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RESISTANCE OF COWPEA (*Vigna unguiculata* L. Walp) CULTIVARS TO SINGLE AND DOUBLE INFECTIONS WITH POTYVIRUS AND SOBEMOVIRUS ISOLATES

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ABSTRACT

Cowpea (*Vigna unguiculata*) is a staple food in sub-Saharan Africa. However, diseases induced by *Cowpea aphid-borne mosaic virus* (CABMV; *Potyvirus*) and *Southern bean mosaic virus* (SBMV; *Sobemovirus*) cause significant yield losses. Cultivation of resistant cowpea varieties remains the most effective and sustainable management approach to these viruses. Eight cowpea cultivars were evaluated for CABMV, SBMV, CAMBV+SBMV, and SBMV+CABMV resistance in Minna, Southern Guinea Savanna of Nigeria during the 2016 cropping season. Virus infected and control plants were evaluated independently using a randomized complete block design with three replicates. In single infections, cowpea seedlings were inoculated at 10 days after sowing (DAS) while in double infections the second virus inoculation was performed at 21DAS. Disease incidence, symptom severity and seed weight per plant were recorded. The data were subjected to analysis of variance and Duncan Multiple Range Test was used for means separation. One hundred percent infection was obtained regardless of the cultivar. Moderate tolerance (symptom score = 3.0) was found only in IT10K-973-1 to CABMV, IT07K-298-9 and IT10K-817-7 to SBMV, and in IT07K-298-9, IT10K-817-7 and IT10K-973-1 to SBMV+CABMV. Seed weight per plant was significantly ($p<0.05$) highest in the healthy plants of IT10K-843. In virus infected, seed weight per plant was significantly highest in IT10K-843 (3.0 g) infected with CABMV; IT07K-298-9 (1.9 g) inoculated with SBMV, IT10K-973-1 (2.6) under CABMV+SBMV infections; and in IT07K-299-6 (3.0 g) infected with SBMV+CABMV. The cowpea cultivars IT10K-843 and IT10K-973-1 which produced appreciable yield under single and double virus infections are recommended to cowpea farmers.

Keywords: Virus infections, disease incidence, disease severity, seed weight, cowpea

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an important pulse in sub-Saharan Africa and most widely used legume crop in the tropical world. It is a major food for millions of people and also insures the availability of high quality hay for livestock feed in dry and wet seasons (Agbogidi, 2010). Cowpea is commonly intercropped with cereal crops such as maize and sorghum because it fixes atmospheric nitrogen into the soil. In the developing world where soil infertility is high; it gives to the soil a huge supply of nitrogen and permits its cultivation without the use of nitrogenous fertilizer (Timko *et. al.*, 2007). It also suppresses weeds and prevents soil erosion. Cowpeas are grown extensively throughout savanna regions of the tropics and sub-tropics, between 35ºN and 30ºS, especially in Western and Central African countries,

across Asia and Oceania, the Middle East, southern Europe, southern USA, and Central and South America. In 2015, the African continent produced almost 95 % of the global cowpea production on a surface area of more than 11 million hectares, followed by Asia (3.2 %), the Americas (1.3 %) and Europe (0.5 %) (FAO, 2015).

Viruses constitute major constraints in all agro-ecologies in Nigeria where cowpea is grown and could induce 100 % yield losses in vulnerable cultivars (Aliyu *et al.*, 2012). *Cowpea aphid borne mosaic virus* (CABMV), a member of the genus *Potyvirus* is an important virus disease of cowpea and can cause a yield loss of 13 - 87 % under field condition depending on crop susceptibility, virus strain and the environmental conditions. It is readily transmitted by mechanical inoculation, several aphid species and through cowpea seeds. Similarly, *Southern bean mosaic virus* (SBMV) a member of the genus *Sobemoviruses* is highly prevalent in cowpea fields causing severe yield losses too (Taiwo *et al.*, 2007). Cowpea plants may be infected by more than one virus disease, resulting in serious economic losses in agricultural production (Byoung-Cheorl *et al.*, 2005). Mixed infections in crops involving two or more unrelated or closely related viruses can induce a series of within-host interactions and the outcome may be synergistic or antagonistic. In a study, there was no significant increase in virus titre in *Nicotiana benthamiana* plants co-infected with *Potato virus Y* (PVY) and *Tobacco etch virus* (TEV) or *Plum pox virus* (PPV), although severe symptoms and plant death were recorded (González-Jara *et al.*, 2004). Recently, maize lethal necrosis disease induced by a synergistic interaction of *Maize chlorotic mottle virus* (MCMV, genus *Machlomovirus*) and *Sugarcane mosaic virus* (SCMV, genus *Potyvirus*) or other *Potyviruses* (e.g. *Maize dwarf mosaic virus* and *Wheat streak mosaic virus*) has suddenly emerged in eastern Africa (Wangi *et al.*, 2012) with attendant huge losses to farmers and seed companies.

Earlier studies on mixed virus infections suggested that the interactions can occur at different levels and factors such as type and age of host, order of virus arrival can also affect the outcome of the interaction (Méndez-Lozano *et al.*, 2003). Breeding efforts for developing resistant cultivars against diseases and evaluation of the locally adapted cowpea cultivars against single and mixed infections of cowpea viruses would provide useful information for breeding cowpea cultivars with multiple resistance to these viruses. Thus, cultivation of these resistant cultivars could prevent severe yield losses in case of disease outbreak and also ensure food security. Therefore, this study was conducted to determine the resistance of selected cowpea cultivars to single and double infections of CABMV and SBMV in Minna, Southern Guinea Savanna of Nigeria.

MATERIALS AND METHODS

Study Location

The study was conducted at the Teaching and Research Farm, Federal University of Technology, Minna (9° 51'N; 6° 44'E; 212 m above sea level), Nigeria. Minna is located in the Southern Guinea Savanna agro-ecology of Nigeria.

Source of Cowpea Seeds

Seeds of eight cowpea cultivars namely IT07K-210-1-1, IT07K-298-9, IT07K-299-6, IT09K-231-1, IT10K-817-7, IT10K-843, IT10K-973-1 and IT11K-61-82 were obtained from the Genetic

Resource Unit of International Institute of Tropical Agriculture, (IITA), Ibadan, Nigeria.

Source and Multiplication of Virus Isolates

The CABMV and SBMV isolates used were obtained from the stock in the Department of Crop Production, Federal University of Technology, Minna, Nigeria. The isolates were maintained on silica gels in vial bottles stored at room temperature. They were multiplied by propagating in a susceptible cowpea cv. Ife Brown through sap transmission in a screen house. Ife Brown seeds were sown in pots of 29.5 cm diameter and 38 cm deep containing sterilized soil. Ten pots each placed in a wooden cage (to protect them from insect and contamination), were used for multiplying CABMV and SBMV inoculum, respectively. Extract for inoculation was prepared by grinding each leaf isolate in extraction 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, pH 7.2) at the rate of 1g/mL. Two microlitres of β-mercapto ethanol was mixed with the extract just before use. Seedlings were inoculated at 10 days after sowing (DAS) by rubbing the virus extract on the upper surface of the leaves dusted with carborundum powder (600-mesh). The inoculated plants were rinsed with sterile distilled water and thereafter left in the screenhouse for symptom expression.

Treatments and Experimental Design, Sowing and Inoculation

Five treatments consisting of mock inoculated plots (control), CABMV inoculated, SBMV inoculated, CABMV+SBMV inoculated, and SBMV+CABMV inoculated plots were used. Each treatment was tested separately on the eight cowpea cultivars. Treatments were arranged in a randomized complete block design with three replicates. Three seeds were sown after dressing with Apron – plus at the rate 3 g per 10 kg seeds to protect them against soil borne pathogens, on 19th August, 2016. Seedlings were thinned to one plant per stand at one week after sowing (WAS). Seedlings of the control plots were mock inoculated (no virus in the buffer), seedlings of the other four treatments were inoculated with their respective virus isolates (CABMV and SBMV) at 10 DAS. For mixed infections, seedlings were inoculated with the second virus isolate at 21 DAS.

Data Collection and Analysis

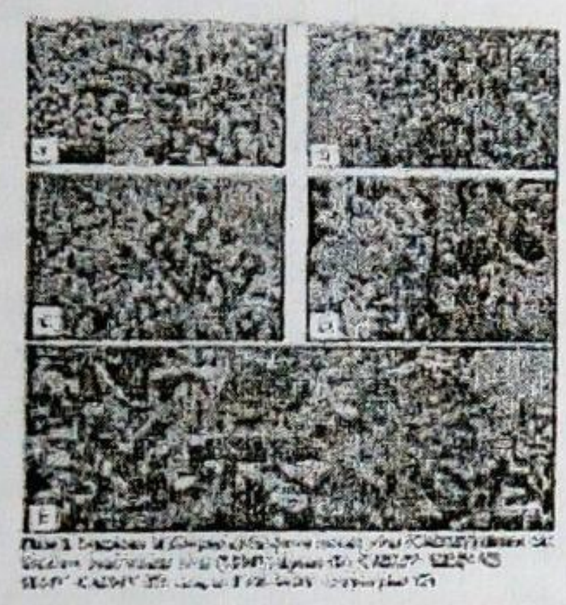
Disease incidence was observed at 1 and 2 weeks after inoculation (WAI) as percentage of infected plants, disease severity was recorded at 8 WAI, while seed weight per plant was determined at harvest. Disease severity was evaluated based on a visual scale of 1-5 as described by Arif and Hassan (2002), where 1 = no symptoms (apparently healthy plants); 2 = slight mosaic; 3 = moderate mosaic; 4 = severe mosaic, leaf distortion and stunting; 5 = severe mosaic, stunting and plant death. The data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2008) at $p=0.05$. Means were separated using Duncan Multiple Range Test (DMRT).

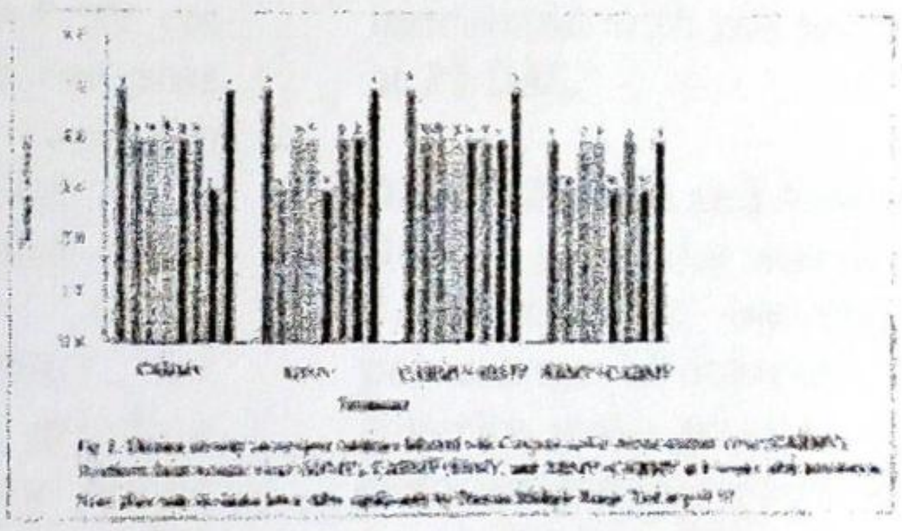
RESULTS

Disease Incidence and Severity

Symptoms became visible on the leaves of inoculated plants irrespective of the virus treatments at 8 days after inoculation. At 2 WAI, 100 % infection was obtained regardless of the cultivar. The symptoms observed on these plants

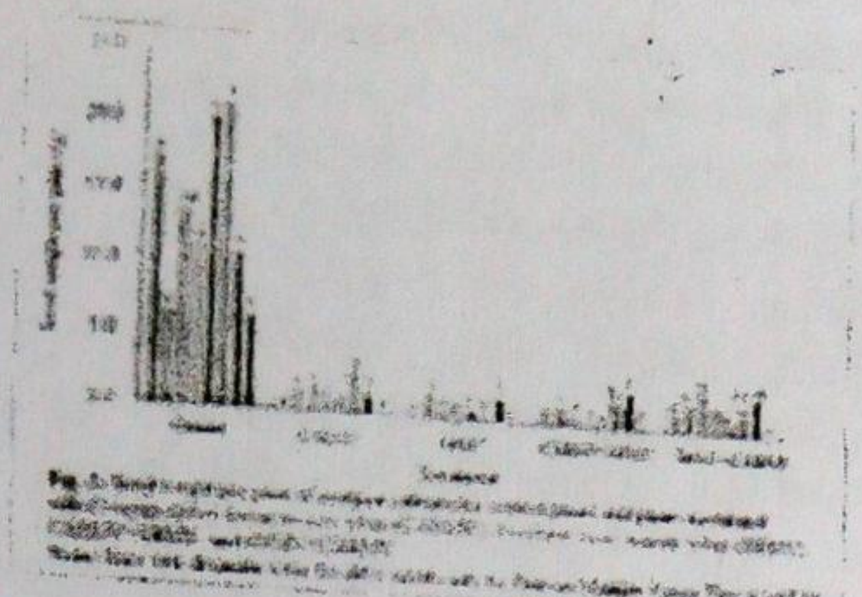
varied based on the virus type they were inoculated with. Disease severity increased progressively after inoculation and the plants inoculated with CABMV showed mottling, mosaic and leaf distortion while those inoculated with SBMV showed vein clearing, mosaic and leaf distortion which were more pronounced on younger leaves. The symptoms observed on plants inoculated with CABMV+SBMV were not much different from those of CABMV alone, and the symptoms observed on SBMV+CABMV were also just like those of SBMV. It was observed that in these four virus treatments, the disease severity differed significantly ($p<0.05$) among the 8 cultivars investigated. At 8 WAI, in CABMV, SBMV and CABMV+SBMV, there were plants with severity rate of 5 (Fig. 1) and this was peculiar to IT07K-210-1-1 and IT11K-61-82 and those plants eventually died. Moderate symptom expression with score of 3.0 was found only in IT10K-973-1 to CABMV, IT07K-298-9 and IT10K-817-7 to SBMV, and in IT07K-298-9, IT10K-817-7 and IT10K-973-1 to SBMV+CABMV. Double infections of CABMV+SBMV induced high symptom severity in all the cultivars





Effect of virus infections on seed weight

Seeds with normal shape were observed in the healthy plants while virus infected plants produced small and wrinkled seeds. Seed weight per plant was significantly ($p<0.05$) highest in the healthy plants of IT10K-843 (21.5 g) which was comparable to the seed weight observed in IT10K-817-7 (20.7 g) (Fig. 2). The seed weight per plant observed in IT07K-210-1-1 (17.5 g) was next. The differences in seed weight among IT07K-299-6 (13.8 g), IT09K-231-1 (11.6 g) and IT10K-973-1 (11.1 g) were not significant ($p>0.05$).



The lowest seed weight was found in IT07K-298-9 (6.6 g) and IT11K-61-82 (6.6 g). In the virus infected, seed weight per plant was significantly ($p<0.05$) highest in IT10K-843 (3.0 g) infected with CABMV; IT07K-298-9 (1.9 g) inoculated with SBMV; IT10K-973-1 (2.6) infected with CABMV+SBMV, and in IT07K-299-6 (3.0 g) under SBMV+CABMV infections. The cultivars IT07K-210-1-1 and IT11K-61-82 infected with CABMV; SBMV, CABMV+SBMV did not produce seeds. The same result was observed in IT09K-231-1 plants infected with SBMV.

DISCUSSION AND CONCLUSION

The differences in response of the cowpea cultivars to virus infections could be attributed to the variability in their genetic background. All the inoculated plants elicited disease symptoms, indicating their susceptibility to the major legume viruses. Nevertheless, the cultivars which showed moderate symptom severity probably contained resistant genes to the respective viruses. The cultivars which failed to produce seeds implied that they were the most susceptible to the test viruses. These observations agree with the findings of Taiwo *et al* (2007) who reported that severity of virus diseases was influenced by the cowpea variety and type of virus treatment. None of the plants infected with SBMV+CABMV died during the study, suggesting that SBMV served as an antagonist to the effects of CABMV. This is supported by Anjos *et al.* (1992) who reported that not all the combinations of unrelated viruses result in increased symptoms severity. Seed weight is an important trait in cowpea breeding because of its direct relationship with overall yield and farmer's income. The cowpea cultivars IT10K-843 and IT10K-973-1 which produced appreciable yield under single and double virus infections are recommended to cowpea farmers as a guarantee against complete crop failure. Adoption of these virus tolerant and high-yielding cultivars would reduce hunger and malnutrition.

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