ARTIFICIAL CULTURE, HOST INFECTION AND PYCnidIAL DEVELOPMENT OF ASCOCHYTA SORGHINA SACC.

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Abstract

Mode of penetration and subsequent development of Ascochyta sorghina Saccardo in the leaf tissues of sorghum (Sorghum vulgare Pers.) were studied under field conditions. Penetration by A. sorghina was observed both directly through the epidermis and indirectly through the stomatal openings in the leaves. The pathogen produced intercellular mycelium, which later became intracellular after death of cells around the infection site through action of toxic metabolites produced by the pathogen. The pycnidia developed through the growth of a subcuticular hyphal crust.

Introduction

Several leaf spot diseases have been reported on sorghum (Sorghum vulgare Pers.) from various parts of India (2, 9). Rough leaf spot disease incited by Ascochyta sorghina Saccardo was first reported on sorghum from Italy in 1878 (7) and has since been found in the U.S.A., India and Tanganyika (3, 5, 6, 8, 11, 12, 13). The disease occurs annually in severe form both on the local and improved sorghum varieties in Varanasi and neighboring districts. Observations on artificial culture, mode of spore germination, host infection and pycnidial development of the pathogen (Ascochyta sorghina Sacc.) are presented to study the etiology of the disease.

Materials and methods

Mature pycnidiospores of Ascochyta sorghina Saccardo easily germinated in sterile tap water at 28–32°C. Spores harvested from pycnidia borne on diseased sorghum (Sorghum vulgare Pers.) leaves were suspended in 5 ml sterile dist. water, serially diluted and evenly spread over 1.5% plated water agar and incubated overnight at room temperature (28–32°C). Single germinating spores transferred to potato dextrose agar (PDA, pH 6.5) were incubated for 3–4 days. Small whitish colonies were transferred to fresh PDA and stored as stock cultures at low temperature.

Mature pycnidiospores of the pathogen (Ascochyta sorghina Sacc.) were suspended in sterile dist. water, adjusted to a concentration of 50 spores/microscopic field, fixed on slides by alternate wetting and drying (10) and the slides incubated at room temperature (28–32°C) in plates lined with moist filter paper/cotton wool.

Adolescent leaves of 4–6 week old sorghum (Sorghum vulgare Sacc.) plants were inoculated by atomizing and/or smearing both surfaces in the evening with a fairly dense suspension of spores of Ascochyta sorghina obtained from 2 weeks old plated cultures on Richards agar medium. Inoculated leaves incubated overnight were sampled twice a day after evaporation of dew deposit in the morning and before dusk in the evening. Epidermal peelings lightly stained with cotton blue in lactophenol were observed for spore germination and mode of penetration in the leaf. Inoculated leaves of sorghum plants were similarly sampled and fixed in FAA and Navashin's solution at 12 hr intervals after germ tube penetration until maturation of pycnidia of the pathogen (8–10 days). Development of the pathogen was studied in serially microtomed sections (10–12 μ), stained with Heidenhain’s iron hematoxylin and counterstained with orange G. Free hand sections from field material were also cut and examined for complementary observations.

Observations

The pathogen

Saccardo (1878) described the causal organism of the
disease as *Ascochyta sorghina* Sacc., a pycnidal fungus containing bicelled, hyaline elliptical pycnidiospores measuring 20 x 8 μ. Tarr (9) reported that the pycnidiospores varied from 14-22.4 x 5.6-9.2 μ and the pycnidia 180-295 μ in diam. In the present studies, the small, black densely gregarious pycnidia measuring 195-322.5 μ (avg. 235.2 μ) were observed on the surface of the lesions giving the characteristic rough feel (Fig. 1). The pycnidiospores were hyaline, 2-celled, oblong to ellipsoidal, pluriguttulate, with the cross wall medianly located, measuring 11.4-22.8 x 3.8-7.6 μ with a mean of 16.9 x 6.1 μ. Comparative morphology of the pycnidia and pycnidiospores of the fungus under study and that described by Saccardo (7) and later by Tarr (9) indicated its identity with *Ascochyta sorghina* Sacc.

**Artificial culture**

Single spore isolates on PDA incubated at room temperature (28-30°C) developed into small whitish colonies in 3–4 days. Cultures of the pathogen grew well on several synthetic and semisynthetic culture media and the topography and composition of the culture pellicle appeared correlated with the composition of the nutrient medium. The colonies growing on PDA were creamy white and smooth with the mycelium gradually changing to greyish white (Fig. 2 a, b) and later turning olive green within 12-15 days. The colony became blackish brown to dull green, leathery in texture (Fig. 2c) with abundant pycnidial development in the aging colony (Fig. 3). The pycnidia were embedded in the hyphae appearing as scattered irregularly shaped knots. They contained uniseptate, occasionally unicellular, hyaline, oblong to ellipsoid, thin-walled pycnidiospores measuring 9.3-21.7 x 2.5-8.2 μ (Fig. 4). The pycnidia developing in culture were slightly larger 140-290 μ in diam. than those produced on the host leaves 102-165 μ in diam. under field conditions.

Figs. 1-4. Disease symptoms and artificial culture of *Ascochyta sorghina*. 1. Field symptoms of rough leaf spot by *Ascochyta sorghina* on sorghum leaf. 2a, b. Plated cultures of *A. sorghina* showing radiating whitish grey mycelial growth; c. 3-week-old olive green growth. 3. Pycnidial development in the plated culture. 4. Uniseptate and few biseptate pycnidiospores. (Figs. 1 x ½ Nat. size. 2 x ½, 3 x 40 x 750).

Figs. 5-13. Host leaf infection by *Ascochyta sorghina*. 5. Typical bicelled pycnidiospores. 6. Pycnidiospores swelling after absorption of water. 7. Septal separation of cells in the pycnidiospores. 8, 9. Germ tubes emerging at both ends initiating separation of cells in the pycnidiospore. 10, 11. Germ tubes from unseparated and separated cells developing into hyphae. 12, 13a, b. Septation of hyphae later.