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Phytoplasmas change the source–sink relationship of field-grown sweet cherry by disturbing leaf function

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ABSTRACT

Changes in the function of field-grown sweet cherry leaves infected with phytoplasma were evaluated through the analysis of photosynthesis, respiration, carbohydrates and hormones. Phytoplasmal infection caused witches' broom, small leaves, leaf yellowing and leaf rolling. The photosynthesis of infected leaves was considerably reduced, and they were unable to produce sufficient carbohydrates for their own needs. In contrast, the starch content of infected leaves was significantly increased. These results demonstrate the change in the role of infected leaves from sources to sinks. Further analysis revealed that the photosynthetic decline was related to a significant decrease in photosynthetic pigments and to marked inactivation of photosystem II (PSII). Furthermore, the loss of PSII function was due to a decrease in chlorophyll content, reduction and closure of active reaction centers, and decline in photochemical efficiency.

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1. Introduction

Phytoplasmas are plant pathogenic bacteria of class Mollicutes [1]. Phytoplasmas are mainly distributed in phloem tissues and spread from plant to plant viasap-sucking insect vectors, grafts or dodder parasitism [1]. Plants infected with phytoplasmas generally exhibit witches' broom, yellowing, phyllody, withering, virescence, stunting and white leaf. Hundreds of plant diseases worldwide, including many diseases of economically important plants, are induced by phytoplasmas.

Recently, it was found that cherry can also be infected and harmed by phytoplasmas, which has been reported in Europe [2–6], Asia [7–9] and America [10]. In cherry, phytoplasmas induce symptoms such as leaf yellowing, leaf reddening, witches' broom, wilting, decline and death [4,9–11]. In China, cherry is most commonly infected by phytoplasmas of the 16SrV-B subgroup [8,9]. Infected Chinese cherries mainly exhibit yellowing, decline and death [8], while infected sweet cherries display classical witches' broom [9]. However, due to the inability to cultivate phytoplasmas in cell-free medium, the mechanisms through which phytoplasmas interact with and harm host plants remain largely unknown.

Phytoplasmas exist mainly in the nutrient-rich phloem tissue of host plants, and their survival depends on the organic matter provided by plants. Phytoplasmas harm host plants by impacting physiological and biochemical processes [12–15] and genetic expression [16–20], with the synthesis, distribution and utilization of carbohydrates being among the most strongly affected processes. Phytoplasmas reduce stomatal conductance, interfere with PSII activity [19,21,22] and inhibit photosynthesis in diseased leaves [23]. However, carbohydrate accumulation increases in diseased leaves [14,23–27], indicating a dramatic change in the carbohydrate distribution in host plants. This change in the carbohydrate distribution may be related to a shift in sink–source relationships in host plants, but the underlying mechanisms cannot be explained by existing studies.

Thus, nutrient metabolism is fundamental in the interaction between phytoplasmas and host plants. It has been hypothesized that phytoplasmas change the source–sink relationship of host sweet cherry trees by reducing photosynthesis and enhancing carbohydrate import in diseased leaves. To obtain support for this hypothesis, we analyzed the synthesis, consumption and accumulation of nutrients in diseased leaves by investigating photosynthesis, respiration and carbohydrate accumulation. Our findings represent an important step towards a better understanding of the interaction mechanisms between phytoplasmas and host plants, and they may be helpful in controlling diseases induced by

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phytoplasma infection.

2. Materials and methods

2.1. Plant material

This research was conducted with 5-year-old healthy and phytoplasma-infected sweet cherry trees (*Prunus avium* L. cv. Summit) planted in an orchard in Nanwangzhuang, Tai'an, Shandong Province, China. The trees were planted at the same time and managed in the same way. We have already demonstrated that the infecting phytoplasma belongs to subgroup B of the elm yellows phytoplasma group (16SrV-B) by conducting a computer-simulated restriction fragment length polymorphism (RFLP) analysis of the 16S rDNA F2nR2 sequence, with a set of 17 restriction enzymes [9]. Nested PCR was carried out using the phytoplasma universal primer pairs P1/P7 and R16F2n/R16R2 to assess the phytoplasma infections of the trees [28]. Three infected trees with a similar disease severity and three non-infected healthy trees with similar growth vigor were selected for the experiments. In the infected trees, witches' broom occurred locally, and the areas without witches' broom showed no visible difference from the healthy trees.

The 4th and 5th mature leaves from the tops of the shoots were selected for analysis. In infected trees, leaves were selected only from witches' broom shoots. The selected leaves were first subjected to measurements of photosynthesis, respiration and chlorophyll fluorescence in vivo, and they were then collected for the determination of pigments, soluble carbohydrates, starch, enzyme activities and hormones. The collected leaves were immediately frozen in liquid nitrogen and then kept at −80 °C for long-term storage. For the measurements of photosynthesis, respiration and chlorophyll fluorescence, three leaves from each tree were tested. For other measurements, samples from each tree were collected and tested as independent biological replicates.

2.2. Pigment analysis

Chlorophyll a (Chl*a*), chlorophyll b (Chl*b*) and carotenoid (Car) levels were determined as described by Arnon [29]: fresh leaves from three shoots were cut and homogenized after the removal of veins. Samples (0.5 g) were ground in 80% acetone and filtered. Then, the filtrates were diluted to 25 ml, and their absorbances (A) were determined at 663 nm, 646 nm and 470 nm.

The pigment concentration was calculated as follows:

$$C_a = 12.21A_{663} - 2.81A_{646};$$

$$C_b = 20.13A_{649} - 5.03A_{663};$$

$$C_{a+b} = C_a + C_b;$$

$$C_{x\cdot c} = (1{,}000A_{470} - 3.27C_a - 104C_b)/245;$$

$$\text{Pigment concentration} = C \times 0.025\ \text{L}/0.2\ \text{g}.$$

C_a, C_b, C_{a+b} and $C_{x\cdot c}$ are expressed as grams per liter of Chl*a*, Chl*b*, total Chl and Car, respectively.

2.3. Photosynthesis and respiration analyses

Photosynthetic and respiratory parameters were determined using a CIRAS-3 portable photosynthesis system (PP Systems, USA). Net photosynthesis was measured under controlled conditions using a light intensity of 1200 μmol photons·m^{-2} s^{-1}, a CO_2 concentration of 390 μmol mol^{-1} and a temperature of 25 °C. Respiration measurements were conducted under similar conditions, except that the light intensity was zero. The leaves were fully light adapted before the measurements were performed. The net photosynthetic rate (Pn), stomatal conductance (Gs), stomatal limitation (Ls), internal CO_2 concentration (Ci), transpiration rate (Tr), water use efficiency (WUE), vapor pressure deficit (VPD) and respiration rate (R) were provided directly by the CIRAS-3 system.

2.4. Chlorophyll fluorescence analysis

Steady-state chlorophyll fluorescence quenching characteristics were determined in vivo at room temperature using an FMS-2 pulse modulated fluorometer (PP Systems, USA) as described by Baerr et al. [30].

The minimal level of chlorophyll fluorescence (F_o), maximal level of chlorophyll fluorescence in the dark-adapted state (F_m), steady-state fluorescence (F_s), maximal chlorophyll fluorescence in the light-adapted state (F'_v/F'_m) and minimal fluorescence in the light-adapted state (F'_o) were determined directly using the fluorometer. The maximal (F_v/F_m) and effective (F'_v/F'_m) quantum yields of PSII photochemistry, antenna quenching (q_o), relative reduction state of PSII ($1 - q_P$), effective quantum yield of PSII electron transport (Φ_{PSII}) and relative linear electron transport rates through PSII (ETR) were calculated as described by Baerr et al. [30].

2.5. Soluble carbohydrate and starch analysis

The extraction and determination of soluble carbohydrates and starch were performed as described by Giorno et al. [27]. The extraction of soluble carbohydrates was performed using 80% EtOH (vol/vol) at 78 °C. Starch was extracted from the insoluble fraction obtained in the 80% EtOH extraction with 10 N KOH at 95 °C and then digested with amyloglucosidase and α-amylase. Analytic measurements were performed using HPLC with DIONEX CarboPac PA20 3 × 150 mm columns.

2.6. Enzyme activity analysis

The activities of six key enzymes involved in starch metabolism, including three involved in synthesis [AGPase (adenosine-5′-diphosphate glucose pyrophosphorylase), SSS (soluble starch synthase) and GBSS (granule-bound starch synthase)] and three involved in degradation (α-amylase, β-amylase and isoamylase), were determined using Plant Enzyme ELISA Kits (Shanghai Bangyi Biotechnology Co. Ltd, China).

Leaf samples were ground to a fine powder in liquid nitrogen. Then, 1 g of leaf powder was homogenized in 4 ml of distilled water and stored at 4 °C for 1 h to achieve complete extraction of the enzymes. The supernatant was collected after centrifugation at 3000 rpm (4 °C) for 20 min and diluted to 50 ml. Enzyme activities were determined according to the supplier's instructions.

2.7. Hormone analysis

The determination of hormones was conducted using methods provided by Qiu (personal communication).

Hormones were extracted from 0.5 g frozen leaf samples. The samples were vacuum freeze-dried and then extracted using cold acetonitrile containing 30 μg ml^{-1} of sodium diethyldithiocarbamate. First, the samples were immersed in 5 ml of acetonitrile and maintained at 0 °C for 12 h in a closed state. After centrifugation, the supernatants were collected, and the sediments were mixed with 4 ml of acetonitrile and shaken at 230 rpm for 2 h. After centrifugation, the supernatant was collected, and the

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