

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

SHUBHENDU SEAL, MANEESH MISHRA, RAJESH PATI, RAMESH CHANDRA AND ALKA SRIVASTAVA

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 dioecious 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°C 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 MgCl₂ (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

Correspondence to:

ALKA SRIVASTAVA, Department of Botany, Faculty of Science, Lucknow University, LUCKNOW (U.P.) INDIA

Authors' affiliations:

SHUBHENDU SEAL, MANEESH MISHRA, RAJESH PATIL AND RAMESH CHANDRA, Biotechnology Laboratory, Crop Improvement Division, Central Institute for Subtropical Horticulture, Rehmankhera, LUCKNOW (U.P.) INDIA