Reduction in carotenoid and chlorophyll content induced by the sweet potato whitefly, Bemisia tabaci

Title

Reduction in carotenoid and chlorophyll content induced by the sweet potato whitefly, *Bemisia tabaci*.

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ABSTRACT

The sweet potato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) B biotype, induces pigmentation changes in fruit and leaves including tomato-irregular-ripening disorder and squash-silverleaf disorder. We compared carotenoid and chlorophyll content of symptomatic and asymptomatic plant tissue. Symptomatic tissue from tomato fruit had 69% and 79% less lycopene (red pigment) and its precursor phytoene, respectively, than asymptomatic tissue. Heavily silvered squash leaves had 63% and 68% less chlorophyll a and b (green pigments), respectively, than healthy leaves. Squash leaves with silvering also had 42-67% lower levels of the carotenoids, β-carotene, all-trans-violaxanthin and lutein. These results suggest that infestation by the sweet potato whitefly inhibits the accumulation of carotenoids and chlorophylls which are both synthesized through the non-mevalonate pathway.

Keywords: Tomato-irregular-ripening; squash-silverleaf; lycopene; non-mevalonate pathway

1. Introduction

The sweet potato whitefly (SPWF), *Bemisia tabaci* (Hemiptera**:** Aleyrodidae) B biotype, is a worldwide pest on many vegetable and ornamental crops (Naranjo et al., 2010). It is well known that the presence of SPWF nymphs causes pigmentation disorders, such as tomato-irregular-ripening disorder (Schuster et al., 1990; Saito and Ozaki, 1991; Hanif-Khan et al., 1996) (Fig. 1a), squash-silverleaf disorder (Yokomi et al., 1990; Hoelmer et al., 1991; Schuster et al., 1991) (Fig. 1b) and soybean-bleaching disorder (Saito, 1992) (Fig. 1c). However, actual evidence for the possible mechanisms of symptom expression is still unknown. In this study, we quantified the carotenoid and/or chlorophyll content in fruits or leaves of plants with typical symptoms of tomato-irregular-ripening disorder or squash-silverleaf disorder, respectively, and compared them with asymptomatic material. Based on the results, we discuss possible mechanisms for symptom expression in relation to pigmentation.

Fig. 1. Tomato-irregular-ripening disorder (a), squash-silverleaf disorder (b), and soybean-bleaching disorder (c).

2. Materials and methods

A laboratory culture of *B. tabaci* B biotype was established from a population infesting cucumber plants, *Cucumis sativus*, in Shizuoka, Japan, in April 2008. The culture was maintained on potted

cabbage plants, *Brassica oleracea* var. *capitata*, at 23 ± 1 °C and a 16L: 8D photoperiod.

Tomato plants (*Solanum lycopersicum* var. Misora, Mikado Kyowa Seed Co. Ltd., Tokyo) were potted individually (dia. 17 cm, height 20 cm) and grown in a glasshouse under ambient conditions. Ten plants, each at the six to eight leaf stage, were placed in a gauze-covered cage (100 cm width, 100 cm depth, 200 cm height) in a glasshouse under ambient conditions, and ca. 1,500 adult whiteflies were released into the cage. After 40 to 50 days, three fruits with typical symptoms of tomato-irregular-ripening disorder were sampled, and each fruit was divided in to red (asymptomatic) and white (symptomatic) tissues. Each tissue type was homogenized with liquid nitrogen using a pestle and mortar. The carotenoid content was determined in 0.2g of each sample following the methods of Kato et al. (2004) using a reverse-phase-high performance liquid chromatography system (LC-2000 plus, Jasco Co., Tokyo) composed of a 250×4.6 mm column (YMC Carotenoid S-5, Yamamura Chem. Co., Kyoto) and a photodiode array detector (MD-2015 plus, Jasco Co., Tokyo). Absorbance was measured three times for each sample and the mean value was used for quantification against the standard curve for each carotenoid.

Squash plants (*Cucurbita maxima* var. Meruhenn, Sakata Seed Co. Ltd., Tokyo) were potted individually (dia. 15 cm, height 13 cm) and grown in a glasshouse under ambient conditions. Ten young plants each at the three to four leaf stage were placed in a small gauze-covered cage (100 cm width, 100 cm depth, 100 cm height) in the glasshouse under ambient conditions, and ca. 600 adult whiteflies were released into the cage. As a control, three plants were placed in another cage without adult whiteflies. After 10 days, silvering leaves were collected and classified into four groups according to the level of silvering that had developed around the veins of the leaves: Healthy = no silvering (collected from the control cage); Level 1 = silvering only on the veins; Level 2 = silvering that had expanded from the veins; and Level 3 = silvering over the entire leaf. Three leaves at each

silvering level were individually homogenized with liquid nitrogen using a pestle and mortar. The chlorophyll content (a and b) was determined in 0.1g of each sample following the method of Porra et al. (1989). Absorbance was measured three times for each sample with a spectrophotometer (U-1900, Hitachi, Tokyo) and the mean value was used for quantification. In addition, 0.2g of each sample was used to determine carotenoid level as described above.

Data obtained from three replicate fruits or leaves were analysed in the software package SPSS (SPSS, 2009).

3. Results and Discussion

In both experiments (i.e. with tomato fruits or squash leaves), nymphs first appeared on the leaves 5 days after release of ovipositing adult whiteflies and nymphal infestation was observed throughout the experiments. To understand the mechanism causing irregular pigmentation in tomatoes, carotenoid contents of red (asymptomatic) and white (symptomatic) tissues of the affected fruits were compared; the red asymptomatic tissue was considered as the control. Symptomatic tissues contained significantly less lycopene (69% reduction) and its precursor phytoene (79% reduction) than the asymptomatic tissues ($F_4 = 0.064$ and $P = 0.038$ for lycopene, $F_4 = 1.958$ and $P = 0.005$ for phytoene; Student's t-test) (Table 1). However, there was no significant difference in the downstream carotenoids, β-carotene and lutein, between asymptomatic and symptomatic tissues ($F_4 = 3.186$ and $P = 0.336$ for β-carotene, $F_4 = 0.390$ and $P = 0.595$ for lutein; Student's t-test) (Table 1). These results suggest that irregular ripening disorder is due to a reduced accumulation of the red pigment lycopene. This study is the first report to determine the carotenoid content of tissue with tomato-irregular-ripening disorder.

Table 1

Carotenoid content in tomato fruit.

Tissue	Content (mean ± SE, μg/g)			
	Phytoene	Lycopene	β-Carotene	Lutein
Asymptomatic	10.92 ± 1.32	0.16 ± 0.03	5.78 ± 0.54	1.53 ± 0.25
Symptomatic	2.31 ± 0.74 ** (79)	0.05 ± 0.02 * (69)	4.44 ± 1.10 (23)	1.70 ± 0.16 (111)

% reduction in each carotenoid compared with asymptomatic tissue is shown in parenthesis. Significant differences from asymptomatic tissues are indicated by asterisks: * ($P < 0.05$) and ** ($P < 0.01$) (Student's t-test).

In squash leaves, there was a significant difference in the content of both chlorophyll a and b amongst all samples ($F_{3,8} = 75.743$ and $P < 0.001$ for chlorophyll a, $F_{3,8} = 69.368$ and $P < 0.001$ for chlorophyll b; ANOVA), and subsequent means tests indicated that leaves with silvering all had significantly less chlorophyll a and b than healthy control leaves ($P < 0.05$; Tukey's HSD test); Level 3 leaves had the least chlorophyll a (63% reduction) and b (68% reduction), respectively, compared with healthy control leaves (Table 3). These results suggest that the silverleaf disorder is due to a reduction in the green pigment chlorophyll, which agrees with previous reports for squash and courgette (*Cucurbita pepo*) (Jiménez et al., 1995; Yokomi et al., 1995; Chen et al., 2004; McAuslane et al., 2004). In addition, squash leaves with silvering had significantly lower levels of the carotenoids, β-carotene, all-trans violaxanthin and lutein, which are known to be the predominant carotenoids in plant leaves (Apel and Bock, 2009); silvered leaves had 42-67% lower levels of carotenoids compared with healthy leaves (Table 3), which occurred alongside the reduction in levels of chlorophylls a and b (Table 2). This is the first record of reduced levels of both chlorophylls and carotenoids in squash-silverleaf disorder.

Table 2

Chlorophyll content in squash leaves.

Silvering level	Content (mean ± SE, mg/g)	
	Chlorophyll a	Chlorophyll b
Healthy	1.41 ± 0.03 a	0.53 ± 0.01 a
Level-1	0.80 ± 0.05 b (43)	0.31 ± 0.03 b (42)
Level-2	0.66 ± 0.02 bc (53)	0.25 ± 0.02 bc (53)
Level-3	0.52 ± 0.06 c (63)	0.17 ± 0.02 c (68)

% reduction in chlorophyll a and b at each silvering level compared with healthy leaves is shown in parenthesis. Within each column values followed by a different lower case letter are significantly different from each other ($P < 0.05$; Tukey's HSD test).

Table 3

Carotenoid content in squash leaves.

Silvering level	Content (mean ± SE, μg/g)		
	β-Carotene	All-trans-violaxanthin	Lutein
Healthy	47.66 ± 1.75 a	7.98 ± 1.16 a	128.54 ± 8.06 a
Level-1	19.07 ± 1.66 b (60)	4.15 ± 0.27 b (48)	48.87 ± 3.68 b (62)
Level-2	20.73 ± 7.02 b (57)	4.64 ± 0.44 b (42)	58.96 ± 11.55 b (54)
Level-3	17.26 ± 2.87 b (64)	2.87 ± 0.21 b (64)	42.60 ± 1.22 b (67)

% reduction in each carotenoid at each silvering level compared with healthy leaves is shown in parenthesis. Within each column values followed by a different lower case letter are significantly different from each other ($P < 0.05$; Tukey's HSD test).

As no disorders of leaves are known in tomato plants infested with whitefly, we only examined fruits in this study and determined the carotenoid content of normal and disordered material. In

contrast both chlorophyll and carotenoid content were determined in squash leaves. While the data obtained from both plants is limited in our study, they are nevertheless new and strongly suggest that expression of SPWF-induced pigmentation disorders is as a result of inhibition of carotenoid and chlorophyll accumulation. This may be as a result of inhibition of the non-mevalonate pathway, because carotenoids and a side chain phytol of chlorophylls are both synthesized through this pathway (Lichtenthaler, 1999; Sandmann et al., 2006). Phloem-feeders, such as SPWF, induce the salicylic acid (SA) defences and suppress the jasmonic acid (JA) defences of the host plant (Kempema et al., 2007; Zarate et al., 2007; Errard et al., 2015). It is possible that plant hormones such as SA and JA may affect carotenoid and chlorophyll synthesis in the non-mevalonate pathway, though further studies are needed to understand the detailed relationship amongst SPWF infestation, plant hormones, and the non-mevalonate pathway.

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