International Journal of Plant Sciences, (July, 2010) Vol. 5 Issue 2 : 682-684

Efficacy of different detergents for leaf extract based sex diagnostics of papaya (*Carica papaya* L.)

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Accepted : April, 2010

Key words : Leaf, Papaya, Detergents

Papaya (*Carica papaya* L.) is an important cash crop of India owing to its economic, nutritional, industrial, pharmaceutical and medicinal values. Papaya is conventionally propagated by seeds and dioeceous papaya varieties do not ensure the right sex type. As a result, 50-60 % of seeds turned out to be male which needs to be uprooted after six to seven months of planting. The resources (fertilizer, water, weeding, land, labour and time) used in development and weeding out male plants makes papaya cultivation cumbersome and uneconomical. Efforts to distinguish sex of papaya at juvenile stage through morphological and biochemical markers have not met with success. In an open pollinated species such as papaya, the selection of the appropriate sex type of the progeny for commercial planting would be beneficial, since only the female and hermaphrodite plants are grown for fruit. The identification of sex types prior to propagation, especially in polygamous plant species with a long juvenile cycle such as papaya, would result in higher fruit production and increased profitability. Knowledge of the sex type of papaya is important in selecting parents for use in hybridization work. Crosses between females and hermaphrodites will give all fruit bearing progenies. In addition for micro propagation, the early detection or identification of the sex type of a particular papaya seedling would be advantageous since the desired sex type can be selected prior to micro propagation. This will ensure that the resulting micro propagated plants are 100% either female or hermaphrodite. India is the largest producer of papaya in the world. However, shortage of planting material from elite varieties in country has impeded its cultivation. To overcome these constraints in cultivation practice and to make it more profitable, there has been a long-standing interest in developing strategies for identifying sex of papaya plants at the juvenile stage. To overcome these constraints in cultivation practice and to make it more profitable, PCR based sex diagnostics in papaya is now available. However, it is expensive. Isolation of plant DNA alone is expensive and labour intensive. Therefore, we have devised an efficient method of sex identification by circumventing the DNA isolation phase and using crude leaf extract directly. We report here efficacy of different detergents for preparation of leaf crude extract.

Around 3-4 discs of very young leaf tissue were collected from the growing papaya plants in 1.5ml centrifuge tubes. 40µl of 0.25N NaOH was added to the centrifuge tubes and they were put in boiling water bath for 1minute. After that 40µl of 0.25N HCl was added followed by addition of 20µl of 0.5M Tris Cl^- (pH8.0) and 25µl of detergent solution. Four different detergents *viz.* PEG (Poly Ethylene Glycol), Igepal CA200 (Sigma Aldrich), Triton X100 and Tween 20 in different concentrations (1.25%, 1.0% and 1.5%) were used for preparation of leaf extract. These were boiled for 2 minutes in hot water bath and than tissues were disintegrated using plastic mortar or pipette tips. The suspension was mixed well and kept at 4^0C for 10-15minutes, followed by pulse centrifugation at room temperature. The supernatant (leaf crude extract) was directly used for PCR reaction. In order to standardize the PCR reactions using the crude extract, different PCR mixes were prepared using different amounts of crude extract (2µl to 16µl), primer concentration (1µl of 15pMol, 20pMol, 25pMol and 30pMol stocks each), Taq polymerase (1µl and 0.5µl of 3U/µl stock), dNTP mix (1µl and 2µl of 10mMol stock) and $MgCl_2$ (2µl and 2.5µl of 25mMol stock). Primers used for sex identification were 'male specific' and 'gender neutral' primers (Paranis *et al*., 2000).

We used four different detergents for preparation of leaf extract of papaya. Our results reveal that out of all the detergents, Tween-20 at 1.25 % concentration in PCR

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Table 1 : Effect of detergents on crude extract based PCR

Band name	Concentration (%)	Variable detergent	DNA in crude extract	Band intensity of PCR reaction
1n	1.25	Igepal	+ + +	+ + + +
2n	1.00	Igepal	+	---------
3n	1.50	Igepal	+ + +	+ +
4n	1.25	PEG	+ + +	+ +
5n	1.00	PEG	+	---------
6n	1.50	PEG	+++	---------
7n	1.25	TritonX100	+ + +	+ + +
8n	1.00	TritonX100	+ + +	+ + +
9n	1.50	TritonX100	+ + +	+ + +
10n	1.25	Tween 20	+ + +	+ + + + +
11n	1.00	Tween 20	++	--------
12n	1.50	Tween 20	+ + +	+ + + +

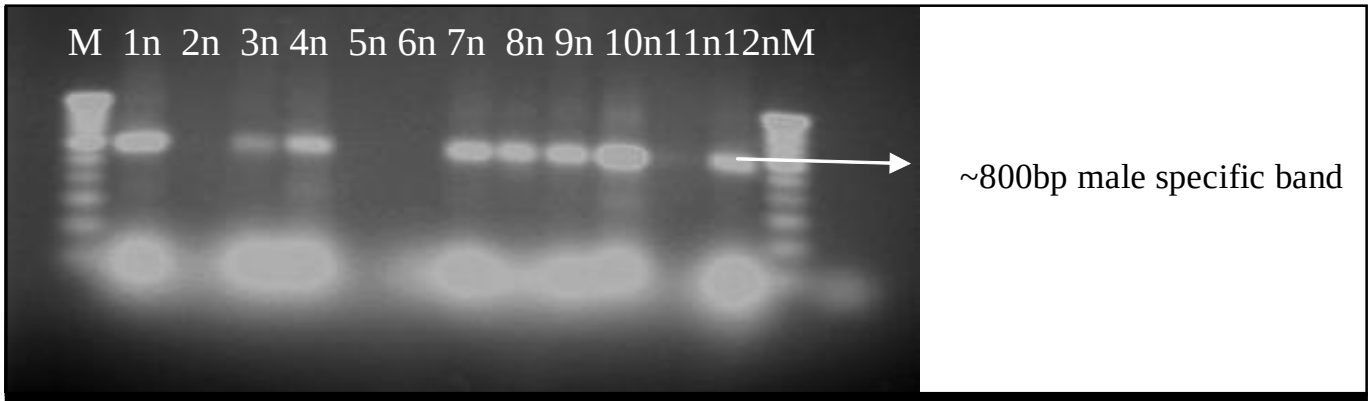

Fig. 1 : PCR result using crude extract from leaves (M= 100 bp ladder; 1n to 12n= male samples)

mixture gave best results in terms of band intensity (Fig.1 and Table 1). Low concentration of detergents (1.0 %) reduces the yield of DNA whereas higher concentration of detergents (1.5%) hinders the PCR mix and thereby affecting the visibility of bands. 5µl of crude extract can be used for PCR reaction. Igepal at 1.25% was also found efficient. However, it is expensive. Tween-20 is cheap and easily available and found effective detergent for PCR mix in terms of higher DNA yield and clarity of bands during electrophoresis. Poly ethylene glycol seems to be the least effective detergents for preparation of leaf extract. We conducted multiplex PCR using crude leaf extracts of 500 papaya seedlings using the male specific primers and the gender neutral primers designed by Parasnis *et al.* (2000) (Fig. 2). Crude extract was prepared using 1.25 % (V/V) Tween-20 as detergent. Out of 500 papaya seedlings tested for detection of sex using leaf extract method, 290 were sorted out as females and were planted in the orchard for flowering. The primers used in this prediction study are highly specific, because of the large number of bases (20-mer nucleotides). The technique of sex prediction by PCR is much more precise than the colorometric test (Jindal and Singh, 1976), chromatographic analysis (Paller, 3) or isozyme markers (Sriprasertsak *et al.*, 1988).

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