421/D

DNA marker based phylogeny for improved resolution of phytoplasma taxonomy

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Phytoplasmas are cell wall-less pleomorphic bacteria that cause numerous phytopathogenic diseases in several hundred plant species worldwide. Due to their inability of *in vitro* growth, the conventional identification and differentiation methods based on biological characteristics used for other cultured prokaryotes are not accessible for phytoplasmas. A classification scheme that identifies the groups and subgroup delineations has been established using highly conserved 16S rRNA sequence with several other genetic markers, based mainly on Neighbor Joining phylograms.In the current study, the phylogeny of phytoplasmas affecting mostly economically important tropical crop plants, i.e. coconut, sugarcane, rice, Bermuda grass and arecanut was investigated using five different genetic markers, highly conserved 16S rRNA, conserved 23S rRNA and 16S-23S IGS and polymorphic *secA* and tRNA-Ile. A total of 253 phytoplasma sequences (for coconut n=109, for sugarcane n=72, for rice n=20, for Bermuda grass n=49, for arecanut n=3) were used to construct the phylogenetic trees using more powerful, robust, simple and fast Maximum Likelihood (ML) method and the genetic variability within and between populations were estimated by calculating population genetic indices. In general, the ML phylogenetic trees revealed similar grouping of selected phytoplasma compared to the existing 16S rRNA gene based classification, with a few number of exceptional conflicting, though clear phylogenetic positioning. For e.g: the exact phylogenetic position of Mexican coconut lethal decline phytoplasma of 16SrXIV-B was depicted only in the tRNA-Ile gene based phylogram. The 16S rRNA gene based ML phylogram classified it under the 16SrXI group, while that based on 23S rRNA gene clustered it in the 16SrIV-A group. The 16S rRNA gene had comparatively lower values for nucleotide diversity (π) and Jukes Cantor nucleotide diversity (π(JC)) and a higher sequence conservation value than the other genetic markers used, which confirms the highly conserved nature of the 16S rRNA gene. This was reiterated by obtaining 16S rRNA based F_{ST} values resulting in less than 0.05 for almost all host plant species.The current study identified close relationships of phytoplasma species not only in the same host plant but also in different plant species in diverse geographical regions globally. Such information is greatly helpful in controlling those closely related diseases and in identifying new disease incidences. It was also revealed that the use of less conserved or polymorphic genetic markers to elucidate the conflicting grouping in the standard phytoplasma taxonomy based on highly conserved 16S rRNA marker, resulted in a more useful output.

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