EVALUATION OF *BLACKEYE COWPEA MOSAIC VIRUS* PATHOGENICITY IN SOYBEAN

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ABSTRACT

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Ten soybean lines were investigated under screenhouse conditions for resistance to Blackeye cowpea mosaic virus (BlCMV) infection. The experiments were laid out in a completely randomised design with five replications. Seedlings were inoculated with the virus at 10 days after sowing by mechanical transmission. Observations were made on percentage disease incidence, symptom severity, growth and yield parameters. Symptom scores were subjected to Area Under Disease Progress Curve (AUDPC) while other parameters were subjected to analysis of variance (ANOVA) at 5 % probability level. All the inoculated plants exhibited disease symptoms (100 %) but AUDPC identified soybean line TGX 2007 - 1F as the most tolerant to BlCMV infection. Among the BlCMV-inoculated plants, highest seed weight (4.7 g per plant) was produced by TGX 1990 – 46F; which also had the lowest seed weight reduction (55.3 %). The genotype TGX 1990 – 46F which produced the highest seed yield under BlCMV infection pressure could be subjected to further yield trials and selected for cultivation in the absence of resistant varieties.

Keywords: BlCMV, growth parameters, yield, infection pressure, disease resistance, soybean

INTRODUCTION

Soybean is one of the major industrial and food crops grown in Africa. A lot of soybean is produced in Nigeria for diverse domestic usage and it is a good source of vegetable oil in the international market. Soybean contains approximately 20 % oil and 34-36 % protein for human consumption (Ajao *et al*., 2012) and also contributes substantially to animal feed sources in the country. It is estimated that 2 % of soybean production is consumed by humans directly as food (Goldsmith, 2008). About 95 % of the oil fraction in soybean is consumed as edible oil while the rest is used for industrial purposes including production of cosmetics, paint removers and plastics (Liu, 2008). Soybean has several other beneficial applications such as dietary supplements for diabetics (Villegas *et al*., 2008), aiding women suffering bone loss (Chen *et al*., 2003), use in weight loss (Maskarinec *et al*., 2008), increasing iron in the blood (Murray-Kolb *et al*., 2003), reducing cholesterol (Rosell *et al*., 2004), and reducing cancer risks (Hamilton-Reeves *et al.*, 2007). The crop can be successfully grown in many States in Nigeria using low agricultural input for so many reasons. One of the remarkable attributes of soybean is its ability to fix atmospheric nitrogen into the soil. Soybean residue (haulm) also improves soil condition, and on decay supplies nutrients to subsequent crops. This is a common practice for soil fertility improvement in the cereal based cropping systems in the Guinea savanna agroecology of Nigeria (Yusuf *et al*., 2006). In the recent years, soybean cultivation has expanded within the savanna agro-ecological zone owing to introduction of the promiscuous soybean cultivars developed by the International Institute of Tropical Agriculture (IITA) (Osunde *et al*., 2003).

Nigeria is the largest producer of soybean in sub-Saharan Africa (SSA), followed by South Africa. In the region, yield per unit area is generally low (1.1 t/ha on farmers fields) (IITA, 2009). Poor yield has been attributed to several factors affecting production activities among these are diseases. Soybean productivity is constrained by diseases such as rust, red leaf blotch, frog-eye leaf spot, bacterial pustule and bacterial blight. Insect pests of economic importance include pod (stink bugs) and foliage feeders, bean flies and nematodes. Other problems include pod shattering which reduces seed longevity, production and distribution difficulties. Dual-purpose improved varieties of soybean are not readily available in many parts of Africa to increase seed yield. Besides, in many countries only a small market exists for soybean (IITA, 2014). The major viruses infecting legumes in sub-Saharan Africa include *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea yellow mosaic virus* (CYMV) genus *Comovirus*, *Southern bean mosaic virus* (SBMV) genus *Sobemovirus*, *Cowpea mottle virus* (CPMoV) genus *Carmovirus*, *Cowpea golden mosaic virus* (CPGMV) genus *Bigeminivirus*, *Cucumber mosaic virus* (CMV) genus *Cucumovirus*, *Cowpea mild mottle virus* (CPMMV) genus *Carlavirus*, *Sunn-hemp mosaic virus* (SHMV) genus *Tobamovirus and Blackeye cowpea mosaic virus* (BlCMV). Soybean is also infected by *Soybean mosaic virus* (SMV) genus *Potyvirus*, *African soybean dwarf virus* (SbDV) (Taiwo and Akinjogunla, 2006; Alegbejo, 2015). These viruses infect soybean although at varying degree of pathogenicity. *Blackeye cowpea mosaic virus* just like other viruses can induce high yield reductions in susceptible cultivars. From time immemorial, breeding and selection for virus-resistant or tolerant cultivars is an effective and sustainable management strategy. Genetic resistance is highly valuable in the control of economically important plant virus diseases as it prevents replication or symptom expression (Salaudeen and Aguguom, 2014). Therefore, evaluation of available germplasm for possible sources of resistance genes is of paramount importance. Availability of sources of BlCMV resistance would be highly valuable to soybean breeders trying to produce varieties that are both virus-resistant and high-yielding. Undoubtedly, this would provide substantial relief to a significant proportion of food insecure populations in the region. The present study was conducted to determine the performance of selected soybean lines as influenced by BlCMV disease.

MATERIALS AND METHODS

Soybean lines and virus isolate

Seeds of 10 soybean lines (TGX 1951 - 3F, TGX 1990 - 46F, TGX 1990 - 57F, TGX 2005 - 1F, TGX 2007 - 1F, TGX 2007 - 3F, TGX2009 - 1F, TGX 2009 - 9F, TGX 2012 - 1F, and TGX 2013 - 1F) were obtained from the Breeding Unit of the National Cereals Research Institute (NCRI), Badeggi in Niger State, Nigeria. These genotypes were among the early maturing lines designated for genetic improvement against major diseases of soybean. The isolate used was obtained from the BLCMV stock at the Department of Crop Production, Federal University of Technology, Minna, Niger State. It was the descent of a severe BlCMV virus previously described by Taiwo (2001). The isolate was maintained in the cowpea cultivar Ife Brown by continuous screenhouse transfer through sap transmission. Virus extract for mechanical inoculation was prepared by grinding the BlCMV-infected leaves with sterilized pestles and mortars in chilled extraction buffer, pH 7.2 (0.1 M sodium phosphate dibasic, 0.1 M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water). Carborundum powder (600 mesh) was added to the extract and this was applied to the youngest (10-day-old) 1 to 2 leaves (first trifoliate) during mechanical inoculation. Virus identity was confirmed using Enzyme-Linked Immunosorbent Assay (ELISA) according to Kumar (2009). The polyclonal antibody developed against Nigerian BlCMV isolate was used.

Treatments/experimental design and seedling inoculation

The 10 soybean lines which constituted the treatments were laid out in a completely randomised design with five replications in a screenhouse. Seeds were sown in plastic pots (23 diameter) filled with heat sterilized soil and seedlings were thinned to five plants per pot after emergence. Plants were inoculated with the virus at 10 days after sowing. The protocol for extracting virus and inoculation were as described above. Uninoculated plants of each genotype served as controls. Plants were assessed for BlCMV disease incidence and severity, growth, and yield characters. Disease incidence was expressed as percentage of the inoculated seedlings showing BlCMV disease symptoms at 7 and 14 days after inoculation. Symptom severity was rated on a visual scale designed by Arif and Hassan (2002), in which 1 = no symptoms (apparently healthy plant); 2 = slightly mosaic leaves (10 – 30 %); 3 = mosaic (31 – 50 %) and leaf distortion; 4 = severe mosaic (51 – 70 %), leaf distortion and stunting; 5 = severe mosaic (>70 %), stunting and death of plants. The experiment was repeated twice.

Statistical analysis

The data on BlCMV severity scores were subjected to Area Under Disease Progress Curve (AUDPC). Other parameters were subjected to analysis of variance (ANOVA) using the General Linear Model (PROC GLM) procedure of SAS (2008) at $p<0.05$. Means separation for significant ANOVA was accomplished using the Least Significant Difference (LSD) or Duncan's Multiple Range Test (DMRT), where applicable. Cluster analysis was performed to determine the relationships among the virus-inoculated soybean lines, based on reductions in their growth and yield data. Similarity coefficients among soybean lines were calculated according to Nei and Li (1979). Dendogram of their relationship was inferred from similarity matrix and Unweighted Pair Group Method with Arithmetic (UPGMA) means clustering procedure.

RESULTS AND DISCUSSION

Incidence and severity of BlCMV infection

Typical leaf mottling and chlorosis of BlCMV disease were observed on the infected plants 6 days after inoculation. Symptoms were observed on the newly emerging leaves of the infected plants, and by two weeks after inoculation, all the inoculated plants were infected. Noninoculated plants were apparently symptomless. Severity of infections was variable but AUDPC estimates indicated that the differences among the soybean lines were not significant (Fig. 1). Despite this observation, disease severity was most pronounced in the inoculated plants of TGX2009 – 1F. The intensity of virus infection was lowest in TGX 2007 - 1F. Earliness to first symptom appearance implied a short incubation period for the virus in susceptible host plants. However, 100 % disease incidence observed in the inoculated plants indicated a virulence and pathogenicity of the isolate used. It also revealed that TGX2009 – 1F was the most susceptible to BlCMV infection, whereas TGX 2007 - 1F was the most tolerant. Studies have shown that one of the factors controlling the level of symptoms elicited by virus-inoculated plants is their genetic background. Therefore, the observed symptoms could be linked to the inherent attributes of the evaluated genotypes. This agrees with the results obtained by Goenaga *et al.* (2011) when some cowpea genotypes were tested with BlCMV.

Effects of BlCMV infection on the growth and yield of soybean lines

The effects of BlCMV infection on plant height and number of branches per plant are presented (Table 1). In all the evaluated soybean lines, uninoculated plants were significantly ($P < 0.05$) taller than the virus-infected plants. Uninoculated plants exhibited normal growth while the BlCMV-inoculated were stunted. Height reduction was most adversely observed in TGX 1990 – 46F. However, height reduction in this genotype was not statistically significant to those observed in TGX 1990 – 57, TGX 2005 – 1F, TGX 2009 – 9F and TGX 2012 – 1F. Similarly, height reduction observed in TGX 1951 - 3F was not significantly different from that in TGX 2007 - 1F and TGX 2013 - 1F. Conversely, the soybean line TGX 2007 - 3F suffered the lowest height reduction. Difference in number of branches per plant between inoculated and uninoculated plants was as observed. However, TGX 1951 - 3F (a variety released in Nigeria) suffered the highest reduction in number of branches per plant while TGX 2013 - 1F showed the lowest. These observations reveal that infected plants were unable to make optimum use of the growth resources. This finding agrees with Bhaduri *et al.* (2014) who stated that the pathogen often hinders nutrient uptake, distribution, and assimilation in virus infected plants.

Fig. 1 Area under disease progress curve in soybean lines inoculated with *Blackeye cowpea mosaic virus*
Soybean 1 = TGX 1951 - 3F; 2 = TGX 1990 - 46F; 3 = TGX 1990 - 57F; 4 = TGX 2005 - 1F; 5 = TGX 2007 - 1F; 6 = TGX 2007 - 3F; 7 = TGX2009 - 1F; 8 = TGX 2009 - 9F; 9 = TGX 2012 - 1F; 10 = TGX 2013 - 1F

Table 1: Plant height and number of branches per plant in soybean lines inoculated with *Blackeye cowpea mosaic virus* and uninoculated ones

Soybean line	Plant height at harvest (cm)		Height reduction (%)	Branches per plant (no.)		Reduction in branches (%)
	Uninoculated	Inoculated		Uninoculated	Inoculated	
TGX 1951 – 3F	97.5[a]	46.0[b]	52.8[b]	20[a]	7[b]	65.0[a]
TGX 1990 – 46F	108.5[a]	34.0[b]	68.7[a]	16[a]	7[a]	56.3[b]
TGX 1990 – 57F	111.0[a]	40.5[b]	63.5[a]	14[a]	7[a]	50.0[c]
TGX 2005 – 1F	113.5[a]	36.5[b]	67.8[a]	14[a]	5[a]	64.3[a]
TGX 2007 – 1F	88.5[a]	41.5[b]	53.1[b]	12[a]	5[a]	58.3[b]
TGX 2007 – 3F	87.5[a]	47.5[b]	45.7[c]	15[a]	6[a]	60.0[a]
TGX2009 – 1F	110.5[a]	49.0[b]	55.7[b]	14[a]	6[a]	57.1[b]
TGX 2009 – 9F	124.0[a]	41.0[b]	66.9[a]	15[a]	5[a]	66.7[a]
TGX 2012 – 1F	99.5[a]	35.0[b]	64.8[a]	12[a]	5[a]	58.3[b]
TGX 2013 – 1F	126.0[a]	54.5[b]	56.7[b]	12[a]	6[a]	50.0[c]

Means followed by dissimilar letters within the row, and along the column differ significantly (p<0.05) by the Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT), respectively

Blackeye cowpea mosaic virus influenced number of days to 50 % flowering significantly. Flowering was earlier in uninoculated plants compared with the virus-inoculated ones (Table 2). Flowering in TGX 2012 - 1F was not seriously affected by the viral attack, though the effect was not significantly different from those observed in virus-inoculated genotypes TGX 1951 - 3F and TGX 2013 - 1F. Number of days to 50 % flowering was prolonged in infected TGX 1990 – 46F and TGX 2007 - 3F. No significant difference was observed for number of days to 50 % flowering in TGX 2005 - 1F and TGX2009 – 1F. Similarly, BlCMV-infected TGX 1990 – 57F and TGX 2007 - 1F did not show significantly difference for number of days to 50 % flowering. Number of days to flowering is an important agronomic trait in soybean production because it plays a critical role in other physiological activities such as number of days to pod formation, maturity and harvesting. Therefore, a genotype with prolonged days to flowering may not be widely adopted by small scale subsistence farmers who usually cultivate for immediate consumption. This is in agreement with Sanginga *et al*. (1999) who reported massive adoption of early maturing soybean cultivars by farmers in the study area.

As observed in other yield components, there was a significant reduction in pod formation in BlCMV-inoculated plants regardless of the genotype (Table 2). Pod reductions in infected plants were generally high (>50 %) relative to uninoculated plants. However, BlCMV-inoculated TGX 1951 - 3F was the most adversely affected. In all the genotypes, uninoculated plants produced significantly higher number of pods compared with their virus-inoculated counterparts. The result observed that uninoculated plants of TGX 1951 - 3F produced the highest number of pods per plant, while its inoculated counterpart suffered the highest reduction in pod production. This supports Chianu *et al*. (2006) who reported that number of pods was one of the major reasons for adopting improved soybean varieties. Also, uninoculated plants generally produced three seeds per pod whereas the virus-infected plants produced one or two seeds per pod. Most of the infected plants produced small and deformed seeds. In contrast, uninoculated plants produced large seeds with normal shape. The difference in seed weight between the uninoculated and inoculated plants was significant in all the evaluated genotypes. Among the BlCMV-inoculated, the highest seed weight was observed in TGX 1990 – 46F; which also had the lowest reduction of seed weight (Table 3). The obvious variation in seed production and weight between BlCMV-infected and uninoculated plants was probably due to cumulative effects of virus infection in the former. Thus, the ability of diseased plants to photosynthesize was drastically retarded and this affected the amount of assimilates translocated to the seeds during pod filling. This is in agreement with the findings of Udayashankar *et al*. (2010) in cowpea seeds infected with *Bean common mosaic virus* strain *blackeye cowpea mosaic* (BCMV-BlCM).

Cluster analysis (Fig. 2) revealed that TGX 1990 - 46F, TGX 1990 - 57F, TGX 2005 - 1F, TGX 2009 - 9F, TGX 2012 - 1F, and TGX 2013 - 1F belonged to the same cluster but TGX 1990 - 46F and TGX 1990 - 57F were more closely related. Similarly, the genotypes TGX 2007 - 1F, TGX 2007 - 3F, and TGX2009 - 1F constituted another group. However, a stronger relationship existed between TGX 2007 - 1F and TGX 2007 - 3F. In contrast, TGX 1951 - 3F was the only member of the third cluster. The different groupings observed among the inoculated plants are obvious indicators of the strong relationships among the members, in term of genetic background and response to BlCMV pathogen attack. This information provides for a rapid method of selection, especially if the evaluated germplasm contains known resistant and susceptible genotypes. Thus, the various clusters generated could be used to identify genotypes with average attributes similar to the checks.

The results herein clearly revealed the susceptibility of the tested soybean lines to BlCMV infection. Therefore, soybean farmers should adopt cultivation of high-yielding BlCMV-resistant varieties in order to avert the significant yield losses posed by the virus. However, the genotype TGX 1990 – 46F which performed best under BlCMV infection pressure could be cultivated in the absence of resistant varieties. Further studies however could be conducted to the response of TGX 1990 – 46F to other major soybean viruses.

Table 2: Number of days to flowering and pod per plant in soybean lines inoculated with and uninoculated *Blackeye cowpea mosaic virus*

	Days to flowering (no.)		Increased days to flowering (no.)	Pod per plant (no.)		Pod reduction (%)
Soybean line	Uninoculated	Inoculated		Uninoculated	Inoculated	
TGX 1951 – 3F	62^{b}	69^{a}	7^{d}	31^{a}	2^{b}	93.5^{a}
TGX 1990 – 46F	48^{b}	66^{a}	18^{a}	19^{a}	7^{b}	63.2^{b}
TGX 1990 – 57F	50^{b}	65^{a}	15^{b}	11^{a}	4^{b}	63.6^{b}
TGX 2005 – 1F	55^{b}	67^{a}	12^{c}	16^{a}	5^{b}	68.8^{ab}
TGX 2007 – 1F	51^{b}	66^{a}	15^{b}	7^{a}	2^{b}	71.4^{ab}
TGX 2007 – 3F	48^{b}	66^{a}	18^{a}	13^{a}	5^{b}	61.5^{b}
TGX2009 – 1F	54^{b}	66^{a}	12^{c}	15^{a}	5^{b}	66.7^{ab}
TGX 2009 – 9F	63^{b}	76^{a}	13^{c}	12^{a}	4^{b}	66.7^{ab}
TGX 2012 – 1F	66^{b}	71^{a}	5^{d}	10^{a}	4^{b}	60.0^{b}
TGX 2013 – 1F	74^{b}	82^{a}	8^{d}	7^{a}	3^{b}	57.1^{b}

Means followed by dissimilar letters within the row, and along the column differ significantly ($p<0.05$) using the Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT), respectively

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Table 3: Number of seeds per pod and seed weight per plant in soybean lines of inoculated and uninoculated with *Blackeye cowpea mosaic virus*

	No of Seeds per pod			Seed weight per plant (g)		
Soybean line	Uninoculated	Inoculated	Seed reduction (%)	Uninoculated	Inoculated	Seed weight reduction (%)
TGX 1951 – 3F	3^{a}	2^{a}	33.3^{b}	8.5^{a}	0.4^{b}	95.3^{a}
TGX 1990 – 46F	3^{a}	1^{b}	66.7^{a}	4.7^{a}	2.1^{b}	55.3^{d}
TGX 1990 – 57F	3^{a}	2^{a}	33.3^{b}	6.8^{a}	0.8^{b}	88.2^{b}
TGX 2005 – 1F	3^{a}	2^{a}	33.3^{b}	4.3^{a}	0.8^{b}	81.4^{c}
TGX 2007 – 1F	3^{a}	1^{b}	66.7^{a}	4.6^{a}	0.2^{b}	95.7^{a}
TGX 2007 – 3F	3^{a}	1^{b}	66.7^{a}	5.4^{a}	0.5^{b}	90.7^{a}
TGX2009 – 1F	3^{a}	2^{b}	33.3^{b}	8.0^{a}	0.9^{b}	88.8^{b}
TGX 2009 – 9F	3^{a}	1^{b}	66.7^{a}	6.8^{a}	0.5^{b}	92.6^{a}
TGX 2012 – 1F	3^{a}	1^{b}	66.7^{a}	6.9^{a}	0.4^{b}	94.2^{a}
TGX 2013 – 1F	3^{a}	1^{b}	66.7^{a}	4.0^{a}	0.4^{b}	90.0^{b}

Means followed by dissimilar letters within the row, and along the column differ significantly ($p<0.05$) by the Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT), respectively.

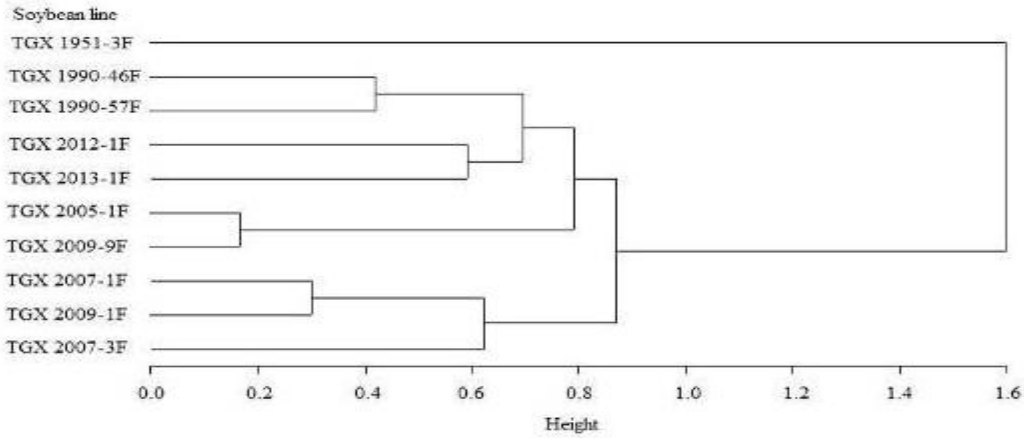

Fig. 2: Dendogram of the percentage reductions in morphological and yield characters of soybean lines inoculated with *Blackeye cowpea mosaic virus*, inferred from Unweighted Pair Group Method with Arithmetic (UPGMA) means

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