

# Bacterial Leaf Streak of Sorghum— a New Report from India

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## Introduction

At least 10 bacterial diseases have been reported on sorghum [*Sorghum bicolor* (L.) Moench] of which bacterial stripe [*Pseudomonas andropogonis* (Smith) Stapp], bacterial leaf streak (BLS) [*Xanthomonas campestris* pv. *holcicola* (Elliott) Starr & Burkholder], and bacterial spot (*Pseudomonas syringae* pv. *syringae* van Hall) Claflin et al. (1992) are considered to be economically important. In southern Africa, BLS of sorghum has reported from Angola, Lesotho, Malawi, Tanzania but the pathogen's identity was not confirmed, whereas from the Republic of South Africa, BLS has been reported with confirmed identity (de Milliano 1992). Occurrence of BLS was also reported from West Africa, South America, Mexico, North America, Iran, and Japan. Noble and Richardson (1968) reported the seedborne nature of BLS; however experimental data were not presented. Rao et al. (1990) have detected *X. campestris* pv. *holcicola* in sorghum leaves by dot immunobinding assay in a sorghum sample imported to India from Yemen in 1987. *X. campestris* pv. *holcicola* is a pathogenic and quarantined organism. In several countries this bacterium is used as trade barrier. Bacterial leaf streak has not previously been reported from India. This paper reports the occurrence of the disease on sorghum in field conditions at the ICRISAT research farm, Patancheru, Andhra Pradesh, India, and in several farmers' fields surveyed in Karnataka, India, from August 1999 to March 2001 and in elite germplasm accessions evaluated for resistance to foliar and panicle diseases during the 2001 rainy season at ICRISAT, Patancheru.

## Material and methods

BLS-infected leaves of cultivar H 112 were collected from the field in the first week of July 2000. Infected portions of leaves were cut in to 1 -cm<sup>2</sup> pieces, surface-sterilized in 1% sodium hypochlorite and washed three times in sterile distilled water. The pieces were plated on nutrient agar medium. The plates were incubated at 25±1°C for 4 days. Subsequent sub-culturing and multiplication was done on sucrose peptone agar medium (sucrose 20 g, peptone 5 g, potassium hydrogen phosphate 0.5 g, magnesium sulphate heptahydrate 0.25 g, and 15 g agar in 1000 mL water). The antiserum to *X. campestris* pv. *holcicola* obtained from L E Claflin, Kansas State University, Manhattan, USA, which had a titer of about 1500, was used to test against the bacterium following an ELISA technique.

To prove the pathogenicity of the bacterium, 30-day-old sorghum seedlings were inoculated with freshly grown bacterium following carborundum powder, vacuum pump, and other methods described below. In the carborundum powder method, 10 30-day-old potted seedlings of variety H 112, were sprayed with carborundum powder (BDH Chemicals, UK, about 300 grit) to create wounds on either side of the leaf surface, and 15 minutes later, absorbent cotton dipped in bacterial suspension was smeared over the leaf surfaces. Seedlings were incubated in a growth chamber for 24 h with a 12-h light cycle. Later the pots were moved to greenhouse benches. Two controls were maintained, one with only carborundum spray, and another with carborundum spray followed by swabbing with sterile distilled water.

In the vacuum pump method, lids of two bottles were marked and cut to an appropriate size to insert a leaf and the plastic pipe of a vacuum pump (Charles Austen Pumps Ltd, 100 Roystan Road, Byfleet Weybridge, Surrey UK). The leaf edges of the seedling were either clipped or left intact. Seedlings with their leaves intact were inserted into a bottle containing bacterial suspension. The container was sealed using wax. The vacuum pump was operated for 3-4 minutes for each leaf dipped in the suspension. Entry of the bacterial suspension into the leaf was ensured by observing of air bubbles emerging from stomata and the cut leaf edges. The leaves were air-dried and incubated in a growth chamber for 24 h. Next day the pots of seedlings were moved to a greenhouse so symptoms could develop. The other methods tried were spray, injection, and drop-inoculation similar to those followed when testing for pearl millet downy mildew [*Sclerospora graminicola* (Sacc.) J. Schrott] (Singh et al. 1997). For control treatments sterile distilled water was used instead of bacterial suspension. The experiment was repeated twice.

## Results and discussion

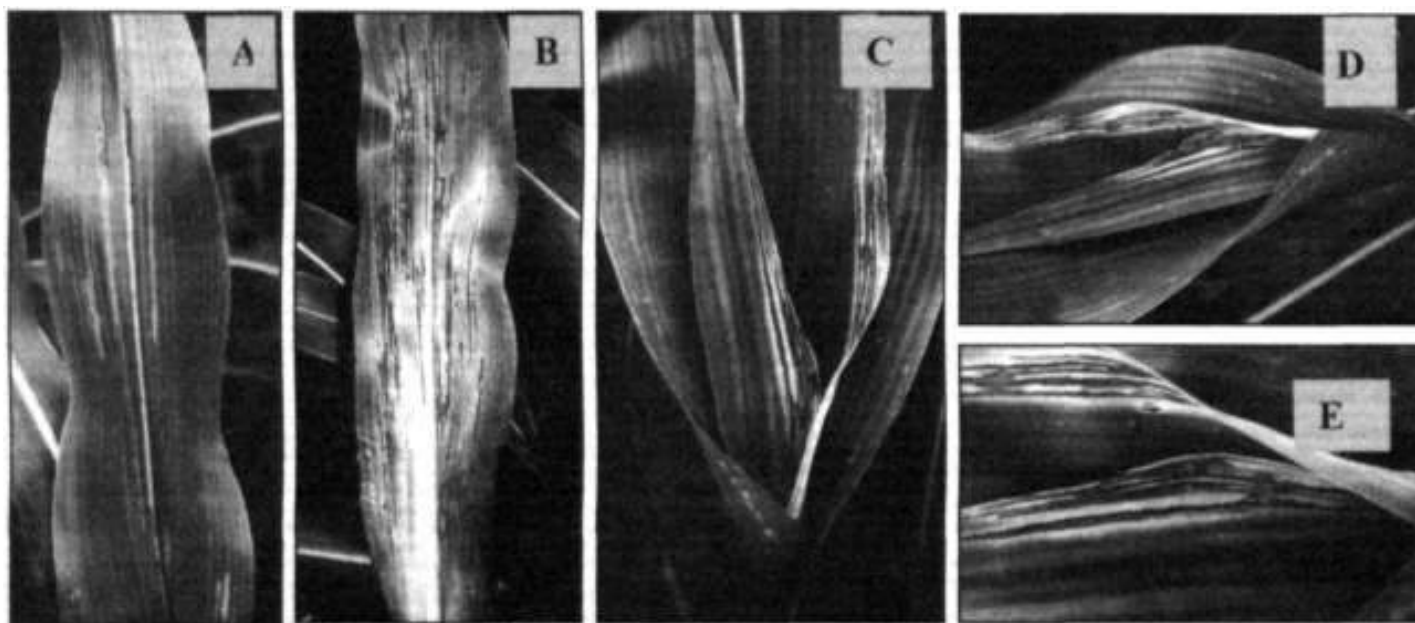
The BLS symptoms observed on H 112 at the ICRISAT research farm were similar those described by Frederiksen and Odvody (2000). Small water-soaked, reddish-brown necrotic streaks appeared in the early stages that later elongated and darkened. Later still the lesions broadened and developed tan centers with a narrow red margin. These symptoms were delimited by the veins (Figure 1a). Occasionally, tiny, yellow, bead-like exudations were found on lesions. The narrow, long or short translucent lesions coalesced forming large patches (Figures 1b and 1c). In severe cases, affected leaf parts acquired a burned appearance (Figures 1d and 1e). At this stage the leaves withered, and turned brown. The most-prominent symptoms were elongate lesions or areas of discoloration, usually of limited length, parallel to the leafveins (Figure 1b). Out of 1014 elite germplasm accessions (originating from 45 countries) evaluated for various foliar and panicle diseases during the 2001 rainy season at ICRISAT, Patancheru, BLS was observed in seven accessions [IS 21977 (India), IS 14008, IS 14305, IS 24497 (South Africa), IS 29389 (Lesotho), IS 13177 (Argentina) and IS 25428 (Kenya)]. The BLS incidence was 4- 10% and the severity 2-20%. BLS was also observed in several farmers' fields in the state of Karnataka, during rainy and postrainy seasons (August 1999 to March 2001) while conducting disease surveys. The locations where BLS was observed were Ainapur

[16° 49' 40" (N), 75° 46' 19" (E)], Kannolli [16°50' 84" (N), 76° 08' 24" (E)] and Bijapur [16° 49' 40" (N), 75° 46' 19" (E)] in Bijapur district; Hunagund [16° 18' 33" (N), 75° 54' 57" (E)] in Bagalakot district, Attigullapura [11° 50' 00" (N), 76° 59' 94" (E)], and Seenappanadoddi [11° 48' 88" (N), 77° 00' 17" (E)] in Chamarajnagar district; Dandinakuru-barahatti [14° 13' 82" (N), 76° 27' 70" (E)] in Chitradurga district; and Sege [13° 06' 69" (N), 76° 04' 73" (E)] in Hassan district. BLS incidence varied from 0.5-20% and its severity from 5-60%. The occurrence of BLS in various farmers' fields and on the research farm appears to be the first record from India (Frederiksen, R A, and Rajasab, A H, personal discussion).

The bacterium was consistently isolated from naturally infected sorghum plants grown in an experimental field at ICRISAT, Patancheru, in June 2000 and was identified as *Xanthomonas campestris* pv. *holcicola* based on the reaction to the lyophilized antiserum to *X. campestris* pv. *holcicola* in ELISA plates. However, attempts to prove pathogenicity following several methods were unsuccessful.

## Acknowledgments

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**Figure 1.** Symptoms of bacterial leaf streak (*Xanthomonas campestris* pv. *holcicola*) of sorghum, 1a. Streak delimited by the veins, 1b. Elongate lesions or areas of discoloration, usually of limited length, parallel to veins, 1c. Narrow, long or short translucent lesions coalesced forming large patches, 1d and e. Burnt appearance similar to that of anthracnose and leaf blight.

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## Usefulness of Non-senescent Parents for Charcoal Rot Resistance Breeding in Sorghum

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## Introduction

Charcoal rot of sorghum (*Sorghum bicolor* (L.) Moench) is caused by the fungus *Macrophomina phaseolina* (Tassi) Goid. The disease has a great destructive potential, particularly in postrainy (rabi)-season sorghum. The ability of non-senescent genotypes to remain

physiologically active during all growth stages of a crop may contribute to the overall tolerance and disease resistance mechanism, by minimizing the predisposition to charcoal rot infection, and its spread inside the stem (Duncan 1984).

## Materials and methods

The Purdue population containing recombinant inbred lines (RILs) derived from a cross between non-senescent parent B 35 and senescent parent TX 7078 (100 lines) and the Queensland population based on non-senescent parent QL 41 and senescent parent QL 39 (144 lines including parents) were evaluated in an Alfisol field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during Nov 1995 Apr 1996. Withholding irrigation before flowering created drought conditions suitable for disease development. Each set of RILs and their parents, along with a few controls were sown in three replications in single-row 4-m long plots with a row spacing of 60 cm. Plant density was maintained at 11 plants m<sup>-2</sup>. Recommended doses of fertilizers was applied to the plots along with carbofuran granules 4 kg ha<sup>-1</sup> to protect the crop from stem borer [*Chilo partellus* (Swinhoe)].

To study plant senescence two criteria, leaf senescence (observations recorded for 6 days during the later phases of maturity till harvest), and the number of green leaves plot<sup>-1</sup>, together with such other parameters as charcoal rot infection (%) and extent of disease spread were recorded at harvest, i.e., 10 days after physiological maturity. Soft stalk and lodging were scored using a 0-9 point disease-rating scale, where 0 is no disease, 1 is 10%, and 9 is 90% disease incidence.

To study the relationship between plant senescence and charcoal rot development, simple correlation and regression estimates were calculated for RILs of both populations. Further, to study the critical relationship between plant senescence and charcoal rot development, the 10 most-green genotypes, and 10 least-green genotypes were considered from each population and simple correlation and regression estimates calculated.

## Results and discussion

In the Purdue population, correlation and regression analyses of different disease resistance characters, leaf senescence, and the number of green leaves plot<sup>-1</sup> revealed positive and significant correlations between lodging, soft stalk, extent of disease spread, charcoal rot (%) and leaf senescence, and negative and significant correlations between lodging, soft stalk, length of spread