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Response of turmeric (*Curcuma domestica* Val.) to *in vivo* and *in vitro* pollination

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Abstract

Seed set and development were obtained through *in vivo* pollination in three combinations of short duration turmeric cultivars and these were not observed in crosses of medium duration cultivars and short x medium duration cultivars. The fruit developed after *in vivo* pollination was a thick walled trilocular capsule. It took 20-22 days for development and ripening. Arilled seeds are triangular with two seed coats. The seeds are filled with massive endosperm and the embryo is seen oriented towards the chalazal end of the seed. In the *in vitro* pollination studies, culture establishment and ovule development were obtained in half strength MS + NAA 0.5 mg l^{-1} with BAP and kinetin at 1 mg l^{-1} each. Among the various methods of pollination tried, ovule $seed^{-1}$ development was observed in the intra ovarian, placental and modified placental pollination techniques. In the *in vitro* developed seed, the endosperm development was not complete. The aforesaid *in vitro* techniques need refinement with respect to medium and other requirements for the germination of *in vitro* produced seeds.

Key words : *Curcuma domestica, in vivo* and *in vitro* pollination, seed structure, turmeric.

Introduction

Cultivated turmeric shows high variability for crop duration, dry yield, curing percentage, curcumin content and disease resistance. Turmeric can be grouped into short, medium and long duration types based on the crop duration. In short duration group quite often good genotypes with exceptionally high curing percentage (>20%) are found. Medium duration group is noted for richness in colouring pigment (curcumin), but low in curing percentage. Internationally known Alleppey turmeric falls in the group of medium duration. Long duration types are good in fresh yield of turmeric but moderate in quality parameters like curing percentage and curcumin. Thus, in turmeric, the desirable economic characters are scattered in various genotypes. They can be assembled in single genotype by a planned hybridization programme.

Short duration types are generally fertile tetraploids (2n=84), while medium and long duration types are sterile triploids (2n=63) (Renjith 1999). Nazeem & Menon (1994) have reported seed set in controlled crosses of short duration types, but encountered difficulty in crossing short duration types with medium and long duration types, as one of the parents involved was triploid. *In vitro* pollination and fertilization is an effective tool for overcoming

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this type of incompatibility reactions hindering seed set. The present study is aimed at developing a hybridisation technique for 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 per cent of cultivated area under turmeric in India.

Materials and methods

Two medium duration turmeric (*Curcuma domestica* Val.) varieties (Kanthi and Sobha) and five short duration types/varieties (VK-70, VK-55, VK-76, Suguna and Sudharsana) used in the study were raised in the field for flowers for conducting pollination studies.

In vivo *pollination*

Controlled *in vivo* stigmatic and stylar pollination were carried out among the selected cultivars in cross combinations. The stigmatic pollination was done as per the method described by Nazeem & Menon (1994). Emasculation was done one day prior to anthesis, in the evening from 4 to 6 pm. The rest of the floral buds including developing ones present in the same bract were 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 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 for few days.

In vivo stylar pollination was done by cutting the styles at different lengths and by applying pollen grains along with pollen germinating medium i.e., modified ME_3 medium (Leduc *et al.* 1990) at the cut end. Seed set and development were monitored. *In vivo* produced fruit and seed are described.

In vitro *pollination*

Flower buds of turmeric one day prior to anthesis, on the day of anthesis and opened flowers were collected and washed thoroughly. They were surface sterilized by immersing in streptocycline (350 mg l^{-1}) for one hour, followed by treatment with 0.1% mercuric chloride for three minutes. Culture establishment was tried in two basal media combinations namely, half and full strength MS (Murashige & Skoog 1962) and SH (Schenk & Hildebrandt 1972) along with BAP 1 mg l^{-1} and kinetin 1 mg l^{-1} and auxins. The pollinated ovaries with intra ovarian pollination technique were incubated and swelling of the ovary was scored 20 days after pollination (DAP).

Different *in vitro* pollination techniques namely, stigmatic, stylar, intra-ovarian, placental, modified placental pollination and ovular/test tube fertilization (Bhojwani & Razdan, 1983) were tried for standardizing an appropriate *in vitro* pollination technique for ovule/seed development. *In vitro* pollination was done with pollen grains suspended in modified ME_3 medium. The ovaries/ovules after pollination were placed in the culture medium and observations were recorded at 10 day intervals.

Results and discussion

In vivo *pollination*

Seed set was obtained only in *in vivo* stigmatic pollination and did not produce seed in stylar pollination. Seed set and development were observed in three of the twelve crosses tried namely, VK-70 x VK-55, VK-70 x VK-76 and VK-70 x Suguna. Seed set was not observed in crosses of VK-55 x VK-76, Suguna x VK-76, Suguna x Sudharsana, Sudharsana x VK-70, Sudharsana x Suguna, eventhough they are tetraploids. The crosses involving medium duration cultivars, Kanthi and Sobha, and vice-versa also failed to set seeds. Similarly, crosses involving short duration cultivar (Suguna) and medium duration cultivar (Kanthi) also failed to set seeds.

Nazeem & Menon (1994) have reported high seed set in crosses involving *Curcuma 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 developing

fruits by bacterial contamination. The presence of open bracts in inflorescence promotes harbouring of bacterial inoculum along with rain water. So the crosses have to be repeated for conclusive results. The causes for absence of seed set in crosses involving medium duration cultivars, Kanthi x Sobha and vice–versa have to be examined thoroughly. Pollen pistil interaction studies can be made to confirm, that the pollen tube growth is sufficient to reach the deeply seated ovules of the female parent. Besides, *in vivo* pollination studies can be made in plants grown under controlled condition. So the prevention of seed set by bacterial and fungal contamination could be controlled. *In vitro* pollination is tried in the absence of seed set under *in vivo* condition.

The fruit developed after *in vivo* pollination was a thick walled trilocular capsule. It took 20-22 days for development and ripening. Arilled seeds are triangular with two seed coats (Fig. 1.a). The seeds are filled with massive endosperm and the embryo is seen oriented towards chalazal end of the seed (Fig. 1.b). The observations made are in confirmation with the earlier reports (Nambiar *et al.* 1982; Lad 1993).

In vitro *pollination*

Flower buds on the day of anthesis only were suitable for *in vitro* pollination. MS basal medium at half and full strength along with supplements were suitable for culture establishment of turmeric ovary. The SH medium with supplements did not promote any ovary and ovule development (Table 1). The percentage of cultures showing ovule development in half MS media combinations ranged from 75.00 to 82.61, while it was 55.56 to 66.67 in full MS. So half strength MS was superior to full strength MS.

In half strength MS, the combination of growth regulators namely, NAA 0.5 mg l^{-1} with BAP 1 mg l^{-1} and kinetin 1 mg l^{-1}, caused more ovule swelling in cultures (82.61%). The cultures with

Table 1. Culture establishment in different basal media

Treatment	Ovule swelling	Cultures showing ovule development* (%)
½ MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	Good	82.61
½ MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	Low	75.00
MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	Low	66.67
MS + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	Low	55.56
SH + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 0.5 mg l^{-1}	Nil	0.00
SH + BAP 1 mg l^{-1} + Kin 1 mg l^{-1} + NAA 1.0 mg l^{-1}	Nil	0.00

*Average of 12 observations

Table 2. Standardisation of *in vitro* pollination technique in turmeric

Method of pollination	Cultures with ovary development*(%)	Cultures with ovule development*(%)
Stigmatic pollination	0.00	0.00
Stylar pollination	0.00	0.00
Intra-ovarian pollination	81.82	45.45
Placental pollination	NA	63.49
Modified Placental pollination	NA	63.49
Ovular or Test tube fertilization	0.00	0.00

*Average of 12 observations

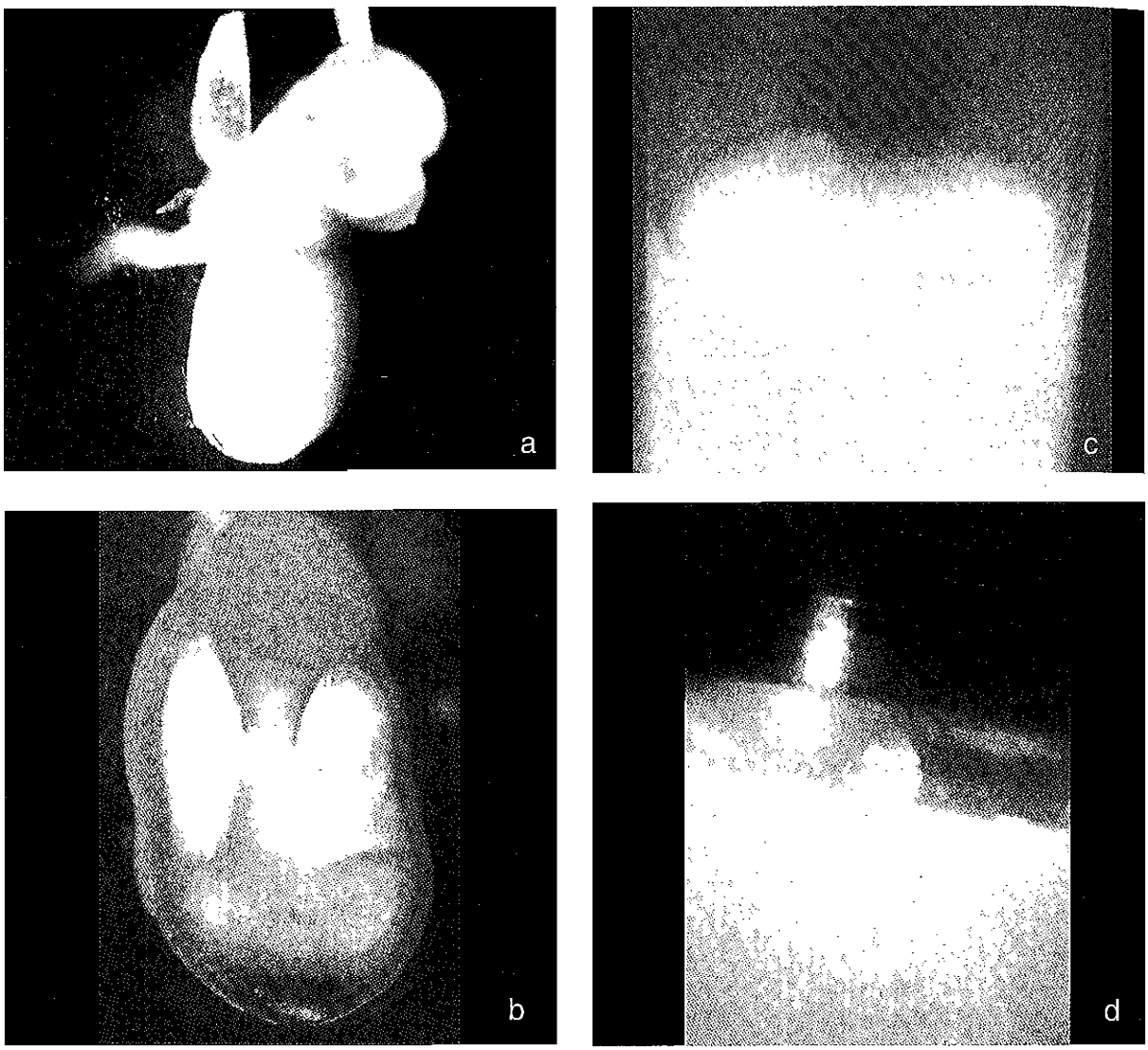

Fig. 1. Development of *in vivo* and *in vitro* pollinated turmeric seeds
a. an arillate seed b. L. S. of a mature seed stained with safranin (X 20) c. *in vitro* fruit development
d. developing ovules 10 days after *in vitro* placental pollination (X 20)

ovule development declined to 75 per cent when NAA concentration was increased from 0.5 to 1 mg l^{-1}. The same trend was observed in full MS media combination (Table 1).

In the different *in vitro* pollination techniques tried, ovules/seeds developed in the intra ovarian, placental and modified placental pollination techniques (Table 2). Culturing of turmeric ovary after intra-ovarian pollination caused ovary development in 81.82 per cent of cultures. The ovule development was 45.45 per cent compared to 63.49 per cent in placental and modified placental pollination. Pollination of individual ovules (ovular or test-tube fertilization) did not cause any ovule development. *In vitro* stigmatic and stylar pollination also did not promote any ovary or ovule development.

Valsala (1994) has also reported seed set and

seed development through placental and modified placental pollination techniques in ginger. Ovule development can be the effect of suitable medium and fertilization. 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 even in the improved pollen germination medium (Renjith 1999).

In the intra-ovarian pollination, ovary developed into fruit and attained a maximum size of 6 mm at 20 DAP under *in vitro* conditions (Fig. 1.c). They were creamy white in diffused light. Ovules developed in the intra ovarian, placental (Fig. 1.d) and modified placental pollination techniques. They 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. This shows that the media compositions for the development of seeds need further refinement. Under *in vivo* condition, the maturity periods for fruit and seed development were 20 and 22 days respectively. Under *in vitro* condition it was 20 to 30 days.

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