Resistance of Soybean Lines Infected with *Blackeye cowpea mosaic virus* Under Controlled Conditions

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Abstract

Ten soybean lines were investigated under screenhouse conditions for resistance to *Blackeye cowpea mosaic virus* (BlCMV) disease. The virus was detected and confirmed using Antigen Coated Plate-Enzyme Linked Immunosorbent Assay (ACP-ELISA). Each genotype was tested as inoculated and healthy control using completely randomized design replicated five times. Seedlings were infected with the virus by sap inoculation at 10 days after sowing. Disease incidence, disease severity, plant yield and yield components were recorded. Data were subjected to Analysis of Variance (ANOVA) at $p<0.05$ probability level. In most of the soybean lines, uninoculated plants gave significantly higher values of the growth and yield parameters. One hundred percent infection was found in the virus inoculated plants at 2 weeks post inoculation (WPI). The soybean line TGX 1990 – 46F was the most tolerant, as it exhibited moderate level of infection (symptom score = 3.0) at 5 WPI, and the lowest seed weight reduction (12.8 %). Genetic improvement of the high-yielding soybean genotypes against BlCMV infection would contribute significantly to sustainable yield and food security. The genotype TGX 1990 – 46F is therefore, recommended for breeding BlCMV-resistant soybean cultivars.

Keywords: Disease incidence, symptom, genotypic tolerance, seed weight

Introduction

Soybean (*Glycine max* [L.] Merr.) is a leguminous crop of global importance. In Nigeria, the land area under soybean cultivation has been on the increase in the last 20 years. This development is attributable to increase in demand for it in human nutrition and animal feed. In 2013, Nigeria produced 600,000 tonnes of soybean estimated from 600,000 ha of land (FAO, 2013). The increased production of soybean in recent years has led to the dominance of soybean oil (about 20 %) among the various vegetable oils available for food use worldwide (FAO, 2008). Soybean is rich in protein, all essential amino acids (except methionine), lipids, vitamins and minerals (Lokuruka, 2010). The water-soluble vitamins of the soybean are niacin, riboflavin, thiamine, pantothenic acid and folic acid. Although a negligible amount of Vitamin C is supplied in the mature beans, an appreciable quantity is present in immature beans. Its major oil-soluble vitamins are A (retinol) and E (tocopherol) (Ponnusha *et al*., 2011). Adequate intake of soybean can ameliorate breast cancer in both pre- and postmenopausal women (Messina and Wood, 2008). Several soybean based feeds are being formulated and soybean haulm is an excellent fodder for the livestock industry. Unfortunately, yield per unit area is usually low in Africa, including Nigeria partly due to incidence of insect pests and diseases.

Soybean is susceptible to over 111 strains of viruses belonging to different virus genera and families (Hartman *et al*., 1999).

BlCMV is one of the major limitations to soybean productivity (Golnaraghi *et al*., 2004). The virus was first observed in Florida (Alegbejo, 2015) but has expanded its geographical coverage to other parts of the world including Nigeria. Symptoms elicited in infected plants vary with genetic background of the host cultivar. The virus induces both local and systemic symptoms in susceptible genotypes. Systemic symptoms include large reddish lesions usually found along the veins. Systemic symptoms are characterized by severe mottling, distortion, yellowing, mosaic and vein necrosis (Thottappilly and Rossel, 1992). BlCMV is transmitted in nature by insect vectors belonging to the family Aphididae. These include *Aphis craccivora* Koch and *Myzus persicae* Sulzer, in a non-persistent manner. Besides, BlCMV is readily sap transmissible and seed transmission has been reported.

Several weeds have also been implicated in the survival and epidemiology of the virus (Alegbejo and Kashina, 1997). In a study conducted by Owolabi *et al*. (1988), total yield loss was reported in some Nigerian commercial cowpea cultivars due to BlCMV infection. Cultivation of resistant or tolerant genotypes is the most effective measure against plant viruses. BlCMV has been reported in Nigeria for more than three decades but most of the research efforts have concentrated on its impacts on cowpea, despite its ability to infect soybean. This phenomenon has resulted in knowledge gap and scarcity of information on the status of the existing soybean genotypes. Information on the varietal reactions to the virus would be valuable to soybean breeders in designing measures against severe losses. Therefore, the objective of this study was to evaluate the level of tolerance of the selected soybean lines to BlCMV infection.

Materials and Methods

Virus Inoculum and Multiplication

The trials were conducted under screenhouse conditions at the Teaching and Research Farm, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna (9^0 51'N, 6^0 44'E and 212 m above sea level), Nigeria. Minna belongs to the Southern Guinea Savanna ecological zone of Nigeria with average annual rainfall of about 1200 mm. The rainfall is distributed between April and early October with peak around September. Temperature ranges from 35 ^oC to 37.5 ^oC while the relative humidity is between 40 and 60 % around January which later increases to between 60 and 80 % towards July. The BlCMV isolate used was obtained from the stock in the Department of Crop Production, Federal University of Technology, Minna, Nigeria. It is filamentous in shape and approximately 750 nm in size (Taiwo, 2001). Virus inoculum was multiplied in 10-day old cowpea (cv. Ife Brown) seedlings by mechanical inoculation. This was accomplished by homogenizing (1g/1mL) BlCMV-infected leaves in inoculation buffer (0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water, adjusted to pH 7.2) using cold sterilized mortar and pestle. Carborundum powder (600-mesh) and 2 μL of β-mercapto ethanol were added to the extract before the extract was rubbed on the upper leaf surface. The inoculated plants were rinsed with distilled water and maintained in a screenhouse for symptom development.

Symptomatic leaves were harvested at two weeks post inoculation and subjected to Antigen Coated Plate-Enzyme-Linked Immunosorbent Assay (ACP-ELISA) as described by Kumar (2009). All the reagents for ELISA were purchased from BDH Chemicals Ltd., England. The antibody used for detecting BlCMV was raised against a Nigerian isolate of BlCMV at the Virology and Molecular Diagnostics Unit, International Institute of Tropical Agriculture, Ibadan. Sample wells with absorbance values (at 405-nm wavelength) more than twice those of healthy soybean control wells were considered positive. Excess leaf tissues from the BlCMV-positive samples were preserved on silica gels and used for subsequent inoculations.

Experimental Design, Sowing and Inoculations

Two trials were conducted simultaneously under screenhouse conditions (28^oC – 39^oC) to determine the level of BlCMV resistance in the tested soybean lines. The experiments were arranged as a completely randomised design with five replications in which 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) constituted the treatments. Seeds were obtained from the breeding Unit of the National Cereal Research Institute (NCRI), Badeggi, Nigeria. These soybean lines were selected from the germplasm designated for evaluation against pests and genetic improvement at the Institute. Each line was tested as inoculated and control (uninoculated). Seeds were sown in 30-cm diameter, 30-cm high plastic pots and seedlings thinned to five plants per pot. Plants were inoculated with the virus at 8-leaf stage (10 days after sowing). Sap preparation for inoculation and inoculation procedure were as detailed above.

Observations and Data Analysis

Disease incidence was calculated as percentage of the plants showing symptoms of BlCMV disease after inoculation. Disease severity was visually assessed based on a 5-point scale (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. Morphological and yield parameters were also recorded. Data were subjected to analysis of variance (ANOVA) using the General Linear Model (PROC GLM) procedure of SAS (2008) at $p<0.05$. For significant F tests, means were separated using either Least Significant Difference (LSD) or Student-Newman Keuls (SNK) test where appropriate.

Results and Discussion

The first symptoms of infection appeared on the inoculated plants at 5 days post inoculation. Symptoms include mottling and chlorosis of the fully expanded topmost trifoliate leaves. At one week post inoculation, disease incidence was significantly ($p<0.05$) highest in the soybean line TGX 2009-9F. Line TGX 2005-1F also exhibited high level of infection, whereas the plants of TGX 2007-1F, TGX 2007-3F, and TGX 2012-1F were apparently symptomless. However, at 2 WPI, 100 % disease incidence was observed in all the inoculated plants. The severity of infection varied significantly among the soybean lines. Symptoms varied from mild to moderate level of infection. At 1 WPI, TGX 2007-1F, TGX 2007-3F, and TGX 2012-1F showed the lowest severity score while TGX 1951-3F exhibited the highest value (Table 1). There was no significant difference in BlCMV infection severity among TGX 1990-57F, TGX 2005-1F, TGX 2009-1F, and TGX 2009-9F. At 3 WPI, 70 % of the lines exhibited moderate level of infection. At 5 WPI, the intensity of symptoms generally declined in almost all the genotypes. At this growth stage, TGX 2005-1F showed the lowest severity score, whereas moderate symptom score was found in TGX 1990-46F and TGX 2012-1F.

The significant differences in percent infection at 1 WPI indicate that the soybean lines were genetically heterogeneous. The lines TGX 2005-1F and TGX 2009-9F which showed high level of infection could be described as the most susceptible. The plants of TGX 2007-1F, TGX 2007-3F, and TGX 2012-1F which did not produce symptom of infection at 1 WPI suggest the presence of BlCMV-tolerant genes. However, the fact that complete infection was observed at 2 week post inoculation across the genotypes implies that none of the soybean lines was immune to BlCMV. Immunity is the highest level of resistance and is partly exhibited as absence of visible symptoms in inoculated plants (McCreight *et al*., 2008). The differences exhibited with respect to symptom severity could also be attributed to varied inherent genetic background of the evaluated genotypes. The severity of infection increased over time in some genotypes, which is in agreement with Aliyu *et al*. (2012) when some cowpeas were inoculated with BlCMV. The ability of infected plants to recover at the later growth stage is in conformity with the result reported by Vanitharani *et al*. (2004) in a study involving *Nicotiana benthamiana* and cassava plants. Recovery is a mechanism of resistance which is regulated by genes acting in an additive manner (Lagat *et al*., 2008).

Table 1: Disease incidence and severity in soybean lines inoculated with *Blackeye cowpea mosaic virus*

	Disease incidence (%) 1 week post inoculation	Disease severity Week post inoculation		
Soybean line	1	1	3	5
TGX 1951-3F	29.2[e]	2.5[a]	2.3[c]	2.3[c]
TGX 1990-46F	8.4[g]	1.5[bc]	3.0[ab]	3.0[a]
TGX 1990-57F	41.7[d]	2.0[ab]	2.9[abc]	2.8[ab]
TGX 2005-1F	80.0[b]	2.0[ab]	2.6[bc]	2.2[c]
TGX 2007-1F	0.0[h]	1.0[c]	2.7[bc]	2.3[c]
TGX 2007-3F	0.0[h]	1.0[c]	3.2[ab]	2.6[ab]
TGX 2009-1F	50.0[c]	2.1[ab]	3.4[a]	2.4[c]
TGX 2009-9F	91.7[a]	2.1[ab]	2.6[bc]	2.7[ab]
TGX 2012-1F	0.0[h]	1.0[c]	2.8[abc]	3.0[a]
TGX 2013-1F	16.7[f]	1.5[bc]	2.6[bc]	2.6[ab]
±SE	4.8	0.3	0.2	0.2

Means followed by dissimilar letter (s) within the column differ significantly ($p<0.05$) by Student-Newman Keuls (SNK) test

Uninoculated plants consistently produced more leaves than the BlCMV-infected plants (Table 2). At 1 WPI, leaf number of uninoculated plants varied between 11 and 13. The lowest number of leaves per plant was found in TGX 2012 - 1F and TGX 2013 - 1F while TGX 1951 - 3F, TGX 1990 - 46F, TGX 1990 - 57F, TGX 2005 - 1F, TGX 2007 - 1F, and TGX 2007 - 3F gave the highest value. The BlCMV-inoculated plants had a range of 4 to 7; the lowest value was observed in TGX 2012 - 1F, whereas the highest was found in TGX 1990 - 46F, TGX 1990 - 57F, TGX 2007 - 3F, TGX2009 - 1F and TGX 2013 - 1F. Two weeks later, the inoculated plants showed slight increase in leaf production (11 to 14) while those without virus treatment exhibited rapid increase in leaf number (18 to 22). This shows that BICMV infection slowed down cell division and expansion which resulted to reduced number of leaves in the inoculated plants. At 5 WPI, the trend in leaf production between BlCMV-infected and uninoculated plants was somewhat similar to the preceding week. Reduction in leaf number was consistently lowest in TGX 2013 - 1F

(Fig. 1) but the values obtained from other genotypes were not stable. At 3 WPI, leaf reduction was less than 50 % in all the genotypes but two weeks later 80 % of them suffered substantial reduction in leaf number (>50 %).

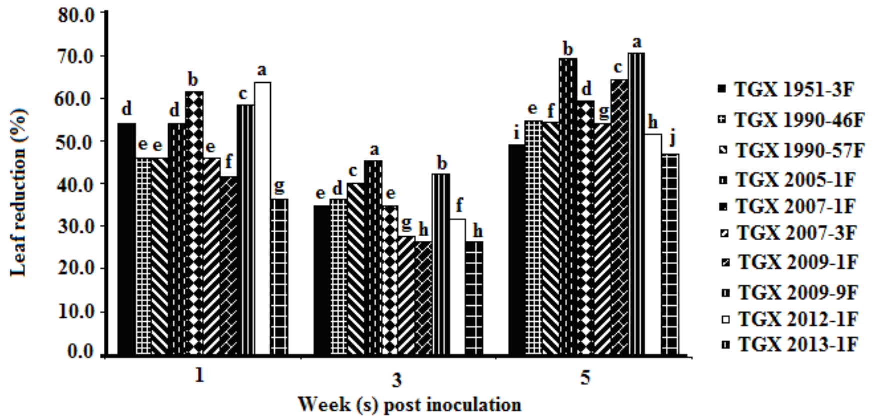

Figure 1: Effect of *Blackeye cowpea mosaic virus* infection on leaf reduction in soybean lines

Means labelled with dissimilar letter differ significantly (p<0.05) by Student-Newman Keuls (SNK) test

The data on leaf diameter are presented in Table 2. Uninoculated plants consistently produced broad leaves contrary to small and narrow leaves produced by their virus-inoculated counterparts. At 1 WPI, leaf diameter of uninoculated plants varied between 2.1 and 3.7 cm while the inoculated plants gave values ranging between 0.9 and 1.7 cm. Two weeks later, leaf diameter of the uninoculated plants varied from 3.4 to 4.4 cm while the inoculated plants gave values ranging between 1.7 and 2.6 cm. At 5 WPI, leaf diameter ranged between 3.8 and 5.6 cm for uninoculated plants as opposed to a range of 2.2 to 3.5 cm observed in infected plants. Reduction in leaf diameter varied significantly among the genotypes as shown in Fig. 2. Apart from TGX 1990 - 57F which exhibited the lowest reduction of 38.1 % at 5 WPI, leaf diameter of other soybean lines was drastically reduced. The better performance of uninoculated plants over the infected ones reveals that BlCMV could be devastating on soybean and could cause significant yield loss. The drastic leaf number reduction in inoculated plants agrees with the results published by Aliyu *et al.* (2012). Although leaf number increased in successive weeks post inoculation, the genotype TGX 1951 - 3F which consistently exhibited the lowest reduction could be described as the most outstanding for this trait. Leaf diameter is an integral component of leaf area which has a strong relationship with the level of photosynthesis. The smaller and narrower leaves produced by infected plants might have retarded their physiological activities. The ability of infection to suppress photosynthesis has been demonstrated by Funayama-Noguchi and Terashima (2005) when *Eupatorium yellow vein virus* was tested on *Eupatorium makinoi* plants.

Table 2: Number of leaves per plant and leaf diameter from soybean lines inoculated with *Blackeye cowpea mosaic virus* and of uninoculated controls

	Leaf per plant (no.) Week post inoculation			Leaf diameter (cm) Week post inoculation		
Soybean line	1	3	5	1	3	5
TGX 1951 - 3F						
Control	13[a]	20[a]	49[a]	2.7[a]	4.0[a]	4.8[a]
Inoculated	6[b]	13[b]	25[b]	1.0[b]	2.2[a]	3.1[a]
±SE	1.3	1.1	2.5	0.3	0.7	0.9
TGX 1990 – 46F						
Control	13[a]	22[a]	42[a]	3.3[a]	4.3[a]	5.1[a]
Inoculated	7[b]	14[b]	19[b]	1.3[b]	2.6[b]	3.0[b]
±SE	0.7	2.1	6.8	0.4	0.3	0.3
TGX 1990 – 57F						
Control	13[a]	20[a]	35[a]	2.1[a]	3.6[a]	3.8[a]
Inoculated	7[b]	12[b]	16[b]	1.3[a]	2.0[b]	2.6[a]
± SE	1.1	0	3.2	0.3	0.3	0.4
TGX 2005 - 1F						
Control	13[a]	22[a]	39[a]	3.7[a]	4.1[a]	5.0[a]
Inoculated	6[b]	12[b]	12[b]	1.7[b]	2.4[a]	3.1[a]
±SE	1.5	1.3	7.8	0.6	0.7	0.4
TGX 2007 - 1F						
Control	13[a]	20[a]	32[a]	3.7[a]	4.1[a]	4.7[a]
Inoculated	5[b]	13[b]	13[b]	1.3[b]	2.0[b]	2.7[b]
±SE	1.5	0.7	1.8	0.6	0.5	0.6
TGX 2007 - 3F						
Control	13[a]	18[a]	39[a]	2.9[a]	3.9[a]	4.7[a]
Inoculated	7[b]	13[b]	18[b]	1.0[b]	1.7[b]	2.2[b]
±SE	1.1	1.1	5.1	0.3	0.1	0.1
TGX2009 - 1F						
Control	12[a]	19[a]	45[a]	3.6[a]	4.4[a]	4.8[a]
Inoculated	7[b]	14[b]	16[b]	1.0[b]	1.8[b]	2.3[b]
±SE	1.6	0.8	2.5	0.4	0.2	0.0
TGX 2009 - 9F						
Control	12[a]	19[a]	44[a]	3.2[a]	4.1[a]	4.6[a]
Inoculated	5[b]	11[b]	13[b]	1.2[b]	2.0[b]	3.0[b]
±SE	0.4	1.4	3.2	0.2	0.1	0.1
TGX 2012 - 1F						
Control	11[a]	19[a]	29[a]	2.9[a]	4.0[a]	5.6[a]
Inoculated	4[b]	13[b]	14[b]	1.2[b]	2.1[b]	3.0[b]
±SE	0	1.1	1.5	0.3	0.5	0.3
TGX 2013 - 1F						
Control	11[a]	19[a]	32[a]	3.3[a]	3.4[a]	4.1[a]
Inoculated	7[b]	14[b]	17[b]	0.9[b]	2.1[b]	3.5[a]
± SE	1.1	1.5	1.9	0.1	0.3	0.4

Means not followed by the same letter within the column differ significantly ($p<0.05$) by the Least Significant Difference (LSD)

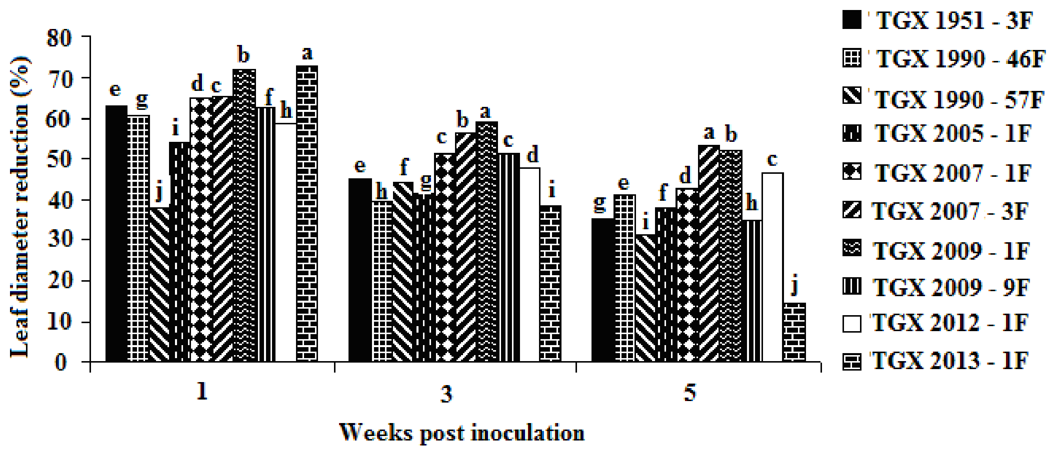

Figure 2: Effect of *Blackeye cowpea mosaic virus* infection on leaf diameter reduction in soybean lines

Means labelled with dissimilar letter differ significantly ($p<0.05$) by Student-Newman Keuls (SNK) test

Plant height was severely impaired by BlCMV infection. Growth and vigour were markedly reduced with characteristic short internodes and reduced heights in susceptible inoculated plants. Conversely, uninoculated plants showed normal growth and development. The data on plant heights are presented in Table 3. At 1 WPI, heights of infected plants varied between 1.5 and 10.0 cm, contrary to a range of 32.5 to 53.5 cm from their uninoculated counterparts. Height reductions were generally high with values ranging between 78.0 and 95.8 % in the inoculated plants (Fig. 3). At 3 WPI, heights of the inoculated plants ranged between 14.5 and 26 cm. Height reductions were still high (>50 %) at this growth stage. The minimum value was observed in TGX 1951 - 3F while TGX 2009 - 9F was the most vulnerable. Two weeks later, the heights of inoculated plants varied from 21.5 to 37 cm which differed significantly from 87.5 to 121.5 cm observed in the healthy plants. The genotype TGX 2009 - 9F was still the most stunted with height reduction as high as 81.9 %. Although the lowest height reduction was obtained in infected TGX 1951 - 3F (62.8 %), plants were evidently stunted. The deleterious effect of BlCMV on plant height resulted from reduction in efficiency of physiological processes and utilization of growth resources in diseased plants. Seeds of some severely diseased plants were markedly small and deformed. In contrast, those of uninoculated plants assumed normal shape. The effect of BlCMV on seed weight per plant is presented in Table 3. With the exception of TGX 1990 – 46F, the seed weights of uninoculated plants were significantly ($p<0.05$) higher than those infected with the virus. The lowest

Table 3: Plant height and number of branches per plant in soybean lines inoculated with *Blackeye cowpea mosaic virus* and of uninoculated controls

	Plant height (cm) Week post inoculation			Seed weight per plant (g)
Soybean line	1	3	5	
TGX 1951 - 3F				
Control	35.0[a]	80.5[a]	99.5[a]	8.5[a]
Inoculated	6.6[b]	24.0[b]	37.0[b]	0.4[b]
±SE	8.5	11.8	16.2	0.9
TGX 1990 – 46F				
Control	39.5[a]	84.5[a]	105.5[a]	4.7[a]
Inoculated	3.0[b]	21.5[b]	31.0[b]	4.1[a]
±SE	6.0	15.2	20.3	1.9
TGX 1990 – 57F				
Control	38.0[a]	85.5[a]	117.0[a]	6.8[a]
Inoculated	4.5[b]	22.0[b]	31.5[b]	0.8[b]
± SE	1.8	4.6	13.5	0.6
TGX 2005 - 1F				
Control	36.0[a]	80.0[a]	100.5[a]	4.3[a]
Inoculated	1.5[b]	14.5[b]	21.5[b]	0.8[b]
±SE	7.1	3.7	7.4	0.1
TGX 2007 - 1F				
Control	32.5[a]	68.0[a]	87.5[a]	4.6[a]
Inoculated	2.5[b]	21.0[b]	30.0[a]	0.2[b]
±SE	6.7	12.8	23.1	0.1
TGX 2007 - 3F				
Control	33.0[a]	72.5[a]	89.5[a]	5.4[a]
Inoculated	6.5[b]	23.5[b]	32.5[b]	0.5[b]
±SE	1.5	1.1	2.5	1.1
TGX2009 - 1F				
Control	45.5[a]	92,0[a]	109.5[a]	8.0[a]
Inoculated	10.0[b]	26.0[b]	36.5[b]	0.9[b]
±SE	5.4	5.7	2.5	1.0
TGX 2009 - 9F				
Control	53.5[a]	97.5[a]	121.5[a]	6.8[a]
Inoculated	4.5[b]	17.5[b]	22.0[b]	0.5[b]
±SE	4.0	1.1	4.0	0.1
TGX 2012 - 1F				
Control	37.0[a]	79.0[a]	102.0[a]	6.9[a]
Inoculated	4.0[b]	18.0[b]	25.0[b]	0.4[b]
±SE	3.2	2.1	1.8	0.2
TGX 2013 - 1F				
Control	41.0[a]	84.0[a]	107.0[a]	4.0[a]
Inoculated	5.0[b]	19.0[b]	25.0[b]	0.4[b]
± SE	4.4	11.7	12.4	0.5

Means not followed by the same letter within the column differ significantly ($p<0.05$) by the Least Significant Difference (LSD)

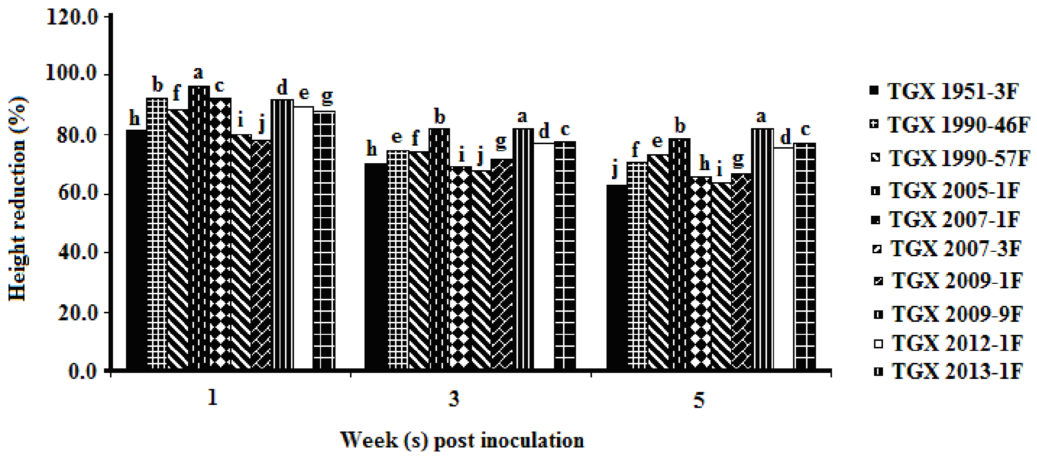

Figure 3: Effect of *Blackeye cowpea mosaic virus* infection on height reduction in soybean lines

Means labelled with dissimilar letter differ significantly (p<0.05) by Student-Newman Keuls (SNK) test

reduction in seed weight was 12.8 % which was found in TGX 1990 – 46F. In other genotypes, seed weight reduction was generally high (>50 %) with the highest value observed in TGX 2007-1F (95.7 %).

The fact that reduction in seed weight was high in the three soybean lines (TGX 2007-1F, TGX 2007-3F, and TGX 2012-1F), which exhibited the lowest disease severity implies a negative correlation between disease resistance and grain yield. This suggests that disease tolerant genes were probably linked with some agronomic characters. This phenomenon is known as linkage drag. Linkage drag is inevitable in genes that are tightly-linked on the same chromosome because they tend to be inherited together (Ali *et al*., 2011). This was probably responsible for the negative relationship between the two parameters. Seed weight is an important trait in soybean breeding because of its direct relationship with productivity. This explains the rationale behind the choice of soybean lines with inherent potential for high-yield during germplasm improvement.

Conclusion and Recommendations

BlCMV is capable of causing severe reduction in soybean productivity. However, incidence and severity depend on genetic background of the cultivar. Based on the observations in this study, the soybean line TGX 1990 – 46F which showed moderate level of infection and exhibited the lowest seed weight reduction was the most tolerant to BlCMV and could be utilized for resistance breeding programmes. This is premised on the fact that genetic improvement of the high-yielding soybean genotypes against BlCMV infection would contribute significantly to sustainable yield and food security.

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