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Interactive effects of virus and *Rhizobium* inocula on nodulation, growth and yield of cowpea

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The effect of separately inoculating cowpea cultivars, 'Ife brown" (IF) and "Owode" (OW) with *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea yellow mosaic virus* (CYMV) and *Rhizobium* IRc 284(RH-284) on nodulation was investigated. Also, the effect of inoculating the cowpea cultivars with RH-284 on the severity of infection caused by the viruses was studied. The interactive effects of inoculating cowpea with RH- 284 and each of CABMV, CYMV, *Southern bean mosaic virus* (SBMV) and *Blackeye cowpea mosaic virus* (BICMV) on nodulation, growth and yield of IF and OW were also investigated. The results showed that infection by CYMV and CABMV significantly reduced nodulation by about 20-30% and 40-45% in IF and OW, respectively. Inoculating with RH-284 alone significantly increased nodulation by about 20% in both cowpea cultivars. In the interactive study involving virus-RH 284 inocula, slight but non-significant increases of 22, 2 and 9% in nodule number were observed in IF inoculated with RH-284 and SBMV, CYMV and CABMV, respectively. The differences observed in the nodule, shoot and seed weights were not significantly different from those of the control. There was a negative correlation between nodule number and severity of symptom. BICMV caused the most severe effect on the two cowpea cultivars. It reduced the number of nodules by 55-66% with or without RH-284. It also caused significant reductions of over 80% in nodule and seed weights of OW. In conclusion, increase in nodulation reduced viral disease severity, the slight but non-significant increases observed in the growth and yield parameters suggest that improved nodulation can be advantageous to cowpea.

Key words: Blackeye cowpea mosaic virus, cowpea aphid-borne mosaic virus, cowpea yellow mosaic virus, southern bean mosaic virus.

INTRODUCTION

Cowpea is an important food and fodder legume in the sub-humid tropics of Africa. As a food, the grain is an important source of dietary protein especially for the West African populace where two-thirds of the world's cowpea grain is produced. The crop has therefore attracted a lot of attention from researchers who have in recent years intensified their efforts at improving its agronomic and nutritional qualities (Rachie, 1985).

Cowpea grain yields vary greatly in different parts of the world. Singh (1980) estimated that the average yield for the crop grown in monoculture is about 1.5 t/ha in the United States of America, 650 kg/ha in South America and Asia, and is often below 400 kg/ha in Africa. The low cowpea yield in Africa is mainly due to pests and

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diseases. Among the numerous pathogens the effect of these viruses can be devastating and they are a major constraint to increased grain production. Although, nine viruses are reported on cowpeas in sub-Saharan Africa (Taiwo, 2003), only *Cowpea aphid- borne mosaic virus* (CABMV) genus *Potyvirus* and *Cowpea yellow mosaic virus* (CYMV) genus *Comovirus* are considered to be very important as far as geographical distribution, pathogenic variability and yield losses are concerned (Thottappilly and Rossel, 1992). *Cowpea mottle virus* (CMeV) genus *Carmovirus, Southern bean mosaic* (SBMV) genus *Sobemovirus*, *Cowpea golden mosaic* (CGMV) genus Begomovirus, Blackeye cowpea mosaic virus (BICMV) genus *Potyvirus* and *Cucumber mosaic* viruses (CMV) genus *Cucumovirus* are considered to be of localized importance (Taiwo, 2003).

Yield reduction attributable to CYMV infections range from 40-100% (Chant, 1960; Wells and Deba, 1961; Shoyinka, 1974; Gilmer et al., 1974) while a virus suspected to be CABMV caused a complete loss in yield in northern Nigeria (Raheja and Leleji, 1974).

Efforts to control viral diseases of cowpea may involve one or more measures intended to reduce sources of infection, roguing of diseased plants and the use of insecticides to prevent virus transmission. Currently, host plant resistance is the most effective method of controlling cowpea viruses in Africa (Thottappilly and Rossel, 1992).

However, like many other legumes, cowpea can symbiose with nodule bacteria present in most tropical soils. Effective cowpea-*Rhizobium* symbiosis fixes more than 150 kg/ha of N_2 and supply a considerable amount of the N_2 requirement of plants (Summerfield et al., 1977). This attribute allows healthy growth and hence optimum yields. Symbiotic N_2 fixation in cowpea-root nodules is a result of the complex biological and biochemical interactions between the host legume and the rhizobial endophyte. If the process is efficient, the plant grows healthily, thereby minimizing the impact of diseases. It is therefore, expected that the extraneous application of *Rhizobium* to cowpea at planting should increase its population in the soil, ensure optimal and rapid growth of the crop, and thus enhance its resistance to phytopathogenic agents, especially viruses.

This study was therefore carried out to assess the effect of rhizobial inoculant on growth and seed yield of cowpea, the impact of viral infection on nodulation, vegetative and seed yield of the crop and determine the extent of amelioration of the impact of viral infection on cowpea by rhizobial inoculant.

MATERIALS AND METHODS

Two greenhouse studies were carried out to determine the effect of inoculating 2 cowpea (*Vigna unguiculata* L. Walp) genotypes (Ife Brown (IF) and Owode (OW)) with cowpea rhizobium IRc 284 (RH-284) and virus. The interaction of the microorganisms on the 2 cowpea genotypes was also evaluated.

Preliminary greenhouse 1

It was a factorial experiment consisting of 2 cowpea genotypes (IF and OW, one rhizobial inoculant (RH-284) I+ and Io and 2 virus strains, CABMV and CYMV, and control. Thus 12 treatment combinations were obtained. The treatments were replicated 3 times and arranged in a randomized complete block design (RCBD). In this study, only nodule number was assessed.

Greenhouse 2

In this experiment, a more detailed study was carried out using the 2 genotypes in the preliminary work as well as rhizobium strain. However, additional viruses, BICMV as well as SBMV were used. Therefore, a factorial design that consisted of 2 cowpea genotypes, 2 rhizobial inoculants I+ and Io and 4 virus strains with a control was set up. The twenty treatment combinations obtained were replicated 8 times.

Rhizobial inoculation

Seeds of IF and OW were inoculated with the cowpea *Rhizobium* IRc 284 (RH-284) (obtained from the culture bank of the International Institute of Tropical Agriculture (IITA)) at a concentration of 10^7 - 10^8 colony forming unit (CFU) before planting in soil at three seeds per pot.

The seeds for the control plants were not inoculated with the rhizobial inoculum. The RH-284 inoculum was prepared in yeast extract mannitol broth (YEM) (Vincent, 1970), (Mannitol, 20.0 g; $(NH_4)_2SO_4$ 1.0g; $MgSO_4.7H_20$, 0.5g; yeast extract, 0.2 g; $FeCl_3$, 2.0 mg; $MnSO_4.H_2O$, 4.0 mg; in 1 L of distilled water pH 6.8). The medium (100 ml/flask) was autoclaved at 121°C for 15 min, cooled, inoculated with RH-284 and incubated for about 7 days on a rotary shaker at 28°C, before being used as inoculum. The number of colony forming units (CFU) on plate count agar was 10^8. Numbers of nodules on 3 plants of the replicates each of RH-284 inoculated and un-inoculated plants were determined after six weeks. A photograph of nodules on roots was also taken.

Virus inoculation

Three seeds were separately sown per pot. The seedlings were later thinned to 2. Seedlings of 8-day old plants of IF OW that were to be inoculated were mechanically inoculated with the viruses. Mechanical transmission was with viral inocula prepared in 0.01 M phosphate buffer pH 7.1 according to Nordam (1973), while buffer inoculated plants served as control. The inoculated plants were labeled and kept in an insect-proof screen house at temperatures of 28-32°C. They were regularly observed for symptom development. The severity of symptoms observed was rated on a scale of 1 - 5, (5 for very severe infection, sometime death, 4 for severe infection, 3 for moderate infection; 2 for mild infection and 1 for very mild infection).

Plants were harvested after six weeks, the plants were carefully uprooted, the soil was washed off and the nodules on the roots counted.

Nodule number and weights, vegetative weights were determined on 3 of the 8 replicated plants while seed yield was determined on the other replicates at maturity.

Analysis of variance (ANOVA) was used to determine significant differences and means of the significantly different sources were separated using Duncan multiple range test.

Table 1. Analysis of variance table for nodule number in preliminary Experiment.

Variation	df	Mean sum of square
Cowpea (block)	1	0.798
Virus	4	16.69**
Inoculant (RH)	1	39.33**
Cowpea x RH	1	0.090
Cowpea x Virus	4	0.040
Virus x RH	4	1.990
Cowpea x Virus x RH	4	0.356
Error	24	5.267

** Significant at P≤0.01.

Table 2. Effect of viral and rhizobial inoculation on nodulation in the preliminary study.

	Treatment	Nodule number
Virus inoculant treatments	control	5.83[a]
	CYMV	4.35[b]
	CABMV	3.87[b]
Rhizobia inoculant treatments	control	3.78[b]
	RH-284	5.59[a]
	Standard error	0.34

Means followed by same letter in a column in each treatment are not significantly different.

Figure 1. Effect of virus on nodulation in roots of A: healthy and B: CABMV = Cowpea aphid-borne mosaic virus-infected cowpea plants.

RESULTS

Analysis of variance in Table 1 showed that the 2 viruses had significant (p<0.01) effect on number of nodules in the 2 cowpea genotypes in the preliminary study. Rhizobium inoculant significantly impacted on the number of nodules. The number of nodules on Ife Brown (IF) and Owode (OW) cowpeas was not significantly different (Table 2). Cowpea genotypes without virus had higher number of nodules when compared with virus infected plant. The number of nodules was significantly higher (p<0.01) than that of plants inoculated with CYMV and CABMV by over 20 and 30%, respectively. Number of nodules on plant inoculated with CABMV and CYMV were not significantly different. Rhizobium significantly (p<0.01) increased nodule number by 30% when compared with uninoculated plant.

Also, in the preliminary study, virus infection adversely affected the number and size of the nodules in the cowpea genotypes. Fewer and smaller nodules were formed and the growths of root hairs as well as the lateral roots were impaired in virus-infected plants (Figure 1).

In the second greenhouse study, nodule numbers were not significantly different from each other in the 2 cowpea genotypes but vary significantly with respect to nodule weight in the analysis of variance (Table 3). The viruses impacted significantly on nodule number and weights. There were also significant (p<0.01) cowpea-virus interaction as well as virus-rhizobium interaction on nodule weight. The 3 factors also interacted on the number of nodules (Table 3).

Root weights in the 2 cowpea genotypes varied significantly from each other but not with the shoot weight (Table 4). There was significant interaction of cowpea and virus on root while virus and rhizobium interaction significantly impacted on weight of shoot. Viruses as well as rhizobium had significant effect on weight of seeds.

Table 5 shows the effect of variety, rhizobium and virus on nodulation, vegetative and seed yield of cowpea. The number of nodules in the 2 cowpea genotypes did not differ significantly but the weight of nodules, root and shoot in Ife Brown were significantly higher than that of Owode variety. Owode genotype however, gave a significantly higher seed yield than IF. Rhizobium significantly increased nodule number but not nodule weight. The nodule weights were also significantly increased by RH-284. Numbers of nodules were significantly reduced by BICMV (IT16) when compared with other viruses and control. Nodule weight in BICMV was significantly reduced by 50% relative to virus-free control. Nodule weight in BICMV-treated cowpea was 30% lower than control and SBMV-treated plants. Weight of shoot and seed were also significantly reduced by 60 and 50% respectively by BICMV relative to control. Seed weights of cowpeas inoculated with CYMV and CABMV were significantly reduced relative to control.

The interactive effect of the 3 factors, namely; variety, rhizobium, rhizobium and viruses on the parameters are

Table 3. Analysis of variance table for nodule number and weight in green house (Experiment 2).

Variation	df	Mean sum of square (number of nodule)	Mean sum of square (weight of nodule)
Cowpea (Block)	1	0.86	2.32**
V	4	129.84**	9.81**
Innoculation (RH)	1	17.7	0.26
Cowpea x RH	1	0.63	0.08
Cowpea x Virus	4	4.11	0.92**
Virus x RH	4	8.14	1.12**
Cowpea x Virus x RH	4	22.41*	0.48
Error	24	10.4167	0.016111

** Significant at P≤0.01, ANOVA; * Significant at P≤0.01, ANOVA

Table 4. Analysis of variance table for weight of shoot and seeds greenhouse (experiment 2).

Variation	df	Shoot weight			Weight of roots		
		Sum of square	Mean sum of square	F	Sum of square	Mean sum of square	F
Cowpea (Block)	1	0.822	0.822	0.32	2.48	2.48	17.6**
V	4	63.84	15.96	6.213**	29.96	7.49	53.1**
Innoculation (RH)	1	0.54	0.543	0.21	0.01	0.01	0.07
Cowpea x RH	1	2.426	2.426	0.95	0.01	0.01	0.07
Cowpea X Virus	4	15.315	3.829	1.491	0.83	0.21	1.5
Virus X RH	4	41.581	10.395	4.05	0.73	0.18	1.3
Cowpea X Virus X RH	4	3.785	0.946	0.37	1.11	0.28	1.987
Error	24	61.65	2.56875		3.3816	0.1409	

**Significant at P≤0.01, ANOVA, * Significant at P≤0.01, ANOVA. V, Virus; RH, rhizobium; Cowp, cowpea and RH, rhizobium.

Table 5. Effects of varietal difference, rhizobial and viral inoculation on nodulation, vegetative and seed yield of cowpea.

Genotypes cowpea	Nodule number	Nodule weight (g)	Weight of sht (g)	Weight of seeds (g)
IF	8.29^{a}	0.36^{a}	2.61^{a}	2.44^{b}
OW	8.09^{a}	0.33^{b}	2.40^{a}	2.80^{a}
Rhizobial inoculant				
RH_0	7.72^{b}	0.35^{a}	2.42^{a}	2.61^{a}
RH	8.66^{a}	0.39^{a}	2.59^{a}	2.62^{a}
Virus inoculant				
V_0	9.88^{a}	0.39^{a}	2.77^{ab}	3.10^{a}
CYMV	9.36^{a}	0.37^{b}	2.31^{b}	2.83^{b}
CABMV	8.75^{a}	0.37^{ab}	2.68^{ab}	2.78^{b}
BICMV(IT16)	3.16^{b}	0.21^{c}	1.00^{c}	1.41^{c}
SBMV-Oyo	9.81^{a}	0.38^{ab}	3.77^{a}	2.95^{ab}
EMS	4.32	0.20	2.54	0.14

CYMV = Cowpea mosaic virus, CABMV = Cowpea aphid-borne mosaic virus, BICMV = Blackeye cowpea mosaic virus, SBMV = Southern bean mosaic virus, RH = Rhizobium, IF = Ife brown and OW = Owode

found in Table 6. Only dry weight of shoot of IF that was free of virus was significantly (p, 0.01) increased by RH-284. Number of nodules as well as weight of seeds of IF inoculated with CYMV was significantly enhanced by rhizobium RH-284. With CABMV on IF, dry weight of shoot was significantly increased by rhizobial inoculant. Even though Ife brown cowpea was infected with BICMV, the number of nodules was increased by RH-284. Number of nodules, weight of shoot and seeds of IF were significantly increased by RH-284 relative to un-inoculated

Table 6. Interactive effects of variety, rhizobial and viral inoculation on nodulation, vegetative and seed yield of cowpea.

Treatments	Nodule number	Nodule weight	Weight of shoot	Weight of seed
1.IFV$_0$RH	11.32^{abc}	0.4^{abc}	4.33^{ab}	2.49^{ef}
2. IFV$_0$RH0	8.98^{bcdef}	0.20^{cd}	3.52^{efg}	2.64^{cdef}
3. IFCYMVRH	7.62^{ef}	0.30^{bcd}	3.46^{efg}	2.56^{def}
4. IFCYMV	3.15^{g}	0.11^{d}	2.91^{g}	1.43^{g}
5. IFCABMVRH	7.60^{ef}	0.2^{cd}	3.99^{abcde}	3.00^{abcde}
6. IFCABMV	6.09^{gf}	0.2^{cd}	3.43^{efg}	3.43^{a}
7. IFBICMVRH	10.81^{abcd}	0.40^{abc}	4.19^{abcd}	2.86^{bcdef}
8. IFBICMV	8.04^{def}	0.28^{bcd}	3.58^{def}	3.14^{abc}
9. IFSBMVRH	3.16^{g}	0.12^{d}	2.12^{h}	1.43^{g}
10. IFSBMV	10.43^{abcde}	0.27^{bcd}	3.51^{efg}	3.13^{abc}
11. OWV0RH	10.27^{abcde}	0.33^{abc}	4.43^{a}	3.25^{ab}
12. OWV0	8.85^{cdef}	0.17^{cd}	3.41^{efg}	2.64^{cdef}
13. OWCYMVRH	9.62^{bcde}	0.28^{bcd}	4.24^{abc}	2.40^{f}
14. OWCYMV	4.14^{g}	0.10^{d}	1.56^{h}	1.41^{g}
15. OWCABMVRH	12.37^{a}	0.55^{a}	4.33^{ab}	2.60^{def}
16. OWCABMV	11.85^{ab}	0.19^{cd}	3.78^{bcdef}	3.25^{ab}
17. OWBICMVRH	8.80^{cdef}	0.17^{cd}	3.55^{efg}	3.19^{ab}
18. OWBICMV	9.69^{abcde}	0.16^{cd}	3.62^{cdef}	3.03^{abcd}
19. OWSBMVRH	3.17^{g}	0.10^{d}	1.67^{h}	1.41^{g}
20. OWSBMV	8.85^{cdef}	0.5^{ab}	3.32^{fg}	3.06^{abcd}

Standard error of mean. All values are averages of 4 replicates. Values followed by the same letters of the alphabet are not significant. CYMV = Cowpea mosaic virus, CABMV = Cowpea aphid-borne mosaic virus, BICMV = Blackeye cowpea mosaic virus, SBMV = Southern bean mosaic virus, RH = Rhizobium.

plant carrying SBMV.

On virus-free Owode genotype, dry weight of shoot and seed were significantly increased by RH-284 relative to uninoculated plant. However with CYMV, number of nodules, dry weight of shoot and seed were significantly increased relative to RH-284 free plant. Weight of shoot and seeds as well as nodule weight of CABMV-inoculated plant were significantly enhanced by rhizobium. Rhizobial inoculation had no positive effect on all the parameters of BICMV inoculated OW genotype but enhanced nodule number and weight as well as weight of shoot and seeds of SBMV inoculated OW.

DISCUSSION

Yield reduction or outright crop failure in cowpea resulting from viral attack are of common occurrence in sub-Saharan African. This has necessitated the need to carry out studies on biological strategy to reduce the incidence of cowpea viruses.

Results in the preliminary study have shown that the 2 viruses used had significant negative impact on the number of nodules in the 2 cowpea genotypes used. While no significant difference was observed in the 2 cowpea genotypes used with respect to their growth. Cowpea yellow mosaic virus (CYMV) and cowpea aphid borne mosaic virus (CABMV) significantly depressed nodule number. Infection of cowpea by these viruses led to impairment of vegetative growth with most of the leaves curling. This indicates that some physiological processes of growth might have been impaired. Nodulation, according to Denarie et al. (1996) and Spaink (2000) is a physiological process that involves the production of diffusible plant and bacterial metabolites such as flavonoids and lipo-chitooligosaccharides respectively, which trigger certain steps of the processes. It is assumed that interference of these processes had caused the reduction in nodule number. Effective nodulation also depends on the population of infective rhizobia in soils. Increasing the soil population through inoculation in this study had led to improved nodulation.

Significant increases in nodule number and root weight were observed in *Rhizobium* inoculated cowpeas. Legume inoculation is a process for the manipulation of rhizobial microflora for improving crop productivity and soil fertility (Keyser and Li, 1992). Although, rhizobial species are as widely distributed as the legumes themselves, there are many soils where suitable strains are absent, or where the population density is as low as to pose a threat to legume establishment and effective nodulation and N_2 fixation. The population of indigenous rhizobial in most tropical soils is very low and these indigenous strains are less efficient in fixing nitrogen

(Ahmad et al., 1981; Ahmad and Mclaughling, 1985). The selection and application of specific rhizobial inoculant as carried out in this study can be exploited in sustainable cowpea production. It was generally observed that nodule number and other growth parameters assessed were significantly reduced by BICMV (IT16). The infection caused by BICMV demonstrated this by resulting in significant growth and yield reductions in the cowpea cultivars used. This confirms previous results by Owolabi et al. (1988) which indicated that BICMV posed a more serious threat than any other virus to the production of cowpea cultivars. This was shown in more severe symptoms including death of plants at an early age in BICMV infections. Viruses such as CABMV and CYMV do cause severe symptoms including mosaic, green vein-banding, stunting and dramatic yield losses. The loss may range between 40-100% depending on the age of the plant at the time of infection (Chant, 1960; Raheja and Leleji, 1974). These results agree with those reported by Tu et al. (1970*)* and Hair and Miller (1982) working with clover and cowpeas, respectively. Patil and Sayyad (1994) reported a greater reduction in nodule number in virus infected cowpea than in *Rhizobium*-virus infected plants. They also reported that the reduction in the fresh weight of the nodules was greater than the reduction in fresh weight of plant due to CYMV.

On the interactive effect of RH-284 and viruses, inoculation of IF and OW with RH-284 significantly increased some parameters in spite of viral infection. This implies that virus infected plants produced more severe symptom of infection when they were not inoculated with rhizobium than *Rhizobium*-virus treated plants. The increase in nodule number and some other parameters of RH-284 inoculated plant relative to un-inoculated control indicated that the inoculant strain contributed significantly to nodulation. Eaglesham (1985) noted that it might be safer to rely on effective inoculant strain than breed plant for the ability to nodulate with indigenous strains of unknown potential. However, rhizobial inoculant can only be successful if it is more competitive than the native rhizobial in nodule formation and N_2 fixation.

Generally, in legume symbiosis, regulation of N_2-fixation is mediated by the host legume rather than by the bacterial symbiont (Giller and Wilson, 1993). In this study, infection by the viruses impaired some growth and yield parameters in spite of rhizobial inoculation. The inference drawn here is that it is only the severity of infection that can be reduced, infection cannot be stopped with rhizobium inoculation and not the infection of virus itself. The actual effect of increased nodulation and N_2-fixation was subsumed in the negative impact of virus attack, leading eventually to the complete suppression of the growth advantage conferred on the plants by the rhizobial inoculants.

There may be the need to experiment with various concentrations of the rhizobial inoculant, in order to derive maximum advantage from the positive impact of rhizobial inoculation.

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