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RESEARCH ARTICLE

VALIDATION OF SCAR MARKERS FOR EARLY SEX DETERMINATION IN PAPAYA (*Carica papaya* L.) UNDER SRI LANKAN CONDITIONS

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Abstract

Papaya (*Carica papaya* L.) is a commercially important tropical fruit crop exhibiting three sex types: male, female and hermaphrodite. Because sex expression cannot be identified morphologically at the seedling stage, excess plants are often maintained until flowering, resulting in inefficient use of land, labour and inputs. This study evaluated the transferability and diagnostic performance of widely used sequence-characterized amplified region (SCAR) markers for early sex identification in a Sri Lankan papaya population. Fifty randomly selected 40–45-day-old seedlings from a controlled hermaphrodite × hermaphrodite (H×H) population were screened using SCAR-W11 and SCAR-T12 markers, with SCAR-T1 used as a positive amplification control. SCAR-W11 and SCAR-T12 amplified a specific ~800 bp fragment in hermaphrodite plants, whereas no amplification was observed in female plants. Morphological assessment at flowering showed complete agreement between molecular marker-based identification and phenotypic sex classification within the evaluated dataset showing complete agreement between molecular and morphological sex identification. These findings indicate that SCAR-W11 and SCAR-T12 are reliable markers for distinguishing hermaphrodite and female papaya seedlings under Sri Lankan conditions and can facilitate more efficient nursery management and breeding programs.

Keywords: *Carica papaya*, Hermaphrodite, Molecular markers, SCAR, Seedling screening, Sex identification

INTRODUCTION

Papaya (*Carica papaya* L.) is an economically important tropical fruit crop cultivated widely in tropical and subtropical regions. The crop is valued for its high nutritional content, rapid growth, and continuous fruit production. In addition to fresh fruit consumption, papaya is an important source of proteolytic enzyme papain, which is widely used in the food and pharmaceutical industries (Sharma *et al.*, 2020).

Papaya is a diploid species (2n = 18) that exhibits a trioecious sexual system consisting of male (XY), female (XX), and hermaphrodite (XY^h) plants. Sex determination in papaya is governed by a recently evolved sex chromosome system characterized by structural divergence and suppressed recombination between the X and Y/Y^h chromosomes (Liu *et al.*, 2004; Ming *et al.*, 2007; Wang *et al.*, 2012). Although male plants contribute to pollination, only female and hermaphrodite plants produce commercially valuable fruits. Hermaphrodite plants are particularly preferred in commercial cultivation because they produce elongated fruits with desirable market characteristics and are capable of self-pollination.

In papaya, hermaphrodite plants possess the genotype XY^h. When hermaphrodite plants are self-pollinated, the expected viable progeny consist of hermaphrodite (XY^h) and female (XX) plants in an approximate 2:1 ratio, because the Y^hY^h genotype is lethal and aborts during embryonic development (Storey,

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1953; Ming *et al.*, 2007). Consequently, male plants (XY) are not expected in progeny derived from hermaphrodite selfing.

A major limitation in papaya cultivation and breeding is that plant sex cannot be determined morphologically at the seedling stage. Consequently, several seedlings are typically planted per pit and maintained until flowering to identify and remove undesired plants. This practice leads to inefficient use of land, labour, water and other agricultural inputs.

Molecular marker-based techniques provide an efficient alternative for early sex identification in papaya. Among different marker systems, sequence-characterized amplified region (SCAR) markers are particularly useful because of their high reproducibility and specificity. Deputy *et al.* (2002) developed several sex-linked SCAR markers, including W11 and T12, which amplify fragments associated with the Y/Y^h chromosomes in male and hermaphrodite plants but not in female plants.

In Sri Lanka, Niroshini *et al.* (2000) reported the identification of sex-linked molecular markers derived from RAPD analysis in local papaya populations, demonstrating the potential of molecular approaches for early sex identification under Sri Lankan conditions. However, the diagnostic performance and transferability of the widely used SCAR marker system developed by Deputy *et al.* (2002) have not been systematically validated in Sri Lankan papaya germplasm.

Papaya cultivated in Sri Lanka represents a genetically heterogeneous population consisting of locally maintained seed stocks as well as introduced cultivars and hybrids. Such diversity may result in variation in Y/Y^h haplotypes among regional germplasm pools. Because papaya sex chromosomes exhibit structural divergence and suppressed recombination (Liu *et al.*, 2004; Ming *et al.*, 2007; Wang *et al.*, 2012), the transferability of sex-linked molecular markers across different populations cannot always be assumed. Therefore, validation of these markers in local germplasm is necessary before their routine application in breeding programs and nursery management.

The present study therefore aimed to evaluate the transferability and diagnostic performance of the widely used SCAR markers W11 and T12 for early sex identification in a Sri Lankan papaya population, using SCAR-T1 as a positive amplification control.

MATERIALS AND METHODS

Experimental site and plant materials

The study was conducted at the Fruit Research and Development Institute (FRDI), Kananwila, Horana, Sri Lanka, from January to May 2024. Known sex-typed male, female, and hermaphrodite plants were included as reference controls to verify the amplification specificity of the SCAR markers.

Fifty papaya seedlings aged 40–45 days were used for molecular analysis. These seedlings were derived from a controlled F_2 population generated through hermaphrodite × hermaphrodite crosses. Self-pollination was ensured by bagging flowers to prevent outcrossing. Seedlings used in this study were selected primarily for validation of SCAR marker performance rather than for estimation of population-level segregation ratios.

DNA extraction and PCR amplification

Genomic DNA was extracted from young, healthy leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method described by Borges *et al.* (2009).

The concentration and quality of extracted genomic DNA were assessed prior to PCR amplification. DNA concentration and purity were determined using spectrophotometric measurements at 260 and 280 nm, and samples with A260/A280 ratios between 1.8 and 2.0 were used for PCR analysis.

Three SCAR markers (T1, T12, and W11) previously reported by Deputy *et al.* (2002) were used for sex identification in papaya (Table 1). Polymerase chain reaction (PCR) was carried out in a total reaction volume of 12.5 µL containing 6.25 µL of 2× GoTaq® colourless master mix (Promega), 0.5 µL each of forward and reverse primers (1 μM), 0.5 μL of template DNA and 4.75 μL of nuclease-free water.

PCR amplification was performed using an initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 1 min, annealing at primer-specific temperatures (Table 1) for 1 min and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. Amplified products were resolved on 1.3% agarose gels stained with diamond dye and visualised using a gel documentation system. A no-template negative control containing nuclease-free water instead of template DNA was included in each PCR run to monitor potential contamination. No amplification was observed in the negative control reactions. PCR amplification was repeated to confirm the reproducibility of the banding patterns, and only clear and consistent amplification profiles were considered for marker interpretation.

Table 1: SCAR markers used for sex determination in papaya (Deputy *et al.*, 2002)

Primer	Sequence (5'-3')	Link trait	Annealing temperature T_A (^oC)	Product size (bp)
W11- F	CTGATGCGTGTGTGGCTCTA	Male and Hermaphrodite	58	800
W11-R	CTGATGCGTGATCATCTACT			
T12- F	GGGTGTGTAGGCACTCTCCTT	Male and Hermaphrodite	57	800
T12 -R	GGGTGTGTAGCATGCATGATA			
T1- F	TGCTCTTGATATGCTCTCTG	All sex types	57	1300
T1-R	TACCTTCGCTCACCTCTGCA			

Morphological validation

All seedlings subjected to molecular analysis were transplanted and maintained in the field until flowering. Sex expression was determined at approximately five months after planting based on floral morphology (Figure 1).

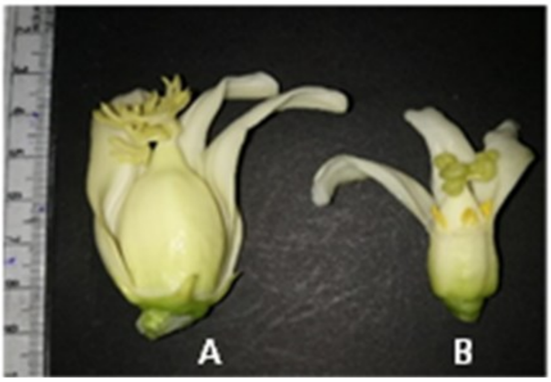

Figure 1: Morphological differentiation of papaya sex types used for validation of SCAR marker results. (A) Female flower showing carpellate structure with prominent stigma. (B) Hermaphrodite flower showing bisexual structure with developed pistil and stamens.

Female flowers were identified by the presence of a well-developed pistil with prominent stigma, whereas hermaphrodite flowers exhibited both functional stamens and pistil structures. These morphological observations were used to validate the sex identification results obtained from SCAR marker analysis.

RESULTS AND DISCUSSION

Validation of SCAR markers

The SCAR markers T1, T12 and W11 were tested using known male, female and hermaphrodite plants to verify their amplification patterns. Agarose gel electrophoresis showed clear and distinct bands at the expected fragment sizes for each marker. Amplification patterns were consistent among samples representing the same sex type, indicating reproducible PCR amplification. No additional bands or smearing were observed, suggesting high specificity of the PCR reactions.

The SCAR-T1 primer pair amplified a fragment of approximately 1300 bp in male, female and hermaphrodite plants, confirming its suitability as a positive amplification control (Figure 2).

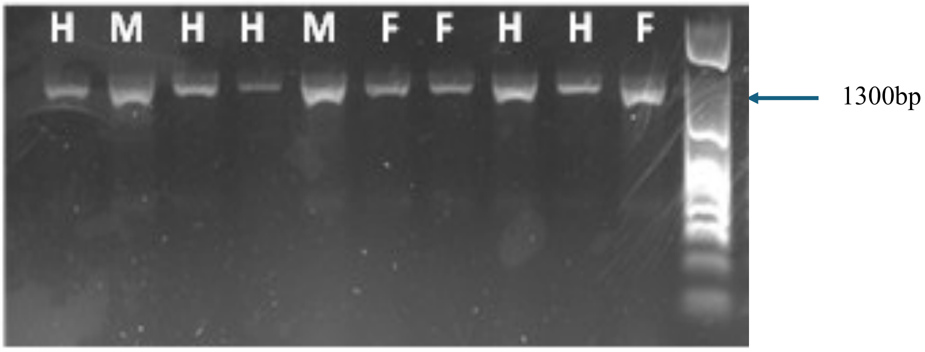

Figure 2: Agarose gel electrophoresis of PCR amplification products obtained using the SCAR-T1 primer pair (positive control) in papaya. A fragment of approximately 1300 bp amplified in male (M), female (F), and hermaphrodite (H) plants. (100 bp DNA ladder was used)

In contrast, the SCAR-T12 and SCAR-W11 markers amplified a specific fragment of approximately 800 bp in hermaphrodite plants, whereas no amplification was detected in female plants (Figures 3). These amplification patterns are consistent with the expected behaviour of sex-linked SCAR markers previously reported for papaya (Deputy *et al*., 2002; Ejaz *et al*., 2015). The SCAR-W11 and SCAR-T12 markers are associated with the Y/Y^h chromosomes, and therefore amplification is expected in male (XY) and hermaphrodite (XY^h) genotypes but not in female plants (XX). Because the experimental population used in this study was derived from hermaphrodite selfing (XY^h × XY^h), only hermaphrodite and female progeny were expected. Consequently, amplification was observed only in hermaphrodite individuals, while female plants showed no amplification.

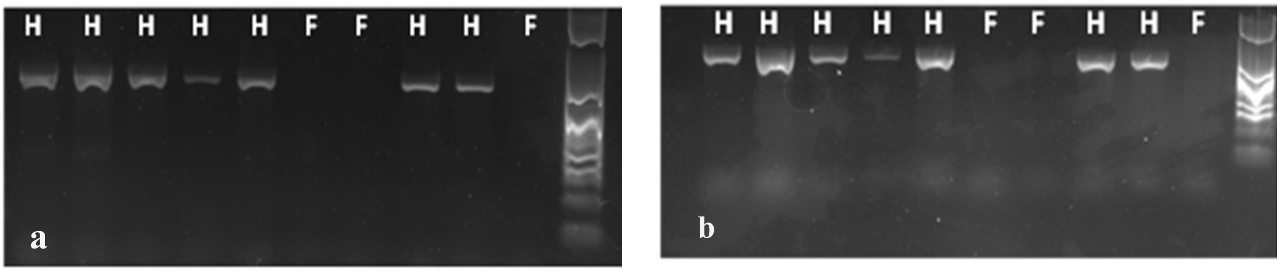

Figure 3: Agarose gel electrophoresis of PCR amplification using the (a) SCAR-T12 marker and (b) SCAR-W11 marker for sex identification in papaya. A fragment of approximately 800 bp amplified in hermaphrodite (H) plants, with no amplification observed in female (F) plants. (100 bp DNA ladder was used)

Morphological confirmation of SCAR-based sex identification

Application of SCAR-T12 and SCAR-W11 markers to fifty papaya seedlings enabled clear discrimination of sex types at the seedling stage. Twenty-two seedlings (44%) produced the ~800 bp fragment and were identified as hermaphrodite plants, whereas the remaining twenty-eight seedlings (56%) showed no amplification and were classified as female plants. Early identification of plant sex is particularly important in papaya breeding and commercial cultivation because sex expression becomes morphologically distinguishable only after flowering. Molecular markers therefore provide an efficient approach for early sex identification, enabling reliable discrimination of sex types before flowering (Modi *et al.*, 2018).

Morphological observations at the flowering stage confirmed the molecular identification results. Female plants produced carpellate flowers with prominent stigmas, whereas hermaphrodite plants produced bisexual flowers containing both functional stamens and pistils (Figure 1). Complete concordance was observed between SCAR marker-based identification and morphological sex determination (50/50), demonstrating the reliability of these markers for early seedling screening.

A chi-square test was conducted to evaluate whether the observed sex ratio differed significantly from the theoretical expectation of hermaphrodite selfing (1 female: 2 hermaphrodite). Among the 50 seedlings analysed, the observed distribution was 22 female and 28 hermaphrodite plants, which does not exactly correspond to the theoretical ratio. However, the chi-square test yielded $\chi^2 = 2.52$ (df = 1; $p > 0.05$), indicating that the observed distribution does not significantly deviate from the expected segregation ratio. The slight difference from the theoretical ratio may be attributed to sampling variation associated with the relatively small population size evaluated in this study.

The amplification patterns observed in this study are consistent with previous reports describing SCAR markers linked to papaya sex chromosomes (Deputy *et al.*, 2002; Ejaz *et al.*, 2015; Sujipuli *et al.*, 2016). Earlier studies have also reported sex-linked molecular markers associated with papaya sex determination (Parasnis *et al.*, 1999). These findings further confirm the usefulness of SCAR markers as reliable tools for early sex identification in papaya.

Validation of molecular markers in local germplasm populations is essential before their routine application in breeding programs because marker performance may vary across different genetic backgrounds. Papaya cultivated in Sri Lanka represents a genetically heterogeneous population composed of locally maintained seed stocks as well as introduced cultivars and hybrids. Therefore, evaluation of marker performance under local germplasm conditions is important before practical application.

Although complete agreement between molecular markers and phenotypic sex was observed in the present study, the population size evaluated was relatively small and primarily intended for marker validation rather than detailed segregation analysis. Therefore, further validation using larger populations and genetically diverse germplasm would strengthen the broader application of these markers in papaya breeding and nursery management.

CONCLUSIONS

The SCAR markers W11 and T12 successfully differentiated hermaphrodite and female papaya seedlings in the evaluated Sri Lankan population, showing complete concordance between molecular marker results and flowering-stage phenotypes within the tested sample set (n = 50; 95% CI: 92.9–100%). These findings demonstrate the suitability of these markers for early sex identification in papaya under Sri Lankan conditions.

Early identification of hermaphrodite plants at the seedling stage can facilitate more efficient nursery management and support papaya breeding programs by enabling timely selection. The slight deviation from the theoretical segregation ratio observed in this study may be attributed to the limited sample size or sampling variation. Larger populations should therefore be evaluated to confirm the expected genetic segregation pattern. Further validation using larger and genetically diverse populations will also strengthen the broader applicability of these markers in papaya improvement and commercial cultivation.

AUTHOR CONTRIBUTION

AGWP and UIPP conceptualized the research project and designed the experiment. AGWP carried out laboratory experiments, data collection, data analysis and preparation of the original manuscript, WTSSW assisted with laboratory experimentation and data

collection, KMM and UIPP provided manuscript editing and supervision.

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