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RESEARCH ARTICLE

Assessment of genetic variation in turmeric (*Curcuma longa* L.) varieties based on morphological and molecular characterization

A. P. Aswathi · S. B. Raghav · D. Prasath

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Abstract Turmeric (*Curcuma longa* L.) is an economically valuable spice crop with a wide range of applications. Crop improvement efforts in this crop had led to identification, selection, and release of several varieties with desirable traits for yield, curcumin content etc. Understanding of genetic variation is a pre-requisite for any crop improvement programme. In this study, we examined the genetic variation present in prominent 18 improved varieties of turmeric in India using morphological and microsatellite markers. We studied 12 morphological characters including 11 quantitative traits, and curcuminoid content following the DUS guidelines of turmeric. For molecular characterization, we used 56 molecular markers including SSRs, and ISSRs to determine the genetic polymorphism among the genotypes. PIC value of these markers ranged from 0 to 0.5. Our study thus confirms the reliability of these molecular markers to study the genetic variation of turmeric varieties. The combination of methods i.e., cluster analysis based on morphological characterization and the UPGMA dendrogram based on molecular characterization revealed both the phenotypic and molecular variation among the improved turmeric varieties. This has helped us to understand pattern of variation, the level of genetic similarity and the genetic divergence among them. It was found that the varieties Varna, Megha Turmeric and Suvarna were the most divergent while Punjab Haldi 1 and Sudarsana were the least divergent among the improved varieties. The results from this study can be used in designing breeding programmes in turmeric.

Keywords Genetic similarity · Morphological evaluation · Molecular characterization · Turmeric · Varieties

Introduction

Turmeric (*Curcuma longa* L.) is an important spice crop belonging to the family Zingiberaceae and widely cultivated in tropical areas mainly Southeast Asia. Being utilized for its culinary and medicinal properties, turmeric finds numerous applications in cosmetic as well as pharmaceutical industry apart from its use as food additive and flavouring agent (Prasath et al. 2019). Turmeric is regarded as rich source of curcumin, a bright yellow bioactive polyphenolic pigment widely known for its powerful and potent antioxidant, anti-inflammatory, neuroprotective, anticancer, and cardioprotective effects (Sharifi-Rad et al. 2020). For India, which is one of

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A. P. Aswathi · S. B. Raghav · D. Prasath (✉)
Division of Crop Improvement and Biotechnology, ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India
e-mail: dprasath@gmail.com

the important producers and a major global exporter of turmeric, the cultivation and crop improvement of this spice crop is of great importance as it is presented with economic prospects and untapped potential (Centre for Advance Trade Research 2019). India is also home to more than 30 different types of turmeric cultivars, all unique to their respective regions, known mostly by the name of locality where they are cultivated. Often this vernacular identity and a lack of clear-cut morphological distinction can result in build-up of duplicates (Shamina et al. 1998). Also, several improved varieties of turmeric are released under the aegis of National Agricultural Research System (NARS) in India (Prasath et al. 2019).

Genetic diversity is an important pre-requisite for any crop improvement programme. It is the variation of heritable characteristics found in a population of same species (Swingland 2013), which determines and express as the morphological and biochemical variation observed in crops. The genomic diversity is defined as diversity at several DNA loci within an individual (Bhandari 2017). Hence, a study of molecular genetic variation in alignment with the observed agro-morphological traits give a broad and substantial understanding of diversity for the crop breeders. In case of turmeric, the agro-morphology of only few of the improved varieties are well studied in different environments. But many of these traits are unstable and show intra-varietal variation in different environment due to genotype × environment interactions (Anandaraj et al. 2014) decreasing their utility as markers and are inefficient to apprehend the real genetic diversity. The DNA sequence-based markers are comparatively stable and not affected by diverse environment (Nadeem et al. 2018). These are based on polymorphisms at the level of DNA sequence. Hence, the molecular characterization would help us not only to better understand the observed agro-morphological diversity of improved varieties but also the real diversity at DNA level among them.

Molecular studies in turmeric have resulted in development and characterization of turmeric genotypes with genomic SSR (Siju et al. 2010a; Sigrist et al. 2010; Senan et al. 2013), EST-SSR (Siju et al. 2010b; Sahoo et al. 2017), apart from characterization with RAPD, ISSR (Nayak et al. 2006; Vijayalatha and Chezhiyan 2008; Hussain et al. 2008; Singh et al. 2012; Verma et al. 2015) and isozyme markers (Shamina et al. 1998). Some of these studies have revealed considerable genetic diversity as well as relatedness among turmeric genotypes (Verma et al. 2015; Singh et al. 2018). All these works have underlined the reliability and efficiency of molecular markers in diversity analysis. Singh et al. (2012) studied the genetic diversity among turmeric genotypes from different agroclimatic zones using ISSR and RAPD markers wherein Nei's genetic diversity index revealed diversity in turmeric accessions from different zones of India. These studies on genetic diversity help us to understand genetic variants which can be utilized in breeding programmes. However, there have not been many studies explicitly addressing the diversity among improved varieties of turmeric in India. This Information about the level of genetic relatedness among these prominent improved varieties of turmeric at the molecular level will help us to understand genetic variation and distinctiveness of these improved varieties.

As far as the agro-morphological traits are concerned, improved varieties possess some of the desirable traits like high curcumin content, increased yield of fresh or dry rhizome, essential oil, oleoresin etc. They can be either short duration or long duration crop based on lifecycle. Some of the improved varieties are similar to each other in many of these aspects. Even though these varieties are superior to other local varieties in terms of desirable traits, it's not clear how diverse they are genetically from each other. Hence, in this study we aim to understand: (1) genetic similarity among the prominent improved varieties in India based on morphological characterization (2) genetic variation and similarity among these improved varieties based on molecular characterization and to find distinct genotypes if any. (3) Finally, to see the parallel between morphological and molecular variation among these varieties. The results obtained from this study can be utilized in choosing genetically distinct or dissimilar parents in breeding programs.

Materials and methods

Morphological characterization

Plant material and experimental methods

For morphological characterization, we have selected 18 improved varieties developed by various research

institutions in India (Supplementary Table 1). These varieties were maintained at the National Active Germplasm Site of ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India, one of the largest and most important turmeric collections in India. Field experiment was carried out in Experimental Farm of ICAR-IISR, located in Peruvannamuzhi, Kozhikode, India (North latitude 11°36′34″; East longitude 75°49′12″; 60 m MSL). The rhizomes of each variety were planted in two replicates in randomized block design (40 plants per replication per variety). The experimental plot was maintained with recommended package of practices (Jayashree et al. 2015). Important characters from DUS guidelines (PPV&FRA 2009) for turmeric and a derived character i.e. percentage of dry yield was chosen for the morphological characterization. The 11 quantitative characters were: plant height (PH), number of shoots (NS), total number of leaves per plant (TP), leaf length (LL), leaf width (LW), length of primary rhizome (PL), thickness of primary rhizome (PT), internodal length (IL), fresh rhizome yield per plant (RY), dry yield of rhizome per plant (DY), percentage of dry yield of rhizome per plant (DYP). In addition to these agro-morphological quantitative traits, economically relevant trait i.e., the curcuminoid content of these varieties was also examined using ASTA method (ASTA 2004). For statistical analysis of morphological data, first a univariate analysis of means by ANOVA was done to find out the characters with statistical significance. Duncan Multiple range test (DMRT) was done to identify the specific varieties with significant differences in their means (Faruq et al. 2014). Then a multivariate hierarchical clustering was done with means of all characters for the improved varieties under study to get the overall pattern of morphological diversity of these varieties (Liu et al. 2015).

Molecular characterization

DNA extraction

For molecular characterization, we isolated DNA from young leaves of all these 18 improved varieties, as per the CTAB based modified method (Doyle and Doyle 1987). We have modified the DNA extraction protocol to get good quality DNA considering the high polyphenolic content in turmeric leaves (Vidya et al. 2021). The absorbance-based concentration of DNA samples were determined using the DeNovix DS-11 spectrophotometer (DeNovix, Wilmington, DE, USA) and further the DNA quality was checked by loading the DNA aliquots from each variety on 0.8% (w/v) agarose gels. The DNA of each sample was diluted to a final concentration of 50 ng/µL for PCR analysis.

Molecular markers-based genotyping

We have utilized a total of 56 primers (Supplementary Tables 2, 3) for the study of genetic variation, which includes 19 genomic SSR primer pairs, 13 EST SSR primer pairs and 24 ISSR primers. Genomic, EST SSR primers were previously developed and characterized in turmeric (Siju et al. 2010a; b; Senan et al. 2013) and ISSR primers were also utilized in previous studies (Giridhari et al. 2020). PCR amplification was performed with 1 µl template genomic DNA (50 ng/ µl), 10 µl of PCR master mix (Emerald Amp GT PCR, Takara), 0.5 µl (10 µM) each forward and reverse primers and 8 µl nuclease-free water in a final reaction volume of 20 µl. The conditions for PCR were: 94 °C for 5 min (initial denaturation), the steps 94 °C for 45 s, annealing temperature for 45 s (depending on the primer used) and extension at 72 °C for 1 min were repeated for 30 cycles followed by a final extension at 72 °C for 10 min. The obtained PCR amplicons were loaded onto a 3.5% agarose gel which was stained with ethidium bromide along with a 100 bp DNA ladder as band size reference and electrophoresis was carried out. The gel was then visualized for the separated PCR products using a gel documentation system (Syngene Gel Doc; Syngene, Synoptics Ltd, UK).

Genetic diversity analysis

The bands obtained after genotyping were scored in a binary format. The polymorphic information content (PIC) of both SSR and ISSR primers were hence calculated by the formula: $PIC = \sum 2\ f_i\ (1 - f_i)$ were f_i is the frequency of i th allele of a marker and $1 - f_i$ is the frequency of null allele (Roldán-Ruiz et al. 2000). A dendrogram was constructed through SAHN clustering method, and similarity coefficients were generated using the unweighted pair group method with arithmetic mean (UPGMA) in NTSYS-pc version 2.0

(Rohlf et al. 2009). Here, the genetic similarity (GS) among the genotypes was calculated by Jaccard's similarity coefficients.

Results

Morphological characterization

The F statistics after ANOVA test (Table 1) had revealed significant differences (P value at 0.01) among the traits of the released turmeric varieties, except for trait TP (total leaves per plant) which was non-significant. The coefficient of variation (CV) ranged from 1.64 to 17.15% for CUR (% of curcumin content) and RY (rhizome yield per plant) respectively (Table 2). The DMRT test showed specific differences among pair of means for the characters of individual varieties which further confirms the morphological and biochemical variability among the varieties (Table 2). Ward's distance based Hierarchical clustering of improved varieties (Fig. 1) divides these varieties into two main clusters. The first cluster includes varieties; Varna, Megha turmeric, Suvarna, IISR Alleppey Supreme, IISR Kedaram, IISR Prabha, IISR Pratibha, BSR 2, CO 2, Punjab Haldi 1 and CO 1. While Suranjana, Rajendra Sonia, IISR Pragati, Suguna, Sudarsana, NDH 3 and NDH 1 falls in second cluster. Ward's distance measures (Supplementary file) indicated highest similarity between Rajendra Sonia and Suranjana (1.47). While the lowest similarity, i.e., highest distance was between IISR Pragati and IISR Kedaram (8.33).

From the hierarchical clustering, we can find that varieties in cluster I are characterized by a higher group means for the character PH (1.24 m), LL (51.8 cm), LW (13.7 cm) than general mean (Table 2) while group means for the same characters in cluster II varieties are: PH (1.06 m), LL (42.4 cm), LW (11.5 cm) comparatively lesser. On the other hand, varieties in Cluster, I show lesser group means compared to general mean for characters; NS (3.14), PL (7.12 cm), PT (6.8 cm), IL (0.92 cm), RY (0.32 kg), DY (0.06 kg), CUR (3.40%). The varieties in cluster II shows a higher group mean for these characters like NS (3.75 cm), PL (7.99 cm), PT (8.68 cm), IL (1.25 cm), RY (0.62 kg), CUR (5.79%) and is on par with general mean for the character DY (0.07 kg).

Molecular characterization

PCR screening with SSR and ISSR markers

It is found that out of the 56 microsatellite markers tested in 18 turmeric varieties, 55 of them were polymorphic. The average number of alleles per genotype per marker ranged from 1 to 3.44. Here the marker with the highest average number of alleles per genotype was CuMiSat 08 and UBC 889 with 3.44 allele; while with the lowest average number of alleles per genotype were UBC 896, CuMisat-01, CuMisat-25, CuMisat-31, CuMisat-33, CuMisat-36, Clest-04, Clest-06, Clest-08, Clest-09, Clest-11, Clest-13, and Clest-17 each with 1 allele. The Polymorphic Information Content (PIC), calculated to estimate the discriminatory power of each marker, ranged from 0 for CuMiSat 36 to 0.5 for CuMisat-33, with a mean PIC of 0.38. PIC value is a measure of the informativeness of a molecular marker as the basis for the classification and selection of markers that were efficient

Table 1 Summary of analysis of variance of 12 characters evaluated in 18 improved turmeric varieties

	df	Mean square											
		TP	PH (m)	NS	LL (cm)	LW (cm)	PL (cm)	PT (cm)	IL (cm)	RY (Kg)	DY (Kg)	DYP (%)	CUR (%)
Varieties	17	6.08 ^{ns}	0.04**	0.71**	149.9**	10.4**	3.15**	2.12**	0.09**	0.06**	0.001**	49.6**	5.1**
Error	18	5.10	0.003	0.06	0.89	0.31	0.30	0.18	0.004	0.005	0.0001	4.24	0.005

NS Non-significant, *df* degrees of freedom, *TP* total number of leaves per plant, *PH* Plant height, *NS* number of ''shoots, *LL* leaf length, *LW* leaf width, *PL* length of primary rhizome, *PT* thickness of primary rhizome, *IL* internodal length, *RY* fresh rhizome yield per plant, DY dry yield of rhizome per plant, DYP percentage of dry yield of rhizome per plant, *CUR* Curcumin content

**Significant at 1%, *Significant at 5%

Table 2 Mean performance of 12 characters in 18 improved turmeric varieties

Varieties	PH (m)	LL (cm)	LW (cm)	NS	PL (cm)	PT (cm)	IL (cm)	RY (Kg)	DY (Kg)	DYP (%)	CUR (%)
IISR Alleppey Supreme	1.13^{EFG}	48.15^{EF}	15.30^{B}	3.66^{BCD}	9.69^{A}	6.79^{FG}	1.15^{BCD}	0.43^{DEF}	0.09^{AB}	21.29^{ABC}	4.65^{G}
BSR 2	1.20^{DE}	53.65^{D}	11.50^{F}	3.33^{CDE}	6.33^{FG}	7.20^{DEF}	0.93^{FG}	0.25^{GHI}	0.03^{E}	12.14^{EFG}	3.54^{K}
CO 1	1.17^{DE}	35.00^{L}	10.00^{G}	3.50^{CDE}	5.26^{G}	6.76^{FG}	0.93^{EFG}	0.29^{FGHI}	0.03^{E}	10.36^{FG}	2.31^{M}
CO 2	1.13^{EFG}	45.80^{GH}	12.80^{CDE}	2.33^{H}	5.86^{G}	7.06^{FG}	0.82^{GHI}	0.16^{I}	0.03^{E}	15.00^{DE}	3.84^{J}
IISR Kedaram	1.44^{AB}	60.15^{B}	18.25^{A}	3.00^{EFG}	5.70^{G}	6.55^{FG}	0.68^{I}	0.22^{HI}	0.06^{D}	24.40^{A}	4.64^{G}
Megha Turmeric	1.53^{A}	62.95^{A}	15.30^{B}	2.66^{FGH}	7.65^{CDE}	6.22^{G}	0.84^{GH}	0.33^{EFGH}	0.08^{BC}	22.78^{AB}	1.70^{N}
NDH 1	1.14^{EF}	48.85^{E}	9.95^{G}	3.17^{DEF}	7.77^{BCDE}	8.04^{CD}	1.27^{B}	0.47^{DE}	0.06^{D}	11.73^{EFG}	4.42^{H}
NDH 3	1.04^{FGH}	41.80^{J}	10.10^{G}	3.50^{CDE}	7.37^{DEF}	9.07^{AB}	1.20^{BC}	0.51^{CD}	0.06^{D}	10.95^{EFG}	6.85^{B}
IISR Prabha	1.16^{DEF}	56.00^{C}	14.80^{B}	2.67^{FGH}	7.26^{DEF}	7.12^{EF}	0.77^{HI}	0.32^{EFGHI}	0.06^{CD}	19.44^{BC}	4.45^{H}
IISR Pragati	0.95^{H}	35.90^{L}	13.15^{C}	3.50^{CDE}	8.78^{ABC}	9.57^{A}	1.43^{A}	0.69^{AB}	0.10^{A}	14.52^{DEF}	6.14^{C}
IISR Pratibha	1.09^{EFG}	46.25^{FG}	12.90^{CDE}	3.17^{DEF}	7.16^{EF}	7.09^{FG}	0.77^{HI}	0.52^{CD}	0.09^{AB}	17.42^{CD}	4.05^{I}
Punjab Haldi 1	1.11^{EFG}	43.85^{HI}	11.75^{EF}	4.33^{A}	5.88^{G}	6.96^{FG}	1.21^{BC}	0.20^{HI}	0.02^{E}	10.26^{FG}	4.01^{I}
Rajendra Sonia	1.12^{EFG}	42.50^{IJ}	9.70^{G}	3.83^{ABC}	8.14^{BCDE}	8.69^{ABC}	1.19^{BC}	0.73^{A}	0.09^{AB}	11.84^{EFG}	5.28^{E}
Sudarsana	1.01^{GH}	38.90^{K}	12.95^{CD}	3.83^{ABC}	8.39^{BCD}	9.04^{AB}	1.23^{BC}	0.65^{ABC}	0.07^{CD}	10.10^{G}	7.14^{A}
Suguna	1.08^{EFG}	44.35^{GHI}	13.35^{C}	4.17^{AB}	7.35^{DEF}	8.01^{CDE}	1.27^{B}	0.56^{BCD}	0.07^{CD}	11.74^{EFG}	5.93^{D}
Suranjana	1.12^{EFG}	44.45^{GHI}	11.90^{DEF}	4.33^{A}	8.16^{BCDE}	8.41^{BC}	1.18^{BCD}	0.73^{A}	0.10^{A}	13.69^{DEFG}	4.80^{F}
Suvarna	1.28^{CD}	55.20^{CD}	15.05^{B}	3.50^{CDE}	8.90^{AB}	6.63^{FG}	1.04^{DEF}	0.45^{DE}	0.10^{A}	21.11^{ABC}	2.60^{L}
Varna	1.38^{BC}	63.40^{A}	13.50^{C}	2.50^{GH}	8.72^{ABC}	6.55^{FG}	1.08^{CDE}	0.41^{DEFG}	0.09^{AB}	22.16^{AB}	1.65^{N}
General mean	1.17	48.18	12.90	3.39	7.46	7.54	1.05	0.44	0.07	15.61	4.33
CV (%)	4.94	1.96	4.36	7.66	7.43	5.68	6.66	17.15	12.66	13.20	1.64

PH Plant height, *NS* number of shoots, *LL* leaf length, *LW* leaf width, *PL* length of primary rhizome, *PT* thickness of primary rhizome, *IL* internodal length, *RY* fresh rhizome yield per plant, *DY* dry yield of rhizome per plant, *DYP* percentage of dry yield of rhizome per plant, *CUR* Curcumin content

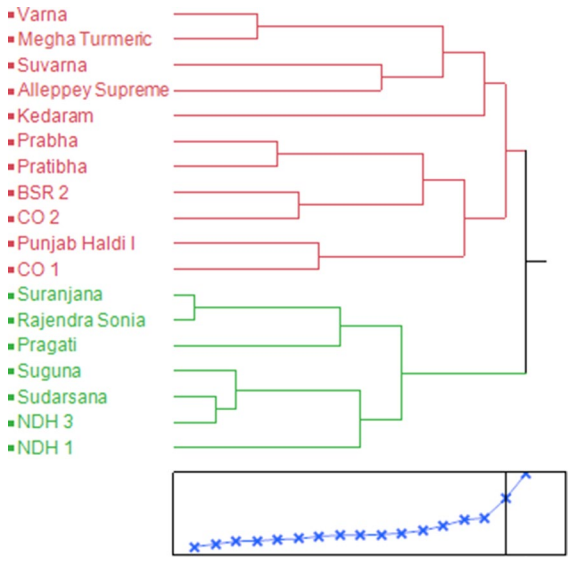

Fig. 1 Hierarchical clustering based on morphological data for the 18 improved varieties using Ward's method

in discriminating among individuals. Therefore, the markers utilized here are very informative and can be recommended for molecular studies in turmeric. The results are summarised in Table 3. Figure 2 shows the PCR amplification profile of a SSR marker i.e., CuMisat 19.

Genetic similarity analysis

In addition to the allelic variation at the marker level, genetic similarity is an important and useful information which help us to understand genetic relatedness, diversity and to find duplicates if any. In this study, similarity matrix generated based on Jaccard index using SIMQUAL module in NTSYSPC was used for sequential, agglomerative, hierarchical clustering as defined by Sneath and Sokal in SAHN module of NTSYSPC. The Jaccard's similarity coefficients ranged between 0.04 and 1 (Fig. 3). The highest similarity coefficient of 1 was observed between Varna and Megha turmeric; Sudarsana and Punjab Haldi. The dendrogram constructed grouped the genotypes into three clusters. Cluster I was the smallest cluster and subdivided into two sub-clusters at similarity of ~ 0.28. Cluster IA comprised of identical genotypes (Varna and Megha Turmeric). Cluster IB contained the variety Suvarna. Cluster II was further splitted and comprised of IISR Kedaram, IISR Alleppey Supreme, IISR Pratibha, IISR Prabha, BSR 2. Cluster III included majority of the variety under study (10 varieties) at ~ 0.76 similarity. Cluster III is subdivided into subclusters III A (Suranjana, Suguna, Rajendra Sonia, IISR Pragati, Sudarsana, Punjab Haldi 1, CO 1, CO 2 and NDH 1) and III B (NDH 3).

Identification of most unique or divergent genotypes

As per (Jamshidi and Jamshidi, 2011), the scored data from PCR based molecular screening of genotypes was further subjected to cluster analysis and dendrogram construction for each of individual markers to identify individuals with least relativeness. Supplementary Table 4 represents the total number of individuals a given individual variety is common in the same clade in each primer's dendrogram which was totalled across all 56 primers. Genotypes are arranged from least relativeness to high relativeness as we move from top to bottom of the table. The same is depicted graphically in Fig. 4. The variety Suvarna shares the least number of clusters with rest of the varieties and hence is the most divergent and unique variety among the varieties under study, followed by Varna and Megha Turmeric. IISR Kedaram, BSR-2, IISR Pratibha, IISR Alleppey Supreme and IISR Prabha which shares an intermediate number of clusters. NDH 3, Suguna, Suranjana, NDH 1, CO 2, Rajendra Sonia, Pragati, CO 1, Sudarsana and Punjab Haldi 1 shares a high number of clusters with the rest of the genotypes and hence have lower divergence. Especially, Sudarsana and Punjab Haldi 1 has the maximum number of shared clusters and hence least unique or divergent of all varieties under study.

Discussion

In this study we have observed that there is genetic variation among released turmeric varieties both at the morphological and molecular level. This study further confirms the utility of earlier developed SSR and ISSR markers (Siju et al. 2010a, b; Senan et al. 2013) in assessment of genetic diversity in turmeric. Morphological characterization is the primary step in the description and classification of any crop species (Smith and Smith 1989). It helps in identification and selection of desirable traits in crop plants (Malek et al. 2014). Previous studies in assessment

Table 3 Characteristics of SSR and ISSR markers obtained after the PCR screening of varieties

Primer	Allele size range	Total number of alleles	Average number of alleles per genotype	Mono-morphic alleles	Poly-morphic alleles	PIC
UBC 815	850–1650	5	1.05	0	5	0.32
UBC 818	875–1350	7	1.27	0	7	0.29
UBC 825	750–1600	4	2.61	0	4	0.40
UBC 826	400–1500	10	1.27	0	10	0.38
UBC 834	600–1500	5	1.55	0	5	0.40
UBC 842	500–1900	9	2.05	0	9	0.35
UBC 845	600–2250	4	1.27	0	4	0.40
UBC 850	500–1600	5	2.11	0	5	0.45
UBC 856	750–850	6	1.72	0	6	0.39
UBC 857	490–2000	2	1.11	0	2	0.44
UBC 860	500–1400	8	2.16	0	8	0.37
UBC 866	730–1450	7	1.27	0	7	0.29
UBC 884	300–1200	7	2.38	0	7	0.43
UBC 889	400–1200	8	3.44	0	8	0.46
UBC 896	600–1700	3	1.00	0	3	0.44
UBC 897	1200–1450	5	1.55	0	5	0.40
ISSR-02	600–1100	7	2.00	0	7	0.40
ISSR-03	400–1200	7	2.44	0	7	0.42
ISSR-06	500–1350	5	1.05	0	5	0.32
ISSR-07	600–1400	4	1.22	0	4	0.40
ISSR 13	350–1200	7	2.38	0	7	0.39
ISSR 14	500–1400	7	2.44	0	7	0.42
ISSR 15	600–1450	10	1.72	0	10	0.27
ISSR 17	875–1350	7	1.27	0	7	0.29
Cumisat 01	238–218	4	1.00	0	4	0.38
Cumisat 02	146–118	7	2.38	0	7	0.42
Cumisat 03	200–140	5	1.55	0	5	0.40
Cumisat 04	204–188	7	1.27	0	7	0.29
Cumisat 05	186–160	8	2.00	0	8	0.38
Cumisat 08	178–132	8	3.44	0	8	0.46
Cumisat 13	268–238	7	2.00	0	7	0.40
Cumisat19	204–154	7	2.44	0	7	0.42
Cumisat 20	158–148	7	1.27	0	7	0.29
Cumisat 22	158–122	5	1.55	0	5	0.40
Cumisat 23	165–132	2	1.05	1	1	0.47
Cumisat 25	146–140	4	1.00	0	4	0.38
Cumisat 28	160–139	5	2.11	0	5	0.45
Cumisat 29	177–150	6	1.72	0	6	0.39
Cumisat 31	169–148	3	1.00	0	3	0.44
Cumisat 32	135–120	6	2.11	0	6	0.40
Cumisat 33	170–140	2	1.00	0	2	0.5
Cumisat 36	220–208	1	1.00	1	0	0.00
Cumisat 37	218–192	4	1.27	0	4	0.40
Clest 02	204–152	6	2.00	0	6	0.44
Clest 03	173–133	5	1.44	0	5	0.39

Table 3 (continued)

Primer	Allele size range	Total number of alleles	Average number of alleles per genotype	Mono-morphic alleles	Poly-morphic alleles	PIC
Clest 04	188–172	4	1.00	0	4	0.38
Clest 06	199–191	4	1.00	0	4	0.38
Clest 08	181–154	4	1.00	0	4	0.38
Clest 09	218–184	4	1.00	0	4	0.38
Clest 10	200–188	3	1.50	0	3	0.44
Clest 11	305–292	5	1.00	0	5	0.32
Clest 12	162–140	6	1.55	0	6	0.34
Clest 13	158–136	3	1.00	0	3	0.44
Clest 15	198–162	4	1.27	0	4	0.41
Clest 16	174–164	6	1.44	0	6	0.35
Clest 17	192–174	3	1.00	0	3	0.44

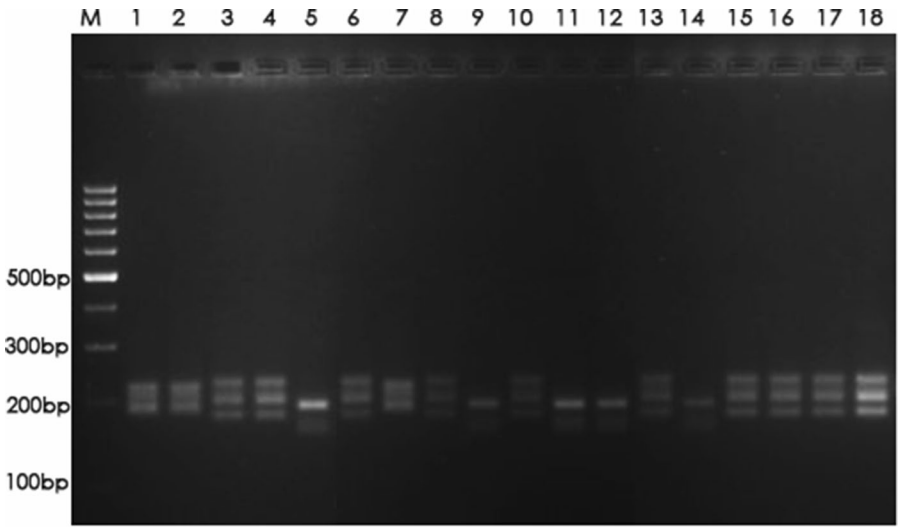

Fig. 2 Amplification profile of 18 improved varieties using CuMisat 19. Loading pattern: M-100 bp DNA ladder; 1-Varna; 2-Megha Turmeric; 3-Suranjana; 4-Suguna; 5-Kedaram; 6-Rajendra Sonia; 7-Suvarna; 8-Pragati; 9-Prabha; 10-Sudarsana; 11-Alleppey Supreme; 12-Pratibha; 13-Punjab Haldi 1; 14-BSR 2; 15-CO 1; 16-C0 2; 17-NDH 1; 18-NDH 3

of morphological diversity in turmeric have reported considerable level of diversity in the crop (Roy et al. 2011; Ullah Jan et al. 2012; Bahadur et al. 2016; Anindita et al. 2020).

Firstly, to understand the morphological variation in improved varieties, we have done a hierarchical clustering based on Wards method on morphological data which revealed the morphological variation based on 11 quantitative characters and curcuminoid content. It was reported that Sudarsana and Suguna (collections from Andhra Pradesh) were similar but very distinct even though these were from the same geographical area, similarly Prabha and Pratibha from Kerala, which were also similar but were very distinct (Vijayalatha and Chezhiyan 2008). But most of the morphological traits are influenced by genotype × environment interactions (Anandaraj et al. 2014). Earlier study on genotypic stability had identified three improved varieties, Mega Turmeric, IISR Kedaram and IISR Pratibha as stable genetic sources for high dry yield and curcumin content (Anandaraj et al. 2014). In our study the statistical analysis and clustering pattern confirms the presence of morphological diversity among improved varieties for the characters under study. Here, the morphological data based hierarchical cluster analysis have resulted in two broad clusters. Cluster 1 include plant varieties with comparatively tall stature, increased leaf length and

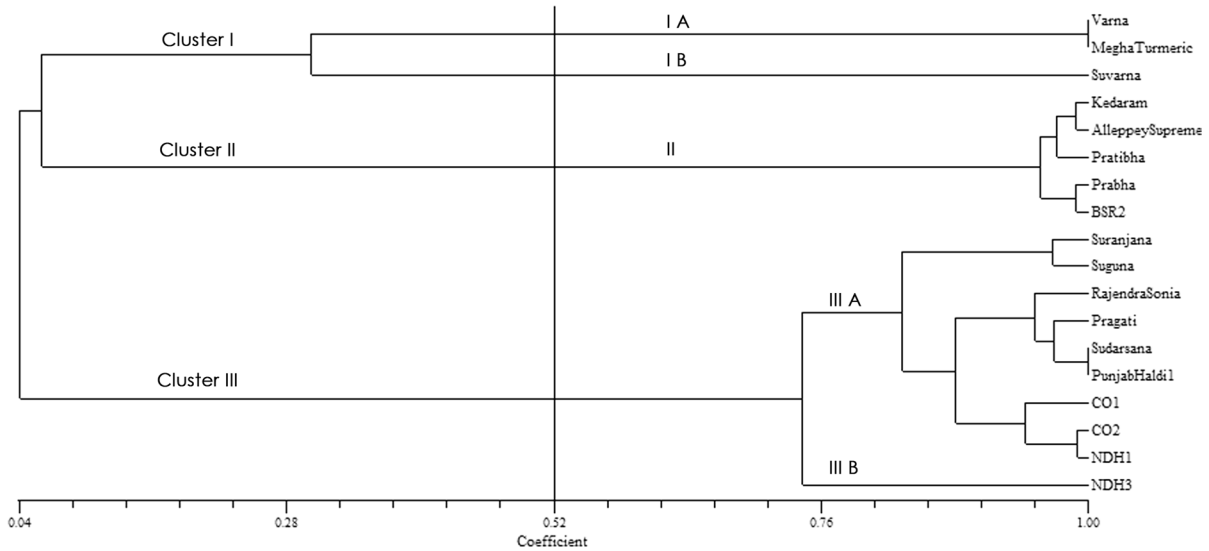

Fig. 3 UPGMA based Dendrogram for scored molecular data from 56 primers in 18 improved varieties

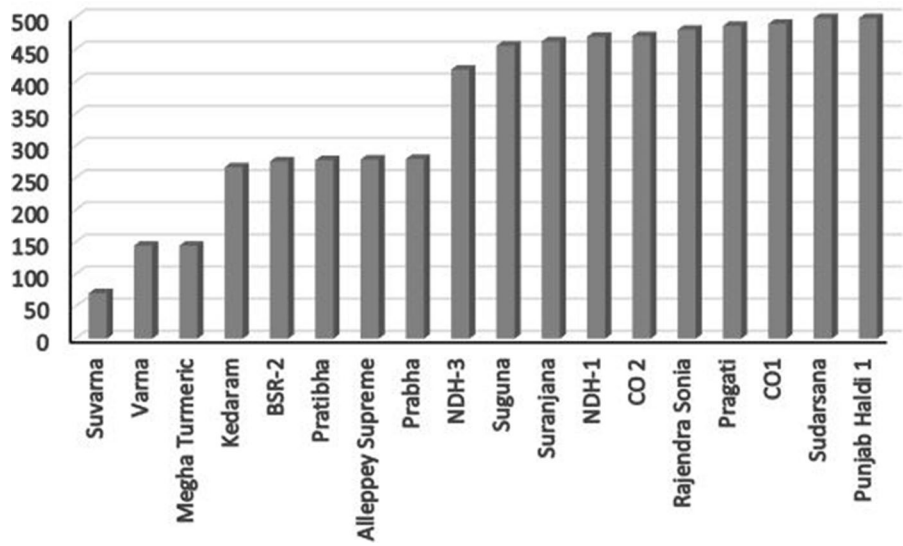

Fig. 4 Improved turmeric genotypes are shown in X-axis and the total number of individual genotypes shared in the same cluster across all primer's dendrograms by the respective genotype in X-axis is given in y-axis

width. Most of which are long duration crops. Cluster 2 includes comparatively shorter turmeric varieties with increased primary finger length and thickness, rhizome yield per plant, curcuminoid content, dry yield per plant etc. and most of which are short duration crops. Some of these characters considered here like plant height, number of leaves, size of primary fingers and number of suckers had showed positive and significant association with rhizome yield (Roy et al. 2011; Bahadur et al. 2016). In our study also (based on morphological clustering of genotypes), we could observe that genotypes with a higher length and thickness of rhizomes, more number of shoots/suckers had higher rhizome yield and curcumin content which hints at the suitability of these characters as possible selective traits for better yield. The same is case with traits required for selection of divergent parents. Here, we have identified Varna, Megha Turmeric and Suvarna as the most divergent genotypes in comparison to other genotypes (based on molecular characterization). These divergent varieties are characterized by a higher plant height, leaf length

and width. These traits can be considered as putative selective traits but it is not conclusive as this may require further study with a larger sample size and validation by field trial to arrive at a confirmed conclusion. This is because most of these traits depend on genotype and genotype × environment interactions (Anandaraj et al. 2014).

Secondly, molecular characterization of these varieties has revealed the presence of molecular genetic variation. Even though we have used codominant SSR markers along with dominant ISSR markers for genotyping, there is no information about the location of targeted loci. SSR Primers may target a locus or in some cases multiple loci owing to the polyploid nature of crop leading to multiple alleles per genotype. Hence, characterization of microsatellite loci is complicated in polyploids as allele dosage of SSRs cannot be determined (Pfeiffer et al. 2011) Here as well, the possibility of multilocus amplification and polyploid nature of turmeric (Sigrist et al. 2011) presents difficulties in scoring and analysis of polyploid marker data if we treat the marker as codominant. So, instead of scoring per locus/primer, we have scored considering each band like a locus and hence scored for the presence (as '1') and absence (as 0) of these bands in a binary format across varieties just as in the case of a dominant marker. Hence, polymorphism information content value of SSR markers were also calculated by treating them as dominant markers like ISSR markers (Rawat et al. 2014). From the dendrogram constructed based on this scored molecular data, we can infer genetic similarity of varieties. Earlier molecular studies involving some of these improved varieties have found that IISR Pratibha, IISR Kedaram, IISR Alleppey Supreme, and Megha were grouped nearby to each other because of the high similarity among them and the varieties Lakadong and Suvarna gave a unique banding profile (Sahoo et al, 2017). Our results also confirm these findings wherein Varna and Megha turmeric which are identical in all loci tested here share a similarity coefficient of ~ 0.28 with Suvarna which showed a unique banding pattern in gel profile for most of the loci tested. These varieties which form Cluster I have the lowest intracluster coefficient of similarity (0.28) and hence broad genetic base compared to genotypes of cluster II (IISR Kedaram, IISR Alleppey Supreme, IISR Pratibha, IISR Prabha and BSR-2) and cluster III (Suranjana, Suguna, Rajendra Sonia, IISR Pragati, Sudarsana, Punjab Haldi 1, CO 1, CO 2, NDH 1 and NDH 3) which have similarity coefficient of ~ 0.95 and ~ 0.76 respectively. This indicates that the genotypes in Cluster II share high relativeness within the cluster (similarity coefficient above 0.9) with highly shared molecular genetic background followed by genotypes in Cluster III and Cluster I, respectively.

Also, it is found that of all the primers tested, varieties Varna and Megha turmeric, Sudarsana and Punjab Haldi 1 respectively had identical genetic background indicating a very close genetic relatedness. Suvarna followed by Varna and Megha turmeric form the most divergent group of genotypes which was confirmed by the analysis to find the unique genotypes (Jamshidi and Jamshidi, 2011). In general, we can understand that of all the loci tested, the genetic similarity of all the improved varieties under study is atleast 0.04 (Jaccards similarity coefficient of 0.04) which hints to a broad genetic base collectively. This can be possibly since these improved genotypes are from diverse locations from India.

In general, from the molecular and morphological characterization, we can infer that (1) they both almost follow a similar pattern except Punjab haldi 1, CO 1 and CO 2 which showed morphological clustering that is quite different from their molecular data-based clustering. This may be due to inadequate number of characters studied which couldn't reveal the molecular diversity present or due to some other factors. (2) molecular analysis reveals the similar genetic background of morphologically distinct/dissimilar varieties (eg: Varna and Megha Turmeric, Sudarsana and Punjab Haldi 1) and (3) molecular characterization helps to find genetically divergent/ unique genotypes. It can be inferred that in general, there was no relation between the origin of the improved variety and its clustering as most of the varieties from the same place of origin are dispersed among different groups. This can be explained by the probable exchange of planting material or rhizome between farmers or stakeholders.

Conclusions

From this study, we inferred the molecular genetic similarity of prominent improved varieties of turmeric and found unique and divergent varieties. We

also examined the major morphological characters of improved varieties to see if they have parallels with the genetic similarity observed. We find that molecular and morphological data-based grouping of genotypes follow a similar pattern. This may imply that most of the morphologically similar varieties have a similar genetic background at the DNA level. The results from this study can be utilized while designing turmeric breeding programmes to choose genetically dissimilar parents among released varieties. Understanding the variation and similarity in improved varieties give insights into the status of crop improvement and can trigger more in depth studies into the dynamics of crop improvement and genetic erosion.

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Author contribution Experiments, analysis, Writing-Original draft preparation was done by APA. DP has done Conceptualization, Resources, Validation, Supervision, Writing- Reviewing and Editing. SBR has conducted a part of the molecular characterization experiments. All authors read and approved the manuscript.

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Data availability Data is contained within the article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication All authors consent to publish.

Ethics approval and consent to participate This work does not involve living animals and no consent is needed.

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