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Graduate School

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A thesis submitted to the Graduate Studies Office in partial fulfillment of the requirements for
the degree of Doctor of Philosophy (PhD) in Plant Pathology

Determination of population genetic structure of *Bipolaris sorokiniana*, the causal agent of wheat root and crown rot in Iran

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October 2022

Abstract

Wheat root and crown rot is one of the most important wheat diseases, caused by the fungus *Bipolaris sorokiniana* in Iran. A collection of 147 *Bipolaris sorokiniana* single conidium isolates was made from different cultivated wheat fields of Iran including West Azerbaijan province (Miyandoab region), Kermanshah province (Kermanshah region), Alborz province (Karaj region), Fars province (Shiraz region) and Golestan province (Gorgan region). Plant specimens were subjected to surface sterilization with distilled 1% sodium hypochlorite solution for 1-2 min then rinsed three times by sterile water. Isolation of the fungal isolates was done by using a blotter method, and purified cultures were yielded by transferring a single spore produced on plant tissues onto 2% water agar (2% WA) and subsequently a hyphal tip onto potato dextrose agar (PDA). Isolates were maintained on PDA slants at 4°C. After morphological and molecular identification by using RABSF primer pair, mating type primers were designed according to each mating type idiomorph of the fungus. BSMAT1-2 and BSMAT1-1 primer pairs were used to amplify a fragment of approximately 1000 bp for *MAT1* and 817 bp for *MAT2* idiomorphs in multiplex PCR method. Out of 147 isolates, *Mat1-1* was amplified for 86 isolates and *Mat1-2* was amplified for 61 isolates. The most frequencies of *MAT1-1* and *MAT1-2* have observed in Shiraz-Gorgan and Miandoab populations, respectively. According to the results of the Chi-square test, Miyandoab, Shiraz and Gorgan populations had not significantly difference ($P > 0.05$) than the Karaj and Kermanshah populations. Therefore, these three populations have the highest potential for sexual reproduction than the two populations of Karaj and Kermanshah. In addition, some isolates were studied to evaluate the sexual fertility status. Such's agar medium with barley straw, seed and young leaves was used for sexual crosses. After two months, pseudothecia without asci and ascospores formed in medium. One hundred forty-seven isolates were analyzed for genetic structure using five simple-sequence repeat (SSR) markers developed from the genome sequence of the *Bipolaris sorokiniana*. A total of 52 alleles were detected for five microsatellite loci across all 147 *B. sorokiniana* isolates. Fungal populations belonged to five sampling regions had a high gene diversity ($H = 0.71-0.87$), high genotypic diversity ($G = 0.95-0.98$), and low clonal fractions ($C = 0.22-0.43$). High gene flow and low genetic differentiation were observed among some populations of Miyandoab and Karaj. Also, linkage disequilibrium tests (I_A and \bar{r}_d) supported the hypothesis of some random mating at the populations.

Keywords: Morphology, taxonomy, phylogeny, mating type, gene flow, genetic diversity