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Research Article

Evaluation of some techniques used for the screening of some cowpea (*Vigna unguiculata* (L.) Walp) genotypes against bacterial blight

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Abstract

The experiment was aimed at evaluating the efficacy of four artificial Inoculation techniques for the screening of some cowpea genotypes against bacterial blight. The experiment comprised of two genotypes of cowpea: Danila and IT97K-819-11 and four treatments of artificial Inoculations. The treatments were seed inoculation, soil inoculation, foliar spray inoculation, stem injection inoculation and un-inoculated control. The experiment was arranged in Completely Randomized Block Design (CRBD) in four replications. The data collected were disease incidence (%), disease severity and chlorophyll SPAD. The results of the study revealed that foliar application on Danila variety produced the highest disease incidence (100%) and disease severity (2.75), while seed inculcation on IT97K-819-11 has the lowest disease incidence (50%) and severity (0.50). The chlorophyll content was highest on seed inoculation (61.9) and lowest in foliar inoculation (41.5). The experiment has shown that foliar spray inoculation method was found most effective in causing higher disease severity and incidence in comparison to other methods. Therefore foliar inoculation artificial technique could be used to screen cowpea varieties against bacterial blight.

Keywords: Bacterial Blight, Cowpea, Screening Techniques, Disease Incidence and severity.

INTRODUCTION

Bacterial blight of cowpea is a disease caused by the bacterial pathogen; *Xanthomonas compestris* Pv. Translucens. It has been known as a disease since the late 19th century. It has a worldwide distribution and can be found in places like China, India, Turkey, Nigeria, Botswana, Egypt, South Africa, Sudan, Tanzania, Zimbabwe, Rico, USA (Moretti *et al*., 2006) and in almost all areas where cowpea is grown (Mehrotra and Aggarwal, 2003; Agrios, 2005). Bacterial blight is a serious disease that can cause complete defoliation of susceptible cowpea cultivars. Symptoms appear on leaves, pods and seeds when the disease is systemic. It leads to pre and post emergence seedling mortality. It also results in loss of crop yield.

The disease appears as brown angular leaf spot with yellow margins on the leaves, pods and stems. The lesions expand and then appear as dry dead spots. The lesions elongate into linear streaks which may eventually extend the full

length of the leaf (Abdulai *et al*., 2003). In severe infections, the leaf becomes yellow, wilt and drop. Pods can also be infected in which case it develop red necrosis. Seeds may also be invaded when the bacterial becomes systemic, the seeds (if naturally white in color) tend to posses yellow spots. In severe cases seeds may shrivel and become small which contribute to yield reduction (Agarios, 2005). Milky gray exudates may be squeezed from the cut end of leaf exhibiting symptoms when the infection becomes severe (Mathre, 2000). The disease is usually seed borne but can also be soil borne. It can cause severe defoliation during periods of high humidity (Abdulai *et al*., 2003). The major hosts of the bacterial blight pathogens are *Vigna unguiculata* (cowpea), *Crotalaria juncea* (Sunn hemp), Lablab purpureus (hyacinth bean), *Phaseolus vulgaris* (Common bean), *Solanum nigrum* (black nightshade), *Vigna mungo* (black grain) (Moretti *et al*., 2006).

This disease is caused by a common bacterium that persists in soil and water and is spread by wind-driven rains (Moretti *et al.*, 2006). The bacterium usually penetrates the host tissue through stomata, hydathodes or wounds created by man or nematodes. After penetration, the organisms invade and destroy spongy and intra vascular tissues (Mehrotra and Aggarwal, 2003). When the pathogen is systemic, it penetrates the stem. In lightly lignified old stems, the bacteria remain restricted to the vascular tissue where they remain viable for long period of time before they are transmitted by seeds (Singh, 2005).

The bacteria overwinter on crop residue, seed, fall-sown cereals and perennial grasses, spring infection may result from any of these sources. Subsequent infections are spread by splashing of bacterial Ooze by rain drops, plant to plant contact and insect (Moretti *et al.*, 2006).

The aim of this research is to assess the efficacy of five artificial inoculation techniques used for the screening of cowpea varieties on the incidences and severity of bacterial blight of cowpea.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in a screen house of the International Institute of Tropical Agriculture (IITA) experimental research Kano station from November, 2012 to February, 2013. Kano is located in the Sudan Savannah Agro-ecological zone, latitude 12^{o} 03'N and longitude 08^{o} 31'E and an altitude of 1500 m above sea level (Kowal and Knabe, 1972).

Inoculum Sample Collection

Diseased leaf samples were collected from a farm considered as a 'hot spot' for many years in the IITA cowpea research farm at Minjibir, Kano State. The leaves were dried under shade for 7 days and then stored at room temperature in an envelope before it was later crushed for inoculation purpose (Moretti *et al*., 2006).

Seed Collection

Two genotypes of cowpea were obtained from seed store of IITA, Kano station. The genotypes were: Local Dan'ila, and IT97K-819-11. Both cowpea varieties are known to be susceptible to bacterial blight (IITA, 1997).

Pot Preparation

The soil used was sterilized loamy soil mixed with animal dung in the ratio of 3:1 soil and animal dung (local manure).The size of pot used was 17 cm length and 17 cm breadth plastic pot. The pots were filled with the sand mixture after creating a hole at the bottom of the pot to allow passage of water so as not to create a water logged soil. The pots were watered and allowed to stand for a day before planting.

Experimental Design

The experiment was set up in a Completely Randomized Block Design (CRBD) with four replications. The genotypes of cowpea used were obtained from IITA Kano station and each was planted in pots and arranged in ten rows with four replications in each row making a total of 40 pots. Four different treatments and a control were made for each variety; each treatment was replicated four times. The treatments were: Seed inoculation, soil inoculation, foliar spray inoculation and stem injection inoculation.

Seed Sowing

A small hole of 3 cm was dug in each pot and 4 seeds per hole were sown and buried under the soil. Irrigation was used as a water supply to the plants for establishment and growth.

Germination

The seed germinated five (5) days after sowing and was thinned to two plants per pots 21 days after sowing.

Seed Inoculation

Two grams of the inoculums were poured into 20ml of distilled water. The content was stirred, and then the seeds were poured in it and mixed in a clean plastic bowl until all the seeds were uniformly coated. The whole inoculation procedure was completed in the shade as sunlight damages the bacteria. The seeds were then dried in shade for an hour before sowing in pots (Bryant *et al.*, 2001).

Soil Inoculation

About 1 kg of dry soil was poured in a bucked and 10 g of inoculants was added to it. The bucket was closed tightly and shaken by hand, it was rolled on the ground until inoculants and sand was thoroughly mixed. The bucket was open to inspect the mixture for uniformity. After uniform mixture of the inoculants and sand, the soil was filled in the pot labeled for soil inoculation. The seeds were sown immediately after inoculation. After placing the seeds into the hole, it was covered with the soil immediately to protect the mixture from sun and heat. The pots were transferred to the screen house after planting (Catroux *et al.*, 2001).

Stem Injection Inoculation

Exactly 2g of the inoculants was placed in 20ml of distilled water and the solution was used to inoculate the growing seedlings using injection method. The inoculums was introduced into the stem with a clean hypodermic needle, by inserting the hypodermic needle gently under the stem and 1ml was inject to each plant and were observed to know the incidence and the severity of the disease (Catroux *et al.*, 2001).

Foliar Inoculation

Exactly 5 g of the inoculants was placed in 50 ml of distilled water and the solution was used to inoculate the growing seedlings using spray pump. The inoculum was poured into the spray pump and it was introduced into the leaves by spraying the leaves until it dried out.

Measurement of Chlorophyll Content

The chlorophyll content was estimated using SPAD meter (model; SPAD 502 PLUS) which determines how green the leaf is and the resultant effect of the disease on the chlorophyll. Three affected leaves were measured per pot and the average of the chlorophyll SPAD was recorded in each pot.

Data Collection

The following data were collected.
1. Incidences of the disease
2. Severity of the disease
3. Chlorophyll content (SPAD Reading)

Determination of Disease Parameters

Disease Incidence
The incidence of the bacterial blight disease was recorded by establishing the proportion of plants showing the symptoms and expressing the result as percentage of total number of plant per pot.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of both healthy and infected plants}} \times 100$$

Disease Severity

The severity was scored at three (3) weeks after inoculation. The severity percentage was calculated using the formula below and then the severity was scored on the infected plant using the severity rating scale as follows:

$$\text{Disease severity (\%)} = \frac{\text{Area of affected leaf}}{\text{Total leaf area}} \times 100$$

0-0% 0 = None infected
1-10% 1 = very slight infection (very few spots on the leaves and a few leaf affected)
11-20% 2 = Slight infection (few spots on the leaves and more visible)
21- 40% 3 =moderate infection (up to four spots per plant and general light spotting i.e appears clearly).
41- 60% 4 = Severe infection (Nearly every leaves with lesions, plant still remaining normal form)
61 – 100% 5 = Lead to death (only few leaves left green, most leaves are dead)

Statistical Analysis

The result obtained were analyzed using analysis of variance (ANOVA), mean separation was by least significant difference LSD at 5% level of probability.

RESULTS

Chlorophyll Content

The results for chlorophyll contents are presented in Table 1. The results showed that, seed inoculation of variety IT97K-819-118 recorded the highest chlorophyll content, while foliar spray inoculation on Danila variety has the lowest chlorophyll content. Treatments on Danila variety have low chlorophyll content, while IT97K-819-118 recorded high chlorophyll content. Stem injection treatment of Danila variety has higher chlorophyll content compared to that of variety IT97K-819-118.

Table 1. Effect of bacterial blight on chlorophyll SPAD of two cowpea Genotypes under four inoculation techniques

Inoculation / Genotype	Dan' ila	IT97K-819-118
Foliar inoculation	41.5	49.7
Stem injection inoculation	53.6	44.5
Seed inoculation	44.6	61.9
Soil inoculation	47	54.4
Control	78.0	76.0
Means		56.5
S.E.±		11.83
LSD		12.24

SE=Standard error, LSD=Least significant difference at 5%

Disease Incidence and Severity

The result for disease incidence is presented in table 2, while that of disease severity is presented in table 3. Two cultivars namely Danila and IT97K-819-118 were used to evaluate the performance of different inoculation methods via foliar spray, stem injection, soil and seed treatment with bacterial blight. The two cultivars used in the evaluation has varied response, the first one was highly susceptible to the bacterial specie, while the second one being moderately susceptible. This was done to know whether different methods of inoculation do affect the reaction of the pathogen. The disease incidence and severity were recorded at three (3) weeks of inoculation.

Table 2. Disease incidence (%) of bacteria blight on two cowpea Genotypes under four Inoculation techniques

Inoculation / Genotype	Dan' ila	IT97K-819-118
Foliar inoculation	100	87.5
Injection inoculation	62.5	100
Seed inoculation	87.5	50
Soil inoculation	75.0	62.5
Control	0.00	0.00
Means		62.5
SE±		24.15
LSD		49.33

SE=Standard error, LSD=Least significant difference at 5%

Table 3: Disease Severity rating of Bacterial Blight on Some Cowpea genotypes Under Four Inoculation Techniques

Inoculation/ Genotype	Dan' ila	IT97K-819-118
Foliar inoculation	2.75	2.00
Injection inoculation	1.50	2.25
Seed inoculation	2.00	0.50
Soil inoculation	2.00	1.00
Control	0.00	0.00
Means		1.40
SE±		0.540
LSD		1.103

SE=Standard error, LSD=Least significant difference at 5%

Symptoms

Typical symptoms of bacterial blight caused by *Xanthomonas campestris* appeared on the cowpea irrespective of the methods of inoculations. The symptoms observed were brown angular leaf spot with yellow margins on the leaves which later expand and appear dry. The uninoculated plants did not show any symptom of the disease. Highest disease incidence was recorded on Danila variety (100%) due to foliar spray, followed by inject inoculation method of IT97K-819-118 variety. The disease incidence was 75% to 100% in the cultivar of Danila where as 50% to 100% in the cultivar of IT97K-819-118.

Cultivar IT97K-819-118 was found moderately susceptible to bacterial blight and much greater disease incidence was noticed in Danila than IT97K-819-118 where foliar inoculation is the highest with 100% followed by stem injection with 62.5%, seed 87.5% and then soil inoculation with 75.5% while the cultivar IT97K-819-118 recorded 87.5% for foliar spray, 100% for stem inject, 50% for seed and 62.5% for soil inoculation. Disease severity due to foliar spray was 2.75 in Danila which is the highest severity rate followed by stem injection method with 2.25 in IT97K-819-118. For seed and soil inoculation methods, the disease severity was lowest in IT97K-819-118 with 0.50 and 1.00, respectively.

Scale of Disease severity (0-5)

0 = No infection
1 = Very slight infection
2 = Slight infection
3 = moderate infection
4 = Sever infection
5 = Lead to death

DISCUSSION

Chlorophyll Content

Total chlorophyll content according to the result obtained showed that seed inoculation of variety IT97K-819-118 recorded the highest chlorophyll content, while foliar treatment on Danila variety recorded the lowest chlorophyll content. Reduction in chlorophyll content could be due to destruction of spongy and intravascular tissue of the plant by the

organism and it could be due to susceptibility of the variety to the disease while high chlorophyll content recorded in variety IT97K-819-118 could be due to its resistance to the disease. The findings agree with that of Allen *et al.,* (1999) who stated that chlorophyll content reduction in cowpea leaves due to bacterial blight occur as a result of increase of the inoculums as well as susceptibility of the variety. Prashant *et al.* (2009) reported that high chlorophyll content in leaves will in turn increase grain yield, while low chlorophyll content reduce grain yield.

Disease Incidence and Severity

According to the result, the cowpea varieties varied in their response to artificial infection with bacterial blight, thus indicating the effect of different inoculation techniques of bacterial blight on cowpea. Characteristics bacterial blight symptom caused by *Xanthomonas campestris Pv*. developed on the plant inoculated through seed, soil, foliar and stem injection has indicated that the methods used were successful in initiating the infection by bacterial blight. However, the degree of severity varied with the method, and significant difference in symptom development were recorded with four method of inoculation tested in the study. The *Xanthomonas* species attacks foliar parts (Hayatu *et al.*, 2013). Foliar spray with the bacterial suspension gave direct access to the spores to susceptible part and tissue resulting to infection in the leaves and latter in stem and pods (Humpherson and Ainsworth, 1984). For this reason, the foliar spray was found to be the most effective method of inoculation to achieve severe disease symptoms among the four different modes of inoculation. The next in effectiveness in causing the disease and its further development was stem injection inoculation. Seed and soil inoculation were not so effective in comparison with foliar spray inoculation or stem injection method.

The analysis between the disease incidence and severity inoculated with different methods has shown stronger relationship in foliar spray inoculation followed by stem injection method. This has shown that foliar inoculation cause disease of the severity that can lead to yield decline greater than other method. Moreover, this effect was observed in both highly susceptible and moderately susceptible cultivars with both species of *Xanthomonas campestris*. The observed susceptibility of the cowpea to bacterial blight disease in artificial infection was similar to the variation in response of cowpea cultivar to natural infection reported by Singh (2004).

CONCLUSION

This study has shown that foliar inoculation method was found relatively more effective in causing higher disease severity and incidence in comparison to other methods. The different method used did not influence the varietal reaction to the bacteria as the variety Danila exhibited blight symptoms greater than variety IT97K-819-118 irrespective of mode of inoculation. Hence foliar method can be used in screening cowpea against bacterial blight.

Farmers are advised therefore to make use of cowpea resistant variety which cannot be affected by the disease and or employ cultural practices such as crop rotation to reduce the disease incidence.

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