ISOENZYME VARIATION IN *Curcuma* WITH SPECIAL REFERENCE TO *Curcuma longa* L.

By

RENU JOSEPH

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
Kerala, India

1999

DECLARATION

I hereby declare that this thesis entitled "**Isoenzyme variation in *Curcuma* with special reference to *Curcuma longa* L.**" is a bona fide 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 to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled "**Isoenzyme variation in *Curcuma* with special reference to *Curcuma longa* L.**" is a record of research work done independently by Mrs. Renu Joseph, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of **Mrs.Renu Joseph,** a candidate for the degree of **Master of Science in Horticulture** with major in Plantation Crops and Spices, agree that the thesis entitled **"Isoenzyme variation in *Curcuma* with special reference to *Curcuma longa* L."** may be submitted by Mrs.Renu Joseph, in partial fulfilment of the requirement for the degree.

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ACKNOWLEDGEMENT

I have great pleasure to place on record my deep sense of gratitude and sincere thanks to ***Dr.V.S.Sujatha****, Assistant Professor, Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur and chairperson of my advisory committee for assigning this problem for study, for her meticulous and gracious guidance, constructive criticism and constant encouragement all through the course of my research work as well as in the preparation of the manuscript.*

I am extremely grateful to ***Dr.E.V.Nybe****, Professor and Head i/c, Department of Plantation Crops and Spices for his propitious support and critical scrutiny of the manuscript.*

I am very much grateful to ***Dr.Alice Kurian,*** *Associate Professor, Department of Plantation Crops and Spices and member of my advisory committee for her valuable suggestions during various stages of the study. Her critical comments indeed helped me a lot in improving the text.*

I am thankful to ***Dr.A.Augustine****, Associate Professor, AICRP on M & AP and member of my advisory committee for his keen interest and the facilities provided during the course of my research work.*

I am thankful to Dr.A.I.Jose, Associate Dean, College of Horticulture for all the facilities provided for my research work.

No words can truely express my profound gratitude to ***Sri.K.C.Velayudhan****, Scientist in charge, NBPGR Regional Station, Vellanikkara, for his ever willing help, scholarly suggestions, sustained interest and for sparing many of the valuable materials for the study.*

With immense pleasure I thank each and every member of Department of Plantation Crops and Spices for their support and encouragement.

I am thankful to Mr.Sreekumar, P.K. (CCRP), Mr.Babu, T.D. and Mr.Achuthan, C.R. (Biotech Centre) for the help given to me for photographic works.

I wish to thank Mr.Preman of Likhitha Arts, Mannuthy for helping me in preparation of graphs.

It is my previlege and fortune to express my sincere thanks to classmates and friends for their help and constant encouragement which helped me a lot to complete this work and accomplish my objective. My cordial thanks are due to my loving friends Thanuja, Sreeja, Sreedevi, Niranjan, Divya, Sulaja, Kavitha, Lalitha and Annie.

A word of thanks to Sri.Joy for the neat and prompt service in typing the thesis.

The award of Junior Fellowship by Kerala Agricultural University is greatly acknowledged.

I am in dearth of words to express my gratitude, appreciation and indebtedness to my-in-laws, parents, brother and sister for their constant encouragement, untiring support and love showered on me, without which, it would have been impossible to complete my study.

Words cannot express my soulful gratitude to my husband for his sacrifice, constant encouragement, immense help and boundless affection throughout the course of study which has helped me in completion of this research on time.

Above all I bow my head before ***Almighty*** *whose constant blessing enabled me to complete this endevour successfully.*

Renu Joseph

RENU JOSEPH

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Dedicated to my loving
Appa and Chachan

Introduction

INTRODUCTION

The genus *Curcuma* belonging to the family Zingiberaceae has a wide spread occurrence in the tropics of Asia and extends to Africa and Australia. The genus contains over 90 species of rhizomatous herbs including turmeric (*Curcuma longa* L. syn. *C. domestica* Val.), which is an important seasonal spice grown in India.

Turmeric is so much identified with human civilization, religion, customs and belief as an important condiment, drug, colouring material and cosmetic. It has been used as an antiseptic from time immemorial and is reported to have anticancerous properties also.

India is the leading producer and exporter of turmeric in the world. Among the spices,turmeric is ranked as third next to black pepper and chillies with respect to the foreign exchange earnings. The major turmeric growing states in India are Andhra Pradesh, Maharashtra, Orissa, Tamil Nadu, Karnataka and Kerala.

Apart from turmeric, many other *Curcuma* species like *C. amada, C. aromatica, C. raktakanta, C. malabarica* and *C. brog* are also economically important and are being used in various medicines, as spice, in cosmetics, pickles etc.

The taxonomic history of the genus give ample evidence of several anomalies and discrepancies with respect to classification, typification, identification and keying out of species.

There is high degree of variation among the species of *C. longa*. The variability among the cultivars are for yield, size of rhizome, number of fingers, colour, aroma, curcumin content, time taken for maturity etc. which led Velayudhan *et al.* (1994) to classify 568 accessions of *C. longa* into 21

morphotypes. Since most of these characters are quantitatively inherited and much influenced by the environment, the real genetic base/diversity in the crop is not understood clearly. Also, only a few types/ varieties flower and set seed.

Studies on phylogeny and evolution are essential prerequisites for scientific crop improvement. Recently, characterization based on isoenzyme variation is being used as a powerful tool to compliment and supplement conventional phylogenetic studies (Rick *et al.*, 1977; Rick and Tanksley, 1981; Gottlieb, 1971, 1977 and 1982; Crawford, 1983; Sujatha *et al.*, 1991 and Sebastian *et al.*, 1996).

The advantage of isoenzyme analysis over phenotypic characters are manyfold. These are basic biochemical constituents which are not affected by direct selection pressure during the course of domestication and evolution of a taxon providing a more accurate picture of the original variation present in the population. Secondly, isoenzyme pattern is not affected by the environment. Moreover, isoenzymes are usually direct product of single locus and relating the phenotypic variation with genotypic character is relatively easier. Allozymes are usually inherited as a co-dominant which help in distinguishing a homozygote from a heterozygote. Besides these, the relative ease in electrophoretic analysis makes isoenzyme studies a powerful tool in phylogenetic studies. In the light of these, isoenzyme analysis was undertaken in the genus Curcuma and *Curcuma longa* with the following major objectives of understanding the

- relationship among different morphotypes of *C. longa* and
- taxonomic relationship among the members of the genus *Curcuma.*

REVIEW OF LITERATURE

2.1 Taxonomy

The genus Curcuma L (*Zingiberaceae*) was first raised by Linnaeus in the year 1793. It is mainly Indo-Malayan in origin and distribution. It includes about 80 species, of which 17 have been reported by Roxburgh (1874) from India, 29 by Baker (1898) from British India, 8 by Fischer (1928) from Madras Presidency, 10 by Haines (1961) from Bihar and Orissa, 4 by Trimen and Hooker (1974) from Ceylon and 5 by Rao (1986) from India.

The earliest known classification of *Curcuma* was by Baker (1898). He divided the genus into three sections or subgenera namely Exantha, Mesantha and Hitchenopsis. The classification was based on flower spike position, nature of bract etc. But a major drawback of his classification was that certain species in both Exantha and Mesantha were reported to have both central and lateral flower spikes.

Valeton (1918) classified the Javan species of the genus by dividing it into two subgenera namely Eucurcuma and Paracurcuma. His classification was based on a good number of characters of above ground and underground parts.

Burt and Smith (1972) clearly established the lectotype of the genus to be *C. angustifolia* which is considered to be one of the oldest species described by Roxburgh.

Botanical term *C. longa* given by Linnaeus (1793) was changed to *C. domestica* by Valeton (1918). Burt (1977) reinstated the botanical term *C. longa* L. leaving *C. domestica* of Valeton as synonym.

Muralidharan *et al.* (1980) divided 221 accessions of *C. longa* and related species into nine groups on the basis of vegetative characters, taste, colour

and smell of the rhizomes. Twentytwo accessions were distinct and their taxonomic status could not be determined.

Mangaly and Sabu (1993) attempted a revision of the South Indian species of the genus. He divided the genus based on the ontogeny of flower spike production into 'Exantha', 'Mesantha', 'Hitchenopsis' and 'Amphiantha'. Sessile tuberising species and non-sessile tuberising species were noticed in Exantha. Species with both central and lateral flowering spikes are included in Amphiantha.

Velayudhan *et al.* (1996) classified the genus based on floral and vegetative characters. He regrouped the genus in India into two subgenera, Paracurcuma and Eucurcuma of Valeton, based on presence or absence of spur on anther. Eucurcuma contains three main sections based on the presence or absence of tubers and stolons. He also identified two new species and included them in the classification.

2.1.1 Description of *Curcuma* species under study

1. *C. latifolia* : Wild species seen in NE India, Erect plant, sessile tubers non stoloniferous, thick, oblong, yellow. Prostrate purplish green leaves, purple mibrib fading, hairy on ventral side, spike lateral, coma present, lip yellow, calyx purple, white corolla, flowering time April-May.

2. *C. aromatica*: Wild species seen in Kerala and Karnataka. Rhizome used in cosmetic industry. Semierect plant, sessile tubers non stoloniferous, thick, oblong, pale yellowish white, semierect green leaves, green midrib without fading, hairy on both sides, spike lateral, coma present, lip yellow, calyx purple, white corolla, flowering time April-May.

3. *C. harita*: Wild species seen in Kerala. Semierect plant, sessile tubers non stoloniferous, thick, oblong, white, semierect light purple leaf, purple midrib fading, hairy on ventral side, spike lateral, coma present, lip yellow, calyx purple, white corolla, flowering time May-June.

4. *C. aeruginosa*: Wild species seen in NE India, semierect plant, sessile tubers non stoloniferous, thick, oblong, verdigris green, semierect purple leaf, glabrous, purple midrib not fading, spike lateral, coma present, purple calyx, white corolla, lip yellow, flowering time May-June.

5. *C. caesia*: Wild species seen in NE India. Erect plant, sessile tubers non stoloniferous, thick, oblong, blue, semierect purple leaf with purple midrib not fading, hairy on dorsal side, spike central/lateral, coma present, lip yellow, calyx purple, corolla purple, flowering time March-June.

6. *C. soloensis*: Wild species seen in NE India, semierect plant, sessile tubers non stoloniferous, thick, oblong, white, semierect purple leaf with green midrib not fading, hairy on ventral side, spike central, coma present, lip yellow, calyx purple, white corolla, flowering time October.

7. *C. brog*: Wild species seen in NE India, semierect plant, sessile tubers non stoloniferous, thick, oblong, white, semierect green leaf with green midrib not fading, hairy on ventral side, spike central, coma present, lip yellow, calyx purple, red corolla, flowering time October.

8. *C. longa*: Widely cultivated all over India mainly as a spice. Erect/semierect plant, sessile tubers non stoloniferous, thick, oblong/cylindrical, orange yellow, semierect green leaves, glabrous on both sides, midrib colour green without fading, spike central, coma present, calyx light purple, corolla white, lip yellow, flowering time September-November.

9. *C. amada*: Widely cultivated all over India. Used as a vegetable and for pickling. Semierect plant, sessile tubers non stoloniferous, thick, oblong, pale yellow with mango flavour, semierect green leaves, hairy on ventral side, midrib colour green without fading, spike lateral, coma present, calyx light purple, corolla pale yellow, lip yellow, flowering time May.

10. *C. zedoaria*: widely cultivated all over India. It is an adultrant of *C. aromatica* in cosmetic industry. Erect plant, sessile tubers non stoloniferous, thick, oblong, light orange yellow, erect/semierect green leaves with purple midrib not fading, glabrous, spike lateral, coma present, calyx purple, corolla light purple, lip yellow, flowering time April-May.

11. *C. malabarica*: Wild type seen in S. India. Erect plant, tubers non stoloniferous, thick, cylindrical, light blue, semierect, green leaves, glabrous, purple midrib fading, spike lateral, coma present, calyx purple, corolla light purple, lip yellow, flowering time April-May.

12. *C. sylvatica*: Wild species seen in NE India. Semi erect plant, sessile tubers non stoloniferous thick, oblong, whitish cream, semierect green leaves, purple midrib fading, glabrous, spike lateral, coma present, lip yellow, calyx light purple, corolla white, flowering time May.

13. *C. raktakanta*: Wild species seen in Kerala. Semi erect plant, sessile tubers non stoloniferous, thick, oblong, pale yellowish, semierect purple leaf, green midrib not fading, hairy on ventral side, spike lateral, coma present, lip yellow, calyx purple, corolla light purple, flowering time April-May.

14. *C. montana*: Wild species seen in Andhra Pradesh. Semierect plant, sessile tubers non stoloniferous, thick, oblong, pale yellow, semierect green leaf with green

midrib not fading, glabrous, spike central, coma present, lip yellow, calyx light purple, corolla white, flowering time August-September.

15. *C. comosa*: Wild species seen in NE India. Semierect plant, sessile tubers non stoloniferous, thick, oblong, orange yellow, semierect purple leaf with purple midrib fading, glabrous, spike lateral, coma present, lip yellow calyx light purple, corolla light purple, flowering time March.

16. *C. vamana*: Wild species seen in Kerala. Semierect plant, sessile tubers stoloniferous, creeping, thin, light orange yellow, semierect green leaf, green midrib not fading, glabrous, spike central, coma absent, lip yellow, corolla white, flowering time June-July.

17. *C. aurantiaca*: Wild species seen in Kerala. Semierect or prostrate plant, sessile tubers absent, semierect green leaf, green midrib not fading, hairy on both sides, spike central, coma present, lip orange yellow, spur absent, calyx light purple, corolla light orange yellow, flowering time July-September.

18. *C. pseudomontana*: Wild species seen in South India. Erect plant, sessile tubers absent, semierect purple leaf, green midrib not fading, hairy on both sides, spike central, coma present, lip bright yellow, spur present, calyx light purple, flowering time August-October.

2.1.2 Key characters for the identification of *Curcuma* species under study (Velayudhan *et al.*, 1994)

Sessile tubers present

Sessile tubers stoloniferous, creeping, thin, coma absent - *C. vamana*

Sessile tubers non stoloniferous, thick, oblong/cylindrical, palmately branched

- Spike central or lateral
  - Spike central
    - Sessile tubers white
      - Leaf sheath purple - *C. soloensis*
      - Leaf sheath green - *C. montana*
    - Sessile tubers not white
      - Sessile tubers orange yellow - *C. longa*
      - Sessile tubers pale yellow - *C. brog*
  - Spike lateral
    - Purple midrib absent on leaf
      - Leaf sheath purple brown - *C. raktakanta*
      - Leaf sheath green
        - Leaf hairy on ventral or dorsal side
          - Leaf hairy on ventral side - *C. amada*
          - Leaf hairy on dorsal side - *C. aromatica*
    - Purple midrib present on leaf
      - Purple midrib fading at maturity
        - Sessile tubers light blue inside - *C. malabarica*
        - Sessile tubers not blue
          - Leaves glabrous
            - Sessile tubers yellow inside - *C. comosa*
            - Sessile tubers pale yellowish or whitish cream - *C. sylvatica*
          - Leaves hairy
            - Sessile fingers yellow - *C. latifolia*
            - Sessile fingers white - *C. harita*
      - Purple midrib not fading
        - Sessile tubers light orange yellow - *C. zedoaria*
        - Sessile tubers verdigris green - *C. aeruginosa*
  - Spike central and lateral - *C. caesia*

Sessile tubers absent

- Spike central
  - Spur absent - *C. aurantiaca*
  - Spur present - *C. pseudomontana*

Plate 1 A. Non-sessile tuberising *Curcuma* species with central spike (*C. aurantiaca*)

B. Sessile tuberising *Curcuma* species with central spike (*C. longa*)

Plate 2 Sessile tuberising *Curcuma* species with lateral spike (*C. aromatica*)

Plate 1

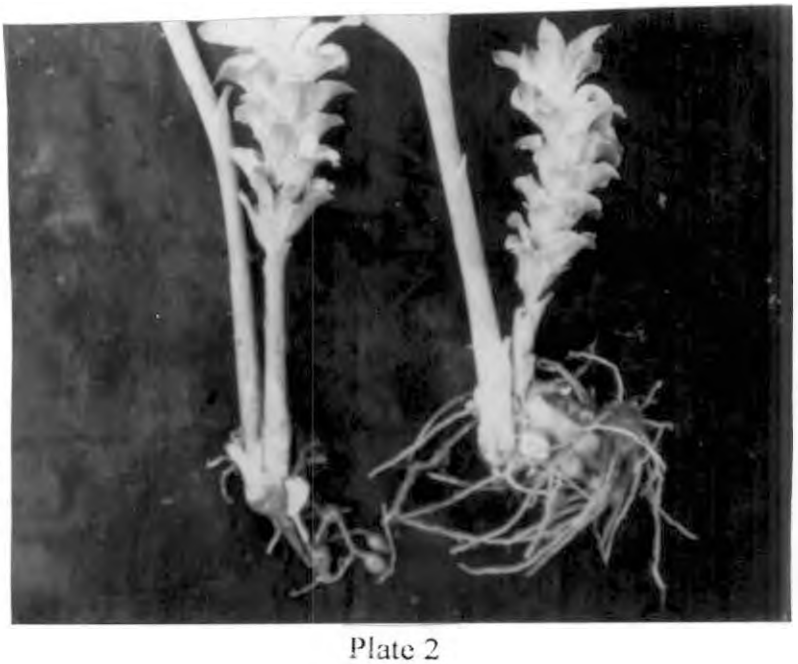

Plate 2

Plate 3A Variation in leaf sheath colour among *Curcuma* species
1. purple leaf sheath (*C. soloensis*) 2. green leaf sheath (*C. longa*)

B Variation in leaf midrib colour among *Curcuma* species
1. green midrib (*C. longa*)
2. purple midrib which fades at maturity (*C. malabarica*)
3. purple midrib (*C. aeruginosa*)

Plate 4 Variation in rhizome flesh colour among *Curcuma* species
1. light yellow (*C. montana*) 2. pale yellowish white (*C. aromatica*)
3. pale yellow (*C. amada*). 4. light orange yellow (*C. zedoaria*)
5. orange yellow (*C. longa*) 6. verdigris green (*C. aeruginosa*)

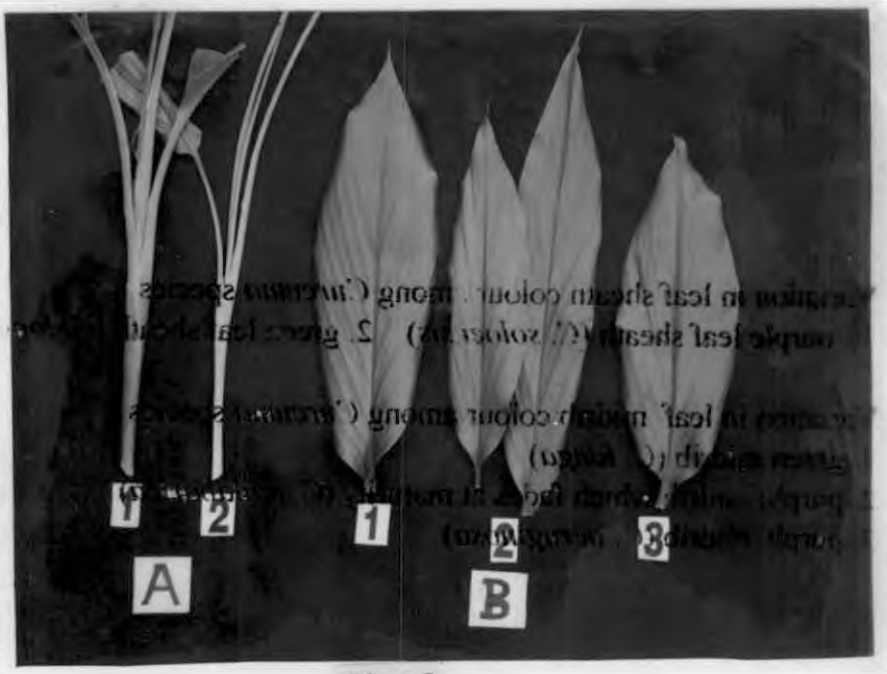

Plate 3

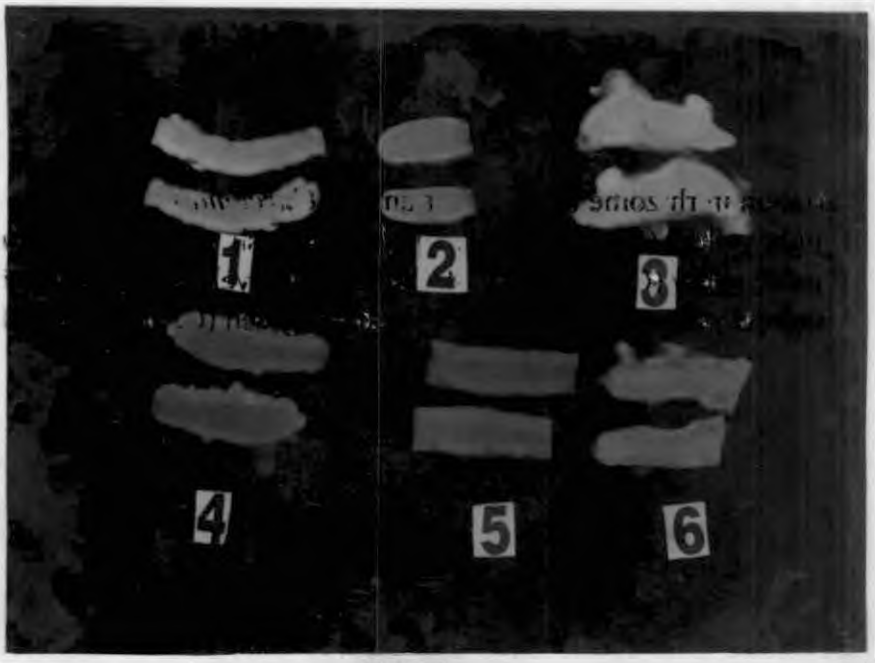

Plate 4

A few key characters used in the identification of species are illustrated in Plates 1, 2, 3 and 4.

2.2 Cytology

Sugiura (1936) was the first to report the chromosome number of *Curcuma longa*. Based on karyomorphology Sato (1948) concluded that *C. longa* is an allotetroploid with a basic chromosome number x = 8.

Ramachandran (1961) reported the tetraploid nature of *C. aromatica* and described the sterility in *C. longa* as due to its auto-triploid nature. He concluded that the genus *Curcuma* might have been derived either by dibasic amphidiploidy (x = 9+12) or by secondary polyploidy (Ramachandran, 1969)

According to Nambiar (1979) the chromosome number in genus *Curcuma* follow a polyploid series with multiples of n = 21. He also reported seed setting in *C. aromatica*.

Nazeem and Menon (1994) reported seed set in *C. longa* x *C. aromatica* crosses which gave new scope for crop improvement.

The inconsistency of chromosome numbers reported in the same species by several workers points out to the existence of both diploidy and triploidy in the same species (Table 1).

2.3 Species grouping

According to Sankaracharya and Natarajan (1973) there are 50 species under the genus *Curcuma* of which *C. longa, C. aromatica, C. angustifolia, C. amada* and *C. caesia* were the economically important. In the family Zingiberaceae only *C. longa* and *C. aromatica* contained the yellow coloured pigment curcumin.

Table 1.Somatic chromosome number reported in *Curcuma* species under study

Name of species	Chromosome No.	Authors and the year
C. amada Roxb.	2n = 42	Raghavan and Venkatasuban, 1943 Chakravorti, 1948 Sharma and Bhattacharyya, 1959 Ramachandran, 1961
C. aromatica Salisb.	2n = 42	Raghavan and Venkatasubban, 1943 Chakravorti, 1948
	2n = 63, 86	Ramachandran, 1961
	2n = 84	Nambiar, 1979
C. longa Linn.	2n = 32	Sato, 1948
	2n = 62	Raghavan and Venkatasubban, 1943 Sharma and Bhattacharyya, 1959
	2n = 62, 63, 64	Chakravorti, 1948 Ramachandran, 1961 Nambiar, 1979
	2n = 64	Sugiura, 1936
C. zedoaria Roxb.	2n = 63	Ramachandran, 1961
	2n = 63, 64	Chakravorti, 1948

2.3.1 D^2 analysis

Nambiar (1979) grouped cultivars of *C. longa* and *C. aromatica* into four clusters based on generalised distance - D^2 statistic. The cultivars belonging to *C. aromatica* were keeping separate identity from the cultivars belonging to *C. longa*.

2.3.2 Cluster analysis and dendrogram analysis

Cluster analysis showed that between the sessile tuber bearing species the correlation coefficient values were much closer in many cases indicating their high relationship. Between the non sessile tubers bearing species, the correlation coefficient values were low indicating their distant relationship. Species were grouped into nine based on dendrogram analysis. *Curcuma amada* and *C. sylvatica*, *C. latifolia* and *C. harita*, *C. aeruginosa*, *C. zedoaria*, *C. comosa* and *C. malabarica* were found to be closely related by dendrogram analysis (Velayudhan *et al.*, 1994).

2.3.3 Quality analysis

Zwaving and Bos (1992) examined the chemical composition of the essential oil from the rhizome of five *Curcuma* species. The characteristic components varied with the species.

Velayudhan *et al.* (1994) studied the arrowroot production potential of 13 *Curcuma* species. Maximum percentage of starch on fresh weight basis was obtained in *C. aeruginosa* and on dry weight basis in *C. harita*.

2.4 Varieties

Aiyer (1954) reported that there were no sharply distinct varieties in the cultivated turmeric. *Curcuma* species were similar in appearance and were likely to

be mistaken one for the other and that *C. longa* was often confused with *C. aromatica*.

Chaurasia *et al.* (1974) described two varieties of *C. longa*, one yielding a hard and bright coloured rhizomes and the other a softer, larger and lighter coloured rhizomes. They noticed camphoraceous odour in *C. aromatica* type as its distinguishing feature.

Menon (1975) found that turmeric produced in different localities varied in quality and 'Alleppey' turmeric, 'Rajpuri' turmeric, 'Guntur' turmeric and 'Madras' turmeric were the popular trade names among Indian turmeric.

Cultivars of turmeric have been classified as long duration (9 months), medium duration (8 months) and short duration (7 months) based on the time taken for the maturity of rhizome (Aiyadurai, 1966).

Short duration types are good yielders of dried turmeric rich in volatile oil but with low curcumin content. Medium duration types yielded more rhizomes and compensated their low curing ability besides rating high for curcumin and volatile oil contents. Long duration types are moderately good both for rhizome yield and other quality constituents (Rao *et al.*, 1975; Subbarayudu *et al.*, 1976; Reddy and Rao, 1988).

Aoi *et al.* (1986) classified the turmeric cultivars into two based on rhizome yield and curcuminoid content. One group characterised by large plant size and a low curcuminoid content and the other group with plant size small and high curcuminoid content.

Pandey *et al.* (1990) reported four distinct morphological complexes on the basis of total yield per plant and weight of primary rhizomes. Within the group morphological variations were of low order.

Velayudhan *et al.* (1994) classified 568 collections of *C. longa* into 21 morphotypes based on subjective analysis of 64 above ground and under ground characters. Numerical taxonomic analysis also supported this. A few characters used in the classification of *C. longa* into 21 morphotypes by Velayudhan *et al.* (1994) is given in next page.

Further attempts have been made by Velayudhan *et al.* (sent for publication) to refine the previous grouping and to classify morphotypes into 6 major groups. Group one contained morphotypes 1, 2, 3 and 4. The accessions had erect leaves which do not fold much. Morphotype 7, 8 and 18 having camphoraceous aroma formed group two. Morphotype 9 and morphotype 12 stood independently forming group three and four respectively. Morphotype 9 had slightly twisted leaf and horizontal leaf was observed in morphotype 12. Group five was represented by morphotype 5, 11, 13, 14, 16, 17, 19, 20 and 21. They formed a complex group with characters intermediate between group one and two. Banana type leaves were observed in morphotype 6, 10 and 15, which formed the sixth group (personal communication, sent for publication).

2.4.1 Genetic variability

Philip *et al.* (1980) evaluated 19 turmeric cultivars and highly significant variation was observed among the cultivars for yield of fresh turmeric, curing percentage, oleoresin and curcumin content.

Philip and Nair (1983) reported highly significant variation among the turmeric types with regard to the morphological and growth characters such as height of the plant, number of leaves per tiller and per plant, leaf characters, number and length of roots, rhizome characters of mother rhizome, primary and secondary fingers. They concluded that the significant variation among the turmeric types grown under the same cultural and agroclimatic conditions can be attributed to the genetic factors.

A few characters used in classifying *C.longa* morphotypes by Velayudhan *et al.* (1994)

Morpho-type	Plant canopy height	Leaf type	Leaf coulor		Hairyness on leaf	Flowering time	Flower exsertion	Fruit setting	Mother rhizome	Tuber colour shape	Tuber taste	Tuber aroma
			Dorsal	Ventral								
1	Medium/high	Erect	Green	Slightly glaucuous	Absent	Oct/Nov	Low	Absent	Hemisperical	Orange yellow	Slightly sweet and bitter	Turmeric aroma
2	Medium/high	Erect	Green	Slightly glaucuous	Absent	Oct/Nov	Low	Absent	Sperical	Light orange yellow	Slightly sweet and bitter	Turmeric aroma
3	Medium/high	Erect	Green	Slightly glaucuous	Absent	Oct/Nov	Low	Absent	Hemisperical	Light orange yellow	Slightly sweet and bitter	Turmeric aroma
4	Medium/high	Erect	Green	Slightly glaucuous	Absent	Oct/Nov	Medium	Absent	Oblong	Light mustard yellow	Slightly bitter	Turmeric aroma
5	Medium	Semierect	Dark green	Slightly glaucuous	Present	Sept/Oct	Medium	Absent	Oblong	Orange yellow	Slightly sweet	Sweet aromatic
6	High	Semierect	Green	Green	Absent	-	-	-	Oblong	Orange yellow	Slightly sweet	Highly aromatic
7	Low	Horizontal	Dark green	Green	Absent	Oct/Nov	Low	Present	Sperical	Light orange yellow	Bitter	Highly aromatic
8	Low	Horizontal	Dark green	Slightly glaucuous	Present	Oct	Low	Present	Sperical	Light orange yellow	Bitter	Highly aromatic
9	Medium	Semierect	Green	Green	Absent	Nov	No exsertion	Absent	Oblong	Dark orange yellow	Slightly acrid	Turmeric aroma
10	Medium	Semierect	Green	Green	Present	-	Low	Absent	Hemisperical	Dark orange yellow	Slightly bitter	Aromatic
11	Medium	Semierect	Green	Green	Present	Oct	Medium	Absent	Oblong	Light orange yellow	Watery	Turmeric aroma
12	Medium	Semierect	Green	Slightly glaucuous	Present	-	-	-	Oblong	Light mustard yellow	Bitter	Turmeric aroma

Contd.

Morpho-type	Plant canopy height	Leaf type	Leaf coulor		Hairyness on leaf	Flowering time	Flower exsertion	Fruit setting	Mother rhizome	Tuber colour shape	Tuber taste	Tuber aroma
			Dorsal	Ventral								
13	Medium	Erect	Green	Slightly glaucuous	Present	Oct	Medium	Absent	Oblong	Light orange yellow	Slightly sweet	Turmeric aroma
14	Medium	Semierect	Green	Green	Present	Sept/Oct	Low	Absent	Hemisperical	Orange yellow	Sweet	Turmeric aroma
15	Low	Semierect	Green	Green	Present	Nov	No exsertion	Absent	Oblong	Mustard yellow	Slightly bitter	Aromatic
16	Low	Semierect	Green	Green	Present	-	-	-	Sperical	Orange yellow	Bitter	Aromatic
17	Medium	Semierect	Dark green	Slightly glaucuous	Absent	-	-	-	Sperical	Light orange yellow	Bitter	Aromatic
18	Low	Horizontal	Dark green	Slightly glaucuous	Absent	Sept	No exsertion	Absent	Oblong	Light orange yellow	Very bitter	Turmeric aroma
19	Medium	Semierect	Green	Green	Absent	-	-	-	Sperical	Mustard yellow	Bitter	Aromatic
20	Medium	Semierect	Green	green	Absent	Oct	Low	Absent	Hemisperical	Orange yellow	Bitter	Aromatic
21	Medium	Semierect	Dark green	Slightly glaucuous	Absent	Oct.	Low	Absent	Oblong	Orange	Slightly acrid	Sweet aroma

Philip and Nair (1986) reported considerable variation in curcumin content, green yield, number of secondary fingers per plant, curing percentage and leaf blotch infection. Heritability estimates were highest for curing percentage, curcumin and oleoresin contents.

Mukhopadhyay *et al.* (1986) revealed significant variation for shoots/clump, leaves per shoot, plant height and yield per plant. Genotypic coefficient of varience was highest for total plot yield.

Geetha and Prabhakaran (1987) reported maximum GCV for rhizome yield followed by height of plant. PCV was also highest for rhizome yield followed by length of secondary fingers and height of the plant. In all cases GCV was lower than the corresponding PCV indicating the profound influence of environment on the expression of the genotypes.

Significant variation was noticed among the different varieties of turmeric in respect of growth characters such as plant height, number of leaves per plant, length of leaves, breadth of leaves and leaf area. However, no significant variation was noticed with respect to number of tillers per plant (Jalgaonkar *et al.*, 1988).

Variability study conducted by Reddy and Rao (1988) revealed that PCV was in general higher than the GCV. The GCV was very high for weight of root tubers followed by rhizome yield, leaf area, number of primary fingers and number of tillers indicating high degree of genetic variability for these characters.

Jalgaonkar *et al.* (1990) reported, good amount of variability, high magnitude of heritability and appreciable expected genetic advance for characters viz., yield of cured turmeric, number of primary fingers and yield of secondary fingers.

Menon *et al.* (1992) evaluated 39 open pollinated progenies of the turmeric variety Nandyal and found significant differences among progenies in respect of all plant characters except the number of tillers per plant, rhizome characters, yield, curing percentage and curcumin content.

Indiresh *et al.* (1992) observed high heritability and genetic advance for rhizome yield, internodal distance of primary and secondary fingers and number of secondary fingers per plant.

Yadav *et al.* (1996) evaluated 17 turmeric genotypes and significant differences were found among the genotypes for all the characters except width of leaves. Yield per plant and plant height exhibited a wide range of variation.

Kurian (1996) evaluated 17 turmeric genotypes and they differed significantly with regard to plant height, cured yield, curing percentage and curcumin content. There was'nt any significant variation among the turmeric types for number of tillers.

2.5 Isoenzyme analysis

Shamina *et al.* (1998) analysed 15 accessions of *C. longa* L. from different geographical areas in India for isoenzyme polymorphism. A high degree of variability was observed in the population studied and accessions showing high similarity were from the same geographical area.

2.5.1 Isoenzyme variation in different plant parts and at different stages of development

Tyson *et al.* (1986) observed that the manifestation range from change in intensity or relative intensity of bands to appearance or disappearance of bands may be correlated with leaf position and developmental stages.

Sebastian (1995) reported the ideal part for analysis of peroxidase was root or mature leaf, and immature leaf for GOT and esterase in pepper.

Marked differences were noted among 18 varieties of spring wheat in the zymograms for peroxidase isoenzyme of similar organs taken at the same growth stage. The enzyme pattern and activity of variety appeared different in different organs at different stages (Li and Li, 1996).

Markose (1996) reported an increase in number of bands for peroxidase with advent of plant growth in chilli. Esterase banding pattern also differed with plant growth.

2.5.2 Isoenzyme variation at inter-specific and intra-specific level

Peroxidase isoenzyme banding patterns were determined in 20 germplasm accessions of *Zizania latifolia*. The patterns were stable between years and could be used for the early identification of varieties and for varietal classification (Cao *et al.*, 1993).

Based on the peroxidase isoenzyme patterns obtained in 41 *Malus* accessions, the systematic positions of the 41 genotypes in the genus *Malus* were examined (Li *et al.*, 1995).

Twentyeight ginger cultivars (*Zingiber officinale*) were compared for peroxidase isoenzyme patterns by fuzzy cluster analysis in Fujian. The cultivars differed in isoenzyme pattern, activity and intensity. They were divided into three types viz., da-fei-jiang, huang-jiang and zhu-zi-jiang (He *et al.*, 1995).

Isoenzyme analysis carried out in 26 varieties and 11 species of pepper showed considerable variation at inter-specific and intra-specific level. They were grouped based on peroxidase, esterase and GOT banding pattern (Sebastian, 1995).

The intra and inter population variation in the reaction of adaptation of individuals of *Elytrigia repens* collected at different altitudes in eastern Germany showed variability of esterase and peroxidase isoenzyme patterns (Guttel and Hartenstein, 1996).

Satrabhandhu *et al.* (1996) reported that esterase isoenzyme could be used to discriminate lime (*C. aurantifolia*) cultivars and peroxidase isoenzyme showed no difference between the genotypes.

MATERIALS AND METHODS

The present study was conducted in the Department of Plantation Crops and Spices and Biochemistry laboratory of College of Horticulture, Vellanikkara, Thrissur from October 1997 to June 1999.

3.1 *Materials*

Materials studied included 18 species of *Curcuma* including *Curcuma longa* Linn. *Curcuma longa* is one of the important spice crops of Kerala showing enormous variation in morphological characters. Therefore, the taxon was studied in detail for within species variation as well. Thirtynine accessions including six released varieties were selected to represent the 21 morphotypes of Velayudhan *et al.*, 1994 (Table 2). These accessions were collected from the germplasm collection maintained at the NBPGR Regional Station, Thrissur. Released varieties were obtained from the collection maintained at the College of Horticulture, which included varieties from Kerala and neighbouring states (Table 3). Species other than *C. longa* were represented by single accessions each and were collected from the germplasm collections at the NBPGR Regional Station, Thrissur (Table 4).

3.2 Methods

For the separation of multiple forms of enzyme, polyacrylamide gel electrophoresis was carried out using vertical slab gel electrophoresis and power supply unit of M/s.Biotech.

Acrylamide monomers ($CH=CHCONH_2$) were co-polymerised with bisacrylamide [$CH_2(NHCONH=CH_2)_2$ bis] to obtain the gel. Freshly prepared ammonium persulphate was used as catalyst and N, N, N', N' tetra methylene diamine (TEMED) as chain initiator.

Table 2. *Curcuma longa* morphotypes used in the study

Sl. No.	Accession No. (NBPGR, Vellanikkara)	Morphotype (Velayudhan *et al.*, 1994)	Number used in the study	IC number (NBPGR)	Place of collection	Status
1	91	M-1	1	*IC-136904	NBPGR, New Delhi	Cultivated
2	94	M-1	1	**NA	Unknown	Cultivated
3	1126	M-2	2	IC-136974	Salem, Tamilnadu	Cultivated
4	92	M-2	2	IC-136975	Nilgiris, Tamilnadu	Cultivated
5	1281	M-3	3	NA	Kerala	Cultivated
6	75	M-4	4	IC-136980	NBPGR, New Delhi	Cultivated
7	1353	M-5	5.1	NA	Assam	-
8	830	M-5	5.2	IC-88879	Wayanad, Kerala	Cultivated
9	199	M-6	6	IC-137010	Arunachal Pradesh	Cultivated
10	190	M-7	7	IC-137023	NBPGR, New Delhi	-
11	158	M-7	7	IC-137011	NBPGR, New Delhi	Cultivated
12	1339	M-8	8	NA	KAU, Kerala	Cultivated
13	1318	M-8	8	NA	NBPGR, Hyderabad	-
14	1352	M-9	9	NA	West-Bengal	-
15	1372	M-9	9	NA	Imphal, Manipur	-
16	171	M-10	10	IC-137050	NBPGR, New Delhi	Cultivated
17	1363	M-10	10	NA	NBPGR, Shillong	-
18	141	M-11	11	IC-137063	NBPGR, New Delhi	Cultivated
19	1371	M-12	12	NA	Imphal, Manipur	-
20	1370	M-12	12	IC-6171	NBPGR, Imphal	-
21	893	M-13	13	IC-137073	Himachal Pradesh	Cultivated
22	897	M-13	13	IC-137076	Himachal Pradesh	Cultivated
23	803	M-14	14	IC-88749	Parambikulam, Kerala	Wild
24	801	M-15	15	IC-88755	Parambikulam, Kerala	Wild
25	1345	M-15	15	NA	Sikkim	-
26	733	M-16	16	IC-88785	Parambikulam, Kerala	Wild
27	197-B	M-16	16	NA	NBPGR, New Delhi	Cultivated
28	436	M-17	17	IC-17177	Unknown	Cultivated
29	739	M-18	18	IC-88779	Parambikulam, Kerala	Wild
30	194-B	M-19	19	IC-137113	NBPGR, New Delhi	Cultivated
31	184	M-19	19	IC-137017	Andhra Pradesh	Cultivated
32	898	M-20	20	IC-137114	Himachal Pradesh	Cultivated
33	1195	M-21	21	IC-137115	Sirmur, Himachal Pradesh	

* Indegeneous collection

** Not alloted

Table 3. *Curcuma longa* varieties used in the study

Sl. No.	Varieties	Number used in the study	Institution responsible for release	Pedigree
1	Kanthi	V-1	KAU, Kerala	Single plant selection from Mydukur, Andhra Pradesh
2	Sobha	V-2	KAU, Kerala	Single plant selection from Methala local, Kerala
3	Prathibha	V-3	IISR, Kerala	Open pollinated progeny selection
4	Prabha	V-4	IISR, Kerala	Open pollinated progeny selection
5	Sudarshana	V-5	IISR, Kerala	A selection from germplasm, collected from Singhat, Manipur
6	Rasmi	V-6	OUAT, Orissa	A clonal selection from Rajpuri local

Table 4. *Curcuma* species used in the study

Sl.No and No used in the study	*Curcuma* species	Sessile tubers present/ absent	Accession No.	IC No.	Place of collection
1	*C. amada* Roxb.	P	571	*NA	NBPGR, Vellanikkara
2	*C. zedoaria* Roxb.	P	999	NA	Nilambur, Kerala
3	*C. malabarica* Vel. *et al.*	P	368	**IC-88846	Kottayam, Kerala
4	*C. aeruginosa* Roxb.	P	228	IC-370090	North-East India
5	*C. caesia* Roxb.	P	224	IC-29968	Arunachal Pradesh
6	*C. soloensis* Val.	P	210	NA	North-East India
7	*C. brog* Val.	P	216	IC-29881	North-East India
8	*C. latifolia* Rosc.	P	1273	NA	North-East India
9	*C. aromatica* Salisb.	P	981	IC-88957	Idukki, Kerala
10	*C. harita* Sabu & Mangaly	P	959	IC-88947	Vellanikkara, Kerala
11	*C. sylvatica*. Val.	P	721	IC-88814	Trichur, Kerala
12	*C. raktakanta* Sabu & Mangaly	P	804	IC-88862	Nelliampathy, Kerala
13	*C. montana* Rosc.	P	1051	NA	Andhra Pradesh
14	*C. comosa* Roxb.	P	989	NA	North East India
15	*C. longa* L.	P	-	-	-
16	*C. vamana* Sabu & Mangaly	Stoloniferous	-	IC-88939	Thrissur, Kerala
17	*C. aurantiaca* Val.	A	-	-	Nilambur
18	*C. pseudomontana* Grah.	A	-	IC-136971	Nelliampathy, Kerala

*Not alloted; ** Indegeneous collection

Polyacrylamide gel was preferred because of its chemical inertness, high resolution, ease in handling and easiness in preparation.

3.2.1 Enzymes assayed

Electrophoresis and isoenzyme variation determination were done for the following enzymes.

1. Peroxidase
2. Esterase
3. Glutamate oxaloacetate transaminase

3.2.2 Preparation of the sample

Individual plants were raised from rhizome bits in polybags filled with potting mixture consisting of sand, soil and farmyard manure in 1:1:1 ratio. To select the ideal part for assay, plant parts like root, rhizome, sprout and leaf were used. In the case of leaf, different maturity stages such as tender and mature were also included. First two leaves from the tip were treated as tender and three basal dark green leaves as mature. For standardising the stage of growth of plant, sampling and analyses were done at three weeks after sprouting and just before senescence of leaf. The plant sample collected was washed to remove dust, dirt and other extraneous matters. It was then rinsed with distilled water. The rinsed material was gently pressed between blotting paper to remove water.

The sample was then weighed and ground in a pre-cooled mortar, along with extraction buffer (0.1M tris hydrochloride pH 7.6) in suitable proportion, around 5-10°C by keeping in an ice tray. From the different proportions tried it was found that a sample buffer ratio of 1:1 to 1:3 was ideal to get sufficient volume of extract in required concentration. During rainy season, since moisture content in leaves was high, the quantity of buffer used was less and during summer due to drying of leaves more buffer was used. Since GOT activity was found weak, less dilution was made with buffer.

The homogenised material was centrifuged at 15000 r.p.m for 20 minutes in a Remi centrifuge below 5°C. After centrifuging, the supernatent was removed into vials, labelled and stored below subzero temperature (refregerator freezer chest). Fresh samples were collected each day to get better results.

3.2.3 Preparation of the gel

Reagents

The analyses of the enzymes were carried out in anionic system. The following stock solutions were prepared.

Solution A

Tris - 36.6 g

TEMED - 0.23 ml

1N HCl - 48 ml

Volume made upto 100 ml with distilled water

pH - 8.9

Solution B

Acrylamide - 28.0g

N'N' methylene bisacrylamide - 0.735 g

Volume made upto 100 ml with distilled water

Solution C

Ammonium persulphate - 0.14 g

Volume made upto 125 ml with distilled water

Solution D

Acrylamide - 18.0 g

Bis-acrylamide - 0.47 g

Made up the volume to 100 ml with distilled water

Working solution	Acrylamide Concentration		
	7.5%	8.5%	10.5%
Solution A (ml)	2	2	2.5
Solution B (ml)	4.285	4.860	7.497
Solution C (ml)	9.715	9.150	10.003

Gel preparation was carried out by mixing the stock solution A, B and C in the above quantities to get the required concentration. Solution C was prepared fresh every time. Solution A and B were stored in amber coloured bottles.

Stacking gel solution

Stacking gel solution contained

Solution A (ml) - 5 ml

Solution B (ml) - 10 ml

Distilled water - 25 ml

Ammonium perulphate 0.1 g in - 300 µl

1 ml distilled water

Electrode buffer

Stock solution

Tris - 6 g

Glycine - 28.8 g

Volume made upto one litre with distilled water keeping the pH at 8.3. The stock buffer was diluted 1:9 before use.

Tracer dye

Bromophenol blue - 25 mg

Make up to 10 ml with tris-chloride buffer pH - 6.7

Tris-chloride buffer solution pH 6.7

Hcl, 1N - 48 ml

Tris - 5.98 g

TEMED - 0.46 ml

Volume made up to 100 ml with distilled water.

Slab gel of size 16 cm x 14 cm was used in the study. After preparing the working solution it was gently poured in between the glass plates kept in polymerisation stand. Polymerisation was achieved within three fourth of an hour to one hour. Stacking gel to a height of 1-1.5 cm was also used for better resolution of bands for all the enzymes.

After polymerisation, the gels along with glass plates were removed to electrophoretic apparatus. The upper and lower tank of the unit were filled with electrode buffer. Upper tank was connected to cathode and the lower one to the anode.

Electrophoresis was carried out at 5°C. A constant current of 25 mA per slab was maintained throughout the run. Bromophenol blue in tris-chloride buffer (pH 6.7) was used as the tracer dye.

3.2.4 Enzyme assays

3.2.4.1 Peroxidase

Gel concentration of 8.5 per cent acrylamide was found best for the peroxidase enzyme separation in *Curcuma spp.*

Gel buffer - Tris hydrochloride pH 8.9

Electrode buffer - Tris-glycine pH 8.3

Staining solution (Conkling and Smith, 1971)

Solution A - 0.05 g 0-dianisidine in 1ml of 1N HCl
0.05M Sodium acetate buffer pH 5.4 - 3 ml
distilled water - 26 ml

Solution B- 0.01% H_2O_2

The gel was incubated in solution A for 30 minutes at 37°C. After half an hour, solution A was removed and solution B was poured into the gel. Orange red bands of peroxidase developed. The reaction was arrested by adding 7 per cent acetic acid. As the bands faded on standing for long time, photographs were taken on the same day of staining.

3.2.4.2 Esterase

Gel concentration : Same as that for peroxidase
Gel buffer and electrode buffer: The same buffer as that for peroxidase was used.
Staining solution (modified from Shaw and Koen, 1968)
100 ml of staining solution contained

Phos A - Na_2HPO_4 (0.2M), pH 8.8	- 10 ml
Phos B - NaH_2PO_4 (0.2M), pH 4.16	- 50 ml
Fast blue RR	- 100 mg
α naphthyl acetate in 50% acetone	- 2 ml
Distilled water - 40 ml	

After the run was over, the gel was taken out and incubated in the staining solution at 37°C for 45-60 minutes till brown bands appeared. The gels were destained in 7 per cent acetic acid. The bands remained stable for 2-3 days.

3.2.4.3 Glutamate oxaloacetate transaminase

Gel concentration: 10.5% acrylamide

Gel and electrode buffer: Same as that for peroxidase

Staining solution (Shaw and Koen, 1968)

100 ml of staining solution contained

L - aspartic acid	- 532 mg
α ketoglutaric acid	- 72 mg
Pyridoxal 5' phosphate	- 50 mg
Fast violet B-salt	- 400 mg
0.1M phosphate buffer pH 7.0	- 100 ml

Fast violet B-salt was added just before use. Gels were incubated in the staining solution for 15 to 20 minutes until reddish orange bands developed. After staining, the gel was fixed in glycerine. The bands faded on standing. So photographs were taken within few hours of staining.

3.3 Nomenclature of isoenzymes adopted in the present study

The enzymes were designated by the following abbreviations.

1. Peroxidase	- PRX
2. Esterase	- EST
3. Glutamate oxaloacetate transaminase	- GOT

Numbering

For numbering of enzymes, all the isoenzymes of an enzyme in the species studied were pooled. The fastest moving anodal band was numbered 1 (eg. PRX-1). The slower ones were given subsequent numbers. Relative mobilities (Rm) of bands were calculated as per the formula

$$Rm = \frac{\text{Distance migrated by band}}{\text{Distance migrated by the dye}}$$

Measurement of similarity

The electrophoretic similarity among varieties and morphotypes of *C. longa* and among *Curcuma spp.* was calculated by making pairwise comparison of the genotypes following the method of Sokel and Sneath (1963) by using the formula

$$SI = \frac{\text{Number of homologous bands}}{\text{Number of homologous bands} + \text{Number of non homologous bands}}$$

Average of similarity indices for all enzymes were computed and also pooled data compared.

RESULTS

The results of the study are presented under three major heads as follows:

1. Isoenzymes in different plant parts and at different stages of development of plant
2. Isoenzyme variation within *Curcuma longa* L.
3. Isoenzyme variation and species relationship in the genus *Curcuma*

4.1 Isoenzyme in different plant parts and at different stages of development of plant

For standardizing the plant part and stage of growth of plant for each enzyme analysis, variety 'Rasmi' was used as a standard. Results of the study are given hereunder.

4.1.1 Peroxidase

In the analysis of isoenzyme variation in different plant parts at different stages of development of leaves, seven isoenzyme bands could be observed in *C. longa*. They were numbered PRX 1 to 7 for convenience. The numbering was later changed based on pooling and numbering the isoenzyme bands in different species.

The peroxidase bands in root were feeble. Only PRX-5, PRX-6 and PRX-7 were visible in root (Fig. 1 and Plate 5). Sprout showed very weak band of peroxidase. Only two isoenzymes viz., PRX-2 and PRX-3 were present in sprouts. PRX-4 and PRX-5 were absent in rhizomes though the other isoenzyme bands were deep and clear. In tender leaf, PRX-1, PRX-2 and PRX-3 were not clearly visible. Mature leaf was found to be the most ideal plant part for the electrophoresis of peroxidase isoenzyme with all bands showing better clarity in separation.

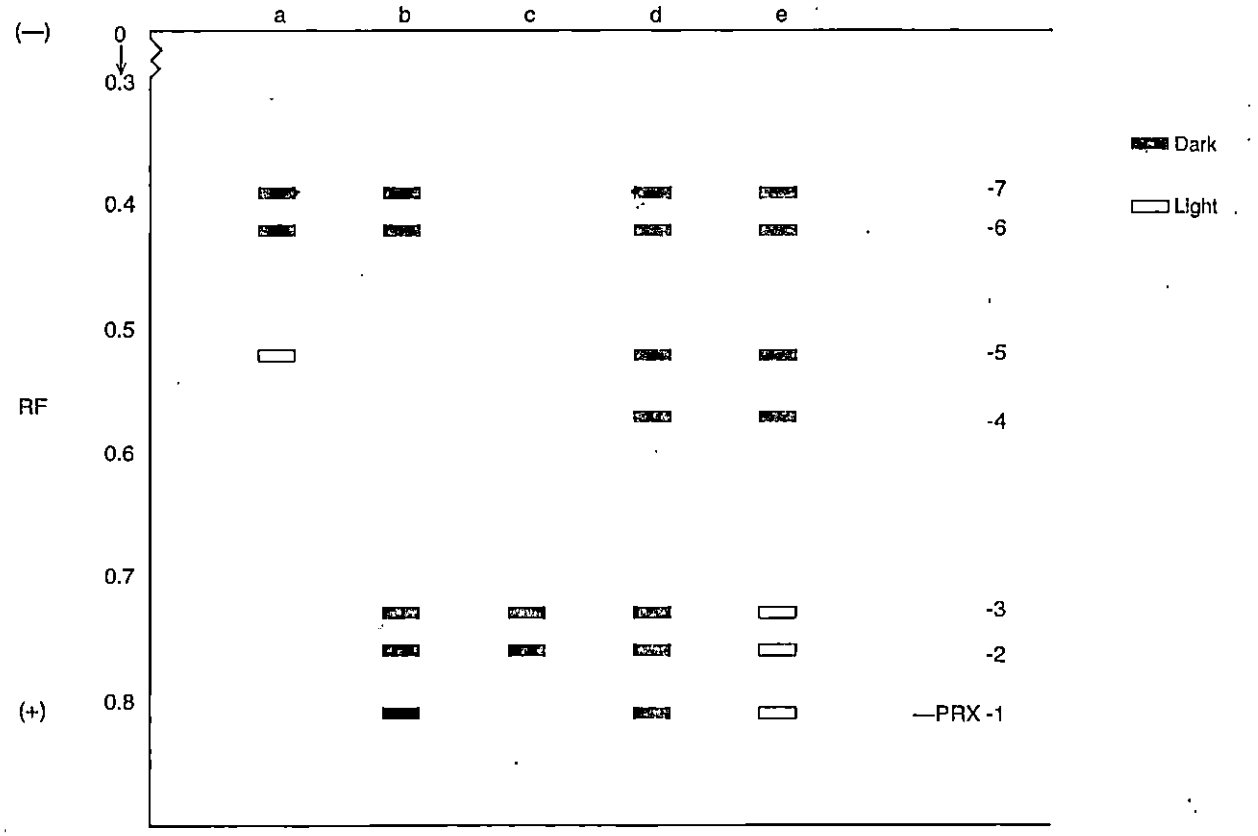

Fig. 1: Peroxidase zymogram in root (a), rhizome (b), sprout (c) mature leaf (d) and immature leaf (e) in *Curcuma longa* var *Rasmi*

The peroxidase bands were found to be increasing with the advent of plant growth. So, near senescence of leaf was the ideal stage for peroxidase isoenzyme analysis.

4.1.2 Esterase

In the case of esterase enzyme, nine bands were observed in *C. longa* and were numbered EST 1 to 9. As in the case of peroxidase, these numbering was changed when species and varieties were compared.

Isoenzyme bands were not observed for root sample. Rhizome had a faint band of EST-9 (Fig. 2 and Plate 6). EST-2, EST-3 and EST-9 were observed in mature leaf. Tender leaves were found to be the most ideal part for esterase enzyme analysis showing better clarity in separation. Sprouts were also found to be good. But due to scarcity of material, tender leaves were used for analysis.

Esterase isoenzymes was found to be more in the early stages of growth. So esterase isoenzyme analysis was done three weeks after sprouting.

4.1.3 Glutamate oxalo acetate transaminase (GOT)

GOT bands were less in the root and sprout and weak in rhizomes. No variation was found in GOT zymograms at different maturity stages of leaf and at different stages of growth of plant. For convenience of analysis, mature leaves were used in the study (Fig. 3 and Plate 7).

4.2 Isoenzyme banding pattern of *Curcuma longa* L.

Thirty nine accessions of *C. longa* including six released varieties were analysed in the study. Accessions were selected representing a wide genetic base with regard to morphological characters. Genotypes from Kerala and other states of India were analysed for variation at the three enzyme loci, viz., esterase, peroxidase

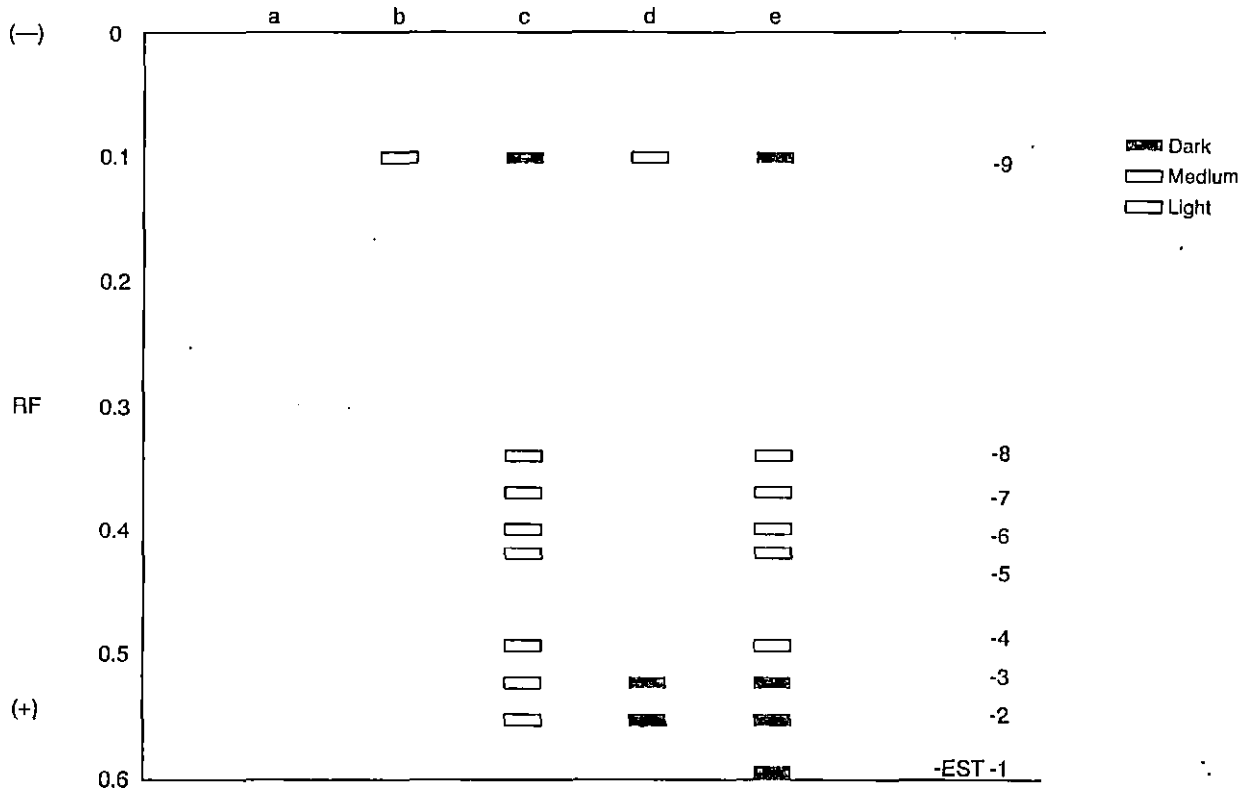

Fig. 2: Esterase zymogram in root (a), rhizome (b), sprout (c), mature leaf (d) and immature leaf (e) in *Curcuma longa* var *Rasmi*

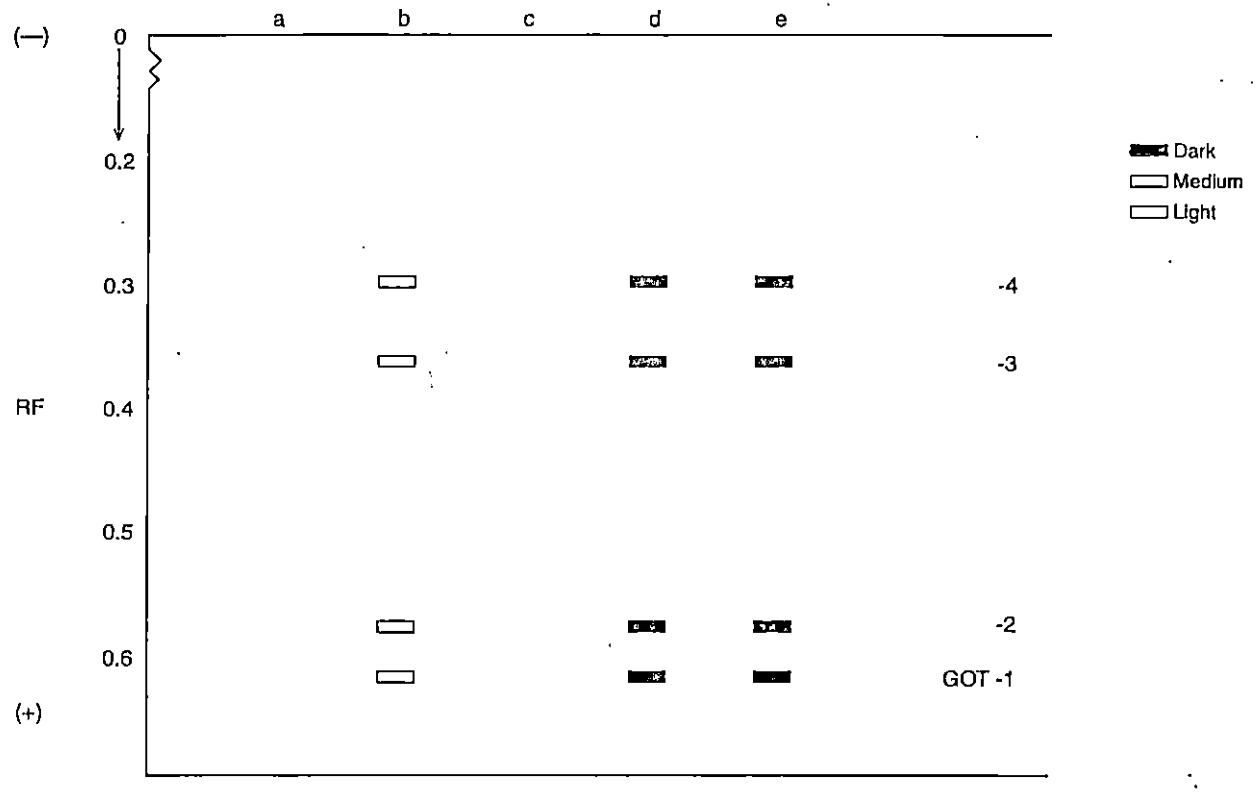

Fig. 3: GOT zymogram in root (a), rhizome (b), sprout (c), mature leaf (d) and immature leaf (e) in *Curcuma longa* var *Rasmi*

Plate 5 Peroxidase banding pattern in root (a), rhizome (c), sprout (b), mature leaf (d) and immature leaf (e) in *C. longa* var. *Rasmi*

Plate 6 Esterase banding pattern in root (a), rhizome (b), sprout (c), mature leaf (d) and immature leaf (e) in *C. longa* var. *Rasmi*

Plate 7 GOT banding pattern in root (a), rhizome (b), sprout (c), mature leaf (d) and immature leaf (e) in *C. longa* var. *Rasmi*

Plate 5

Plate 6

Plate 7

and GOT. Genotypes other than released varieties were selected to represent the 21 morphotypes described by Velayudhan *et al.*, 1994.

4.2.1 Peroxidase

4.2.1.1 Morphotypes (M)

Morphotypes showed considerable variation in the banding pattern of peroxidase isoenzymes. Out of the 51 isoenzyme bands found in the genus *Curcuma* 44 were observed in *C. longa*. Seventeen variant isoenzymes were observed in the 21 morphotypes of *C. longa* analysed (Fig.4 and Plates 8, 9, 10). PRX-11 was common in all the 21 morphotypes. PRX-28 was lacking in M-12 and PRX-36 in M-10. In all other morphotypes these bands were observed. Accessions within a morphotype showed similar banding pattern except in M-5.

Based on the complex pattern of peroxidase all the 33 accessions representing the 21 morphotypes were grouped into 17 (Table 5).

4.2.1.2 Varieties

All the six varieties showed similar banding pattern with PRX-3, 8, 11, 24, 28, 36 and 39 (Plate 11). These varieties fall in the group I based on peroxidase isozyme pattern (Table 5).

4.2.2 Esterase

4.2.2.1 Morphotypes

Tender leaf was observed to be ideal for the analysis of esterase isoenzyme. Out of the 36 esterase bands found in the genus *Curcuma*, 29 were observed in *C. longa*. Sixteen variant isoenzymes were observed in the 33 genotypes of *C. longa* analysed (Fig. 5 and Plates 12, 13, 14). EST-26, 27, 28, 29 were found to be common for all the 21 morphotypes. Accessions in the fifth

Plates 8, 9, 10,11 Peroxidase banding pattern of *C. longa* genotypes
(1 - 33 serial no. of accessions as in Table 2.)
(34-39 varieties V-1 to V-6)

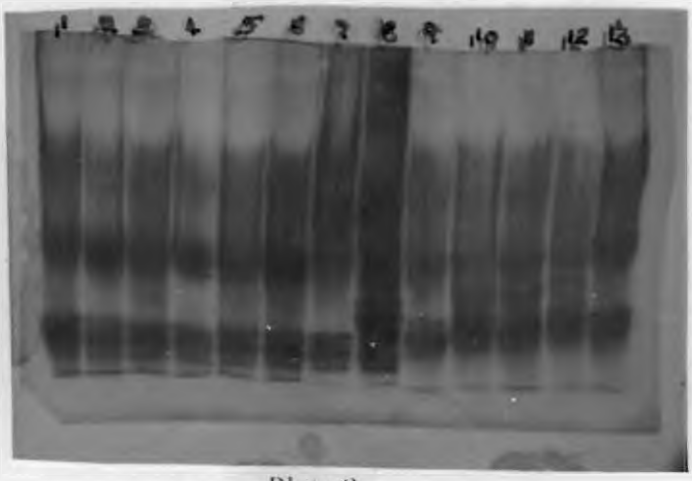

Plate 8

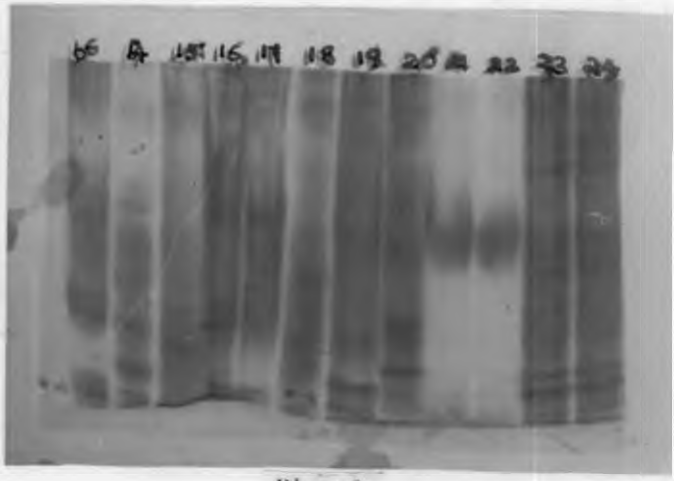

Plate 9

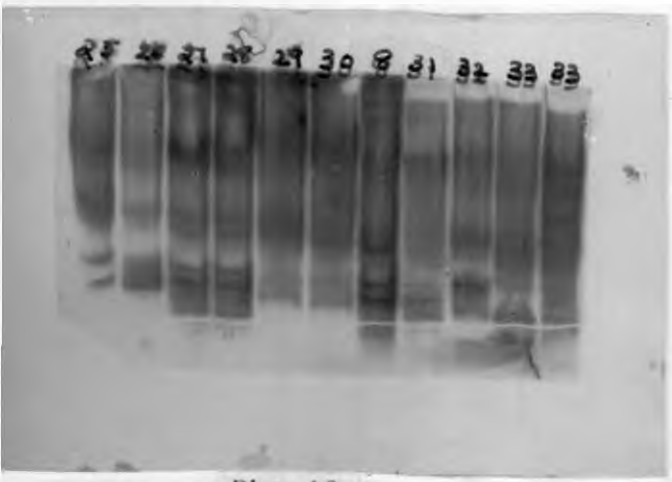

Plate 10

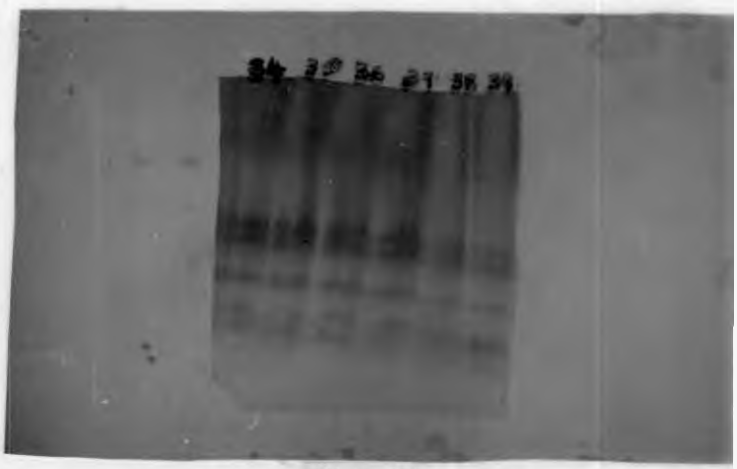

Plate 11

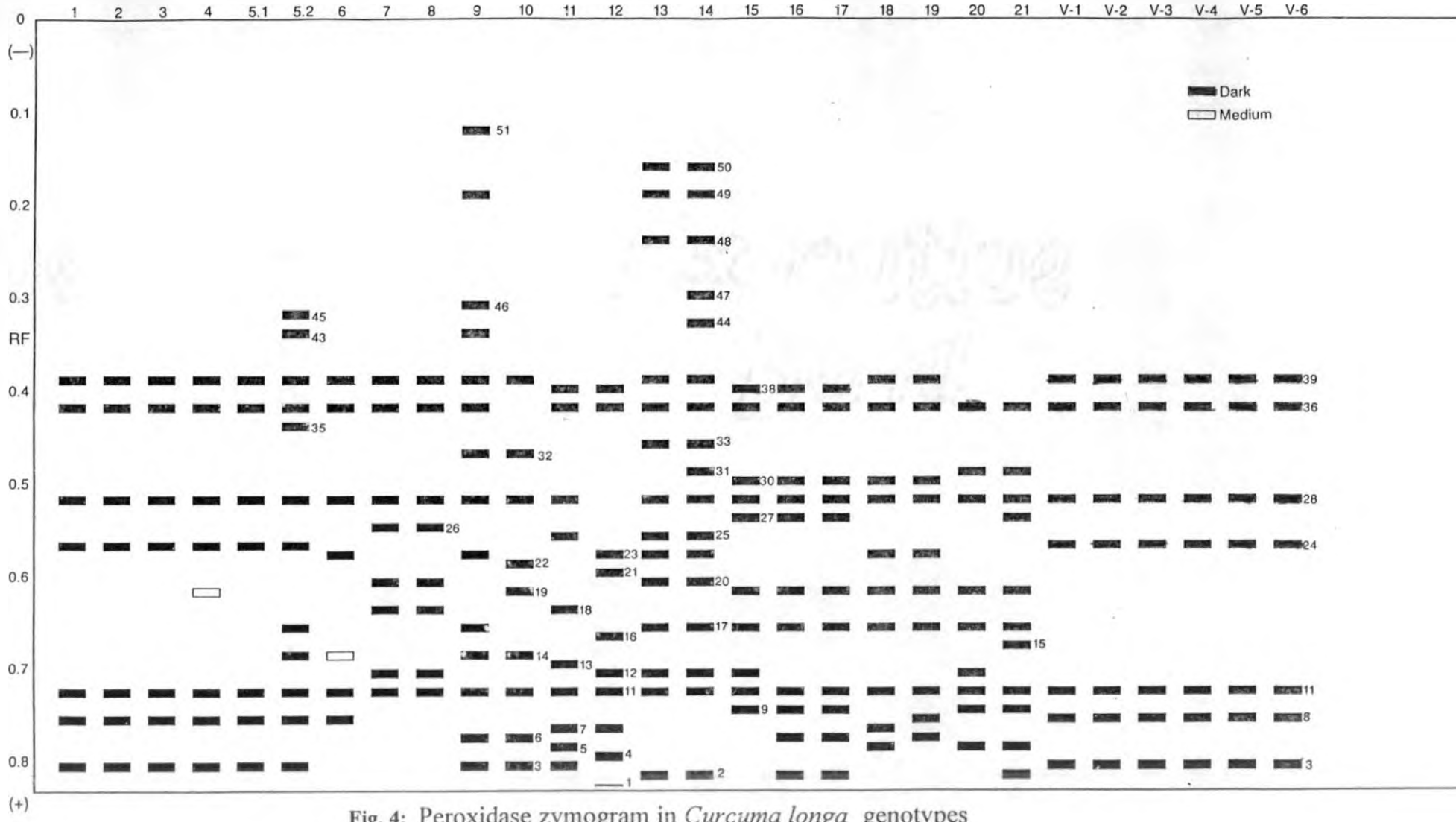

Fig. 4: Peroxidase zymogram in *Curcuma longa* genotypes
(1-21 morphotypes M_1 — M_{21}, V-1 — V-6 varieties)

Table 5. Groups of *C. longa* morphotypes and varieties based on isoenzyme banding pattern of peroxidase

Sl.No.	Accession No.	Morphotype	Group	Sl.No.	Accession No.	Morphotype	Group
1	91	M-1	G-1	21	893	M-13	G-10
2	94	M-1	G-1	22	897	M-13	G-10
3	1126	M-2	G-1	23	803	M-14	G-11
4	92	M-2	G-1	24	801	M-14	G-11
5	1281	M-3	G-1	25	1345	M-15	G-12
6	75	M-4	G-2	26	733	M-15	G-12
7	1353	M-5	G-1	27	197-B	M-16	G-13
8	830	M-5	G-3	28	436	M-17	G-13
9	199	M-6	G-4	29	739	M-18	G-14
10	190	M-7	G-5	30	194-B	M-19	G-15
11	158	M-7	G-5	31	184	M-19	G-15
12	1339	M-8	G-5	32	898	M-20	G-16
13	1318	M-8	G-5	33	1195	M-21	G-17
14	1352	M-9	G-6	34	Kanthi	V-1	G-1
15	1372	M-9	G-6	35	Sobha	V-2	G-1
16	171	M-10	G-7	36	Prathibha	V-3	G-1
17	1363	M-10	G-7	37	Prabha	V-4	G-1
18	141	M-11	G-8	38	Sudarshana	V-5	G-1
19	1371	M-12	G-9	39	Rasmi	V-6	G-1
20	1370	M-12	G-9				

Plates 12, 13, 14 Esterase banding pattern of *C. longa* genotypes
(1 - 33 serial no. of accessions as in Table 2.)
(34-39 varieties V-1 to V-6)

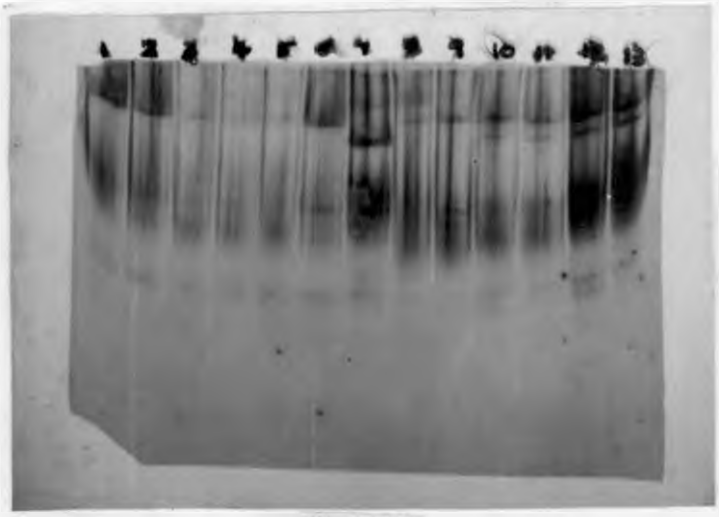

Plate 12

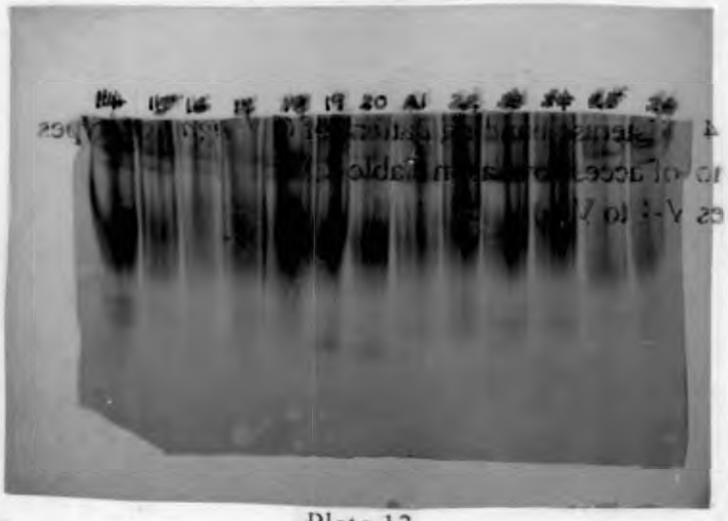

Plate 13

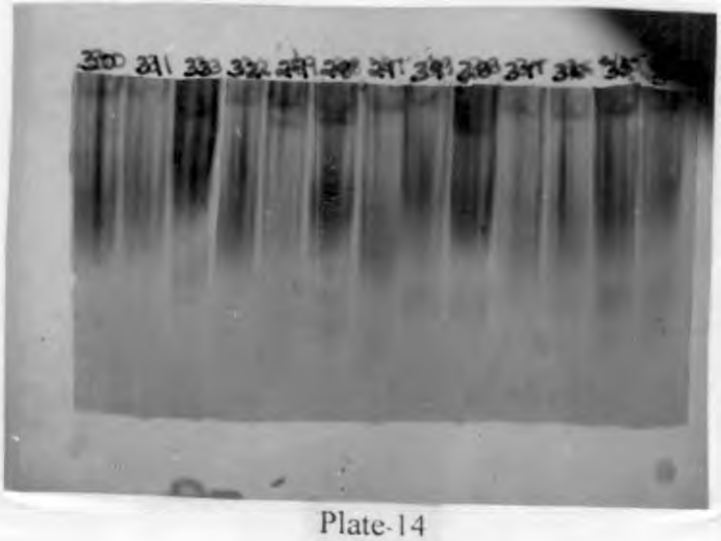

Plate-14

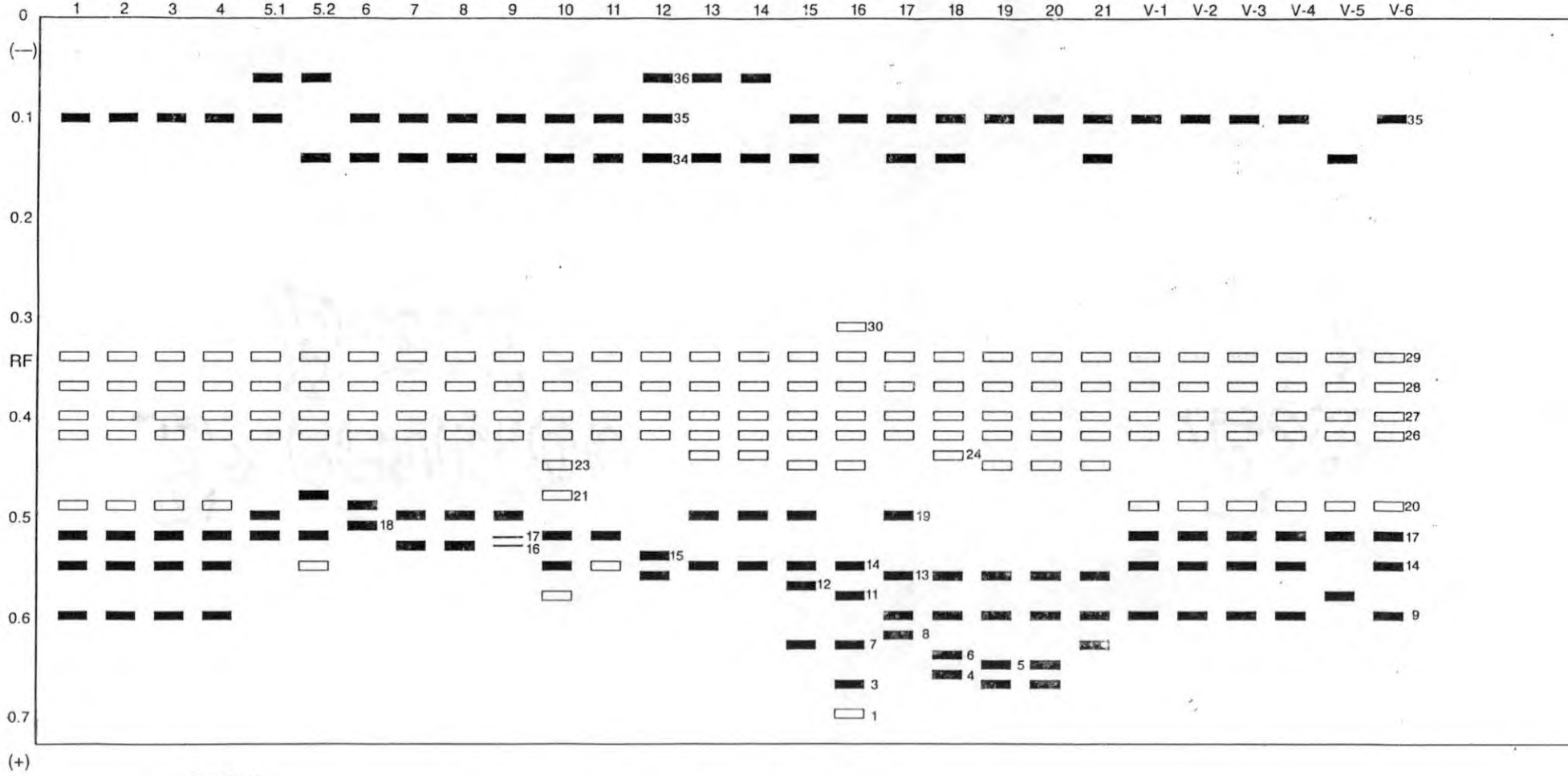

Fig. 5: Esterase zymogram in *Curcuma longa* genotypes
(1-21 morphotypes M_1 — M_{21}, V-1 — V-6 varieties)

Table 6. Groups of *C. longa* morphotypes and varieties based on isoenzyme banding pattern of esterase

Sl.No.	Accession No.	Morphotype	Group	Sl.No.	Accession No.	Morphotype	Group
1	91	M-1	G-1	21	893	M-13	G-10
2	94	M-1	G-1	22	897	M-13	G-10
3	1126	M-2	G-1	23	803	M-14	G-10
4	92	M-2	G-1	24	801	M-14	G-10
5	1281	M-3	G-1	25	1345	M-15	G-11
6	75	M-4	G-1	26	733	M-15	G-11
7	1353	M-5	G-2	27	197-B	M-16	G-12
8	830	M-5	G-3	28	436	M-17	G-13
9	199	M-6	G-4	29	739	M-18	G-14
10	190	M-7	G-5	30	194-B	M-19	G-15
11	158	M-7	G-5	31	184	M-19	G-15
12	1339	M-8	G-5	32	898	M-20	G-15
13	1318	M-8	G-5	33	1195	M-21	G-16
14	1352	M-9	G-6	34	Kanthi	V-1	G-1
15	1372	M-9	G-6	35	Sobha	V-2	G-1
16	171	M-10	G-7	36	Prathibha	V-3	G-1
17	1363	M-10	G-7	37	Prabha	V-4	G-1
18	141	M-11	G-8	38	Sudarshana	V-5	G-17
19	1371	M-12	G-9	39	Rasmi	V-6	G-1
20	1370	M-12	G-9				

Plates 15, 16, 17 GOT banding pattern of *C. longa* genotypes
(1 - 33 serial no. of accessions as in Table 2.)
(34-39 varieties V-1 to V-6)

morphotype showed different banding patterns. Morphotypes could be grouped into 16 based on the esterase banding pattern (Table 6).

4.2.2.2 Varieties

Kanthi, Sobha, Prathibha, Prabha and Rasmi showed similar banding pattern with nine isoenzyme bands. Their zymograms were similar to that of morphotypes 1, 2, 3 and 4. Sudarshana was different from these and formed a separate group (Table 6).

4.2.3 GOT

4.2.3.1 Morphotypes

Twelve bands were observed in the 21 morphotypes studied (Fig. 6 and Plates 15, 16, 17). GOT-1 and 2 were common in all the 21 morphotypes. Thirty three genotypes representing the 21 morphotypes could be grouped into five based on the isoenzyme banding pattern of GOT enzyme (Table 7). Accessions belonging to the same morphotype showed similar banding pattern for GOT.

4.2.3.2 Varieties

Kanthi, Sobha, Prathibha and Prabha could be included in the first group based on GOT zymogram. Four bands were observed in Sudarshana and Rasmi which were numbered GOT-1, 2, 8 and 12. They formed a sixth group different from the morphotypes.

4.2.4 Similarity within *Curcuma longa*

Similarity index, among and between the morphotypes and varieties for isoenzyme banding pattern was calculated according to Sokel and Sneath (1963). The similarity index values for the three enzymes separately as well as the pooled data are presented hereunder.

Plates 15, 16, 17 GOT banding pattern of *C. longa* genotypes
(1 - 33 serial no. of accessions as in Table 2.)
(34-39 varieties V-1 to V-6)

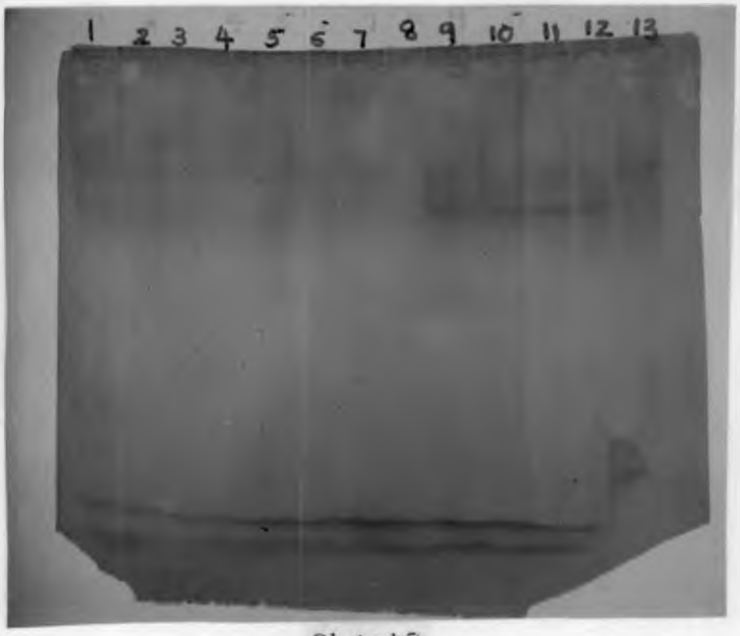

Plate 15

Plate 16

Plate 17

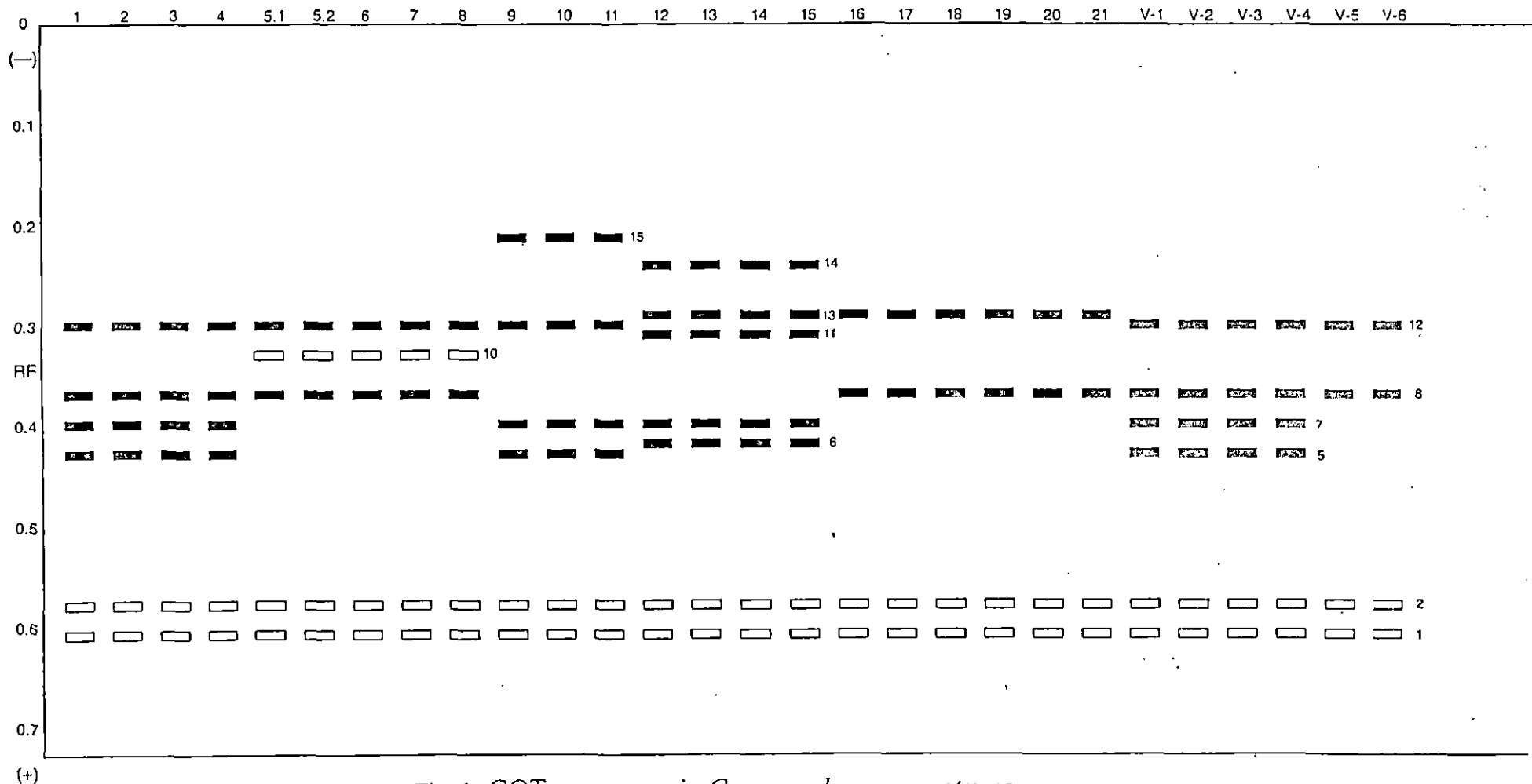

Fig. 6: GOT zymogram in *Curcuma longa* genotypes
(1-21 morphotypes M_1 — M_{21}, V-1 — V-6 varieties)

Table 7. Groups of *C. longa* morphotypes and varieties based on isoenzyme banding pattern of GOT

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
M-1	M-5	M-9	M-12	M-16	Sudarshana
M-2	M-6	M-10	M-13	M-17	Rasmi
M-3	M-7	M-11	M-14	M-18	
M-4	M-8		M-15	M-19	
Kanthi				M-20	
Sobha				M-21	
Prathibha					
Prabha					

4.2.4.1 Peroxidase

4.2.4.1.1 Morphotypes

Range of similarity among the morphotypes for peroxidase zymogram was from 0.21 to 1 (Table 8). Accessions of the same morphotype showed a similarity index one except in the case of morphotype 5. Here similarity within morphotype was 0.74. Three groups of morphotypes were obtained with similarity index one among the members (Table 9). Similarity index was more with the succeeding or preceeding morphotypes. Least similarity index of 0.21 was observed between morphotype 12 and 10.

4.2.4.1.2 Varieties

All the varieties under study showed a similarity index of one among them.

4.2.4.1.3 Morphotypes and varieties

Range of similarity among the morphotypes and varieties for peroxidase zymogram was from 0.24 to 1. Highest similarity of one based on peroxidase banding pattern was with the first group of morphotypes which included morphotype 1, 2 and 3. Least similarity index of 0.24 was observed with morphotype 12.

4.2.4.2 Esterase

4.2.4.2.1 Morphotypes

Range of similarity among morphotypes for esterase enzyme was from 0.42 to 1 (Table 10). Based on similarity, morphotypes could be grouped into 4 as furnished in Table 11. Similarity index was more with the succeeding or preceeding morphotype except for M-12. M-12 showed more similarity with M-17. Least

Table 8. Similarity index for peroxidase in *C. longa* genotypes

	1	2	3	4	5/1	5/2	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	V-1	V-2	V-3	V-4	V-5	V-6
1	1																											
2	1	1																										
3	1	1	1																									
4	0.93	0.93	0.93	1																								
5/1	1	1	1	0.93	1																							
5/2	0.74	0.74	0.74	0.70	0.74	1																						
6	0.71	0.71	0.71	0.71	0.71	0.63	1																					
7	0.53	0.53	0.53	0.53	0.53	0.40	0.53	1																				
8	0.53	0.53	0.53	0.53	0.53	0.40	0.53	1	1																			
9	0.48	0.48	0.48	0.48	0.48	0.62	0.57	0.36	0.36	1																		
10	0.50	0.50	0.50	0.63	0.50	0.48	0.50	0.35	0.35	0.61	1																	
11	0.47	0.47	0.47	0.47	0.47	0.36	0.35	0.44	0.44	0.33	0.32	1																
12	0.24	0.24	0.24	0.24	0.24	0.27	0.35	0.33	0.33	0.33	0.21	0.50	1															
13	0.38	0.38	0.38	0.30	0.38	0.39	0.48	0.46	0.46	0.43	0.26	0.33	0.50	1														
14	0.33	0.33	0.33	0.33	0.33	0.35	0.42	0.40	0.40	0.39	0.23	0.30	0.44	0.90	1													
15	0.35	0.35	0.35	0.47	0.35	0.36	0.35	0.44	0.44	0.33	0.32	0.40	0.60	0.42	0.37	1												
16	0.33	0.33	0.33	0.44	0.33	0.35	0.33	0.32	0.32	0.40	0.40	0.38	0.48	0.40	0.36	0.76	1											
17	0.33	0.33	0.33	0.44	0.33	0.35	0.33	0.32	0.32	0.40	0.40	0.38	0.48	0.40	0.36	0.76	1	1										
18	0.59	0.59	0.59	0.71	0.59	0.55	0.71	0.44	0.44	0.50	0.42	0.50	0.40	0.42	0.37	0.60	0.57	0.57	1									
19	0.59	0.59	0.59	0.71	0.59	0.55	0.71	0.47	0.47	0.58	0.53	0.30	0.50	0.42	0.37	0.60	0.67	0.67	0.80	1								
20	0.38	0.38	0.38	0.50	0.38	0.38	0.38	0.47	0.47	0.35	0.33	0.42	0.53	0.40	0.46	0.74	0.60	0.60	0.63	0.53	1							
21	0.44	0.44	0.44	0.56	0.44	0.44	0.33	0.32	0.32	0.40	0.40	0.48	0.38	0.32	0.36	0.66	0.64	0.64	0.57	0.48	0.80	1						
V-1	1	1	1	0.93	1	0.74	0.71	0.53	0.53	0.48	0.50	0.47	0.24	0.38	0.33	0.35	0.33	0.33	0.59	0.59	0.38	0.44	1					
V-2	1	1	1	0.93	1	0.74	0.71	0.53	0.53	0.48	0.50	0.47	0.24	0.38	0.33	0.35	0.33	0.33	0.59	0.59	0.38	0.44	1	1				
V-3	1	1	1	0.93	1	0.74	0.71	0.53	0.53	0.48	0.50	0.47	0.24	0.38	0.33	0.35	0.33	0.33	0.59	0.59	0.38	0.44	1	1	1			
V-4	1	1	1	0.93	1	0.74	0.71	0.53	0.53	0.48	0.50	0.47	0.24	0.38	0.33	0.35	0.33	0.33	0.59	0.59	0.38	0.44	1	1	1	1		
V-5	1	1	1	0.93	1	0.74	0.71	0.53	0.53	0.48	0.50	0.47	0.24	0.38	0.33	0.35	0.33	0.33	0.59	0.59	0.38	0.44	1	1	1	1	1	
V-6	1	1	1	0.93	1	0.74	0.71	0.53	0.53	0.48	0.50	0.47	0.24	0.38	0.33	0.35	0.33	0.33	0.59	0.59	0.38	0.44	1	1	1	1	1	1

Table 9. Groups of *C. longa* morphotypes and varieties having similarity index one among the members for peroxidase zymogram

Group 1		Group 2		Group 3	
Morphotype	Accession No.	Morphotype	Accession No.	Morphotype	Accession No.
M-1	91	M-7	190	M-16	197-B
M-1	94	M-7	158	M-17	436
M-2	1126	M-8	1339		
M-2	92	M-8	1318		
M-3	1281				
M-5	1353				
V-1	Kanthi				
V-2	Sobha				
V-3	Prathibha				
V-4	Prabha				
V-5	Sudarshana				
V-6	Rasmi				

Table 10. Similarity index for esterase in *C. longa* genotypes

	1	2	3	4	5/1	5/2	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	V-1	V-2	V-3	V-4	V-5	V-6
1	1																											
2	1	1																										
3	1	1	1																									
4	1	1	1	1																								
5/1	0.71	0.71	0.71	0.71	1																							
5/2	0.67	0.67	0.67	0.67	0.71	1																						
6	0.71	0.71	0.71	0.71	0.63	0.59	1																					
7	0.59	0.59	0.59	0.59	0.75	0.59	0.75	1																				
8	0.59	0.59	0.59	0.59	0.75	0.59	0.75	1	1																			
9	0.63	0.63	0.63	0.63	0.78	0.63	0.67	0.89	0.89	1																		
10	0.74	0.74	0.74	0.74	0.67	0.84	0.67	0.67	0.67	0.70	1																	
11	0.71	0.71	0.71	0.71	0.75	0.82	0.67	0.67	0.67	0.78	0.89	1																
12	0.56	0.56	0.56	0.56	0.71	0.67	0.71	0.71	0.71	0.63	0.63	0.71	1															
13	0.56	0.56	0.56	0.56	0.71	0.78	0.59	0.71	0.71	0.74	0.63	0.71	0.67	1														
14	0.56	0.56	0.56	0.56	0.71	0.78	0.58	0.71	0.71	0.74	0.63	0.71	0.67	1	1													
15	0.60	0.60	0.60	0.60	0.63	0.67	0.63	0.74	0.74	0.76	0.67	0.74	0.60	0.80	0.80	1												
16	0.57	0.57	0.57	0.57	0.50	0.48	0.50	0.50	0.50	0.55	0.64	0.60	0.57	0.48	0.48	0.70	1											
17	0.63	0.63	0.63	0.63	0.67	0.53	0.67	0.78	0.78	0.70	0.60	0.67	0.74	0.63	0.63	0.67	0.46	1										
18	0.70	0.70	0.70	0.70	0.53	0.50	0.63	0.63	0.63	0.57	0.57	0.63	0.70	0.60	0.60	0.55	0.44	0.76	1									
19	0.74	0.74	0.74	0.74	0.56	0.42	0.56	0.56	0.56	0.60	0.50	0.56	0.63	0.42	0.42	0.57	0.64	0.70	0.76	1								
20	0.74	0.74	0.74	0.74	0.56	0.42	0.56	0.56	0.56	0.60	0.50	0.56	0.63	0.42	0.42	0.57	0.64	0.70	0.76	1	1							
21	0.74	0.74	0.74	0.74	0.56	0.53	0.67	0.67	0.67	0.70	0.60	0.67	0.74	0.53	0.53	0.67	0.64	0.80	0.76	0.80	0.80	1						
V-1	1	1	1	1	0.71	0.67	0.71	0.59	0.59	0.63	0.74	0.71	0.56	0.56	0.56	0.60	0.57	0.63	0.70	0.74	0.74	0.74	1					
V-2	1	1	1	1	0.71	0.67	0.71	0.59	0.59	0.63	0.74	0.71	0.56	0.56	0.56	0.60	0.57	0.63	0.70	0.74	0.74	0.74	1	1				
V-3	1	1	1	1	0.71	0.67	0.71	0.59	0.59	0.63	0.74	0.71	0.56	0.56	0.56	0.60	0.57	0.63	0.70	0.74	0.74	0.74	1	1	1			
V-4	1	1	1	1	0.71	0.67	0.71	0.59	0.59	0.63	0.74	0.71	0.56	0.56	0.56	0.60	0.57	0.63	0.70	0.74	0.74	0.74	1	1	1	1		
V-5	0.82	0.82	0.82	0.82	0.63	0.71	0.75	0.63	0.63	0.67	0.78	0.75	0.59	0.59	0.59	0.53	0.50	0.56	0.53	0.44	0.44	0.56	0.82	0.82	0.82	0.82	1	
V-6	1	1	1	1	0.71	0.67	0.71	0.59	0.59	0.63	0.74	0.71	0.56	0.56	0.56	0.60	0.57	0.63	0.70	0.74	0.74	0.74	1	1	1	1	0.82	1

Table 11. Groups of *C. longa* morphotypes and varieties having similarity index one among the members for esterase zymogram

Group 1	Group 2	Group 3	Group 4
M-1	M-7	M-13	M-19
M-2	M-8	M-14	M-20
M-3			
M-4			
Kanthi			
Sobha			
Prathibha			
Prabha			
Rasmi			

similarity index was between morphotypes 13 and 14 with morphotype 19 and 20 (SI - 0.42).

4.2.4.2.2 Varieties

Except Sudarshana all other varieties showed a similarity index of one among them. Similarity index of Sudarshana with other varieties was 0.82.

4.2.4.2.3 Morphotypes and varieties

Range of similarity among morphotypes and varieties for esterase zymogram was 0.44 to 1. Highest similarity index was between Kanthi, Sobha, Prathibha, Prabha with the first group of morphotypes based on esterase banding pattern. Sudarshana showed a similarity index of 0.82 with the first group of morphotype. Least similarity was observed between Sudarshana and fifteenth group of morphotype M-19 and M-20 (SI - 0.44).

4.2.4.3 Glutamate oxaloacetate transaminase (GOT)

4.2.4.3.1 Morphotypes

In the case of GOT, a similarity index range of 0.33 to 1 was observed among the morphotypes (Table 12). Five groups of morphotypes were observed with similarity index one among the members of the group (Table 7). The least similarity was observed between group 2 and group 4 members.

4.2.4.3.2 Varieties

The varieties could be grouped into two with similarity index one among the members of the group Kanthi, Sobha, Prathibha and Prabha formed the first group. and Sudarshana and Rasmi formed the second group. Similarity index of 0.8 was observed between the two groups of varieties.

Table 12. Similarity index for GOT in *C. longa* genotypes

	1	2	3	4	5/1	5/2	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	V-1	V-2	V-3	V-4	V-5	V-6
1	1																											
2	1	1																										
3	1	1	1																									
4	1	1	1	1																								
5/1	0.73	0.73	0.73	0.73	1																							
5/2	0.73	0.73	0.73	0.73	1	1																						
6	0.73	0.73	0.73	0.73	1	1	1																					
7	0.73	0.73	0.73	0.73	1	1	1	1																				
8	0.73	0.73	0.73	0.73	1	1	1	1	1																			
9	0.83	0.83	0.83	0.83	0.55	0.55	0.55	0.55	0.55	1																		
10	0.83	0.83	0.83	0.83	0.55	0.55	0.55	0.55	0.55	1	1																	
11	0.83	0.83	0.83	0.83	0.55	0.55	0.55	0.55	0.55	1	1	1																
12	0.46	0.46	0.46	0.46	0.33	0.33	0.33	0.33	0.33	0.46	0.46	0.46	1															
13	0.46	0.46	0.46	0.46	0.33	0.33	0.33	0.33	0.33	0.46	0.46	0.46	1	1														
14	0.46	0.46	0.46	0.46	0.33	0.33	0.33	0.33	0.33	0.46	0.46	0.46	1	1	1													
15	0.46	0.46	0.46	0.46	0.33	0.33	0.33	0.33	0.33	0.46	0.46	0.46	1	1	1	1												
16	0.60	0.60	0.60	0.60	0.67	0.67	0.67	0.67	0.67	0.40	0.40	0.40	0.55	0.55	0.55	0.55	1											
17	0.60	0.60	0.60	0.60	0.67	0.67	0.67	0.67	0.67	0.40	0.40	0.40	0.55	0.55	0.55	0.55	1	1										
18	0.60	0.60	0.60	0.60	0.67	0.67	0.67	0.67	0.67	0.40	0.40	0.40	0.55	0.55	0.55	0.55	1	1	1									
19	0.60	0.60	0.60	0.60	0.67	0.67	0.67	0.67	0.67	0.40	0.40	0.40	0.55	0.55	0.55	0.55	1	1	1	1								
20	0.60	0.60	0.60	0.60	0.67	0.67	0.67	0.67	0.67	0.40	0.40	0.40	0.55	0.55	0.55	0.55	1	1	1	1	1							
21	0.60	0.60	0.60	0.60	0.67	0.67	0.67	0.67	0.67	0.40	0.40	0.40	0.55	0.55	0.55	0.55	1	1	1	1	1	1						
V-1	1	1	1	1	0.73	0.73	0.73	0.73	0.73	0.83	0.83	0.83	0.46	0.46	0.46	0.46	0.60	0.60	0.60	0.60	0.60	0.60	1					
V-2	1	1	1	1	0.73	0.73	0.73	0.73	0.73	0.83	0.83	0.83	0.46	0.46	0.46	0.46	0.60	0.60	0.60	0.60	0.60	0.60	1	1				
V-3	1	1	1	1	0.73	0.73	0.73	0.73	0.73	0.83	0.83	0.83	0.46	0.46	0.46	0.46	0.60	0.60	0.60	0.60	0.60	0.60	1	1	1			
V-4	1	1	1	1	0.73	0.73	0.73	0.73	0.73	0.83	0.83	0.83	0.46	0.46	0.46	0.46	0.60	0.60	0.60	0.60	0.60	0.60	1	1	1	1		
V-5	0.80	0.80	0.80	0.80	0.89	0.89	0.89	0.89	0.89	0.60	0.60	0.60	0.36	0.36	0.36	0.36	0.75	0.75	0.75	0.75	0.75	0.75	0.80	0.80	0.80	0.80	1	
V-6	0.80	0.80	0.80	0.80	0.89	0.89	0.89	0.89	0.89	0.60	0.60	0.60	0.36	0.36	0.36	0.36	0.75	0.75	0.75	0.75	0.75	0.75	0.80	0.80	0.80	0.80	1	1

4.2.4.3.3 Morphotypes and varieties

Group one of morphotypes and group one of varieties showed a similarity index of one among its members. Group two of varieties showed the highest similarity index of 0.89 with group two of morphotypes. Least similarity was observed between the group four of morphotypes and the varieties (SI - 0.36 to 0.46).

4.2.5 Similarity index among *C. longa* genotypes for isoenzyme banding pattern

4.2.5.1 Morphotypes

Similarity index for three isoenzymes put together ranged from 0.41 to 1 (Table 13). Highest similarity index of one was seen in two groups of morphotypes (Table 14). Accessions in the morphotype 5 showed a similarity index of only 0.82 between them. Accessions in all other morphotypes showed a similarity index of one among them. More similarity was observed between succeeding or preceeding morphotype except in M-15. M-15 was more similar to M-13. The least similarity (SI - 0.41) was between M-20 and M-10.

4.2.5.2 Varieties

Similarity index ranged from 0.87 to 1 among the varieties. Kanthi, Sobha, Prathibha and Prabha showed a similarity index of one among them and also with M-1, M-2 and M-3. Lowest similarity index of 0.87 was between Sudarshana and above group of varieties.

4.2.5.3 Morphotypes and varieties

Range of similarity among the 39 genotypes of *C. longa* was from 0.39 to 1. All the varieties showed a similarity index in the range of 0.87 to 1 with the first group of morphotypes. Least similarity was between M-12 and varieties (SI - 0.39 to 0.42).

Table 13. Average similarity among *Curcuma longa* genotypes for peroxidase, esterase and GOT

	1	2	3	4	5/1	5/2	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	V-1	V-2	V-3	V-4	V-5	V-6
1	1																											
2	1	1																										
3	1	1	1																									
4	0.98	0.98	0.98	1																								
5/1	0.81	0.81	0.81	0.79	1																							
5/2	0.71	0.71	0.71	0.70	0.82	1																						
6	0.72	0.72	0.72	0.72	0.78	0.74	1																					
7	0.62	0.62	0.62	0.62	0.76	0.66	0.76	1																				
8	0.62	0.62	0.62	0.62	0.76	0.66	0.76	1	1																			
9	0.65	0.65	0.65	0.65	0.60	0.60	0.60	0.60	0.60	1																		
10	0.69	0.69	0.69	0.73	0.57	0.62	0.57	0.52	0.52	0.77	1																	
11	0.67	0.67	0.67	0.67	0.59	0.58	0.52	0.55	0.55	0.70	0.70	1																
12	0.42	0.42	0.42	0.42	0.43	0.42	0.45	0.46	0.46	0.47	0.43	0.56	1															
13	0.46	0.46	0.46	0.46	0.47	0.50	0.53	0.50	0.50	0.54	0.45	0.50	0.72	1														
14	0.45	0.45	0.45	0.45	0.46	0.49	0.51	0.48	0.48	0.53	0.44	0.49	0.70	0.96	1													
15	0.47	0.47	0.47	0.51	0.44	0.45	0.45	0.50	0.50	0.52	0.48	0.53	0.73	0.74	0.72	1												
16	0.50	0.50	0.50	0.54	0.50	0.50	0.49	0.50	0.50	0.45	0.48	0.46	0.53	0.48	0.46	0.66	1											
17	0.52	0.52	0.52	0.56	0.56	0.52	0.56	0.50	0.50	0.50	0.47	0.48	0.59	0.53	0.51	0.66	0.82	1										
18	0.63	0.63	0.63	0.67	0.60	0.57	0.63	0.58	0.58	0.49	0.46	0.51	0.55	0.52	0.51	0.57	0.67	0.76	1									
19	0.64	0.64	0.64	0.68	0.61	0.55	0.60	0.56	0.56	0.53	0.48	0.59	0.56	0.46	0.45	0.57	0.77	0.79	0.85	1								
20	0.57	0.57	0.57	0.61	0.54	0.49	0.49	0.57	0.57	0.45	0.41	0.46	0.57	0.46	0.48	0.62	0.75	0.77	0.80	0.84	1							
21	0.59	0.59	0.59	0.63	0.56	0.55	0.51	0.55	0.55	0.50	0.47	0.52	0.56	0.47	0.48	0.63	0.76	0.81	0.78	0.76	0.87	1						
V-1	1	1	1	0.98	0.81	0.71	0.70	0.62	0.62	0.65	0.69	0.67	0.42	0.47	0.45	0.47	0.50	0.52	0.63	0.64	0.57	0.58	1					
V-2	1	1	1	0.98	0.81	0.71	0.70	0.62	0.62	0.65	0.69	0.67	0.42	0.47	0.45	0.47	0.50	0.52	0.63	0.64	0.57	0.58	1	1				
V-3	1	1	1	0.98	0.81	0.71	0.70	0.62	0.62	0.65	0.69	0.67	0.42	0.47	0.45	0.47	0.50	0.52	0.63	0.64	0.57	0.58	1	1	1			
V-4	1	1	1	0.98	0.81	0.71	0.70	0.62	0.62	0.65	0.69	0.67	0.42	0.47	0.45	0.47	0.50	0.52	0.63	0.64	0.57	0.58	1	1	1	1		
V-5	0.87	0.87	0.87	0.85	0.84	0.78	0.77	0.68	0.68	0.68	0.63	0.61	0.40	0.47	0.43	0.41	0.53	0.54	0.62	0.59	0.52	0.58	0.87	0.87	0.87	0.87	1	
V-6	0.93	0.93	0.93	0.91	0.86	0.77	0.76	0.67	0.67	0.67	0.61	0.59	0.39	0.47	0.42	0.44	0.55	0.57	0.68	0.69	0.62	0.63	0.93	0.93	0.93	0.93	0.94	1

Table 14. Groups of *C. longa* genotypes having similarity index 'one' among the members for esterase, peroxidase and GOT zymogram

Group 1		Group 2	
Accession No.	Morphotype	Accession No.	Morphotype
91	M-1	190	M-7
94	M-1	158	M-7
1126	M-2	1339	M-8
92	M-2	1318	M-8
1281	M-3		
V-1	Kanthi		
V-2	Sobha		
V-3	Prathibha		
V-4	Prabha		

Table 15. Grouping of 39 genotypes of *C. longa* based on esterase, peroxidase and GOT enzyme

Sl.No.	Accession No.	Morphotype	Group	Sl.No.	Accession No.	Morphotype	Group
1	91	M-1	G-1	21	893	M-13	G-11
2	94	M-1	G-1	22	897	M-13	G-11
3	1126	M-2	G-1	23	803	M-14	G-12
4	92	M-2	G-1	24	801	M-14	G-12
5	1281	M-3	G-1	25	1345	M-15	G-13
6	75	M-4	G-2	26	733	M-15	G-13
7	1353	M-5	G-3	27	197-B	M-16	G-14
8	830	M-5	G-4	28	436	M-17	G-15
9	199	M-6	G-5	29	739	M-18	G-16
10	190	M-7	G-6	30	194-B	M-19	G-17
11	158	M-7	G-6	31	184	M-19	G-17
12	1339	M-8	G-6	32	898	M-20	G-18
13	1318	M-8	G-6	33	1195	M-21	G-19
14	1352	M-9	G-7	34	Kanthi	V-1	G-1
15	1372	M-9	G-7	35	Sobha	V-2	G-1
16	171	M-10	G-8	36	Prathibha	V-3	G-1
17	1363	M-10	G-8	37	Prabha	V-4	G-1
18	141	M-11	G-9	38	Sudarshana	V-5	G-20
19	1371	M-12	G-10	39	Rasmi	V-6	G-21
20	1370	M-12	G-10				

Based on the pooled data of three enzymes, thirtynine genotypes of *C. longa* L. were grouped into 21. Thirty three accessions representing the 21 morphotypes formed 19 groups. Prathibha, Prabha, Kanthi and Sobha fell in the first group with a similarity index of one among the members. Sudarshana and Rasmi formed separate groups (Table 15).

4.3 Isoenzyme variation and species relationship in the genus *Curcuma*

Eighteen species of the genus *Curcuma* were compared for three enzyme systems namely peroxidase, esterase and GOT. Eighteen species included 15 sessile tuberising, two non-sessile tuberising and one stoloniferous type. *Curcuma longa* was represented by the morphotype one.

4.3.1 Peroxidase

A total of 34 isoenzyme bands were observed in the genus *Curcuma* for peroxidase zymogram (Fig.7, Plates 18, 19, 20). Different species differed much widely in peroxidase banding pattern. Maximum number of isoenzyme bands were observed in *C. longa* (7 bands) and the least was observed in *C. aurantiaca, C. aeruginosa* and *C. zedoaria* (3 bands). Some bands were found specific for certain species (Table 19). Rest of the bands were observed in more than one species. Similarity index among the species ranged from zero to 0.75 (Table 16). Maximum similarity index was noticed between *C. aeruginosa* and *C. comosa*. The non-sessile tuberising species showed more similarity with sessile tuberising species than among themselves. The stoloniferous type *C. vamana* showed maximum similarity with *C. latifolia* and *C. raktakanta* (SI - 0.44).

4.3.2 Esterase

A total of 29 isoenzyme bands were observed in the genus curcuma for esterase zymogram (Fig. 8 and Plates 21, 22, 23). EST-27, 28 and 29 were found in all sessile tuberising species. Some bands were found specific to certain species

Plates 18, 19, 20 Peroxidase banding pattern of *Curcuma* species
(1-18 serial no. of species as in Table 4.)

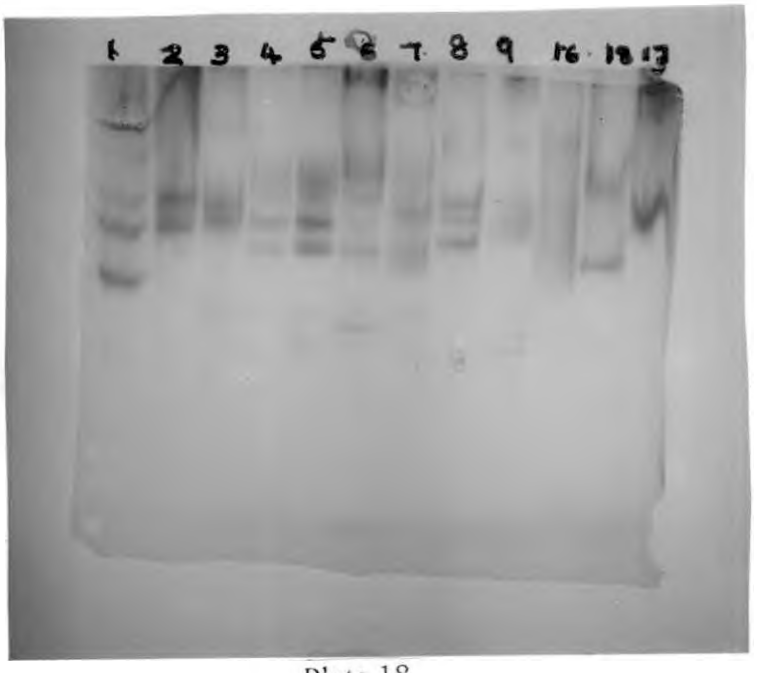

Plate 18

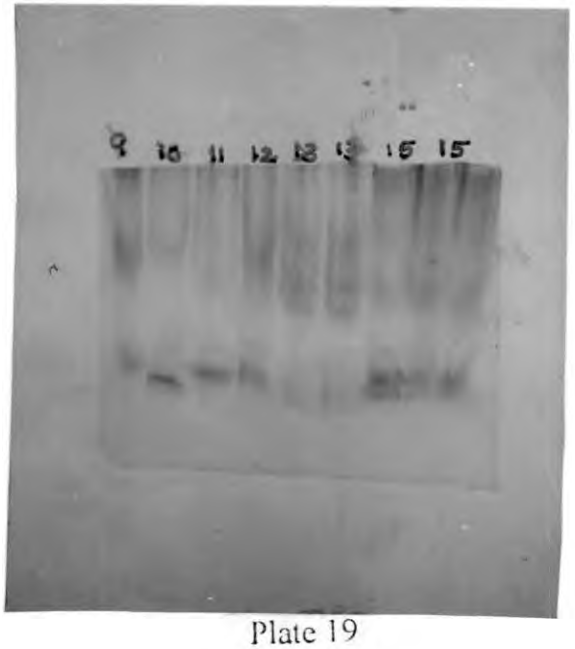

Plate 19

Plate 20

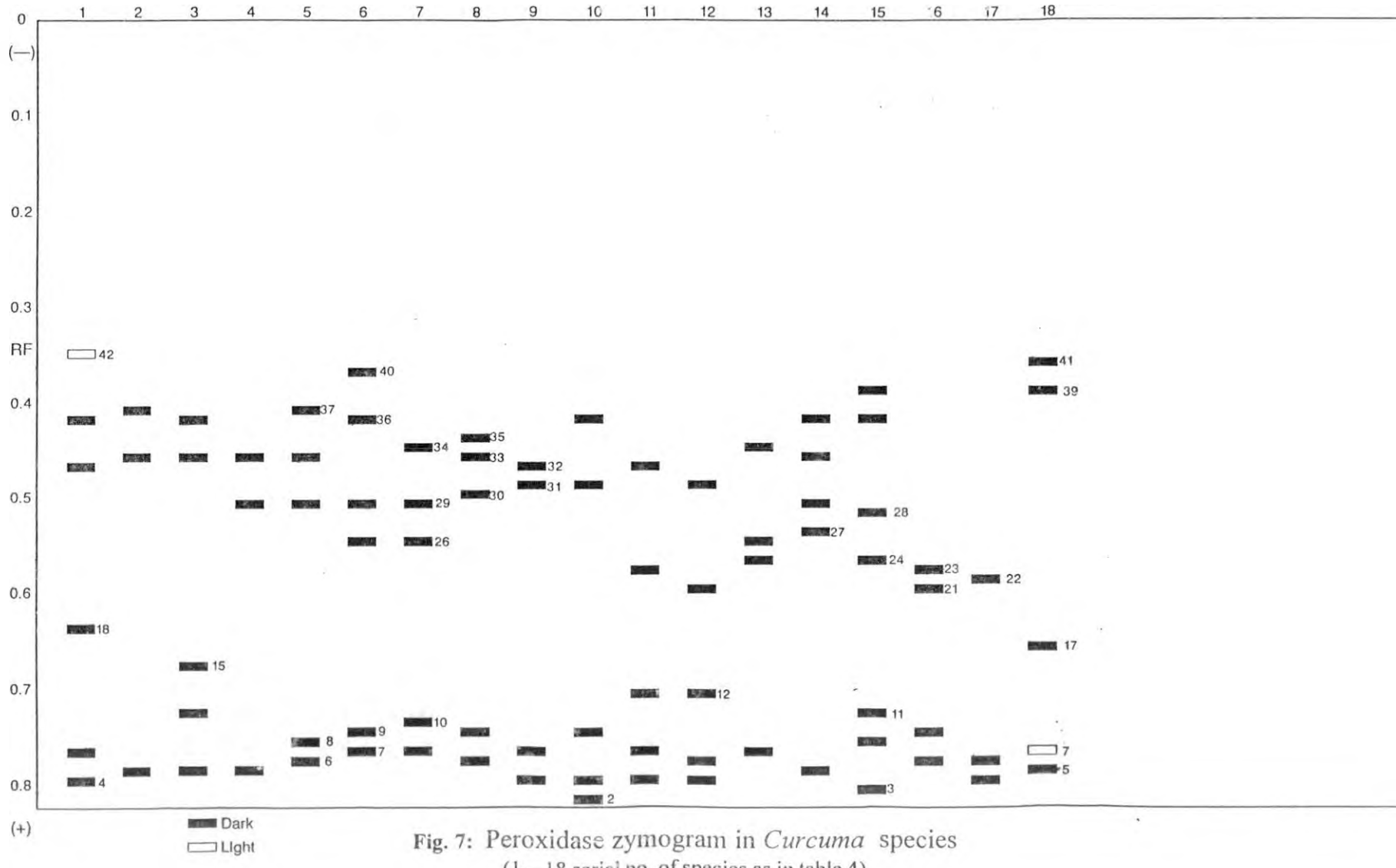

Fig. 7: Peroxidase zymogram in *Curcuma* species
(1—18 serial no. of species as in table 4)

Plates 21, 22 Esterase banding pattern of *Curcuma* species

Plate 23 Esterase and GOT banding pattern of *Curcuma* species
(1-18 serial no. of species as in Table 4.)

Table 16. Similarity Index for peroxidase in *Curcuma* species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1																	
2	0.00	1																
3	0.18	0.50	1															
4	0.00	0.67	0.50	1														
5	0.00	0.50	0.25	0.50	1													
6	0.33	0.00	0.18	0.22	0.18	1												
7	0.18	0.00	0.00	0.25	0.20	0.55	1											
8	0.00	0.25	0.20	0.25	0.40	0.18	0.00	1										
9	0.60	0.00	0.00	0.00	0.00	0.20	0.22	0.00	1									
10	0.36	0.00	0.20	0.00	0.00	0.36	0.00	0.20	0.44	1								
11	0.55	0.00	0.00	0.00	0.00	0.18	0.20	0.00	0.67	0.20	1							
12	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.20	0.44	0.40	0.4	1						
13	0.20	0.00	0.00	0.00	0.00	0.40	0.66	0.00	0.25	0.00	0.22	0.00	1					
14	0.18	0.50	0.60	0.75	0.40	0.36	0.20	0.20	0.00	0.00	0.00	0.00	0.00	1				
15	0.15	0.00	0.33	0.00	0.17	0.15	0.00	0.00	0.00	0.17	0.17	0.00	0.18	0.17	1			
16	0.00	0.00	0.00	0.00	0.22	0.20	0.00	0.44	0.00	0.22	0.22	0.44	0.00	0.00	0.18	1		
17	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.25	0.00	0.00	0.25	0.50	0.00	0.00	0.20	0.29	1	
18	0.18	0.25	0.20	0.25	0.00	0.18	0.20	0.00	0.22	0.00	0.20	0.00	0.22	0.20	0.17	0.00	0	1

Plates 21, 22 Esterase banding pattern of *Curcuma* species

Plate 23 Esterase and GOT banding pattern of *Curcuma* species
(1-18 serial no. of species as in Table 4.)

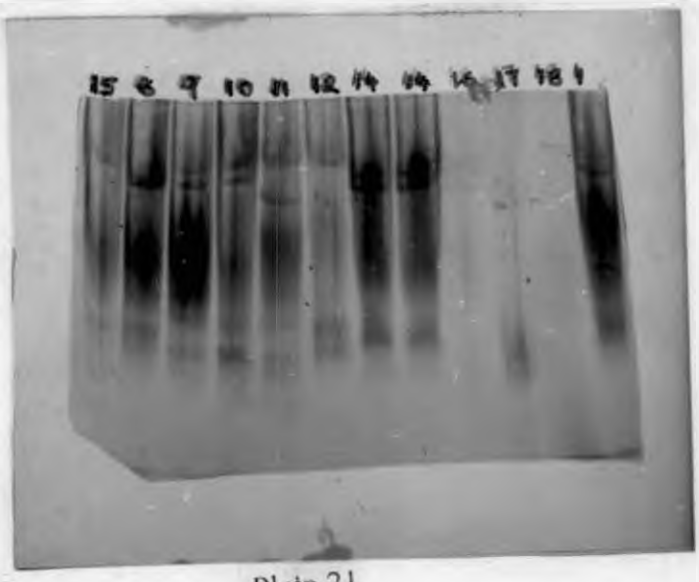

Plate 21

Plate 22

Plate 23

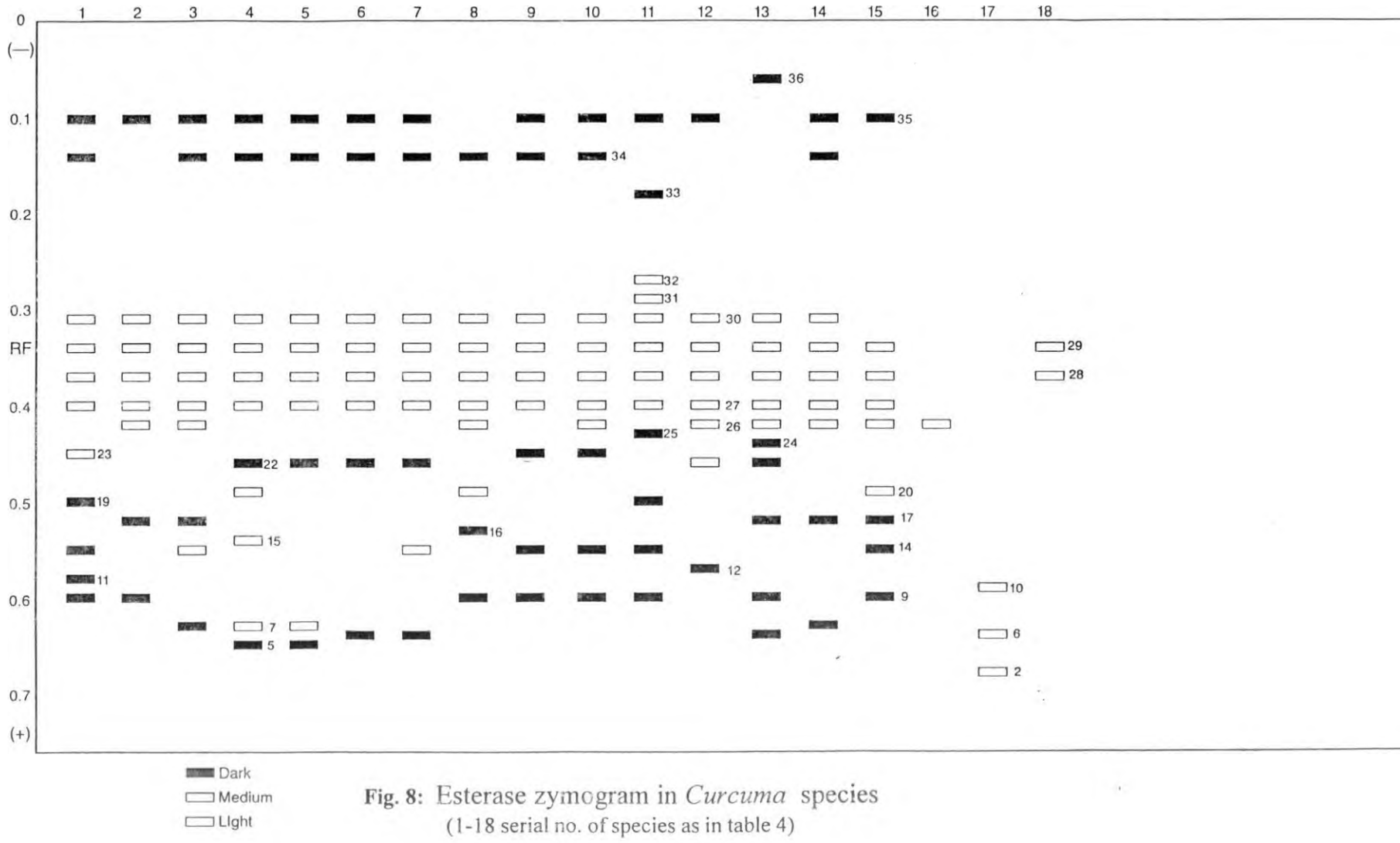

Fig. 8: Esterase zymogram in *Curcuma* species
(1-18 serial no. of species as in table 4)

(Table 19) whereas others were present in more than one species. The number of bands in stoloniferous and non-sessile tuberising species were in the range of 1 to 3.

Similarity index among the species for esterase enzyme ranged from 0 to 0.95 (Table 17). In the sessile tuberising species alone the similarity index ranged from 0.44 to 0.95. Maximum similarity was observed between *C. aromatica* and *C. harita*, *C. malabarica* and *C. comosa*. The least similarity was between *C. aeruginosa* and *C. sylvatica* (SI-0.44). Similarity index was zero among non-sessile tuberising species. The stoloniferous type showed more similarity with sessile tuberising species.

4.3.3 Glutamate oxaloacetate transaminase

A total of 10 isoenzyme bands were observed in GOT zymogram for *Curcuma* species. They were numbered GOT-1, 2, 3, 4, 5, 7, 8, 9, 10 and 12. GOT-1 and 2 were common for all species (Fig.9 and Plates 23, 24, 25). Similarity index for GOT zymogram ranged from 0.4 to 1 among the species (Table 18). All the sessile tuberising species except *C. longa* and *C. comosa* showed the highest similarity index of one among them. These species showed a similarity index of 0.8 with *C. longa* and 0.75 with *C. comosa*. Similar result was observed in the stoloniferous species *C. vamana*. Similarity index between sessile and non-sessile tuberising species was in the range of 0.4 to 0.5. Between the non-sessile tuberising species the similarity index was 0.5.

4.3.4 Similarity index among *Curcuma* spp. for isoenzyme banding pattern

Average similarity indices for the three enzymes were computed among the eighteen *Curcuma* species (Table 20) to study the relative closeness and distinctness of different species.

Similarity index for three isoenzymes put together ranged from 0.16 to 0.83. In the sessile tuberising species alone the similarity index ranged from 0.2 to

Table 17. Similarity Index for estrase in *Curcuma* species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1																	
2	0.63	1																
3	0.48	0.78	1															
4	0.55	0.53	0.76	1														
5	0.60	0.59	0.74	0.90	1													
6	0.63	0.63	0.67	0.74	0.82	1												
7	0.70	0.59	0.63	0.70	0.78	0.94	1											
8	0.60	0.71	0.63	0.60	0.56	0.59	0.56	1										
9	0.90	0.71	0.74	0.60	0.67	0.71	0.77	0.67	1									
10	0.86	0.78	0.70	0.57	0.63	0.67	0.74	0.74	0.95	1								
11	0.70	0.60	0.55	0.44	0.48	0.50	0.57	0.48	0.67	0.64	1							
12	0.53	0.75	0.67	0.63	0.71	0.75	0.71	0.59	0.59	0.67	0.5	1						
13	0.45	0.74	0.57	0.45	0.5	0.63	0.60	0.67	0.50	0.57	0.43	0.63	1					
14	0.55	0.82	0.95	0.70	0.77	0.71	0.71	0.67	0.67	0.74	0.48	0.71	0.60	1				
15	0.60	0.82	0.63	0.50	0.44	0.47	0.56	0.67	0.67	0.74	0.57	0.59	0.60	0.67	1			
16	0.00	0.22	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.22	0.16	0.20	0.20	1		
17	0.00	0.00	0.00	0.00	0.00	0.18	0.16	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0	1	
18	0.29	0.37	0.31	0.29	0.33	0.37	0.29	0.29	0.29	0.31	0.27	0.37	0.31	0.36	0.29	0	0	1

Plates 24, 25 **GOT** banding pattern of *Curcuma* species
(1-18 serial no. of species as in Table 4.)

Plate 24

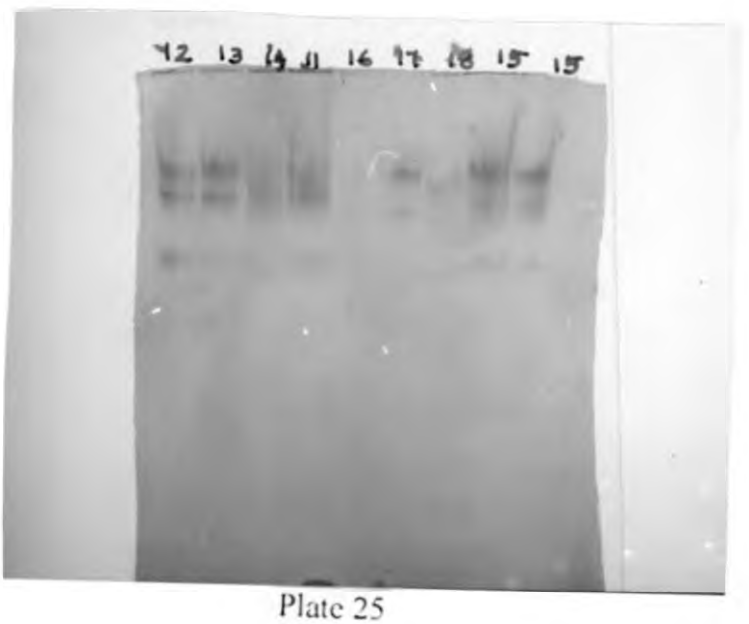

Plate 25

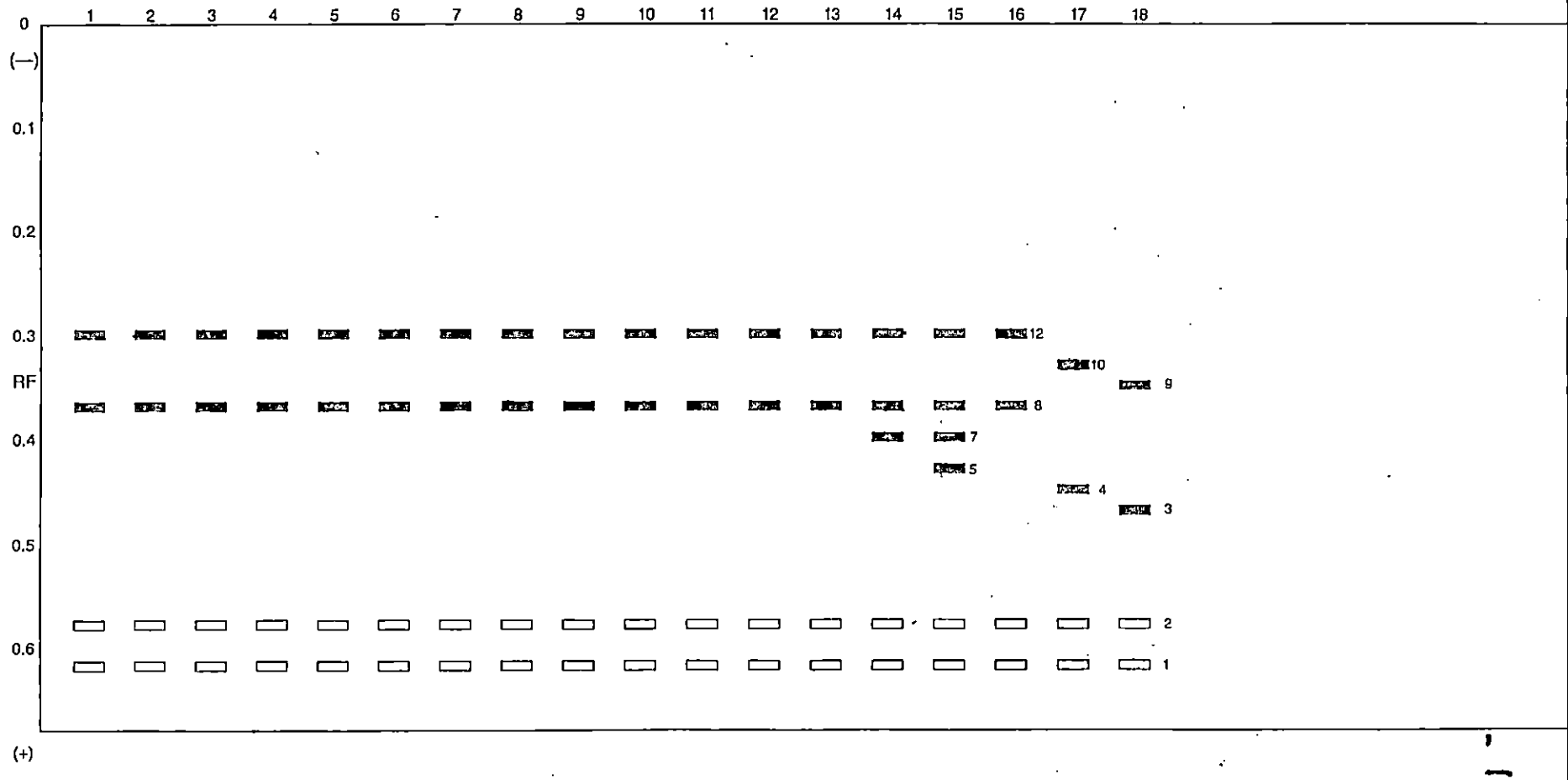

Fig. 9: GOT zymogram in *Curcuma* species
(1-18 serial no. of species as in table 4)

Table 18. Similarity Index for GOT in *Curcuma* species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1																	
2	1	1																
3	1	1	1															
4	1	1	1	1														
5	1	1	1	1	1													
6	1	1	1	1	1													
7	1	1	1	1	1	1	1											
8	1	1	1	1	1	1	1	1										
9	1	1	1	1	1	1	1	1	1									
10	1	1	1	1	1	1		1	1	1								
11	1	1	1	1	1	1	1	1	1	1	1							
12	1	1	1	1	1	1	1	1	1	1	1	1						
13	1	1	1	1	1	1	1	1	1	1	1	1	1					
14	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	1				
15	0.80	0.80	0.30	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	1			
16	1	1	1	1	1	1	1	1	1	1	1	1	1	0.75	0.80	1		
17	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.40	0.50	1	
18	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.40	0.50	0.50	1

Table 19. Isoenzyme bands of peroxidase esterase and GOT specific for *Curcuma* species

Sl.No.	*Curcuma* species	Peroxidase	Esterase	GOT
1	*C. amada*	PRX-42,18	EST-11	-
2	*C. malabarica*	PRX-15	-	-
3	*C. aeruginosa*	-	EST-15	-
4	*C. soloensis*	PRX-40	-	-
5	*C. broq*	PRX-10	-	-
6	*C. latifolia*	PRX-35,30	EST-16	-
7	*C. harita*	PRX-2	-	-
8	*C. sylvatica*	-	EST-33,32,31 25	-
9	*C. raktakanta*	-	EST-12	-
10	*C. montana*	-	EST-24	-
11	*C. comosa*	PRX-27	-	-
12	*C. longa*	PRX-39.28,3	-	GOT-5
13	*C. aurantiaca*	PRX-22	EST-10,2	GOT-4,10
14	*C. pseudomontana*	PRX-41,17	-	GOT-3,9

Table 20. Average similarity among *Curcuma* species for peroxidase, esterase and GOT

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1																	
2	0.54	1																
3	0.55	0.76	1															
4	0.52	0.73	0.75	1														
5	0.53	0.70	0.66	0.80	1													
6	0.65	0.54	0.62	0.65	0.64	1												
7	0.63	0.53	0.54	0.65	0.66	0.83	1											
8	0.53	0.65	0.61	0.62	0.65	0.59	0.52	1										
9	0.83	0.57	0.58	0.53	0.56	0.64	0.66	0.56	1									
10	0.74	0.59	0.63	0.52	0.54	0.68	0.58	0.65	0.80	1								
11	0.75	0.53	0.52	0.48	0.49	0.56	0.59	0.49	0.78	0.61	1							
12	0.51	0.58	0.56	0.54	0.64	0.58	0.57	0.60	0.68	0.69	0.63	1						
13	0.55	0.58	0.52	0.48	0.50	0.68	0.75	0.56	0.58	0.52	0.55	0.54	1					
14	0.49	0.69	0.77	0.73	0.64	0.61	0.55	0.54	0.47	0.5	0.40	0.49	0.45	1				
15	0.52	0.54	0.59	0.43	0.47	0.20	0.45	0.49	0.49	0.57	0.51	0.46	0.53	0.55	1			
16	0.33	0.41	0.39	0.33	0.41	0.40	0.33	0.55	0.33	0.47	0.41	0.55	0.39	0.32	0.39	1		
17	0.17	0.16	0.17	0.17	0.25	0.23	0.22	0.25	0.17	0.17	0.25	0.33	0.21	0.17	0.23	0.26	1	
18	0.32	0.37	0.34	0.35	0.28	0.31	0.33	0.26	0.34	0.27	0.32	0.29	0.34	0.35	0.32	0.17	0.17	1

0.83. Maximum similarity was observed between *C. soloensis* and *C. brog* and *C. aromatica* and *C. caesia* (SI-0.83). Among the sessile tuberising species the least similarity was between *C. soloensis* and *C. longa* (SI-0.2). When the 18 species were considered, the least similarity was between *C. aurantiaca* and *C. zedoaria* (SI-0.16).

C. longa was found similar to *C. amada, C. zedoaria, C. malabarica, C. sylvatica, C. montana, C. comosa* and *C. harita* in a range of 0.51 to 0.59 when M-1 was taken as standard. Similar results were observed with almost all morphotypes of *C. longa.*

Similarity index range of *C. vamana* was 0.17 to 0.55 with high similarity to *C. raktakanta* and *C. latifolia.* The two non-sessile tuberising species showed a similarity index of 0.17 between them. Range of similarity between sessile and non-sessile tuberising species was 0.16 to 0.33.

Discussion

DISCUSSION

5.1 Isoenzyme variation in different plant parts at different stages of development

Much variation was observed for various isoenzymes in different plant parts at different growth stages in genus *Curcuma*. Tyson *et al.* (1986) reported that change in banding pattern of isoenzymes may be correlated with leaf position and developmental stages. With different plant parts and growth stages, much variation was observed in banding pattern of peroxidase in spring wheat (Liand Li, 1996), of esterase in chilli (Markose, 1996) and of peroxidase, esterase and GOT in pepper (Sebastian, 1995).

Ideal part for stable and distinct peroxidase banding pattern was mature leaf and for esterase immature leaf was more suitable. For GOT both mature and immature leaves were found ideal. Similar observations were made by Sebastian (1995) in black pepper. Similar to the report in chilli by Markose (1996), in genus *Curcuma* also the number of bands of peroxidase increased with the advent of plant growth.

5.2 Isoenzyme variation within *C. longa* L.

In the present study on isoenzyme analysis of *Curcuma longa* genotypes, large amount of variation was observed within the species. The similarity index ranged from 0.39 to 1. Eventhough *C. longa* is very shy in seed setting and is vegetatively propagated, high degree of genetic variability exists in the species for morphological characters, yield and processing qualities. This within species variability has been reported by many workers like Philip *et al.* (1980), Philip and Nair (1983, 1986), Mukhopadhyay *et al.*, (1986), Jalgaonkar *et al.* (1988, 1990),

Reddy and Rao (1988), Menon *et al.* (1992), Indiresh *et al.* (1992), Kurian and Nair (1996) and Yadav *et al.* (1996). Isoenzyme variation in the population of *C. longa* was also reported to be high (Shamina *et al.*, 1998).

Results obtained by isoenzyme analysis for three enzymes viz., peroxidase, esterase and GOT, largely agreed with the observations of Velayudhan *et al.* (1994) in grouping the 568 *C. longa* accessions into 21 morphotypes based on subjective analysis. Accession within a morphotype showed a similarity index one between then in all the morphotypes except M-5. Accessions in M-5 showed a similarity of only 0.87. On comparison of the morphotypes it was observed that morphotype 1, 2 and 3 shared a similarity index one among them. Also, morphotype 7 and 8 formed another group with similarity index one among the members of group. Thus results of study suggests that the first three morphotypes of Velayudhan *et al.* (1994) could be grouped together. M-7 and M-8 also could be put in a single group. Thus based on isozyme studies it is suggested that 21 morphotypes of Velayudhan *et al.* (1994) could be reduced to 19 with a similarity index one within the groups. On examination of the released varieties it was found that the variety Kanthi, Sobha, Prathibha and Prabha shared a similarity index one at isozyme level and also with the newly formed group one. Sudarshana and Rasmi showed a slightly lower similarity with this group and were put in two separate groups, 20 and 21.

The isozyme data was further used to examine the newly proposed classification of *Curcuma longa* genotypes into six groups by Velayudhan *et al.* (sent for publication).

Morphotype 1, 2 and 3 showed a similarity index of one among them. However, M-4 had a similarity index of only 0.98 with M-1, 2 and 3. Velayudhan *et al.* (1994) also observed greater similarity in morphological characters among the morphotype 1, 2 and 3. M-4 differed from M-1, 2 and 3 in rhizome colour and

flower exsertion. Coefficient of association between the four morphotypes were found to be very high by Velayudhan *et al.*, 1994. These four morphotypes were grouped as Alleppey type of turmeric of commerce which is highly preferred in the national and international markets. However, since differences were observed at isozyme level between M-4 and other three morphotypes, it is suggested that the first three morphotypes may be grouped into one and M-4 may be put in a separate group.

Morphotypes 7 and 8 showed a similarity index of one between them. They were also reported to be quite similar in their morphological characters such as fruit setting and highly pleasant aromatic rhizomes. It is therefore suggested that M-7 and M-8 may be grouped together. However, isozyme similarity index between M-7 and M-8 with M-18 was only 0.58. M-18 was found to be more similar to M-19 in isozyme study. This was contradictory to morphological observations and grouping of M-7, M-8 and M-18 into one group.

Turmeric morphotypes 7 and 8 and similar types having seed setting and pleasant aroma were treated as *Curcuma aromatica* by some workers (Nambiar *et al.*, 1982 and George, 1981). Isoenzyme banding pattern of M-7 and M-8 for peroxidase, esterase and GOT were compared with *C. aromatica* Salisb and *C. longa*. The similarity of M-7 and M-8 with *C. aromatica* was only 0.49. They showed a high similarity of 0.76 with some *C. longa* morphotypes. Thus the results of the present study support the view of Mangaly and Sabu (1993) and Velayudhan *et al.* (1990b) that the aromatic morphotypes M-7 and M-8 are infact *C. longa* types and different from the original *C. aromatica*.

Morphotype 9 showed specific bands such as EST-16, PRX-51 and PRX-46. Similarity index of this morphotype ranged from 0.49 to 0.77 with other morphotypes. M-9 represented by semierrect and dwarf to medium tall plants with

slightly twisted leaves formed the third major group of morphotypes, now identified as a separate cultivar (Velayudhan *et al.* sent for publication).

Velayudhan *et al.* (1994) described M-12 as a unique type with mixed aroma of turmeric and kasturi. In the present study M-12 was found to be distinct based on isoenzyme analysis. Presence of PRX-1 and absence of PRX-28 were observed only in this morphotype. EST-34, 35 and 36 occurred together only in this morphotype. Similarity indices of M-12 with other morphotypes were in the range of 0.42 to 0.73. Morphotypes 1 to 8 had the lowest similarity index with M-12. So grouping of M-12 into a separate class and identifying it as a distinct cultivar is justifiable.

In the group 5 of new classification, M-13 and M-14 with similar morphological characters had a high similarity index of 0.96. Both the morphotypes were highly susceptible to Tephrina leaf spot also. M-16, 17, 18, 19, 20 and 21 showed a similarity index in the range of 0.75 to 0.87 among them except between M-16 and M-18 (SI = 0.67). Eventhough, M-5, 11, 13, 14, 16, 17, 19, 20 and 21 were grouped as the fifth major group of morphotypes by Velayudhan *et al.* (sent for publication), similarity index among the members of the group ranged from 0.41 to 0.96. Velayudhan *et al.* (1994) reported that the similarity and dissimilarity of the members of the group to other morphotypes and coefficient of correlation among the members were not stable making them a complex group. Based on complexity of the group and the wide variation in the isozyme similarity index, the grouping of these nine morphotypes into one class could not be approved.

M-6, M-10 and M-15 formed the sixth major group of morphotypes with distinct characters such as erect tall nature, broad banana type leaves, bend sessile tubers and flower bract with purple tinge (Velayudhan *et al.*, 1994). On isoenzyme analysis M-6, M-10 and M-15 showed a similarity index in the range of only 0.45 to

0.58 among them. The study does not agree with grouping of these morphotypes into one group.

Kanthi, Sobha, Prathibha and Prabha showed a similarity index of 'one' among them and also with M-1, 2 and 3. With M-4, a similarity index of 0.93 was observed. All these are selections from South India which showed more similarity to M-1, 2 and 3 in morphological characters also. Sudarshana, which is a selection from Manipur also showed a low level of morphological similarity with M-1, 2 and 3 compared to other released varieties. This was also supported by isozyme studies (SI = 0.87).

Present study agrees with the classification of *C. longa* into 21 morphotypes by Velayudhan *et al.* (1994) in large. Accessions within a morphotype were identical with similarity index one among them except in M-5 where the similarity was only 0.87. So a regrouping of M-5 was suggested based on the result.

However, the recent classification and taxonomic studies by Velayudhan *et al.* (sent for publication) in *C. longa* by clubbing different morphotypes into six major classes based on subjective analysis first, supported by numerical taxonomic studies does not take into consideration all the morphological and other parameters in total. The results of the present isozyme study could not agree with the new classification into six major groups fully as discussed in detail earlier. So present study cannot support such a grouping of widely differing accessions into one group based on a few morphological characters.

Thus to conclude, the original classification of 568 *Curcuma longa* accessions into 21 morphotypes by Velayudhan *et al.* (1994) is in agreement with the results of the present study provided M-5 is regrouped. However, based on isozyme analysis, it is suggested that M-1, 2 and 3 could be grouped together. So

also M-7 and 8 could be clubbed together. This is supported in the new classification by the same authors. However, the recent classification of *C. longa* into six major groups is not fully supported in the present study and warrants further examination.

High amount of variation within the species *C. longa* at electrophoretic loci has been observed in the present study. This could be due to vegetative propagation in the species where variability once created get fixed. Such variation in isoenzyme banding pattern is observed in other vegetatively propagated crops like banana (Bhat *et al.*, 1992a and b), pineapple (DeWald *et al.*, 1988) and ginger (He *et al.*, 1995).

Isoenzyme analysis has been used as a tool for differentiating accessions in a species and for better classification and identification of varieties as in *Zizania latifolia* (Cao *et al.*, 1993), Malus genotypes (Li *et al.*, 1995), lime cultivars (Satrabhandhu *et al.*, 1996) and black pepper (Sebastian *et al.*, 1996).

The present study has supplemented the morphological classification and helped in clarifying many confusions at morphological level in *Curcuma longa*.

5.3 Isoenzyme variation and species relation within the genus *Curcuma*

High amount of variation was observed among the *Curcuma* species based on isozyme analysis. Based on the similarity index, species which were found to be closely related were grouped as follows:

Group I - *C. zedoaria, C. aeruginosa, C. malabarica, C. comosa* and *C. caesia*

SI = 0.64 to 0.80

Group II a - *C. aromatica, C. amada, C. sylvatica*

SI = 0.75 to 0.83

b - *C. aromatica, C. amada, C. harita*

SI = 0.74 to 0.83

Group III - *C. soloensis, C. brog, C. montana*

SI = 0.68 to 0.83

In group I, species included were *C. zedoaria, C. aeruginosa, C. malabarica, C. comosa* and *C. caesia*. Highest similarity index of 0.8 was between *C. aeruginosa* and *C. caesia*. Similarity index of *C. caesia* with *C. malabarica* and *C. comosa* was 0.66 and 0.64 respectively. Similarity between *c. aeruginosa, C. zedoaria, C. comosa* and *C. malabarica* was in the range of 0.69 to 0.77. Velayudhan *et al.* (1994) reported high similarity between *C. aeruginosa, C. zedoaria, C. comosa* and *C. malabarica* based on dendrogram analysis. *Curcuma malabarica* and *C. caesia* are treated as one in the revision work of Mangaly and Sabu (1993). However, Velayudhan *et al.* (1990a) was of the opinion that they were different species with close resemblence. Isozyme analysis supports the view of Velayudhan *et al.* (1990a). All the species in group one were morphologically similar in several characters such as purple streak on the leaf midrib, variously coloured rhizomes, and lateral flower spikes produced at the end of summer season (Velayudhan *et al.*, 1994). The results of the isozyme analysis in these five species support the morphological observations of Velayudhan *et al.* (1994) and that the five species are closely related.

In group II, the species included are *C. aromatica, C. amada, C. sylvatica* and *C. harita*. They were sub grouped into group IIa and IIb. Group IIa included species *C. aromatica, C. amada* and *C. sylvatica* and IIb included *C. aromatica, C. amada* and *C. harita. C. harita* and *C. sylvatica* could not be grouped together as they were having a low similarity between them (SI = 0.61). However, both *C. harita* and *C. sylvatica* showed high similarity with *C. amada* and *C. aromatica. Curcuma aromatica* and *C. amada* are highly similar in morphological characters and at isozyme level (SI = 0.83). Major difference in morphology was in the smell of rhizome and hairyness of leaf (Velayudhan *et al.*, 1994). They have also reported the close relationship of *C. amada and C. sylvatica* by dendrogram analysis. Close similarity between *C. harita* and *C. aromatica* was reported by Mangaly and Sabu (1993) in his taxonomic revision work of South Indian species of *Curcuma*. Results of isoenzyme analysis support the observations of Velayudhan *et al.* (1994) and Mangaly and Sabu (1993) to a great extent. Grouping of *C. amada, C. aromatica, C. sylvatica* and *C. harita* into one group with two sub groups based on isozyme similarity index is supported by morphological records.

Curcuma soloensis, C. brog and *C. montana* formed the third group with a similarity index of 0.68 to 0.83. *Curcuma soloensis* and *C. brog* had a similarity index of 0.83 between them. They have the same area of distribution also. Similarity indices of *C. montana* with *C. soloensis* and *C. brog* were 0.68 and 0.75 respectively. *Curcuma montana, C. brog* and *C. soloensis* have similar plant type, central spike and flowering period thus forming a single group according to Velayudhan *et al.*, 1994. Isoenzyme analysis supported this.

Curcuma latifolia and *C. raktakanta* showed variable similarity with other species and did not fall in any group. *Curcuma latifolia* showed more

similarity to *C. zedoaria*, *C. caesia* and *C. harita* (SI = 0.65). *Curcuma latifolia* was found similar to *C. harita* by Velayudhan *et al.* (1994) in his morphological studies and dendrogram analysis. *Curcuma raktakanta* showed maximum similarity with *C. aromatica* and *C. harita* (SI = 0.68 to 0.69). *Curcuma latifolia* and *C. raktakanta* showed a similarity index of 0.6 between them, 0.49 to 0.65 with the first group, 0.49 to 0.68 with the second group and 0.49 to 0.59 with the third group.

Cultivated type *C. longa* did not fall in any of the groups. *Curcuma longa* was found similar to *C. amada*, *C. zedoaria*, *C. malabarica*, *C. sylvatica*, *C. montana*, *C. comosa* and *C. harita* in a range of 0.51 to 0.59. Mangaly and Sabu (1993) reported the close similarity of *C. longa* and *C. amada* in many morphological characters. Cytological similarity of *C. longa* with *C. zedoaria* was reported by Ramachandran (1961). Similarity index of *C. longa* with the fourth group was only in the range of 0.2 to 0.53. Velayudhan *et al.* (1994) grouped *C. longa* with *C. brog*, *C. montana* and *C. soloensis* of the fourth group based on morphological studies. Results of isozyme analysis did not support this.

The stoloniferous type *C. vamana* showed more similarity with the sessile tuberising species (SI = 0.32 to 0.55) than with the non-sessile tuberising species (SI = 0.26 to 0.17). *Curcuma vamana* showed high similarity to *C. raktakanta*, *C. latifolia* and *C. harita* (SI = 0.47 to 0.55).

The present study also revealed the distinctness of non-sessile tuberising species *C. aurantiaca* and *C. pseudomontana* from the rest of the species as they stood individually having only low similarity index with others (SI = 0.16 to 0.33).

Correlation coefficient values between the non-sessile tuberising species was found to be low, which indicated their distant relationship. Correlation coefficient values among the sessile tuberising species was much higher indicating

their closer relationship (Velayudhan *et al.*, 1994). Results of isozyme analysis also supported these.

The present study had been successful in comparing the similarity between different *Curcuma* species. The study has helped in identifying three closely related groups among *Curcuma* species eventhough all the species examined could not be included into these.

Thus to conclude isoenzyme analysis in 18 species of *Curcuma* has helped in establishing the identity of the species at isoenzyme level. It has also brought out relationship among the different species. A grouping of 12 species into three major groups has also been possible in the present study. *Curcuma longa* which is the commonly cultivated species is expected to have evolved much ahead of the other species, has retained its individuality showing a lower similarity with the other species of *Curcuma*.

Within species variation in *C. longa* was high supporting the morphological variability observed in the species. The classification of *C. longa* based on morphological analysis could be verified, supported and supplemented to a large extent.

A further detailed analysis involving more isoenzymes and more species and accessions is expected to give a clearer picture of the variability in *Curcuma*. Analysis of the genetic polymorphism using the recent molecular techniques also could be rewarding.

Summary

SUMMARY

The present study was undertaken with the objective of analysing isoenzyme variation in the genus *Curcuma* for understanding the taxonomic relationship. The cultivated turmeric *C. longa* was studied in detail to find out the relationship among the different morphotypes of *C. longa*. Eighteen species of the genus *Curcuma* including *C. longa* were included in the study. Thirtynine accessions including six released varieties of *C. longa* were selected to represent the 21 morphotypes proposed by Velayudhan *et al.* (1994).

The selection of ideal plant part for analysis was done based on observations of isoenzyme banding pattern of root, rhizome, stem and leaf. With respect to leaf, different maturity stages were also analysed to get a clear picture of isoenzyme variation at different stages of growth of leaf. The study was undertaken with respect to peroxidase, esterase and GOT. For peroxidase, mature leaf was selected as the best plant part and for esterase tender leaf was found to be ideal. In the case of GOT, leaf was found to be the best sample material. GOT zymogram in *Curcuma* did not differ at different stages of maturity. Here for convenience of analysis, mature leaf was selected as sample.

For standardising the stage of growth of plant, sampling and analyses were done at three weeks after sprouting and just before senescence of leaf.

For peroxidase, the number of bands increased with the advent of plant growth. Esterase banding was high during the initial stages of growth and GOT zymogram was same through out the entire phase of growth of plant. So peroxidase and GOT analyses were done during the stage just before the senescence of leaf and esterase analysis was done three weeks after sprouting.

Isoenzyme banding pattern for peroxidase in *C. longa* genotypes showed 17 variant isoenzymes. PRX-11 was common in all the 39 genotypes. In the case of esterase, EST-26, 27, 28 and 29 were found common in all the 39 genotypes and the genotypes could be grouped into 17. For GOT, GOT-1 and 2 were common and genotypes were grouped into five based on banding pattern.

Similarity index among the genotypes based on individual enzymes as well as pooled data for three enzymes were found. Similarity indices for peroxidase in *C. longa* ranged from 0.21 to 1. Least similarity index of 0.21 was observed between M-12 and M-10. Based on peroxidase zymogram, three groups of genotypes were identified with similarity index 'one' among the members in the group. For esterase, similarity index range was from 0.42 to 1. Among the *C. longa* genotypes, M-13 and 14 showed the least similarity index with M-19 and 20. Four groups of *C. longa* genotypes showed similarity index one among the members. In the case of GOT banding pattern of *C. longa* genotypes, similarity index ranged from 0.33 to 1. Five groups of genotypes were observed with similarity index one among the members within the group. Least similarity was between group 2 and group 4 members. Accessions in all the morphotypes showed a similarity index of 'one' among the members for peroxidase, esterase and GOT except M-5 for esterase and peroxidase. Similarity indices for three enzymes put together ranged from 0.39 to 1. Least similarity was between Rasmi and M-12. Highest similarity index of one was observed in 2 groups of genotypes of *C. longa*. They are M-1, M-2, M-3, Kanthi, Sobha, Prathibha and Prabha in one group and M-7 and M-8 in the next group. Based on the pooled data of three enzymes, thirtynine genotypes of *C. longa* were grouped into 21. Varieties under studies showed more similarity to M-1, 2 and 3.

Results of the present study agree with the classification of 568 accessions of *C. longa* into 21 morphotypes by Velayudhan *et al.* (1994) to a great extent. However, the number of groups has to be reduced to 19 and the morphotype 5 has to be rechecked. Recent revision of these morphotypes into six major groups by the same authors is not fully agreeable with the results of present study.

Much variation was observed in the peroxidase banding pattern in the 18 *Curcuma* species under study. Similarity index among the species ranged from zero to 0.75. Maximum similarity index was noticed between *C. aeruginosa* and *C. comosa*. For esterase, EST-27, 28 and 29 were found common in all the sessile tuberising species. Banding pattern varied with the species and similarity index ranged between 0 to 0.95. Maximum similarity was between *C. aromatica* and *C. harita, C. malabarica* and *C. comosa*. In the case of GOT, GOT-1 and 2 were found common in all the species. Similarity indices ranged from 0.5 to 1. All the species except *C. longa, C. comosa, C. aurentiaca* and *C. pseudomontana* showed a similarity index of one among each other. Pooled similarity indices ranged from 0.16 to 0.83 whereas maximum similarity was observed between *C. soloensis* and *C. brog* and *C. aromatica* and *C. caesia*. The least similarity was between *C. aurantiaca* and *C. zedoaria*.

Pooled analysis of similarity indices have shown that the 18 species studied could be grouped into three groups. *Curcuma zedoaria, C. malabarica, C. aeruginosa, C. comosa* and *C. caesia* formed the first group with similarity index ranging from 0.64 to 0.8. *Curcuma aromatica, C. amada, C. harita* and *C. sylvatica* formed the second group with two sub groups. Group IIa includes *C. aromatica, C. amada* and *C. harita* (SI = 74 to 83) and IIb includes *C. aromatica, C. amada* and *C. sylvatica* (SI = 0.75 to 0.83). Similarity between *C. sylvatica* and *C. harita* was 0.61. The third group included *C. montana, C. brog* and *C. soloensis* with

similarity index ranging from 0.68 to 0.83. Members within a single group showed a high level of morphological similarity.

Curcuma latifolia and *C. raktakanta* showed variable similarity with other species in the range of 0.49 to 0.68. *Curcuma longa*, the cultivated species was found similar to *C. amada, C. zedoaria, C. malabarica, C. sylvatica, C. montana, C. comosa* and *C. harita* in a range of 0.51 to 0.59.

Stoloniferous type *C. vamana* showed more similarity with sessile tuberising species (SI = 0.32 to 0.55) than with the non-sessile tuberising species (SI = 0.26 to 0.17). The non-sessile tuberising species *C. aurantiaca* and *C. pseudomontana* stood individually showing their distinctness from rest of the species.

References

REFERENCES

*Aiyadurai, S.G. 1966. *A Review of Research on Spices and Cashewnut in India.* Regional office (Spices and Cashewnut), Indian Council of Agricultural Research, Ernakulam, p.228

Aiyer, Y.A.K. 1954. *Field Crops in India.* 4th ed. Bangalore Printing and Publishing Co. Ltd. p.319-326

*Aoi, K., Kaburagi, K., Seki, T., Tobata, T., Satake, M. and Kuroyanagi, M. 1986. Studies on the cultivation of turmeric (*Curcuma longa* L.). 1. Varietal differences in rhizome yield and curcuminoid content. *Bull. Nat. Inst. Hygenic Sci.* **104:**124-128

Baker, J.G. 1898. *Scitamineae. The Flora of British India.* Vol.6 (ed. Hooker, J.D.). Periodical Experts Book Agency, London, Indian reprint 1982. p.198-264

Bhat, K.V., Bhat, S.R. and Chandel, K.P.S. 1992a. Survey of isozyme polymorphism for clonal identification in Musa-I. Esterase, acid phosphatase and catalase. *J. hort. Sci.* **67**(4):501-507

Bhat, K.V., Bhat, S.R. and Chandel, K.P.S. 1992b. Survey of isozyme polymorphism for clonal identification in Musa-II. Peroxidase, superoxide dismutase and malate dehydrogenase. *J. hort. Sci.* **67**(6):737-745

* Burt, B.L. 1977. Cited by Velayudhan, K.C., Muralidharan, V.K., Amalraj, V.A., Rana, R.S., Singh, B. and Thomas, T.A. 1994. *Genetic Resources of Curcuma.* National Bureau of Plant Genetic Resources, ICAR, New Delhi.

*Burt, B.L. and R.M. Smith. 1972. Cited by Velayudhan, K.C., Muralidharan, V.K., Amalraj, V.A., Rana, R.S., Singh, B. and Thomas, T.A. 1994. *Genetic Resources of Curcuma.* National Bureau of Plant Genetic Resources, ICAR, New Delhi.

Cao, P.S., Li, W., Jiang, J.Z., Chen, J.X. and Zhou, X.H. 1993. Classification of water bamboo varieties by means of peroxidase banding patterns. *Chinese Vegetables.* **4**:11-14

* Chakravorti, A.K. 1948. Multiplication of chromosome numbers in relation to speciation in Zingiberaceae. *Sci. Cult.* **14**:137-140

* Chaurasia, L.O., Kulkarni, B.M., Nair, K.N.G. and Mathew, T.V. 1974. Curcumin content in Indian turmeric. *Proc. Symp. Dev. Prospects of Spice Ind. in India.* Association of Food Science and Technology, India, p.55-56

Conkling, M.E. and Smith, H.H. 1971. Peroxidase Isozymes - A measure of molecular variation in ten herbaceaus species of *Datura. Ann. J. Bot.* **58**:688-696

Crawford, D.J. 1983. Phylogenetic and systematic inferences from electrophoretic studies. *Isozymes in Plant Genetics and Breeding* - Part A (eds. Tanksley, S.D. and Orton, T.J.). Elsevier, Amsterdam. p.257-287

De Wald, M.G., Moore, G.A. and Sherman, W.B. 1988. Identification of pineapple cultivars by isozyme genotypes. *J. Amer. Soc. Hort. Sci.* **113**(6):935-938

*Fischer, C.E.C. 1928. *Scitamineae. Flora of the Presidency of Madras.* Vol.3 (ed. Gamble, J.S.). Adlard and Son Ltd, London. p.1481-1483

Geetha, U. and Prabhakaran, P.V. 1987. Genotypic variability correlation and path-coefficient analysis in turmeric. *agric. Res. J.* **25**(2):249-254

George, H. 1981. Variability in the open pollinated progenies of Turmeric (*Curcuma aromatics* Salisb). M.Sc.(Hort.) thesis, Kerala Agricultural University, Thrissur, p.88-103

Gottlieb, L.D. 1971. Gel electrophoresis: new approach to the study of evolution. *BioScience.* **21**:939-944

Gottlieb, L.D. 1977. Electrophoretic evidence and plant systematics. *Ann. Mo. Bot. Gard.* **64**:161-180

Gottlieb, L.D. 1982. Conservation and duplication of isozymes. *Pl. Sci.* **216**:373-380

Guttel, F. and Hartenstein, G. 1996. Variability of enzymatic activity and of isozyme patterns in populations of *Elytrigia repens* (L.) Desv. *Archieves of Phytopathology and Pl. Protection.* **30**(4):353-363

* Haines, H.H. 1961. *The Botany of Bihar and Orissa*. Botanical Survey of India, p.1181

He, C., Li, J., Guo, S., Zheng, M., Chen, W., He, C.K., Li, J.S., Guo, S.Z., Zheng, M., Chen, W.S. and Zhu, D. 1995. The relationship between geographic distribution and the genetic difference of peroxidase isozyme of ginger germplasm in Fujian. *Acta Horticulturae.* **402**:125-132

Indiresh, K.M., Uthaiah, B.C., Reddy, M.J. and Rao, K.B. 1992. Genetic variability and heritability studies in turmeric. *Indian Cocoa, Arecanut and Spices J.* **16**(2):52-54

Jalgaonkar, R., Jamadagni, B.M. and Salvi, M.J. 1990. Genetic variability and correlation studies in turmeric. *Indian Cocoa, Arecanut and Spices J.* **14**(1):20-21

Jalgaonkar, R., Patil, M.M. and Rajput, J.C. 1988. Performance of different varieties of turmeric (*Curcuma longa* L.) under Kokan conditions of Maharashtra. *Proc. Nat. Sem. Chillies, Ginger and Turmeric.* (eds. Satyanarayana, G., Reddy, M.S., Rao, M.R., Azam, K.M. and Naidu, R.) Hyderabad, p.102-106

Kurian, A. and Nair, G.S. 1996. Evaluation of turmeric germplasm for yield and quality. *Indian J. Pl. Genet. Resour.* 9(2):323-327

* Linnaeus, C. 1793. *Species Plantarum* cited by Velayudhan, K.C., Muralidharan, V.K., Amalraj, V.A., Rana, R.S., Singh, B. and Thomas, T.A. 1994. *Genetic Resources of Curcuma*. National Bureau of Plant Genetic Resources, ICAR, New Delhi.

Li, S. and Li, S.H. 1996. Zymogram analysis of peroxidase isoenzyme of spring wheat varieties in Ningxia. *Ningxia J. of agric. and For. Sci. Technol.* **2**:10-13

Li, Y., Li, X., Li, Y.N. and Li, X.L. 1995. Studies on peroxidase isoenzyme band patterns of the plants in the genus *Malus*. *J. Southwest agric Univ.* **17**(5):371-377

Mangaly, J.K. and Sabu, M. 1993. A taxonomic revision of the South Indian species of *Curcuma* Linn (*Zingiberaceae*). *Rheedea*. **3**(2):139-171

Markose, B.L. 1996. Genetic and biochemical basis of resistance to bacterial wilt in Chilli. Ph.D. thesis, Kerala Agricultural University, Thrissur, p.90-110

Menon, K.P.G. 1975. Indian turmeric wins world market. *Indian Spices*. **12**(2):6-13

Menon, R., Valsala, P.A. and Nair, G.S. 1992. Evaluation of open pollinated progenies of turmeric. *S. Indian Hort.* **40**(2):90-92

Mukhopadhyay, S., Roy, K. and Som, M.G. 1986. Variability in turmeric (*Curcuma longa* L.). *Experimental Genet.* **2**(2):10-12

Muralidharan, V.K., Velayudhan, K.C. and Thomas, T.A. 1980. Preliminary studies on the germplasm collection of *Curcuma*. *Proc. Nat. Sem.* Ginger and Turmeric (eds. Nair, M.K., Premkumar, T., Ravindran, P.N. and Sarma, Y.R.). Central Plantation Crops Research Institute, Kasargod, p.55-57

* Nambiar, M.C. 1979. Morphological and cytological investigations in the genus *Curcuma* Linn. Ph.D. thesis, University of Bombay, p.95-97

Nambiar, M.C., Thankamma, P.P.K. and Sarma, Y.S. 1982. Seedling propagation in turmeric (*Curcuma aromatica* Salisb.). *J. Pln Crops.* **10**(2):81-85

Nazeem, P.A. and Menon, R. 1994. Blossom biological and hybridisation studies in turmeric (*Curcuma* spp.). *S. Indian Hort.* **42**(3):161-167

Pandey, G., Sharma, B.D. and Hore, D.K. 1990. Metroglyph and index score analysis of turmeric germplasm in North East region of India. *Indian J. Pl. Genet. Resour.* **3**(2):59-66

Philip, J. and Nair, P.C.S. 1983. Morphological and yield characters of turmeric types. *Indian Cocoa, Arecanut and Spices J.* **6**(3):61-63

Philip, J. and Nair, P.C.S. 1986. Studies on variability heritability and genetic advance in turmeric. *Indian Cocoa, Arecanut and Spices J.* **10**(2):29-30

Philip, J., Nair, P.C.S., Nybe, E.V. and Mohankumaran, N. 1980. Variation of yield and quality of turmeric cultivars. *Proc. Nat. Sem.* Ginger and Turmeric. (eds. Nair, M.K., Premkumar, T., Ravindran, P.N. and Sarma, Y.R.) Central Plantation Crops Research Institute, Kasargod, p.43-46

* Raghavan, T.S. and Venkatasubban, K.R. 1943. Cytological study in the family Zingiberaceae with special reference to chromosome number and cytotaxonomy. *Proc. Indian Acad. Sci.* **17B**:118-132

Ramachandran, K. 1961. Chromosome numbers in the genus *Curcuma* Linn. *Curr. Sci.* **30**:194-196

Ramachandran, K. 1969. Chromosome numbers in *Zingiberaceae. Cytologia.* **34**:213-221

* Rao, M.R., Reddy, R.C. and N. Subbarayudu. 1975. Promising turmeric types of Andhra Pradesh. *Arecanut and Spices Bull.* **6**(3):59-62

Rao, R.S. 1986. *Flora of India*. Vol.2. Botanical Survey of India. p.425-427

Reddy, N.L.N. and Rao, D.V.R. 1988. Genetic variability and association in turmeric. *Proc. Nat. Sem. Chillies Ginger and Turmeric.* (eds. Satyanarayana, G., Reddy, N.S., Rao, N.R., Azam, K.N. and Naidu, R.) Hyderabad, p.84-97

Rick, C.N., Fobes, J.F. and Holle, N. 1977. Genetic variation in *Lycopersicon pimpinellifolium* evidence of evolutionary change in mating systems. *Pl. Syst. Evol.* **127**:137-170

Rick, C.M. and Tanksley, S.D. 1981. Genetic variation in *Solanum penellii*. Comparison with two other sympatric tomato species. *Pl. Syst. Evol.* **139**:11-45

* Roxburgh, M.D. 1874. *Flora Indica*. Todays and Tomorrows Printers and Publishers, New Delhi. p.6-13

* Sankaracharya, N.B. and Natarajan, C.P. 1973. Turmeric chemistry, technology and uses. *Indian Spices*. **10**(4):8-11

* Sato, D. 1948. *Jap.* J. Genet. 23: 44

Satrabhandhu, A., Sahavacharian, O., Nangnai, V., Sethpakdee, R. and Pongtongkam, P. 1996. Identification of lime cultivars and hybrids by isozyme patterns. *Kasetsart J. Natural Sci.* **30**(2):249-253

Sebastian, A. 1995. Isoenzyme variation in *Piper* spp. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p.25-49

Sebastian, A., Sujatha, V.S., Nybe, E.V., Augustine, A. and Nair, G.S. 1996. Isoenzyme variation and species relationship in the genus *Piper*. *J. trop. Agric.* **34**:85-92

Shamina, A., Zachariah, T.J., Sasikumar, B., and George, J.K. 1998. Biochemical variation in turmeric (*Curcuma longa* Linn.) accessions based on isozyme polymorphism. *J. hort. Sci. and Biotech.* **73**(4):429-483

* Sharma, A.K. and Bhattacharyya, N.K. 1959. Cytology of several members of Zingiberaceae and a study of the inconsistency of their chromosome complement. *La Cellule.* **59**:279-349

Shaw, C.R. and Koen, A.L. 1968. Starch gel zone electrophoresis of enzymes. *Chromatographic and Electrophoretic Technique.* Vol.2. 2nd ed. (ed. Smith, I.) John Wiley, New York. p.325-359

Sokel, R.R. and Sneath, P.H.A. 1963. *Principles of Numerical Taxonomy.* W.H. Freeman and Company, San Francisco.

Subbarayudu, N., Reddy, K.R. and Rao, N.R. 1976. Studies on varietal performance of turmeric. *Andhra agric. J.* **23**(6):195-199

* Sugiura, T. 1936. Studies on the chromosome numbers in higher plants. *Cytologia.* **7**:544-595

Sujatha, V.S., Seshadri, V.S., Srivastava, K.N. and More, T.A. 1991. Isoenzyme variation in *Cucumis* spp. *Indian J. Genet.* **57**:252-259

Trimen, H. and Hooker, J.D. 1974. *A Handbook to the Flora of Ceylon.* Part IV. Periodical Experts, New Delhi. p.240-243

Tyson, H., Fields, M.A. and Starobin, J. 1986. Genetic control of acid phosphatase Rm and its relation to control of peroxidase Rm in flax (Linum) genotrophs. *Biochem. Genet.* **24**:369-383

Valeton, T.H. 1918. New notes on the Zingiberaceae of Java and Malaya. *Bull. Jard. Buitenzorg.* Ser **11**, **27**:1-81

Velayudhan, K.C., Amalraj, V.A. and Muralidharan, V.K. 1990a. *Curcuma malabarica* (Zingiberaceae). A new species from Westcoast of South India. *J. Econ. Tax. Bot.* **14**(1):189-191

Velayudhan, K.C., Amalraj, V.A. and Muralidharan, V.K. 1990b. Identity of *Curcuma aromatica* Salisb. from South India. *J. Econ. Tax. Bot.* **14**(3):579-582

Velayudhan, K.C., Amalraj, V.A. and Muralidharan, V.K. 1996. The conspects of the genus *Curcuma* in India. *J. Econ. Tax. Bot.* **20**(2):375-382

Velayudhan, K.C., Muralidharan, V.K., Amalraj, V.A., Rana, R.S., Singh, B. and Thomas, T.A. 1994. *Genetic Resources of Curcuma*. National Bureau of Plant Genetic Resources, ICAR, New Delhi.

Yadav, D.S., Ramesh, S. and Singh, R. 1996. Studies on genetic variability in turmeric (*C. longa*). *J. Hill Res.* 9(1):33-36

Zwaving, J.H. and Bos, R. 1992. Analysis of the essential oil of five *Curcuma species*. *Flavour and Fragrance J.* 7(1):19-22

*Originals not seen

ISOENZYME VARIATION IN *Curcuma* WITH SPECIAL REFERENCE TO *Curcuma longa* L.

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

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1999

ABSTRACT

The study on 'Isoenzyme variation in *Curcuma* with special reference to *C. longa* was conducted in the Department of Plantation Crops and Spices and Biochemistry laboratory at College of Horticulture, Vellanikkara, Thrissur from October 1997 to June 1999. Eighteen species of genus *Curcuma*, including *C. longa* were included in the study. Thirty three accessions of *C. longa* were selected to represent 21 morphotypes of Velayudhan *et al.* (1994). Six released varieties were also studied. These were analysed for three enzymes viz. peroxidase, esterase and GOT using polyacrylamide gel electrophoresis.

The selection of ideal part for electrophoresis for each enzyme was done based on observations of banding pattern of root, rhizome, sprout and different maturity stages of leaf. Sampling and analysis was done during three weeks after sprouting and just before senescence of leaf to standardise the stage of growth of plant. Mature leaf just before its senescence was selected for peroxidase and GOT analysis. Tender leaf three weeks after sprouting was found to be ideal for esterase.

Isoenzyme banding pattern of 39 genotypes of *C. longa* were studied for esterase, peroxidase and GOT. The similarity index among the genotypes ranged from 0.39 to one. The genotypes were classified into 21 groups with similarity index one among the members. Morphotypes M-1, M-2 and M-3 of Velayudhan *et al.* (1994) formed one group with similarity index one. M-7 and M-8 were also grouped into one. Released varieties under study showed more similarity with first group of morphotypes.

On grouping the 18 species of *Curcuma* based on isoenzyme similarity *C. zedoaria*, *C. aeruginosa*, *C. malabarica*, *C. comosa* and *C. caesia* formed one group, *C. amada*, *C. sylvatica*, *C. aromatica* and *C. harita* the second group and

C. soloensis, *C. brog* and *C. montana* the third group. Cultivated type *C. longa* and other sessile tuberising species *C. latifolia* and *C. raktakanta* showed variable similarity with other species and stood independently. Stoloniferous type *C. vamana* showed more similarity with sessile tuberising species than with non-sessile tuberising species. Non-sessile tuberising species *C. aurantiaca* and *C. pseudomontana* stood individually showing their distinctness from rest of the species.