DOI : 10.5958/0974-0112.2017.00067.6

Indian J. Hort. 74(3), September 2017: 334-339

Efficacy of gene-based markers associated with sex expression in papaya

Anjali Soni, Jai Prakash*, S.K. Singh, A.K. Goswami, N.C. Gupta and A.K. Singh

Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

The papaya (*Carica papaya* L.) is a polygamous plant, has three types of sex forms, *viz.* male, female and hermaphrodite. Efforts were made to identify sex expression and validation of gene-linked molecular markers associated with it. Molecular markers (ISSR and SCAR) were employed to distinguish male, female and hermaphrodite sex forms in seedlings and well differentiated adult plant types. The important markers which have been found linked with sex forms in papaya were used for validation in population of six papaya genotypes. Total 10 sex linked DNA markers (9 SCAR and 1 ISSR) were employed for validation of sexes in 6 papaya genotypes (dioceious and gynodioecious) . Out of 10 only five (T12, W11, SCAR SDSP, C09/20 and GACA4) were amplified for the sex expression. However, five SCAR (SCARp, SCARpm, T1, STS 807 and STS 831) marker did not get amplified with the all sex genotypes. Of the two marker systems tested, SCAR markers were most consistent. Markers, namely, T12, CFW+CRV and W11 were most informative to predict cent per cent sex forms. These markers can be used by the breeders and commercial papaya growers to identify desired sex types at seedling stage in nursery for establishing a productive plantation.

Key words: Papaya, sex form identifican, molecular markers.

INTRODUCTION

The papaya, *Carica papaya* L., is a native of Central and South America and belongs to the family Caricaceae. Papaya is a commercial fruit crop cultivated throughout the tropical and sub-tropical regions of the India and ranks fifth with regards to area and production among different fruit crops. Propagation of papaya by seed is still the most practical method of raising commercial crop. There are several reasons why a desirable sex type of papaya plant needs to be identified prior to planting. Generally, the number of male plants outnumbers the females in a plantation, which renders it unproductive.

The papaya generally flowers 75 to 150 days after transplanting and identification of the desirable plants at seedling stage would help in raising the orchard with appropriate design. In subtropical region the dioecious varieties like Pusa Nanha and Pusa Dwarf are preferred in over gynodioecious once due higher number of stable female plants in a population, besides their dwarf stature and high yields. Conversely in the tropical areas, gynodioecious varieties are preferred because of their high yield potential. Sex expression in papaya is controlled by a single gene with three alleles, which have a pleiotropic effect (Hofmeyr, 8; Storey, 15). With the advancement of the science and techniques new facts has been explored, *i.e.* physical mapping and sample sequencing of the non-recombination region led to the conclusion that sex determination is controlled by a pair of primitive sex chromosomes with a small male-specific region (MSY) of the Y chromosome (Ming *et al.*, 10).

The leaf extracts of a large number of sexually undifferentiated seedlings at nursery stage, with modified Almen reagent were analysed. A colorimetric test for total phenols can differentiate females (86%) from males (77%), but was unable to detect the bisexual plants (Jindal and Singh, 9). Paper chromatography also indicated that trans-cinnamic acid is dominantly expressed in the leaves of hermaphroditic seedlings, but females and males could not be differentiated (Poller, 13). In addition, isozymes have also been exploited to identify markers that could co-inherit with sex types in papaya using the banding patterns of cationic peroxidase, males could be differentiated from females, but females could not be distinguished from hermaphrodites (Sriprasertsak *et al.*, 14).

The failure of morphological tags, cytological evidences and isozyme markers to determine the sex types in papaya at the seedling stage has led to use of DNA markers for determining the sex differences. Hence, the present study was undertaken with an objective to validate the efficacy of gene based markers associated with sex expression in papaya.

MATERIALS AND METHODS

Seedling of six papaya genotyes (dioecious and gynodioecious), namely, Pusa Nanha, Red Lady, P-7-2, P-9-5, P-9-12 and P-7-2 x SAM were raised

*Corresponding author e-mail: singhjai2001@rediffmial.com

to examine the sex expression at the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi during 2014-2015. Genomic DNA extraction was done at seedling stage and same tagged plants were planted in the net house to validate the male, female and hermaphvrodite type of sex expression at the flowering. Polymorphism study was carried out using Inter Simple Sequence Repeats (ISSR) and Sequence Characterized Amplified Region (SCAR). Total 10 primers (Table 1) were employed to distinguish male, female and hermaphrodite sex forms in seedlings and well differentiated adult plants.

From tender leaves of papaya seedling, genomic DNA was extracted using the protocol and the steps involved in CTAB method of DNA isolation as given by Murray and Thomson (11). Two hundred milligram of young, succulent and healthy leaves were taken and grind in liquid nitrogen to a fine powder and transferred to a 1.5 ml Eppendorf tube. Quantification of DNA was done by analysing the purified DNA on 0.8% agarose gel with Hind III-cut λ DNA as standard. The concentration of DNA in individual sample was determined based on the intensity of the bands in the λ DNA ladder. Part of the DNA samples was diluted with appropriate amount of TE buffer to yield a working concentration of 20 ng/µl and stored at 4°C. The reaction (Touchdown PCR) with initial denaturation at 94^0C for 5 min., denaturation 94^0C for 1 min., primer annealing at 55^0C for 1 min. 35 cycles, primer extension at 72^0C for 1 min., final extension at 72^0C for 8 min., touch down of 0.5^oC for the first 20 cycles were carried out. The amplified fragments were resolved on 2 per cent agarose gel. Number of polymorphic bands generated by each primer was scored as 0 or 1 for absence or presence of band, respectively.

RESULTS AND DISCUSSION

Out of 10 only five (T12, W11, SCAR SDSP, C09/20 and $GACA_4$) were amplified for the sex expression, while rest five SCAR (SCARp, SCARpm, T1, STS 807 and STS 831) markers did not get amplified with the all the sex types. Among the amplified markers some of them did show ambiguity in the results particularly with the validation of hermaphrodite plants. Though, some of the markers were consistent with particular genotypes.

The Fig. 1 exhibited PCR amplification of SCAR T12 DNA markers showing segregation for male, female and hermaphrodite sexes of Pusa Nanha,

Table 1. List of 10 primers (9 SCAR and 1 ISSR) were used to validate male, female and hermaphrodite sex forms in six papaya genotypes.

Name	Primer sequence (5'……..3')	Reference
SCAR marker		
T12	T12F:GGGTGTGTAGGCACTCTCCTT T12R:GGGTGTGTAGCATGCATGATA	Deputy *et al.* (5)
W11	W11F:CTGATGCGTGTGTGGCTCTA W11R:CTGATGCGTGATCATCTACT	Deputy *et al.* (5)
SCAR SDSP	CFW:AAACTACCGTGCCATTATCA CRV:AGAGATGGGTTGTGTCACTG	Chaves-Bedoya and Nunez (2)
C09/20	FP:CTCACCGTCCATTTTAATTA RP:CTCACCGTCCGCGGCATCAATGTA	Niroshini *et al.* (12)
SCARp	SDP-1:GCACGATTTAGATTAGATGT SDP-2:GGATAGCTTGCCCAGGTCAC	Urasaki *et al.* (17)
SCARpm	SDP-2-F: GGATAGCTTGCCCAGGTCAC SDP-2-R: GGTAAGAGTTTTTCCCAAGC	Urasaki *et al.* (16)
T1	T1F:TGCTCTTGATATGCTCTCTG T1R:TACCTTCGCTCACCTCTGCA	Deputy *et al.* (5)
STS 807	STS 807-F ATTAGCCCCCAAAACAGAGC STS 807-R ATGGAGGGGGAGGACTCTAA	Conomikes *et al.* (3)
STS 831	STS 831- F ATA TAT ATA TAT ATA TYA STS 831-R ATA TAT ATA TAT ATA TYC	Conomikes *et al.* (3)
ISSR marker		
(GACA)4	(GACA)4:GACAGACAGACAGACA	Gangopadhyay *et al.* (7)

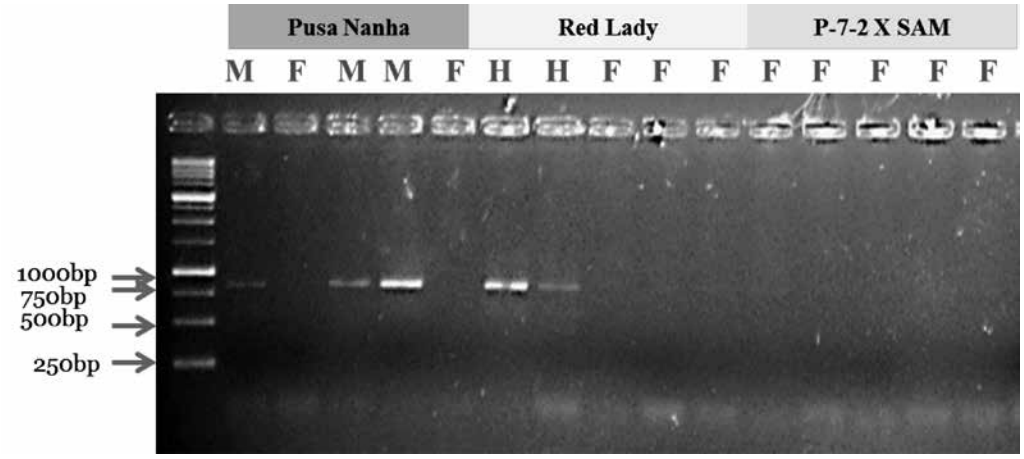

Fig. 1. PCR amplification showing segregation of SCAR T12 with papaya sex. Genotype names are depicted in coloured boxes, M = male, F = female, H = hermaphrodite, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples and their sexes.

Red Lady and P-7-2 × SAM genotypes. PCR was run on genomic DNA from papaya genotypes Pusa Nanha, Red Lady and P-7-2 × SAM using primers of SCART12 marker. The desired size of band (~800 bp) is present in male and hermaphrodite plants but not in female plants among the dioecious and gynodioecious genotypes. It was most effective DNA marker could be useful to identify to the desired sex across the genotypes the male and hermaphrodite. The similar results were reported by Deputy *et al.* (5) markers tightly linked to Sex1, the gene, which determines type of plant sex in papaya with a population of 182 F_2 plants from a SunUp × Kapoho.

PCR amplification and segregation of SCAR SDSP (CFW, CRV) with female and hermaphrodite types of papaya sexes (Fig. 2). PCR was run on genomic DNA from papaya genotypes Red Lady, P-9-12 and P-7-15 using CFW and CRV primers of SCAR SDSP marker. The desired size of band (~375bp SCAR SDSP) is present only in hermaphrodite plants but not in female plants among studied genotypes. PCR was run on genomic DNA from Red Lady, P-9-12 and P-7-15 using primers for C09/20 SCAR marker (Fig. 3). SCAR C09/20 with ~1000 bp band size is present only in hermaphrodite plants but not in female plants. SCAR C09/20 was able to validate hermaphrodite plants of the two gyndioecious genotypes indicate that it is very effective for P-9-12 and P-7-15 but presence of too faint band in female Red Lady indicates human errors. The findings of SCAR C09/ 20 are in accordance with the earlier report of Deputy *et al.* (5); Urasaki *et al.* (16) identified after screening of 25 arbitrary

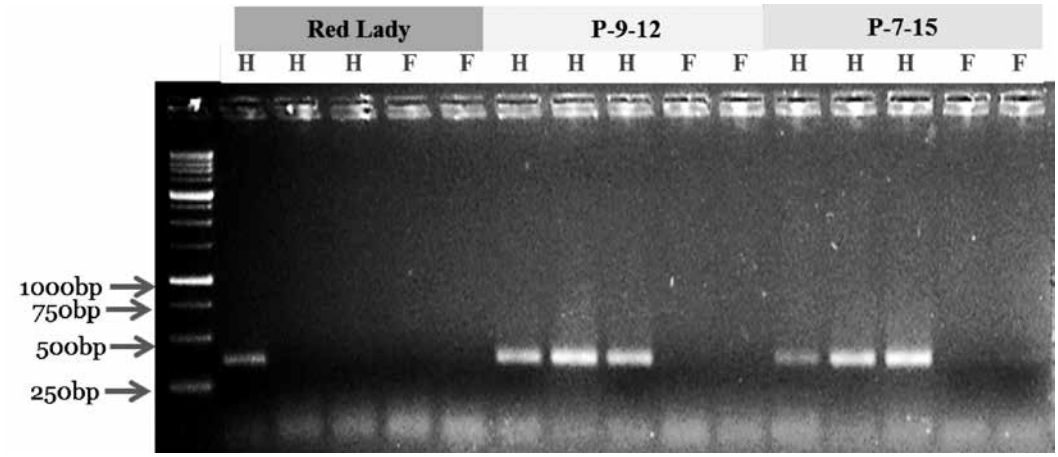

Fig. 2. PCR amplification showing segregation of SCAR SDSP (CFW, CRV) with papaya sex. Genotypes names are depicted in coloured boxes, H = hermaphrodite, F = female, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples with their type of sexes.

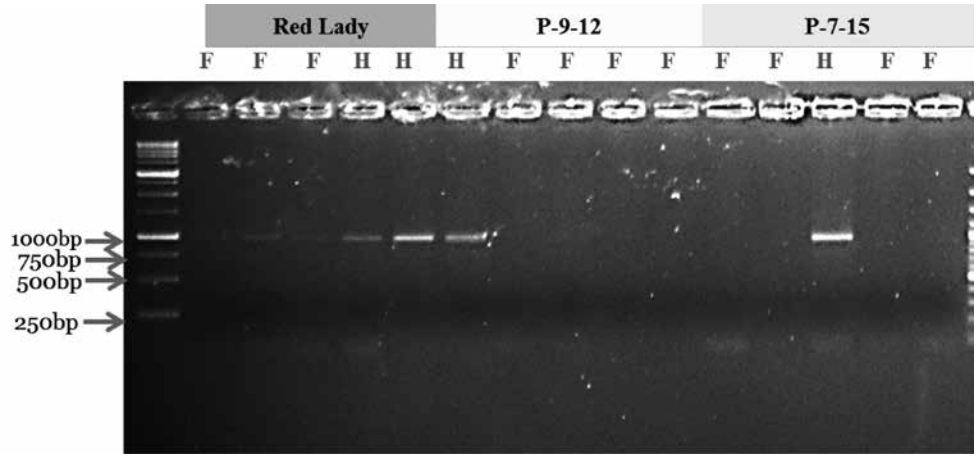

Fig. 3. PCR amplification showing segregation of SCAR C09/20 with papaya sex. Names of the genotypes are depicted in boxes, H = hermaphrodite, F = female, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples.

primers in Sunrise Solo papaya and De Oliveira *et al*. (6), Niroshini *et al.* (12) from 100 arbitrary decamer primers in 10 plants of papaya from Sri Lanka for type of sexes and Chaturvedi *et al.* (1) with six sequences of SCAR from the 84 F_1 plants of papaya. DNA is extracted from the leaves of the papaya plants and is subjected to PCR amplification showing segregation of SCAR W11 with papaya sex (Fig. 4). PCR was run on genomic DNA from papaya varieties Red Lady, P-7-2 and P-9-5 using primers for W11 SCAR marker. SCAR W11 with a band size of ~825 bp is present only in hermaphrodite plants but not in female plants. The results are in accordance to Urasaki *et al.* (16) who had tested these primers on three papaya genotypes.

The Fig. 5 exhibits PCR amplification showing segregation of ISSR in male, female and hermaphrodite types. The PCR was run on genomic DNA from papaya varieties Pusa Nanha, Red Lady and P-7-2 × SAM using $(GACA)_4$ primers for ISSR marker. The P1 (~ 600 bp) amplicon was present in male and female plants but not in hermaphrodite plants. The P2 (~2 kb) amplicon is present only in female plants but not in male and hermaphrodite plants. The P3 (~ 3 kb) amplicon present only in P-7-2 × SAM genotype but not in others. The $(GACA)_4$ primer was also very effective to validate male, female and hermaphrodite plants among the diverse genotypes for sexes. The similar finding have been reported by Parasnis *et al*. (1999) identification of male sex specific markers in 8 dioecious papaya varieties of papaya using $(GATA)_4$ and $(GACA)_4$ simple sequence repeats, Gangopadhyay *et al.* (7) differentiated sex expression

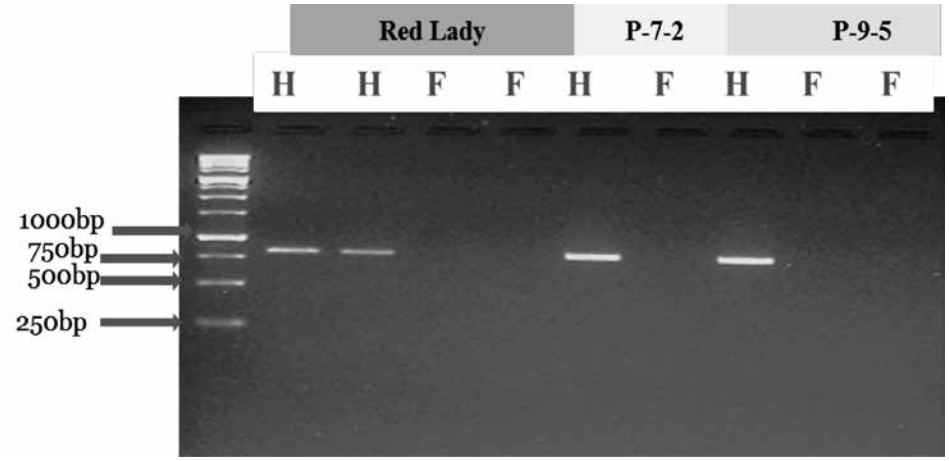

Fig. 4. PCR amplification showing segregation of SCAR W11 with papaya sex. Genotypes names are depicted in boxes, H = hermaphrodite, F = female, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples.

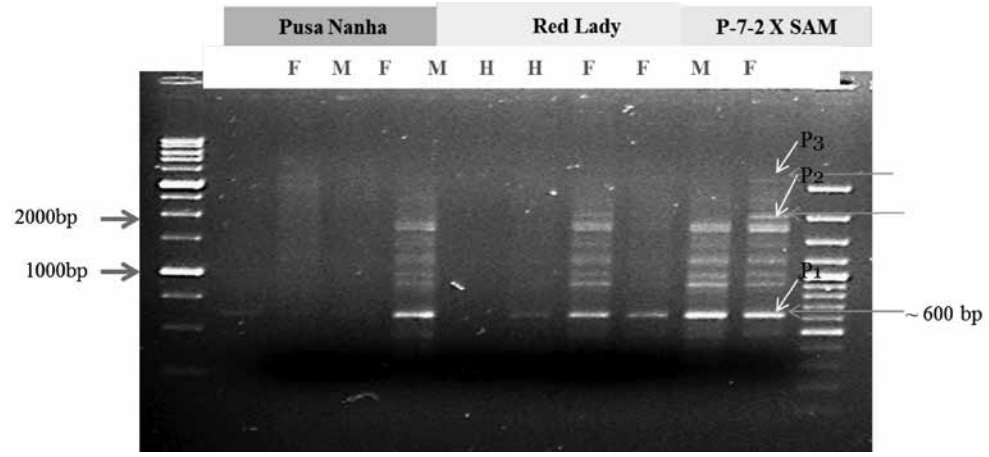

Fig. 5. PCR amplification showing segregation of ISSR with papaya sex. The genotypes names are depicted in coloured boxes, M = male, H = hermaphrodite, F = female, L = 1 kb DNA ladder, L2 = 100 bp DNA ladder and numeric over the agarose gel represent different samples of the papaya.

in *Carica papaya* and *Cycus ciranilis*. Costa *et al*. (4) established sex differentiation through ISSR marker at 500 bp in *Carica* and *Vasconcellea* spp. Among the five amplified sex-linked DNA markers some of them have shown the ambiguity in the results particularly with the validation of hermaphrodite plants. ISSR markers were consistent with P-7-2 × SAM genotypes. Of the two marker systems tested, SCAR markers were most consistent. Markers, namely, T12, CFW+CRV and W11 were most informative to predict 100% sex forms. These markers can be used by the breeders and commercial papaya growers to identify desired seedlings at early stage.

REFERENCES

1. Chaturvedi, K., Bommisetty, P., Pattanaik, A., Chinnaiyan, V., Ramachandra, D.M. And Chennareddy, A. 2014. PCR detection assay for sex determination in papaya using SCAR marker. *Acta Bot. Croatica*, **73**: 291-98.
2. Chaves-Bedoya, G. and Nenez, V. 2007. A SCAR marker for the sex types determination in Colombian genotypes of *Carica papaya* L. *Euphytica,* **153**: 215-20.
3. Conomikes, M., Wright, M. and Delpratt, J. 2011. Sex discrimination of Buloke (*Allocasuarina luehmannii*) for selective revegetation. *Muelleria,* **29**: 104-09.
4. Costa, F.R.D., Santana, T.N., Paula, P.A., Gabriel, C. and Pereira, M.G. 2011. ISSR markers for genetic relationships in Caricaceae and sex differentiation in papaya crop. *Breed. App. Biotech.* **11**: 352-57.
5. Deputy, J., Ming, R., Ma, H., Liu, Z., Fitch, M.M., Wang, M., Manshardt, R. and Stiles, J.I. 2002. Molecular markers for sex determination in papaya (*Carica papaya* L.). *Theor. Appl. Genet.* **106**: 107-11.
6. De Oliveira, E.J., Dantas, J.L.L., Castellen, M.D.S., De Lima, D.S., Barbosa, H.D. and Motta, T.B.N. 2007. Molecular markers for sex identification in papaya. *Pesquisa Agropecuaria Brasileira,* **42**: 1747-54.
7. Gangopadhyay, G., Roy, S.K., Ghosh, K., Poddar, R., Bandyopadhyay, T., Basu, D. and Mukherjee, K.K. 2007. Sex detection of *Carica papaya* and *Cycas circinalis* in pre-flowering stage by ISSR and RAPD. *Curr. Sci.* **92**: 525-26.
8. Hofmeyr, J.D.J. 1941. Genetics of *Carica papaya* L. *Chron. Bot*. **6**: 245-47.
9. Jindal, K.K. and Singh, R.N. 1976. Sex determination in vegetative seedling of *Carica papaya* L. by phenolic test. *Scientia Hort.* **4**: 33-39.
10. Ming, R., Yu, Q. and Moore, P.H. 2007. Sex determination in papaya. *Sem. Cell. Dev. Biol.* **18**: 401-08.
11. Murray, H.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight DNA. *Nucl. Acids Res.* **8**: 4321-25.
12. Niroshini, E., Everard, J.M.D.T., Karunanayake, E.H. and Tirimanne, T.L.S. 2000. Sex specific random amplified DNA (RAPD) markers in *Carica papaya* L. *Tropical Agril. Res.* **12**: 41-49.

13. Poller, E. 1988. Differences in phenol content of the male, female and hermaphrsssssodite tree of rambutan (*Nephelium lappaceum* L.), pili (*Canarium ovatum* Engl.) and papaya (*Carica papaya* L.) through paper chromatographic analysis. University of the Philippines at Los Banos, Philippines. B.Sc. thesis.

14. Sriprasertsak, P., Burikam, S., Attathom, S. and Piriyasurawong, S. 1988. Determination of cultivar and sex of papaya tissues derived from tissue culture. *Kasetsart J. Nat. Sci.* **22**: 24-29.

15. Storey, W.B. 1953. Genetics of papaya. *J. Hered.* **44**: 70-78.

16. Urasaki, N., Tarora, K., Uehara, T., Chinen, I., Terauchi, R. and Tokumoto, M. 2002. Rapid and highly reliable sex diagnostic PCR assay for papaya (*Carica papaya* L.). *Breed. Sci.* **52**: 333-35.

17. Urasaki, N., Tokumoto, M., Tarora, K., Ban, Y., Kayano, T., Tanaka, H., Oku, H., Chinen, I. and Terauchi, R. 2002. A male and hermaphrodite specific RAPD marker for papaya (*Carica papaya* L.). *Theor. Appl. Genet.* **104**: 281-85.

Received : December, 2015; Revised : September, 2016; Accepted : March, 2017