

Evaluation of opposite Type Heterologous MAT Genes Transfer, in the Filamentous Fungi *Neofusicoccum mediterraneum* and *Verticillium dahliae*

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Abstract—Mating type genes are present in most filamentous fungi, even though teleomorphs for all species have not been recorded. Our study, tries to explore the effect of different growth conditions on the expression of MAT genes in *Neofusicoccum mediterraneum*. As such, selected isolates were grown in potato dextrose broth or in Water Agar supplemented with pine needles under a 12 h photoperiod, as well as in constant darkness. Mycelia and spores were collected at different time points and RNA extraction was performed, with the extracted product being used for cDNA synthesis. New primers for MAT gene expression were designed, while qPCR results are underway.

The second part of the study involved the isolation and cloning in a selected pGEM-T vector, of the *Botryosphaeria dothidea* MAT1-1-1 and MAT1-2-1 mating genes including flanking regions. As a next step, the genes were amplified using newly designed primers with engineered restriction sites. Amplicons were excised and subsequently sub-cloned in appropriate binary vectors. The constructs were afterwards inserted into *Agrobacterium tumefaciens* and utilized for *Agrobacterium*-mediated transformation (ATMT) of *Neofusicoccum mediterraneum*. At the same time, transformation of a *Verticillium dahliae* tomato race 1 strain (70V) was performed as control. While the procedure was successful in regards to *V. dahliae*, transformed strains of *N. mediterraneum* could not be obtained. At present, a new transformation protocol, which utilizes a combination of protoplast and Agro transformation, is being evaluated.

Keywords—Anamorph, heterothallism, perithecia, pycnidia, sexual stage.

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