REACTION STUDIES OF A SELECT GROUP OF SORGHUM LINES TO LEAF STREAK DISEASE (*XANTHOMONAS HOLCICOLA*)

A Thesis
Presented to the
School of Graduate Studies
Addis Ababa University

in partial fulfillment
of the Requirements for the Degree of
Master of Science in Biology

---

by
Yeshi Andenow
May, 1984

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. Mengistu Hulluka; my advisor and Dr. Brhanu A. Gashe, my co-advisor, for their guidance throughout the course of this research.

I would like to thank Dr. Biru Abebe for his generous help in the selection of sorghum lines.

My acknowledgement also goes to the staff members of Scientific Phytopathological Laboratory at Ambo for their help in providing me their laboratories and greenhouses.

I am grateful to Dr. Desta Hamito, Ato Assefa Admasse and staff members of the Statstics Department, Science Faculty. AAU, for their help in the statistical analysis of the data.

Thanks are due to Dr. Yilma Kebede and Endashaw Bekele who were kind enough to supply me with information relevant to my work.

All those who contributed to the completion of this thesis are gratefully acknowledged and also my deepest appreciation is extended to my husband who encouraged and helped me when I was preparing this dissertation.

Finally, I am thankful to the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for financial assistance towards the costs incurred in this research.

CONTENTS

LIST OF TABLES

Table | Page

ABSTRACT

From infected sorghum leaves brought from three different place, 17 yellow coloured bacterial colonies were isolated. After having conducted hypersensitivity, morphological, physiological and biochemical tests, including pathogenicity test using sorghum line WS 1297 as a susceptible host, only one isolate was found to be Xanthomonas holcicola, the causal organism of bacterial streak disease of sorghum. Six different inoculation techniques were compared to achieve an effective method of screening together with pathogenicity test. The isolated organism induced typical symptomatic response when the rub and spray method of inoculation was used.

Using the rub and spray method, 31 sorghum lines, 7 from highland (about 2000 metres), 8 from intermediate (1600-1900 metres), 15 from lowland (800-1600 metres) and sorghum WS 1297 as a control were examined for resistance to bacterial streak disease caused by X. holcicola. High resistance to the disease was shown by sorghum lines 80 K 6056, Gambella 1107, 80 K 6088, 81 ESIP 21, 81 ESIP 25, Melkamash 79, ETS 0601, ETS 4946. Of these 75% are from lowland and 25% are from highland groups. Sorghum lines IS 9294, IS 9302, IS 9379, 81 ESIP 29, Dobbs, IS 8686, Muyra white, IS 9323 and the control WS 1297 showed susceptible reactions. Of the susceptible sorghum lines 62,5% are from intermediate, 25% from lowland and 12.5% from highland groups. The reaction of the other lines range from moderately resistant to moderately susceptible.

INTRODUCTION

In Ethiopia, sorghum grows under a wide range of altitude and rainfall conditions. It is important both in highlands and lowlands. Normally, it is cultivated in altitudes as high as 2500 metres and as low as near sea level. Often it is the only crop grown in moisture stressed areas and in the lowlands covering a total of over a million hectares in the country(ESIP, 1976; Brhane, 1977; ESIP, 1978; Brhane and Yilma, 1979; Brhane, 1981a).

Sorghum is used as a grain crop for human consumption, for brewing local beers and other drinks. The leaves and stalks are used as a forage for animals, as fuel, for fences, for constructing n local huts, baskets, mats etc, (Tarr, 1962; Tadelle and Brhane 1975; Brhane, 1979).

Yearly production of sorghum is estimated to be one million tons(ESIP, 1978; Brhane and Belainesh, 1981).

Sorghum is second to teff (Eragrostis teff) for making injera (the traditional unleavened bread) both in quality and quantity (Brhane, 1977; Brhane and Yilma, 1979; Brhane and Belainesh, 1981). Sorghum ranks fourth in importance following wheat, rice and maize in the world(Brhane and Yilma, 1979). It is estimated that nearly 50% of the arable land in the world is cultivated with sorghum (Rangaswami and Rajagopalan, 1973). They also have reported that sorghum consistently outyields other crops grown in these areas.

Though tolerant to a wide range of climatic and soil conditions, sorghum is known to be attacked by both fungal and bacterial diseases (Tarr,1962; Rao and House, 1972). According to Rangaswami and Rajagopalan(1973), seven bacterial diseases are reported that attack sorghum; Pseudomonas andropogoni, Pseudomonas alboprecipitans, Pseudomonas syringae, Xanthomonas holcicola, Xanthomonas albilineans, Xanthomonas rubrilineans and Xanthomonas vasculorum. Out of these, bacterial stripe caused by Pseudomonas andropogoni, bacterial spot caused by Pseudomonas syringae and bacterial streak caused by Xanthomonas holcicola are best known and wide spread foliar bacterial diseases(Rao and House, 1972; Mengistu and Brhane, 1978).

The present study deals with bacterial streak disease(X.holcicola) the most commonly occuring bacterial disease in Ethiopia(Mengistu and Brhane, 1978).

Little has been reported on bacterial streak disease regarding extent of damage, dissemination mechanism, establishment of artificial infection and varietal resistance(Tarr, 1962; Rao and House, 1972; ICRISAT, 1978). In Ethiopia too, little information is available on varietal resistance and other aspects of the disease. Though it is widely distributed in Ethiopia and other parts of the world, it has not been considered as a major disease of sorghum. However, Frohlich and Rodewald(1969), FAO(1972) and Nikolaeva(1977) have reported the incidence of the disease is increasing and also that

some popular cultivars such as Wonder Kaffir, European Milo, Farr's Dwarf were found susceptible(ICRISAT,1978).

The present investigation, therfore, had the following objectives:

1) To study the diagnostic characterstic of the pathogen(X.holcicola)
2) To evaluate sorghum cultivars for their reaction to bacterial streak disease caused by X.holcicola.

LITERATURE REVIEW

General Characterstics of Xanthomonas holcicola

Classification

The term Xanthomonas is derived from two Greek words:xanthus (yellow) and monas(unit or monad); hence it is a yellow monad. Dowson(1939) in Starr and Stephens (1964) emphasized that the yellow colour is one of the most significant characterstics. This emphasis upon the production of the yellow colour, and its use as a primary determinative feature for these bacterial group has also promoted a general study of the yellow colouring matter of Xanthomonas. The yellow substance was found to be carotenoid in nature and non-water soluble(Walter and Starr,1948; Buchanan and Buchanan, 1952; Breed et al;1957).

The genus Xanthomonas is very closely related to the genus Pseudomonas. In fact, Xanthomonas initially was formed by splitting the genus Pseudomonas into two, so as to include a considerable group of phytopathogenic bacteria in the genus Xanthomonas. However, due to some variability in characterstics, some purely saprophytic species are included in this genus(Buchanan and Buchanan, 1952). As reported by Vidaver(1976) nomenclature of phytopathogenic bacteria is unsettled, particularly for Pseudomonas and Xanthomonas. According to Breed et al.,(1957) and Rangaswami and Rajagopalan,

(1973), the genus Xanthomonas is grouped in the family Pseudomonadaceae and in the order Pseudomonadales.

Xanthomonas speciation has been based on proved or assumed host specificity. Neverthless, as summarized by Gibbs and Skinner (1966) this concept is widely held to be unreliable because of the following reasons.

a) Xanthomonads are relatively homogeneous and there is no consistent physiological basis for species differenciation. It is usually impossible to identify a species when it is separated from the host of origin(Walter and Starr, 1948). Species from diverse hosts are difficult to be distinguished in the laboratory.

b) It appears that virulence and pathogenicity may not be st stable characters, although the evidence on this point is conflicting.

c) Species of Xanthomonas have been shown by numerical taxonomy to form a group distinct at the generic level from Pseudomonas, but too closely clustered to justify the numerous species found in the literature.

In the proposal of Young et al.,(1978) for nomenclature and classification of plant pathogenic bacteria, X. holcicola is included as a pathovar of X. campestris for it is generally indistinguishable from X.campestris except by its host range.

It is most likely that some of the species of the genus Xanthomonas will be united or recogized as varieties having special adap-

the maximum 36 -37^{o}C, and the thermal death point is 51^{o}C.

Symptoms, Epidemiology and host susceptibility

The appearance of bacterial streak(X. holcicola) in sorghum fields is related to the stage of plant development(FAO, 1972). Similarly, the colour of the streaks varies according to the variety of sorghum(FAO, 1972). The initial symptoms of infection appear as narrow water-soaked streaks 2 -3 mm wide by 2-15cm long surrounded by red, purple or brown colour appearing as early as the second leaf stage of the seedlings. As the plant grows old. the lesions soon turn red, become opaque, and at intervals may broaden into somewhat irregularly oval spots with tan centres and narrow red margins. In severe attacks these coalesce to form long irregular streaks and blotches extending across all ormuch of the leaf blade, with more or less dead tissue bordered by narrow dark margins between the reddish brown streaks. Bacterial exudate is produced as light yellow droplets which dry to thin white or cream coloured scales(Dagnachew, 1967; Ganga et al., 1971; FAO, 1972; Rao and House, 1972; Weber, 1973; ARS, 1975; Williams et al., 1978).

Bacterial streak (X.holcicola) was first discovered on sorghum leaves in Texas and was subsequently found on many sorghum varieties in Kansas, Oklama and Montana (Tarr, 1962). Although apparently reported from the United States of America, Australia, South Africa

and Argentina(Tarr,1962;FAO, 1972), the disease is widely distributed where sorghum are cultivated(Johnson, 1953; Ganga et al., 1971) especially in USSR, India and Phillipines(ICRISAT,1978). The disease has recently been reported to prevail in Argentina (Frezzi and Teyssandier,1978), in Bangladesh(Mian and Ahmed,1978), in Mexico (Vallejo, 1978) and in New Zealand (Watson, 1971). In ESIP (1978) it is reported that X. holcicola occurs at Kobo, Nazareth, Arsi Negelle, Alamaya and Dakata which were ESIP's research stations.

Bacterial diseases of plants occur in almost every place that is reasonably moist and warm (Wood, 1953). X. holcicola is found in climates with lower temperatures but more favoured between 75 to 85°F (Leukel and Martin, 1953). However, it is limited in host range(Ganga et al.,1971; Rao and House, 1972; Weber,1973). Leukel and Martin(1953), Tarr (1962), Ganga et al.,(1971), Rao and House (1972), ICRISAT(1978) and FAO (1980) have recorded X.holcicola on sorghum species which include grain sorghum, sorgho(or sweet sorghum), broomcorn, sudangrass and johnsongrass. This disease did not appear to attack broomcorn in the United States, however, broomcorn is reported to be susceptible in Argentina (Tarr,1962;ICRISAT, 1978). Vel'sovskii (1978) also reported that X. holcicola is known to be a pathogen on millet (Fanicum miliaceum).

Bacteria may be carried over from one season to another on seeds, on infected plant materials, in or on the soil, and occasionally on plants that overwinter (Leukel and Martin, 1953;Cafati and Saettler, 1980). Chumaevakaya and Nikolaeva (1975) made a study on the three bacterial diseases of sorghum that include X. holcicola. On buruing infected leaves or placing them on the soil surfaces, infection was found on the surface and at 5 cm depth after 5 months. they also found that infection was higher in plots where sorghum was grown in two successive years. Evidently, bacteria persist between crop seasons on infected sorghum trash and plants remaining after harvesting (Tarr, 1962).

Streaks may develop on sorghum crops any time between the seedling stage and their maturity (ARS, 1975). The appearance of streak on the leaf of sorghum seedlings suggests that transmission of the disease is possibly, from seeds or soil. It was not possible to isolate X. holcicola from seeds (Tarr, 1962). However, Avezdzhanova and Sotochenko (1978) isolated 35 bacterial isolates from seeds and leaves of 150 specimens of Penicum miliaceum.

Bacteria, in general, may also be spread from leaf to leaf or from plant to plant by wind, rain splash or insects (Leukel and Martin, 1953). Though little has been studied on X. holcicola, it most likely spreads by rain and wind (Tarr, 1962).

Physiological and Biochemical Tests

Tests conducted in the laboratory were: i) liquefaction of gelatin ii) nitrification iii) fermentation of starch and other carbohydrates iv) production of acid, gas, ammonia, hydrogen sulphide and indol v) coagulation of milk vi) production of catalase and oxidase.

Pathogenicity Test

To achieve an effective methid of screening of sorghum lines, six methods of inoculations employed in phytopathological experiments were compared (Table 6) simultaneously with the test of pathogenicity. The susceptible host plant used for this test was WS1297 obtained from Plant Genetic Resource Centre in Ethiopia. For inoculation purposes, 48-hour-old cultures were used to make bacterial suspension of approximately 10^9 cells/ml (Howell and Frederiksen, 1983). The average greenhouse day and night temperatures were approximately 81 and 38^{o}F respectively (Table 1) and the humidity level was maintained almost near saturation point at the time of inoculation. After inoculation the seedlings were kept in a moist chamber, under plastic bags, till symptoms of infection appeared in at least one or more of the methods.

Bacteria were reisolated from the artificially infected leaves and were characterized in laboratory tests for similarity with those isolated from the naturally- infected leaves.

Screening of Sorghum Lines in the Greenhouse

A total of 31 sorghum lines, 7 highland, 8 intermediate, 15 lowland lines and one selection WS 1297, as a susceptible control were obtained from the Ethiopian Sorghum Improvement Project (ESIP). Five to 10 seeds were planted in a pot using sterilized garden soil taken from the compound of Addis Ababa University, Science Faculty and were placed in a greenhouse. Three seedlings in a pot were kept under high humidity condition for a period of 24 hours so as to predispose the seedlings for infection (Tuite, 1969; Rangaswami and Rajagopalan, 1973; ICRISAT, 1978).

Three weeks after planting, artificial inoculation of sorghum lines was performed in the evenings (Watson, 1971; Rao and Davadath, 1977) using the rub and spray method which was found to be the most effective. The leaves were rubbed with sterilized fine sand (mesh size 0.365mm) and were sprayed with aqueous bacterial suspemsion of approximately 10^9 cells/ml using a paint sprayer. The principle involved in this technique was the forcing of inoculum through the wounds formed by the fine sand. They were replicated three times and the controls were inoculated with sterile distilled water by using the same method. After inoculation the infected plants and the controls were kept in moist chamber under plastic bags and were placed under greenhouse benches for 24 hours to maintain high humidity (Haygood and Strider, 1979). Then they were kept on the greenhouse

benches where the average day and night temperatures are 81 and 38°F respectively.

Disease reactions were recorded 7 days after inoculation. A rating scale of 0 -5 , 0 being highly resistant and 5 being highly susceptible was used to evaluate disease reaction as indicated below.

Disease Score	Infection Type	Reaction
0	No sign of streaks	Resistant
1	Small dots of streaks	Resistant
2	Larger dots of streaks	Resistant
3	Lesion length ¼ -½ of leaf	Moderately resistant
4	Lesion length greater than ½ of leaf	Moderately susceptible
5	Streak covers the whole size of the leaf	Susceptible

Lesion width and length were also measured and the disease scores were converted to disease index by the procedure adopted from Kaufmann et al., (1973).

Disease Index = Disease Score X Length of lesion X Width of lesion

Using the above scoring method, seedlings in each pot were scored for disease reaction. The scoring was determined by dividing the score sum of each reading by the number of replicates. The screen-

ing experiment was repeated three times. Finally the mean disease index was used to evaluate disease reaction.

Statistical analysis of the data was performed and the Duncan Multiple Range test was used to compare individual means.

EXPERIMENTAL RESULTS

Isolation of the Pathogen

Seventeen yellow bacterial isolates (four from Alamaya, seven from Nazareth and six from Ambo) were isolated from infected leaves grown on media, Potatto Dextrose Agar (Table 2). Of these only seven bacterial isolates, Al_3, Al_4 (from Alamaya), N_1 and N_3 (from Nazareth) and Am_3, Am_4 and Am_6 (from Ambo) were showing positive response to hypersensitive reaction test on broad bean leaves (Table 2).

Characterization of the Pathogen

In morphological, physiological and biochemical tests, all seven bacterial isolates possessed the majority of the diagnostic features of the genus Xanthomonas. Cells were Gram negative, straight rods, motile and produced a yellow pigment (on PDA). All were oxidase negative, catalase positive and produced ammonia. All have oxidative metabolism of carbohydrates such as arabinose, cellobiose, fructose, galactose, mannose, saccharose, trehalose and aesculin. The isolates showed 70% similarity with each other (Table 3). Only two bacterial isolates were identified as possible Xanthomonas holcicola when compared with the simplified scheme prepared for the identification of Xanthomonas holcicola (Table 4).

Table 1. Maximum and Minimum temperatures in ^{o}F in the greenhouse during the test periods after inoculation.

	Expt. 1				Expt. 2				Expt. 3			
	Day		Night		Day		Night		Day		Night	
No	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
1	96	64	62	38	98	64	62	38	82	68	62	38
2	98	72	60	38	98	66	64	38	82	66	64	38
3	98	72	62	38	98	72	64	38	82	68	66	38
4	98	66	64	38	98	72	64	38	84	68	64	38
5	96	72	64	36	98	72	62	38	82	68	64	38
6	98	72	62	36	98	70	64	38	84	68	64	38
7	98	72	62	38	98	72	64	38	84	68	64	38
Mean	97.4	70	62.2	37.4	98	69.7	63.4	38	82.8	67.7	64	38

Table 2. Hypersensitive reaction for 17 yellow coloured bacterial isolates from Alamaya (Al), Nazareth (N) and Ambo (Am).

Isolates	Al_1	Al_2	Al_3	Al_4	N_1	N_2	N_3	N_4	N_5	N_6	N_7	Am_1	Am_2	Am_3	Am_4	Am_5	Am_6
Reaction	-	-	+	+	+	-	+	-	-	-	-	-	-	+	+	-	+

+ = positive

- = negative

Table 3. Morphological, physiological and biochemical characterstics of bacterial isolates which showed positive reaction with hypersensitivity test on broad bean-leaves.

Kinds of Tests	Isolates						
	Al_3	Al_4	N_1	N_3	Am_3	Am_4	Am_6
Gram reaction	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+
Gelatin liquefaction	+	$+^S$	(+)	(+)	+	+	(+)
Nitrate reduction	-	-	-	-	-	-	-
Indol production	-	(+)	+	(+)	-	-	-
H_2S production	+	+	(+)	(+)	+	(+)	+
Ammonia production	+	+	+	+	+	+	+
Catalase production	+	+	+	+	+	+	+
Oxidase production	-	-	-	-	-	-	-
Starch hydrolysis	+	+	-	-	+	(+)	+
Milk coagulation	-	-	+	-	-	-	-
Acid but no gas with							
Dextrose	+	+	+	(d)	+	-	+
Arabinose	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+
Saccharose	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+
Aesculin	+	+	+	+	+	+	+

Note: $+^S$ strongly positive; (+) slight positive (not defined zone in the case of starch hydrolysis and gelatin liquefaction). (d) doubtful (becomes positive but delayed); + positive, - negative.

Table 4. Simplified scheme for the identification of *Xanthomonas holcicola* (summarized from Breed *et al.*, 1957, Tarr, 1962; Weber, 1975; Gibbs and Skinner, 1966).

Kinds of Tests	Reaction
Gram reaction	-
Motility	+
Gelatin liquefaction	+
Nitrate reduction	(d)
Indol production	-
H_2S production	+
Ammonia production	+
Catalase production	+
Oxidase production	-
Starch hydrolysis	(p)
Milk coagulation	-
Acid but no gas with	
Dextrose	-
Arabinose	+
Cellobiose	+
Fructose	+
Galactose	+
Mannose	+
Saccharose	+
Trehalose	+
Aesculin	+

Note: - = negative
+ = positive
(d) = doubtful
(p) = partial(zone of clearing not well defined

Pathogenicity Test

Six inoculation techniques were tested to effect a reliable infection (Table 5). However, the rub and spray inoculation technique was found to be effective in creating an avenue for the multiplication of the bacteria. When the injured leaves were sprayed with the aqueous bacterial suspensions, characterstic symtoms were produced on those inoculated with culture of Am_4. No symptom was found on the controls injured but sprayed with sterile distilled water. Small water soaked lesions appeared 48 hours after inoculation and these were enlarged to narrow water soaked areas as the disease prgressed. After taking out the plants from the moist chamber, the colour of the water soaked area gradually changed into a brownish lesion and extended almost to one -fourth of the blade.

Few tests were made for the reisolated bacteria and they were found to be the same with the original isolate inoculated but only the zone of clearing seems more defined (Table 6).

Screening for Resistance

A total of 31 sorghum lines, 7 highland, 8 intermediate and 15 lowland lines with selection WS 1297 as a control were used in artificial inoculation experiments.

Table 5. Extent of infection using different techniques of inoculation compared during pathogenicity test.

Types of Techniques	Extent of Infection
Hypodermic Injection*	Infection localized
Pin Prick Inoculation*	Infection localized
Rub and Spray Inoculation	Streak formed
Spray Inoculation	No infection
Fine Brush Inoculation	No infection
Leaf Clipping Inoculation*	Infection localized

* Localized, non-specific necrosis and poor development of symptoms

Table 6. Morphological, physiological and biochemical characterstics of the reisolated Xanthomonas holcicola from the artificially infected sorghum leaves.

Kinds of Tests	Reaction
Gram reaction	-
Motility	-
Starch hydrolysis°	+
Ammonia production	+
Acid but no gas with	
Dextrose	-
Sucrose	+

Note: - = negative

+ = positive

° The zone of clearing seems more defined

Seven days after inoculation disease scores were taken (Table 7) and the length and width of the lesions were measured (Table 8). Average disease index was calculated for each experiment (Table 9) and the mean disease index was used for reaction t test (Appendix 1)

The time for symptom development did not vary much among the lines. Size of the lesions appearing after 7 days of inoculation was highly variable and significant differences were occuring among most of the sorghum selections used in the experiment.

Low disease index reflecting highly resistant reaction was observed in 8 sorghum lines, 80 K 6056, Gambella, 1107, 81 ESIP 21, 80 K 6088, 81 ESIP 25, Melkamash 79, ETS 0601 and ETS 4946. Nine lines, IS 9294, IS 9302, IS 9379, IS 9323, 81 ESIP 29 , Dobbs, IS 8686, Muyra white and the control WS 1297 have showed high disease indices, which reflect highly susceptible reaction. The reaction of the other lines range from moderately resistant to moderately susceptible (Appendix 1).

The Duncan Multiple Range test (Appendix 1) showed that the mean indices of 80 K 6056, Gambella 1107, 80 K 6088, 81 ESIP 21, 81 ESIP 25, Melkamash 79, ETS 0601 and ETS 4946 (having low indices)are significantly different from those which are mentioned to be susceptible (having high disease indices). Muyra white was a line having the highest index and is rated to be the most suscep-

Table 7. Average disease score of bacterial streak on sorghum lines in three experiments.

Entry No	Line	AVERAGE SCORE Expt. 1	Expt.2	Expt.3
1	Alamaya 70	1.33	1.67	1.00
2	ETS 2752	1.00	1.00	1.00
3	ETS 3235	0.67	1.00	1.00
4	ETS 4946	0.67	1.00	0.89
5	ETS 0601	0.67	1.33	1.00
6	Muyra white	2.67	4.00	4.00
7	ETS 2624	1.67	1.67	1.00
8	IS 8686	3.33	3.00	2.56
9	IS 9293	1.67	1.67	1.00
10	IS 9294	2.00	2.33	1.44
11	IS 9302	2.00	2.33	1.44
12	IS 9323	3.00	2.33	2.33
13	IS 9379	2.00	3.00	2.00
14	IS 9466	1.67	1.33	1.56
15	IS 9521	2.00	1.67	1.22
16	Dobbs	2.00	2.33	2.33
17	Gambella 1107	0.00	0.00	0.00
18	Melkamash 79	1.00	1.00	1.00
19	N - 13	1.67	1.33	1.11
20	80 K 5044	1.00	1.00	1.11
21	80 K 6056	0.00	0.00	0.00
22	80 K 6085	1.00	0.67	0.55
23	80 K 6088	0.33	0.33	0.00
24	81 ESIP 21	0.33	0.00	0.33
25	81 ESIP 25	0.39	0.67	1.00
26	81 ESIP 29	1.00	2.33	2.22
27	81 ESIP 30	1.33	1.33	1.11
28	81 ESIP 31	1.33	1.00	1.22
29	76 T_1 # 23	1.33	1.67	1.56
30	RS_1 X VGC	1.67	1.67	2.22
31	WS 1297	2.56	3.00	2.33

Note: Entry No 1-7 are highland lines, 8-15 intermediate & 16-30 lowland's.

Table 8. Average length(L) and(W) measurements of lesions formed on sorghum lines by Xanthomonas holcicola (cm)

		Average length & width measurement		
Entry No	Line	Expt.1	Expt.2	Expt.3
1	Alamaya 70	0.85	0.97	0.91
2	ETS 2752	1.19	0.99	1.26
3	ETS 3235	1.32	1.08	1.00
4	ETS 4946	0.86	0.67	0.89
5	ETS 0601	0.63	0.79	0.55
6	Muyra white	6.66	8.93	7.42
7	ETS 2624	1.30	1.41	0.71
8	IS 8686	3.70	3.85	3.50
9	IS 9293	2.10	2.38	2.35
10	IS 9294	2.98	3.01	2.11
11	IS 9302	3.25	3.89	2.14
12	IS 9323	3.90	2.01	2.33
13	IS 9379	2.24	3.57	2.63
14	IS 9466	1.42	0.74	1.92
15	IS 9521	1.48	1.13	1.29
16	Dobbs	2.28	4.21	2.19
17	Gambella 1107	0.00	0.00	0.00
18	Melkamash 79	0.65	0.72	0.41
19	N - 13	1.24	1.05	1.21
20	80 K 5044	0.79	0.64	1.52
21	80 K 6056	0.00	0.00	0.00
22	80 K 6085	1.80	1.00	1.09
23	80 K 6088	0.07	0.08	0.00
24	81 ESIP 21	0.10	0.00	0.33
25	81 ESIP 25	0.05	0.03	0.51
26	81 ESIP 29	2.85	4.15	2.22
27	81 ESIP 30	1.17	1.18	1.00
28	81 ESIP 31	1.95	1.06	1.45
29	76 T_1 # 23	0.94	1.93	0.98
30	RS_1 X VGC	1.29	1.29	2.89
31	WS 1297	2.33	3.35	3.62

Table 9. Disease indices of sorghum lines in three experiments.

Entry No	Lines	Disease Index			
		Expt. 1	Expt. 2	Expt. 3	Mean
1	Alamaya 70	11.30	16.20	12.10	13.10
2	ETS 2752	11.90	9.90	12.60	11.47
3	ETS 3235	8.84	10.80	10.00	9.88
4	ETS 4946	5.76	6.70	7.92	6.79^{r}
5	ETS 0601	4.22	10.50	5.50	6.74^{r}
6	Muyra white	177.82	357.20	296.80	277.27^{s}
7	ETS 2624	21.71	23.55	7.10	17.45
8	IS 8686	123.21	115.50	89.60	109.44^{s}
9	IS 9293	35.07	39.75	23.50	32.77
10	IS 9294	59.60	70.13	30.38	53.37^{s}
11	IS 9302	65.00	90.64	30.82	62.15^{s}
12	IS 9323	117.00	46.83	80.39	81.41^{s}
13	IS 9379	44.80	107.10	52.60	68.17^{s}
14	IS 9466	23.71	9.84	29.95	21.17
15	IS 9521	29.60	18.08	15.74	21.14
16	Dobbs	45.60	98.09	74.33	72.67^{s}
17	Gambella 1107	0.00	0.00	0.00	0.00^{r}
18	Melkamash 79	6.50	7.20	4.10	5.93^{r}
19	N -13	20.71	13.97	13.43	16.64
20	80 K 5044	7.90	6.40	16.87	10.39
21	80 K 6056	0.00	0.00	0.00	0.00^{r}
22	80 K 6085	18.00	6.70	5.45	10.05
23	80 K 6088	0.23	0.26	0.00	0.16^{r}
24	81 ESIP 21	0.33	0.26	0.00	0.20^{r}
25	81 ESIP 25	0.17	0.20	5.10	1.82^{r}
26	81 ESIP 29	28.50	96.70	80.36	68.52^{s}
27	81 ESIP 30	15.56	15.69	11.10	14.12
28	81 ESIP 31	25.94	10.60	17.69	18.08
29	76 T [illegible] #23	12.50	32.23	14.89	19.87
30	RS [illegible] X VGC	21.54	21.54	64.16	35.75
31	WS 1297	59.65	100.50	84.34	81.49^{s}

Note: means followed by a letter r are resistant, & by a letter s susceptible.

Table 10. Disease index and reaction of sorghum lines recommended for highland zones when infected with bacterial streak disease (Xanthomonas holcicola).

Lines	Mean Disease Index	Reaction
Alamaya 70	13.10	Moderately resistant
ETS 2752	11. 47	Moderately resistant
ETS 3235	9.88	Moderately resistant
ETS 4946	6.79	Resistant
ETS 0601	6.74	Resistant
Muyra white	277. 27	Resistant
ETS 2624	17. 45	Moderately resistant

Table 11. Disease index and reaction of sorghum lines recommended for intermediate zones when infected with bacterial streak disease (Xanthomonas holcicola).

Line	Mean Disease Index	Reaction
IS 8686	109.44	Susceptible
IS 9323	81.41	Susceptible
IS 9379	68.17	Susceptible
IS 9302	62.15	Susceptible
IS 9294	53.37	Susceptible
IS 9293	32.77	Moderately susceptible
IS 9466	21.17	Moderately susceptible
IS 9521	21.14	Moderately susceptible

Table 12. Disease index and reaction of sorghum lines recommended for lowland zones when infected with bacterial streak disease (Xanthomonas holcicola)

Line	Mean Disease Index	Reaction
Dobbs	72.67	Susceptible
81 ESIP 29	68.52	Susceptible
RS_1XVGC	35.75	Moderately susceptible
76 T_1 #23	19.87	Moderately susceptible
81 ESIP 31	18.08	Moderately resistant
N - 13	16.04	Moderately resistant
81 ESIP 30	14.12	Moderately resistant
80 K 5044	10.39	Moderately resistant
80 K 6085	10.05	Moderately resistant
Melkamash 79	5.93	Resistant
81 ESIP 25	1.82	Resistant
81 ESIP 21	0.20	Resistant
80 K 6088	0.16	Resistant
Gambella 1107	0.00	Resistant
80 K 6056	0.00	Resistant

tible while Gambella 1107 and 80 K 6056 were found to be totally immune for the bacterial streak disease.

Among the resistant lines 75% are form the lowland group and 25% from the highland groups. None of the intermediate group were showing resistant reaction. Among the lines showing susceptible reactions, 62.5% are from intermediate groups, 25% lowland and 12.5% from highland lines (Table 10, Table 11, Table 12).

DISCUSSION

The inability to isolate Xanthomonas holcicola from most specimens is supported by the work of Watson (1971). In his work, bacteria were seen in preparations of damaged leaf tissue examined microscopically, but occured infrequently and in low numbers. He detected the organisms mixed with non-pathogenic bacteria.

In this experiment, characterstic symptoms appeared when the leaves were injured and sprayed with the bacterial suspension. Likewise, Watson (1971) found streaks appearing on the emerging leaves of inoculated plants on both wounded and unwounded areas, when the seedlings were sprayed with sufficient force.

Disease resistance in plant cultivars, in general, is indicated when the host excludes the entrance, curtails the establishment, restricts the reproduction, and/or prevents the spread of pathogens (Derege, 1975). Although some sorghums acquire resistance as they age, little is known of the nature of mechanism of resistance. Whether known sources of resistance restrict penetration and /or colonization and subsequent growth by the pathogen has not been established (Ying and Frederiksen, 1980). Information on the genetics of resistance is very useful in the choice of appropriate breeding and screening methods (Raj and Patel,1978). Recent screening tests at ICRISAT have shown that sorghum has sources of resistance for

downy mildew, leaf blight and rust (ICRISAT, 1982). Limited work suggests that sorghum cultivars differ in reaction to streak (ICRISAT, 1978; Sundaram, 1978). There is also little information on efforts to breed for resistance against Xanthomonas holicicola (Rao and House, 1972). However, several workers have presented several sources of resistance for a whole range of diseases in sorghum (ICRISAT, 1982). One important plant characterstics that has been used effectively in breeding and screening programmes is tan colour (Rao et al., 1978; ICRISAT, 1982). Tan plant types have generally higher degrees of resistance to foliar diseases.

Some of the sorghum lines under screening experiment in this study are collections from Ethiopia, some are introductions from ICRISAT and Near East and a few are hybrids.

In considering the most resistant lines in this, Gambella 1107 is a tan coloured plant (Yilma, personal communication) and it has high level of disease resistance, good grain and excellent agronomic backgrounds (Brhane, 1981 b). It possibly has gene for resistance against most diseases.

Gambella is typical of the lowlands (500 metres) with high rainfall (1000 mm/annum), high humidity (mostly greater than 50% relative humidity) and high temperature (greater than 86^{o}F all the year round). Such an environment is the most ideal for disease development. Therfore, lines which are susceptible for most diseases

cannot survive under this condition (Yilma, personal communication).

80 K 6056 Which also another most resistant line in this study is a cross between NES-830 x 705 X NES-8847 ESIP, 1977). Both the parents are introduced from Near East as best parents for the low-land programme (Brhane, 1981). The male parent NES -8847 is a clean-leaved plant probably showing resistance for most foliar diseases. Hence, 80 K 6056 had possibly inherited its resistance to streak disease from its male parent (Yilma, personal communication).

80 K 6080 and 81 ESIP 21 which are the next resistant lines are crosses of 76 T_4 #432 X NES- 635 and 76 T_4 # 432-1 / 269 X 76 T_4 # 478 (ESIP, 1977). In these two cases 76 T_4 #432 seems to have complimentary effect in giving resistance to the lines (Yilma, personal communication).

Again 76 T_4 # 441 X NES -8835 are found to be the parents of 81 ESIP 25(ESIP, 1977), one of the resistant line in this study. The male parent (NES -8835) is a clean-leaved cultivar which is resistant to most foliar diseases. Hence the gene for resistance in 81 ESIP 25 is possibly inherited from the male parent (Yilma, personal communication).

Melkamash 79 is selected from ICRISAT Nursery and is known to be resistant against most diseases while line ETS 0601 is released as a better line of intermediate and highland zones, ETS 4946 has been recommended for highland regions (Yilma, personal communication).

On the other hand, Muyra white, IS 8686, IS 9323, Dobbs, 81 ESIP 29, IS 9379, IS 9302, and IS 9294 have shown a susceptible r reaction to bacterial streak disease. It is not surprising that Muyra white is found to show susceptible reaction, because it has never been selected for any disease resistance. It is simply a local Hararge selection. IS 8686, another most susceptible line has been introduced from ICRISAT for it was tolerant to a parasitic weed known as "striga", hence it may not be resistant to diseases (Yilma, personal communication). It has been reported that certain Kafirs are resistant to bacterial streak disease and some others to be moderately resistant (ICRISAT, 1978;Sundaram, 1978). However, all Kafir types, IS 9323, IS 9379, IS 9202, Is 9294, and IS 8686 have shown a susceptible reaction to our condition. All these lines are selected for intermediate altitudinal regions. Intermediate altitude (1600 -1900metres) with high rainfall (greater than 1000mm) is known to be favourable for disease development (Yilma, personal communication).

Bacterial diseases of plants occur in almost every place that is reasonably moist and warm (Wood, 1953). Xanthomonas holcicola is found in climates with lower temperatures but more favoured between 75 and 85^{o}F (Leukel and Martin, 1953). The higher the rainfall with such warm condition, the more severe the disease problems particularly leaf diseases (Yilma, personal communication). In addi-

tion, IS 9323 has been severely attacked by blight and rust. IS 9294 is attacked by severe rust and moderate blight and IS 9302 is attacked by slight rust and moderate blight. Therefore, there is a probability that streak may be found;but IS 9302 is released as a better line for production for intermediate and zones and there was less streak by the time it was released (Yilma, personal communication).

Nothing is known about the disease resistance of Dobbs. It is introduced from East Africa for its characterstics not to be attacked by birds due to its bitter seeds. Besides, it is a good yielder (Yilma, personal communication).

Sorghum cultivars collected by Ethiopian Sorghum Improvement Project are not selected on the basis of disease resistance per se. But good grain qualities and some other agronomic backgrounds are also considered. Besides, selection against disease resistance is on the basis of natural infestation not on artificial inoculation (Yilma, personal communication).

Younger materials are often more susceptible than older ones (Tuite, 1969; Zaumeyer and Meiners, 1975; Hazwl et al., 1980). Younger leaves of sorghum are also reported relatively to be more susceptible to bacterial streak disease (Rao and Davadath, 1977). As mentioned above, the intermediate groups which are all Kafir types are found to be susceptible which, in fact, is contrary to the reports of other researchers. It is most likely that greenhouse

results may not be similar with that of the field. For instance, many dry bean cultivars were found to be highly resistant to halo blight infection (Pseudomonas phaseolicola)in the field but showed small necrotic spots in the greenhouse (Zaumeyer and Meiners, 1975). Many conditions such as microclimate, age, temperature, humidity, light, mineral nutrition, wounding, inoculum concentration, etc can alter the susceptibility of a plant to infection (Whitney, 1976). Therfore, it is always necessary to study these materials in the field under natural epidemic condition. As a follow-up to this experiment it may be necessary to undertake a study to identify the genetic factor responsible for disease resistance in sorghum.

REFERENCES

Agricultural Research Service. (1975). Sorghum disease in the United States and their control. Agricultural-Handbook No. 467. Department of Agriculture. U.S. Washington D.C. pp. 1 - 13.

Al - Issa, A.N. and Sigee, D.C. (1982). The hypersensitive reaction in tobacco leaftissue infilterated with Pseudomonas pisi. 3. Changes in the synthesis of DNA in bacteria and mesophyll cells. J. Phytopath.,105: 198 - 213.

Avezdzhanova, G.P. and Sotochenko, L.V. (1978). Results of evaluating the resistance of millet specimens to bacterial diseases. Selektsiya i semenovodstova (1978). No. 5, 43 - 44. In: Rev. Plant Path. (1980).,59(9): 409 (abstr.)

Breed, R.S., Murray, E.G.D. and Smith, N.R. (eds). (1957). Bergey's Manual of Determinative Bacteriology. 7th ed. The Williams Company. Baltimore. pp. 152 - 158.

Brhane Gebrekidan. (1977). Sorghum legume intercropping in the Chercher highlands of Ethiopia. J. Assoc. Adv. Agri. Sc. Afri., 4(2): 39 - 46.

Brhane Geberkidan. (1979). Sorghum genetic resources in Africa. J. Agri. Sci., 1(2): 108 - 115.

Brhane Gebrekidan. (1981 a). Salient features of the sorghum breeding strategies used in Ethiopia. Eth. J. Agri. Sci.,3(2): 97 -105.

Brhane Gebrekidan. (1981). Utilization of germplasm in sorghum improvement. Proceedings of the international symposium on sorghum, 2 - 7 Nov 81. ICRISAT 1982. Patancheru, A.P., India: ICRISAT. pp. 335 - 345.

Brhane Gebrekidan and Belainesh Gebrehiwot. (1981). Sorghum injera preparations and quality parameters. Proceedings of the international symposium on sorghum grain quality, 28 -31 October 1981. ICRISAT 1982. Patancheru, A.P., India: ICRISAT. pp. 55 -65.

Brhane Gebrekidan and Yilma kebede. (1979). The traditional culture and yield potential of the Ethiopian high lisine sorghum. Eth. J. Agri. Sci., 1(1): 29 -40.

Buchanan, R.E. and Buchanan, E.D. (eds). (1952). Bacteriology. 5th ed. The Macmillan Company. New York. pp. 543 - 544.

Cafati, C.R. and Saettler, A.W. (1980). Role of non-host species as an alternate inoculum sources of Xanthomonas phaseoli. Plant Dis.,64: 194 -196.

Chumaevskaya,M.A. and Nikolaeva, N.F (1975). Results of a study of bacterial diseases of sorghum in the Stavropol region. Vestinik Moscovskogo Universiteta. 30(30): 120-122. In: Rev.Plt. Path., (1976). 55(11): 1007 (Abstr.).

Cruickshank, R., Duguid, J.P., Marmion, B.P., and Swain, R.H.A. (1975). Medical Microbiology: The proactice of medical microbiology. 12th ed. vol.2. Longman Group Ltd. New York. pp. 19-20 170-189.

Dagnatchew Yirgou. (1967). Plant disease on economic importance crops in Ethiopia. Expt. Stat. Bull. 50 Hailesellassie I University, College of Agriculture. Debrezeit. Ethiopia. pp. 9 - 11.

Derege Ashagari. (1975). Post penetration phenomena in wheat cultivars with low receptivity to infection by Puccinia graminis tritici. Ph. D Thesis. University of Minnesota. pp. 1 - 2.

Ethiopian Sorghum Improvement Project. (1976). Progress Report No 4. Addis Ababa University, College of Agriculture. Institue of Agricultural Research. p. 1.

Ethiopian Sorghum Improvement Project. (1977). Progress Report No.5. Addis Ababa University, College of Agriculture. Institute of Agricultural Research. pp. 1, 61, 63.

Ethiopian Sorghum Improvement Project. (1978). Progress Report No.6. Addis Ababa university, College of Agriculture. Institute of Agricultural Research. p.1.

Food and Agricultural Organization. (1972). Improvement and Production of maize, sorghum and millet. FAO. Rome. pp. 469 -470.

Food and Agricultural Organization. (1980). Problems and prospects of chemical control. FAO. International society for plant pathology. pp. 92 - 106.

Frezzi, M. and Teyssandier, E.E. (1978). Summary and horizontal review of sorghum in Argentina. International workshop on sorghum diseases. Hyderabad. India. 1 - 12.

Frohlich, G. and Rodewald, W. (1969). Pests and diseases of tropical crops and their control. pp. 107 - 170.

Ganga, N., Prasada, R. and House, L.R. (1971). Sorghum in Seventies. Oxford and IBM publishing Co. New Delhi, Bombay. pp. 421 - 422.

Gibbs, B.M. and Skinner, F.A. (1966). Identification Methos for Microbiologists. London, pp. 9-13.

Granada, G.A. and Sequeira, L. (1975) A hypersensitve reaction induced in tobacco leaves by a compatible (Race 1) isolate of Pseudomomnas solanacearum. Phytopath., 65: 731 - 733.

Haygood, R.A. and Strider, D.L. (1979). Influence of temperature, inoculum concentration and wounding on infection of Philodendron selloum by Erwinia chrysanthemi. Plant Dis. Reptr., 63: 578 - 580.

Hazel, W.J., Civerolo, E.L. and Bean, G.A. (1980). Procedure for growth and inoculation of Xanthomonas fragariae, causal organism of Angular leaf spot of strawberry. Plant Dis., 64(2): 178 -181.

Howell, M.L. and Frederiksen, R.A. (1983). Screening sorghum for resistance to bacterial stripe and streak. Phytopath., 73(3): 504. (abstr.)

International Crops Research Institute for the Semi Arid Tropics. (1978). Proceedings of the International Workshop on Sorghum Diseases. ICRISAT. Patancheru, P.O., Hyderabad, India. pp. 249 - 279, 324, 502.

International Crops Research Institute for the Semi Arid Tropics. (1982). Sorghum in Eighties. Proceedings of the International Workshop of Sorghum diseases. ICRISAT 2 - 7 nov 81.,Patancheru, A.P., India. pp 335 -345.

Johnson, H.W. (1953). Leaf diseases in grasses in the South, Plant Diseases. The yearbook of agriculture. Oxfored and IBM. U.S. Department of Agriculture. pp. 368 - 376.

Kauffman, H.E., Reddy, A.P.K., Hsien, S.P.Y. and Merca, S.D. (1973). An improved technique for evaluating resistance of rice varieties to Xanthomonas oryzae. Plant Dis. Reptr, 57: 537 -541.

Lawrence, G.J., Mayo, G.M.E. and Shepherd, K.W. (1981) Interaction between genes controlling pathogenicity in the flask rust fungus, Phytopath., 7(1): 12 -15.

Leukel, H.W. and Martin, J.H. (1953). Four enemies of sorghum. Plant Diseases. The yearbook of agriculture. Oxfored and IBM. U.S. Department of Agriculture. pp. 368 - 376.

Mengistu Hulluka. (1972). Population trends of three varieties of bean wilt bacterium (Corynebacterium flaccumfaciens) in three bean cultivars. Ph.D. Thesis. University of Nebraska. Lincholn. pp. 8 -9.

Mengistu Hulluka and Brhane Gebrekidan. (1978). Diseases of Sorghum in Ethiopia. International Workshop on Sorghum Diseases. Hyderabad. India. pp. 1 - 8.

Mian, M.I.H. and Ahmed, A. (1978). Sorghum diseases in Bangladesh . International Workshop on Sorghum Diseases. Hyderabad. India. pp. 1-7.

Mitruka, B.M. (1976). Methods of detection and identification of bacteria. 1st ed. Philadelphia. Pennsylavania. pp. 13 - 23.

Nikitina, K.V., Yakushevskii, M.S. and Sukhotskay, N.P. (1976). Bacterial diseases of Sorghum and Sudangrass under conditions of Kuban. Trudy Po Prikladnoi Botanike, Genetike i Seleksii (1976)., 57(3): 119 - 132. In: Rev. Plant Path., (1977). 56(12): 1137 (abstr.)

Sundaram, N.V. (1978). Bacterial leaf diseases. International Workshop on Sorghum Diseases. Hyderabad. India. pp. 5 -6.

Suslow,T.V., Schroth, M.N. and Isaka, m. (1982). Application of a rapid method for Gram differenciation of plant pathogenic and saprophytic bacteria without staining. Phytopath.,72: 917-918.

Tadelle Gebre Sallassie and Brhane Gebrekidan. (1975). Effects of defoliation on grain yield and other characters of sorghum. E.A.Agri.For.J.,40: 337 - 341.

Tarr, S,A.J. (1962). Diseases of sorghum, sudangrass and broomcorn. The Common Wealth Mycological Institute. Kew. Surrey. pp. 226-268.

Tuite,J. (1969). Plant pathological method: Fungi and Bacteria. Burgress Publishing Company. Minneapolis. pp. 176 - 193.

Vallejo, A.B. (1978). Sorghum diseases in Mexico. International Workshop on Sorghum Diseases. Hyderbad. India. pp. 1 - 16.

Vel'sovskii,V. (1978). The problems of breeding millet for resistance to melanosis. Referativnyi Zhurnal (1979). 6(65): 252. In: Rev. Plant Path., (1980). 59(4): 183. (abstr.)

Vidaver, R. (1976). Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. Ann.Rev. of Phytopath., 14: 451 - 455.

Walter, H.B. and Starr, M.P. (1948). The genetic and specific characters of phytopathogenic species of Pseudomonas and Xanthomonas. Phytopath., 38: 494 - 501.

Watson,D.R.W. (1971). A bacterial pathogen of sorghum in New Zealand. J.of Agri. Res., 14: 944 -947.

Appendix:- Duncan Multiple Range test showing results of individual means' comparison.