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Experimental Transmission of Grapevine Leafroll Virus 1-3 (GLRaV1-3) by Citrus mealybug, *Planococcus* citri (Risso) (Hemiptera, Pseudococcidae) and Grape leafhopper, *Arboridia adanae* Dlab (Hemiptera, Cicadellidae)

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ABSTRACT

This study was conducted to investigate the experimental transmission ability of Grapevine Leafroll Virus 1-3 (GLRaV1-3) by Citrus mealybug, *Planococcus citri* (Risso) (Hemiptera, Pseudococcidae) and Grape leafhopper, *Arboridia adanea* Dlab (Hemiptera, Cicadellidae). Grapevine plants (*Vitis vinifera* L.) cv. "Antep karası" exhibited typical leaf symptoms of GLRV disease was selected as virus source during the surveys in the vineyards of Hatay province. GLRaV-1 and GLRaV-3 were detected in source plants according to Double antibody sandwich- Enzym linked immunosorbent assay (DAS-ELISA) tests and Transmission Electron Microscopy (TEM) observations. *P. citri* and *A. adanae* cultures were maintained on the young shoots of germinated potato tubers and healthy grapevine seedlings, respectively. In groups of 10 individual insects from both species were tested by ELISA for presence of GLRaV-1 and/or GLRaV-3, before using in transmission trials. Nymphs and adults of *P. citri* were fed on infected plants for 2 hours for an acquisition access period (AAP) in non-persistent transmission experiments. After that, they have been transferred in groups of 25 nymphs or adults of *P. citri* onto healthy grapevine and tobacco (*Nicotiana clevelandii* and *N. benthamiana*) seedlings for 3 hours. Plants were observed for symptoms and tested at regular intervals by ELISA. The same experimental conditions were applied in non-persistent transmission trials by *A. adanae.* In persistent transmission experiments, AAP and IAP were applied for 30 days for both insects**.** Grapevine plants infected with GLRV-1 and GLRV-3 exhibited typical symptoms and leaf reddening was the most obvious symptom in all the symptomatic plants. In non-persistent transmission experiments for both insects, GLRaV-1 and GLRaV-3 were not found in grapevine and tobacco plants. However %13.3 and %35.0 infection rate of GLRV-3 was detected when adult individuals and group of nymphs of *P. citri* were used in persistent transmission experiments, respectively. Positive results were not found in tobacco plants by ELISA. According to the results of this study, *P. citri* can experimentally transmit GLRaV-3 in a persistent manner. Therefore, it is suggested that *P. citri* might

play a role in the spread of the GLRV in vineyards of Turkiye. Therefore, further investigations on the effects of other potential mealybug vectors on transmission of GLRaVs are necessary to improve the current management for Grapevine leafroll disease (GLD).

Key words: ELISA, grapevine, insect vector, leafroll virus, experimental transmission.

INTRODUCTION

Grapevine leafroll disease (GLD) is one of the most serious viral diseases of grapevine, occurring in all grapevine-growing areas worldwide (Bovey *et al.,* 1980; Martelli 1986; Bovey and Martelli, 1992). The disease causes important losses to yield and quality (Goheen and Cook 1959; Credi and Babini, 1997; Cabaleiro et al. 1999), and has spread rapidly, infecting new vineyard areas within a few years (Krake et al. 1999). GLD is associated with a group of at least nine closely related viruses belonging to the family *Closteroviridae* (Gugerli *et al*., 1984; Boscia *et al*., 1995; Martelli et al. 2002; Alkowni et al. 2004). Grapevine leafroll-associated virus 3 (GLRaV-3) is one of the most common of the leafroll-associated viruses (Martin et al. 2005; Pietersen 2006; Akbas et al., 2007). GLRaV-2 can be mechanically transmitted to herbaceous plant species (e.g. *Nicotiana benthamiana*) (Boscia *et al*., 1995; Goszczynski *et al*., 1996).

Some mealybug species are known to transmit important viruses in grapevines and to be responsible in the natural spread of the viruses (Golino et al. 2002). GLD is transmitted by insect vectors as mealybugs (Homoptera, Pseudococcidae) and soft scales (Homoptera, Coccidae) (Tanne et al. 1989; Engelbrecht and Kasdorf 1990; Belli et al. 1994; Cabaleiro and Segura 1997; Petersen and Charles 1997; Golino et al. 2002; Sforza et al. 2003). The vine mealybug, *Planococcus ficus* and the longtailed mealybug, *Pseudococcus longispinus* are known as vectors of GLRaV in several grapevine growing countries (Engelbrecht and Kasdorf 1990; Petersen and Charles 1997). *P. ficus* was also determined to be an efficient vector of GLRaV-3 in South Africa (Walton and Pringle 2004).

GLRaV-3 can be transmitted by at least 8 mealybug species *inluding* Citrus mealybug, *Planoccocus citri* (Risso) in the vineyards (Golino et al. 2002; Sforza et al. 2003; Charles et al. 2006).

This study was conducted to investigate of the experimental transmission status of Grapevine leafroll Virus 1-3 (GLRaV1-3) by *P. citri* (Risso) (Hemiptera, Pseudococcidae), occasionally emerged in some vineyard areas and Grape leafhopper, *Arboridia adanae* Dlab. (Hemiptera, Cicadellidae), a monophagous pest which can be serious problem if it is not controlled in grapevine-growing areas in Turkiye (Günaydın, 1972; İren, 1972; Lodos 1982; Yiğit and Erkılıç, 1987; Caglayan, 1997).

MATERIAL AND METHODS

Plant and Insect Cultures: Grapevine plants (*Vitis vinifera* L.) of cv. "Antep karası" was selected as the source of virus which were exhibited typical leaf symptoms of Grapevine leafroll disease (Figure 1) during the surveys in the vineyards of Hatay province. They have been identified to be infected with GLRaV-1 and GLRaV-3 by previous study conducted by Çağlayan (1997) using Double antibody sandwich-Enzyme linked immunosorbent assay (DAS-ELISA) and Transmission Electron Microscopy (TEM) technique during the years of 1997-2001 (Figure 2).

Figure 1. Typical symptoms of Grapevine leafroll disease: reddening of the lamina, interveinal discoloration and downward rolling of the leaf margins.

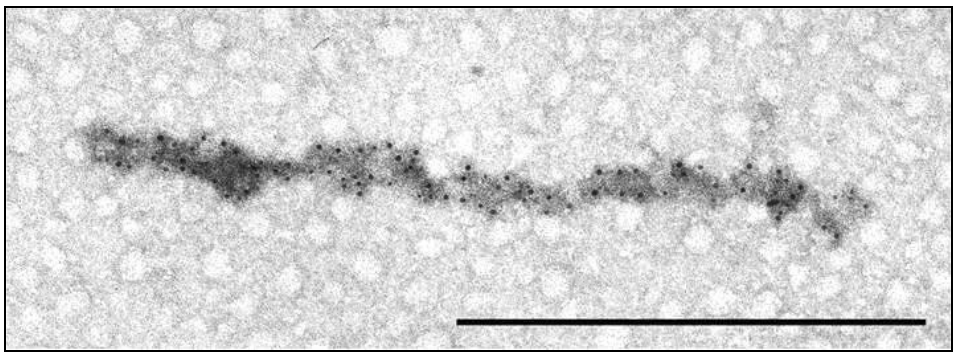

Figure 2. Electron micrograph of Grapevine leafroll virus (GLRV) particle decorated with rabbit antiserum of GLRV1-3 and probed with gold-goat anti rabbit IgG. Gold clusters can be seen as the very dark spots in these images (bar=50µm) (Çağlayan, 1997).

One-year old grapevine plants propagated from the source plant and detected for presence of GLRaV-1 and GLRaV-3 by DAS-ELISA. The plants showed high absorbance (A405) values in ELISA tests were used as donor plants (Figure 3).

Figure 3. Producing of grapevine plants from seeds and rooting of cuttings taken from healthy and GLRV-infected plants.

The healthy plants were produced by cuttings and seeds of the same cultivar, and used in transmission experiments. *P. citri* cultures were maintained on the young shoots of germinated potato tubers (Figure 4). Field-collected gravid females of *P. citri* with ovisacs were transferred on sprouted potatoes and reared for several generations throughout the years at 25 ± 1°C, and 70 ± 10% R. H.

Figure 4. Rearing of *Planococcus citri* on germinated potato tubers and GLRV-transmission assays on grapevine plants by using adults of *P. citri*

Field-collected adult and nymphes of *A. adanae* were transferred onto healthy grapevine seedlings, planted in pots were placed in 50 x 110 x 80 cm growth cages, the sides of which were covered with cheese-cloth and the top with a glass pane (Figure 5). The cages were maintained in a constant temperature room at 25 ± 2°C under 16 h illumination and 70 ± 10% R. H.

Both insect species in groups of 10 individuals were tested by ELISA for presence of GLRaV-1 and/or GLRaV-3, before using in transmission assays. Healthy plants were grown in an insect-proof room, at 24±2°C and a photoperiod of 16:8 h (L:D).

The presence of viruses in all the plant and insect materials were confirmed by DAS–ELISA at the initiation of the experiments. GLRV-1 and GLRaV-3 infections have not been determined in *A. adanae* individuals in groups of 25-30 adults and grapevine plants which were randomly sampled at regular intervals and tested by ELISA.

Figure 5. GLRV-transmission assays on grapevine plants by using adults of *Arboridia adanae.*

Non-persistent transmission experiments: Nymphs and adults of *P. citri* cultured on potato plants were transferred onto infected plants for an acquisition access period (AAP) of 2 hours. After AAP, they transferred in groups of 25 nymphs or adults of *P. citri* onto healthy seedlings for an inoculation access period (IAP) of 3 hours. All *P. citri* individuals were then removed. The plants used in transmission experiments were placed at 24±2°C and a photoperiod of 16:8 h (Light:Dark).

The same experimental conditions were applied in non-persistent transmission trials of GLRV by *A. adanae.*

Persistent transmission experiments: *P. citri* and *A. adanae* individuals were fed on grapevine plants seedlings infected with GLRV-1 and GLRV-3 for an AAP of 30 days. Then, they were transferred on to healthy grapevine plants in groups of 25 nymphs and/or adults of *P. citri* and/or *A. adanae* for 30 days (IAP).

Healthy grapevine plants (1 year-old) produced from cuttings (Figure 3) were used as acceptor plants in transmission assays. Due to some grapevine viruses such as GVA and GVB can be transmitted by mealybugs and can also be transmitted by sap inoculation to several tobacco species (Garau et al. 1995; Yoshikawa et al. 1997), tobacco (*Nicotiana clevelandii* ve *N. benthamiana*) seedlings in 6-8 leaf stage were also used in the transmission experiments with *P. citri*.

The plants used in the experiments were placed in insect-proof room conditions at 24±2°C and a photoperiod of 16:8 h (L: D). The leaf samples collected from these plants were tested at regular intervals (2, 4, 8, 10, 24 and 30 months after transmission) by ELISA.

RESULTS AND DISCUSSION

Symptoms included reddening of the lamina, interveinal discoloration and downward rolling of the leaf margins were observed on the plants produced from

grapevine plants infected with GLRV-1 and GLRV-3. Leaf reddening was the most obvious symptom in all the young plants.

In previous studies, symptoms of GLRaV-3 were visually monitored on susceptible indicator rootstock, such as *C. sauvignon* and LN33**,** 12 weeks after inoculated by green-grafting technique (Pathirana and McKenzie, 2005).GLRV-1 and GLRaV-3 have been reported to be found in a high titres in midvein instead of other part of leaf tissue and virus antigen titers vary in different tissues or seasons in grapevines (Monis and Bestwick, 1996; Pekekti *et al*. 2001). Therefore, the plants were examined by two-month intervals for a long period in this study. Transmission of GLRV-3 by insect groups containing of 1, 5, 10, 20 and 40 nymphs of *P. ficus* and *P. longispinus* were studied by Douglas and Krüger (2008). After AAPs of 5 days on virus source plants and IAPs of 5 days on virus indicator plants, the plants were tested for GLRaV-3 starting 8 weeks after transmission and then in intervals for up to 11 months. There was a discrepancy between the percentage of nymphs infected within a group and the infection rate of the corresponding plant groups. For example, even though none of the *P. longispinus* nymphs of the '1-nymph group' tested positive for GLRaV-3 after 5-day AAPs and 5-day IAPs, 70% of the grapevine plants in this group tested positive. In addition, significantly more nymphs tested positive for GLRaV-3 in the '40-nymph group,' whereas the number of positive plants at 20% was the lowest of all groups for *P. longispinus*. However, when excluding the '40-nymphs' *P. longispinus* group, the number of positive nymphs of *P. ficus* and *P. longispinus* was lower after AAPs and IAPs of 5 days each than the respective controls after 5-day AAPs only. It was reported that these differences could possibly be due to nymphs having moulted during the 10-day period (5 days AAP and 5 days IAP), loss of infectivity, and/or the sensitivity and detection levels of the PCR being insufficient to detect GLRaV-3 (Douglas and Krüger, 2008). In a similar study, transmission efficiency of *P. ficus* and *P. longispinus* using 20 nymphs per plant reported to be ranged between means of 65 to 100% and 40%, respectively (Krüger *et al*. 2006).

In non-persistent transmission experiments by *P. citri* and *A. adanae*, positive results were not found in tobacco plants (*N. clevelandii* and *N. benthamiana*) for presence of the GLRV-1 and GLRV-3 at the end of the 10th month by ELISA applied within two months intervals. Red color formation was observed on a grapvine seedling produced by cuttings 16 months after transmission experiments by *P. citri*. However, none of the plants including the suspected plant was found to be infected with GLRV-1 and GLRV-3 by ELISA 20 and 30 months after transmission assay. GLRV-3 was reported to be determinable in plants two months before symptom expression on leaves (Matthews,1996).

Results of DAS-ELISA in samples taken from test plants used in non-persistent transmission experiment of GLRV-1 and GLRV-3 by *P. citri* and *A. adanae*, 30 months after transmission assays were given in Table 1 and Table 2.

Table 1. Results of DAS-ELISA in samples taken from grapevine seedlings used in non-persistent transmission of GLRaV-1 and GLRaV-3 by *Planoccocus citri* 30 months after transmission assays

Biological stage of insects	**The number of infected/ tested plants (% Infection rate)**					
	Grapevine (*Vitis vinifera* L.)		***Nicotiana clevelandii***		***Nicotiana benthamiana***	
	GLRaV-1	**GLRaV-3**	**GLRaV-1**	**GLRaV-3**	**GLRaV-1**	**GLRaV-3**
Nymph	**0/15** (%0.0)	**0/15** (%0.0)	**0/15** (%0.0)	**0/15** (%0.0)	**0/15** (%0.0)	**0/15** (%0.0)
	0/10[a] (%0.0)	**0/10** (%0.0)				
Adult	**0/10** (%0.0)	0/10 (%0.0)	**0/10** (%0.0)	**0/10** (%0.0)	**0/10** (%0.0)	**0/10** (%0.0)
	0/10[a] (%0.0)	**0/10** (%0.0)				

[a]Plant produced by seed.

Table 2. Results of DAS-ELISA in samples taken from grapevine **(*Vitis vinifera* L.)** seedlings used in non-persistent transmission of GLRaV-1 and GLRaV-3 by *Arboridia adanae* 30 months after transmission assays

Biological stage of insects	**The number of infected /tested plants (% Infection rate)**			
	Grapevine (*Vitis vinifera* L.) (produced by cutting)		**Grapevine (*Vitis vinifera* L.) (produced by seed)**	
	GLRaV-1	**GLRaV-3**	**GLRaV-1**	**GLRaV-3**
Nymph	**0/10** (0.0%)	**0/10** (%0.0)	**0/10** (%0.0)	**0/10** (%0.0)
Adult	**0/10** (0.0%)	**0/10** (%0.0)	**0/10** (%0.0)	**0/10** (%0.0)

GLRaV-1 and GLRaV-3 were not detected in the test plants at the end of the non-persistent transmission assays, used by both *P. citri* and *A. adanae*. Although, many studies have been reported on transmission of GLRaV by *P. citri* (Gugerli, 2003; Pathirana and McKenzie 2005; Cabaleiro and Segura, 1997a, 1997b; Cid et al., 2006; 2007; Douglas and Krüger 2008), there was not any report for the virus transmission by *A. adanea*.

GLRaV-3 was reported to be transmitted to only 1/10 test plants each of which had been exposed to a groups of five *P. citri*, fed on virus-infected plants for at least three days, eventhough more than 80% of the insect groups were expected to contain viruliferous individuals under these conditions. According to results of the same research, the virus was suggested to be remained latent or undetectable by ELISA for at least 13 months (Cabaleiro and Segura, 1997a).

Although it is known that GLRaV-1 is transmitted by soft scale insects such as *Parthenolecanium corni* (Bouché) and *Neopulvinaria innumerabilis* (Rathvon), but there was not any report of transmission by mealybugs (Martelli 2000). On the other hand transmission of GLRaV, especially GLRaV-3 by mealybugs (obscure, longtailed, citrus or grape mealybug) and by the soft scale, *Pulvinaria vitis* (Linnaeus) was reported (Rosciglione and Castellano, 1985; Engelbrecht and Kasdorf 1987; Engelbrecht and Kasdorf 1990; Boscia et al. 1993; Jordan 1993; Digiaro *et al.* 1994; Minafra and Hadidi 1994; Garau *et al.* 1995; Cabaleiro and Segura 1997a, b; La Notte et al. 1997; Petersen and Charles 1997; Borgo and Michielini 2000; Sforza and Greif, 2000; Golino et al. 2002; Sforza et al. 2003).

GLRV-3 infected plants were obtained at a rate of 2/15 by the group of adult individuals, and infected plants were obtained in a higher rate (7/20) conducted by the group of nymphs in persistent transmission experiments by *P. citri*. 10 months after IAP, positive results were not detected in samples of tobacco plants inoculated with randomly selected groups of five insects (nymph and adults) which were fed on GLRV-1 and GLRV-3 infected plants for an AAP of 30 days. Results of DAS-ELISA**,** in samples taken from grapevine seedlings used in persistent transmission of GLRaV-1 and GLRaV-3 by *P. citri* 30 months after transmission assays were given in Table 3.

Table 3. Results of DAS-ELISA**,** in samples taken from grapevine seedlings used in persistent transmission of GLRaV-1 and GLRaV-3 by *Planoccocus citri* 30 months after transmission assays

Biological stage of insects	**The number of infected / tested plants (% Infection rates)**					
	Grapevine (*Vitis vinifera* L.)		***Nicotiana clevelandii***		***Nicotiana benthamiana***	
	GLRaV-1	**GLRaV-3**	**GLRaV-1**	**GLRaV-3**	**GLRaV-1**	**GLRaV-3**
Nymph	**0 /15** (%0.0)	**6 /15** (%40.0)	**0/15** (%0.0)	**0/15** (%0.0)	**0/15** (%0.0)	**0/15** (%0.0)
	0 / 5[a] (%0.0)	**1 / 5[a]** (%20.0)				
Adult	**0 /15** (%0.0)	**2 /15** (%13.3)	**0/10** (%0.0)	**0/10** (%0.0)	**0/10** (%0.0)	**0/10** (%0.0)
	0 / 5[a] (%0.0)	**0 / 5[a]** (%0.0)				

[a] Plants produced by seeds.

There have been several studies of the ability to detect GLRaV-3 in different tissues collected from known infected vines over the course of a season (Teliz et al., 1987; Matthews, 1996, Pekekti ve ark., 2001). In New Zealand it has been found that if vines become infected late in the growing season, infection may not be detected in the following autumn, or winter using ELISA, possibly because of uneven virus distribution in newly infected vines (Charles et al., 2006).

In this study, single or mixed infected test plants with GLRV-1 were not found in any transmission experiments. GLRV-3 transmissions occured at the rate of 7/20 (%35) in the assays carried out by *P. citri* (especially by nymphs). The results of this study indicated that GLRV-3 can be transmitted to grapevine plants by *P. citri*. GLRaV-3 was reported to be detected 3 years after planting in vineyards established with virus-free material, and the incidence of virus symptoms rised from 33 to 83%, *P. citri* was suggested to be associated with the infected plants (Cabaleiro and Segura, 1997b). It has been reported that nymphs of different mealybug species were to be more effective vectors of GLRV-3, due to being more active than adults (Petersen and Charles, 1997; Cabaleiro and Segura, 1997a; Krüger *et al.* 2006, Cid *et al.* 2007; Douglas, and Krüger, 2008; Tsai et al, 2008; Barrass et al., 1994, Charles et al. 2006).

GLRaV-3 belongs to the genus Ampelovirus in the family *Closteroviridae* and is thought to be transmitted in a semi-persistent manner by several species of mealybugs and soft-scale insects from grapevine to grapevine (Martelli et al., 2002; Gugerli, 2003). Though the transmission mechanism has been described as semipersistent on the basis of temporal parameters, definitive proof of this mechanism has never been provided. A circulative transmission mechanism was suggested which is based on a specific transportation route for the viral particles from the midgut or hindgut to the salivary glands (Cid et al., 2007). Similarly, the study on the transmission of GLRaV-3 by the vine mealybug, *P. ficus* showed that transmission of the virus occurs in a semi-persistent manner (Tsai *et al*., 2008).

Results of GLRaV-1 and GLRaV-3 transmissions in persistent manner by *A. adanae* were given in Table 4.

Table 4. Results of DAS-ELISA in samples taken from grapevine seedlings used in persistent transmission of GLRaV-1 and GLRaV-3 by *Arboridia adanae* 30 months after transmission assays

Biological stage of insects	**The number of infected plants / tested plants (% Infection rate)**			
	Grapevine (*Vitis vinifera* L.) (produced by cutting)		**Grapevine (*Vitis vinifera* L.) (produced by seed)**	
	GLRaV-1	**GLRaV-3**	**GLRaV-1**	**GLRaV-3**
Nymph	**0/15** (%0.0)	**0/15** (%0.0)	**0/3** (%0.0)	**0/3** (%0.0)
Adult	**0/15** (%0.0)	**0/15** (%0.0)	**0/3** (%0.0)	**0/3** (%0.0)

No virus transmission was observed in the grapevine plants 30 months after transmission assays. GLRaV-1 and GLRaV-3 were not transmitted by nymphs and adults of *A. adanae* in persistent transmission experiments. According to the results of this study, *P. citri* can transmit GLRaV-3, one of the most common viruses in

vineyards. Therefore, it is suggested that *P. citri* has a role in the spread of the disease caused by the virus in vineyards of Turkiye.

It was reported that the lower abundance of *P. longispinus* compared to *P. ficus* on grapevines in South Africa indicated that this species is not as well adapted to grapevine. However, *P. ficus* and *P. longispinus* were suggested to be equally efficient as vectors of GLRaV-3, and a single individual of either *P. ficus* or *P. longispinus* is capable of transmitting GLRaV-3 (Douglas and Krüger, 2008).

Mealybug management in vineyards of Turkey has gained more importance, due *to P. citri* does not only cause losses to yield and quality as a result of feeding damage, but also revealed as a vector of GLRaV-3, one of the most important viruses in grapevines. Therefore, further investigations on the effects of other potential mealybug vectors on transmission of GLRaVs are necessary to improve the current management for Grapevine leafroll disease (GLD).

ÖZET

Asma Yaprak Kıvırcıklık Virüslerinin (GLRaV 1-3) Turunçgil Unlubiti, *Planococcus citri* (Risso) (Hemiptera, Pseudococcidae) ve Bağ Üvezi, *Arboridia adanae* Dlab. (Hemiptera, Cicadellidae) ile Taşınma Durumu

Bu çalışma, Asma yaprak kıvırcıklık virüsü-1 ve 3 (Grapevine leafroll-associated virus 1-3: GLRaV1-3)'nün Turunçgil unlubiti, *Planococcus citri* (Risso) (Hemiptera, Pseudococcidae) ve Bağ üvezi, *Arboridia adanae* Dlab. (Hemiptera, Cicadellidae) ile ülkemizde asma bitkisinde taşınma durumunu incelemek üzere yürütülmüştür. Hatay ili bağ alanlarında yapılan survey çalışmaları sırasında Asma yaprak kıvırcıklık (AYK) hastalığının tipik belirtilerini sergileyen "Antep karası" çeşidine ait asma bitkileri (*Vitis vinifera* L.) seçilmiştir. Bu bitkiler arasından DAS-ELISA (Double antibody sandwich-Enzym linked immunosorbent assay) ve TEM (Transmission Electron Microscopy) yöntemleri kullanılarak GLRaV-1 ve GLRaV-3 ile tek olarak enfekteli olduğu belirlenenler taşıma çalışmaları için kaynak bitki olarak ayrılmıştır. *P. citri* bireyleri çimlenmiş patates yumrularının genç sürgünler üzerinde kültüre alınmış olup, *A. adanae* nimf ve erginleri ise sağlıklı asma bitkileri üzerinde çoğaltılmıştır. Non-persistent taşıma denemelerinde *P. citri* nimf ve erginleri virüsü alma dönemi (VAD) için enfekteli bitkiler üzerinde 2 saatlik bir besleme süresi uygulanmıştır. Bu süreden sonra sağlıklı asma ve tütün (*Nicotiana clevelandii* ve *N. benthamiana*) bitkileri üzerine 25 bireylik nimf ve ergin grupları aktarılarak 3 saat süre ile virüs inokulasyon dönemi (VİD) için beslenmiştir. Bitkiler simptomlar yönünden gözlenmiş ve düzenli aralıklarla ELISA ile incelenmiştir. Aynı deneysel koşullar *A. adanae* ile yapılan non-persistent taşıma çalışmalarında da uygulanmıştır. Persistent taşıma denemelerinde, *P. citri* ve *A. adanae* bireyleri VAD ve VİD için enfekteli ve sağlıklı bitkiler üzerinde 30 gün sure ile beslenmiştir. GLRV-1 ve GLRV-3 ile enfekteli asma bitkileri AYK hastalığının tipik simptomlarını sergilemiş ve en belirgin simptom olarak yaprakların kırmızılaşması

gözlenmiştir. *P. citri* ve *A. adanae* ile non-persistent taşıma çalışmalarında asma ve tütün bitkilerine GLRaV-1 ve GLRaV-3 taşıması gerçekleştirilememiştir. Bununla birlikte *P. citri* ile asma bitkilerinde yapılan persistent taşıma çalışmalarında, VİD'den 10 ay sonra *P. citri*'nin yetişkin bireyleri ile %13.3 oranında, nimf grupları ile ise daha yüksek bir oranda (%35.0) GLRV-3 ile enfekteli bitki elde edilmiştir. Tütün bitkilerinde pozitif sonuç alınamamıştır. Bu çalışmanın sonuçlarına göre, *P. citri* bağlarda en yaygın virüslerden biri olan GLRaV-3'ü taşıyabilmektedir. Buna göre, *P. citri*'nin Türkiye'deki bağlarda AYK hastalığı etmeni virüsün yayılmasında rol oynayabileceği düşünülmektedir. *P. citri*'nin bağlarda beslenme sonucu verim ve kalite kayıplarına yol açması yanı sıra, GLRaV-3'ün bir vektörü olabileceğinin belirlenmesi dolayısıyla, ülkemiz bağ alanlarında zararları görülen unlubit savaşının yönetimi daha da önem kazanmaktadır. AYK hastalığının mevcut mücadele yönetimlerini geliştirmek için *P. citri* ve diğer potansiyel unlubit türlerinin asma yaprak kıvırcıklık virüslerinin yayılmasına etkileri konusunda daha ayrıntılı araştırma yapılması yararlı olacaktır.

Anahtar kelimeler: Asma, bağ, böcekle taşınma, ELISA, kıvırcıklık virüsü

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