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Cyto-morphological Study of Mango Malformation in India

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Abstract: With view to identify the causes of Malformation, Cyto-morphological studies were undertaken on malformation in relation to normal in two cultivars of (*Mangifera indica* L.) which is widely cultivated for its fruits. Malformation reduced the panicle length and its emergence as compared to normal; panicle length as well as number of flowers reached its optimum on 12 days in normal, while in malformed tissue, these two processes continued up to 33 days. Very high frequency of flowers emergence in malformed as compared to normal irrespective of the cultivars was also noted. The presence of high frequency of multicellular bulbous trichome in the malformed tissue of both the cultivars was probably noted for the first time. Further, meiotic cell division was found to be highly disturbed leading to many meiotic irregularities, such as, laggards, bridges, non orientation of spindles, cell cycle variations, variation in nucleolus number etc., in the malformed tissues. Meiotic abnormalities and presence of high frequency of multicellular bulbous trichome may be attributed to the reduction in growth of panicle as well as flowering pattern, resulting in poor fertility and fruit setting.

Key words: *Mangifera indica*, malformation, trichome, meiotic abnormality

INTRODUCTION

The mango (*Mangifera indica* L.) is one of the premier choice fruit crops of tropical and sub-tropical regions of the world, especially in Asia. Its popularity and importance provided it name ‘King of fruits’ in the tropical world (Kumar *et al*., 2011). India is the leading mango producing country i.e., 19.22 million tons (mt) which is 41.0% of the total world production (Negi, 2000). Mango production is highest in India i.e., 13,692,000 mt from 2,205,000 ha. Its global significance has increased because of great demand in international market; about 54531 mt of mango exported from India to other countries which have approx. value of Rs. 127.42 crores (NBH, National Horticulture Database, 2008). Albeit, highest production covering highest area (22,05,000 ha) in the world, productivity of mango is very low i.e., 6.3 mt ha^{-1}. This is because due to its susceptibility to various biotic and abiotic diseases (Shahbaz *et al*., 2009). Amongst biotic diseases of mango malformation is very nuisance; It causes deformation of vegetative and floral tissues in mango (Chakrabarti, 2011). Yield may be losses as much as 90% (Ploetz, 2001).

The complex nature of malady was obvious by diverse claim made by different workers from India and different countries about its causes e.g., Physiological (Bains and Pant, 2003; Singh, 2006), Biochemicals (Singh, 2006), Mites (Malo and Mcmillan, 1972); Virus (Giani, 1965); Nutritional and cultural practices; Fungus (Misra and Singh, 2002; Zheng and Ploetz, 2002). Recently, isolates of *Fusarium moniliforme* var. Subglutinans were found to be members of a new species, *F. mangiferae* Britz, Wingfield and *Marasas* sp. nov. (Britz *et al*., 2002). None of the above causes and information appeared to be solely responsible/authentic for malformation in mango (Akhtar and Alam, 2002). Moreover, the Cytogenetical aspect of mango malformation which has not received the attention of scientist as yet, as felt immediate attention and hence the investigation, was carried out to mining the reason behind this malady.

MATERIALS AND METHODS

Studies were conducted on 10-year-old tree of two mango cultivars, Amrapali and Dasehari at Central Institute for Subtropical Horticulture, Lucknow, located at latitude 26.55° N and longitude 85.59° E in the subtropical zone of India. Ambient average temperatures were 32.5°C (maximum) and 12.5°C (minimum) during the course of the experiments. Twenty normal and twenty malformed panicle were tagged on each side of plants of cv Amrapali

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and Dasehari. Panicle initiation and flowering data were noted with the initiation of panicle emergence on the tree. Total length of panicle and total number of flowers were counted on panicles tagged every fourth days throughout the flowering period; all counted flowers were detached after counting so that actual number might not be inflated; counting was done once a day (10 am to 1 pm) as per Asif *et al.* (2002).

Meiotic analysis: Flower buds of suitable sizes from 10 randomly plants of malformed and normal were taken and fixed in chilled Carnoy's fixative (3:1) and placed in a refrigerated at 4°C for about 24 h. Buds were then separated and thoroughly washed with water and then taken in 45% acetic acid in a watch glass and a pinch of ferric chloride was added. Anther was then plucked with a forceps and placed on the slide and squashed in the acetocarmine stain with simultaneous warming for few seconds and cover slip was placed on the material and tapped gently. The slide was then observed under light microscope for divisional stages and data tabulated. Pollens were assessed from anther squash preparations in acetocarmine solution. Fully stained pollen grains were considered fertile and data recorded.

Mitotic cell study: For mitotic cell study shoot buds of each malformed and normal plants were prefixed in 5% colchicines for 5 h and fixed in chilled Carnoy's fixative (3:1), the hydrolyzed (with NHCl at 40°C for 1 min) shoot buds were kept in 2% acetic orcein. Small part of the middle portion of the growing shoot bud was squashed in 45% acetic acid on the slide and visualized under microscope and data recorded.

RESULTS AND DISCUSSION

A comparison was made on the pattern of blossom buds emergence and development of full panicles in malformed and normal panicles of the cultivars Dasehari and Amrapali. The development of average length of panicles and flowering were found to be continuous process. This lasted for approximately a fortnight (1st week of March to 3rd week of March) in normal, whereas, slightly delayed in malformed and lasted for a month (1st week of March to 2nd week of April). Comparative study of panicle length elongation in both normal and malformed plants revealed that initially, extension of normal panicles was very rapid (grew from 26.1 to 35.3 cm), consequently took less number of days for its full length completion. In contrast growth in malformed panicle was very moderate and consistent which followed slow pattern of increasement (from 14 cm in 1st week of March to 22.3 cm in 2nd week of April), consequently took more number of days (more than one month) for its completion. Notwithstanding, normal panicles showed the final average length up to 35.3 cm in less number of days, while malformed panicle showed the final average length up to 22.3 cm in more number of days. Studies also revealed that there was significant difference (significant t value, Table 1).

Similarly, study on blossom bud emergence revealed that flowering was also continuous process. This lasted for approximately a fortnight (1st week of March to 3rd week of March) in normal, whereas, flowering were slightly delayed in malformed panicle and lasted for a month (2nd week of March to 2nd week of April). Albeit, maximum number of flowers emerged on single day, coincided in both the samples (normal/ malformed) and after a quite less flowering, again maximum flowering was noted before last counting (significant t value, Table 1). In present study on cv. Amrapali and cv. Dasehari, blossom emergence and panicle extension were found to be truly a genetic character as reported earlier by Singh (1954). However, the response of different varieties to the disease was variable observed by Ahmad *et al.* (2002).

Meiosis was studied in the PMC's obtained from anthers excised from unopened flowers. The malformed anthers, in which meiosis was underway, were smaller in size. Different stages of meiosis were studied in malformed as well as normal buds, however in malformed tissues Metaphase-I could not be recorded. The PMC's (Pollen Mother Cells) were invariably in Metaphase-II or Anaphase-II stages (Table 2). Lots of meiotic abnormalities were recorded which include laggards, bridges, non orientation of spindles, cell cycle variations, variation in nucleolus number etc. (Fig. 1a, b; Table 3).

Studies in different plant species have revealed the role of meiotic irregularities for declined seed setting and production capabilities (Bajpai and Singh, 2006; Arabbeigi *et al*., 2010).

Kaur and Singhal (2010) observed that pollen grains resulting due to dense cytoplasm are inviable for fertilization. In our study too, pollen abnormalities in both varieties was the major contributing factor towards impaired pollen fertility.

It was interesting to record the presence of high frequency of multicellular bulbous trichomes in malformed shoot buds (Fig. 1c). The frequency of these structures were 9.44±0.7 in malformed as compared to 5.1±0.55 in normal tissues and t value in paired t-test significantly showed differences. Size of these structures range from 32.5 to 72.5 μm in malformed tissues while it was 22.2 to 72.5 μm in normal tissues (Table 4). Cell division was also recorded in these structures (Fig. 1c) the branch formation

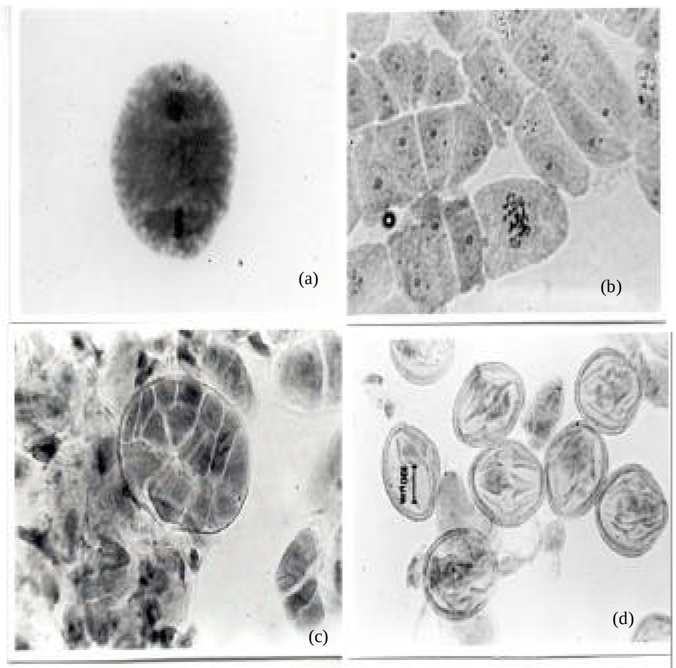

Fig. 1(a-d): Cytological studies of mango (a) cell cycle variation in malformed tissue of mango (b) c-metaphase observed in malformed tissue of mango (c) multicellular bulbous trichome with anaphase in its one cell in malformed tissue of mango (d) rhomboid pollen grain in malformed flower

Table 1: Emergence of panicle length and blossom buds

No. of countings	Length of panicle (Avg.) Normal	Length of panicle (Avg.) Malformed	Total no. of flower (Avg.) Normal	Total no. of flower (Avg.) Malformed
1st	26.1±0.52	14.0±0.09	7.6±0.3	0±0
2nd	29.6±0.15	17.1±0.09	25.0±0.3	9±0.4
3rd	33.1±0.39	18.6±0.06	86±0.5	89±0.7
4th	34.8±0.29	19.8±0.18	253±0.6	283±0.8
5th	35.3±0.18	20.3±0.24	127±0.7	153±0.9
6th		21.0±0.24	61.6±0.6	200±0.6
7th		21.0±0.24	8.3±0.9	184±0.8
8th		21.6±0.22		57.6±0.5
9th		22.0±0.18		95±0.6
10th		22.3±0.11		237±0.7
11th				148±0.8
12th				28±0.7
T value at 5%	3.6		2.47	

Table 2: Frequency of cells in different stages of meiotic division

Types of tissue	Total No. of PMC'S	MI (%)	M II (%)	A I (%)	AII (%)	TI (%)	T II (%)
Normal	92	17.3	32.6	Nil	8.6	19.5	32.6
Malformed	38	Nil	42.1	Nil	57.8	Nil	Nil

is derived from mechanisms employed in cell divisions speculated by Schnittger and Hulskamp (2002). Previous study on tobacco by Meyberg *et al.* (1991) revealed that trichomes also had a gland which exude nicotine and believed to produce diterpenoid. Similarly Hulskamp *et al.*

Table 3: Percent Abnormalities in Malformed floral buds

Meiotic deviations	Frequency (%) Malformed	Frequency (%) Normal
Laggards (A II)	5	Nil
M II plates non-oriented	40	1-2
Cell cycle variation	2-5	Nil
Variation in nucleolus No.	5-10	1
Persistent nucleolus	2-5	Nil
Pollen grains with dense cytoplasm	80-95	4-5
Total no. of pollen	40.8	27.3

Table 4: Multicellular bulbous structure (Trichome)

Types of mitotic cells	Total no. of analyzed cells	Size in μm (Range)	No. of trichome (Avg.) /microscopic field
Normal	208	22.2-72.5	5.1±0.55
Malformed	240	32.5-72.5	9.44±0.7
T value			2.40

(1994) observed that Arabidopsis multicellularity allows trichomes to function as miniature organs and glandular which secrete a variety of compounds viz. excess salt and chemicals. What biochemical and ultra-structural changes occur at the hypha-trichome interface? Do the trichomes themselves play a role in attracting the hyphae, perhaps along carbohydrate gradients, or is association with *Fusarium* species *Fusarium moniliforme* var.

subglutinans, causal organism of mango malformation as claimed by (Misra and Singh, 2002; Zheng and Ploetz, 2002). Answers to these questions will assist in to find exact reason behind mango malformation and its remedy.

CONCLUSION

The irregularity in panicle length elongation and delayed consistence flowering behaviour of malformed mango plants which often resultant in reduced fruit setting in comparison to normal one (which properly set seed). Somehow, due to presence of peculiar structure in high frequency which here used to called as multicellular bulbous trichome whose role is still enigma, along with that impact of rather cytological abnormalities at some extent viz. Non-orientation of spindles, secondary association of bivalents, nucleolus number etc. Therefore, some more exercise are required for mining the reason behind peculiar structure (Multicellular bulbous trichome) and need of intensification of crop regulation technique and crop management strategies which could contribute in proper seed setting and fruit production of mango.

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