TITLE: IN SILICO AND IN VITRO VALIDATION OF NEW AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) FINGERPRINTING FOR GENETIC STUDIES OF CLINICAL *FUSARIUM* SPECIES.

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ABSTRACT:
Fusariosis is an emerging mycosis worldwide. *Fusarium* spp. cause superficial infections characterized by onychomycosis, dermatitis, and keratitis, while invasive and systemic fusariosis involve sepsis and lesions of different organs. *Fusarium* spp. diversity and their molecular epidemiology are well explored in phytopathology using various strategies such as Amplified Fragment Length Polymorphisms (AFLPs) and multilocus sequence analysis (MLSA), but it remains neglected in medically relevant fusaria. While MLSA schemes usually limit variability to two genes (e.g., *tef1α* and *rpb2*), AFLPs allow genome-wide mapping variability. Here, we report using AFLP markers to determine the degree of intraspecific variability among medically relevant *Fusarium* species. We used 20 whole-genome sequences from *Fusarium* species to generate 5,120 virtual AFLP fingerprints. *In silico* screening highlighted eight primer pair combinations to be tested *in vitro* (C1-C8). Forty (n=40) clinical isolates belonging to the *F. solani* (n=26), *F. oxysporum* (n=6), *F. fujikuroi* (n=4), and *F. dimerum* (n=4) species complexes (SC) were used for *in vitro* AFLP, and an MLSA scheme using *tef1α* and *rpb2* was performed for molecular identification and haplotype network analysis. A total of 678 loci were amplified using the selective primers EcoRI+2 and MseI+2, among them 93, 93, 85, 84, 90, 78, 78, and 77polymorphic fragments, for C1-C8, respectively. Each AFLP fingerprint was exported to BioNumerics for UPGMA, PCA, MST, and congruence analysis. Reproducibility was tested for duplicates, and the error rate was calculated. Using MLSA, all species were identified by high bootstrap-values (>99%), and the haplotype network revealed 33 haplotypes (Hd=0.99, π=0.130). Clusterization of AFLP profiles rendered well-supported dendrograms (cophenetic values >90%). Diversity indexes were satisfactory to all combinations, although better indexes were observed in C1 and C2 (H=0.4910, PIC=0.3704, E=40.2500, MI=0.0053, D=0.8128). All combinations had low error rates (1.08-1.95%) and were highly reproducible. PCA was better reproduced by C6 (PCA Σ=34.1%) and C8 (PCA Σ=33.8%), while MST had a better reproduction in C5 (tree length=1,728.30). Congruence revealed an important correlation between C1 and C6 (r=0.8051), and C2 and C5 (r=0.8278). All AFLP markers tested allowed to

access diversity in all species complexes studied, mainly C2 and C5, while C1 and C6 are better to FOSC and FDSC.

Keywords: Diversity, sequencing, fusariosis, AFLP, neglected mycosis

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