11-1966

Use of Shoot-Root Grafts in Case studies of Site of *o*-Hydroxycinnamic Acid Synthesis in *Melilotus alba*

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Published in Crop Science (November-December 1966) v. 6, no. 6

Use of Shoot-Root Grafts in Studies of Site of *o*-Hydroxycinnamic Acid Synthesis in *Melilotus alba*[1]

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ABSTRACT

The content of *o*-hydroxycinnamic acid was measured in shoot-root grafts involving four combinations of *Melilotus alba* plants of the *CuCuBB* and *cucuBB* genotypes. Six portions from each grafted plant were assayed. Results provide evidence that *o*-hydroxycinnamic acid synthesis occurs in the aboveground parts of sweetclover plants, with limited translocation to the roots. No support was obtained for the view that the root plays an important role in *o*-hydroxycinnamic acid synthesis.

IN SWEETCLOVER (*Melilotus alba* Desr.), the contents of the *trans* and *cis* isomers of β-D-glucosyl-*o*-hydroxycinnamic acid are highest in young leaves, intermediate in stems, and lowest in roots (1). This pattern of distribution, viewed in conjunction with data indicating that only limited translocation occurs in stem grafts (3), may be interpreted as evidence that the primary site of glucosidically bound *o*-hydroxycinnamic acid ("bound coumarin") synthesis is in the young, actively growing leaves.

In recent years, however, conflicting reports have appeared concerning the relative importance of the shoot and root as the site of synthesis. Mothes (7), noting the poor development of *Pisum* and *Tetragonolobus* scions when grafted to rootstocks of high-coumarin *Melilotus*, concluded that the poor growth of the scions was due to the formation of coumarin in the *Melilotus* root with subsequent translocation to the aboveground tissues. This explanation seemed logical inasmuch as Mothes and Romeike (8) had previously observed that substances formed in the root were responsible for the incompatibility of some grafts, as in low-alkaloid scions grafted to high-alkaloid rootstocks. Reppel and Wagenbreth (9), using *Trigonella-Melilotus* grafts, concluded that the root was the primary site of coumarin synthesis, but that the stem was able to synthesize coumarin when suitable precursors were available. This view concerning the importance of the root as the site of coumarin synthesis was supported by the observation of Weygand and Wendt (11) that labeled phenylalanine was incorporated into coumarin in root cultures of *M. officinalis*. However, in a similar study by Williams et al. (12), no net synthesis of *o*-hydroxycinnamic acid was detected in cultured roots. Other recent investigations of synthesis in grafted plants have indicated that coumarin synthesis occurs in the shoot (2, 10).

In the present study, shoot-root grafts were utilized in an investigation of the relative importance of the aboveground parts and the roots as the site of *o*-hydroxycinnamic acid synthesis in grafted sweetclover plants. Evidence to be presented supports the conclusion that virtually all *o*-hydroxycinnamic acid is synthesized in the shoot.

MATERIALS AND METHODS

Highly inbred plants of sweetclover (*Melilotus alba*) of the *CuCuBB* (subsequently referred to as H) and *cucuBB* (hereafter referred to as L) genotypes were used in these experiments. The derivation of these genotypes has been described elsewhere (6). Plants of the *CuCu* genotype are high and *cucu* plants are low in content of glucosidically bound *o*-hydroxycinnamic acid. Preparations of *BB* plants display β-glucosidase activity.

Plants to be grafted were grown in flats in a greenhouse without supplemental light. Prior to grafting, each plant was tested qualitatively for *o*-hydroxycinnamic acid using a method based on the fact that *o*-hydroxycinnamic acid on filter paper exhibits bright fluorescence when treated with alkali and exposed to ultraviolet light.[3] At the time of grafting, most plants were approximately 3 months of age, although a few plants 1½ months of age also were used.

For rootstocks, plants were removed from the flat with the root system essentially intact in a block of soil. The upper portion of soil was removed to expose approximately 2 cm of tap root, but fibrous roots were left intact in the soil. A sharp razor blade was used to make a 1-cm longitudinal slit in the white, exposed portion of the tap root.

Scions approximately 1 cm in length were obtained from the terminal growing point of young stems. The stem of each scion was carefully trimmed to a wedge shape with a razor blade and inserted into the slit in the root. The scion was secured with the apex upright by wrapping with thin strips of self-sticking latex rubber. The grafted plant, with soil and roots intact, was transferred to a 4-inch clay pot, which was filled to the level of the graft union with sand. Ten days after grafting, the entire aboveground portion of the host plant was removed as close to the graft union as possible. All grafted plants were kept under an 18-hour photoperiod in the greenhouse until sampled at the late bud to early flower stage. No root growth from the scions was observed in any of the grafted plants.

Approximately 20 grafts of each of the 4 possible combinations of the 2 plant genotypes were made. The plant combinations included high scion on low root (H/L), low scion on high root (L/H), with controls consisting of high scion on high root (H/H) and low scion on low root (L/L). In all subsequent references to graft combinations, the first letter will refer to the scion; the second to the rootstock. The numbers of grafted plants that survived and grew to the flowering stage were distributed among the 4 graft combinations as follows: L/L - 13, L/H - 15, H/L - 8, and H/H - 13. Of these totals, 10, 13, 7, and 10 grafted plants, respectively, were assayed for content of *o*-hydroxycinnamic acid.

Six portions were taken from each grafted plant for assay of *o*-hydroxycinnamic acid content. The portions are described below in sequence from the upper to the lower part of the plant: (a) youngest, fully expanded leaf at the apex of the main stem, (b) a 3-cm section of stem immediately above portion c, (c) a 3-cm section of stem immediately above portion d, (d) a 1.5-cm section that included the graft union, (e) a 2-cm section of tap root immediately below portion d, and (f) all remaining root tissue, consisting primarily of fibrous roots.

Nine grafted plants, including 3, 2, 1, and 3 plants of the L/L, L/H, H/L, and H/H graft combinations, respectively, were sampled as described above and the portions were used for determination of percentage dry matter. Because differences between similar portions from the various graft combinations were slight, an average dry matter percentage was calculated from the 9 plants for each of the 6 portions. These 6 averages were used in calculating *o*-hydroxycinnamic acid percentages for appropriate assay samples.

[1] Contribution from the Crops Research Division, ARS, USDA, and the Nebraska Agricultural Experiment Station, Lincoln, Nebraska. Supported in part by the National Science Foundation (Grant No. GB-1148). Published with the approval of the Director as Paper No. 1895, Journal Series, Nebraska Agr. Exp. Sta. Received May 5, 1966.

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Published in Crop Science (November-December 1966) v. 6, no. 6

Previously published procedures (4, 5) were followed for extraction and assay of the *o*-hydroxycinnamic acid glucosides, except that extracts were assayed only for total *o*-hydroxycinnamic acid, rather than for both isomeric forms of this compound.

RESULTS AND DISCUSSION

Average contents of *o*-hydroxycinnamic acid in sampled portions of the four types of grafted plants are given in Table 1. A cursory examination of the data indicates that contents in scion tissue (portions a, b, and c) depended to a much greater extent on the genotype of the scion than on that of the stock. Contents in the tissues of the stocks (portions e and f) also were highly dependent on the genotype of the scion. Thus, the scion appears to be of much greater importance than the root in determining the *o*-hydroxycinnamic acid content of the various tissues sampled.

The data are more readily evaluated for evidence of site of *o*-hydroxycinnamic acid synthesis if contents are expressed as percentages of the contents found in the control grafts (Table 2). Thus, for comparative purposes, contents of all samples from L/L and H/H control grafts would have a numerical value of 100. Roots from L/L grafts contained only 5% as much *o*-hydroxycinnamic acid as was found in H/H roots, whereas roots from H/L grafts contained approximately 75% as much. Thus, the L roots from H/L grafts contained approximately 10 to 15 times as much *o*-hydroxycinnamic acid as was found in L roots from L/L plants. These data provide evidence that most of the content in the root is determined by translocation from the aboveground portions of the plant. Although contents of stem and leaf samples of H/L plants were almost identical to those of H/H plants, they were from 40 to 58 times greater than those of L/L plants, indicating that the genotype of the root had no observable influence on levels in the aboveground parts of the plant.

Roots of L/H grafts had only 1/4 to 1/3 as much *o*-hydroxycinnamic acid as was found in H/H controls, but this was several times as much as was found in L/L controls. This result would be expected if the normal content of H roots at the time of grafting was slowly dissipated during the growth of the grafted plants, leaving a residual amount that was somewhat greater than the amount in L/L controls but less than levels found in H/H controls.

Evidence that slow dissipation of *o*-hydroxycinnamic acid does occur in roots of L/H plants is presented in Table 3. Plants assayed for *o*-hydroxycinnamic content from each of the 4 graft combinations were divided into 2 groups. Grafted plants in which less than 60 days elapsed between grafting and sampling for assay comprised the "younger" group; those in which more than 60 days elapsed made up the "older" group. Contents in roots from older plants of H/H grafts were higher than comparable samples in younger plants, whereas contents in roots from older L/H grafts were lower. Sections from the stem and the graft union of older L/H plants also were lower in content of *o*-hydroxycinnamic acid than sections from younger L/H plants. These observations support the conclusion that little if any *o*-hydroxycinnamic acid synthesis occurs in roots but that a portion of the compound present in H roots, when grafted to L scions, is gradually lost with increasing age of the grafted plant. Additional evidence that synthesis occurs primarily in the shoot is provided by the higher contents found in roots from older H/L plants, a situation similar to that observed in H/H plants.

Some diffusion of *o*-hydroxycinnamic acid from roots of L/H grafts to stem-sections immediately above the graft union was indicated by slightly higher values in stem sections from L/H grafts than in L/L controls. Diffusion apparently did not extend to the upper leaves. Contents in leaves and stems of L/H plants were less than 5% as great as those in H/H controls (Table 2).

Schlosser-Szigat (10) used stem grafts on field-grown sweetclover plants to demonstrate downward translocation of *o*-hydroxycinnamic acid in the fall of the year. She also investigated *o*-hydroxycinnamic acid content in roots of stem-grafted plants whose rootstocks or scions were subjected to defoliation treatments. Her observations, like those resulting from the present study, support the conclusion that the major site of synthesis is in the aboveground plant parts, with limited translocation to the roots. No evidence was found to support the hypothesis, presented by Mothes (7) and Reppel and Wagenbreth (9), that the root is the primary site of synthesis. In the present study, the root appeared to have no significant influence on the synthesis of *o*-hydroxycinnamic acid.

[3] Gorz, H. J., and F. A. Haskins. Unpublished data. 1964.

Table 1. Mean contents of *o*-hydroxycinnamic acid (% of dry weight) in various portions of grafted sweetclover plants. The numbers of grafted plants assayed were as follows: L/L — 10, L/H — 13, H/L — 7, H/H — 10.

Portion assayed*	*o*-Hydroxycinnamic acid levels in 4 graft combinations			
	L/L	L/H	H/L	H/H
	% ± S.E.			
Leaf-a	0.107 ± .032	0.063 ± .013	4.331 ± .727	4.661 ± .572
Stem-b	0.009 ± .001	0.016 ± .003	0.527 ± .040	0.497 ± .027
Stem-c	0.010 ± .002	0.022 ± .003	0.446 ± .043	0.453 ± .025
Graft-d	0.012 ± .001	0.054 ± .007	0.253 ± .047	0.318 ± .024
Root-e	0.020 ± .004	0.149 ± .019	0.312 ± .073	0.424 ± .034
Root-f	0.022 ± .002	0.075 ± .012	0.242 ± .060	0.298 ± .028

* See text for description of the portions assayed.

Table 2. Contents of *o*-hydroxycinnamic acid in portions of grafted plants expressed as the percentage of contents in L/L and H/H control grafts.

Portion assayed	Contents of grafts expressed as percentage of contents in					
	L/L controls			H/H controls		
	L/H	H/L	H/H	L/L	L/H	H/L
Leaf-a	59	4048	4356	2	1	93
Stem-b	178	5856	5522	2	3	106
Stem-c	220	4460	4530	2	5	98
Graft-d	450	2108	2650	4	17	80
Root-e	745	1560	2120	5	35	74
Root-f	341	1100	1355	7	25	81

Table 3. Contents of *o*-hydroxycinnamic acid in various portions of grafted sweetclover plants that were sampled prior to ("younger") or following ("older") 60 days of growth after grafting.

Graft combinations	Age of plants		No. of plants	*o*-Hydroxycinnamic acid (mean % of dry wt.)					
	Relative	Avg (days)		Leaf (a)	Stem (b)	Stem (c)	Graft (d)	Root (e)	Root (f)
L/L	Younger	50	6	.059	.010	.013	.013	.025	.021
	Older	90	4	.180	.009	.007	.011	.013	.023
L/H	Younger	54	11	.046	.016	.023	.060	.166	.080
	Older	75	2	.155	.012	.015	.026	.057	.050
H/L	Younger	48	5	3.388	.475	.433	.202	.247	.156
	Older	86	2	6.686	.657	.478	.379	.475	.457
H/H	Younger	49	6	3.654	.461	.462	.284	.368	.251
	Older	72	4	6.172	.551	.441	.370	.508	.370

Published in Crop Science (November-December 1966) v. 6, no. 6

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