RESPONSE OF TURMERIC *Curcuma domestica* Val. TO *IN VIVO* AND *IN VITRO* POLLINATION

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**Response of turmeric *Curcuma domestica* Val. to *in vivo* and *in vitro* pollination**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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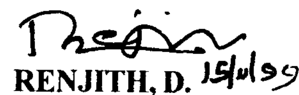

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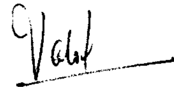

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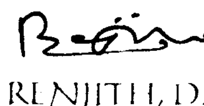

RENJITH, D.

LIST OF ABBREVIATIONS

GCV	- Genotypic coefficient of variation
PCV	- Phenotypic coefficient of variation
IAA	- Indole-3-Acetic Acid
IBA	- Indole Butyric Acid
NAA	- α Naphthalene Acetic Acid
2,4-D	- 2,4-Dichloro Phenoxy Acetic Acid
BAP	- Benzylaminopurine
GA	- Gibberellic acid
2ip	- Isopentenyl adenine
Kin	- Kinetin, N6-furfuryl acetone
CH	- Casein hydrolysate
CW	- Coconut water
FAA	- Formalin acetic acid mixture
PEG	- Poly ethylene glycol – 4000
MS	- Murashige and Skoog's (1962) medium
SH	- Schenk and Hildebrandt (1972) medium
μm	- micrometer
mm	- millimeter
mg l^{-1}	- milligram per litre
kg	- kilogram
t	- tonne
v/v	- volume in volume
DAP	- Days after pollination
ppm	- parts per million

LIST OF TABLES

Table No.	Title	Page No.
1	Source of different cultivars of turmeric	29
2	Composition of Brewbaker and Kwack's medium	32
3	Composition of modified ME_3 medium	33
4	*In vitro* sensitivity of bacterial population to antibiotics in culture establishment	37
5	Composition of various culture media used for culture establishment	38
6	Cytokinin concentrations tried in the ovule development	39
7	Auxin concentrations tried in the ovule development	40
8	Cytokinin and auxin combinations tried in the ovule development	40
9	Organic media supplements tried in the ovule development	41
10	Cytokinin and auxin combinations tried in the *in vitro* seed germination	43
11	Variability in pseudostem characters of turmeric cultivars	45
12	Variability in rhizome and root characters of turmeric cultivars	48
13	Variability in curcumin content and curing percentage of turmeric cultivars	48
14	Floral biology and morphology of turmeric cultivars	49
15	Pollen germination of turmeric cultivars in different media	52
16	Effect of position of flowers in the inflorescence on the fertility and viability of pollen grains and pollen tube length of turmeric cultivars	54
17	Natural seed set in turmeric cultivars	57

18	Controlled *in vivo* crosses in turmeric cultivars	57
19	Culture establishment in basal media combinations of ½ MS full MS and SH	61
20	*In vitro* sensitivity of bacterial population to antibiotics in culture establishment	62
21	Influence of cytokinins in the ovule development of turmeric after modified placental pollination	63
22	Influence of auxins in the ovule development of turmeric after modified placental pollination	63
23	Influence of cytokinins and auxins in the ovule development of turmeric after modified placental pollination	65
24	Influence of sucrose in ovule development of turmeric after *in vitro* pollination	67
25	Influence of coconut water on ovary and ovule development in turmeric after modified placental pollination	67
26	Influence of casein hydrolysate in ovule development of turmeric after *in vitro* placental pollination	68
27	Standardisation of *in vitro* pollination techniques in turmeric for crossing	69
28	Effect of pre-sowing treatments on germination of turmeric seeds	70

LIST OF FIGURES

Fig. No.	Title
1	Longitudinal section of turmeric seed
2	Cross section of turmeric seed

LIST OF PLATES

Plate No.	Title
1a, b, c, d, e, f	Short duration cultivars – VK 70, VK 55, VK 76, Suguna, Suvarna and Sudharsana
1g, h	Medium duration cultivars – Kanthi and Sobha
2	Turmeric cultivar Kanthi on flowering
3a	Inflorescence of *Curcuma domestica* Val.
3b	Flower of *Curcuma domestica* Val.
3c	Ovules of *Curcuma domestica* Val.
4	Pollen germination and tube growth in modified ME3 medium
5	Pollen grains stained with acetocarmine
6a	Late prophase in the cultivar VK 70 showing 2n=84
6b	Late prophase in the cultivar Suvarna showing 2n=84
6c	Late prophase in the cultivar Kanthi showing 2n=63
7a	*In vivo* stigmatic pollination in turmeric
7b	*In vivo* stylar pollination in turmeric
8a	Pollen germination on the stigma and tube growth through the style in controlled *in vivo* cross of VK 70 x VK 76
8b	Pollen tube covering the ovule length
9	Developed fruit of turmeric 20 DAP under *in vivo* condition
10a	Arillate seed of turmeric
10b	L.S. mature seed of turmeric stained with saffranin
11a	Developing ovules ten days after *in vitro* placental pollination
11b	Callusing from the ovules 10 DAP

11c *In vitro* placental pollination

11d Fruit of turmeric developed under *in vitro* condition

11e Black seed of turmeric 20 days after *in vitro* placental pollination

11f L.S. of seed produced under *in vitro*

12 Seed germination of turmeric under *in vitro* condition

CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1-2
2	REVIEW OF LITERATURE	3-27
3	MATERIALS AND METHODS	28-43
4	RESULTS	44-70
5	DISCUSSION	71-87
6	SUMMARY	88-92
	REFERENCES	i-xi
	APPENDIX	
	ABSTRACT	

Affectionately dedicated to

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INTRODUCTION

INTRODUCTION

Turmeric, the sacred spice of Asian countries is obtained from the rhizome of *Curcuma domestica* Val./*Curcuma longa* L. (Zingiberaceae), a herbaceous plant native to Indo Malayan region.

Turmeric is the third important spice crop of India, being next to chillies and black pepper. Here, it is cultivated in an area of 1.47 lakh ha with an annual production of 6.59 lakh tonnes. In the world, India is by far the largest producer of the same contributing 82 percentage of the production and 45 percentage of export (Peter, 1997).

The foreign exchange earning by way of turmeric export comes to the tune of Rs.103.87 crores annually (spice India, 1999). Andhra Pradesh, the lead state of turmeric cultivation in the country shares 39 percentage of the area and 57 percentage of the production (Spices Statistics, 1998). The area and production of turmeric in Kerala is only 0.03 percentage (3800 ha) and 0.01 percentage (8400 tonnes), respectively of the national figures. However, the Alleppey turmeric from Kerala is much valued in the international market for its rich yellow colouring matter, curcumin (6 to 7%).

Turmeric is valued globally as a condiment, colourant, dyestuff and medicine. The yellow colouring factor curcumin is gaining importance in food industry, pharmaceuticals preservatives and in body care. With the ban of artificial colours in food industry, the use of curcumin has become wide spread. The estimated global demand of turmeric by 2000 AD is 0.31 lakh tonnes (dry) with an annual growth rate of 10 per cent in demand. These facts point to the future prospects of turmeric cultivation in the country. To take up cultivation in a competitive manner, high yielding varieties with high quality and disease resistance is a must.

So far, crop improvement in turmeric is limited to clonal selection and mutation breeding only. The existing population of turmeric shows tremendous variability for dry yield, curing percentage, curcumin content and disease resistance. The available gene pool is not so far fully exploited for crop improvement as hybridization technique is not yet perfected for this crop.

Cultivated turmeric consists of short, medium and long duration types. Short duration types are generally fertile tetraploids (2n=84) while medium and long duration types are sterile triploids (2n=63). Nazeem and Menon (1994) have reported seed set in controlled crosses of short duration types, but posed difficulty in crossing short duration types with medium and long duration types as one of the parents involved is triploid. *In vitro* pollination and fertilization is an effective tool for overcoming this type of incompatibility reactions hindering seed set.

Present study "Response of turmeric (*Curcuma domestica* Val.) to *in vivo* and *in vitro* pollination" aims at perfecting a hybridisation technique in turmeric either through *in vivo* or *in vitro* pollination. Success in this line will open up new vistas of crop improvement in turmeric, especially for the improvement of medium and long duration types which occupy 93 percentage of cultivated area of turmeric. Alleppey turmeric, which is rich in curcumin content can be further improved for curing percentage. Establishment of hybrid population will be easier as micropropagation is standardized in this crop. Maintenance of hybrids will be easier as the crop is vegetatively propagated in cultivation.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Turmeric (*Curcuma domestica* Val.) has been described by many as a species producing flowers and seeds scarcely. In the conventional breeding programme seed set was obtained in crosses involving short duration cultivars but failed with medium and long duration cultivars. Present study aims at perfecting a hybridisation technique in turmeric through *in vivo* and *in vitro* pollination techniques. The cytology of turmeric has to be examined to ascertain the triploidy of long duration types and tetraploidy of short duration types. The accumulated literature on various aspects relevant to the subject matter of the present investigation is reviewed here.

2.1 Nomenclature

According to Purseglove *et al.* (1981), *Curcuma longa* of Linnaeus was based on a plant observed by Hermann in Srilanka and is not this species. *C. longa* of Koenig is probably the true turmeric but as this name belongs to another, Valeton proposed the new name of *C. domestica* in 1918. Valeton observed that the types cultivated in the name of turmeric might contain different entities. The name was subsequently reinstated as *C. longa* L. by Burt, 1977 and Velayudhan, *et al.* 1994). Hence *C. longa* L. and *C. domestica* Val. are synonyms of turmeric.

2.2 Origin and distribution

Turmeric might have originated in the Indo-Malayan region (Harlan, 1975, Purseglove, 1978 and Velayudhan *et al.*, 1994). In the Indo-Malayan region India can be the centre of origin as over 40 species of the genus are indigenous to this region (Velayudhan *et al.*, 1994)

At present the crop is distributed in India, Pakistan, Malaysia, Indonesia, Myanmar, Vietnam, Thailand, Philippines, Japan, China, Korea, Srilanka, Nepal,

South Pacific Islands, East and West African nations, Malagassay, Caribean Islands and Central America (Velayudhan *et al.*, 1994).

Thus the turmeric belt now extends from South East Asia to Central America which also includes some islands in South Pacific.

2.3 Classification

Turmeric belongs to the family Zingiberaceae. The family Zingiberaceae consists of two subfamilies ie aromatic zingiberoideae and non aromatic costoideae. The subfamily zingiberoideae include important genera like *Aframomum, Amomum, Curcuma, Elettaria, Hedychium, Kaempferia* and *Zingiber*. The principal genus of costoideae is *Costus* (Purseglove, 1978)

2.4 Species

The genus *Curcuma* has got about 70-80 species of rhizomatous herbs distributed in the Indo-Malaysian region (Purseglove *et al.*, 1981 and Velayudhan *et al.*, 1994). In India about 40 species are found (Velayudhan *et al.*, 1994). Among them, *C. longa* L./*C. domestica* Val., *C. aromatica* Salisb., *C. amada* Roxb., *C. angustifolia* Roxb., *C. caesia* Roxb., *C. mangga* Val. and *C. zeodoria* (Beng.) Rosc. are some of the economically important ones (Purseglove *et al.*, 1981).

C. longa L./*C. domestica* Val. is the source of turmeric which is used as a spice, food colourant, dye and in medicine. *C. aromatica* Salisb. Is the wild turmeric or yellow zeodory, used as a dye, cosmetic and drug but not as a condiment. This species is found wild in India. The rhizomes have the smell of camphor (Purseglove *et al.*, 1981).

Regarding the identity of *C. aromatica* Salisb. some confusion exists. Many Indian researchers have used this species as the name for short duration types

of turmeric having pleasant aroma (Philip, 1978, George, 1981, Nazeem and Menon, 1994 and Rao and Rao, 1994).

According to Velayudhan *et al.* (1990) and Mangaly and Sabu (1993), this is incorrect. They supported the view of Purseglove *et al.*, 1981 i.e., *C. aromatic* Salisb. is a wild species occurring in India and its rhizome is not used a spice or condiment.

Short duration type of turmeric is only a morphotype of *C. longa* Syn., *C. domestica* (Velayudhan *et al.*, 1994).

2.5 Genetic variability

Evaluation of 19 turmeric types collected from different parts of the country revealed the presence of significant variation among types with regard to morphological characters, yield of fresh turmeric, curing percentage, oleoresin, curcumin content, leaf blotch, leaf spot and shoot borer infection. The yield of cured produce varied between 8558.4 kg ha^{-1} (VK 5 - Mannuthy local) and 1504.1 kg ha^{-1} (Thekurpet). The curing percentage ranged from 14.06 (Nandyal) to 28.17 per cent (Dindrigam Ca 69). The oleoresin content varied from 12.1 (Kasturi Tanuka) to 21.1 per cent (Duggirala Cll-325) and the variation in curcumin content was from 2.33 (Duggirala) to 6.55 per cent (VK 5 - Mannuthy local). The incidence of leaf blotch disease ranged from 1.16 (G-L Puram II) to 5.27 per cent (Armoor Cll-325), leaf spot from 1.54 (VK 5 - Mannuthy local) to 4.07 (Rajpuri) per cent and shoot borer infection from 18.1 (VK 5) to 84.5 (G.L. Puram). Heritability estimates were higher for curing percentage, curcumin and oleoresin content (Philip, 1978, Philip *et al.*, 1980, Philip and Nair, 1983 and Philip and Nair, 1986). The investigations concluded that significant variation among the turmeric types grown under the same cultural and agroclimatic conditions can be attributed to the genetic factors.

Occurrence of genetic variability in turmeric for morphological characters, yield, curing percentage, curcumin content and disease resistance were

reported by Philip, 1978, Mohanty, 1979, Mukhopadhyay *et al.*, 1986, Pujari *et al.*, 1987, Jalgaonkar *et al.*, 1988, Reddy and Rao, 1988, Jalgaonkar *et al.*, 1990, Yadav *et al.*, 1996, Kurien and Valsala, 1995 and Kurien and Nair, 1996).

The open pollinated seedlings of turmeric *C. aromatica* viz. Amalapuram and Dindrigram showed variability with respect to fresh yield, curing percentage, curcumin and oleoresin content (George, 1981).

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

2.6 Cultivars

A number of cultivars are distinguished in India by the names of their localities in which they are grown (Purseglove *et al.*, 1981). Menon (1975) found that turmeric produced in different localities vary in quality and Alleppey, Rajpuri, Guntur and Madras are popular trade names of Indian turmeric.

Turmeric types can be grouped into three, based on the time taken for harvest as short, medium and long duration types. Short duration types are known as Kasturi. They mature in seven months, rhizomes possess pleasant aroma, good yielders of dried turmeric and rich in volatile oil content but low in curcumin and used in culinary preparation. Flowering is common in these types and seeds can produce gametic seedlings. Medium duration Kesari types (Bontha) mature in eight months referred as intermediary types and are high yielders of fresh rhizomes than Kasturi types and moderately rich in curcumin and volatile oil. They are susceptible to leaf blotch and rhizome rot. Long duration types mature in nine months, which are superior to the above groups in rhizome yields and other quality parameters. Flowering is rare in these types (Rao *et al.*, 1975 and Rao and Rao, 1988).

All short duration types are not in confirmity with the above statements. Exceptions occur. Released short duration varieties, Suguna and Sudharsana are low in curing percentate (12 to 14) but some what high in curcumin content (>5%) (Edison *et al.*, 1991).

Long duration types are economically more important accounting for 96.4 per cent of total area under turmeric cultivation and the remaining 3.6 being under short duration types (Pruthy, 1976).

Some of the popular Kasturi types of Andhra Pradesh are Kasturi Kothapeta, Kasturi Tanuka, Kasturi Amalapuram, Chayapasupu, Gummalakshmipuram etc. They have an yield potential of 10-15 t of raw turmeric per ha. The curing percentage of Kasturi Kothapeta, Kasturi Tanuka and Kasturi Amalapuram is about 24-25 per cent while in Chayapasupu it is about 19 percentage and in Gummalakshmipuram 14 percentage. Gummalakshmipuram has a high curcumin content of 5 percentage (Rao and Rao, 1994).

Popular Kesari types of Andhra Pradesh are Kesari Deevur and Amrutapani Kothapeta. (Rao and Rao, 1994) Popular long duration types are Duggirala, Tekurpet, Mydukur, Armoor, Sugandham, Vontimitta, Nandyal, Avanigadda. They have an yield potential ranging from 15 to 30 t ha^{-1} and curing percentage 15 to 22.4 (Rao and Rao, 1994).

Erode and Salem are popular cultivars of Tamil Nadu with curcumin 3.9 and 4.75 per cent respectively (Muthuswamy and Ahmed Shah, 1982).

Shillong and Tall Karbi are popular cultivars of Assam (Rathiah, 1986). Shillong is a high yielder of fresh rhizomes (40 t ha^{-1}) and low in curing percentage (14) but good in curcumin (4.1%). Tall Karbi has a good yield potential (30-40 t ha^{-1}) but low in curing percentage (17.6%) and curcumin content (3.06%).

In Maharashtra and Gujarat, the popular cultivars are Rajpuri and Evaigon. Rajpuri is a moderate yielder (20 t ha^{-1}) with 3.4 per cent curcumin content, while Evaigon has low curing percentage of 17 and high green yield of 45 t ha^{-1} (Pujari *et al.*, 1986).

The popular cultivars in Orissa are Duhgi, Jabedi and Katingia. Duhgi is a low yielder (10 t ha^{-1}) but has good curing percentage (26%) and poor in curcumin content (1.2%). Jabedi is low in curcumin content (1.2%), While Katingia has high curing percentage (36%) but poor in yield (8 t ha^{-1}) and curcumin content (1%) (Rao and Rao, 1994).

Gorakhpur, the popular cultivar of Uttar Pradesh has a green yield of 15 t ha^{-1} and moderate curing outturn (18%) and curcumin content (3.5%) (Rao and Rao, 1994).

Alleppey turmeric has a yield potential of 25 t ha^{-1} and high curcumin content of 6.5 per cent but with slightly low curing percentage of 19 (Lewis, 1973 and Purseglove, *et al.* 1981).

Mannuthy local from Central Kerala is a good yielder of rhizome with 24 t ha^{-1} and rich in curcumin content of 7.58 per cent (Philip, 1978).

Kurian and Valsala (1995) have also reported the superiority of Mannuthy local cultivar. They also found that the local cultivars from Kuruppampady (Ernakulam District) and Mupliyam (Thrissur district) are rich in curcumin content with 7.82 and 7.69 per cent respectively.

Turmeric produced in different localities vary in quality and price. Alleppey, Salem, Erode, Rajpuri and Nizamabad fingers are popular trade names of Indian turmeric (Spices Market, 1999).

Velayudhan *et al.* (1994) classified 568 collections of *C. longa* into 21 morphotypes based on the above ground and underground characters.

2.7 Crop improvement in turmeric

So far, the crop improvement in turmeric is limited to clonal selection and mutation breeding. The genepool of cultivated turmeric consists of high yielding types like Dindrigam and G.L. Puram with high curing percentage of 35 and high curcumin types like Sobha and Mannuthy local (>7 per cent). The available wide variability for yield and quality characters are not so far exploited for crop improvement, as hybridisation technique is not perfected.

2.7.1 Clonal selection

Varietal improvement through clonal selection has resulted in the release of few high yielding culivars with good quality.

Suguna, Suvarna and Sudharsana are high yielding varieties released for Kerala (Rao and Rao, 1994).

Suvarna has a green yield potential of 43.5 t ha^{-1}, curing percentage 20 and curcumin content 4 per cent. suguna has a raw yield potential of 60.3 t ha^{-1}, curcumin content of 4.9 per cent and curing percentage 24.6. Sudharsana has a high yield potential of 54.8 t ha^{-1} with curcumin content of 7.9 and curing percentage 25.3 (Edison *et al.*, 1991).

Kanthi is a clonal selection from Mydukur cultivar of Andhra Pradesh and has a curing percentage 20.15 and curcumin content 7.18 per cent. Sobha is a clonal selection from the local cultivar of Methala region of South Kerala. It has a curing percentage of 19.28 and curcumin content of 7.39 per cent (Mohanakumaran and Rao, 1996).

The other released varieties for the various states of the country are Krishna, Sugandham, BSR-1, Roma, Suroma, Rajendra Sonia, Ranga and Rasmi (Edison *et al.*, 1991).

2.7.2 Hybridisation

Nazeem and Menon (1994) have reported seed set for the first time in controlled crosses of *C. aromatica* types (short duration) and *C. domestica* (long duration) types. Seed set was noticed in eight of the eleven crosses tried. The hybrids are of the crosses Amalapuram x Nandyal, Amalapuram x Amrithapani Kothapetta and its reciprocal, Kodur and Amalapuram.

2.7.3 Mutation breeding

CO-1 and BSR-1 are the two varieties recommended for cultivation in Tamil Nadu, which were developed from Erode local by X-ray irradiation (Khader and Vedamuthu, 1989).

2.8 Cytology

Sugiura (1936) was the first to report the chromosome number in *C. longa* as 2n=64.

Raghavan and Venkatasubban (1943) reported chromosome number for *C. longa* as 2n=62 and for *C. aromatica* as 2n=42.

A chromosome number of 2n = 32 for *C. longa* was reported by Sato (1960) and based on the karyomorphology he concluded that the species seems to be on allotetraploid with a basic number of x = 8. According to Nair *et al.* (1980) this can be a miscount as later works suggest a chromosome number of 63.

Cytology of six species of *Curcuma* and seven cultivars of *C. longa* was reported by Ramachandran (1961). A chromosome number of 2n = 86 for

C. aromatica (short duration types) was reported by him and he concluded that the species is a tetraploid. He also studied in detail the meiosis of two species *C. decipiens* (2n = 42) and *C. longa* (2n = 63) and concluded that the sterility in *C. longa* was probably due to its auto-triploid nature.

A further report on the chromosome numbers in Zingiberaceae and genus *Curcuma* was published by Ramachandran (1969), where in he reported the chromosome number for *C. aromatica* (2n = 63,86) and *C. longa* (2n = 63). A high basic number of n = 21 was assumed for *Curcuma* and Ramachandran (1969) concluded that the genus *Curcuma* might have been derived either by dibasic amphidiploidy (x = 9 + 12) or by secondary polyploidy.

Detailed cytological investigations by Nambiar (1979) revealed that all the cultivars of *C. aromatica* had chromosome numbers of 2n = 84, and *C. longa* 2n = 63. This along with 2n = 42 for *C. amada* indicated a polyploid series with multiples of n = 21.

2.9 Flowering

2.9.1 Flowering behaviour

Valeton (1918) reported that all the cultivars in Java came to flowering, but the fruitset was observed in only two. Aiyer (1954) mentioned that flowering was scarce in turmeric.

Patnaik *et al.* (1960) and Pai (1961) described the flowering behaviour of *Curcuma longa*. The flowering period ranged from June to October.

Aiyadurai (1966) stated that in Andhra Pradesh cultivar of the *longa* species flowered very rarely and viable seeds could be collected from the flowering types.

Pillai and Nambiar (1975) noticed flowering and fruitset in 9 *longa* types and 8 *aromatica* types.

Philip (1978) observed that out of the 19 types studied 15 types had flowered under Vellanikkara conditions. The percentage of flowering ranged from 1.2 to 95.28 per cent with the maximum in VK-14 (Dindrigam Ca – 69) and the minimum in VK-16 (Rajpuri). Flowering was high in Amalapuram (92.7%) also.

Aiyadurai (1966) also mentioned that the climatic conditions influenced flowering to a great extent.

Under Kasaragod conditions the flowering period was July to September in *C. aromatica* and September to December in *C. longa* (Nambiar *et al.*, 1982).

Nazeem and Menon (1994) recorded observations on the various aspects of flowering in turmeric.

2.9.2 Inflorescence

The inflorescence in turmeric is a compound spike which is terminal on the leafy shoot. The bracts were large sized, greenish white and the upper most tinged with pink and were spirally arranged. The lower bracts enclosed 18 to 41 flowers which opened in succession. The upper three to seven and lower five to eight bracts were sterile with no flowers. The rest carried one to three flowers and very rarely four in their axils. The mean number of flowers per inflorescence ranged between 26 to 35.2 (Philip, 1978, Lad, 1993, Nazeem and Menon, 1994).

2.9.3 Floral Morphology

The flowers of turmeric are yellowish white in colour, zygomorphic, bisexual, bracteolate and epigynous. The perianth is in two whorls, consisting of six segments, making a gamosepalous calyx and a gamopetalous corolla. Calyx, tubular

and short dividing above into three short teeth. Corolla tube funnel shaped having three ovate/oblong lobes.

Androecium consists of six stamens which are epipetalous arising from the throat of the corolla tube in two whorls. Of the three stamens in the outer whorl one was suppressed and the other two were reduced to staminodes. In the inner whorl the posterior stamen is fertile and had a prominent two lobed anther having broad connective projected beyond the anther. The ether two were sterile petalloid and fuse together to form a labellum, the most conspicuous part of the flower. The anther is two lobed with a sickle shaped spur. Style is elongated and slender lying in the channel. The fertile stamen and stigma were projected above the anther and two lipped. Ovary is inferior, syncarpous, trilocular and with axile placentation. Fruit is a capsule with numerous arillate seeds (Philip, 1978, Purseglove *et al.*, 1981 and Lad, 1993).

2.9.4 Floral Biology

Patnaik *et al.* (1960) and Pai (1961) described the floral biology of *C. longa*.

The anthesis commenced 7.6 to 11.6 days after the emergence of the inflorecence. The duration of flower opening within an infloresence lasted 8 to 11 days. The anthesis started from 7 am and continued upto 9 am. Maximum number of flowers (72.0%) opened before 8 am (Nazeem and Menon, 1994).

2.10 Pollen studies

2.10.1 Pollen production and morphology

The pollen grains of turmeric are described as ovoid to spherical in shape, light yellow in colour and slightly sticky. The mean pollen length ranged from 7.0 μ (Amalapuram and Kodur) to 7.2 μ (Dindrigam and Nandyal) while the

breadth varied between 4.2 μ (Kodur) and 4.95 μ (Mannuthy local) (Nazeem and Menon, 1994).

2.10.2 Pollen fertility and germination

In turmeric pollen grains are reported to have high degree of fertility.

Nambiar *et al.* (1982) observed that pollen stainability in acetocarmine varied from 45-48 per cent in *C. longa* and 68-75 per cent in *C. aromatica*.

Stain test indicated pollen fertility ranging from 71.1 per cent (Kodur) to 84.46 per cent (Kuchipudi) (Nazeem and Menon, 1994).

In turmeric germination of pollen grains under *in vitro* conditions is not reported so far.

2.10.3 Pollination and seed set

Patnaik *et al.* (1960) reported that in turmeric pollination is brought about by insects.

Seed setting in turmeric has been reported by Aiyadurai (1966) and Pillai and Nambiar (1975).

Seed set was noticed in *C. aromatica* types while in the triploid species *C. longa* types, fruitset as well as seed set was not observed (Nambiar *et al.*, 1982).

Nazeem and Menon (1994) observed seed set in crosses involving *Curcuma domestica* and *C. aromatica* types. In crosses involving the aromatica types, seed set was high. While none of the varieties produced seeds on selfing.

Two distinct type of seeds, dark heavy and light brown were extracted from mature capsules of *C. aromatica*. The seeds had a white aril, smooth surface and an apical micropylar ring with a wavy line (Nambiar *et al.*, 1982).

Lad (1993) observed seed setting in turmeric cultivars Ca-92/1 and Ca-17/1. Ca-92/1 produced 27 seeds/plant whereas Ca-17/1 only 4-5 seeds/plant. According to him turmeric seeds are triangular and of bright chocolate, lustrous colour with white placenta persisting at the base.

2.11 Seed germination studies

Nazeem *et al.* (1993) observed the seed germination in turmeric 17 to 26 days after sowing and its duration ranged from 10 to 44 days in the various crosses.

Nambiar *et al.* (1982) found that percentage of germination varied from cultivar to cultivar and even plant to plant. 70 to 90 per cent germination was recorded in 8-20 days after sowing. There was practically no germination, after the 20th day. Seedlings raised from seeds of individual plants had remarkable morphological similarities to the parental clones.

Seed germination ranged from 17.22 to 100 per cent and seedlings produced only root tubers during the first year of growth and the weight ranged between 14.18 and 49.4 g (Nazeem and Menon, 1994).

2.12 *In vitro* pollination and fertilization

The technique of *in vitro* pollination and fertilization is an effective tool for getting seed set in species where pre fertilization barriers blocks seed set. Some of the barriers to fertilization are a) inability of pollen to germinate on foreign stigma, b) failure of the pollen tube to reach the ovule due to excessive length of the style, or slow growth of the pollen tube which fails to reach the base of the style before the ovary absices, c) bursting of the pollen tube in the style. The most important application of the technique is the production of rare hybrids (Bhojwani and Razdan, 1983).

2.12 1 *In vitro* pollination and fertilization technique

The pre-requisits and procedures to be adopted for the successful *in vitro* pollination had been described by Kanta and Maheswari (1963).

The technique consists of (1) A detailed study of floral biology of the crop i.e., time of anthesis, anther dehiscence, mode of pollination etc. (2) Pollen germination and pollen tube growth has to be determined. (3) Standardisation of a surface sterilization technique for flowers and flower buds so that the viability of pollen grains and receptivity of gynoecium is not affected. (4) Identification of a suitable medium which support, the development of the ovule or ovary into mature seed or fruit. (5) Standardisation of appropriate pollination technique so that fertilization and growth of the ovule takes place. (6) Identification of suitable culture conditions which will promote the growth of the ovule to viable seeds. (7) Histological examination of ovule or ovary at various stages to know the fertilization and development of embryo and endosperm. (8) Standardisation of culture conditions for germination of seeds.

2.12.2 Different kinds of *in vitro* pollination

The different kinds of *in vitro* pollination as per Bhojwani and Razdan (1983) are *in vitro* stigmatic pollination, intra ovarian pollination, *in vitro* placental pollination and *in vitro* ovular pollination. *In vitro* stylar pollination was reported by Niimi (1976). Tuyl *et al.* (1991) have reported *in vitro* grafted style pollination.

2.12.2.1 *In vitro* stigmatic pollination

The pollen grains are applied on the surface of the stigma. Usha (1965) obtained seeds in *Antirrhinum majus* by *in vitro* stigmatic pollination. In Lilium interspecific hybrids were successfully obtained through *in vitro* stigmatic pollination (Tuyl *et al.*, 1991).

2.12.2.2 *In vitro* stylar pollination

In the *in vitro* stylar pollination style is cut at various heights and the pollen grains are deposited on the cut surface of the style. *In vitro* seed production has been susscessfully reported in *Petunia hybrida* (Niimi, 1976) and in maize (Gengenbach, 1977a and Hauptli and Williams, 1988).

2.12.2.3 Grafted style pollination

Here pollen is applied in a compatible stigma while the style with the germinated pollen is attached to the ovary of another plant which is incompatible. In Lilium crosses style and stigma were joined using a piece of drinking straw filled with stigmatic exudate or were stuck together only with the exudate (Tuyl *et al.*, 1991).

2.12.2.4 Intra-ovarian pollination

Pollen grains are directly applied into the ovary in intra ovarian pollination.

In *Papaver rhoeas*, Kanta (1960) developed seeds through modified intra-ovarian pollination. In this technique the pollen suspended in distilled water having boric acid (100 ppm) was injected into the ovary through a hole using a hypodermic syringe. This technique was also successfully adopted in *Papaver somniferum* and *Eschscholzia california*.

The pollen grain can also be introduced into the ovary through a slit made on the ovary wall. This technique has given positive result both in *Argemone mexicana* and in *Argemone ochroleuca*. By adopting this technique interspecific hybrids between these two species could be produced (Kanta and Maheswari, 1963).

2.12.2.5 *In vitro* placental pollination

In, *in vitro* placental pollination the whole placenta bearing ovules attached to short pedicel is generally used (Rangaswamy and Shivanna, 1967 and 1971a). The placenta may be cut into two or more pieces each carrying a certain number of ovules.

Rangaswamy and Shivanna (1971b) developed a modification to the placental pollination technique. They cultured the entire pistils after exposing the placenta bearing ovules by removing the ovary wall partially. Pollination was done on the exposed ovules.

2.12.2.6 *In vitro* ovular pollination/test tube fertilization

Pollen is directly applied to the excised ovules in *in vitro* ovular pollination.

This technique has successfully adopted in *Papaver somniferum, Argemone mexicana, Eschscholzia california, Nicotiana rustica* and *Nicotiana tabacum* (Kanta and Maheswari, 1963).

Mature embryoids and plants were obtained after *in vitro* ovular pollination of the cruciferous species (Zenkteler, 1980).

By this technique embryos were developed in plums. (Lech *et al.*, 1994).

Slusarkiewicz (1984) also reported the test tube fertilization of ovules in some species of Solanaceae.

2.12.3 Factors affecting seed set

2.12.3.1 Age of flower buds

Generally the flower buds on the day of anthesis or one or two days after anthesis respond to *in vitro* pollination. The occurrence of seed set was reported to

be higher when the ovules were excised 1-2 days after anthesis than on the day of anthesis (Kanta *et al.*, 1962, Kanta and Maheswari, 1963, Rangaswamy and Shivanna, 1967 and Balatkova *et al.*, 1977a).

In maize the spike, 3-4 days after silking is found suitable for *in vitro* pollination (Gengenbach, 1977a). The flower buds one day prior to anthesis were ideal for *in vitro* pollination in Gossypium (Refaat *et al.*, 1984).

In lilium, for the production of interspecific hybrids, the seed set was observed when the flowers pollinated three days prior to anthesis. Success rate was often reduced when pollination was done two days prior to anthesis.

2.12.3.2 Surface sterilization

A suitable surface sterilization technique, harmless to pollen viability and receptivity of the ovules, is necessary for the success of *in vitro* pollination.

Surface sterilization of the ovaries of *Papaver somniferum* which is relatively hard is done by dipping in rectified spirit and flaming. The ovaries of *Argemone mexicana, Eschcholzia californica, Nicotiana rustica* and *Nicotiana tabacum* which are more delicate were surface sterilized with chlorine water (Kanta and Maheswari, 1963).

Usha (1965) reported surface sterilization of flower buds of *Antirrhinum majus*, prior to anthesis by dipping first in 70 per cent alcohol and later in strong chlorine water followed by washing with sterile distilled water.

In *Vaccinium* with 1 per cent sodium hypochlorite solution for 15 min, satisfactory surface sterilization was achieved (Munoz and Lyrene, 1985).

In lilium, flower buds in ethyl alcohol (70%) for one minute followed by a commercial bleach (2% Cl) treatment for 15 min give proper surface sterilization (Tuyl *et al.*, 1991).

In maize surface sterilization was found unnecessary since the ovaries are protected by several layers of husks. The removal of inner husks with sterile forceps in the laminar flow chamber is sufficient (Gengenbach, 1977a).

2.12.3.3 Nature of the explant

In the intra ovarian pollination of *Papaver rhoeas* L., the retention of petals and sepals were found to accelerate the development of the ovary (Kanta, 1960).

In placental or ovular pollination wetting the surface of the ovules should be avoided as it causes poor pollen germination, bursting of pollen tube and finally poor seed set (Balatkova and Tupy, 1968 and Zenkteler, 1980).

In *Petunia axillaris in vitro* pollination on excised ovules or a group of ovules attached to a piece of placenta did not result in seed set. The pollen grains germinated normally, but it failed to enter the ovules. Normal seed development resulted when pollination was done on intact placenta with undisturbed ovules (Rangaswamy and Shivanna, 1971a).

In *Petunia hybrida* complete removal of the style had a deleterious effect on seed set following placental pollination. Consequently the entire pistil with exposed ovules by peeling the ovary wall alone was used for *in vitro* pollination(Wagner and Hess, 1973).

In maize the ovaries attached to the cob tissue produced favourable development than single ovaries (Sladky and Havel, 1976; Gengenbach, 1977a; Dhaliwal and King, 1978).

In vitro pollination of unfertilized ovules excised from pollinated pistils with its own pollen or that of *Malus* sp. resulted in good seed set than the ovules from unpollinated pistils (Balatkova *et al.*, 1977a).

2.12.3.4 The culture medium

An appropriate culture media combination is necessary for the successful development of *in vitro* fertilized ovule into mature seed.

In the initial years of *in vitro* pollination and fertilization technique, the Nitsch medium (1951) identified for ovary development was used (Kanta and Maheswari, 1963; Usha, 1965; Rangaswamy and Shivanna, 1967 and Jarzina and Zenkteler, 1983).

In maize Gengenbach and Green (1975) reported modified LS medium to support *in vitro* seed development. Later found that MS medium is also suitable for the development of maize karyopsis (Dhaliwal and King, 1978; Bajaj, 1979 and Havel and Novak, 1981). MS medium was also suitable for the production of interspecific hybrids of *Gossypium, Nicotiana* and lilium following the *in vitro* pollination (Refaat *et al.*, 1984; Slusarkiewicz, 1984; Zhou *et al.*, 1991 and Tuyl *et al.*, 1991). It was suitable for plum also (Lech *et al.*, 1994).

2.12.3.5 Sucrose concentrations

The sucrose concentration influences the development of ovary, ovule and germination of seeds after *in vitro* pollination.

Generally sucrose has been used at a concentration of 4-5 per cent (Kanta and Maheswari, 1963; Usha, 1965 and Rangaswamy and Shivanna, 1967).

In maize a high concentration of 15-17 per cent has given successful results (Sladky and Havel, 1976; Gengenbach, 1977a). But Dhaliwal and King (1978) could obtain viable seeds even with 5 per cent sucrose. Bajaj (1979) found that 7 per cent sucrose is optimum for maize.

In the production of interspecific hybrids of lilium by *in vitro* pollination it was found that the sucrose concentration affect the capsule development. The

swelling of the capsule was highest at a sucrose concentration of 10 per cent but embryos were mostly found in ovaries cultured at 6-8 per cent sucrose. Best results were obtained with MS medium supplemented with 7 per cent sucrose (Tuyl *et al.*, 1991).

Lech *et al.* (1994) observed that in plum, 2 per cent sucrose along with other supplements can support embryo development consequent to *in vitro* fertilization.

2.12.3.6 Effect of growth regulators and supplements

Tuyl *et al.* (1991) reported that in allium, *auxin* is essential at the initial stages of ovule development.

In plum, GA_3 0.5 ppm promoted the development of zygote after *in vitro* pollination (Lech *et al.*, 1994).

Casein hydolysate at 500 ppm was found to enhance ovule development (Kanta and Maheswari, 1963; Wagner and Hess, 1974 and Zubkova and Sladky, 1975).

Usha (1965) could obtain seed set in *Antirrhinum majus* through *in vitro* pollination, on addition of 25 per cent coconut water in Nitsch medium.

Balatkova *et al.* (1977a) observed the influence of growth regulators like IAA, kinetin, tomato juice, coconut milk and yeast extract on seed development in tobacco after placental pollination. Coconut milk, tomato juice and yeast extract inhibited the seed development while the growth regulators like IAA 10 ppm or kinetin 0.1 ppm improved the number of seeds per ovary. Kinetin at a higher concentration (1 ppm) had an inhibitory effect.

2.12.3.7 Culture conditions

Cultures were usually stored in darkness or near darkness (Rangaswamy and Shivanna, 1967; Balatkova *et al.*, 1977a and Dhaliwal and King, 1978).

In petunia diffusa daylight (10-12 ft candle at 250±2°C) is optimum for *in vitro* seed development (Rangaswamy and Shivanna, 1967). The result of *in vitro* pollination was same whether the cultures were incubated in light or dark (Zenkteler, 1969).

Lech *et al.* (1994) reported that embryo development in plum after *in vitro* pollination was not influenced by light conditions.

2.12.4 *In vitro* pollination in ginger

Seed set and seed germination in ginger through *in vitro* pollination and fertilization has been reported for the first time by Valsala (1994), Valsala and Nair (1996) and Valsala *et al.* (1996). The protocol standardized could be summarized as given below.

Flower buds on the day of anthesis and one day after anthesis were suitable for *in vitro* pollination.

Flower buds had to be surface sterilized before anthesis i.e., 2.30 pm. Surface sterilization was done by dipping the flower buds in 500 mg l^{-1} for one hour followed by wipping with 70 per cent alcohol and rinsing with mercuric chloride 0.1 per cent for 3 minutes.

In vitro pollination could be done by 5.30 pm as by this time pollen grains from anthers could be easily scooped out for pollination. Placental, modified placental and ovular pollination along with pollen germination medium (modified

ME_3 medium) brought out fertilization. Placental pollination was the best as it registered maximum number of seeds per culture.

Seed set and seed development could be obtained in the medium of ½ MS + 6% sucrose + NAA 0.5 mg l^{-1} + BAP 2.5 mg l^{-1} + 15% v/v coconut water. The effect of coconut water could be replaced by casein hydrolysate.

The *in vitro* produced seeds of ginger germinated, when 80 days old seeds were incubated initially in the medium of half MS with 2,4-D 8 ppm for two months and then in hormone combination of BAP 9 mg l^{-1} and 2,4-D 0.2 mg l^{-1}.

Nazeem *et al.* (1996) have reported the protocol for rapid multiplication of *in vitro* germinated ginger seedlings.

2.12.5 Germination of *in vitro* produced seeds

The *in vitro* raised seeds may often require special pre-treatments, media and culture conditions for its germination.

In vitro seeds of *Papaver rhoeas* soaked in tap water for 24 h germinated in modified Whites (1943) medium (Kanta, 1960).

Seeds of *Papaver somniferum* did not germinate when they attached to the placenta. If separated from the placenta, they germinated within 15 days after planting in new medium. Even though the endosperm development was not normal, *Argemone mexicana* germinated within three months after starting the culture. The seeds of *Nicotiana rustica* and *N. tabacum* germinated *in situ* as well as in basal medium (Kanta *et al.*, 1962).

The seeds produced by *in vitro* pollination in *Antirrhinum majus* germinated in basal medium (Nitsch, 1951) with 4 per cent sucrose (Usha, 1965).

In vitro produced seeds of *Petunia* were germinated in water soaked filter paper and in the same nutrient agar medium on which it was developed. The germination was more in water soaked filter paper (Rangaswamy and Shivanna, 1967).

The time taken by *in vitro* produced seeds of *Nicotiana tabacum* for germination which sown *in vitro* was found to vary. Sometimes the seeds dried out and passed to a dormant state (Balatkova *et al.*, 1976)

The karyopsis of maize produced under *in vitro* condition were dried at room temperature for several days and then made to germinate on moist filter paper at 28°C. They germinated within 7 days after incubation (Gengenbach, 1977a).

2.13 Micropropagation

Micropropagation of *Curcuma* spp. was first reported by Nadgauda *et al.* (1978). He got success with young sprouting buds in Murashige and Skoog medium supplemented with coconut milk 10.0 per cent, kinetin 0.1 ppm and BAP 0.2 ppm. Smith's medium with above supplements was also suitable. Rooting was obtained in White's liquid medium supplemented with 2 per cent sucrose.

Kuruvinashetty *et al.* (1982) reported successful micropropagation in modified Murashige and Skoog's medium containing sucrose 4 per cent and kinetin 0.2 to 0.5 ppm. Kuruvinashetty and Iyer (1982) was able to get a multiplication rate of eight plantlets from every bud cultured for two months which works out to over two lakh plantlets per bud per year. The medium used was MS supplemented with 0.2 ppm kinetin and 0.4 ppm BAP.

Winnaar and Winnaar (1989) cultured emerging buds of 5-10 mm long, trimmed to contain the meristem with a few leaf primordia in a nutrient medium containing BAP 1 ppm and sucrose 2 per cent for one month. Then they were

subcultured to fresh medium of the same composition. Shoot growth as well as multiple shoots and root formation occurred after 3-4 weeks.

Young sprouting buds of turmeric cultivars Co-1 and BSR-1 were cultured on MS medium supplemented with kinetin 1 ppm, BAP 1 ppm and 40 per cent sucrose. Multiple shoots were obtained on an average 2.11 and 2.50 respectively in BSR-1 and Co-1 (Keshavachandran and Khader, 1989).

In vitro clonal multiplication of turmeric was reported by Balachandran *et al.* (1990). Rhizome buds excised from *C. domestica*, *C. aeruginosa* and *C. caesia* were inoculated on MS medium with different combinations of benzyladinine and kinetin. For shoot multiplication 3 ppm BAP was found to be optimum for all the species.

Vidya *et al.* (1989) conducted micropropagation studies in turmeric and enhanced release of axillary buds were recorded, when inoculated on a medium containing cytokinins. A multiplication ratio of 1:2240 was obtained from single rhizome in a year as against 5-6 plants under normal field conditions.

Rajan (1995) reported micropropagation of turmeric by *in vitro* microrhizomes. MS medium supplemented with 0.3 ppm BAP, 0.1 ppm NAA, 0.5 ppm ancymidol and 10 per cent sucrose was best for induction of microrhizomes.

Sit and Tiwari (1996) reported that best sprouting in turmeric obtained through axillary bud culture in half MS media supplemented with BAP and kinetin (both at 1 ppm).

2.13.1 Callus mediated organogenesis

Kuruvinashetty and Iyer (1982) reported initiation of callus cultures, when buds were grown on MS medium supplemented with 2 ppm IAA or 0.5 ppm

2,4-D in dark. These calli were soft and friable. When exposed to light, IAA induced calli underwent differentiation to produce several plantlets.

Rejuvenation of plantlets from the calli separated from the base of the shoot bud explant, were obtained when the separated calli inoculated on MS medium supplemented with kinetin 0.2 ppm, 0.4 ppm BAP, 0.01 ppm GA_3 and sucrose 4 per cent (Kurvinashetty *et al.*, 1982).

Nadgauda *et al.* (1980) reported that, on repeated subculture of shoots, a swelling was observed at the base of the leaves. These swelling when carefully removed and cultured on MS medium supplemented with 0.1 ppm kinetin, 0.2 ppm BAP and 10 per cent coconut water, formed a callus under dark incubation. This callus readily differentiated into shoots, which incubated in light and could be maintained indefinitely by monthly sub culture.

Yasuda *et al.* (1988) reported that callus was successfully induced from rhizome tissues of *C. zeodoria, C. domestica* and *C. aromatica* on MS medium supplemented with 1 ppm NAA and 0.1 ppm kinetin.

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigations on "Response of turmeric (*Curcuma domestica* Val.) to *in vivo* and *in vitro* pollination" were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during May, 1997 to May, 1999. The details regarding the experimental materials and methodology adopted for conducting various aspects of the study are presented in this chapter.

3.1 Plant materials

Eight cultivars of turmeric viz., VK 70, VK 55, VK 76, Suguna, Suvarna, Sudharsana, Kanthi and Sobha having variation in the crop duration, curcumin content and curing percentage were selected for the study. The cultivars represent different pedigree and geographic region (Table 1). The cultivars were examined for the variation in salient morphological characters, curcumin content and curing percentage. Observation were recorded on twelve plants of each cultivar six months after planting.

Height of the plant was measured from the ground level to the top of the longest leaf. Observations were also taken with respect to the number of tillers/plants, number of leaves/plant, number of leaves/tiller and length of petiole in cm. The breadth of the leaf was measured at the middle portion of the leaf, at the middle region of the plant.

The root and rhizome characters like number of roots, length of roots, number of mother rhizome, girth of mother rhizome, number of nodes, length of internodes, number of primary, secondary and tertiary fingers, length of primary, secondary and tertiary fingers were also observed in each cultivar. Girth of mother rhizome was taken from the mid portion of the mother rhizome. Number of nodes were counted on mother rhizome and length of internode was measured from the middle of mother rhizome.

The curcumin content in each cultivar was quantitatively extracted by refluxing the material in alcohol and is estimated spectrometrically at 425 nm (Sadasivan and Manikam, 1991).

Suvarna was not included for *in vivo* and *in vitro* pollination studies since it was not flowered during the course of investigations.

Table 1. Source of different cultivars of turmeric

Sl.No.	Cultivar	Pedigree	Source
1	VK-70	Dindrigam D-182	Andhra Pradesh
2	VK-76	Dindrigam CL No.625	Andhra Pradesh
3	VK-55	Amalapuram Cl 320	Andhra Pradesh
4	Suguna	PCT-13	Andhra Pradesh
5	Suvarna	PCT-8	Assam
6	Sudharsana	PCT-14	Manipur
7	Kanthi	Single plant selection from Mydukur	Andhra Pradesh
8	Sobha	Single plant selection from Methala Local	Kerala

3.2 Management of turmeric in the field

Two crops were raised for the study, the first crop was planted in the last week of May, 1997 and the second one was planted in the first week of May, 1998. The cropping field was prepared and beds of 2 x 1 m size were taken at 50 cm spacing. The selected cultivars were planted at random. Seed bits of 15-20 g weight were used for planting. The cultural, manurial and other plant protection operations were adopted according to package of practices recommendations (KAU, 1996). The crop was given a streptocyclin spray of 500 mg l^{-1} at the time of flowering for controlling bacterial infection.

3.3 Floral biology and morphology

3.3.1 Flowering season

The flowering season of the selected turmeric cultivars were examined.

3.3.2 Inflorescence development and floral biology

The infloresence development of turmeric was studied by observing the development of first inflorescence of the seven cultivars viz., VK 70, VK 55, VK 76, Suguna, Sudharsana, Kanthi and Sobha. The stage at which the inflorescence primordium was 4 to 5 cm long was taken as the date of visual initiation of flowering. The duration from initiation of inflorescence to blooming and the blooming period of an inflorescence were recorded by daily monitoring flower anthesis. The time of anthesis and anther dehiscence in various cultivars were also observed.

3.3.3 Morphology of flowers

The floral characters of different cultivars were examined. The length of the style was measured in cm. The length and diameter of the ovary were expressed in mm with the help of a vernier caliper. The number of ovules per ovary in each cultivar was also counted. The length and breadth of the ovules in various cultivars were also recorded in µm using a calibrated ocular micrometer.

3.4 Pollen studies

3.4.1 Pollen fertility and viability studies

Studies were carried out in order to determine the pollen fertility and viability of all the selected cultivars. Fertility and viability of the pollen grains of the flowers at lower, middle and upper portions of the inflorescences were also estimated. The observations recorded were statistically analysed for interpretation.

3.4.2 Estimation of pollen fertility

The pollen fertility was estimated using acetocarmine stain. Opened flowers on the day of anthesis were collected and used for the study. The pollen grains were stained and viewed at 40x magnification. All the pollen grains, that were well filled and stained were counted as fertile and others as sterile. The means of a sample from 10 microscopic field served as a replication. The fertility per cent was calculated using the formula

$$\frac{\text{Number of well stained pollen grains}}{\text{Total number of pollen grains in the field}} \times 100$$

In order to estimate the heterogenity in pollen size, the length and breadth of seven pollen grains in a microscopic field were recorded using a calibrated ocular micrometer and expressed in µm. Photomicrographs of pollen grains were taken showing fertile and sterile pollen grains of different size ranges.

3.4.3 Standardisation of medium for pollen germination and pollen tube growth

The *in vitro* pollen germination and tube growth of various turmeric cultivars viz., VK 70, VK 55, VK 76, Suguna, Sudharsana, Kanthi and Sobha were tried in the following media.

1) Brewbaker and Kwack's medium (1963)
2) Modified ME_3 medium with 12% PEG 4000 (Leduc *et al.*, 1990) at pH 4
3) ,, ,, at pH 5
4) ,, ,, at pH 6
5) ,, ,, at pH 8
6) Control (distilled water)

The composition of Brewbaker and Kwack medium and modified ME_3 medium are given in Table 2 and Table 3 respectively.

Table 2. Composition of Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963)

Constituents	Concentration (mg l^{-1})
Sucrose	1,00,000.00
$Ca(NO_3)_2.4H_2O$	300.00
$MgSO_4.7H_2O$	200.00
KNO_3	100.00

The different media were prepared by dissolving the required quantity of chemicals in distilled water. The prepared media were sterilized before use. The pH of the modified ME_3 medium was adjusted before sterilization.

The pollen grains were collected from the flower buds at the time of anthesis and were incubated in a desicator along with a drop of medium tested for germination. The desicator served as a moisture chamber. The observations were recorded 24 h after incubation. The number of germinated pollen grains and also the total number of pollen grains were counted on 10 microscopic fields and the mean germination per cent was calculated. Mean and maximum pollen tube growth attained also measured using a calibrated ocular micrometer. Photomicrographs of the germinated pollen grains were also taken.

3.5 Cytology

The turmeric cultivars viz. VK 70, Suvarna and Kanthi were subjected to cytological study and chromosome number was counted.

The rhizomes were germinated in sand filled plastic trays. The roots were collected when they attain 1-2 cm length at half an hour interval. They were washed

Table 3. Composition of modified ME_3 medium (Leduc *et al.*, 1990)

Constituents	Concentration (mg l^{-1})
Macronutrients	
$MgSO_4.7H_2O$	370.00
KNO_3	950.00
H_2PO_4K	85.00
$CaCl_2.2H_2O$	880.00
NH_4NO_3	412.50
KCl	175.00
Na_2EDTA	7.45
$FeSO_4.7H_2O$	5.55
Micronutrients	
H_3BO_3	50.00
$MnSO_4.H_2O$	16.80
$ZnSO_4.7H_2O$	10.50
KI	0.83
$Na_2MO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Vitamins	
Thiamine	1.00
Pyridoxine1.00	
PEG-4000	120,000

and pre-treated with colchicine 0.2 to 0.5 per cent for 1 h. They were again washed with distilled water and fixed in a fixative containing three parts of ethyl alcohol and one part glacial acetic acid. The glacial acetic acid had saturated with ferric chloride. The root tips were fixed for one hour and then stored in 70 per cent alcohol. The meristematic tips were squashed in one per cent acetocarmine. The slides were sealed with nailpolish and observed under a microscope to study the mitotic stages. Chromosome counting was done from temporary slides thus prepared. Photomicrographs were taken in Leitz Biomed Research Microscope at 1000 x.

3.6 Natural seed set

The cultivars were observed for natural seed set. The mode of natural pollination was also examined. The time taken by the different cultivars to fruit set and maturity was also recorded.

3.7 Controlled *In vivo* pollination

Controlled *in vivo* stigmatic and stylar pollination were carried out among the selected turmeric cultivars in cross combinations.

The stigmatic pollination was done as per the method described by Nazeem and Menon (1994). Emasculation was done one day prior to anthesis, in the evening, from 4 to 6 pm using a needle and forceps. The rest of the floral buds including developing ones present in the same bract were pulled out and removed. The inflorescence was bagged with butter paper cover. Next day morning from 6 to 8 am pollen from the opened flowers of the desired male parent was collected using a needle and applied to the receptive stigma of the emasculated female parent. The pollinated flower was labelled and tagged. The whole inflorescence was bagged after pollination.

In vivo stylar pollination was done by cutting the styles at different length and by applying pollen grains along with pollen germinating medium at the cut end. *In vivo* produced turmeric fruit and seed were described.

3.7.1 Pollen pistil interaction after *in vivo* pollination

Pollen germination and tube growth through stigmatic and stylar tissues, after *in vivo* pollination were studied using fluorescence technique proposed by Kho and Baer (1968) and Kho *et al.* (1980). The pollinated flowers were fixed in FAA mixture (Formalin 10 ml, Acetic acid 10 ml and Ethyl Alcohol 80 ml) at 24 h after pollination. After 24 h of fixation, the materials were transferred into glass vials containing 1N NaOH for 8 h at room temperature in order to soften the tissue. The softened material was washed thoroughly with distilled water, then transferred to another glass vial containing 0.1 per cent Anilin Blue in 0.1N K_2HPO_4 for 18 h. After staining, the gynoecium with style and stigma was mounted on a microscopic slide and viewed through fluorescence microscopy and photomicrographs were taken.

3.8 *In vitro* pollination

3.8.1 collection of explant

Flower buds prior to the day of anthesis, on the day of anthesis and opened flowers were collected for *in vitro* pollination The flower buds and flowers were scooped out carefully using a forceps and needle without injuring the ovary. The calyx surrounding the base of the corolla tube was removed.

3.8.2 Surface sterilization

Surface sterilization was carried out under perfect aseptic conditions in order to exclude the micro-organisms present in the explant. The washed explants were put into the sterilant viz. Streptocycline 350 ppm and kept immersed for 1 hr and were continuously agitated manually. Explants were then surface sterilized in

0.1 per cent $HgCl_2$ for 3 minutes. They were then rinsed with sterile distilled water thrice to remove traces of the sterilant.

3.8.3 Culture establishment

A preliminary study was undertaken using two basal media combinations i.e., MS (Murashige and Skoog, 1962) and SH (Schenk and Hildebrandt, 1972). The composition of the media are given in Table 5. The based media supplemented with BAP 1 mg l^{-1} and kinetin 1 mg l^{-1} served as the treatments. The pollinated ovaries with intra ovarian pollination techniques were cultured and swelling of the ovary was scored 20 DAP. The cultures were also observed for microbial interference.

3.8.3.1 Management of bacterial interference in the cultures

In order to identify the antibiotic, most suitable for the control of bacterial occurrence in the culture, a sensitivity test was done. The antibiotics concentrations tested as treatments are given in Table 4.

3.8.4 Culture medium

Murashige and Skoog's (MS) medium (1962) and Schenk and Hilderbrandt (SH) medium (1972) were used as basal media in the present study. The composition of these media are given in the Table 5. The basal media were supplemented with different levels of auxins, cytokinins, casein hydrolysate, coconut water etc. as different treatments.

3.8.4.1 Preparation of media

The chemicals used for preparing various media were analytical grade obtained from British Drug House (BDH), Sisco Research Laboratories (SRL), Merck or Sigma. Standard procedures were adopted (Gamborg and Shyluk, 1981) for the preparation of media. The pH of the medium was adjusted to 5.7. Semi solid

Table 4. *In vitro* sensitivity of bacterial population to antibiotics in culture establishment

Antibiotics	Concentration (μl)
Gentamycin	50
,,	100
Kanamycin	50
,,	100
Ampicillin	50
,,	100
Ambystrin S	50
,,	100
Dicrystacin	100
,,	100
,,	150
,,	300
,,	500
Control (YEM)	

Table 5. Composition of various culture media used for culture estabilishment

Constraints	Quantity (mg l^{-1})	
	MS	SH
Macronutrients		
KNO_3	1900.0	2500.0
NH_4NO_3	1650.0	-
$(NH_4)_2\ SO_4$	-	-
KH_2PO_4	170.0	1250.0
$NaH_2PO_4.H_2O$	-	-
$NH_4.H_2PO_4$	-	300.0
$MgSO_4.7H_2O$	370.0	400.0
$CaCl_2.2H_2O$	440.0	200.0
$Ca(NO_3)_2.4H_2O$	440.0	200.0
Micronutrients		
H_3BO_3	6.200	5.0
$MnSO_4.H_2O$	-	10.0
$MnSO_4.4H_2O$	22.300	-
$ZnSO_4.7H_2O$	8.600	1.0
$Na_2MO_4.2H_2O$	0.250	0.10
$CaSO_4.5H_2O$	0.025	0.20
$CoCl_2.6H_2O$	0.025	0.10
KI	0.830	15.00
Na_2EDTA	33.600	20.00
Vitamins		
Thiamine, HCl	0.10	5.00
Pyridoxine, HCl	0.50	0.50
Nicotinic acid	0.05	5.00
Niacin	-	-
Others		
Glycine	2.00	-
Myo-inositol	100.00	1000.00
Sucrose	30000.00	30000.00
pH	5.8	5.8

media were prepared by adding good quality agar (0.75 per cent). Sterilization of medium was done by subjecting the media to a temperature of 121°C at a pressure of 1.06 kg cm² for 20 minutes (Dodds and Roberts, 1981). After sterilization the media were stored in air conditioned culture room.

3.8.5 Standardisation of media

3.8.5.1 Cytokinins/auxin

The effect of cytokinin and auxin alone and in combination were studied.

3.8.5.1.1 Influence of cytokinin

The basal media ½ MS was supplemented with BAP and kinetin at various concentrations (Table 6).

Table 6. Cytokinin concentrations tried in the ovule development

Basal Medium	Treatments	
	Cytokinin (mg l^{-1})	
	BAP	Kinetin
½ MS	1	-
,,	4	-
,,	-	1
,,	-	3
,,	-	4
,,	1	1
,,	1	3

3.8.5.1.2 Influence of auxins

The treatment details are given in Table 7.

Table 7. Auxin concentrations tried in the ovule development

Basal media	Treatments	
	Auxins	Combinations
½ MS	NAA	0.5 mg l^{-1} and 1 mg l^{-1}
	IAA	0.2 mg l^{-1}
	IBA	0.2 ,,
	2,4-D	0.2 ,, and 1 mg l^{-1}

sucrose 3%

3.8.5.1.3 Cytokinin and auxin combinations

The treatment details were described in Table 8.

Table 8. Cytokinin and auxin combinations tried in the ovule development

Basal Media	Treatments		
	Cytokinins (mg l^{-1})		Auxins (mg l^{-1})
	BAP	Kinetin	
½ MS	1	3	IAA (0.2), NAA (0.05), IBA (0.2), 2,4-D (0.2)
	1	1	NAA (0.05)
	4	-	2,4-D (0.2), IAA (0.2)
	-	4	IAA (0.2), NAA (0.5, 0.2), IBA (0.2) 2,4-D (0.2)

Sucrose 3%

3.8.5.2 Influence of carbon source

The influence of different levels of sucrose in 3.0 and 6.0 per cent in ovary and ovule development was studied. The medium of ½ MS + BAP 1 mg l^{-1}, kinetin 1 mg l^{-1} and Naa 0.5 mg l^{-1} was used for supplementing with different concentration of sucrose.

3.8.5.3 Effect of organic media supplements tried in the ovule development

The influence of organic supplements such as CW, CH in the development of ovule was studied. The treatment details are given in Table 9.

Table 9. Organic media supplements tried in the ovule development

Supplements	Concentration
C.W	15 per cent v/v
C.H	200 mg l^{-1}

The basal media used were ½ MS with BAP 1 mg l^{-1}, kinetin 1 mg l^{-1} and NAA (0.5 mg l^{-1} and 1 mg l^{-1}) and MS with BAP (2.5 mg l^{-1} and 1 mg l^{-1}) and NAA (0.5 mg l^{-1}). Placental, modified as well as intra ovarian pollination were followed in these treatments.

3.8.6 Standardisation of *in vitro* pollination techniques

Different *in vitro* pollination techniques as per Bhojwani and Razdan (1983) were tried for standardizing an appropriate *in vitro* pollination technique for turmeric breeding. Flowers and flower buds collected on the day of anthesis were surface sterilized. The pollen grains were scooped out from the anthers using a needle and applied on the specific portion of the gynoecium along with a drop of pollen germination medium. The ovary/ovule after pollination were placed in the culture medium and observations were recorded at ten days interval for swelling. The different pollination techniques tried are described below:

3.8.6.1 Stigmatic pollination

In this method the pollen grains were applied on the stigma, along with pollen germination medium after the partial removal of floral appendages like corolla and anther.

3.8.6.2 Stylar pollination

The style was cut at various levels and pollen grains were deposited on the style along with pollen germination medium.

3.8.6.3 Intra-ovarian pollination

Ovary was cut just below the junction of the style and ovary and pollination was done on the opened surface of the ovary.

3.8.6.4 Placental pollination

Placenta with the ovules were exposed by completely peeling the ovary wall and pollination was done.

3.8.6.5 Modified placental pollination

A portion of ovary wall is retained and pollination was done on exposed ovules.

3.8.6.6 Test tube/ovular fertilization

The ovules were separated and the pollen grains were applied on individual ovules.

3.8.7 Post pollination changes

Observations were recorded at ten days interval for the ovary and ovule changes in the media combination.

3.9 Seed germination studies

3.9.1 *In vivo* studies

Seeds obtained through *in vivo* pollination were kept in moist sterile sand for germination.

3.9.1.1 Pre-sowing treatments on germination of turmeric seeds

Seeds were subjected to various pre-sowing treatments like soaking seeds in water for 24 h and acid scarification (25% HNO_3 for 10 min) followed by water soaking for 24 h.

3.9.2 *In vitro* studies

Seeds obtained were subjected to various treatments like keeping on moist filter paper, moist sand, moist sand with vermiculite (1:1) and basal medium for germination.

The seeds obtained were incubated in various media combinations for germination. The combination of growth regulators tested are furnished in Table 10.

Table 10. Cytokinin and auxin combinations tried in the *in vitro* seed germination

Basal media	Treatments		
	Cytokinin (mg l^{-1})		Auxin (mg l^{-1})
	BAP	Kinetin	
½ MS + 3% sucrose	2	2	0.5
,,	4	4	0.5
½ MS + 0.6% sucrose	1	1	0.5
,,	Nil	Nil	Nil

3.10 Statistical analysis

The data generated from the various experiments were subjected to statistical analysis in completely randomised design wherever possible as prescribed by Panse and Sukhatme (1985).

RESULTS

RESULTS

The results of the experiments on "Response of turmeric *Curcuma domestica* Val. to *in vivo* and *in vitro* pollination" are described in this chapter.

4.1 Variability in plant materials

The plant materials selected for the study were VK 70, VK 55, VK 76, Suguna, Suvarna, Sudharsana (short duration cultivars), Kanthi and Sobha (medium duration cultivars) (Plates 1a to h).

4.1.1 Pseudostem characters

The selected cultivars differed significantly with respect to height, number of tillers/plant, number of leaves/tiller/plant, number of leaves/plant, length of leaf and leaf area (Table 11). With regard to height, the cultivar Suvarna has recorded the maximum value of 165.5 cm and VK 70 the minimum of 114.4 cm. Maximum number of tillers was observed in Kanthi (4.4) and minimum in Suguna (1.9). Suvarna has recorded maximum number of leaves/tiller/plant (6.4) and the minimum was observed in Kanthi (4.4). Maximum number of leaves/plant was noticed in VK 55 (19.3) and the minimum in Suguna (9.9). The cultivars Sobha and Kanthi had the longer petiole (37.3 cm) and VK 55 showed the shortest (26.1 cm). Maximum length for leaves was observed in Suvarna (74.4 cm) and the minimum in VK 55 (51.5 cm). Kanthi has recorded maximum breadth (16.2 cm) for leaves and the minimum in VK 76 (12.7 cm).

4.1.2 Rhizome characters

4.1.2.1 Mother rhizome characters

There was no significant difference among cultivars regarding the number of mother rhizome, but has shown significant variation with respect to length and girth of mother rhizome (Plate 1a to 1h). Sobha has recorded maximum

Plate 1a, b, c, d, e, f	Short duration cultivars – VK 70, VK 55, VK 76, Suguna, Suvarna and Sudharsana

Vk-70

Vk-55

Plate 1c VK 76

Suvarna

Sudharsana

Plate 1g, h   Medium duration cultivars – Kanthi and Sobha

Kanthi

Sobha

Table 11. Variability in pseudostem characters of turmeric cultivars

Sl. No.	Cultivars	Height of the plant at 6 months after planting (cm)	No. of tillers/plant	No. of leaves/plant	No. of leaves/ tiller/plant	Length of petiole (cm)	Length of leaf (cm)	Breadth of leaf (cm)	Area of leaf (cm²)
1	VK 70	114.400	3.200	18.900	6.192	24.800	54.500	12.730	700.810
2	VK 55	127.000	3.900	19.300	5.309	26.100	51.500	12.850	667.150
3	VK 76	124.800	3.400	18.900	5.613	27.800	57.900	12.700	738.150
4	Sudharsana	139.100	3.500	18.800	5.443	32.100	61.700	13.550	847.350
5	Suvarna	165.500	2.900	18.300	6.433	33.100	74.400	14.450	1081.400
6	Suguna	129.780	1.900	9.900	5.608	34.250	35.860	12.740	716.000
7	Sobha	146.970	3.600	17.900	5.168	37.300	64.550	13.980	914.100
8	Kanthi	148.750	4.400	19.200	4.398	37.300	60.400	16.170	982.455
	Mean	137.04	3.35	17.65	5.52	31.59	60.10	13.65	830.93
	CD at 5%	12.14	0.97	4.82	1.053	4.62	6.015	1.913	175.098

Table 12. Variability in rhizome and root characters of turmeric cultivars

Sl. No.	Cultivars	No. of roots	Length of roots (cm)	No. of mother rhizome	Length of mother rhizome (cm)	Girth of mother rhizome (cm)	No. of nodes	Length of internodes (cm)	No. of primary fingers	No. of second-ary fingers	No. of tertiary fingers	Length of primary fingers (cm)	Length of second-ary fingers (cm)	Length of tertiary fingers (cm)
1	VK 70	20.714	21.857	1.571	9.000	7.857	10.857	0.643	4.429	11.429	7.857	12.000	5.857	2.714
2	VK 55	20.857	14.000	1.571	6.000	5.000	7.857	0.986	5.571	12.429	4.571	9.429	5.814	2.643
3	Sobha	44.000	18.417	2.000	10.033	6.700	13.667	1.033	6.833	20.000	10.000	10.550	6.417	2.217
4	Sudharsana	21.857	19.500	1.857	6.571	6.500	12.000	1.457	5.571	12.429	10.286	12.286	7.786	2.714
5	Suvarna	22.500	17.875	1.625	7.650	6.675	13.000	1.275	7.000	16.625	12.625	13.188	7.837	2.125
6	Kanthi	46.000	20.500	2.571	9.500	8.429	12.000	0.814	8.571	18.000	13.571	9.714	5.500	2.500
7	VK 76	28.875	27.813	1.625	7.563	6.375	12.500	1.250	5.625	17.875	13.750	12.250	7.500	2.938
8	Suguna	22.286	17.143	1.571	6.743	5.429	10.429	1.357	6.286	14.714	9.571	11.643	7.357	2.357
Mean		28.386	19.638	1.798	7.882	6.620	11.538	1.018	6.235	15.437	82.231	11.382	6.758	2.526
SEm±		4.24	1.78	0.33	0.74	0.48	0.70	0.12	0.82	2.06	1.80	0.67	0.57	0.33

length for mother rhizome (10.0 cm) and VK 55 minimum (5.0 cm). The cultivar Kanthi has maximum girth (9.5 cm) while VK 55 minimum (5.0 cm). Significant difference existed with regard to number of nodes and length of internodes. Maximum number of nodes was observed in Sobha (13.6) while minimum in VK 76 (7.8). The internodal length was maximum in Sudharsana (1.5 cm) while minimum in VK 70 (0.6 cm) (Table 12).

4.1.2.2 Finger characters

There was no significant difference among cultivars with respect to number of primary and secondary fingers (Plate 1a to 1h). But there was significant difference with respect to tertiary fingers. The cultivar VK 76 recorded the maximum number of tertiary fingers (13.7) and minimum number was observed in VK 55 (4.6). But there was no significant difference with regard to length of tertiary fingers. The variation was significant with regard to the length of primary and secondary fingers. Suvarna recorded the maximum length for primary fingers (13.2 cm) and secondary fingers (7.8 cm). Minimum length for primary fingers was observed in VK 55 (9.43 cm). Kanthi recorded minimum secondary finger length (5.5 cm) (Table 12).

4.1.3 Variability in quality characters

The curcumin content in the selected cultivars ranged from 2.13 in VK 70 to 7.43 in Sobha. Curcumin content in Kanthi and VK 76 were 7.02 and 6.38 respectively, while all others recorded less than 5.13 percentage. Curing percentage ranged from 9.5 in VK 55 to 21.5 in VK 70. The cultivars VK 76 and Suvarna also recorded high curing percentage of 20 and 19.5 respectively. In Kanthi and Sobha it ranged from 14 to 15 per cent (Table 13).

Table 13. Variability in curcumin content and curing percentage of turmeric cultivars

Cultivars	Curcumin (%)	Curing (%)
VK 70	2.13	21.5
VK 55	5.13	9.5
VK 76	6.38	20.0
Suguna	4.80	12.0
Suvarna	4.47	19.5
Sudharsana	5.23	11.5
Kanthi	7.02	15.0
Sobha	7.43	14.0
Mean	5.32	15.38

4.2 Study of floral biology and floral morphology

4.2.1 Flowering season

The flowering season ranged from July to October. Turmeric generally flowers within a period of 109 to 155 days. VK 70 was the earliest flowering cultivar (109 days). Kanthi and Sobha were late flowering types (155 days and 153 days) respectively (Plate 2). The cultivars VK - 55 came to flowering in 125 days, VK -76 (117 days), Suguna (141 days) and Sudharsana (148 days) (Table 14).

4.2.2 The inflorescence development and floral biology

The inflorescence primordium of 4 to 5 cm length developed into a blooming inflorescence within a period of 9.6 days (Table 14).

The inflorecence of turmeric is a symmetrical compound spike born terminally on leafyshoot and partially enclosed by leafsheath (Plate 2). The spike bears bracts with spiral arrangement and are dark green at base which are fertile ie. carry flowers while the bracts on the top are pale green and sterile ie. do not carry flowers (Plate 3a). Lower fertile bracts carry three flowers which open in succession. Flowering was noticed in all the cultivars except Suvarna.

Table 14. Floral biology and morphology of turmeric cultivars

Characters	VK 70	VK 55	VK 76	Kanthi	Sobha	Suguna	Sudharsana	Mean
	Cultivars							
1. Crop duration for the initiation of flowering (days)	109	127	117	155	153	141	148	135.7
2. Duration from initiation of inflorescence to blooming (days)	9.8	9.6	9.4	10.0	8.8	9.6	10.0	9.6
3. Blooming period of an inflorescence	8.8	9.8	9.2	10.8	10.2	9.4	9.8	9.71
4 Time of anthesis (am)	5.30-6	5.30-6	5-5.30	5-6	5-6	5.30-6	5-6	
5 Anther dehiscence (am)	7.15-7.45	7.15-7.45	7.15-7.45	7.15-7.45	7.15-7.45	7.-15-7.45	7.15-7.45	
6 Style length (cm)	4.164	4.24	4.228	4.596	4.726	4.658	4.376	4.426
7 Ovary length (mm)	2.64	2.54	2.40	2.42	2.80	2.50	2.66	2.565
8 Ovary diameter (mm)	2.32	2.28	2.38	2.48	2.34	2.46	2.52	2.397
9 No. of ovules per ovary	31.75	27.6	32.8	26.8	28.2	28.0	30.0	29.307
10. Length of ovule (μm)	641.043	601.391	581.565	476.791	664.1739	677.391	637.739	611.44
11. Breadth of ovule (μm)	449.39	409.739	446.086	393.217	462.608	485.739	409.739	436.645
12. Length of pollen grain (μm)	82.608	79.85	77.10	59.478	70.807	88.091	79.304	76.75
13. Breadth of pollen grain (μm)	56.1739	63.33	55.06	46.26	54.2857	63.866	62.782	57.39

Plate 2. Turmeric cultivar Kanthi on flowering

KANTHI

Plate 3a. Inflorescence of *Curcuma domestica* Val.

Plate 3b. Flower of *Curcuma domestica* Val.

- 171557 -

4.2.3 Morphology of Flowers

The flowers are zygomorphic, bisexual and epigynous (Plate 3b). Calyx is short and tubular, dividing above into three short teeth. Corolla is gamopetalous, tubular at base and separated into three whitish and translusent lobes.

Stamens which are epipetalous arouse from the throat of the corolla tube in two whorls of three each. The outer whorl of three stamens is represented by petaloid, trilobed labellum, the most conspicuous part of the flower. Of the inner whorl, the posterior stamen was fertile, and had a prominent two lobed anther having broad connectives projected beyond the anther. The other two were reduced to two staminodes and seen at the base of the flower. Honey secretion the present at the base of the staminodes.

The style passes through the groove formed by the anther lobes and ends in stigma. The flowers are characterized by a long style, recorded a mean length of 4.43 cm in cultivars. The ovary measurement of the cultivars had a mean length of 0.26 cm and diameter of 0.24 cm at the middle. The mean number of ovules noticed in an ovary was 29.31. Microscopic measurement of ovule with ocular micrometer showed a mean length of 611.44 μm and 436.65 μm at the middle (Table 14 and Plate 3c).

The blooming of flowers in a spike was completed within a period of 9.71 days and ranged from 8.8 (VK-70) to 10.8 (Kanthi) days.

Anthesis in different cultivars occurred between 5 am to 6 am. Anther dehiscence was not found to occur simultaneously with flower opening and was noticed between 7.15 and 7.45 am.

Plate 3c. Ovules of *Curcuma domestica* Val.

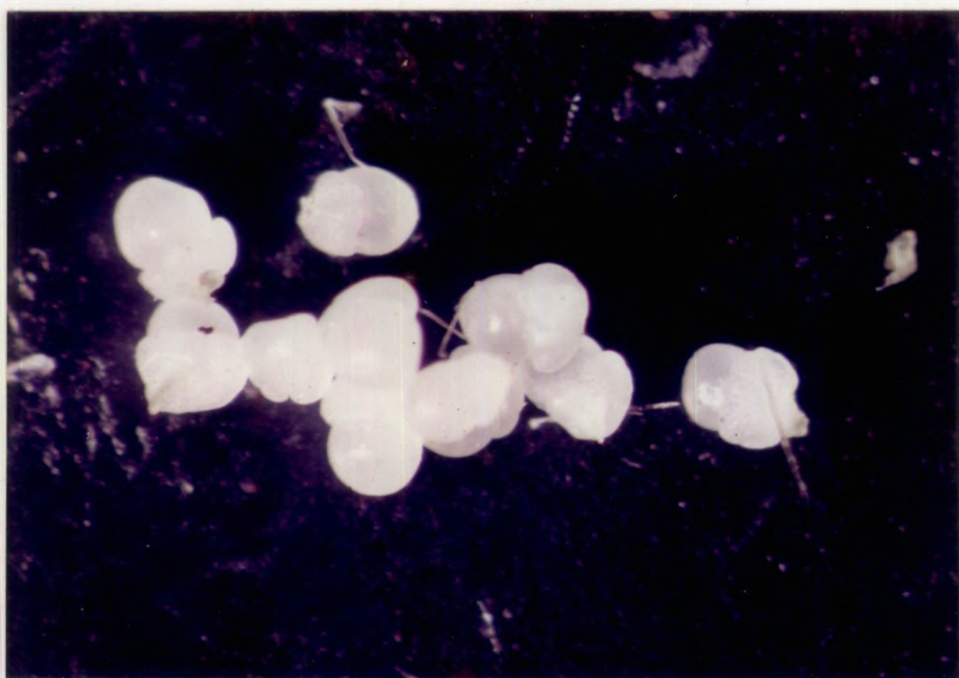
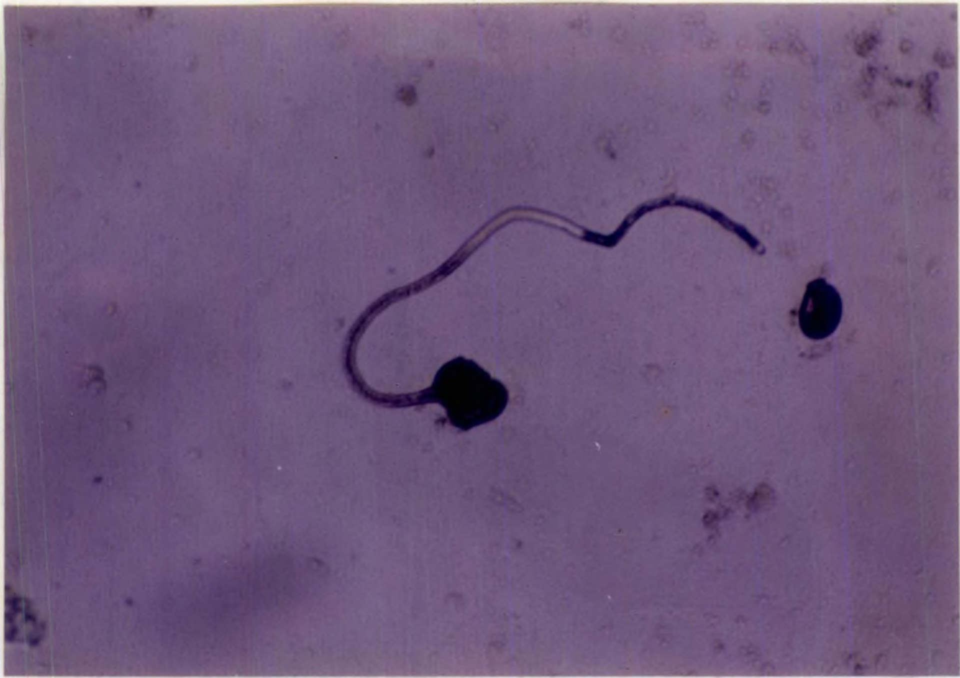
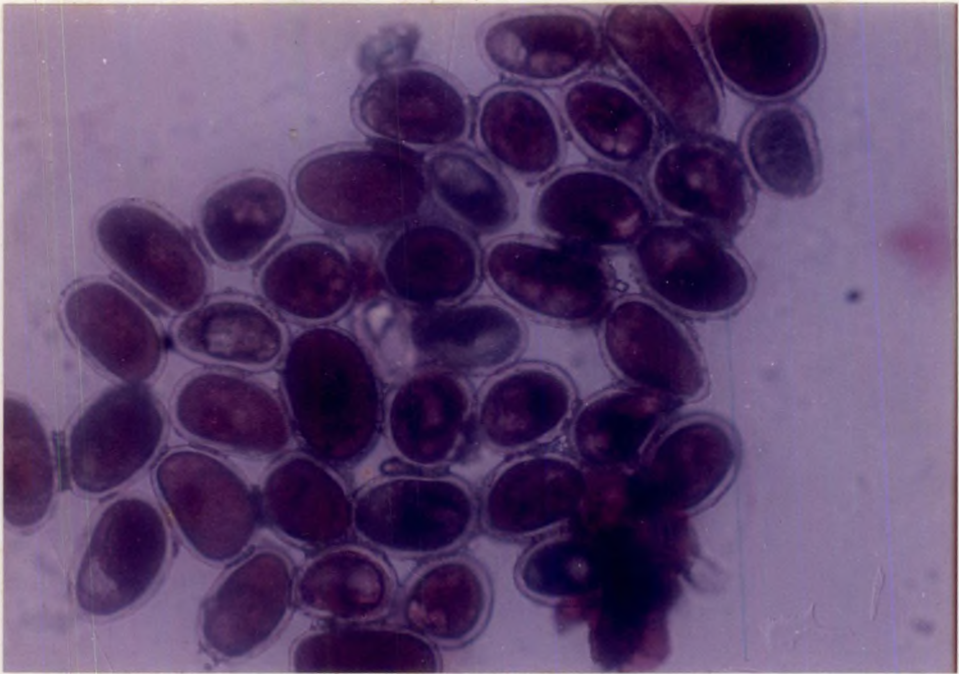


Plate 4. Pollen germination and tube growth in modified ME3 medium (x 200)

Plate 5. Pollen grains stained with acetocarmine (x 200)





4.3 Pollen studies

4.3.1 Pollen morphology

Turmeric flowers have enough of pollen grains present in anthers. The pollen grains were ovoid to spherical in shape, highly heterogenous in size and light yellow in colour.

Microscopic measurements of pollen grains with ocular micrometer recorded that pollen grains of different turmeric cultivars are highly heterogenous in size among cultivars with length ranging from 59.48 µm in Kanthi to 82.61 µm in VK 70 and breadth ranging from 54.29 µm in Sobha to 63.87 µm in Suguna (Table 14).

4.3.2 Standardisation of medium for pollen germination and pollen tube growth

The pollen grains incubated in different media were observed for germination after 24 h and the data are presented in Table 15. There was no pollen germination in distilled water and Brewbacker and Kwack's medium. Pollen grains germinated only in the ME_3 medium. ME_3 medium at different pH range viz. pH 4, 5, 6, 8 showed various levels of germination percentage. ME_3 medium of pH 6 recorded the highest germination percentage (32.55) while pH 4 recorded the lowest value (10.72).

The pollen grains germinated after 24 h were observed for pollen tube growth (Plate 4). Maximum pollen tube growth was observed in the cultivar Suguna (576.22 µm) and minimum in the cultivar Kanthi (218.72 µm). The pollen tube growth among short duration cultivars ranged from 268.48 µm to 576.30 µm. In medium duration cultivars it ranged from 218.72 µm to 245.47 µm. Pollen tube growth was higher in cultivars like VK 70 (576.30 µm), Suguna (576.22 µm) and VK 76 (432.85 µm).

Table 15. Pollen germination of turmeric cultivars in different media

Treatments	Pollen germination (%)							Mean
	VK 70	VK 55	VK 76	Suguna	Sudharsana	Kanthi	Sobha	
T_1 ME_3 medium at pH 4	13.39	8.20	15.32	17.84	8.12	5.97	6.13	10.72
T_2 ME_3 medium at pH 5	21.62	16.00	23.33	27.87	17.66	6.65	7.63	17.25
T_3 ME_3 medium at pH 6	53.2	22.74	37.86	61.66	28.44	10.33	13.62	32.55
T_4 ME_3 medium at pH 8	36.32	20.49	35.32	40.30	19.83	10.00	11.99	24.89
T_5 Brewbaker and Kwack medium	0	0	0	0	0	0	0	0
T_6 Distilled water	0	0	0	0	0	0	0	0

4.3.3 Pollen fertility and viability in turmeric cultivars

Pollen fertility was determined using the acetocarmine stain (Plate 5). The mean pollen fertility in the turmeric cultivars was 78.51 percentage. The pollen fertility among cultivars ranged from 67.07 percentage in Sobha to 86.79 percentage in Sudharsana. Statistical analysis showed that there was no significant difference among turmeric cultivars with respect to pollen fertility (Table 16).

The cultivars differed significantly in pollen germination. Maximum pollen germination was recorded in the cultivar Suguna (46.08) while Kanthi recorded the minimum (8.22). In short duration cultivars the germination percentage ranged from 23.75 to 46.08. The germination percentage was comparatively high in cultivars like Suguna (46.08), VK 70 (44.65) and VK 76 (35.77). The germination percentage in medium duration cultivars ranged from 8.22 (Kanthi) to 11.51 (Sobha) (Table 16).

4.3.4. Influence of position of flowers on pollen on fertility and viability

The pollen fertility of the flowers from different parts of the inflorescence i.e lower middle and upper region are presented in Table 16. Statistical analysis revealed that there exists significant difference in pollen fertility of flowers. The pollen fertility was high in the flowers at the lower portion of the inflorescence (90.32%) which progressively reduced in the flowers in the middle (82.38%) and upper portion (63.13%).

The outcome of the influence of position of flowers in the inflorescence on viability of pollen grains are presented in Table 16. The statistical analysis showed that germination percentage vary with respect to the position of flowers in the inflorescence. The flowers at the lower portion of the inflorescence recorded a germination percentage of 32.55 while it was only 26.57 at middle and 22.93 at the upper portion.

Table 16. Effect of position of flowers in the inflorescence on the fertility and viability of pollen grains and pollen tube length of turmeric cultivars

Cultivars	Position of flowers in the inflorescence								Maximum pollen tube length (μm)
	Lower		Middle		Upper		Mean		
	Fertility %	Viability%	Fertility %	Viability%	Fertility %	Viability%	Fertility %	Viability%	
VK 70	86.185	53.20	81.01	44.66	63.30	44.65	76.83	44.65	576.30
VK 55	90.63	22.74	80.21	22.02	74.34	19.70	81.73	21.49	262.77
VK 76	84.41	37.86	80.64	36.64	64.57	32.80	76.54	35.77	432.85
Suguna	93.71	61.66	85.40	41.17	76.428	35.41	84.51	46.08	576.22
Sudharsana	94.33	28.44	87.60	22.02	78.43	20.80	86.79	23.75	268.48
Kanthi	89.97	10.33	83.33	7.90	55.09	6.43	76.10	8.22	218.72
Sobha	93.00	13.62	78.46	11.60	29.75	9.30	67.07	11.51	245.47
Mean (%)	90.32	32.55	82.38	26.57	63.13	22.93	78.51	27.35	368.69
SEm±							9.52	3.77	

4.4 Cytology

The results of the investigation showed that the optimum time for collection of roots for mitotic studies in turmeric was between 6.30-7.00 am. The somatic chromosome number of cultivars VK 70 and Suvarna were found to 2n=84 (Plate 6a and 6b) and that of Kanthi as is 2n=63 (Plate 6c).

4.5 Natural seed set

Natural fruit set and seed set were observed in short duration cultivars viz. VK 70, VK 55, VK 76, Suguna and Sudharsana. The natural pollinating agent is insects i.e., ants. No seed set was observed in medium duration cultivars Kanthi and Sobha.

Natural fruit set was observed in cultivars VK 70, VK 76 and VK 55. The fruits matured in VK 76 within 22 days while it was only 20 in VK 70 and VK 55. The cultivar VK 70 produced six seeds/fruit while it was five in VK 55 and four in VK 76. The occurrence of seed set was high in VK 70 while in other cultivars the set was found to be limited.

4.6 Controlled *in vivo* pollination

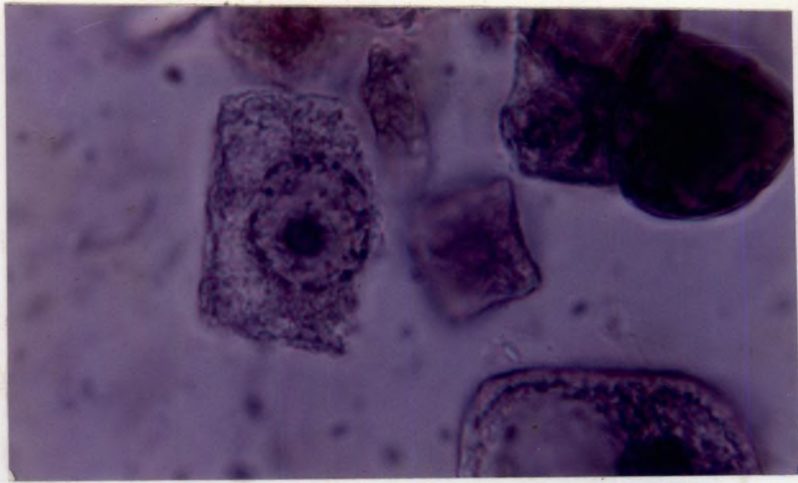
Seed set was obtained only in stigmatic pollination (Plate 7a). Observations recorded with respect to *in vivo* cross pollination are presented in Table 18. Seed set was observed in three of the twelve crosses tried viz. VK 70 x VK 76, VK 70 x VK 55 and VK 70 x Suguna. But it failed to yield seeds in combination with medium duration cultivars viz. Kanthi and Sobha. The combinations involving VK 70 as the female parent only yielded seed set. No set was observed in the crosses other than VK 70.

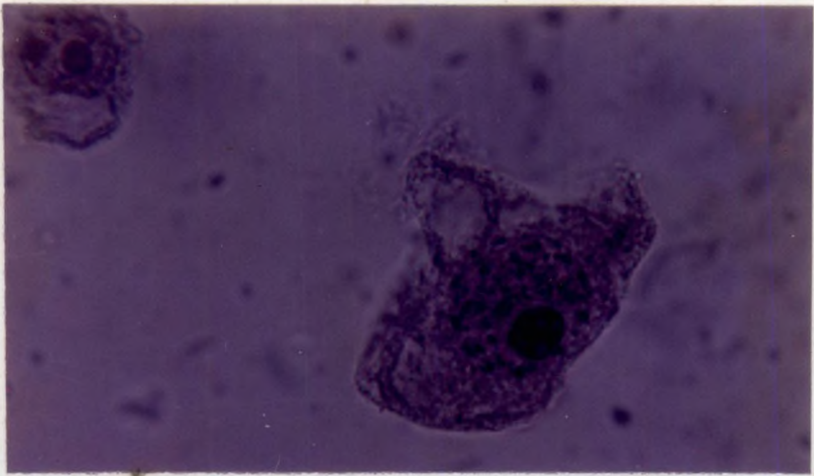
In vivo stylar pollination did not produce any seed set (Plate 7b).

Plate 6a. Late prophase in the cultivar VK 70 showing 2n=84 (x 1000)

Plate 6b. Late prophase in the cultivar Suvarna showing 2n=84 (x 1000)

Plate 6c. Late prophase in the cultivar Kanthi showing 2n=63 (x 1000)





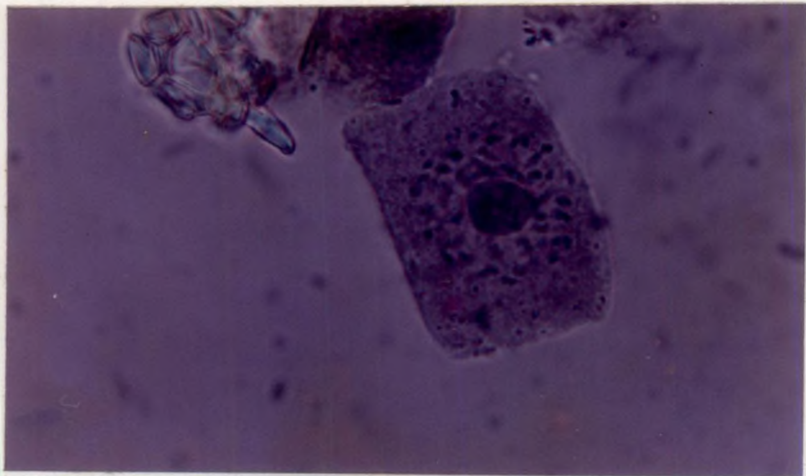


Table 17. Natural seed set in turmeric cultivars

Characters	Cultivars						
	VK 70	VK 55	VK 76	Suguna	Sudharsana	Kanthi	Sobha
1. Natural seed set	Yes	Yes	Yes	Yes	Yes	Nil	Nil
2. Mode of natural pollination	IP	IP	IP	IP	IP		
3. Days to fruit set	13	14	12	NA	NA	NA	NA
4. Days to fruit maturity	20	20	22	NA	NA	NA	NA
5. No. of seeds/fruit	6	5	4	NA	NA	NA	NA
6. Percentage of germination	Nil	Nil	Nil	-	-	-	-

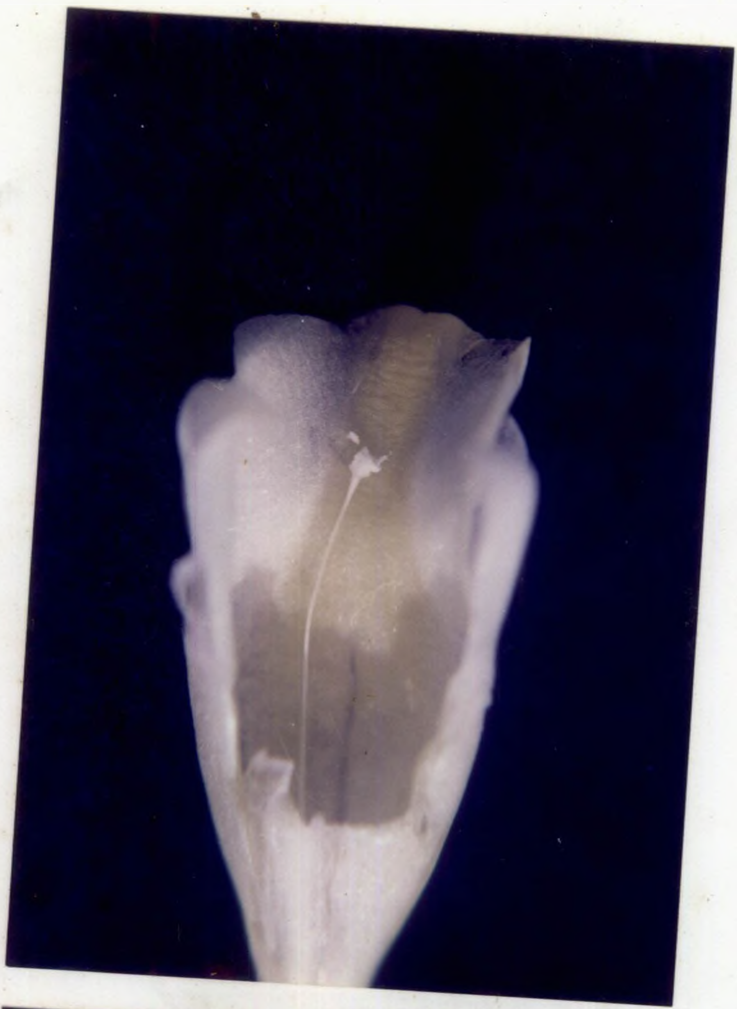
IP - Insect pollination
NA – Not applicable

Table 18. Controlled *in vivo* crosses in turmeric cultivars

Crosses	Success/failure*
VK 70 x VK 76	Success
,, x VK 55	,,
,, x Suguna	,,
VK 76 x VK 55	Failure
VK 55 x VK 76	,,
Suguna x VK 76	,,
,, x Sudharsana	,,
Sudharsana x VK 70	,,
,, x Suguna	,,
Kanthi x Sobha	,,
Sobha x Kanthi	,,
Suguna x ,,	,,

Plate 7a. *In vivo* stigmatic pollination in turmeric (x 20)

Plate 7b. *In vivo* stylar pollination in turmeric (x 20)



4.6.1 Pollen pistil interaction in short duration cultivars after *in vivo* cross pollination

Pollinated gynoecium along with style and stigma were observed for pollen germination under fluorescence microscopy. Pollen tube penetration and growth through the stigmatic and stylar tissues were observed. It reached and covered the ovules. Plates (8a and 8b) support the results.

4.6.2 Description of turmeric fruit and seed produced in *in vivo*

The turmeric fruit developed after *in vivo* pollination was a thick walled trilocular capsule (Plate 9).

Seeds are triangular with two seed coats (Plate 10a and Fig.1 and 2). The outer layer of seed coat is hardened, highly lignified and thickned. While the inner seed coat consists of few layers of cells which are radially arranged. Seeds are filled with massive endosperm and the embryo is oriented towards the upper side of the ovule. Plumule of the embryo is oriented towards the upper side and radicle is oriented towards lower side of the seed (Plate 10b). Endosperm cells are large parenchymatous cells all filled with starch grains.

4.7 *In vitro* pollination

4.7.1 Standardisation of media

4.7.1.1 Basal medium for culture establishment

In order to get the initial *in vitro* establishment, surface sterilized pollinated ovaries with intra ovarian pollination technique, were incubated in three different basal media combinations i.e., half strength MS, full MS and SH media with BAP 1 mg l^{-1} and kinetin 1 mg l^{-1}. Most of the cultures were destroyed by bacterial contamination. A few cultures showed ovary development in ½ MS and MS media with BAP and kinetin. The results of comparative efficiency of half strength MS and full strength MS media with placental pollination on culture

Plate 8a. Pollen germination on the stigma and tube growth through the style in controlled *in vivo* cross of VK 70 x VK 76 (x 200)

Plate 8b. Pollen tube covering the ovule length (x 200)

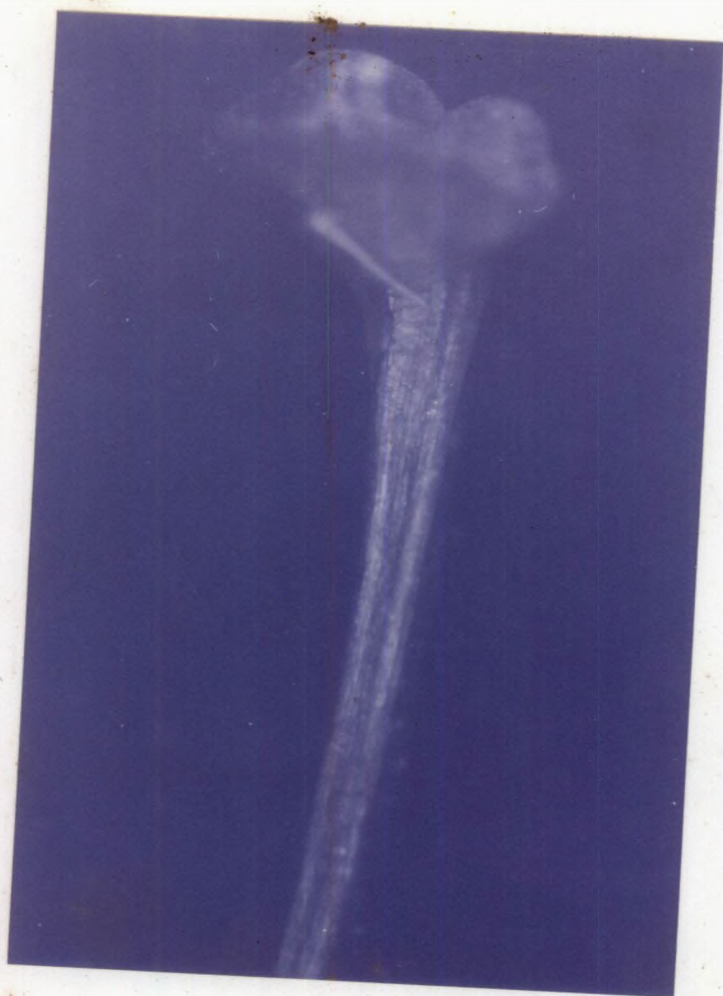


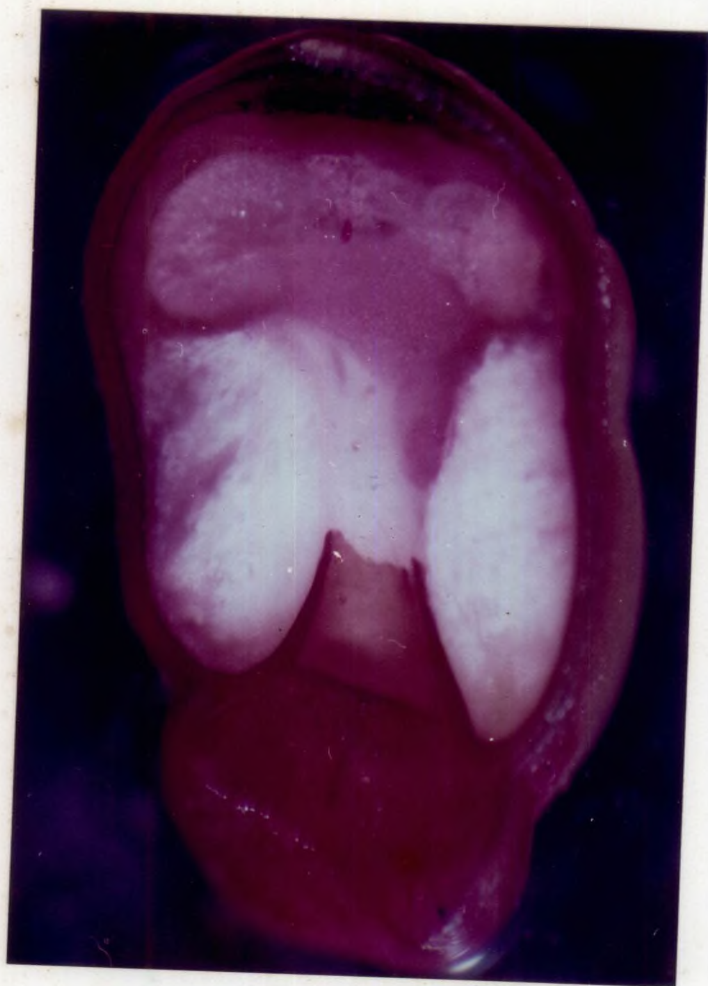
Plate 9. Developed fruit of turmeric 20 DAP under *in vivo* condition (x 40)

Plate 10a. Arillate seed of turmeric (x 40)

Plate 10b. L.S. mature seed of turmeric stained with saffranin (x 20)

es – endosperm

em - embryo



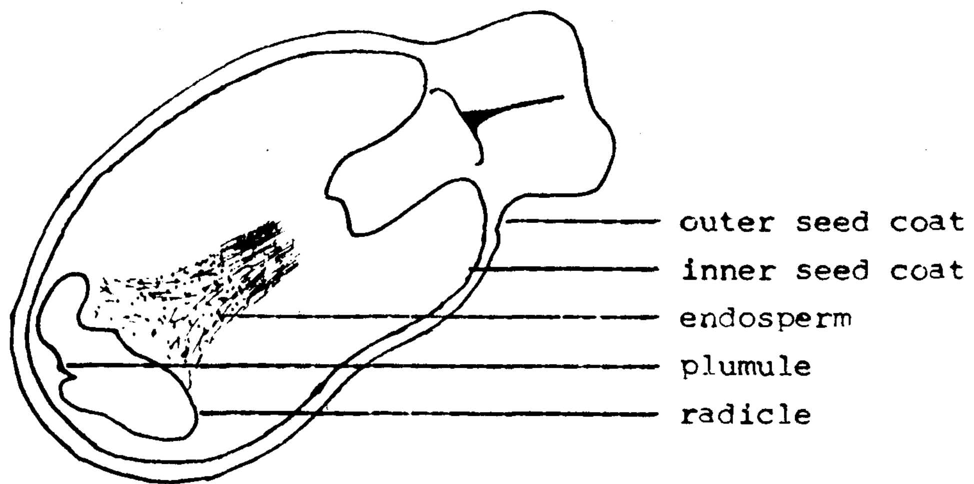


Fig.1. Longitudinal section of turmeric seed

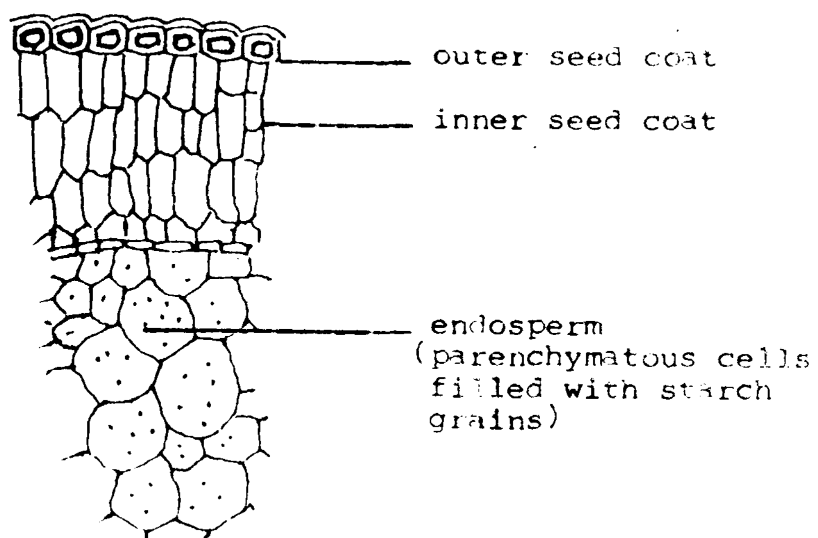


Fig.2. Cross section of turmeric seed

establishment are presented in Table 19. The ovules developed in both media combinations. But half strength MS medium was superior to full strength. The percentage of cultures showing ovule development in ½ MS media combinations ranged from 75 to 82.61 cultures while it was 55.56 to 66.67 in full MS. Maximum ovule swelling was also observed in ½ MS medium when supplemented with BAP 1 mg l^{-1}, Kin 1 mg l^{-1} and NAA 0.5 mg l^{-1}. The ovule development got reduced when NAA concentration was increased from 0.5 mg l^{-1} to 1 mg l^{-1}

4.7.1.1.1 Management of bacterial interference in the cultures

The *in vitro* sensitivity of bacterial population to various concentrations of antibiotics in culture establishment were studied. The results showed that ambystryn-S (50-100 µg l^{-1}), ampicillin (50-100 µg l^{-1}), dicrystacin (100-500 µg l^{-1}), gentamycin (50-100 µg l^{-1}) and kanamycin (50-100 µg l^{-1}) were not effective in controlling the bacterial interference in the culture establishment (Table 20).

4.7.1.2 Influence of cytokinins in ovule development

The results (Table 21) showed that in the basal medium of ½ MS with 3.0 per cent sucrose level both BAP and kinetin favoured ovule development individually as well as in combination. BAP 1 to 4 mg l^{-1} favoured ovule development in 58.33 to 64.29 per cent of cultures. Kinetin 1 to 3 mg l^{-1} also favoured ovule development. The combination treatment of BAP 1 mg l^{-1} along with Kin 1 to 3 mg l^{-1} also gave positive results of the treatment. Response was noticed in about 60 to 61.11 per cent of cultures.

4.7.1.3 Influence of auxins (NAA, IAA, IBA and 2,4-D)

The results (Table 22) indicated that auxins can induce ovule development in the basal medium of ½ MS with 3.0 per cent sucrose level. The NAA concentration 0.5 mg l^{-1} to 1 mg l^{-1} favoured ovule swelling in 66.67 to 67.86 percentage of cultures. The growth regulators IAA (0.2 mg l^{-1}), IBA (0.2 mg l^{-1}),

Table 19. Culture establishment in basal media combinations of ½ MS, full MS and SH

Sl.No.	Treatments	Ovule swelling	Percentage of cultures showing ovule development*
1	½ MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	+++	82.61
2	½ MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	+	75.00
3	MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	+	66.67
4	MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	+	55.56
5	SH + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	Nil	0.00
5	SH + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	Nil	0.00

*Average of 12 observations
Sucrose 3%
Scoring: + - low ovule swelling
+++ - good ovule swelling
Explant – Ovary after placental pollination

Table 20. *In vitro* sensitivity of bacterial population to antibiotics in culture establishment

Antibiotics (µ/l)		Sensitivity
Gentamycin	50	+ve
,,	100	+ve
Kanamycin	50	+ve
,,	100	+ve
Ampicillin	50	+ve
,,	100	+ve
Ambystryns	50	+ve
,,	100	+ve
Dicrystracin	100	+ve
,,	150	+ve
,,	300	+ve
,,	500	+ve
Control (YEM)		+ve

Table 21. Influence of cytokinins in the ovule development of turmeric after *in vitro* modified placental pollination

Sl.No.	Treatments	Ovule swelling	Percentage of cultures showing ovule development*
1	BAP 1 mg l^{-1}	+	64.29
2	,, 4 ,,	+	58.33
3	Kinetin 1 mg l^{-1}	+	64.00
4	,, 3 ,,	+	54.55
5	,, 4 ,,	+	51.72
6	BAP 1 mg l^{-1} + Kin 1 mg l^{-1}	+	65.71
7	,, + ,, 3 ,,	+	54.55

*Average of 12 observations
Basal medium - ½ MS + 3% sucrose
Scoring - + - low ovule swelling
Explant – Ovary after modified placental pollination

Table 22. Influence of auxins in the ovule development of turmeric after *in vitro* modified placental pollination

Sl. No.	Treatments	Ovule swelling	Percentage of cultures showing ovule development*
1	NAA 0.5 mg l^{-1}	+	67.86
2	,, 1.0 ,,	+	66.67
3	IAA 0.2 ,,	+	50.00
4	IBA 0.2 ,,	+	47.61
5	2,4-D 0.2 ,,	+	61.11
6	,, 1.0 ,,	+	60.00

*Average of 12 observations
Basal medium - ½ MS + 3% sucrose
Scoring - + - low ovule swelling
Explant – Ovary after modified placental pollination

2,4-D (0.2 mg l^{-1} and 1 mg l^{-1}) also caused ovule swelling in 50.00, 47.61, 61.11 and 60.00 percentage of cultures.

4.7.1.4 Influence of auxins and cytokinins

The combination of cytokinins (BAP and kinetin) and auxins (NAA, BA, 2,4-D) supported ovule development (Table 23) (Plate 11a). The BAP 1mg l^{-1} along with NAA (0.5 to1 mg l^{-1}) favoured ovule development in 75 to 80 per cent of cultures. The percentage of success was reduced to 33 per cent when the BAP concentration was increased from 1 mg l^{-1} to 2 mg l^{-1}.

The combination of BAP 1 mg l^{-1} along with 2,4-D 0.2 mg l^{-1} produced ovule swelling in 75 per cent of cultures. The percentage of cultures showing ovule development was reduced to 50 per cent when the BAP concentration was increased from 1 mg l^{-1} to 4 mg l^{-1}. Similarly, the combination of BAP 1 mg l^{-1} along with IAA 0.2 mg l^{-1} produced ovule development in 80 per cent of cultures. But an increase of BAP from 1 mg l^{-1} to 4 mg l^{-1} produced callusing (Plate 11b) instead of ovule swelling. The combination treatment of BAP and IBA also resulted in positive response. The combination of BAP 1 mg l^{-1} to 4 mg l^{-1} along with IBA 0.2 mg l^{-1} produced ovule development in 50 to 57 per cent of cultures.

The cytokinin, kinetin 1 mg l^{-1} along with NAA 0.5 to 1 mg l^{-1} favoured ovule development in 73.91 to 75 per cent of cultures. An increase of kinetin from 1 mg l^{-1} to 4 mg l^{-1} reduced the success rate to 33.33 per cent. Similarly, the kinetin (3 mg l^{-1} to 4 mg l^{-1}) along with 2,4-D (0.2 mg l to 1 mg l^{-1}) caused ovule development in 33.33 to 75.00 percentage of cultures. Here also an increase of 2,4-D from 3 to 4 mg l^{-1} reduced the success rate from 75 to 33 per cent.

The kinetin combinations (3 mg l^{-1} to 4 mg l^{-1}) with IAA 0.2 mg l^{-1} favoured ovule development in 62.50 to 80.00 percentage of cultures and with IBA 0.2 mg l^{-1} caused ovule swelling in 37.50 to 57.14 percentage of cultures.

Table 23. Influence of cytokinins and auxins in the ovule development of turmeric after *in vitro* placental pollination

Sl. No.	Basal medium	Treatments	Ovule swelling	Percentage of cultures showing ovule development*
1	½ MS + 3% sucrose	BAP 1 mg l^{-1} + NAA 0.5 mg l^{-1}	+	80.00
2	,,	,, + ,, 1.0 ,,	+	75.00
3	,,	,, 2 ,, + ,, 0.5 ,,	+	33.33
4	,,	,, 1 ,, + 2,4-D 0.2 ,,	+	75.00
5	,,	,, + ,, 1.0 ,,	+	50.00
6	,,	,, 4 ,, + ,, 0.2 ,,	+	50.00
7	,,	,, 1 ,, + IAA 0.2 ,,	+	80.00
8		,, 4 ,, + ,, 0.2 ,,	Callusing	00.00
9	,,	,, 1 ,, + IBA 0.2 ,,	+	57.14
10	,,	,, 4 ,, + ,, 0.2 ,,	+	50.00
11	,,	Kin 1 ,, + NAA 0.5 ,,	+	73.91
12	,,	,, + ,, 1.0 ,,	+	75.00
13	,,	,, 4 ,, + ,, 0.5 ,,	+	33.33
14	,,	,, 3 ,, + 2,4-D 0.2 ,,	+	75.00
15	,,	,, + ,, 1.0 ,,	+	50.00
16	,,	,, 4 ,, + ,, 0.2 ,,	+	33.33
17	,,	,, 3 ,, + IAA 0.2 ,,	+	80.00
18	,,	,, 4 ,, + ,, 0.2 ,,	+	62.50
19	,,	,, 3 ,, + IBA 0.2 ,,	+	57.14
20	,,	,, 4 ,, + ,, 0.2 ,,	+	37.50
21	,,	BAP 1 mg l^{-1} + Kin 1.0 mg l^{-1} + NAA 0.5 mg l^{-1}	+++	82.61
22	,,	BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	+	75.0

*Average of 12 observations

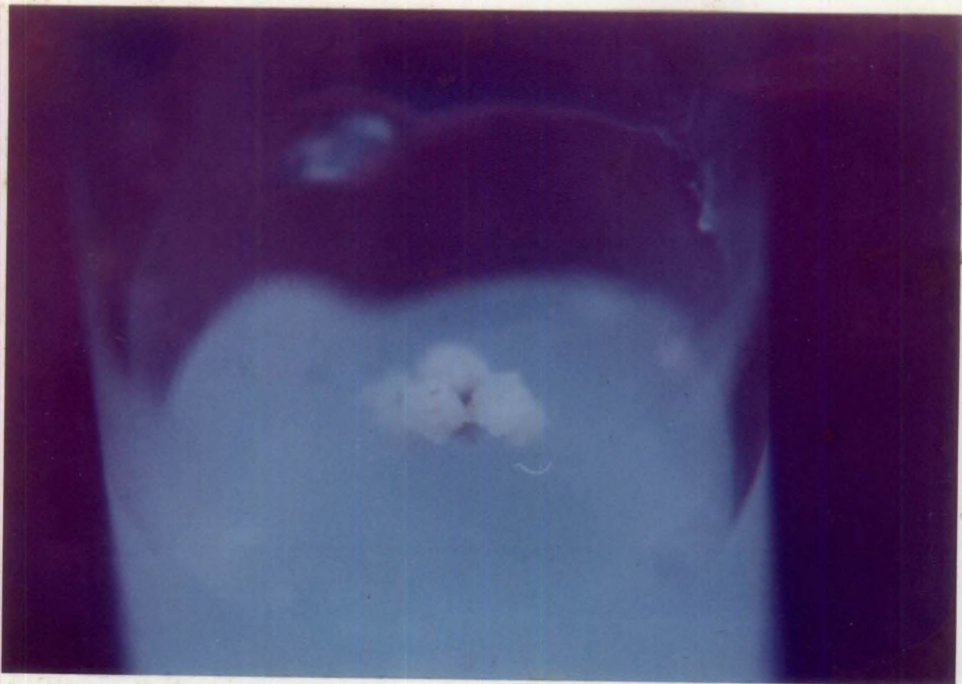
Scoring: + - low ovule swelling

+++ - good ovule swelling

Explant – Ovary after placental pollination

Plate 11a. Developing ovules ten days after *in vitro* placental pollination (x 20)

Plate 11b. Callusing from the ovules 10 DAP (x 20)



4.7.1.5 Influence of carbon source

Ovule development was found to attain maximum at 3 per cent sucrose when basal media was supplemented with BAP 1 mg l^{-1}, kinetin 1 mg l^{-1} and NAA 0.5 mg l^{-1}. The positive response was observed in 82.61 per cent of cultures. But in the same media combination an increase of NAA from 0.5 mg l^{-1} to 1 mg l^{-1} reduced ovule swelling. An increase of sucrose level from 3 to 6 per cent reduced ovule swelling and the percentage of cultures showing response were also reduced from 44.44 to 66.66 (Table 24).

4.7.1.6 Influence of coconut water in ovary and ovule development

The coconut water at 15 per cent v/v supported ovary and ovule development (Table 25). The effect was positive in the half strength MS medium along with hormones at 3 to 6 per cent level of sucrose. The mean diameter of ovary on the day of pollination was 2.4 mm.

The ovaries after intra-ovarian pollination developed into a size of 3 mm to 6 mm diameter at 3 per cent sucrose level. The positive effect was observed in 75 per cent cultures. At 6 per cent sucrose level, maximum ovary development was 5 mm only. Coconut water supported ovule swelling in modified placental pollination at 3 to 6 per cent level of sucrose and the positive response was observed in 66.67 to 75.00 per cent of cultures.

4.7.1.7 Influence of casein hydrolysate in ovule development

In the half strength MS along with the hormones casein hydrolysate 200 mg l^{-1} promoted ovule development (Table 26). The favourable influence was observed in 12.50 to 70.37 percentage of cultures at 3 to 6 per cent level of sucrose.

4.7.2 Standardisation of *in vitro* pollination technique

Different *in vitro* pollination techniques, stigmatic, stylar, intra-ovarian,

Table 24. Influence of sucrose in the ovule development of turmeric after *in vitro* placental pollination

Sl.No.	Treatments	Ovule swelling	Percentage of cultures showing ovule development*
1	Sucrose 3% + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	+++	82.61
2	Sucrose 3% + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	+	75.00
3	Sucrose 6% + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	+	66.66
4	Sucrose 6% + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	+	44.44

*Average of 12 observations
Basal medium: ½ MS
Scoring: + - low ovule swelling
+++ - good ovule swelling
Explant – Ovary after placental pollination

Table 25. Influence of coconut water on ovary and ovule development in turmeric after *in vitro* modified placental pollination

Sl.No.	Treatments	Diameter of ovary in intra ovarian pollination		Percentage of cultures showing ovary development*	Ovule swelling	Percentage of cultures showing ovule development*
		Max mm	Min mm			
1	3% sucrose + coconut water 15% v/v	6	3	75.00	+	75.00
2	6% sucrose + coconut water 15% v/v	5	3	50.40	+	66.67

*Average of 12 observations
Basal medium: ½ MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}
Scoring: + - low ovule swelling
Explant – Ovary after modified placental pollination

Table 26. Influence of casein hydrolysate in ovule development of turmeric after *in vitro* placental pollination

Sl.No.	Treatments	Ovule swelling	Percentage of cultures showing ovule development*
1	3% sucrose + casein hydrolysate 200 mg l^{-1}	+	12.50
2	6% ,, + ,, 200 ,,	+	70.37

*Average of 12 observations
Basal medium: ½ MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}
Scoring: + - low ovule swelling
Explant – Ovary after placental pollination

Table 27. Standardisationof *in vitro* pollination technique in turmeric for crossing

Sl. No.	Method of pollination	% of cultures with ovary development*	% of cultures with ovule development*
1	Stigmatic pollination	0.00	0.00
2	Stylar pollination	0.00	0.00
3	Intra-ovarian pollination	81.82	45.45
4	Placental pollination	NA	63.49
5	Modified Placental pollination	NA	63.49
6	Ovular or Test tube fertilization	0.00	0.00

*Average of 12 observations
Basal medium: ½ MS + BAP 1 mg l^{-1} + Kinetin 1 mg l^{-1} + NAA 0.5 mg l^{-1} + sucrose 3%
Explant – Pollinated gynoecium or part of it

placental, modified placental and ovular pollination as described by Bhojwani and Razdan (1983) were attempted. Among these, ovules developed in intra-ovarian, placental (Plate 11c) and modified placental pollination techniques (Table 27). In the intra-ovarian pollination technique, ovary as well as ovules developed. Ovary development was noticed in 81.82 per cent of cultures while ovule development was observed only in 45.45 per cent of cultures. Success in placental and modified placental pollination was noticed in 63.49 per cent of cultures.

4.7.3 Post pollination changes

In the intra-ovarian pollination technique, ovary developed into fruit and attained a maximum size of 6 mm 20 DAP (Plate 11d). They were creamy white in diffused light. Ovules/seeds developed in the intra-ovarian, placental and modified placental pollination technique. They were creamy white during the initial stage of development and changed to dark brown colour within a period of 20 to 30 DAP (Plate 11e). The *in vitro* developed seed had two seed coats and a cavity which was partly filled with endosperm. The embryo development was not distinct (Plate 11f).

4.8 Seed germination studies in turmeric

4.8.1 *In vivo* studies

The seeds kept in moist sterile sand did not germinate.

4.8.1.1 Influence of pre-sowing treatments on germination of turmeric seeds

The results of various pre-sowing treatments tried on germination are listed in Table 28. The pre-sowing treatments had no influence on germination of turmeric seeds.

Plate 11c. *In vitro* placental pollination (x 40)

Plate 11d. Fruit of turmeric developed under *in vitro* condition (x 20)

Plate 11e. Black seed of turmeric 20 days after *in vitro* placental pollination (x 20)

Plate 11f. L.S. of seed produced under *in vitro* (x 200)

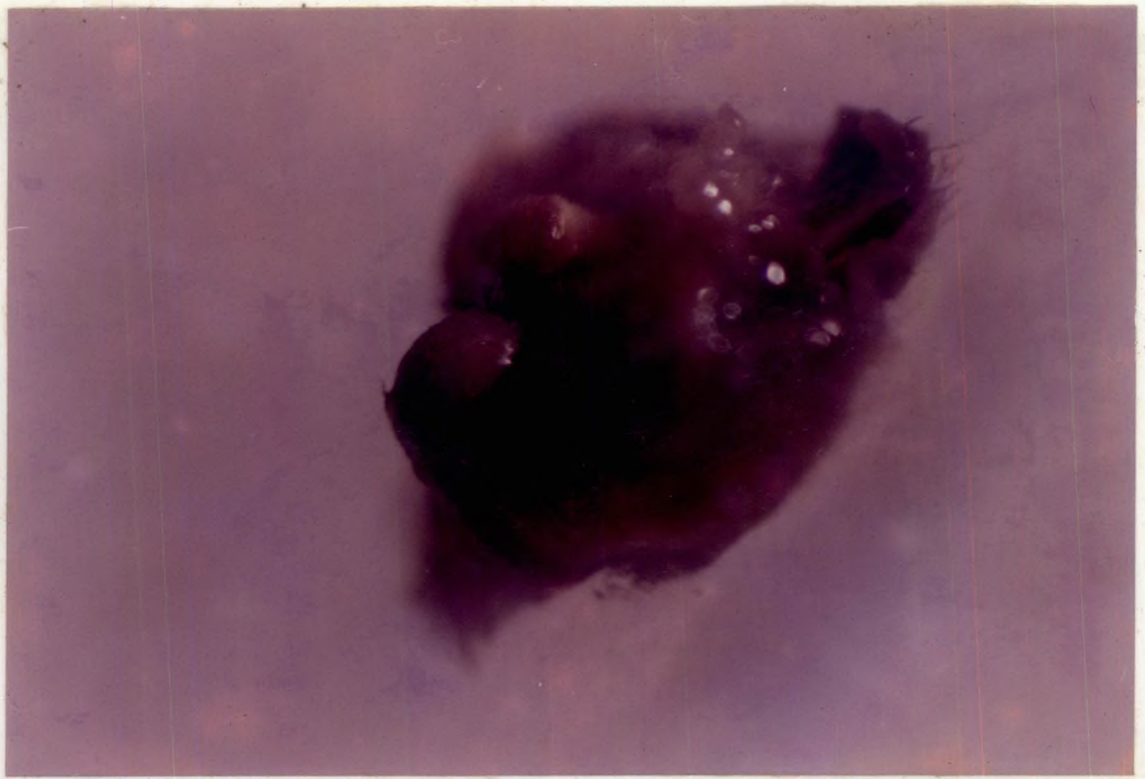


Plate 12. Seed germination of turmeric under *in vitro* condition (x 40)

Table 28. Effect of pre-sowing treatments on germination of turmeric seeds

Sl.No.	Treatments	Germination (%)
1	Control	Nil
2	Soaking seeds in water for 24 h	Nil
3	Acid scarification (25% HNO_3 for 10 min)	Nil
4	Acid scarification (25% HNO_3 for 10 min) + water soaking for 24 h	Nil

4.8.2 *In vitro* studies

The seeds kept in moist filter paper only germinated (Plate 12) while those kept in moist sand, mixture of moist sand and vermiculite at 1:1 proportion and basal medium did not germinate.

Incubating seeds in the basal medium of ½ MS + 3 per cent sucrose along with BAP and Kinetin 2 to 4 mg l^{-1} and NAA 0.5 mg l^{-1} did not favour seed germination. Reducing sucrose from 3 per cent to 0.6 per cent, BAP and kinetin from 4 to 1 mg l^{-1} also did not favour seed germination. Basal media with 0.6 per cent sucrose also was not effective for seed germination.

DISCUSSION

DISCUSSION

The investigations on "Response of turmeric *Curcuma domestica* Val. to *in vivo* and *in vitro* pollination" were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1997-1999.

Turmeric (*C. domestica* Val.) a monocot belonging to the family Zingiberaceae. At present the rhizome of turmeric is valued as a spice, food colourant, medicine, cosmetic and dye in addition to its use in religious ceremonies.

Turmeric is exclusively propagated by vegetative means. Even then tremendous variability is available in the crop and the population shows variation for yield from 2 tonnes to 8 tonnes, for curing precentage from 14 to 31 and for curcumin content 3 to 7.5 percent (Philip, 1978). This available variability is not fully exploited for crop improvement as hybridisation programme is not perfected in this crop. Cultivated turmeric consists of fertile tetraploids (2n=84) and sterile triploids (3n=63). Generally tetraploids are high yielders of cured turmeric and low in curcumin content but exceptions are also available. Long duration types are high yielders but moderate in curing percentage and curcumin content. Medium duration types are rich in curcumin content but low in curing percentage. Theoritically seed set could be obtained in crosess involving short duration types as they are tetraploids. There can be difficulty in getting seed set in crossess involving short and medium duration cultivars.

Nazeem and Menon (1993) have reported successfull *in vivo* pollination in turmeric. Seed set could also be obtained in different parental combinations of short duration types. But in crossess involving short and long duration types, only one particular combination has given seed set. So it can be assumed that certain incompatibility mechanism is operating and seed set is not naturally occuring. The mechanism of incompatibility reaction has to be examined.

In vitro pollination and fertilization is an effective tool in situations where incompatibility reactions prevent seed set. In ginger, another important spice crop of zingiberaceous family, natural seed set is not occuring because of the incompatibility reactions operating in stigma and style. Valsala (1994) was able to get seed set in this crop through *in vitro* pollination and fertilization.

The present investigations were aimed at standardizing a technique for successful seed set through *in vivo* or *in vitro* pollination among turmeric cultivars. Success in this line will open up new vistas of crop improvement in turmeric, especially for the improvement of medium and long duration types, which occupies 93 percentage of cultivated area of turmeric. Alleppey turmeric which is rich in curcumin content can be further improved for curing percentage. Similarly, short duration types with high cured yield and curcumin content suitable for specific climatic condition can also be developed.

The major aspects of investigations were

1. Variability studies in the selected plant materials
2. Floral morphology and biology
3. Pollen fertility and viability studies
4. Cytogenetics of short and medium duration types
5. Response of selected cultivars to *in vivo* pollination
6. Standardisation of media for culture establishment
7. Response of selected cultivars to *in vitro* pollination
8. Seed germination studies

The results obtained from the studies are discussed in this chapter.

5.1 Variability in plant materials

The variability in selected cultivars was examined for salient morphological characters, curcumin and curing percentage.

The results showed that the cultivars differed significantly with respect to various pseudostem characters (Table 11), mother rhizome characters and finger rhizome characters (Table 12 and Plate 1a to 1h). The curcumin content in selected cultivars ranged from 2.13 in VK 70 to 7.43 in sobha (Table 13). It was also high in medium duration cultivar Kanthi (7.02) and short duration cultivar VK 76 (6.38) while all others recorded <5.13 percentage. The curing percentage in the cultivars ranged from 9.5 in VK 55 to 21.5 in VK 70. The curing percentage was high in short duration types VK 76 (20.0) and Suvarna (19.5). In the released medium duration varieties ie., Sobha and Kanthi it was only 14 to 15 per cent.

Significant variation among turmeric cultivars with regard to morphological and quality characters were reported earlier by Philip (1978), Geetha and Prabhakaran (1987), Jalgaonkar *et al.* (1986), Mukhopadhyay *et al.* (1986), Reddy and Rao (1988), Menon *et al.* (1992), Yadav *et al.*, (1996) and Kurian and Nair (1996).

As per Rao *et al.* (1975) short duration types are low in curcumin content but VK 76, Sudharsana and VK 55 are exceptions to this. They contained >5.1 per cent curcumin content.

The cultivar Sobha take its pedigree to the Alleppey turmeric and high curcumin content can be expected.

Above study revealed that plant materials selected for the study are having adequate variability for the exploitation of hybrid vigour.

It also revealed that VK 76, Kanthi and and Sobha can be identified as the source of genetic material for high curcumin and Suvarna and VK 70 for high curing percentage. It also enlightens that the released medium duration varieties Kanthi and Sobha can be improved for curing percentage and curcumin content by conducting suitable crosses with VK 70 and VK 76. The released short duration cultivars Suguna, Suvarna and Sudharsana can also be improved for the curcumin content and curing percentage by conducting suitable crosses with Sobha, Kanthi,

VK 76 and VK 70. Since VK 76 possesses high curing percentage and curcumin content, yield potential of the same has to be assessed and if it is low, it can be improved by crossing with other high yielding cultivars. Suvarna is a high yielding cultivar with high curing percentage (19.5) and the crossing of the same with VK 76 for improving curcumin can be thought off if it flowers.

5.2 Study of floral biology and floral morphology

Turmeric flowers within a period of 105 to 155 days. The cultivars VK 70 and VK 76 were too early to flower while Kanthi and Sobha were late flowering (Plate 2). The flowering season ranges from July to October.

The inflorescence of turmeric is a symmetrical compound spike, which bears flowers in bracts, open in succession (Plate 3a).

Stamens are epipetalous in nature represented by two whorls of three each. The outer whorl is represented by labellum and the inner whorl by two staminodes and one fertile stamen. Honey secretion is present at the base of the staminode (Plate 3c).

The pistil of the flower is characterized by a long style of mean length 4.43 cm, which passes through the groove present between two anther lobes. The stigma protrudes just above the anther lobes. Ovary recorded a mean length of 0.26 cm and diameter of 0.24 cm in the middle. The mean number of ovules in ovary was 29.31. The ovule measured a length of 611.44 μm and 436.65 μm at the middle (Table 14).

The flower opening occurred between 5 am to 6 am and anther dehiscence between 7.15 am and 7.45 am.

The observations on floral biology and morphology are in conformation with the previous reports (Philip, 1978, Purseglove *et al.*, 1981 and Nazeem and Menon, 1994) except androecium details.

As per the present study the androecium consists of two whorls of three stamens each. The outer whorl is represented by a trilobed, petalloid labellum. Inner whorl is represented by one fertile stamen and two staminodes.

According to Purseglove *et al.* (1981) labellum is the modification of two stamens of the inner whorl. This may not be correct as the labellum is trilobed. Present observation is also supported by the fact that, in Cardamom, the labellum has been described as the modification of three stamens (Purseglove *et al.*, 1981).

The observations on flowering season is confirmation with earlier reports (Patnaik *et al.*, 1960, Pai, 1961 and Nambiar *et al.*, 1982). Nambiar *et al.* (1982) have also observed that *C. aromatica* (short duration types) are early to flower i.e., July to September and *C. longa* (medium and long duration cultivars) types are late to flower i.e., September to December.

The selected cultivars for study include early flowering and late flowering types. Synchronization of flowering in specified cultivars is essential for conducting particular crosses. This can be achieved either through late planting of early flowering cultivars or through pollen storage.

As the stigma is just protruding above the fertile anther lobes, emasculation has to be done in crossing programmes under *in vivo*.

As the flower opening is during 5 am to 6 am controlled *in vivo* pollination has to be done in the early morning itself.

In the *in vitro* pollination work flower buds to be used as the male gamete have to be surface sterilized before flower opening. This is advisable as the

contact of pollen grain with sterilant may reduce the pollen viability. So the possibility of using unopened flower buds one day prior to anthesis can be thought of. The pollen viability of the same has to be acertained. *In vitro* pollination work can also be scheduled in the forenoon hours as the anthesis and pollen shedding takes place during that period.

5.3 Pollen studies

5.3.1 Pollen morphology

The pollen grains are ovoid to spherical in shape, sticky and light yellow in colour.

5.3.2 Standardisation of medium for pollen germination and pollen tube growth

Modified ME_3 medium of pH 6 recorded improved pollen germination and pollen tube growth in turmeric cultivars (Table 15).

Valsala (1994) and Bindu (1997) had also reported improved pollen germination for ginger pollen grains in the ME_3 medium. The ME_3 medium is characterised by high osmotic potential due to the presence of 12 per cent PEG 4000. The presence of optimum concentration of ions i.e., B, Ca^{++}, Mg^{++}, K^+ and Na^+ in the ME_3 medium may be supporting pollen germination and tube growth. There are previous reports that pollen grains of species which are difficult to germinate may respond to osmotic effect of PEG (Robert *et al.*, 1983, Leduc *et al.*, 1990, Goldhirsh *et al.*, 1991).

Since improved pollen germination and tube growth was recorded in ME_3 medium at pH 6 this can be used in pollination under *in vivo* and *in vitro* conditions.

5.3.3 Influence of position of flowers on polllen fertility and viability

The pollen fertility and viability were high in flowers at the lower portion of the inflorescence compared to middle and upper portion (Table 16). Bindu (1997) had also made similar observation in ginger.

The afforesaid finding indicates that the chances of seed set will be more when pollen grains were collected from the flowers of the lower portion.

5.3.4 Pollen fertility, viability and pollen tube growth in turmeric cultivars

The pollen fertility was high in turmeric cultivars (78.51%) as per the acetocarmine stain test (Table 16 and Plate 5). The fertility of pollen grains ranged from 67.07 percentage in Sobha to 86.79 percentage in Sudharsana. But viability in the ME_3 medium was only 27.35 per cent. The viability was high in short duration types (21.49 to 46.08%) while low in medium duration cultivars (8.22 to 11.51%) (Table 16).

Pollen tube growth for various cultivars in ME_3 medium ranged from 244.47 µm to 576.3 µm (Plate 4). Pollen tube growth was comparatively high for short duration types (268.48 µm to 576.30 µm) and was low for medium duration types (218.72 to 244.4 µm) (Table 16).

Even if pollen fertility was fairly high in turmeric cultivars the viability in ME_3 medium was not correspondingly high. This may due to the fact that acetocarmine stain test may not be fully efficient in revealing fertility of pollen grains or ME_3 medium may not be fully efficient in bringing about full viability. Therefore, for assessing pollen fertility tests other than with acetocarmine may be thought off.

The improved pollen germination and tube growth justifies seed set in short duration types. The study also brings out the fact that seed set will be more in

crosses involving Suguna, VK 70 and VK 76 as male parents and these cultivars could be effectively utilised for future breeding programmes.

5.4 Cytology

The results revealed chromosome number 2n=84 in VK 70 and Suvarna and 2n=63 in Kanthi (Plate 6a, b and c). The basic chromosome number of turmeric is n=21. So it can be concluded that cultivated turmeric consists of triploid and tetraploid cultivars. This is in confirmation with previous reports of chromosome number in turmeric by Ramachandran (1969) and Nambiar (1978).

Kanthi is a medium duration cultivar with no natural seed set. This must be due to its triploidy as found in the presentstudy. VK 70 is a short duration cultivar with profuse seed set. Owing to the balanced tetraploidy, the cytological study explains the occurrence of seed set in short duration cultivar and lack of seed set in medium duration cultivar.

5.5 Natural seed set

Natural fruit set and seed set were observed in short duration cultivars viz. VK 70, VK 55, VK 76, Suguna and Sudharsana and the natural pollinating agent is insects (Table 17). Seed set was high in VK 70. Since they are tetraploids with high pollen viability natural seed set can occur. Fruit set and seed set was not noticed in long duration cultivars i.e., Kanthi and Sobha, which are said to be triploid species.

The afforesaid results are in agreement with the reports of Nambiar *et al.* (1982). He observed seed set in *C. aromatica* types and no seed set in *C. longa* types.

The fruit of turmeric matured in 20-22 days. There were 5-6 seeds in the fruit. This is in agreement with previous report of Lad (1993). Seed set was high in VK 70 but in other cultivars the set was limited.

5.6 Controlled *in vivo* pollination

Eventhough stylar and stigmatic pollination were done seed set was obtained only in stigmatic pollination (Plate 7a and b). Seed set was observed in three of the twelve crosses tried viz. VK 70 x VK 55, VK 70 x VK 76 and VK 70 x Suguna (Table 18).

Seed set was not observed in crosses VK 55 x VK 76, Suguna x VK 76, Suguna x Sudharsana, Sudharsana x VK 70 and Sudharsana x Suguna, eventhough they are tetraploids. The crosses involving medium duration cultivars i.e., Kanthi and Sobha and vice-versa also failed to set seeds. Similarly, crosses involving short duration cultivar (Suguna) and medium duration cultivar (Kanthi) failed to set seeds.

Nazeem and Menon (1994) have reported high seed set in crosses involving *C. aromatica* cultivars. But in the present investigation, only some crosses were successful. Since they are tetraploids seed set could be obtained. The failure may be due to the decay of the developing fruits due to bacterial infestation, as the inflorescence structure i.e., presence of open bracts promote harbouring of bacterial inoculum along with rain water. So that the crosses have to be repeated for conclusive results. *In vitro* pollination and fertilization may be tried in the absence of seed set under *in vivo* condition.

The hybrids of the combination VK 70 x VK 76 could be promising as they can combine high curing percentage of VK 70 (21.5) and high curcumin content of VK 76 (6.38). Crossing Kanthi with Sobha and vice-versa can also be promising as it will combine high yield potential of Kanthi with high curcumin content of Sobha. The improvement of released varieties Suguna, Sudharsana and high yielding accession VK 55 poses difficulty as they registers curcumin content below 5.5 per cent and curing below 13.0 per cent.

The combinations involving VK 70 as the female parent yielded seed set. These results indicate that VK 70 could be successfully employed as a female parent in the future hybridisation programme, since the cultivar easily set seeds and possess good curing percentage. Hence an effective hybridisation programme could be chalked out with this cultivar with that of Sobha and VK 76, which are rich in curcumin content.

5.6.1 Pollen pistil interaction

The pollen pistil interaction studies in crosses of VK 70 x VK 76 confirmed the chances of seed set in controlled crosses of short duration cultivars (Plate 8a and b).

5.6.2 Description of turmeric fruit and seed

The turmeric fruit developed after *in vivo* pollination was a thin walled trilocular capsule with small black arillate seeds (Plate 9).

The small black arillate seeds had two seed coats (Plate 10a and Fig.1 and 2). The outer layer being thick and the inner being thin consists of a few layers of radially arranged cells. Seeds are filled with massive endosperm and embryo is seen towards the upper side of the ovule (Plate 10b). This is in confirmation with earlier reports (Nambiar *et al.*, 1982 and Lad, 1993).

5.7 *In vitro* pollination

5.7.1 Standardisation of media

5.7.1.1 Basal medium for culture establishment

Murashige and Skoog (1962) basal medium at half and full strength along with plant growth regulators supported the development of the ovary. The medium of SH (1972) was not found to be promising.

The suitability of MS medium with plant growth substances for *in vitro* culture of turmeric has been reported earlier (Nadgauda *et al.*, 1978; Kuruvinashetty *et al.*, 1982, Kuruvinashetty and Iyer, 1982; Winnaar and Winnar, 1989; Keshavachandran and Abdul Khader, 1989, Balachandran *et al.*, 1990; Vidya *et al.*, 1989 and Rajan, 1995).

The results on comparative influence of the basal media on culture establishment revealed that maximum ovule swelling was observed in MS media at half strength supplied with BAP, Kinetin and NAA (Table 19).

This is supported by earlier reports of Sit and Tiwari (1996), that half strength of MS was more favourable than full strength in micropropagation of turmeric.

Hence the basal medium MS at half strength supplemented with BAP, Kinetin and NAA can be used for *in vitro* pollination studies in turmeric.

5.7.1.1.1 Management of bacterial interference in the cultures

The various antibiotics (Ambystryn-S 50 to 100 µg, Ampicillin 50 to 100 µg, Dicrystacin 100 to 500 µg l^{-1}, Gentamycin 50 to 100 µg l^{-1} and Kanamycin 50 to 100 µg l^{-1}) tested in controlling the bacterial interference in the culture were ineffective (Table 20).

The concentration of antibiotics could not be increased further as they lead to the distruction of cells. Hence alternative ways like growing the stock plants for supply of flowers in controlled condition can be thought of to eradicate the menace.

5.7.1.2 Influence of cytokinins and auxins in ovule development

In the basal medium of half MS with 3 per cent sucrose level, the plant growth regulators i.e., cytokinins and auxins were required for ovule development

following *in vitro* pollination. The individual effect of cytokinins, auxins and combination effect of both were also studied. In the basal medium of half MS with 3 per cent sucrose level, BAP (1 to 4 mg l^{-1}) or kinetin (1 to 3 mg l^{-1}) caused little ovule development. The combination of BAP 1 mg l^{-1} along with kinetin (1 to 3 mg l^{-1}) also produced similar effect. Auxins (NAA 0.5 to 1 mg l^{-1}, IAA 0.2 mg l^{-1}, IBA 0.2 mg l^{-1} and 2,4-D 0.2 to 1 mg l^{-1}) also produced the same degree of ovule development. The combination of cytokinins (BAP, kinetin) and auxins (NAA, BAP and 2,4-D) supported more ovule development (Plate 11a). The combination of BAP (1 to 3 mg l^{-1}) or kinetin (1 to 4 mg l^{-1}) with NAA (0.5 to 1 mg l^{-1}) or 2,4-D 0.2 mg l^{-1} orr IAA 0.2 mg l^{-1} were effective for ovule development. But an increase of BAP from 3 to 4 mg l^{-1} with IAA 0.2 mg l^{-1} produced callusing instead ovule swelling (Plate 11b). Maximum ovule swelling was obtained in the combination of BAP and kinetin both at 1 mg l^{-1} with NAA 0.5 mg l^{-1} (Table 23). In this combination the requirement of cytokinin is met by both BAP and kinetin.

Valsala (1994) reported that the combination of BAP ranging from 2.0 to 10 mg l^{-1} with 0.5 to 1 mg l^{-1} NAA promoted ovule development in ginger under *in vitro*..

Quartrano (1987) reported that hormones play an important role in the stages of seed development and germination. The cytokinins, auxins, gibberellin (GA) and abscisic acid (ABA) were found relatively high in extracts from seeds of different development stages.

The cytokinin activity at the early stage of embryogenesis is responsible for enhanced seed size by increasing cell number (Michael and Kelbitsch, 1972). Studies with isogenic mutants of barley which vary in grain weight demonstrated that large grain lines contain higher amount of cytokinin at the very early stage of seed development than small grain lines (Kelbitsch *et al.*, 1975). According to Tollenaar (1977), cytokinins are found in relatively high concentrations in the liquid

endosperm stage of early seed growth and their presence coincides with the higher rate of mitosis.

Eeuwens and Schwale (1975) have reported that, in general high auxins (IAA) and GA levels have been associated with active seed growth and fruit growth by cell expansion. In a number of plants, both GA and auxins are highest during early to mid embryo development, at a stage when cytokinins are decreasing rapidly.

The afforesaid reports explain the requirement of auxins and cytokinins for proper development of ovules.

The combination of BAP and kinetin both at 1 mg l^{-1} with NAA 0.5 mg l^{-1} caused maximum ovule swelling. In this treatment the cytokinin required for ovule development is partly met by BAP and kinetin. From this, it can be assumed that combination effect of both BAP and kinetin is more favourable for ovule development than individual effect. For optimizing the media requirement for ovule development, an increased level of both BAP and kinetin upto 2 mg l^{-1} each and replacement of NAA with IAA 0.2 mg l^{-1} or 2,4-D 0.2 mg l^{-1} or IBA 0.2 mg l^{-1} can be thought of. As flowering season was limited to two months, this media combination could not be tried.

5.7.1.3 Influence of carbon source

The ovule development was maximum at 3 per cent sucrose level along with plant growth substances BAP, kinetin and NAA (Table 24) compared to 6 per cent sucrose level.

The sucrose concentration has been reported to influence ovary development, ovule development and germination of seeds following *in vitro* pollination. In most cases, sucrose has been used at a concentration of 4.0-5.0 per cent (Kanta and Maheswari, 1963; Usha, 1965; Rangaswamy and Shivanna, 1967

and Dhaliwal and King, 1978). According to Valsala (1994) ovary development was maximum in ginger at sucrose levels of 3.0 and 6.0 per cent.

From the present study, the sucrose concentration at 3 per cent can be considered as favourable for the ovule development. But this inference was mainly drawn based on visual assessment of ovule development. The endosperm and embryo development at various sucrose concentrations have to be examined, for conclusive results.

5.7.1.4 Influence of coconut water

The coconut water favoured ovary and ovule development at 15 per cent v/v (Table 25). Coconut water contains a number of factors favouring cell division and a large number of free amino acids (Shantz and Steward, 1952 and Letham, 1974), which might have favoured ovule development. Valsala (1994) reported the favourable influence of coconut water in the ovule development of ginger.

In the present study the influence of coconut water at 15 per cent v/v only was examined. Hence lower and higher levels may also be tested.

5.7.1.5 Influence of casein hydrolysate

Casein hydrolysate (200 mg l^{-1}) promoted ovule development in cultures (Table 26). The favourable effect of casein hydrolysate in ovule development has been reported earlier (Kanta and Maheswari, 1963; Wagner and Hess, 1973 and Zubkova and Sladky, 1975 and Valsala, 1994).

Due to the scarcity of flowers, the effect of casein hydrolysate (200 mg l^{-1}) alone was studied. So the concentrations above and below may also be tested.

5.7.2 Standardization of *in vitro* pollination technique

Ovule developed in intra ovarian, placental and modified placental pollination (Table 27 and Plate 11c). Pollen grains along with modified ME_3 pollen germination medium were applied over the ovules or ovary as the case may be.

Valsala (1994) has also reported seed set and seed development through placental and modified placental pollination technique in ginger.

Ovule development can be the effect of suitable medium and fertilization. Pollination along with ME_3 medium enhances the chances of seed set. Fertilization and seed set can be further confirmed by pollen pistil interaction studies and cytological examination of pollinated ovules at various time intervals.

The aforesaid pollination technique can bring about seed set in turmeric in crosses involving short duration types. It can also be used for crossing short and medium duration cultivars, provided medium duration types are used as female parents. The use of medium duration cultivars as female parents is suggested due to limited pollen tube growth of the same even in the improved pollen germination medium. For confirming fertilization and seed set pollen pistil interaction and cytological work can also be undertaken.

Owing to the limited flowering season, scarcity of flowers and nonsynchronisation of flowering in short and medium duration types, elaborate study could not be taken up.

5.7.3 Post pollination changes

In the intra-ovarian pollination technique ovary developed into fruit and attained a maximum size of 6 mm 20 DAP (Plate 11d). They were creamy white in diffused light. Ovules developed in the intra-ovarian, placental and modified placental pollination technique. They were creamy white during the initial stage of

development and changed to dark brown colour within a period of 20 to 30 DAP (Plate 11e). In the *in vitro* developed seed, the endosperm development was not complete. This shows that the media components for the development of seeds need further refinement.

Under *in vitro* condition, the maturity period for fruit and seed development were 20 and 22 days respectively. Under *in vitro* condition it was 20 to 30 days.

5.8 Seed germination studies in turmeric

Seeds kept in moist sterile sand did not germinate under *in vivo*. Pre-sowing treatments like soaking seeds in water and acid scarification with 25 per cent HNO_3 also did not favour seed germination. But there are previous reports of successful seed germination in turmeric (Nambiar *et al.*, 1982 and Nazeem and Menon, 1994).

Under *in vitro* condition, the seeds kept in moist filter paper only germinated and only 17 per cent seed germination was recorded (Plate 12).

This shows that the seeds produced under *in vivo* can be germinated under *in vitro* condition successfully and the conditions required for germination are very simple i.e., adequate moisture and air. There was no necessity of any complex media requirements and growth regulators. The reasons for low germination percentage has to be examined i.e., embryo and endosperm development. The seed viability test can also be undertaken.

The reasons for lack of seed germination under *in vivo* condition may be due to the absence of satisfactory conditions required for germination or high percentage of defective seeds.

Future lines of work suggested

Short duration types of turmeric can be improved for economic characters through *in vivo* pollination technique. The gene pool of turmeric has cultivars for high yield, curing percentage, curcumin content and disease resistance.

In vitro pollination technique could be used for the improvement of medium and long duration cultivars, provided they are used as female parents and short duration cultivars as male parents. The technique need refinement with respect to media requirements, for the development of seeds. The conditions required for the germination of seeds also should be found out.

SUMMARY

SUMMARY

Investigations were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1997 to 1999 on "Response of turmeric *Curcuma domestica* Val. to *in vivo* and *in vitro* pollination". The salient findings of the study are summarized as follows.

1. The selected cultivars of turmeric viz short duration cultivars VK 70, VK 55, VK 76, Suguna, Sudharsana, Suvarna and medium duration cultivars Kanthi and Sobha differed significantly with respect to pseudostem, rhizome and quality characters. The curcumin content in the selected cultivars ranged from 2.13 per cent in VK 70 to 7.43 per cent in Sobha. The curcumin content was high in Kanthi i.e 7.02 per cent and VK 76 i.e 6.38 per cent while all others recorded < 5.10 percentage. Curing percentage ranged from 9.50 in VK 55 to 21.50 in VK 70. The cultivars VK 76 and Suvarna recorded a curing percentage of 20.0 and 19.50 respectively. In Kanthi and Sobha it ranged from 14 to 15 percent.

2. In turmeric the flowering season ranged from July to October.

3. Turmeric cultivars flowered within a period of 105 to 155 days. VK 70 was the earliest flowering cultivar while Kanthi and Sobha were late flowering types.

4. The turmeric inflorescence took 9.6 days from visual initiation to flower opening and the blooming completed within 9.71 days.

5. The flowers are characterized by long style of mean length 4.43 cm.

6. The ovary measured a mean length of 2.6 mm and diameter of 2.4 mm and recorded a mean ovule number of 29.31. The ovules measured a mean length of 611.44 µm and breadth of 436.65 µm at the middle.

7. The anthesis in different turmeric cultivars started by 5 am and continued up to 6 am. Anther dehiscence took place between 7.15 am and 7.45 am.

8. The pollen grains are sticky, light yellow and ovoid to spherical in shape and measured a mean length of 76.675 μm and breadth of 57.390 μm. Among the cultivars studied, pollen grains were highly heterogenous in size.

9. Among the various artificial media tried for pollen germination under *in vitro* in modified ME_3 medium was the best. The pH reactions 4 to 8 influenced pollen germination and maximum pollen germination (32.55%) was recorded at pH 6.

10. The mean pollen fertility in turmeric cultivars as per acetocarmine stain test was 78.51 per cent. There was no significant difference among cultivars with respect to pollen fertility. Pollen fertility was high in the flowers at the lower portion (90.32%) and low at the middle (82.38%) and upper portions (63.13%).

11. Cultivars differed significantly with respect to pollen germination percentage. Germination percentage in short duration cultivars ranged from 23.75 to 46.08 while in medium duration cultivars it ranged from 8.22 to 11.5.

12. Pollen viability was influenced by the position of flowers in the inflorescence. It was high (32.55%) in the flowers at the lower portion and low in the upper portion (22.93%) of the inflorescence.

13. The optimum time for collection of roots for mitotic studies in turmeric was between 6.30 am and 7 am. The somatic chromosome number of short duration cultivars viz. VK 70 and Suvarna was found to be 2n = 84 and that of medium duration cultivar Kanthi as 2n = 63.

14. Natural fruitset and seedset were observed in short duration cultivars namely VK 70, VK 55, VK 76, Suguna and Sudharsana. No seedset was observed in medium duration cultivars Kanthi and Sobha. The fruits matured within a period of 20 to 22 days. The number of seeds per fruit ranged from 4 to 6.

15. In controlled *in vivo* pollination, seedset was obtained only through stigmatic pollination. Seedset was obtained in three of the twelve crosses tried viz VK 70 x VK 76, VK 70 x VK 55 and VK 70 x Suguna. Seedset was failure in crosses involving short and medium duration cultivars and medium and medium duration cultivars. *In vivo* stylar pollination did not produced any seedset.

16. The fruit of turmeric is a thick walled trilocular capsule with small black arillate seeds. The arillate seeds had two seed coats, the outer being thick and the inner one being thin. Seeds are filled with massive endosperm and embryo is seen towards the upper side of the ovule.

17. Murashige and Skoog (1962) basal medium at half and full strength along with supplements are suitable for culture establishment of turmeric ovary. Half strength MS was superior to full strength MS.

18. Occurrence of a mixture of endophytic bacteria caused difficulty in culture establishment. The various antibiotics, gentamycin (50 – 100 μg l^{-1}), kanamycin (50 – 100 μg l^{-1}), ampicillin (50 – 100 μg l^{-1}), ambystryn-S (50 – 100 μg l^{-1}), dicrystracin (100 – 500 μg l^{-1}) were not effective in controlling the bacterial population in the cultures.

19. In the basal medium of half MS with three percent sucrose, cytokinins, BAP 1-4 mg l^{-1} and kinetin 1-3 mg l^{-1} induced some ovule development after *in vitro* placental pollination. In the same way, auxins NAA 0.5–1 mg l^{-1}, IAA

0.2 mg l^{-1}, IBA 0.2 mg l^{-1} and 2,4-D 0.2–1 mg l^{-1} also induced some ovule development. The ovule development was more when auxins and cytokinins together were given. The combination of BAP (1-3 mg l^{-1}) or kinetin (1-4 mg l^{-1}) with NAA (0.5-1 mg l^{-1}) of 2,4-D 0.2 mg l^{-1} or IAA 0.2 mg l^{-1} were effective for ovule development. But the combination of BAP 4 mg l^{-1} with IAA 0.2 mg l^{-1} produced callusing instead of ovule swelling. The combination of NAA 0.5 mg l^{-1} with BAP 1 mg l^{-1} and kinetin 1 mg l^{-1} showed maximum ovule swelling in cultures. Hence the medium of half MS + 3 per cent sucrose + BAP 1 mg l^{-1} + kinetin 1 mg l^{-1} + NAA 0.5 mg l^{-1} can be taken as a template for the refinement of media requirements.

20. The ovules developed at three and six per cent levels of sucrose after *in vitro* pollination. On visual assessment of ovule development, three per cent sucrose concentration was found superior to six percent level.

21. The organic supplements CW 15 per cent v/v and CH 200 mg l^{-1} supported ovary and ovule development.

22. Different *in vitro* pollination techniques, stigmatic, stylar, intra ovarian, placental, modified placental and test-tube fertilization were attempted. Ovules/seeds developed in intra ovarian, placental and modified placental pollination techniques. These techniques can be used for conducting crosses involving short duration cultivars, short and medium duration cultivars provided medium duration cultivars used as female parents.

23. In the intra-ovarian pollination, the ovary developed in to fruit and attained a maximum size of six mm 20 DAP under *in vitro* condition.

24. Ovules/seeds developed after *in vitro* pollination were creamy white during the initial stage of development and changed to dark brown colour within a

period of 20 to 30 DAP. The *in vitro* developed seed had two seed coat and a cavity, partly filled with endosperm. Embryo development was not distinct.

25. Under *in vivo* condition, various treatments like keeping on moist sterile sand, soaking seeds in water for 24 h and acid scarification with 25 per cent HNO_3 failed to induce seed germination.

26. The seeds produced under *in vivo* germinated under *in vitro*, on moist filter paper. But failed to germinate in the medium of moist sand, mixture of moist sand and vermiculite at 1:1 proportion, basal medium and basal medium with BAP and kinetin.

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*Originals not seen

APPENDIX

APPENDIX-1

Abstract of analysis of variance for the effect of different treatments

Sl.No.	Table details and characters	Treatment MSS	Error MSS	Level of significance percentage
I	Table 11 – Variability in pseudostem characters			
1	Height in cm for plants of different cultivars	2665.486	191.24	1
2	Number of tillers per plant	5.457	1.22	1
3	Number of leaves per plant	100.171	30.18	1
4	Number of leaves per tiller per plant	3.906	1.478	5
5	Length of petiole (cm)	236.332	27.857	1
6	Length of leaf (cm)	507.257	47.205	1
7	Breadth of leaf (cm)	14.752	4.725	1
8	Area of leaf (cm^2)	226362.92	39908.864	1
II	Table 12 – Variability in rhizome and root characters of different turmeric cultivars			
1	No. of roots	753.362	144.152	1
2	Length of roots (cm)	124.753	25.261	1
3	No. of mother rhizome	0.858	0.887	NS
4	Length of mother rhizome (cm)	14.552	4.353	1
5	Girth of mother rhizome (cm)	8.937	1.828	1
6	No. of nodes	23.054	3.934	1
7	Length of internodes (cm)	0.561	0.119	1
8	No. of primary fingers	11.006	5.408	NS
9	No. of secondary fingers	67.510	34.027	NS
10	No. of tertiary fingers	69.615	25.894	5
11	Length of primary fingers (cm)	13.013	3.634	1
12	Length of secondary fingers (cm)	6.749	2.614	5
13	Length of tertiary fingers (cm)	0.573	0.850	NS
III	Table 15 – Effect of position of flowers in the inflorescence on the fertility and viability of pollen grains and pollen tube growth of turmeric cultivars			
1	Pollen fertility	13.250	271.716	NS
2	Pollen viability	692.652	42.614	1

RESPONSE OF TURMERIC *Curcuma domestica* Val. TO *IN VIVO* AND *IN VITRO* POLLINATION

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Investigations on "Response of turmeric *Curcuma domestica* Val. to *in vivo* and *in vitro* pollination" were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1997 to 1999.

Research was carried out with turmeric cultivars viz. VK 70, VK 55, VK 76, Suguna, Sudharsana, Suvarna, Kanthi and Sobha. Among them, Kanthi and Sobha were medium duration types and the rest were short duration types. The selected cultivars differed significantly with respect to morphological and quality characters. The curcurmin content was high in Sobha (7.43%), Kanthi (7.02%) and in VK 76 (6.38%) while all others recorded <5.1 percentage. The cultivars VK 70 (21.5%), VK 76 (20.0%) and Suvarna (19.5%) were noted for high curing percentage.

The floral biology and morphology of turmeric were studied. Turmeric cultivars took 105 to 155 days for flowering and the flowering season ranged from July to October.

The anthesis started by 5 am and continued up to 6 am. Anther dehiscence took place between 7.15 am and 7.45 am. Androecium consists of six stamens in two whorls of three each. The outer whorl is modified as labellum. The inner whorl is represented by two staminodes and one fertile stamen. Gynoecium has a long style of mean length 4.43 cm. The ovary measured a mean length of 2.6 mm and diameter of 2.4 mm and recorded a mean ovule number of 29.31. The ovules recorded a mean length of 611.44 μm and breadth of 436.65 μm at the middle. At the base of the flower honey secretion is present and ants are the pollinating agents.

The mean pollen fertility with acetocarmine stain in the studied cultivars were 78.51 per cent.

Attempts to develop a medium which will support pollen germination and tube growth in turmeric resulted in the identification of modified ME_3 medium of pH 6 (Leduc *et al.*, 1990). The pH reactions of the medium influenced the pollen germination.

Pollen germination was high in short duration type (23.75 to 46.08%) compared to medium duration types (8.22 to 11.50%). The pollen tube length also was higher in short duration cultivars (268.48 µm to 576.3 µm) compared to medium duration cultivars (218.72 µm to 245.45 µm).

Pollen germination was influenced by the position of flowers in the inflorescence. The germination was high in the flowers at the lower portion (32.55%) and low in the upper portion (22.93%) of the inflorescence.

The optimum time for collection of roots for mitotic studies in turmeric was between 6.30 am and 7 am. The somatic chromosome number of short duration cultivars viz. VK 70 and Suvarna were found to be 2n = 84 and that of medium duration cultivar Kanthi as 2n = 63.

Natural fruitset and seed set were observed in short duration cultivars and not noticed in medium duration cultivars. The natural pollinating agent in turmeric is insects i.e., ants.

Through controlled *in vivo* pollination, seed set was obtained in crosses involving short duration cultivars but failed in crosses involving short and medium duration cultivars.

The fruit of turmeric is a thick walled trilocular capsule with small black arillate seeds. Seeds are filled with massive endosperm and embryo is seen towards the upper side of the ovule.

Initial studies for culture establishment showed that the basal medium of ½ MS and MS are suitable. The half strength MS being superior to full strength MS.

Various antibiotics tried for controlling bacterial contamination in cultures were in effective.

Studies were made to standardize optimum media components for ovule development. The combination of NAA 0.5 mg l^{-1} with BAP and kinetin both at 1 mg l^{-1} induced maximum ovule swelling. On visual assessment, 3 per cent sucrose concentration was superior to 6 per cent level for ovule development. Organic supplements, coconut water (15% v/v) and CH (200 mg l^{-1}) enhanced ovule development.

In vitro pollination was done by pollen grains suspended in modified ME_3 medium. Among the various methods of pollination tried, ovules/seeds development were observed in the intra ovarian, placental and modified placental pollination techniques. These techniques can be used for conducting crosses involving short and medium duration cultivars provided medium duration cultivars used as female parents.

In the intra-ovarian pollination fruit development occurred. The ovary developed into fruit and attained a maximum size of 6 mm 20 DAP under *in vitro* condition. Ovules/seeds developed after *in vitro* pollination were creamy white during the initial stage of development and changed to dark brown colour within a period of 20 to 30 DAP. In the *in vitro* developed seed the endosperm development was not complete.

The seeds developed under *in vivo*, germinated under *in vitro* on moist filter paper.