The Pathogenicity of Soybean Mosaic Virus and Cowpea Mild Mottle Virus on Soybean Variety TGX 14428-2E

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Abstract

A greenhouse experiment was performed to investigate the pathogenicity of soybean mosaic virus (SMV) and cowpea mild mottle virus (CPMMV) on Soybean cultivar TGX 1448-2E. The viral isolates which served as the viral inoculum were extracted by homogenisation from infected leaves at the rate of 1 g, leaf sample to 5ml buffer solution. A total of 15 plastic pots were filled with sandy-loam soil previously steam-sterilized for 12 hours at 100^{o}C. Four seeds of the soybean cultivar were sown in each plastic pot prior to mechanical inoculation 14 days after. Inoculation with the two viral inoculums and phosphate buffer solution which served as the control was carried out on 5 pots (20 plants) per treatment. The results indicated increasing disease severity for both viruses over time. However at the end of observation, plants inoculated with SMV manifested higher infection rate (14.1 %), followed by CPMMV (13.6 %) when compared with the control (8.6 %). The virus inoculated plants produced relatively short and reduced number of leaves compared to control. In terms of yield, SMV inoculated plants had reduced pod (11.5 g) and seed (3.9 g) weights in comparison to control (19.6 g and 3.9 g respectively). The results from the study showed that Soybean variety TGX1448-2E is susceptible to both viruses with SMV more pathogenic. This is an indication that adequate control measures for viruses should be deployed to increase soybean production with a view to improving farmer income and enhancing food security in Nigeria.

Keywords: Greenhouse, Viral isolate, Buffer solution, Inoculation, Severity, Food security

1.0 Introduction

Soybean, *Glycine max* (L), is one of the oldest of cultivated leguminous oil seeds belonging to the family *Fabaceae*. It is among the major industrial and food crops grown in every continent and is one of the most important sources of oil and protein commonly used in both human and animal diets [1]. It is also an important crop component in the farming system of most parts of Nigeria [2]. Soybean seeds contain about 18% oil and 38% protein and of the oil fraction, 95% is consumed as edible oil with the rest used for industrial products such as cosmetics and hygiene products [3]. It is a versatile food plant that is used in various forms for it is capable of supplying most nutrients [4]. Soybeans are also a rich source of bioflavonoids, lecithins, oligosaccharides, phytosterols, saponins, and tocopherols which have been shown to benefit human health in reducing protein malnutrition [5]. Modest reductions in serum cholesterol levels have been achieved with soy intake, especially for subjects with hypercholesterolemia [6]. Soybean is not only high quality protein, but it is now thought to play preventive and therapeutic roles for several diseases [7].

Some of the challenges faced by farmers in soybean production include unpredictable weather, diseases, pests, weeds and variable soil quality [8]. This generally leads to low levels of soybean production arising from biotic and abiotic factors [9]. The abiotic factors include drought and low soil fertility status. Low native soil phosphorus availability coupled with poor utilization efficiency of added phosphorous have been shown to be a major constraint limiting the productivity of soybean [10]. Biotic constraints such as pathogens, pests and weeds, can be detrimental to soybean production and result in significant negative impacts on yield. The extent of economic plant damage however depends upon the type of pathogen/pest, plant tissue being attacked, number of plants affected, severity of attack, environmental conditions, host plant susceptibility, plant stress level and stage of plant development [11].

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Soybean mosaic virus (SMV) is a member of the genus *Potyvirus* in the family *Potyviridae*. It is the most prevalent virus and is recognized as the most serious, long-standing problem in many soybean producing areas of the world [12]. Infection by SMV usually results in severe yield losses and seed quality reduction. It has been reported that yield losses usually range from 8 to 50% under natural field conditions [13] and reach up to 100% in severe outbreaks [14]. Cowpea mild mottle virus (CPMMV) is a member of genus *Carlavirus* which has recently been classified under the plant virus family *Flexiviridae* [15]. CPMMV causes mosaic, chlorosis, necrosis and distortion in a range of indicator host plants [16]. The virus is reported to be transmitted by the whitefly, *Bemisia tabaci* in a non-persistent manner and infects beans, soybeans and peanuts [17].

In the facing challenges of shortages of food and access to a better life by farmers and rural communities, there is the need to increase food production. This could be achieved by identifying sources of damage to crops on the field by pathogens especially viral diseases. This research was therefore conducted to examine the severity of soybean mosaic virus (SMV) and cowpea mild mottle virus (CPMMV) on a widely grown soybean cultivar TGX 1448-2E. The objective was to investigate the effect of the two viruses on some growth and yield attributes of the crop under greenhouse conditions.

2.0 Materials and methods

2.1 Experimental site

The experiment was conducted at the Crop Protection Research Laboratory and the greenhouse pavilion of the Crop Protection Department of the Faculty of Agriculture, University of Ilorin.

2.2 Seed variety, plant propagation and experimental layout

The soybean cultivar TGX 1448-2E was obtained from International Institute of Tropical Agriculture (IITA) Ibadan. Four seeds were sown in each of the 15 (50 cm diameter) plastic buckets filled with sandy-loam soil that was previously steam-sterilized at 121^{o}C for 120 minutes.

2.3 Sourcing of inoculum and inoculation procedure

Soybean mosaic **v**irus and cowpea mild mottle virus isolates were separately extracted from infected leaves obtained from the stock of the Plant Pathology Laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State Nigeria. The infected leaf samples were extracted by homogenization according to Balogun and Aliyu [18]. The inoculation procedure was carried out 14 days after planting when the plants were at the 2nd leaf stage. The procedure involved slight dusting of the two primary leaves with carborundum to act as slight abrasive agent, after which the leaves were then rubbed with the extracted juice of either SMV, CPMMV or phosphate buffer solution (control). The plants where thereafter rinsed with water to reduce inoculation stress. Each treatment was replicated five times with each pot containing 4 plants. The seedlings were constantly watered and weeds were manually removed when necessary.

2.4 Data collection

From the 3rd to the 8th week after inoculation, data were collected for plant height, number of leaves per plant, number of diseased leaves per plant and the number of pods per plant. The percentage disease severity was measured by the number of diseased leaves relative to the total number of leaves on any given plant.

2.5 Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using the statistical package for the social sciences SPSS version 15.0. The treatment means where significant, were separated using New Duncan Multiple Range Test at 5% level of probability [19].

3.0 Results

3.1 Effect of treatments on percentage disease severity

Table 1 shows the effect of the treatments on percentage disease severity over 8 weeks. The results showed that soybean variety TGX 1448-2E was susceptible to both soybean mosaic virus and cowpea mild mottle virus. The percentage disease severity increased significantly from the 3rd to the 8th week after inoculation (WAI). However, there were variations in the values obtained for both viral inoculums.

At the 3rd WAI, soybean plants inoculated with SMV had the highest disease severity of 2.9 %, which was not significantly different from the CPMMV inoculated plants (2.5 %). The control plants had the lowest disease severity (1.2 %). By the 4th WAI, SMV inoculated plants still possessed the significantly highest infected rates (5.1 %), followed by CPMMV (3.2 %) and control (2.9 %). This trend was continuous to the 8th WAI with the SMV inoculated plants manifesting the highest disease severity (14.1 %), followed by CPMMV (13.6 %). The data indicate that in all cases, the control plants had the lowest infection severity.

3.2 Effect of treatments on plant height

The effect of the application of treatments on soybean variety TGX 1448- 2E is presented in Table 2. The results showed that application of treatments had significant effect on plant height. It was observed that the plants inoculated with the viral inoculums were significantly shorter than the buffer inoculations. There were no significant differences in plant heights across the treatments at the 3rd WAI and the effect only became manifest from the 4th WAI. The control plants exhibited significant increase in height (14.3 cm) at 28 days after inoculation. The plants inoculated with the two viruses (SMV and CPMMV) experienced significant reduction in height. Specifically, at 5 WAI, SMV inoculated plants were the shortest (12.2 cm), followed by CPMMV (13.3 cm) and control plants (19.5 cm). The viral inoculated plants of SMV (10.2 cm) and CPMMV (10.5 cm) were similar and the significantly shortest. At 5 WAI, the significantly affected were the SMV inoculated plants (12.2 cm), followed by CPMMV (13.3 cm). The control plants significantly improved in height (19.5 cm). From the 6th to the 8th WAI, a similar trend of results unfolded whereby the SMV inoculated plants manifested significantly reduced height followed by CPMMV inoculated plants. In this case, the control plants experienced significant increase in height (41.7 cm).

3.3 Effect of treatments on number of leaves

Table 3 shows the effect of the treatments on the average number of leaves per plant. The results indicated that the treatment effect was significant from the 4th to the 8th WAI. At the 4th WAI the plants that were inoculated with SMV had the lowest number of leaves per plant (3.5), which was significantly reduced compared to the values obtained for CPMMV inoculated plants (4.3), while the control plants had the highest number of leaves (6.1). At 5th WAI, the control plants had the highest number of leaves per plant (10.3), while the SMV inoculated plants had the lowest (4.6). The same situation was observable from the 6th to the 8th WAI.

3.4 Effect of treatments on number of pods per plant

Table 4 presents the effect of treatments on the average number of pods per plant of the soybean plants. The data indicated that viral pathogens significantly influenced number of pods. At the 7th WAI, the plants inoculated with SMV had significantly reduced number of pods per plant (9.7), while the CPMMV inoculations produced an average of 11.4 pods. The control plants in this instance produced significantly high number of pod (15.4). The observed phenomenon was also noticed through to the 8th WAI with the SMV inoculations having an average of 11.5 pods, followed by the CPMMV inoculations (13.3) and the control plants (19.6).

3.5 Effect of treatments on pod weight and seed weight at harvest

The effect of the treatments on dry pod weight and dry seed weight per plant at harvest is presented in Table 5. The result indicated that viral inoculation resulted in significantly reduced number of dry pod and seed pod weight compared to the control. However, the values were significantly reduced in SMV inoculated plants (2.9 g and 1.2 g), followed by the CPMMV inoculated plants (4.0 g and 2.3 g), while the highest yield parameters for dry pod weight (5.2 g) and dry seed weight (3.9 g) was obtained in the control plants.

Table 1: Effect of treatments on percentage disease severity over eight weeks

Treatment	Week3	Week 4	Week 5	Week 6	Week 7	Week 8
SMV	2.9^{a}	5.1^{a}	8.6^{a}	10.3^{a}	12.2^{a}	14.1^{a}
CPMMV	2.5^{ab}	3.2^{b}	7.3^{b}	9.4^{b}	11.4^{b}	13.6^{ab}
CONTROL	1.2^{c}	2.9^{c}	3.1^{c}	4.8^{c}	6.4^{c}	8.6^{c}
SEM	0.280	0.380	0.601	0.617	0.533	1.197

Means within a column followed by the same letter(s) are not significantly different using Duncan Multiple Range Test at P≥0.05.

Table 2: Effect of treatments on plant height over eight weeks

Treatment	Week3	Week 4	Week 5	Week 6	Week 7	Week 8
SMV	9.7^{a}	10.2^{b}	12.2^{c}	17.4^{c}	22.2^{c}	23.3^{c}
CPMMV	9.8^{a}	10.5^{b}	13.3^{b}	19.2^{b}	30.3^{b}	31.9^{b}
CONTROL	9.8^{a}	14.3^{a}	19.5^{a}	28.6^{a}	34.7^{a}	41.7^{a}
SEM	0.002	0.380	0.601	0.519	0.781	0.736

Means within a column followed by the same letter(s) are not significantly different using Duncan multiple Range Test at P$\geq$0.05.

Table 3: Effect of treatments on number of leaves over eight weeks

Treatment	Week3	Week 4	Week 5	Week 6	Week 7	Week 8
SMV	2.0^{a}	3.5^{c}	4.6^{c}	6.0^{c}	10.6^{c}	13.9^{c}
CPMMV	2.1^{a}	4.3^{b}	7.8^{b}	11.7^{b}	15.3^{b}	17.6^{b}
CONTROL	2.1^{a}	6.1^{a}	10.3^{a}	19.1^{a}	24.3^{a}	26.8^{a}
SEM	0.135	0.261	0.156	0.532	0.778	0.815

Means within a column followed by the same letter(s) are not significantly different using Duncan multiple Range Test at P$\geq$0.05.

Table 4: Effect of treatments on number of pods

Treatment	Week 7	Week 8
SMV	9.7^{c}	11.5^{c}
CPMMV	11.4^{b}	13.3^{b}
CONTROL	15.4^{a}	19.6^{a}
SEM	1.653	0.261

Means within a column followed by the same letter(s) are not significantly different using Duncan multiple Range Test at P$\geq$0.05.

Table 5: Effect of treatments on pod and seed weights at harvest

Treatment	Dry pod weight (g)	Dry seed weight (g)
SMV	2.9^{c}	1.2^{c}
CPMMV	4.0^{b}	2.3^{b}
CONTROL	5.2^{a}	3.9^{a}
SEM	0.111	0.216

Means within a column followed by the same letter(s) are not significantly different Duncan multiple Range Test at P$\geq$0.05.

4.0 Discussion

Virus diseases have been shown to cause most damage to soybean especially when infection occurs in the early stages of growth. Previous studies have shown that physiological and photosynthetic properties, as well as growth of plants are negatively influenced in crops infected by virus [20]. The results presented in this study clearly agree with this view and confirms the susceptibility of soybean variety TGX 1448- 2E to cowpea mild mottle virus and soybean mosaic virus. The soybean response to the virus infection ranged from mosaic patterns to characteristic leaf mottling, resulting in reduced growth and yields compared to the non-infected plants. These symptoms are similar to those described in previous study [21].

According to Lesemann [22], plant virus infections induce changes in the fine structure of host cell. These changes comprise pathological reactions of the plant cells, resulting from virus induced disturbed balance of the host plant physiology and virus specific cytological alterations directly associated to virus propagation. It is therefore probable that the two viruses exerted some adverse effects on plant physiological responses and photosynthetic ability. Zoltan *et al*. [23] reported that virus infection induces changes in host plant metabolic processes, including the most basic one, photosynthesis. The findings from the present study is in consonance with Suryawanshi *et al*. [24], that reduced crop growth and yield losses are both consequent effects of virus infection.

Further findings from the study indicated that soybean mosaic virus infection was more pathogenic than cowpea mild mottle virus infection. The basis for severity of the symptoms of a viral disease in plants results from the combination of expression of the viral genes controlling pathogenicity, physiological response of the infected plants and genetic makeup of the plants in response to the virus. The higher severity of SMV infection could be attributed to these factors, as the virus has been reported to be one of the most common diseases of soybean [25]. The susceptibility of soybean cultivar TGX 1448-2E to CPMMV in this study indicates that the virus is prevalent and pathogenic to some varieties of soybean [26]. The infection rate is however dependent on the soybean variety, time of infection and inoculums strain.

5.0 Conclusion

The study concludes that soybean mosaic virus and cowpea mild mottle virus are pathogenic and cause severe growth and yield reductions in soybean cultivar TGX 14482-E. However, the relative ability of the viruses to cause diseases was higher in soybean mosaic virus. The utilization of soybean cultivars resistant to soybean mosaic virus and cowpea mild mottle virus should be explored as a way of combating diseases that may result from these viruses.

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