Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 2(10) pp. 276-282, October, 2013.
Available online http://garj.org/garjas/index.htm

Full Length Research Paper

Screening of some genotypes of cowpea (*Vigna unguiculata* L. (Walp) against Bacterial Blight caused by *Xanthomonas compestris* Pv. Translucens

*** Hayatu[1] M., [2]Kutama, A. S, [2]Aisha, W.A., and S., [3]Nura**

[1]Department of Plant Biology, Bayero University, Kano
[2]Department of Biological Sciences, Fedaral University, Dutse
[3]School of Remedial Sciences, Ahmadu Bello University, Funtua

Accepted 31 October, 2013

An experiment was conducted in the screen house of IITA, Kano State to screen some cowpea genotypes for bacterial blight. The following parameters were measured during the trial; chlorophyll content, number days to 50% flowering, disease incident (%) and disease severity. From the results it is clear that at 42 days after inoculation (when the disease was more severe) variety IT08K-180-11 recorded the least chlorophyll SPAD values and IT07K-187-55 had the highest chlorophyll SPAD values. The results also showed that bacterial blight does not have effect (p≥0.05) on number of days to flowering of the cowpea genotypes because the flowering days of both inoculated and non inoculated varieties were very close. It was also concluded that the disease incidence was not directly related to the disease severity because some genotypes with 100% incidence only have 2% severity by rank the scale of 1-5 in the severity score and some genotypes have 4 (severe) while some genotypes had 1 (free) in the severity score.

Keywords: Bacterial Blight, Cowpea, Screening,

INTRODUCTION

Cowpea *(Vigna unguiculata* (L.) Walp.) Is an important crop grown mainly in the savanna zones of the tropics and sub tropics. It is grown for its grains, green pods and leaves and is also used for forage in Nigeria (Oyekan, 1977). World cowpea production in 1994 was estimated at 3.53 million metric tonnes of which 1.75 million metric tonnes was produced in Nigeria (Adejumo, 1997). In West Africa, cowpea is second in importance after groundnut, with Nigeria accounting for over 70% of the total world production (Singh and Ajeigbe, 2002). Unfortunately, in Africa, most of the cowpea is produced under small scale subsistence agriculture where low grain yield of about 88 kg ha^{-1} may be the maximum obtained in the lowland tropics of West Africa (Summerfield *et al.,* 1985).

Cowpea (*Vigna unguiculata* L. Walp) is a major source of protein and of considerable importance for human nutrition in tropical regions of Africa (Gowda *et al.,* 2000). Cowpea contains about 24% protein, 62% soluble carbohydrates and small amounts of other nutrients (Elias *et al.,* 1964). Cowpea constitutes the cheapest source of dietary protein

*Corresponding Author's Email: haymoh2001@yahoo.com; Tel:+2348065504101

for low income sector of the population (Rachie, 1985). It was further observed that of all the leguminous crops, cowpea appears to be one of the most important in sustainable soil fertility management (IITA, 1990), as it can fix up to 88 kg Nha-1 (Fatokun *et al.,* 2002).

Cowpea yields, especially among the subsistence farmers, are generally low due to several factors, but diseases such as leaf virus, bacterial blight and smut remain major constraints to sustained high cowpea grain yields (Soyinka *et al*., 1997).

Bacterial blight induced by *Xanthomonas axono-podis* pv *vignicola* has been reported to have wide spread in areas where cowpea is grown (Ajeigbe *et al.,* 2008). Bacterial blight is caused by *Xanthomonas campestris* pv. *vignicola* (Patel 1981). The symptoms of bacterial blight of cowpea do not vary although the description may sound a little different from different authors (Preston 1949; Patel and Jindal 1970; Williams 1975; Emechebe and Shoyinka,1985). Preston (1949) described three types of symptoms: blight, pod symptoms and canker. In the blight phase, water-soaked spots appear on the cotyledon and primary leaves of young seedlings. They begin to turn reddish brown after a few days and then to light yellow-brown as the infected parts dry out. The spots range from the size of a pinpoint to nearly half an inch (1.25 cm) in diameter. Spots can enlarge and cover more of the surface of the older leaves. Severely blighted leaves usually drop from the plant. In pod phase, spots appear on pods raised or swollen, reddish-brown and distorted. Symptoms may be masked on pods with dark coloration. In severe cases there is poor pod development, most of the seeds are shriveled and will not germinate. In the canker phase, reddish brown swollen cankers or elongated cracks appear anywhere from the ground line to the top of the plant. It is very common for severely cankered stems to break just above the crown. Stem cankers are usually found on older plants, but may be present on stems of younger plants as well in which case the plants seldom reach maturity.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the screen house of the International Institute of Tropical Agriculture (IITA) Kano station, Kano state of Nigeria in October 2011 to Feb 2012. Kano is located in sudan savannah on longitude 08 31E and latitude 12 03N and an altitude of 1500m (Kowal and Knabe, 1972)

Seed Collection

Fifteen genotypes of cowpea were collected from seed store of IITA, Kano office. The genotypes are; IT08K-180-11, IT07K-213-3-1, IT07K-187-55, IT04K-267-8, IT08K-180-5, IT09K-269-1, IT08K-138-6, IT95K-238-3, IT09K-321-1, IT97K-499-35, IT99K-573-1-1, IT98K-506-1, IT97K-568-18, IT98K-131-2, IT97K-1092-2.

Pot Preparation

The soil used was mixed with animal dung at the ratio of 3:1 i.e 3 parts of the soil mixed with 1 part of the animal dung (local fertilizer). The size of the pot used is 17cm length and 17cm breadth. They 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 some hours before planting.

Seed Sowing

A small hole was dug in the pot and 3 seeds per hole were sown and buried under the soil. Iirrigation was used as a water supply to the plants.

Experimental Design

The experiment was set up in a complete randomized block design with three replications. The genotypes were planted in pots with each replication having 30 pots respectively, making a total of 90 pots. Two treatments were done i.e inoculated and non inoculated with bacteria.

Isolation of the Inoculum

The inoculums were from infected cowpea leaves obtained from Minjibir farm of IITA and cultured on nutrient agar (NA) and incubated at 28^0C for 48 hours, A yellow colony was observed, picked and recultured for 24 hours to obtain a pure sample of the bacterium. The yellow colony was picked and placed on a glass slide, gram stained and viewed under electron microscope. A rod shaped gram negative was obtained indicating the presence of the bacterium in the leave.

Culturally, yellow colony indicates the presence of the bacteria (*Xanthomonas campestris* **pv.** *Vignicola)* and microscopically gram negative rod shaped confirms the presence of the bacteria. (Tika and Sundar, 1989).

Inoculation of Seedlings

Stem injection artificial inoculation technique described by Sundaman and modified by Kuatama *et al*. (2011) was used to inoculate all the cowpea genotypes. About 1ml of the bacteria suspension was introduced into the plant with a pediatric syringe by inserting the needle gently into the stem or the growing tissues of the plant, while carefully

Table 1. Mean square values of chlorophyll SPAD at different days after inoculation

		Mean square (chlorophyll Spad)			
Source of Variation	df	21d	28d	35d	42d
Genotypes	14	448.58**	481.67**	460.12**	343.91**
Treatment	1	1622.23**	2401.47**	4091.18**	8263.71**
Genotypes X Treatment interaction	14	138.83**	128.7**	109.06**	126.92*
Residual	58	25	33	33.5	45.02

** Significant at p<0.0001, * significant at P<0.003

holding and supporting the whole plant with a hand to prevent damage of the plant tissue (Kutama *et al*., 2010).

DATA COLLECTION

Chlorophyll content

Chlorophyll content was taken using Minolto chlorohpyll SPAD 502 meter. Measurements were done by randomly selecting any three leaves, average SPAD reading were recorded (Feruse and Arkersivora, 2001).

Number of days to flower opening

First day of flowering, was taken by counting from the day the genotypes were planted to the day the first flower appeared and 50% flowering was done also by counting from the day the varieties were planted to when five or six flowers appear (Davis *et al.,*1991)

Disease incidence

Disease incidence was assessed by counting the number of diseased plant multiply by 100 and divide by the total number of plant per pot (Kutama *et al*., 2010).

$$\text{Incidence \%} = \frac{\text{Number of disease plant}}{\text{Total number of plant per pot}} \times 100$$

Severity assessment

Disease severity was taken using visual scale of 1-5 (Kutama *et al*., 2010).
Where: 1= free
2 = slightly severe
3= moderately severe
4= severe
5= very severe

Statistical Analysis

The results obtained were analyzed using analysis of variance (ANOVA). Mean separation was done by least significant difference (LSD) at 5%.

RESULTS AND DISCUSSION

The mean square for the chlorophyll SPAD at different days after inoculation showed that geotypes, treatmet and genotypes by treatment interaction were significant at p<0.0001(Table 1)

Total chlorophyll content according to the results obtained showed that at 42 days after inoculation, when the disease was more severe, genotype IT08K-180-11 recorded least chlorophyll content while IT07K-187-55 had the highest chlorophyll content (Tabel 2). Reduction in chlorophyll content could probably be due to destruction of spongy and intravascular tissue of the plant by the organism, and it could be due to susceptibility of the genotype to the disease. While high chlorophyll content recorded in IT07K-187-55 could be due to its resistance to the disease. These findings agrees with that of Allen *et al.* (1999) who stated that reduction in chlorophyll content in cowpea leaves could be due to the bacterial blight and susceptibility of the genotype. Also Prashant *et al,* (2009) reported that high chlorophyll contents were obtained in the leaves of tolerant genotypes which will in turn increased grain yield as a result of higher photosynthetic activities.

The mean square for number of days to first flower opening and number of days to 50% flower are presented in Table 3. The result showed that number of days to first flower openong were significant (p<0.0001) at genotypes, treatments and genotypes by treatment interactions.

Number of days to flowering, according to the results showed that the first day of flowering and 50% flowering for inoculated and control genotypes are very close, probably because bacterial blight does not affect the flowering date of cowpea. According Bhattarai *et al,* (1996) flowering

Table 2. Chlorophyll content (SPAD) of some genotypes of cowpea at differents days after inoculation

	21 D		28 D		35 D		42 D	
genotypes	unino	ino	unino	ino	unino	ino	unino	ino
IT04K-267-8	60.03	47.40	60.73	46.08	60.77	47.77	64.23	51.04
IT07K-187-55	71.07	58.03	71.08	58.03	77.05	63.93	78.73	60.77
IT07K-213-3-1	65.47	55.23	66.97	55.43	68.93	58.23	74.03	51.43
IT08K-138-6	75.03	63.97	78.53	63.87	79.05	59.57	79.04	46.43
IT08K-180-11	60.33	56.07	62.77	54.47	64.83	50.83	69.00	42.83
IT08K-180-5	65.07	64.33	66.03	64.03	67.97	59.47	70.04	56.07
IT09K-269-1	69.53	67.02	70.93	68.03	73.23	67.97	74.05	55.07
IT09K-321-1	65.33	56.06	69.37	55.13	70.23	51.07	71.02	45.04
IT95K-238-3	53.02	56.33	56.43	57.01	57.87	56.13	62.01	44.07
IT97K-1092-2	52.93	49.63	52.93	50.97	54.77	50.03	58.03	53.27
IT97K-499-35	65.08	66.43	68.04	68.05	72.47	60.93	77.01	54.57
IT97K-568-18	61.83	54.09	64.73	61.23	64.08	50.67	66.27	53.07
IT98K-131-2	64.83	53.77	68.07	53.04	69.93	53.00	73.87	46.47
IT98K-506-1	55.93	49.04	57.05	48.07	59.06	49.23	59.27	59.06
IT99K-573-1-1	59.03	53.04	61.01	51.09	62.03	56.07	63.43	46.13
Mean	63.05	54.56	65.15	54.82	66.98	53.05	69.44	50.27

Table 3. Mean Square values of days to first flower opening and days to 50% flowering of some cowpea genotypes at different inoculation of bacterial blight.

source of variation	df	first flower	50% flower
Genotypes	14	1056.90**	1368.34**
Treatment	1	51.38**	144.4^{ns}
Genotypes X Treatment	14	116.09**	168.04**
Residual	58	34.19	50.12

** significant at $p<0.0001$, ^{ns} not significant

Table 4. Number of days to flowering of some genotypes of cowpea inoculated with bacterial blight.

	Days to first of Flowering		Days to 50% flowering	
genotypes	unino	ino	unino	ino
IT04K-267-8	50.67	50.33	58.00	60.00
IT07K-187-55	46.00	48.67	61.00	56.67
IT07K-213-3-1	49.00	48.67	57.00	57.00
IT08K-138-6	49.33	52.67	57.00	56.67
IT08K-180-11	51.67	49.67	54.67	56.67
IT08K-180-5	0.67	0.00	0.33	0.00
IT09K-269-1	54.00	54.33	62.00	62.33
IT09K-321-1	54.00	54.00	63.00	62.33
IT95K-238-3	47.67	51.00	57.33	56.00
IT97K-1092-2	51.00	48.33	62.67	60.33
IT97K-499-35	44.33	44.33	49.00	49.67

Table 4. Continue

IT97K-568-18	45.33	44.33	48.33	50.33
IT98K-131-2	34.00	37.33	54.00	54.33
IT98K-506-1	44.00	43.33	46.67	48.67
IT99K-573-1-1	44.33	46.33	51.00	51.00
Mean	44.04	42.89	52.13	49.6

unino; uninoculated, ino; inoculated.

Table 5. Incidence of bacterial blight of some genotypes of cowpea

Genotypes	unino	Ino
IT04K-267-8	0.0	100.0
IT07K-187-55	0.0	100.0
IT07K-213-3-1	0.0	100.0
IT08K-138-6	0.0	66.7
IT08K-180-11	0.0	83.3
IT08K-180-5	0.0	50.0
IT09K-269-1	0.0	83.3
IT09K-321-1	0.0	100.0
IT95K-238-3	0.0	100.0
IT97K-1092-2	0.0	0.0
IT97K-499-35	0.0	100.0
IT97K-568-18	0.0	0.0
IT98K-131-2	0.0	0.0
IT98K-506-1	0.0	66.7
IT99K-573-1-1	0.0	100.0
Mean	0.0	70.0
L S D (5%)		
G		12.39
T		4.52
T by G interaction		17.52

LSD, Least significant difference, G; genotype, T; treatment, unino; uninoculated, ino; inoculated.

Table 6. Severity scores (1-5) of some genotypes of cowpea against bacterial blight infestation

Genotypes	unino	ino
IT04K-267-8	1.00	3.00
IT07K-187-55	1.00	2.67
IT07K-213-3-1	1.00	2.67
IT08K-138-6	1.00	4.00
IT08K-180-11	1.00	3.00
IT08K-180-5	1.00	3.00
IT09K-269-1	1.00	3.67
IT09K-321-1	1.00	2.00
IT95K-238-3	1.00	4.00
IT97K-1092-2	1.00	0.33

Table 6. Continue

IT97K-499-35	1.00	2.00
IT97K-568-18	1.00	1.00
IT98K-131-2	1.00	1.00
IT98K-506-1	1.00	2.00
IT99K-573-1-1	1.00	2.00
Mean	1.00	2.42
L S D(5%)		
G		0.4322
T		0.1578
T by G interaction		0.6112

LSD, Least significant difference, G; genotype, T; treatment, unino; uninoculated, ino; inoculated.

difference of cowpea varieties may be due to varietal character, sowing time and growing environment

The research shows that some varieties such as IT04K-26-8, IT07K-187-55, IT07K-213-3-1, IT08K-180-11, IT90K-269-1, IT09K-321-1, IT95K-238-3, IT97K-499-35 and IT99K-573-1-1 all shows 100% incidence and could be susceptible or tolerant bacterial blight. IT08K-138-6, IT08K-180-5, IT97K-499-35, IT99K-573-1-1 all shows 50% incidence and could also be tolerant or resistance to bacterial blight while IT097K-1092-2, IT97K-568-18, IT98K-131-2 shows 0% incidence because they are resistance to bacterial blight and all the varieties that are not inoculated remain blight free and this finding agree with Okechukwu *et al*,(2010) who stated that disease incidence in plants derived from inoculated seeds increased with time while plants from uninoculated seeds remained blight-free.

According to the result the disease severity is high in IT08K-138-6, IT95K-238-2 and they are said to be susceptible to bacterial blight. IT04K-267-8, IT08K-180-11, IT08K-180-55, IT09K-269-1 are moderately severe and can be said to tolerant to bacterial blight. IT07K-187-55, IT07K-213-3-1, IT09K-321-1, IT97K-499-35, IT98K-506-1, IT99K-573-1-1, are slightly severe and they could probably be resistance to bacterial blight and IT97K-1092-2, IT97K-568-18, IT98K-131-2 are free and they are said to be resistance to bacterial blight (Adejumo, 1997).

CONCLUSION

From the result, at 42 days when the disease becomes more severe, least chlorophyll content was recorded in susceptible genotypes. IT08K-180-11 and IT07k-187-55 had the highest chlorophyll content. The result also shows that bacterial blight does not have effect on the number of days to flowering of cowpea, because there is no significant difference between the inoculated and non inoculated genotypes in the flowering period. IT08K-138-6, IT95K-238-2 recorded the highest severity score of 4%(severe) in the scale of 1-5. It can also be concluded that the disease incidence is not directly related to the severity because some varieties with 100% incidence only have 2% in the scale of 1-5 in the severity score.

REFERENCES

Adejumo TO (1997). Identification, incidence, severity and methods of control of the causal organism of false smut disease of cowpea *Vigna unguiculata*(L.) Walp. PhD. Thesis. University of Ibadan, Ibadan, Nigeria.

Adejumoh TO (1997). Nutritional value of cowpea *Vigna unguiculata*(L.) Walp. PhD. Thesis. University of Ibadan, Ibadan, Nigeria

Ajeigbe HA, Singh BB, Amechebe AM (2008). Field evaluation of improved cowpea lines

Allen DJ, Neberic CLN, Raji JA (1999). Screening for resistance to the diseases of the African savannas. Tropical pest management 27: Pp: 137-133

Bhattarai SP, Subedi PP (1996). Varietal trial on vegetable pea and varieties by plant density trials on vegetable bean and cowpea at outreach research sites during 1993/94 season. *LARC Working Paper No. 96/35.* Lumle Agriculture Research Centre, Kaski.

Davis DW, Oelke EA, Oplimger ES, Doll DJ (1991). Alternative plant and animals products. Programs in information exchange and research In: Alternative field crop manual. Janick and J.E Simon(eds). New crops. John Wiley and Sons New York, Pp: 133-143. USA.

Ellas LG, Colindres R, Bressani R (1964). The nutritive value of eight varieties of cowpea *(Vigna sinensis).* J. Food Sci., 29: 118-122.

Emechebe AM, Shoyinka SA (1985). Fungal and bacterial diseases of cowpeas in Africa. In: Cowpea Research, Production and Utilization. Singh SR and Rachie KO (eds.). John Wiley & Sons Ltd. Great Britain: 173-197. IAR 1980 Soil Characteristics of Agricultural Research Station Ahmadu Bello University, Minjibir Local Government Area Kano.

Fatokun CA, Tarawali SA, Singh BB, Kormarawa PM, Tamo M (eds) (2002). Challenges and opportunities for enhancing sustainable cowpea production. IITA. Ibadan, Nigeria.

Gowda BS, Miller JL, Rubin SS, Sham DL, Timko MP (2000). Isolation, sequencing and mapping of resistnace gene analogs from cowpea (Vigna unguicualta L. Walp). In: C.A Fatokun, S.A. Tarawali, B. B. Singh, P. M. Kormowa and M. Tawo (Eds).Challenges and opportunities for enhancing sustainable cowpea production. Proc. of the world cowpea conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 4-8 September, 200 IITA, Ibadan, Nigeria. Pp: 167-184.

IITA (1990). Manual for resistance to bacterial blight, virus and Striga under natural infestation in the West African; Grain legumes improvement work shop, IITA Ibadan Nigeria Pp: 270-273.

Kowal JM, Knabe DT (1972). An Agroclimatiological Atlas of the northern states of Nigeria with explanatory notes. Ahmadu Bello University Zaria, Nigeria. ABU Press 128 PP:

Kutama AS, Emechebe AM, Aliyu BS (2010b). Incidence and distribution of head and loose smut s of sorghum (sorghum bicolor L Moench) in Nigeria Sudan savanna. Bayero journal of pure and applied science, 3 (2):142-147

Okechukwu RU, Ekpo EJA, Okechukwu OC (2010). African J. Agric. Res. Vol. 5 (6), pp: 431-435,

Oyekan PO (1977). Reaction of some cowpea varieties to *Fusarium oxysporum* f.sp. *tracheiphilum*

Patel PN (1981). Pathogen variability and host resistance in bacterial pustle disease of cowpea in Africa. Tropical Agriculture (Trinidad) 58(3): 275-280

Patel PN, Jindal JK (1970). Studies on resistance of bacterial disease in India: II. Resistance in cowpea to bacterial blight (canker) disease. Indian Phytopathology Society Bulletin. 6: 28- 34. savannas

Prashant R, Ninganyr BT, Chetti MB, Hiremath SM (2009). Karnataka J. Agric. Sci. volume 22 no 2

Preston DA (1949). Bacterial canker of cowpeas. Oklahoma Agricultural Experiment Station Bulletin No. B-334, 11 pp:

Rachie KO (1985). Introduction of cowpea research, production and utilization, edited by Singh, S. R. Rachie, John Wiley and Sons, Chichester, U. K.

Singh BB, Ajeigbe HA (2002). Improving cowpea-cereal based systems in the dry Savannas of West Africa. In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4-8 September 200. IITA, Ibadan Nigeria. pp. 278-286

Soyinka SA, Thottappilly G, Anno-Nyako FO (1997). Survey of virus incidence and distribution in Nigeria. International Journal of Pest Management. 43(2): 127-132.

Summerfield RJ, Pate JS, Roberts EH, Wien HC (1985). The physiology of cowpeas. In: Cowpea research, production and utilization. Singh SR and Rachie KO (eds.). John Wiley and Sons Ltd. Great Britain: 65-101. Williams RJ 1975 Diseases of cowpea *(Vigna unguiculata* (L.) Walp.) in Nigeria. PANS 21(3):

Tika B, Adhikari S, Shrestha M (1989). Laboratory manual of plant pathology, Institute of agriculture and animal science.

Williams RJ (1975). Diseases of cowpea (*Vigna unguiculata* (L.) Walp.) in Nigeria. Pest Articles and News Summaries 21(3): 253-267