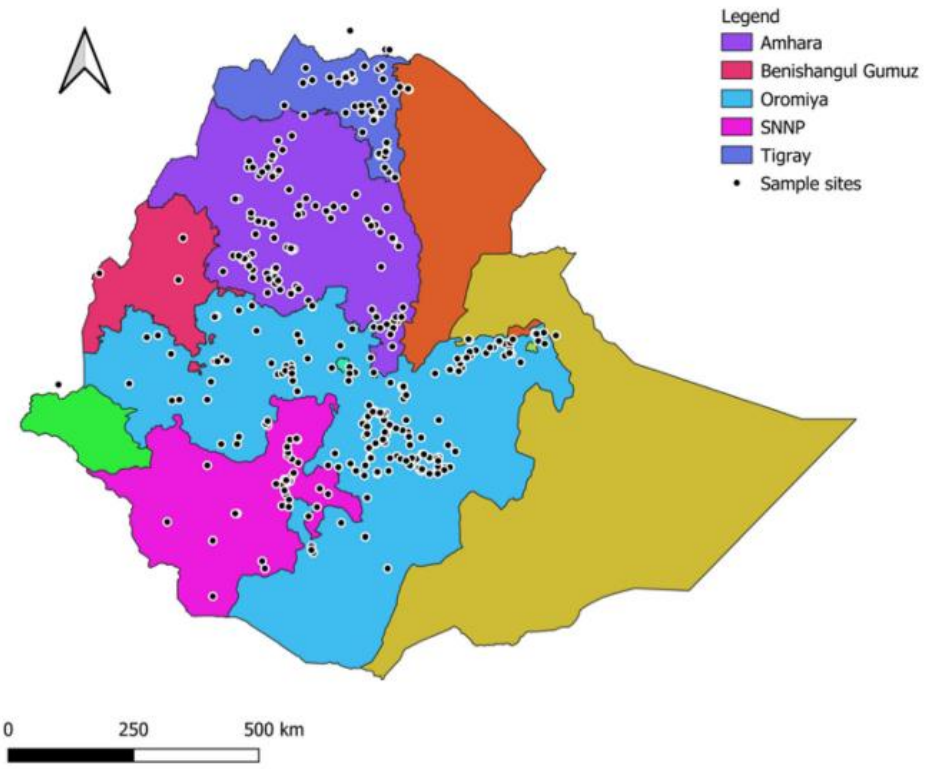

Figure 1. Map of Ethiopia showing the collection sites of barley landraces based on Ethiopian National Regional States. The map was constructed using the QGIS-OSGeo4W v. 3.8.0 software.

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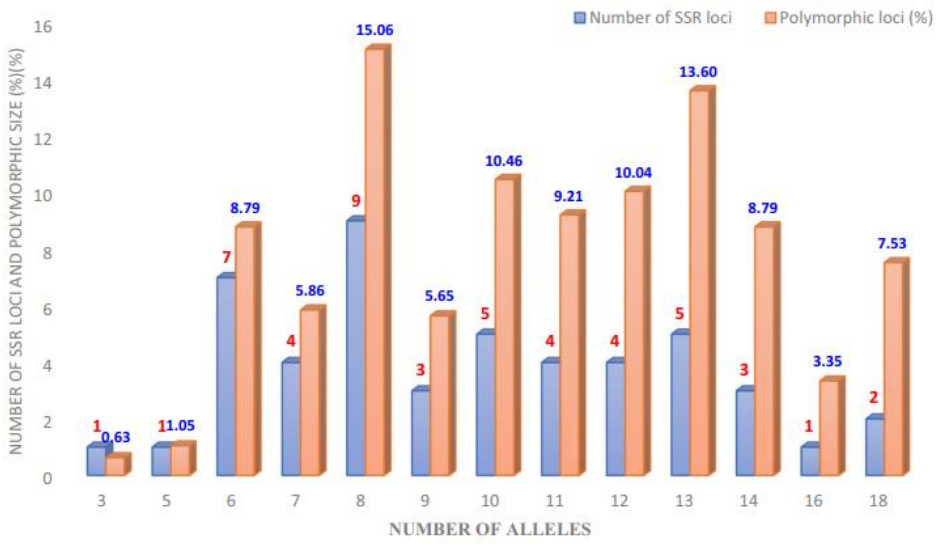
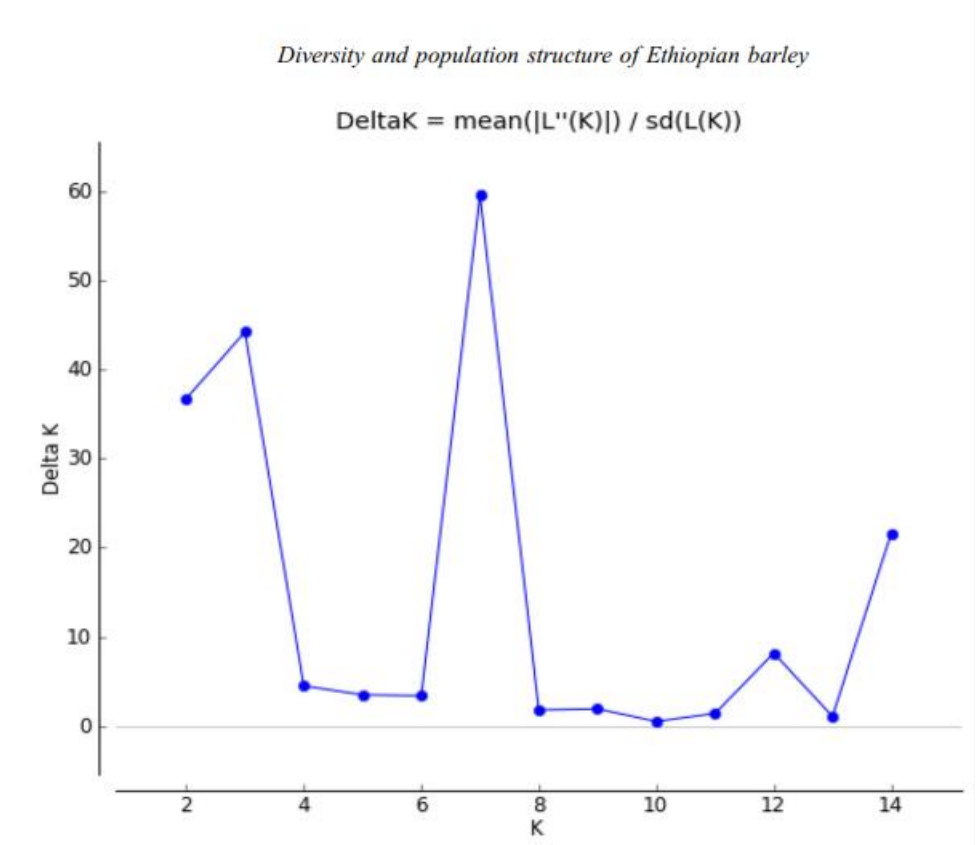


Figure 2. Distribution of allelic variation in 49 polymorphic SSR loci.



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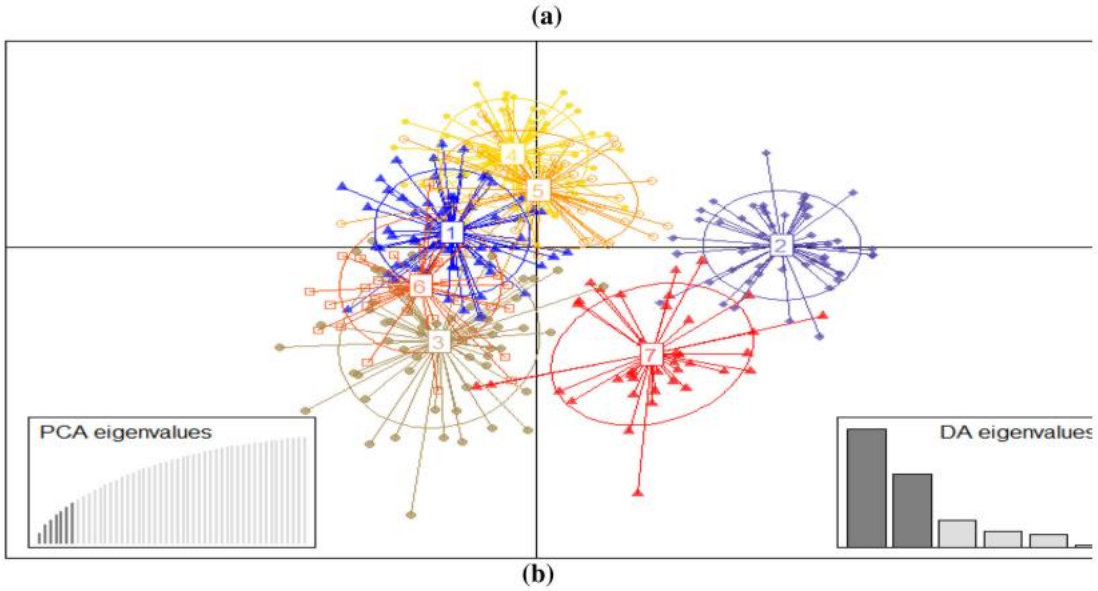

Figure 3. (a) ΔK plot showing its maximum value at K = 7 suggesting the optimal number of genetic clusters of seven, (b) scattered plot of DAPC, each colour represents one cluster.

Conclusion:

The genetic information obtained in this study provides essential data on the genetic diversity and population structure of Ethiopian barley (H. vulgare L.) populations across various agro-ecologies. This data is crucial for developing conservation and improvement strategies to enhance the productivity of the crop. Among the 15 populations from different barley-producing zones in Ethiopia, a total of 478 alleles were identified, with an average of 9.755 alleles per locus. The natural populations exhibited moderate genetic diversity, high gene flow, and low genetic differentiation among populations, indicating significant seed exchange.

The AMOVA analysis further confirmed the high variation within populations and low variation among populations, largely influenced by gene flow due to seed exchange. The model-based STRUCTURE analysis grouped the 15 populations into seven clusters, which could serve as management groups for conservation purposes. Conservation measures, both on-farm (in situ) and ex situ, are crucial for preserving the largest number of populations, with a focus on those with high genetic diversity.

The utilization of SSR markers in this study enabled the investigation of genetic diversity patterns, population structure, and germplasm collection and conservation strategies for barley landraces. This valuable information can aid in crop breeding to enhance productivity. The

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findings, particularly the identification of private (unique) alleles, support conservation strategies and the use of SSR markers for molecular breeding in future improvement efforts.

Overall, the barley accessions analyzed in this study exhibited significant genetic and phenotypic diversity, suggesting strong genetic structure. These results will be valuable for future association mapping studies using the Ethiopian barley collection.

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