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HOST RANGE AND VIRUS -VECTOR RELATIONSHIP OF A LUTEOVIRUS CAUSING THE NAMAMARAKO (NMK) SYNDROME OF AMPALAYA

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ABSTRACT

A total of 36 host plant species from 13 families of plants were tested for their reaction to the Cucurbit aphidborne yellows virus (CABYV) luteovirus using viruliferous aphids (*Aphis gossyppii)* in persistent manner. Of these, only three cucurbitaceous species namely, *Cucurbita pepo* (Zucchini), *Luffa acutangula* (patola ridge), and *Trichosanthes anguina* (snakegourd) showed viral symptoms 4 wk after inoculation. No weed species developed the viral symptoms upon inoculation but ELISA results yielded positive for five weed host species viz., *Trianthema portulacastrum* L. (Horse purslane), *Eclipta prostata (*L) (False daisy), *Cyperus rotundus* L. (Purple nutsedge), *Echinocloa colona* (L.) Link (Jungle rice) and *Portulaca oleracea* L. (common purslane).

Results on virus-vector relationship showed that the aphids can acquire the virus as early as 24 hr and can effectively transmit it at 24-36 hr after inoculation. Results also showed that 10-15 aphids can already cause an effective virus transmission but 20 aphids gave 100% transmission. For serial transmission and latent period, the aphids were able to transmit the virus with high percentage within 2 days, but infection went down to 50% in the next 3rd and 4th day. It was also noted that as the aphids were transferred from one plant to the other, a decrease in the number of insects was observed to have been retained.

Key words: luteovirus, cucurbit aphidborne yellows virus (CABYV), namamarako, virus – vector, host range

INTRODUCTION

Ampalaya is among the cucurbits of economic importance to vegetable industry. It is presently considered as one of the top moneymakers among vegetable growers, especially in Luzon. Ampalaya ranks second to squash in terms of hectarage planted and total production in the country. In 1999, the area planted to ampalaya was 8,129 ha as compared to squash at 8,512 ha (BAS, 1999). This statistics may not have accounted for the small and backyard growers. However, the increasing interest in ampalaya production coincided with the occurrence of malady that affects a lot of farmers in Luzon. This problem commonly termed namamarako results to very low yield because the affected plants become stunted. Has poor flowering and bears few fruits. The namamarako

syndrome may have existed in the past remained undetectable due to its very low incidence. The East-West Seed Company (EWSC) reported the first severe incidence of the syndrome in farmers' fields in Palayan and Laur, Nueva Ecija in the dry season of 1996 and later observed in Pangasinan, La Union, Laguna, Batangas, Cavite and Quezon.

Initial identification of the namamarako syndrome suggested that namamarako was not due to known viral infection. Several studies including the physiological aspects have been initiated to stress out the problem of the namamarako malady. A research done at the Institute of Plant Breeding (IPB), UPLB has suggested micronutrient deficiency (boron, zinc and iron) as one factor contributing to the malady. To deal with the problem, the EWSC concentrated on preventive measures by improving the cultural management practices for ampalaya production. This led to reduction in the incidence of NMK in EWSC plantings.

With the continuous effort of the East West Seed Company, the NMK problem was elucidated and identified as a disease caused by cucurbit aphidborne yellows (CABYV) luteovirus transmitted by aphids in persistent manner. (East west Seed Company, 2003).

The first report of natural infection of cultivated cucurbits by a luteovirus was done by Leqoc *et al.* (1992). The disease was exhibited as severe yellowing in older leaves of cucurbits (melon, cucumber and squash) transmitted by aphids in persistent manner. The causal pathogen, cucurbit aphid borne yellows virus (CABYV) (D'Arcy *et al.*, 2000; Guilley *et al.*, 1994) is serologically related to but distinct from beet western yellows virus (BWYV), a virus commonly found in vegetable crops (Lecoq, 1990; Ashby et al., 1979).

Knowledge on its host range and relationship with the aphid vector is very important in understanding the epidemiology of the NMK disease and at the same time significant parameters that can be exploited in NMK disease management and breeding for disease resistance. Hence, this study was conducted to determine the host range and virus-vector relationship associated with NMK disease of ampalaya.

MATERIALS AND METHODS

Host Range and Transmission of a Luteovirus Causing NMK in Ampalaya

The procedures for host range and transmission involved the use of aphids, *Aphis gossypii* in persistent manner. The virus was maintained in susceptible ampalaya genotype, Jadestar L, by periodically transmitting them to young seedlings via aphids. Test plants were raised from seeds sown directly into clay pots containing sterilized soil. Test seedlings were inoculated within 5-7 days of germination. A total of 36 host plant species from 13 host plant families were used in this study. Ten to 25 test plants from each host species belonging to Amaranthaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Leguminosae, Solanaceae, Aizoceae, Asteraceae, Cyperaceae, Passifloraceae, Poaceae and Portulacaceae families were used in each experiment and were repeated at least three times.

After the aphids were allowed an Acquisition Access Period (AAP) of 24 hr from the virus source, inoculation was performed by exposing each test plant to 20-25 viruliferous aphids for 48 hr Inoculation Access Period (IAP). Aphids were killed with insecticide after inoculation and plants were observed for symptoms. Uninoculated control plants were used for each test. Symptoms were recorded and symptomless plants were subjected to DAS-ELISA (Clark and Adams, 1977) test in collaboration with the East West Seed Company and/or by graft inoculation to healthy ampalaya seedlings to confirm the presence of NMK luteovirus.

Virus-Vector Relationship of CABYV Luteovirus Associated with NMK Syndrome

Mass rearing of Aphis gossypii. Colonies of aphids, *Aphis gossypii*, were collected from ampalaya, eggplant and pepper (Fig. 1). Mass rearing of aphids using seedlings of eggplant was successful. Rearing of aphids was also tried on "kundol" plants, however, the insects did not survive long.

Aphid adults used for infestation were collected and transferred to detached leaves of eggplant. The leaves were placed in pans lined with moistened tissue paper. The detached stems were covered or rolled with wet cotton to keep the leaves fresh for at least 3 days.

To test if the reared aphids were free of NMK, about 100 nymphs were infested on healthy ampalaya seedlings and allowed to feed on the plants for 24 hr. Disease symptom was observed on the plant and negative infection would mean that the aphids were virus–free. The virus-free insects were used throughout the experiment.

Determination of Most Effective Acquisition Access Period

Batches of first to second instar non-viruliferous aphids were allowed to feed on NMK infected seedling for the periods: 0.5, 1, 8 and 24 hr. After feeding, 10 nymphs, 5 for each leaf (first two leaves), were allowed to feed on test plants. Only the AAP was varied while IAP was set for 24 hr. All the aphids were removed after IAP and the plants were observed for NMK symptoms. Ten seedlings (4 day-old) were used per treatment. Ten ampalaya seedlings were also infested with NMK-free aphids and served as control.

A second batch of infestation was conducted. As suggested, the IAP was changed from 24 to 36 hr because of the "persistent" characteristic of the virus. The number of aphids was also increased from 10 to 20 individuals per plant, to ensure a more efficient inoculation of the pathogen. Aphids were allowed to feed (AAP) on NMK infected plant for 24, 36, 48 and 60 hr and were introduced to the ampalaya seedlings for an IAP of 36 hr.

Determination of Inoculation Access Period

The experiment was conducted with the following IAP: 0.08, 0.33, 0.67, 2, 6, 12, 24 and 36 hr. The AAP was set at 24 hr. Leaf samples from these treatments were submitted for confirmation of the virus using ELISA.

Another set of experiment was conducted which include the following IAP: 0.5, 1, 12, 24, 26 and 48 hr. Twenty aphids instead of ten per plant were used.

Vector Number and Transmission

Determination of effective number of vector for NMK transmission, was conducted using the following treatments: one, five, ten and 15 aphids. Results obtained from these experiment was the basis for the conduct of the experiment on latent period and transmission of NMK.

Latent Period and Retention

Twenty second instar nymphs were allowed an AAP of 36 hr. The aphids were individually caged on separate test seedlings and were serially transferred at 24-hr interval to fresh sets of plants. From each set of transfers, the cumulative percentage of first transmissions were recorded and retention of persistence was noted as the time period from leaving source plants to the last successful transmission in the serial transfer.

RESULTS AND DISCUSSION

Host Range and Transmission of Cucurbit Aphidborne Yellows Virus (CABYV)-