THE EFFECT OF BLOCKING METHYLATION OF SESQUITERPENOIDS BY OMT RNAi ON DISEASE RESISTANCE IN COTTON

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Abstract

Gossypol and related terpenoid compounds play an important role in defense against microbial pathogens and insect pests in cotton. Previously, we have identified and cloned an enzyme, desoxyhemigossypol-6-*O*-methyltransferase (dHG-6-OMT), that is responsible for converting non-methylated form of these compounds into methylated form, which are less toxic to plant insect pests and pathogens than the non-methylated counterparts. Three independent RNAi lines were generated in *Gossypium hirsutum* backgrounds. Blocking this enzyme by RNAi resulted in more than 94% reduction of methylated terpenoid compounds, but the amount of the non-methylated compounds remained almost unchanged. The total terpenoid compounds in the roots were reduced by half in the RNAi plants compared to their wild type siblings. The effect of these altered defense compounds profile in the RNAi plants on disease resistance was assessed by inoculating plants with a Fusarium wilt pathogen Fov race 1 isolate in the presence of root-knot nematodes. Two of the RNAi lines had less shoot weight reductions compared to the wild type siblings, while one of the RNAi lines had similar shoot weight reductions compared to the wild type siblings in the first two trials. This trend is reversed for shoot and root weight reductions in the third trial. Inconsistent results may indicate that the methylated terpenoid compounds contribute minor role in the disease resistance, consistent with their toxicity being only half of the toxicity of the unmethylated terpenoids. Analysis of infected stem stele tissues showed that almost no methylation occurred even in the wild type sibling lines. Therefore, OMT RNAi trait is not expected to contribute to disease resistance in this tissue. Whether the RNAi traits will be beneficial to disease resistance in *G. barbadense*, in whose stem high proportions of terpenoids were methylated upon infection, needs to be further explored.

Introduction

Gossypol and related terpenoid compounds play an important role in defense against microbial pathogens and insect pests in cotton. Previously, we have identified and cloned an enzyme, desoxyhemigossypol-6-*O*-methyltransferase (dHG-6-OMT), that is responsible for converting non-methylated form of these compounds into methylated form, which are less toxic to plant insect pests and pathogens than the non-methylated counterparts (Figure 1; Liu et al., 2002). Blocking this enzyme by RNAi resulted in more than 90% reduction of methylated terpenoid compounds. However, the amount of the non-methylated compounds remained almost unchanged in the roots of RNAi plants compared to that of the wild type siblings. The aim of this study is to assess the effect of these altered defense compound profiles in the RNAi plants on disease resistance by inoculating plants with a Fusarium wilt race 1 pathogen Fov11 in the presence of root-knot nematodes (RKN).

Materials and Methods

Pathogenicity assay

Three independent transgenic OMT RNAi cotton (*Gossypium hirsutum*) lines, 7BR, 7JAR, 7JBR and their corresponding wild type sibling lines, 7BW, 7JAW, 7JBW, were used in this study. Treatments consisted of control, Fov, Fov/RKN, and RKN. Seed were germinated in germination papers. For Trial 1, the seedlings were planted into soil cups (1 plant/cup) and either 6 ml of water or 6 ml of Fov11 ($1x10^7$ conidia/ml) was added around the plant

using a syringe. Plants were grown in a growth chamber at 25 °C: 20 °C (13 h light : 11 h dark). After 1 week, 6 ml of RKN (1000 eggs/ml) was added to the cups for the Fov/RKN and RKN treatment groups. There were between 6-9 plants/treatment/line. After a further 6 weeks, shoot weight, plant height, total leaf number, diseased leaf number, and nematode number were measured or counted. Trial 2 is similar to Trial 1, except that measurements were taken 5 weeks after nematode treatments. In Trial 3, 9990 eggs were used to infest the soil/cup, and only control and Fov/RKN treatments were included. There are between 9-11 plants/line for control treatment and 14-16 plants/line for Fov/RKN treatment. The disease responses also included root weights, and all response variables were measured 5 weeks after nematode infestation.

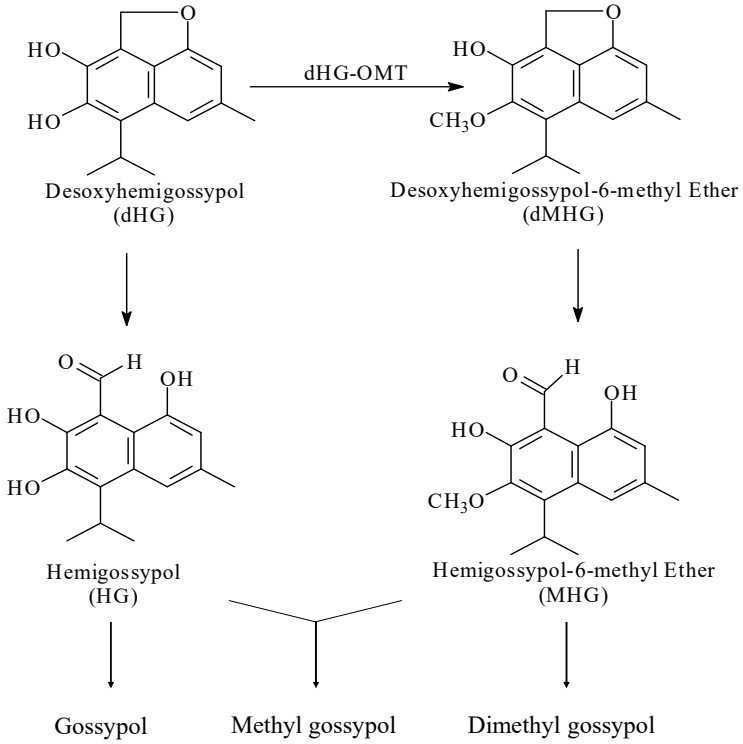

Figure 1. Cotton defense gossypol pathway compounds in root and stem tissues of cotton.

Quantification of gossypol pathway compounds

OMT RNAi and their wild type sibling lines were grown in 9:1 sand:loam soils for 21 or 24 days in two separate experiments. The root tissues were then freeze dried, and their gossypol pathway terpenoid compound contents were extracted and analyzed by HPLC. Induced gossypol pathway compounds in stem stele tissue were also determined. When the fourth true leaf still not yet fully expanded, the plants were stem-puncture inoculated with a suspension of Fov11 conidia just below the cotyledonary node using a syringe needle. Nine days later, the first internodes were excised, and the stele tissues were freeze dried. The gossypol pathway compound contents were extracted and analyzed by HPLC.

Results and Discussion

Responses of OMT RNAi lines to infection by Fov/RKN

Disease assays in Trial 1 and 2 showed that Fov only or RKN only treatments failed to cause disease as expected. Therefore, only results from Fov/RKN treatment compared to control will be given (Table 1). Shoot weight reductions compared to control treatment for the RNAi lines, 7BR and 7JAR, were less than that of their corresponding sister lines in Trial 1. The shoot weight reductions were similar between the sibling pair 7JBR and 7JBW. In trial 2, a similar trend was observed except that sibling pairs 7JAR and 7JAW had similar shoot weight reductions. Overall, wild type sibling lines had greater shoot weight reductions than the RNAi lines in these two trials. In Trial 3, about 65% more RKN eggs were used for the Fov/RKN treatment and disease symptoms were

more severe than the first 2 trials. Overall, wild type sibling lines had less shoot weight and root weight reductions than the RNAi lines in this trial.

Gossypol pathway terpenoid compound profile

We have previously shown that roots of cotton plants contain high level of gossypol pathway compounds, and no further induction of these compounds occur upon infection by Fov (Wagner et al., 2020). Therefore, the profiles of these compounds were analyzed from the roots of uninfected RNAi and their wild type sibling line plants (Table 2). All three RNAi lines had less than 3% methylated terpenoids compared to about 50% methylated terpenoids in their corresponding wild type sibling lines, representing a greater than 94% blockage of methylated pathway. The blockage failed to increase the unmethylated terpenoids and resulted in decrease in total terpenoid contents by half in the RNAi lines relative to their wild type sibling lines. Healthy stem stele tissues are devoid of gossypol terpenoid compounds and induction of these compounds occur upon infection with wilt pathogens. In the Fov11 infected stem stele tissue, the terpenoid profile differed from that in the roots (Table 3). No methylation occurred even in the wild type sibling lines and less than 2% terpenoids were in methylated form in this tissue. This tissue is similar to the leaf tissue of *G. hirsutum* which also is devoid of methylated gossypol pathway compounds. The 7BW and 7BR sibling pair had almost identical terpenoid profiles while the total terpenoid content is lower in the 7JBR line than in its sibling line in the stem tissues. Therefore, the wild type sibling lines may possess more protective power than the corresponding RNAi lines in the roots but not in the stem. However, this protective power in the roots probably is also limited due to fact that the methylated terpenoids are only half as toxic as the unmethylated terpenoids. Inconsistent resistance levels of RNAi lines compared to their wild type sibling lines in different trials are in accordance with these hypotheses. Whether the RNAi traits will be beneficial to disease resistance in *G. barbadense*, in whose stem high proportions of terpenoids were methylated upon infection, needs to be further explored.

Table 1. Comparison of the effect of Fov/RKN treatment on shoot or root weight of RNAi OMT lines compared to wild type sibling lines. Values are means (µg/g fresh shoot or root weight) for the number of plants used for each of the lines as indicated in the Materials and Methods section. Percent reductions of the Fov/RKN treated plants compared to control mock inoculated plants are given.

Treatment	7BW	7BR	7JAW	7JAR	7JBW	7JBR
Trial 1 (shoot weight)						
Control	29.2	26.8	34.2	29.4	26.9	33.6
Fov/RKN	13.6	23.7	13.8	20.6	15.6	16.7
% reduction	53.4	11.7	59.6	29.9	41.9	50.3
Trial 2 (shoot weight)						
Control	18.1	19.9	19.3	18.9	18.7	18.2
Fov/RKN	11.5	15.9	15.7	14.6	9.4	11.8
% reduction	36.5	20.1	18.6	23.0	49.7	34.9
Trial 3 (shoot weight)						
Control	18.7	18.5	19.6	20.2	20.8	19.4
Fov/RKN	4.1	3.4	8.1	3.4	1.9	2.7
% reduction	78.0	81.6	58.9	83.3	91.0	86.1
Trial 3 (root weight)						
Control	6.1	6.7	6.1	6.9	7.3	5.8
Fov/RKN	4.0	3.6	5.5	3.2	2.3	2.9
% reduction	34.5	46.6	9.4	53.8	68.2	50.6

Table 2. HPLC analysis of the gossypol pathway compounds present in the roots of wild type and RNAi plants that were grown in 9:1 sand:loam for 24 days (7JAW, 7JAR, 7JBW and 7JBR plants) or 21 days (7BW and 7BR plants). Values are means for the number of plants given the parenthesis (µg/g dry root tissue).

Compound	7BW (6)	7BR (11)	7JAW (7)	7JAR (7)	7JBW (7)	7JBR (7)
HG	107.3	172.3	300.3	498.7	227.5	628.6
dHG	3.5	6.5	12.9	13.2	8.7	18.6
HGAL	0.0	28.0	0.0	0.0	0.0	0.0
G	958	1299	1639	2186	1805	2999
MHG	213.0	0.0	360.2	0.0	330.5	0.0
dMHG	13.9	0.0	27.7	0.0	27.7	0.0
MG	1123	99	2217	151	2363	187
DMG	951	0	1605	7	1738	0
Total unmethylated	1068.8	1505.8	1952.2	2697.9	2041.2	3646.2
Total	3369.7	1604.8	6162.1	2855.9	6500.4	3833.2
% methylation	50.7	3.0	49.5	2.8	49.6	2.4

Table 3. Induced cotton defense gossypol pathway compounds (µg/g fresh stele from the first internode) 9 days after Fov11 hypocotyl stem inoculation. Values are means from 8 plants for each line.

Gossypol pathway compound	7BW	7BR	7JBW	7JBR
HG	86	81	118	60
dHG	42	29	45	23
HGAL	10	20	18	11
G	18	25	28	17
MHG	0.1	0	1	0
dMHG	1	0	2	0
MG	0	0	1	0
DMG	0	0	0	0
Total	157	155	213	111
% methylation	0.7	0	1.9	0

Acknowledgements

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References

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