Drought reduces transmission of *Turnip yellows virus*, an insect-vectored circulative virus

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ARTICLE INFO

ABSTRACT

Application of a severe water deficit to *Arabidopsis thaliana* plants infected with a mutant of *Turnip yellows virus* (TuYV, Family *Luteoviridae*) triggers a significant alteration of several plant phenology traits and strongly reduces the transmission efficiency of the virus by aphids. Although virus accumulation in water-stressed plants was similar to that in plants grown under well-watered conditions, virus accumulation was reduced in aphids fed on plants under water deficit. These results suggest alteration of the aphid feeding behavior on plants under water deficit.

1. Introduction

Plants suffer from a broad range of abiotic and biotic stresses that do not occur in isolation but are commonly present simultaneously (Mittler, 2006; Suzuki et al., 2014). On-going climate changes, mainly characterized by altered precipitation patterns, increased temperatures and levels of atmospheric CO_2, are already affecting animal and plant populations (Parmesan and Hanley, 2015; Zhang et al., 2015), as well as agricultural productivity and human health (Sutherst, 2004; IPCC, 2014; Yusa et al., 2015). Phytoviruses also represent highly prevalent constraints in cultivated and wild species (Pagan et al., 2012; Roossinck, 2012) causing an estimated US$60 billion losses in crop yield worldwide each year. Most of plant viruses rely on arthropods vector for transmission between host plants, and aphid vectors are by far outperforming all other members of the class *Insecta* (Ng and Perry, 2004). However, virus transmission is mediated by critical successive processes starting from virus acquisition on an infected plant, then virus retention in the alimentary apparatus or in the aphid's body, and finally virus inoculation to a new plant (Bragard et al., 2013). Considering the major challenges posed by environmental changes to predict epidemiology of viral diseases, it is important to evaluate the effect of abiotic stresses on virus transmission rate by vectors, and particularly aphids. Global warming was shown to increase the abundance and geographic distribution of plant viruses and vectors (Gautam et al., 2013; Maino et al., 2016; Shaw and Osborne, 2011) and consequently a number of studies have been published on the influence of climate change on

vector-borne diseases of plants and on their spread. Among these studies, those concerning viruses have mostly focused either on vector biology (e.g. developmental time, longevity, fecundity, migration) and ecology (Nancarrow et al., 2014; Scherm, 2004; Xie et al., 2014), or on virus accumulation and symptom expression *in planta* (Cronin et al., 2010; Fu et al., 2010; Trebicki et al., 2015). While most of these studies speculated on a possible impact of environmental changes on the rate of virus transmission, direct experimental support was only brought very recently (Chung et al., 2016; Dader et al., 2016; Nachappa et al., 2016).

Concerning the influence of a water deficit, we have recently shown that a severe water-deprivation treatment applied to *Brassica rapa* infected with *Cauliflower mosaic virus* (CaMV) or *Turnip mosaic virus* (TuMV), two non-circulative transmitted viruses, dramatically enhanced transmission rate by around 34% and 100%, respectively (van Munster et al., 2017). Non-circulative transmission is the predominant strategy for plant virus-vector interaction in which the virus is taken up by a vector on an infected plant, attaches on the inner part of the cuticle lining the feeding apparatus (Uzest et al., 2007), and is subsequently released and inoculated into a new host plant. All three steps, acquisition, retention and inoculation, occur generally within seconds to minutes and do not require a passage within the vector's body. These viruses are mainly acquired during probing and transient puncturing of epidermal and mesophyll cells of infected leaf tissues (See for review Whitfield et al., 2015).

In case of circulative viruses, transmission is characterized by longer acquisition and inoculation periods (from hours to days), and long

retention time in vector's body that can last several weeks, and often until the vector dies. These circulative viruses must cross several epithelia at the gut and salivary glands levels. This mode of transmission is found mainly for phloem-limited viruses belonging to the families *Luteoviridae*, *Geminiviridae*, and *Nanoviridae* (Blanc et al., 2014; Gray and Gildow, 2003; Sicard et al., 2015). Phloem-limited viruses are therefore acquired and inoculated by aphid vectors during long-lasting sap ingestions phases in sieve tubes (See for review Whitfield et al., 2015).

Due to these major differences between circulative vs. non-circulative transmission (e.g. acquisition and inoculation of the virus in different cell types, retention of the virus in the vector), information on impact of abiotic stresses on a large panel of plant-virus-vector system is desirable.

Turnip yellows virus (TuYV) is a circulative non-propagative aphid transmitted plant virus of the *Luteoviridae* family (genus *Polerovirus*). This virus is a major threat for oilseed rape (*Brassica napus*) where average yield losses can reach 30% (Juergens et al., 2010) but is asymptomatic in *Arabidopsis thaliana*. Here, an engineered virus derived from TuYV and inducing leaf vein yellowing was used to evaluate the ability of the aphid *Myzus persicae* to transmit the virus from water-stressed *A. thaliana* plants compared to unstressed ones.

2. Material and methods

2.1. Plant growth and drought stress protocol

Seeds of *Arabidopsis thaliana* (L.) Columbia wild type (Col-0) were sown into individual $7 \times 7 \times 7$ cm pots containing equal amount of organic substrate (Huminsubstrat N2, Neuhaus, pH 5.5–6.5; Klasmann-Delmann GmBH, Geeste, Germany). Soil water content was controlled before sowing to estimate the amount of dry soil and water in each pot. Subsequent changes in pot weight were due mainly due to changes in water status. Dynamic changes in plant size could not be taken into account in calculations. These changes were negligible under WW but may have led to a maximum of 20% over-estimation under WD (data not shown). Plants were maintained in an insect-free walk-in growth chamber with 9 h light (3600 lx) set at 21/18 °C (day/night) with 53% air relative humidity. Plants were irrigated with a nutritive solution (N 168 mg L^{-1}, P 115 mg L^{-1}, K 336 mg L^{-1}, CaO 162 mg L^{-1}, MgO 19 mg L^{-1}), maintaining the soil water content between 1.74 and 1.79 g H_20 g^{-1} dry soil (depending on plant size) until virus agroinoculation.

A water-deprivation treatment (WD) was applied to half of the pots starting after virus inoculation (Day 0; see Section 2.2). Water-deprivation treatment was standardized as follows: no watering from Day 0 until reaching a soil water content between 0.14 and 0.18 g H_20 g^{-1} dry soil, depending on plant size. Soil water content of WD plants was then maintained to this value through an adequate supply of water until the transmission assays. We performed preliminary experiments to test that this level of soil water content was above permanent wilting point (growth recovered upon re-watering) for this plant species. The WD condition was reached after 17 days and maintained for 6 days until transmission experiments (Supplementary Fig. 1). In the well-watered treatment (WW) soil water content was maintained at 1.74–1.79 g H_20 g^{-1} dry soil.

Three consecutive experiments were carried out following the same experimental procedure.

2.2. Virus inoculation of source plants by agroinoculation

To produce virus-infected source plants, we used a modified TuYV virus, which induces vein clearing in *A. thaliana* (TuYV-SUL) while the wild-type virus is symptomless in this plant species (V. Ziegler-Graff, kind gift, unpublished results). This engineered virus allows direct visualization of the infection of plants. The modified sequence of the virus

was placed under the control of the *Cauliflower mosaic virus* 35S promotor in a binary vector (pBinTuYV-SUL) and transformed into *Agrobacterium tumefaciens* strain GV3101 for agroinoculation (Leiser et al., 1992; V. Ziegler-Graff unpublished results). Briefly, Agrobacteria harboring pBinTuYV-SUL were grown for 48 h at 28 °C, pelleted and diluted in a buffer containing 10 mM MES (pH 5.6), 10 mM MgCl₂ and 0.15 mM acetosyringone at an optical density (OD) at 600 nm of 0.5. Agrobacteria suspension was incubated for 2 h at room temperature before being infiltrated to 5 week-old *A. thaliana* Col-0 plants.

2.3. Aphid rearing

The colony of aphid-vector species *M. persicae* was maintained on eggplants (*Solanum melongena*) in insect-proof cages, in a growth chamber at 23/18 °C (day/night) with a photoperiod of 14/10 h (day/night), in conditions ensuring clonal reproduction. Aphids were transferred to new cages and to new host plants every two weeks, in order to avoid overcrowding and induction of the development of winged morphs.

2.4. Measurements of plant source traits

2.4.1. Plant size

Plant size was estimated 23 days after the start of WD treatment by measuring rosette diameter (mm) on TuYV- source plants (WW or WD).

2.4.2. Fresh and dry weight, and water content measurements

Aboveground tissues from each source plant used for transmission assays were collected and weighted to estimate the total fresh weight (FW) depending on the treatment (WW or WD). Aboveground tissues were then placed in an oven at 65 °C for 24 h for dry weight (DW) estimation. Water content (WC), i.e. the amount of water in leaves relative to its dry weight, was calculated as follows:

WC (g/g) = (FW − DW)/DW.

2.5. Aphid transmission assays

For transmission experiments, batches of twenty nymphs of *M. persicae* (L1-L2 stage) were starved for 1 h and then placed on a TuYV-infected source plant for 24 h. Aphids were then collected and transferred individually to one month-old *A. thaliana* plantlets (test plants) grown and maintained under the WW condition for all the experiment. Aphids were confined on test plants by Falcon® tube cages for an inoculation period of 3 days before insecticide treatment. Vein clearing symptoms were recorded three weeks later by visual inspection and the experiment was repeated three times.

Noteworthy, one biological replicate included the use of two aphids per test plant for the inoculation step. Estimated transmission rates by single aphid was then determined by the following formula: $Y = 1- \sqrt[n]{(1-T)}$, where Y = estimated transmission rate for one insect/plant, n = number insects per test plant (n = 2), T = experimental transmission rate (Nault et al., 1978).

2.6. RNA extraction from A. thaliana source plants and M. persicae

A. thaliana infected leaves were stored at −80 °C before RNA extraction. Total plant RNA was extracted according to a modified Edwards protocol (Edwards et al., 1991) including an additional washing step with 70% ethanol, followed by a DNAse treatment (RQ1 RNase-free DNase, Promega).

Total RNA was extracted from whole *M. persicae* (15 aphids were pooled per sample) that had fed on TuYV-infected WW or WD plants for 24 h. Aphids were then transferred to healthy plants for 2 days to clear the gut content from non-internalized virus particles. Aphids were