Cure, transmission and modifications on dsRNA'S purification protocol in *Guignardia citricarpa*

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Guignardia citricarpa is an ascomycete of extreme importance for the Brazilian citrus culture since it's the causal agent of Citrus Black Spot (CBS). Some isolates from this species are infected by a double stranded RNA virus causing unknown effects on the fungal host. One of the ways to analyze the influence of dsRNA in *G. citricarpa* is to obtain isogenic strains that differ from each other only by the presence or absence of this genetic material. Two methods were tested to obtain isogenic strains: cure of dsRNA from the infected isolate, and infection of a dsRNA-free isolate. Several cure processes described on literature were tested with no success for *G. citricarpa*. The first positive results were obtained after some isolates were cultured under stressing conditions (35ºC). After that, when grown under normal conditions, they developed sector-like regions in their colonies possibly due to a genetic material reorganization. These sector-like regions had their total nucleic acids purified and, in some cases, it was confirmed that dsRNA was eliminated. DsRNA transmission occurs by hyphal anastomosis after two strains, one bearing the virus and the other with no viral infection, reach each other in a petri dish. The isolates used in this essay were chosen based on the presence or absence of dsRNA and also on a morphological marker unrelated to the presence or absence of dsRNA, described for *G. citricarpa.* This marker corresponds to the appearance of a yellow halo around the fungal colony, when grown on oatmeal medium. Selected strains were: 1) positive for dsRNA infection and positive for the morphological marker. 2) Negative for dsRNA infection and negative for the morphological marker. These contrasting isolates were inoculated in pairs. In some cases it was possible to observe incompatibility between strains since there was no contact between hyphae. When compatibility was observed, monosporic colonies were obtained from that region and essayed regarding the presence of a yellow halo. Isolates with no halo formation are being investigated regarding the presence of dsRNA. Identification of a dsRNA band in agarosis gel after nucleic acids purification is not always easy and precise due to the need of a large amount of total nucleic acids to make it visible. Phenol-chloroform extraction is normally used. In this study, instead of using pH 8,0 phenol, it was used an acid phenol (pH 4,7). Acidification was obtained with sodium acetate. In this condition, genomic DNA tends to precipitate into the organic phase while RNA is solubilized with the supernatant. Purifications with acid phenol showed a fainter genomic DNA band when compared to regular pH 8,0 phenol. This provided a more efficient DNase treatment that facilitates identification of a dsRNA band.
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