

## Polymerase chain reaction detection of *Candidatus liberibacter asiatic* associated with citrus huanglongbing

G.P. Jagtap and Utpal Dey

Department of Plant Pathology, College of Agriculture, Marathwada Krishi Vidyapeeth, India

Citrus is one of the most important tropical fruit crops of the world. Citriculture is the largest fruit industry in India which occupies important place in economy of the country. Diseases are known as one of the important factors in low productivity of citrus fruits in India. Among the diseases of citrus, viral diseases cause heavy economic losses in varying proportion. Around 30 viral diseases are known to infect citrus worldwide. In India, the major pathogens of economic importance in citrus are Citrus tristeza (CTV), Citrus yellow mosaic badna virus (CYMV), Indian citrus ring spot virus (ICRSV), viroids disease like citrus exocortis viroid and a fastidious prokaryote causing citrus greening disease. Detection by DNA probes though an accurate method for detection but requires handling of radioactive elements and is being discouraged now a days. Moreover, these are not practically feasible methods for handling a large sampling unit.

Polymerase chain reaction diagnosis of *Candidatus liberibacter asiatic* associated with citrus Huanglongbing disease is molecular technique which is used for detection of disease when pathogen present is very low concentration in disease sample. Among these three DNA isolation methods viz., commercial kit method, sodium sulphite method and membrane bard nucleic acid technique, sodium sulphite method is cost effective for commercial use. In nucleic acid membrane method for DNA extraction isolation there is no use of liquid nitrogen. Polymerase chain reaction detection of disease is based on principal of thermal cycling in which PCR instrument allow to run generally 60-65 thermal cycle, during PCR operation it allow different stages of cycle at different temperatures for different period of time i.e. initiation (94°C), denaturation (94°C), primer annealing (60°C), extension/elongation step (72°C), final elongation (72°C) and holding temperature (4°C). PCR based diagnosis system is developed for detection of greening bacteria. The comparative cost of detection by various combinations of reagent and sampling time was determined and cost effective technology was standardized and validated.

**Keywords:** Citrus, *Candidatus liberibacter asiatic*, Polymerase Chain Reaction.

### Biography

G.P.Jagtap M.Sc. (Agri), Ph.D NET (ICAR) is Norman Borlaug Fellow 2008 (USA). He is a recipient of Norman Borlaug International Fellowship - 2008 and worked at Plant Disease Diagnostic Laboratory, Texas; A&M University, USA. He did his doctorate degree from GBPUA&T, Pantnagar with specialization in Plant Pathology and presently working as a Assistant Professor in the Department of Plant Pathology, MKV, Parbhani. He has published 20 research papers, 40 popular articles, 2 Books and 6 practical manuals. He has participated in several International and National conferences and guided 10 M.Sc. (Agri) students. He has 11 years experience in Teaching, Research and Extension work.

drpjjagtap@gmail.com

## Plant regeneration in *Litsaea salicifolia* Roxb through callus culture

G.C. Dev Goswami

Department of Botany & Biotechnology, Goalpara College, India

*Litsaea salicifolia* Roxb. (Lauraceae) commonly known as 'Dighaloti' in Assam, India, is a secondary food plant of 'muga silkworm' (*Antheraea assama* Westwood), is a wild bushy shrub, unisexual dioecious plant seen growing mostly in riverbanks in Assam, India. Viable seeds of 'Dighaloti' (*Litsaea salicifolia*) were grown aseptically in MS media containing 0.1 mg l<sup>-1</sup> BAP. Hypocotyls segments were excised from the seedlings and cultured on culture media containing various concentrations of 2, 4D and BAP. Friable callus was obtained on medium containing 2 mg l<sup>-1</sup> 2, 4-D and 0.5 mg l<sup>-1</sup> BAP. When transferred to MS medium supplemented with BAP (2 mg l<sup>-1</sup>) and NAA (0.1 mg l<sup>-1</sup>) became nodular and developed 30-40 shoots within 30 days. Among amino acids tested, lysine (2 mg l<sup>-1</sup>) was found effective in more shoot bud induction. Rooting on the shoots was achieved when cultured on MS basal medium supplemented with NAA (0.5 mg l<sup>-1</sup>). Hardening of the plants was done in sugar free MS basal medium and subsequently in vermiculite. The 39 plants thus developed were successfully established in earthen pots under green house conditions with 80% survival and are under screening for somaclonal variation with beneficial characters.

**Keywords:** Dighaloti, *Litsaea salicifolia*, Regeneration, Somaclonal.

goswamigakul@gmail.com