CHARACTERIZATION OF KASTHURI TURMERIC (*Curcuma aromatica* Salisb.)

MANUEL ALEX

2005

Department of Plantation Crops and Spices
COLLEGE OF AGRICULTURE
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DECLARATION

I hereby declare that this thesis entitled **"Characterization of kasthuri turmeric (*Curcuma aromatica* Salisb.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled **"Characterization of kasthuri turmeric (*Curcuma aromatica* Salisb.)"** is a record of research work done independently by **Mr. Manuel Alex (2002-12-05)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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EXTERNAL EXAMINER

Dedicated
to

My Beloved Chachan and Amma

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LIST OF ABBREVIATIONS

µm	–	Micrometre
µM	–	Micromolar
AFLP	–	Amplified fragment length polymorphic DNA
CD	–	Critical difference
cm	–	Centimetre
DNA	–	Deoxy ribonucleic acid
dNTPs	–	Deoxy nucleotides
ECV	–	Environmental coefficient of variation
EDTA	–	Ethylene diamino tetra acetic acid disodium salt
Fig.	–	Figure
GCV	–	Genotypic coefficient of variation
GV	–	Genotypic variance
IISR	–	Indian Institute of Spices Research
ISSR	–	Inter simple sequence repeats
KCl	–	Potassium chloride
$MgCl_2$	–	Magnesium chloride
mM	–	Millimolar
ng	–	Nanogram
OD	–	Optical density
PCR	–	Polymerase chain reaction
PCV	–	Phenotypic coefficient of variation
pM	–	Picomolar
PV	–	Phenotypic variance
PVP	–	Poly vinyl pyrrollidone
RAPD	–	Randomly amplified polymorphic DNAs
RFLP	–	Restriction fragment length polymorphism
TAE	–	Tris acetic acid EDTA
TE	–	Tris HCL-EDTA
Tris HCl	–	Tris (hydroxy methyl) aminomethane hydrochloride
UPGMA	–	Un weighted pair group method for arithmetic average

Introduction

1. INTRODUCTION

The basic character of life is its unlimited diversity. Biodiversity is the outcome of natural selection which has been going on for the last 3.5 billion years when life originated (Narain, 2000). An important component for efficient and effective management of plant genetic resources as well as their utilization is the characterization of germplasm. Such a characterization is essential not only for the identification of various species, but also to determine their genetic relatedness. This also assumes relevance in the present context of intellectual property rights and trade agreements (Ravishankar *et al*., 2000).

Kasthuri turmeric (*Curcuma aromatica* Salisb.) is a medicinal and aromatic plant with multiple uses. Many cosmetics and ayurvedic preparations contain kasthuri turmeric. Skin care is the major domain of application of this crop. Rhizomes of *Curcuma aromatica* are used in medicines as a stomachic, a carminative, an emmenagogue, for skin diseases and recently as a health food in Japan (Kojima *et al*., 1998).

Eventhough, kasthuri turmeric has got wide range of applications, it is getting slowly depleted from cultivation due to various reasons. The ignorance about the true identity of the crop is the major reason for the decline in the cultivation of this crop. This also makes it easy for the vendors to sell any turmeric in the disguise of kasthuri turmeric. Easily available *Curcuma zedoaria* Roxb. [Manjakoova (Mal.)] is the common *Curcuma* spp. sold at an exorbitant price as kasthuri turmeric by many vendors (Sasikumar, 2000).

The genus *Curcuma* is mainly Indo-Malayan in distribution and includes about 100 species of which 40 species are found in India (Sasikumar *et al.*, 1999). Species diversity, morphotype diversity and varietal diversity are characteristic of this genus (Velayudhan *et al*.,

1994). Detailed study regarding morphological, physiological, biochemical and molecular characters of different accessions of kasthuri turmeric, in comparison with ordinary turmeric and zedoary is required for its conservation, multiplication and popularization. Therefore well defined characterization and differentiation of the under exploited species, *Curcuma aromatica* having commercial and economic importance is essential.

Characterization of plants has traditionally been done based upon morphological, biochemical and cytological characters. However, these approaches are subject to environmental influences and hence their effectiveness is debatable (Simi, 2001).

Molecular characterization of organisms using DNA-based molecular markers has been widely accepted as an ideal method as the molecular markers are not affected by environmental conditions. Several molecular markers, namely, RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism) etc. are being utilized for this purpose. RAPD marker technique used in this study is quick, reliable and widely applicable. It is used for characterization of genetic variability, determination of somaclonal variants and hybrids, taxonomic studies, sex determination etc.

In this context, the present study was undertaken with the following objectives:

- To characterize various accessions of kasthuri turmeric collected from different parts of Kerala in comparison with ordinary turmeric (*Curcuma longa* L.) and zedoary (*Curcuma zedoaria* Rosc.) through morphological, biochemical, anatomical and physiological traits.
- To characterize the different accessions through molecular markers using RAPD technique to analyse the genetic make up of kasthuri turmeric accessions in comparison with ordinary turmeric and zedoary.

Review of Literature

2. REVIEW OF LITERATURE

Kasthuri turmeric (*Curcuma aromatica* Salisb.) is a medicinal and aromatic plant having great importance in cosmetic industry as well as in indigenous systems of medicine. The plant belongs to the genus *Curcuma* whose 40 species are reported as occurring in India. Species diversity, morphotype diversity and varietal diversity are characteristic of this genus. The present study is concentrated on characterization of kasthuri turmeric in comparison with ordinary turmeric and zedoary. Variabilities in morphological, physiological, biochemical, anatomical and molecular characters were evaluated in order to establish the true identity of kasthuri turmeric. The available literature related to the study is reviewed under the following subheads.

2.1 VARIABILITY IN GROWTH AND PHYSIOLOGICAL CHARACTERS

Morphological characters have been the main criteria by which plant genotypes have been identified and it is also employed to assess the variability between genotypes. Most of the crop plants are essentially classified into varieties on the basis of characters such as growth habit, leaf and floral morphology.

Indiresh *et al*. (1992) studied the genetic variability in turmeric. Analysis of variance revealed highly significant variations in characters such as plant height, petiole length, fresh rhizome yield, length of primary and secondary fingers per plant, girth of primary and secondary fingers and weight of mother rhizome. The other characters studied were not significant.

Prakash and Krishnan (1994) observed variation in various accessions and inter varietal hybrids of *C. forskholii* at different stages of growth.

Korla and Tiwari (1999) evaluated twenty four genotypes of ginger (*Zingiber officinale*) for yield and yield components under rainfed and irrigated conditions at Solan. Study revealed significant effects of rainfed and irrigated conditions on pseudostem length, tillers per plant, leaf length, leaf breadth and yield per plot. Significant genotype differences were observed for pseudostem length, rhizome length, rhizome breadth and yield per plant.

Lal *et al*. (1999) collected more than 100 diverse accessions of vetiver (*Vetiveria zizanioides*) from wild / cultivated sources. A considerable amount of natural variability for nine metric traits (plant height, tillers/plant, leaf width, root depth, root girth, fresh and dry root yield, oil content and oil yield) was recorded among these collections.

Singh *et al*. (1999) evaluated eighteen ginger cultivars for growth, yield and quality in Nagaland during 1992. The cultivars Thinglaidum, Nadia and Khasi local were the tallest and had most tillers per plant. They also had the highest rhizome yields.

Lynrah and Chakrabarthy (2000) evaluated the performance of 25 genotypes of turmeric including *C. longa*, *C. aromatica* and *C. caesia* during 1994-95 which revealed significant variation with respect to growth, yield and quality parameters due to genotypes. Among the genotypes, black turmeric (*C. caesia*), a semi wild type, showed the most vigorous growth and yield with highest number of tillers, leaves and leaf area per clump.

Poduval *et al*. (2001) studied *C. aromatica* cv. Kasthuri, *C. zedoaria* cv. Manjakoova and 13 cultivars of *C. domestica* for yield in a field experiment conducted in West Bengal during 1997 revealed that the *C. aromatica* and *C. zedoaria* yielded more than the cultivars of *C. domestica*.

Narayanpur *et al*. (2003) analysed 16 cultivars of turmeric for morphological and yield characteristics. Plant height, number of leaves, number of tillers and leaf area index differed significantly. High significant variations were noticed among the cultivars for fresh and cured rhizome yield for which the reason was attributed to genetic characteristics and their response for particular agroclimatic conditions.

Kurian *et al*. (2004) reported that the dry rhizome yield of the turmeric varieties from KAU *viz*., Kanthi, Sobha, Sona and Varna ranged from 4.02 to 8.27 t ha^{-1} with a driage ranging from 18.88 to 20.15 per cent.

2.2 BIOCHEMICAL VARIATION

Preliminary screening of 14 species of ginger family by Zakaria and Ibrahim (1987) showed the presence of alkaloids terpenoids, flavanoids and saponins in trace amounts in the rhizomes of turmeric and ginger.

Pathania *et al*. (1990) observed greatest variation for curcumin content (0.28 to 8.76 per cent) while examining 23 collections of C*urcuma longa* for agronomic and quality characters. Variation for volatile oil content was also determined to classify genotypes into four groups.

The essential oil from five *Curcuma* spp. including *C. domestica* and *C. aromatica* were analysed by Zwaving and Bos (1992). The results showed that *C. domestica* yielded 3.50 per cent and *C. aromatica* yielded 9.40 per cent of essential oil.

Shahi *et al*. (1994a) evaluated 40 genotypes of turmeric and data were collected on dry matter, oleoresin and curcumin content. Significant differences were observed due to genotypes and genotype x environment interactions. High yielding genotypes showed high dry matter content with wide adaptability and stability.

Jena and Das (1997) studied the influence of microbial inoculants on quality of turmeric. Study revealed that the microbial inoculation

resulted in a higher protein content (8.47 %) which was 7.9 per cent more than that of the lowest 7.85 per cent in uninoculated control.

Garg *et al*. (1999) studied the essential oil and curcumin contents of 27 accessions of *Curcuma longa* from the Taraibelt. The oil content of the rhizomes varied between 0.16 and 1.94 per cent on a fresh weight basis. The curcumin content was also found to vary from 0.61 to 1.45 per cent on dry weight basis.

Korla *et al*. (1999) evaluated twenty four ginger clones under rainfed and irrigated conditions. The analysis of variance indicated significant differences among the clones for ginger oil, oleoresin and crude fibre content. However growing conditions (rainfed and irrigated) exerted no significant effects except on crude fibre content.

Narayanpur *et al*. (2003) observed significant variation in curcumin content and suggested the variation attributed due to genetic character of the cultivars.

Menon *et al*. (2003) analysed four major cultivars of black pepper from Thevanmundi, Poonjaranmunda, Valiakaniakadan and Subhakara from Kerala for their oil composition. Fifty five compounds were identified in the oils by gas chromatography. The main components of the oil were sabinene (4.5 – 16 %), β-pinene (3.7 – 8.7 %), limonene (8.3 – 18.0 %) and β-caryophyllene (20.3 – 34.7%).

Niranjan *et al*. (2003) analysed the foliage of three species of *Curcuma* (*C. longa, C. zedoaria* and *C. amada*) cultivated on sodic soil and found out a total chlorophyll (0.346 to 1.207 mg g^{-1}), essential oil (3.7 to 5.3 per cent) and protein content (3.6 to 6.8 per cent).

Twenty four genotypes of ginger were evaluated for yield and quality attributes under rainfed and irrigated conditions at Solan, by Tiwari (2003). Significant differences among the genotypes were observed

for ginger oil, oleoresin, crude fibre and dry matter content, irrespective of the two growing conditions.

Kurian *et al*. (2004) reported that the unique feature of turmeric varieties of KAU *viz.,* Kanthi, Sobha, Sona and Varna is that they have more than 7.0 per cent curcumin in composite sample and 5.0 per cent in fingers. The volatile oil content ranged from 4.24 to 5.15 per cent.

Padmapriya and Chezhiyan (2004) analysed turmeric rhizomes and reported the chemical composition as moisture (13.1 %), protein (6.3 %, fat (5.1 %), minerals (3.5 %), fibre (2.6 %) and carbohydrates (69.4 %).

2.3 ANATOMICAL VARIABILITY

Adaptation to different environmental conditions usually result in extreme modifications of organs particularly those of the vegetative body. In order to get detailed information about the population, diagnostic characters other than gross morphology are needed. Root and leaf anatomical characters have been used extensively in taxonomic and phylogenetic studies because they are very informative and reliable.

Micro morphological characters of the leaf surface have been used widely in taxonomic and phylogenetic studies. Several leaf epidermal characters associated with systematic consideration includes number of stomata, type of stomata, morphology of trichomes, leaf thickness, cuticle etc.

Ghahreman *et al*. (1998) studied the characteristics of the foliar epidermis of twelve species of the genus *Hyoscyamus* occurring in Iran. The study revealed that the most useful anatomical characters for taxonomic purposes were stomatal occurrence, stomatal index, pattern of anticlinal walls and density and type of trichomes.

Sherlija *et al*. (1998) studied the anatomy of rhizomes of four commercially important species of *Curcuma viz., C. longa, C. aromatica, C. amada* and *C. zedoaria*. Though all the species basically had similar

anatomical characters, variations were noticed in the number and arrangement of primary and secondary vascular bundles, orientation of endodermoid layer, number and shape of starch grains and curcumin cells.

Stern and Judd (1999) compared the anatomy of 17 species of *Vanilla* and found enormous variation in the occurrence of uniseriate hypodermis, crystals in the foliar epidermis, raphide bundles in the mesophyll tissue and size of the stomatal apparatus.

According to Edeoga (2001) eight wild species of *Dioscorea* could be differentiated from each other based on the difference in foliar epidermal cells, trichomes, vascular bundle structure and stomatal size, shape and index.

Baruah and Nath (2002) studied micro morphological characters namely the epidermal and venation character of the two variants of *Cinnamomum parthenoxylon* and recorded no remarkable variation in quantitative data for the variants. In qualitative characters, the stomata in both the variants were hypostomatic, paracytic, randomly distributed and irregularly oriented. The epidermal cells were tetragonal to polygonal in nature.

The morphology of epidermal cell walls, nature and number of epidermal cells per unit area, stomatal frequency, trichome length, and stomatal index were identified as useful parameters for the differentiations of twelve species of Zingiberaceae (Gogoi *et al.*, 2002).

Nartunai *et al.* (2003) examined the leaf anatomy of *Cyphostemma setosum* and found that, the upper and lower epidermis are made up of polygonal, straight and thin walled cells with anomocytic stomata.

2.4 GENETIC VARIABILITY AND CORRELATION

Gopal *et al.* (1990) conducted correlation studies in three types of cardamom and reported that panicles per plant, capsule fresh weight per

plant, nodes per panicle and inter nodal length within the panicle were useful characters in selection of cardamom for yield improvement.

Twelve yield components in 10 genotypes of *Curcuma longa* were evaluated for genetic variance and yield correlations by Jalgaonkar *et al*. (1990). Cured yield of all the genotypes was found significantly and positively correlated with yield of secondary fingers.

In turmeric, phenotypic coefficient of variation was higher than the genotypic coefficient of variation in general. GCV was very high for fresh rhizome yield (63.36), indicating the high degree of genetic variability for this character (Indiresh *et al*., 1992).

Ali *et al*. (1994) studied genotypic coefficient of variation in ginger genotypes. The result showed genotypic coefficient of variation was high for length and weight of secondary and primary rhizomes and rhizome yield per plant.

Stability in rhizome yield and its determining characters in turmeric were evaluated by Shahi *et al*. (1994b). Study showed that stability in rhizome yield was associated with length and girth of rhizome, number of leaves and tillers per plant.

Fifteen ginger cultivars were studied for variability and association of characters among them by Prasad *et al*. (1998). High coefficient of variability was observed for number of tillers followed by number of leaves. Moderate to low variability was noticed for length and breadth of rhizomes, breadth of leaves, number of primary fingers, rhizome weight per plant, plant height and length of leaves.

Chandra *et al*. (1999) evaluated the performance of 25 genotypes of turmeric at Meghalaya for three consecutive years. Among the 19 characters studied, weight of primary finger rhizomes, number of primary and secondary finger rhizomes per clump. Plant height, length of leaf, diameter and weight of primary finger rhizome, internodal distance of

primary finger rhizome and rhizome yield per hectare were significantly and positively associated with fresh rhizome yield per clump. A negative correlation between dry rhizome recovery and fresh rhizome yield per clump was observed.

Korikanthimath *et al*. (1999) evaluated 12 elite clones of cardamom along with a local control for yield during 1994. All the five yield components studied were positively correlated with yield.

The coefficient of variation for 18 important horticultural traits was estimated in 26 accessions of ginger by Yadav (1999) during the period 1997. The study observed that the genotypic coefficient of variation was high for length and weight of secondary rhizome, weight of primary rhizome, number of secondary and primary rhizomes and rhizome yield per plant.

An investigation was carried out on 22 genotypes of turmeric (*Curcuma longa* L.) by Hazra *et al*. (2000) to elucidate the role of different growth characters components of rhizome yield. Genetic variability and correlations were studied to assess the direct and indirect relationships in respect of growth characters and yield. Of the growth characters only leaves per clump at 180 DAP exhibited significantly positive phenotypic correlation with rhizome yield.

Singh *et al*. (2000) reported significant association of essential oil content with rhizome yield per plant and per cent oleoresin content in ginger.

Correlation studies conducted in turmeric cultivars indicated that, weight of primary, secondary and mother rhizomes had direct effect on yield (Mohanty, 1979). The genotypic correlation coefficients were in general higher than the phenotypic correlation coefficients, thus revealing strong association at genotypic level between the characters (Reddy, 1987; Shanmugasundaram *et al.*, 2001).

In *Curcuma longa* number of leaves per clump, leaf area, leaf area index, number of primary and secondary fingers had positive strong association with rhizome yield at both genotypic and phenotypic levels (Jana *et al*., 2001).

Singh *et al*. (2003) studied the genetic variation for rhizome yield and yield components in 65 turmeric genotypes grown in Palampur, Himachal Pradesh during 1998. The greatest variation was recorded for yield, followed by weight of mother rhizome per plant, plant height, weight of primary rhizome per plant and number of leaves. The phenotypic coefficient of variation was generally higher than the genotypic coefficient of variation. The results suggested that superior genotypes may be obtained through selection based on the number and weight of mother, primary and secondary rhizomes.

Genotypic coefficient of variation were studied in ginger genotypes (Yadav, 1999; Singh and Mittal, 2003) The genotypic coefficient of variation was high for length and weight of secondary and primary rhizomes and rhizome yield per plant.

In *Curcuma longa* correlation studies showed that number of leaves per clump, leaf area, leaf area index, number of primary and secondary fingers had positive strong association with rhizome yield at both genotypic and phenotypic levels (Narayanpur and Hanashetti, 2003; Singh *et al*., 2003).

2.5 MOLECULAR MARKERS

Traditionally, majority of the plant taxonomic and variability studies have been conducted based on morphological, anatomical and physiological features. In view of the advancement of scientific techniques which are capable of critically analyzing the biological material at molecular level, the potential of molecular tools for taxonomic and variability refinement can be very well visualized. The macro molecules which can be studied for generating distinct molecular profiles of an individual include DNA, RNA,

proteins, lipids and carbohydrates. The techniques of RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism) etc., are being utilized practically in various plant species for such molecular marking. The important application of molecular markers include genome mapping and gene tagging, taxonomic studies, cultivar identification, genetic diversity analysis, sex differentiation, genetic fidelity analysis etc (Suresh, 2004).

2.5.1 Isozyme Markers

Pooler and Simon (1993) employed morphological and isozyme assay method for intra specific classification of 110 garlic cultivars. Though isozyme markers provide the basis for relatively simple genetic analysis, the small number of consistently resoluable loci limits their utility. Moreover, isozymes are influenced by stages of development and the tissue used for extraction.

Mabb and Klass (1995) studied the intra specific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers.

Phylogenetic relationship of *Allium* species and their intra generic, intra specific differentiation and grouping by chloroplast DNA sequence coding and restriction enzyme analysis was reported by Havey (1991); Linne *et al*. (1996); Klass (1998).

Seven early flowering and two unidentified *Curcuma* species was studied for isoenzyme polymorphism using polyacrylamide gel electrophoresis (PAGE). Based on the polymorphism phylogenetic relationships were compared (Apavatjrut *et al.*, 1999).

Fifteen accessions of *C. longa* collected from different geographic areas in India along with a few seedling progenies were studied for variation based on isozyme polymorphism (Babu *et al*., 2001). A high degree of variability (63.8 – 96 per cent similarity) was seen in the population studied.

Phenotypic analysis revealed several groups with distinctive features. Two seedling progenies which showed maximum similarity, stood distinctly from the clonally propagated material.

High diversity was observed from cultivated and wild populations of *Curcuma alismatifolia* in Thailand by alloenzyme polymorphism (Paisooksantivatana *et al*., 2002).

2.5.2 Protein Markers

The use of protein markers is based on protein polymorphism. These markers code for specific proteins and they can be visualized through gel electrophoresis.

Agarwal and Kaul (1993) studied seed protein analysis in eight varieties of dill (*Anethum graveolens)* by gel electrophoresis and found significant variation between varieties in seed protein content which ranged from 10.8 to 23.8. Proteins were also qualitatively different between varieties.

Bank *et al*. (1995) observed low levels of genetic variation within species and high degree of differentiation between species for four morphologically distinct populations of *Aloe ferox* and one population of *A. marlothii* through gel electrophoresis and the variation was assessed at 22 protein coding loci.

According to Sharma *et al*. (2000) protein markers could not delineate *Podophyllum hexandrum* populations into region specific groups.

Electrophoretic analysis of seed proteins from seven induced morphological mutants and their parental line of *Nigella sativa* revealed a wide range of variation among genotypes for band number and intensity. In a quantitative analysis, buffer soluble and acid soluble protein contents ranged from 0.0084 to 0.0189 (g/ dry seed), 0.0069 to 0.0111 respectively, most of the mutants having higher contents than the parental line (Datta *et al*., 2003).

2.5.3 DNA Markers

The term DNA fingerprinting was introduced by Jeffrey *et al.* (1985). Presently the term DNA fingerprinting/profiling is used to describe the combined use of several single locus detection systems and are being used as versatile tools for investigating various aspects of plant genomes. These included characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, plant breeding and diagnostics.

With the advent of molecular biology techniques, DNA based markers have replaced enzyme markers in germplasm identification and characterization as well as in gene mapping because of its plasticity, ubiquity and stability. DNA is the ideal molecule for such analysis (Caetano–Anolles *et al.*, 1991). Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified as hybridization based markers and polymerase chain reaction (PCR) based markers (Joshi *et al.*, 1999).

2.5.3.1 Restriction Fragment Length polymorphism (RFLP)

In this method, the genomic DNAs are cleaved using restriction endonucleases followed by fractionating the fragments electrophoretically and then detecting the fragments containing homologous sequences by hybridizing them to specific DNA probes. RFLP technique provides a potentially infinite number of variable markers for the development of genetic linkage maps.

Mess *et al*. (1998) included 29 species of *Allium* and seven species of related genera in a phylogenetic study using amplified chloroplast DNA, PCR – RFLP.

PCR –RFLP successfully differentiated medicinal species *Codonopsis pilosula, C. langshen, C. modesta and C. nervosa* var. *macrantha* from two

adulterants *Campaumoea javanica and Platycodon grandiflorus* (Zhao *et al*., 1999).

The RFLP probes are locus specific, resulting in an easy to screen co – dominant behaviour. However, it is relatively labour intensive, expensive and involves the use of radioactive chemicals, which are harmful to the users.

2.5.3.2 Amplified Fragment Length Polymorphism (AFLP)

This technique is also called "Selective Restriction Fragment Amplification". It is a combination of RFLP and PCR used for obtaining highly informative fingerprints. The technique involves restriction of the DNA and ligation of oligonucleotide adapters, selective amplification of sets of restriction fragments and gel analysis of the amplified fragments.

Morphological descriptors, quantitative, phytochemical analyses and DNA finger printing using AFLP were utilized to define the extent of variation existing between Hawaiian cultivars of *Piper methysticum* and extremely narrow genetic base was observed among the accessions (Lebot *et al*., 1999).

AFLP finger printing was employed to differentiate or to identity *Actaea racemosa* from its closely related species *A. pachypoda*, *A. cordifolia* and *A. podocarpa* (Zerega *et al*., 2002).

RAPD and AFLP profiling of *Aloe* germplasm (*Aloe vera, A. perryii, A. orborescens, A. saponaria)* were carried out for estimating the extent of diversity and establishing phylogenetic relationship (Darokar *et al*., 2003). The pattern of phylogeny remained parallel to RAPD pattern, and the degree of diversity revealed by AFLP showed zooming effect over RAPD analysis.

Genetic diversity of 62 *Mentha* accessions from different geographic origins representing five species and three hybrids was assessed through AFLP method and the analysis supports taxonomic classification established among *Mentha* species by conventional methods (Gobert *et al*., 2003).

Lal *et al*. (2003) conducted genetic diversity analysis using AFLP technique in 14 accessions of *Vetiveria zizanioides* and obtained 65 per cent divergence due to polymorphism with nine primer combinations.

2.5.3.3 Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA method was first developed by Welsh and McClelland (1990) and Williams *et al*. (1990). This method utilizes single short oligo nucleotide primers of arbitrary sequence for the amplification of DNA segments distributed randomly throughout the genome, using polymerase chain reaction (PCR). In this reaction the primers of arbitrary sequence bind to perfect or imperfect sites in the genomic DNA, so that a subset of them will lie in inverted orientation to each other. As a result a number of bands are amplified through the action of DNA polymerase. The reaction products are conveniently analysed on agarose gels. Since this technique is being adopted in the present study and the literature on kasthuri turmeric is very limited, available literature from medicinal and aromatic crops is reviewed.

2.5.3.3.1 Genetic Diversity Analysis

Mabb and Klass (1995) studied the intraspecific differentiation of garlic *Allium sativum* by isozyme and RAPD markers, whereas, Bradley *et al*. (1996) studied the usefulness of RAPD markers for determining the distribution of genetic variability within and among 20 taxonomic units of Australian garlic cultivars.

The relationship of common onion to different types of shallot was investigated by RAPD markers and morphological traits (Dennequin *et al.*, 1997).

RAPD markers were used by Rout *et al*. (1998) to evaluate the genetic stability of micropropagated plants of *Zingiber officianale*. All RAPD profiles from micropropagated plants were monomorphic and similar to those

of field grown control plants. The study revealed that the use of RAPD markers in determining the genetic stability of micropropagated plants.

Padmesh *et al*. (1999) observed high genetic variability among 35 accessions of *Andrograrphis paniculata* collected from different phyto geographical regions.

Cheng *et al*. (2000) carried out the RAPD analysis of *Lycium barbarum*, a medicinal plant popular in Taiwan. The results showed two types of random amplified polymorphic DNA (RAPD) bands among the 20 *Lycium* samples with 15 samples belonging to one type and five samples to the other. A low genetic diversity among the samples was revealed by the RAPD analysis.

RAPD profiles for band similarity indices clearly differentiated five of the *Mentha arvensis* accessions analysed, from the rest of the accessions and found them belonging to another species of the genus *Mentha* (Khanuja *et al*., 2000).

Diversity among three female varieties of *Piper longum* was noticed through RAPD analysis (Philip *et al*., 2000).

Darokar *et al*. (2001) examined narrow genetic base among various geographically distinct accessions of *Bacopa monnieri* by RAPD analysis. Low level of polymorphism was observed and the range of similarity was 0.8 to 1.0.

RAPD profiles distinguished eight peppermint accessions of different geographical origin. However, only limited polymorphism was observed among the most widely grown pepper mint and scotch spearmint cultivars (Fenwick and Ward, 2001).

Tochika and Asaka (2001) used RAPD for characterising a high quality *Panax ginseng* in which they obtained a 725 base pair band for a selected elite strain Aizu K-111, while the other strains did not necessarily show this band.

RAPD analysis was conducted in two morphologically distinct varieties of *Digitalis* namely *D. minor* var. *minor* (pubescent) and *D. minor* var. Palavi (glabrous) and found restricted population variability (Sales *et al*., 2001).

Zhang *et al*. (2001) applied RAPD technique to distinguish *Lycium barbarum* from other closely related species of the same genus and obtained distinctive DNA finger prints corresponding to different *Lycium* species and higher similarity values between intra species than inter species.

Bazina *et al*. (2002) studied the genetic pattern and volatile oil composition of *Salvia officinalis* clones of various geographical origin by using random amplified polymorphic DNA (RAPD) analysis. The banding pattern obtained by RAPD analysis confirmed the distinct genetic variability among various clones of *S. officinalis*.

Klocke *et al*. (2002) conducted DNA finger printing studies through RAPD analysis in three progenies of marjoram (*Origanum majorana* L.) pollinator lines and found heterogeneity among them.

RAPD profiles of six species of *Curcuma* and four species of *Zingiber* were developed using five primers (IISR, 2002) and relatively good polymorphism was evident at species level.

Shasany *et al*. (2002) assessed the genetic diversity of *Mentha spicata* germplasm through RAPD analysis and observed high level of polymorphism.

Comparative studies of four elite genotypes of *Vetiveria zizanioides* through RAPD analysis showed a wide range of variability among them (Shasany *et al*., 1998). According to Lal *et al*. (2003) 40 per cent diversity was observed among 51 accessions of vetiver through RAPD profiling.

Elite and popular cultivars of *Cymbopogon martinii* were examined for genomic variability through RAPD, enzyme and SDS-PAGE protein

polymorphisms (Sangwan, *et al*., 2003). They observed wide range of molecular diversity among the cultivars in all type of analysis.

RAPD analysis was used to access genetic diversity in basil (Vieira *et al.,* 2003). Means of genetic similarities with in *Ocimum* spp. indicated that, the domesticated species showed the lowest similarity.

RAPD analysis provided inter and intra specific variations in *Aloe* species (Darokar *et al*., 2003).

Shiod *et al*. (2003) characterized *Aloe* species using random amplified polymorphic DNA (RAPD) analysis. The study revealed that by the comparison of the characteristic bands of PCR (Polymerase chain reaction) products on agarose gel, it was possible to distinguish the four *Aloe* species.

Besse *et al*. (2003) conducted a genetic diversity study of endangered *Psiadia* species endemic from Mauritius Island using PCR markers. The RAPD analysis revealed a relatively high intra-specific variability and the existence of four major phenetic groups in the accessions studied which were in consistence with the chemical composition of the essential oils of the as well as with their floral characteristics.

2.5.3.3.2 RAPD for Genome Mapping and Gene Tagging

Plant improvement, either by natural selection or through the efforts of breeders, has always relied upon creating, evaluating and selecting the right combination of alleles. With the use of molecular markers it is now a routine to trace valuable alleles in a segregating population and mapping them. Once mapped, these markers are efficiently employed in tagging several individual traits that are extremely important for a breeding programme like yield, disease resistance, stress tolerance, seed quality, secondary metabolite production etc.

Grothe *et al*. (2001) characterized salutaridinol 7-0 acetyl transferase which involved in morphine biosynthesis in opium poppy (*Papaver somniferum*).

Peng *et al.* (2002) conducted studies to analyse the sequences of the nuclear ribosomal RNA small subunit gene (18 Sr RNA) and the chloroplast mat K gene of crude drug patchouli to provide molecular evidence for the identification of patchouli drug.

Straka and Nothnagel (2002) conducted linkage analysis and genetic map construction in *Papaver somniferum* based on molecular and morphological markers. RAPD and AFLP technique were employed and 125 molecular markers were detected, 77 as AFLP and 48 as RAPD markers.

2.5.3.3.3 Cultivar Identification

Khandka *et al*. (1996) studied the polymorphism exhibited between *Asparagus* cultivars by random amplified polymorphic DNA.

Nine accessions of three species of the medicinal plant genus *Scutellaria* were analysed by RAPD in an effort to distinguish between members of these three species. RAPD markers produced species specific bands and this could be useful for the future identification of members of the three species (Hosokawa, 2000).

Two accessions of *Mentha spicata viz.,* CIMAP C 33 and CIMAP C 32 were distinguished clearly from each other by RAPD analysis (Khanuja *et al*., 2000).

Pradeepkumar *et al*. (2001) analysed 24 accessions including 13 land races and nine advanced cultivars of pepper through RAPD technique and found cultivar specific bands and this could be successfully utilized for cultivar identification in pepper.

2.5.3.3.4 Taxonomic Studies

Molecular taxonomy is the fast emerging branch of biosystematics today for precise and finer analysis to establish phylogenetic relationships

among plant species. The rate of evolution of specific markers certainly discloses the type of classification that the ecotypes or forms or subspecies need. Further more these studies hold a great promise for revealing more about the pattern of genetic variation within species or forms.

According to Khanuja *et al.* (1998) *Mentha gracilis* var. *cardiaca* showed a high similarity to *M. spicata* and *M. arvensis,* which themselves showed a greater distance in RAPD analysis indicating that *M. gracilis* var. *cardiaca* might have evolved as a natural hybrid between *M. arvensis* and *M. spicata.*

RAPD markers were used to study inter specific variation among six species of the genus *Digitalis*. The species relationships revealed were fully consistent with those previously obtained using morphological affinities. The hybrid *D. exelsior* seems to have stronger affinity to the section *Digitalis* than to *Grandiflora* (Nebauer *et al*., 2000).

Calculation of genetic distance, based on the number of shared bands through RAPD analysis indicated that *M. spicata* is more closely related to *M. gracilis* than to *M. piperita* (Fenwick and Ward, 2001).

While analyzing 15 accessions of *Asparagus* spp. through RAPD technique, Shasany *et al.* (2003) found that two of the accessions from *A. racemosus* were associated with morphologically unrelated species.

Materials and Methods

3. MATERIALS AND METHODS

The study “Characterization of kasthuri turmeric” was carried out at the Departments of Plantation Crops and Spices and Plant Biotechnology, College of Agriculture, Vellayani during 2003-‘04. The materials and methods followed for the study of morphological, physiological, anatomical, biochemical and molecular characters are described in this chapter.

Field experiments were conducted at the Instructional Farm, College of Agriculture, Vellayani situated at 8°5' North latitude and 77°1' East longitude at an altitude of 29 m above the mean sea level. Predominant soil type of the experimental site was red loam belonging to Vellayani series, texturally classified as sandy clay loam.

The study consisted of the following experiments.

3.1 Morphological characterization

3.2 Physiological characterization

3.3 Anatomical characterization

3.4 Biochemical characterization

3.5 Molecular characterization

3.1 MORPHOLOGICAL CHARACTERIZATION

3.1.1 Materials

The materials for the study included 11 accessions of kasthuri turmeric collected from Western Ghats and other parts of Kerala, two local accessions of ordinary turmeric and two accessions of zedoary. The details of the 15 accessions with their names indicating their sources are presented in Table 1 and Plate 1.

Table 1. List of accessions of *Curcuma* spp. used for the study

Sl. No.	Accession number	Accession Name
1	T_1	Vellanikkara accession
2	T_2	Vellayani accession
3	T_3	IISR accession
4	T_4	Nedumangad accession
5	T_5	Vellanikkara local
6	T_6	Kumily local
7	T_7	Kottoor wild
8	T_8	Pala wild
9	T_9	Thodupuzha accession
10	T_{10}	Pala White (Wild)
11	T_{11}	Kozhikode accession
12	T_{12}	Turmeric – Alleppey
13	T_{13}	Turmeric – Vellayani local
14	T_{14}	Zedoary – yellow
15	T_{15}	Zedoary – black

Plate 1. General view of the experimental field

3.1.2 Methods

3.1.2.1 Design and Layout

Healthy, disease and pest free rhizome bits weighing 15-20 g were planted in randomized block design with three replications. In each replication ten plants were maintained. The crop was raised organically adopting cultural practices commonly followed for raising rhizomatous crops.

3.1.2.2 Biometric Observations

For recording different biometric observations at bimonthly intervals, five plants were selected at random from each replication as observational plants. Pre-harvest observations started at 2 MAP and continued upto 8 MAP.

3.1.2.2.1 Growth characters

1. Plant Height

The height of the plants were measured at bimonthly intervals from 2 MAP from the base of the main pseudostem to the tip of the topmost leaf and was expressed in cm.

2. Number of Tillers

The number of aerial shoots arising around a single plant were recorded at bimonthly intervals from 2 MAP.

3. Number of Leaves

Number of leaves were recorded by counting the number of leaves of all the tillers at bimonthly intervals from 2 MAP.

4. Leaf Area

The length and width of leaves were measured at bimonthly intervals from 2 MAP and the leaf area in cm^2 was calculated based on the length and breadth method.

The following relationship was used for computing the leaf area (Randhawa *et al*., 1985).

Y = 4.09 + 0.564 (Length x Breadth)

where, Y = Leaf area

Length = Length of the leaf in cm

Breadth = Breadth of the leaf in cm

5. Rhizome Spread

The horizontal spread of rhizomes were measured at 8 MAP and expressed in cm.

6. Rhizome Thickness

Rhizome thickness was measured at 8 MAP using screwguage and expressed in cm.

7. Root Length

The plants were uprooted at 8 MAP and maximum length of roots was measured and mean length expressed in cm.

8. Root Spread

Root spread was measured at 8 MAP by spreading the root system on a marked paper and measuring the spread of the root system at its broadest part and expressed in cm.

9. Root Weight Plant^{-1}

Roots separated from individual plants at 8 MAP were taken and dried in hot air oven at 70°C and its weight was taken and expressed in g plant^{-1}.

3.1.2.2.2 Yield and Yield Components

1. Rhizome Yield

The yield of fresh rhizome from each treatment was recorded at 8 MAP and expressed as g plant^{-1}.

2. Dry Rhizome Yield

Immediately after the harvest at 8 MAP, the rhizomes were washed, weighed and kept to dry under sun for one week. After this, it was kept in hot air oven at 70°C. The dry weight of the rhizome was expressed in g plant^{-1}.

3. Top Yield

The yield of above ground portion in individual treatment was recorded at 8 MAP and expressed in g plant^{-1} on dry weight basis.

4. Crop duration

The number of days from planting to harvest in case of each treatment was recorded as the maturity period.

3.1.2.2.3 Scoring for pests and diseases

The incidence of various pests and diseases were recorded under field conditions and timely control measures were adopted. Though the incidence of fruit borer was noticed at early growth stages of the crop, scoring was not required due to effective control measures.

3.1.2.2.4 Morphological Cataloguing

A detailed description of various accessions based on the morphological characters was prepared and used for cataloguing (Appendix I).

3.2 PHYSIOLOGICAL CHARACTERIZATION

3.2.1 Dry Matter Production (DMP)

Leaves, petioles, pseudostem, rhizomes and roots of the uprooted plants were separated and dried to a constant weight at 105°C in a hot air oven at 8 MAP. The sum of these individual components gave the total dry matter yield of the plant and expressed as g plant^{-1}.

3.2.2 Drying percentage of Rhizome

To workout the drying percentage, a sample of one kg fresh rhizomes from each treatment was boiled. After boiling rhizomes were dried under sun for 10days. The dry weight was recorded after sun drying and drying percentage was worked out as below:

$$\text{Drying percentage} = \frac{\text{Dry weight of rhizomes after drying (kg)}}{\text{Fresh weight of rhizomes (kg)}} \times 100$$

3.2.3 Leaf Area Index (LAI)

Leaf area index was calculated bimonthly intervals from 2 MAP. Five sample plants were randomly selected for each treatment and the number of leaves on each plant was counted. Maximum length and width of leaves from all the sample plants were recorded separately and leaf area was calculated based on length and breadth method.

$$\text{LAI} = \frac{\text{Sum of leaf area of N sample plants (cm}^2\text{)}}{\text{Area of land covered by N plants (cm}^2\text{)}}$$

3.2.4 Leaf Area Duration (LAD)

LAD was calculated using the formula given by Power *et al*. (1967) at bimonthly intervals from 60 DAP.

$$\text{LAD} = \frac{\text{Li} + (\text{Li} + 1) \text{ x } (t_2 - t_1)}{2}$$

where, Li = LAI at first stage

Li + 1 = LAI at second stage

$t_2 - t_1$ = Time interval between these stages

3.2.5 Harvest Index (HI)

HI was calculated at final harvest as

$$HI = \frac{Y\ econ}{Y\ biol}$$

where, Y econ = total dry weight of rhizome

Y biol = total dry weight of the plant

3.3 ANATOMICAL CHARACTERIZATION

3.3.1 Leaf Cuticle Thickness

Third leaf from the top at 4 MAP were collected for anatomical studies. Very thin free hand cross sections of three randomly selected leaves from each replication were taken at 4 MAP and the cuticle thickness was observed using 40 x objective and 10 x eyepiece and measured using micrometer and the values are expressed in micrometers.

3.3.2 Number of Vascular Bundles in Rhizomes and Roots

Free hand thin cross sections of medium sized finger rhizomes and roots were taken and observed under 10 x objective and the number of vascular bundles were recorded.

3.3.3 Stomatal Frequency

Stomatal frequency refers to the number of stomata per unit area of leaf. Stomatal frequency was recorded for both upper and lower surface of three randomly selected leaves from each replication. Leaf imprints were prepared for the purpose using the adhesive, ‘Quick fix’. It was uniformly applied on the surface of the leaf and after five minutes, the dried membrane was carefully peeled off and mounted on a microscope slide with a drop of water. The stomata were observed and counted using 40 x objective and 10 x eyepiece. The field of the microscope was

measured using a stage micrometer and the number of stomata per unit area was calculated (Taylor *et al*., 1997).

3.4 BIOCHEMICAL CHARACTERIZATION

3.4.1 Curcumin Content

Curcumin is quantitatively extracted by refluxing the material in alcohol and is estimated spectrometrically at 425 nm. For this, dissolved 0.2 – 0.5g of moisture-free rhizome powder in 250 ml absolute ethanol. Refluxed the contents in the flask fitted with an air condenser over a heating mantle for 3-5 hours. Cooled and decanted the extract into a volumetric flask and made up the volume. Then diluted a suitable aliquot of 1-2 ml to 10 ml with absolute alcohol. Measured the intensity of yellow colour at 425 nm in a spectrometer (Sadasivam and Manikam, 1991).

$$\text{Percentage of cucurmin} = \frac{0.0025 \times A_{425} \times \text{Volume made up} \times \text{dilution factor} \times 100}{0.42 \times \text{weight of the sample (g)} \times 1000}$$

Since 0.42 absorbance at 425 nm = 0.0025 g curcumin.

3.4.2 Volatile Oil

Coarsely ground powder of dried rhizomes was used for estimates of volatile oil. The method adopted was hydro-distillation using Clevenger distillation apparatus for four hours. The oil content was expressed in percentage (v/w) on dry weight basis.

3.4.3 Crude Fibre

The fibre content of the rhizomes was estimated by acid and alkali digestion method (Sadasivam and Manikam, 1991).

3.4.4 Ash Content

To estimate the ash content, a known weight of the rhizome samples were ignited to destroy the organic fractions and the inorganic

residue left out is weighed as ash, as per the procedure given by Muthuvel and Udayasooriyan (1998).

Percentage of ash in the sample on moisture free basis

$$= \frac{C - A}{B - A} \times 100 \times \frac{100}{(100 - M)}$$

where,

A = weight of the empty silica crucible in grams

B = Weight of crucible + sample

C = Weight of crucible + ash

M = Moisture percentage of the sample

3.4.5 Starch

Starch content in dried rhizomes was estimated by the method suggested by Sadasivam and Manikam (1991).

3.4.6 Total Carbohydrate

Total carbohydrate was estimated by Anthrone method suggested by Sadasivam and Manikam (1991).

3.4.7 Estimation of Protein

Protein content of fresh rhizomes was estimated by using the method developed by Lowry *et al*. (1951).

3.4.8 Chlorophyll Content (chlorophyll a, chlorophyll b and total chlorophyll)

Photosynthetic pigments namely chlorophyll a, chlorophyll b and total chlorophyll were estimated by following the method described by Starnes and Hadley (1965).

3.4.9 Crude Alkaloid Content

The crude alkaloid content of the rhizomes was estimated gravimetrically by solvent extraction of the powdered material (Sunitha, 1996).

3.4.10 Moisture Content

Moisture content was determined by heating a known weight of the dried rhizome sample to a constant weight in an electric oven at 70°C and finding the loss in weight (Muthuvel and Udayasooriyan, 1998).

$$\text{Percentage of moisture in the sample} = \frac{B - C}{B - A} \times 100$$

where,

Weight of moisture bottle alone = A_g

Weight of moisture bottle + sample = B_g

Weight of moisture bottle + sample after drying in the oven C_g.

3.4.11 Statistical Analysis

1. Analysis of variance (ANOVA) and covariance (ANCOVA) for Randomized Block Design (RBD) in respect of the various characters was done as per Panse and Sukhatme (1967).

2. Mean : The mean of the i^{th} character $\overline{Xi}$ (xi) was worked out.

3. Variability components at phenotypic and genotypic levels for different characters were estimated as suggested by Kempthorne (1977).

(a) The variance and covariance components were calculated as per the following formulae :

For the character X_i,

$$\text{Environmental variance, } \sigma_{ei}^2 = MSE$$

$$\text{Genotypic variance, } \sigma_{gi}^2 = \frac{MST - MSE}{r}$$

Phenotypic variance, $\sigma_{pi}^2 = \sigma_{gi}^2 + \sigma_{ei}^2$

where, MST and MSE are respectively, the mean sum of squares for treatment and error respectively from ANOVA and r, the number of replications.

For two characters X_i and X_j,

Environmental covariance, $\sigma_{eij} = MSPE$

Genotypic covariance, $\sigma_{gij} = \frac{MSPT - MSPE}{r}$

Phenotypic covariance, $\sigma_{pij} = \sigma_{gij} + \sigma_{eij}$

where, MSPT and MSPE are respectively, the mean sum of products between the i^{th} and j^{th} characters for genotype and environment respectively from Analysis of Covariance (ANCOVA).

(b) Coefficient of variation

Variability that existed in the population for various characters were apportioned using the estimates of coefficient of variation (Singh and Chaudhary, 1979).

For the character X_i,

Phenotypic coefficient of variation, $PCV = \frac{\sigma_{pi}}{\overline{X}_i} \times 100$

Genotypic coefficient of variation, $GCV = \frac{\sigma_{gi}}{\overline{X}_i} \times 100$

Environmental coefficient of variation, $ECV = \frac{\sigma_{ei}}{\overline{X}_i} \times 100$

where, σ_{pi}, σ_{gi} and σ_{ei} are respectively the phenotypic, genotypic and environmental standard deviations with respect to each character.

6. Correlation analysis

Phenotypic, genotypic and environmental correlation coefficients were worked out according to the procedure suggested by Singh and Chaudhary (1979).

$$\text{Genotypic correlation coefficient } r_g(ij) = \frac{\sigma_g ij}{\sigma_g i \times \sigma_g j}$$

$$\text{Phenotypic correlation coefficient } r_p(ij) = \frac{\sigma_p ij}{\sigma_p i \times \sigma_p j}$$

$$\text{Environmental correlation coefficient } r_e(ij) = \frac{\sigma_e ij}{\sigma_e i \times \sigma_e j}$$

3.5 MOLECULAR CHARACTERIZATION

Fifteen accessions of various *Curcuma* spp. were used for the isolation of genomic DNA and molecular characterization.

3.5.1 Isolation of Genomic DNA

The genomic DNA was isolated using the procedure suggested by Murray and Thompson (1980). The following steps were involved.

1. Emerging young leaves of grown *Curcuma* spp. accessions were collected in the morning hours
2. They were first washed in running tap water followed by distilled water, two to three times after chopping the leaves coarsely. Then the leaves were wiped using tissue paper to remove the water on the surface.

3. One gram of chopped leaf sample was weighed out and placed in a cool dry porcelain mortar and were ground well to a fine powder in liquid nitrogen.
4. The powdered tissue was then transferred to a 15 ml polypropylene centrifuge tube containing 5 ml of pre-warmed CTAB extraction buffer with the help of a sterile spatula (extraction buffer:CTAB, 100 mM Tris. HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.2 per cent mercapto ethanol). One ml of 1 per cent PVP solution was also added along with the sample.
5. The samples were incubated at 60°C for 45 minutes in water bath with occasional mixing by gentle swirling.
6. After 45 minutes, the samples were taken and kept at room temperature for 10 minutes.
7. An equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) solution was added and mixed by inversion and centrifuged at 10,000 rpm for 10 minutes at 4.0°C.
8. After collecting the upper phase, the phenol : chloroform : isoamyl alcohol extraction was repeated until the interphase disappeared.
9. After that, the aqueous phase was collected and an equal volume of chloroform : isoamyl alcohol (24:1) solution was added and the two phases were mixed gently.
10. Centrifugation was done at 10,000 rpm for 10 minutes at 4.0°C.
11. To the upper phase collected, $1/10^{th}$ volume of 3.0 M sodium acetate and double the volume of cold absolute ethanol were added.
12. It was then mixed by quick gentle inversion to precipitate the DNA. Then the precipitated DNA was pelleted by centrifugation at 10,000 rpm for 5 minutes at a temperature of 4°C and the supernatant was decanted carefully.

13. The DNA pellet was washed with 70 per cent cold ethanol and air dried.
14. The dried DNA pellet was dissolved in 50-100 µl. TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) and stored at 4.0°C.

All the materials used in the preparation and storage of reagents including reagent bottles, conical flasks, centrifuge tubes, spatula, glass rods, funnels and tips of micro pipettes were washed with Labolin solution and rinsed with distilled water and autoclaved for 45 minutes before use. Phenol used was saturated and equilibrated using Tris buffer and pH adjusted to 8.0.

3.5.2 Quantification of DNA

Reliable quantification of DNA concentration is important for many applications in molecular biology including amplification of target DNA by polymerase chain reaction. DNA quantification was carried out with the help of a spectrophotometer (Spectronic Genesis 5).

The spectrophotometer was calibrated at 260 and 280 nm wave lengths using TE buffer, in which the isolated DNA was dissolved. The optical density (O.D.) of the DNA sample dissolved in the buffer was recorded at both 260 and 280 nm.

Since an OD of 1.0 at 260 nm represent 50 µg ml^{-1} of DNA, the quantity of DNA in the sample was estimated by employing the following formula.

Amount of DNA (µg ml^{-1}) = A_{260} x 50 x dilution factor

where, A_{260} = absorbance at 260 nm

The quality of DNA could be judged from the ratio of the OD values recorded at 260 and 280 nm. A ratio between 1.8 and 2.0 indicates good quality of DNA.

3.5.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit supplied by the Bangalore Genei. The required amount of agarose was weighed out (0.9 per cent for visualizing the genomic DNA and 1.4 per cent for visualizing the PCR products) and added to 1 x TAE buffer. Agarose was dissolved by boiling. After cooling to about 50°C, ethidium bromide was added to a final concentration of 0.5 μg ml^{-1}. The mixture was poured immediately to preset template with appropriate combination. After solidification, the combination and the sealing tapes were removed and the gel was mounted in electrophoresis tank filled with 1 x TAE running buffer, so that it just covered the entire gel. The DNA sample was mixed with required volume of gel loading dye (0.25 per cent bromophenol blue). Each well was loaded with 12 μl (10 μl sample DNA + 2 μl loading dye) of sample DNA mixture.

For PCR products, each well was loaded with 25 μl (20 μl PCR sample + 5 μl loading dye) of amplified product mixture. One of the wells was loaded with 5 μl of molecular weight marker (2 μl of ladder + 1 μl of loading dye + 3 μl of double distilled water). Electrophoresis was performed at 75 volts until the loading dye reached $^{3}/_{4}^{th}$ of the length of the gel. The gel was visualized using an ultraviolet visible (UV-Vis) transilluminator. The amplified products were documented using Alpha Imager 1200 (Alpha Innotech Inc., USA).

3.5.4 Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA analysis was performed using forty arbitrarily designed decamer primers (Operon Inc., CA, USA) adopting the procedure of Williams *et al*. (1990) with required modifications.

Polymerase chain reactions were carried out in volume of 25 μl reaction mixture containing 2.5 μl 10x PCR buffer [10 mM Tris HCl,

(pH 9.0), 1.50 mM $MgCl_2$, 50mM KCl and 0.01 per cent gelatin], 10 pM of primer, 200 μM each of deoxynucleotides (dNTPs), 0.8 unit of Taq DNA polymerase and 20 ng of genomic DNA. Amplifications was performed in a Programmable Thermocycler (MJ Research Inc., USA) set for the following programme: An initial denaturation at 93°C for 3.0 minutes, followed by 43 cycles of denaturation at 95°C for 1.0 minute, annealing at 35°C for 1.0 minute and extension at 72°C for 1.5 minutes. The synthesis step for extension at 72°C for by 7.0 minutes was included after the last cycle. Finally the products of amplification were cooled to 4.0°C. A negative control containing sterile water, instead of template DNA was included in each reaction set.

After completion of the PCR, amplified products along with DNA molecular weight marker supplied by US Biochemicals were electrophoresed in 1.2 per cent agrose gel, stained with ethidium bromide for 2.5 hours at 75 volts. After completion, the gel was immediately visualized on a UV trans illuminator and documented using Alpha Imager.

The number of monomorphic bands, number of polymorphic bands and intensity of bands were recorded. Those primers which when used for amplification produced the maximum number of bands were used to amplify the DNA isolated from all the *Curcuma* accessions. The RAPD bands were represented as ‘1’ for presence and ‘0’ for absence and recorded. The PCR was repeated twice in order to confirm the reproducibility. The primers which could produce amplification for most of the accessions alone were used for further analysis.

3.5.5 Data Analysis

The reproducible bands were scored for their presence (+) or absence (-) for all the *Curcuma* spp. accessions. A genetic similarity matrix was constructed using the Jaccard’s Co-efficient Method (Jaccard, 1908).

$S=j = a / (a+b+c)$

where, S_j – Similarity coefficient

a – Number of bands present in both the accessions in a pair

b – Number of bands present in the first one, but not in the second one

c – Number of bands in the second one, but not in the first.

Based on the similarity coefficient, the distance between the accession with the help of the software package NTSYS (Version 2.02). Using these values of distances between genotypes, a dendrogram was constructed by following the UPGMA (Un weighted pair group method for arithmetic average) method. Association between the various accessions was found out from the dendrogram.

Results

4. RESULTS

Eleven accessions of kasthuri turmeric (*Curcuma aromatica* Salisb.) and two accessions each of ordinary turmeric (*Curcuma longa* L.) and zedoary (*Curcuma zedoaria* Rosc.) were evaluated during 2003-'04 in the Departments of Plantation Crops and Spices and Plant Biotechnology. Morphological, physiological, anatomical, biochemical and molecular characterization of kasthuri turmeric accessions were carried out. Comparison of these accessions with turmeric and zedoary is also attempted.

4.1 MEAN PERFORMANCE OF THE ACCESSIONS

Mean performance of the accessions in terms of growth characters, quality characters, anatomical characters, physiological characters and yield characters are presented in Tables 2, 3, 4, 5 and 6 respectively.

4.1.1 Growth Characters

Variability was observed among the accessions for the growth characters like plant height, number of tillers, number of leaves, leaf area, rhizome spread, rhizome thickness, root length, root spread and root weight per plant (Plates 2 to 16).

Plant height, number of tillers and leaves were recorded at bimonthly intervals from 2 MAP to 8 MAP

4.1.1.1 Plant Height

At all the four growth stages studied, plant height was maximum in T_{14} (59.71, 101.64, 112.64 and 112.64 cm respectively), followed by T_{15} (53.23, 100.69, 105.42 and 105.42 respectively). Plant height was observed minimum in T_{13} (20.67 cm) at 2 MAP, T_7 (59.70 cm) at 4 MAP, T_{13} (68.33) at 6 MAP and T_1 (79.73 cm) at 8 MAP respectively. The accessions T_5, T_6, T_7 and T_9 exceeded the general mean (32.23 cm) for this trait at 2 MAP.

Table 2. Mean values for various growth characters of the *Curcuma* spp. accessions

Accession No.	Plant height (cm)				Number of tillers				Number of leaves			
	2 MAP	4 MAP	6 MAP	8 MAP	2 MAP	4 MAP	6 MAP	8 MAP	2 MAP	4 MAP	6 MAP	8 MAP
T_1	22.63	70.45	79.73	79.73	1.06	1.28	2.13	2.13	4.532	9.675	10.693	10.69
T_2	21.36	76.30	82.47	82.47	1.20	1.26	2.07	2.07	5.013	10.135	11.317	11.32
T_3	24.95	70.43	81.11	88.66	1.40	1.53	2.81	3.00	5.343	11.236	13.86	14.32
T_4	22.57	78.28	79.33	90.48	1.46	1.59	3.00	3.24	4.236	11.565	14.32	15.45
T_5	41.50	90.63	93.71	97.33	1.53	2.97	5.16	5.71	6.023	15.68	19.65	21.86
T_6	33.82	91.00	92.90	96.35	1.53	2.13	2.65	3.32	5.236	12.38	15.532	16.66
T_7	35.78	59.70	80.88	89.80	2.10	4.11	6.22	6.22	7.235	15.25	18.67	21.44
T_8	31.62	76.40	80.48	87.85	1.60	2.26	2.87	3.41	5.672	12.34	16.53	17.49
T_9	36.07	73.79	82.23	92.43	1.73	2.83	3.25	3.52	4.534	13.23	17.25	20.62
T_{10}	30.52	77.33	80.53	90.24	1.85	3.20	5.42	6.20	7.565	14.67	21.23	23.14
T_{11}	27.36	68.31	74.37	86.71	1.20	2.02	3.43	3.51	6.023	12.32	15.96	17.45
T_{12}	21.67	74.33	78.47	85.71	1.11	2.21	5.51	5.94	4.343	13.68	18.68	20.57
T_{13}	20.67	72.53	68.83	84.64	1.06	1.84	4.60	5.55	4.534	11.38	17.42	18.91
T_{14}	59.71	101.64	112.64	112.64	1.21	1.45	3.21	3.87	3.836	10.57	15.23	17.37
T_{15}	53.24	100.69	105.42	105.42	1.26	2.86	4.71	4.67	4.615	13.62	17.67	19.96
Mean	32.23	78.79	84.87	91.36	1.42	2.24	3.80	4.15	5.249	12.515	16.267	17.82
CD (0.05)	2.893	3.242	4.666	4.145	0.143	0.564	0.436	0.672	0.002	0.012	0.012	3.55

Table 2. Continued

Accession No.	Leaf area (cm^2)	Rhizome spread (cm)	Root spread (cm)	Rhizome thickness (cm)	Dry root weight (g)	Root length (cm)
T_1	2656.00	20.50	14.04	1.27	1.46	17.65
T_2	2812.33	20.02	14.24	1.30	1.73	18.13
T_3	5879.67	26.25	18.36	1.54	2.36	20.43
T_4	5004.67	21.74	11.83	1.30	0.92	15.97
T_5	6825.00	26.68	18.75	1.36	2.84	22.48
T_6	5200.67	18.74	13.26	1.42	1.23	17.50
T_7	4966.00	27.37	16.13	1.41	2.19	18.48
T_8	6020.00	26.51	17.27	1.51	2.24	19.86
T_9	5856.00	18.52	12.96	1.46	1.42	16.73
T_{10}	4889.33	26.79	16.57	1.45	2.63	19.02
T_{11}	6198.00	26.24	17.74	1.53	2.52	20.63
T_{12}	6020.00	17.96	12.76	1.52	0.97	16.29
T_{13}	6111.67	18.24	11.81	1.52	1.22	15.33
T_{14}	7680.00	26.46	17.62	1.57	2.52	20.61
T_{15}	8526.67	27.59	18.86	1.64	1.88	19.15
Mean	5643.07	23.31	15.48	1.45	1.88	18.55
CD (0.05)	269.18	4.26	0.62	0.16	0.36	0.59

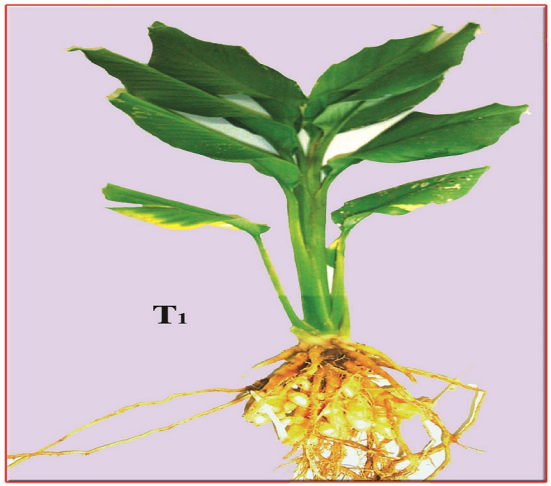

Plate 2. Vellanikkara accession

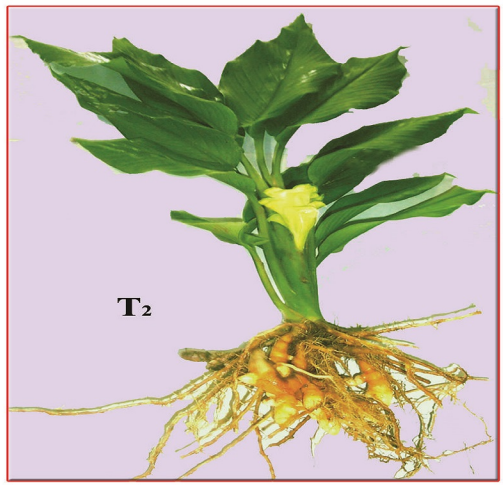

Plate 3. Vellayani accession

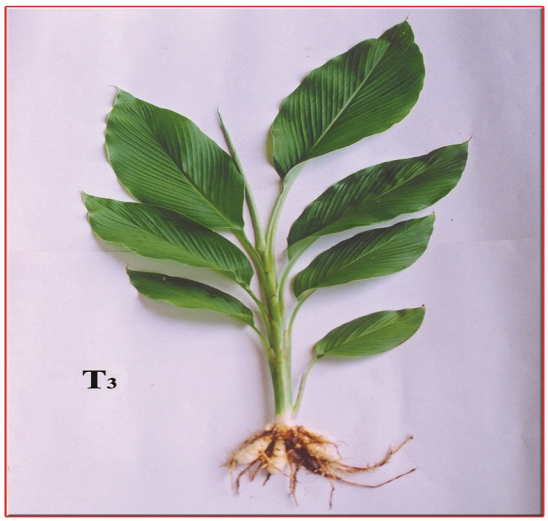

Plate 4. IISR accession

Plate 5. Nedumangad accession

Plate 6. Vellanikkara local

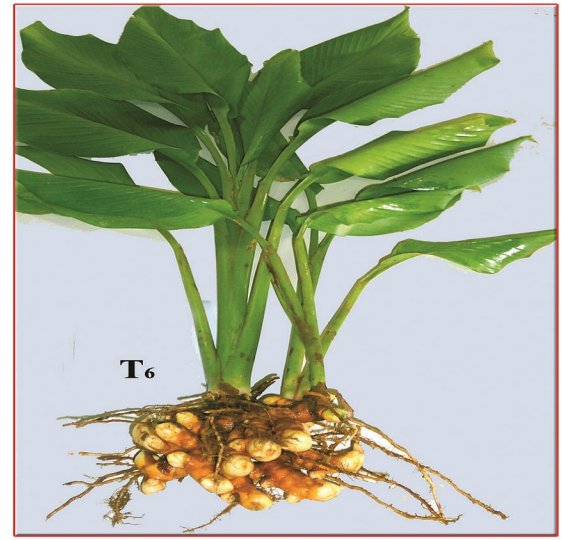

Plate 7. Kumily local

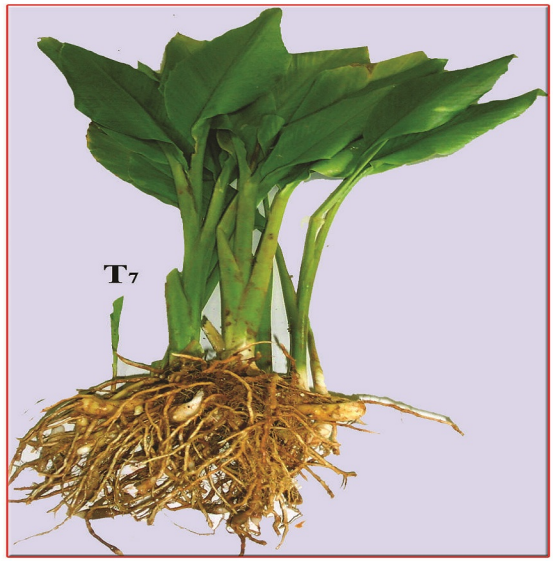

Plate 8. Kottoor wild

Plate 9. Pala wild

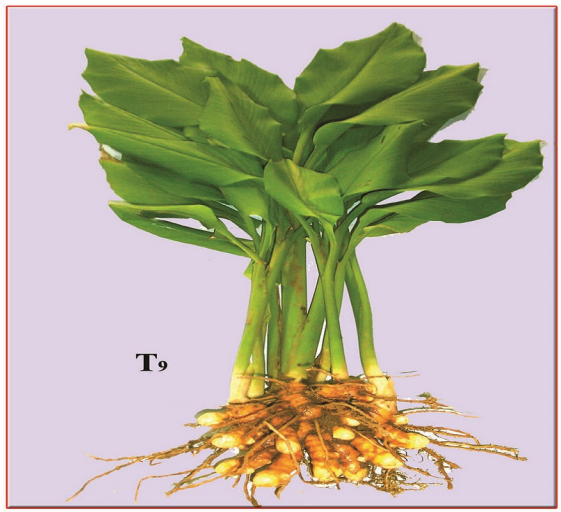

Plate 10. Thodupuzha accession

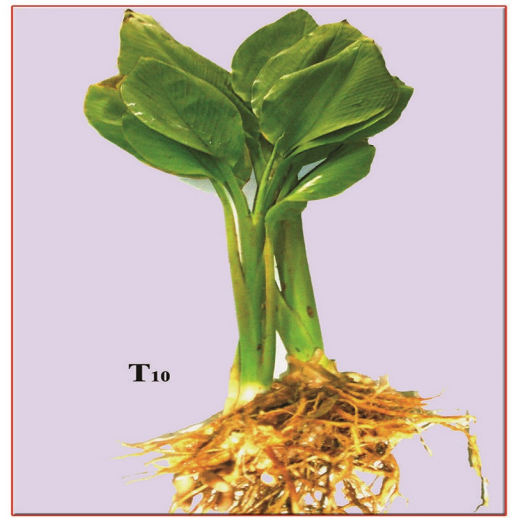

Plate 11. Pala white (wild)

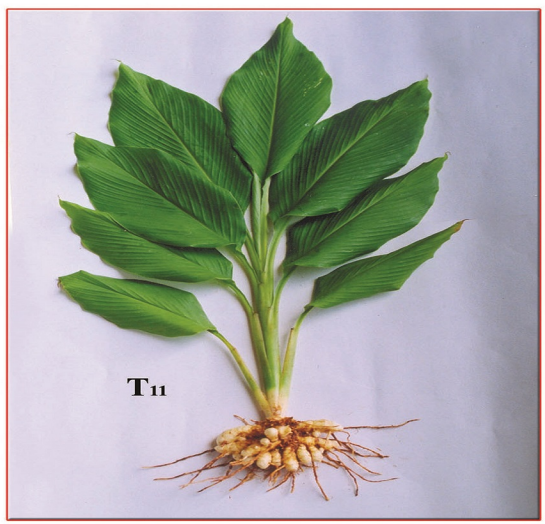

Plate 12. Kozhikode accession

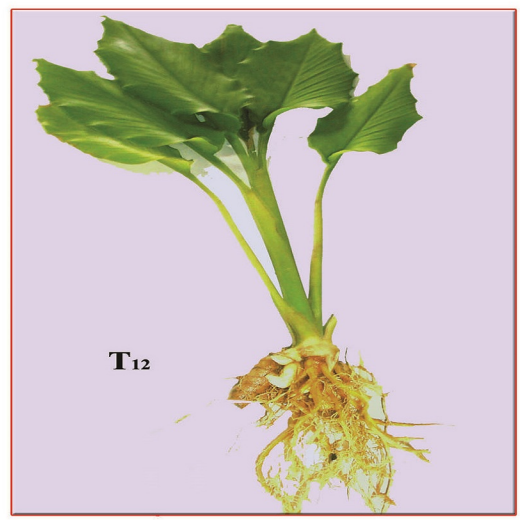

Plate 13. Turmeric - Alleppey

Plate 14. Turmeric - Vellayani local

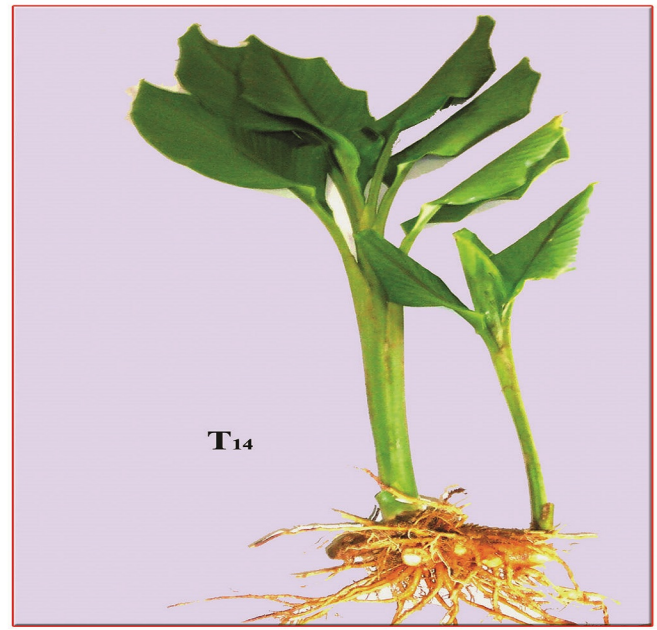

Plate 15. Zedoary - yellow

Plate 16. Zeadoary - black

During 4 MAP, 6 MAP and 8 MAP, the accessions T_5 and T_6 recorded values above the general mean which are 78.79, 84.87 and 91.36 cm respectively. During 8 MAP, T_9 also recorded plant height above the general mean value.

4.1.1.2 Number of Tillers

The accession T_7 produced the highest number of tillers (2.1, 4.11, 6.22 and 6.22) at all the four growth stages followed by T_{10} (1.80, 3.20, 5.42 and 6.20 each). The accessions T_1 and T_{13} recorded the least number (1.06 each) at 2 MAP, whereas accession T_2 recorded the minimum number of tillers (1.26, 2.07 and 2.07 each) at 4 MAP, 6 MAP and 8 MAP respectively. Number of tillers above the general mean (1.42) was recorded in accessions T_4, T_5, T_6, T_8 and T_9 at 2 MAP. At 4 MAP, tiller number above general mean (2.23) was recorded by T_5, T_8, T_9 and T_{15}. At 6 MAP, above general mean value (3.80) was recorded by T_5, T_{12}, T_{13} and T_{15}. At 8 MAP, accessions T_5, T_{12}, T_{13} and T_{15} recorded values above the general mean (4.15).

4.1.1.3 Number of Leaves

At 2 MAP the accession T_{10} recorded maximum number of leaves (7.56) whereas at 4 MAP it goes to T_5 and the rest of the two stages (6 MAP and 8 MAP) the first one recorded the highest number of leaves. At 2 MAP, the accession T_4 produced least number of leaves while during the remaining three stages accession T_1 produced least number of leaves.

The accessions T_5, T_7 and T_{10} recorded values above the grand mean at all stages, while T_9, T_{12} and T_{15} exceeded the grand mean in the last three stages. Accession T_3 exceed the grand mean at 2 MAP only. The general mean values during the four stages were 5.24, 12.51, 16.26 and 17.82 respectively. The variation in number of tillers and leaves among various accessions are shown in Fig. 1.

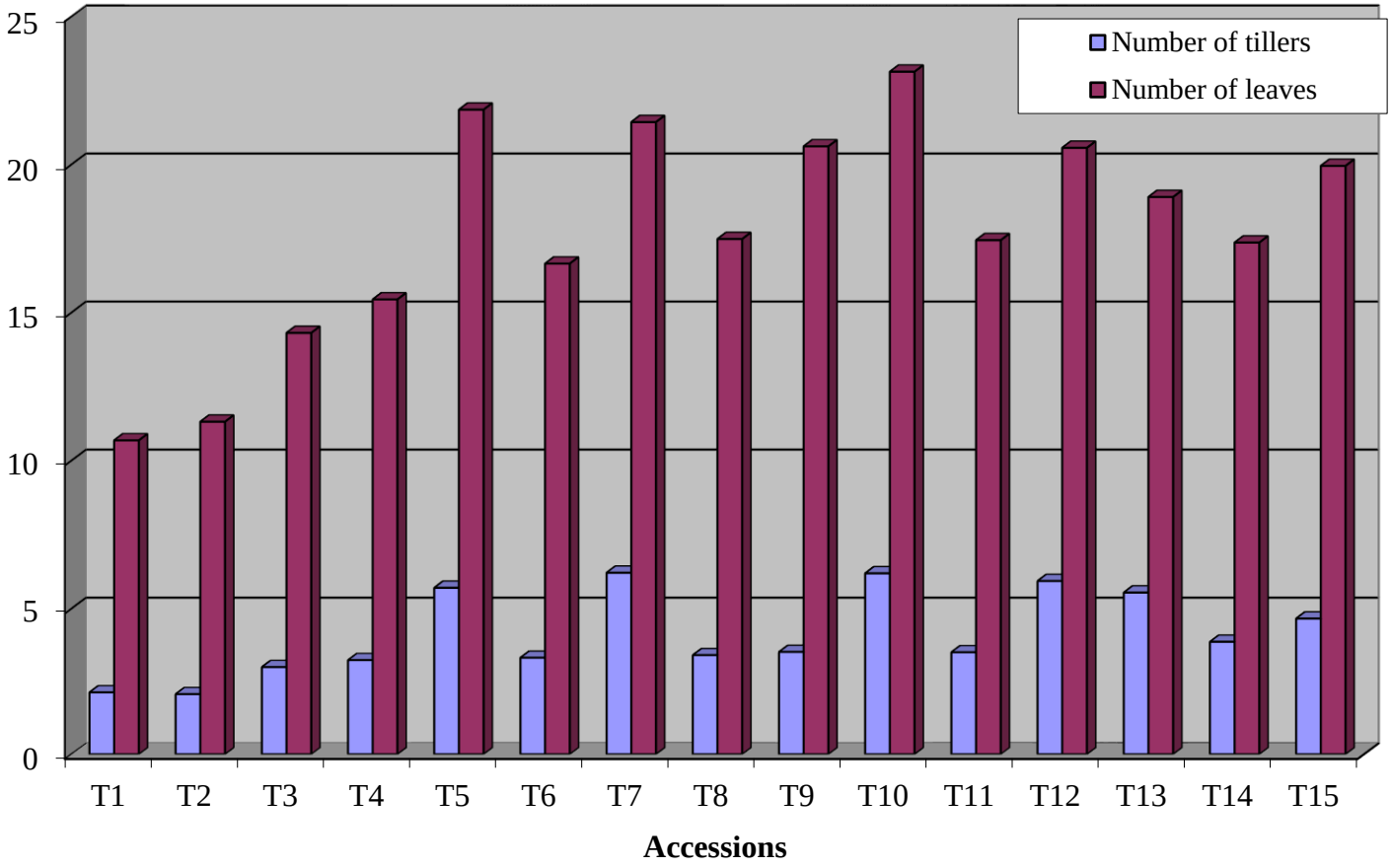

Fig. 1. Number of tillers and leaves of various accessions of *Curcuma* spp.

4.1.1.4 Leaf Area

The accession T_{15} (8526.66 cm^2) recorded the largest leaf area followed by T_{14} (7680.00 cm^2) and T_5 (6825.00 cm^2) whereas the accession T_1 (2656.00 cm^2) had the least leaf area. Leaf area above the general mean (5643.00 cm^2) was observed in accessions T_3, T_8, T_9, T_{11}, T_{12} and T_{13}.

4.1.1.5 Rhizome Spread

Maximum rhizome spread was observed in accession T_{15} (27.59 cm) followed by accessions T_{14} (26.46 cm) and T_5 (26.68 cm), whereas the least rhizome spread was recorded in accession T_{12} (17.96 cm). Rhizome spread values above the general mean value (23.31 cm) were observed in accessions T_{11}, T_{10}, T_8, T_7 and T_3 (Fig. 2 and Plates 17 to 31).

4.1.1.6 Root Spread

Root spread recorded a maximum value in T_{15} (18.86 cm) followed by T_5 (18.75 cm) and T_3 (18.36 cm) while the least value was observed in T_{13} (11.81 cm). Root spread values above the general mean value (15.48 cm) were recorded in T_7, T_8, T_{10}, T_{11} and T_{14} respectively.

4.1.1.7 Rhizome Thickness

The highest value for rhizome thickness was recorded in accession T_{15} (1.64 cm) followed by T_{14} (1.57 cm) and T_3 (1.54 cm). Least value for rhizome thickness was seen in T_1 (1.27 cm). Rhizome thickness above the general mean value (1.45 cm) were recorded in accessions T_8, T_9, T_{11}, T_{12} and T_{13} respectively.

4.1.1.8 Dry Root Weight per Plant

Maximum values for dry root weight was recorded by accession T_5 (2.84 g) followed by T_{10} (2.63 g) and T_{11} and T_{14} (2.52 g each). Dry root weight was found least in the accession T_4 (0.92 g). Accessions T_3, T_7, T_8

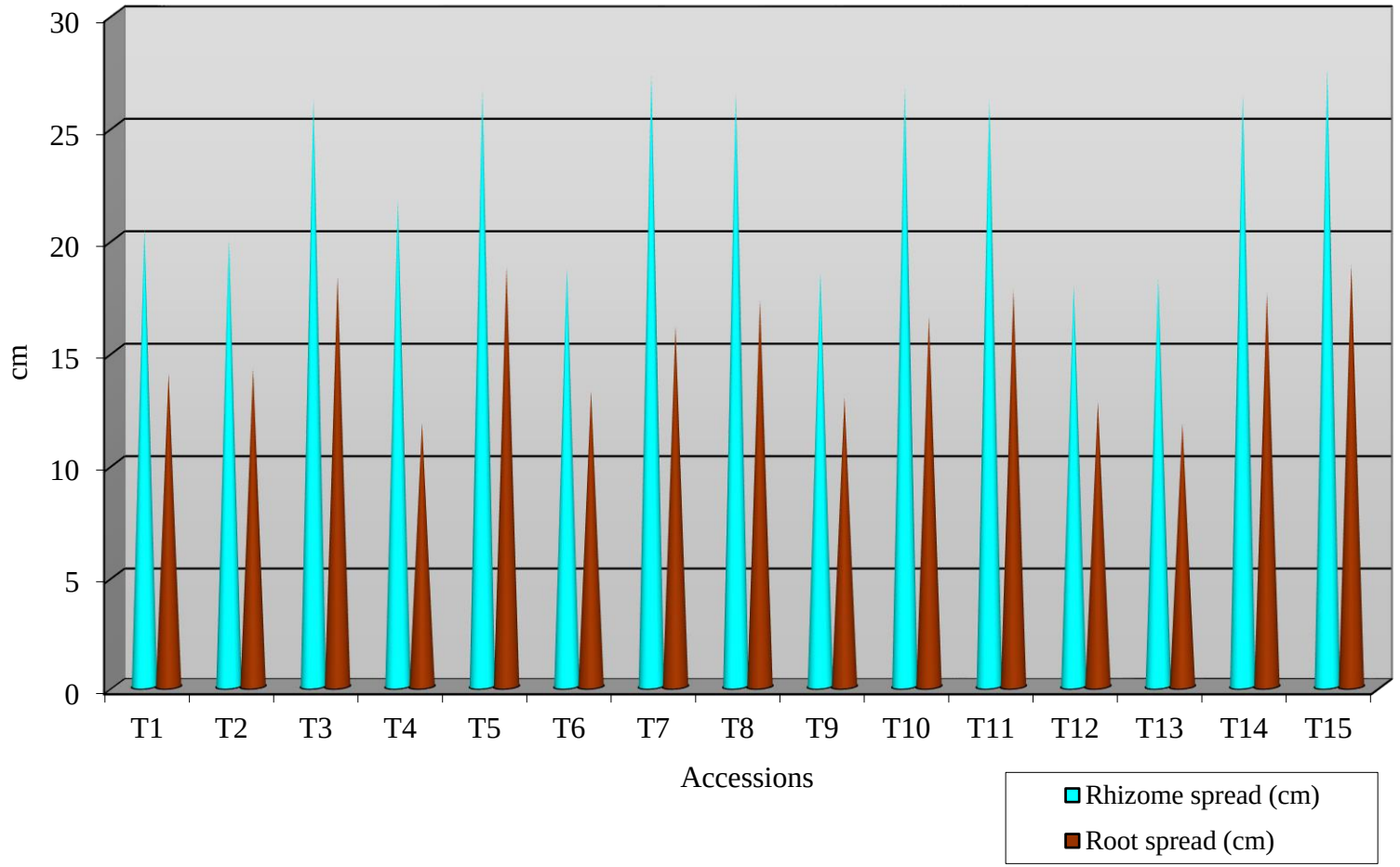

Fig. 2. Rhizome spread and root spread of various accessions of *Curcuma* spp.

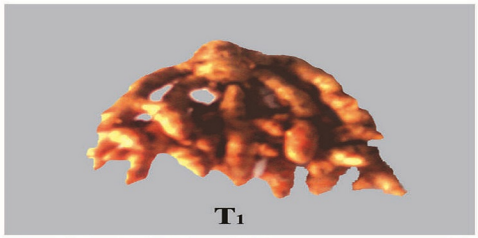

Plate 17. Vellanikkara accession

Plate 18. Vellayani accession

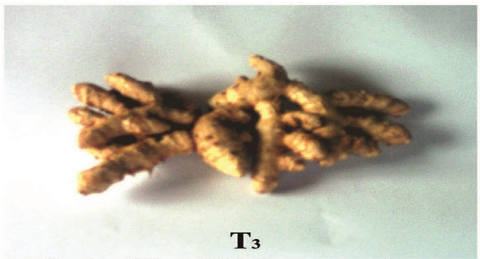

Plate 19. IISR accession

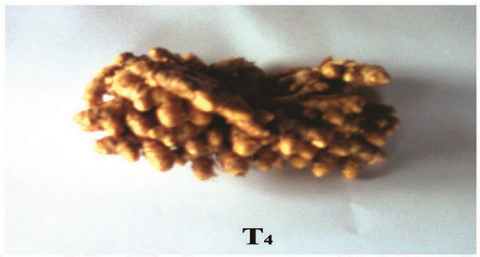

Plate 20. Nedumangad accession

Plate 21. Vellanikkara local

Plate 22. Kumily local

Plate 23. Kottoor wild

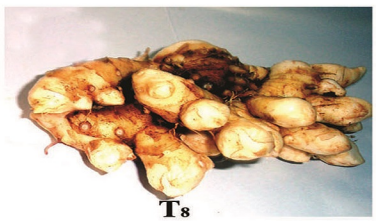

Plate 24. Pala wild

Plate 25. Thodupuzha accession

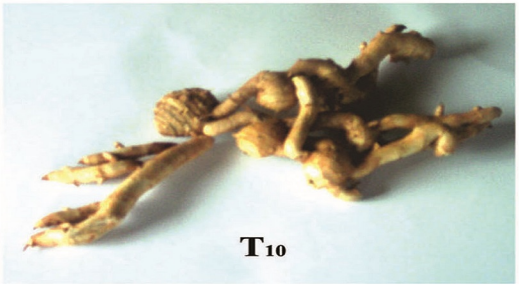

Plate 26. Pala white (wild)

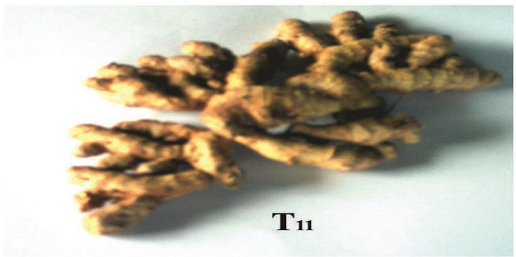

Plate 27. Kozhikode accession

Plate 28. Turmeric - Alleppey

Plate 29. Turmeric - Vellayani local

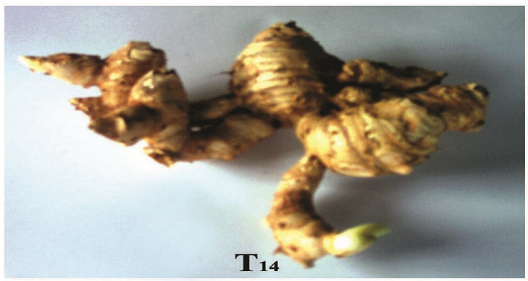

Plate 30. Zedoary - yellow

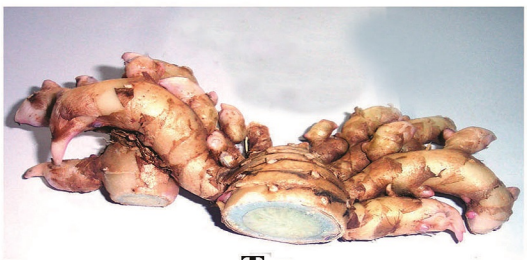

Plate 31. Zedoary - black

and T_{15} recorded dry root weight values above the general mean value (1.88 g).

4.1.1.9 Root Length

Root length was found highest in accession T_5 (22.47 cm) followed by T_{11} (20.62 cm) and T_{14} (20.61 cm). Root length values above the general mean value (18.55 cm) were found in accessions T_3, T_8, T_{10} and T_{15}.

4.1.1.10 Pest and Disease Incidence

There was no severe incidence of any pests or diseases. However, a minor attack of shoot borer was observed. Since the incidence of pests and diseases were below threshold level, no scoring was done.

4.1.1.11 Morphological Cataloguing

All the fifteen accessions were described based on seventeen selected morphological characters (Appendix I). Characters like leaf pubescence and leaf midrib colour were observed as the most easily identifiable characters to distinguish between the various accessions. The accessions T_3, T_7, T_8, T_{10} and T_{11} were found to have pubescence at the under leaf surface while all other accessions did not show this character (Plates 32 and 33). Out of these, T_3, T_8 and T_{11} were densely pubescent and T_7 and T_{10} were thinly pubescent.

Leaf midrib was deep pink in T_5, purple in T_{14} and pinkish upto the distal half of the midrib in T_{15}. All the other accessions had midrib with green colour. All other characters studied were present at varying levels in all the accessions.

4.1.2 Biochemical Characters

Variability was observed among the accessions for biochemical characters like curcumin content, chlorophyll content, essential oil content, crude fibre, ash, sugar, starch, protein, alkaloid and moisture

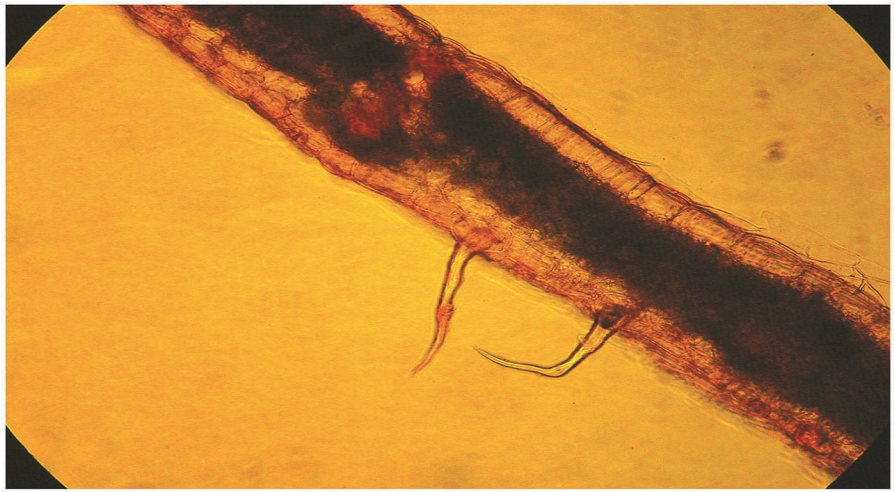

Plate 32. Leaf cross section of kasthuri turmeric with leaf hair

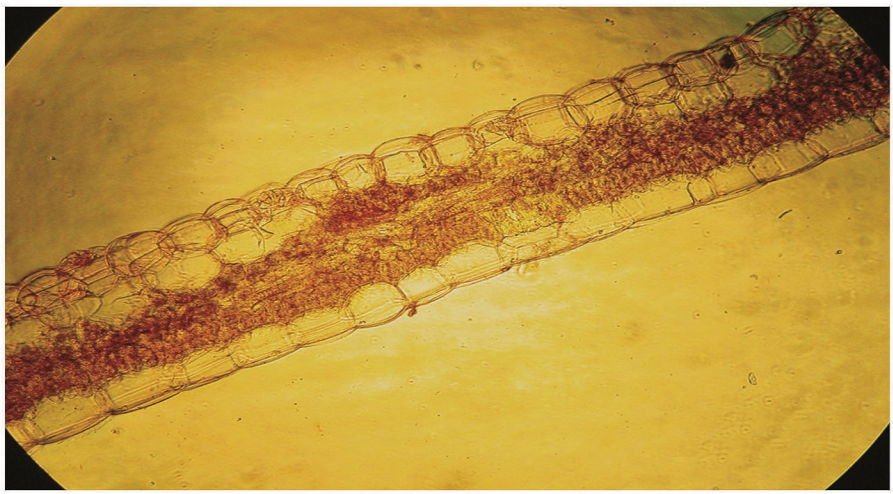

Plate 33. Leaf cross section of ordinary turmeric without leaf hair

content. These characters were analysed in order to differentiate the kasthuri turmeric from other *Curcuma* spp. and the results are presented in Table 3.

4.1.2.1 Curcumin Content

It is one of the most important biochemical character determining the quality in case of turmeric varieties, for the use as a spice. Among the 15 accessions studied, curcumin content was found maximum in the accessions T_{13} (5.94 %) followed by T_{12} (5.78 %) which are selected as local turmeric cultivars for comparative study (Fig. 3). The curcumin content was found least in T_{15} (0.012 %) which is a zedoary accession used for comparative study with bluish grey rhizome. The accessions T_3 (0.05 %), T_5 (0.98 %), T_7 (0.026 %), T_8 (0.04 %), T_{10} (0.033 %), T_{11} (0.04 %) also had a very low curcumin content below 1.00 per cent. The accessions T_1 (5.13 %), T_2 (4.92 %), T_6 (3.67 %), T_9 (4.41 %) had curcumin content values above the general mean (2.27 %).

4.1.2.2 Chlorophyll 'a' Content

Maximum values for chlorophyll 'a' content was recorded in accession T_8 (0.91 mg g^{-1}) followed by T_{11} (0.88 mg g^{-1}). The least value was recorded by T_{10} (0.68 mg g^{-1}). Accessions T_4, T_6, T_9, T_{14} and T_{15} recorded values above the general mean (0.79 mg g^{-1}).

4.1.2.3 Chlorophyll 'b' Content

Accession T_8 (0.78 mg g^{-1}) recorded the maximum value for chlorophyll 'b' content followed by T_5 (0.37). Least value was recorded by T_{14} (0.27 mg g^{-1}). The accessions T_2, T_3, T_7, T_{10}, T_{11}, T_{13} and T_{15} recorded values above the general mean (0.33 mg g^{-1}).

4.1.2.4 Total Chlorophyll Content

Total content of chlorophyll was found maximum in T_8 (1.29 mg g^{-1}) followed by T_{11} (1.22 mg g^{-1}). Least value was recorded by T_{10} (0.99 mg g^{-1}).

Table 3. Mean values for various biochemical characters of the *Curcuma* spp. accessions

Accession No.	Curcumin content (%)	Chlorophyll 'a' (mg g^{-1})	Chlorophyll 'b' (mg g^{-1})	Total chlorophyll (mg g^{-1})	Essential oil (%)	Crude fibre (%)	Ash content (%)	Sugar (%)	Starch (%)	Protein (%)	Alkaloid (%)	moisture content (%)
T_1	5.13	0.68	0.32	1.00	3.32	2.34	5.46	1.34	22.49	5.90	0.009	11.01
T_2	4.92	0.77	0.34	1.11	3.23	2.41	5.83	1.61	23.68	5.63	0.009	11.63
T_3	0.05	0.83	0.36	1.19	5.83	3.10	6.60	1.71	22.93	6.43	0.012	12.38
T_4	1.46	0.85	0.32	1.17	3.11	2.47	6.54	1.46	23.02	5.34	0.008	11.65
T_5	0.98	0.76	0.37	1.13	4.29	2.99	6.48	1.67	24.44	4.90	0.011	12.65
T_6	3.67	0.90	0.28	1.18	4.02	2.27	5.93	1.90	23.79	4.71	0.007	10.43
T_7	0.02	0.69	0.34	1.03	0.25	2.62	6.28	1.03	24.66	4.50	0.005	11.95
T_8	0.04	0.91	0.38	1.29	5.53	3.10	6.20	1.44	22.86	6.14	0.013	12.25
T_9	4.41	0.82	0.30	1.12	4.06	2.12	6.05	1.47	22.47	5.40	0.012	11.47
T_{10}	0.03	0.68	0.34	0.99	0.27	2.56	6.31	1.02	24.22	4.51	0.004	11.92
T_{11}	0.04	0.88	0.34	1.22	5.80	2.98	6.64	1.68	22.63	6.04	0.012	12.19
T_{12}	5.78	0.69	0.33	1.01	4.26	2.44	5.95	1.54	24.48	5.46	0.014	11.14
T_{13}	5.94	0.68	0.34	1.02	5.80	2.23	6.04	2.08	23.09	6.09	0.004	11.58
T_{14}	1.54	0.84	0.27	1.20	2.03	2.58	6.12	1.87	24.92	4.85	0.006	13.07
T_{15}	0.01	0.83	0.33	1.16	1.63	2.42	6.26	1.41	25.39	5.45	0.008	13.28
Mean	2.27	0.79	0.33	1.12	3.56	2.58	6.18	1.55	23.59	5.42	0.008	11.91
CD (0.05)	0.23	0.10	0.04	0.09	1.19	0.30	NS	0.43	2.18	0.85	NS	1.24

NS – Not significant

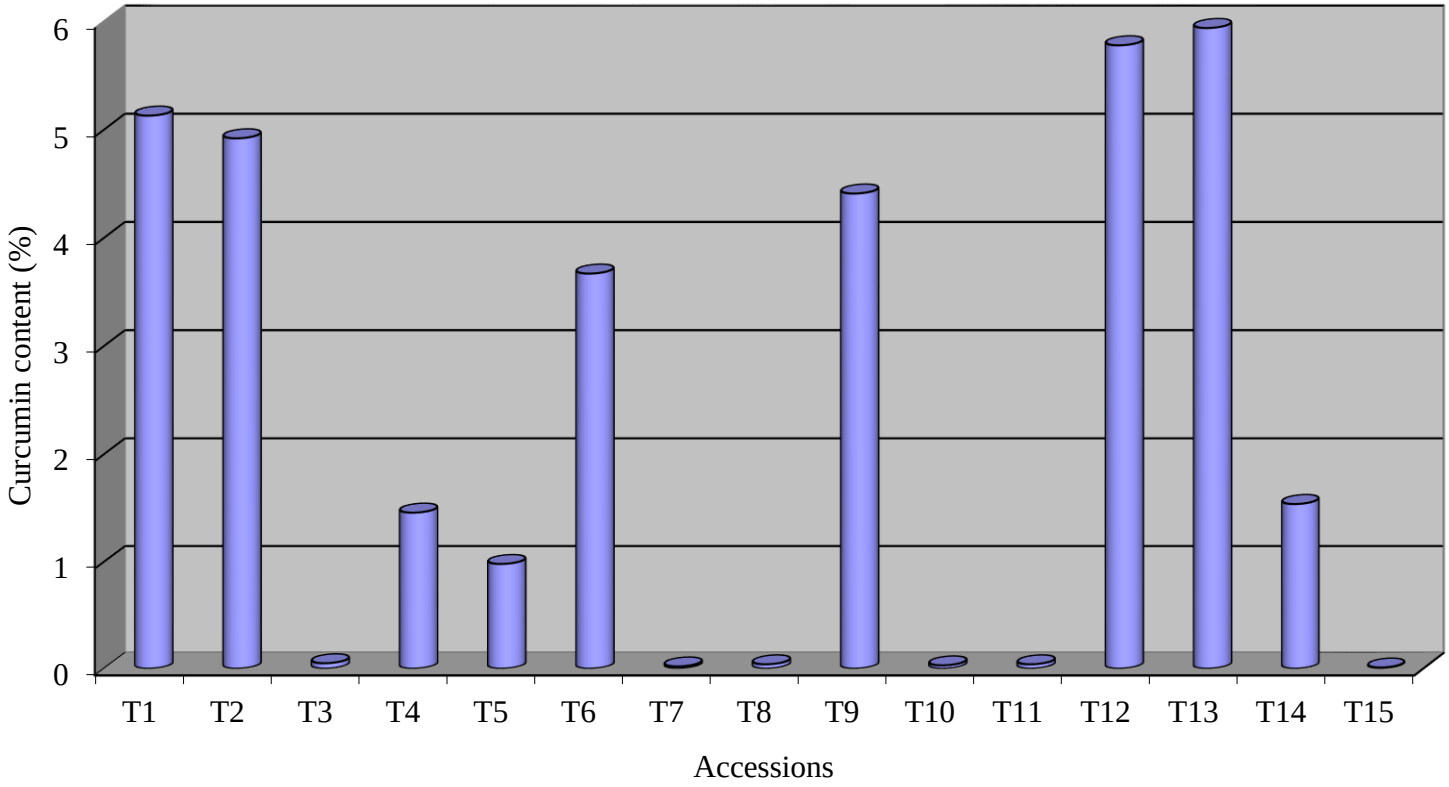

Fig. 3. Curcumin contents (%) of various accessions of *Curcuma* spp.

The accessions T_3, T_4, T_5, T_6, T_9, T_{14} and T_{15} showed values above the general mean (1.12 mg g^{-1}).

4.1.2.5 Essential Oil Content

It is an important quality attribute which determines the aroma of the rhizomes. Various accessions exhibited wide variations in essential oil content (Fig. 4). The maximum content of essential oil was observed in the accession, T_3 (5.83 %) followed by accessions T_{11} (5.80 %) and T_8 (5.53 %). The least values were recorded by T_7 (0.25 %) and T_{10} (0.27 %). The accession T_5, T_6, T_9, T_{12} and T_{13} recorded values above the general mean (3.56 %).

4.1.2.6 Crude Fibre Content

Maximum values for crude fibre content was observed in accession T_8 (3.10 %) followed by T_3 (3.10 %) and T_{11} (2.98 %), whereas minimum value was recorded in T_9 (2.12 %). The accessions T_5, T_7 and T_{14} recorded values above general mean value (2.58 %).

4.1.2.7 Ash Content

Values for ash content was found maximum in accession T_{11} (6.64 %) followed by T_3 (6.60 %) and T_4 (6.54 %), whereas the least content of ash recorded in accession T_1 (5.46 %). The accession T_5, T_7, T_8 and T_{10} recorded values above general mean (6.18 %).

4.1.2.8 Sugar Content

The maximum values were recorded in accession T_{13} (2.10 %) followed by T_6 (1.90 %), whereas the least value was observed in T_{10} (1.02 %). The accessions T_2, T_3, T_5, T_{11} and T_{14} recorded values above the general mean value (1.54 %).

4.1.2.9 Starch Content

Maximum content of starch was recorded in accession T_{15} (25.39 %) followed by T_{14} (24.92 %), while the least value was recorded in accession

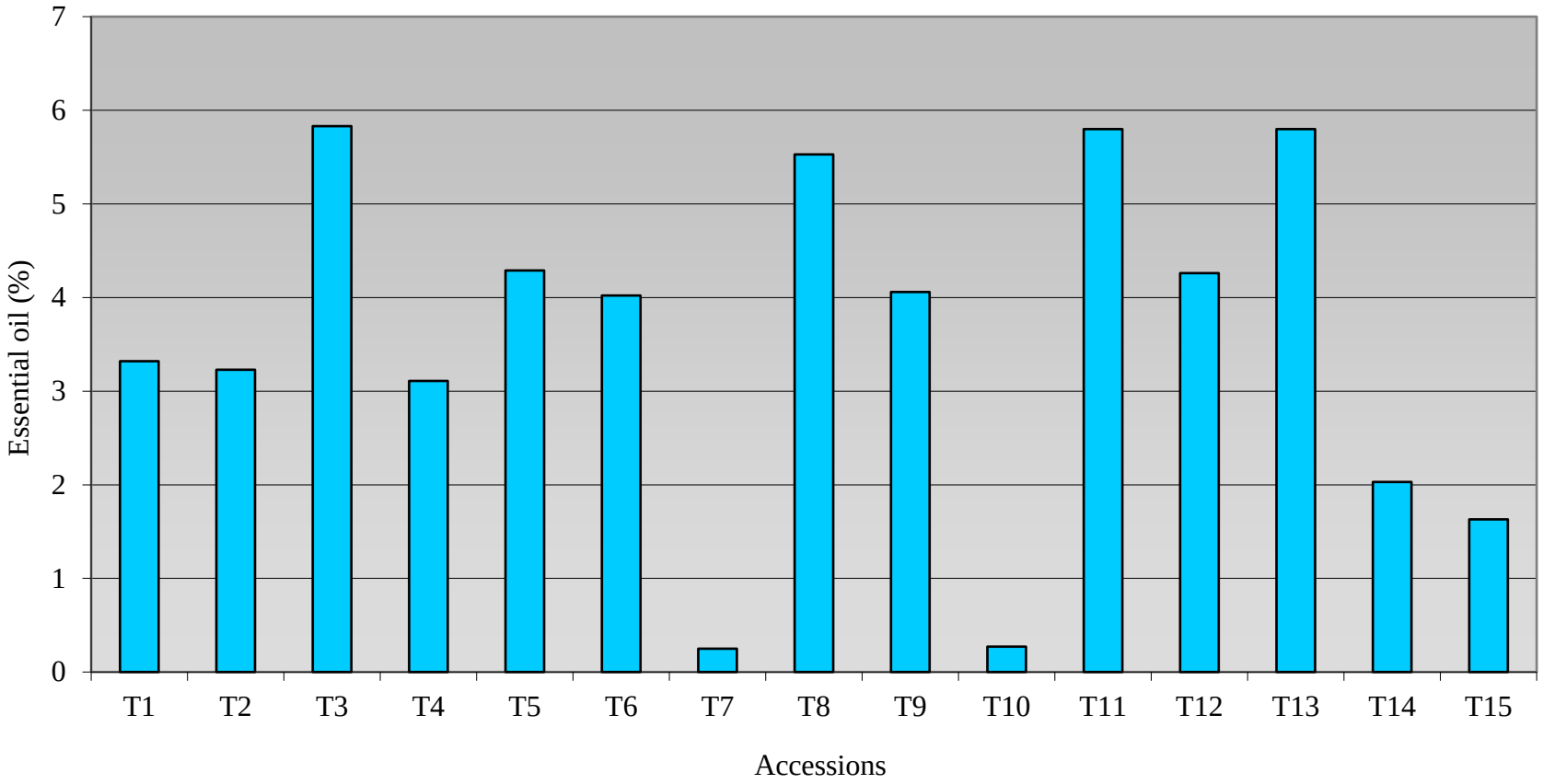

Fig. 4. Essential oil content (%) of various accessions of *Curcuma* spp.

T_9 (22.47 %). The accession T_2, T_5, T_6, T_{10}, T_{12} and T_{14} recorded values above the general mean value (23.59 %).

4.1.2.10 Protein Content

Protein content was found highest in the accession T_3 (6.42 %) followed by T_8 (6.14 %) and T_{13} (6.09 %). The least value was recorded in T_7 (4.50 %). The accession T_1, T_2, T_{11}, T_{12} and T_{15} recorded values above general mean (5.42 %).

4.1.2.11 Alkaloid Content

Alkaloids were found only in trace amounts in various accessions. Among these the highest value was recorded in T_{12} (0.014 %) followed by T_8 (0.013 %) whereas the least value was observed in T_{10} (0.004 %). The accessions T_3, T_5, T_9 and T_{11} recorded values above the general mean (0.01 %).

4.1.2.12 Moisture Content

Maximum moisture content in dried rhizomes was observed in accession T_{15} (13.28 %) followed by T_{14} (13.07 %), whereas the least content was in T_6 (10.43 %). The accession T_3, T_5, T_7, T_8, T_{10} and T_{11} recorded values above the general mean value (11.91 %).

4.1.3 Yield Characters

The data presented in Table 4 shows the variation in yield characters of various *Curcuma* spp. studied.

4.1.3.1 Fresh Rhizome Yield

Wide range of variation was observed among various accessions with respect to yield per plant (Fig. 5). The highest fresh rhizome yield per plant was obtained in accession T_{14} (478.31 g) followed by T_{15} (453.33 g) and T_5 (450.70 g). The lowest yield per plant was in accession T_2 (255.46 g). The accession T_3, T_7, T_8, T_{10} and T_{11} recorded fresh rhizome yield above the general mean (358.34 g).

Table 4. Mean values of yield characters of the *Curcuma* spp. accessions

Accession No.	Fresh rhizome yield (g plant^{-1})	Dry rhizome yield (g plant^{-1})	Dry top yield (g plant^{-1})	Crop duration (days)
T_1	260.15	55.72	11.09	207
T_2	255.46	53.10	11.45	208
T_3	424.52	118.45	17.65	221
T_4	272.76	61.14	13.63	221
T_5	450.70	121.77	20.07	207
T_6	292.70	63.83	14.48	226
T_7	399.07	112.78	16.33	230
T_8	419.78	116.96	18.98	223
T_9	292.52	62.34	13.67	231
T_{10}	396.22	110.11	14.69	234
T_{11}	424.16	116.09	18.12	226
T_{12}	275.20	56.61	13.66	236
T_{13}	280.26	59.52	14.18	238
T_{14}	478.31	127.26	22.82	215
T_{15}	453.33	119.90	21.68	223
Mean	358.34	88.11	16.17	223
CD (0.05)	11.903	26.042	1.826	8.145

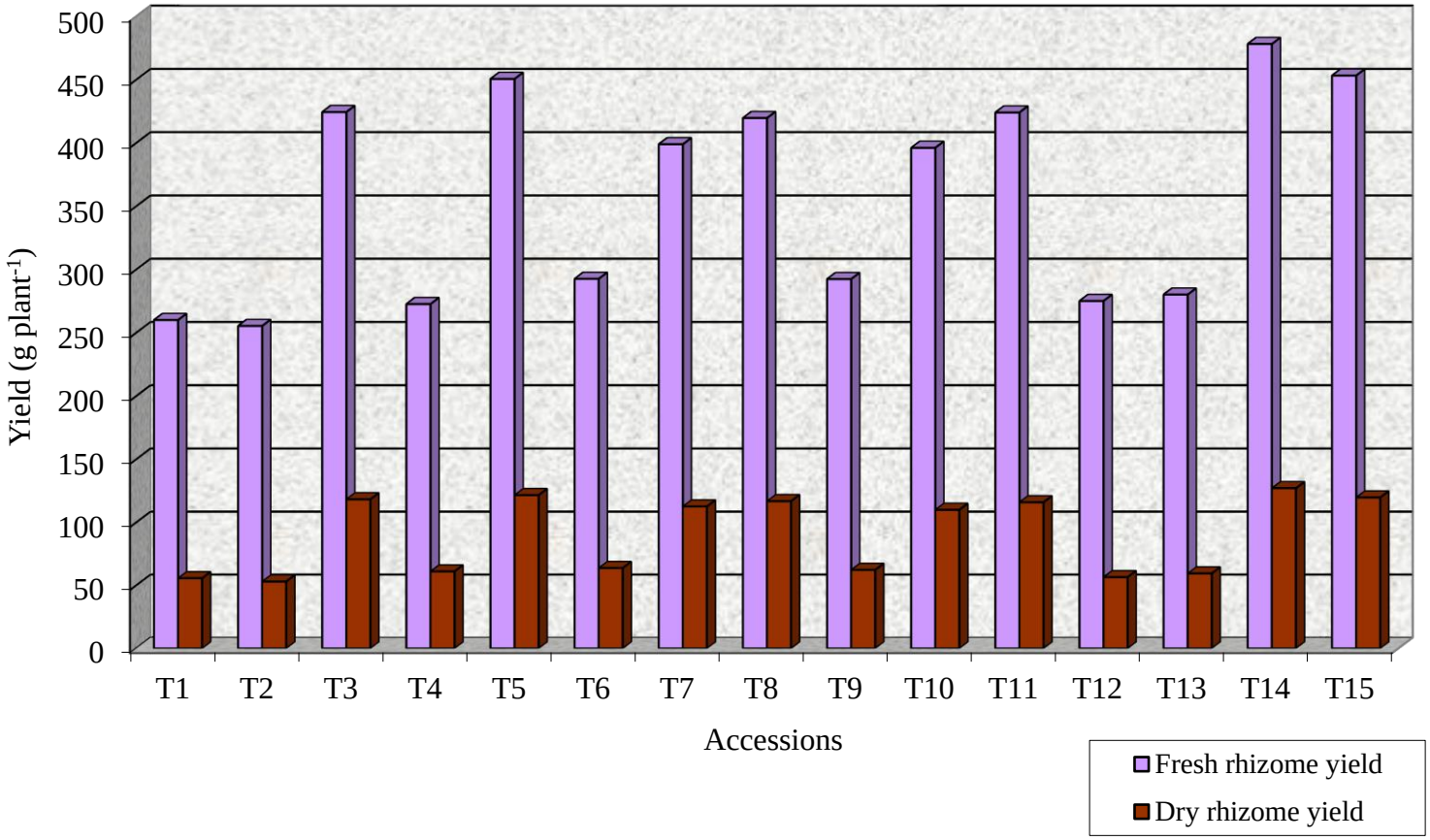

Fig. 5. Fresh and dry rhizome yields of various accessions of *Curcuma* spp.

4.1.3.2 Dry Rhizome Yield

Dried rhizome yield was in proportion to the fresh yield obtained. It was found maximum in T_{14} (127.26 g) followed by T_5 (121.76 g), while the least dry rhizome yield was in T_2 (53.10 g). The accessions T_3, T_7, T_8, T_{10}, T_{11} and T_{15} had dry rhizome yields above the general mean (88.11 g).

4.1.3.3 Dry Top Yield

Dry top yield was highest in T_{14} (22.82 g) followed by T_{15} (21.68 g) whereas the lowest top yield was in accession T_1 (11.10 g). The accessions T_3, T_5, T_7, T_8, T_{11} had top yield values above the general mean (16.17 g).

4.1.3.4 Crop Duration

Variation was observed among various accessions with respect to crop duration also. It was found highest in accession T_{13} (231.67 days) followed by T_{12} (228.67 days), whereas the least time was taken by accession T_1 (193.33 days). The accessions T_6, T_7, T_9, T_{10}, T_{11} and T_{15} also had crop duration above the general mean value (214 days).

4.1.4 Physiological Characters

The various physiological characters evaluated have shown considerable variation among the accessions and are represented in Table 5.

4.1.4.1 Dry Matter Production

The dry matter production was found maximum in accession T_{14} (126.58 g) followed by T_{15} (124.36 g), whereas the least value was in the accession T_2 (51.28 g). The accessions T_3, T_5, T_7, T_8, T_{10} and T_{11} recorded values above the general mean (89.18 g).

4.1.4.2 Drying Percentage of Rhizomes

Drying percentage determines the dry yield of rhizomes in various accessions. The drying percentage was found maximum in accession T_7 (28.26 %) followed by T_3 (27.90 %) whereas the least value was recorded

Table 5. Mean values of various physiological parameters of the *Curcuma* spp. accessions

Accession No.	Dry matter production (g plant^{-1})	Curing percentage of rhizomes	Leaf area index	Leaf area duration (days)	Harvest index
T_1	53.27	21.42	4.25	155.76	0.62
T_2	51.28	20.79	4.50	157.32	0.62
T_3	120.46	27.90	9.41	288.01	0.88
T_4	60.69	22.42	8.02	260.47	0.63
T_5	112.65	27.02	10.76	297.46	0.92
T_6	64.54	21.81	8.32	260.82	0.69
T_7	108.30	28.26	7.92	255.75	0.85
T_8	110.46	27.86	9.61	288.92	0.87
T_9	63.47	21.31	9.33	281.77	0.71
T_{10}	104.38	27.79	7.82	252.80	0.84
T_{11}	118.65	27.37	9.92	296.90	0.88
T_{12}	58.42	20.57	9.65	288.36	0.63
T_{13}	60.27	21.24	9.74	290.02	0.64
T_{14}	126.58	26.61	12.26	302.83	0.94
T_{15}	124.36	26.45	13.54	305.71	0.92
Mean	89.18	24.59	9.00	265.53	0.78
CD (0.05)	2.901	0.555	0.739	10.959	0.029

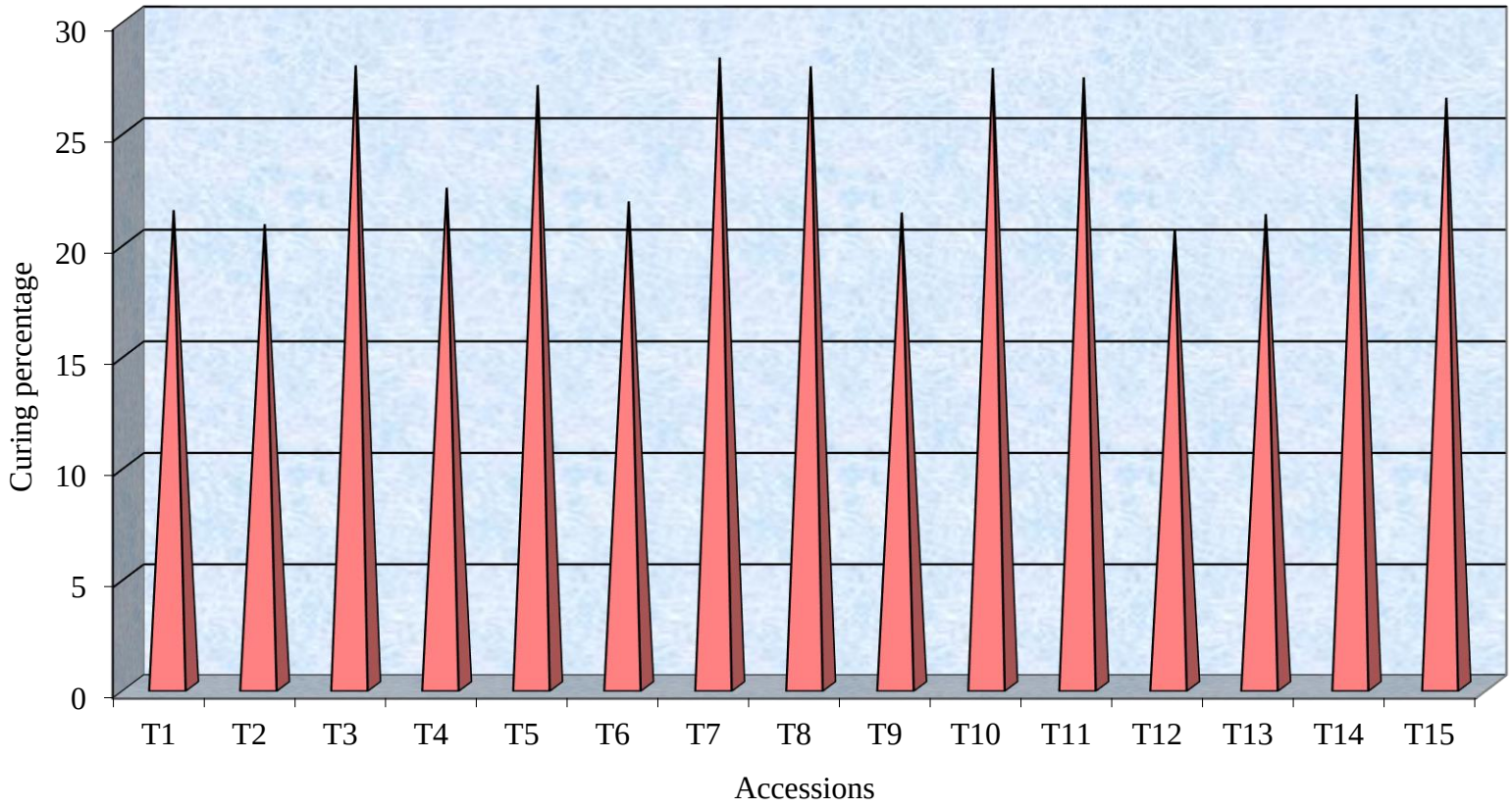

Fig. 6. Curing percentage of various accessions of *Curcuma* spp.

by accession T_{12} (20.57 %). The accessions T_5, T_7, T_8, T_{10}, T_{11}, T_{14} and T_{15} recorded values above the general mean value (24.58 %) (Fig. 6).

4.1.4.3 Leaf Area Index (LAI)

LAI was found maximum in accessions T_{15} (13.54) followed by T_{14} (12.26) while the least value was recorded by T_1 (4.25). The accessions T_3, T_5, T_8, T_9, T_{11}, T_{12} and T_{13} recorded values above the general mean (9.004).

4.1.4.4 Leaf Area Duration (LAD)

LAD was recorded maximum in T_{15} (305.71) followed by T_{14} (302.83) whereas the least value was recorded by T_1 (155.76). The accessions T_3, T_5, T_8, T_9, T_{11}, T_{12} and T_{13} recorded values above the general mean (265.53).

4.1.4.5 Harvest Index

Harvest index indicates the yield potential of a crop. It was recorded maximum in T_{14} (0.94) followed by T_5 and T_{15} (0.92 each) whereas the least value was recorded in accession T_2 (0.62). The accessions T_3, T_7, T_8, T_{10}, T_{11} recorded values above the general mean (0.78).

4.1.5 Anatomical Characters

The various anatomical characters studied to characterize the kasthuri turmeric accessions were presented in Table 6.

4.1.5.1 Leaf Cuticle Thickness

Leaf cuticle thickness was observed maximum in accession T_5 (0.42 μm) followed by T_{15} (0.41 μm) whereas the least value was observed in T_3 (0.30 μm). The accessions T_6, T_7, T_9, T_{10}, T_{12} and T_{14} recorded values above the general mean value (0.35 μm).

Table 6. Mean values of anatomical characters of *Curcuma* spp. accessions

Accession No.	Leaf cuticle thickness (μm)	Number of stomata on abaxial leaf surface (mm^{-2})	Number of stomata on adaxial leaf surface (mm^{-2})	Number of vascular bundles in roots
T_1	0.32	19.33	4.33	18.67
T_2	0.32	20.00	4.00	20.33
T_3	0.30	20.00	5.00	20.00
T_4	0.33	19.33	5.00	19.33
T_5	0.42	17.00	6.00	18.00
T_6	0.39	20.67	5.00	18.67
T_7	0.36	21.00	4.00	20.33
T_8	0.31	19.33	5.33	18.33
T_9	0.37	20.33	5.00	19.67
T_{10}	0.37	19.33	6.33	19.67
T_{11}	0.30	19.67	5.00	20.00
T_{12}	0.35	20.00	6.33	18.67
T_{13}	0.35	20.33	6.00	21.00
T_{14}	0.39	17.33	4.67	17.67
T_{15}	0.41	19.67	5.33	20.67
Mean	0.35	19.56	5.16	19.40
CD (0.05)	0.036	NS	NS	NS

NS – Not significant

4.1.5.2 Number of Stomata on Abaxial Surface of Leaf

No significant difference was found in the number of stomata among the various accessions. The number of stomata per mm^2 on abaxial surface of leaf was observed maximum in accession T_7 (21.00) followed by T_6 (20.66) whereas least value was recorded by accession T_4 (17.33). The accessions T_2, T_3, T_6, T_9, T_{11}, T_{12}, T_{13} and T_{15} recorded values above the general mean value (19.56).

4.1.5.3 Number of Stomata on Adaxial Surface of Leaf

It was found maximum in accessions T_{10} and T_{12} (6.33 each) followed by T_{13} and T_5 (6.10) whereas the least value was recorded in accession T_2 and T_7 (4.00 each). The accessions T_8, T_{10}, T_{12} and T_{13} recorded values above the general mean (5.16).

4.1.5.4 Number of Vascular Bundles in Roots

There was no significant difference in the number of vascular bundles in roots among the various accessions. It was found maximum in accession T_{13} (21) followed by T_{15} (20.67) whereas the least number was recorded in accession T_{14} (17.67). The accession T_2, T_3, T_7, T_9, T_{10}, T_{11} and T_{15} recorded number of vascular bundles above the general mean (19.4).

4.2 VARIABILITY STUDIES

The phenotypic and genotypic variance and coefficient of variation for the 14 selected characters are presented in Table 7 and Fig. 7.

The highest genotypic (131.29) and phenotypic variance (150.75) recorded for crop duration followed by plant height and fresh rhizome yield, while the lowest value recorded in harvest index for both cases.

Maximum GCV and PCV in per cent was observed in curcumin content (105.58 and 105.74) followed by essential oil content (51.08 and 54.86), number of tillers (35.01 and 35.67), LAI (27.00 and 27.44) and

Table 7. Phenotypic, genotypic and environmental coefficient of variation, phenotypic and genotypic variance for the 14 selected characters of the *Curcuma* spp. accessions

Characters	PCV	GCV	ECV	PV	GV
1	9.73	9.35	2.71	79.05	72.91
2	35.67	35.01	6.85	1.84	1.77
3	22.94	19.60	11.92	16.70	12.19
4	19.03	15.59	10.92	19.68	13.20
5	9.16	6.59	6.37	0.02	0.01
6	105.74	105.57	6.01	5.76	5.74
7	13.71	11.76	7.04	0.13	0.09
8	54.86	51.08	20.01	3.82	3.31
9	22.84	21.82	6.75	13.63	12.44
10	5.74	5.36	2.03	150.75	131.79
11	27.44	27.00	4.91	6.10	5.91
12	16.70	16.54	2.27	0.02	0.02
13	12.99	12.92	1.35	10.19	10.08
14	23.25	23.17	1.99	56.24	55.83

1. Plant height (cm)
2. Number of tillers
3. Number of leaves
4. Rhizome spread (cm)
5. Rhizome thickness (cm)
6. Curcumin content (%)
7. Crude fibre content (%)
8. Essential oil (%)
9. Dry top yield (g plant^{-1})
10. Crop duration (days)
11. Leaf area index
12. Harvest index
13. Curing percentage of rhizomes (%)
14. Fresh rhizome yield (g plant^{-1})

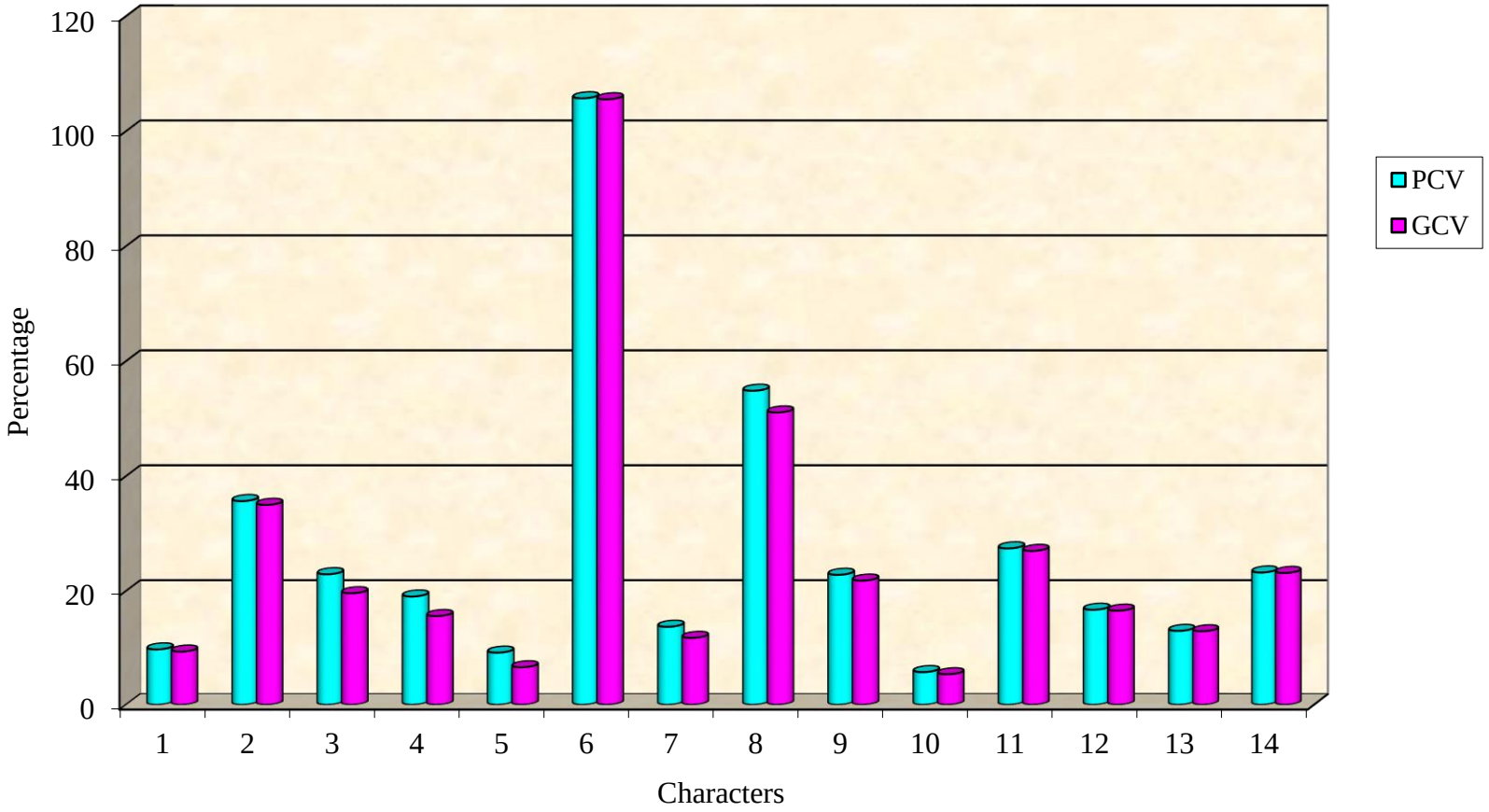

Fig. 7. Coefficient of variation for 14 characters in 15 accessions of *Curcuma* spp.

fresh rhizome yield (23.17 and 13.25). However the crop duration recorded lowest GCV (5.36) and PCV (5.737).

4.3 CORRELATION STUDIES

The phenotypic, genotypic and environmental correlation among 14 characters were worked out and are presented in Tables 8, 9 and 10.

4.3.1 Phenotypic Correlation Coefficients

Fresh rhizome yield per plant showed high positive correlation with HI (0.99), drying percentage of rhizomes (0.91), and dry top yield (0.90). It is also positively correlated with rhizome spread (0.81), LAI (0.67), Crude fibre content (0.625), plant height (0.62), rhizome thickness (0.49) and number of tillers (0.30). Fresh rhizome yield was negatively correlated with curcumin content (-0.83), essential oil (-0.16) and crop duration (-0.07).

Curcumin content had positive correlation with percentage of essential oil content. Other characters were negatively correlated with curcumin content. However essential oil content was positively associated with crude fibre content, crop duration and leaf area index.

Crude fibre content had high positive relationship with drying percentage of rhizome (0.70), rhizome spread (0.62) and harvest index (0.61). Its relationship with number of tillers, number of leaves, rhizome thickness, essential oil content, dry top yield and LAI are also positive.

Drying percentage of rhizome expressed positive association with HI (0.91), rhizome spread (0.84), dry top yield (0.71) and crude fibre content (0.70). It was also positively associated with plant height, number of tillers, number of leaves, rhizome thickness and LAI.

Dry top yield (0.88), rhizome spread (0.80), leaf area index (0.65) and plant height (0.71) showed high positive correlation with harvest index.

Table 8. Phenotypic correlation among 14 selected characters of the *Curcuma* spp. accessions

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000													
2	0.143	1.000												
3	0.332	0.768	1.000											
4	0.401	0.256	0.293	1.000										
5	0.411	0.249	0.358	0.225	1.000									
6	-0.387	-0.216	-0.276	-0.821	-0.260	1.000								
7	-0.025	0.033	0.052	0.619	0.096	-0.646	1.000							
8	-0.322	-0.408	-0.244	-0.294	0.107	0.247	0.241	1.000						
9	0.740	0.236	0.374	0.664	0.581	-0.663	0.487	-0.054	1.000					
10	-0.014	0.486	0.550	-0.166	0.419	0.032	-0.213	0.066	-0.027	1.000				
11	0.727	0.379	0.526	0.375	0.687	-0.414	0.216	0.049	0.814	0.347	1.000			
12	0.607	0.285	0.385	0.802	0.481	-0.838	0.607	-0.166	0.875	-0.063	0.647	1.000		
13	0.349	0.319	0.339	0.841	0.321	-0.931	0.701	-0.204	0.713	-0.028	0.434	0.913	1.000	
14	0.616	0.297	0.367	0.814	0.486	-0.830	0.625	-0.155	0.900	-0.071	0.673	0.991	0.913	1.000

1. Plant height (cm)
2. Number of tillers
3. Number of leaves
4. Rhizome spread (cm)
5. Rhizome thickness (cm)
6. Curcumin content (%)
7. Crude fibre content (%)
8. Essential oil (%)
9. Dry top yield (g plant^{-1})
10. Crop duration (days)
11. Leaf area index
12. Harvest index
13. Curing percentage of rhizomes (%)
14. Fresh rhizome yield (g plant^{-1})

Table 9. Genotypic correlation among 14 selected characters of the *Curcuma* spp. accessions

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000													
2	0.156	1.000												
3	0.381	0.913	1.000											
4	0.450	0.315	0.288	1.000										
5	0.560	0.345	0.512	0.432	1.000									
6	-0.407	-0.220	-0.325	-0.011	-0.355	1.000								
7	0.042	0.035	0.029	0.794	0.276	-0.753	1.000							
8	-0.372	-0.422	-0.339	-0.343	0.145	0.260	0.345	1.000						
9	0.808	0.233	0.373	0.823	0.745	-0.694	0.585	-0.048	1.000					
10	-0.007	0.542	0.653	-0.189	0.633	0.035	-0.247	0.019	-0.033	1.000				
11	0.769	0.387	0.608	0.459	0.955	-0.418	0.253	0.076	0.871	0.378	1.000			
12	0.638	0.301	0.458	0.985	0.627	-0.846	0.707	-0.173	0.923	-0.067	0.672	1.000		
13	0.376	0.332	0.393	0.950	0.462	-0.937	0.820	-0.216	0.751	-0.028	0.438	0.927	1.000	
14	0.640	0.305	0.430	0.987	0.652	-0.835	0.726	-0.157	0.944	-0.074	0.690	0.995	0.921	1.000

1. Plant height (cm)
2. Number of tillers
3. Number of leaves
4. Rhizome spread (cm)
5. Rhizome thickness (cm)
6. Curcumin content (%)
7. Crude fibre content (%)
8. Essential oil (%)
9. Dry top yield (g plant^{-1})
10. Crop duration (days)
11. Leaf area index
12. Harvest index
13. Curing percentage of rhizomes (%)
14. Fresh rhizome yield (g plant^{-1})

Table 10. Environmental correlation among 14 selected characters of the *Curcuma* spp. accessions

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000													
2	-0.073	1.000												
3	0.130	0.031	1.000											
4	0.297	0.020	0.307	1.000										
5	0.128	0.044	0.122	-0.074	1.000									
6	0.213	-0.032	0.066	0.184	-0.135	1.000								
7	-0.413	0.036	0.115	0.209	-0.209	-0.030	1.000							
8	0.104	-0.323	0.133	-0.153	0.039	0.260	-0.188	1.000						
9	-0.024	0.302	0.449	0.118	0.340	-0.064	0.047	-0.112	1.000					
10	-0.085	-0.171	0.153	-0.106	-0.029	-0.036	-0.080	0.381	0.028	1.000				
11	-0.002	0.148	0.156	0.057	0.097	-0.269	0.024	-0.314	-0.096	-0.021	1.000			
12	0.002	-0.292	-0.043	0.037	0.364	-0.095	0.077	-0.120	0.033	-0.022	-0.313	1.000		
13	-0.362	-0.249	0.094	-0.235	-0.129	-0.155	0.016	-0.093	-0.031	-0.052	0.295	-0.008	1.000	
14	0.157	-0.121	0.022	0.177	0.315	0.037	0.107	-0.314	0.093	-0.076	-0.201	0.747	-0.036	1.000

1. Plant height (cm)
2. Number of tillers
3. Number of leaves
4. Rhizome spread (cm)
5. Rhizome thickness (cm)
6. Curcumin content (%)
7. Crude fibre content (%)
8. Essential oil (%)
9. Dry top yield (g plant^{-1})
10. Crop duration (days)
11. Leaf area index
12. Harvest index
13. Curing percentage of rhizomes (%)
14. Fresh rhizome yield (g plant^{-1})

4.3.2 Genotypic Correlation Coefficients

Harvest index (1.00), rhizome spread (0.99), dry top yield (0.94) and drying percentage of rhizomes were highly and positively correlated with fresh rhizome yield. Fresh rhizome yield also positively associated with crude fibre content (0.73), LAI (0.69), rhizome thickness (0.65), plant height (0.64), number of leaves (0.43) and number of tillers (0.31). It had negative relation with crop duration, essential oil content and curcumin content.

Drying percentage of rhizome had positive correlation with rhizome spread (0.95), HI (0.93) and dry top yield (0.75). It was also positively associated with plant height, number of tillers, number of leaves, rhizome thickness, crude fibre content and LAI.

Essential oil content (0.26) and crop duration (0.04) had positive correlation with curcumin content, while the other characters are negatively associated with curcumin content.

Crude fibre content showed high positive correlation with drying percentage of rhizome (0.820), rhizome spread (0.79) and HI (0.71). Curcumin content and crop duration are negatively associated with crude fibre content.

Essential oil content had positive correlation with rhizome thickness, crude fibre content, crop duration and LAI, while other characters are negatively associated with essential oil content.

Rhizome spread (0.99), dry top yield (0.92), LAI (0.67), plant height (0.68) and rhizome thickness (0.627) showed high positive correlation with harvest index.

Crop duration was negatively associated with plant height. Rhizome spread, crude fibre content, dry top yield, harvest index and drying percentage of rhizome, number of leaves, rhizome thickness and number of tillers had high positive correlation with crop duration.

4.3.3 Environmental Correlation Coefficients

Most of the correlation coefficients were very low indicating that the effect of environment on expression of the association between the characters was not so strong as to alter it markedly. However the environment influence the association between fresh rhizome yield and harvest index (0.747).

4.4 MOLECULAR CHARACTERIZATION

The result of the experiments carried out for characterizing the *Curcuma* spp. accessions using RAPD markers are presented in this chapter.

4.4.1 DNA Isolation

The genomic DNA was isolated using the procedure suggested by Murray and Thompson (1980). Emerging leaves before fully unfurling were used for DNA isolation.

The DNA yield of 15 accessions estimated using UV-Vis spectrophotometer ranged from 1.26 ng/µl (T_2) to 6.39 ng/µl (T_{13}). The purity of DNA estimated using the O.D. ratio (A_{260}/A_{280}) ranged from 1.52 (T_9) to 1.92 (T_{12}) as shown in Table 11.

4.4.2 Gel Electrophoresis

The quality of DNA was assessed by gel electrophoresis. In some of the samples smearing was observed indicating shearing of DNA. The rest of the DNA was observed as a crisp single band. For those samples showing sheared DNA, the isolation process was repeated and electrophoresed. The bands indicated unsheared good quality DNA. RNA was observed as a thick band below the genomic DNA.

4.4.3 Polymerase Chain Reaction (PCR)

DNA amplification was carried out for the 15 accessions of *Curcuma* spp., adopting the procedure of Williams *et al*. (1990) with

Table 11. Quality and quantity of DNA isolated from different accessions of *Curcuma* spp.

Accessions	260 nm	280 nm	A_{260}/A_{280}	DNA yield ng / µl
T_1	0.097	0.053	1.76	2.91
T_2	0.042	0.024	1.75	1.26
T_3	0.110	0.068	1.61	3.30
T_4	0.165	0.101	1.63	4.95
T_5	0.075	0.043	1.74	2.25
T_6	0.170	0.101	1.68	5.10
T_7	0.155	0.086	1.80	4.65
T_8	0.145	0.079	1.83	4.35
T_9	0.110	0.072	1.52	3.30
T_{10}	0.149	0.087	1.71	4.47
T_{11}	0.172	0.099	1.73	5.16
T_{12}	0.146	0.076	1.92	4.38
T_{13}	0.213	0.111	1.91	6.39
T_{14}	0.166	0.096	1.72	4.98
T_{15}	0.148	0.079	1.87	4.44

required modifications. PCR reactions were carried out in a volume of 25 µl reaction mixture containing 40 ng genomic DNA, 200 µM each of the four dNTPs, 0.8 unit of Taq DNA polymerase and 10 pM of primer, 2.5 µl of 10 x PCR buffer (10 mM Tris-HCl, pH 9.0, 1.5 mM KCl and 0.01 % gelatin) and 3 mM $MgCl_2$.

Forty decamer primers were screened for their efficiency using the DNA isolated from T_3. Out of the forty primers screened, 22 yielded amplification products. The total number of bands, number of faint bands and number of intense bands produced by the primers are given in Table 12 and Fig. 8 to 11.

A total of 59 RAPDs (average 0f 1.47 bands per primer) were generated of which 56 bands were polymorphic.

The highest number of RAPDs were produced by the primer OPB-18 (6 bands) followed by OPB-17 (5 bands) and OPA-4 (4 bands). Of these primers, the highest number of intense bands (4 bands) were produced by OPB-17 and OPB-18. OPA-4 produced three intense bands and one faint band and OPA-3 produced one intense band and three faint bands. OPA-2, OPA-7, OPA-11, OPA-14, OPB-1, OPB-15 and OPB-20 produced three bands each.

Two bands each were obtained when OPA-1, OPA-10, OPA-19, OPB-2, OPB-8, OPB-10, OPB-12 and OPB-13 were used for amplification. The primers OPA-20, OPB-5 and OPB-9 produced one band each. Among these all the three were intense bands.

Out of the forty primers screened, three (OPA-04, OPB-17 and OPB-18) were selected for amplifying DNA from all the *Curcuma* accessions (Plates 34 to 36). PCR reaction was repeated twice for each sample in order to check the reproducibility. The number of bands resolved per amplification was primer dependent and varied from a minimum of 6 (OPB-17 and OPB-18) to a maximum of 7 (OPA-4). The nucleotide

Table 12. Primer associated banding patterns with the DNA of the *Curcuma* spp. accession T_3 using forty primers supplied by the Operon Inc., CA, USA

Sl. No.	Primers	Total number of bands	Number of intense bands	Number of faint bands
1	OPA-1	2	2	0
2	OPA-2	3	0	3
3	OPA-3	4	1	3
4	OPA-4	4	3	1
5	OPA-5	0	0	0
6	OPA-6	0	0	0
7	OPA-7	3	1	2
8	OPA-8	0	0	0
9	OPA-9	0	0	0
10	OPA-10	2	2	0
11	OPA-11	3	2	1
12	OPA-12	0	0	0
13	OPA-13	0	0	0
14	OPA-14	3	0	3
15	OPA-15	0	0	0
16	OPA-16	0	0	0
17	OPA-17	0	0	0
18	OPA-18	0	0	0
19	OPA-19	2	2	0
20	OPA-20	1	1	0
21	OPB-1	3	1	2
22	OPB-2	2	0	2
23	OPB-3	0	0	0
24	OPB-4	0	0	0
25	OPB-5	1	1	0
26	OPB-6	0	0	0
27	OPB-7	0	0	0
28	OPB-8	2	1	1
29	OPB-9	1	1	0
30	OPB-10	2	0	2
31	OPB-11	0	0	0
32	OPB-12	2	0	2
33	OPB-13	2	1	1
34	OPB-14	0	0	0
35	OPB-15	3	1	2
36	OPB-16	0	0	0
37	OPB-17	5	4	1
38	OPB-18	6	4	2
39	OPB-19	0	0	0
40	OPB-20	3	0	3

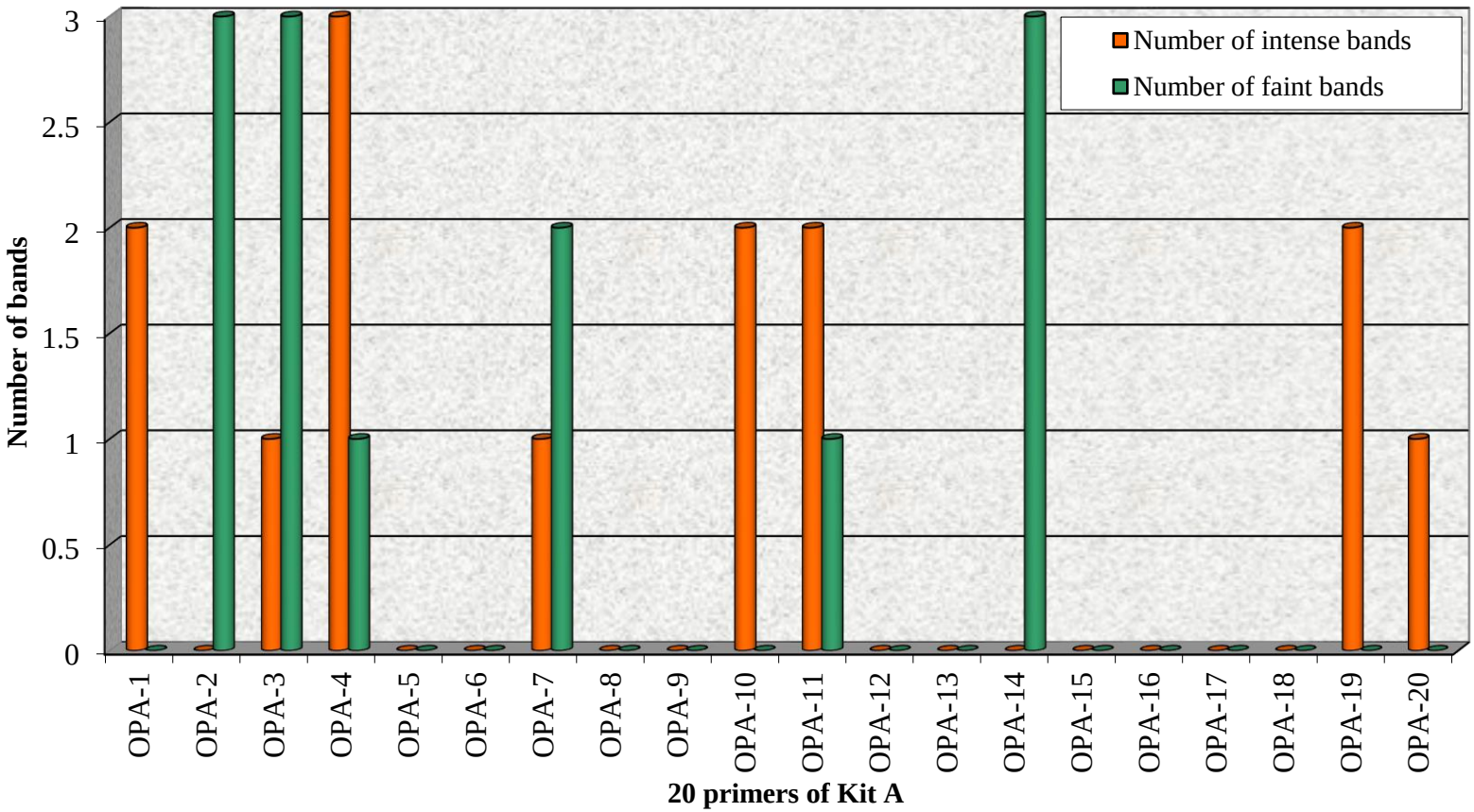

Fig. 8. Amplification profiles (intense and faint bands) of the DNA of T_3 (IISR accession of kasthuri turmeric) using 20 primers of Kit A

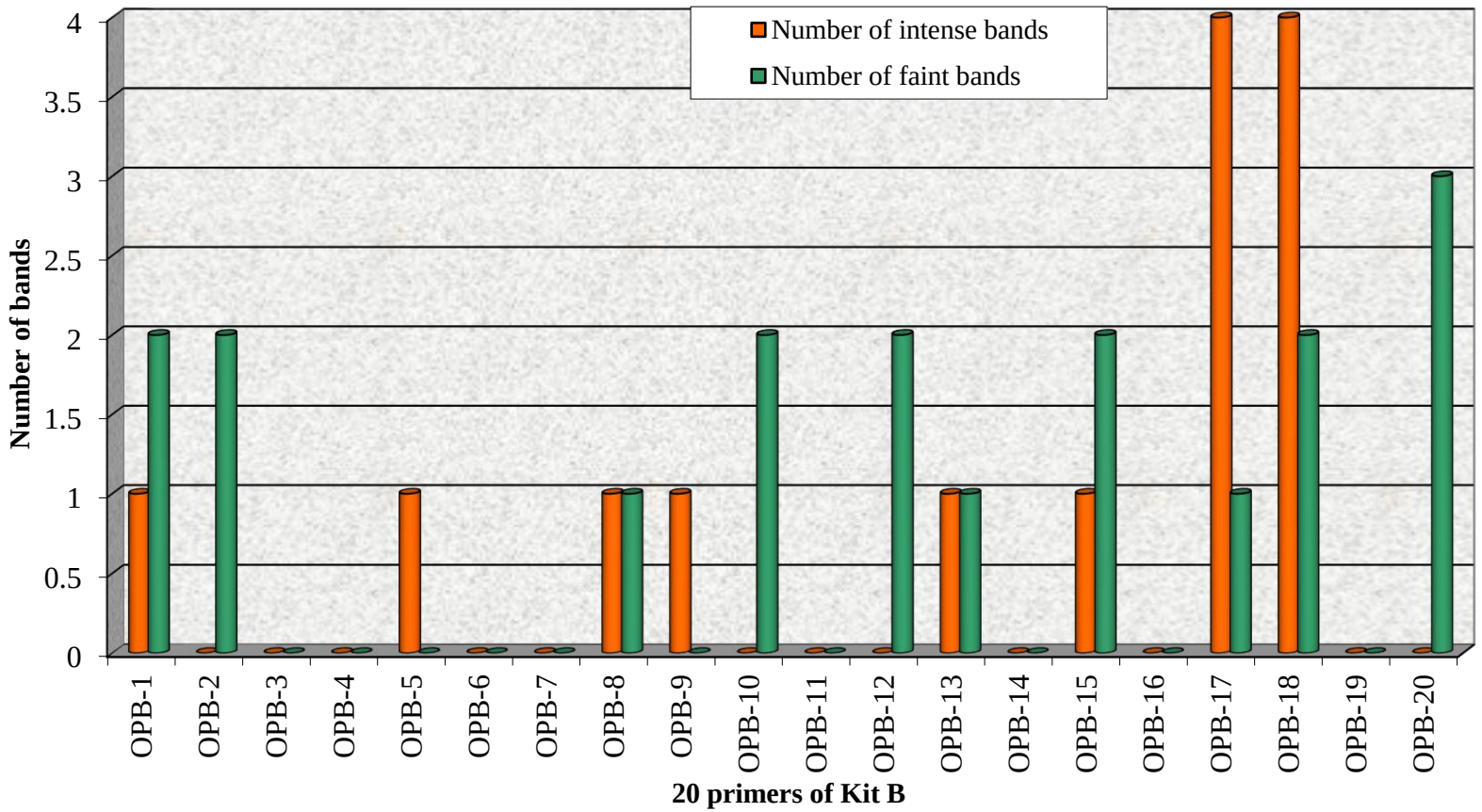

Fig. 9. Amplification profiles (intense and faint bands) of the DNA of T_3 (IISR accession of kasthuri turmeric) using 20 primers of Kit B

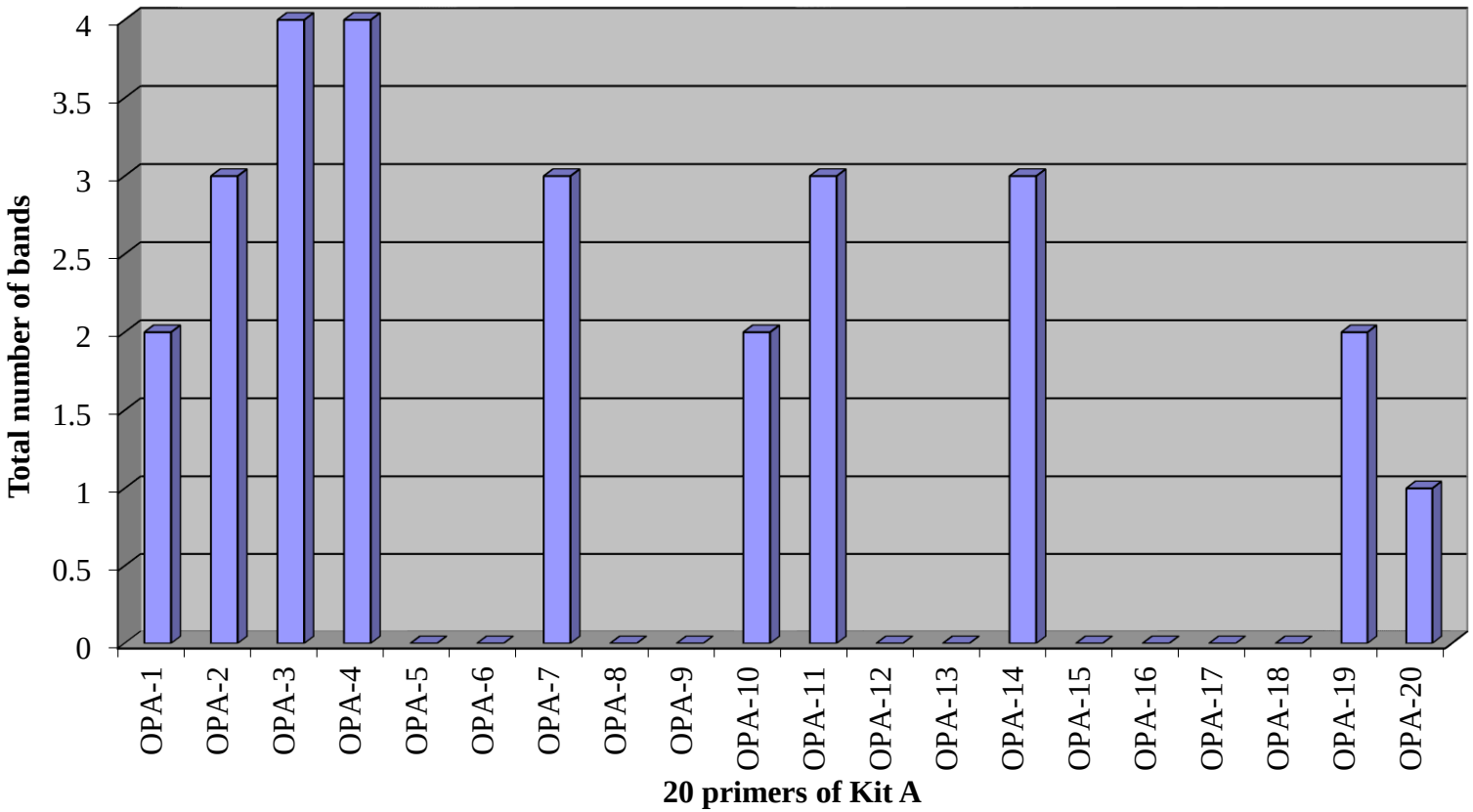

Fig. 10. Amplification profiles (total bands) of the DNA of T_3 (IISR accession of kasthuri turmeric) using 20 primers of Kit A

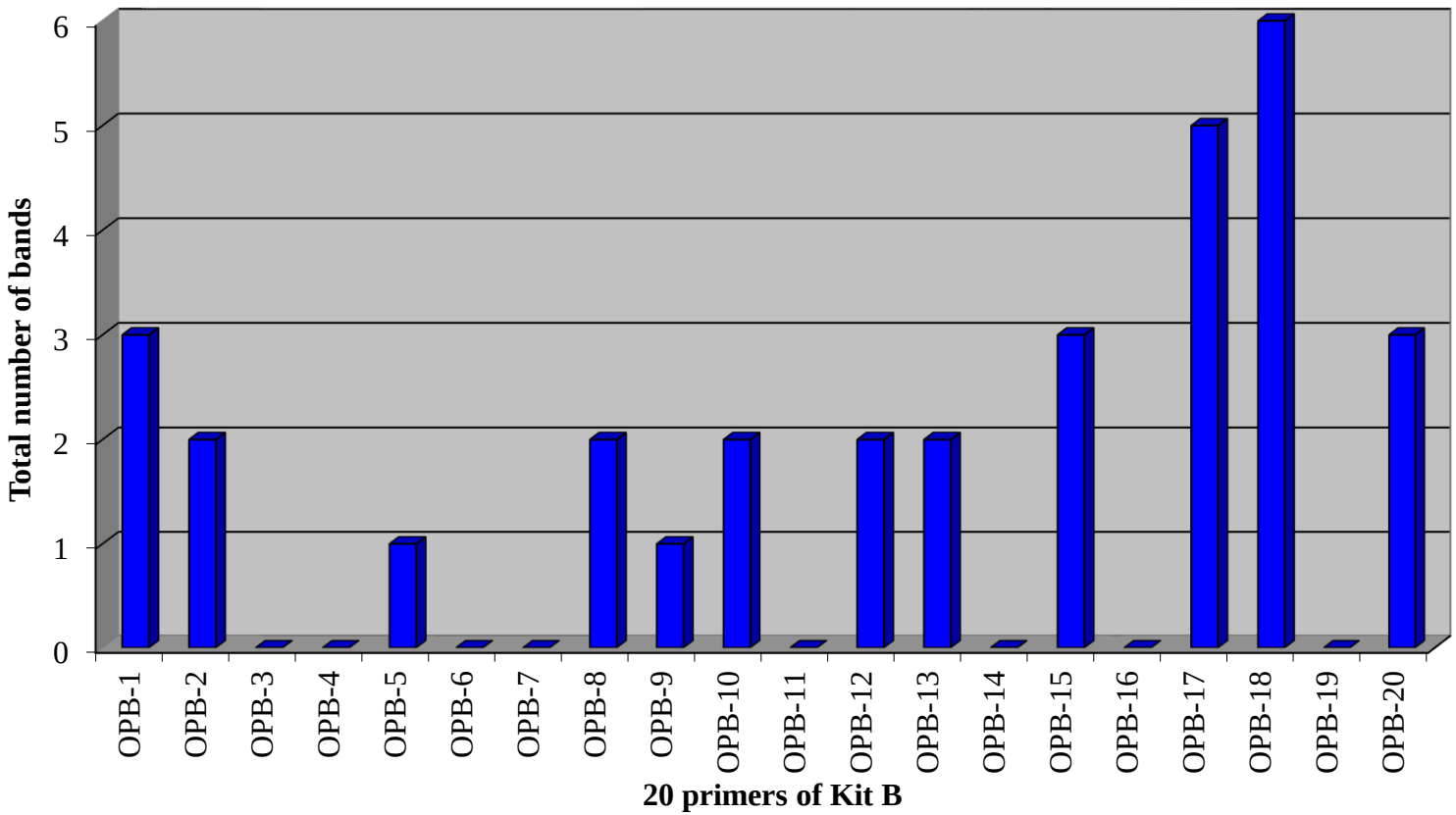

Fig. 11. Amplification profiles (total bands) of the DNA of T_3 (IISR accession of kasthuri turmeric) using 20 primers of Kit B

Plate 34

Amplification profiles of the DNA of 15 *Curcuma* spp. accessions using the primer OPA-04

M : DNA molecular weight marker (U.S. Biochemicals)

1 : Vellanikkara accession

2 : Vellayani accession

3 : IISR accession

4 : Nedumangad accession

5 : Vellanikkara local

6 : Kumily local

7 : Kottoor wild

8 : Pala wild

9 : Thodupuzha accession

10 : Pala White (Wild)

11 : Calicut accession

12 : Turmeric – Alleppey

13 : Turmeric – Vellayani local

14 : Zedoary – yellow

15 : Zedoary – black

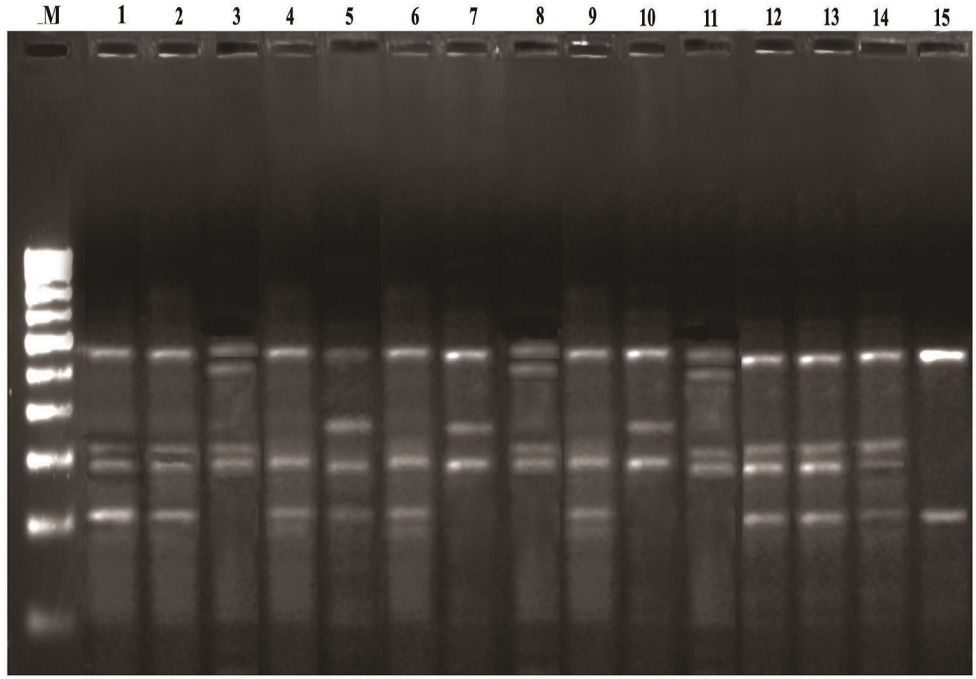

Plate 34. Amplification profiles of the DNA of fifteen *Curcuma* spp. accessions using the primer OPA-4

Plate 35

Amplification profiles of the DNA of 15 *Curcuma* spp. accessions using the primer OPB-17

M : PCR molecular weight marker (U.S. Biochemicals)

1 : Vellanikkara accession

2 : Vellayani accession

3 : IISR accession

4 : Nedumangad accession

5 : Vellanikkara local

6 : Kumily local

7 : Kottoor wild

8 : Pala wild

9 : Thodupuzha accession

10 : Pala White (Wild)

11 : Calicut accession

12 : Turmeric – Alleppey

13 : Turmeric – Vellayani local

14 : Zedoary – yellow

15 : Zedoary – black

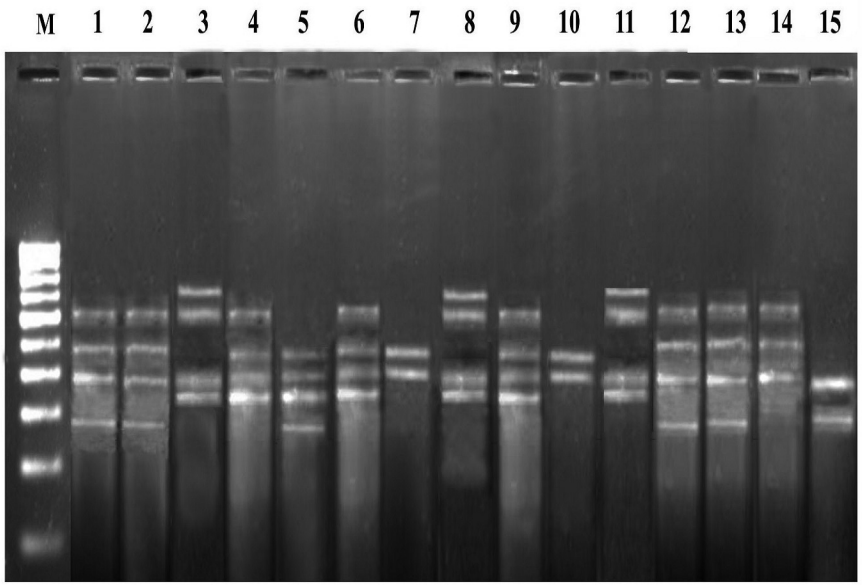

Plate 35. Amplification profiles of the DNA of fifteen accessions of *Curcuma* spp. using the primer OPB-17

Plate 36

Amplification profiles of the DNA of 15 *Curcuma* spp. accessions using the primer OPB-18

M : PCR molecular weight marker (U.S. Biochemicals)

1 : Vellanikkara accession

2 : Vellayani accession

3 : IISR accession

4 : Nedumangad accession

5 : Vellanikkara local

6 : Kumily local

7 : Kottoor wild

8 : Pala wild

9 : Thodupuzha accession

10 : Pala White (Wild)

11 : Calicut accession

12 : Turmeric – Alleppey

13 : Turmeric – Vellayani local

14 : Zedoary – yellow

15 : Zedoary – black

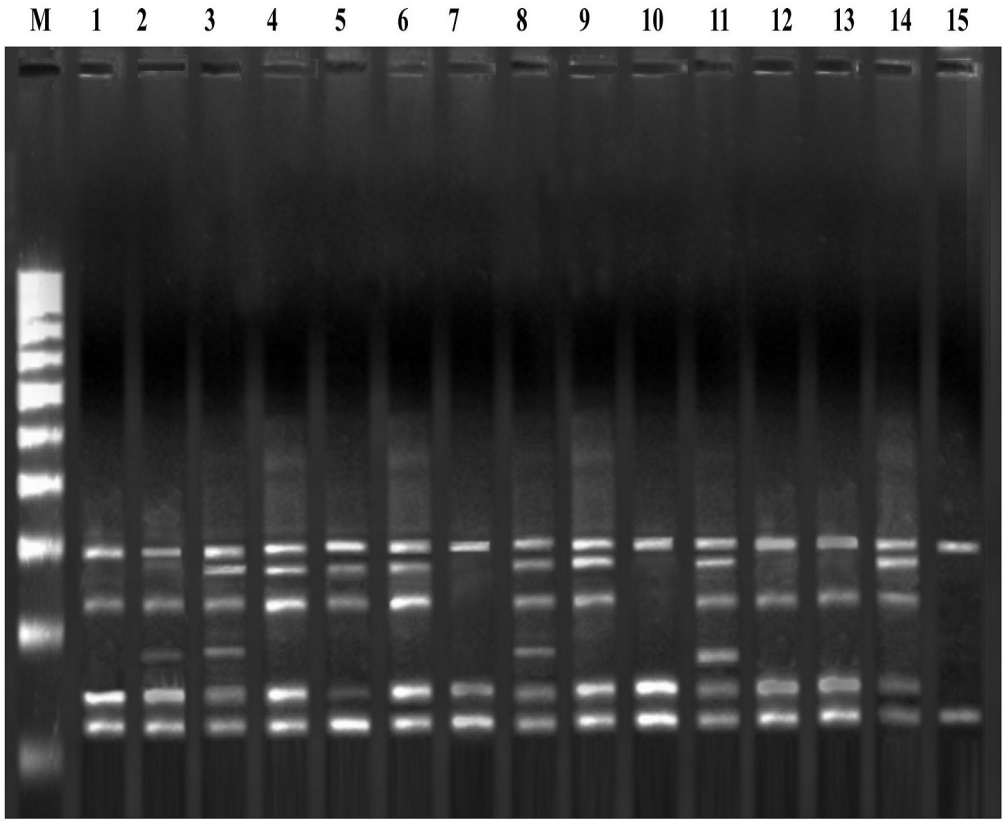

Plate 36. Amplification profiles of the DNA of fifteen *Curcuma* spp. accessions using the primer OPB-18

sequence of informative RAPD markers given by each primer is shown in Table 13. The GC content of the primers used varied from 60 to 70 per cent.

All the three primers selected together produced 19 scorable bands. Of these 19 bands, four bands were monomorphic for all the accessions (21 %). Remaining were polymorphic (79 %).

The highest number of scorable bands (7 bands) was given by the primer OPA-4. The accession T_{15} gave two bands and accessions T_7 and T_{10} gave three bands each. All other accessions gave four bands each when OPA-04 was used for amplification (Fig. 12). Among the seven scorable bands one was monomorphic for all the accessions and six were polymorphic (85.75 %).

Six scorable bands were obtained on amplification using the primer OPB-17. One band produced by this primer was monomorphic for all accessions. Lowest number of bands (2 bands) were produced by the accessions T_7, T_{10} and T_{15} followed by T_{14} (3 bands) whereas all other accessions produced four bands each (Fig. 13).

When OPB-18 was used for amplification, two bands were monomorphic in all accessions (33 %). Remaining were polymorphic (67 %). Highest number of bands were produced by the accessions T_3, T_8 and T_{11} (6 bands) whereas the accession T_{15} produced lowest number of bands (2 bands). Accessions T_4, T_5, T_6, T_9 and T_{14} produced five bands each (Fig. 14).

Statistical Analysis

Reproducible bands were scored for their presence (1) or absence (0) for all accessions studied. RAPD marker data were analysed by using NTSYS (version 2.02) statistical package. A genetic similarity matrix was constructed using the Jaccard's similarity coefficient method. The pair wise coefficient values varied between 0.250 and 1.000. The least

Table 13. Nucleotide sequences of primers and total number of informative RAPD markers amplified with them in the *Curcuma* accessions used in the study

Primer	Sequence (5' – 3')	Number of informative RAPD markers
OPA-04	5' – AATCGGGCTG - 3'	7
OPB-17	5' – AGGGAACGAG - 3'	6
OPB-18	5' – CCACAGCAGT - 3'	6

Fig. 12. Representation of the amplification profile of the DNA of 15 accessions of *Curcuma* spp. using the primer OPA-04

T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
0	0	0	0	1	0	1	0	0	1	0	0	0	0	0
1	1	1	0	0	0	0	1	0	0	1	1	1	1	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
1	1	0	1	1	1	0	0	1	0	0	1	1	1	1
0	0	0	1	0	1	0	0	1	0	0	0	0	0	0

1 – Presence of band 0 – Absence of band

Fig. 13. Representation of the amplification profile of the DNA of 15 accessions of *Curcuma* spp. using the primer OPB-17

T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
1	1	1	1	0	1	0	1	1	0	1	1	1	1	0
1	1	0	1	1	1	1	0	1	1	0	1	1	1	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	1	1	1	1	0	1	1	0	1	0	0	0	0
1	1	0	0	1	0	0	0	0	0	0	1	1	0	1

1 – Presence of band 0 – Absence of band

Fig. 14. Representation of the amplification profile of the DNA of 15 accessions of *Curcuma* spp. using the primer OPB-18

T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	1	1	1	1	0	1	1	0	1	0	0	1	0
1	1	1	1	1	1	0	1	1	0	1	1	1	1	0
0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1 – Presence of band 0 – Absence of band

similarity values were those of accessions T_{15} and T_3, T_8 and T_{15} and T_{11} and T_{15}.

The cent per cent similarity index was obtained for accession T_{12} and T_1, T_{12} and T_{13}, T_8 and T_3, T_{11} and T_3, T_6 and T_4, T_9 and T_4, T_6 and T_9, T_7 and T_{10}, T_8 and T_{11} and T_{12} and T_{13}. Similarity matrix obtained for all the accessions is shown in Table 14.

The estimation of similarity coefficient and construction of dendrogram using the UPGMA (Unweighted Pair Group Method for Arithmetic average) method revealed the presence and extent of genetic similarity among the fifteen *Curcuma* accessions examined.

On drawing a vertical line in the dendrogram along the point corresponding a similarity coefficient value of 0.63, all the 15 accessions got divided into four clusters. Accessions T_5, T_9, T_6, T_4, T_{14}, T_2, T_{13}, T_{12} and T_1 together formed the largest cluster. Within this cluster, accessions T_1, T_{12} and T_{13} and T_4, T_6 and T_9 are having cent per cent similarity (Fig. 15).

Again a vertical line in the dendrogram drawn along the point corresponding to a similarity coefficient value of 0.69, all the 15 accession got divided into five clusters. Accessions, T_1, T_{12}, T_{13}, T_2 and T_{14} together formed the largest cluster followed by the accessions T_4, T_6, T_9 and T_5 which together formed the second largest cluster. Accessions T_3, T_8 and T_{11} together formed a cluster. Accessions T_7 and T_{10} formed another cluster. The accession T_{15} and formed a single separate cluster at both the distances.

Table 14. Similarity matrix among fifteen accessions of *Curcuma* spp. obtained by RAPD analysis using three primer combinations

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.000														
2	0.923	1.000													
3	0.529	0.588	1.000												
4	0.667	0.625	0.588	1.000											
5	0.667	0.625	0.500	0.733	1.000										
6	0.667	0.625	0.588	1.000	0.733	1.000									
7	0.538	0.500	0.375	0.500	0.615	0.500	1.000								
8	0.529	0.588	1.000	0.588	0.500	0.588	0.375	1.000							
9	0.667	0.625	0.588	1.000	0.733	1.000	0.500	0.588	1.000						
10	0.538	0.500	0.375	0.500	0.615	0.500	1.000	0.375	0.500	1.000					
11	0.529	0.588	1.000	0.588	0.500	0.588	0.375	1.000	0.588	0.375	1.000				
12	1.000	0.923	0.529	0.667	0.667	0.667	0.538	0.529	0.667	0.538	0.529	1.000			
13	1.000	0.923	0.529	0.667	0.667	0.667	0.538	0.529	0.667	0.538	0.529	1.000	1.000		
14	0.846	0.786	0.625	0.786	0.667	0.786	0.538	0.625	0.786	0.538	0.625	0.846	0.846	1.000	
15	0.500	0.462	0.250	0.357	0.462	0.357	0.400	0.250	0.357	0.400	0.250	0.500	0.500	0.385	1.000

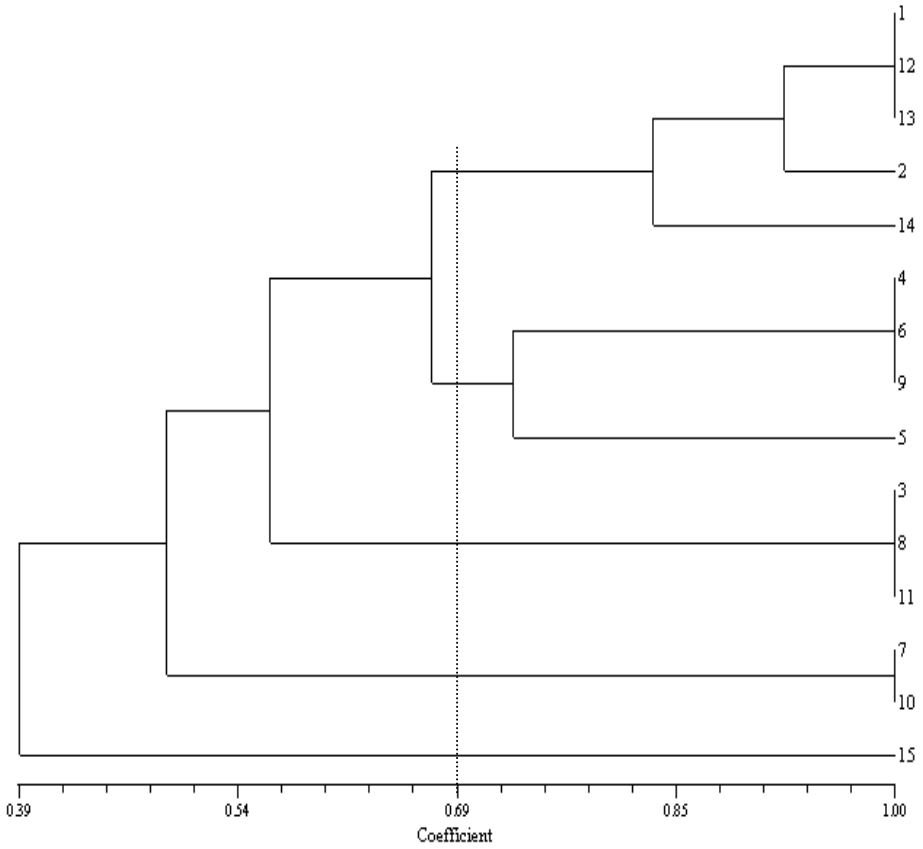

Fig. 15. Dendrogram obtained from RAPD analysis using UPGMA method

Discussion

5. DISCUSSION

Kasthuri turmeric (*Curcuma aromatica* Salisb.) is a medicinal and aromatic plant with multiple uses. Many cosmetics and ayurvedic preparations claim to contain kasthuri turmeric. Eventhough kasthuri turmeric has got wide range of applications, it is getting slowly depleted from cultivation and production due to various reasons. The ignorance about the true identity of the crop is the major reason for decline in cultivation of this crop. This has also led to the use of any turmeric, especially zedoary (*Curcuma zedoaria* Roxb.) as kasthuri turmeric.

Wide variation is noticed in morphological features, quality and physiological aspects and yield of various accessions of kasthuri turmeric, zedoary and ordinary turmeric used for the comparative study and characterization. An insight into to the magnitude of variability and characterization based on every aspects of the crop is essential for determining the true identity of the crop and for further studies for crop improvement.

Though characterization based on morphological attributes was done by few workers, the results are not convincing as it is influenced by environment. As it is a vegetatively propagated crop, molecular characterization and classification deserves priority in terms of improvement. Information generated through DNA profiling using RAPD markers would give a comprehensive picture on diversity and relatedness among the accessions of various *Curcuma* species evaluated.

Fifteen accessions of *Curcuma* spp. were evaluated in the field of which eleven were kasthuri turmeric accessions, two were ordinary turmeric and two were zedoary accessions. Morphological, anatomical, physiological, biochemical and molecular characterization of these

cultivars were done. The accessions were grouped into clusters based on the results of molecular characterization.

The results of the study are discussed below:

5.1 MEAN PERFORMANCE OF THE ACCESSIONS

5.1.1 Growth Characters

Analysis of variance showed significant difference among the accessions for most of the characters studied. The morphological characters evaluated were plant height, number of tillers, number of leaves, leaf area, rhizome spread, root spread, rhizome thickness, dry root weight, and root length. All these characters varied significantly such that kasthuri turmeric, ordinary turmeric and zedoary differed markedly among them.

In the present study the accession T_{14} recorded maximum plant height, followed by T_{15} at all growth stages, which were two accessions of zedoary used for comparative study. The least plant height was recorded in the accessions T_{13} and T_1 at different growth stages of which T_{13} was an ordinary turmeric variety and T_1 was a local accession of kasthuri turmeric which closely resembled ordinary turmeric. Among the kasthuri turmeric accessions T_5 and T_6 recorded maximum plant height. The results indicated that the zedoary accessions have more vigourous growth at all the growth stages which can be attributed to the genetic makeup of the crop. This finding is in consonance with the findings of Lynrah and Chakrabarthy (2000) who recorded most vigorous growth in zedoary when compared with ordinary turmeric and kasthuri turmeric.

A high variability was recorded with respect to the number of tillers. The highest number of tillers (6.22) was recorded in T_7 followed by T_{10} (6.20) which were two wild accessions of kasthuri turmeric with almost similar characters. A similar finding in ginger where varietal variation in the number of tillers was reported by Singh *et al*. (1999).

Number of leaves among the various accessions also showed considerable variation during all the growth stages which ranged from 23.14 (T_{10}) to 10.69 (T_1) with a mean value of 17.82. The results of the study conducted by Singh *et al*. (2003) also confirmed a great variation in the number of leaves when 65 ordinary turmeric accessions alone were evaluated. Similar results were obtained by Narayanpur *et al*. (2003) when they evaluated 16 cultivars of turmeric. Yadav (2002) also reported considerable variation in leaves per clump among six cultivars of turmeric evaluated.

The role of leaf area in plant vigour and productivity is very important as the leaves are the prime organs for synthesis of carbohydrates and other organic substances. Leaf area recorded maximum in T_{15} (8226.66 cm^2) and T_{14} (7680.00 cm^2) which were zedoary accessions and the minimum recorded in T_1 (2656.00 cm^2) which was a local accession of kasthuri turmeric which closely resembled ordinary turmeric. This finding is in consonance with the results derived by Subramanian and Abdulkhader (1988) on leaf area of certain turmeric accessions where it ranged between 4716 to 5292 cm^2. Many of the kasthuri turmeric accessions also had a leaf area above the general mean and had a medium leaf size.

Values with respect to rhizome spread have also showed significant variation among the three species compared. It was found maximum in the zedoary accessions T_{15} (27.59 cm) and T_{14} (26.46) and the least in the turmeric accessions used for comparison, T_{12} (17.96 cm). The kasthuri turmeric accessions were found to have rhizome spread values in between the other two species. This finding is in accordance with the observation that significant genotypic differences are there for rhizome spread in ginger as observed by Korla *et al*. (1999). Indiresh *et al*. (1992) also reported similar findings in turmeric. Values with respect to root spread also exhibited the same trend. Rhizome thickness values were also found

maximum in the zedoary accessions and kasthuri turmeric accessions had rhizomes with medium thickness ranging with the 1.27 to 1.54 cm.

Dry root weight recorded maximum in the kasthuri turmeric accession T_5 followed by the wild accession T_{10} and the Kozhikode accession T_{11}. Root length was also highest in T_5 (22.47 cm) followed by T_{11} (20.62 cm) and found least in the ordinary turmeric accession T_{13} (15.33 cm).

Morphological cataloguing based on selected characters revealed that some of these characters are specific to certain accession only. Leaf pubescence was observed only in the kasthuri turmeric accessions T_3, T_7, T_8, T_{10} and T_{11}. Among these, T_3, T_8 and T_{11} were densely pubescent while T_7 and T_{10} were thinly pubescent. Mangaly and Sabu (1993) reported similar finding regarding leaf pubescence in kasthuri turmeric. This character can be taken up as a morphological marker for easy identification of kasthuri turmeric.

Pigmentation of the leaf midrib was observed only in the zedoary accessions T_{14} and T_{15} and in the kasthuri turmeric accession T_5. Similar observations in zedoary was earlier reported by Mangaly and Sabu (1993). Leaf midrib pigmentation can be considered as a distinguishing feature of zedoary.

Analysis of the results of morphological studies show that most of the characters vary considerably among the three species of *Curcuma* which were compared. Growth characters like plant height, leaf area, rhizome spread, root spread and rhizome thickness were found to be maximum in the zedoary accessions. This shows that zedoary is more vigorous in above growth characters compared to kasthuri turmeric and it can be used as a distinguishing feature between the two. These characters were found minimum in the accessions T_{12}, T_{13}, T_1 and T_2 of which the first two were ordinary turmeric accessions and the latter two local kasthuri turmeric accessions which closely resembled ordinary turmeric.

All other kasthuri turmeric accessions performed in between the zedoary and ordinary turmeric accessions used for comparative study.

The two wild accessions of kasthuri turmeric, T_7 and T_{10} recorded maximum number of tillers (6.22 and 6.20) and number of leaves (21.44 and 23.14) indicating that they will form a separate group among the kasthuri turmeric accessions studied.

5.1.2 Biochemical Characters

Analysis of biochemical characters have also showed significant variation among kasthuri turmeric, zedoary and ordinary turmeric accessions. Curcumin content was the major biochemical character analysed and was found maximum in the ordinary turmeric accessions T_{13} (5.94) and T_{12} (5.78) and was least in the zedoary accession T_{15} (0.01) which is commonly referred as black zedoary having a bluish grey rhizome. The wild accessions of kasthuri turmeric, T_7 and T_{10} recorded very low curcumin content of 0.026 and 0.033 per cent respectively. Their rhizomes were white in colour indicating very low curcumin content. Kasthuri turmeric accessions from IISR, Kozhikode (T_3), Pala (T_8) and Kozhikode (T_{11}) recorded curcumin contents 0.05, 0.04 and 0.04 per cent respectively indicating their similarity regarding this character and had similar light pale yellowish or creamy rhizomes. This similarity coupled with the similarity in case of morphological features can be used to conclude that these three accessions belong to a single group.

The findings Poduval *et al*. (2001) also confirm that although *C. aromatica* and *C. zedoaria* produced rhizomes more than the cultivars of *C. domestica*, the two species had the least curcumin contents. The variation in curcumin content among the various accessions was earlier noted by Garg *et al*. (1999). Narayanpur *et al*. (2003) attributed the reason for this variation among the genotypes in curcumin content to the genetic character of the cultivars. Hegde and Venkatesha (1997) have also

reported that variation in curcumin content among the cultivars can be attributed to genetic factors.

The total chlorophyll content in the leaves ranged between 0.99 to 1.29 mg g^{-1} and the variation observed is not so distinctive to demarcate between the accessions. This finding is in line with the chlorophyll content estimates in various *Curcuma* spp. by Niranjan *et al*. (2003). However the highest chlorophyll contents were observed in the kasthuri turmeric accessions T_8, T_{11} and T_3 which showed uniqueness in this character also.

The essential oil content, which determines the characteristic aroma of rhizomes also varied significantly among the various accessions. The maximum oil content was observed in the accessions T_3 (5.83 %) followed by the accessions T_{11} (5.80 %) and T_8 (5.53 %). These three kasthuri turmeric accessions were having similar rhizome characters and aroma differing from the other accessions. T_7 and T_{10} recorded least values whose rhizomes were almost odourless. The ordinary turmeric accessions recorded essential oil content above the general mean value (3.56 %), while the zedoary accessions were low in essential oil content.

The high essential oil content in the three kasthuri turmeric accessions is in agreement with the findings of Zwaving and Bos (1992), who recorded 3.5 per cent essential oil in *C. domestica*, while *C. aromatica* recorded a higher content of 9.4 per cent. Higher oil content of six per cent in *C. aromtica* with flowerly camphoraceous odour as reported by CSIR (1950) is also in line with the present finding. Similar reports on essential oil content of *Curcuma* spp. were given by Nirnjan *et al*. (2003) and Chattopadhyay *et al*. (2004).

Crude fibre content was also found maximum in the three kasthuri turmeric accessions T_8, T_3 and T_{11} in which earlier examined characters were also similar. All other accessions had a lesser fibre content and note

varied considerably. Similar report on crude fibre content was earlier given by Padmapriya and Chezhiyan (2004).

The ash content in the rhizomes varied slightly among the accessions and did not show any specificity with any of the species. Reports on the ash content of ordinary turmeric rhizomes by Peter (1999) and Niranjan (*et al*. (2003) are in line with the above finding. Sugar content in the rhizomes varied only slightly among various accessions and the maximum content was recorded in the ordinary turmeric accession, T_{13}.

Starch content was found maximum in the zedoary accession T_{15} (25.39) and minimum in Thodupuzha accession, T_9 (22.47). The average starch content in the rhizomes (23.59 %) is in agreement with the starch content in kasthuri turmeric rhizomes recorded by CSIR (1950).

The protein content was recorded maximum in the kasthuri turmeric accessions T_3 and T_8 and the average protein content was 5.42 per cent which was in agreement with the finding of Niranjan *et al*. (2003) and Padmapriya and Chezhiyan (2004). Alkaloids were found only in trace quantities and the variation was also very less. This finding is in consonance with the findings of Zakaria and Ibrahim (1987), who reported trace amounts of alkaloids in ginger and turmeric. Moisture content in the dried rhizomes was found maximum in he zedoary accessions T_{15} (13.28 %) and T_{14} (13.07 %), whereas most of kasthuri turmeric accessions recorded values around the general mean value (11.91 %). Similar values on moisture content of turmeric rhizome was reported by Padmapriya and Chezhiyan (2004).

The major outcome of the biochemical studies was that the three accessions of kasthuri turmeric, T_3 (IISR accession), T_8 (Pala wild) and T_{11} (Kozhikode accession) performed uniquely and was superior in terms of essential oil content and chlorophyll content. These accession also recorded a higher protein and fibre content. Curcumin content was very

less, below, one per cent in these accessions. But the yellow zedoary and ordinary turmeric accessions used for comparative study recorded a higher curcumin content, less essential oil and crude fibre contents. Other characters studied also varied significantly among the accessions and a clear difference was established between these three accessions of kasthuri turmeric, ordinary turmeric and the zedoary used for the comparative study and characterization.

5.1.3 Yield Characters

Yield characters varied significantly among all the accessions. Highest fresh rhizome yield per plant was recorded in zedoary accessions T_{14} (478.31 g) and T_{15} (453.33 g), while the turmeric accessions yielded much below the general mean yield (358.34 g). Most of the kasthuri turmeric accessions especially T_3, T_8 and T_{11} yielded more than the general mean yield. The higher yield in zedoary and kasthuri turmeric is in agreement with the findings of Poduval *et al*. (2001) and Lynrah and Chakrabarthy (2000). Singh *et al*. (2003) observed greatest variation in the yield character in 65 genotypes of turmeric evaluated. Korla *et al*. (1999) observed genotype differences in the rhizome size and yield per plant in ginger. The dry rhizome yield per plant also showed the same pattern of fresh rhizome yield with slight variations depending upon the drying percentage. The dry top yield of all the accessions varied and followed the same pattern of variation as in fresh rhizome yield.

Crop duration varied significantly among the accessions and was found highest in the ordinary turmeric accessions T_{13} (231.67 days) and T_{12} (228.67 days). Least crop duration was observed in a local accession of kasthuri turmeric, T_1 (193.33 days). The crop duration ranged from 215 to 232 days in six cultivars of turmeric evaluated by Yadav (2002) is also in agreement with the present findings on crop duration.

Analysis of the yield characters shows that zedoary accessions are superior in rhizome yield and the kasthuri turmeric accessions yielded

higher compared to the ordinary turmeric accessions. Crop duration of kasthuri turmeric was lesser compared to ordinary turmeric.

5.1.4 Physiological Characters

The physiological characters analysed also varied significantly among the different *Curcuma* accessions. The dry matter production varied among the accessions and was found to be proportionate to the dry rhizome and top yield. Drying percentage of rhizomes also varied from a maximum of 28.26 per cent in T_7, followed by 27.90 per cent in T_3 to a minimum of 20.57 per cent in T_{12}. The maximum values recorded were in kasthuri turmeric accessions and minimum was in ordinary turmeric. The variation in recovery percentage among various turmeric cultivars was earlier studied by Radhakrishnan *et al*. (1995) and they attributed the reason for the variation to genetic factors rather than the environmental conditions under which they are grown. Drying percentage in 16 turmeric accessions varying from 18 to 24 per cent was also reported by Narayanpur *et al*. (2003). Similar reports were recorded by Choudhari and Hore (2004) and Sasikumar (2004).

Leaf area index recorded maximum in the two zedoary accessions T_{15} and T_{14} and was least in the kasthuri turmeric accession T_1. Most of the kasthuri turmeric accessions and ordinary turmeric accessions recorded LAI above the general mean value (9.004). Significant variation in the LAI of turmeric cultivars were earlier reported by Narayanpur *et al*. (2003).

Leaf area duration was also found maximum in the zedoary accessions and leas in the kasthuri turmeric accession T_1. Most of the other kasthuri turmeric accessions and ordinary turmeric accessions recorded LAD above the general mean. Harvest index is the indicator of economic yield of a crop and it was found varying among most of the accessions evaluated. It was found maximum in the zedoary accessions T_{14} (0.94) followed by T_{15} (0.92) and the kasthuri turmeric accession

T_5 (0.92). Least HI was recorded in T_2 (0.62). Harvest index varying in the range of 0.91 to 0.95 was earlier reported by Subramanian and Abdulkhader (1988).

Analysis of the physiological characters revealed distinct variation among the various accessions of *Curcuma* compared. Dry matter production, LAI, LAD and HI were found higher in the zedoary accessions differentiating them from the other accessions. The kasthuri turmeric accessions T_3, T_5, T_8, T_9, T_{11} and ordinary turmeric accessions recorded LAI and LAD values above the general mean value. The drying percentage of rhizomes was found high in the kasthuri turmeric accessions T_7 and T_3 which recorded higher yield also. LAI, LAD and HI were found least in the accessions T_1 and T_2 which were short statured, low yielding and shorter duration accessions.

5.1.5 Anatomical Characters

Some of the anatomical characters were also analysed to characterize the kasthuri turmeric accessions. Chandurkar (1989) reported that, taken along with other characters like morphology, cytology and embryology, anatomical characters help in taxonomic studies considerably. Foliar epidermal structures were used by Baruah and Nath (2002) to identify the taxonomic status of certain plants. The leaf cuticle thickness ranged from 0.42 to 0.30 μm. Samasya (2000) reported leaf cuticle thickness ranging from 0.5 to 2 μm in orchid. In the present study, number of stomata was found more on the lower leaf surface than the upper surface. But the variation observed among various accessions were found to be very less to distinguish them based on this character. But Ghahreman *et al*. (1998) reported that stomatal occurrence, and stomatal index are most useful anatomical characters for taxonomic purposes.

The vascular bundles observed in the root consisted of alternating strands of xylem and phloem embedded in the conjunctive tissue. Xylem was arranged in two rings, one made of large metaxylem vessels towards

the centre and the other made of small protoxylem vessels towards the outside. Xylem vessels were circular in outline. The number of vascular bundles in roots did not vary considerably among the accessions and it ranged between 21 to 17.67 numbers. The anatomical characters analysed were more or less similar in all the accessions and any profound species specific variation was not observed.

5.2 VARIABILITY STUDIES

As the preliminary step in any crop improvement programme, the knowledge of the genetic variability of different characters is very essential. The variations may be due to genetic and environmental effects.

Coefficient of variation – PCV and GCV are better indices for comparison of characters with different units of measurements, than estimates of quantitative variations like range and variation around mean.

Magnitude of genotypic and environmental components of phenotypic variance and coefficient of variance at genotypic and phenotypic levels in the accessions are also evaluated.

PCV ranged from 5.737 to 15.744 and GCV ranged from 5.364 to 105.573. Among the different characters, curcumin content, essential oil content, number of tillers, leaf area index and fresh rhizome yield had higher PCV and GCV values. Yadav (1999) reported a high GCV for length and weight of secondary rhizome, weight of primary rhizome, number of secondary and primary rhizome and rhizome yield per plant. Similar reports were given by Prasad *et al*. (1998) showing high to moderate coefficient of variability for number of tillers, number of leaves, leaf area, number of primary rhizomes, rhizome weight per plant and plant height..

Higher GCV and PCV for most of the characters revealed great extent of variability for these characters suggesting good scope for improvement through selection. Furthermore, the magnitude of genetic

variation nearly approached phenotypic variation, in all the characters, indicating that the selection on phenotypic basis will hold good scope on genetic upgradation.

5.3 CORRELATION STUDIES

The degree and direction of the inherent association (genotypic correlation) of characters apart from the observable correlation (phenotypic correlation) between two characters are important for the simultaneous selection of characters for genetic improvement. Correlation coefficient gives an idea about the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement in yield. The presence of genotypic correlation may be either due to pleiotropic action of genes or due to linkage or more likely both. If a positive genotypic correlation was observed for a pair of characters, certainly the improvement in one character on the correlated response to selection. If the improvement in one of the characters results in a decrease in another character, this will also help the breeder in the selection of characters if necessary.

Correlation coefficient is a statistical measure used to find out the degree and direction of relationships between two or more variables. So this helps in understanding the change caused in one character by doing selection based on another character.

The results showed that genotypic correlation is higher than the phenotypic correlation and the environmental correlation was less, revealing strong association at genotypic level between the characters. In the current experiment, harvest index, dry percentage recovery of rhizomes, dry top yield, rhizome spread, LAI, plant height, rhizome thickness and number of leaves showed positive genotypic correlation with fresh rhizome yield. From this, it is evident that selection based on these characters will result in higher yield. Similar results were obtained by Hazra *et al*. (2000) and Jana *et al*. (2001). Correlation studies

conducted by Mohanty (1979) and Reddy (1987) in turmeric indicated that weight of primary, secondary and mother rhizomes had a direct effect on yield.

Selection based on rhizome spread, HI and dry top yield will result in an increase in dry percentage recovery of rhizomes, because these three characters have high genotypic correlation with dry recovery percentage of rhizomes.

Curcumin content was found to be positively correlated with essential oil content and maturity period. All other characters are negatively correlated with curcumin content. A similar report on positive correlation with the essential oil content and oleoresin content in ginger was given by Singh *et al*. (2000).

Dry recovery percentage of rhizomes, rhizome spread and HI have positive genotypic correlation with crude fibre content. Essential oil and crude fibre contents were found to have a positive correlation. Characters like rhizome spread, dry top yield, LAI, plant height and rhizome thickness were found positively correlated with HI of the crop. Cured yield positively correlated with yield of secondary fingers, as reported by Jalgaonkar *et al*. (1990) is in line with this finding. Correlation studies also revealed that number of leaves, number of tillers and rhizome thickness were positively correlated with crop duration.

The phenotypic correlation includes both genetic and environmental effects. In the present study, the magnitude of genotypic correlation was higher than the corresponding phenotypic correlation indicating that environment had negligible effect on these characters.

5.4 MOLECULAR CHARACTERIZATION

The present study was undertaken to characterize the fifteen accessions of *Curcuma* using RAPD markers. Out of the fifteen accessions, eleven belonged to *C. aromatica*, two belonged to *C. zedoaria*,

and two belonged to *C. longa*. The results obtained are discussed in detailed hereunder.

Isolation of genomic DNA of *Curcuma* spp. was done using modified method of Murray and Thompson (1980). The quantity and quality of isolated DNA depend on the source as well as efficient disruption of plant cell wall. Emerging leaves before fully unfurling were used for the isolation of DNA from *Curcuma* accessions. The yield of DNA and its purity varied with accessions. The yield ranged from 1.26 ng μl^{-1} (T_2) to 6.39 ng μl^{-1} (T_{13}). The purity of the DNA (A_{260}/A_{280}) of various accessions ranged from 1.52 (T_9) to 1.92 (T_{12}).

The DNA obtained using modified Murray and Thompson method was white in colour indicating that there is no interference of phenols during isolation.

Agarose gel electrophoresis was used for analyzing the genomic DNA isolated from different accessions as well as for the RAPD products. The concentration of gel is an important factor for the separation of DNA fragment. A low concentration of agarose is ideal for the separation of genomic DNA, which are of high molecular weight while small DNA fragments can give good separation in high concentration of agarose gel. In this study 0.90 per cent agarose was used for genomic DNA and 1.20 per cent for RAPD analysis, which were found satisfactory for the resolution of bands.

5.4.1 RAPD Analysis

The PCR amplification was carried out using 40 decamer primers (Operon Inc., CA, USA) of kit A and kit B with the DNA of accession T_3. The procedure standardized by Williams *et al*. (1990) with required modifications was used for amplification. Out of the 40 primers screened, 22 yielded amplification products. The total number of bands ranged from one to six.

The primers OPA-5, OPA-6, OPA-8, OPA-9, OPA-12, OPA-13, OPA-15, OPA-16, OPA-17, OPA-18, OPB-3, OPB-4, OPB-6, OPB-7, OPB-11, OPB14, OPB-16 and OPB-19 did not yield any bands. This indicated that there is no sequence complementary to the sequence of these primers in the DNA of T_3.

A total of 59 RAPDs (average of 1.47 bands per primer) were generated of which, 56 bands were polymorphic.

In the present study, three primers were finally selected for the RAPD analysis for all the accessions based on the number of intense bands obtained. They were OPB-17, OPB-18 and OPA-4. According to Weising *et al*. (1995) primers with a GC content of at least 50 per cent should be used. In this study the GC content of the primers varied from 60 to 70 per cent. The PCR reaction was repeated twice in order to check the reproducibility.

The primers used in the analysis yielded 19 scorable bands with an average of 6.33 bands per primer. The number of bands resolved per amplification was primer dependent and varied from six to seven.

The highest number of scorable bands was given by the primer OPA-04 (7 bands), among these one band was monomorphic and six bands were polymorphic (85.75 %). Except the accessions T_{15}, T_7 and T_{10}, all others produced four bands each. The primer OPB-17 produced six scorable bands when used for amplification. One band produced by this primer was monomorphic for all the accessions.

The primer OPB-18 produced six scorable bands among which 33 per cent were monomorphic (2 bands) and 67 per cent were polymorphic (4 bands).

All the three primers together produced four monomorphic (21.05 %) and 15 polymorphic bands (78.94 %). These primers together produced

highest number of polymorphic bands, serving well in fine differentiation of the 15 accessions of *Curcuma* spp. studied.

5.4.2 Statistical Analysis

Jaccard's pair-wise similarity coefficient values for 15 accessions of *Curcuma* spp. were calculated and presented in Table 14. The range of genetic similarity was found to be between 0.250 to 1.00. Cent per cent similarity were obtained between the accessions T_{12} and T_1, T_{13} and T_1, T_8 and T_3, T_3 and T_{11}, T_6 and T_4, T_9 and T_6, T_{10} and T_7, T_{11} and T_9 and T_3 and T_{12}. Accessions having cent per cent similarity are having similar genomic constitution and can be originated from the same ancestry.

A dendrogram was generated by UPGMA cluster analysis based on Jaccard's similarity coefficients (Fig. 15). Cluster analysis revealed that at 63 per cent similarity the 15 accessions got divided into four groups.

The first cluster consisted of only one accession *i.e.,* T_{15} which is the black zedoary accession. The accessions T_7 and T_{10} together formed the second group. Both are collected from Pala (Kottayam) and Kottoor (Thiruvananthapuram) are having cent per cent similarity.

The third cluster included the three accessions (T_3, T_8, T_{11}). The three accessions coming under this cluster are having cent per cent similarity.

The fourth cluster consisted of nine accessions at 63 per cent similarity. At 69 per cent similarity this cluster again got divided into two sub clusters. One sub cluster having four accessions *i.e.,* T_4, T_6, T_9 and T_5. Second cluster is having five accessions. The accessions are T_{14}, T_1, T_2, T_{12} and T_{13} are coming under this cluster.

The accessions of *C. zedoaria* are coming under different clusters. Phenotypically also they look different. The rhizomes of the accession T_{15} was bluish grey in colour with low curcumin content, whereas as the other

one (T_{14}) having yellowish rhizomes and a comparatively high curcumin content.

The accessions T_{12} and T_{13} of *C. longa* are grouped into one cluster thereby revealing a close similarity.

The accessions coming under *C. aromatica* are spread under different clusters. The accessions T_3, T_8 and T_{11} are having cent per cent similarity and they formed a single cluster. These three accessions are found to have dense pubescence under the leaf surface and had exactly similar plant habit. They also had high essential oil content. These accessions were collected from IISR, Pala and Kozhikode. By considering the high essential oil content, camphoraceous aroma of the rhizomes, low curcumin content and the cent per cent similarity obtained in statistical analysis, these three accessions can be concluded as the true kasthuri turmeric.

The accessions T_1 and T_2 come under same cluster along with ordinary turmeric. Morphologically both accessions were similar and found very close to the ordinary turmeric in their morphological features. Thus these two accessions are supposed to have originated from the same source.

Accessions T_4, T_6 and T_9 are having cent per cent similarity and these three are having high amount of curcumin content (compared to T_3, T_8 and T_{11}) and similar morphological features also. Among these, T_6 and T_9 were collected from Idukki district. These three accessions were found to be different from other kasthuri turmeric accessions and more resembling ordinary turmeric.

A high degree of variation at the DNA level was observed among the different species, though the genetic difference within the species was found to be low.

Zhang *et al*. (2001) applied RAPD technique to distinguish *Lycium barbarum* from other closely related species of the same genus and obtained distinctive DNA prints corresponding to different *Lycium* spp. and higher similarity values were obtained between intra species than at inter species level.

Shasany *et al*. (2000) observed low level of diversity among 23 accessions of *Allium sativum* from different geographical parts of India through RAPD profiling.

Darokar *et al*. (2001) examined narrow genetic base among various geographically distinct accessions of *Bacopa monnieri* by RAPD analysis.

Thus, the study on molecular characterization revealed a great difference within the various kasthuri turmeric accessions. As kasthuri turmeric accessions formed different clusters among them, it can be concluded that kasthuri turmeric accessions which are being used at various parts of the state, as kasthuri turmeric, are different.

From the present study using RAPD, it is observed that the accessions T_3, T_8 and T_{11} are coming under the same cluster with cent per cent similarity. Supporting this finding, these three accessions were having similar morphological features and had dense pubescence under the leaf surface. The physiological and yield characters also found to be similar in these accessions. High essential oil content with a camphoraceous aroma of the rhizome, low curcumin content and a higher percentage of crude fibre were characteristics of these accessions revealed by the biochemical studies. The available literature about kasthuri turmeric regarding characters like leaf pubescence, essential oil content, low curcumin content and yield were also in line with the present findings. From the present study, it is evident that accessions T_3 (IISR accession), T_8 (Pala) and T_{11} (Kozhikode accession) exhibited unique features of kasthuri turmeric, leading to the conclusion that these accessions are the true kasthuri turmeric.

Morpho-molecular, biochemical, physiological and anatomical studies were carried out to establish the true identity of kasthuri turmeric. Among the kasthuri turmeric accessions, the morphological character *viz*., pubescence on the under surface of leaves can be considered as a morphological marker for easy and field level identification of true kasthuri turmeric from most of the other accessions now being used as kasthuri turmeric.

Biochemical characters like high essential oil content and low curcumin content are also found as unique features of kasthuri turmeric. Moreover, molecular characterization using RAPD clearly distinguished the accessions with the above mentioned characters by giving similar banding patterns and by forming a single cluster. It is evident that the characterization using morphological, biochemical, physiological and anatomical characters will help to characterize kasthuri turmeric at varying levels of precision. Combing the above characterization methods with molecular characterization using RAPD provided the most distinct characterization of kasthuri turmeric. Further studies by collecting more number of accessions from various parts of the country and by using other molecular markers like RFLP, AFLP or IISR will provide more information on the subject.

Summary

6. SUMMARY

The study entitled “Characterization of kasthuri turmeric (*Curcuma aromatica* Salisb.)” was conducted at the Department of Plantation Crops and Spices and Department of Plant Biotechnology, College of Agriculture, Vellayani during the period 2003-'04. The objective of the study was to characterise kasthuri turmeric in comparison with ordinary turmeric (*C. longa* L.) and zedoary (*C. zedoaria* Rosc.). For this eleven accessions of kasthuri turmeric collected from various sources like IISR, Kozhikode, various locally grown accessions and few wild types were grown along with two accessions each of ordinary turmeric and zedoary. Variability among these accessions in morphology, biochemical aspects, yield characters, physiological and anatomical characters were evaluated. Molecular characterization using RAPD technique was also carried out in order to clearly demarcate between the various accessions. The salient results of the study are summarized hereunder.

Analysis of variance showed significant difference between the accessions for all the characters studied. Among the morphological characters, the zedoary accession T_{14} recorded maximum plant height (112.64 cm) and the least by T_1 (79.73 cm). The highest number of tillers and leaves were recorded in T_7 (6.22 and 21.44) and T_{10} (6.20 and 23.14) respectively indicating their uniqueness in these characters. But the leaf area was found to be maximum in the zedoary accessions T_{15} and T_{14} (8226.66 and 7680.00 cm^2) and the least in T_1 (2656.00 cm^2). The zedoary accessions were found to have the highest rhizome, root spread and rhizome thickness. These characters were found to be the least in the ordinary turmeric accessions and in the two kasthuri turmeric accessions T_1 and T_2. Root length and dry root weight were found maximum in the kasthuri turmeric accession T_5 and was least in the ordinary turmeric accessions used for the comparative study.

Analysis of growth characters like plant height, leaf area, rhizome spread, root spread and rhizome thickness show that zedoary is more vigorous in above growth characters compared to kasthuri turmeric and ordinary turmeric and these characters can be used to distinguish between them. These characters were found to be the least in the accessions $T_{12,}$ T_{13}, T_1 and T_2 of which the first two were turmeric accessions and the latter two were kasthuri turmeric accessions, which closely resembled ordinary turmeric. All other kasthuri turmeric accessions performed in between the zedoary and ordinary turmeric accessions used for comparative study for most of the growth characters.

Biochemical studies revealed that the three accessions of kasthuri turmeric, T_3 (IISR accession), T_8 (Pala wild) and T_{11} (Kozhikode accession) performed uniquely and was superior in terms of essential oil content, crude fibre and chlorophyll content. They recorded the highest protein content also. But the curcumin content was very less, below one per cent in these three accessions. The zedoary and ordinary turmeric accessions used for comparative study recorded a higher curcumin content, less essential oil and crude fibre contents. A clear difference was established between these three accessions of kasthuri turmeric, ordinary turmeric and the zedoary used for characterization in terms of biochemical characters.

Analysis of yield characters shows that the zedoary accessions are superior in rhizome yield and the kasthuri turmeric accessions yielded higher compared to the ordinary turmeric accessions. The maturity period of kasthuri turmeric accessions was lesser compared to ordinary turmeric.

Physiological characters also revealed distinct variation among various accessions of *Curcuma* spp. Dry matter production, LAI, LAD and HI were found higher in the zedoary accessions differentiating them from kasthuri turmeric and ordinary turmeric accessions. The kasthuri turmeric accessions T_3, T_5, T_8, T_9, T_{11} and ordinary turmeric accessions recorded

LAI and LAD values above the general mean value. The drying percentage of rhizomes was found to be high in the kasthuri turmeric accessions, T_7 and T_3, which recorded higher yield also. LAI, LAD and HI were found least in the accessions T_1 and T_2 which were short statured, low yielding and shorter duration accessions. The anatomical characters studied were more or less similar and did not show any significant variation among various accessions.

As the preliminary step in any crop improvement programme, the knowledge of the genetic variability of different characters is very essential. The variations may be due to genetic and environmental effects. High GCV and PCV for most of the characters revealed great extent of variability for these characters suggesting good scope for improvement through selection. Furthermore, the magnitude of genetic variation nearly approached phenotypic variation, in all the characters, indicating that the selection on phenotypic basis will hold good scope on genetic upgradation.

Correlation coefficient gives an idea about the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement in yield. The results showed that genotypic correlation is higher than the phenotypic correlation and the environmental correlation was less, revealing strong association at genotypic level between the characters. The phenotypic correlation includes both genetic and environmental effects. In the present study, the magnitude of genotypic correlation was higher than the corresponding phenotypic correlation indicating that environment had negligible effect on the characters analysed.

For the molecular characterization of kasthuri turmeric, DNA was isolated from young leaves of various accessions using Murray and Thompson method. The yield of DNA ranged from 1.26 to 6.39 ng μl^{-1}.

The purity of DNA estimated using the O.D. ratio (A_{260}/A_{280}) ranged from 1.52 to 1.92.

The quality of DNA was assessed using gel electrophoresis. The concentrations of agarose were 0.9 per cent and 1.4 per cent for identification of genomic DNA and PCR products respectively.

For PCR amplification of DNA, 40 ng of DNA, 200 μM each of the four dNTPs, 0.8 unit of Taq DNA polymerase and 10 pM of primer were used. The programme consisted of an initial denaturation at 93°C for 3.0 minutes, followed by 43 cycles of denaturation at 45°C for one minute, annealing at 35°C for one minute and extension at 72°C for 1.5 minutes. The synthesis step for extension at 72°C for seven minutes was also included after the last cycle. The products of the amplification were kept at 4°C until attended.

A total of 59 RAPDs (average of 1.47 bands per primer) were generated when PCR amplification was carried out using 40 decamer primers (Operon Inc., CA, USA) of Kit A and Kit B. Of these 56 bands were polymorphic. Out of the 40 primers screened, three primers (OPA-04, OPB-17 and OPB-18) were selected for amplifying DNA from all the *Curcuma* accessions. These three selected primers together produced 19 scorable bands. The number of bands resolved per amplification was primer dependent and varied from a minimum of six (OPB-17 and OPB-18) to a maximum of seven (OPA-04).

The similarity coefficient values ranged from 0.250 and 1.00. The estimation of similarity coefficient and construction of dendrogram revealed the presence and extent of genetic similarity among the 15 *Curcuma* accessions examined. At 63 per cent similarity, the 15 accessions got divided into four clusters. First cluster consisted of only one accession, T_{15} which is the black zedoary accession. The accessions

T_7 and T_{10} together formed the second cluster. The third cluster included the three accessions T_3, T_8 and T_{11}.

The fourth cluster consisted of nine accessions at 63 per cent similarity. At 69 per cent similarity this cluster again got divided into two sub clusters. One sub cluster consisted of four accessions *i.e.*, T_4, T_6, T_9 and T_5. Second cluster consisted of five accessions, *i.e.*, T_{14}, T_1, T_2, T_{12} and T_{13} are coming under this cluster.

The various accessions of kasthuri turmeric are spread under different clusters. The accessions T_3, T_8 and T_{11} having cent per cent similarity formed a single cluster. These three accessions are found to have dense pubescence under the leaf surface and had exactly similar plant habit. They also had high essential oil content. These accessions are collected from IISR, Pala and Kozhikode. By considering the high essential oil content, camphoraceous aroma of the rhizomes, low curcumin content and the cent per cent similarity obtained from the molecular characterization studies, these three accessions can be concluded as the true kasthuri turmeric.

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*Original not seen

Appendices

APPENDIX – I

Morphological descriptor for *Curcuma* spp.

Sl. No.	Characters	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1.	**Shoot characters**								
1.1	Plant height (cm)	65 – 95 Medium tall	68 – 94 Medium tall	70 – 90 Medium tall	80 – 92 Medium tall	80 – 105 Tall	85 – 102 Tall	75 – 95 Medium tall	70-90 Medium tall
1.2.	Leaf shape	Oblong – lanceolate	Oblong – lanceolate	Broadly lanceolate	Oblong – lanceolate	Narrow lanceolate	Broadly lanceolate	Narrow lanceolate	Broadly lanceolate
1.3	Number of tillers per clump	1 – 3	1 – 3	2 – 4	2 – 4	4 – 6	2 – 4	4 – 7	2 – 4
1.4	Number of leaves per shoot	4 – 6	4 – 6	4 – 7	5 – 8	5 – 7	4 – 6	4 – 6	5 – 8
1.5	Leaf midrib colour	Green	Green	Green	Green	Deep pink	Green	Green	Green
1.6	Leaf pubescence	Absent	Absent	Densely pubescent at under leaf surface	Absent	Absent	Absent	Thinly pubescent at under leaf surface	Densely pubescent at under leaf surface
1.7	Lea length (cm)	35 – 40	35 – 39	40 – 60	42 – 61	45 – 64	43 – 58	40 – 53	40 – 61
1.8	Leaf width (cm)	10 – 15	10 – 16	12 – 16	12 – 15	10 – 14	12 – 17	10 – 15	12 – 18
1.9	Petiole length (cm)	30 – 35	30 – 36	21 – 27	18 – 29	30 – 38	30 – 37	20 – 33	18 – 27
2.	**Rhizome characters**								
2.1	Rhizome thickness (cm)	1.12–1.43 Slender	1.25–1.48 Slender	1.34–1.73 Thick	1.24–1.48 Slender	1.28–1.42 Thick	1.30–1.49 Thick	1.35–1.44 Slender	1.32–1.63 Thick
2.2	Rhizome spread (cm)	18 – 23 Low spreading	16 – 24 Low spreading	16 – 28 Highly spreading	16- 2 1 Low spreading	28 – 34 Highly spreading	15 – 21 Low spreading	23 – 32 Highly spreading	16 – 29 Highly spreading

APPENDIX – I Continued

Sl. No.	Characters	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
2.3	Rhizome colour	Deep orange yellow	Deep orange yellow	Creamy or light yellow	Light yellow	Fluorescent yellow	Deep orange yellow	Dull white	Creamy or light yellow
2.4	Rhizome aroma	Strong aroma of ordinary turmeric	Strong aroma of ordinary turmeric	Intense camphoraceous aroma	Mild aroma of turmeric	Mild camphoraceous aroma	Mild aroma of turmeric	No distinct aroma	Intense camphoraceous aroma
2.5	Finger rhizomes	Sessile, many and branching	Sessile, many and branching	Thick sessile, many and branching	Short sessile, many , branching	Sessile, many and elongated	Sessile, few, branching	Sessile, few , elongated	Thick sessile, many and branching
3.	**Root Characters**								
3.1	Root type	Narrow fibrous	Narrow fibrous	Fleshy fibrous	Narrow fibrous	Fleshy fibrous	Narrow fibrous	Fleshy fibrous	Fleshy fibrous
3.2	Root tubers	Rare	Rare	Common	Rare	Many	Rare	Many	Common
3.3	Root length (cm)	13- 1 8 Medium	12-18 Medium	18 – 21 Long	12 – 16 Short	18 – 25 Long	15 – 20 Medium	14 – 21 medium	18-22 Long
3.4	Root spread (cm)	12 – 15 Medium spreading	11 – 17 Medium spreading	15 – 21 High spreading	10 – 14 Low spreading	16 – 19 High spreading	10 – 15 Low spreading	13 – 18 Medium spreading	15 – 19 High spreading.

APPENDIX – I Continued

Sl. No.	Characters	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
1.	**Shoot characters**							
1.1	Plant height (measured in cm)	76 – 97 Tall	75 – 90 Medium tall	70 – 92 medium tall	76 – 94 medium tall	76 – 93 medium tall	100 – 120 Very tall	90 – 112 Very tall
1.2.	Leaf shape	Broadly lanceolate	Narrow lanceolate	Broadly lanceolate	Oblong lanceolate	Oblong lanceolate	Oblong lanceolate	Oblong lanceolate
1.3	Number of tillers per clump	2 – 4	5 – 7	2 – 5	3 – 6	4 – 6	2 – 5	3 – 6
1.4	Number of leaves per shoot	5 – 7	4 – 6	4 – 7	4 – 6	4 – 7	4 – 6	4 – 6
1.5	Leaf midrib colour	Green	Green	Green	Green	Green	Purple along entire midrib	Pinkish along distal half
1.6	Leaf pubescence	Absent	Thinly pubescent at under leaf surface	Densely pubescent at under leaf surface	Absent	Absent	Absent	Absent
1.7	Lea length (cm)	40 – 62	42 – 53	43 –59	41 – 54	42 – 55	51 – 70	50 – 70
1.8	Leaf width (cm)	12 – 17	10 – 15	12 – 16	12 – 15	12 – 14	10 – 18	12 – 17
1.9	Petiole length (cm)	30 – 35	20 – 31	20 – 26	24 – 33	23 – 34	31 – 38	30 – 41
2.	**Rhizome characters**							
2.1	Rhizome thickness	1.38–1.62 Thick	1.33–1.46 Slender	1.48–1.70 Thick	1.43–1.68 Thick	1.40–1.65 Thick	1.49–1.72 Thick	1.52–1.75 Thick
2.2	Rhizome spread (cm)	15-20 Low spreading	20 – 32 Highly spreading	17 – 30 highly spreading	15 – 20 Low spreading	15 – 20 Low spreading	20 – 35 Highly spreading	24 – 34 Highly spreading

APPENDIX – I Continued

Sl. No.	Characters	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
2.3	Rhizome colour	Deep orange yellow	Dull white	Creamy or light yellow	Deep orange yellow	Deep orange yellow	Deep yellow	Bluish at centre, grayish at periphery.
2.4	Rhizome aroma	Strong aroma of ordinary turmeric	No distinct aroma	Intense camphoraceous aroma	Turmeric aroma	Turmeric aroma	Mild camphoraceous aroma	Mild camphoraceous aroma
2.5	Finger rhizomes	Many, Sessile, branching.	Sessile, few elongated	Thick sessile, many and branching	Sessile, few branched	Sessile, few branched	Sessile, few branched	Sessile, many branched
3.	**Root Characters**							
3.1	Root type	Narrow fibrous	Fleshy fibrous	Fleshy fibrous	Narrow fibrous	Narrow fibrous	Thick, fleshy fibrous	Sessile, many branched
3.2	Root tubers	Rare	Many	Common	Rare	Rare	Common	Many
3.3	Root length (cm)	13 – 18 medium	15 – 21 medium	18 – 22 Long	12 – 18 medium	11 – 17 Medium	18 – 23 Long	16- 24 Long
3.4	Root spread (cm)	10 – 14 Low spreading	13 – 18 medium spreading	15 – 21 high spreading	10 – 14 Low spreading	10 – 14 Low spreading	15 – 22 High spreading	14 – 21 High spreading

CHARACTERIZATION OF

KASTHURI TURMERIC (*Curcuma aromatica* Salisb.)

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ABSTRACT

The study entitled “Characterization of kasthuri turmeric (*Curcuma aromatica* Salisib.)” was conducted at the Department of Plantation Crops and Spices and Department of Plant Biotechnology, College of Agriculture, Vellayani during the period 2003-'04. The objective of the study was to characterize kasthuri turmeric in comparison with ordinary turmeric (*C. longa* L.) and zedoary (*C. zedoaria* Rosc.). Studies were carried out using eleven accessions of kasthuri turmeric and two accessions each of ordinary turmeric and zedoary. Characterization in terms of morphological, biochemical, physiological, anatomical and yield characters were carried out. Molecular characterization using RAPD technique was also used in this study.

Analysis of variance of the observations showed significant difference among the accessions for most of the characters. Growth characters like plant height, leaf area, rhizome spread, root spread and rhizome thickness showed that zedoary is more vigorous and superior in above growth characters compared to kasthuri turmeric and ordinary turmeric. Most of the kasthuri turmeric accessions performed in between the zedoary and ordinary turmeric accessions for most of the growth characters analysed.

Biochemical studies revealed that the three accessions of kasthuri turmeric, T_3 (IISR accession), T_8 (Pala wild) and T_{11} (Kozhikode accession) performed uniquely and was superior in terms of essential oil content, crude fibre, protein and chlorophyll content. But the curcumin content was less than one per cent in these accessions. The zedoary and ordinary turmeric accessions used for comparative study recorded a higher curcumin content, less essential oil and crude fibre contents. A clear

difference was established between these three accessions of kasthuri turmeric, ordinary turmeric and the zedoary.

Analysis of yield characters revealed that zedoary accessions are superior in rhizome yield and kasthuri turmeric accessions yielded higher compared to ordinary turmeric accessions. Physiological characters like dry matter production, lead are index, leaf area duration and harvest index were found higher in the zedoary accessions differentiating them from kasthuri turmeric and ordinary turmeric accessions. The anatomical characters studied were found to be similar and did not show any significant variations among various accessions.

Variability study showed high genotypic coefficient of variance and phenotypic coefficient of variance for most of the characters, revealing great extent of variability for these characters, suggesting good scope for improvement through selection. Correlation studies showed that genotypic correlation is higher than the phenotypic correlation and the environmental correlation was less, revealing strong association at genotypic level between the characters.

For the molecular characterization of kasthuri turmeric, DNA was isolated from young leaves of various accessions using Murray and Thompson method. The yield of DNA ranged from 1.26 to 6.39 ng μl^{-1}. The purity of DNA estimated using the O.D. ratio (A_{260}/A_{280}) ranged from 1.52 to 1.92. PCR amplification was carried out using 40 decamer primers (Operon Inc., CA, USA) of Kit A and Kit B and a total of 59 RAPDs were generated. Of these, 56 bands were polymorphic.

Out of the 40 primers screened, three primers (OPA-04, OPB-17 and OPB-18) were selected for amplifying DNA from all the *Curcuma* accessions. The estimation of similarity coefficient and construction of dendrogram revealed the presence and extent of genetic similarity among the 15 *Curcuma* accessions examined. The similarity coefficient values ranged from 0.25 and 1.00.

At 63 per cent similarity, the 15 accessions got divided into four clusters. First cluster consisted of only one accession, T_{15} which is the black zedoary accession. The accessions T_7 and T_{10} together formed the second cluster. The third cluster included the three accessions T_3, T_8 and T_{11}. The fourth cluster consisted of nine accessions at 63 per cent similarity. At 69 per cent similarity this cluster again got divided into two sub clusters. One sub cluster consisted of four accessions *i.e.*, T_4, T_6, T_9 and T_5. Second cluster consisted of five accessions, *i.e.*, T_{14}, T_1, T_2, T_{12} and T_{13} are coming under this cluster.

The various accessions of kasthuri turmeric are spread under different clusters. The accessions T_3, T_8 and T_{11} having cent per cent similarity formed a single cluster. These three accessions are found to have dense pubescence under the leaf surface and had exactly similar plant habit. By considering the high essential oil content, camphoraceous aroma of the rhizomes, low curcumin content and the cent per cent similarity obtained from the molecular characterization studies, these three accessions can be concluded as the true kasthuri turmeric.