Occurrence of seed decay caused by *Diaporthe longicolla* on soybean in Colombia

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Soybean (*Glycine max* L. Merr.) is an important raw material to manufacture balanced concentrates for the animal feed industry in Colombia, particularly for the poultry and pig (swine) production chains. In 2018, approximately 40% soybean plants (cultivar BRS Serena) presented pod and seed decay (Figure 1) at a commercial field in the locality of Puerto Lopez (Meta, Colombia).

Specimen collection was conducted according to the permit granted to AGROSAVIA under ANLAS' Resolution No. 1466 of December 03, 2014, Colombia. The affected seeds were cracked, shriveled and covered with chalky white mold (Figure 1). Small tissue pieces (5-mm diameter) from the margins of necrotic lesions on pods were surface disinfested, placed on potato dextrose agar (PDA; pH=4.5), and incubated at 25ºC in darkness. Likewise, infected seeds were used to isolate fungal pathogens according to the protocols of the International Seed Testing Association (2). Three isolates from pods (AGSV-7 to AGSV-9) and four isolates from seeds (AGSV-10 to AGSV-12, and 283-107) were obtained and purified by culturing single hyphal tips. On PDA media, all isolates produced compact fluffy white colonies with aerial mycelium and numerous black clusters of stromata and pycnidia. Single-celled alpha conidia (n =50), observed after 20-day growth on PDA, measured 5.3 – 7.2 µm (6.4 ± 0.4 µm) × 2.0 – 3.0 µm (2.5± 0.4 µm), were fusiform to ellipsoidal and had two guttules at each end. Beta-conidia (n=50), observed after 40-day growth on PDA, measured 22.0 – 30.0 µm (25.30 ± 3.4 µm) × ١,٠ – 1.2 µm (1.16 ± 0.2 µm). On 1.5% water-agar added of soybean stem pieces, alpha conidia (n =50) were abundantly produced after 15-day growth; they measured 5.3 – 7.2 µm (6.4 ± 0.4 µm) × 2.0 – 3.0 µm (2.5± 0.4 µm), were aseptate, hyaline, smooth, ellipsoidal and often biguttulate, and had subtruncate base. No perithecia were observed either on PDA or on water-agar added of soybean stem pieces. The above-described characters correspond to those of *Diaporthe* spp. as described by Udayanga et al. (4). Genomic DNA was obtained from each of the fungal isolates to sequence the internal transcribed spacer region (ITS) using the primer pairs ITS5/ITS4 (5). Results from an NCBI-BLASTn revelated that ITS sequences of the seven isolates (GenBank accessions MN298743 to MN298748 and MW566592) had 100% (579, 579, 586, 585, 581, 584 and 592 bp) identity with *Diaporthe longicolla* (KX977489 and AY857868). The translation elongation factor 1-α (TEF1) gene was additionally obtained for isolate 283-107 (GenBank accession MW597409), using the primer pair EF1-728F/EF1- 986R (1), and also showed 100% (342/342 bp) identity with strain FAU599 of *D. longicolla* (GenBank accession KJ590767). Phylogenetic analysis, by maximum likelihood, using ITS and TEF1 sequences from type specimens available in GenBank, showed that isolate 283-107 clustered in a clade together with the reference type strain FAU599 of *D. longicolla* (4). To fulfill Koch's postulates, an inoculation method involving the spraying of mycelium (3) was used to inoculate the isolate 283-107 in mature soybean seeds cv. BRS Serena. Ten seeds were treated with sterile distilled water and served as non-inoculated control. Inoculated seeds were incubated under high humidity for seven days at 28°C. Soybean seeds inoculated with

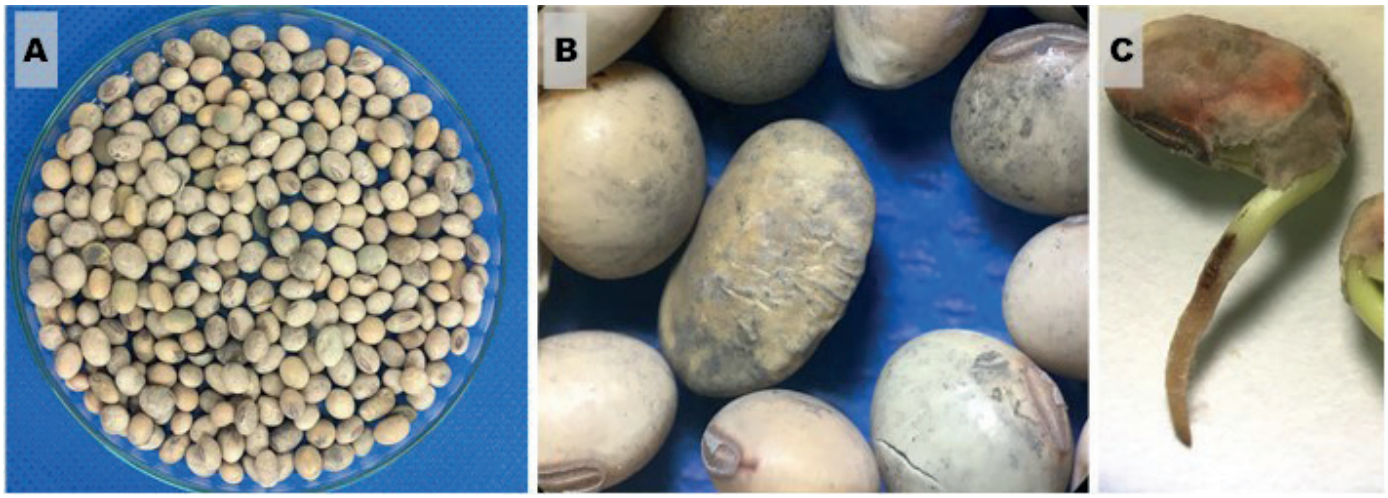

Figure 1. Seed decay caused by *Diaporthe longicolla* on soybean cultivar BRS Serena. A,B. Cracked and shriveled seeds covered with chalky white mold. C. Seedling showing blight symptoms at 7 days after inoculation of germinated seeds with strain 283-107 (GenBank accession MW597409) of *Diaporthe longicolla*. *Figure: Own authorship

isolate 283-107 showed decay symptoms similar to those observed in the field (Figure 1). Disease symptoms were not observed in the non-inoculated controls. Fungal cultures were recovered from symptomatic seeds and their morphological characteristics were similar to those of the originally inoculated isolate. This is the first report of soybean seed decay caused by *Diaporthe longicolla* in Colombia. Our results provide critical information to determine the causes of seed decay and allow the selection of the best soybean seed treatments in the Colombian eastern plains region.

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REFERENCES

1. Carbone, I.; Kohn, L. M. A method for designing primer sets for speciation studies in filamentous ascomycetes. **Mycologia**, Madison, v.91, n.3, p.553-556, 1999. DOI: 10.1080/00275514.1999.12061051. Available at: <https://www.tandfonline.com/doi/abs/10.1080/00275514.1999.12061051>. Accessed on: May. 1999.
2. Walcott, R. 7-016: Detection of *Phomopsis* complex in *Glycine max* (soybean, soya bean) seed. **International Seed Testing Association**, Zurich, chap.7, n.16, p.1-6, 2022. Available at: <https://www.seedtest.org/en/international--rules-for-seed-testing/seed-health-methods-product-1014.html>. Accessed on: Oct. 2022.
3. Mena, E.; Stewart, S.; Montesano, M.; Ponce de León, I. Soybean stem canker caused by *Diaporthe caulivora*; pathogen diversity, colonization process, and plant defense activation. **Frontiers in Plant Science**, Israel v.10, n.1733, p.1-21, 2020. DOI: 10.3389/fpls.2019.01733. Available at: < https://www.frontiersin.org/articles/10.3389/fpls.2019.01733/full>. Accessed on: 23 Jan. 2020.
4. Udayanga, D.; Castlebury, L.A.; Rossman, A.Y.; Chukeatirote, E.; Hyde, K.D. The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. **Fungal Biology**, United Kingdom, v.119, n.5, p.383-407, 2015. DOI: 10.1016/j.funbio.2014.10. Available at: < https://www.sciencedirect.com/science/article/abs/pii/S1878614614001627>. Accessed on: May. 2015. 0 1.
5. White, T.J.; Bruns, T.; Lee, S.J.W.T.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A.; Gelfand, D.H.; Sninsky, J.J.; White, T.J. (ed.). **PCR protocols**: a guide to methods and applications, New York: Academic Press, 1990. p.315-322. DOI: 10.1016/B978-0-12-372180-8.50042-1. Available at: < https://www.sciencedirect.com/science/article/pii/B9780123721808500421?via%3Dihub>. Accessed on: Jan. 1990.