BACTERIAL BLIGHT DISEASE OF
PASPALUM SCROBICULATUM L. AND
PANICUM MILIACEUM L.
Paspalum scrobiculatum L. commonly known as kodra or kodo millet, is one of the most important small millets of Gujarat. The cultivation of this crop is more or less confined to the Peninsular India, including Gujarat, Mysore and parts of Madras state. According to the statistics of 1980-81 kodra occupies an area of about 74300 hectares in Gujarat State. In Gujarat this crop is grown in the districts of Bulsar, Dangs, Panchmahals, Sabarkantha and Surat.

Kodra is a minor drought-resistant food crop suited to the conditions of moderate rainfalls, ranging from about 10" - 30". It can be grown in a variety of soils but thrives best on loamy soils and sandy loams. It is grown in kharif season. Kodra is a suitable food grain for the poor classes and substitutes rice.

Very few diseases caused by fungi on this crop have been studied. They are: (i) Head smut caused by Sorosporium paspali McAlp; the fungus is systemic, transforming the entire ear into sorus. The fungus
is mainly externally seed-borne and is controlled by chemical seed treatment, (ii) Rust caused by *Puccinia substriata* Ellis and Barth and (iii) Ergot caused by *Claviceps paspali* Stev and Hall.

Systemic bacterial disease of kodra causing yellowing and premature wilting of plants has been observed by the author since 1973. In the districts of Bulsar, Dangs and Surat in South Gujarat, the bacterial blight of kodra occurs extensively causing considerable damage to the crop. The disease is easy to recognise as a bacterial one by examining the bacterial ooze from the cut diseased portions. In the present work isolation of the causal organism was carried out. The morphological, cultural and biochemical characteristics of the organism have been studied and the pathogen was named *Xanthomonas campestris* f.sp. *paspali*. The bacterial blight of kodra has been studied with reference to host symptoms, pathogenicity and etiology of the organism.

A Symptoms of the Disease

The lesion appears first as a small, water-soaked, dark green streak limited by veins on a young leaf.
The lesion gradually elongates along the veins and also somewhat widens, and eventually elongates upto 15 cms length and 0.5 - 2.0 mm in width (Plate 1). Gradually it turns yellow in colour. Minute yellow bacterial oozes can be seen on it early in the morning or under humid condition. The lesion further turns brown and coalesce with other ones, so that the affected leaf looks blighted when a large number of lesions appear on the leaf. Yellow halo sometimes appears around the streaks on susceptible varieties. The infection is chiefly vascular in nature and the pale yellow to brown streaks on the leaf blade are the result of infection of several parallel veins.

**B Cultural Studies on**

*Xanthomonas campestris* f.sp. *paspali*

**Isolation of the causal organism**

Isolation of bacterial pathogens from cereals is found to be difficult. Difficulties in the isolation of pathogenic bacteria from the diseased leaves of rice and *ragi* were experienced by Srinivasan (1958) and by Desai (1968). Similarly, in the present study, isolation of the bacterial pathogen was found
Plate 1

Bacterial blight on leaves of

*Paspalum scrobiculatum* L. incited by

*X. campestris* f.sp. *paspali*.
to be difficult. Unlike other bacterial phytopathogens, the bacterium under study could not be isolated on nutrient agar from the diseased material. On the basis of further studies isolations were tried on sucrose-peptone-agar.

For the isolation of the pathogen young lesions-leaf spots at the dark-greenish black watersoaked stage-were chosen. Selected specimens were handled as aseptically as possible. The diseased-portion was divided into two portions. One portion was left unsterilised. In the other portion, surface sterilisation was carried out by immersing the material for a minute or two in the solution of 1: 1000 mercuric chloride. It was ensured that the surface was completely wetted by the solution and that adherent air bubbles were removed. This was done by adding a wetting agent and agitating thoroughly. After sterilising, a very thorough rinse in sterile water was given. Suspensions were made from sterilised and unsterilised leaf lesion in 9 ml of sterile distilled water. The bacteria were allowed to diffuse into water for at least thirty minutes and then a loopful of suspension was streaked onto dried plates of sucrose-peptone-agar (SPA) (Hayward, 1960) containing
litre$^{-1}$, sucrose 20.0 g, peptone 5.0 g, dipotassium hydrogen phosphate 0.5 g, magnesium sulphate 0.25 g, agar 25.0 g, pH 7.2 - 7.4. The medium was sterilised under 15 psi at 121°C for 15 minutes. Plates were incubated at 28° - 30°C, room temperature. Circular, raised, mucilaginous colonies with an entire margin and a water-insoluble, yellow pigment appeared after 72 hours. A single colony was subcultured and subsequently maintained at 4°C in refrigerator on glucose yeast chalk agar (GYCA) slants containing litre$^{-1}$, glucose 20.0 g, yeast extract 10.0 g, calcium carbonate 20.0 g, agar 25.0 g. The medium was sterilised under 15 psi at 121°C for 15 minutes.

Inoculation experiments

The water suspension of a 48 hour old culture grown on glucose yeast chalk agar slants containing about $1 \times 10^9$ cells ml$^{-1}$ was atomized on the seedlings as well as the mature plants of *Paspalum scrobiculatum* L. The plants were placed in a humid chamber for 4 hours prior to inoculation and for 24 hours after inoculation. Plants were then transferred to normal condition to await symptom development. Typical disease symptoms developed in
ten days and reisolation of the same pathogen from it confirmed the pathogenicity of the isolate.

Infected specimens from Balsar, Dangs, Surat and Panchmahals districts of Gujarat State were collected and the pathogens isolated were found to be similar in all respects. This indicates that pathogen is the same at different locations.


Characterisation of the pathogen

Morphology and staining reactions

The organism is a short rod with rounded ends measuring 0.5 - 0.7 X 1.5 - 1.8 μm; gram negative, encapsulated, non-spore former, non-acid fast and motile by a single polar flagellum.
Cultural characters

Colonies on nutrient agar plates are smooth, glistening, raised, round, yellow and butyrous.
Colonies on sucrose-peptone-agar plates are circular, smooth, raised, mucilaginous with an entire margin and a water insoluble, yellow pigment. Colonies on potato dextrose agar plates are circular, with entire margin, smooth, pulvinate, butyrous and glistening yellow. Colonies on glucose yeast chalk agar plates are circular, raised, deep yellow and glistening.

Biochemical and physiological characters

a) Lipolytic activity

The medium containing 10 g peptone, 5.0 g yeast extract, 25 ml oil emulsion (25%), 50 ml alcoholic solution of nile blue sulphate (0.3%), 20.0 g agar and distilled water 1000 ml was sterilized under 15 psi at 121°C for 15 minutes and poured into the petri dishes. The organism was streaked on dried plates and incubated at 28° - 30°C, room temperature for 96 hours. Deep blue precipitated zone developed around the growth of the organism indicating lipolytic activity.
b) Hydrolysis of aesculin

Nutrient agar mixed with 1.0% aesculin was sterilized by steaming for 30 minutes on three successive days and poured in plates. In the control plate, only nutrient agar was poured. Both the plates were streaked with the organism. In the control plate there was no change in pigmentation of the organism whereas in the plate containing aesculin, there was blackening of the colony due to hydrolysis of aesculin.

c) Utilization of carbon compounds

Utilization of carbohydrates

The basal medium was prepared as suggested by Burnett et al. (1957). The medium contains:

\[0.1\% \left(\text{NH}_4\right)_2\text{PO}_4, 0.02\% \text{KCl}, 0.02\% \text{MgSO}_4 \cdot 7\text{H}_2\text{O}\] and 1.6% alcoholic solution of bromothymol blue (1 ml per 1000 ml), pH was adjusted to 7.0 by the addition of 0.1 N sodium carbonate. This basal medium was sterilized under 15 psi at 121°C for 5 minutes.

The different carbon compounds were dissolved separately in distilled water. A 10% (w/v) aqueous
solution of all the carbohydrates was prepared and fractionally sterilised for three consecutive days at 100°C for 20 minutes. This was then added aseptically to sterile basal medium to give a final concentration of 1.0% (w/v). The basal medium containing different carbohydrates was inoculated with 0.1 ml of the homogeneous suspension of the bacteria. The organism utilised glucose, galactose, mannitol, lactose, trehalose, cellobiose, maltose, xylose, fructose, mannose and sucrose with the production of acid only but it did not utilise raffinose, rhamnose, sorbitol, inositol, salicin, dulcitol, glycerol and inulin.

**Utilisation of organic acids**

The basal medium was used with the addition of 1% of different organic acids and the pH of the medium was adjusted to 7.2. The organism utilised citric, formic, acetic and ascorbic acids but failed to utilise lactic and oxalic acids.

d) **Utilisation of nitrogen compounds**

**Utilisation of amino acids**

The method as described by Ketasthane et al.
(1965) was followed. Basal medium containing
\((\text{NH}_4)_2\text{HPO}_4\) 1.0 g; KCl 0.2 g; MgSO\(_4\cdot7\text{H}_2\text{O}\) 0.2 g
and glucose (sterile solution added aseptically
after sterilization) 1.0 g litre\(^{-1}\); pH adjusted at
7.0, was employed. Basal medium was sterilized
under 15 psi at 121°C for 15 minutes. While testing
an amino acid as a sole source of nitrogen, \((\text{NH}_4)_2\text{HPO}_4\)
from the medium was emitted and the amino acid
equivalent to 0.012% nitrogen was added. Similarly,
while testing an amino acid as a sole source of
carbon glucose from the medium was emitted and amino
acid equivalent to 0.04% carbon was added.

It was found that the organism utilized
DL-alanine, L-glutamic acid, L-proline as a source
of carbon and nitrogen. All other amino acids except
DL-serine, DL-leucine and L-tyrosine were utilized as
a source of nitrogen.

Utilization of inorganic nitrogen

The basal medium was used without the addition
of \((\text{NH}_4)_2\text{HPO}_4\) and the deficiency of the phosphate
radical was made good by addition of an equivalent
amount of potassium basic and acid phosphate to the
medium. 1% each of nine different inorganic nitrogen salts vis., (NH₄)H₂PO₄, NH₄NO₃, NH₄Cl, (NH₄)₂SO₄, (NH₄)₂MPO₄, NH₄-citrate, (NH₄)₆Mo₇O₂₄·4H₂O, NaNO₃ and KNO₃ was added to the different tubes containing the basal medium. After sterilisation, 0.1% glucose was added aseptically. The tubes were inoculated with the organism and incubated for 96 hours at 28° - 30°C, room temperature. The organism utilised ammonium dihydrogen phosphate, ammonium nitrate, ammonium chloride and ammonium sulphate as a source of nitrogen but did not utilize diammonium hydrogen phosphate, ammonium citrate, ammonium molybdate, sodium nitrate and potassium nitrate as a source of nitrogen.

e) Other characters

Litmus milk reduced, gelatin liquefied, hydrogen sulphide produced, urease negative, indole not produced, starch hydrolysed, nitrates not reduced, M.R. and V.P. tests negative, catalase test positive but oxidase test negative, citrate utilised as a carbon source in Kosser's medium, Kosser's uric acid not utilised, 3% sodium chloride tolerated.
The organism was facultative aerobe. Optimum temperature for its growth was 27° - 30°C. The thermal death point of the organism was found to be 51° - 52°C.

Technical Description of the Pathogen

*X. campestris* f.sp. *paspali*

Short rods, 0.5 - 0.7 μm X 1.5 - 1.8 μm, gram negative, capsulated, no endospores, non-acid fast and motile by a polar flagellum. Colonies on potato dextrose agar plates are circular with entire margin, smooth, pulvinate, butyrous and glistening yellow. Gelatin liquified, starch hydrolysed, tween-80 hydrolysed, milk peptenised and litmus reduced, ammonia and hydrogen sulphide produced from peptone, nitrates not reduced, indole not produced, M.R. and V.P. tests negative, citrate utilised, tolerates 3.0% sodium chloride, acid without gas produced from arabinose, xylose, glucose, fructose, galactose, mannose, lactose, maltose, sucrose, cellobiose, trehalose, and mannitol but not from raffinose, rhamnose, sorbitol, inocitol, salicin, dulcitol, glycerol and inulin; citric and formic acids utilised but not oxalic and lactic acids; DL-alanine, L-glutamic acid and L-proline utilised as source of
both carbon and nitrogen, catalase positive, facultative aerobe, optimum temperature for growth 27°C - 30°C, thermal death point 51°C - 52°C.

According to the recent classification of the genus *Xanthomonas* given by Dye and Lelliot (1974), *Xanthomonas* is grouped into five groups according to physiological and biochemical characters. Host specificity is considered to be one of the major criteria. Though the organism shows all the characters similar to those of *X. campestris* group, it is host specific only to *Paspalum scrobiculatum* L and hence it is proposed to name the organism as *Xanthomonas campestris* f.sp. *paspali*.

The culture has been deposited with the National Collection of Plant Pathogenic Bacteria, Harpenden, England (NCPBP).
BACTERIAL BLIGHT DISEASE OF
PANICUM MILIACEUM L.

*Panicum miliaceum* L. commonly known as vari, is widely cultivated in many places of Gujarat. It is an annual hairy grass, 2-4 feet high with leafy erect stem and a much branched panicle and believed to be a native of Egypt and Arabia. In Gujarat, it forms the food of the poor people, particularly the hill tribes.

Only one fungal disease, downy mildew caused by *Sclerospora graminicola* (Sacc.) Schroet has been reported on this millet. The common symptoms are downy growth on the leaves and leaf shredding, the green-ear symptoms being mostly absent. Amongst the reported bacterial diseases on this millet are leaf stripe disease caused by *Pseudomonas panici-miliacei* occurring in Japan and bacterial stripe disease caused by *Xanthomonas panici* (Elliot) Savul. occurring in the U.S.A. (Elliot, 1951).

The occurrence of the bacterial disease systemic in the vascular strands on vari has been observed in Gujarat by the author since 1973. The bacterial
nature of the disease is easy to recognise, since by sectioning the infected plant parts, the bacterial ooze from the cut portions of the vascular strands is quite conspicuous under the microscope. In the districts of Panchmahals and Mehsana in north and Bulsar, Dangs and Surat in south Gujarat, the bacterial blight of vari occurs extensively causing considerable damage to the crop. In the present studies, attempts have been made to isolate the causal organism in pure culture in order to carry out the subsequent cultural and inoculation studies and to establish the etiology of the disease. The pathogen was named *Xanthomonas campestris* f.sp. *paniculata*.

**Symptoms of the Disease**

In the initial stage of the disease the stripes are from 0.5 - 1.0 mm wide and from 3-5 mm long, running parallel to the mid-rib of the lamina. These linear lesions of variable length frequently coalesce with adjacent lesions forming elongated streaks. These lesions, at first translucent and water-soaked, become light tan in colour and later necrotic. In the later stages of disease development, the entire leaf turns brown and withers away. The infection is chiefly
vascular in nature and the pale yellow streaks on the leaf blade are a result of infection of the several parallel veins (Plate 2).

B Cultural Studies on

*Xanthomonas campestris* f.sp. *panici*

Materials and methods

Methods of isolation, inoculation and testing the bacteriological characteristics were the same as those described in the case of *Xanthomonas campestris* f.sp. *paspali*.

The test isolates were isolated from the infected specimens collected from Panchmahals, Bulsar, Dangs and Surat districts. They were isolated on sucrose-peptone-agar plates and maintained on glucose yeast chalk agar slants, kept in a refrigerator at 4°C and transplanted to fresh medium once a month.

Results

Inoculation experiments

Vari plants inoculated with the test isolates showed the same symptoms as those resulted from natural
Plate 2

Bacterial blight on leaves of
*Panicum miliaceum* L. incited by
*X. graminea* f.sp. *panici*. 
infection. The same bacterium as the original isolate was successfully reisolated from those plants, confirming the pathogenicity of the organism.

Inoculation experiments carried out on
Oryza sativa L., Paspalum scrobiculatum L.,
Sorghum vulgare (L) Pers., Setaria italic L.,
Pennisetum typhoides Rich., Zea mays L.,
Eleusine coracana Gaertn and Hordeum vulgare L.
showed that these were not susceptible.

Characterisation of the pathogen

Morphology and staining reactions

The organism is short rod with rounded ends measuring 1.4 X 0.5 μm and motile with a single polar flagellum. It is gram negative, encapsulated, non-spore former, non-acid fast.

Cultural characters

Colonies on nutrient agar plates are circular, with entire margin, convex, smooth, glistening and yellow in colour. Colonies on potato-dextrose-agar
plates are circular with entire margin, smooth, pulvinate, butyrous and glistening yellow. Colonies on sucrose peptone agar are circular, raised, butyrous with entire margin and with water insoluble yellow pigments.

Biochemical characters

It liquefied gelatin, hydrolysed starch, casein. Milk was peptonized completely within 6-7 days. Gradual change in colour of litmus in milk ultimately fading indicated complete reduction. Ammonia and hydrogen sulphide were produced from peptone. Nitrates were not reduced to nitrites. Indole was not produced. M.R. and V.P. tests were negative. Citrate was utilised as a carbon source in Koser's medium. It tolerated 3% sodium chloride.

In a synthetic medium containing 1% arabinose, xylose, glucose, fructose, galactose, mannose, lactose, maltose, sucrose, cellobiose, starch and mannitol, acid was produced but no gas. Rhamnose, inulin, salicin, sorbitol, dulcitol and inositol were not fermented.
Seventeen amino acids supported growth as source of nitrogen while DL-serine, DL-nor-leucine and L-tyrosine failed to do so. DL-alanine, L-glutamic acid, L-proline and L-hydroxyproline supported growth while sixteen other amino acids failed to support growth as source of carbon.

The organism was facultative aerobe. Optimum temperature for its growth was 27° - 30°C. The thermal death point of the organism was found to be 52°C.

C Technical Description of the Pathogen

*Xanthomonas campestris* f.sp. *panic*.

Short rods with rounded ends, 1.4 X 0.5 μm, gram negative, capsulated, no endospores, non-acid fast and motile by a polar flagellum. Colonies on potato dextrose agar plates are circular with entire margin, smooth, pulvinate, butyrous and glistening yellow. Gelatin liquefied, starch hydrolysed, casein digested, milk peptonised and litmus reduced, ammonia and hydrogen sulphide produced from peptone, nitrate not reduced to nitrite, indole not produced, M.R. and V.P. tests negative, citrates utilised, tolerates 3% sodium chloride, acid without gas produced from
arabinose, xylose, glucose, fructose, mannose, lactose, galactose, maltose, sucrose, cellobiose, starch and mannitol but not from rhamnose, inulin, salicin, sorbitol, dulcitol and inocitol; catalase positive; facultative aerobe; optimum temperature for growth 27° - 30°C and thermal death point 52°C.

According to the eighth edition of the Bergey's Manual of Determinative Bacteriology, the genus Xanthomonas comprises of five species. Host specificity is considered to be one of the major criteria. Though the organism shows all the characters similar to those of X. campestris group, it is host specific only to Panicum miliaceum L. and hence it is proposed to name the organism as Xanthomonas campestris f.sp. panici.

The culture has been deposited with the National Collection of Plant Pathogenic Bacteria, Herpenden, England (NCPPB).