

Variability of *Agave amica* in India Using SRAP Markers and Multivariate Analysis of Morphological Traits

Jadhav PR

ICAR-Directorate of Floricultural Research

Jagtap AY

ICAR-Directorate of Floricultural Research

Shingote PR

Vasantrao Naik College of Agricultural Biotechnology (Dr. PDKV, Akola), Waghapur Road, Yavatmal, Maharashtra-445001, India.

Solanke AU

ICAR-National Institute for Plant Biotechnology

Pagariya MC

BJS Arts, Science and Commerce College

PN Kumar

ICAR-Indian Institute of Horticultural Research

Prasad KV

ICAR-Directorate of Floricultural Research

Kawar PG

pkawar@gmail.com

ICAR-Directorate of Floricultural Research

Research Article

Keywords: Genetic diversity, SRAP, Tuberose, Cluster analysis, Resolving power (Rp), PIC value

Posted Date: September 30th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4991024/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The genus *Agave*, indigenous to Mexico, comprises approximately 15 species, 3 varieties, and a few commercially cultivated cultivars. Despite its ornamental value and global economic importance, the development of new cultivars has been limited, with only two primary varieties and around 20 single and double-flower cultivars currently in cultivation, restricting the genetic diversity available to breeders.

This study investigates the genetic and morphological diversity among thirteen tuberose (*Agave amica*) cultivars using Sequence-Related Amplified Polymorphism (SRAP) markers and morphological assessments. SRAP analysis generated 63 scorable bands, with 54 polymorphic and 9 monomorphic bands, resulting in about 81% polymorphism. Cluster analysis via the NTSYS-pc program grouped the cultivars into two major clusters, with genetic similarity coefficients ranging from 0.51 to 0.89, indicating significant genetic variation. Within Cluster I, Hyderabad Single and Arka Prajwal showed 85.93% similarity, while Arka Sugandhi shared 84.37% similarity with both. Arka Nirantara formed a distinct subgroup, showing 84.37% similarity with Arka Prajwal and 78.12% with Arka Sugandhi. In Cluster II, Bidhan Ujjwal and Arka Vaibhav exhibited high relatedness with 89.06% similarity. Sikkim Selection and Mexican Single formed a subgroup within this cluster. SRAP primers showed a resolving power ranging from 6 to 25, with an average of 3.85 polymorphic bands per primer pair and a PIC value of 0.528, demonstrating their effectiveness in distinguishing among cultivars.

Morphological evaluation of 11 traits, including plant height, leaf dimensions, flowering period, and spike characteristics, revealed significant variability. Multifactorial Analysis (MFA) and Agglomerative Hierarchical Clustering (AHC) identified key traits contributing to this diversity. The integration of molecular and morphological data offers a thorough understanding of the genetic and phenotypic diversity in tuberose, crucial for breeding, conservation, and the development of improved cultivars, as well as the effective conservation of its germplasm.

INTRODUCTION

The genus *Agave*, indigenous to Mexico, comprises approximately 15 species, 3 varieties, and a few cultivars suitable for commercial cultivation. All species within this genus are wild, with the exception of *Agave amica* (Medikus) Thiede & Govaerts (synonym: *Polianthes tuberosa*; family Asparagaceae), which exists solely under cultivation. Commonly referred to as tuberose, *Agave amica* is cultivated for its ornamental appeal and strong fragrance in tropical and subtropical regions worldwide. Tuberose, a popular ornamental and aromatic plant, is a half-hardy perennial with waxy and luminous flowers which are either classified as single or double type based on their whorl arrangement (Biswas et al. 2002; Sadhukhan et al. 2021). Double-petalled cultivars are referred to as double types, while single-petalled cultivars are referred to as single types. The flowers are fragrant characterized by a funnel-shaped perianth, waxy white, and blooms from bottom to top of the spike (Hutchinson et al. 2004; Naz et al. 2012). Each flower is about 25mm long, has six stamens, a 3-locular ovary, numerous ovules, the fruit is a capsule and its spike elongates up to 45 cm (Kumar et al. 2007). The bulbs of the tuberose are

composed of scales from the leaf base, with the stem concealed within these scales. Its roots are primarily adventitious and shallow.

Tuberose, popularly known as Rajanigandha or Nishigandha, is renowned for its sweet fragrance and attractive beauty. The flower's serene beauty is attributed to its tall, straight spike, bright white flowers, and captivating fragrance making it excellent cut flower ideal for floral arrangements such as bouquets and table decorations (Barba-Gonzalez et al. 2012). Loose flowers are a source of tuberose oil, which is known for its medicinal properties. Tuberose symbolizes sensuality and is used in aromatherapy for its ability to open the heart, calm the nerves, and restore joy and harmony, thereby improving an individual's emotional depth. It can stimulate the right side of the brain, bringing gaiety to the mind and heart. Commercially, tuberose is one of the most important flowers in the cut flower trade worldwide (Safeena et al. 2015).

A significant amount of variability exists in tuberose with respect to fragrance, vase-life, flowering behavior, wide adaptability to varied climates and soils, growth habits and more. Despite this variability, only a few cultivars possess desirable characteristics in terms of yield and quality (Ranchana et al. 2013) due to limited gene pool. Despite its significant global economic value as an ornamental plant, the development of new cultivars has been largely unsuccessful. At present, only two primary varieties, encompassing around 20 cultivars of both single and double flower types, are grown worldwide. This limited genetic diversity constrains the gene pool available to flower breeders (Datta, 2017). Furthermore genetic variability in tuberose is constrained by dichogamy and self-incompatibility, presenting significant challenges for conventional breeding (Verhock-Williams, 1975). Additionally, there is extensive uncertainty in naming the cultivars and improper nomenclature for various double and single tuberose is found to be followed in different states of India. Therefore, maintaining the purity of these different tuberose cultivars is crucial for their proper identification (Bharti et al. 2015).

Morphological characterization is a useful traditional method for examining the genetic diversity among species. It is successfully used in systems such as the DUS (Distinctness, Uniformity, and Stability) testing technique, which relies on morphological traits and standardized unified characteristics or agronomic traits. However, while effective, this method can be time-consuming, land-intensive, and influenced by environmental factors (Cooke, 1995; Van Beuningen and Busch, 1997; Korir et al. 2013). Despite these challenges, morphological evaluation remains crucial for understanding the diversity and potential of tuberose genotypes. Such evaluations help in identifying superior varieties with desirable traits such as flower size, fragrance, yield, and resistance to diseases and pests. The genetic variability within tuberose genotypes can be exploited through breeding programs to enhance crop performance and meet market demands. This study, conducted under Pune conditions, utilized multifactorial analysis (MFA) to examine eleven key morphological traits, revealing patterns of agro-morphological diversity among tuberose accessions. Several previous morphometric studies have demonstrated that multivariate discriminant analyses of morphological traits effectively enable the precise and objective differentiation of various plant varieties. This has been reported for various species, including wheat (*Triticum aestivum* L.) (Oumata et al. 2023), Kale (*Brassica oleracea. acephala*) (Pipan et al. 2023)

Both morphological and molecular characterizations of plant germplasm are critical for the effective utilization of plant collections in breeding and the preservation of valuable crop diversity in crop improvement programs (ZeybekoGLu et al. 2019). As the number of tuberose cultivars continues to grow, it becomes increasingly necessary to monitor these cultivars for novelty, distinctness, uniformity, and stability. Additionally, identifying molecular markers that can protect Plant Breeder's Rights is essential. Efficiently identifying and assessing the genetic relationships among diverse varieties makes it easier to choose the best candidates for breeding programs, ensuring the ongoing development and improvement of tuberose and other important crops (Geeta et al. 2014).

DNA fingerprinting-based testing offers a simpler and more accurate alternative for identifying species differences that are challenging to discern through phenotypic characters (Lanka et al. 2023). It has been effectively utilized for cultivar identification and genetic diversity studies in various ornamental plants like rose, bougainvillea and jasminum (Chatterjee et al. 2007; Baydar et al. 2004; Mahmood et al. 2013). In recent times it has also been applied in chrysanthemum, gladiolus, and tuberose (Baliyan et al. 2014; Chaudhary et al. 2018; Sirohi et al. 2017a). However, in case of tuberose, only RAPD and ISSR markers have been employed for diversity analysis (Sarkar et al. 2010; Kameswari et al. 2014; Khandagale et al. 2014; Sirohi et al. 2017a, b). Overall, bulbous crops have received limited attention in terms of molecular characterization.

The DNA-based markers enable direct analysis of the genome, providing reliable information on genetic uniformity and overcome the limitations of morphological and biochemical methods (Caetano et al. 1991; Zhang et al. 2014; Fajardo et al. 2017; Tian et al. 2018). These markers are independent of tissue type or age and are not affected by ecological dynamics, giving them a high capacity to distinguish genotypes. They offer numerous descriptors that can complement morphological data, particularly when distinguishing between varieties or germplasm is challenging.

Among the various markers available, Sequence-Related Amplified Polymorphism (SRAP) markers stand out for their simplicity, polymorphism and informativeness comparable to AFLP, reliability, reasonable throughput ratio, and ease of sequencing specific bands. (Li and Quiros, 2001; Aneja et al. 2012). The SRAP markers are highly reproducible and can be used for gene tagging and mapping. SRAP is a PCR-based marker system utilizing two primers: a 17-base forward primer and an 18-base reverse primer, designed to amplify open reading frames (ORFs). The forward primer targets exonic regions, while the reverse primer amplifies intronic regions with the promoter (Agyenim-Boateng et al. 2019). Compared to other marker systems, SRAP markers are easy to use, highly reproducible, and provide multiple markers through combinations of primer reactions. It has been used successfully in examining genetic diversity and relations within various species (Ferriol et al. 2003). Furthermore, it has been shown to be more revealing in identifying genetic diversity compared to other PCR-based techniques (Budak et al. 2004). Both morphological and molecular characterization of plant germplasm are essential for effectively utilizing plant collections in breeding and preserving valuable crop diversity in crop improvement programs (Zeybekoglu et al., 2019).

Hence this study employs SRAP markers to estimate genetic similarity and diversity among thirteen tuberose cultivars, demonstrating their effectiveness in detecting polymorphism and identifying genotypes for breeding programs. Additionally, a field study conducted at the ICAR-DFR research farm in Pune to assess the morphological diversity of tuberose genotypes by evaluating eleven key traits. Advanced statistical methods, including Multifactorial Analysis (MFA) and Agglomerative Hierarchical Clustering (AHC), are utilized to identify patterns of agro-morphological differentiation and explain the variability among accessions. This comprehensive analysis aims to improve the selection and development of superior tuberose varieties suitable for diverse agro-climatic conditions in India and worldwide.

MATERIALS AND METHODS

Plant Materials

A total of thirteen tuberose cultivars (Arka Nirantara, Hyderabad Single, Arka Prajwal, Arka Sugandhi, Phule Rajani, Local Double, Arka Suvasini, Local Single, GKTC-4, Mexican Single, Bidhan Ujjwal, Arka Vaibhav, Sikkim Selection) representing majority under cultivation in India were analyzed in the present study (Table 1). A total of 80 combinations of SRAP were tried. The combinations that gave reproducible polymorphic amplification were selected and used in analysis of the genetic polymorphism study (Table 2).

Genomic DNA isolation and quantification

Total genomic DNA from fresh young leaves of tuberose cultivars were isolated by using CTAB method with minor modifications (Doyle and Doyle 1987). The DNA quality was estimated on 0.8% agarose stained with ethidium bromide (EtBr). The genomic DNAs were stored at -20°C until further use. A total of 80 combinations of SRAP primers were used to assess polymorphism among the 13 tuberose cultivars (Table 2).

DNA profiling of tuberose cultivars using SRAP

The SRAP PCR was carried out by using reactions mixture contained 20–40 ng template DNA, primer (0.05–1 µM), 2.5mM MgCl₂, 200µM each dNTP, 1.0 U *Taq* DNA polymerase in 1× assay buffer in a final volume of 20µL with thermal profile: Initial denaturation (94°C:4 min), 10 cycles (94°C:1 min, 35°C:1 min, 72°C:1 min), 30 cycles (94°C:1 min, 50°C:1 min, 72°C:1 min) and final extension (72°C:5 min) (Liu et al. 2012).

All the PCR amplified products were resolved on 2.0% agarose gel prepared in Tris Borate Electrophoresis (TBE) buffer and stained with 0.5µg/ml ethidium bromide. SRAP profile scoring of clearly visible amplified allele (band) was recorded as presence (1) and absence (0), the binary scored data was subjected to NTSYS-pc version 2.02i to compute the similarity matrix based on the Dice coefficient (Rohlf 1990), also known as the similarity coefficient of (Nei and Li 1979). Clustering analysis was conducted using the Unweighted Pair-Group Method with Arithmetic averages (UPGMA) to construct the

dendrogram. Besides, PIC (polymorphism information content), marker index (MI), and Resolving Power (Rp) were also calculated.

Morphological evaluation

The study conducted at the ICAR-DFR research farm in Pune involved cultivating and evaluating tuberose genotypes for various morphological traits. Eleven key morphological characteristics were assessed: Plant Height (cm), Spike Length (cm), Rachis Length (cm), Leaf Length (cm), Leaf Width (cm), Number of Leaves, Flower Length (cm), Flower Diameter (cm), Total Number of Flowers, Total Number of Spikes, and Inter-Floret Length (cm) (Table 3). A multifactorial analysis (MFA) was performed using XLSTAT software (Version 2023.3.1) to analyze the collected data (Gonne et al. 2013). This analysis aimed to identify patterns of agro-morphological differentiation and determine the factors and traits that explain variability among the accessions. Following this, Agglomerative Hierarchical Clustering (AHC) was used to construct a dendrogram, which grouped the thirteen genotypes into distinct classes based on observed similarities. Additionally, Pearson Correlation coefficients were calculated to establish relationships among the various descriptors (Kassambara and Mundt 2020). This comprehensive approach facilitated a thorough examination of the morphological diversity and interrelationships within the tuberose germplasm under Pune conditions.

RESULTS AND DISCUSSION

SRAP (Sequence-Related Amplified Polymorphism) markers have attracted attention in genetic diversity analysis and cultivar identification due to their beneficial characteristics. They integrate the advantages of RAPD (Random Amplified Polymorphic DNA) and AFLP (Amplified Fragment Length Polymorphism) markers, making them particularly suitable for practical applications. This marker system uncovers genetic variation in coding regions which are more homogenous among cultivars and have a relatively low genetic variation, revealing relatively more informative bands (Liu et al. 2008). Consequently, SRAP markers are valuable tools in plant breeding and genetic studies, often preferred for their balance between cost-effectiveness and accuracy in genetic diversity assessments (Li and Quiros 2001).

Molecular profiling of thirteen tuberose cultivars utilizing SRAP markers

In the present study, out of eighty SRAP primer combinations, fourteen primer combinations that produced consistent, scorable, and reproducible amplification patterns were selected for molecular profiling of 13 tuberose cultivars (Table 3). These 14 SRAP primer combinations gave 63 alleles of which 54 were polymorphic and 9 were monomorphic, resulting in approximately 81% polymorphism (Table 3; Figs. 1 & 2). The extent of polymorphism is crucial for accurately identifying associations among cultivars. This level of polymorphism is higher than that reported in previous SRAP-based studies on other crops, such as 66.2% in pumpkin (Ferriol et al. 2004) and 75.72% in *Chrysanthemum morifolium* (Shao et al. 2010). The polymorphism level of SRAP in tuberose in the present study is 81%, which is also

higher than the polymorphism levels revealed by RAPD and ISSR markers, which were 53% and 73%, respectively (Khandagale et al. 2014). This difference may be attributed to RAPD and ISSR markers amplifying both coding and non-coding regions of the genome, while SRAP markers amplify only coding regions (Bargish and Rahmani 2016). The high marker polymorphism indicates significant variation among the studied cultivars.

The number of amplicons ranged from 2 to 8, with a mean of 4.5 amplicons per primer combination, which is higher than in SRAP marker-based studies on *Silene* species, where the number of amplicons ranged from 2 to 6 with an average of 3.2 amplicons per primer (Bargish and Rahmani 2016). This variation is due to differences in primer binding sites, resulting in each primer producing a different number of amplicons. The size of the amplified products ranged from 50 bp to 1200 bp. The SRAP primer combination m3e7 produced the highest number of alleles (8 alleles), followed by m1e6 (7 alleles). The m2e2 and m5e15 combinations produced the fewest alleles (2 alleles each). Species-specific bands were obtained for the cultivars Local Double, Arka Vaibhav, Mexican Single, Sikkim Selection, Local Single, and Arka Suvasini, with a maximum of six specific bands observed in Arka Suvasini.

Polymorphic information content (PIC) reflects the diagnostic capacity of a marker, associated with both the presence of polymorphic alleles and their frequencies. According to Botstein et al. (1980), values greater than 0.5 are classified as highly informative diversity loci. In the present study, the average PIC value was 0.52, with a range from 0.07 (m2e2) to 0.92 (m1e7), indicating the high efficiency of the primers used. The marker index varied from 0.032 (m2e5) to 6.19 (m3e7), with an average of 2.30. The resolving power of the primers ranged from 6 (m1e7) to 25 (m2e2), with an average of 15.43. Resolving power and the SRAP primer index were both noticeably higher than those reported in other crops (Bhatt et al. 2017). Prevost and Wilkinson (1999) described resolving power (RP) as a measure of the discriminatory ability of molecular markers. The most informative marker for distinguishing tuberose cultivars was selected based on the RP values obtained. The RP values in this study ranged from 6 to 25 (Table 3).

Genetic relationships between tuberose cultivars were assessed using Jaccard's Pairwise Similarity Coefficients, which provide a quantitative measure of genetic similarity. Higher coefficients indicate greater genetic similarity, while lower coefficients suggest greater genetic distinctness. The analysis revealed significant genetic variation among the tuberose cultivars, with coefficients ranging from 0.51 to 0.89. The cultivars Bidhan Ujjwal and Arka Vaibhav exhibited the highest similarity with a coefficient of 0.89, indicating a high genetic resemblance. This was followed by Hyderabad Single and Arka Prajwal, which showed a coefficient of 0.859, indicating a strong genetic connection. Conversely, the cultivars Suvasini, Arka Nirantara, and Arka Vaibhav showed the lowest pairwise similarity of 0.515, indicating they are genetically more distinct from each other (Table 4).

The UPGMA-based clustering, depicted by the dendrogram, showed that all 13 tuberose cultivars were grouped into two major clusters (Cluster I and II) at a similarity coefficient of 0.65 (Fig. 3). Cluster I

contained the majority of cultivars (9 out of 13) and was further divided into two subgroups, Ia and Ib. Subgroup Ia was further distinguished into two subgroups (Ia.1 and Ia.2). Subgroup Ia.1 included only one cultivar, Local Single. Subgroup Ia.2 comprised seven cultivars: four single-petaled varieties (Arka Nirantara, Hyderabad Single, Arka Prajwal, and Arka Sugandhi), where Hyderabad Single and Arka Prajwal were closely related and clustered with Arka Sugandhi. Arka Nirantara, with its parental cross of single and double petal varieties, was distinct from the other three single-petaled varieties. Phule Rajani, a single-petaled cultivar, was closely related to Local Double, a double-petaled cultivar, and both clustered closely with Suvasini, another double-petaled cultivar. Suvasini was clustered closely with its parental single and double petal crosses. Subgroup Ib contained only one single-petaled cultivar, GKTC-4, it is a distinct cultivar within this subgroup, suggesting genetic differences from the cultivars in subgroup Ia. The genetic differences observed may be attributed to the structural origins of each marker and the influence of various factors such as plant development stage, agricultural management, and environmental conditions on morphological expression (phenotype) (Sirohi et al. 2017).

The cluster II comprise of four cultivars including 3 single petaled cultivar namely Mexican Single, Bidhan Ujjwal, Sikkim Selection, and Arka Vaibhav a Semi-double petaled cultivar. Both Bidhan Ujjwal and Arka Vaibhav showed the highest similarity with no common parent. They both were also closely related to Sikkim Selection and Mexican Single. This is because Bidhan Ujjwal has Sikkim Selection as its one parent while Arka Vaibhav has Mexican Single as one of its parents and shared one cluster with a 0.75 similarity coefficient. Their parents Sikkim Selection and Mexican Single are closely related with 0.76 similarity, this may be the reason Bidhan Ujjwal and Arka Vaibhav are closely related. Whereas, Arka Prajwal was placed away from its parent Mexican Single with a 0.67 similarity coefficient. This may be due to the presence of a major genetic background of Shringar, its other parent.

This hierarchical clustering analysis highlighted the genetic diversity and morphological variation among the tuberose cultivars. The identification of distinct genetic groupings and close relationships among certain cultivars provides valuable insights for breeding programs and cultivation strategies. Understanding these genetic relationships is crucial for enhancing desirable traits and maintaining genetic diversity within the tuberose germplasm.

Patterns of agro-morphological differentiation among tuberose genotypes

Multifactorial analysis (MFA) was used together with quantitative data analysis to examine patterns in agro-morphological characteristics and identify the main sources of variation among tuberose genotypes. Descriptive statistics, such as maximum, minimum, mean values, and standard deviation for the estimated quantitative parameters, are provided in Table 6.

The correlations between variables and factors grouped the thirteen genotypes into several factors, with the first four factors explaining approximately 84.103% of the total observed variation (Table 7). According to the results from Tables 7 and 8, Factor 1 (F1) accounted for about 32.086% of the total variation, with plant height, spike length, and flower length, traits related to agronomic characteristics,

being the primary contributors to genotype separation along this axis. Factor 2 (F2), associated with Rachis Length, Leaf Length, Total Number of Flowers, Total Number of Spikes, and Inter-Floret Length explained 24.496% of the total variation. Factor 3 (F3) accounted for around 16.295% of the total variation and differentiated genotypes based on Leaf Width and Flower Diameter. The final Factor (F4) accounted for 11.225% of the total variation and primarily involved the number of leaves. This factor analysis effectively identified the main sources of morphological variation among the studied tuberose genotypes.

The Multifactorial Analysis (MFA) plot in Fig. 4 illustrates the relationships among various genotypes based on attributes such as Plant Height, Spike Length, Rachis Length, Leaf Length, Leaf Width, Number of Leaves, Flower Length, Flower Diameter, Total Number of Flowers, Total Number of Spikes, and Inter-Floret Length. The plot shows that genotypes with similar characteristics cluster together, while those with differing attributes are positioned further apart. In the first and second quadrants of the MFA plot, genotypes like Arka Prajwal, Suvasini, Phule Rajani, Mexican Single, Sikkim Selection, and Local Single are grouped together (Fig. 4). These genotypes share similarities in attributes such as Number of Leaves, Leaf Length, Total Number of Spikes, Plant Height, Spike Length, and Flower Length (Fig. 5). For instance, Suvasini and Phule Rajani are closely positioned in quadrant one due to their similar Number of Leaves and Total Number of Spikes. Similarly, Arka Prajwal is placed alone, sharing similarities in Leaf Length. Mexican Single and Sikkim Selection are closely associated in quadrant two, showing resemblances in traits such as Plant Height and Spike Length. Additionally, Local Single is positioned alone in quadrant two, displaying similarity in Flower Length. Conversely, genotypes in the opposite quadrants, such as Arka Vaibhav, Arka Nirantara, Local Double, Arka Sugandhi, GKTC-4, Hyderabad Single, and Bidhan Ujjwal, are characterized by attributes related to Total Number of Flowers, Rachis Length, Inter-Floret Length, Leaf Width, and Flower Diameter (Figs. 4 & 5). Thus, the MFA plot provides a clear visualization of the relationships between different genotypes based on various attributes, aiding in the identification of patterns and associations within the dataset.

Agglomerative hierarchical clustering (AHC) analysis was conducted to investigate the agromorphological differentiation among various tuberose genotypes. The degree of dissimilarity was estimated using the distance to the centroid based on phenotypic data of all traits, and the resulting dendrogram was divided into four main clusters (Fig. 6). Cluster 1 includes six genotypes: Arka Nirantara, Phule Rajani, Arka Vaibhav, Local Double, Hyderabad Single, and Arka Sugandhi. Within this cluster, Arka Vaibhav and Local Double are closely related despite Arka Vaibhav having semi-double flowers and Local Double having double florets. Arka Nirantara and Phule Rajani are also closely related to each other and are clustered near Arka Sugandhi. These three genotypes, in turn, are closely related to Hyderabad Single, with all four genotypes sharing similarities in terms of single floret type. Cluster 2 consists of three genotypes: Mexican Single, Sikkim Selection, and Local Single. Mexican Single and Sikkim Selection are closely related to each other and are clustered near Local Single, all sharing the single floret type. Cluster 3 contains two genotypes: Bidhan Ujjwal and GKTC-4, which are closely related and show similarities in terms of single flower type. Cluster 4 includes Suvasini and Arka Prajwal. Suvasini, a multi-whorled flower type variety, while Arka Prajwal is a single flower type variety. Despite

these differences, they are placed together in this cluster, likely due to sharing a similar genetic background.

Conclusion

Continuous advancements in both genotyping and phenotyping technologies contribute to improving the accuracy of genotype-phenotype correlations in plant studies. In the present study, SRAP markers demonstrated remarkable potential in differentiating tuberose cultivars, revealing high polymorphism levels among thirteen cultivars. These markers effectively assessed genetic diversity, aiding breeding and germplasm conservation efforts. Genetic relationships among the cultivars were unveiled, informing crossbreeding and hybridization decisions to improve tuberose varieties. Additionally, the study used Multifactorial Analysis (MFA) and Agglomerative Hierarchical Clustering (AHC) to examine agro-morphological differentiation among tuberose genotypes. Four main factors explained 84.103% of the total variation, with traits like plant height, spike length, and flower length being significant contributors. The MFA plot grouped similar genotypes together, revealing patterns in their morphological traits, while AHC analysis categorized the genotypes into four clusters, highlighting genetic relationships despite differences in flower types. This comprehensive analysis underscores the value of SRAP markers and advanced phenotyping in understanding genetic diversity, informing breeding programs, and enhancing conservation efforts for tuberose cultivars.

Declarations

Author contribution statement

Jadhav PR: Methodology, Formal analysis, Writing - original draft, final editing. **Jagtap AY:** Writing - original draft, Writing - review and editing and data analysis **Shingote PR:** Formal analysis, Investigation, Writing - editing. **Solanke AU:** Conceptualization, Methodology, formal analysis. **Pagariaya MC:** data analysis and editing of draft. **PN Kumar:** Resources, morphological analysis. **Prasad KV:** Resources, Supervision. **Kawar PG:** Conceptualization, Methodology, Formal analysis, Writing - review and final editing; Supervision & coordination.

Acknowledgement

The authors gratefully acknowledge the DG ICAR & Secretary DARE, New Delhi, India DDG (HS) ICAR, New Delhi ADG (HS) ICAR, New Delhi India. Director, ICAR-NIPB, New Delhi and Director, ICAR-IIHR, Bengaluru India, for financial and research support. The authors whose work formed the basis for this work and anonymous reviewers for critically reviewing and suggestions are also acknowledged.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Statements and Declarations

Funding: Institute funded.

Ethics approval: NA

Consent to participate (include appropriate consent statements): NA

Consent for publication (appropriate statements regarding publishing an individual's data or image): NA

Availability of data and material - (data transparency):

Data will be made available on reasonable request.

Code availability - (software application or custom code): NA

References

1. Agyenim-Boateng KG, Lu J, Shi Y, Zhang D, Yin X (2019) SRAP analysis of the genetic diversity of wild castor (*Ricinus communis* L.) in South China. PLoS One, 14(7), e0219667.
2. Aneja B, Yadav NR, Chawla V, Yadav RC (2012) Sequence-related amplified polymorphism (SRAP) molecular marker system and its applications in crop improvement. Molecular breeding, 30, 1635–1648.
3. Baliyan D, Sirohi A, Kumar M, Kumar V, Malik S, Sharma S, Sharma S (2014) Comparative genetic diversity analysis in chrysanthemum: A pilot study based on morpho-agronomic traits and ISSR markers. Scientia Horticulturae 167, 164–168.
4. Barba-Gonzalez R, Rodríguez-Domínguez JM, Castañeda-Saucedo MC, Rodríguez A, Van Tuyl JM, Tapia-Campos E (2012) Mexican geophytes I. the genus *Polianthes*. Floriculture and Ornamental Biotechnology 6(1), 122–130.
5. Bargish TA, Rahmani F (2016) SRAP markers based genetic analysis of *Silene* species. Journal of Tropical Biology & Conservation (JTBC) 13.
6. Baydar NG, Baydar H, Debener T (2004) Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. Journal of biotechnology 111(3), 263–267.
7. Baydar H, Akçay UÇ, Kazaz S, Baydar NG (2021) Floral and molecular characterization of gamma rays induced mutants in oil-bearing rose (*Rosa damascena* Mill.). Ekin Journal of Crop Breeding and Genetics 7(1), 21–33.
8. Bhatt J, Kumar S, Patel S, Solanki R (2017) Sequence-related amplified polymorphism (SRAP) markers based genetic diversity analysis of cumin genotypes. Annals of Agrarian Science 15(4), 434–438.

9. Bharti H, Singh KP, Singh MC (2015) Morphological characterization of tuberose (*Polianthes tuberosa* Linn.) germplasms using DUS testing. *Progressive Horticulture* 47(2), 280–287.
10. Biswas B, Kumar NP, Bhattacharjee SK (2002) Tuberose. *Tech. Bulletin, All India Co. Res. Proj. Flori*, New Delhi, Pp. 1–25.
11. Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics* 32(3), 314.
12. Budak H, Shearman RC, Parmaksiz I, Dweikat I (2004). Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theoretical and Applied Genetics* 109, 280–288.
13. Caetano-Anollés G, Bassam BJ, Gresshoff PM (1991) DNA amplification fingerprinting: a strategy for genome analysis. *Plant Molecular Biology Reporter* 9, 294–307.
14. Chatterjee J, Mandal AKA, Chakrabarty D, Datta SK (2007) Use of RAPD Analysis to Determine Genetic Diversity and Relationships among Bougainvillea Cultivars at Intra-and Inter-Specific Levels. *Horticulture Environment and Biotechnology* 48(1), 43.
15. Chaudhary V, Kumar M, Sharma S, Kumar N, Kumar V, Yadav HK, Sirohi U (2018) Assessment of genetic diversity and population structure in gladiolus (*Gladiolus hybridus* Hort.) by ISSR markers. *Physiology and Molecular Biology of Plants* 24, 493–501.
16. Cooke RJ (1995) Varietal identification of crop plants. *New diagnostics in crop sciences* 33–63.
17. Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*.
18. Dutta SK, (2017) Breeding of ornamentals: tuberose (*Polianthes tuberosa* L.). *Cur. Sci.*, 113 (7) 1255–1263.
19. Fajardo CG, de Almeida Vieira F, Felix LP, Molina WF (2017) Negligence in the Atlantic Forest, northern Brazil: a case study of an endangered orchid. *Biodiversity and Conservation* 26, 1047–1063.
20. Ferriol M, Picó B, Nuez F (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* L. using SRAP and AFLP markers. *Theoretical and Applied Genetics* 107, 271–282.
21. Ferriol M, Pico B, de Cordova PF, Nuez F (2004) Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. *Crop Science* 44(2), 653–664.
22. Geeta SV, Shirol AM, Nishani S, Shiragur M, Varuna KJ (2014) Assessing genetic diversity of gladiolus varieties using SRAP markers. *Research Journal of Agricultural Science*, 5(4), 658–661.
23. Gonné S, Félix-Alain W, Benoît KB (2013) Assessment of twenty bambara groundnut (*Vigna subterranea* (L.) *Verdcourt*) landraces using quantitative morphological traits. *International Journal of Plant Research*, 3(3), 39–45.
24. Hutchinson MJ, Onamu R, Obukosia S (2004) Effect of thidiazuron, benzylaminopurine and naphthalene acetic acid on *in vitro* propagation of tuberose (*Polianthes tuberosa* L.) from shoot tip

- explants. *Journal of Agriculture, Science and Technology* 6(1), 48–59.
25. ICAR-DFR Annual Report (2020) ICAR-Directorate of Floricultural Research, College of Agriculture Campus, Shivajinagar Pune-411005, Maharashtra, India
 26. Kameswari PL, Girwani A, RadhaRani K (2014) Genetic diversity in tuberose (*Polianthes tuberosa* L.) using morphological and ISSR markers. *Electronic Journal of Plant Breeding* 5(1), 52–57.
 27. Kassambara A, Mundt F (2020) Factoextra: Extract and visualize the results of multivariate data analyses. v1. 0.7. CRAN. [r-project.org/package = factoextra](https://r-project.org/package=factoextra).
 28. Khandagale K, Padmakar B, Reddy DL, Sane A, Aswath C (2014) Genetic diversity analysis and barcoding in tuberose (*Polianthes tuberosa* L.) cultivars using RAPD and ISSR markers. *Journal of Horticultural Sciences* 9(1), 5–11.
 29. Korir NK, Han J, Shangguan L, Wang C, Kayesh E, Zhang Y, Fang J (2013) Plant variety and cultivar identification: advances and prospects. *Critical reviews in biotechnology*, 33(2), 111–125.
 30. Kumar B, Malik S, Kumar M, Singh MK, Kumar V (2007) Postharvest Quality and Vase Life of Tuberose (*Polianthes Tuberosa* Linn.) Cut Spikes as Affected by Vase Chemicals. *Journal of Ornamental Horticulture* 10(4), 274–275.
 31. Lanka, C. L., Ram, M., & Krishna, S. M. (2023). DNA Fingerprinting of Crops and Its Significance in Crop Improvement. *International Journal of Plant & Soil Science*, 35(16), 232–242.
 32. Liu LW, Zhao LP, Gong YQ, Wang MX, Chen LM, Yang JL, Wang LZ (2008) DNA fingerprinting and genetic diversity analysis of late-bolting radish cultivars with RAPD, ISSR and SRAP markers. *Scientia Horticulturae* 116(3), 240–247.
 33. Liu L, Liu Z, Chen H, Zhou L, Liu Y, Luo L (2012) SRAP markers and morphological traits could be used in the test of distinctiveness, uniformity, and stability (DUS) of lettuce (*Lactuca sativa*) varieties. *Journal of Agricultural Science* 4(3), 227.
 34. Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and applied genetics* 103, 455–461.
 35. Mahmood MA, Hafiz IA, Abbasi NA, Faheem M (2013) Detection of genetic diversity in *Jasminum* species through RAPD techniques. *International Journal of Agriculture and Biology* 15(3).
 36. Naz S, Aslam F, Ilyas S, Shahzadi K, Tariq A (2012) *In vitro* propagation of tuberose (*Polianthes tuberosa*). *Journal of Medicinal Plants Research*, 6(24), 4107–4112.
 37. Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences* 76(10), 5269–5273.
 38. Oumata S, Monneveux P, Zaharieva M, Mekliche-Hanifi L, David J (2023) Variation of morphological traits among wheat (*Triticum aestivum* L.) landraces from two regions of the Algerian Sahara. Potential interest for wheat breeding. *Genetic Resources and Crop Evolution*, 70(1), 235–250.
 39. Pipan B, Neji M, Meglič V, Sinkovič L (2023) Genetic diversity of kale (*Brassica oleracea* L. var *acephala*) using agro-morphological and SSR markers.

40. Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and applied Genetics* 98, 107–112.
41. Ranchana P, Kannan M, Jawaharlal M (2013) Evaluation of tuberose (*Polianthes tuberosa*) genotypes (Double) for yield and genetic variability. *Journal of Ornamental Horticulture* 16(1and2), 10–14.
42. Rohlf FJ (1990) NTSYS-pc. Numerical taxonomy and multivariate analysis system.
43. Rose JN (1905) Studies of Mexican and Central American plants—No. 4. Contributions from the United States National Herbarium 8(4), 281–339.
44. Safeena SA, Thangam M, Devi SP, Desai AR, Singh NP (2015) Ready reckoner on cultivation of tuberose. Technical Bulletin, 50, 1–33, ICAR-Central Coastal Agricultural Research Institute (Indian Council of Agricultural Research), Ela, Old Goa-403 402, Goa, India.
45. Sarkar J, Misra RL, Bhat KV, Singh A, Singh SK (2010) Genetic diversity analysis in tuberose (*Polianthes tuberosa*) genotypes through Randomly Amplified Polymorphic DNA. *Indian Journal of Genetics and Plant Breeding* 70(02), 182–188.
46. Sadhukhan R, Chowdhuri TK, Datta SK (2021) Tuberose (*Polyanthes tuberosa* Linn./*Agave amica*). In *Floriculture and Ornamental Plants* (pp. 1–52). Singapore: Springer Singapore.
47. Shao QS, Guo QS, Deng YM, Guo HP (2010) A comparative analysis of genetic diversity in medicinal *Chrysanthemum morifolium* based on morphology, ISSR and SRAP markers. *Biochemical Systematics and Ecology* 38(6), 1160–1169.
48. Shingote PR, Amitha Mithra SV, Sharma P, Devanna NB, Arora K, Holkar SK, Solanke AU (2019) LTR retrotransposons and highly informative ISSRs in combination are potential markers for genetic fidelity testing of tissue culture-raised plants in sugarcane. *Molecular Breeding* 39, 1–13.
49. Sirohi U, Kumar M, Chauhan P, Kumar N, Prakash S, Chand P, Chaudhary V (2017a) Genetic diversity in tuberose (*Polianthes tuberosa* L.) germplasm using Inter Simple Sequence Repeat (ISSR) markers. *Int. J. Curr. Microbiol. App. Sci* 6(5), 1313–1321.
50. Sirohi U, Kumar M, Kumar P, Singh MK, Kumar N, Prakash S, Chaudhary V (2017b) Genetic diversity in single and double type of tuberose (*Polianthes tuberosa* L.) germplasm using randomly amplified polymorphic DNA (RAPD) markers. *Int. J Agric. Stat. Sci* 13(2), 519–526.
51. Tian HZ, Han LX, Zhang JL, Li XL, Kawahara T, Yukawa T, Chung MY (2018) Genetic diversity in the endangered terrestrial orchid *Cypripedium japonicum* in East Asia: Insights into population history and implications for conservation. *Scientific Reports* 8(1), 6467.
52. Van Beuningen LT, Busch RH (1997) Genetic diversity among North American spring wheat cultivars: I. Analysis of the coefficient of parentage matrix. *Crop Science* 37(2), 570–579.
53. Zeybekoğlu E, Ascioğlu TK, Hulya ILBI, Ozzambak ME (2019) Genetic diversity of some daffodil (*Narcissus* L. spp.) genotypes in Turkey by using SRAP markers. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47(4), 1293–1298.
54. Zhang Y, Dai S, Hong Y, Song X (2014) Application of genomic SSR locus polymorphisms on the identification and classification of chrysanthemum cultivars in China. *PLoS One* 9(8), e104856.

Tables

Tables 1 to 8 are available in the Supplementary Files section.

Figures

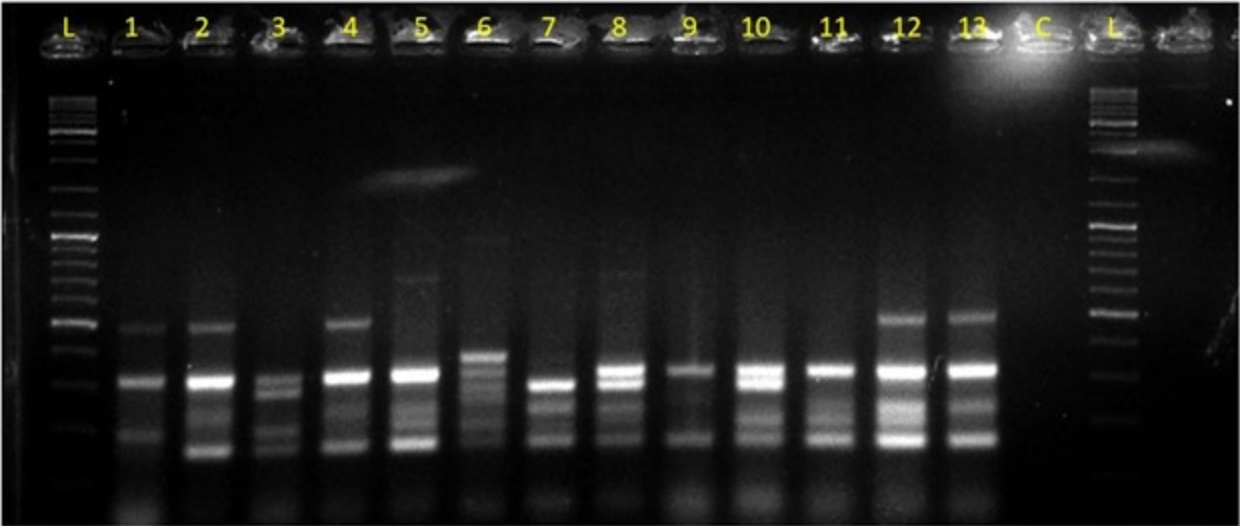


Figure 1

Representative SRAP profile of 13 tuberose varieties using m4e7 primer combination.

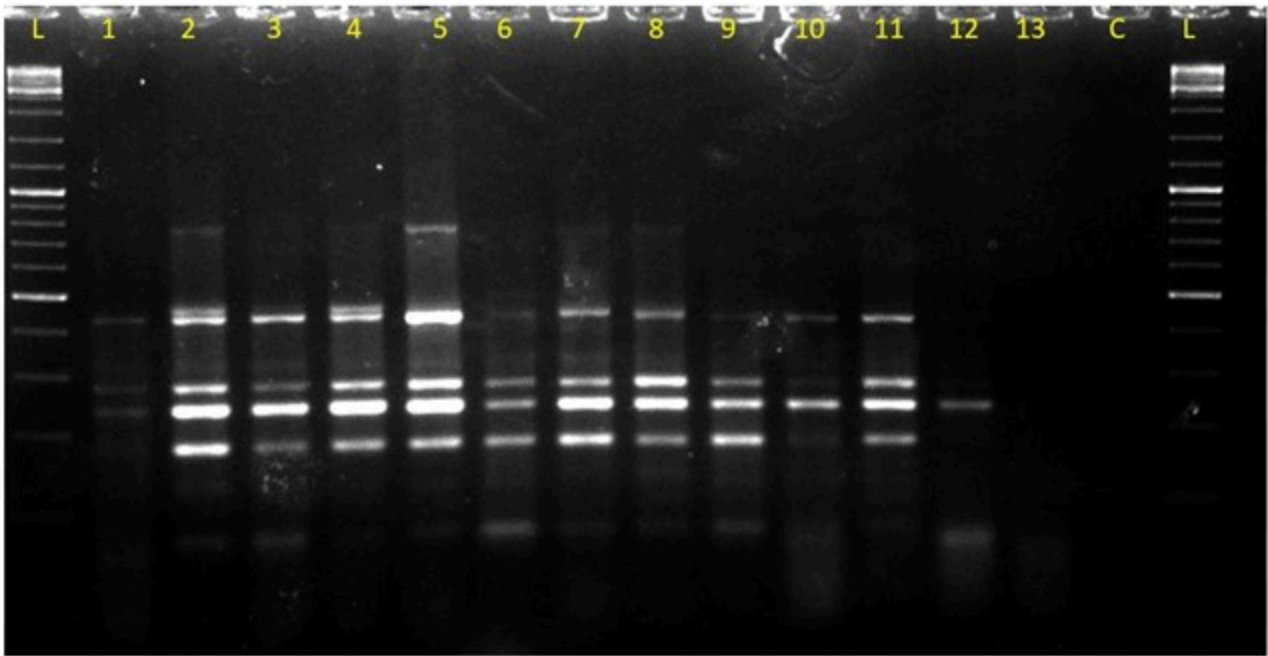


Figure 2

Representative SRAP profile of 13 tuberose varieties using m5e4 primer combination.

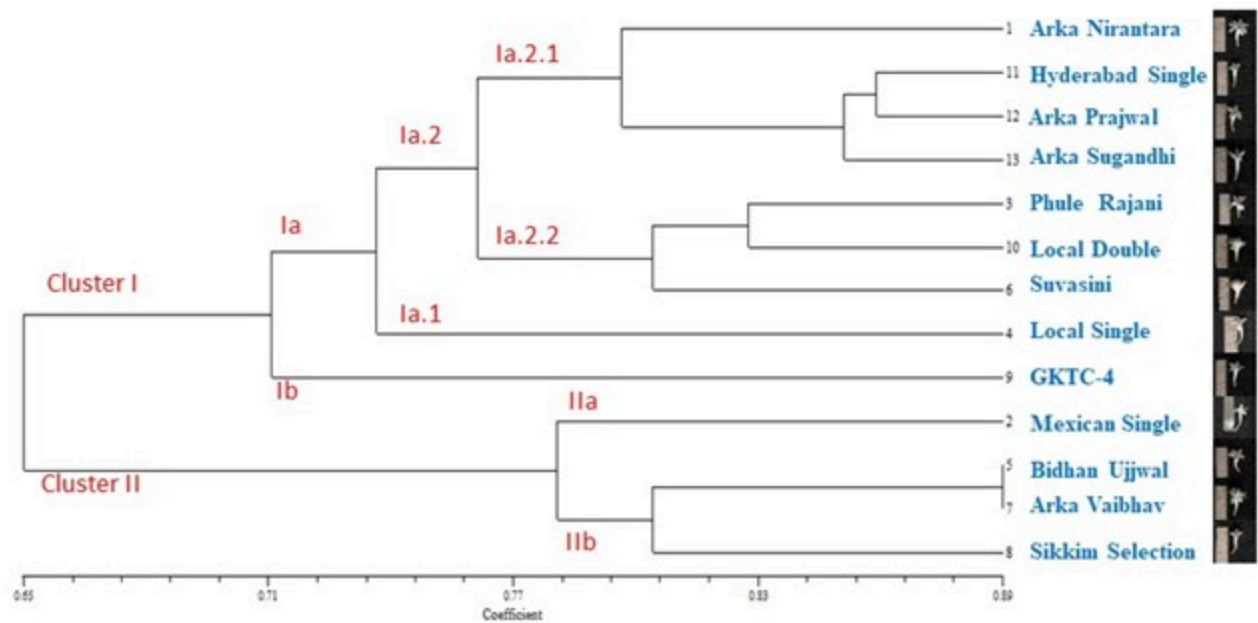


Figure 3

Dendrogram based on SRAP marker analysis showing similarity among 13 tuberose accessions.

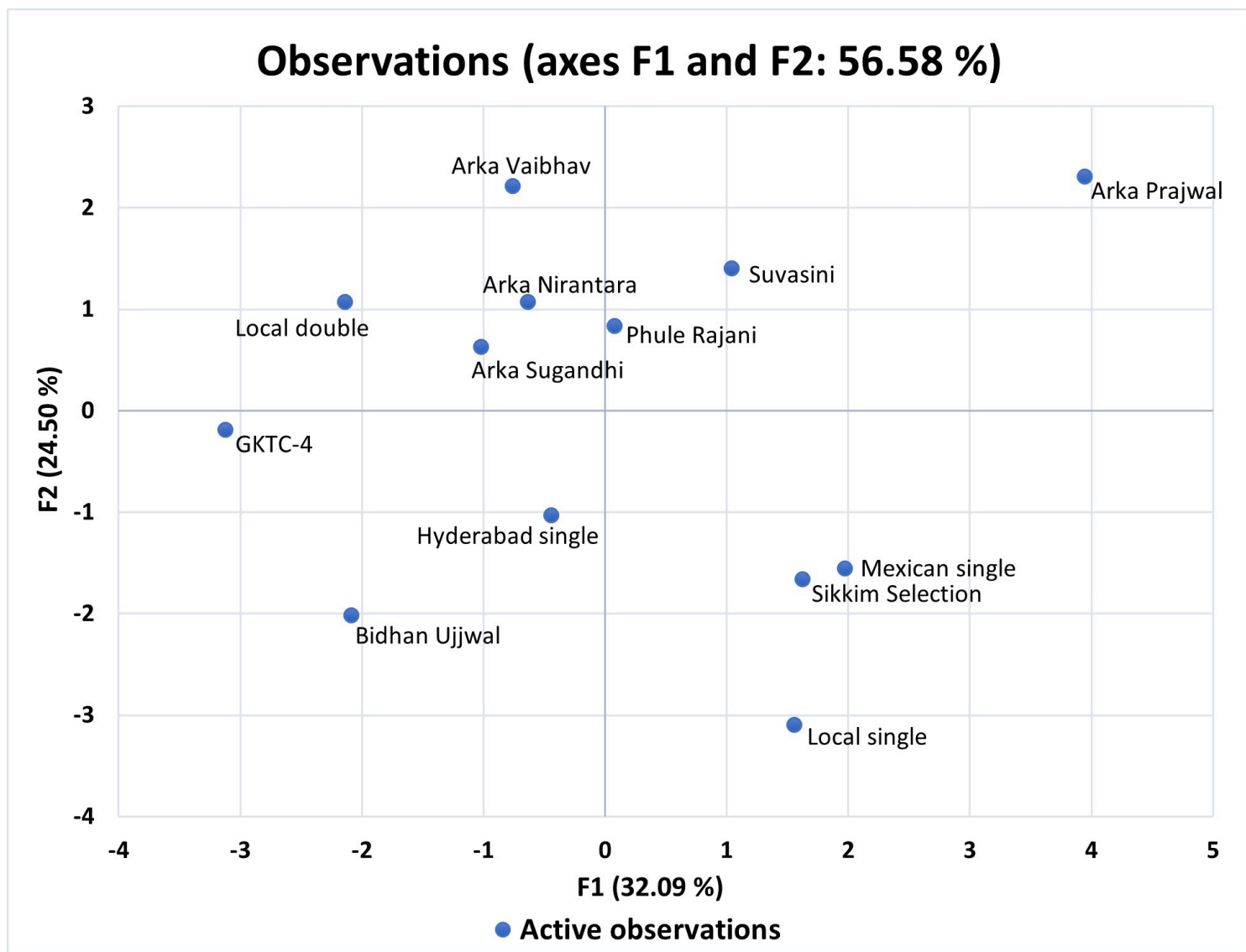


Figure 4

Two-dimensional multifactorial analysis plots showing the relationship patterns between all 13 Tuberose Genotypes

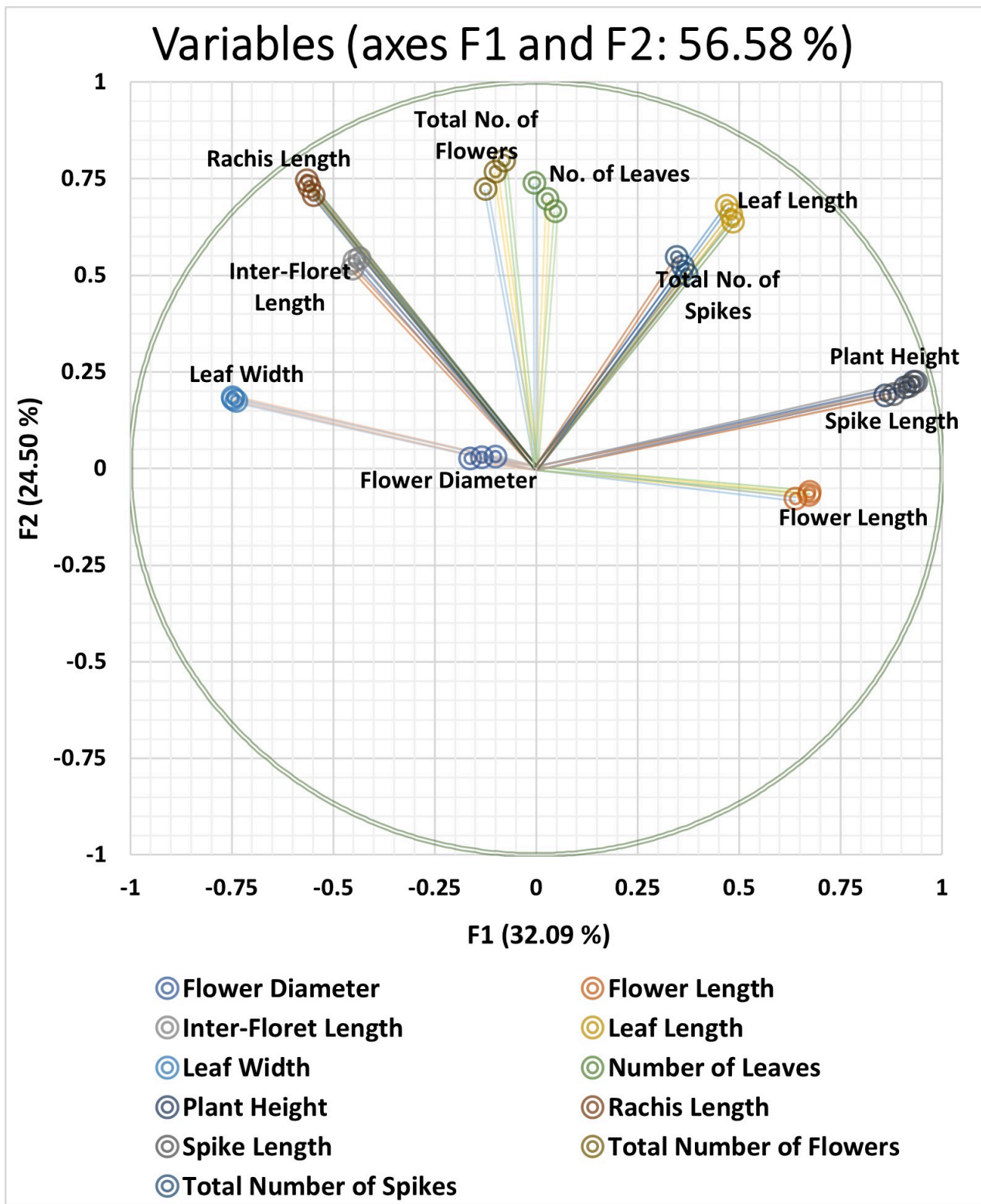


Figure 5

Correlation map of the variables

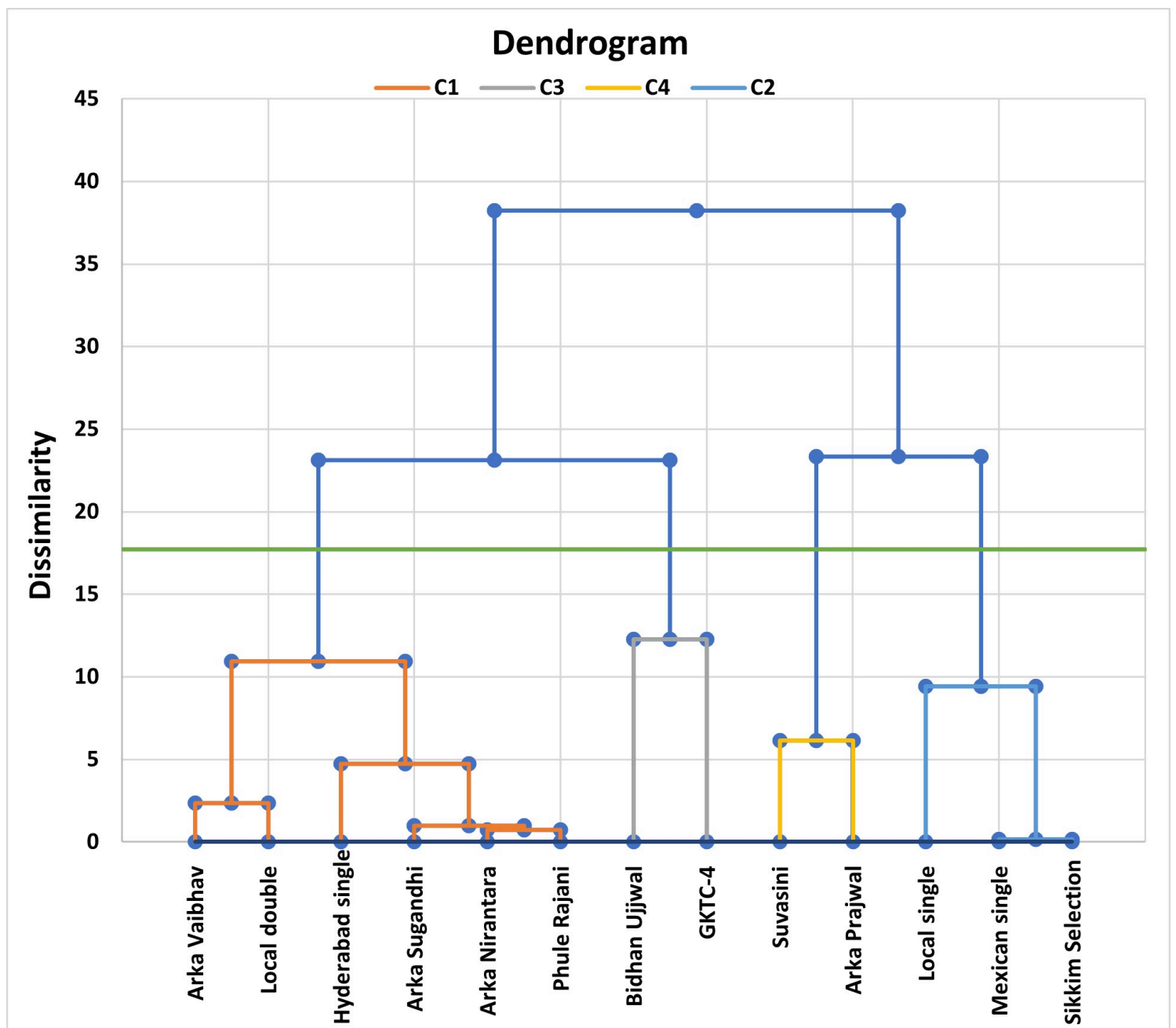


Figure 6

Agglomerative hierarchical clustering (AHC) illustrating the four distinct classes based on the estimated quantitative morphological characters

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TablesJadhavetal.docx](#)