A Pictorial Technique for Mass Screening of Sorghum Germplasm for Anthracnose (*Colletotrichum sublineolum*) Resistance

Louis K. Prom[*,1], Ramasamy Perumal[2], John Erpelding[3], Thomas Isakeit[2], Noe Montes-Garcia[4] and Clint W. Magill[2]

[1]*USDA-ARS, Southern Plains Agricultural Research Center, College Station, Texas, USA*

[2]*Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843, USA*

[3]*USDA-ARS, Tropical Agriculture Research Station, Mayaguez, Puerto Rico 00680-5470, USA*

[4]*Instituto Nacional de Investigacuones Forestales, Agricolas y Pecuarias, Centro de Investigación Regional del Noreste, Rio Bravo, Tamaulipas, CP 88900, Mexico*

Abstract: Globally, the foliar phase of anthracnose is one of the most destructive diseases of sorghum. In most cases, anthracnose resistance screening relies on the use of a spore suspension. This method is usually conducted after sundown and when there is the possibility of dew formation the following morning. Using a spore suspension for sorghum anthracnose field evaluation in College Station, Texas over five years (1996, 1997, 1999-2001) yielded inconsistent linkage results and failed to identify any closely linked molecular markers. For large scale screening of sorghum germplasm for anthracnose (*Colletotrichum sublineolum*) resistance, plants are inoculated in the field or in the green house at either 30 d after planting or at the 8-10 leaf-stage. In field inoculation, the use of *C. sublineolum*-colonized sorghum grains was shown to be the most efficient and effective in identifying resistant sources. For effective, efficient, fast and accurate infection, approximately 10-20 seeds are placed in each plant leaf whorl and it takes about 16.7 kg of colonized grains to cover a 0.4 ha area. In the greenhouse, though colonized grains are equally effective, spray inoculation is preferred for easy and uniform coverage. Using this method of inoculum preparation, spore suspension was extracted and sprayed (10^6 conidia·ml^{-1}), followed by 10 hr/d misting for 30 sec at 30-45 min interval continuously for a period of one month resulted in effective infection.

Keywords: Sorghum, anthracnose, inoculation.

INTRODUCTION

Anthracnose caused by the fungus *Colletotrichum sublineolum* P. Henn., in Kabat. and Bubák (syn. *C. graminicola* (Ces.) G. W. Wilson [1, 2], is one of the most damaging diseases of sorghum [3-5]. Estimating grain yield losses due to anthracnose can often be difficult [6], but losses as high as 50% have been reported in susceptible cultivars [7-9]. Foliar infection can occur at any stage of plant development, but symptoms are generally observed 40 days after seedling emergence. Characteristic symptoms on susceptible cultivars include small circular to elliptical spots or elongated lesions and as the fungus sporulates, fruiting bodies (acervuli) appear as black spots in the center of the lesions [10]. For identifying disease resistant sources in a large scale germplasm evaluation, it is imperative to identify and follow an effective and efficient inoculation method, both in the field and under green house conditions. To address this problem, an alternate inoculation system which has proven to be extremely effective [11] was used. In this study, *C. sublineolum*-colonized sorghum grains were used to evaluate sorghum exotic germplasm accessions and advanced breeding lines in the field. In addition, a total of 320 germplasm accessions received from ICRISAT, India were evaluated under green house condition by using the method of inoculum preparation, spore suspension extraction and spraying to identify anthracnose resistance sources.

*Address correspondence to this author at the The USDA-ARS, Southern Plains Agricultural Research Center, Crop Germplasm Research, 2881 F&B Road, College Station, Texas, TX 77845, USA;
E-mail: louis.prom@ars.usda.gov

MATERIALS AND METHODS

Inoculum Preparation

Single spore cultures of *Colletotrichum .sublineolum* on potato dextrose agar plates, method of large scale inoculum preparation using sorghum grain and microscopic view of *C. sublineolum* conidia are detailed in Fig. (**1**).

Field Evaluation

A total of 100 advanced breeding lines in set I, and 76 exotic (17 from Uganda, 25 from Sudan, 13 from Mali, 15 from Ethiopia and 6 from India) sorghum germplasm lines in set II were evaluated in replicated trials along with BTx623 (susceptible check), and SC748-5 (resistant check) at the research farm, College Station, Texas in 2007. Materials used for field evaluation and method of colonized grains application for anthracnose disease screening is given in Fig. (**2**).

Green House Evaluation

A total of 302 (245 - mini-core, 35– high biomass sweet stalk, 15- grain mold tolerant cultivars, 3- high biomass

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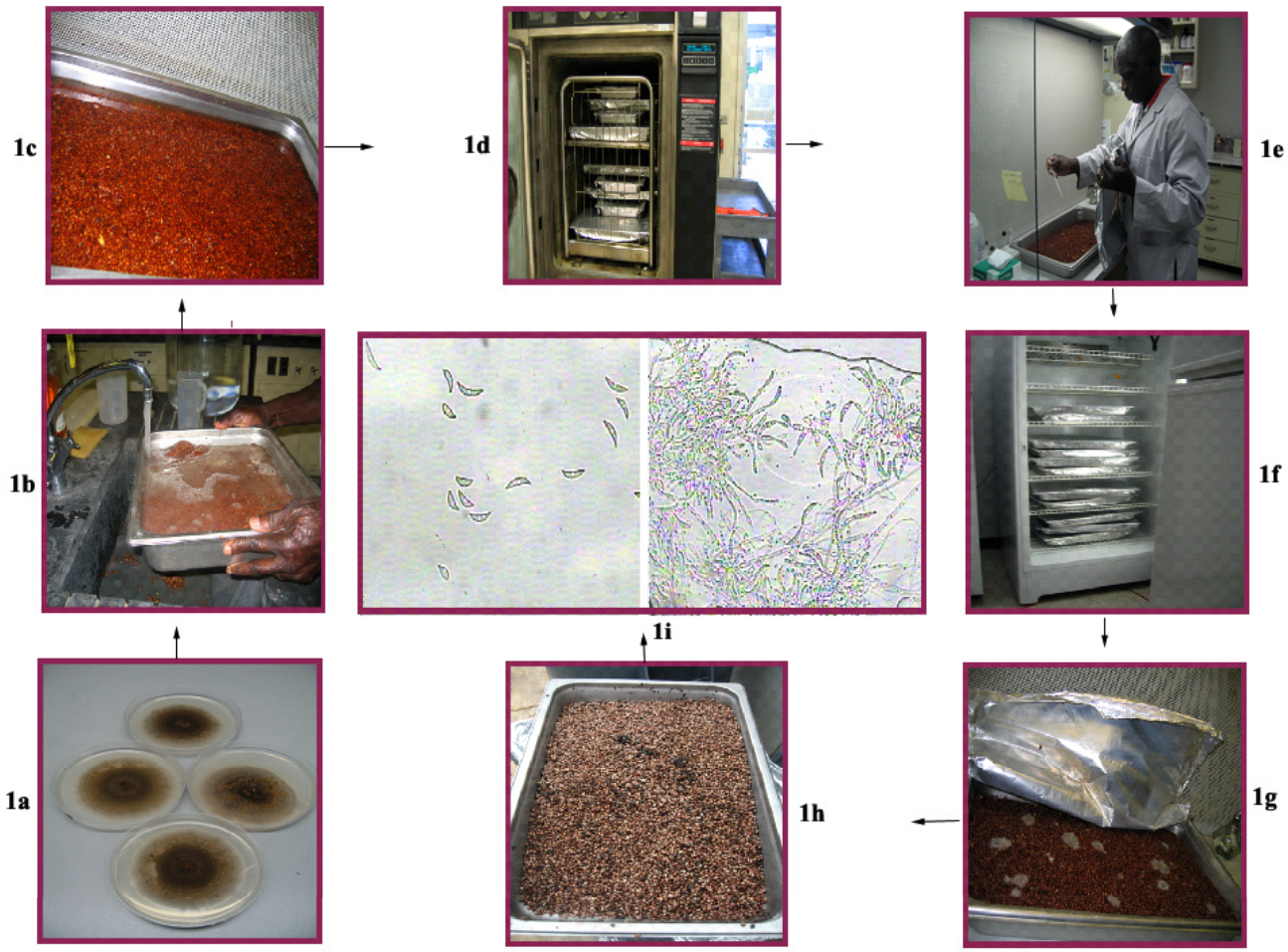

Fig. (1). ***Colletotrichum sublineolum*** **- Large scale inoculum preparation.** (**a**) Single spore isolates of *Colletotrichum sublineolum* grown at 25°C on half-strength potato dextrose agar. (**b**, **c**) Sorghum grains in stainless steel pans are washed thoroughly under tap water and then soaked for 48 h. (**d**) After 48 h, the water is drained and the pans are covered with aluminum foil. The pans are autoclaved at 121°C for 30 min on two consecutive days. (**e**) Conidial suspensions or agar plugs containing the fungal isolates are obtained from two-week old culture plates. Under the hood, several drops (10-12) of the conidial suspension or agar plugs containing the fungal isolates are placed on the sterilized sorghum grains and then covered with aluminum foil. (**f**) Pans containing the mixture of sterilized sorghum grains and fungal isolates are incubated at 25°C for 14-21 d. (**g**) To ensure complete colonization, the inoculated grains are mixed every 3 to 4 d either by using a sterile spatula under the hood or by shaking the pans without opening the aluminum foil. (**h**) Colonized sorghum grains are stored in the cold room until used. (**i**) *C. sublineolum* conidia as viewed under microscope.

forage and 4-ergot tolerant types) germplasm sorghum lines received from ICRISAT, India along with 16 differentials and BTx623 (susceptible check), and SC748-5 (resistant check) were evaluated two times consecutively in September 2007 and January 2008 in the USDA-ARS green house, College Station, Texas. Detailed method of spray inoculation followed by misting in the green house with photographic illustrations are presented in Fig. (**3**).

Disease assessments are conducted 30 d post-inoculation and thereafter on a weekly basis for four consecutive weeks until flowering (Fig. **4**). Scoring multiple times enhanced the probability of detecting sporulating lesions. The difference between a resistant and susceptible response is the presence of acervuli on the leaves, which indicates successful reproduction of the pathogen. Ratings are based on a scale of 1-to-5 modified from Pande *et al.* [13] by Erpelding and Prom [14]. Thakur and Mathur [15] followed 1-5 severity rating scale and suggested a 1-9 severity rating scale for differentiating between lines with minor differences in resistance.

RESULTS AND DISCUSSION

In a detailed examination of disease development by sorghum anthracnose, Wharton *et al.* [16] observed that early infection processes (spore germination, differentiation of appresoria, and penetration of sorghum leaf sheaths epidermal cells) were the same in compatible and incompatible interactions. Host responses in both resistant and susceptible cultivars lead to the accumulation of pigments around the sites of infection. These conditions tend to cause mis-scoring of plants, with escapes being assigned a resistant rating. Pande *et al.* [13] inoculated both leaf surfaces of the plant with a conidial suspension of *C. sublineolum* using a hand sprayer. One hour after inoculation humidifiers were run continuously for 18 hr to create 100% humidity. Further to promote disease development humidifiers were run continuously for 6 days for 8 hr/day.

Fig. (2). Field inoculation. (**a**) Two sets of experiments, one with 100 advanced breeding lines and another with 76 exotic germplasm accessions along with BTx623 (susceptible check), and SC748-5 (resistant check) were evaluated at the College Station Research Farm in 2007 under randomized block design with 3 replicates. Field inoculations are made during the transition from growth stage 1 to growth stage 2 [12], when approximately 8 to 10 leaves are fully developed. (**b**) *C. sublineolum* colonized sorghum grains (approximately 10-20 seeds) are placed in each plant leaf whorl. (**c**) Colonized grains placed in the leaf whorl infect foliar tissues reducing the amount of photosynthate accumulation. Infection of the stalk leads to lodging, a detriment to maximizing harvestable biomass. (**d**) Sorghum plants are being inoculated with the colonized grains. It takes approx. 16.7 kg of colonized grains to inoculate a 0.4 ha area.

To confirm disease infection, the author conducted the experiments five times. Mehta [17, 18] followed the same inoculation method by spraying approximately 3-5 mL of a conidial suspension (10^6 conidia ml^{-1}) onto the leaves and the whorl of each plant, using backpack sprayers or a tractor-mount sprayer at night or in the early morning so as to have low light intensity. Based solely on anthracnose field scores obtained in this manner (College Station, 1996, 1997, 1999, 2000 and 2001), the author was not able to identify closely linked molecular markers, due to inconsistent phenotyping results for anthracnose resistance from the segregating F_2 progeny of the cross BTx623*SC748-5. Mehta [17] reported that the degree of infection on susceptible plants within susceptible or segregating $F_{2:3}$ lines were very low compared to the infection observed on susceptible checks indicating a lower level of viable inoculum throughout the evaluated field plots. This made accurate scoring difficult.

To alleviate this problem, an alternate inoculation system, as detailed above, has proven to be extremely effective. Its use here and in the field greatly enhanced the ability to differentiate resistant, segregating and susceptible families [11]. This reclassification of the mapping population clearly revealed the mis-classification in earlier disease phenotyping of the cross BTx623*SC748-5, and also permitted the identification of molecular markers that co-segregate with the anthracnose resistance gene [19]. In a 2007 field evaluation at College Station, Texas, 95% of the

Fig. (3). Green house inoculation. (**a**) A total of 320 sorghum germplasm lines were evaluated consecutively, in September 2007 and January 2008, along with BTx623 as susceptible and SC748-5 resistant checks under a randomized block design replicated four times. For easy handling, four germplasm accessions, each with five plants, were accommodated in a five gallon pot. (**b**) Either colonized grains or spray inoculation have been used in the greenhouse to identify new resistance sources with limited spacing between plants and pots in the green house, plant growth won't be as vigorous when compared to field conditions, and makes difficult the placement of colonized grains on individual plant whorls. Hence, for easy and uniform coverage, spray inoculation is preferred for green house screening, though colonized grains application is equally effective. (**c**) The protocol for colonized grains inoculation is similar to that of the field with the spray inoculation technique, approx. 3-5 ml conidial suspension (10^6 conidia·mL^{-1}) is deposited on the leaves of each plant. Tween 20 (wetting agent) is added to the inoculum (0.5 ml/L). (**d**) Shortly after spraying the inoculum, plants are misted for 30 sec at 30-45 min intervals, 10 hr/d for one month. This misting regime provides favorable environment for infection and disease development.

accessions from 100 advanced germplasm lines and 55% from the exotic lines were found to be susceptible indicating the effectiveness of the alternate inoculation method developed by Erpelding and Prom [11].

Upadhyaya *et al.* [20] developed a minicore sorghum germplam (242 accessions from 57 countries) which was representative of entire 2246 germplam collection (10% of core, 1% of entire collection) being maintained as Genetic Resources at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. Based on many statistical analyses, mini-core germplasm captured about 90% of the diversity and, majority of the co-adapted gene complexes present in the core. Due to its greatly reduced size and its representation of the entire diversity, the sorghum mini-core was economically evaluated extensively under green house conditions for anthracnose resistance two consecutive times (September 2007 and January 2008). From the screening results, 200 accessions were identified as resistant, 120 accessions were identified as susceptible

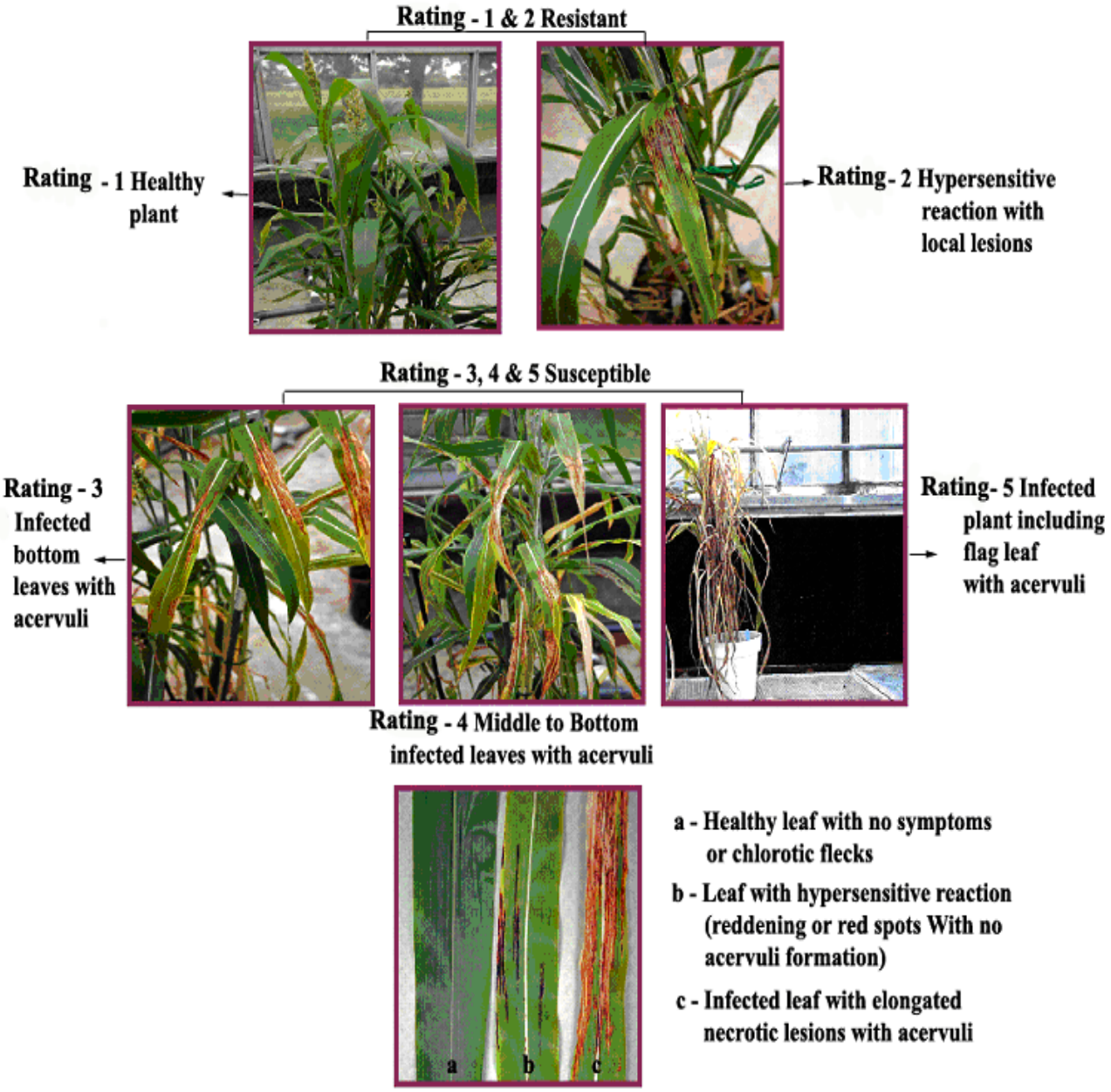

Fig. (4). Disease ratings.

(foliar infection with acervuli), and 11 accessions were identified as susceptible to mid-rib infection. Using the colonized sorghum grain inoculation method, the identified resistance sources will be further evaluated under field conditions in different locations to confirm the stability of this resistance performance and for use in further breeding programs.

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