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Research Article

CROSSABILITY STUDIES IN MANGO (*Mangifera indica* L.) MALFORMATION IN A SUBTROPICAL CLIMATE

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Abstract- With a view to mine the reason behind mango malformation malady and its causes, a cytological study has been conducted. In this study, reproductive biology of normal and malformed flowers was studied in the subtropical conditions of India, using the cultivars 'Dasehari' and 'Amrapali'. In this regard, characteristics of flower, pollen biology and cross compatibility of normal and malformed flowers were analyzed, using microscopy techniques from the beginning of the blooming season. Results showed large variation in flower characteristics of malformed panicles. The flower size (diameter) was large and arrangement of sepals were varied. Carpel number and curved style shape were other major abnormalities. Whereas, pollen grains from malformed tissues were large rhomboid, inaperculate and had large pollen size with the varied germ pores in different frequencies. Acetocarmine and FDA staining also evidenced pollen sterility. In crossability study, pollen adhesion and germinability was poor of malformed pollen grains also the germinating pollen tubes were blocked by callose plugs. The malformed flowers when taken as female flowers were unable to affect pollen adhesion, probably due to their curved/hooked structure. These results showed that low fruit set in mango in subtropical climates is due to abnormal pollen and flowering pattern.

Key words- Crossability, Pollen, Incompatibility, Floral, Malformation

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Introduction

The cultivation of Mango (*Mangifera indica* L.), is as old as Indian civilization i.e., four to six thousand years. The genus Mangifera originated in the lower hills of northern India and Myanmar region [1]. India is the leading mango producing country followed by China, Thailand and Pakistan [2]. Unfortunately, this fruit crop is exacerbated by the menaces of malformation. This is most destructive in nature leading to great economic losses ranging from 5-30%. Mango malformation is of two types, vegetative and floral. Normally, floral malformation is more prevalent. Generally, malformed inflorescence produces no fruit, or aborts at early stage and is directly responsible for reduction in yield [3]. Malformation is very complex malady and different researcher claimed different cause of this *e.g.* Physiological, Mites, Virus, Fungus, Nutritional, Trichomes and cultural practices *etc*. [4]. Nevertheless, exact region behind this malady is unknown or any single factor appear to be responsible for malformation in mango. The knowledge of reproductive biology of species of agronomic interest is utmost important [5]. However, none of the studies has been done to identify reason behind reproductive biology of malformation. In the present study, in order to study the causes behind this massive malady under a subtropical region, we examined the flowers and crossability in normal and malformed mango plants under the microscope.

Material and methods

Research site

The investigations were conducted in orchard of Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow, India, located at latitude 26.55°N and longitude 85.59°E. Recorded maximum temperature during the experiment conducted was 32.5°C and minimum was 12.5°C.

Experiments for Floral biology

Twenty flowers selected from normal and malformed panicles were collected from panicles of Var. Amrapali and comparison was made for floral biology characters *viz.* flowers size, petal number, petal size, carpel number, stamens no. and style shape *etc.*

Evaluation of pollen biology using Standard Acetocarmine and Fluorochromatic Reaction (Fcr) Test

Anthers from fixed flower buds of different lines were taken on a clean slide. A drop of acetocarmine was placed on squashed anthers and cover slip placed with gentle tapping. The slide was then observed under light microscope for fertility and data tabulated.

Stock solution of Fluorescein diacetate (FDA) was prepared in acetone (2 mg/ml) to 2-5 ml of sucrose solution in small glass vial/tube drops of stock solution of FDA was added until the resulting mixture shows persistent turbidity. A drop of sucrose - FDA mixture was taken on a slide. Sufficient amount of pollen grains was suspended in the drop and uniform distribution of pollen in the preparation was ensured.

Evaluation of Crossability

For experiment an old mango trees cultivars Dasehari showing incidence of malformation in the orchard was selected. The panicles of both malformed and normal were tagged, after removing all the opened flowers, hermaphrodite flowers on healthy and malformed panicles of Dasehari prior to their dehiscence. The anthers were kept in shade for their dehiscence.

The emasculated flowers were pollinated between 11am and 1pm by brushing the stigmatic surface of healthy and malformed flowers. The samples of 20 pollinated healthy and malformed flowers were collected 3 and 24 hours. Post pollination and fixed in Carnoy's (3:1) in different vials as different crossed flowers N×N, N×M, M×N and M×M (Where N=Normal and M=Malformed flower). The fixed pistils were transferred to 1N NaOH for clearing. After clearing, the pistils were washed in water in a petridishes and rinsed carefully. Mounted the water rinsed material in a 1:1 V/V mixture of Aniline blue and glycerine. Gentle pressure was applied on the cover glass to achieve required degree of spreading of tissue. The preparations were observed under fluorescence microscope and data tabulated.

Results and discussion

Floral Biology

Large variations were recorded in flower characteristics of malformed panicles. The flower size (diameter) was large (0.8-1.0 cm vs 0.4-0.6 cm). The sepals were fairly uniform however variations and their arrangement also recorded. Carpel number and curved style shape were other major abnormalities [Table-1]. The opening of flower buds was similar initially in both the tissues however; the malformed panicles, recorded continuous flowering and more bursts of flowers bud opening (anthesis) were displayed. Major variation that were recorded, include abnormal growth in petal size, enhanced petal number, their arrangement, carpel number, shape of style *etc* [Table-1]. Some such variations are reported in Dasehari and Langra also [6], but at a very low frequency. The malformed panicles comprise completely of flowers have one or other such abnormalities, leading to sterility and no fruit set.

Table-1 Floral Biology Normal vs Malformation in Mango

S	Characteristics	Normal	Malformed
1	Flower size	0.4 mm-0.6 mm	0.8 - 1.0 mm.
2	Petal Number	5	5 - 6
3	Petal size	0.2 - 0.4 cm.	0.4 - 0.6 cm.
4	Carpel Number	1	1 - 3 fused
5	Stamens Number	1	1 - 2
6	Style Shape	Straight	curved, hooked

Table-2 Pollen Biology Normal vs Malformation in Mango

S			Normal	Malformed
1	Total no. of Pollen		273	408
2	Pollen size (Avg.)		24.1	25.8
3	Pollen Shape	Normal	All	188
		Rhomboid	NIL	220
4	Pollen size (range)		22.5-25	15-35
5	Germ pore Number	One	NIL	62
		Three	All	55
		NIL	NIL	291
6	Furrows (Colpi)		3	NIL

Table-3 Crossability studies in Normal vs Malformation in Mango

S	Cross combination	Time after flowers taken (hr.)	Number of flowers	Number of pollens on stigma	Number of germinating pollen
1	N×N	3	9	11	Nil
		24	10	30	12
2	N×M	3	8	1	Nil
		24	11	4	2*
3	M×N	3	11	-**	Nil
		24	9	Nil	Nil
4	M×M	3	9	Nil	Nil
		24	8	Nil	Nil

*with callose plug, **Large number of pollen grains found inside the hollowed stylar tissue in malformed flower

Pollen Biology

The pollen shape was elliptical in dry state in both samples normal and malformed fixed buds. However, the malformed tissues had slightly larger pollen grains when the pollen grains were in hydrated condition, the pollen grains from normal flowers exhibited normal-circular-triangular shape, with three germ pores /colpi size being 22.5-25. Whereas, pollen grains from malformed tissues were large rhomboid, pollen size being 15-35. The germ pores varied in number being 0, 1 and 2 in different frequencies. Pollen grains were largely inaperculate (no aperture) [Table-2]. Aceto carmine and FDA staining also evidenced pollen sterility. The pollen grains of malformed flowers were filled up with some substances, accounting for dark staining when examined under microscope. Mukherjee (1949) demonstrated ample pollen fertility in Dasehari (93%) and Langra (88.2). However, the pollen fertility scenario, in the event of malformation was not documented in the literature. Poor pollen fertility was recorded in the present study by acetocarmine and FDA test. The FDA test revealed poor fluorescence indicative of abnormal cell membrane and poor esterase activity. The pollen grain size reported by Singh (1954) was variable for different varieties. In our study the average pollen size of variety Amrapali was 24.1 μ, whereas, the pollen grain size of malformed flowers ranged between 15-35 μ. The pollen grains in malformed flowers were largely swollen. The pollen shapes described by many authors [6] have distinct oblong shape in dry and change shape when moistened. Similarly, in our study the pollen shape was elliptical oblong in dry state, whereas when moistened, the normal pollen grains were spherical, triangular and malformed ones were rhomboidal. Pollen grains of all varieties having three long tapering furrows (tricolpate type) with germ pore centre was reported [8]; whereas Singh (1954) reported lack of furrows. In our study, the mature pollen grains of cv. Amrapali (normal flowers) invariably possessed three germ pores and tapering furrows. Whereas, the pollen grains derived from malformed panicles possessed no furrows and no/1-2 germ pores. The pollen from malformed flowers was large inaperculate [Table-2].

Crossability studies

Malformed and normal flowers of cultivar Dasehari were crossed in various combinations. [Table-3] for recording the germinability of the malformed pollens (Where N=Normal and M=Malformed flower) in combination N×N, has after pollination pollen deposition on stigma was recorded. However, the germination of pollen tube was evidenced only in flowers fixed 24 hours after pollination. Nevertheless, when malformed pollen grains were used to cross normal flowers, the pollen adhesion and germinability was poor, the germinating pollen tubes were blocked by callose plugs [Fig-1]. The malformed flowers when taken as female flowers were unable to affect pollen adhesion, probably due to their curved/hooked structure. Interestingly, deposition of pollen grains inside hollow stylar tissue was recorded in malformed female flowers. The crossability studies conducted to check the germinability demonstrated that malformed pollen grains derived after dehiscence and hand pollination in normal stigma had some adhesion and poor germinability, whereas malformed style had no pollen adhesion in its stigma. The curved stigma poor pin like stigma probably was responsible for it. Similar results were reported by [9] in Punjab, where degeneration of stylar tissue, and deformed stigmatic surface was found. Interestingly, in our studies, degeneration of stylar tissue indicated by presence of hollow tube like style, where large pollen deposition was recorded. Therefore, the results indicated the presence of a mysterious self-defensive system in mango that would prevent cross-fertilization especially with abnormal entities.

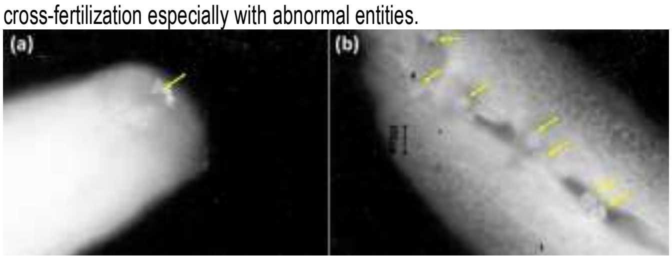

Fig-1 Crossability studies of mango (a) the germinating pollen tubes were blocked by callose plugs in N×M crosses in 3 hours (b) deposition of pollen grains inside hollow stylar tissue in malformed female flowers in M×N crosses in 24 hours (yellow arrow indicates pollen position inside the pistil)

Conclusion

These results presented here showed that low fruit set in mango in subtropical climates is due to mysterious causes.

The low number of germinability observed due to the malformed flowering and pollen quality. Finally, incompatibility of pollen-pistil is due to many factors like pollen abnormalities, callose plugs on malformed pollen tubes, and curved/hooked structure of stigma *etc.* This type of defense mechanism followed by any species probably to protect themselves from any further more anomalies or disease and this affect the fruit setting in plant species like mango.

Application of research: This research article highlights the impact of malformation on floral, pollen biology and crossability of flowers of mango and its role in fruit setting and productivity.

Research Category: Cytology, Mango malformation

Abbreviations:
% – Percentage
Cm. – Centimeter
mm. – millimeter
1N – One Normal
NaOH – Sodium hydroxide
Vs. – versus
V/V – Volume by volume
mg/ml – milligram per millilitre
°C – Degree centigrade
°N – Degree North
°E – Degree East

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