Revealing Latent Infections of Imported Seed Potato by Next Generation Sequencing

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Abstract

Transmission of tuber borne diseases was identified as a major cause of yield reduction in number of crops. Seed tubers are usually the costliest single input in potato production. In Sri Lanka, majority of the seed potato requirement is fulfilled through importation from different countries. In many districts imported seed potato is used by the farmers to produce the Yala season crop. It has been estimated that 21.8% of the total potato production of the world is lost before the harvest due to various diseases. The reduction in tuber yield in Sri Lanka was also identified as positively correlated with the destructive diseases coming with imported seed potatoes. Detection of the causal organisms at the latent stage of a disease is a challenging task as the symptoms are not clearly observable. Meta-genomic approach using Next generation Sequencing in microbial detection is important as it generates the whole microbial profile even in asymptomatic samples with low abundance of pathogens.

Imported seed potato samples intercepted at the entry ports of Sri Lanka were collected from the National Plant Quarantine Service. Microbial DNA was extracted from the seed potato tubers. Ion Torrent Next Generation Sequencing (NGS) technology was used for the detection of both bacterial and fungal infections associated with the analysed samples amplifying V1-V2 region of 16S rRNA gene for bacteria identification and ITS1 region for fungal diversity analysis. Multistep computational analysis was performed for the detection of diversity of available bacteria and fungi species. Further, sub species level of the detected quarantine pathogens was confirmed by PCR amplification with the species specific primers.

Average 16-80 bacterial species per sample were detected by using NGS which revealed a greater prevalence and diversity of microbes in the analysed seed potato. The sequence analysis resulted the presence of quarantine bacterial pathogens *Pectobacterium caratovorum* subsp. *caratovorum* and *Streptomyces scabies* which were confirmed by NGS and followed by species specific PCR for sub species level detection. Among the detected fungal pathogens, two quarantine important fungi

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Phoma and *Phytophthora* were identified only at genus level however, it has to be further tested for identification upto species levels.

Pectobacterium carotovorum subsp. *carotovorum* is one of the most significant bacterial pathogens of various horticultural crops around the world which is responsible for Black leg and Soft rot diseases. *Streptomyces scabies* is responsible for the common scab disease of potatoes. Therefore detection of quarantine important pathogens is needed to limit the repeated pathogen introductions via import consignments.

Keywords: Next Generation Sequencing; Pectobacterium caratovorum; Quarantine; Streptomyces scabies; Seed potato

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