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Climate-Smart Agriculture in the Post COVID Era:

A Gate Way to Food Security in Africa

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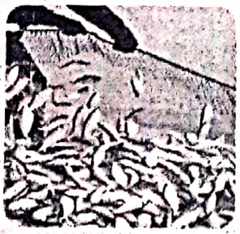


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EVALUATION OF SINGLE AND MIXED VIRUS-INOCULATED BAMBARA GROUNDNUT LANDRACES FOR NODULATION AND NITROGEN FIXATION

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ABSTRACT

Bambara groundnut (Vigna subterranea [L.] Verdc) enriches soils by forming a symbiotic relationship with nitrogen-fixing and stem-nodulating bacteria. Virus infections, however, limit the plant's ability to fix nitrogen. The study evaluated the nodulation and nitrogen-fixing ability of Bambara groundnut landraces (Vg 01, Vg 02, Vg 03, Vg 04, and Vg 05) under single and mixed virus infections of Blackeye cowpea mosaic virus (BlCMV; Potyvirus), Cowpea mild mottle virus (CPMMV; Carlavirus), BlCMV+CPMMV, and CPMMV+BlCMV. The experiment was conducted under screenhouse conditions, using a completely randomised design with three replications. The results showed that the landraces varied significantly (p<0.05) in their nodulation and symbiotic effectiveness (SE). The highest number of nodules (7 per plant) was observed in Vg_04 followed by Vg_05 (6 per plant), and Vg_01 had the lowest of 5 nodules per plant. The highest reduction in the number of nodules per plant was 54.1 %, dry weight of shoot 48.6 % and root 43.8 %; length of shoot and root reduced by 43.1 % and 59.3 %, respectively in BlCMV+CPMMV treated plants. Nitrogen-fixing efficiency of the landraces varied from ineffective (IE), in BlCMV (SE = 29.1 %) and BlCMV+CPMMV (SE = 32.0 %) to poorly effective (PE) for CPMMV (SE = 35.6 %) and CPMMV+BlCMV (SE = 36.4 %) while the control treatment was effective with average percentage SE of 70.7 %. These results suggest the need to protect plants from virus infections to guarantee desirable yield and food security.

Keywords: Bambara groundnut, Landraces, Nitrogen Fixation, Nodulation, Virus

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an indigenous legume of Africa; cultivated across the Sub-Saharan and Semi-Arid region of Africa, Southeast Asia in regions of Indonesia and Thailand (Mayes *et al.*, 2019). The crop is the third most important food legume in Africa after groundnut and cowpea, both in consumption and land area under cultivation (Puozaa *et al.*, 2017). It is a hardy, drought-tolerant legume which thrives well in nutrient deficient soils largely due to its ability to form effective root nodules with compatible soil rhizobia that convert atmospheric nitrogen (N) to Ammonia for bacterial and plant use, thereby contributing to soil fertility (Mbosso *et al.*, 2020). However, the ability of a plant to form nodules along with the subsequent capacity of fixing nitrogen (symbiotic effectiveness, SE) could be hampered when infected with a virus, resulting in significant yield reductions. Also, systemic virus infections of grain legumes severely hamper N2 fixation and the effectiveness of the symbiosis by controlling the plant cellular machinery for replication (López *et al.*, 2017). Among the viruses infecting Bambara groundnut on the fields in Nigeria *Blackeye Cowpea mosaic virus* (BICMV) *Potyvirus* and *Cowpea mild mottle virus* (CPMMV) *Carlavirus* have emerged in the past two decades as an important threat ravaging the crop. The exploitation of the genetic variation in the available landraces of Bambara groundnut for multiple virus resistance with high nodulation and nitrogen-fixing ability could contribute to the improvement of the crop and selection of elite genotypes against the viruses. Also, the availability of seeds of cultivars with a high level of multiple virus resistance could aid in the effective management of virus diseases. Hence, this study was carried out to determine the effects of single and mixed infections on nodulation and nitrogen fixation in virus-inoculated Bambara groundnut plants.

MATERIALS AND METHODS

Source of experimental materials and virus isolates

The five Bambara groundnut landraces used were obtained from the local farmers in Minna, Niger State and the nitrogen fertilizer was obtained from a reputable Agro-chemical Store in Minna. The isolates of BICMV and CPMMV were obtained from the stock in the Department of Crop Production, Federal University of Technology (FUT), Minna, maintained and multiplied on cowpea plants (Ife Brown) to ensure sufficient inoculum.

Virus multiplication

Multiplication of the viruses was done with three plastic pots (30 cm diameter and 30 cm deep) at the rate of two seedlings per pot. The collected virus inoculum in the infected leaf tissues was extracted by grinding the isolate in an extraction buffer (pH 7.2) (0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetraacetic acid and 0.001M L-cysteine per litre of distilled water) at the rate of 1 g/mL. One microlitre of β-mercaptoethanol was dispensed into the extract just before being used. Before inoculation, the plants were dusted with carborundum powder (600 mesh) to facilitate the entry of the virus into the hosts. Inoculation was done mechanically by using a pestle to rub sap over the upper leaf surface from base to top of the leaf and incubated at screenhouse temperatures of 23 – 32° C.

Experimental site and design

The experiment was conducted under screenhouse conditions at the Teaching and Research Farm (9.51752o N, 6.44190 E and altitude 203 masl), FUT, Minna. Top loamy soil (0–15 cm) was collected from the Teaching and Research Farm, bulked together and thoroughly mixed to form a composite. A sub-sample (10 g) of the homogeneous composite soil was air-dried and passed through a 2 mm diameter sieve for physical and chemical analysis. Soil analysis was carried out according to the procedures described by Okalebo *et al.* (2002). The set-up for the assessment of nodulation and nitrogen fixation in Bambara groundnut landraces inoculated with the virus treatment combinations consisted of five landraces of Bambara groundnut, two different viruses (BlCMV and CPMMV) in 4 virus treatment combinations and control, 2 Nitrogen levels (starter N and without starter N) with each replicated two times (5 × 5 × 2 × 2) making a total of 100 experimental bags arranged in a completely randomised design (CRD).

Sowing and seedling inoculation

A total of 100 experimental bags (30 cm diameter and 30 cm deep) were filled with 20 kg of soil and labelled properly based on the treatment. Seeds of the landrace were sown in the bags at the rate of three seeds per bag and seedlings thinned to one plant per bag a week after emergence. After thinning of samples, 1.75 g of nitrate fertilizer was applied to the soil of each N starter treatment sample. The seedlings were then mechanically inoculated with respective virus treatment at 2 and 3 weeks after emergence as presented below: i) Single virus inoculation: The landraces were inoculated singly with each virus BICMV and CPMMV; ii) Mixed virus inoculation: For mixed virus treatments a set of the landraces were first pre-inoculated with one of the viruses singly and 7 days later with the second and vice-versa (Nsa and Kareem, 2015). The plants were irrigated daily with 500 mL and observed for symptom development.

Data collection and analysis

At 45 days after inoculation, the plants were carefully uprooted and rinsed under running tap water through a 40-mesh screen to remove all traces of soil. The number of nodules per plant was counted and the dry weight of the root and the shoot were recorded after drying at 70 °C for 48 hours (Kabede *et al.*, 2020). Data collected were subjected to analysis of variance (ANOVA) using the statistical analysis system (SAS, 2008). Symbiotic effectiveness percentage (SE %) was calculated by comparing the inoculated plant with the N-fertilized positive control (Purcino *et al.*, 2000). Nitrogen-fixing efficiency (NFE) was classified as highly effective (SE %> 80%), effective (SE %= 50-80%), poorly effective (SE % = 35-50% and ineffective (SE % <35%).

RESULTS

Symptomatically, all the inoculated plants exhibited varying degrees of foliar symptoms of both single and mixed virus infections. The mixed infection had high effects on the morphological indices and nodulation when compared to the single inoculated plant. Significant ($p<0.05$) lowest dry weight of shoot (0.57 g) and root (0.09 g) were obtained in BICMV+CPMMV treated plants with parentage reduction of 48.6 and 43.8, respectively (Table 1). Similarly, the shortest shoot (10.61 cm) and root (6.15 cm) length was recorded in BICMV+CPMMV.

The highest average number of nodules (9 per plant) was obtained in the control plant, followed by 6.2 nodules per plant in CPMMV and the lowest of 4.13 nodules per plant in BICMV+CPMMV. However, the lowest nodules per plant were not significantly different ($p>0.05$) from the values of CPMMV+BICMV (4.8 per plant) and BICMV (4.87 per plant (Table 1). Significant ($p<0.05$) differences were observed among the landraces for all the morphological parameters and number of nodules per plant with Vg_05 having the highest dry weight (0.89 g), length of the shoot (15.25 cm) and root (11.92 cm) while Vg_04 had the highest average number of nodules per plant with the value of 6.93 followed by 6.13 in Vg_05. However, there were no significant differences ($p>0.05$) among Vg_01, Vg_02 and Vg_03 with values of 5, 5.33 and 5.6, respectively (Table 1). The mean Nitrogen-fixing efficiency varied from ineffective (SE< 35 %) in BICMV and BICMV+CPMMV to poorly effective (SE = 35-50 %) in CPMMV and CPMMV+BICMV (Table 2). *Blackeye cowpea mosaic virus* exhibited considerable deleterious effects on symbiotic activities of Vg_01 (26.05 %) and Vg_02 (25.91 %) while CPMMV had a higher negative effect on Vg_03 and Vg_04 with a percentage symbiotic effectiveness value of 22.13 % and 25.25 %, respectively. Percentage symbiotic effectiveness for BICMV+CPMMV and CPMMV+BICMV infected plants ranged from 24.21 – 43.96 % and 25.10 – 46.48 %, respectively (Table 2). However, the percentage of symbiotic effectiveness of the control plots varied from 56.42 % in Vg_01 (Effective, SE = 50-80 %) to 91.22 in Vg_05 (Highly effective, SE>80 %).

Table 1: Growth indices and nodulation of Bambara groundnut infected with single and mixed *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mild mottle virus* (CPMMV)

Factor	Dry Weight				Length				Number of Nodules
	Shoot (g)	% Red	Root (g)	% Red	Shoot (cm)	% Red	Root (cm)	% Red	
Treatment									
BICMV	0.83b	25.2	0.11c	31.3	11.38d	38.9	7.65b	49.3	4.87c

CPMMV	0.62c	44.1	0.15b	06.3	16.97b	09.0	14.63a	03.1	6.20b
BICMV+CPMMV	0.57c	48.6	0.09c	43.8	10.61d	43.1	6.15c	59.3	4.13c
CPMMV+BICMV	0.59c	46.8	0.14b	12.5	12.55c	32.7	8.82b	41.6	4.80c
Control	1.11a	-	0.16a	-	18.64a	-	15.10a	-	9.00a
SEM	0.06		0.01		0.38		0.51		0.36

Landrace					
Vg_01	0.76a	0.16a	13.77bc	7.82c	5.00b
Vg_02	0.55b	0.14ab	14.51ab	11.67a	5.33b
Vg_03	0.68ab	0.13ab	13.84bc	9.73b	5.60b
Vg_04	0.79a	0.09c	12.79c	11.21a	6.93a
Vg_05	0.89a	0.12b	15.25a	11.92a	6.13ab
SEM	0.06	0.01	0.38	0.51	0.36

Means followed by similar alphabet letters are not significantly different by Student-Newman-Keuls (SNK);
% Red = Percentage reduction

Table 2: Symbiotic effectiveness of Bambara groundnut infected with single and mixed *Blackeye cowpea mosaic virus* (BlCMV) and *Cowpea mild mottle virus* (CPMMV)

Treatment	Vg_01	Vg_02	Vg_03	Vg_04	Vg_05	Mean	NFE
BICMV	26.05	25.91	33.02	34.50	26.00	29.10	IE
CPMMV	45.92	36.91	22.13	25.25	47.59	35.56	PE
BICMV+CPMMV	37.68	28.27	43.96	26.07	24.21	32.04	IE
CPMMV+BICMV	45.16	46.48	25.10	25.32	39.88	36.39	PE
Control	56.42	68.23	70.35	67.44	91.22	70.73	E
Mean	42.25	41.16	38.91	35.72	45.78	40.76	

NFE = Nitrogen fixing efficiency, HE = Highly effective, E = Effective, PE = Poorly effective, IE = Ineffective. Highly effective (SE>80 %), Effective (SE= 50-80 %), Poorly effective (SE = 35-50 % and Ineffective (SE<35 %)

DISCUSSION

The expression of typical foliar symptoms of BICMV, CPMMV or both viruses by all the inoculated plants indicated that none of the Bambara groundnut landraces was immune to the disease. The low growth (weight and length of shoot and root) parameters values and the number of nodules recorded in the inoculated plants of this study could be attributed to the proliferation of particles from replicating virus (es), resulting in alteration of growth pathways. In support of this assertion, Salaudeen and Aguguom (2014) reported that a series of physiological changes are triggered as soon as a virus is introduced into a host plant depending largely on the genetic composition of the infected plant. Similar to this result, López *et al.* (2017) who worked on the plants pre-infected with *Southern bean mosaic virus* (SBMV) attributed the greater magnitude of the deleterious effects on growth masses to the virus' presence in the radicles. The authors further ascribed this to the virus hampering the catabolism of ureides in the leaves.

The N2 fixation by bacteroids within the nodules has great importance in agriculture since leguminous crop yields are highly enhanced in Rhizobium-nodulated plants grown in low nutrient soils (Mabrouk and Belhadj, 2016). The ability of a plant to form nodules along with the subsequent capacity to fix nitrogen (symbiotic effectiveness) is widely used as means of evaluating the inherent links between rhizobia and respective hosts (Osei *et al*., 2018). In conformity with the result of this study, a drastic reduction in root nodulation of virus-infected plants had been reported by López *et al*. (2017). The variation in the number of nodules among the landraces indicated that responses to nodule efficiency differed from one landrace to another. The high number of nodules recorded in the control plants as well as Vg_04 and Vg_05 indicated sufficiently establishment of the Rhizobium–host relationship for nodulation. The poor to ineffectiveness in symbiotic activities of all the landraces when inoculated with either or both viruses could be attributed to an alteration in symbiotic signalling molecules.

CONCLUSION

The study confirmed that all tested viruses reduced growth indices and nodulation of the infected plans. It was also found that either BICM and CPMMV or their combination decreased nitrogen fixation with greater deleterious effects exerted by the mixed viruses. Though the landraces showed different symbiotic characteristics, Vg_05 had proven to be more effective among the landraces and could be explored further to determine its potential for crop improvement.

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