

A COMPARATIVE STUDY OF DIFFERENT COWPEA GENOTYPES IN BREEDING FOR MOTTLE VIRUS DISEASE RESISTANCE

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ABSTRACT

An experiment was conducted in a screen house at International Institute for Tropical Agriculture, Kano station in 2008/2009 with an objective to compare the response of 20 different cowpea genotypes to mottle virus disease and to select the most suitable cowpea germplasm for resistance to CMV. The experiment was laid out in a completely randomized design and replicated three times. All collected data were subjected to the analysis of variance using statistical package Genstat, and the means were separated using least significant difference (LSD). The result shows that there was no significant difference of the effect of disease on the number of pods per plant, pod weight per plant, seed per pod and seed weight per pod. However, it affected all the treatments significantly ($P < 0.05$) in terms of plant height, flowery and pod development. This confirmed the susceptibility of these genotypes to cowpea mottle virus infection which resulted in poor flowering, pod setting and reduced plant height which was notably reflected in seed yield (With a reduction of about 10 - 75% loss).

KEYWORDS: cowpea, mottle virus disease, response comparative study

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is an important food legume in semi-humid tropics. The crop has low grain yield, which is 25-50 % and below that as compared with other cereals. Yield stability is a complex product of genetic yield potential and tolerance to stress conditions (Smith and Francis, 1986), and therefore farmers identify insects and diseases particularly virus, bacterial and fungal diseases as major constraints of cowpea production. The old method of disease control using organic and inorganic chemical has now posed hazardous effect on plant and animal health. The most suitable alternative method, pollution free and non phytotoxic is the biological control method, a technique of breeding for disease resistance. This can be easily achieved by exploiting genetic resistance through search for sources of disease resistance. The 20 genotypes were screened in this study with the aim of finding sources of resistance against mottle virus disease. The growing conditions of all the genotype were

made uniform to allow the actual level of observed resistance reveal due in accordance with their response to the virus. This experiment set a specific objective as follows:

- i. To assess the reactions of 20 different cowpea genotype to mottle virus disease.
- ii. To compare the effect of the viral disease on the productivity and yield of the 20 different cowpea varieties.
- iii. To select the most suitable germplasm for resistance to CMV disease

MATERIALS AND METHODS

The experimental site for the study was the screen house of International Institute for Tropical Agriculture (IITA) Kano Station located at latitude 11°59N, longitude 8°35E and altitude 4866m/1595F in the Sudan savannah ecological zone of Nigeria. The following twenty different cowpea varieties were selected from the stock at IITA Kano sub-station:

IT97K-461-4	IT07K-293-18
IT97K-499-35	IT07K-293-3
IT98K-131-2	IT07K-299-4
IT98K-568-18	IT98K-300-12
IT06K-133	IT06K-301-3
IT07K-178-1	IT07K-302-20
IT07K-187-24	IT07K-302-27
IT07K-290-73	IT07K-308-1
IT07K-292-10	IT07K-311-1
IT07K-293-16	Aloka local

These varieties were developed at the station and subjected to various growing conditions for adaptability to a suitable production zone. The experiment was designed to assess the response of these varieties to mottle viral disease and its significant effect on crop yield and productivity.

It was carried out in a completely randomized design (CRD) and replicated three times. Sixty plastic pots (17.0cm in diameter) were used in the experiment and they were all filled up with sterilized fine sand and arranged in the screen house in a completely randomized design (CRD).

Four grams (4g) of elemental phosphorous fertilizer was applied before planting to each plastic pot and followed by a periodic irrigation as pre-sowing operations. Seeds were planted in the second week of June in plastic pots at a depth of 2-3cm a week after pot filling. All the raised seedlings were thinned to one plant per pot and the crop was irrigated three times fortnightly.

Mechanical Inoculation of the Plant Virus

Young infected plant tissues (leaves) showing primary symptoms of the virus were obtained from IITA and kept in a freezer (-4°C) before the inoculums preparation. 100g of the sample of infected plant tissues were then weighed on a weighing balance and prepared viral inocula by grinding infected leaves systematically in a sterilized mortar with pestle. One litter of distilled water was added into the sap (making a solution of 1ppm) and filtered with a filter paper to separate the residue from the prepared inoculums. The inoculums were then kept in a freezer (-4°C) until the end of the experiment. Mechanical inoculation with the virus inoculums was carried out 10 days after sowing (DAS). Prior to this, the whole varieties were dusted with sterilized fine river sand (2mm size) before inoculation.

All the twenty varieties were monitored regularly from the date of inoculation up to the emergence of the first symptom. Then, some quantitative characters (plant height, number of flowers per plant, number of pods per plant), disease expression, severity of the disease were observed and recorded at a weak interval until the end of the experiment (9 WAS). The number of seeds per pods, number of pod and seed weight were also measured and disease severity was determined using

$$\text{Severity} = \frac{\sum n}{N \times H} \times 100$$

Where:

\sum =summation

n=individual assessment

N=number of assessed plant

H=highest score in the scale

Analysis of variance of the result was carried out to test the treatments' response and to reveal any significant difference among the treatments using Genstat discovery third edition (Developed by USN international), and all the means were compared using the least significant difference (LSD).

Results

Reaction of some early cowpea varieties to cowpea mottle virus infection recorded from 4WAS to 9WAS showed significant difference in 6 and 8 WAS, while there was no significant difference at 4, 5, 7 and 9 WAS (Table.1a). This showed that almost all the cowpea varieties used were I significantly (P<0.05) susceptible to the virus at 6 whereas IT07k-290-73 had lower susceptibility at 8 WAS with a Systemic symptoms which varied from green-vein, mottling, shortening of the internodes, apical necroses and reduction in leaf size, all were induced to plants that were inoculated mechanically with the cowpea mottle virus (Table 1a). This is an indication that 6 and 8 WAS were the periods that virus infects the crop gnificantly, stage t which the crop need to be protected and to control the disease vector.

The crop started flowering at 5th WAS and some scores of effect of cowpea mottle virus were recorded on number of flowers per plant and there was significant difference at 5WAS, 8WAS, and 10WAS. However no significant difference was observed at 6WAS, 7WAS and 9WAS (Table 2). On the other hand the effect of cowpea mottle virus on plant height (cm) recorded from 4WAS to 9WAS was not significantly different (Table 3). Pod development at 6WAS to 10WAS showed only significant response to cowpea mottle virus at 5WAS while there was none at 7WAS, 8WAS, 9WAS and 10WAS (Table 4)

On the other hand, the effect of cowpea mottle virus infection on harvested pod: pod weight per plant, seeds per pod, and seed weight per pod was also observed and the result shows that there were

Table 1a: Reaction of some early cowpea varieties to cowpea mottle virus infection at IITA kano sub-station

<u>Week after sowing</u> Varieties	4	5	6	7	8	9
Aloka Local	1.57	1.57	2.00a	2.00	2.00b	2.00
IT06K-133	2.00	2.00	2.00a	2.00	2.00b	2.33
IT07K-178-1	2.00	2.00	2.00a	2.00	2.00b	2.33
IT07K-187-24	1.67	1.67	2.00a	2.00	2.00b	2.00
IT07K-290-73	1.67	1.67	2.67b	2.00	2.33a	2.33
IT07K-292-10	2.00	2.00	2.00a	2.00	2.00b	2.00
IT07K-293-16	1.67	2.00	2.00a	2.00	2.00b	2.00
IT07K-293-18	1.67	2.00	2.00a	2.00	2.00b	2.00
IT07K-293-3	2.00	2.00	2.00a	2.00	2.00b	2.00
IT07K-299-4	1.00	1.67	1.67b	2.00	2.00b	2.00
IT07K-300-12	1.00	1.67	2.00a	2.00	2.00b	2.00
IT07K-301-3	1.67	1.67	2.00a	2.00	2.00b	2.00
IT07K-302-20	1.67	2.00	2.00a	2.00	2.00b	2.00
IT07K-302-27	1.67	2.00	2.00a	2.00	2.00b	2.00
IT07K-308-1	2.00	2.00	2.00a	2.00	2.00b	2.00
IT07K-311-1	1.33	1.67	2.00a	2.00	2.00b	2.00
IT97K-461-4	1.67	1.67	2.00a	2.00	2.33a	2.33
IT97K-499-35	1.00	1.33	2.00a	2.00	2.00b	2.00
IT98K-131-2	1.00	2.00	2.00a	2.00	2.00b	2.33
IT98K-568-18	1.67	2.00	2.00a	2.00	2.00b	2.00
Level of significance	NS	NS	*	NS	*	NS
LSD (0.05%)			0.30b		0.301	
SE ±	0.369	0.279	0.151	0.00	0.149	0.236

NS = Not Significant

* = Significant at 5%

1.00= the plant is free from disease

1.0 < 2.0= the plant is susceptible

> 2.0 = plant was dead

Table 1b Severity of cowpea mottle virus disease (%) at IITA Kano sub - station,

<u>Week after sowing</u> Varieties	4	5	6	7	8	9
Aloka Local	55.5b	55.5b	66.6a	66.6a	66.6b	66.6b
IT06K-133	66.6a	66.6a	66.6a	66.6a	66.6b	77.7a
IT07K-178-1	66.6a	66.6a	66.6a	66.6a	66.6b	77.7a
IT07K-187-24	55.5b	55.5b	66.6a	66.6a	66.6b	66.6b
IT07K-290-73	55.5b	55.5b	55.5b	66.6a	77.7a	77.7a
IT07K-292-10	66.6a	66.6a	66.6a	66.6a	66.6b	66.6b
IT07K-293-16	55.5b	66.6a	66.6a	66.6a	66.6b	66.6b
IT07K-293-18	55.5b	66.6a	66.6a	66.6a	77.7a	66.6b
IT07K-293-3	66.6a	66.6a	66.6a	66.6a	66.6b	66.6b
IT07K-299-4	0.0d	55.5b	55.5b	66.6a	66.6b	66.6b
IT07K-300-12	0.0d	55.5b	66.6a	66.6a	66.6b	66.6b
IT07K-301-3	55.5b	55.5b	66.6a	66.6a	66.6b	66.6b
IT07K-302-20	55.5b	66.6a	66.6a	66.6a	66.6b	66.6b
IT07K-302-27	55.5b	66.6a	66.6a	66.6a	66.6b	66.6b
IT07K-308-1	66.6a	66.6a	66.6a	66.6a	66.6b	66.6b
IT07K-311-1	44.4c	55.5b	66.6a	66.6a	66.6b	66.6b
IT97K-461-4	55.5b	55.5b	66.6a	66.6a	66.6b	77.7a
IT97K-499-35	0.0d	44.4c	66.6a	66.6a	66.6b	66.6b
IT98K-131-2	0.0d	66.6a	66.6a	66.6a	66.6b	77.7a
IT98K-568-18	55.5b	66.6a	66.6a	66.6a	66.6b	66.6b
Level of significance	*	*	*	*	*	*
LSD(0.005%)	10.99	3.01	1.53	0.00	1.53	2.21
SE+	5.50	1.51	0.76	0.00	0.76	1.10

* = Significant at 5%

Table 2: Effect of cowpea mottle virus on number of flowers per plant at IITA Kano sub-station

<u>Week after sowing</u> Varieties	5	6	7	8	9	10
Aloka Local	0b	0	0	0b	1	0b
IT07K-133	0b	0.333	2.67	0b	0	0b
IT07K-178-1	0b	0	0.67	0b	0	0b
IT07K-187-24	0b	0	3	0b	0	0b
IT07K-290-73	0.333a	0.333	0	0b	0	0b
IT07K-292-10	0b	0	0	0b	0	0b
IT07K-293-16	0b	0	1	0b	0	0b
IT07K-293-18	0b	1	2	0b	0	0b
IT07K-293-3	0b	0	4	0b	0	0b
IT07K-299-4	0b	0	0	0.333b	1	0b
IT07K-300-12	0b	0	0.67	0.333b	0	0b
IT07K-301-3	0b	0	0	1.677a	0.667	0b
IT07K-302-20	0b	0	0	0b	0	0b
IT07K-302-27	0b	0	0	0b	0	0b
IT07K-308-1	0b	0	1	0b	0	0b
IT07K-311-1	0b	0	2	0b	0	0b
IT97K-461-4	0.33a	1.667	0	0b	0	0b
IT97K-499-35	0b	0	1	0b	0	0b
IT98K-131-2	0b	0	0	0b	0.333	0b
IT98K-568-18	0b	0	1.33	0b	0	0.333a
Level of significance	*	NS	NS	*	NS	*
LSD (0.05%)	0.3057			0.825		0.2134
SE ±	0.151	0.4582	1.08	0.4075	0.517	0.1054

NS = Not Significant

* = Significant at 5%

Table 3: Effect of cowpea mottle virus on plant height

<u>Week after sowing</u> Varieties	4	5	6	7	8	9
Aloka Local	11.27	13.3	15.4	19.67	22.6	25.1
IT07K-133	19.23	2.6	22.7	22.97	23.1	21.8
IT07K-178-1	13.17	16.3	15.8	15.93	15.9	15.8
IT07K-187-24	15.07	15.8	16.83	18.5	22.5	24.2
IT07K-290-73	18.7	19.8	20.77	20.97	20.8	21.1
IT07K-292-10	17.67	19.6	21.57	28.27	31.3	36.9
IT07K-293-16	18.67	20.1	20.43	20.93	21.5	21.5
IT07K-293-18	17.73	18.4	20.27	20.2	20.6	20.5
IT07K-293-3	17.4	18.8	21.17	22.67	22.6	22.7
IT07K-299-4	15.6	17.5	19.33	24.23	26	26.6
IT07K-300-12	14.67	13.8	17.33	19.27	19.4	19.6
IT07K-301-3	15.1	16	17.43	22.93	31.5	42.9
IT07K-302-20	15.47	17.3	19.43	22.7	31.7	37.8
IT07K-302-27	15.23	17.2	18.43	23.5	27.7	28.4
IT07K-308-1	15.17	16.3	16.97	20.03	26.4	31.4
IT07K-311-1	15.93	18.5	21.07	31.9	41.9	44
IT97K-461-4	15.93	18.5	18.73	19.33	15.79	17.7
IT97K-499-35	16.27	18.2	19.17	23.53	23.9	33.9
IT98K-131-2	15.17	17.7	20.7	29.73	37.5	43.5
IT98K-568-18	15.23	16.4	18.37	24.47	27.5	31.4
Level of significance	NS	NS	NS	NS	NS	NS
SE ±	1.425	1.397	1.379	3.625	8.03	12.01

NS = Not Significant

Table 4: Effect of cowpea mottle virus on pod development at IITA kano sub-station

Week after sowing	6	7	8	9	10
Varieties					
Aloka Local	0b	0	0	0	0.67
IT07K-133	0b	0	2.67	3.67	3.33
IT07K-178-1	0b	0	2.67	3.67	2
IT07K-187-24	0b	0	1	0.67	1.33
IT07K-290-73	0.667a	2.333	2	2.33	2.33
IT07K-292-10	0b	0	0	0.67	0.67
IT07K-293-16	0b	1.667	2	3.67	1.67
IT07K-293-18	0b	0.667	2	3.67	1.67
IT07K-293-3	0b	0	1	4	2
IT07K-299-4	0b	0	0	0.33	2.33
IT07K-300-12	0b	0	1	2.67	2.67
IT07K-301-3	0b	0	0	0.67	0
IT07K-302-20	0b	0	0	0	0
IT07K-302-27	0b	0	0	0	0
IT07K-308-1	0b	0	1.33	1.67	1.67
IT07K-311-1	0b	0	1	1.33	1.33
IT97K-461-4	0b	2.667	1.33	1.33	1.33
IT97K-499-35	0b	0	1.33	1.67	1
IT98K-131-2	0b	0	0	0	0
IT98K-568-18	0b	0	0	0	0
Level of significance	*	NS	NS	NS	NS
LSD (0.05%)	0.4268				
SE ±	0.2108	0.5656	0.99	1.216	0.989

NS = Not Significant

* = Significant at 5%

Table 5: Effect of cowpea mottle virus on pod and seed weight: IITA Kano

Variety	Pods Harvested	Pod weight Per Plant	Seeds per pod	Seed weight Per Pod
Aloka Local	1	1.88	9.17	1.6
IT07K-133	2.33	2.43	15.17	2.34
IT07K-178-1	2	4.16	22.73	3.26
IT07K-187-24	1.67	1.16	7.1	0.87
IT07K-290-73	2	1.72	7.67	1.35
IT07K-292-10	1.67	2.68	12	2.14
IT07K-293-16	2	4.01	13.23	2.84
IT07K-293-18	2	2.13	9	1.82
IT07K-293-3	3	2.86	18.1	3.46
IT07K-299-4	2.67	2.89	14	2.42
IT07K-300-12	2.33	2.25	11.33	1.69
IT07K-301-3	2	3.42	12.67	2.87
IT07K-302-20	1.67	2.62	13.23	1.9
IT07K-302-27	1.33	2.28	13.17	1.83
IT07K-308-1	2.67	2.01	8.67	1.56
IT07K-311-1	2.33	2.38	11.33	1.7
IT97K-461-4	1.33	0.92	6.33	0.74
IT97K-499-35	2.67	2.66	12.67	2.02
IT98K-131-2	0.67	1.97	14.53	1.68
IT98K-568-18	3.67	2.33	14.33	1.86
Level of significance	NS	NS	NS	NS
SE ±	0.968	0.71	3.618	0.638

NS = Not Significant

DISCUSSION

Reaction of Cowpea varieties to cowpea mottle virus infection.

The result of this investigation showed that, the twenty cowpea varieties used in this experiment

were susceptible to cowpea mottle virus at particular period of vegetative growth. This is in agreement with the previous finding of Owolabi et al (1988).

At 4WAS, only four varieties IT07K-299-4, IT07K-300-12, IT97K-499-35 and IT98K - 131 - 2 were found free from infection, even though, they were mechanically inoculated at the same day with the other varieties but they did not show any symptoms of infection at this week despite the systemic action of the virus. At 5WAS, 6WAS, and 7WAS all the varieties showed response to disease from mild to severe infection. At 8WAS the infection on two varieties (IT07K - 290-73 and IT97K - 461-4) was significant due to the increase in severity of the disease. These increase in severity, results in the death of some varieties (IT06K - 133, IT07- 187-24, IT07-290-73, IT97K - 461 - 4 and IT98K - 131 - 2) at 8WAS and 9WAS. (Table 1a & b)

Effect of cowpea mottle virus on flowering

The analysed data revealed that, the highest flowering was recorded at 7WAS in IT07K - 293 - 3, followed by IT07K - 187 - 24 and IT06K- 133, however varieties IT07k - 292 - 10, IT07K - 302 -20 and IT07K - 302- 27 even though they developed a reproductive flower throughout the period of this study, most of their flowers were prematurely aborted, this was due to the effect of the virus. Variety with zero (0) value indicated that it did not flower at that particular week of experiment. Therefore, these two varieties were mostly affected by the disease. (Table 2).

Effect of cowpea mottle virus on plant height

Inoculation of varieties with the cowpea mottle virus at 10 days after planting resulted in reduced plant height. This showed that the virus had significant effect on suppressing plant height, this is in conformity with the finding of Agrios et al 1985. A considerable stunting growth were observed on varieties like IT07K - 133, IT07K - 293 - 18, IT07K - 300-12, IT97K - 461 - 4, IT07K - 178 -1, IT07K -290 -73 and IT07K-293-16. Pio Ribeiro (1978) has reported that the stunting growth in cowpea affected by mottle virus disease may be as the result of the virus in utilizing most of the amino acid and protein synthesis in their replication rather than being utilized by the plants in their vegetative growth. (table 3.)

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Effect of cowpea mottle virus on pod development

This study also revealed the fact that, the highest number of pods were recorded at the latter stage of growth of the varieties (9WAS) where the highest average number of pods were 4.3 pods recorded on the variety IT07K - 293 -16 followed by IT07K - 293 - 3 with average number of pods of 4.0. Some varieties did not produce pod at all even few that produced pod were prematurely aborted. However, a significance difference in pod development was observed at 6WAS. This was due to the effect of the mottle virus in prolonging the fruiting as well as the flowering period of the varieties affected (table 4)

Effect of Cowpea Mottle virus infection on yield

The result of the effect of the virus on yield was so significant which lead to the drastic yield reduction of about 10 - 75% this agrees with the finding of Rachie (1985) and Robertson (1963). That mottle virus disease significantly reduces the yield of cowpea.

CONCLUSION

A screen house experiment on the comparative study of cowpea genotypes (*Vigna unguiculata* L.) response to mottle virus disease was conducted at the International Institute for Tropical agriculture (IITA) kano sub - station. The experiment was aimed at comparing the reaction of different cowpea varieties to mottle virus disease and to investigate the effect of the viral disease on the crop vegetative growth and yield. The results of this study confirmed the susceptibility of some of the cowpea varieties to mottle virus infection. Early infection of the varieties by the virus resulted in poor flowering, pod setting and reduced plant height. The implication of these was a notable yield reduction of about 10 - 75% yield loss and the death of some plants. Thus, necessitate the development of a resistant or even tolerant variety to this virus.

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