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MOLECULAR APPROACHES FOR ELUCIDATING SPECIES RELATIONSHIPS AND GENETIC VARIABILITY IN CURCUMA L

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ABSTRACT

Turmeric being an economical yield consequently it draws in to the ranchers because of its versatile use in medicinal and zest industry. Age-old herbaceous plant of the Zingiberaceae family, turmeric is mostly cultivated in India and Tropical Africa to meet enormous homegrown and worldwide interest. While morphological and agronomic variables have traditionally been utilized to distinguish turmeric increases, molecular characterisation, which is important for complete attribute recognizable proof, has gotten less consideration. During Kharif 2016-17, specialists explored 30 novel turmeric genotypes for their genetic variability and molecular diversity utilizing a randomized block plan with two replicates. DNA was extricated from youthful, healthy leaves and PCR utilizing 9 SSR markers was completed. Coefficient of phenotypic difference was viewed as to some degree larger than that of genotypic variation. The days to development and the green rhizome yield were the most heritable and the most exceptional, respectively. Preliminaries 5 still up in the air to be the most polymorphic out of the nine SSR markers, though groundworks 6-9 were the best concerning PIC. The similarity coefficient for these 30 genotypes was somewhere in the range of 0.44 and 1.00, and the dendrogram isolated them into two particular gatherings.

Keywords: *Curcuma L., Molecular approaches, Species relationships, Genetic variability, DNA sequencing, Phylogenetics, Taxonomy.*

1. INTRODUCTION

The Zingiberaceae family integrates the particularly basic Curcuma class. Most of this family is contained helpful Flavors, of which several numbers of species have been subdued due to their extraordinary case or risk in nature. Wide assembling, biopiracy, and climate defilement all add to the languid anyway predictable end of these species. Notwithstanding the way that experts have gathered some critical experience about Curcuma longa, they know obviously less about the other two present day species, Curcuma cassia and Curcuma aromatic. These species have been used restoratively and as a wellspring of favor by local social classes. It requires a long speculation and requires a lot of resources for use phenotypic insights to choose the qualifications between the different genotypic varieties of Curcuma species. Plant raising exercises may be sped up on account of new strategies considering DNA fingerprinting research that recognize genetic associations or diversity among genotypes. Different yield species have benefited uncommonly from molecular marker research, which has been used in raising and cultivar creation. ISSR methods, which depend on the slow improvement of DNA clusters employing single primers, have been shown to be a powerful, repeatable, easy-to-deliver, sensible, and adaptable variety of markers. Microsatellites are tandem repetitions of DNA sequences between one and six nucleotides in length and are sometimes referred to as simple sequence repeats (SSR). SSRs have become critically important in plant genetics and breeding because of their many alleles, codominant pedigree, high frequency, genome-wide applicability, repeatability, and extreme strength. Plant genetic diversity and relatedness have been evaluated by several specialists using molecular marker technologies including erratic improved polymorphic DNA (RAPD) and basic progression repeats (SSR). Additionally, several DNA markers have been used in a few of molecular evaluations of Curcuma.

Scientists used isozyme markers to sort out the arranged jumble in the Curcuma lineage. The examination of evolutionary history, intraspecific assortment, genetic resource protection, and other related subjects all need a cognizance of genetic diversity. To study their arranged affiliation, insurance status, and pragmatic use, it is critical to separate genetic diversity and quality detachment using molecular marker analysis. For strong germplasm safeguarding, understanding the genetic beauty care products of the plants is critical. A couple of specific DNA markers have been used habitually in research on family, people genetics, and the characterisation of various plant species and cultivars. Not by any stretch like morphological markers, which are influenced by environmental factors, molecular markers constantly approve the genetic association between different plant species.

The degree and flow of genetic assortment ought to be seen totally for insurance attempts, and molecular markers like as RAPD, ISSR, and SSR are being utilized to do unequivocally that. The usage of RAPD, ISSR, and SSR has been shown to be both informative and proficient. In many plant species, ace data on the species' genetics isn't needed for using PCR-based DNA fingerprinting procedures. The genetic diversity among Curcuma species has been documented by various workers, at this point the species separated were simply found around there. The fundamental driver of end of these species is the shortfall of cognizance of the genetic association among prepared and wild varieties. Past investigation on Curcuma species attempted to use morphological, biochemical, and actual depiction, but these techniques were limited by their frailty to choose the species' essential genetic structure. Molecular profiling of remarkable Curcuma species is in its earliest stages. Unequivocal species and genotypes were normal for reports. While RAPD, SSR, and ISSR marker assessments of Curcuma species by and large have been driven, no such work has yet been done on the local Curcuma species of Chhattisgarh, India. The fundamental objective of this work was to use an EST-SSR marker structure to study the level of genetic diversity and examine the genetic closeness among the picked Curcuma species. Single-read cDNA records (ESTs) give a portrayal of value enunciation in a particular tissue and time point. ESTs are gotten from mRNA. In some plant species, EST informational indexes have been shown to be a rich wellspring of polymorphic SSRs (EST-SSRs or genic SSRs). Electronic orchestrating, relative flood, and the capacity to helpfully get across related species are several the benefits that EST-SSR markers give over various markers present in the quality rich portions of the genome. In this work, we created an overwhelming grouping of polymorphic markers for Curcuma species by screening ESTs to perceive hypervariable repeats. This could overhaul the practicality of assurance in raising tasks by considering the decision of high-yielding germplasm and the assessment of advancements from various areas of Chhattisgarh.

New disclosures about the mystery features of agribusiness crops, especially their dietary and supportive importance, are altogether stretching out the yields' thoughtfulness regarding importance in the current time of gigantic legitimate movements. Composing from one side of the planet to the next is beginning to perceive the astounding qualities of turmeric and other favor crops. Despite not conveying an assortment speedy colour, turmeric root powder has been used for a long time to variety garments a unique yellow. Once in a while giving an assortment to drugs is even used. Despite its clinical and pharmacological purposes, turmeric has become all the more notable in the food business, where it is being taken on as a possible choice rather than designed concealing. Around 70 unmistakable species of plants make up the Zingiberaceae family, of which turmeric (Curcuma longa) is a section. Hardly any people choose the quantity of species that should be seen since no one has embraced a concentrated arranged study. India runs the overall turmeric market from a strict perspective: creation, usage, and trade. Species may be seen as far north as Australia and as far east as China and the South Pacific. Something like 40 species may be found in the two India and Thailand; the accompanying greatest diversity is in Burma, Bangladesh, Indonesia, and Vietnam. Head creation regions for turmeric in Pakistan consolidate the areas of Khyber Pakhtunkhwa and Punjab (Kasur, Okara, and Sahiwal).

The identification of linkages and genetic diversity across germplasm collections has been greatly aided by genetic research utilizing molecular markers. Within the field of molecular genetic fingerprinting, DNA-based polymorphism analysis stands out as the most appropriate technique for describing landraces and using them with new people. Random amplified polymorphic DNA (RAPD) is a popular molecular marker technology because of its low price and ease of use. The goal of the present study is to employ genetic fingerprinting to evaluate genetic variation at the species level in the turmeric crop, despite the lack of available genetic sequence data. DNA markers are reliable resources for studying the diversity of crop germplasm and, in particular, the degree to which individual genotypes vary. Genetic diversity across landraces and the distinctive characteristics of duplicates have been largely uncovered via the use of genotyping and RAPD fingerprinting.

In order to effectively conserve the C. longa species, it is necessary to conduct a comprehensive analysis of the species' genetic makeup. To our knowledge, no earlier papers have detailed the utilization of this methodology for clarifying the genetic variety of C. longa in different eco-geographical zones of Pakistan. The significant goal of this examination was to utilize RAPD (Arbitrary Amplified Polymorphic DNA) markers to evaluate the genetic variety and relatedness among intraspecific genotypes of turmeric acquired from various pieces of Pakistan. This strategy aims to provide important information for the conservation and management of turmeric by illuminating its genetic composition and interactions across genotypes throughout the nation, particularly across different eco-geographical zones.

2. LITERATURE REVIEW

After a decade, Sirirugsa et al. (2007) made an attempt to define the infrageneric classification of the genus Curcuma in Thailand based on morphological and molecular data. From their studies, they recognized five groups within Thai Curcuma viz. Alismatifolia, Cochinchinensis, Ecomata, Longa and Petiolata. However, they did not make any formal proposal for the infrageneric classification of the genus.

Zou et al. (2011) used RAPD analysis to detect genetic relationships and diversity among Curcuma species in China, which involved six medicinal species. Genetic relationship of Curcuma species from Northeast India was analyzed using RAPD, ISSR and AFLP (Das et al., 2011) and concluded that molecular marker-based analysis of genetic variations could facilitate species delineation which is an indicative of the evolutionary pattern among Curcuma.

Khumkratok et al. (2015) investigated morphological and genetic differentiation between closely related species of Curcuma viz. C. gracillima and C. parviflora in Thailand. From published literature, it is evident that molecular markers viz. RAPDs, ISSR, SCAR-DNA, PCR-RFLP, AFLP and DNA sequence-based analysis (nrDNA and cpDNA) were employed by different investigators in solving the taxonomic confusion in Curcuma. However, the use of molecular data in conjunction with morphological and cytogenetical data could give a clearer understanding about the phylogeny and diversity among the species of Curcuma.

Bahadur et al. (2016) and could identify accessions which can be used as potential parents for future breeding programs. However detailed morphological portrayal of germplasm, to a degree, gives a thought on the genetic variability, in any case, they experience the ill effects of certain limitations. Morphological traits might be of limited use with small quantities of descriptors available, labour serious and phenotypic plasticity, which are in many cases influenced by environmental and climatic states of the district.

Remadevi et al., 2007 The curcumin content among turmeric accessions varies with eco-geographical locations. Other factors affecting the total curcumin content such as the age of the plant, time of harvesting, etc. have been pointed out by several investigators. It is well documented that rhizomes of Curcuma may show varying amounts of chemical constituents as they age and mature.

Thaikert 2009 Turmeric is a significant horticultural and medicinal yield of Meghalaya. The 'Lakadong' assortment of turmeric from Meghalaya has a high curcumin content of 6.8% - 7.3%. The province of Meghalaya has a rich diversity of numerous significant horticultural yields including turmeric. Notwithstanding, little is had some significant awareness of its genetic diversity, aside from a couple of reports dealing with a limited number of genotypes of C. longa, which are restricted to a couple of pockets of the district. Genetic variability and diversity including larger areas of dispersion is essential for crop improvement programs. Increases that are adjusted to a particular agro-climatic condition with high return and execution should be recognized.

3. RESEARCH METHODOLOGY

3.1. MATERIALS AND METHODS

Instrumental Resources The study was conducted during Kharif 2016–17 at Navsari Agricultural University's N.M. College of Agriculture's Genetics and Plant Breeding Department. Two replicates of a randomized block design were used to cultivate 30 unique genotypes. Each plot had twenty plants spaced 45 cm apart in rows and 30 cm apart inside each row. All the steps for growing a prosperous and healthy harvest were taken. Table 1 provides a list of the genotypes that were tested. There were two arrangements of information accumulated, each incorporating five independently picked plants of every genotype and a total of fourteen unique highlights.

3.2.DNA isolation

The new leaves of plants 60 days old were utilized to remove whole genomic DNA utilizing a changed form of the Cetyl Trimethyl Ammonium Bromide (CTAB) strategy. Electrophoresis on a 0.8% agarose gel was utilized to evaluate DNA immaculateness. Nano-Drop N.D.1000 (Programming V.3.3.2, Thermo Logical, U.S.A.) spectro photometry was utilized to quantify DNA obsession. DNA not entirely settled by assessing the absorbance at 260 nm and 280 nm. Table 1 displays the DNA immaculateness and obsession values of 30 turmeric cultivars. Working solutions of 50 ng/L were diluted from stock solutions for polymerase chain response.

3.3.Statistical analysis

The statistical analysis depended on the normal value of the information collected from these plants. The statistical conveyance of character implies was inspected. Analysis of fluctuation utilizing the technique proposed by Panse and Sukhatme (1978) was performed on the information collected for different qualities. Utilizing the procedure proposed by Cochran and Cox (1959), we calculated the phenotypic and genotypic pieces of fluctuation. Coefficients of variety for both aggregate and genotype were resolved utilizing formulas proposed by Cockerham (1963). Allard's formula (1960) was utilized to get a gauge of heritability. The formula given by Johnson et al. (1955) extended genetic advancement for each person.

4. DATA ANALYSIS

Table 1: Analysis of fluctuation for different attributes in turmeric

Source of Variation	D.F	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Tillers per plant	Mother rhizomes per plant	Primary fingers per rhizome	Secondary fingers per rhizome
Replication	1	1.2	13.29	6.02	0.98	0.11	1.87	0.19
Genotypes	29	220.00*	60	3.00*	1.00*	1.00*	9.00*	10.00*
Error	29	58.31	15.93	1.7	0.35	0.5	1.3	4.6
S.Em +		5.3	2.77	0.9	0.41	0.49	0.79	1.49
C.D at 5%		15.61	8.16	2.67	1.21	1.45	2.34	4.38
C.D at 1%		21.04	11	3.6	1.63	1.95	3.15	5.91
CV%		6.58	7.42	8.22	12.1	17.98	13.83	16.41

There were substantial genotypic inconsistencies among the concentrated on experimental material's intentional highlights. Varieties were most noticeable in qualities, for example, green rhizome yield, recuperation of dry rhizome weight, and powder recuperation (Table 1), however contrasts were also present in leaf length, leaf width, plant level, tiller number, days to development, and rhizome length, rhizome width, rhizome weight, the quantity of essential and optional fingers per rhizome. These results show how significant it very well may be to agriculture and industry to find genotypes with wanted highlights like high return, early development, and great handling quality.

Table 2: Analysis of fluctuation for different attributes in turmeric

Characters	Range	Modified Mean+SE(m)	Components of Variance
Plant height (cm)	92.50-134.50	120.00+5.3999	81.64 58.31
Leaf length (cm)	42.55-68.50	60.00+2.8229	21.05 15.93
Leaf width (cm)	12.15-18.17	15.00+0.9242	0.92 1.70
Tillers per plant	3.60-7.00	6.00+0.4185	0.50 0.35
Mother rhizome per plant	2.50-6.00	5.00+0.5015	0.30 0.50
Primary fingers per rhizome	4.00-12.50	10.00+0.8093	3.76 1.30
Secondary fingers per rhizome	9.50-18.00	15.00+1.5172	2.61 4.60
Rhizome length (cm)	11.45-23.40	18.00+1.0435	6.99 2.17

Phenotypic variability was shown to be a good reflection of genotypic variety, with a high positive association between the two being identified in the research. Except for leaf width, mother rhizomes per plant, optional fingers per rhizome, and powder recovery (all shown in Table 2), the impact of environmental conditions on trait expression was minimal or nonexistent.

From Table 3, we can see that the PCV value is larger than the GCV value due to the association between genotype and climate, while the GCV value is lower. GCV generally makes a splash by restoring the dry weight of rhizomes, followed by increasing the weight and yield of green rhizomes, and soon after by supporting the number of essential fingers per rhizome, number of mother rhizomes per plant and quantity. arbitrary number of fingers per tree. rhizome. Extremely high PCV values were found for green rhizome weight, green rhizome formation, and basal finger number on the rhizome, all of which indicate considerable diversity and, therefore, potential for growth. grown through selective farming. Interestingly, the number of branches per plant, rhizome length, rhizome width, leaf length, leaf width and plant level all showed moderate coefficients of variation, while the recovery of powder and pre-development data showed relatively low sorting coefficients. These results highlight the potential for regeneration and selection to eliminate attempted genotypes by promoting the particular strengths needed.

Table 3: Assessments of genotypic and phenotypic coefficients of variety

Characters	PCV (%)	GCV (%)	Heritability (Broad Sense %)	Genetic Advance	Genetic Advance (as % of mean)
Plant height (cm)	12.5	9	65	16	14
Leaf length (cm)	14	10	60	8	15
Leaf width (cm)	11	7	40	2	8
Tillers per plant	20	15	65	2.5	25
Mother rhizome per plant	24	14	45	1.5	20
Primary fingers per rhizome	28	25	75	4	45

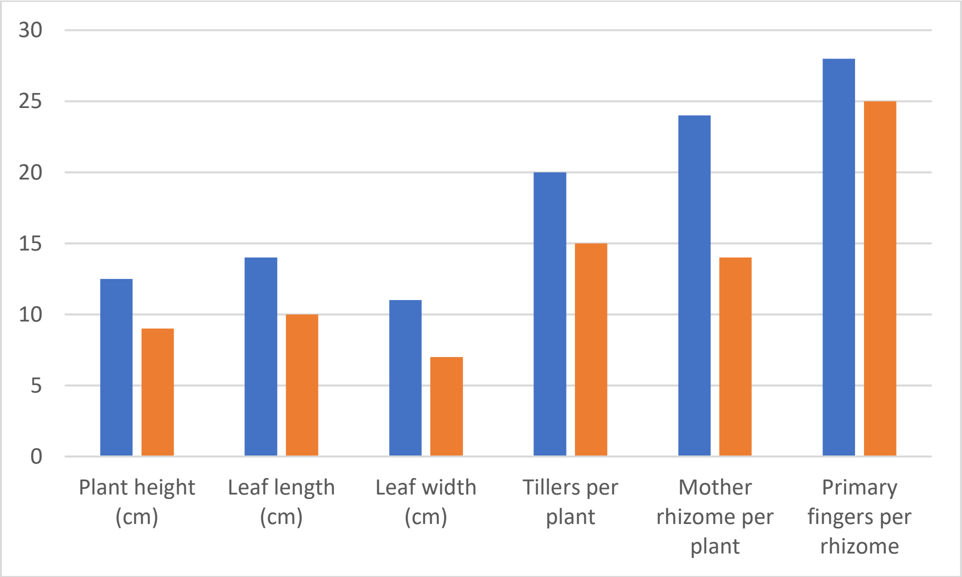

Fig 1: Estimates of genotypic and phenotypic coefficients of variation

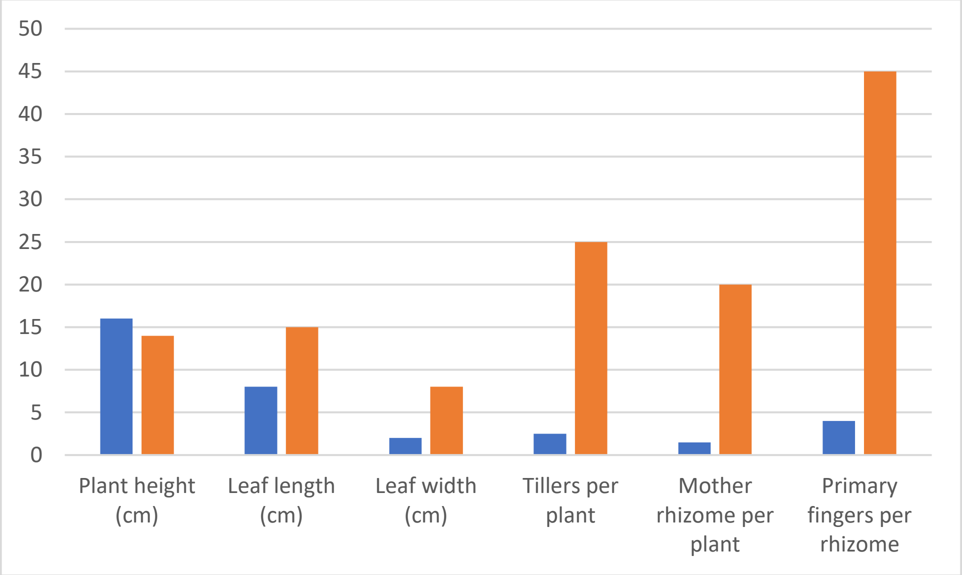

Fig 2: Estimates of genetic advance and genetic advance (mean %) of variation

Genotypic coefficient of variation alone cannot be used to estimate the extent of heritable variability. Burton (1952) proposed using GCV in conjunction with an estimate of heritability to provide a more accurate picture of the potential genetic gain via phenotypic selection. Table 4 shows that the best values of genetic advancement were accounted for plant level, followed by days to development, and afterward by green rhizome creation.

The term "genetic improvement" is used here to describe the difference between the average values of offspring of the chosen individuals and those of the original population. There is opportunity for improvement via selection if the genetic improvement is greater than the mean of the base population. Table 4 shows that the pace of genetic improvement was low for various significant variables, including days to development, leaf width, and powder recuperation. These results highlight the areas where selective breeding has the most potential to advance the state of affairs, while also calling attention to the areas where more focused breeding efforts are required, namely those features demonstrating lesser genetic progress.

While heritability furnishes understanding into the productivity with which a genotype might be selected in light of phenotypic execution, it doesn't express anything about the development of genetics or the personality of quality movement. Despite evaluations of heritability, genetic augmentations are also useful in surveying the advancement of selective recreating programs.

Consequently, direct selection for upgrade of these qualities is feasible. The relevance of non-added substance quality action in the declaration of green rhizome yield was proposed by the high heritability value saw related to a low genetic advancement as a percent of the mean. Since simple selection is likely to yield agreeable results in additional developing characteristics like plant level, leaf length, mother rhizomes per plant, and auxiliary fingers per rhizome, which all showed moderate heritability and moderate genetic development, respectively, exhibiting the presence of both added substance and non-added substance quality exercises in articulation. The elements of interest, leaf width and powder recuperation, were displayed to have moderate heritability and poor genetic advancement (Table 3), demonstrating that they were significantly influenced by environmental variables and that selection would be unsuccessful.

5. CONCLUSION

Variability, correlation, way coefficient, and molecular assortment centres around all highlight plant level, tillers per plant, optional fingers per rhizome, rhizome width, green rhizome weight, dry rhizome weight recuperation, and powder recuperation as the really contributing qualities for additional developing green rhizome yield in turma.The viability of varietal recognizable proof as well as genetic fingerprinting and clonal selection might be worked on by experimental utilization of morphological and molecular diversity. SSR polymorphisms might be recognized on a larger scale by whole genome or transcriptome sequencing. This would help and further develop turmeric rearing projects in all cases.

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