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Research Article

CHANGES IN THE LEVEL OF GROWTH HORMONES DURING FRUIT BUD DIFFERENTIATION AND IDENTIFYING THE MORPHOLOGICAL AND HISTOLOGICAL DIFFERENCES OF HEALTHY AND MALFORMED SHOOTS AND BUDS OF MANGO

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Abstract

The present study was carried out to find out the changes in the level of phytohormones in three mango hybrids namely Amrapali, Pusa Arunima and Mallika during the period from last week of August to the last week of December in 2003-04 and 2004-05 and the study was also carried out to find out the morphological and histological differences between the malformed shoots and or buds of Amrapali and Bhadauran. Changes in the quantity of phytohormone during the study period was analyzed by HPLC.To identify the differences between healthy and malformed shoot and or bud, dissected buds and or shoots were observed under microscope. From the first part of the study it was found that the Indole Acetic Acid and the Abscisic acid have been found to be increasing and the trend was opposite in case of Gibberellic acid like substances during the period of fruit bud differentiation i.e. from 15th October to 15th December in all the hybrids during the study period. From the second part of the study, morphologically healthy inflorescence axis has been found long, slender having normal light green color and normal buds whereas the malformed inflorescence was thicker, short having dark green color with larger buds in comparison to the healthy ones. Histologically the diameter of the main axis of malformed inflorescence of the Amrapali was found bigger than the healthy inflorescence. The area occupied by various tissues like cortex, vascular bundles and pith in the main axis of inflorescence appeared thicker in malformed inflorescence of Amrapali in comparison to the healthy inflorescence of Bhadauran. The healthy buds had been found symmetrical

whereas, the main axis of malformed buds had asymmetrical shape. The malformed buds were appeared generally distorted and leaf primordia were not clearly visible as against the healthy buds of Bhadauran.

Key words: Phytohormones, fruit bud differentiation, malformation, mango hybrids and histology and morphology.

Introduction

The time of fruit-bud-differentiation in mango is known to be governed by local weather factors and also by varietals factors the mango. The knowledge of time of fruit-bud-differentiation under a particular set of climatic conditions for a given variety would enable the orchardists to schedule the fertilization, irrigation and other cultural operations and hence to have a better yield. The fruit-bud-differentiation process is a critical event in the growth and development of mango, as it marks the partitioning of metabolites between the vegetative and reproductive organs. Different physiological and biochemical factors affect the transport of metabolites from source to sink. These factors governing fruit-bud-differentiation in mango have not been adequately studied especially the role of naturally occurring growth substances. Such studies assumes more important, since these naturally occurring growth substances are now recognized as important factors controlling the ontogeny of flowering in higher plants. The newly developed hybrids/varieties are excellent material for the study of fruit-bud-differentiation to establish the time period of fruit bud differentiation and the associated growth hormones during this period. Mango malformation is one of the menaces for the cultivars grown in northern India which is responsible for the considerable yield loss. Even though many causal factors such as virus, bacteria, mites and fungi have been reported, the proper causal agent is not known so far. Some histological studies on malformed bud and/or shoot samples may be helpful in finding out a solution to this malady. The present study was aimed to observe the changes in the level of growth hormone during the period of fruit bud differentiation in three hybrids and malformation study was done with cultivar Bhadauran and one of the hybrids Amarpalli.

Materials and Methods

The present investigations were carried out at the experimental orchard of the Division of Fruits and Horticultural Tecnology, Indian Agricultural Research Institue, New Delhi during 2003-04 and 2004-05.

Estimation of Phyto-Hormones: Shoot and / or bud samples were collected, rinsed with distilled water and preserved in ethanol (80% v/v) and stored in a deep freeze (-20˚C). The method of extraction, purification was done by the method suggested by Takahashi and Yamaguchi (9) with slight modifications.

Ten grammes of the shoots and / or bud sample was ground in cold aqueous ethanol (80% v/v) with pestle and mortar. The total volume was made up to 50 ml with ethanol (80% v/v). Then, it was left for 24 hours at 0˚C for extraction of hormones. The extracts were filtered through filter paper Whatman No.42. The filtrates were combined and taken for further analysis, while the residue was discarded. The ethanol extract was evaporated to dryness under vacuum in water bath (35˚C). The aqueous phase was added with distilled water and residue was discarded. The pH of the filtered aqueous phase was adjusted to 8.6 with 1% NaoH. The extract was treated three times with equal volume of ethyl acetate. The resulting treatment separated the extract into two fractions i.e. upper ethyl acetate fraction and lower aqueous fraction. The upper ethyl acetate fraction was separated by micro-pipette in another tube. The lower aqueous fraction was adjusted to pH 2.8 with 1% HCI and again extracted three times with equal volume of ethyl acetate and allowed to separate into two fractions. The upper ethyl acetate was allowed to separate in two fractions. The upper ethyl acetate fraction was separated and evaporated to dryness under vacuum and dissolved in one ml of HPLC grade methanol for further analysis using High Performance Liquid chromatography (HPLC). A reverse phase high performance liquid chromatographic technique was carried out. (Waters Associate Model-244 Liquid Chromatography, Waters Associates, Milford, MA, USA equipped with 6000 psi pumps, Model-600 Solvent programmer and Model-46 K universal factor).

The chromatographic conditions were:-

1. Mobile phase : Water: methanol (80:20).

2. Flow rate : 0.7ml min-1.

3. λ Max : 274 nm.

4. Injection volume : 20 ul.

5. Column and solvent temperature : Ambient (25 to 28˚C).

Retention time of different hormone

Standard hormone	**:**	**Retention time**
Indole-actic acid	:	7.80
Gibberellic acid (GA_3)	:	11.30
Abscisic acid	:	8.69

For the quantification of amount of hormones, respective peak area of individual hormone at its designated retention time was recorded by the integrator and the amount was calculated with the help of standard peak area. The quantity of the hormone was expressed as ug/g on fresh weight basis.

Histological and morphological studies of healthy and malformed shoot

For the purpose of comparison of healthy buds and / or shoots with malformed bud and /or shoots, samples of the healthy buds and / or shoots from the variety Bhadauran (reported to be resistant to the malformation) and malformed buds and / or shoots from Amrapali (susceptible to malformation) were collected before and after flowering (with inflorescence) from both the cultivars. The malformed and healthy buds were dissected longitudinally and horizontal sections of shoots to get thin sections using microtome and observations were made to find out the histological differences between the healthy and malformed buds and / or shoots samples.

Statistical analysis

The experiments were laid out in factorial completely randomized design with five replications and the data were analyzed.

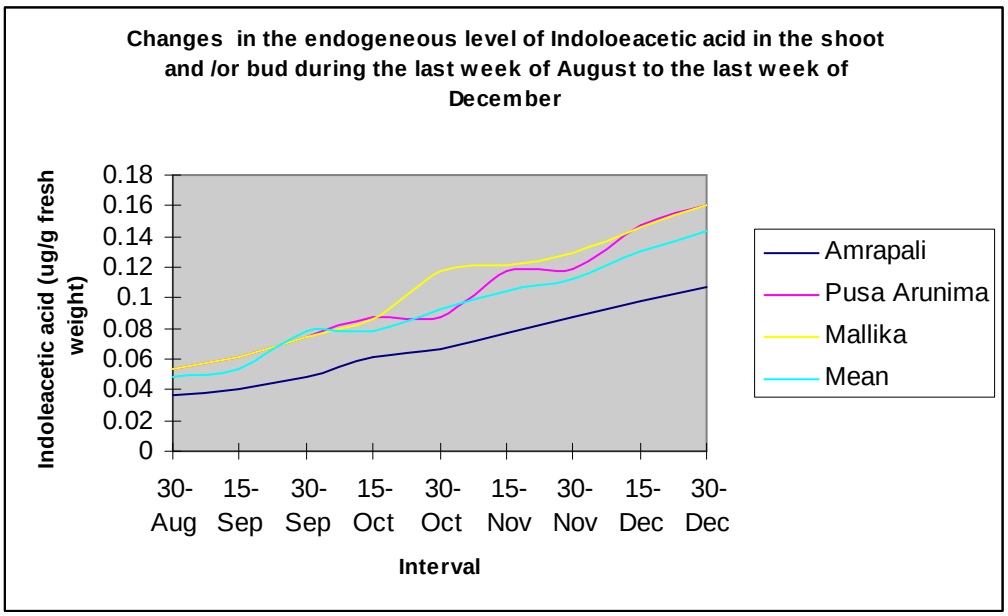
Results and Discussion

I. Changes in the level of the endogenous phyto hormone.

A. Changes in the endogenous level of the indole-acetic acid.

It was very much interesting to note that the endogenous levels of indoleacetic acid (**Fig.1**) started increasing from the first date of sampling i.e. 30th August and continued increasing up to the last date of sampling i.e. 30th December during the study period in all the three hybrids. There were some variations in indoleacetic acid contents in different varieties. The figures ranged form 0.3034 to 0.104 ug/g on fresh weight basis in Amrapali, from 0.053 to 0.161 in Pusa Arunima and from 0.053 to 0.159 ug/g on fresh weight basis in Mallika during the study period. The endogenous level of indoleacetic acid started increasing from the first date of sampling, i.e. 30th August and continued increasing up to the last date of sampling, i.e. 30th December in both the years in all the hybrids. The specific concentration of indoleacetic acid in the tissues might be responsible for the production of a putative floral stimulus. Furthermore, the alternation in the ratio of florigenic and antiflorigenic compounds might be translocated to the target cells in meristem. Apart from it, a critical ratio of cytokinin and auxin has been found useful for the bud initiation and proliferation (Chacko, 1). Kashmirilal and Ram (5) and Ram and Sirohi (7) have also reported an increase in the levels of auxin like substances in shoot tips at flower-bud-initiation, flower-bud-differentiation and panicle emergence stages.

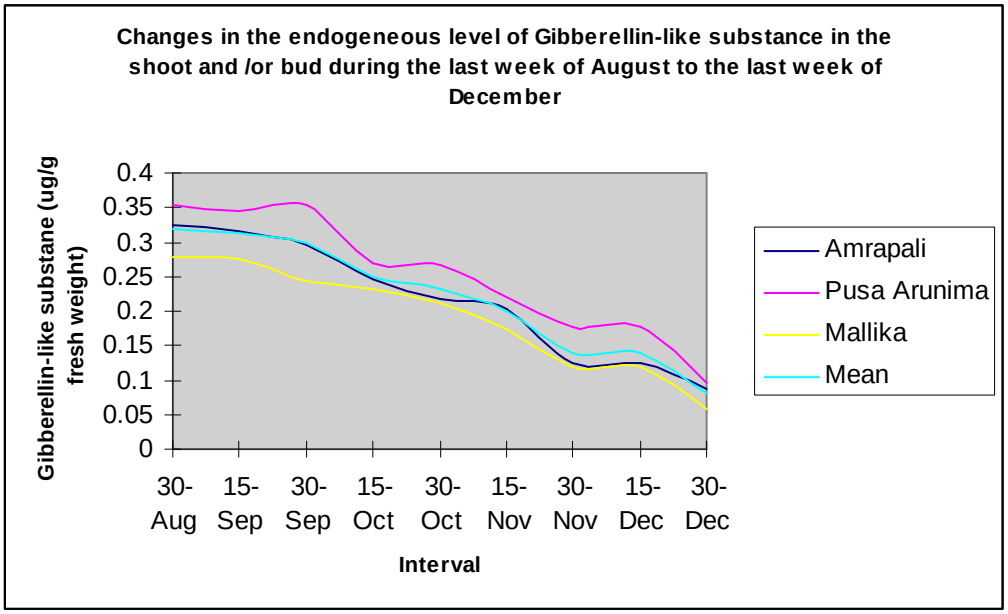
Fig.1 Indole Acetic Acid.



B. Changes in the level of endogenous gibberellin-like substance

Unlike indoleacetic and contents, the mean value of endogenous gibberellins-like substance decreased from 0.320 ug/g on fresh weight basis to 0.080 ug/g on fresh weight basis from 30th August to 30th December during the study period (**Fig.2**). The maximum gibberellin-like substance (0.325 ug/g on fresh weight basis) were found at the start of sampling date and continued to decrease up to 30th December and they reached at the lowest value of 0.088 ug/g on fresh weight basis in Amrapali. The figures ranged from 0.355 to 0.096 in Pusa Arunima. Whereas, the gibberellin-like substances varied from 0.279 to 0.055 µg/g on fresh weight basis in Mallika. The endogenous level of gibberellin-like substances started decreasing from the 30th August i.e. much more earlier than the process of initiation of fruit bud, and continued up to 30th December in both the in all the hybrids during the study period.. It is well established fact that the meristematic cells reduce the production of gibberellin-like substances during the process of fruit-bud-differentiation. The reducing level of gibberellin-like substances favours the initiation of reproductive shoots. An antagonistic relationship in the endogenous gibberellin-like substances and flower-bud-differentiation has been observed by several workers like**,** Karchru et al. (4), Kashmirilal and Ram (5), Pal and Ram (6) and Ram and Sirohi (7). All of them observed that the gibberellin-like substances inhibited flowering in mango.

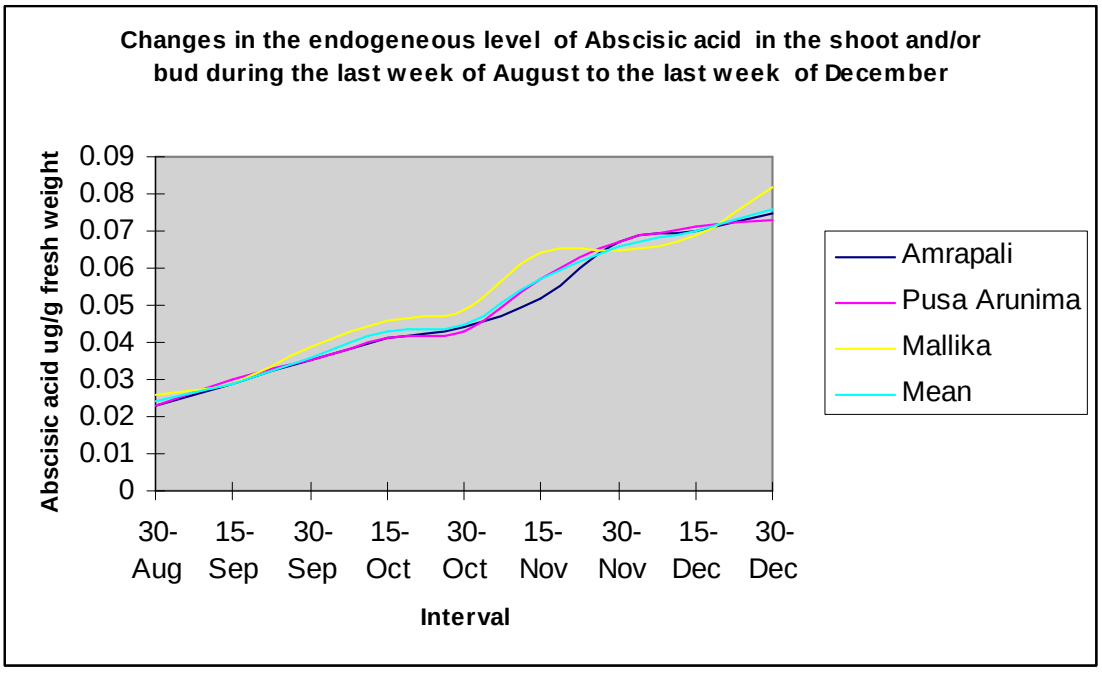
Fig.2 Gibberellic acid like substances.



C. Changes in the level of the endogenous abscisic acid

Unlike gibberellins-like substance, the endogenous abscisic acid contents of mean values increased from 0.024 µg/g on fresh weight basis to 0.076 µg/g on fresh weight basis from 30th August 30th December (**Fig.3**). The minimum endogenous abscisic acid contents (0.023 µg/g on fresh weight basis) were recorded at the first date of sampling and continued to increase up to 0 30th December and it reached to the maximum value of 0.075 µg/g on fresh weight basis in Amrapali. The figures of the minimum and maximum ranged between 0.023 to 0.073 µg/g on fresh weight basis in Mallika. The endogenous Abscisic acid contents increased from 30th August to 30th December during the study period in all the hybrids unlike gibberellin-like substances. Other inhibitors similar to the abscisic acid were relatively higher in comparison to the gibberellin-like substances in the shoots of mango during flower-bud-initiation. The abscisic acid had been reported to keep the vegetative growth under control in higher plants, which provided congenial for the flower-bud-initiation. Similar results have also been reported by Chacko (1), Stino et al. (8), and Jogdande and Chowdhary (3) in mango.

Fig.3 Abscisic acid.



II.Morphological and histological differences between malformed and healthy shoots and or buds.

Morphologically, the main axis of the malformed inflorescence of the hybrid Amraplli appeared thicker, short, greenish structure with bigger green buds whereas the healthy inflorescence of Bhadauran, axis of the inflorescence was slender, normal having light green color.

The histological aspects of the main axis of the normal and malformed inflorescence for Amrapali and Bhadauran are illustrated in **Fig.4 and Fig. 5.** It was realized that the diameter of the main axis of the malformed inflorescence of the Amrapali is decidedly bigger than the healthy one. The detailed structure of the main axis of the malformed inflorescence was not found different from the healthy inflorescence of Bhadauran. The area occupied by various tissues like cortex, vascular bundle and pith appeared thicker in the malformed inflorescence of Amrapali than the healthy inflorescence of Bhadauran. Therefore, it seems that the tissues like cortex, vascular bundles, pith etc might have contributed significantly for the bigger size of abnormal rachis. Histologically, the main axis of malformed inflorescence had abnormal shape in comparison to the healthy inflorescence, which has been found symmetrical.

Fig. 4 Transverse section of the main axis of the healthy inflorescence of Bhaduran.

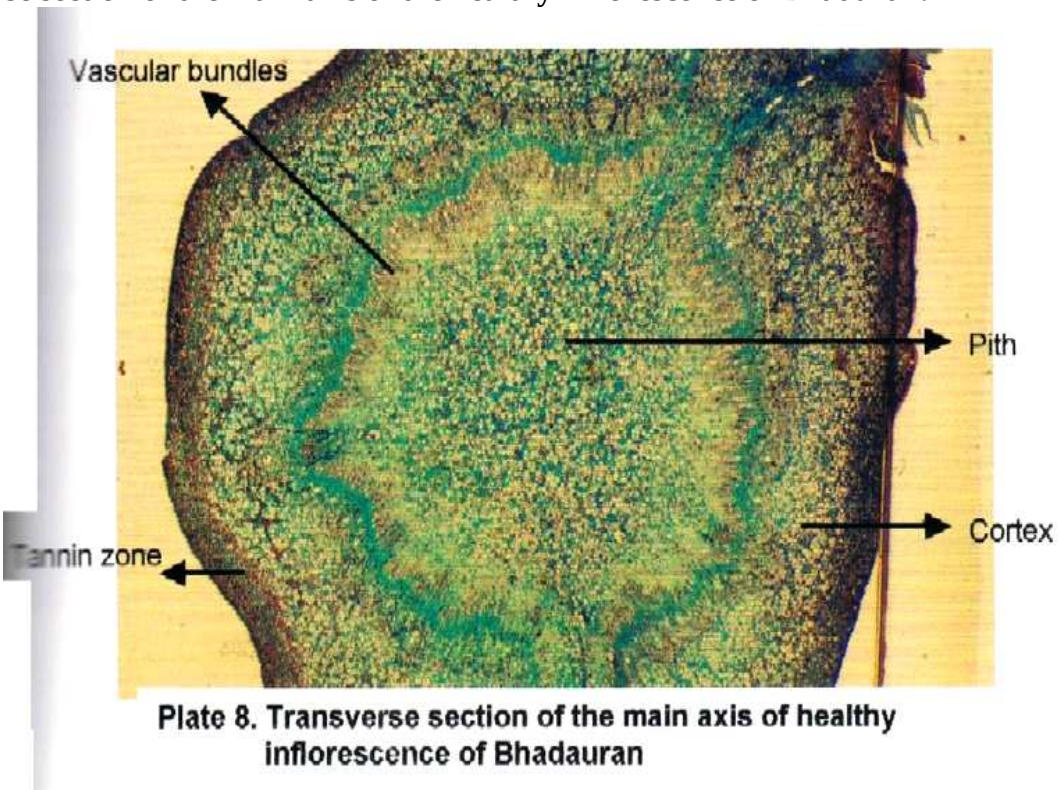


Plate 8. Transverse section of the main axis of healthy inflorescence of Bhadauran

Fig. 5 Transverse section of the main axis of the malformed inflorescence of Amrapali

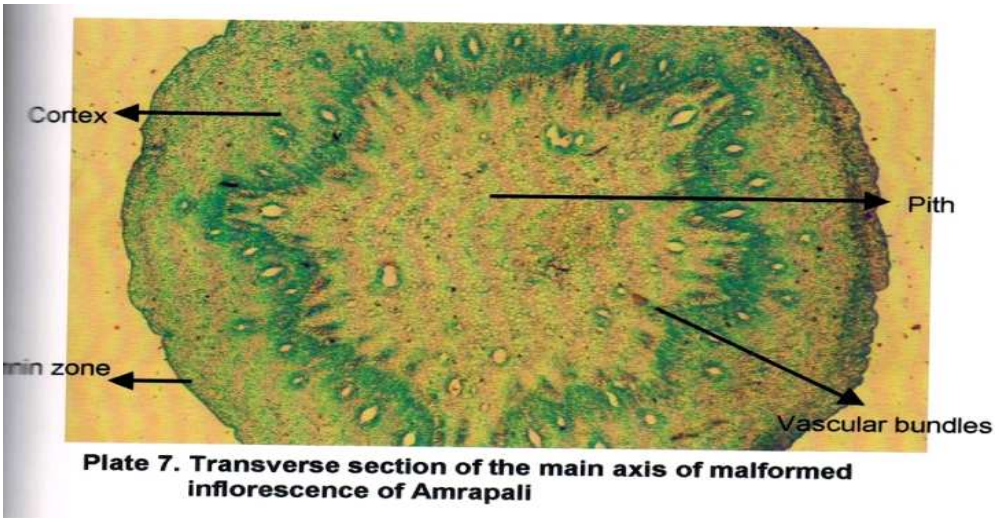


Plate 7. Transverse section of the main axis of malformed inflorescence of Amrapali

Longitudinal sections of the malformed (Amrapali) and healthy (Bhadauran) buds are illustrated in **Fig. 6 and 7**. The normal buds had dome shape structure, which was lacking in malformed buds. Furthermore, the malformed buds are generally distorted. Apart from this the leaf primordial was not clearly visible in malformed buds and they looked like. Similar histological differences have been reported by Ibrahim and Foad (2) in malformed and healthy shoot and/ or bud.

Fig. 6 Longitudinal section of healthy bud of Bhadauran.

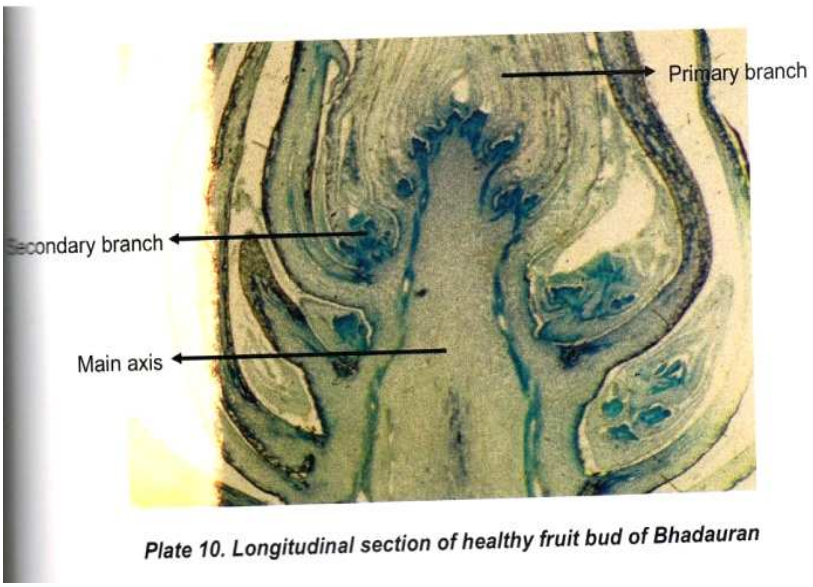


Plate 10. Longitudinal section of healthy fruit bud of Bhadauran

Fig.7 Longitudinal section of malformed bud of Amarapli.

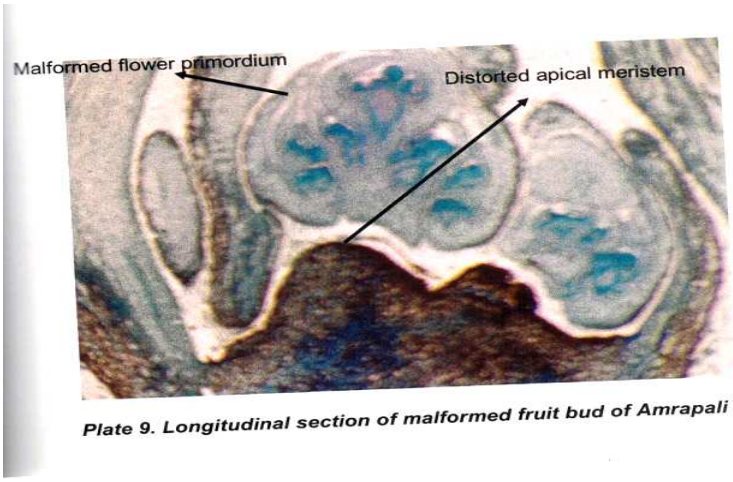


Plate 9. Longitudinal section of malformed fruit bud of Amrapali

Conclusion

First part of the study was carried out to find out the changes in the level of phytohormone during the time period of fruit bud differentiation in three mango hybrids namely Amrapalli, Pusa Arunima and Mallika.It was found that the endogenous Indole Acetic Acid and the natural inhibitor Abscisic acid have been found increasing during fruit-bud-differentiation on the other hand the endogenous gibberellins-like substances have been found decreasing during fruit-bud-differentiation. Second part of the study was done to find out the morphological and histological differences between the main axis of the healthy inflorescence and buds of malformation resistant cultivar Bhadauran and of susceptible hybrid Amrapalli.Morphohlogically, healthy inflorescence axis has been found long slender having normal light green color and normal buds whereas the malformed inflorescence was thicker, short having dark green color with larger buds in comparison to the healthy ones. Histologicallly the diameter of the main axis of malformed inflorescence of the Amrapali was found certainly bigger than the healthy inflorescence. The area occupied by various tissues like cortex, vascular bundles and pith in the main axis of the appeared thicker in malformed inflorescence of Amrapali in comparison to the healthy inflorescence of Bhadauran. The healthy buds had been found symmetrical whereas, the main axis of malformed buds had asymmetrical shape. The malformed buds were appeared generally distorted and leaf primordia were not clearly visible as against the healthy buds of Bhadauran.

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References

1. Chacko, E. K. 1968. Studies on the physiology of flowering and fruit growth in mango (Mangifera indica L.). Ph.D. thesis submitted to P. G. School of IARI.
2. Ibrahim, I. M. and Foad, M. R. 1981. Histological studies on mango Malformation. Egypation J. Hort. 1: 34-44.
3. Jogdande, N. D. and Chowdhary, K. G. 2001. Seasonal changes in Abscisic acid content and its role in flowering in mango (Mangifera indica L.). Orissa J.f Hort., 29(1): 46-49
4. Kachru, R. B., Singh, R. N. and Chacko, E. K. 1971. Inhibition of flowering in mango (Mangifera indica L) by gibberellic acid. Hort Sci. 6: 140-41.
5. Kashmirilal and Ram, S. 1977. Natually occurring auxins in mango shoot-tips and their significance in flowering. Pantnagar. J. Res. 2: 31-35.
6. Pal, S. and Ram, S. 1978. Endogenous gibberellins of mango shoot-tips and their significance in flowering. Scientia Hort. 9: 369-79.
7. Ram, S. and Sirohi, S.C. 1986. Hormonal control of alternate bearing in mango (Mangifera indica L.). Programme and Abstract Int. Nat. hort. Cong. 83rd Ann. Meet. Of the American Soc. Hort. Sci. pp. 673.
8. Stino, G.R., Fayer, K.M., Khattar, M.M. and Works, M.B.B. 1981. The Physiology of biennial bearing of mango tree. Annal. Agril. Sci. mostahar. 16: 193-207.

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