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Characterisation of phytoplasma that cause phyllody in *Sesamum indicum L.* (Sesame) by PCR-RFLP

H A C K Ariyarathne[1*], E Jayamanna[2] and E H Karunanayeke[1]
[1] *IBMBB, Cummarathunga Munidasa Mawatha, Colombo 3*
[2] *Coconut Research Institute, Lunuwilla*

Sesame is cultivated in all agro-economic zones of Sri Lanka, particularly in the intermediate and dry zone. Sesame phyllody (SP) caused by phloem-limiting phytoplasma, is a common occurrence in all agro–economic zones where sesame is cultivated. Infected plants produce large number of leaves of reduced size; and short internodes resulting rosette-like branches. Flower buds produce vegetative parts and no flowering or fruiting occur in infected plants causing substantial loss of harvest. This

paper communicates results of a preliminary investigation made towards characterisation of the causative phytoplasma strains of sesame phyllody (PS) by PCR-RFLP technique.

Plant samples, leaves and young twigs, were collected from farmer fields at Ampara. Total DNA was extracted following CTAB protocol. DNA quantity and Quality was estimated by 0.8% agarose gel electrophoresis and by the Genequant (Pharmacia Biotech). DNA obtained from phyllody sesame (PS); asymptomatic sesame (Non-PS) (negative control), and Japanese Hydrangea phyllody (JHP) (positive control) was subjected to PCR using the universal phytoplasma primer pair Pc399/P1694 (Skzeczkowski,2001). PCR products were purified by ethanol precipitation and subsequencely digested with a series of restriction enzymes in order to determine the restriction enzyme digestion profiles for the particular phytoplasma strain.

CTAB protocol yielded sufficient amount of high quality DNA; PS (200 ngμl^{-1}, DNA / protein=1.76). Non-PS(200 ngμl^{-1}, DNA / protein =1.84), and JHP (200 ngμl^{-1}, DNA / protein =1.87). The primer pair P399 and Pc1694, amplified target DNA from all SP symptomatic samples as well as DNA from JHP. The PCR product appeared as a unique band around 1.2 kb on 1% agarose gel. However, the primer pair did not amplify DNA from asymptomatic sesame samples.

The restriction enzyme digestion profiles, of the PCR products show that both enzymes results the same profile for JHP while contrasting profiles for SP. Further, the enzmes EcoR I, and AluI distinguish SP uniquely; from JHP.

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* ckariyarathna@yahoo.com Tel: 051-2222601