Genetic Diversity Analysis Revealed the Hot Spot of *Acmella Paniculata* Existing in Natural Populations of Gujarat

Sveta Patel
Gujarat University
Nikisha Purohit
Gujarat University
Palak Sapra
Gujarat University
Hitesh Solanki
Gujarat University
Ashok Kumar Bishoyi (ashokkumar.bishoyi@marwadieducation.edu.in)
Marwadi University https://orcid.org/0000-0001-6810-2511

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Abstract

Genetic diversity analysis contributes to the conservation, protection and utilization of genetic resources toward efficient management of germplasm. In this investigation, Inter Simple Sequence Repeats (ISSR) markers were used to analyze the genetic diversity of 32 genotypes of *Acmella paniculata* collected from 12 different states of Gujarat, India. There were 113 loci amplified by 15 different ISSR markers with an average of 7.53 loci per primer. From the total 113 amplicons; 81 were polymorphic, 43 were monomorphic and 9 were found to be unique showing an average of 60.18% polymorphism. The primer $(CT)_8T$ produced a high value (0.65) of polymorphic information content (PIC); whereas, primer $(TC)_8RA$ produced a low value (0.21). The resolving power (RP) value of the primers ranged from 5.25 in primer $(TC)_8RA$ to 23.19 in primer $(AC)_8YT$ with an average of 12.17. The Jaccard's similarity coefficients value ranged from 0.48 to 0.94. The highest percentage of similarity (94%) was found between accessions AP6 and AP16 which were collected from Panchmahal and Narmada respectively, as they are geographically closely located. Whereas, accessions AP2 from Banaskantha and AP7 from Panchmahal were most distantly related with the similarity value of 48%, as expected to their collection locations. The study revealed that there is no correlation between geographical distance and genetic diversity among the populations studied. The resultant data generated from ISSR molecular marker-based genetic diversity analysis of *A. paniculata* in Gujarat can provide a reference for the conservation and efficient management of the important Indian genetic resources.

Introduction

Acmella paniculata (Wall Ex. DC.) R. K. Jansen is commonly known as the "Toothache Plant" because of its wide use to cure toothache and gum infection. The genus *Acmella* (*Spilanthes*) has more than 30 species, generally occurring in the tropics and subtropical parts of the world (Reshmi et al. 2016). In India, it is distributed in Andhra Pradesh, Assam, Chattisgarh, Goa, Gujarat, Himachal Pradesh, Kerala, Karnataka, Meghalaya, Maharastra, Madhya Pradesh, Rajasthan, and Tamil Nadu (Cook 1996). The species possesses a wide range of phytoconstituents such as alkaloids, tannins, saponins, and flavonoids (Mamidala and Gujjeti 2013; Rajeshwar and Lalitha 2013). *Acmella paniculata* known to possess a vast range of pharmacological activities like antimicrobial (Arora et al. 2011; Sharma et al. 2012), antioxidant (Sana et al. 2014), anti-inflammatory (Urankar et al. 2013), insecticidal (Borges-Del-Castillo et al. 1984), antipyretic, antiulcer & anticancer (Paulraj et al. 2013), a local anesthetic (Chakraborty et al. 2002), larvicidal (Saraf and Dixit 2002), etc. The plant has a great source of spilanthol (alkylamides); the principal bioactive compound which has been used as medicine to cure bacterial and fungal infections, rheumatism, urinary tract infection, kidney and gall stone pulverization, cancer, etc (Patel et al. 2019).

Medicinal plants face a high risk of genetic diversity erosion due to their destructive collecting patterns from the wild. In this situation, it is essential to catalog the medicinal plant resources and maintain their germplasm through various strategies. Evaluation of genetic diversity using molecular markers aids in realistic conservation & crop improvement strategies and helps in deciding which germplasm to conserve on a priority basis (Morikawa and Leggett 1990; Nybom and Bartish 2000; Bishoyi et al. 2014). A better understanding of genetic diversity and its distribution in the natural population is essential for plant conservation and its efficient utilization towards human welfare (Morikawa and Leggett 1990; Bishoyi et al. 2016a; Bishoyi et al. 2018).

In India; six species are reported from the genus *Acmella* are, *A. ciliata*, *A. oleracea*, *A. paniculata*, *A. radicans*, *A. uliginosa* and *A. calva*. Identification of these species becomes confusing because of more or less similar morphological characteristics and these are highly influenced by the environmental modulations. In this investigation, two species of *Acmella* have been taken for identification and genetic diversity analysis. The traditional identification method becomes less effective for *A. paniculata* and *A. radicans* because of similarities in morphological features except for flower color (the white color flower is *A. paniculata* and the yellow flower colour is *A radicans*), flowering season, and its habitat. Hence

we opted for the DNA barcoding technique; which has been proved as a more reliable, authenticate and accurate tool for the identification of an organism (Kress et al. 2007; Hebert et al. 2009; Bishoyi et al. 2017; Li et al. 2021)

Molecular markers are employed to assess genetic diversity which will reveal the distribution and extent of genetic variation within the species (Nybom and Bartish 2000). DNA-based molecular markers are the best choice for accurately assessing genetic diversity among the population (Bishoyi et al. 2018; Nadeem et al. 2018). Although a large number of molecular marker techniques exist, each marker technique has its strengths and weaknesses (Powell et al. 1996). Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) are basic approaches used for molecular authentication and validation as they are faster, cheaper and require no prior information of DNA sequence (Bishoyi et al. 2016; Gupta et al. 2017). Genetic diversity evaluation of *Acmella paniculata* populations from Gujarat state has not been recorded yet. Hence, the present study aims to evaluate the genetic diversity analysis of *A. paniculata* using ISSR markers collected from different regions of Gujarat State. Due to the lack of DNA sequence information of *A. paniculata*, in the present investigation; ISSRs make a perfect choice for carrying forward the investigation.

Materials And Methods

Plant Material Collection:

Five samples of each *Acmella paniculata* and *Acmella radicans* were collected from Navsari and Sabarkantha district, Gujarat respectively. Both the species were morphologically identified at the Department of Botany, Gujarat University and processed for DNA barcoding experiments.

Around 90 plant samples of *Acmella paniculata* (Wall ex. DC) R. K. Jansen was collected from various locations of 12 districts of Gujarat. All samples were identified at the Department of Botany, Gujarat University. Freshly collected healthy and tender young leaves were wiped with ethanol to assure contamination-free specimen collection. The leaves were stored in a plastic bag filled with silica gel and tagged with sample ID. The specimens were stored in -20°C for further utilization. Voucher specimen has been made for future reference and submitted to the department of botany herbarium. From the above samples, 32 samples representing each and all populations were taken for DNA isolation followed by genetic diversity analysis. Details of the sample collection, locations with passport data are given in Figure 1 & Table 1. The species are surveyed throughout the Gujarat, but it was available at the river side of some districts as shown in Figure 1 & Table 1.

Table 1
Details of populations of *A. paniculata* collected from different locations of Gujarat

Sample No.	Sample ID	District	Location
1	AP1	Banaskantha	24˚17'39.87"N 72˚37'22.93"E
2	AP2		24˚20'08.87"N 72˚52'50.07"E
3	AP3	Sabarkantha	23˚35'10.70"N 72˚57'10.80"E
4	AP4		24˚00'24.27"N 73˚26'92.70"E
5	AP5	Panchmahal	22˚68'39.68"N 73˚71'54.10"E
6	AP6		22˚70'42.55"N 73˚77'04.94"E
7	AP7		22˚35'05.39"N 73˚70'50.67"E
8	AP8		22˚40'24.44"N 73˚64'88.71"E
9	AP9	Dahod	22˚68'78.92"N 73˚90'17.12"E
10	AP10		22˚61'15.42"N 73˚96'38.81"E
11	AP11	Chotaudepur	22˚34'98.74"N 74˚05'84.35"E
12	AP12		22˚30'21.17"N 73˚99'10.03"E
13	AP13	Narmada	21˚60'30.29"N 73˚49'31.15"E
14	AP14		21˚51'93.30"N 73˚35'63.86"E
15	AP15		21˚86'36.44"N 73˚63'55.17"E
16	AP16		21˚51'52.81"N 73˚64'01.52"E
17	AP17		21˚57'70.63"N 73˚68'06.83"E
18	AP18	Bharuch	21˚63'51.24"N 73˚36'59.29"E
19	AP19		21˚58'30.44"N 73˚35'54.65"E
20	AP20	Surat	21˚38'67.70"N 73˚32'97.03"E
21	AP21		21˚44'85.81"N 73˚32'02.41"E
22	AP22	Tapi	21˚05'26.27"N 73˚23'59.63"E
23	AP23		21˚15'04.8"N 73˚22'40.7"E
24	AP24		20°58'38.7"N 73°37'24.8"E
25	AP25		20°58'11.8"N 73°29'18.4"E
26	AP26	Dang	20˚54'06.0"N 73˚35'26.0"E
27	AP27		20˚48'21.26"N 73˚34'04.37"E

Sample No.	Sample ID	District	Location
28	AP28		20°58'01.9"N 73°29'36.6"E
29	AP29	Valsad	20°59'38.17"N 73°23'51.99"E
30	AP30		20°34'76.29"N 72°98'95.52"E
31	AP31		20°52'56.43"N 73°17'79.68"E
32	AP32	Navsari	20°46'31.5"N 73°21'51.4"E

Genomic Dna Extraction:

The collected leaves were ground to fine powder in mortar and pestle using liquid nitrogen. DNA extraction was carried out according to the modified CTAB protocol of Bishoyi et al. (2016b). The quality of DNA was checked by performing on 0.8% agarose gel electrophoresis. Quantification of DNA was done with a BioTek™ Epoch™ Microplate Spectrophotometer (US). Isolated DNA was stored in -80° C temperatures for further usage in the polymerase chain reaction.

Barcode Gene Amplification And Sequencing:

Universal *rbcl* primers (*rbcl* 1F & *rbcl* 724R) reported by Fay et al. (1998) were taken for barcode gene amplification. PCR reactions were carried out in 25 µl volumes containing ~40 ng of template DNA, 2 µm of each of the four dNTPs, 1X PCR buffer (10 mm Tris, 50 mm KCl), 1.5 mm $MgCl_2$, 1unit Taq DNA polymerase and 10 picomols of each primer. PCR profile was kept at initial denaturation at 95°C for 5 mins followed by 35 cycles of 95°C for 30sec, annealing at 50° C for 30 sec and strand extension at 72°C for 1 min, followed by final extension 72°C for 10min. PCR amplification was carried out in Veriti Thermal Cycler (Applied Biosystems, US). The amplified PCR products were sequenced using the BigDye Terminator V3.1 cycle sequencing kit according to the provided protocol. Cycle sequencing reaction mixture consisted of 4 µl of Terminator ready reaction mix, 120-300 ng of the amplicon, 10 picomols of primer, 1 µl of Big dye sequencing buffer and deionized water to make the volume up to 10 µl. Two individual reaction tubes were prepared for forward and reverse primers. PCR amplification was carried out in Veriti Thermal Cycler (Applied Biosystems, US). Cycle sequencing conditions were kept at 4 min at 96̊ C; followed by 25 cycles of 10 sec at 96̊ C, 5 sec at 55̊ C, 4 min at 60̊ C, and 4̊ C for infinity time. Cycle sequencing purification was done using Big Dye Terminator purification kit by following provided instructions. Sequencing of DNA was done using 3500XL Genetic Analyzer (Applied Biosystems, US). Codoncode aligner 5.0.2 was used for sequence assembly, alignment and contig preparation. Resulted sequences were further analyzed in National Center for Biotechnology Information (NCBI) blast and submitted in the NCBI gene bank & Barcode of Life Data System (BOLD system) for public access.

Issr -pcr Analysis:

Fifty-five synthesized Inter Simple Sequence Repeats primers (synthesized from Xcleris, Ahmedabad) were taken for initial screening. Optimum amplification conditions and their annealing temperature were standardized with the help of gradient PCR. Out of 55 primers taken, 15 primers were selected for further analysis due to the generation of clear and reproducible amplification. The DNA amplification protocol was carried out by following the procedure of Zeitkiewicz et al (1994) with suitable modifications. Each PCR amplification was carried out with 25 µl reaction mixture containing 2.5 µl of 10X Taq buffer having $(NH_4)_2SO_4$ (Thermo scientific, USA), 2 µl of $MgCl_2$ (25 mM), 0.3 µl of 10 mM dNTPs (Thermo scientific, USA), 0.3 µl of Taq polymerase (5 U/µl; Thermo Scientific, USA), 1.5 µl of primer (10 pmol/µl), 2 µl of genomic DNA (50 ng) and

final volume was made up with ddH_2O. PCR reactions were carried out in the Veriti^{TM} Thermal Cycler (Applied Biosystems, US) with optimum amplification conditions. To perform ISSR amplification; PCR condition consisted of denaturation at 94° C for 5 mins, 35 cycles at 94° C for 1.30 mins, annealing was optimized according to the primer for 1.30 mins and extension at 72° C for 2 mins, followed by a final extension at 72° C for 10 mins. Amplified products were stored at 4° C until they were subjected to gel electrophoresis. The reproducibility of DNA profiles was examined by repeating twice all the PCR amplification with each selected individual primers. Only reproducible bands were considered for further analysis. Negative control was incorporated in all PCR reactions to check the cross-contamination of the samples.

Agarose Gel Electrophoresis:

PCR products of the DNAs were separated by using 1.5% gel agarose electrophoresis (Genei Banglore, India) containing ethidium bromide (SRL) in 1X TBE buffer (40 mM tris base, 20 mM boric acid, 1 mM EDTA) at 100 V for 80 mins. 100 bp DNA ladder (Thermo Fisher Scientific, US) was run alongside PCR products to determine the molecular weight of amplified products. Ethidium bromide-stained gels were documented by using the UVITEK (Cleaver Scientific, UK), gel documentation system.

Data Analysis:

Gel images were used to score data for ISSR markers. Each DNA amplicon given by primer was considered as a unit character and the fragments were scored as a binary variable (1,0) for presence and absence, respectively. Documentation of the total no of bands, monomorphic bands, polymorphic bands and Unique bands has been done from the binary data matrix box. Data were analyzed by NTSYS 2.02 software (Rohlf 1998). Jaccard's coefficient similarity was calculated using the SIMQUAL (Similarity for quantitative data) program (Jaccard 1908). A dendrogram was constructed based on UPGMA (Unweighted Pair Group Method using Arithmetic average) and SAHN (Sequential agglomerative hierarchical Non-overlapping) clustering. RP value (Resolving Power) of the primers was calculated as per Prevost and Wilkinson (1999) formula. Marker index of each primer was also calculated according to Powell et al (1996) and Polymorphic information content (PIC) was determined with the formula given by Anderson et al (1993).

Results And Discussion

Barcode gene amplification and sequencing:

The present investigation was able to accurately sequence 707 bp and 723 bp of the *rbcl* gene for *Acmella paniculata* and *Acmella radicans*, respectively. Species identification has been confirmed in NCBI- BLAST which shows 99-100% homology with 99% query cover. More details of the barcode analysis are shown in Table 2. In this investigation, *rbcl* alone was able to identify both the studied species; which has been recently proved by similar studies across different parts of the globe (Enan et al. 2017; Chandrasekara et al. 2021; Ho et al. 2021; Li et al. 2021).

Table 2
Details of the barcode gene sequencing

Sl No	Name of the gene	Nucleotide length	Query cover	% Identity	E value	Name of the plant	Gene Bank Accession No (NCBI & BOLD)
1.	*rbcl*	707	99	99.72	0	*Acmella paniculata*	NCBI: OM127877 BOLD: gi|313770794| GU724226.1
2.	*rbcl*	723	99	99.86	0	*Acmella radicans*	NCBI: OM127877 BOLD: gi|313770795| GU724226.1

Issr-pcr Analysis:

There were 113 loci amplified by 15 various ISSR markers with an average of 7.53 loci per primer. Out of the total 113 amplicons, 81 were polymorphic, 43 were monomorphic and 9 were found to be unique showing an average of 60.18% polymorphism. The number of produced bands by various markers varied from 3 in primer $(TC)_8RA$ to 14 in $(AC)_8YT$ with a mean amplicon size of 150- 3000 bp (Table 3). The highest number of polymorphic bands (14) was amplified by the primer $(AC)_8YT$, whereas the lowest number of polymorphic bands (3) was obtained with primer $(TC)_8RA$. The primer $(CT)_8T$ produced a high value (0.65) of polymorphic information content (PIC), while primer $(TC)_8RA$ produced a low value (0.21). The resolving power (RP) value of the primers ranged from 5.25 [$(TC)_8RA$] to 23.19 [$(AC)_8YT$] with an average of 12.17. Marker index (MI) values of studied primers were reported as low as 0.22 in primer $(TC)_8RA$ and highest value of 0.50 in $(CT)_8T$ and $(CT)_9G$ with an average of 0.39. The moderately high average values of %P, RP, MI, and PIC of ISSR markers analysis advocate the efficiency of the selected markers used for the present investigation. Details of the marker analysis are shown in Table 3. Moreover, the resolution of the selected ISSR primer amplification was found to be good quality as revealed by gel images indicating the usefulness of the markers for this investigation. The obtained results were strongly supported by the proven hypothesis of earlier reports, i.e., ISSR markers are verified as more relevant for assessing the genetic diversity where genome information of the said species is unknown (Bishoyi et al. 2014; Bishoyi et al. 2016a; Gupta et al. 2017). The present study also revealed that occurrence CT/TC repeats are more frequent in *A. paniculata* genome in comparison to other possible repeats (out of a total of 15 primers, 8 primers are CT/ TC repeats) and these repeats contribute more than 60% to the total number of amplicons analyzed. This may be due to the more common and random existence of CT/TC repeats in the herbaceous plant genome followed by any other repeats. This result is in agreement with the similar study reported from *Allium* cultivars (Mishra et al. 2015), *Cymbopogon* species (Bishoyi et al. 2016) and these repeats are may arbitrarily be distributed in the non-coding region of the genome of a particular species (Lu et al. 2019; Yang et al. 2020; Bhattarai et al. 2021).

Table 3
Details of ISSR primers used for the study

Sr. No.	Primer	Sequence (5'-3')	AFS (bp)	TB	PB	MB	UB	%P	RP	MI	PIC
1	$(CT)_8T$	CTCTCTCTCTCTCTCTT	200-1000	11	6	3	2	54.55	10.19	0.50	0.65
2	$(CT)_8A$	CTCTCTCTCTCTCTCTA	300-900	9	5	4	0	55.56	15.31	0.25	0.24
3	$(CA)_8T$	CACACACACACACACAT	400-3000	7	5	2	0	71.43	11.13	0.33	0.35
4	$(TC)_8C$	TCTCTCTCTCTCTCTCC	350-1000	9	7	2	0	77.78	14.44	0.32	0.33
5	$(AC)_8C$	ACACACACACACACACC	500-2000	9	4	5	0	44.44	14.69	0.30	0.29
6	$(GA)_8YC$	GAGAGAGAGAGAGAGAYC	150-2000	9	7	2	0	77.78	10.25	0.49	0.60
7	$(CT)_8RA$	CTCTCTCTCTCTCTCTRA	200-1000	9	2	6	1	22.22	14.25	0.33	0.23
8	$(CT)_8RC$	CTCTCTCTCTCTCTCTRC	250-800	5	2	2	1	40.00	6.31	0.47	0.45
9	$(CA)_8RT$	CACACACACACACACART	200-1200	10	7	3	0	70.00	13.50	0.44	0.44
10	$(CA)_8RC$	CACACACACACACACARC	250-1200	5	3	1	1	60.00	5.75	0.49	0.57
11	$(TC)_8RA$	TCTCTCTCTCTCTCTCRA	600-900	3	2	1	0	66.67	5.25	0.22	0.21
12	$(AC)_8YT$	ACACACACACACACACYT	200-3000	14	10	4	0	71.43	23.19	0.28	0.29
13	$(AC)_8YG$	ACACACACACACACACYG	250-2000	11	8	3	0	72.73	12.88	0.49	0.53
14	$(CTC)_6$	CTCCTCCTCCTCCTCCTC	400-2000	11	4	4	3	36.36	14.06	0.46	0.43
15	$(CT)_9G$	CTCTCTCTCTCTCTCTCTG	300-900	11	9	1	1	81.82	11.38	0.50	0.62
Total				133	81	43	9	*60.18	*12.17	*0.39	*0.41

Abbreviations - AFS (bp): approximate fragment size in base pairs, TB: total number of bands, PB: polymorphic bands, MB: monomorphic bands, UB: unique bands, % P: percentage of polymorphism, RP: resolving power, MI: marker index, PIC: polymorphic information content, * average of the column

Issr Cluster And Genetic Diversity Analysis:

The ISSR based dendrogram discriminated all the 32 genotypes into two major clusters, *i.e.*, A and B (Figure 2). The first cluster A was further divided into four groups, i.e, sub-cluster AI, AII, AIII and AIV. The sub-cluster AI included two genotypes AP1 and AP31 collected from Banaskantha and Valsad respectively. Sub-cluster AII has consisted of 15 genotypes, AP3, AP21, AP19, AP28, AP23, AP27, AP8, AP25, AP6, AP16, AP17, AP5, AP10, AP15 and AP7. The sub-cluster AIII included two

accessions AP13 and AP22 collected from Narmada and Tapi districts, respectively; whose genomic background is from nearby geographic locations. Sub-cluster AIV included only one accession namely, AP20 from Surat district. The highest numbers of genotypes were found to be from sub-cluster AII. The second major cluster B was again divided into four sub-clusters, BI, BII, BIII and BIV. Sub-cluster BI included two genotypes from Banaskantha (AP2) and Bharuch (AP18). The sub-cluster BII has consisted of six accessions, i.e, AP4, AP26, AP9, AP29, AP14 and AP30. Sub-cluster BIII included three accessions, two (AP11, AP12) from Chota-Udaipur and one (AP32) from Navsari district. The sub-cluster BIV included only one genotype AP24 from Tapi district. All the accessions from Panchmahal district clustered into sub-cluster AII only which shows its genetic similarity by virtue of its geographical locations. The clustering patterns of accessions from Banaskantha and Sabarkantha were quite different to our expectations. The accessions were separated into different clusters A and B irrespective of their geographical locations. This indicated its ancestry to the accessions from South Gujarat. It might be due to the anthropogenic movement of plant material of South Gujarat to North Gujarat because of its medicinal and ethnobotanical value.

The Jaccard's similarity coefficients ranged from 0.48 to 0.94. The highest percentage of similarity (94%) was found between accessions AP6 and AP16 collected from Rinchhwani, Panchmahal and Chikada, Narmada respectively. The geographical distance between both locations is 193 km which suggests that both populations might be evolved from the same ancestral population. 93% similarity was found between accession AP19 from Netrang, Bharuch and accession AP28 from Bhenskatri, Dang. The geographical distance between the two locations is 92 km. Accessions AP2 from Koteshwar, Banaskantha and AP7 from Paniyara, Panchmahal were most distantly related with the similarity value of 48%. The geographical distance between both locations is 212 km. The ISSR based genetic similarity of the studied genotypes is given in Table 4.

The clustering patterns also revealed that accessions from South Gujarat were scattered in all the sub-cluster, which indicated that it might be the ancestor population and site of origin of *Acmella paniculta* species in Gujarat state. This higher level of diversity among accessions of south Gujarat made them evade more and more possible habitats all over Gujarat, which can also be viewed from their distribution patterns i.e, abundant in South Gujarat which constantly decrease from South to North Gujarat (Figure 2 and Table 4). The genotypes of the hot spots are generally scattered throughout their natural habitats and it has been proved by several researchers using different plant species (Nudin et al. 2017; Bishoyi et al. 2018; Zhou et al. 2020). The present study also revealed that there is no correlation between geographical distance and genetic diversity among the populations of *A. paniculata*. The possible reasons are its center of origin of the plant and anthropogenic activities followed by unrestricted plant material transfer within the geographical boundaries. The obtained results were also supported by similar trend of results are reported in many plant species concerning different geographical locations (Rajendran et al. 2016; Kelkar et al. 2017; Zhou et al. 2020; Venkatesan et al 2021). This type of unrestricted genetic resources migration within and between geographical boundaries may enhance the adaptability of the species in harsh climates leading to the development of better genotypes.

Conclusion

In the present investigation first and the fruitful attempt has been made to assess genetic diversity among the genotypes of *Acmella paniculata* collected from Gujarat state using ISSR markers. Clustering analysis of the genotypes revealed that scattering occurrence of genotypes of south Gujarat genotypes in all the sub-clusters. This indicates south Gujarat is the hot spot of *Acmella paniculata* from where the species has been migrated to other regions of the state and neutralized.

The resultant of the study may helpful for germplasm management, conservation of biodiversity and efficient breeding programme management.

Declarations

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Conflict of interest

All authors declare that they do not have any conflict of interest.

Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Sveta Patel, Nikisha Purohit, Palak Sapra, Archana Mankad, Hitesh Solanki and Ashok Kumar Bishoyi. The first draft of the manuscript was written by Sveta Patel and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Table

Table 4 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Plant sampling locations within Gujarat state

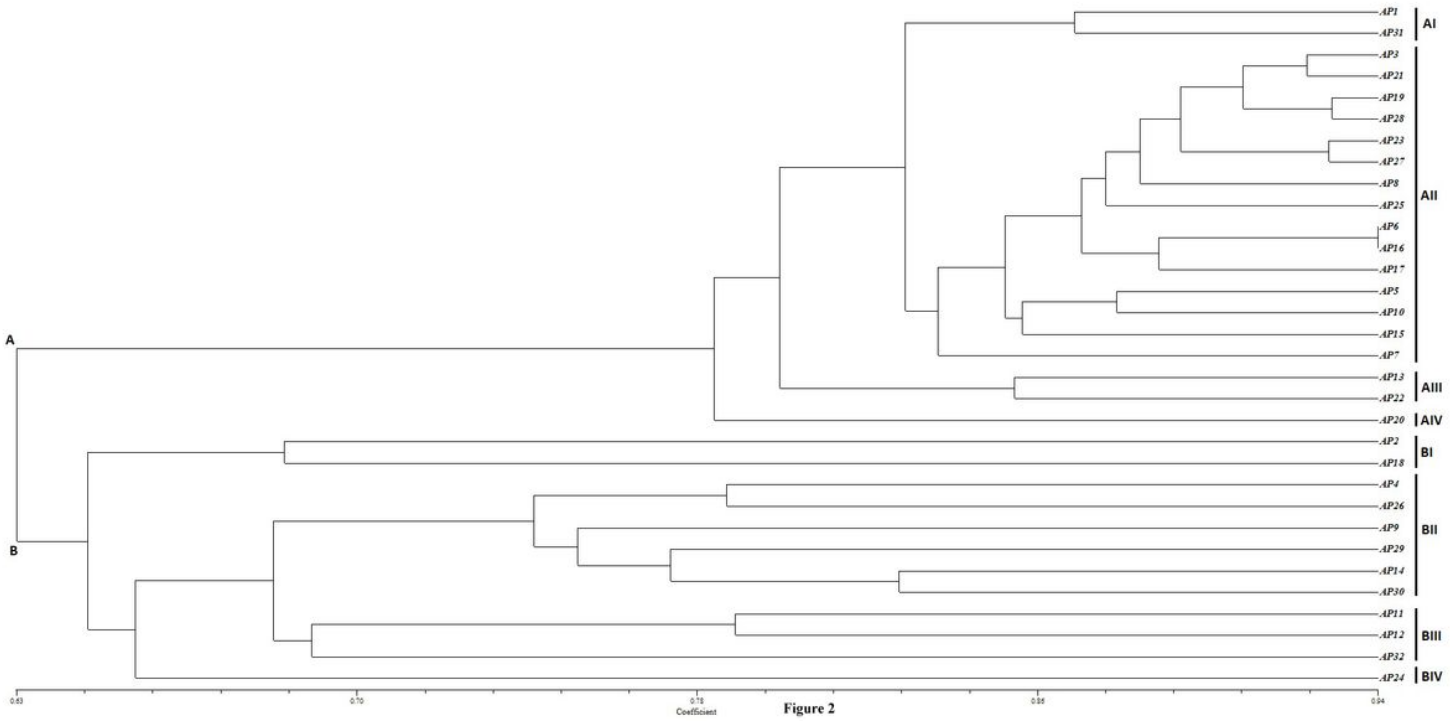

Figure 2

UGPMA Dendrogram with Jaccard's Similarity Coefficient among 32 Accessions of *A. paniculata* based on ISSR Markers

Supplementary Files

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- Table4.docx