Indian J. Microbiol, 31 (4) December, 1991, pp. 435–441

Stalk and Top Rot of Sorghum caused by *Erwinia chrysanthemi* in India

S.C. SAXENA[1], L.K. MUGHOGHO[2] and S. PANDE[2]

[1] Department of Plant Pathology, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttar Pradesh 263 145, India.

[2] Cereals Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

Abstract : A stalk and top rot disease of sorghum was observed at Pantnagar, India. The disease was severe on a number of genotypes. Infected plants showed signs of wilting of apical leaves and yellowing of lower leaves. Stalks turned brown, were full of bacterial exudate, and collapsed from rotten internodes. Rotting was fast and plants were killed within 2–3 days after symptom appearance. A bacterium consistently associated with disease symptoms was identified as *Erwinia chrysanthemi* pv. *zeae*. This is the first report of this bacterium as a cause of stalk and top rot of sorghum under natural conditions in India.

Key words : *Erwinia chrysanthemi*, Stalk rot, Sorghum.

Introduction

Sorghum (*Sorghum bicolor* L. Moench.) is one of the world's major food crops (1). Because of the wide range of environments in which it is cultivated, it is affected by a wide range of diseases (2). Soft rot, caused by *Erwinia chrysanthemi*, is one of the 14 bacterial diseases reported on sorghum (3). During the 1987 and 1988 crop seasons a similar disease was observed in sorghum fields at Pantnagar, India. The disease was widespread and affected 60–80% of plants in different sorghum genotypes. The disease is characterised by wilting of apical leaves giving an ashy appearance to the infected plant. Lower leaves and leaf sheaths covering the internodes are chlorotic, and the rind is pale-straw instead of green in colour. The stalk is soft and the pith is destroyed leaving the vascular bundles in a disorganised state. The affected plants collapse and die within 2–3 days after the symptoms appear. The rot progresses in either direction from an infected internode. Top rot extends from the top to the base of the plant and basal stalk rot extends upwards from the base of the plant. In split opened stalks the vascular bundles are greyish in colour, emit a strong putrid odour, and the internodes are filled with sticky bacterial exudate. The disease appears before the onset of flowering. Cloudy weather, relatively high temperature (>30°C) and frequent rainfall favours disease epidemic.

In this paper we report the isolation, pathogenicity, and identification of the bacterium causing stalk and top rot of sorghum based on morphological gnd physiological tests.

Materials and Methods

Isolation : Sorghum plants showing early symptoms were cut 30 cm above ground level, leaves were clipped off, stalks with 3 internodes were thoroughly cleaned, surface sterilized with 95% alcohol and then flamed. A small opening was asceptically made with a knife in the middle internode, one or two 5 mm pieces from the pith were removed, placed in a culture tube containing 5 ml sterile distilled water and rinsed three times. Each piece was cut into two halves,

ICRISAT Journal Article No. 1020.

transferred to culture tubes containing 5 ml sterile distilled water and incubated for 15 min at 30°C to allow the bacterial cells to diffuse into water. Isolations were made from serial dilutions of the bacterial suspension by streak plating on nutrient yeast dextrose agar (NYDA, nutrient agar 28 g, yeast extract 5 g, dextrose 10 g and distilled water 1 L adjusted to pH 7.0). Plates were incubated at 30°C for 24 h. The isolates were purified by single colony transfers on to NYDA slants.

Pathogenicity tests : Patnogenicity tests were conducted on a susceptible sorghum cultivar SPV 86, and a maize (*Zea mays* L.) hybrid Ganga 5 in a controlled environment chamber with a high relative humidity (>90%) and temperature set at 32±2°C. One loopful of an actively growing culture on NYDA was transferred to 250 ml nutrient broth (NB, peptone 10 g, beef extract 5 g, glucose 10 g and distilled water 1 L) and incubated at 30°C for 18-20 h. Bacterial suspension of each isolate was inject-inoculated (2 ml/plant) with a 21 G hypodermic needle into the vicinity of a growing point of a 21-day-old plant. Plants were grown in 30.5-cm-diameter plastic pots filled with vertisol. A sterile moist cotton pad was wrapped around the inoculated point to maintain high humidity. With another set of plants, bacterial suspension was supplemented with 5 ml/l of tween 20 (polyoxyethylene sorbitan monolaurate) and poured into leaf whorls (2 ml/whorl) without causing injury. Care was taken not to disturb the plants after inoculation so that maximum inoculum was retained in the whorls. Plants injected with sterile distilled water served as control. After inoculations, pots were shifted to the controlled environment chamber and incubated for 7 days for disease development.

For species identification, two known 'Erwinia' isolates, *E. carotovora* pv. *carotovora* (ECC) and *E. chrysanthemi* pv. *zeae* (EchrZ) causing soft rot of potato (*Solanum tuberosum* L.) and Erwinia stalk rot of maize, respectively, were included for comparison. One nonpathogenic sorghum bacterium obtained during isolations of the pathogen was also included in these tests as a check isolate.

Characterization of isolates on selective media : Each test isolate was plated on the selective media D3 (4) and crystal violet pectate (CPV) (5). The potato softening test (6) was conducted using 8-10 mm thick slices cut asceptically from surface sterilized tubers (immersed in 1:1000 mercuric chloride solution for 2 min). The slices were put in Petri plates (8-10 per plate) containing sterile water and the upper level of water touched the edge of the slices. All the potato slices in a dish were inoculated at the center with an actively growing 24-h-old culture isolate, incubated at 30°C for 48-72 h, and then examined for softening.

Physiological and morphological tests : Standard procedures for identifying plant pathogenic bacteria (7) were followed. Colony characteristics were determined 2 days after incubation at 30°C on NYDA. Hucker's modification was followed for Gram stain reaction. Growth and pattern of cavity formation was determined on nutrient agar and CVP medium, respectively, at 37°C, and 5% salt tolerance in nutrient broth (8). Hydrogen sulphide (H_2S) production (9), starch hydrolysis, gelatin liquefaction (10), indole, nitrate, methyl red and gas from glucose were detected following Lelliott (11). Pectolytic activity was determined on CVP medium (5). Acid production from arabinose, cellobiose, dulcitol, fructose, galactose, glucose, glycerol, lactose, mannose, melibiose, maltose, rhamnose, ribose, sorbitol, sorbose, starch, sucrose and xylose was determined in 1% peptone water with bromothymol blue indicator (12). Sensitivity to erythromycin (25, 50 ug/ml)

was detected on nutrient agar in 100 × 15 mm disposable Petri plates at 28, 32 and 37°C and, *in vitro* inhibition to sodium hypochlorite (5.25%) at 1, 5, 10, 25, 50 and 100 μg/ml, using the filter disc method (13) at 30°C.

Results

Two types of bacterial colonies were predominant on isolations made from infected stalks after 48 h incubation in NYDA. Creamy white to white mucoid colonies, raised with undulated margins were separated from those that were creamy light yellow, round but flat with irregular margins. Based on colony morphology the two sorghum soft rot isolates were marked as isolate 1 (SSR1) and isolate 2 (SSR2), respectively, and used in all subsequent tests.

In pathogenicity tests typical disease symptoms developed in both inject and whorl-inoculated plants with SSR1 and EchrZ isolates within 5 days of inoculation. Wilting of upper and inner whorl leaves, which later became necrotic was the first symptom (Fig. 1A). Infected whorl leaves could be pulled out easily because of extensive rotting of tissues at the base. A fermenting odour was emitted by the rotten tissues. The rot extended downward and the basal part of the plant became brown and developed rot after 7 days (Fig. 1B). EEC and SSR2 strains failed to produce any symptoms.

Fig. 1. Series of symptoms developing on 21 day-old sorghum plants 5 days of inoculations. (A) Note (L to R) initial wilting of inner four apical leaves due to soft rot establishment, infold of leaves and wilting extending to lower leaves. Leaves look deteriorated and the lower ones show flaccidity, withered apical leaves and plants toppling due to softening of pith region. (B) Inner whorl separated from stalk due to intense rot near growing point exhibiting rotten base.

Isolate characterization : Both the test strains (SSR1 and SSR2) were Gram negative, straight rods occurring singly or in pairs and motile. Growth on selective D3 medium produced characteristic red colouration. Isolates ECC, EchrZ and SSR1 produced a more intensive red colour within 24 h than SSR2 isolate which required >48 h to produce any noticeable reaction. Typical deep cup-like depressions formed within 36 h on CVP medium (Table 1). Delayed but

very shallow depressions were formed by SSR2 isolate. Isolates ECC, EchrZ and SSR1 produced rapid and marked potato slice rot with intensive fermenting odour at 30°C. Rot was slight and delayed with SSR2 isolate. These tests indicated a close identity of SSR1 isolate to known *Erwinia* strains ECC and EchrZ.

Table 1. Reaction of *Erwinia* and unknown isolates on D3 medium, cavity formation on crystal violet pectate (CVP) medium and potato slice rot at 30°C.

Erwinia and unknown isolates	D3 medium[a]	CVP medium	Potato-slices
ECC[b] (Potato)	+[c]	+	+
EChrZ[d] (Maize)	+	+	+
SSR1[e] (So ghum)	+	+	+
SRR2[f] (Sorghum)	delayed	delayed	delayed

a : Kado and Heskett, 1970.
b : *Erwinia carotovora* pv *carotovora*.
c : Positive
d : *Erwinia chrysanthemi* pv *zeae*
e : Sorghum soft rot isolate 1.
f : Sorghum soft rot isolate 2.

Isolates SSR1 and EchrZ produced gas from glucose aerobically and anaerobically, but gave negative results with methyl red and starch hydrolysis tests (Table 2). These two isolates also reduced nitrates, produced H_2S, liquefied gelatin, and showed a positive indole reaction. Acid was produced from 1% concentration of various carbon sources arabinose, cellobiose, fructose, galactose, glucose, mannose, melibiose, ribose, sorbitol, sorbose, sucrose and xylose, in peptone water with bromothymol blue as indicator within 7–10 days at 30°C but not from dulcitol, glycerol, lactose, maltose, rhamnose and starch. ECC and SSR2 showed more variable responses than EchrZ and SSR1 in utilization of different carbon sources.

Table 2. Reaction of *Erwinia* and unknown isolates to various tests 30°C.

Tests	*Erwinia* isolates		Unknown isolates	
	ECC[a] (Potato)	EChrZ[b] (Maize)	SSR1[c] (Sorghum)	SSR2[d] (Sorghum)
Gas from glucose	−[e]	+[f]	—	—
Methyl red	—	—	—	—
Nitrate reduction	+	+	+	+
Starch hydrolysis	—	—	—	+
H_2S production	+	+	+	+
Indole production	—	+	+	—
Gelatin liquefication	+	+	+	+

a : *Erwinia carotovora* pv *carotovora*.
b : *Erwinia chrysanthemi* pv *zeae*.
c : Sorghum soft rot isolate 1.
d : Sorghum soft rot isolate 2.
e : No noticeable reaction.
f : Positive within 14 days.

Erwinia isolates EchrZ and SSR1 were inhibited in 5% salt but formed typical irridescent depressions on CVP at 37°C within 18 h, whereas ECC formed fewer colonies at higher temperatures and produced shallow cavities on CVP (Table 3). The pathogenic isolate (SSR1) was highly sensitive to erythromycin at 25 and 50 ug/ml to the extent of 24 mm and 52 mm inhibition zone around the disc. Sodium hypochlorite inhibited the bacterium completely at and above 25 ppm *in vitro*. The inhibition was 10 mm at 10 ppm, and no inhibition occurred at 5 and 1 ppm (Table 4).

Table 3. Growth and cavity formation on crystal voilet pectate medium by *Erwinia* and unknown isolates at different temperatures

Temperature °C	*Erwinia* isolates		Unknown isolates	
	ECC[a] (Potato)	EChrZ[b] (Maize)	SSR1[c] (Sorghum)	SRR2[p] (Sorghum)
28	+[e]	+	+	Vf
32	+	+	+	—[g]
37	—	+	+	NG[h]

a : *Erwinia carotovora* pv *carotovora*.
b : *Erwinia chrysanthemi* pv *zeae*.
c : Sorghum soft rot isolate 1.
d : Sorghum soft rot isolate 2.
e : Positive
f : A few shallow cavities formed
g : Few colonies and shallow cavities formed.
h : No growth.

Table 4. Zones of inhibition (mm) of *Erwinia* and unknown isolates by erythromycin and sodium hypochlorite at 30°C.

Test chemical	Conc. (μg/ml)	*Erwinia* isolates		Unknown isolates	
		ECC[a] (Potato)	EChrZ[b] (Maize)	SSR1[c] (Sorghum)	SSR2[d] (Sorghum)
Erythromycin	25	0	28	24	0
	50	0	50	52	0
Sodium hypochlorite	1	1	0	0	0
	5	0	0	0	0
	10	0	15	10	0
	25	0	NG[e]	NG	0
	50	18	NG	NG	0
	100	28	NG	NG	0

a : *Erwinia carotovora* pv *carotovora*.
b : *Erwinia chrysanthemi* pv *zeae*.
c : Sorghum soft rot isolate 1.
d : Sorghum soft rot Isolate 2.
e : No growth.

Discussion

Erwinia stalk rot of sorghum has been reported from Israel (14), New Zealand (15), the Philippines (16), Puerto Rico (17), and USA (18, 19, 20). The bacterial stalk rot and top rot disease described without naming the pathogen in Australia (21), USA (22), and USSR (23) is probably incited by an *Erwinia* spp. It is quite possible that the disease also occurs in other sorghum growing countries. Symptoms of Erwinia stalk rot in sorghum appear any time from just before booting to half bloom growth stage. At Pantnagar, these growth stages often coincide with the prevalance of high temperatures and high relative humidity due to frequent rainfall; the environment within the crop canopy is thus hot and humid favouring infection and spread of the bacterium (24, 20). Susceptibility to infection decreases with increased age of the plant and changes in weather conditions.

In artificial inoculations typical wilting and rotting of apical leaves occurred and the dead leaves could easily be pulled out. Rotten tissues at the base of such leaves emitted a foul-smelling odour (17, 25). Hingorani *et al.* (26) reported *E. carotovora* f. sp. *zeae* as the cause of stalk rot of maize in India and that sorghum was one of the hosts in artificial inoculations. Symptoms of stalk rot and wilting of sorghum caused by bacteria in India were reported but without a detailed study of the disease and diagnosis of the pathogen (27, 28). In the present study the two isolates obtained from infected sorghum stalks varied greatly in their colony characteristics. Isolate SSR1 caused soft rot on sorghum similar to EchrZ (29). Positive reaction on D3 medium, ability to soften potato slices and degradation of CVP by forming characteristically deep cup-like depressions further, indicate that the isolate SSR1 belonged to the genus *Erwinia*.

Positive reaction in indole test, gas from glucose, lack of acid production from lactose and maltose, and lack of growth in 5% salt solution indicated that the causal bacterium belongs to *E. chrysanthemi* (30). Complete tolerance to growth and formation of typical cup-like cavities on CVP medium at 37°C and high sensitivity to erythromycin confirmed its identification (31). Based on these tests, the bacterial isolates from infected sorghum plants (SSR1) had identical characteristics to *E. chrysanthemi* pv. *zeae* Burk., McFadden and Dimock which causes soft rot of maize. This is the first report of this bacterium as the cause of stalk and top rot of sorghum under natural infection conditions in India.

Acknowledgement : We are thankful to International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for providing the Research Fellowship to the senior author and facilities to conduct this research at ICRISAT Center. We are also thankful to the Director of Research, G.B. Pant University of Agriculture and Technology, Pantnagar for providing land and facilities.

References

1. Dogget H, 1988, Sorghum, Longman London.
2. Frederiksen RA, 1986, Compendium of sorghum diseases, American Phytopathological Society, St. Paul, Minnesota.

3. Bradbury JF, 1986, Guide to Plant Pathogenic Bacteria, CAB International Farnham Royal.
4. Kado CI and Heskett MG, 1970, Phytopathology **60** : 969.
5. Cupples D and Kelman A, 1974, Phytopathology **64** : 468.
6. Prasad M and Sinha SK, 1976, Science and Culture **42** : 327.
7. Schaad NW, 1988, Laboratory guide for identification of plant pathogenic bacteria. 2nd Edition. The American Phytopathological Society St. Paul., Minnesota.
8. Dickey RS, 1979, Phytopathology **69** : 324.
9. Skerman VBD, 1967, A Guide to the Identification of the Genera of Bacteria. 2nd Edition. Williams and Wilkins, Baltimore.
10. Dye DW, 1968, New Zealand J Sci **11** : 590.
11. Lelliott RA, 1974, In Bergey's Manual of Determinative Bacteriology Eighth Edition. Buchanan RE and Gibbons NE (Ed) Williams and Wilkins, Baltimore 332.
12. Society of American Bacteriologists, 1957, Manual of Microbiological Methods. McGraw-Hill. New York.
13. Thornberry HH, 1950, Phytopathology **40** : 419.
14. Zutra D and Kenneth R, 1978, Hassadeh **58** : 830 (in Hebrew).
15. Watson DRW, 1971, New Zealand J Agric Res **14** : 944.
16. Dalmacio SC, 1978, In sorghum Diseases a World Review Williams RJ, Frederiksen RA and Mughogho LK (Ed) 70–71. ICRISAT, Hyderabad, India.
17. Hepperly PR and Ramos-Davila E, 1987, J Agric. Puerto Rico **71** : 265.
18. Jardin DJ, 1986, Crop Extension Service Kansas State University **4** : 741.
19. Jensen SG, Mayberry WR and Obrigawitech JA, 1982, Phytopathology **72** : 990.
20. Zummo N, 1969, Phytopathology **59** : 119.
21. Ludbrook WP, 1942 J Coun Sci Ind Res Australia **15** : 213.
22. Hsi CH, 1956, Plant Disease Reporter **40** : 369.
23. Pastushenko LL, 1962, J Microbi l Kiev **24** : 34 (in Russian).
24. Jensen SG, Mayberry WR and Obrigawitech JA, 1986, Plant Disease **70** : 593.
25. Hoppe PE and Kelman A, 1969, Plant Disease Reporter **53** : 66.
26. Hingorani MK, Grant UJ and Singh NJ, 1959, Phytopathology **12** : 151.
27. Anahosur KH and Patil SH, 1979, Sorghum Newsletter **22** : 121.
28. Koteswara Rao G and Lakshman Rao S, 1974, Sorghum Newsletter **17** : 27.
29. Janse JD and Ruissen MA, 1988, Phytopatology **78** : 800.
30. Thomson SV, Hilderbrand DC and Schroth MN, 1981, Phytopathology **71** : 1037.
31. Perombelon MCM and Hyman LJ, 1986, J Appl Bacteriol **60** : 61.