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Sex Differentiation in Papaya (*Carica papaya* L.)

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Papaya (*Carica papaya L.*) is a fast-growing tropical fruit tree valued for its nutritious fruits and milky latex. It has three sex types, male, female and hermaphrodite with only female and hermaphrodite plants producing fruit. However, sex cannot be identified at early growth stages, which poses a challenge for commercial cultivation. Early sex identification using morphological traits and gene-linked markers can boost productivity and profitability.

Papaya (*Carica papaya L.*), native to Central and South America, is the most important species of the Caricaceae family, widely grown in tropical and subtropical regions for its nutrient-rich fruits and milky latex. With increasing demand due to rising health awareness and purchasing power, especially among India's middle and upper classes, boosting productivity is essential. Papaya is a trioecious species with male, female and hermaphrodite plants but only female and hermaphrodite plants bear marketable fruit. Since propagation is mostly through seeds, sex cannot be determined until 5–8 months of growth. Early identification of sex is vital to improve yield and aid breeding efforts. Dioecious varieties like Pusa Nanha and Pusa Dwarf are preferred in subtropical regions due to a higher proportion of stable female plants, compact size, and good yield. In tropical regions, gynodioecious papaya varieties are preferred for their high yield in mild climates. However, the inability to identify plant sex at the seedling stage limits commercial cultivation. If sex can be determined early, growers can establish plantations with an ideal 95% female to 5% male ratio, saving land, water, labor, and inputs for fruit-bearing plants. Currently, farmers plant 3–5 seedlings per hill, wait 4–6 months to identify sex, and then remove unwanted plants. Male plants often outnumber females, reducing productivity and increasing costs, as male plants have little commercial value. Kumar (1951) reported morphological traits like seed coat color and root type linked to papaya sex. Choudhary *et al*. (1957) found male leaves had more carbohydrates, phosphorus, and chlorophyll, while female leaves had higher nitrogen and potash.

Various methods have been explored to identify papaya sex types early. Cytological studies sought chromosomal differences (Datta, 1971). Colorimetric tests using leaf extracts showed moderate accuracy for males (67%) and females (87%) (Singh *et al*., 1961), while phenol tests differentiated females (86%) and males (77%) but not hermaphrodites (Jindal & Singh, 1976). Paper chromatography detected trans-cinnamic acid in hermaphrodites only (Poller, 1988). Isozyme analysis using cationic peroxidase distinguished males from females, but not females from hermaphrodites (Sriprasertsak *et al*., 1988). Due to the limitations of morphological, cytological, and isozyme markers, DNA markers using PCR offer a more accurate method for early sex identification in papaya. This is crucial for selecting parent plants in hybridization and ensuring desired sex types in micropropagation.

Genetics of Papaya

Papaya (*Carica papaya)* is the only species in its genus and has a small genome (372 Mb) with nine chromosomes. It is a polygamous species with sex determined by nascent sex chromosomes: XX (female), XY (male), and XYh (hermaphrodite). Sex is controlled by dominant alleles, with homozygous dominants being lethal. Molecular studies revealed a male-specific region (MSY) on the Y chromosome involved in sex determination. Papaya sex determination likely involves two genes: one suppressing stamens (feminizing) and the other suppressing carpels (masculinizing) (Ming *et al*., 2007). Van Buren *et al*. (2015) sequenced the male-specific Y region (MSY), showing only 0.4% divergence from the hermaphrodite Y (HSY). Although seven genes were found in the sex region, none could distinguish all sex types (Yu *et al*., 2008). Thus, DNA markers remain crucial for early, efficient sex identification in papaya production.

Markers related to sex expression in papaya

Despite progress in understanding papaya sex determination, the expression and variation of sexual forms remain poorly understood and impact fruit production. Dominant alleles linked to hermaphroditism and maleness can be lethal, and environmental factors (e.g., temperature, moisture, soil nitrogen) further affect sex expression. Hermaphrodite plants are unstable, often showing floral abnormalities or sex reversals, which reduce yield and fruit quality. These issues cause seasonal production shifts and market price fluctuations. Several morphological, physiological, biochemical, and molecular methods are used to identify papaya sex at the seedling stage.

Morphological marker: Morphological traits have been linked to sex expression in papaya. Kumar (1951) noted traits like seed coat color and root morphology. Reddy *et al*. (2012) found that males typically have more three-lobed leaves and slower growth, while females show five-lobed leaves and grow faster. Soni *et al*. (2017) identified seed color as a reliable marker: in dioecious types, dark brown seeds relate to males and black seeds to females; in gynodioecious types, brown indicates hermaphrodites and black indicates females.

Physiological and biochemical markers: Biochemical traits have been used to identify sex in papaya seedlings. Bojappa (1969) suggested pH, nitrogen levels, photoperiod, and growth regulators influence sex expression. Jindal and Singh (1976) found similar phenolics in male and female plants but with differences in concentration across tissues. Begum *et al*. (2010) noted higher peroxidase activity in females. Sánchez-Vilas and Retuerto (2009) observed sex-specific eco-physiological responses to water stress, with females showing better recovery. Soni *et al*. (2017) reported that females had higher phenolic content, stomatal conductance, and photosynthesis rates, while hermaphrodites showed higher chlorophyll ‘a’, indicating these traits can aid in early sex identification.

Plant growth regulators play key roles in papaya development and sex expression. Hasdiseve *et al*. (1989) found auxin and gibberellin-like substances increased with age in males but peaked early and then declined in females and hermaphrodites, with males having the highest levels overall. Ghosh and Sen (1975) reported higher nitrogen in male leaves and that applying NAA and CCC increased female-to-male ratios, while MH and GA3 promoted maleness. Han *et al*. (2014) showed that gibberellic acid (GA3) boosted flower and branch numbers and plant height in most papaya varieties but did not cause sex reversal.

Molecular marker for sex expression: Molecular technology, especially PCR-based markers, offers a fast and reliable way to identify papaya sex types at the seedling stage. These markers help breeders select desired genotypes early, improving precision and efficiency in plant breeding (Collard and Mackill, 2008). Several male, hermaphrodite-specific markers, initially developed using RAPD or AFLP, were later converted into SCAR markers like T12, W11, Napf, and PSDM to distinguish hermaphrodites from female seedlings (Deputy *et al*., 2002; Parasnis *et al*., 2000; Urasaki *et al*., 2002). These markers have been effectively used in papaya production (Sobir & Pandia, 2008; Matsumoto *et al*., 2010). While PCR-based sex-diagnostic methods are effective, they require costly lab equipment and are challenging for field use (Rigano *et al*., 2010). To address this, simpler and more accessible methods are needed. Sondur *et al*. (1996) cloned RAPD products and developed SCAR primers; two RAPD markers, OPT1C and OPT12, were found to flank the SEX1 gene associated with papaya sex determination. Parasnis *et al*. (1999) screened commercial papaya genotypes and a wild species (V. cauliflora) using restriction enzymes and microsatellite probes. The $(GATA)_4$ repeat showed clear male/hermaphrodite-specific bands (5 kb with HinfI and 4 kb with HaeIII), while $(GAA)_6$ detected sex polymorphisms in only some genotypes. Parasnis *et al*. (2000) screened 80 RAPD primers and identified OPF2-0.8 kb as a male-specific marker in papaya. Lemos *et al*. (2002) used RAPD markers to differentiate sex in three Solo group genotypes, finding primer BC210, particularly BC210438, effective in identifying hermaphrodites. This 438 bp marker was consistently present in hermaphrodite plants but absent in females across 195 samples from various genotypes. Deputy *et al*. (2002) developed molecular markers closely linked to the Sex1 gene in papaya. They cloned RAPD products and designed SCAR primers: T12 and W11 identified male and hermaphrodite plants, while T1 served as a positive control, amplifying in all sex types. These markers showed no recombination in 182 F2 plants and allowed for sex prediction with 99.2% accuracy. SCAR W11 and T12 are now widely used for reliable early sex identification in papaya. SCAR marker T1, designed from the T1 interval sequence, amplifies all papaya sex types at 1,300 bp and serves as a positive PCR control. SCAR markers T12 and W11, each producing an 800 bp band, specifically identify male and hermaphrodite plants, and were tightly linked to the SEX1 gene (0.3 cM) in a SunUp × Kapoho F_2 population. Urasaki *et al*. (2002) used RAPD to identify a 450 bp fragment (PSDM) present only in male and hermaphrodite plants, which led to the development of SCARps, a 225 bp marker. A multiplex PCR combining SCARps with a papain gene marker was successfully used to distinguish male/hermaphrodite from female plants in the 'Sunrise Solo' cultivar. De Oliveira *et al*. (2007) found that RAPD marker BC210 accurately identified all hermaphrodite and female plants in Brazilian commercial papaya genotypes, showing strong potential for marker-assisted selection. However, SCAR markers showed false positives and negatives in these genotypes. Gangopadhyay *et al*. (2007) used ISSR and RAPD techniques and identified a female-specific band with ISSR primer $(GACA)_4$ at the pre-flowering stage. Somsri and Bussabakornkul (2008) used DAF to analyze 14 Thai papaya genotypes and identified primer OPA06 as effective for sex determination. It produced a 365 bp band for hermaphrodites and a 360 bp band for males, with no bands in females. Testing on 254 plants showed 88.18% accuracy overall, and 100% accuracy in identifying 47 hermaphrodite plants of the 'Khaeg Dum' cultivar from tissue culture. Niroshini *et al*. (2008) screened 100 RAPD primers in papaya and identified two OPC09 and OPE03 that produced male/hermaphrodite-specific bands at 1.7 kb and 0.4 kb, respectively. SCAR primer C09/20, developed from OPC09, successfully amplified 1.7 kb and 978 bp fragments in male and hermaphrodite plants. However, SCAR primers from OPE03 (E03/20FP and E03/20RP) failed to distinguish between sex types. Costa *et al*. (2011) used ISSR markers to study genetic diversity in Caricaceae and identify markers for early sex differentiation in papaya. They found a 500 bp ISSR marker present in 25% of papaya genotypes, which could distinguish female from hermaphrodite plants. The study also showed Jacaranda spinosa is genetically closer to Vasconcellea than to Carica, with C. papaya more distantly related to both. Hsu *et al*. (2012) developed six male/hermaphrodite-specific markers using multiplex loop-mediated isothermal amplification (mLAMP) for rapid, efficient sex identification in papaya seedlings. This method is fast (under 1 hour), requires minimal equipment, uses small DNA amounts (0.5 mg) and is cost-effective (5 µl reaction volume). Reddy *et al*. (2012) analyzed papaya genetic diversity using 20 RAPD primers. Among them, OPL-9 amplified DNA only from male plants, while OPL-13 clearly distinguished male and female plants. OPL-12 showed the highest polymorphism but could not differentiate sexes. Chaturvedi *et al*. (2014) validated the sex-linked SCAR marker W11 using PCR across different papaya cultivars—dioecious (female or male) and gynodioecious (female or hermaphrodite). In a double-blind test on 84 F1 seedlings, the assay accurately identified sex by producing an 800 bp band in male and hermaphrodite plants. Tsai *et al*. (2016) used the loop-mediated isothermal amplification (LAMP) method for papaya sex determination, which doesn't require DNA purification. This approach proved to be easy, efficient, and cost-effective for identifying papaya sex. Soni *et al*. (2017) used ISSR and SCAR markers to identify those linked to sex expression in papaya. Out of 10 tested markers (9 SCAR, 1 ISSR), five (T12, W11, SCAR SDSP, C09/20, and GACA4) were confirmed to be associated with sex. SCAR markers proved more consistent, enabling breeders and growers to accurately identify desired sex types at the seedling stage, improving plantation productivity and speeding up papaya breeding programs. Liao *et al*. (2017) developed two male-specific papaya markers, PMSM1 and PMSM2, which amplify 585 bp and 548 bp fragments, respectively. These markers target a large male-specific retrotransposon insertion and were validated across 11 gynodioecious and 4 dioecious papaya genotypes. Modi *et al*. (2018) evaluated sex-specific RAPD and SCAR markers in papaya, finding that both marker systems accurately identified sex types at the seedling stage with equal precision and eliminated false negatives.

Conclusion

Significant progress has been made in understanding sex determination in plants, especially in model species like *Carica papaya*. Molecular markers have proven to be the most reliable method for early sex identification distinguishing male, female, and hermaphrodite seedlings more accurately than morphological or physiological traits. Reducing the cost of sex identification is crucial to enable commercial growers to benefit from this technology, improving farm productivity and profitability. Advances in science are paving the way for developing affordable, user-friendly kits for quick and efficient sex determination in papaya in the near future.

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