Communication

Leaf Traits Associated with Flavonol Glycoside Genes in Soybean

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ABSTRACT

In the soybean (Glycine max [L.] Merr.), the gene combination Fg1 Fg3 is responsible for the glycosylation in the biosynthesis of kaempferol triglucoside (K9) in leaves. The presence of K9 is associated with reduction in chlorophyll content, specific leaf mass, photosynthetic rate and stomatal frequency. Blocking the action of Fg1 Fg3 with the magenta flower gene was prevents formation of K9 and restores leaf traits to normal. A direct effect of K9 on leaf development is postulated.

In the soybean (Glycine max [L.] Merr.) a complex genetic system controls leaf flavonol glycoside formation (3) with a kaempferol tri-glycoside, K9, being associated with a reduction in specific leaf mass, Chl content and leaf photosynthetic rate (4). Although K9 appeared to have a direct effect on leaf development and photosynthesis, the possible involvement of the Fg1 and Fg3 genes in some other associated metabolic process could not be completely excluded. Cosio and McClure (8) did not detect K9 or other flavonol glycosides in mesophyll cells isolated from mature soybean leaves of Fg1 Fg3 line and concluded that K9 is a marker that has no direct inhibitory effect on photosynthesis. There are two possibilities for K9 to be a marker. Either Fg1 and Fg3 could result in the formation of other glycosidic compounds which cause the observed effects or there could be another pair of complementary genes that are linked with Fg1 and Fg3 and when brought together result in deleterious effects. If K9 is not a marker, it could be having an indirect effect on photosynthesis.

We proceeded to test the hypothesis that flavonol glycosides have an effect on leaf traits associated with photosynthesis. In the Wm/wm genotype flavonol glycoside formation is practically nil but flavonol accumulation in the pod pubescence is not affected (4, 5). Wm/wm isolines were developed and tested with the objective of determining whether blocking flavonol glycoside formation would influence the apparent effect of K9 on leaf traits. Leaves were evaluated for mechanisms that might be controlling the observed effects.

MATERIALS AND METHODS

In the presence of genes t, fg2, and Fg4, gene combinations Fg1 Fg3, Fg1 fg3, and fg1 Fg3 result in flavonol classes 3t, 5t, and 7t, respectively (2). K9 is present in leaves of class 3t but not in 5t or 7t; K3 is present in 3t and 5t, whereas K6 occurs in 3t and 7t. Four Fg1-derived isolines involving Wm/wm were obtained from the cross of OX281 (a line carrying Wm Wm) with OX922 (a 3t line carrying Wm Wm). With Wm/wm being closely linked with W1/w1 (5), purple (W1) and white (w1) flowers were used as markers for Wm and w1, respectively. OX941 (3t) and OX942 (3t w1) were obtained from an F1 (Fg1 Fg1 Fg3 Fg3 Wm w1 W1 w1) plant and OX943 (3t) and OX944 (3t w1) were obtained from an F1 (Fg1 Fg1 Fg3 Fg3 Wm Wm W1 W1) plant as homozygous lines. Genotypes were confirmed by TLC.

Experimental plants were grown in the field (a fertile sandy loam) at Harrow, Ontario, machine planted in rows 3.7 m long, 0.6 m apart. Photosynthetic rate (Pn) was measured by a 14CO2 method (4) on the youngest fully mature leaves in August 1985 and 1986. Stomatal numbers were counted in leaf epidermal strips secured to glass slides by a cyanoacrylate adhesive (9) at a magnification of 400× using five random fields of 0.184 mm2 for each leaf. Chl was determined in 80% acetone extracts (1). Flavonol glycosides were routinely monitored by cellulose TLC. For hydrolysis of these glycosides, an equal volume of 2 N HCl was added to the acetone extracts used for Chl determinations and the mixture heated for 30 min on a boiling water bath. Kaempferol was determined quantitatively in the hydrolysate using a Hewlett Packard 1090 HPLC equipped with a Hypersil MOS 5 μm column (100 × 2.1 mm). Kaempferol had a retention time of 17 min at a flow rate of 0.5 ml min⁻¹, an oven temperature of 38°C, and a methanol gradient between 30 and 50% MeOH in 10% HCOOH in H2O. Elution was monitored at 365 nm.

RESULTS

The 3t OX941 and the 5t OX943 contained several hundred nanograms of kaempferol (after hydrolysis) per mg of leaf tissue, while OX942 and OX944 with the w1 gene contained very little (Table I). The principal glycoside is K9 in OX941, and K3 in OX943 (data not shown). OX941 had the lowest values of SLM, Chl, and photosynthetic rate both per unit area and per unit of Chl (Table I).

In a test the following year OX941 again had a lower rate of photosynthesis than OX942, OX943, and OX944 (Table II). Examination of leaf surfaces showed that OX941 had a very much reduced number of stomata on the upper and somewhat reduced number on the lower, compared to OX942, OX943, and OX944. Another 3t line, OX922, likewise had a much reduced number of stomata compared to its 7t isolate OX921 (Table II).

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1 Abbreviations: K9, kaempferol 3-O-2'-glycosyl-gentiobioside; K3, kaempferol 3-O-gentiobioside; K6, kaempferol 3-O-rosohoroside; SLM, specific leaf mass; Pn, photosynthetic rate per unit leaf area.
FLAVONOLS AND SOYBEAN LEAF TRAITS

Table 1. Leaf Traits of Flavonol Glycoside Isolines Grown in Field Test, 1985, and Sampled August 16 during Pod Fill

<table>
<thead>
<tr>
<th>Lines</th>
<th>Genes</th>
<th>Flavonol Class</th>
<th>Kaempferol*</th>
<th>SLM*</th>
<th>Chl* (a + b)</th>
<th>Photosynthetic rate mg CO₂ h⁻¹</th>
<th>Photosynthetic rate mg CO₂·dm⁻² h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX941</td>
<td>Fg1Fg3Wm</td>
<td>3t</td>
<td>440 y</td>
<td>5.20 z</td>
<td>50.9 z</td>
<td>15.6 z</td>
<td>3.1 z</td>
</tr>
<tr>
<td>OX942</td>
<td>Fg1Fg3wm</td>
<td>3twm</td>
<td>14 z</td>
<td>6.06 y</td>
<td>67.2 x</td>
<td>29.6 y</td>
<td>4.5 y</td>
</tr>
<tr>
<td>OX943</td>
<td>Fg1gFg3Wm</td>
<td>5t</td>
<td>650 y</td>
<td>6.18 y</td>
<td>57.1 yz</td>
<td>26.7 y</td>
<td>4.7 y</td>
</tr>
<tr>
<td>OX944</td>
<td>Fg1gFg3wm</td>
<td>5twm</td>
<td>12 z</td>
<td>6.15 y</td>
<td>64.8 y xy</td>
<td>29.1 y</td>
<td>4.5 y</td>
</tr>
</tbody>
</table>

* Values in columns followed by the same letter do not differ at P = 0.05 level in Duncan's Range Test.

Table II. Leaf Traits of Flavonol Glycoside Isolines Grown in Field Test, 1986, and Sampled 26 to 28 August during Pod Fill

<table>
<thead>
<tr>
<th>Lines</th>
<th>Flavonol Class</th>
<th>Glycosides*</th>
<th>Stomatal numbers*</th>
<th>Photosynthetic rate mg CO₂ h⁻¹</th>
<th>Photosynthetic rate mg CO₂·dm⁻² h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K3</td>
<td>K6</td>
<td>K9</td>
<td>Upper*</td>
</tr>
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<td>3t</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>3 z</td>
</tr>
<tr>
<td>OX942</td>
<td>3twm</td>
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<td>-</td>
<td>-</td>
<td>146 xy</td>
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<tr>
<td>OX943</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>130 y</td>
</tr>
<tr>
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<td>5twm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>154 x</td>
</tr>
<tr>
<td>OX921</td>
<td>7t</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>164 x</td>
</tr>
<tr>
<td>OX922</td>
<td>3t</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>5 z</td>
</tr>
</tbody>
</table>

* Based on TLC separations of methanolic leaf extracts: +++ = high level; + = medium level; - = absent. Measurements were made on five plants per line, using the youngest fully mature leaf. * Values in columns followed by the same letter do not differ at the P = 0.05 level in Duncan's Range Test. * It has previously been demonstrated that OX922 has a lower photosynthetic rate than OX921 (4).

DISCUSSION

These data are consistent with a previous report (4) that plants with high levels of K9 in their leaves have reduced levels of photosynthesis, Chl and SLM. It is further evident that the presence of Fg1 Fg3 only results in reduced photosynthesis, etc., when 'normal' levels of flavonols are being produced, i.e., in the presence of Wm (OX941 and OX942). When flavonol synthesis is severely inhibited in the presence of Wm (OX942), photosynthesis is on a par with OX943. In other words, the presence of K9 seems to be involved in the suppression of the reduced photosynthesis and associated characters.

Cosio and McClure (8) have demonstrated that K9 does not occur in the mesophyll of a 3t line (OX913) and does not directly interfere with CO₂ fixation in mesophyll cells. The simplest hypothesis is that K9 acts by interfering with leaf development in such a way that stomatal numbers are much reduced. This could account for reduced photosynthesis and stomatal SLM. It is also possible that K9 could have a more generalized effect on leaf development leading directly to thinner, paler leaves as well as reduced stomatal numbers.

The alternative explanation, that the gene combination Fg1 Fg3 is responsible for the biosynthesis of K9, but that another undiscovered function (or a function of very closely linked genes) is responsible for the lower P₄, Chl, SLM, and stomatal numbers, seems to be ruled out by the effect of the Wm gene in blocking flavonol glycoside formation. It is unlikely that Wm would inhibit flavonol glycoside formation and some other function (or other genes) at the same time and to the same degree. However, other flavonol glycosides may affect leaf development and stomatal numbers to a lesser extent than K9; the blocking of Fg1 with Wm in OX944 resulted in significantly (P = 0.05) more stomata than in OX943. The two 3t lines (OX941 and OX922) differed in stomatal frequency in the lower leaf surface indicating that other factors may be involved. Thus, various combinations of flavonol glycoside genes need to be evaluated in different genetic backgrounds. The Wm/wm isolines used in our research carried W1/w1 as markers which could have contributed a nonisogenic effect; however, W1 is a phenolase gene for anthocyanidins and not for flavonol (6). On the average in the work of Chia and Brun (7), purple-flowered cultivars had a similar adaxial stomatal frequency as white-flowered cultivars.

A number of questions are suggested by these results. Why are the effects of K9 so much greater on the upper than on the lower epidermis? Does K9 interfere with supply or activity of growth regulators in the developing leaves? What are the effects of different stomatal frequencies on transpiration and water use efficiency? We hope to answer some of these questions in the near future.

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LITERATURE CITED

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