International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Transmission Characteristics of Bean Common Mosaic Virus (BCMV) in Yard Long Bean

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Abstract: ***Pole sitao also known as yard long bean is the most important and popular legume crop in the Philippines because of its nutritional benefits and the pods, young shoots as well as the beans are available throughout the year. However, yard long bean are commonly attack by pest and diseases which lessens the production and quality of harvest. BCMV is an important disease of beans worldwide that caused significant yield losses that can reach up to 80%. This study was conducted to determine the efficiency and symptom characteristics of the different BCMV transmission. The study was lay-outed using CRD, replicated three times with six treatments: T1-5 starved aphids, T2-10 starved aphids, T3-5 unstarved aphids, T4-10 unstarved aphids, T5-Mechanical Transmission, T6-Control. The plant height, number of leaves and symptom appearance were assessed for a period of 21 Days After Inoculation (DAI). Results showed significant differences among varied treatments on the plant height at 14 DAI and number of leaves at 21 DAI. Symptoms of BCMV inT5 showed vein banding, distortion, and decrease of leaf size and mosaic pattern 9 DAI, T2 and T4 showed vein banding, distortion, and reduction of leaf size 12 DAI, and same symptoms were observed in T1 and T3 at 15 DAI while T6 exhibited no symptoms of BCMV.***

Keywords: Pole sitao, BCMV, Transmission characteristics, Insect transmission, Mechanical transmission

1. Introduction

Pole sitao also known as yard long bean is scientifically known as *Vignaunquiculata and belongs to* Leguminaceae family. It is the most important and popular legume crop in the Philippines because the pods, young shoots as well as the beans are available throughout the year. It is used in favorite Filipino dishes such as adobongsitaw, pinakbet and kare-kare.

Bureau of Agricultural Statistics (BAS) [18] showed that the total land area planted to pole sitao is 14, 681 hectares with a total volume of production of 119, 453.02 metric tons. Pole sitao has several benefits to health since it is a good source of protein, vitamin A, thiamin, riblofavin, iron, phosphorus and potassium. In addition, it is a good source of vitamin C, folate, magnesium and manganese [16].

However, pole sitao are commonly attack by pest and diseases which lessens the production and quality of harvest. Plant viruses are among the major constraints in bean production in the Philippines.

Bean common mosaic virus (BCMV) is still an important disease of beans worldwide. Crop losses caused by BCMV impact severely not only on commercial scale cultivation of this high-value crop but also on production by smallholder farmers in the developing world, where bean serves as a key source of dietary protein and mineral nutrition [15]. According to Morales [11] BCMV and BCMNV caused significant yield losses that can reach up to 80%.

BCMV belongs to the family Potyviridae under the genus Potyvirus. The virions of BCMV are non-enveloped flexuous filaments, with 12–15 nm wide, and 847–886 nm long. In addition, virions are composed of a single stranded RNA of *c*.10 000 nt (accounting for 5% of the particle weight) encapsulated in about 1700–2000 monomer units of one CP polypeptide species with a molecular mass of *c*.33 kDa for BCMV (comprising 95% of the particle weight) [7].

A BCMV strain symptom on beans is “common mosaic” characterized by green vein banding and leaf malformation [11, 14]. It is seed-transmitted up to 83% in *P. vulgaris*. Seed-borne inoculum contributes to primary spread, and secondary spread occurs through aphid vectors which transmit both viruses [10] but they can be transmitted mechanically [17] and through an aphid vector. A single *Aphis craccivora* was capable to transmit the BCMV with the highest incidence (53%) and the transmission efficiency using 10 aphids reached 100% [1]. The BCMV type of transmission is generally characterized by a short Acquisition Access Period (AAP) of a few seconds to minutes, absence of a distinct latent period and a short Inoculation Access Period (IAP). Therefore, the virus is acquired during brief exploratory probes in the process of host finding and is transmitted to a healthy bean plant within a few seconds to minutes.

In this study, the efficiency and characteristics in disease transmission was determined based on the number of aphids, time of starvation/unstarvation and the mechanical transmission on the healthy yard long bean. Specifically, the study determined the occurrence of symptoms of the disease after inoculation, evaluated the effect of BCMV on plant height and number of leaves and disease incidence of plants.

2. Methodology

Experimental Design and Treatments

The study was lay-outed using Completely Randomized Design (CRD) with three replications. The Analysis of Variance (ANOVA) for CRD was used to determine the significant differences between treatments. There were six treatments in this study: T1-5 starved aphids, T2-10 starved aphids, T3-5 unstarved aphids, T4-10 unstarved aphids, T5-Mechanical Transmission, T6-Control.

Survey and Collection BCMV and Aphids vector

A survey was done in a nearby yard long bean farm or garden to locate the source of aphids transmitting BCMV. The Aphids were collected by cutting the yard long bean infected with BCMV and were gathered using soft paint brush. Leaves showing BCMV symptoms were also collected (Figure 1).

Figure 1: Collected yard long bean infected with BCMV (a); Collected aphids from the Yard long bean infected with BCMV (b).

Preparation of the Soil media and sowing of seeds

Soil media used was the mixture of organic fertilizer, rice hull and garden soil with 1: 1: 1 proportion. The Montenegro seeds from Kaneko company was used as test crop. It is prolific and high quality seeds with at least 85% germination and 99% purity. The seeds were sown with one seed per hill at 3 cm deep.

Inoculation/ Transmission

Insect transmission was done during the 5 leaf stage of plants or 15 days after planting. The aphids were allowed to starved or unstarved for 30 minutes following the treatments (Figure 2). Then aphids were inoculated to each treatment group for 24 hours inoculation access feeding (IAF). An insect proof screen cage was used to cover the plants preventing the escape of aphids. After 24 hours insecticides was sprayed to control aphids

Ten infected leaves with BCMV were macerated using mortar and pestle with sterile water (Figure 2). Then the extracted sap was mechanically inoculated to yard long bean leaves at 5 leaf stage or 15 days after planting. A sponge was used to make wounds in the leaves and sap was rubbed lightly using the tip of the middle finger (Figure 3).

Figure 2: Treatments preparation: starving of 5 aphids in T1 (a); Starving of 10 aphids in T2 (b); Feeding/unstarving of 5 aphids in T3 (c); feeding/unstarving of 10 aphids in T4 (d); Maceration of leaves (e) and the extracted sap in T5 (f).

Figure 3: Aphid transmission (a); putting of proof screen cage (a1); brushing of leaves using sponge (c); rubbing of macerated leaves in the leaves.

3. Results and Discussion

Effects of BCMV on the height of Yard long bean

Plant height of pole sitao as affected by the varied BCMV inoculation is presented in Table 1. The results revealed no significant differences on the plant height increment before inoculation, 7 days and 21 days after inoculation. However, at 14 days after inoculation, height of pole sitao differs significantly. T6 (control) obtained the tallest height with a mean of 49.97 cm, followed by T4 (10 unstarved aphids) with a mean height of 45.30 cm and obtained the lowest growth reduction of 9.35%. However, T4 is not statistically different from T3 (5 unstarved aphids) and T1 (5 starved aphids) with a mean height of 44.71 cm and 41.50 cm with growth reduction of 10.53% and 16.95%, respectively. The height of plants in T1 does not differ significantly with the T2 with a mean height of 41.50 and a growth reduction of 36.27%. The shortest height and the highest growth reduction was observed in T5 (Mechanical transmission) with a mean height of 33.53 cm and 32.90% growth reduction.

Result implies that regardless of any type of BCMV inoculation used it can significantly reduce the height of the plants. The successful damage of the plant's cuticle and epidermis, leads to the inoculation of the BCMV, resulted in a reduction in height in mechanically transmitted plants. While, the efficiency of 5-10 aphids either starved/unstarved in reducing plant height was in agreement with the report of Damayanti et al. [1] which stated that single *Aphis craccivora* was capable to transmit the BCMV with the highest incidence (53%) and the transmission efficiency

Paper ID: SR211102090451 DOI: 10.21275/SR211102090451

using 10 aphids reached 100%. The increasing growth inhibition in the plants as the number of aphids increases implies that the more aphids, the higher the efficiency of transmission.

Thus, the reduced growth of pole sitao inoculated by BCMV indicates that virus mediated interference with physiological and metabolic process of plants and that BCMV had a detrimental effect on the height of pole sitao. This result conforms to the study of Nanjunadappa et al. [12].

Table 1: Mean height of pole sitao as affected by BCMV

Treatments	Before Inoculation ns	7 DAI ns	14 DAI ** (% growth inhibition)	21 DAI ns
T1-5 starved aphids	21.13	27.50	41.50^{abc} (16.95%)	80.47
T2-10 starved aphids	19.92	31.83	36.27^{bc} (27.42%)	76.67
T3-5 unstarved aphids	20.31	33.22	44.71^{ab} (10.53%)	79.26
T4-10 unstarved aphids	20.99	34.62	45.30^{ab} (9.35%)	75.86
T5-Mechanical transmission	20.81	28.52	33.53^{c} (32.90%)	78.81
T6-control	21.03	39.60	49.97^{a}	93.60
Cv (%)	10.77	27.99	8.70	8.33

*In a column with the same letters indicate that the values are insignificantly different by Least Significant Difference Post Hoc Test (P>0.05)

Effects of BCMV on the number of leaves of Yard long bean

Table 2 shows the number of leaves in pole sitao as a result of the BCMV infection. There were no significant variations in treatment means before inoculation, 7 days after inoculation, or 14 days after inoculation, according to the findings. However, after 21 days of inoculation, a substantial difference was seen among various treatments.

T6 had the most leaves, with an average of 15.40, followed by T3, T1, T2, and T4 with averages of 15.00, 14.87, 13.97, and 13.73, respectively. The differences between these treatments, however, were not statistically significant when compared to T6. With a mean of 13.30, only T5 considerably reduced the number of leaves. As a result, mechanical transmission proved to be the most effective in all types of inoculation, infecting all sampling plants and significantly reducing the number of leaves.

The reduction in the number of leaves in inoculated plants can be attributed to the virus reducing photosynthetic production in infected plants, which would be a primary factor explaining the host plants poor performance [5]. This result conforms to the study of Fuchs *et al.* [4] and Laughlin *et al.* [9] that virus inoculation also resulted in a 27–37% drop in productiveness, although previous studies of wild *Cucurbita pepo* have found that virus infection can reduce productiveness by up to 80–100%. The same result was observed to the study of Gergerich and Dolja [6] that virus infection caused a reduction in plant growth, lower yield, and inferior product quality.

Table 2: Mean number of leaves of pole sitao as affected by BCMV

Treatments	Before Inoculation ns	7 DAI ns	14 DAI ns	21 DAI *
T1-5 starved aphids	5.43	7.83	10.30	14.87^{ab}
T2-10 starved aphids	5.20	8.40	10.60	13.97^{ab}
T3-5 unstarved aphids	5.53	8.30	11.13	15.00^{ab}
T4-10 unstarved aphids	5.66	8.53	11.60	13.73^{ab}
T5-Mechanical transmission	5.60	7.23	9.67	13.30^{b}
T6-control	5.93	8.93	12.00	15.40^{a}
Cv				

*In a column with the same letters indicate that the values are insignificantly different by Least Significant Difference Post Hoc Test (P>0.05)

BCMV Symptoms Observed Days After Inoculation (DAI)

The result on the observed symptom appearance days after inoculation is presented in Table 3. Results revealed that there were no symptoms observed in T6. However, symptoms in T5 was first observed at 9 DAI and the observable symptoms were vein banding, distortion and reduction of leaves at the 3rd-5th nodes of 8 plants and stunted growth in 2 plants. However, after 21 DAI, the 7th nodes revealed mosaic patterns, and two stunted plants gradually recovered and grew.

The symptoms of BCMV on T2 and T4 were only observed on 12 DAI. Vein banding, distortion and leaf size reduction symptoms were observed in T2 until 21 DAI at the 4th-6th nodes of four plants (4/10 plants exposed). In T4, the same symptoms and a portion of the nodes were observed, but only for three plants (3/10 plants exposed). In the 5th-6th nodes of the two plants (2/10 plants exposed), the identical symptoms were noticed in T1 and T3 which started at 15 DAI.

In terms of transmission types, results showed that mechanical transmission is the most efficient in inoculating BCMV where all sampling plants showed symptoms mosaic and distortion of the leaves. However, 10 starved aphids have potential in inoculating the virus but in 4 sampling plants only. On the other hand, the inoculation potential of 5 starved/unstarved and 10 unstarved aphids ranges from 2-3 sampling plants only (Figure 4).

Thus, it can be surmised that regardless of the different transmission types, number of starved/unstarved aphids, symptoms could appear which means that BCMV could be transmitted. Moreover, the symptoms coincides with the study of Frate et al. [3] that BCMV causes mosaic patterns of light green-yellow leaf tissue, dark green tissue, or both light and dark mosaics together on the trifoliolate leaves. Leaf discoloration is usually accompanied by green vein banding and leaf malformation [2] and the intensity and severity of the symptoms depends on the strain of BCMV, the bean variety, and the age of the plant when infected but plants infected at a young age may be stunted and distorted [3].

Figure 4: Distortion of leaves and mosaic symptoms in T1 (a); T2 (b); T3 (c); T4 (d); T5 (e); and a healthy leaves from T6 (control) (f).

Symptoms of BCMV observed Days after Inoculation

Treatment	Days after Inoculation (DAI)																				
	3			6			9			12			15			18			21		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
T1- 5 starved aphids													■	■	■	■	■	■	■	■	■
T2- 10 starved aphids										■	■	■	■	■	■	■	■	■	■	■	■
T3- 5 unstarved aphids													■	■	■	■	■	■	■	■	■
T4- 10 unstarved aphids										■	■	■	■	■	■	■	■	■	■	■	■
T5- Mechanical Transmission							■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
T6- Control																					

Legend:

■ Symptoms observed

□ No symptoms observed

4. Conclusion

The height of infected plants significantly reduced at 14 DAI as well as the number of leaves at 21 DAI. Symptoms observed showed vein banding, distortion and reduction of leaves in aphid transmission at the 4^{th}-6^{th} portion of the nodes started at 12 DAI and vein banding, distortion and reduced leaves and mosaic pattern in mechanical transmission at the 3^{rd}-7^{th} portion of the nodes started at 9 DAI. Based on the results, it is therefore concluded that transmission through aphid vector either 5-10 numbers in a population, starved/unstarved were efficient in inoculating BCMV as well as in mechanical transmission.

The knowledge on the symptom characteristics on BCMV will serve as baseline information for the farmers to initially assess and identify the efficiency of aphid population/natural wounds in transmitting BCMV in their yard long bean and can set a threshold level in their IPM strategy.

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