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Transmission efficiency of Tunisian *Potato leaf-roll virus* isolates by Tunisian clones of the *Myzus persicae* complex (Hemiptera: Aphididae)

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Potato leafroll virus (PLRV) is naturally transmitted by aphids, especially by the *Myzus persicae* complex (Hemiptera: Aphididae), in a persistent manner. The transmission efficiency of Tunisian PLRV isolates by Tunisian clones of *M. persicae* is unknown. Thus, six Tunisian and two French control clones (MP3 and LCSA) of *M. persicae* complex were used to assess the aphid transmission of nine PLRV isolates collected from three different Tunisian field regions. The transmission values were compared to the transmission efficiency of two PLRV isolates characterized as highly (CU87) and poorly (14.2) aphid-transmissible. Experiments conducted under controlled conditions showed that the transmission rates obtained were in general high. All tested PLRV isolates were good transmitted (40-100%) except for the PLRV14.2 isolate characterized as poorly transmissible which was not transmitted by all Tunisian *M. persicae* clones. The relation between the transmission rate and the mean value of antigen titre was also reported.

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Key words: aphid clones, DAS-ELISA, infection, inoculation, *Physalis floridana* (Rydb), PLRV isolates, transmission.

INTRODUCTION

Potato leafroll virus (PLRV) is an important pathogen of potato (*Solanum tuberosum* L.) crops worldwide and is causing significant damage (ROBERT & LEMAIRE, 1999). In Tunisia, PLRV was identified as one of the main constraints for potato seed multiplication and production in Tunisia (MNARI *et al.*, 1994).

Like the other members of the genus *Polerovirus* (family *Luteoviridae*) (MAYO & d'ARCY, 1999), PLRV is obligatory transmitted in a persistent, circulative and non replicative manner (Sylvester, 1988), by a limited number of aphid species principally by *Myzus persicae* (Sulzer) (KENNEDY *et al.*, 1962) which transmits PLRV more efficiently from *P. floridana* than from potato, and usually infects *P. floridana* more efficiently than potato (KIRKPATRICK & ROSS, 1952).

During feeding in phloem tissues of an infected plant (SHEPARDSON *et al.*, 1980), *M. persicae* ingests PLRV virions which circulate through the aphid body (reviewed by

Table 1.- **Characteristics of Tunisian PLRV isolates and the protocols of their first transfer (to homogenate the virus source) from vegetal collected potato material (tuber sprouts or leaves) to *P. floridana* by LCSA clone.**

Tunisian PLRV isolates	Geographic origin	Collection date	Collected material	First transmission protocol
is_4, is_5, is_6 and is_{16}	Mahdia (central east)	01- 2002	tuber sprouts	Plant to plant
is_{SAM}	Bizerte (North)	02- 2002	tuber sprouts	Plant to plant
is_{24}, is_{26}, is_{29} and is_{30}	Tunis (Northern east)	03- 2002	leaves	Membrane feeding

GILDOW *et al.*, 1999). Specific interactions between the virus and some membrane and/or haemolymph components of the vector are essential for virus circulation and transmission (van den HEUVEL *et al.*, 1994; HOGENHOUT *et al.*, 1996). Moreover, virus transmission depends on many factors including the aphid biotypes, species, clones, morphs and genotype and virus isolates (BJÖRLING & OSSIANNILSSON, 1958; JOLLY & MAYO, 1994; BOURDIN *et al.*, 1998; TERRADOT *et al.*, 1999; ROUZÉ-JOUAN *et al.*, 2001). PLRV variability is rather limited, so strains were first identified according to the severity of symptoms they induced on *P. floridana* (HARRISON, 1984). Then, highly aphid-transmissible (HAT) and poorly aphid-transmissible (PAT) isolates were distinguished irrespective of their different transmissibility (TAMADA *et al.*, 1984). Lastly, SINGH *et al.* (1982) used both symptom intensity and transmissibility by clones of *M. persicae* as parameters for strain identification.

This study aims at determining the transmission efficiency of the Tunisian PLRV isolates by the Tunisian clones of *M. persicae* under laboratory conditions. A relation between the PLRV transmission and ELISA OD values of inoculated plants was also reported.

MATERIALS AND METHODS

Virus isolates. Tuber-son or leaves were collected from naturally secondarily PLRV-infected potato plants (cv. *Spunta*) growing in different Tunisian fields. A collection of nine Tunisian PLRV isolates was obtained after their first transfer to *P. floridana* by LCSA transmission (Table 1). Two isolates previously collected in the north of France in 1985 (PLRV14.2) and in Cuba in 1987 (PLRVCU87) and maintained for years on *P. floridana* by vegetative propagation (Bourdin *et al.*, 1998) were chosen for the present study as controls and to compare their transmission efficiency to the transmission rates of Tunisian PLRV isolates. PLRVCU87 is considered as a HAT (highly aphid transmissible) reference isolate, whereas PLRV 14.2 is considered as PAT (poorly aphid transmissible) reference isolate by most clones of aphids belonging to the *M. persicae* complex (BOURDIN *et al.*, 1998).

Aphid clones. Six green Tunisian populations of *M. persicae* were collected at the beginning of the growing season (Table 2). Each nonviruliferous (virus-free) clone was derived from a single apterous virginiparous aphid. Before virus transmission experiments, aphids were multiplied on PLRV-immune Chinese cabbage (*Brassica campes-*

Table 2.- **Characteristics of Tunisian aphid clones of *M. persicae* complex**

Tunisian aphid clones	Sampling date	Plant origin	Geographical origin
VeB1, VeB3 and VeB7	April, 2002	*Solanum tuberosum*	Tekelsa (Northern east)
VeT3, VeT6 and VeT8	May, 2002	*Convolvulus arvensis*	Tunis (Northern east)

tris L. var. *pekinensis*) under controlled conditions (ROBERT *et al.*, 1969) to avoid PLRV contamination and to keep them free of PLRV (CHUQUILLANQUI & JONES, 1980). LCSA (*M. persicae* taxa) and MP3 (*M. antirrhinii* taxa) clones belonging to the *M. persicae* complex (TERRADOT *et al.*, 1999) were used in this study as control since they transmit efficiently several PLRV isolates except for the poorly transmitted PLRV14.2 isolate by the MP3 clone (BOURDIN *et al.*, 1998). LCSA was also used for the first transfer of all Tunisian PLRV isolates to *P. floridana* test plant.

Virus purification. Since PLRV is_{24}, is_{26}, is_{29} and is_{30} isolates are available in the form of frozen potato leaves, their first transfer to *P. floridana* necessities their purification and their transfer by membrane feeding. The purification protocol was done following the method described by TAMADA & HARRISON (1980) and modified by ROUZÉ-JOUAN *et al.* (2001). Virus yields were 200-300 μg/kg leaf.

Virus transmission. In order to homogenate the virus source, all Tunisian PLRV isolates were firstly transferred to *P. floridana* source plants by plant to plant or by membrane feeding protocols (Table 1). These protocols were carried out under controlled laboratory and glasshouse condition (20°C; 60% relative humidity; 16h light/ 8h dark photoperiod). Once all PLRV isolates transferred on *P. floridana* source plants, each PLRV-isolate/aphid-clone combination was tested by plant to plant transmission.

For plant to plant virus transmission from infected to healthy *P. floridana*, 90 –120 fourth instar apterous nymphs or young adults were given a 3 day acquisition access period (AAP) on infected *P. floridana*. Then, aphids were transferred in groups of 3 to 20 *P. floridana* seedlings (one leaf stage) for a 3 day inoculation access period (IAP). A total of about 1760 test plants (20 inoculated *P. floridana* for each PLRV-isolate/aphid-clone combination) in over 88 different experiments were used in this work in addition to the non infected indicator plants of the same age used as healthy control for symptom comparison. All PLRV inoculated plants were checked for virus infection, 15 to 20 days after IAP, by typical symptoms and ELISA test.

The membrane feeding protocol (ROUZÉ-JOUAN et al, 2001) was used for the first transfer of PLRV is_{24}, is_{26}, is_{29} and is_{30} isolates to *P. floridana*. In that, young adult aphids of the LCSA clone were fed for a 24 h AAP through a stretched Parafilm membrane, on purified PLRV suspensions (50μg/ml of virus). Aphids were then transferred to healthy *P. floridana* plants for a 3 day IAP (three aphids/ test plant) as previously described for the plant to plant transmission tests.

Double Antibody Sandwich-ELISA (DAS-ELISA). Collected potato leaves, tuber-son and inoculated *P. floridana* test plants were checked for PLRV infection by DAS-ELISA (CLARK & ADAMS, 1977). The tests were done with 0.5 μg/ml solution of immunoglobuline (Igs). Alkaline phosphatase-conjugated monoclonal Igs were used as a second antibody layer at 1/1000 dilution. The ELISA tests were done at the same time after infection and with leaves of the same development stage.

Data analysis. For each virus isolate/aphid clone combination, transmission rate (Arcsin $\sqrt{x}$ transformed ACPT) and mean ELISA OD values were analysed by a two-way ANOVA with GLM (General Linear Model) procedure of the SAS (Statistical Analysis Software) package and compared with Duncan's multiple range test (SAS Institute Inc., 1995).

RESULTS

Plant to plant transmission experiments showed that the PLRVCU87 isolate was very well transmitted by Tunisian aphid clones (Table 3). The transmission rates were above 60% with most of the tested aphid clones, except for VeT8 (40%) ($Pr>0.05$) (Table 4). In contrast, the PLRV14.2 isolate was not transmitted by Tunisian aphid clones and poorly transmitted by LCSA (10%) and MP3

Table 3.- **Transmission percentages of each tested virus isolate/aphid clone combination**

PLRV isolates	Aphid clones							
	MP3	LCSA	VeB1	VeB3	VeB7	VeT3	VeT6	VeT8
CU87	95	100	60	85	65	90	95	40
14.2	5	10	0	0	0	0	0	0
is_4	95	55	80	55	75	100	100	80
is_5	75	95	100	90	95	100	95	95
is_6	100	90	100	95	75	100	95	85
is_{16}	95	95	100	70	75	90	100	100
is_{SAM}	90	75	90	95	95	100	95	90
is_{24}	90	95	80	70	95	80	70	70
is_{26}	60	95	85	100	80	95	90	90
is_{29}	80	80	95	85	50	95	80	90
is_{30}	60	90	90	90	80	100	95	90

(5%) (Table 3) ($Pr<0.001$). The Tunisian PLRV isolates were efficiently transmitted overall by Tunisian *M. persicae* clones. The transmission rates ranging from 50 to 100% showed that Tunisian aphid clones were consistently efficient vectors for all Tunisian isolates throughout the experiment (Table 3). In fact, there is no significant difference between the transmission % means of Tunisian PLRV isolates. The Duncan's test resulted in defining an isolate effect for which the means transmission % and ELISA OD values were statistically different and the test is highly significant (Pr<0.001) (Table 4). The difference between transmission % means analysed by Duncan's test showed that the PLRV14.2 isolate can be distinguished from all Tunisian and PLRVCU87 isolates. Thus, this test resulted in defining 2 groups: A group consisting of PLRV Tunisian and CU87 isolates and B group consisting of the PLRV14.2 isolate (Figure 1). In contrast, there were no statistical differences between aphid clones and all tests were non-significant (Pr>0.05). The Duncan's test didn't show in this case any vector effect because of the overlapping groups defined among the 8 tested aphid clones. The VeT3 aphid clone belonged to A group; the VeB3, VeB7 and VeT8 aphid clones belonged to B group and the remaining aphid clones overlapped into A and B groups (Figure 2).

Table 4.- **Effects of vectors and isolates on the mean transmission % and mean OD values using ANOVA and SAS package.**

Dependent variable: Mean OD value			
Source	DF	F value	Pr>F
Vector	7	0.21	0.98
Isolate	10	10.33	0.0001 ***
Dependent variable: Mean transmission % (transformed ACPT)			
Source	DF	F value	Pr>F
Vector	7	2.07	0.058
Isolate	10	27.61	0.0001 ***

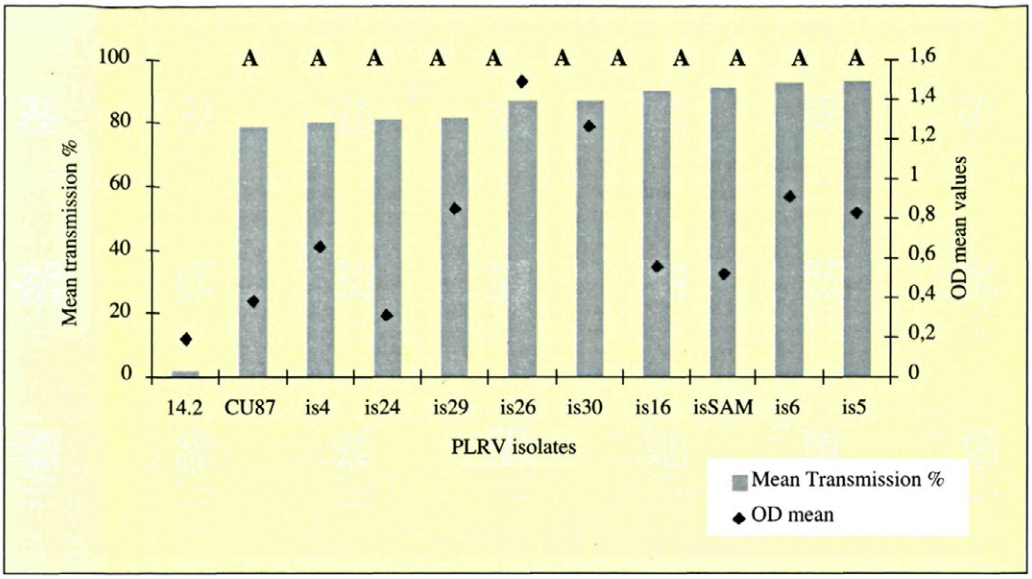

Figure 1: Mean transmission values and mean OD values of each of the tested 11 PLRV isolates by all tested aphid clones.

Lastly, MP3 and LCSA aphid clones could transmit Tunisian isolates at 75% efficiency or more, except for is_4/LCSA (55%), is_{26}/MP3 and is_{30}/MP3 (60%) ($Pr<0.001$) (Table 3 and 4). $PLRVis_5$ is the best transmitted Tunisian isolate by all tested aphid clones (mean transmission value: 93%) (Figure 1), especially by the VeT3 clone that

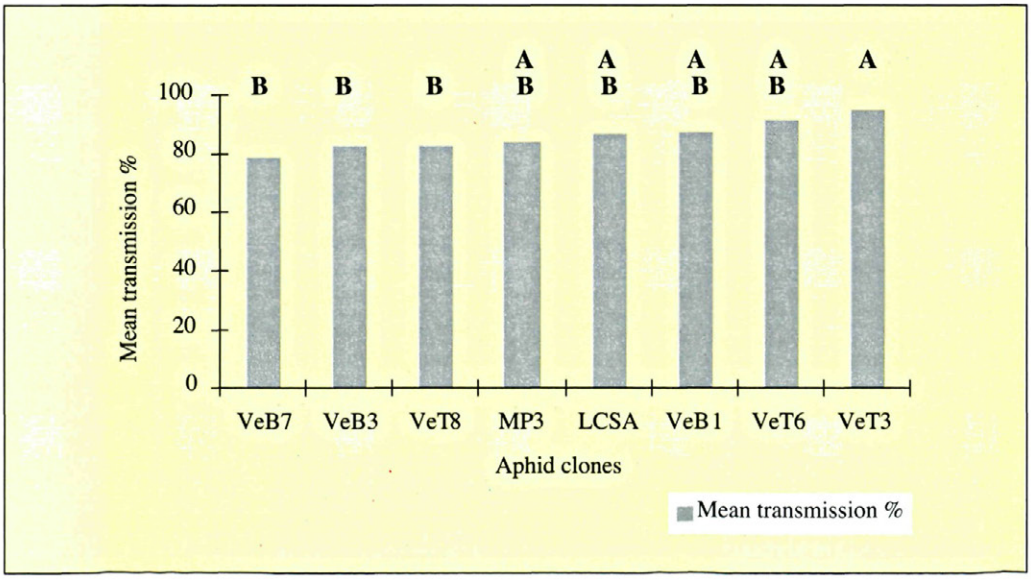

Figure 2: Mean transmission values of all tested PLRV isolates for each of the 8 tested aphid clones.

could be considered as the most efficient aphid clone in this study (mean transmission value: 95%) (Figure 2).

As shown in Figures 1 and 2, mean OD values are not very well related with mean transmission %. In fact PLRVCU87 and is_{24} are well transmitted by all aphid clones (78.75 and 81.25% respectively) but are poorly confined in infected plants (0.38 and 0.31 mean OD values, respectively). Moreover, $PLRVis_{SAM}$ (91.25%) is better transmitted than $PLRVis_{26}$ (86.87%), nevertheless it was less concentrated in the infected plants (Figure 1).

DISCUSSION

This work represents the first report in Tunisia and North Africa of PLRV transmission experiment under laboratory conditions. The transmissibility to *P. floridana* of a collection of nine Tunisian PLRV isolates was assessed by six Tunisian aphid clones of the *M. persicae* complex.

The PLRVCU87 isolate was well transmitted by almost the tested Tunisian aphid clones (Table 3). This confirms the overall high transmissibility of this isolate previously showed by BOURDIN *et al.* (1998). Moreover, none of the Tunisian clones transmitted the PLRV14.2 isolate. Our results appear to be in agreement with loss of transmissibility of the PLRV14.2 isolate by MP3 clone following acquisition on *P. floridana* and/or membrane feeding of purified virus (Bourdin *et al.*, 1988). ROUZÉ-JOUAN *et al.* (2001) demonstrate by microinjection of purified PLRV14.2 in the aphid abdomen the important role of the gut membrane as a barrier in the blockage of virions in the transmission process. Furthermore, this loss of transmissibility was shown to be due to some amino acid changes in the CP (capsid protein) and RTP (readthrough protein) of the PLRV14.2 isolate reducing virus recognition by aphid gut receptors (ROUZÉ-JOUAN *et al.*, 2001) and to the genotype of aphids (TERRADOT *et al.*, 1999). Moreover, GILDOW (1982) observed that PLRV particles attached specifically to accessory salivary gland (ASG) membrane. Moreover, it has been shown that variation in PLRV transmission by aphids depends more on the interaction between aphid clones and virus isolates (BOURDIN *et al.*, 1998) than on the properties of solely the virus or the vectors (JOLLY & MAYO, 1994). However, it's not possible to say in our case, if PLRV14.2 virions were blocked in the Tunisian aphid clones at the gut membrane or ASG membrane level. This highlights that the interactions between luteoviruses and aphids evolved into complex associations explaining the complexity and the specificity of the transmission process. Moreover, most of the poor vector clones belonged to the *M. antirrhinii* taxon of the *M. persicae* complex, showing that aphid genotype variation can affect virus transmission (TERRADOT *et al.* 1999).

Transmission rates above 70% of Tunisian PLRV isolates by Tunisian aphid vectors were obtained except for is_4/VeB3 (55%) and is_{29}/VeB7 (50%) (Table 3). Only small and inconsistent differences in aphid transmissibility were detected between the nine Tunisian PLRV isolates since all these isolates were transmitted readily. These isolates were so considered to be highly transmissible. The VeT3 aphid clone was the most efficient Tunisian aphid vector clone in this study (Figure 3). This seems to be in according to the molecular typing results of these Tunisian aphid clones by microsatellite markers showing that these aphid clones genetically clustered into two groups: *M. persicae* taxa comprising the VeB3, VeT3, VeT6 and VeT8 clones, and another group, genetically so distant from *M. antirrhinii* than from *M. persicae* taxa, comprising VeB1 and VeB7 having an identical genotype (data not shown). Moreover, VeT3 clone was genetically the most distant from *M. antirrhinii* taxa that was identified by TERRADOT *et al.* (1999) as inefficient vector in transmitting the PLRV14.2 isolate.

This high transmission % obtained overall could be due to the presence of vector/isolate affinity. More over, *M. persicae* complex

has previously been found to be the most abundant aphid on potato crop in Tunisia (CHERIF & BOUTHIR, 1990). This could explain the rapid degeneration of seed potato quality in Tunisia attributed also to the year-round presence of the efficient *M. persicae* vector and their primary and secondary host plants, which serve as reservoirs of this aphid (CHERIF & BOUTHIR, 1990; BEN HALIMA & BEN HAMOUDA, 1998). Tunisian climate is also considered to be strongly favourable to aphid transmission. In fact, temperature both before and after aphid inoculation with PLRV, greatly influenced the susceptibility of potato plants to infection and virus accumulation (SYLLER, 1991). Thus, the ability with which all tested aphid clones transmitted all the Tunisian isolates and the CU87 isolate suggests that they all share some properties allowing them to transmit PLRV efficiently. The MP3 and LCSA aphid clones were shown to be efficient vectors for almost the Tunisian isolates. Thus, there could not be any virus/aphid specific affinity related to the geographic origin of the epidemiological complex protagonists.

The serological test (DAS-ELISA) of the inoculated plants was assessed to look for any relation between transmission efficiency and virus accumulation. In this study, the high transmission values were not correlated to virus concentration in inoculated plants (Figures 1 and 2). This is according to the previous results of TAMADA *et al.* (1984) showing that the poor transmission of isolate PLRV-15 was not caused by a low virus concentration in source plants. Furthermore, the aphid transmissibility of PLRV under uniform conditions can reflect fluctuations in the source plants virus concentration (SYLLER, 1987). In contrast, BARKER & HARRISON (1986) have found a correlation between the PLRV titre in the plants and the transmission efficacy.

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RESUMEN

DJILANI KHOUADJA F., J. ROUZÉ-JOUAN, JP. GAUTHIER, S. BOUHACHEM, M. MARRAKCHI, H. FAKHFAKH. 2004. Eficiencia en la transmisión de aislados de *Potato leaf-roll virus* de Túnez por diferentes clones tunecinos de *Myzus persicae*. *Bol. San. Veg. Plagas*, **30**: 47-55.

Eficiencia en la transmisión de aislados de *Potato leaf-roll virus* de Túnez por diferentes clones tunecinos de *Myzus persicae*.

Potato leafroll virus (PLRV) se transmite en la naturaleza por áfidos, especialmente por el complejo *Myzus persicae* (Hemiptera: Aphididae), de manera persistente. La eficiencia de la transmisión de los aislados de PLRV procedentes de Túnez por clones de *Myzus persicae* de este pais no ha sido estudiada. Seis clones de *Myzus persicae* procedentes de Túnez y dos clones control franceses (MP3 y LCSA) se utilizaron en la transmisión de nueve aislados de campo de PLRV recolectados de tres regiones diferentes de Túnez. La eficiencia en la transmisión, por los áfidos vectores, de estos aislados se valoró frente a la obtenida con dos aislados, conocidos, caracterizados por presentar alta capacidad de transmisión por áfidos (CU87) y baja transmisión por áfidos (14.2). Los experimentos realizados bajo condiciones controladas muestran que la relación de transmisión obtenida fue en general alta. Todos los aislados de PLRV presentaron buena transmisión (40-100%), excepto el aislado 14.2 caracterizado por su baja transmisión ya que

no fue transmitido por todos los clones de *M. Persicae* tunecinos. La relación entre la tasa de transmisión y el valor medio del título del antígeno ha sido señalada.

Palabras clave: aislados PLRV, clones de áfidos, DAS-ELISA, infeccion, inoculation, *Physalis floridana* (Rydb), transmisión.

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