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First Report of *Diaporthe novem*, *D. foeniculina*, and *D. rudis* Associated With Soybean Seed Decay in Serbia

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Seed decay is one of the most important diseases of soybean (*Glycine max* (L.) Merr.), which can greatly affect seed quality. Some of the most damaging causal agents of soybean seed decay are *Diaporthe longicolla*, *D. sojae*, *D. caulivora*, and *D. aspalathi* (Li 2011). A total of 94 single-conidial isolates were obtained from soybean seeds collected throughout the soybean-producing area in Serbia from 1996 to 2015. Isolates were separated into two groups (A and B) according to their morphological features. In group A, 70 isolates were assigned with the following morphological characteristics: white mycelium, black stromatic structures, pycnidial conidiomata with alpha conidia and rarely beta conidia, and absence of perithecia (Hobbs et al. 1985). Twenty-four isolates were assigned to group B with culture characteristics that included white mycelium, pycnidial conidiomata with alpha and beta conidia, and perithecia (Udayanga et al. 2015). Using sequence comparison of the internal transcribed spacer (ITS1-5.8S-ITS2) region of the rDNA, partial translation elongation factor 1 alpha (EF-1 alpha), and partial large ribosomal subunit (LSU), five different *Diaporthe* species were identified. Using BLAST analyses, five isolates from group A (PL42, PL44, PL68, PL75,

and PL/KR6) showed 100% sequence identity to *D. novem* (CBS117165) with the following sequences deposited in GenBank: ITS sequences JQ697841 to JQ697843, JF704181, and KU672724; EF-1 alpha sequences JQ697854 to JQ697856, JF704182, and KU672725; and LSU sequences JQ697867 to JQ697869, JF704180, and KU672726. The remaining isolates assigned to group A were identified as *D. longicolla*. From group B, 21 isolates were identified as *D. sojae*, and two isolates (PS22 and PS48) had 100% sequence identity (ITS: JF430495 to JF430496; EF-1 alpha: JF461481 to JF461482; LSU: JF704179, JQ697865) with *D. foeniculina* (CBS123209). One isolate, PS76 (ITS: JQ697840; EF-1 alpha: JQ697853; LSU: JQ697866), showed 100% identity to *D. rudis* (CBS113201). To verify the pathogenicity, 25 soybean plants (cv. Sava) were inoculated with five isolates at the V2 growth stage by the plug method (Vidić et al. 2013). Isolates identified as *D. novem* (PL68 and PL75) and an isolate identified as *D. foeniculina* (PS22) caused wilting symptoms in 100% of the plants. Plants infected with *D. rudis* (PS76) and *D. foeniculina* (PS48) did not show any disease symptoms. Soybean seeds were inoculated using a conidial suspension (10^6 conidia/ml). Twenty-five seeds were placed on wet filter paper in 90 mm petri dishes and incubated at 24°C in the dark. The test was set up in four replications. All decayed seeds were counted after 7 days. The results showed that *D. novem* (PL68 and PL75) was pathogenic causing seed decay with incidence ranging between 82 and 92%. *D. rudis* caused seed decay on 62% of seed tested, while *D. foeniculina* (PS48 and PS22) caused seed decay at incidence levels ranging from 30 to 42%. The control plants and seeds were symptomless. Koch's postulates were fulfilled by reisolation and molecular identification of *Diaporthe* isolates from the symptomatic stems and seeds. To our knowledge, this is the first report of soybean seed decay caused by *D. novem*, *D. foeniculina*, and *D. rudis* in Serbia, and the first report of *D. rudis* on soybean seeds in the world.

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