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HYDROXYCINNAMIC ACID AMIDES IN FERTILE AND CYTOPLASMIC MALE STERILE LINES OF MAIZE

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Key Word Index—*Zea mays*: Gramineae; hydroxycinnamic acid amides; reproductive organs; grains; cobs; cytoplasmic male sterility; development.

Abstract—Hydroxycinnamic acid (HCA) amides in fertile and cytoplasmic male sterile lines of maize were determined in reproductive organs, developing grains and cobs. HCA amides occurred in large amounts in the anthers of fertile plants (line F7N) and were absent from the anthers of cytoplasmic male sterile lines (lines F7T and F7C). Restoration of fertility was associated with the production of these compounds (line FC31). Considerable variations were observed in the concentrations of HCA amides at different stages of growth and grain maturation. Changes of HCA amides in the grains which were to produce sterile plants followed a pattern similar to that obtained with the grains which were to produce fertile plants. Accumulation of HCA amides was substantially higher in fertile lines whatever their genotype (F7N, FC31 and F7T × FC31) than in sterile lines. Marked changes occurred in the HCA amide content of embryo and endosperm during grain development. Many changes in HCA amides were observed in cobs during development and maturation, but no substantial differences could be observed between fertile and sterile lines.

INTRODUCTION

Earlier findings, obtained in our laboratory, indicated the presence of large amounts of hydroxycinnamic acid (HCA) amides in a number of species of flowering plants, representing 20 families[1]. Within the plant, these compounds were found only in meristems, reproductive organs and seeds where they appear to be the main phenolic compounds[1–5]. HCA amides disappeared after germination[1, 3–5]. Analysis of HCA amides in whole plants, has revealed a relationship between HCA amide accumulation and the flowering state[2, 3, 5, 6]. Previous work has shown that HCA amides were absent from sterile reproductive organs in several plants[4–7], and HCA amides appeared to constitute biochemical markers of pollen and ovule fertility[4–8]. We recently demonstrated that HCA amides were absent from the anthers of maize with Texas male sterile cytoplasm[4]. Restoration of fertility was associated with the production of these substances[4]. All the mature grains which were to produce male fertile plants, whatever their genotype, contained large amounts of HCA amides[4]. On the other hand, in the grain which were to produce sterile plants, with Texas cytoplasm, much smaller amounts of HCA amides were found[4]. During germination, the content of these substances decreased drastically[4]. Of particular interest was the relation between synthesis of large amounts of HCA amides, initiation of floral development, and processes controlling reproduction in many plants.

Here we report on the quantitative analysis of HCA amides in reproductive organs, developing grains and cobs in fertile and cytoplasmic male sterile lines of maize.

RESULTS

HCA amides in reproductive organs of male fertile and male sterile lines of maize

HCA amide content and distribution in reproductive organs of male fertile and male sterile lines of maize are shown in Table 1. HCA amides were found in large quantities in the anthers of male fertile maize (line F7N). These compounds were absent in the anthers of the two male sterile lines one with cytoplasm Texas (F7T), and the other with cytoplasm C (F7C). In parallel with HCA amide accumulation, fertile anthers contained large amounts of tyramine. Restoration of fertility (line FC31) was associated with the production of these substances. These compounds were localized in the pollen grains. They appeared to constitute biochemical markers of pollen fertility. Female organs contained mainly neutral HCA amides and there were no differences in the HCA amide contents of female organs, irrespective of the presence or absence of T or C cytoplasm and the restorer genes.

Changes in HCA amides during grain development of a fertile line of maize (line F7N)

The changes in the amounts of HCA amides during grain development are shown in Fig. 1. Fertilization was achieved at *ca* day 4 after pollination. Considerable variation was observed in the concentration of HCA amides at different stages of growth and grain maturation.

The initial phase of grain development until 40 days after pollination was characterized by a rapid and substantial accumulation of feruloylputrescine and by

Table 1. HCA amide content and distribution in reproductive organs of male fertile and male sterile lines of maize

	Basic HCA amides (nmol/g.fr.wt)		Neutral HCA amides (nmol/g.fr.wt.)					
	Ferpne	Tyr aromatic amine (nmol/g.fr.wt.)	Diferpne	Diferspd	Diferspm	Fertyr	Fer x*	Fer y*
Male fertile maize								
Line F7N [male non-sterile maize (N)] cytoplasm								
Anthers	6000	2000	2600	130	40	1000	—	—
Ovaries	90	70	270	550	—	150	****	***
Line FC31 [male fertile maize (restored male fertile)]								
Anthers	6500	1800	3000	100	70	1200	—	—
Ovaries	80	50	300	700	—	100	****	***
Male sterile maize								
Line F7T (maize with T male sterile cytoplasm)								
Anthers	50	—	90	—	—	20	—	—
Ovaries	80	60	250	500	—	50	****	***
Line F7C (maize with C male sterile cytoplasm)								
Anthers	90	—	50	—	—	50	—	—
Ovaries	90	70	250	500	—	150	****	***

Abbreviations: ferpne, feruloylputrescine; Tyr, tyramine; diferpne, diferuloylputrescine, diferspd, diferuloylspermidine; diferspm, diferuloylspermine; fertyr, feruloyltyramine.

*These neutral HCA amides were found in large amounts in the ovaries (x was an aliphatic amine and y an aromatic amine).

small amounts of neutral HCA amides (diferuloylputrescine, diferuloylspermidine, diferuloylspermine, feruloyltyramine). Feruloylputrescine content was maximal at this stage. During the second phase from day 50 to maturity, feruloylputrescine decreased, *p*-coumaroylspermidine was synthesized, and high concentrations of neutral compounds were found.

The feruloylputrescine accumulation appeared to be biphasic with an initial accumulation at day 15 of development (milky stage) and a subsequent one occurring on day 50. On day 15 feruloylputrescine was 13 times higher than on day 4. On day 50, accumulation was less pronounced: there was six times the amount present on day 4. After this period, feruloylputrescine showed a marked decrease during the later stages of development. Diferuloylputrescine was initially low between days 4 and 15 after pollination. Diferuloylputrescine content then increased rapidly, reaching a maximum within 50 days after pollination. On day 50, diferuloylputrescine was 55 times higher than the value on day 4. On day 55, the diferuloylputrescine content decreased and then remained constant during the later stages of grain maturation. At maturity, diferuloylputrescine was 34 times higher than the value on day 4.

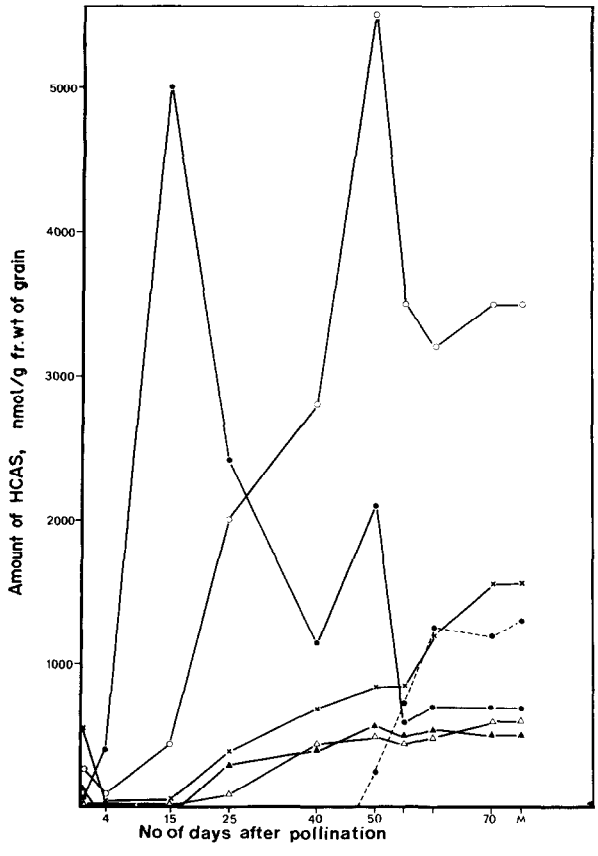

Fig. 1. Changes in HCA amides during grain development of fertile line of maize (F7N). (●—●) Feruloylputrescine, (○—○) diferuloylputrescine, (●----●) *p*-coumaroylspermidine, (×—×) diferuloylspermidine, (△—△) diferuloylspermine, (▲—▲) feruloyltyramine, M = Maturity.

The high level of feruloylputrescine in the early stages of grain development, its progressive decline as the rate of diferuloylputrescine accumulation increased, and the low level at maturity, might be explained by the conversion of feruloylputrescine into diferuloylputrescine.

p-Coumaroylspermidine was synthesized within 50 days after pollination. The maximum value occurred between 60 days and maturity. Diferuloylspermidine was found in small amounts initially. The maximum value occurred during the final stage of maturation. Diferuloylspermine and feruloyltyramine were present in very small amounts at the beginning and increased slowly during grain development.

Marked changes were observed in the HCA amide content of embryo and endosperm during grain development. Comparative evolution of HCA amides in embryo and endosperm on day 40 and at maturity is shown in Table 2. In the embryo on day 40, spermidine derivatives occurred in larger amounts than the putrescine derivatives. On the other hand, this stage of grain development was characterized by a substantial accumulation of putrescine derivatives in endosperm. During the initial period (4–40 days) feruloylputrescine was not detected in the embryo. The diferuloylputrescine level was *ca* twice as high in the endosperm as in the embryo. Production of diferuloylspermidine in the embryo was *ca* four times that in the endosperm.

In the embryo, from day 40 to maturity, a progressive increase in the concentrations of HCA amides occurred, the level being greatest for *p*-coumaroylspermidine, feruloylputrescine, diferuloylputrescine (×4), followed by diferuloylspermidine (×2.5). Levels of HCA amides in the embryo were comparatively higher than these found in mature endosperm (*ca* three times higher than the endosperm values). The spermidine derivatives became *ca* twice as abundant as the putrescine derivatives. The mature embryo was also characterized by large amounts of tyramine and phenethylamine.

With the onset of maturation a rise in diferuloylspermidine was observed in the endosperm (2.5 times the amount present on day 40). Diferuloylputrescine increased *ca* 1.5 times, *p*-coumaroylspermidine appeared and feruloylputrescine decreased with maturation of grain. Only small changes in the concentrations of diferuloylspermine and feruloyltyramine were observed at maturity in both embryo and endosperm.

The further increases in HCA amides which occurred during the final maturation of the embryo could result from the synthesis *in situ* or from the translocation of these compounds from the endosperm into the embryo.

Changes in HCA amides during grain development of cytoplasmic male sterile lines of maize (lines F7T and F7C)

The changes in the amounts of HCA amides during grain development of sterile lines of maize are shown in Figs. 2 and 3. Biosynthesis of HCA amides in developing grains of male sterile lines followed similar profiles the sequences being similar to those observed with F7N. During grain development the

Table 2. HCA amides and aromatic amines in the embryo on day 40 and at maturity in fertile line of maize (F7N)

	Basic HCA amides (nmol/g.fr.wt)		Aromatic amines (nmol/g.fr.wt)		Neutral HCA amides (nmol/g.fr.wt)			
	Ferpne	Pcspd	Tyr	Phe	Diferpne	Diferspd	Diferspm	Fertyr
On day 40								
Embryo	50	40	—	—	1250	2700	850	220
Endosperm	1100	150	—	—	2700	700	400	400
At maturity								
Embryo	1400	5250	1200	1500	5100	6900	900	250
Endosperm	600	1300	—	—	3400	1700	700	650

Abbreviations: phe, phenethylamine; pcspd, *p*-coumaroylspermidine. See Table 1 for others.

HCA amide content in F7T did not differ much from that of F7C.

Feruloylputrescine reached a maximum content on day 15 (9.5 times higher than the value on day 4). The maximum value of diferuloylputrescine occurred on day 50 (35 times the amount on day 4). *p*-Coumaroylspermidine was synthesized within 50 days after pollination the maximum value being reached between 60 days and maturity. The maximal value of diferuloylspermidine occurred during the final maturation. Diferuloylspermine and feruloyltyramine increased slowly throughout the developmental stages. In general, at all stages of grain development F7N accumulated *ca* twice as much of the HCA amides as F7T and F7C.

On day 15, the feruloylputrescine content in both the sterile varieties (F7T and F7C) was 40% that of the fertile line (F7N). The maximum difference in the rates of feruloylputrescine accumulation between F7N and F7T or FTC was found on day 40. At this stage of grain development the level of feruloylputrescine in F7N was 9.5 times greater than that of feruloylputrescine found in F7T and F7C. At maturity the feruloylputrescine content was in F7N *ca* six times the amount observed in grain from sterile lines.

The distribution of HCA amides between embryo and endosperm at the two different stages of grain development in sterile lines is shown in Table 3. Changes and distribution in HCA amides in embryo and endosperm of sterile lines followed a pattern similar to that obtained with F7N. No substantial difference could be observed between sterile lines. In the embryo, during the initial period (4–40 days) spermidine derivatives occurred in larger amounts than putrescine derivatives. This stage of development was characterized by an accumulation of diferuloylputrescine in the endosperm.

At maturity, the levels of HCA amides in the embryo were higher than in the endosperm. Compared with the sterile lines, the HCA amide content in the grain from the fertile line was higher in both the embryo

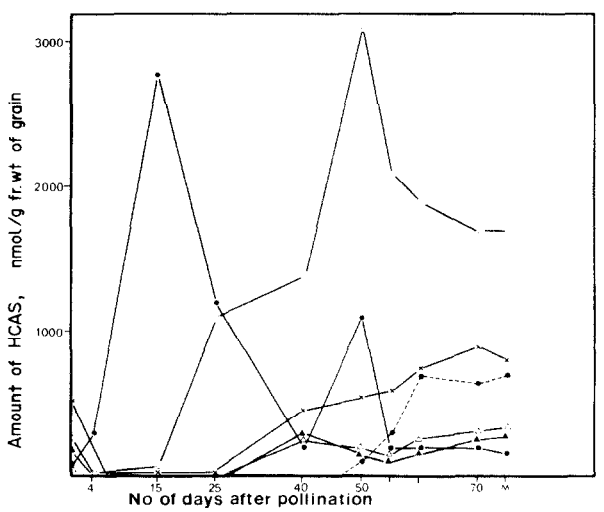

Fig. 2. Changes in HCAs during grain development of maize with T male sterile cytoplasm (line F7T). Key as for Fig. 1.

Table 3. HCA amides and aromatic amines in embryo and endosperm on day 40 and at maturity in cytoplasmic sterile lines of maize (lines F7T and F7C)

	Basic HCA amides (nmol/g.fr.wt)		Aromatic amines (nmol/g.fr.wt)		Neutral HCA amides (nmol/g.fr.wt)			
	Ferpne	Pcspd	Tyr	Phe	Diferpne	Diferspd	Diferspm	Fertyr
Line F7T								
At day 40								
Embryo	—	—	—	—	650	1300	350	50
Endosperm	200	—	—	—	1300	450	200	200
At maturity								
Embryo	700	2200	850	900	3000	4000	300	100
Endosperm	150	650	—	—	1700	700	300	300
Line F7C								
At day 40								
Embryo	—	—	—	—	600	1400	250	15
Endosperm	100	—	—	—	1400	350	200	250
At maturity								
Embryo	650	2350	700	800	2800	3800	200	100
Endosperm	150	600	—	—	1800	800	300	200

Abbreviations: see Tables 1 and 2.

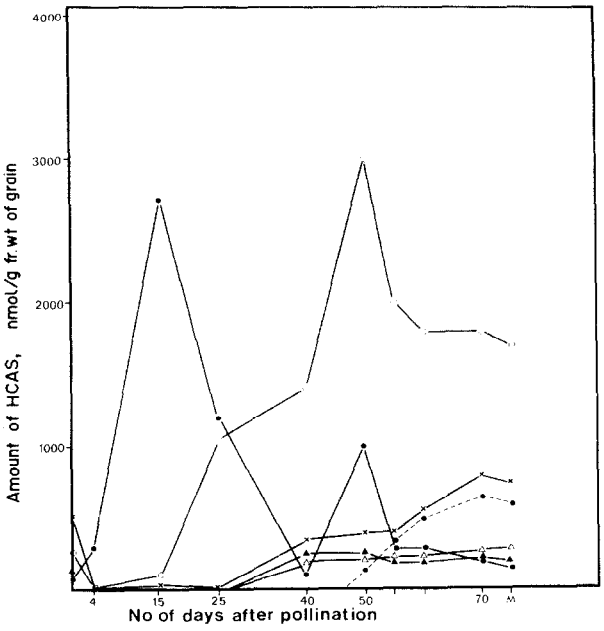

Fig. 3. Changes in HCAs during grain development of maize with C male sterile cytoplasm (line F7C). Key as for Fig. 1.

and endosperm. At all stages of grain development, restoration of fertility (grain from lines FC31 and F7T × FC31) was characterized by similar variations and quantitative changes in HCA amides as observed in grains of the fertile line (Table 1).

HCA amide changes in the cob during development and maturation of fertile and sterile lines of maize (Fig. 4)

During development and maturation many changes in HCA amides were observed in the cob of maize. Maximal values of feruloylputrescine and diferuloylputrescine contents in the cob occurred at the same stages of development as those observed in the developing grain. Initially, just after pollination, the feruloylputrescine content was low, but began to increase soon after and by day 15 it had increased 10 times. After this, it decreases sharply, and on day 40, feruloylputrescine disappeared. The decline in feruloylputrescine was accompanied by a rapid rise in diferuloylputrescine content. On day 50, there was 34 times the amount present on day 4. Thereafter the diferuloylputrescine content decreased and then remained constant during the later stages of maturation. At maturity diferuloylputrescine was 12 times higher than the value on day 4. Small changes in the concentrations of diferuloylspermine and feruloyltyramine were observed during development.

Putrescine derivatives occurred in abundance in the cobs compared with spermidine derivatives. The high level of feruloylputrescine in the early stages of development, and its loss as the level of diferuloylputrescine accumulated might be explained by the conversion of feruloylputrescine to diferuloylputrescine. No important differences could be observed between fertile and sterile lines of maize.

DISCUSSION

Considerable and similar variations were observed in the concentration and distribution of HCA amides at the different stages of growth and maturation in the grain and cobs of fertile and cytoplasmic male sterile lines of maize. This report suggests that in maize, cytoplasmic male sterility affects HCA amide synthesis in both anthers and grain. In the anthers, the presence of male sterile cytoplasm led to the inhibition of HCA amide accumulation. Was the presence of HCA amides a result rather than a cause of pollen fertility? All the grain for producing male fertile plants, whatever their genotype (F7N, FC31, and F7T × FC31) contained larger amounts of HCA amides than those producing sterile plants. The larger amounts of HCA amides in grain for producing fertile plants were not due to a maternal effect, but rather to the presence of the two restorer genes. F7T × FC31 grains did not show any difference in HCA amides compared to FC31 grains. Cytoplasmic male sterility could affect both HCA amide synthesis and/or degradation.

Given the large amounts of HCA amides present within the cells at all stages of the reproductive cycle of maize, it is probable that a number of the cellular processes involved in the developmental phases of reproductive organs and grain depend on HCA amides.

Two examples may be drawn from the correlation between our results on HCA amides and other results in the literature. Ingle *et al.*[9] have studied the changes in RNA, DNA and protein which occurred during the development and maturation of maize grain. There is a close correlation between the most rapid increase in the content of HCA amides and the maximal rates of RNA, DNA and protein synthesis, in such a way that our figures parallel those of Ingle.

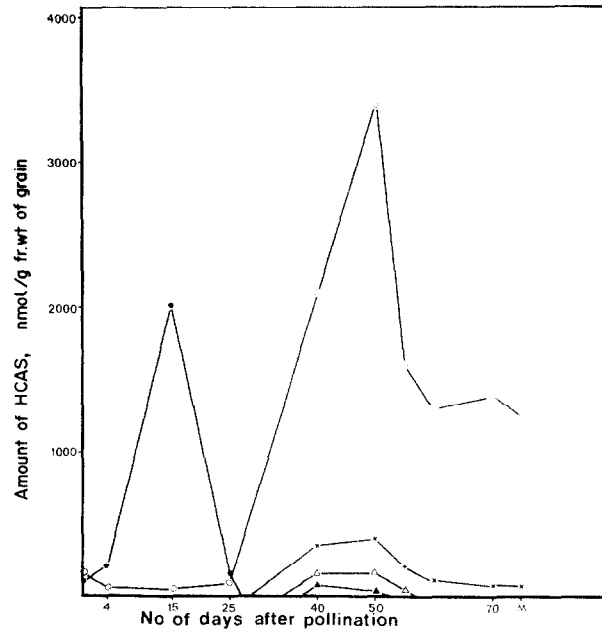

Fig. 4. HCA in cob during development and maturation of fertile and sterile lines of maize. Key as for Fig. 1.

Such a coincidence has already been observed between nucleic acids or protein synthesis and polyamines in fast growing cells of different biological systems [10–18].

On the other hand, many authors [19–25] have shown that maize endosperm synthesizes active growth factors during the first phase (8–18 days) of grain development, during which maize endosperm could grow on a simple medium without growth regulators. Afterwards mature endosperm was unable to grow in tissue culture. This could be correlated with the presence of basic HCA amides (particularly feruloylputrescine) during the first phase, and by contrast, with the predominance of neutral HCA amides in mature grains.

EXPERIMENTAL

The fertile and cytoplasmic male sterile lines of maize, chosen for this study were F7N, F7T and F7C: they are isogenic and had normal, Texas and C cytoplasms respectively. A two-locus system restores the normal fertility of plants with Texas cytoplasm. Line FC31, used here, had Texas cytoplasm and was homozygous for the two dominant genes Rf1 and Rf2; this it was male fertile. Grains from a cross between F7T and FC31 (as a male parent) were also analysed. Cytoplasmic male sterility was characterized both in F7T and F7C by pollen abortion and normally developed tassels. Lines of maize were planted in a greenhouse at the Experimental Station at Epoisses (Dijon, Côte d'Or, France) in the spring of 1980. For analysis of HCA amides in these lines, tassels were cut off before anther dehiscence and the ears some days after emerging from the silks. Plants were pollinated in July. Collection of the developing grains was started just after pollination and was continued at 15, 25, 40, 50, 55, 60, 70 and 75 days after pollination.

At each sampling, three ears were selected from each line and taken directly to the laboratory. Grain was removed from the axial part (cob) of each ear, mixed, and used for the experimental determinations. Cobs were examined. Duplicate lots of 30–50 grains were used for each of the determinations. Embryos were dissected from the grain sampled on day 40 and at maturity. The embryo (scutellum, plus embryo axis) and the remainder of the grain, which by this stage of development was largely endosperm were analysed separately. Duplicate lots of 50 endosperms and 100 embryos were used. Extraction of plant tissue was according to refs. [1, 3, 5, 6]. HCA amides were isolated, purified and identified using the methods of refs. [1, 3, 5–8, 26]. HCA amides were estimated by the methods of refs. [3, 5–8].

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