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TISSUE AGE AND CAFFEOYLQUINIC ACID CONCENTRATION IN SUNFLOWER

D. E. KOEPPE,* L. M. ROHRBAUGH, E. L. RICE and S. H. WENDER

Departments of Botany–Microbiology and of Chemistry, University of Oklahoma, Norman, Oklahoma 73069, U.S.A.

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Abstract—Through the use of one-dimensional paper chromatographic separation, followed by spectrophotometric analysis, chlorogenic acid, neochlorogenic acid and 4-*O*-caffeoylquinic acid concentrations have been determined in sections of field-grown *Helianthus annuus* L. Using similar procedures, relative concentrations of "isochlorogenic acid" were also determined. All these compounds decreased in concentration basipetally in stem and leaf sections except in leaves from the first six nodes where the monocaffeoylquinic acids increased in concentration down from the apex. These concentration changes are seen to correlate with the postulated role of caffeoylquinic acids in lignification and in influencing internal regulatory mechanisms.

INTRODUCTION

IN RECENT papers[1–6] it has been reported that in tobacco and sunflower the concentration of the caffeoylquinic acids and scopolin is affected by both environment and the age of the plant tissue. These concentration changes correlate well with the biochemical gradient studies of Lavee and Galston[7] and Kerstetter and Keitt.[8] With reported evidence indicating the probability that these compounds, or their immediate precursors, may have a synergistic effect on indoleacetic acid (IAA) action, possibly acting to suppress an "IAA oxidase" system,[9–11] and with reports that they may play a role in lignin synthesis;[12–14] it seems that a comprehensive analysis of these phenolic compounds in a plant where they are known to exist in high quantities might reveal new clues as to their possible roles. The native sunflower (*Helianthus annuus* L.) found in the early stages of abandoned field succession in Oklahoma is of additional interest because of its production of phenolic phytotoxic substances,[15] including the chlorogenic acids studied in this paper.

* Present address: Department of Agronomy, University of Illinois, Urbana, Illinois 61801.

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RESULTS

Environmental Effects

A valid comparison of environmental effects would be that of young plants (6–10 cm tall) harvested on 2 May and 24 May (see Table 1). Concentrations of chlorogenic acid (3-*O*-caffeoylquinic acid) (CGA), 4-*O*-caffeoylquinic acid ("band 510") (4 CQ), neochlorogenic acid (5-*O*-caffeoylquinic acid) (neo CGA), and "isochlorogenic acid" (a mixture, including some dicaffeoylquinic acids) (iso CGA) all were higher in those plants harvested on 24 May, with the greatest increase in the older leaves (Table 1). Similar comparative patterns do not hold for older and taller plants harvested on the two dates, most likely because of the large difference in the height and size of the two groups of plants. In young

TABLE 1. CONCENTRATION OF CAFFEOYLQUINIC ACIDS IN NATIVE SUNFLOWER

	μg/g fresh weight*				
	CGA	4CQ	neo CGA	Relative iso CGA†	Ratio CGA:iso CGA
2 May Harvest					
Apex leaves	1883	176	193	178·4	10·6
Three and four node leaves	1540	248	194	60·8	25·3
Five and six node leaves	3384	262	205	98·8	34·2
Stems	926	86	81	49·8	18·6
24 May Harvest					
Apex leaves	2000	238	218	238·6	8·4
Three and four node leaves	2692	326	274	229·2	11·7
Five and six node leaves	4870	410	324	297·2	16·4
Stems	1550	160	124	86·2	18·0

Young plants (6–10 cm tall) harvested on 2 and 24 May 1968.

* CGA = Chlorogenic acid; neo CGA = neochlorogenic acid; 4CQ = 4-*O*-caffeoylquinic acid.
† Only a relative value calculated, as given in Experimental section.

plants, the relative ratios of CGA to iso CGA changed little in apex leaves and stem sections, but were considerably less in the older leaves of the plants harvested on 24 May.

Age Effects

Stem Sections. In plants harvested on 2 May there is little change in CGA, 4CQ, neo CGA or iso CGA concentrations when the three sections (1, 2 and 3) are compared (Table 2). Taller plants harvested on 24 May exhibited pronounced concentration changes basipetally in the eight sections analyzed (Table 2). All four compounds examined, decreased in concentration from the top down to the fifth section, increasing slightly in the remaining bottom sections. Changes in the ratio of CGA : iso CGA roughly paralleled the concentration changes, being lowest in the middle sections.

TABLE 2. CONCENTRATION OF CAFFEOYLQUINIC ACIDS IN STEM SECTIONS OF NATIVE SUNFLOWER

Stem section	μg/g fresh weight*				
	CGA	4CQ	neo CGA	Relative iso CGA†	Ratio CGA:iso CGA
2 *May Harvest*					
1‡	914	64	65	37·2	24·6
2	956	76	62	24·6	38·9
3	1065	82	79	32·0	33·3
24 *May Harvest*					
1	842	121	86	50·1	16·8
2	422	41	41	20·9	20·2
3	288	25	25	18·6	15·5
4	202	30	19	17·0	11·9
5	100	21	21	7·9	12·7
6	143	27	25	8·6	16·6
7	174	24	28	11·7	14·9
8	159	23	25	12·0	13·2

Older plants harvested on 2 May (16–24 cm tall) and 24 May (48–64 cm tall).

* As in Table 1.
† As in Table 1.
‡ Top stem section, others numbered in sequence basipetally (each section 8 cm in length).

Leaves

All plants which were analyzed showed increases in CGA, 4CQ and neo CGA basipetally to the fifth and sixth node (Tables 1 and 3). In the tall plants harvested on 24 May (Table 3)

TABLE 3. CONCENTRATIONS OF CAFFEOYLQUINIC ACIDS IN LEAVES OF NATIVE SUNFLOWER

	μg/g fresh weight*				
	CGA	4CQ	neo CGA	Relative iso CGA†	Ratio CGA:iso CGA
2 *May Harvest*					
Apex leaves‡	2172	141	152	157·3	13·8
Three and four node leaves	2674	240	180	109·2	24·5
Five and six node leaves	3366	321	207	66·4	50·7
24 *May Harvest*					
Apex leaves	1532	196	164	182·5	8·4
Three and four node leaves	1525	245	190	146·0	10·4
Sixth-node leaves	2082	308	213	159·2	13·1
Eighth-node leaves	1834	377	234	100·8	18·2
Tenth-node leaves	1604	276	202	88·2	18·2
Twelfth-node leaves	710	124	91	30·6	23·2
Fourteenth-node leaves	636	130	97	28·4	22·4

Older plants harvested on 2 May (16–24 cm tall) and 24 May (48–64 cm tall).

* As in Table 1.
† As in Table 1.
‡ Top leaves, others numbered in sequence basipetally.

leaves were harvested basipetally to the fourteenth node. CGA, 4CQ and neo CGA were found to decrease in concentration basipetally after the sixth or eighth node maximal levels. Contrary to the other chlorogenic acids, the concentration of iso CGA decreased basipetally in all leaves except those from the young plants harvested on 24 May (Tables 1 and 3). Ratios of CGA:iso CGA increased down from the apex in all leaves analyzed, even in the young plants of 24 May (Tables 1 and 3). While all data reported here are on the basis of fresh weight, dry weights were also obtained for all samples, with data so compiled showing no differences from that of the fresh weight.

DISCUSSION

In previous work with the Russian Mammoth variety of sunflower[3] we observed in leaves a CGA concentration change with age which differed from that observed with tobacco,[2,5,6] *Theobroma cacao*,[16] cotton phenolics[17] and coffee.[18] Concentrations of the CGA, 4CQ, and neo CGA were found to increase with age in sunflower leaf tissue; whereas tobacco showed a decrease in these concentrations with increased age. The present work with the native sunflower indicates further that the sunflower does exhibit an inverse effect of aging when different leaf tissues from the same plant are compared, but only to a point (sixth to eighth node). Other than the CGA, 4CQ and neo CGA concentration increase from the apex approximately to the middle leaves, there is a general decrease basipetally. The stem section data obtained from the more mature plants show no concentration increase at all with age for any of the isomers. From this, one might infer, as with other species analyzed, that the decrease in CGA concentration basipetally relates to increased IAA oxidase activity. Our finding that in stem sections the least concentrations are found slightly below the mid-stem corresponds well to the curves of peroxidase activity shown for tobacco pith by Lavee and Galston.[7]

In *Xanthium*, Taylor has found that 3,5-dicaffeoylquinic acid is a primary precursor of lignin,[13] and Taylor and Zucker reported that CGA and iso CGA are in a constant state of turnover.[14] The decrease in iso CGA concentration with age of both stem sections and leaves leads to the inference that it is being incorporated into lignin as is the case with *Xanthium*. Since the decrease in iso CGA concentration in stems with age is somewhat greater than that of CGA might indicate that iso CGA is more readily converted into lignin than CGA. A comparison of tobacco and sunflower shows interesting differences in possible lignin precursors. 4CQ and neo CGA concentrations are much higher proportionally in tobacco than in sunflower.[2, 3, 19] But tobacco contains little, if any, iso CGA in comparison to the relatively huge quantities found in sunflower. Could it be that iso CGA is a preferred intermediate lignin precursor in sunflower, but not in tobacco? As in the other plants analyzed, it is possible that the role of the chlorogenic acids in sunflower may be dual: first as a metabolic inhibitor in young tissues, and then as precursors to lignin in older tissue.

The comparison of young plants harvested on 2 and 24 May shows higher concentrations of all the chlorogenic acids in the plants of the latter harvest. Since these plants were selected from their native habitat, their growing conditions obviously were not controlled. It does seem likely however, that increased light intensity and higher temperatures are responsible

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for these higher concentrations. Both intensity[4] and duration of light[19] have already been shown in tobacco to affect chlorogenic acid concentrations.

Wilson and Rice reported that the chlorogenic acids from native sunflower are phytotoxic. They could find no evidence, however, of the release of these depsides from the plant.[15] They also observed that the ground around the plants in the Oklahoma prairie was inhibitory to certain other native species. Our evidence could indicate that enzyme systems responsible for the conversion of the chlorogenic acids, possibly to other phytotoxins (or to lignin), are already at work in these relatively young plants since there is appreciable drop in the concentration of all the chlorogenic acids with increased tissue age. There do not appear to be the concentration increases in naturally growing sunflowers with age that were seen with the Russian Mammoth variety[3] in the growth chamber.

Even though Wilson and Rice[15] identified scopolin (7-glucoside of scopoletin) in the leachate obtained from leaves of native sunflower, it was not found in measurable amounts in any of our extracts. This, and our previous observation of scopolin in extracts of sunflowers of the Russian Mammoth variety grown in the growth chamber,[3] may indicate that the production of scopolin is age and/or environment affected in this plant, and that its biochemical production may be stimulated under leaching conditions.

EXPERIMENTAL

Native sunflower plants (*Helianthus annuus* L.) were harvested on 2 and 24 May 1968 from an abandoned field at the south city limits of Norman, Oklahoma. All plants were brought into the laboratory and divided into individual parts as indicated in the results; then weighed, fixed, and extracted as previously described.[20]

Separation and quantitation of CGA, 4CQ, and neo CGA were accomplished through a procedure developed by Koeppe *et al.*[3] Separation of iso CGA was carried out through one-dimensional chromatography on Whatman No. 1 paper (9¼ × 22 in.), freshly washed with *ca.* 50 ml 5% MeOH and dried. The paper was developed for 20 hr using IFW (isopropyl alcohol:formic acid:water, 5:0·1:95, v/v/v). Three iso CGA bands were located within 6–10 in. of the origin under u.v. light without exposure to NH_3. Each band was cut out and estimated in a fashion similar to that reported for the other caffeoylquinic acids. Positive identification of the iso CGA bands was obtained through co-chromatographic and u.v. spectra comparison to authentic standards. The relative iso CGA data reported were obtained by multiplying the O.D. reading × the product of the sample volume divided by the gram fresh weight of the sample × the eluate volume. All reported data are the average obtained from two replicates (7–10 plants/replicate) with quantitative analysis run in duplicate for each replicate.

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