

Research Article

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Genetic Diversity in Ginger Accessions from Pakistan Using ISSR Markers

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ABSTRACT:

Ginger is an economically important crop with significant culinary and medicinal value. As a relatively novel crop in Pakistan, its production remains limited, with most germplasm imported from major ginger-producing countries, resulting in a narrow genetic base. This study assessed the genetic diversity of eight ginger accessions collected from different sources across Pakistan using Inter Simple Sequence Repeat (ISSR) markers. Fourteen ISSR primers generated 64 reproducible bands ranging from 150–1000 bp, of which 50 (74.4%) were polymorphic. Several primers, including UBC811, UBC808, ISSR-21, ISSR-4, and ISSR-1, exhibited 100% polymorphism, confirming the high-resolution power of ISSR markers. Cluster analysis (UPGMA) grouped the accessions into two major clusters primarily reflecting their trade origins, while Principal Coordinate Analysis (PCoA) supported these relationships. Notably, the Turkish accession (C7) was genetically distinct, indicating the presence of unique germplasm. Although basic morphological traits were recorded, molecular markers provided a more robust approach for assessing genetic relationships. The high level of polymorphism detected demonstrates the utility of ISSR markers for analyzing ginger diversity. This study establishes the first molecular baseline for local ginger accessions in Pakistan, providing valuable resources for germplasm conservation, breeding, and future genetic improvement programs.

KEYWORDS:

Ginger, Genetic diversity, ISSR markers, Cluster analysis.

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INTRODUCTION

Ginger (*Zingiber officinale*) is a perennial herbaceous plant belonging to the family Zingiberaceae (Prasath et al., 2024). It is better known for its culinary and medicinal properties. Its rhizomes are rich in carbohydrates (60-70%), proteins, fibers, essential oils, phenolics, and terpenes. It has some key bioactive constituents which include; gingerol, shogaol, paradols, zingiberene, and flavonoids such as luteolin and rutin (Mao et al., 2019). It is also a good source of nutraceutical chemicals utilized in traditional Eastern medicine, which provide vital health benefits, such as disease prevention and therapy (AlAli et al., 2021). It has recently been suggested as a treatment for COVID-19-related lung inflammation due to its anti-inflammatory and immune-modulatory qualities (Jafarzadeh et al., 2021). Globally, ginger is a significant cash crop, widely cultivated in countries such as India, China, Nepal, Nigeria, and Indonesia (Nia et al., 2024). These countries export

significant quantities of ginger to meet the heavy demand for this spice in various forms, including fresh, dried, and processed ginger products. In Pakistan, although ginger production is limited, there is increasing interest in its local cultivation, especially in Punjab and Khyber Pakhtunkhwa (Sabir & Malik, 2023). The yield per hectare (0.45 t/ha) is considerably lower than global averages, which highlights the need for improved cultivars and production practices (Fayyaz et al., 2023). Due to small-scale production, it remained mostly an imported commodity, with over \$42.9 million (75.9 metric tonnes) of worth imported in 2023. The plant typically reaches a height of approximately 1.2 meters, characterized by its erect, slender leaves and fibrous, fleshy rhizomes with a striated texture (Kumari et al., 2020). Ginger is a diploid plant and reproduces vegetatively due to self-incompatibility (Dhamayanthi et al., 2003). This greatly limits its genetic variability, and major sources of genetic

variation in this crop are spontaneous mutation and natural selection (Yusuff et al., 2024). Molecular markers provide a direct means to identify and characterize plant genotypes by accessing their genome (Amiteye, 2021). These include inter-simple sequence repeats (ISSR), simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP), among others. These markers are particularly valuable as they are widely applied in various aspects of crop species, including breeding programs (Hasan et al., 2021). ISSR markers are cost-effective, relatively simple, and do not require previous knowledge of the genome sequence (Gemmell & Grierson, 2021). Since ginger is primarily propagated vegetatively, its genetic base is considered narrow. However, variations observed in local ginger types suggest the presence of untapped diversity that requires further investigation. Currently, there is no established molecular reference data to guide breeding, conservation, or varietal improvement in the country (Ucer et al., 2025; Šučur et al., 2025). Therefore, this research aims to assess the genetic variability among locally available ginger accessions using ISSR markers, providing insights that can contribute to conservation and crop improvement strategies.

MATERIALS AND METHODS

Germplasm collection and identification

A total of eight accessions were collected from diverse sources across Pakistan, including local markets, research institutions, and farms. For reference, the accessions were tagged based on their site or source of collection (Table 1).

Table 1. Accession, their source, and common names

S. No.	Accession Code	Source	Common name
1	C1	Islamabad	Indian
2	C2	NIGAB	Chinese
3	C3	Faisalabad	Indian
4	C4	Imported	Thailand
5	C5	Nishtar Farm	Chinese
6	C6	NIGAB	Local Ginger
7	C7	Haripur	Turkish
8	C8	Nishtar Farm	Indonesian

Rhizome sowing and germination

Pot (6 inches) containing peat moss was prepared for sowing the rhizomes and placed in a screen-house under controlled environmental conditions (Rymbai et al., 2018) including temperature ranging from 24–34 °C, humidity (70–85%), and light were ensured. The optimal moisture level was maintained by watering periodically.

Morphological data collection

The rhizomes of each accession were harvested and evaluated for basic morphological traits, including rhizome width (cm), rhizome length (cm), rhizome weight (g), and

number of days to sprouting. Rhizome dimensions were measured using a vernier caliper with a least count of 0.01 cm, and weight was measured using digital weight scales. The sprouting days were recorded from the date of sowing until the appearance of the first visible shoot.

DNA Extraction and quantification

Young healthy sprouts from each accession were excised using a sterilized scalpel. These samples are initially crushed and ground into a fine powder using mortars and pestles, with the aid of liquid nitrogen. For DNA extraction, 100 mg of the powdered tissue was used. The CTAB (Cetyltrimethylammonium bromide) technique (Abbas et al., 2021), with some modifications, was used for DNA extraction. The CTAB buffer was added to the samples, and the mixture was incubated at 65°C for 60 minutes. Chloroform: Isoamyl alcohol (24:1) was poured into the samples, mixed thoroughly, and centrifuged to separate the aqueous and organic phases. The supernatant containing genomic DNA was transferred to a new Eppendorf tube and precipitated with isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, and dissolved in Tris-EDTA buffer (Gupta, 2019). The quality of the extracted DNA was determined by gel electrophoresis using 1% agarose gel stained with ethidium bromide at a constant voltage of 100v for 45 minutes. The gel was then visualized in a gel documentation system (gelLITE, Germany), and the DNA bands were compared to the 50kb DNA ladder (MBP Inc., PK) in order to estimate the concentration and assess the quality of the DNA (Wittmeier & Hummel, 2022).

Inter Simple Sequence Repeat (ISSR)-PCR analysis

ISSR primers (Table 2) were selected based on their reported success in ginger studies or related plant species (Das et al., 2017; Ismail et al., 2019). PCR reactions were prepared in a total volume of 12 µL such that they contain 3.5 µL PCR Master Mix (Thermo Fisher Scientific Inc., US), ISSR Primer (2 µL), ddH₂O (4.5 µL), and DNA (2 µL) for each reaction (Akhtar et al., 2021). PCR amplification was achieved in a thermal cycler (Thermo Fisher Scientific Inc. US) in a way that Initial denaturation is given at 94°C for 8 mins, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at the primer-specific temperature (in table) for 40 seconds, and extension at 72°C for 50 seconds. Finally, extension at 72°C for 10 mins and the sample is cooled to 4°C at the end of the reaction (Khehra et al., 2025).

Statistical analysis

Microsoft Excel 2013 was used to analyze the morphological data, including rhizome width, length, weight, and days to sprouting. For molecular data, clear and reproducible ISSR bands were scored in a binary format, with '1' representing the presence and '0' the absence of a band for each primer

across all accessions. Genetic similarity coefficients were calculated using Jaccard's similarity index based on the binary matrix. Principal Coordinate Analysis (PCoA) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering were performed to assess genetic relationships among the accessions, and a dendrogram was constructed using DARwin software version 5.0.158 (Dissimilarity Analysis and Representation for Windows).

RESULTS

Rhizome width

Among the eight ginger accessions (Figure 1), C5 has the highest rhizome width (7.1 cm), followed by C1 (7.0 cm) and C6 (7.0 cm). The rhizome width was statistically significant ($P < 0.05$). C3 also has a greater rhizome width of 6.5 cm. The accessions C3, C7, and C2 have intermediate rhizome widths of 6.5 cm, 5.5 cm, and 5.0 cm, respectively. C8 has the lowest rhizome width (3.5 cm) of all.

Rhizome length

The highest rhizome length (7.1 cm) was observed in accession C1, and the lowest (7.0 cm) in C8. All the other accessions have intermediate rhizome lengths within the range of 11 cm – 13 cm (Figure 2).

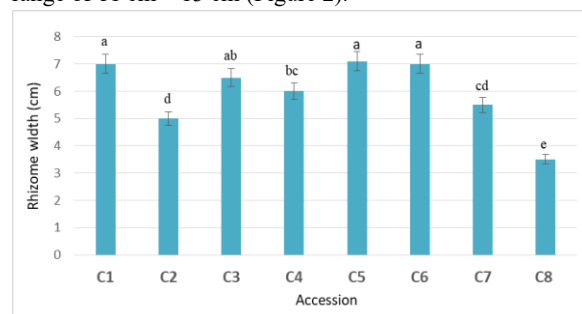


Figure 1. Rhizome width across eight ginger accessions

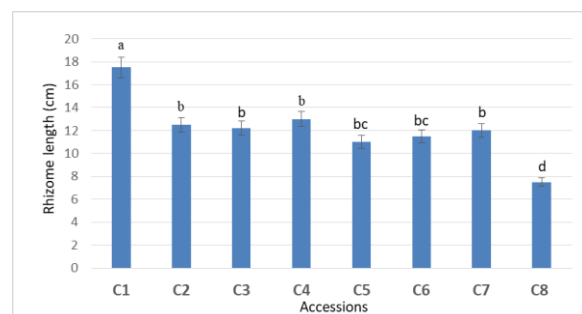


Figure 2. Rhizome length across eight ginger accessions

Rhizome weight

A significantly higher mean weight (19.5 g) was observed in C8 (Figure 3), closely followed by C5 (108 g), and both indicated superior performance. C1 (102.3 g), C4 (92 g), and C8 (91.6 g) reflected moderate potential. Lower mean rhizome weight was shown by C7 (86.9 g) and C3 (80.5 g)

as compared to the leading accessions. The lowest mean rhizome weight was recorded in C2 (68.8 g).

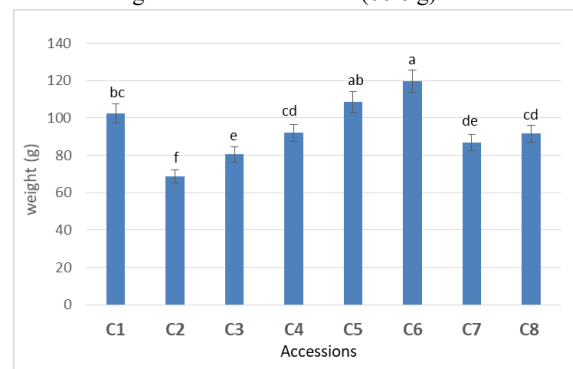


Figure 3. Rhizome weight across eight ginger accessions

Days to Sprout

There is a significant variation in the days to sprouting among the accessions (Figure 4). C7 sprouted in the least days (≈ 10 days). Accession C3 and C6 (approximately 11 days) also showed relatively faster sprouting compared to the rest. In accessions C1, C4, and C5, intermediate sprouting (≈ 12 days) was recorded. The slowest sprouting was observed in C2 and C8 (≈ 13 -14 days). These results showed a diverse sprouting behavior, with specific accessions demonstrating notable potential for rapid field establishment.

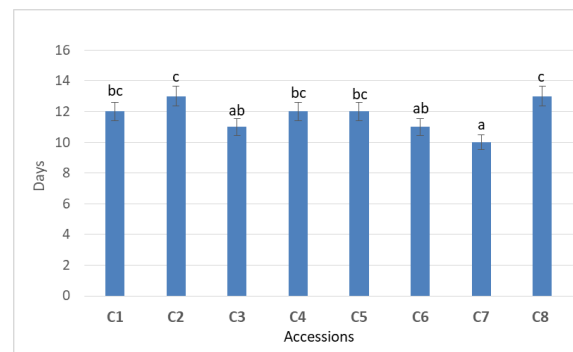


Figure 4. Days to sprout across eight ginger accessions

ISSR Profiling

A total of 64 bands were obtained using 14 ISSR Markers (Table 2), out of which 50 bands are polymorphic, and 10 are monomorphic. All the bands were produced in the size range between 150 bp and 1000 bp. The average number of bands produced per primer was 4.6. The present study reveals high genetic diversity, with an average of 78.1% polymorphic bands and 21.9% monomorphic bands. Out of 14 ISSR primers, five primers (UBC811, UBC808, ISSR-21, ISSR-4, and ISSR-1) showed 100% polymorphism and produced the highest number of bands (5). The lowest polymorphism (33.3%) was observed with primers ISSR-18 and ISSR-8. The average percentage of polymorphism among the studied accessions was 74.4.

Dendrogram analysis

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis, using Jaccard's similarity coefficient, constructed a dendrogram (Figure 5) based on ISSR marker data, grouping the eight accessions into two major clusters that reflect their genetic relationships. Cluster I included four accessions: C2 (Chinese), C5 (Chinese), C6 (local ginger), and C4 (Thailand), indicating a close genetic relationship between Chinese from different locations, local-type and Thai origin samples, suggesting a possible shared genetic background, which may be due to similar agro-climatic adaptation or familiar sources of import. Cluster II included C1 (Indian), C3 (Indian), and C8 (Indonesian), which also clustered closely, demonstrating a genetic relationship despite their different trade origins. The accession C7 (Turkish) appeared distinct and formed a separate out-group, highlighting its high genetic divergence from all other accessions.

Principal coordinate analysis

The Principal Coordinate Analysis (PCoA) provided a clear visualization of genetic variation among the eight accessions based on binary band scoring data. The first two coordinates explained the majority of the variation in the dataset. The accessions C1 (Indian) and C3 (Indian) from Islamabad and Faisalabad, respectively, clustered closely, indicating high genetic similarity. Similarly, the close grouping of accessions C2 (Chinese), C5 (Chinese), C6 (local type), and C4 (Thai) suggests a narrow genetic base or a possible shared origin. In contrast, accession C8 (Indonesian type) and C7 (Turkish type) were distinctly separated from the rest, showing greater genetic divergence. The position of C7 in the far lower right quadrant highlights its considerable genetic uniqueness, which is consistent with its separate clustering in the dendrogram.

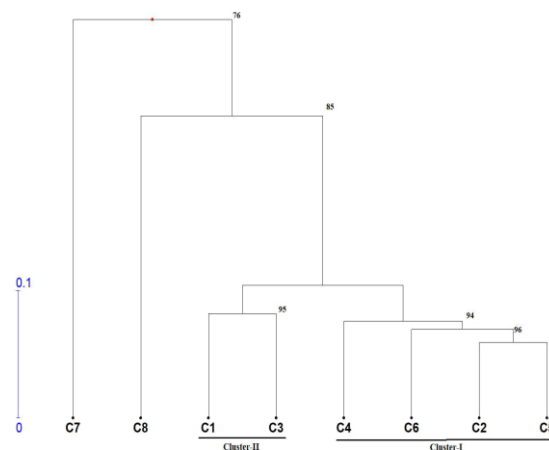


Figure 5. Dendrogram of eight ginger accessions based on hierarchical analysis using Unweighted pair group method with arithmetic mean (UPGMA)

DISCUSSION

Understanding the degree of genetic variation within and among populations is crucial for assessing crop diversity and, consequently, plays a pivotal role in guiding selection and improvement strategies for crops. Nowadays, the development of molecular tools has made DNA-based markers indispensable for characterizing genetic diversity due to their speed, specificity, sensitivity, and accuracy. Among these, ISSR markers have been successfully used to distinguish between genotypes and evaluate genetic relationships in a variety of horticulture crops, including ginger (Mujeeb et al., 2017; Luz et al., 2020; Vyas et al., 2023). Being a vegetatively propagated crop, it is difficult to distinguish between the genotypes of ginger using morphological markers; therefore, molecular approaches are highly useful for the characterization of ginger genotypes. Sprouted buds of ginger rhizome were used to extract genomic DNA through a modified CTAB method, which is reported to yield higher quality and purity than other methods that use leaf samples of ginger (Dalgıç et al., 2025; Qureshi et al., 2025). The results of genetic diversity indicators used in this study are consistent with the previous investigations on the genetic diversity of ginger. Profiling of 14 ISSR primers showed 64 total bands, of which 74.4% are polymorphic, which is slightly higher than the earlier study by (Das et al., 2017) 55%, 55%. The same study reported a band size range of 180bp-1000bp, which coincides with the current study (150bp-1000bp). The minimum (33.3%) and maximum (100%) polymorphic bands were shown by ISSR primers; similar results were reported by (Das et al., 2020). The dendrogram using Jaccard's similarity coefficient broadly grouped all the accessions into two clusters (I, II) except accessions C7 and C8. Cluster-I consists of four accessions, out of which two are of the Chinese type, one is

of the local type, and one is of the Thai type. Cluster-II comprises two Indian-type. This clustering pattern shows a geographical bias and is consistent with the findings of Kizhakkayil and Sasikumar (2010), who reported geographical grouping among ginger genotypes using ISSR markers. Similarly, (Ceyhan et al., 2025; Madenova et al., 2024) also observed that molecular clustering of accessions reflects their geographical origin. These findings support the idea that the imported nature of ginger in Pakistan has introduced genetically diverse material, with clustering patterns mainly linked to trade origins. The genetic variability

observed in this study could serve as a valuable resource for germplasm conservation and breeding programs. The Principal Coordinate Analysis (PCoA) of accessions, based on the genetic distance matrix, showed a similar number of clusters as

indicated by the dendrogram. The accessions C1 (Indian) and C3 (Indian) are grouped in Cluster-I. Similarly, Cluster-II contains accession C2 (Chinese), C5 (Chinese), C6 (local-type), and C4 (Thai), suggesting a potential common genetic base or possible shared geographical origin. The accessions C7 (Turkish-type) and C8 (Indonesian-type) are positioned far from each other in different quadrants and also not included in any cluster. This spatial distribution further confirms the results of cluster analysis. These findings highlight both genetic diversity and relationships among imported ginger germplasm in Pakistan. These findings support the idea that the imported nature of ginger in Pakistan has introduced genetically diverse material, with clustering patterns mainly linked to trade origins. The genetic variability observed in this study could serve as a valuable resource for germplasm conservation and breeding programs.

CONCLUSION AND FUTURE PERSPECTIVES

Our study provides the first molecular baseline of ginger accessions cultivated in Pakistan, highlighting significant genetic variability among imported germplasm. The use of

ISSR markers proved highly effective, revealing a high level of polymorphism and enabling clear differentiation among accessions that could not be achieved solely through morphological traits. Cluster analysis and PCoA demonstrated distinct grouping patterns primarily associated with trade origins, with the Turkish accession (C7) standing out as genetically unique. Looking ahead, broadening the genetic base of ginger in Pakistan is essential to reduce dependence on imported material and enhance local adaptability. Future research should expand the sampling of germplasm from diverse geographical origins, including

Table 2: Diversity assessment parameters of eight ginger accessions based on ISSR primers

Sr.No.	Primer	Total Bands	Polymorphic Bands	Monomorphic Bands	Percentage of polymorphic bands	Range of amplicon
1	UBC809	4	4	0	100	150bp-900bp
2	UBC817	4	4	0	100	250bp-1000bp
3	UBC855	4	3	1	75	200bp-700bp
4	UBC880	4	3	1	75	270bp-600bp
5	UBC830	4	3	1	75	250bp-750bp
6	UBC811	5	4	1	80	200bp-750bp
7	UBC808	5	3	2	60	200bp-600bp
8	ISSR-20	4	4	0	100	200bp-550bp
9	ISSR-21	5	4	1	80	250bp-750bp
10	ISSR-18	3	1	2	33.3	300bp-650bp
11	ISSR-8	3	1		33.3	200bp-600bp
12	ISSR-4	5	4	1	80	200bp-800bp
13	ISSR-2	4	2	2	50	150bp-700bp
14	ISSR-1	5	5	0	100	250bp-900bp
ISSR		64	50	14	74.4	150bp-1000pb

landraces and wild relatives, to capture untapped variation. Integrating ISSR with high-resolution molecular tools such as SSRs, SNP genotyping, or next-generation sequencing approaches will provide deeper insights into genome-wide diversity and trait-associated markers.

DECLARATIONS

AI Usage Declaration

In line with COPE guidelines, AI-assisted tools were used only for language editing and formatting and did not contribute to scientific content, data, analysis, or conclusions. All responsibility for the manuscript rests with the authors.

Conflict of Interest Statement

There is no conflict of interest in publishing this article

Author contribution statement

Muhammad Arif: Data curation, investigation, methodology and writing- original draft. **Aqleem Abbas:** Supervision, writing- review & editing. **Mughees ul Hassan:** Visualization, writing- review & editing. **Abid Khan:**

Conceptualization, writing- review & editing. **Talha Shafique** and **Muhammad Asif Shabbir**: Software, formal analysis, writing- review & editing.

Data Availability

Data will be available on demand.

Conflict of interest

The authors declare that they don't have any competing interests.

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