Detection of *Puccinia kuehnii* and *P. melanocephala* in sugarcane using specific primers

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Abstract

Sugarcane (*Saccharum* spp. hybrids) is the most important industrial crop in Mexico, in terms of its yield in field and factory. Producers have been very concerned since brown rust appeared in 1979 in cv. B-4362 in Tabasco State, and in 2008, when orange rust was detected in cv. CP 72-2086 in Chiapas State. Both states are in southeast Mexico. With the presence of these two rusts caused by *Puccinia* spp. a breeding program was established during 2010/12 to obtain resistant cultivars. With the aim of determining resistance levels to rusts, 36 cultivars from advanced selection stages have been evaluated under field conditions in Tabasco State. Plants from 120 days old with early pustules emerged were subjected to molecular analysis. Small pieces of leaf tissue were crushed for DNA extraction. Amplification and sequencing of 28S rDNA fragments were performed using the NL1/NL4 set of primers. Detection of *P. kuehnii* and *P. melanocephala*, was carried out with Pk1F/Pk1R and Pm1F/Pm1R specific primers, respectively. Maximum parsimony method was used for phylogenetic analysis; it confirmed the presence of *P. Kuehnii* and *P. melanocephala* in Tabasco State. As previously reported, specific primers also detected *P. melanocephala* in cv. B-4362, and new infections in cv. PR-1048 whose sequences were 100% identical to accession no. GU564429 (Florida). On the other hand, *P. Kuehnii* was detected in cvs. MEX 06-474 and CP 72-2086 with a maximum identity of 99% to GU564421 (Florida) and with GU564409 (Costa Rica). This rust has not previously been detected in any cultivar in Tabasco State. Results indicate that the movement of species of *Puccinia* should be managed to minimise the dispersal of rust through propagative material. It is impossible reduce spores dispersal in the atmosphere. The development of genetic resistance is one of the most important strategies for improving yield and maintaining the phytosanitary quality of the Mexican sugar cane crop.