

CYTOMORPHOLOGICAL BARRIERS IN SEED SET OF CULTIVATED GINGER (*ZINGIBER OFFICINALE* ROSC.)

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Studies on floral biology, meiotic behaviour and pollen sterility in four cultivars of ginger (*Zingiber officinale* Rosc.) revealed that anthesis in the green house and open field conditions took place between 1-2 P. M. and 9-10 A. M. respectively. The hermaphrodite flowers had "pin" and "thrum" type of incompatibility and the dehisced pollen grains did not reach the stigma head. No seed set of ginger during selfing or cross pollination was recorded. Meiotic studies revealed 30.35 to 40.50% of meiotic index in the studied cultivars of ginger namely Bhaisey, Ernad Chanad, Gorubathaney and Turia Local. Pollen Mother Cells (PMCs) showed incomplete homologous pairing in metaphase I and spindle abnormalities (late separation, laggard chromosomes, sticky bridge and etc.) in anaphase I leading to pollen sterility (60.80%). Lack of seed set inspite of selfing and cross pollination might be due to non-homology in bivalents with irregular segregation of genomic complements leading to sterile gamet formation. Failure of germination of pollen in artificial media (2-15% sucrose solution) was due to the absence of pollen germinating pores and which was confirmed by Scanning Electron Microscopic studies.

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Key words. Crossing, floral biology, ginger, meiosis, pollen, scanning electron microscopy, *Zingiber*.

موانع تشکیل بذر در زنجبیل (*Zingiber officinale*)

پرماناندا داس، اس رای و آنات باندهو داس

مطالعات زیست‌شناسی گل، رفتار کروموزومی و ناباروری دانه‌گرده در چهار رقم زنجبیل نشان داد که گلدهی در شرایط گلخانه و محیط باز به ترتیب بین یک تا دو بعد از ظهر و نه تا ده قبل از ظهر رخ می‌دهد. گل‌های نر ماده ناسازگاری از نوع *pin* و *thrum* داشتند و دانه‌های گرده به کلالة نمی‌رسیدند. تشکیل بذر زنجبیل در گرده‌افشانی‌های خودگشن و دگرگشن مشاهده نگردید. مطالعات میوز، اندکس ۳۵/۳۰ تا ۵۰/۴۰ را در ارقام زیر نشان داد.

"Bhaisey"; "Ernad Chanad"; "Gorubathaney"; "Turia Local".

در سلول‌های مادر دانه‌گرده در مرحله متافاز اول کروموزومها به طور ناقص جفت شدند و در آنافاز اول دوکهای غیرعادی مشاهده گردید که همین عوامل موجب ناباروری دانه‌گرده (۶۰/۸۰٪) می‌گردد. عدم تشکیل بذر علیرغم خودگشنی و یا دگرگشنی ممکن است به علت ناهمگنی زوج کروموزومها باشد که منجر به تشکیل گامت نابارور می‌شود. عدم تنش دانه‌گرده در محیط کشت مصنوعی (۲ تا ۱۵٪ محلول سوکروز) به علت نبود مجاری تنشی بود که از طریق انجام مطالعات میکروسکوپ الکترونی اسکن تأیید گردید.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.), a vegetatively propagated perennial herb of the family *Zingiberaceae* is a major spice cultivated in India both for internal consumption and export. It is principally used as an ingredient in various spice blends in food processing and beverage industries. In the West, ginger biscuits, ginger cakes, ginger bread and ginger puddings are favourite products and ginger is main ingredient in chutneys, jams and preservatives. Ginger is an essential flavourant in Chinese cuisine and the pungent essence of ginger is due to gingerole, zingerone and shogaols (Leung 1984). Dry ginger is commonly used for the extraction of oleoresin and essential oil. It is used in folk medicine since centuries both in the East and the West and has diaphoretic effect. It is carminative, appetite stimulant, and is used in veterinary medicine for treatment of indigestion in horses and cattle (Ravindran & al. 1994).

Till today, crop improvement in ginger is limited to germplasm selection and mutation breeding (Ravindran & al. 1994). Sasikumar & al. (1994) reported normal flowering with moderate pollen fertility: 8-45% and P. A. Okwuowulu in Nigeria

observed 60-80% (personal communication) fertile pollen grains through aceto-carmin test. However, there is no report on seed set in ginger which is the major bottleneck in recombination breeding. The present thrust area of breeding is for a resistant cultivar against rhizome rot disease of ginger which is the most serious problem throughout ginger growing areas in the world. Routine plant protection practices have not helped much to protect ginger against the deadly rhizome rot disease causing loss of hundreds of tons of ginger every year around the world. Production of viable seeds, if possible, might make a major breakthrough in recombination breeding of ginger. The somatic chromosome number of ginger $2n=22$ was reported by Sugiura (1928), Sharma and Bhattacharya (1959) and Ramachandran (1969) but $2n=24$ and 66 was reported by Kihara & al. (1931) and Bison & al. (1968) respectively. Detailed karyotype analysis and $4C$ DNA estimation in different cultivars were carried out by Rai & al. (1997). To achieve viable seed set, detail morphological study of the flower and meiotic behaviour during gamet formation are essential. In the present

investigation, the floral biology, meiotic compatibility in chiasma formation, pollen sterility and Scanning Electron Microscopic (SEM) studies on pollen morphology and meiotic abnormalities in relation to seed set are reported.

MATERIALS AND METHODS

Study of floral biology

The inflorescence of four ginger cultivars i. e., "Bhaisey", "Ernad Charnad", "Gorubathaney", and "Tural Local" were studied for floral biology in both the field and green house grown plants. The morphological characters of the flowers were recorded in the laboratory with the help of Nikon Stereo Zoom research microscope.

Selfing and cross pollination

For selfing, the flowers were selected at the time of opening and the pollen grains were collected carefully with the help of camel hair brush (from its own anther) and dusted on the stigma lobe having hair like projections all around the wall with a hollow centre which runs down to the ovary through the style. The selfing was

done at 10.00, 12.00, 14.00, 16.00, 18.00 and 19.00 hrs. to know the exact time of receptivity. The selfed flowers were tagged and immediately covered with a perforated butter paper bags to avoid further pollination.

The flowers in four cultivars studied were selected for cross pollination in all possible combinations. The flowers were carefully emasculated; protected against cross pollination or damage by other external agencies like insects, rain etc. with the help of a perforated butter paper bag and were tagged. The emasculated flowers were pollinated with pollen grains collected from the selected pollen parent during 10.00, 12.00, 14.00, 16.00, 18.00 and 19.00 hrs.

Meiotic studies

Fresh flower buds were collected around 9 A. M. from the inflorescence and fixed in modified Carnoy's solution (1 part propionic acid: 3 parts chloroform: 6 parts ethanol) for 3 hr and transferred to 70% ethanol. The flower buds of suitable size were smeared in 2% propionic-carmin and observed under oil immersion objectives.

Study of pollen fertility

For pollen fertility studies, the anthers were collected at the time of anthesis and the pollens were dusted on clean slides in a drop of 2% aceto-carmin. The stained pollens were counted as viable; for each cultivar, 800 pollens were studied for their fertility count.

Scanning Electron Microscopy

Mature anthers were collected and sun dried. Pollens of each cultivar were dusted on separated metal stub precoated with adhesive tape. Each sample was coated with a (100-200° A) thick layer of gold-palladium in a rotating and tilting vacuum coating apparatus. The gold coated pollens were scanned with the Philips SEM 500 (PSM 500) Scanning Electron Microscope operating at 25 KV at the Regional Sophisticated Instrumentation Centre, Bose Institute, Calcutta. Photographs were taken with indicator scales at appropriate magnifications.

OBSERVATION AND DISCUSSION

Floral biology

Ginger grown under both the green house and the open field conditions flowered normally. The anthesis was recorded at

9-10 A. M. in the open field while those kept in the green house flowered only between 1-2 P. M. under Bhubaneswar conditions. An inflorescence had 12-18 flowers on an average and flowers opened in the upward direction beginning from the lowermost floret (Fig. 1). Normally only one flower opens in a day, however, occasionally opening of two flowers were also recorded. The flowers are irregular and the most distinctive part being a conspicuous two-lobed lip (labellum) produced by the fusion of two staminodes. There is only one functional stamen, corresponding to one of the members of the inner whorl of stamens; the other two members were absent altogether. The flower is enclosed by three outer perianth segments fused into a tubular calyx, and three inner segments which are petal like, more or less united with the posterior segment large. The whole is subtended by a sheathing green bract (Fig. 2). The style is so thin that it can not support itself. It is fused to a groove along the length of the anther and only the pin headed long stigma protrudes above it (Fig. 3). The ovary is inferior with fused carpels and axil placentation. There are numerous ovules in each locule. The ginger flower has pin and

thrum type of incompatibility, where one out of three anther is fertile and is wrapped by modified filament of the anther which opens for pollen dispersal when matured. The modified filament wrap thin style which is 3-4 cm long and leaves only the stigma head open. The stigma head is slightly bigger in size than the thin style body with feathery structure all around the wall leaving central part open and hollow. The position of male gamete and the female receptive part (stigma head) indicated positional sterility and even if this plant would had been producing viable seeds, ginger could very well be placed under the cross pollinated crops. We assumed that the rain water which accumulated in the bracts of the inflorescence much before flowering interfered with the seed set. Although it did not affect normal flowering, it might cause rotting of embryo. With this idea, we removed all the bracts several days before flowering to prevent water accumulation, but we failed to get seed set by both selfing and crossing (Fig. 4). P. A. Okwuowulu of National Root Crop Research Institute, Umudike, Nigeria observed difficulties in synchronisation in the receptive period of the ovule and anthesis in cross pollination

(personal communication).

Selfing and cross pollination

Ginger stigma head is dry and hairy without any sticky material, the pollenkitt or tryphine (Fig. 5). However, in the four cultivars viz., Bhaisey, Ernad Charnad, Gorubathaney and Turia Local, selfing and crossing every two hours intervals from 10.00 A. M. to 7.00 P. M. failed to set seed (Fig. 4). The failure in seed set might be due to sexual incompatibility at cultivar level. However, crossing between four cultivars in all possible combinations (Table 1) did not yield any positive result. During selfing or cross pollination accumulation of rain water though sometimes rotted the flowers, removal of the bracts to prevent water accumulation did not help in seed set of ginger which suggest that these might be genetic incompatibility of the species during its micro-evolution.

Meiotic study and pollen fertility

Studies on the meiotic behaviour of the ginger cultivars showed incomplete pairing of the 11 bivalents in metaphase I (Fig. 6). The meiotic index varied from 30.35% in "Turial Local" to 40.50% in "Bhaisey"

(table 2). Different types of spindle abnormalities like late separation, clumping, early separation, laggards, fragmented chromosomes, sticky bridge were found in the first meiotic anaphase division (Figs. 7 & 8). The average abnormal cells in PMC's showed the minimum (51.02%) in "Bhaisey" and the maximum (71.31%) "Gorubathaney". Perhaps, the abnormal PMC's ultimately produced the nonfertile, abortive pollen grains during macrosporogenesis (Fig. 9). Pollen sterility were high which confirmed high meiotic abnormalities observed by Sasikumar & al. (1994). The ginger pollen grain failed to germinate in 2-15% sucrose solution. Further investigation is needed to find out suitable media for germination of ginger pollen grains.

SEM study of the pollen

Scanning Electron Microscopic (SEM) studies of pollen grains of the different cultivars showed two types of pollen grains. Very few round, globose so-called fertile pollen grains and large number of wrinkled and flat nonfertile pollen grains were found in the cultivars studied (Figs. 10-13). In most plant species the outer wall of the pollen grains contain one or more

apertures, designated areas for pollen germination and tube growth. In few plant species including ginger however, such germinal apertures are absent (Knox and Williams 1986) which was confirmed by scanning electron microscopic studies of ginger pollen grains (Fig. 11). Knox (1984 a, b) reported that in plant species which do not have such germinal aperture, an opening is produced de novo by the pollen tube at germination.

In conclusion, beside its "pin" and "thrum" type of incompatibility, genetic incompatibility caused major hindrance in seed set in ginger. Meiotic abnormalities like late separation, laggard chromosomes, sticky bridge and etc. were responsible for high pollen sterility. Lack of pollen germination in sucrose solution suggest the absence of pollen germinating pores which was confirmed by SEM study. Studies on mutation breeding, somatic hybridization and *Agrobacterium* mediated genetic transformation might help in developing soft-rot resistant/tolerant cultivars in ginger.

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Table: 1. Selfing and possible combination of crossing in four cultivars of ginger; V1 = "Bhaisey", V2 = "Ernad Charand", V3 = "Gorubathaney", V4 = "Turia Local".

Cultivars	Cross combinations and selfed			
	V1	V2	V3	V4
V1	V1×V1	V1×V2	V1×V3	V1×V4
V2	V2×V1	V2×V2	V2×V3	V2×V4
V3	V3×V1	V3×V2	V3×V3	V3×V4
V4	V4×V1	V4×V2	V4×V3	V4×V4

Table 2. Meiotic index, meiotic abnormalities and pollen sterility percentage in 4 cultivars of ginger.

Cultivars	Meiotic index (%)	Meiotic abnormalities (%)	Pollen sterility (%)
Bhaisey	40.50	51.02	40.88
Ernad Charnad	33.37	67.08	57.13
Gorubathaney	35.01	71.31	60.80
Turia Local	30.35	51.81	41.37

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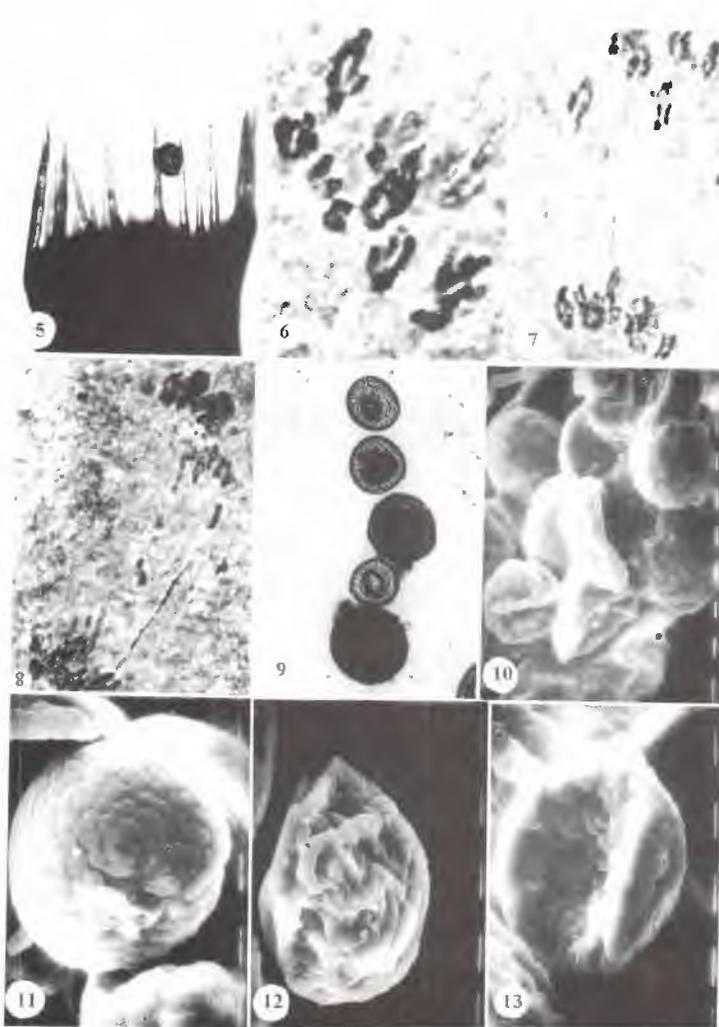
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Figs. 1-4: *Zingiber officinale*. -1: Inflorescence. -2: Inflorescence showing a flower with stigma head, labellum and elongated pistil. -3: The pin and thrum type of incompatibility. -4: The inflorescence after removal of bracts and the stigma head of gynoecium.



Figs. 5-13: *Zingiber officinale*. -5: Meiotic metaphase I showing irregular pairing of the homologous chromosome complement. -6: Meiotic anaphase I showing late separation of the chromosomes. -7: Late meiotic anaphase I showing laggard chromosomes and sticky bridge formation. -8: Light microscopic photographs of pollen grains after carmine staining showing fertile and nonfertile pollen grains. -9-13: SEM of pollen grains; 9, fertile and abortive pollen grains; 10, fertile pollen without germinal aperture; 11-13, abortive pollen grains.