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SEED SET IN GINGER (*ZINGIBER OFFICINALE* ROSC.) THROUGH *IN VITRO* POLLINATION

P. A. Valsala, G. Sreekandan Nair and P. A. Nazeem
College of Horticulture, Vellanikkara 680 654, Trichur, India

Abstract: Different *in vitro* pollination techniques viz., stigmatic, stylar, intra-ovarian, placental, modified placental pollination and test-tube fertilization were tried in ginger flowers. The pollen grains suspended in ME, medium were used for pollination. The pollinated pistil or its part was cultured in the medium of 1/2 MS + NAA 0.5 mg l^{-1} + BAP 2.5 mg l^{-1} + coconut water 15 per cent v/v. Seed development was obtained in placental, modified placental pollination and test tube fertilization. The seed germinated under *in vitro* condition on supply of appropriate combination of 2,4-D, BAP and NAA.

Key words: Culture medium, ME_3 medium, placental pollination, seeds.

INTRODUCTION

Natural seed set does not occur in ginger and thus genetic variability is very much limited in this crop on account of its exclusive vegetative propagation. Conventional *in vivo* methods like artificial cross pollination on stigma and on style cut at various heights, bud pollination, chemically aided pollination, mixed pollination and mentor pollination tried to induce seed set, did not give any positive response so far. Investigations carried out by Sathiabhama (1988) for the lack of seed set in ginger revealed that the pollen tube growth is not sufficient to fertilize the deeply seated ovules. According to Bhojwani and Razdan (1983) the technique of *in vitro* pollination and fertilization is very promising for overcoming pre-fertilization barriers to compatibility and for raising new genotypes through seed propagation. Hence *in vitro* pollination and fertilization techniques were attempted to get viable seeds so as to generate a variable population of ginger.

MATERIALS AND METHODS

Different *in vitro* pollination techniques as compiled by Bhojwani and Razdan (1983) were carried out using flower buds of popular cultivars of ginger namely, Rio-de-Janeiro and Maran. The flower buds on the day of anthesis, prior to flower opening were surface sterilized. Surface sterilization was effected by dipping the flower buds in 500 mg l^{-1} streptocylcin for 1 h followed by wiping with 70 per cent alcohol and rinsing with mercuric chloride 0.1 per cent for 3 min. The gynoecium was prepared depending up on the pollination technique followed. The pollen grains were scooped out with a scalpel and were deposited on the specific part of the gynoecium along with a drop of ME_3 medium of pH 6 (Leduc *et al.*, 1990).

The following techniques of *in vitro* pollination were tried:

1. *Stigmatic pollination:* The surface sterilized flower buds ready for opening were allowed to dehisce naturally and pollen grains were deposited on the stigma with the help of a needle.

2. *Stylar pollination:* The style was cut at different levels and pollen grains were applied on the style along with pollen germinating medium.

3. *Intra-ovarian pollination:* Intra-ovarian pollination was done in three ways. In all the cases, the style and corolk tube were removed just above the ovary and only the ovary was used for pollination. In intra-ovarian pollination-1, pollen grains were applied on the top of the ovary at the cut surface. In intra-ovarian pollination-2, a slit was made on the top of the ovary by inserting a surgical blade and pollen grains were applied in the slit. Intra-ovarian pollination-3 was done by injecting the pollen suspension in pollen germinating medium into the ovary with the help of a syringe.

4. *Placental pollination:* Placental pollination was tried in two ways. In placental pollination-1, placenta bearing the ovules were exposed by completely peeling the ovary wall and

Table 1. Effect of method of pollen application in *in vitro* cross pollination of ginger cv. Maran x Rio-de-Janeiro

Sl. No.	Methods of pollen application	Cultures with ovary development, %	Cultures with developed ovules, %	No. of ovules developed / culture
1	Stigmatic pollination	83.3	0.0	0.0
2	Stylar pollination	77.8	0.0	0.0
3	Intra-ovarian pollination-1	83.3	0.0	0.0
4	Intra-ovarian pollination-2	77.8	0.0	0.0
5	Intra-ovarian pollination-3	80.0	0.0	0.0
6	Placental pollination-1	NA	83.3	15.8
7	Placental pollination-2	NA	77.7	6.8
8	Modified placental pollination	77.7	77.7	5.2
9	Ovular or test-tube fertilization	NA	83.3	14.5

NA - Not applicable; Medium: 1/2 MS + 6% sucrose + BAP 2.5 rag l^{-1} + NAA 0.5 mg l^{-1} + coconut water 15% v/v; Explant: Pollinated gynoecium or part of it

Table 2. Effect of method of pollen application in *in vitro* self pollination of ginger cv. Maran

Sl. No.	Methods of pollen application	Cultures with ovary development, %	Cultures with developed ovules, %	No. of ovules developed / culture
1	Stigmatic pollination	77.8	0.0	0.0
2	Stylar pollination	83.3	0.0	0.0
3	Intra-ovarian pollination-1	77.8	0.0	0.0
4	Intra-ovarian pollination-2	72.2	0.0	0.0
5	Intra-ovarian pollination-3	72.2	0.0	0.0
6	Placental pollination-1	NA	83.3	15.7
7	Placental pollination-2	NA	77.7	8.9
8	Modified placental pollination	77.8	83.3	6.2
9	Ovular or test-tube fertilization	NA	77.7	15.6

NA - Not applicable; Medium: 1/2 MS + 6% sucrose + BAP 2.5 mg l^{-1} + NAA 0.5 mg l^{-1} + coconut water 15% v/v; Explant: Pollinated gynoecium or part of it

pollination was done on it. In placental pollination-2, placenta bearing the ovules were cut into two pieces longitudinally and pollination was done.

5. *Modified placental pollination:* Ovary wall of the locule was peeled away and pollination was done on the exposed ovules.

6. *Ovular pollination / test-tube fertilization:* The ovules were separated and put in a cavity slide containing pollen suspension in pollen germinating medium. After 24 hours, this was transferred to culture medium.

In all cases, self as well as cross pollination was tried. In cross pollination, cultivar Maran was used as female and Rio-de-Janeiro as male parent. Self pollination was done in Maran only. The pollinated gynoecium immediately cultured in half strength MS medium supplemented with BAP 2.5, NAA 0.5 mg l^{-1} and coconut water 15 per cent (v/v). Observations were recorded for swelling of the ovules.

Table 3. Effect of treatments on the germination of ginger seeds

Treatments	Sucrose % in the medium	Germination %
Distilled water	-	-
Sand and vermiculite mixture	-	-
Basal medium, 1/2 MS	3.0	-
GA 0.2, 1, 2, 5 mg l^{-1}	3.0	-
BAP 2, 5, 10, 25 mg l^{1}	3.0	-
BAP 2.5 mg 1 ' with 0.1, 0.5 mg l^{-1} NAA	3.0	-
BAP 2 mg 1^{1} with 0.05 mg l^{-1} IAA	3.0	-
BAP 2 mg l^{-1} with 0.1 mg l^{1} 2,4-D	3.0	-
BAP 5 mg 1 ' with 0.1 mg 1^{-1} NAA	3.0	-
BAP 5 mg 1^{-1} with 0.1 mg l^{-1} 2,4-D	3.0	-
BAP 2.5 mg 1^{1} with 0.1 rag l^{1} NAA	8.0	-
BAP 2.5 mg 1 ^{1} with 0.05 mg l^{1} IAA	8.0	-
BAP 2.5 mg l^{-1} with 0.1 mg 1^{-1} 2,4-D	8.0	-
BAP 5 mg l^{1} with 0.1 mg 1^{-1} 2,4-D	8.0	-
BAP 5 mg l^{-1} with 0.05 rag 1^{-1} IAA	8.0	-
BAP 5 mg l^{-1} with 0.1 mg 1^{-1} 2,4-D	8.0	-
2,4-D 8 mg l^{1} and BAP 9 rag l^{-1} + 2,4-D 0.1 mg l^{-1} (subculture)	.3.0	5.0

*Average of 20 observations

The seeds were subcultured at 20 days interval to the same medium of 1/2 MS + BAP 2.5 mg 1^{-1} + NAA 0.5 mg l^{-1} + 15 per cent CW (v/v). The seeds of 80 DAP were incubated in various culture media for germination as detailed in Table 3. In all the treatments except with distilled water as well as sand and vermiculite mixture, solid and liquid forms of media were tested. The cultures were kept in dark and diffused light at 26 ± 2°C and were observed for germination for 80 days.

RESULTSANDDISCUSSION

Among the various *in vitro* cross pollination methods tried, ovules were found to develop in placental pollination, modified placental pollination and test-tube fertilization (Table 1). Both types of placental pollination have resulted in ovule development. About 77.8 to 83.3 per cent of cultures showed ovule development in the different pollination methods. The number of ovules per culture was maximum in placental pollination-1 (15.8) followed by test-tube fertilization (14.5). It was minimum in modified placental pollination technique (5.2).

Ovaries developed into fruit in stigmatic, stylar, intra-ovarian and modified placental pollination techniques, but none of the developed ovaries contained seeds except in modified placental pollination. There also ovules developed on the exposed side only. About 77.8 to 83.3 per cent of the total cultures showed ovary response.

With self pollination also, ovule development was observed in placental pollination, modified placental pollination and ovular or test-tube fertilization (Table 2). About 77.8 to 83.3 per cent of cultures showed ovule development. Here also maximum ovule development per culture was observed in placental pollination-1 (15.7), followed by test-tube or ovular fertilization (15.6). Ovule development was minimum in modified placental pollination (6.2).

In vitro seed set was obtained only in placental pollination, modified placental pollination and ovular or test-tube fertilization (Table 1 and 2). In all the three cases pollen grains along with pollen germinating medium were applied over the ovules. The reason attributed by Sathiabhama (1988) for lack of seed set in ginger under *in vivo* is the lengthy style and limited pollen tube growth. The maximum pollen tube growth obtained in her study was only 108 µm while the mean style length of the ginger flowers were 3.5 cm. Since in the

successful pollination techniques the pollen grains are applied over the ovules, the distance to be traversed for effecting the pollination is very little. Hence it could be assured that ovule development / seed development occurs only after fertilization.

Among the various successful *in vitro* fertilization techniques, maximum number of seeds i.e., 15.8 was recorded in placental pollination-1. The mean number of ovules in ginger flowers was found to be 24.4. Hence theoretically, from a single ovary 24 seeds will be obtained. This shows under *in vitro* condition all the 24 ovules did not develop into seeds. The mean pollen fertility reported in ginger flowers was 22.67 per cent (Valsala, 1994). Hence some male and female infertility might have contributed for the non-development of some ovules.

Dark coloured arillate mature seeds were obtained 80 days after pollination. The *in vitro* produced seed of ginger germinated, when 80 days old seeds were incubated initially in the medium of 1/2 MS with 2,4-D 8 mg l^{-1} for two months and then in hormone combination of BAP 9 mg l^{-1} and 2,4-D 0.1 mg l^{-1} (Table 3). The ginger seeds did not germinate in distilled water, sand and vermiculite mixture. It did not germinate in the basal medium alone but germinated when appropriate quantity of 2,4-D and BAP were given. This shows that for the development of ginger embryo into seedling hormones are required.

This is the first report of formation of seeds in ginger, through *in vitro* pollination and fertilization. According to Hooker (1894) ginger is a species never setting seeds. Natural seed set is not occurring in this crop due to problems of spiny stigma, long style and limited pollen tube growth as described earlier by Sathiabhama (1988). The technique of *in vitro* placental pollination is a potential tool for overcoming pre-fertilization barriers in selfing and crossing programmes. *Petunia axillaris* and *Petunia hybrida* are two self incompatible species, where seed set could be obtained through *in vitro* placental pollination (Rangaswamy and Shivanna, 1971; Nimi, 1970). Dhaliwal and King (1978) have raised interspecific hybrids of *Zea mays* and *Zea mexicana* using this technique. The results of the present study can have tremendous potentiality in ginger breeding as it opens the path for hybridization between cultivars to induce genetic variability.

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