HYDROXYCINNAMIC ACID AMIDES (HCA) IN *ZEA MAYS*
Distribution and changes with cytoplasmic male sterility

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1. Introduction

Earlier findings, obtained in our laboratory, indicated the presence of large amounts of hydroxycinnamic acid amides (HCA) in a number of species of flowering plants, representing 13 different families [1]. HCA are hydroxycinnamic acid amine conjugates. The link between these molecules is an amide bound. They occur as basic (water-soluble) or as neutral (water-insoluble) forms. In the basic forms, only one amine group of an aliphatic amine (diamine or polyamine) is linked with a phenolic acid. Neutral forms can be divided into two classes. In class 1, each terminal amine group of an aliphatic amine is neutralized by a phenolic acid. In class 2, the amine group of an aromatic amine is linked with a cinnamic acid. Some 24 hydroxycinnamic acid derivatives of the 5 amines: putrescine, spermidine, spermine, cadaverine and tyramine have been characterized [1]. Thus, HCA appear to be common constituents of flowering plants. Within the plant, these compounds were found to occur only in the reproductive organs, where they appear to be the main phenolic compounds. They are apparently absent from the green parts (leaves, stems) petals and sepals [1]. For example, in *Nicotiana tabacum*, we have reported the presence of caffeylputrescine, caffeylspermidine in the meristems [2] and demonstrated that there is an increase in the amount of these HCA in the apical part of tobacco plants at the time of floral induction [2,3]. Accumulation of HCA can also be induced in lower leaves by the topping of flowering plants [3]; This indicates that their production may be correlated with floral induction. In *Nicotiana tabacum* cv. *Xanthi* n.c., an increase in temperature which inhibits the flowering process suppresses the accumulation of HCA [2]. These compounds were found in large amounts in the reproductive organs of tobacco: caffeylputrescine and caffeylspermidine being present in the ovaries, and neutral compounds like di-*p*-coumaryl-putrescine, di-*p*-coumarylspermidine and *p*-coumaryltyramine in the anthers [3]. Furthermore, similar compounds were found in the seeds and disappeared after germination [1,3].

Of particular interest is the relation between the synthesis of large amounts of HCA, initiation of floral development, and processes controlling reproduction in many plants. The present work deals with the identification of HCA in the reproductive organs and seeds of maize. In addition, a correlation between the amount of the compounds and cytoplasmic male sterility is reported.

2. Materials and methods

The fertile and cytoplasmic male-sterile lines of maize, respectively, chosen for this study were F7N and F7T: they are isogenic lines and have normal and Texas cytoplasms, respectively.

A two-locus system restores the normal fertility of plants with Texas cytoplasm. Line FC31, used here has Texas cytoplasm and is homozygote for the two dominant genes *Rf1* and *Rf2*; thus, it is male fertile.

For analysis of HCA in these 3 lines, tassels were cut off before anther dehiscence and ears some days after emerging from the silks. Analyses were carried out on dry, mature seeds and, separately, on embryos together with scutellum and endosperm. Seeds and

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embryos from a cross between F7T and FC31 (as a male parent) were also analyzed.

Extraction of plant tissue was done as in [1]. HCA were isolated, purified and identified by using the methods in [1–3].

3. Results

3.1. *Reproductive organs*

3.1.1. Anthers

Basic and neutral HCA were found in large amounts in the anthers of F7N. They contained ferulylputrescine, di-ferulylputrescine, di-ferulylspermidine, di-ferulylspermine and ferulyltyramine. Ferulylputrescine, di-ferulylputrescine and ferulyltyramine were present in the highest quantities. Further analysis showed that these compounds were localized in the pollen grains only. HCA were absent in the anthers of maize of the cytoplasmic male sterile line F7T. Restoration of fertility (line FC31) was associated with the production of these substances.

3.1.2. Ovaries

Similar, neutral HCA such as di-ferulylspermidine, di-ferulylcadaverine and ferulyltyramine were found in all the lines tested (F7N, F7T and FC31).

3.2. *Seeds*

All the seeds which were to produce male fertile plants, whatever their genotype (F7N, FC31 and F7T × FC31), contained large amounts of ferulylputrescine, *p*-coumarylspermidine, di-ferulylputrescine, di-ferulylspermidine, di-ferulylspermine, di-ferulylcadaverine and ferulyltyramine.

On the other hand, in the seeds which were to produce sterile plants, with Texas cytoplasm, ferulylputrescine was completely absent; also, in these seeds much smaller amounts of neutral HCA, especially di-ferulylputrescine and ferulyltyramine, were found. The relative decrease in the concentration of these compounds was ~80%. HCA were only detected in embryos and scutella. During germination, the content of these substances decreased drastically.

The results are summarized in table 1.

Table 1
Hydroxycinnamic acid amides (HCA) in the reproductive organs and seeds of male fertile and male sterile maize

HCA	Male fertile maize Line F7N = male non-sterile maize (N) cytoplasm Line FC31 = male fertile maize (restored male fertile)			Male sterile maize Line F7T = maize with Texas male sterile (T) cytoplasm		
	Anthers (pollen)	Ovaries	Seeds (embryo + scutellum)	Anthers	Ovaries	Seeds (embryo + scutellum)
Basic compounds	ferpne	–	ferpne	–	–	–
	–	–	p-cspd	–	–	p-cspd
Neutral compounds	di-ferpne	–	di-ferpne[a]	–	–	di-ferpne
	di-ferspd	di-ferspd	di-ferspd	–	di-ferspd	di-ferspd
	di-ferspm	–	di-ferspm	–	–	di-ferspm
		di-fercd	di-fercd	–	di-fercd	di-fercd
	fertyr	fertyr	fertyr[a]	–	fertyr	fertyr

[a] These neutral HCA were present in higher concentration in seeds which were to produce male fertile maize than in those producing plants with male sterile (T) cytoplasm

Abbreviations: ferpne, ferulylputrescine; di-ferpne, diferulylputrescine; di-ferspd, di-ferulylspermidine; di-ferspm, diferulylspermine; di-fercd, diferulylcadaverine; fertyr, ferulyltyramine; p-cspd, coumaryl-spermidine

4. Discussion

The results presented show that HCA are concentrated in the reproductive organs. In maize, the female organs contain only the neutral compounds. There were no differences in the HCA contents of female organs, irrespective of the presence or absence of Texas cytoplasm and the restorer genes. Only male fertile plants contain HCA in their male reproductive organs. Among the compounds found, feruloylputrescine seems to be of particular significance: it appears to constitute a biochemical marker of pollen fertility. It is present not only in plants having normal cytoplasm but also in those having Texas cytoplasm in the presence of the restorer genes *Rf1* and *Rf2*. The analysis of seeds has shown the same correlation. According to the analytical data, the presence of feruloylputrescine is not due to a maternal effect, but rather to the presence of the two restorer genes. F7T × FC31 seeds do not show any difference in HCA content, as compared to FC31 seeds.

The results described here refer only to maize lines, F7 and FC31. However, similar results were obtained, as to their HCA content, for two other male-sterile lines, one with cytoplasm T (A 188), and the other with cytoplasm C (F7C). In both cases, we have found no HCA in the anthers: in the seeds, which were to produce sterile plants, feruloylputrescine was absent and the amount of neutral HCA (diferuloylputrescine and feruloyltyramine) was very much decreased. The restoration of fertility has led to the production of HCA in the anthers, to that of feruloylputrescine and a higher amount of neutral HCA in the seeds.

Acknowledgements

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References

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