J.Natn.Sci.Foundation Sri Lanka 2020 48 (1): 81 - 92
DOI: http://dx.doi.org/10.4038/jnsfsr.v48i1.8704

RESEARCH ARTICLE

Transmission and host range of *Horsegram yellow mosaic virus* (HgYMV) causing common bean (*Phaseolus vulgaris* L.) yellowing disease in Sri Lanka

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Submitted: 28 January 2019; Revised: 30 September 2019; Accepted: 25 October 2019

Abstract: The common bean (*Phaseolus vulgaris* L.) yellowing disease (BYD) is caused by the *Horsegram yellow mosaic virus* (HgYMV), which is a begomovirus transmitted by whiteflies (*Bemisia tabaci* Genn.). It is a serious threat to the common bean cultivation in Sri Lanka. Despite the fact that the disease has been affecting bean production for a long time, its etiology and virus vector relationships are not understood well. Therefore, the present work was carried out to identify major aspects of the etiology of the virus. Initially a survey was conducted to determine the prevalence of the virus in representative bean growing locations in Sri Lanka, followed by a host range study using 54 plant species. Characteristics of virus-vector relationships were determined by conducting a series of virus transmission tests under glass-house conditions. The survey results revealed the incidence of BYD at some time or the other in most of the sampled localities, which are scattered around all four major districts where the common bean is grown. Through the host range study, it was demonstrated that *Ageratum* sp., *Glycine max* Merrill., *Macrotyloma uniflorum* (Lam.) Verdc., and *Phaseolus lunatus* L. are susceptible to HgYMV, indicating a narrow host range. In addition to the symptomatology, the successful entry of the virus following inoculation was confirmed through PCR using degenerate primers. Relative transmission efficiency was found to be 96.7 %, which corresponds to 0.24 of the estimated probability of transmission through a single whitefly (p*). The virus could be transmitted through probing by a single viruliferous whitefly with an incidence of 36.7 % (p* = 0.37). The minimum acquisition access period and inoculation access period were 20 min in each case and the respective disease incidence values were 30.0 % (p* = 0.04) and 36.7 % (p* = 0.05). The whiteflies could retain the virus in an active state for up to 9 days.

Keywords: Begomovirus, *Bemisia tabaci* Genn., common bean yellowing disease, *Horsegram yellow mosaic virus* (HgYMV), host range, virus transmission.

INTRODUCTION

Begomoviruses transmitted by the whitefly, *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) (*Bt*), are becoming serious threats to agriculture in the world due to the devastating effect they have on an array of economically important crops (Rojas *et al*., 2018). An attack by any of these viruses can result in yield losses that can range from 0 % to 100 % (Briddon *et al*., 2001; Legg & Fauquet, 2004; Navas-Castillo *et al*., 2011). Adaptability to diverse geographical regions and climates (Costa, 1976; Andret-Link & Fuchus, 2005; Navas-Castillo *et al*., 2011) and to a broad host range (Nene, 1972; Harrison, 1985), has favoured the spread of begomoviruses throughout the world. Not only do the characteristics of the vector favour the distribution, but also the ability of the virus to mutate could be the reason behind the increasing host range with the passage of time. *Horsegram yellow mosaic virus* (HgYMV) infection in horsegram was first reported in India by

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William *et al.* (1968). HgYMV was known to be a major virus that affected horsegram but now it is found to be infecting legume crops including common beans (*Phaseolus vulgaris* L.) (Muniyappa *et al*., 1987). In Sri Lanka, common beans are extensively grown in different agro climatic regions as a cash crop because it is popular among people due to its nutritional properties. Badulla, Nuwara-Eliya, Matale and Kandy districts have been identified as the major common bean growing districts due to their climate being favourable for the crop. Although systematic statistics on crop losses caused by particular plant viruses are not available in Sri Lanka, HgYMV is considered as a major virus affecting common beans, with losses that can go up to 50 % (Wickramaarachchi *et al*., 2012; Rienzie *et al*., 2016). Common bean yellowing disease (BYD) is currently widespread throughout the traditional bean growing areas. BYD was first recorded in Sri Lanka in 1999 in the Balangoda area, but the precise causal agent was identified only about a decade later. Monger *et al.* (2010) confirmed that the causal agent was HgYMV, which is a whitefly transmitted begomovirus of the family Geminiviridae. The disease initiates with the mild yellowing of immature leaves, followed by progressing into a mosaic pattern, which appears as irregular mottling interspersed with greenish areas. In time, these mottles develop and turn into large patches. Subsequently, the leaves become smaller and the pod length and size also reduce. Overall, the plant becomes stunted (Muniyappa & Reddy, 1976; Muniyappa *et al*., 1987; Prema, 2013).

Information on the distribution and etiology of a disease can be useful to develop management packages designed to control the spread of a disease. Accordingly, the present research was designed to determine the occurrence of the virus in common bean cultivations, and the relationships between the virus, vector and its hosts, by performing a series of experiments. This research would be the first attempt at conducting a detailed study, revealing the nature of transmission of HgYMV in Sri Lanka.

METHODOLOGY

Survey on common bean yellowing disease (BYD) in Sri Lanka

A survey was conducted to determine the prevalence and distribution of BYD in Sri Lanka during the period 2016–2018. Tender leaf samples of the common bean, of both pole and bush type bean cultivars, showing typical BYD symptoms were collected from 20 commercial farmer fields located in different administrative districts of Sri Lanka, namely, Kandy, Matale, Nuwara Eliya and Badulla. A purposive sampling procedure was employed using lists of infected fields provided by the Agriculture Officers of the respective areas. A single plant was considered as a sampling unit. A total of five samples, each comprising two tender leaves, were collected from each of the 20 farmer fields (total of 100 samples). Leaf samples from apparently healthy plants of the same cultivars were also collected from the same locations as control samples. All the leaf samples were collected irrespective of their growth stage but from those plants grown within the same growing season.

Study sites and establishment of *Bemisia tabaci* colonies for transmission tests

Bemisia tabaci were reared in the divisions of Plant Pathology and Entomology of the Horticultural Crops Research and Development Institute (HoRDI) in Gannoruwa, Sri Lanka. All transmission experiments were done in the glass houses and insect cages of HoRDI and the Department of Agricultural Biology, University of Peradeniya, Sri Lanka. The sites are located in the mid country wet zone (WM_{2b}) with a height above mean sea level of 576 m, mean annual temperature of 24° C and mean annual rainfall of 2131 mm. The colonies were maintained at 79–82 % relative humidity and temperature 24–27° C, under 12L (~ 4000 Lux):12D lighting throughout the experiment period. The whiteflies were reared on cotton seedlings as they are immune to HgYMV.

Test plants for inoculation purposes

Seeds of all the weed and crop species and common bean plants used for all the transmission tests were sown in pots, which contained sterilised soil and then maintained inside insect-proof cages at the Division of Plant Pathology, Horticultural Crop Research and Development Institute, Gannoruwa, Sri Lanka.

Testing for cross transmission ability of the virus among different crop and weed plants

Testing for susceptibility to BYD was conducted using 33 crop species and 14 weed species that belong to various families. Both weed and crop species were selected based on the abundance, growth patterns (perennial or annual) and previous reports on begomovirus infestations. All the healthy test plants were inoculated with viruliferous *Bt* fed on HgYMV-infected common bean plants, which were maintained as source plants for 24 h at a rate of 10 *Bt* per plant and kept under insect proof conditions

for another 24 h. The time periods (24 h) for inoculation access period (IAP) and acquisition access period (AAP) mentioned here and the tests that are described hereafter were selected considering the minimum acquisition and inoculation access periods reported on begomoviruses. The methods employed here are similar to those used by other researchers in this field (Nene, 1972; Inoue-Nagata *et al*., 2007).

Extraction of total DNA, amplification of target DNA and partial sequencing

Total DNA from plant tissues were extracted according to the CTAB (cetyl trimethyl ammonium bromide) extraction protocol as described by Lodhi *et al.* (1994) with a few modifications and subjected to PCR amplification. Previously optimised Begomovirus specific degenerate primer pair, Deng 540/541 (Deng *et al*., 1994) (Promega, WI, USA) was used at a concentration of 1/25 (DNA: sterile water). The amplified DNA resulting from the initial DNA collected from symptomatic host plants (legumes) was tested for virus transmissibility and subjected to further analysis through single directional sequencing.

PCR amplification mixture (1 X)

A 25 µL reaction mixture that contained 2.0 µL of total DNA extracted from infected common bean leaf tissues was prepared. This mixture was diluted to 1/25 (80–100 ng) and to this was added 0.2 µL Taq DNA polymerase (5 U/µL), 2.5 µL of 10X PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 0.5 µL of 25 mM $MgCl_2$, 2.0 µL each primer (10 mM), 2.0 µL dNTPs mix (2.5 mM each) and sterile water to make up the volume.

PCR conditions

PCRs with Deng primers were conducted in a thermo-cycler (Labnet Inc., USA, Model: Labnet Gradient). The mixture was subjected to one cycle of initial denaturation at 94˚ C for 5 min followed by 30 cycles of denaturation at 94˚ C for 30s, annealing at 58˚C for 30s, extension at 72˚C for 45s and a final extension at 72˚C for 10 min. The PCR products were analysed by performing 1.0 % agarose gel electrophoresis at 60V for 1 hr in 1X TBE buffer [100 mM Tris (pH 8), boric acid, 0.5 M EDTA (pH 8)] with a loading of 5.0 µL of product per well. The gel was previously stained with ethidium bromide at 0.5 µg/ mL. The size of the amplicons was estimated with a 100 bp DNA ladder (Vivantis, Germany). DNA from healthy plants and double distilled water were used instead of template DNA as experimental controls.

Relative transmission efficiency

Non-viruliferous *Bt* were allowed to feed on BYD infected common bean plants inserted into a transmission cage for 24 h. This feeding time was determined based on the maximum time taken to acquire the viruses. After 24 h, the insects were released onto ten 12-day-old healthy common bean seedlings (per replicate) inserted into individual transmission cages at a rate of 10 *Bt* per seedling and the test was triplicated. After 24 h, the *Bt* were removed from individual plants and the plants were sprayed with Imidachloprid (Admire®, Bayer Crop Sci.) at 200 g/L SL (1 mL/L). Plants were maintained under insect-proof conditions until the development of virus infection-like symptoms.

Minimum number of *Bemisia tabaci* adults required for transmission

This experiment was designed to determine the minimum number of *Bt* individuals required for successful transmission of the virus and the effects of different numbers of *Bt* to the rate of transmission. Non-viruliferous *Bt* were first fed on diseased common bean leaves for 24 h as in the previous experiment. Here the *Bt* were released onto seven 12-day-old healthy common bean seedlings inserted into transmission cages at rates of 1, 3, 5, 10, 15, 20, and 25 insects per seedling at the rate of three replicates each (total of 21 plants). Next, they were allowed to feed on inoculated seedlings for another 24 h. On the following day (after 24 h), the cages were removed and the plants were sprayed with Imidachloprid (Admire®, Bayer Crop Sci.) at 200 g/L SL (1 mL/L) to kill the *Bt*. Plants were kept inside an insect-proof glass-house until the symptoms developed.

Minimum acquisition access period (AAP)

This experiment was aimed at determining the minimum time taken by non-viruliferous *Bt* to acquire the virus from a virus-infected plant and the effects of different acquisition access periods on the rate of transmission. *Bt* were fed on infected common bean leaves for periods of 5, 10, 15, 20 and 30 min and 1, 2, 4, 6, 8, 24 and 48 h. After the prescribed feeding period, *Bt* were transferred at the rate of 10 *Bt* per seedling onto twelve healthy 12-day-old common bean seedlings covered with micro transmission cages and left for feeding for 24 h with three replicates. This test was conducted with a total of 36 plants (12 time periods × 3 replicates). Thereafter, the plants were sprayed with Imidachloprid at the same rate as given in the above test and kept for symptom development. The control experiment consisted of 10 plants inoculated with non-viruliferous *Bt* at the rate of 10 *Bt* per seedling.

Minimum inoculation access period (IAP)

The aim of this experiment was to determine the minimum time taken by viruliferous *Bt* to inoculate the virus into healthy common bean plants and the influence of different IAPs on transmission of BYD. First, the non-viruliferous whitefly individuals were given acquisition feeding on diseased common bean leaves for a period of 24 h. Then, the resulting viruliferous *Bt* were transferred onto twelve, 12-day-old healthy common bean seedlings and covered with transmission cages for periods of 5, 10, 15, 20 and 30 min and 1, 2, 3, 6, 8, 24 and 48 h at a rate of 10 *Bt* per seedling with three replicates. This test was also done with a total of 36 plants (12 time periods × 3 replicates). After each feeding period, the cages were removed and the plants sprayed with Imidachloprid as in previous tests and maintained in an insect-proof glass-house until the appearance of symptoms.

Persistence of the virus in the vector

To determine the persistence of the virus in *Bt*, a non-viruliferous experiment was designed in which the inoculation of healthy common bean plants was conducted through serial transmission using groups of *Bt* infected with HgYMV. *Bt* were first given an AAP of 24 h on BYD infected common beans. Then *Bt* from 12 groups were transferred at the rate of 10 *Bt* from each group onto 12 common bean plants (each plant was 14 days old at the time of transferring) over a period of 24 h for each plant. After 24 h, each group was transferred to another set of 12 new and healthy common bean plants. In this manner, the serial transmission procedure was employed for all groups while maintaining all plants inside an insect-proof glass-house until the development of symptoms. Serial transferring was carried out until all the insects of each group were dead. The serial transmission efficiency was calculated using the formula, (Number of infected bean plants in the series/ Number of inoculated plants) × 100. The rate of infection per group of *Bt* was determined by simply calculating the average of the sum of serial transmission rates for all groups. Further, the transmission rates corresponding to each date were also calculated by dividing the sum of transmission by the number of groups (12).

Calculation of transmission rate

Transmission rate was calculated by the formula, Number of infected plants/ Number of test plants × 100. Probability of HgYMV transmission through a single whitefly was calculated using the formula $p^* = 1-(1-R/N)^{1/i}$ suggested by Gibbs and Gower (1960) (where p^* = Estimated transmission rate for a single whitefly, R = Number of infected plants, N = Number of test plants, and i = Number of *Bt* per receptor plant). The means were analysed using the least significant difference method (LSD) at ($p < 0.05$).

RESULTS AND DISCUSSION

BYD survey

The survey was conducted to detect the disease using symptomatological features such as severity of yellowing (e.g. bright yellowing or mild yellowing), rugosity and reduced leaf size, which are characteristics of BYD. The results are summarised in Table 1 with sampling locations, which are also mapped in Figure 1.

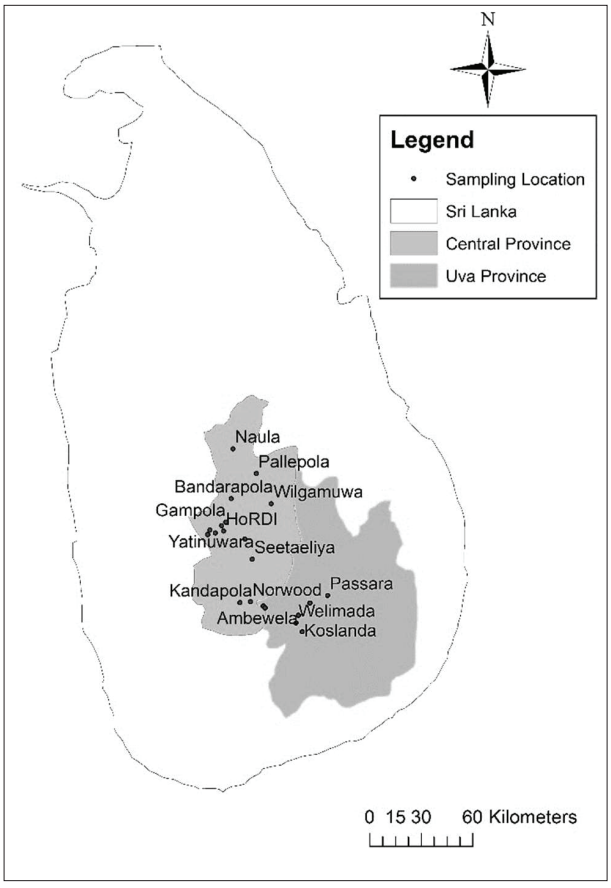

Figure 1: Sampling locations for collecting BYD in Sri Lanka during 2016–2018 period

Table 1: Results of the diagnosis based on symptomatology of commercial varieties of the common bean

District	Location	Variety	Symptoms
Kandy	Kadugannawa	Contender	BY, R, RL
Kandy	Yatinuwara	Contender	BY, R, RL
Kandy	Ambilmeegama	Keppetipola Nil	BY, R, RL
Kandy	Katugasthota	Top Crop	BY, R, RL
Kandy	Gampola	Keppetipola Nil	MY, RL
Matale	Bandarapola	Top Crop	MY, RL
Matale	Naula	Top Crop	MY, RL
Matale	Pallegama	Unknown	BY, R, RL
Matale	Wilgamuwa	Unknown	BY, R, RL
Matale	Pallepola	Sanjaya	MY, RL
Nuwara Eliya	Seetaeliya	Lanka Butter	MY, RL
Nuwara Eliya	Norwood	Lanka Butter	BY, R, RL
Nuwara Eliya	Kandapola	Lanka Butter	BY, R, RL
Nuwara Eliya	Ambewela	Top Crop	MY, RL
Nuwara Eliya	Pattipola	Contender	MY, RL
Badulla	Bandarawela	Unknown	BY, R, RL
Badulla	Passara	Unknown	MY
Badulla	Demodara	Sanjaya	BY, R, RL
Badulla	Koslanda	Unknown	BY, R, RL
Badulla	Welimada	Top Crop	BY, R, RL

BY- bright yellow; R- rugose; MY- mild yellow; RL- reduced leaf size

Host range study

Among the species tested for susceptibility, only *Ageratum* sp., *Glycine max*, *Macrotyloma uniflorum*, *Phaseolus lunatus* and *P. vulgaris* L. (Table 2) showed symptoms characteristic of virus disease. Among them, *G. max*, *M. uniflorum*, *P. lunatus* and *P. vulgaris* showed incipient symptoms of yellow mosaic virus on leaves, while *Ageratum* spp. showed mild crinkling of leaves with no progression towards yellowing. The single directional sequence data of the DNA extracted from these hosts showing symptoms confirmed that HgYMV had been successfully transmitted from the original host. The sequence data were identical to the data reported previously by the same authors (Rienzie *et al*., 2016), and therefore there was no need to repeat it in the current work.

Host range of a virus is determined by two major factors, specifically those intrinsic factors such as genetic traits that determine the fitness of the virus in different hosts, and extrinsic factors independent of the virus such as ecological factors like distribution, abundance and interaction of species. As a result of the interaction between intrinsic and extrinsic factors, virus emergence and adaptation to new hosts takes place (McLeish *et al*., 2018). Almost all the plant families tested in this study for cross transmission have been reported by many workers as likely hosts of begomoviruses (Nene, 1972; Shivanathan, 1983; Harrison, 1985). In this study, HgYMV was able to transmit the virus to *Ageratum* spp. According to Saunders *et al.* (2002), *Ageratum conizoides* behaves as a host to the *Sri Lankan cassava mosaic virus*. However, Muniyappa *et al.* (2003) reported that *Ageratum conizoides* does not host *Pumpkin yellow vein mosaic virus* (PYVMV), implying that *Ageratum* spp. does not host certain begomoviruses, showing selectivity towards hosting. Currently, *Ageratum* spp. with virus-like-symptoms is typical throughout Sri Lankan crop fields. However, the virus transmission behaviour from *Ageratum* to any other crop has not yet been studied in Sri Lanka. Unlike in the case of dicot plants, there is no evidence of infection of monocots by begomoviruses in literature. Supporting evidence on the cross transmission of HgYMV has been revealed through several studies. For instance, Rienzie *et al*. (2017) detected HgYMV in soybean (*Glycine max*) plants grown in experimental fields in Gannoruwa, Sri Lanka, and further identified *Hedyotis corymbosa* as a weed that harboured HgYMV (Rienzie *et al*., 2016). Moreover, Abarshi *et al*. (2017) detected the same virus in lima bean (*Phaseolus lunatus*) in Bangalore, India. Although only a minimum number

Table 2: Results of the host range experiment for transmission of HgYMV

Plant species	Family	Number of plants infected/inoculated (percent infection)	Control [c] (percent infection)
Abelmoschus esculentus	Malvaceae		
var. Haritha		0/5 (0%)	0/5 (0%)
var. MI 7		0/5 (0%)	0/5 (0%)
Acalypha indica	Euphorbiaceae	0/15 (0%)	0/5 (0%)
Ageratum spp.	Compositae	2/10[a] (20%)	0/5 (0%)
Allium cepa L.	Liliaceae	0/20 (0%)	0/5 (0%)
Amaranthus caudatus L.	Amaranthaceae	0/15 (0%)	0/5 (0%)
Arachis hypogaea L.	Fabaceae	0/20 (0%)	0/5 (0%)
Benincasa hispida (Thunb.) Cogn.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Beta vulgaris L.	Chenopodiaceae	0/10 (0%)	0/5 (0%)
Borreria spp.	Rubiaceae	0/10 (0%)	0/5 (0%)
Brassica oleraceae L.	Brassicaceae	0/10 (0%)	0/5 (0%)
Cajanus cajan L.	Fabaceae	0/10 (0%)	0/5 (0%)
Calopogonium mucunoides	Fabaceae	0/10 (0%)	0/5 (0%)
Canavalia spp.	Fabaceae	0/10 (0%)	0/5 (0%)
Capsicum annuum L.	Solanaceae	0/10 (0%)	0/5 (0%)
Centrocema pubescence	Fabaceae	0/10 (0%)	0/5 (0%)
Citrullus vulgaris Schrad.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Crotolaria juncea Neck.	Fabaceae	0/10 (0%)	0/5 (0%)
Cucumis melo L.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Cucumis pubescens	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Cucumis sativus L.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Cucurbita maxima Duch.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Cucurbita moschata Duch. Ex Poir	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Cucurbita pepo L.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Daucus carota L.	Apiaceae	0/10 (0%)	0/5 (0%)
Dolichos trilobus	Fabaceae	0/10 (0%)	0/5 (0%)
Eleusine coracana Gaertn.	Poaceae	0/10 (0%)	0/5 (0%)
Emilia sonchifolia	Asteraceae	0/10 (0%)	0/5 (0%)
Euphorbia heterophylla	Euphorbiaceae	0/10 (0%)	0/5 (0%)
Glycine max Merrill.	Fabaceae	11/15[a] (73.3%)	0/5 (0%)
Gossypium barbadense L.	Malvaceae	0/10 (0%)	0/5 (0%)
Helianthus annuus L.	Asteraceae	0/10 (0%)	0/5 (0%)
Lablab purpureus L.	Fabaceae	0/10 (0%)	0/5 (0%)
Lagenaria siceraria (Mol.) Standl.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Luffa acutangula (L.) Roxb.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Luffa cylindrica (L.) Roem.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Lycopersicon esculentum Mill.	Solanaceae		
var. Thilina		0/10 (0%)	0/5 (0%)
var. Rajitha		0/10 (0%)	0/5 (0%)
var. T 245		0/10 (0%)	0/5 (0%)
Macrotyloma uniflorum (Lam.) Verdc.	Fabaceae	13/15[a] (86.7%)	0/5 (0%)
Manihot esculenta Crantz	Euphorbiaceae	0/8 (0%)	0/5 (0%)
Momordica charantia L.	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Mucuna spp.	Fabaceae	0/10 (0%)	0/5 (0%)
Phaseolus lunatus L.	Fabaceae	3/5 (60%)	0/5 (0%)

Continued -

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Plant species	Family	Number of plants infected/inoculated (percent infection)	Control [c] (percent infection)
Phaseolus vulgaris L.	Fabaceae		
var. Keppetipola Nil		10/10[b] (100%)	0/5 (0%)
var. Contender		9/10[a] (90%)	0/5 (0%)
var. Sanjaya		10/10[b] (100%)	0/5 (0%)
var. Top Crop		10/10[a] (100%)	0/5 (0%)
Phorocarpus tetragonolobus	Fabaceae	0/10 (0%)	0/5 (0%)
Raphanus sativus L.	Brassicaceae	0/10 (0%)	0/5 (0%)
Sesamum indicum L.	Pedaliaceae	0/10 (0%)	0/5 (0%)
Sida spp.	Malvaceae	0/10 (0%)	0/5 (0%)
Solanum melongena L.	Solanaceae	0/10 (0%)	0/5 (0%)
Sorghum bicolor L.	Poaceae	0/10 (0%)	0/5 (0%)
Trichosanthes cucumerina	Cucurbitaceae	0/10 (0%)	0/5 (0%)
Vigna mungo L.	Fabaceae	0/10 (0%)	0/5 (0%)
Vigna radiata (L.) Wilczek	Fabaceae	0/10 (0%)	0/5 (0%)
Vigna ungiculata (L.) Walp.	Fabaceae	0/10 (0%)	0/5 (0%)
Vigna ungiculata (L.)	Fabaceae	0/10 (0%)	0/5 (0%)
Vigna ungiculata (L.) var. sesquipedalis	Fabaceae	0/10 (0%)	0/5 (0%)

[a] Confirmed through PCR only; [b] confirmed through both PCR followed by partial sequencing; [c] control experiment was performed by inoculation with non-viruliferous *Bt* at a rate of 10 *Bt* per plant

of inoculated test plants was infected with HgYMV in this study, further studies are needed to understand the reaction of other legume weeds to HgYMV, since there is a possibility of new plant species becoming hosts for the begomovirus.

Relative transmission efficiency

Relative transmission efficiency was tested by inoculating healthy common bean plants with viruliferous *Bt* and the result was calculated as 96.7 % (S.E. = ±5.77) with p* = 0.24. Available literature provides details about different efficiencies with respect to different *Bemisia tabaci* transmitted viruses. Prema (2013) observed that HgYMV transmission occurs at a range of 6–86% under field conditions. According to Mware *et al.* (2009), the transmission percentage of *Cassava brown streak virus* is 22% although the *Bt* were allowed 48 hours AAP. Reasons for higher transmission efficiency for *Bt* in the present experiment could be due to forced feeding under confined spaces. Based on the results of the present study, it could be observed that the incidence of disease increases with the increased numbers of *Bt* per common bean plant. Similar results have been reported with the same whitefly species for *Mung bean yellow mosaic virus* (MYMV) in India (Nene, 1972), for leaf curl virus of *Zinnia elegans* in India (Mathur, 1933), for *Bhindi yellow vein mosaic virus* (BYVMV) in India (Varma, 1952), and for *Tomato yellow leaf curl virus* (TYLCV) (Cohen & Nitzany, 1966).

Table 3: Transmission percentages with different numbers of *Bt*

Number of *Bt* per plant	Average percent of infected plants	p*
1	36.7 [a] (± 3.3)	0.37
3	53.3 [b] (± 6.7)	0.22
5	86.7 [c] (± 6.7)	0.33
10	100 [d] (± 0.0)	1.00
15	100 [d] (± 0.0)	1.00
20	100 [d] (± 0.0)	1.00
25	100 [d] (± 0.0)	1.00
CV	7.96	
LSD	11.48	

Means with the same letter along the column are not significantly different from each other according to least significant difference (LSD) at $p < 0.05$. Data expressed as mean (±) standard error (n = 3), CV = coefficient of variation, p^* = estimated transmission rate for a single whitefly.

Minimum number of *Bemisia tabaci* required for successful transmission

Results for the different numbers of *Bt* insects ranging from 1 to 25, which were fed on infected common bean leaves for 24 hours AAP and then transferred onto healthy common bean seedlings for another 24 h IAP are given in Table 3.

When the plants were inoculated with a single viruliferous whitefly, the disease incidence was 36.7 % (p* = 0.37), indicating that the disease could be transmitted even due to the feeding of a single viruliferous whitefly. Similar results have been reported by Nene (1972), Muniyappa *et al.* (2003), and Reddy (2006) for MYMV, PYVMV and *Tomato leaf curl virus* (ToLCV), respectively. Light intensity may change the behaviour of insects, probably by affecting the feeding efficiency (Mware *et al.,* 2009) and the desire to feed. When plants were inoculated with 15 or more viruliferous *Bt* per plant, initial symptoms appeared within 12–13 days. However, with 10 or fewer viruliferous *Bt* per plant, it took more than 14 days for symptoms to appear. In plants inoculated with a single whitefly, the amount of virus particles entering at a time is lower, whereas increased numbers of *Bt* lead to the accumulation of more virus particles, resulting in increased viral loading within the tissues. Therefore, plants inoculated with a single viruliferous *Bt* may show symptoms with a lower severity and lower incidence than those inoculated with more viruliferous *Bt.* Generally, plant defense mechanisms trigger reactions against the invasion of any virus. Such mechanisms cause the production of different chemical compounds by plant tissues that inhibit the activity of the virus.

Minimum acquisition access period (AAP) of *Bemisia tabaci* for HgYMV

Viruliferous *Bt* with different AAPs of 5 minutes to 48 hours were released onto healthy seedlings and percent infections were recorded in all three replicates. Disease incidences resulting from different AAPs as given in Table 4 indicate that the *Bt* can effectively acquire the virus from infected common beans within a minimum period of 20 minutes. No symptoms appeared on healthy bean plants inoculated for 5, 10 and 15 minutes, indicating that the whiteflies were not able to acquire the virus by feeding on infected bean plants for less than 15 minutes.

Table 4: Acquisition access periods (AAPs) of *Bemisia tabaci* for HgYMV

Feeding period on infected beans	Percent infection	p*
5 min	0.0 [a]	0.00
10 min	0.0 [a]	0.00
15 min	0.0 [a]	0.00
20 min	30.0 [b] (± 10.0)	0.04
30 min	43.3 [cb] (± 3.3)	0.06
1 hour	56.7 [c] (± 10.0)	0.09
2 hours	60.0 [c] (± 8.80)	0.08
4 hours	80.0 [d] (± 5.8)	0.15
6 hours	86.7 [ed] (± 8.8)	0.18
8 hours	86.7 [ed] (± 3.3)	0.18
24 hours	93.3 [ed] (± 3.3)	0.24
48 hours	93.3 [ed] (± 3.3)	1.00
LSD	19.12	
CV	16.64	

Means with the same letter are not significantly different according to least significant difference (LSD) at ($p < 0.05$). Data expressed as mean (±) standard error (n = 3); CV = coefficient of variation; p* = estimated transmission rate for a single whitefly.

Table 5: Inoculation access periods (IAPs) of *Bemisia tabaci* for HgYMV

Feeding period on healthy beans	Percent infection	p*
5 min	0.0 [a]	0.00
10 min	0.0 [a]	0.00
15 min	0.0 [a]	0.00
20 min	36.7 [b] (± 3.3)	0.05
30 min	40.0 [bc] (± 5.8)	0.05
1 hour	53.3 [ed] (± 8.8)	0.07
2 hours	63.3 [dc] (± 8.8)	0.01
4 hours	66.7 [ed] (± 3.)	0.10
6 hours	76.7 [fe] (± 8.8)	0.14
8 hours	83.3 [gf] (± 8.8)	0.16
24 hours	100.0 [h] (± 0.0)	1.00
48 hours	96.7 [hg] (± 3.3)	0.29
LSD	17.31	
CV	14.73	

Means with the same letter are not significantly different from each other according to least significant difference (LSD) at ($p < 0.05$). Data are expressed as mean (±) standard error (n = 3); CV = coefficient of variation; p* = estimated transmission rate for a single whitefly.

Minimum inoculation access period (IAP)

Results revealed that the viruliferous *Bt* are able to transmit the virus into a healthy common bean seedling within a minimum of 20 minutes (Table 5). The disease incidence reached 100 % with an IAP of 6 hours. The failure to develop the disease when the whiteflies feed for 5–15 minutes on healthy bean plants indicates that the vectors are unable to transmit the virus when feeding time is less than 15 minutes.

Many researchers have reported that AAPs and IAPs fall within the range of 15–30 minutes for various *Bt* transmitted viruses and that they can notice regional differences. Accordingly, a minimum of 15 minutes was reported for MYMV in terms of both AAP and IAP (Nene, 1972) in India. In another case of HgYMV, 30 minutes each in AAP and IAP were reported by Muniyappa and Reddy (1976), also from India. Similarly, 30 minutes each for AAP and IAP have been reported by Mansur and Al-Musa (1992) from Jordan, Ioannou (1985) from the Middle East, Caciagli *et al.* (1995) in Italy, and Muniyappa *et al.* (2000) in India for TYLCV. Furthermore, Reddy and Yaraguntaiah (1981) for ToLCV and Muniyappa *et al.* (2003) for PYVMV have also reported similar results in India.

In contrast to the above results, minimum AAP and minimum IAP have been reported as 1 hour and 2 hours, respectively for TYLCV by Brown and Nelson (1988). Interestingly, Senanayake *et al*. (2012) reported minimum AAP and IAP of 3 hours and 1 hour, respectively for *Chilli leaf curl virus* in India. The values in the above two cases are much higher than the present experimental values obtained. No symptoms were observed in the healthy bean plants fed by *Bt* for less than the minimum AAP reported in this experiment (20 minutes). However, various researchers have confirmed the presence of certain begomoviruses in *Bt* insects even when they fed on the infected plants for only 5–10 minutes; but this happened mainly when they used either molecular or serological means for assessment (Navot *et al*., 1992; Atzmon *et al*., 1998; Ghanim *et al*., 2001).

In most cases, the reported AAPs are higher than the IAP. According to Costa (1976), one reason for this might be insufficient time for the stylet of the vector to reach into the susceptible tissues in the phloem; another reason could be the long time feeding requirement of *Bt* vector to become infective and increase its chance of transmission.

Table 6: Persistence of virus in *Bemisia tabaci* during a serial transfer of HgYMV to common bean plants

Test group	Serial transfer of *Bemisia tabaci* insects at 24 hour intervals (D_1–D_{12})											
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}	D_{11}	% Infection
1	¬	+	-	+	+	-	+	-	d			57.1
2	+	+	+	+	+	+	-	-	d			75.0
3	+	+	+	+	+	+	+	+	+	d		100
4	+	+	+	+	-	+	+	+	-	d		77.8
5	+	+	+	+	+	+	+	d				100.0
6	+	+	+	+	+	+	+	-	+	d		88.9
7	+	-	+	+	+	+	d					83.3
8	+	-	-	+	-	+	+	d				57.1
9	+	+	+	-	+	+	-	d				71.4
10	+	-	+	-	+	d						60.0
11	+	-	+	+	+	-	-	+	+	-	d	45.5
12	+	+	+	+	+	-	+	-	d			75.0
Daily average % infection	100	58.3	91.6	83.3	75.0	81.8	50.0	50.0	50.0	-	-	

+ Symptoms appeared; - Symptoms did not appear; d - Death of last *Bt* of 12 *Bt* group

Persistence of the virus in the *Bemisia tabaci* insects

In this study, the virus persisted in the whitefly vector for a maximum of 9 days irrespective of the sex of the insects, further confirming the possibility of horizontal transfer of the virus. Similarly, Muniyappa *et al*. (2003) also reported the persistence of PYVMV for 8 days. When comparing the serial transmission rates between test groups it was found that they ranged from 45.4 % (minimum) to 100 % (maximum). The average infection rate per group was calculated to be 74.3 % per group of *Bt* insects. Furthermore, when comparing the time lapsed with infection strength, it varied from 50 % (minimum resulted on 8th day and 9th day) to 100% (maximum resulted on 1st day). An overall pattern of decreasing trend in infection was observed with the increase in time (Table 6). Higher persistence of the virus, lasting for more than 9 days would have been achieved if there were only female insects or a high proportion of females in the transfer groups and with higher survival rates as observed by Nene (1972) and Muniyappa *et al.* (2003). As the whiteflies were used without segregating them by sex, its influence on infection could not be clearly determined. Furthermore, it must be mentioned that in this study, the vertical transfer ability was not studied. Except for one or two cases including *Tomato yellow leaf curl virus* (TYLCV), which had been widely studied for transovarial transmission (Wei *et al*., 2017; Bosco *et al*., 2004), there is no evidence of the same phenomenon occurring in the majority of begomoviruses transmitted by *B. tabaci.* Accordingly, it has been demonstrated that transmission of viral DNA through vector eggs and subsequent nymphal stages is a relatively common phenomenon (Accotto & Sardo, 2010; Bosco *et al*., 2014; Guo *et al*., 2019).

CONCLUSION

Among the 54 plant species tested for their hosting ability, only a few plant species, namely, *Ageratum* sp., *G. max*, and *M. uniflorum* were susceptible to HgYMV while none of the tested monocots showed susceptibility to it. *Bemisia tabaci* Genn. was efficient at transmitting HgYMV causing yellowing disease in common beans, with successful transmission being achieved even through a single viruliferous whitefly. Minimum acquisition access period and minimum inoculation access period were 20 minutes in both cases and the virus could be retained by *Bemisia tabaci* for up to nine days after acquisition. The transmission characteristics lay within the typical ranges reported for begomoviruses around the world, especially in India but were lower than those reported in the Middle East and Mediterranean countries. The characteristics determined in the present study may differ if tests are repeated under field conditions. Furthermore, the present work emphasises the importance of considering all relevant aspects including the nature of hosts when managing viral diseases.

Acknowledgements

The authors gratefully acknowledge the assistance of the research staff of both the Plant Pathology and Entomology Divisions, Horticultural Crop Research and Development Institute, Department of Agriculture, Gannoruwa, Peradeniya. The authors also express their thanks to the technical staff and research students of the Department of Agricultural Biology Faculty of Agriculture, University of Peradeniya, for the immense technical support they provided.

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