Evaluation of Inoculation Methods and Resistance of some Sorghum Genotypes to Bacterial Leaf Streak (*Xanthomonas campestris* pv. *holcicola*)

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Abstract

Six methodologies of inoculation were compared under field condition. Application of finely ground-infected leaves in leaf whorls and spraying with bacterial suspension after wounding leaves with sterile fine sand gave the most uniform and consistent results. Using these methods of inoculation, 121 sorghum genotypes were screened for resistance to *Xanthomonas campestris* pv. *holcicola (Xch)* under field and greenhouse conditions at the Alemaya University Research Center (eastern Ethiopia) after insuring effective inoculation method. Out of the 121 sorghum genotypes examined in the greenhouse, 31, 35 and 54 lines were resistant, moderately resistant and susceptible, respectively. In the field study, 87.5%, 5% and 7.5% of the genotypes were resistant, moderately resistant and susceptible, respectively. In this study it was possible to identify that 25 sorghum genotypes were found to be resistant to sorghum bacterial leaf streak both at the seedling and adult stages of growth with the desirable agronomic characteristics. These genotypes could form the basis for a breeding program to develop sorghum cultivars more resistant to this disease under cool growing conditions of Hararghe Highlands.

Introduction

Sorghum is susceptible to a number of pathogens and insect pests. Among the many diseases, bacterial leaf streak, incited by *Xanthomonas campestris* pv. *holcicola* (Elliott) Dye (*Xhc*) is a problem of higher magnitude in most sorghum growing countries, including Ethiopia (Tarr 1962 Claflin and Howell 1986, Temam 1990). Sudangrass, broomcorn, and johnson grass are additional hosts (Claflin and Howell 1986).

In Ethiopia, this particular disease has been reported to be endemic in many sorghum fields from year to year and has wide distribution in all sorghum growing areas of the country Severe infection of this disease occurs naturally on susceptible sorghum genotypes in the experimental fields of the Alemaya University (Mengistu 1982, Temam 1990). The loss due to this disease has not been quantified in Ethiopia. However, considering the leaf damage it can cause, grain losses could be considerable under favorable weather conditions.

Though bacterial leaf streak has the potential to cause heavy losses in sorghum in Ethiopia, little is done to control the disease. To minimize the possibility that improved high yielding sorghum will be vulnerable to epidemics of this disease, stable broad-spectrum resistance must be located and incorporated into the high yielding genotypes. In an earlier study (Yeshi and Mengistu 1985) on varietal resistance der greenhouse condition, the presence of resistant factors in agronomically acceptable sorghum varieties as been reported.

In breeding crops for resistance to bacterial or fungal diseases, it is important to utilize effective and reliable inoculation technique(s). The success of breeding, therefore, depends on the availability of practical and effective inoculation (screening) technique(s) that should be developed through carefully designed experiments. Detailed study on the epidemiology of sorghum bacterial streak is lacking; specific method for establishing artificial epiphytotic is also not available.

Researchers testing plants for resistance to bacterial pathogens usually apply several inoculation techniques (Winsead and Kelman 1952, Klement and Goodman 1967). Suspensions of bacterial cells or preparations of infected host tissues can be applied to plant surface in many ways. According to the available literature (Dhingra and Sinclai, 1995), propagules can be applied to leaves, etc. by spraying or dusting, or by direct placement. The most widely used technique for inoculating plants with foliar pathogens is spraying. But the most appropriate method for a particular pathogen needs to be determined experimentally. This study was undertaken with the following objectives: (i) identification of effective method(s) of inoculating sorghum genotypes with *Xhc* for resistance screening in the field; (ii) investigation and selection of sorghum genotypes resistant to bacterial leaf streak using the effective inoculation method(s) such that these materials could be incorporated in the sorghum-breeding program to create broader and stable sorghum genetic base.

Materials and Methods

Isolation and characterization of the pathogen

Isolation of the pathogen from sorghum leaves with bacterial streak symptom was carried out following the method described by Schaad (1988). Small slices of tissue (approximately 1 cm^2) were removed from the advanced portion of an infected leaf. The slices were surface-sterilized for 3 min. in a 1:9 dilution of household bleach (5.25% active sodium hypochlorite) and washed in sterile distilled water. These sections were crushed in 5 ml of sterile water using a sterile mortar and pestle. The resulting suspension was serially diluted and streaked with a wire loop onto petriplates containing yeast extract-dextrose-calcium carbonate agar (YDCA) medium (Schaad, 1988). The plates were incubated at 27 $\pm$ 1°C for 72 hours and were examined for type of colonies. Creamy and light yellow colored single typical colonies were selected and transferred to fresh YDCA slants.

The inoculum was prepared by growing cells from pure slant cultures in nutrient broth with vigorous agitation at 27°C and incubated for 72 hours. Cells were suspended in phosphate (PO_4) buffer and cells concentration was adjusted to 10^9 cells/ml prior to inoculation (Schaad 1988).

Verification of pathogenicity was conducted by inoculating 4-weeks-old sorghum seedlings of the susceptible cultivar, ETS 2111, (Yeshi and Mengistu 1985) grown under greenhouse conditions (11 to 28°C) (Sharm, 1980). The results were recorded 2 weeks after inoculation. The pathogenic isolates were maintained on YDCA slants at 4°C. Pathogenic isolates were used for characterization of the bacterium.

Isolates of the pathogen were characterized using the following tests; Gram reaction starch, casein and gelatin hydrolysis (Schaad and Stall, 1988), arginine dihydrolase production, oxidase reaction, nitrate reduction, malonate and citrate utilization and acid production from carbohydrates (Schaad 1988).

Greenhouse screening for resistance

One hundred nineteen sorghum genotypes consisting of improved varieties, lines derived from either exotic x exotic or exotic x indigenous crosses and indigenous collections plus two susceptible checks were tested. Seeds were planted in clay pots (20-cm diameter) filled with an autoclaved mixture of soil:sand:manure (3:1:1) and 400 mg urea per pot. About 10 seeds were planted in drills and thinned to 5 plants/pot 15 days after planting.

Four weeks-old seedlings were inoculated with mixed bacterial suspensions (10^9 cells/ml) of selected virulent isolates of *Xhc*, namely, AL-27-1, AL-27-3, BAB-3-1, and JIG-4-2 collected from Alemaya, Babille, and Jijiga areas. Leaves were rubbed with carborandum and sprayed with

bacterial suspension by an atomizer until run off. Control plants were inoculated with sterile distilled water using the same method. Thereafter, the potted seedlings were arranged in an 11 X 11-simple lattice design in the greenhouse and covered with a transparent perforated plastic sheet. The seedlings were sprayed with water every 2 hr to maintain high humidity until maximum disease developed. All inoculations were performed and plants were kept in the greenhouse maintained at an average day and night temperatures of 28°C and 11°C, respectively and relative humidity ranging from 44 to 90.5 % and a mean of 67.25%.

Disease reactions were recorded two weeks after inoculation on a 1 to 9 scale (Sharma, 1980) where 1 = no sign of streaks; 2 = 2.5 %; 3 = 5 %; 4 = 10 %; 5 = 20 %; 6 = 35 %; 7 = 75 %; 9 = 100 % of leaf area infected. Individual disease ratings of plants in the two replications were averaged to derive the treatment means. Cultivars receiving 1 to 3 grades were considered resistant; those scoring 4 to 5 were moderately resistant and those with grades of =6were susceptible (Sharma, 1980). This experiment was repeated two times.

Field screening for resistance

Evaluation of inoculation methods

To establish a reliable and efficient inoculation method of screening for resistance, six methodologies described in phytopathological literature (Kiraly et al. 1970, Dhingra and Sinclair 1995, Winsead and Kelman 1952, Klement and Goodman 1967) were tested under field conditions using a susceptible sorghum variety, ETS 2111. A randomized complete block design was used with 4 replications. Spacing between rows and plants were 75 and 25 cms, respectively (Berhane and Yilma 1979). The experiment was conducted in plots of 5.00 X 3.00 m in sorghum-bean rotated field.

Naturally infected sorghum leaves were collected in cloth bags from Alemaya University (AU) experimental field on the main Campus at the end of the main cropping seasons. Pathogen identity and viability was checked right after collection and every 10 days thereafter until the materials were used for field inoculation.

Seeds were sown mechanically by hand and thinned to the appropriate spacing four weeks after emergence. Fertilizer was applied at the rate of 150 kg/ha DAP and 100 kg/ha urea at planting and at knee-heightt stage, respectively (Berhane and Yilma 1979). Other cultural practices were accomplished by standard recommended methods. Rainfall, air temperature, soil temperature, and relative humidity for the growing seasons were recorded.

The following six inoculation methods were compared:

Inoculation with infected debris: About 1 g of finely ground infected leaves were applied in leaf whorls of young seedlings with a hand.

- Wounding and spraying: Plants were physically struck with very fine sterile sand and then sprayed with aqueous bacterial suspension using a hand-operated knapsack sprayer. The principle lying behind this technique is the forcing of bacteria through the wounds caused by the sand.
- Leaf tip clipping inoculation: This consists of clipping off the tips of sorghum seedlings with a pair of sterile/flamed scissors whose blades have been dipped in aqueous bacterial suspension containing 10^9 CFU.
- Injection with hypodermic syringe: A hypodermic syringe needle was used to introduce the inoculum into the sub-epidermal space of young leaves.
- Needle-prick inoculation: The upper part of petioles of young leaves were punctured in several locations with a sharp sterile/flamed needle dipped in a freshly prepared bacterial suspension.
- Spray inoculation: Using a hand-operated knapsack sprayer, the whole plant was lightly sprayed with aqueous bacterial suspension.
- Control: This consisted of uninoculated plots.

All inoculations were done late in the afternoon at about 4.00 to 5.00 p.m. 40 days after planting and the plants were periodically sprayed with sterile water to establish the disease most effectively. Ten plants in the middle rows of each plot were randomly selected and tagged for disease assessment. Disease severity (percent leaf area infected) were estimated at 10-day intervals starting at about 3 weeks after inoculation until maximum disease development was obtained. Final observations were taken 51 days after inoculation. These severity scales were converted into disease index (DI) using the formula given below (James 1974). Finally, a combined analysis of variance for the three years was computed for recent disease intensity.

Field screening

The materials tested in the greenhouse were also

evaluated for resistance to bacterial leaf streak using infector-row technique under artificial infection in the field (Sharma 1980). Entries were planted in two rows of 3.75-m length in an 11 X 11-simple lattice design with two replications (Gomez and Gomez 1984) and pacing of 75 x 25 cm. Fertilizer was applied at the rate of 150-kg DAP/ha during planting and 100 kg urea/ha at knee height. Cultivation, weed and insect control was done following the standard recommendations (Berhane and Yilma 1979).

Disease epidemics were enhanced by growing a thick population of two susceptible varieties AL-70 and ETS-2111, around the test entries. ETS-2111 was also sown as an indicator row between plots at approximately 6-m length. Inoculations were performed at three growth stages (Sharma 1980): at the start of tillering (growth stage 20), stem elongation (growth stage 30), and booting (growth stage 40) with the pathogen. Inoculation was performed in two ways: by applying approximately 1 g of dry finely ground infected leaves in the whorl of each plant and by spraying with bacterial leaf streak suspension of local isolates collected from naturally infected leaves. The suspension was sprayed using a hand-operated knapsack sprayer two days after the first inoculation after wounding tissue with sterilized fine sand. All inoculations were done late in the afternoon at about 4.00 to 5.00 p.m. The weather data (temperature, rainfall and relative humidity) during the season were recorded.

Disease evaluations were made twice during the season, at flowering (growth stage 60) and at soft dough (growth stage 80) development stages using the 1-9 disease rating scale (Sharma 1980). The number of plants per replications varied depending on availability and variability of seeds of each entry. Total plants tested per entry ranged from 30 to 60/ plot. Data were subjected to analysis of variance using MSTAT-C software (MSU 1998) and mean separation was done using Duncan's multiple range test (Gomez and Gomez 1984). Cultivars were selected not only on the basis of disease resistance but also on their desirable agronomic characteristics using the 1 to 5 scale: 1 = very good; 2 = good; 3 = average; 4 = below average and 5 = poor (Sharma 1980).

Results

Isolation and characterization of the pathogen

Results of morphological, physiological and biochemical tests indicated that only 4 isolates; namely, AL-27-1, AL-27-3, BAB-3-1 and Jij-4-2 fit the characteristics of *X. campestris* pv. *holcicola*y (Table 1). Pathogenicity results measured as disease severity on a 1-9 scale indicated that the Alemaya isolates scored 6 while the Babile and Jijiga isolates scored 7 or more. This indicated that isolates from Babile and Jijiga were more virulent as compared with those from Alemaya.

Greenhouse screening for resistance

Under greenhouse conditions, about 26 % (31 entries) of the material tested were found resistant; 29 % (35 entries) were moderately resistant and the rest were susceptible. The difference in disease intensity among the 121 sorghum lines was significant ($P < 0.05$). Pairwise comparison between test entries and the control indicated that 35 percent of the test entries had lower levels of infection as compared with the susceptible control (ETS-1).

Field screening for resistance

In the field, favorable conditions for disease development were observed throughout the test period (about 800-mm precipitation, and temperature range of 10 to 29° C). Final observations on disease severity taken 51 days post inoculation are given in Table 2. Differences among the disease indices for the various inoculation techniques were compared statistically using Duncan's multiple range tests (Table 2). As shown in Table 2, all the 6 inoculation methods tested were effective in producing infection. No significant differences were found among leaf tip clipping, hypodermic injection, and needle prick methods of inoculation that scored 34.57, 27.67, and 24.46 infection intensities, respectively. Application of finely ground leaves in leaf whorls scored the highest (67.81) followed by wounding tissues and spraying with bacterial suspension that scored 46.98 disease intensities. Most of the genotypes (87.5 percent) were apparently resistant (reaction type, < 3). Five percent of the materials were classified as moderately resistant and the rest

(7.5 %) were susceptible. The mean disease rating ranged from 5 to 6.30 on the 1 to 9 scale. on the susceptible checks, AL-70 and ETS 2111

Table 1. Biochemical and physiological characteristics of *X. campestris* pv. *holcicola*

Characteristics	Isolates			
	AL-27-1	AL-27-3	BAB-3-1	JIJ-4-2
Gram reaction	-	-	-	-
Motility	+	+	+	+
Yellow colonies on YDC	+	+	+	+
Mucoid growth on YDC	+	+	+	+
Starch hydrolysis	-	-	-	-
Casein hydrolysis	+	+	+	+
Gelatin liquifaction	-	-	-	-
Arginine dihydrolase	-	-	-	-
Oxidase reaction	-	-	-	-
Nitrate reduction	-	-	-	-
Citrate utilization	-	-	-	-
Malonate utilization	+	+	+	0
Acid production from:				
Arabinose	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Glucose	+	+	+	+
Glycerol	-	-	-	-
Maltose	-	-	-	-
Mannose	+	+	+	+
Sucrose	+	+	+	+

+ = Positive reaction; - = Negative reaction

Table 2. A comparison of inoculation methods to test sorghum genotypes for resistance to *Xanthomonas campestris* pv. *holcicola* under field conditions.

Techniques used for inoculation	Mean Disease Index [a]
Application of finely ground infected leaves in leaf whorls	67.81 [a]
Wounding tissues and spraying with bacterial suspension	46.98 [b]
Leaf-tip clipping inoculation	34.57 [c]
Hypodermic syringe inoculation	27.67 [c d]
Needle-prick inoculation	24.46 [c d]
Spray inoculation without wounding tissues	20.43 [d]
Control	18.55 [d]

S.E. ± 3.2, LSD = 9.49, C.V. (%) = 23.15

[a] Data are the average of 3 years trials; each treatment replicated 4 times. Means followed by a common letter in column do not differ significantly at the 0.05 level of significance, according to Duncan's multiple range test.

Table 3. Reactions of 25 selected sorghum genotypes to *Xanthomonas campestris* pv Holcicola under green house and field conditions at Alemaya

Identification/ pedigree	Disease score (1-9 scale)* Greenhouse**	Field***	Agronomic characteristics (1-5 scale) ****
ETS-02454	1.00 ^{h}	2.00 ^{a}	2.0
87 AL-4069	1.00 ^{h}	2.00 ^{a}	1.5
Jaru# 5	1.00 ^{h}	1.00 ^{c}	2.5
G.P.C.AL-1987 #460	1.00 ^{h}	2.00 ^{a}	2.0
IS 158 x (ETS 3235)[4]	1.00 ^{h}	2.00 ^{a}	3.0
ETS - 3235	1.00 ^{h}	2.00 ^{a}	3.0
IS 158x (ETS 2113)[4]	1.00 ^{h}	1.00 ^{c}	1.0
85 PGRC/E Acc. # 105	1.00 ^{h}	1.00 ^{c}	2.0
86 MW # 5228 (YE-126x17 BGM 117 x IS 9439)	1.00 ^{h}	2.00 ^{a}	2.0
85 MW # 5354 (RS/R-20-8614-x IS 9521)	1.00 ^{h}	2.00 ^{a}	2.0
85 MW # 5606 (RS/R-20-8614-x IS 9293)	1.00 ^{h}	2.00 ^{a}	2.0
85 PGRC/E Acc. # 166	2.00 ^{fg}	1.00 ^{c}	2.5
ETS - 2247	2.00 ^{efg}	2.00 ^{a}	1.5
G.P.C AL-1987 # 54	2.30 ^{efg}	2.33 ^{a}	3.0
G.P.C.AL.-1987 # 715	1.80 ^{g}	1.00 ^{c}	2.0
G.P.C. AL- 1987 # 716	2.30 ^{efg}	1.88 ^{ab}	2.5
G.P.C. AL-1987 # 725	2.40 ^{efg}	2.00 ^{a}	2.0
AL - 1988 NVT - 2 # 5	2.60 ^{def}	1.02 ^{c}	2.5
AL - 1988 NVT - 2 # 8	2.00 ^{fg}	1.00 ^{c}	2.5
85 PGRC/E Acc.# 278	2.80 ^{cde}	1.00 ^{c}	2.0
1989 AL - HESPVT-2 # 1	3.22 ^{cd}	2.00 ^{a}	2.0
AL - 1988 NVT - 2 # 11	2.90 ^{cde}	2.00 ^{a}	2.0
Muyra Red	3.56 ^{bc}	1.88 ^{ab}	2.0
AL - 1988 HESPVT - 1 # 21	4.24 ^{b}	1.00 ^{c}	2.0
AL - 1988 HESPVT - 1 # 15	5.30 ^{a}	1.30 ^{bc}	2.0

The mean disease ratings on the susceptible checks, AL 70 and ETS 2111, were 5 and 6.3, respectively.
G.P.C. = Germplasm Collection
** = Disease scores are based on the 1-9 scale (Sharma, 1980) where 1 = no sign of streaks, 2 = 2.5 %, 3 = 5 %, 4 = 10 %, 5 = 20 %, 6 = 35 %, 7= 75 %, 9 = 100 % leaf area covered*
*** = Averages of two experiments*
*** = Averages of two replications in three years test
**** = Score is based on the 1-5 scale where 1 = excellent, 2 = very good, 3 = good, 4 = poor, 5 = very poor
Means followed by the same letter in a column are not significantly different at the 0.05 level of significance.

Discussion

As shown in Table 2, all the 6 methods tested were effective in producing infection. No significant differences were found among leaf-tip clipping, hypodermic injection, and needleprick inoculations. Application of finely ground infected leaves in leaf whorls and wounding and spraying were either significant ($P < 0.05$) or highly significant ($P < 0.01$) from the other inoculation techniques. It is apparent from the results of the comparison of inoculation techniques that the highest infection intensity (67.81) was obtained by inoculating plants with finely ground infected plant debris followed by the second method, wounding leaf tissues and spraying, which resulted in disease intensity of 46.98 % (Table 2).

In several studies made earlier, a number of successful inoculation techniques have been reported. In one study good plant infection with *X. campestris* pv. *phaseoli* was obtained by pricking bean stems at the cotyledonary node. In screening for common bacterial blight resistance, multiple needle primary leaf inoculation was found to be effective (Dhingra and Sinclair 1995). Sharma (1980) obtained good results by applying finely ground-infected leaves into the leaf whorls of sorghum seedlings in screening for resistance to bacterial diseases of sorghum. In the

present study, among the several inoculation techniques tested, application of finely ground infected sorghum leaves in leaf whorls of seedlings effectively induced infection by bacterial leaf streak. This technique alone or in combination with wounding and inoculating plants can be used on a large scale to screen for varietal resistance to bacterial leaf streak.. This techniques has many advantages over the rest of the tested techniques in that it is easy to store and apply the inoculum, does not require multiplication of inoculum in the laboratory, etc. It was also more efficient and resulted in more reliable evaluation of infection. Disease scores from leaf to leaf and from plant to plant also varied less for the plants inoculated by this technique than those inoculated by the rest of the techniques. In addition, the present study has revealed the possibility of standardizing inoculation technique to achieve effective method of screening for resistance to this pathogen.

Among the 31 sorghum genotype selected for their resistance in the greenhouse 5 lines showed highly resistant reaction while 26 lines were resistant under field conditions. In this study it was noted that the assessment for resistance in seedlings was positively correlated ($r = 0.60$) with ratings of adult plants in the field. All the genotypes that were resistant under greenhouse condition were also resistant under field condition. However, those that were susceptible under greenhouse condition showed variable degrees of reaction, ranging from resistant to susceptible, under field conditions. The higher percentage of resistant reaction observed under field condition as compared with that of greenhouse test, could be due to the use of less virulent isolates from Alemaya which were used for field inoculation as opposed to the mixture of virulent isolates from two locations (Babile and Jijiga) used for greenhouse inoculation.

Since Ethiopia is a major center of diversity of sorghum, the amount of indigenous genetic diversity available in the crop in the country is tremendous. This genetic diversity has been the initial starting ground for sorghum improvement work in Ethiopia. The indigenous Ethiopian sorghum germplasm has been useful not only to the Ethiopian national program but also to many other sorghum researchers worldwide.

According to Frederikson and Franklin (1980), characters such as tan plant color, thick waxy leaves, erect leaves and the like, are believed to be associated with leaf disease resistance. These characters, particularly tan plant color, has effectively been used in sorghum breeding.

In the present study, the majority of the resistant materials with the above characters were those selected for cool growing conditions of the highlands (> 1900 m.a.s.l) of Ethiopia. One cultivar, IS 158 x (ETS 2113)[4], with highly resistant reaction was among advanced sorghum lines selected for agronomic excellence and apparent resistance to most of foliar diseases at different locations including Alemaya (Berhane and Yilma 1979). The exotic sorghums were lowland types introduced from ICRISAT, Middle East, and Near East countries and were characterized by tan plant color. This is in agreement with earlier reports (Frederiksen and Franklin 1980).

The susceptible genotypes included cross derivatives from exotic x exotic and exotic x indigenous crosses as well as local collections. The highly susceptible accessions were purely local materials, susceptible introductions or crosses between the two groups. For instance, WB77, Muyra White, PGRC/E Acc. No. 262, Meta Hara Collection, Wegere, AL-70, etc. were among the local collections while (Hafukagne x Hirna 305/547 x (ETS 2111)[3], NES 8827 x (ETS 3235)[3], NES 8827 x (WB 77)[3] were cross derivatives from exotic x exotic and exotic x indigenous collections.

In the present study, as shown by the pedigrees or parental lines of some of the resistant genotypes, sorghums such as IS 158, 79 SEPON # 391, etc. and the local collections 17 BGM 117, etc. appear to be good sources of resistance against bacterial leaf streak.

In general this study had revealed 25 sorghum genotypes (Table 3) with desirable agronomic characteristics as well as seedling and adult plant resistance to sorghum bacterial leaf streak. These genotypes could form the basis for a breeding program to develop sorghum cultivars resistant to *Xch* under cool growing conditions of Hararghe Highlands.

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References

Berhane G/Kidan, Yilma Kebede. 1979. The traditional culture and yield potential of the Ethiopian high lysine sorghum. Ethiopian Journal of Agricultural Sciences 1:29-40.

Claflin LE, Howell ML. 1986. Diseases caused by bacteria. In compendium of sorghum diseases, ed. Frederiksen RA, pp. 5-7. The American Phytopathological Society, St. Paul, Minnesota. 164 pp.

Dhingra OD, Sinclair JB.1995. Establishment of disease and testing for resistance. In basic plant pathology methods (2nd ed.), eds. Dhingra OD, Sinclair JB, pp. 151-215. Lewis Publishers, Boca Raton. 434 pp.

Frederiksen RA, Franklin D.1980. Sources of resistance to foliar diseases of sorghum in the International Disease and Insect Nursery: proceedings of the international workshop on sorghum diseases, 11-5 December 1978. Hyderabad, India. 469 pp.

Gomez KA, Gomez AA. 1984. Statistical procedures for agricultural research (2nd ed.).

James WC. 1974. Assessment of plant diseases and losses. Annual Review of Phytopathology 12:27-48. John Wiley and Sons. New York.

Kiraly Z, Klement Z., Solymosy F. 1970. Methods in plant pathology with special reference to breeding for disease resistance, ed. Kiray Z. Academica Kiado, Budapest. 509 pp.

Klement Z, Goodman RN.1967. The hypersensitive reaction to infection by bacterial plant pathogens. Annual Review of Phytopathology 5:17-30.

Mengistu Hullluka. 1982. Diseases of sorghum at some locations in Ethiopia. Ethiopian Journal of Agricultural Sciences 4:45-53.

Michigan State University (MSU). 1998. User's guide to MSTAT-C. Michigan State University, Michigan, USA. 35 pp.

Schaad NW, Stall R.E. 1988. Xanthomonas. In laboratory guide for identification of plant pathogenic bacteria (2nd ed.), ed. Schaad NW, pp. 81-94. The American Phytopathological Society, St.Paul Minnesota.164 pp.

Sharma HC. 1980. Screening of sorghum for leaf disease resistance in India: proceedings of the international workshop on sorghum diseases, 11-15 December 1978. Hyderabad, India. 469 pp.

Tarr SAJ. 1962. Diseases of sorghum, sudan grass and broom corn. Commonwealth Mycological Institute, Kew, England. 380 pp.

Temam Hussien. 1990. A review of sorghum disease research in Ethiopia. In proceedings of a regional workshop on cereals of the semi-arid tropics, 12-16 September, 1989. Garoua, Cameroon. 321 pp.

Winstead NN, Kelman A. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology 42:628-630.

Yeshi Adnew, Mengistu Huluka. 1985. Characterization of isolates of bacterial streak (*Xanthomonas campestris* pv. *holcicola* (Elliot) Starr and Bruk.) and reaction of some sorghum varieties to the disease. Ethiopian Journal of Agricultural Sciences 7: 99-107.