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Research article

Photosynthetic responses to phytoplasma infection in Chinese jujube

Zhiguo Liu [a], Jin Zhao [b,*], Mengjun Liu [a,**]

[a] Research Center of Chinese Jujube, Agricultural University of Hebei, Baoding, 071001, China
[b] College of Life Science, Agricultural University of Hebei, Baoding, 071000, China

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ABSTRACT

Phytoplasma is one of the most devastating plant pathogens. Jujube witches' broom (JWB) is a typical and highly fatal phytoplasma disease of Chinese jujube (*Ziziphus jujuba* Mill.), which is widely cultivated in Asia. To further elucidate the mechanism of plant-phytoplasma interaction, we first compared the effects of phytoplasma infection on photosynthetic pigments and activities between a JWB-resistant cultivar (Xingguang) and a susceptible cultivar (Pozao). Total chlorophyll and carotenoid levels were significantly decreased in the susceptible cultivar at later stages of infection, but were remarkably increased in the resistant cultivar at the earlier stages. Compared to uninfected plant, a significant decrease in the main photochemical parameters (F_v/F_m, Φ_{PSII} and qP) was recorded at the initial stages of infection in the resistant cultivar, but occurred at later stages in the susceptible cultivar. Meanwhile, the qRT-PCR results of four key photosynthesis-related genes (*ZjGluTR*, *ZjCBP*, *ZjRubisco* and *ZjRCA2*) demonstrated that the expression patterns were similar in uninfected cultivars, but up-regulated in resistant cultivar and down-regulated in the susceptible one at 12 wks after grafting inoculation. Collectively, our data indicated that the resistant cultivar 'Xingguang' undergoes a decrease in initial stage (inhibiting phytoplasma multiplication) and then a rapid enhancement of photosynthetic activity (helping jujube recovery) in response to phytoplasma infection, while the susceptible cultivar 'Pozao' displays a later decrease in photosynthetic activity. The novel photosynthetic response pattern of the resistant cultivar may contribute to its stronger immunity to phytoplasma infection, which provides new insights into plant-phytoplasma interactions.

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1. Introduction

Chinese jujube (*Ziziphus jujuba* Mill.), an economically important fruit tree, is widely grown in the temperate zone of the Northern Hemisphere (Liu and Wang, 2009). Since the 1990s, Jujube witches' broom (JWB) has become the most serious and destructive disease of Chinese jujube in China (Liu et al., 2010), Korea (Lee, 1988) and Japan (Ohashi et al., 1996). JWB was first described as a graft-transmissible viral disease in Korea (Kim, 1965) and then demonstrated to be associated with a phytoplasma (Yi and La, 1973).

Phytoplasmas, members of the class Mollicutes, are minute cell-wall-less prokaryotes generally confined to the phloem of plants (Strauss, 2009). They are important agricultural pathogens which result in significant economic impacts and have been identified in over 1000 plant species (Seemüller et al., 1998; Lee et al., 2000; Streten and Gibb, 2006). Plants infected by phytoplasmas exhibit an array of symptoms, such as yellowing, stunting (reduction of internodes and leaf size), virescence (the development of green flowers and the loss of normal pigments), phyllody (the development of floral parts into leafy structures), sterility of flowers, witches' broom, and so on (Lee et al., 2000). At the same time, previous studies have reported that phytoplasma infection impaired photosynthesis, increase of sugar and starch concentration in leaves, and decrease of soluble carbohydrates and starch in roots (León et al., 1996; Lepka et al., 1999; Tan and Whitlow, 2001; Bertamini et al., 2002a, 2002b; Maust et al., 2003; Giorno et al., 2013). However, there is no information about the variation of photosynthetic characteristics in Chinese jujube infected by phytoplasma.

Yellowing was the typical symptom of JWB. Meanwhile, in previous studies, we found that the photosynthetic-related genes were differentially expressed in Chinese jujube after infected by

* Corresponding author.
** Corresponding author.
E-mail addresses: zhaojinbd@126.com (J. Zhao), lmj1234567@aliyun.com (M. Liu).

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JWB phytoplasma (Liu et al., 2014). So, in this work, we take advantage of a JWB-resistant cultivar (Liu et al., 2006) and a susceptible cultivar, the photosynthetic characteristics of both cultivars were monitored under phytoplasma infection by using Chlorophyll fluorescence quenching analysis, which was widely used to examine the energy fluxes in the photosynthetic apparatus and photoinhibition (Krause and Weis, 1991). At the same time, four key photosynthesis-related genes (*ZjGluTR*, *ZjCBP*, *ZjRubisco* and *ZjRCA2*) were cloned and their expression patterns were examined. Our study aimed to reveal the differences between resistant and susceptible cultivar in photosynthetic responses under phytoplasma infection. The results will provide new insights into the plant-phytoplasma interaction.

2. Materials and methods

2.1. Plant material and treatment design

A JWB-resistant cultivar (*Z. jujuba* Mill. 'Xingguang') and a susceptible cultivar (*Z. jujuba* Mill. 'Pozao') were used as scions. The rootstock cultivar was 'Zanhuangdazao'. The scions of 'Xingguang' and 'Pozao' were grafted on JWB- diseased rootstocks and healthy ones growing in the field. All grafting treatments were conducted with three replicates. The mature leaves in the middle of bearing shoot were collected from sprouted scions at five key stages (6, 9, 12, 15 and 18 weeks after grafting (WAG)). Some of the sampled leaves were used to detect pigment contents and chlorophyll fluorescence, and the others were frozen by liquid nitrogen rapidly and kept at −80 °C for DNA and RNA extraction.

2.2. Pigment analysis

Chlorophyll (Chl) and carotenoids (Car) were extracted with 100% acetone (10 ml) from leaf discs (0.05 g) under dark condition for 24 h. Chl and Car contents were analyzed by spectrophotometer according to the method of Lichtenthaler (1987).

2.3. Extract and assay of Rubisco and Rubisco activase activity

Rubisco and Rubisco activase (RCA) activity were determined by the method of continuous circulation colorimetry of GENMED plant Rubisco carboxylation activity (GMS16015.1 v.A, GENMED SCIENTIFICS INC. U.S.A) and GENMED plant RCA activity (GMS16016 v.A, GENMED SCIENTIFICS INC. U.S.A).

3. Chlorophyll fluorescence measurements

All measurements of Chl fluorescence on detached leaves were performed with a MINI-IMAGING-PAM fluorometer (Walz, Effeltrich, Germany) at ambient CO_2 and temperature. All treatments were repeated three times. Before each measurement, the leaves were dark-adapted for 30 min with leaf clips provided by Walz Company. Maximal fluorescence (F_m) was induced by a saturation pulse of light (approx. 4800 μmol m^{-2} s^{-1}, 0.8 s). Minimal fluorescence (F_o) was measured as the average of the fluorescence signal under a weak measuring light (0.1 μmol m^{-2} s^{-1}). The maximum quantum efficiency of PSII was determined as $F_v/F_m = (F_m - F_o)/F_m$. An actinic light source (290 μmol m^{-2} s^{-1}) was applied for 400 s to achieve steady state photosynthesis and to obtain F_s (steady state fluorescence yield), and then a second saturation pulse was applied for 0.8 s to obtain F'_m (light adapted maximum fluorescence). The actual quantum efficiency of PSII (Φ_{PSII}) was determined as $\Phi_{PSII} = (F'_m - F_s)/F'_m$ (Genty et al., 1989; Tan and Whitlow, 2001). Other fluorescent parameters were calculated according to the dark adapted and light adapted fluorescence measurements: photochemical quenching coefficient (q_p)=$(F'_m - F_s)/(F'_m - F_o)$; non-photochemical quenching coefficient (NPQ) = $F_m/F'_m - 1$. The absorption coefficient (*Abs*), which reflects the light-harvesting capacity of PSII, was determined as well.

3.1. Extraction of DNA and phytoplasma determination

Total DNA of leaves was extracted by modified CTAB method (Gu et al., 2005). Taking the advantage of relative quantitative real-time PCR (qRT-PCR) technology, total DNA of phytoplasma infected 'Xingguang' and 'Pozao' leaves were used as template, the relative expression of phytoplasma *TMKZ* gene to jujube *ZjH3* gene (Sun et al., 2009) was employed to represent pathogen quantity (Yuan, 2014). The relative value was calculated by the equation $Y = (1 + E\ (TMKZ))^{-Ct\ (TMKZ)}/(1 + E\ (ZjH3))^{-Ct\ (ZjH3)}$. E is the amplification efficiency which calculated by the LinRegPCR software. Ct (cycle threshold), defined as the PCR cycle at which a statistically significant increase of reporter fluorescence was first detected, was used as a measure of the starting copy number of the gene. Primer sequences for qRT-PCR were shown in Table 1.

3.2. Total RNA extraction

Isolation of total RNA was carried out according to the instructions of improved CTAB method (Zhao et al., 2009). DNase I treatment was applied to remove contaminating genomic DNA. TaKaRa RNA PCR Kit (AMV) Ver.3.0 (TaKaRa) was used in the synthesis of double-stranded cDNA according to the manufacturer's instructions.

3.3. Cloning and expression analysis of four photosynthetic-related genes

Four photosynthetic genes, i.e. *GluTR* (Glutamyl-tRNA reductase gene), *CBP* (Light-harvesting chlorophyll A/B binding protein gene), *Rubisco* (Ribulose-1, 5-bisphosphate carboxylase gene) and *RCA2* (Rubisco activase 2 gene), were isolated by homology-based

Table 1
Primer sequences for phytoplasma determination, homology-based cloning and qRT-PCR.

Name of primer	No.	Primer sequence (5′–3′)
GluTR1	1	ATGGCCGTGTCGACCAGTTTC
GluTR2	2	CTATTTCTGATTTTGTTCTAC
CBP1	1	ATGGCCACCTCTGCCATC
CBP2	2	TCATTTTCCAGGCACGAAG
Rubisco1	1	ATGTCACCACAAATAGAGAC
Rubisco2	2	TTACAAAGTATCCATTGCTTC
RCA2-1	1	ATGGCTGCCTCCGTCTCC
RCA2-2	2	CTAACCGTAGAAAGATCCAC
ZjH3-1	1	TCGCTCAGGATTTCAAGAC
ZjH3-2	2	GAACAGACCGACCAAGTAA
TMKZ1	1	GCAACAAATCCAAGAAGAGGAAA
TMKZ2	2	TTGCGAGGATAAGCTTGATAGG
ZjGluTR1	1	TTGTTGGCTTTGGCAGGAATA
ZjGluTR2	2	CCCAGCAGCAATGTTTGTCTC
ZjCBP1	1	CAGGTGAGTTCCCAGGTGAC
ZjCBP2	2	CCAGCCTTGAACCAAACTGC
ZjRubisco1	1	AGCACAGGCTGAAACAGGTGA
ZjRubisco2	2	GGCAATAATGAGCCAAGGTAG
ZjRCA2-1	1	CGAGCACCTGTGAGAATAAATGG
ZjRCA2-2	2	TATCTTGTTGGTCGTCCGAAATG

Note: *GluTR1*, *GluTR2*, *CBP1*, *CBP2*, *Rubisco1*, *Rubisco2*, *RCA2-1* and *RCA2-2* were used for homology-based cloning; *ZjH3-1* and *ZjH3-2* were used for internal control; *TMKZ1* and *TMKZ2* were used for phytoplasma determination; *ZjGluTR1*, *ZjGluTR2*, *ZjCBP1*, *ZjCBP2*, *ZjRubisco1*, *ZjRubisco2*, *ZjRCA2-1* and *ZjRCA2-2* were used for qRT-PCR.

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