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Review article

Regulation of sex expression and flowering in papaya (*Carica papaya*)

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Abstract
Sex expression in dioecious species such as *Carica papaya* is result of genetic constitution of individual and is determined genetically. However, it is subjected to be influenced by the various environmental factors including temperature and plant growth regulators (PGRs). The sex reversal in papaya is encouraged due to application of ethrel (2-chloroethylphosphonic acid) and chlorflurenol (2-chloro-9-hydroxy-9H-fluorene-9-carboxylic acid) at different doses and it was reported that not only sex expression in this species was under the control of hormonal influence but the genetically male flower primordia transformed into female ones and behaved completely into female flower and developed into fruit. Application of ethephon, NAA, CCC, and TIBA in *Carica papaya* at various doses reported that the sexual character of the plant become more female and resulted in a higher female:male ratio, whereas use of maleic hydrazide (MH) and gibberellic acid (GA_3) at different doses enhanced maleness.

Keywords: *Carica*, chlorflurenol, ethrel, CCC, NAA, Maleic hydrazide, TIBA

Introduction
The papaya (*Carica papaya* L.) is one of the delicious and important fruit crops which is grown in tropical and sub-tropical part of India. It is one of the major fruit crops which has fast growing and early bearing habits. In southern part of India, it comes in bearing within a year whereas in northern part of India it comes in bearing within 15-16 months due to climatic variations. The papaya fruit is one of the best sources of vitamin A and vitamin C and also it contains papain as an enzyme which act as essential ingredient in various textile and pharmaceutical industries.

In nature, papaya is dioecious plant where both male and female flowers are separately located but due to interference and proper selection by human, gynodioecious plants have been originated with female, male and perfect flower which is also known as hermaphrodite flower. Female flower bears fruit in papaya so female ratio should be high as compare with male plant for proper and high production. In India, gynodioecious cultivars are mostly preferred for plantation of papaya due to high female:male ratio but constraint regarding gynodioecious cultivars are that these are costly but dioecious plants or cultivars which are very cheap in cost as compared with gynodioecious plants are less preferred for plantation due to low female:male ratio.

Several workers have worked to overcome difference between male and female ratio at pre-flowering stage on the basis of vegetative characters but practically they failed. Later on, various experiments were conducted to convert male plants into female plants by using various growth regulators and nutrients in papaya (Ghosh and Sen, 1975; Biswas *et al*., 1989) [3, 2]. Jindal and Singh (1976a) [11] reported more femaleness in dioecious plants of papaya by using Ethephon and TIBA. NAA and GA_3 were also used by Subhadrabandhu *et al.* (1997) [20] who had observed that NAA treatment reduced the proportion of male plant whereas GA_3 treatment did not alter the proportion of male plant. Except Morphactin and gibberellic acid, all chemicals played vital role in successfully transformation of male papaya to female papaya. Kumar (1998) [13] also worked on papaya for getting more femaleness by using Ethrel and Chlorflurenol. The main objective of the current review work is to elaborate importance to dioecious cultivars of papaya over gynodioecious one, as it is cheap and bears good attributes, by treating with some of chemicals; also, to impart dwarfness as well as early bearing for more fruiting. Thus, grower can grow papaya in less input cost and can achieve high production with high economic output.

Sex form in *Carica*

All members of Caricaceae are dioecious except *C. monoica* (Monoecious), *Carica papaya and C. pubescens* having three sex forms-Pistillate, staminate and hermaphrodite where hermaphrodites are ambivalent and show seasonal sex reversal to produce all the three sex forms at different seasons of year. Thus, *Carica papaya* is trioecious plant species where sex expression is defined by sex chromosomes. Hofmeyr's hypothesis based on Genic balance theory (1939, 1967) [7, 8] confirmed triallelic genic regulation of sex form in *Carica* where M_1 is dominant for maleness, M_2 is dominant for hermaphroditism and m is recessive for femaleness. Thus, M_1m is staminate; M_2m is hermaphrodite; and mm is pistilate whereas, M_1M_1, M_2M_2, and M_1M_2 are lethal. Storey (1938, 1941, 1958) [17, 18, 19] had also proposed a similar finding for sex expression and determination in *Carica papaya*.

The classical XY chromosome hypothesis of sex expression in Carica can be supported by physical mapping and gene sequencing of non-recombinant regions. Ming *et al.* (2007) [15] concluded that sex determination in papaya is regulated by a pair of sex chromosomes and two suppressor genes involved in this pathway. The stamen suppressor or feminizing genes induces abortion of stamen while the carpel suppressor or masculinizing gene results in abortion of carpels. Zerpa-Catanho (2019) [22] had used RNA-seq to identify the expression of differentially expressed genes for various sex forms and the result was verified by using RT-q PCR. They also reported a Male sterility 1 gene (CpMS1) which was expressed in male and hermaphrodite flowers at early stages and might be associated with development of male flowers; however, it was not accepted as a sex determination gene.

Sex-reversal in *Carica*

Hofmeyr (1938) [6] has concluded that sex reversal in papaya occurs due to genetical and environmental factor in which plant changes into femaleness by favourable growing condition where as poor growing condition result in maleness of a plant. He also concluded that with respect to sex change pistillate and hermaphrodite plants are stable and staminate plants are intermediate in stability. Jindal and Singh (1976b) [12] carried an experiment by using total phenolic or the Prussian blue test to determine sex in vegetative seedlings of *Carica papaya* and concluded that male plants are rich in phenolic content then of female plants and on these bases, plants can be forecasted as male or a female. They also concluded that Prussian blue test followed by sodium cobat-nitrate is a best and more accurate result giving test than that of phenolic test. Lin *et al.* (2016) [14] studied about gene which play vital role in transcriptome of male to hermaphrodite sex reversal at low temperature in papaya and concluded that male to hermaphrodite reversal was due to silencing gynoecium suppression function on the sex determination pathway through epigenetic modification.

Growth regulators to regulate sex expression

Ghosh and Sen (1975) [3] carried an experiment by using nitrogen, NAA (1-napthalene acetic acid), CCC (2-chloroethyel-trimethyl ammonium chloride), malic hydrazide (MH), gibberallic acid (GA_3) and the animal sex hormone stilboesterol dipropionate and testosterone chemicals which were applied at 5th and 6th leaf stage and second after 3 to 4 months later and concluded that application of NAA and CCC promotes femaleness in treated plant whereas MH and GA_3 enhanced maleness in papaya, the animal sex hormone stilboesterol dipropionate had not affected sex expression of papaya whereas high dose of application of nitrogen fertilizer promoted femaleness.

Jindal and Singh (1976a) [11] carried experiment with 4 replication in RBD by using morphactin (methylester of 2-chloro-9-hydroxy fluerene-(9)-carboxylic acid), ethephone (2-chloro-ethane phosphoric acid) and TIBA (2,3,5-triodobenzoic acid) at different doses *viz.* 100ppm, 200ppm, 300ppm, 400ppm, 500ppm applied at 4th to 6th leaf stage of a plant and concluded that TIBA, ethephone had promoted more femaleness but in case of morphactin femaleness was promoted by lower concentration, they also concluded that dwarfness in plant is also observed by the application of chemicals.

Jaiswal and Kumar (1982) [10] carried experiment on *Morus nigra* L. by using chlorflurenol at three different concentration of 50ppm, 100ppm, 200ppm, applied on small bud which give rise to new branches after shading in December the 1st application was given in 1st week of January followed by 2nd application after 2-3 weeks and concluded that higher concentration of chlorflurenol changes more male flowers into female while the lower concentration of chloroflurenol male primordial remains same.

Allan *et al.* (1987) [1] carried an experiment on clonal female and male plant of papaya under 6 different environmental condition and concluded that for good and optimum growth of papaya the temperature range should be 25-30^{0}C at day and 11-16^{0}C at night. The vital thing they observed that the sex reversal take place in male papaya when the night temperature was about 12^{0}C with 11hrs day length where male plant is converted into elongate-type hermaphrodite.

Hore *et al.* (1989) [9] studied effect of chemicals on growth and yield of papaya (*Carica papaya*) by using MH, CCC, NAA, B-9, GA_3, at various doses and concluded that plant height attained 202.52cm with GA_3 200ppm and MH 500ppm and maximum number of leaves were obtained with ethrel 500ppm.

Subhadrabandhu *et al.* (1997) [20] carried an experiment on papaya plant by using 100ppm NAA, 500ppm GA_3 and distilled water sprayed until runoff, firstly at 30 days after transplanting and then repeated at 60 days and concluded that NAA treatment reduced the percentage of male plants and did not change the proportion of female and hermaphroditic plants and also dwarfness with less vegetative growth was reported. Whereas GA_3 treatment had not induced any difference over male:female:hermaphrodite ratio but seedlings had grown more vigorously then controlled one.

Kumar (1998) [13] carried an experiment by ethrel (2-chloro ethylphosphonic acid) at 240ppm, 480ppm, 960ppm and chloroflurenol (2-chloro-9-hydroxyflurene(9)carboxylic acid) at juvenile stage and second when plants entered into reproductive stage and concluded that ethrel is more effective than a chlorflurenol as 90% of male plants were successfully converted into female or intersexual flower. Although chloreflurenol also played a vital role in reversal of male into female but fruits developed by the flowers were abnormal in shape and size while taste was similar to the normal papaya plant.

Mitra and Ghanta (2000) [16] carried an experiment by spraying TIBA (25 and 50ppm), GA_3 (25 and 560ppm), ethrel (200 and 500ppm), MH (200 and 500ppm) and NAA (50 and 100ppm) at 45 days and at 75 days after transplanting and concluded that ethrel increased femaleness in plant whereas GA_3 improved quality, yield, size of fruits of papaya. Similarly, Syamal *et al.* (2010) [21] carried experiment by using GA_3 (100 and 150ppm), TIBA (100 and 150ppm) and

ethrel (200 and 300ppm) and concluded that TIBA and ethrel promoted femaleness in papaya while GA_3 increased the vegetative growth in papaya plant (Hazarika *et al.*, 2016) [5]. Han *et al.* (2014) [4] had observed that application of GA_3 did not have influenced sex reversal on female and hermaphrodite flowers; however, peduncle elongation and branching in inflorescence were reported in Sun Up, its mutant and AV9 cultivars. Further, increase in plant height and number of female flowers was reported.

Conclusion

Dioecious varieties of papaya are cheaper than that of gynodioecious but the main problem regarding dioecious plants are that the female:male ratio which is lower and growers who grow dioecious plant comes in big loss. The studies have proved that spraying of ethephon, TIBA, NAA and CCC at various doses in 4th to 6th leaf stage can convert male plant in female plant and hence increased the production in dioecious plant. Further, by spraying these chemicals dwarfness in papaya can also be induced hence this can be very useful and helpful to papaya growers as input cost will be less and production will be high.

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