Phytochemistry, Vol 24, No 5, pp 929–932, 1985
Printed in Great Britain

0031–9422/85 $3 00 + 0 00

TISSUE COMPARTMENTATION OF PHENYLPROPANOID METABOLISM IN TOMATOES DURING GROWTH AND MATURATION

ANNIE FLEURIET and JEAN-JACQUES MACHEIX

Laboratoire de Physiologie Végétale Appliquée, Université des Sciences et Techniques du Languedoc, 34060 Montpellier Cédex, France

(*Revised received* 20 *September* 1984)

Key Word Index—*Lycopersicon esculentum* var *cerasiforme*, Solanaceae, cherry tomato, accumulation and biosynthesis, compartmentation, hydroxycinnamic acid derivatives

Abstract—Marked changes in the metabolism of hydroxycinnamic acid derivatives were observed in pulp and pericarp of tomato fruit (*Lycopersicon esculentum* var *cerasiforme*) during its development During fruit growth, biosynthesis and accumulation of chlorogenic acid were especially active in the pulp, whereas the formation of glucose derivatives occurred during maturation in the pericarp There was a clear difference between the two compartments of the fruit concerning hydroxycinnamate CoA ligase, *O*-methyltransferase and glucosyltransferase activities The first two enzymes were high in the pulp during growth and the latter one was high in the pericarp during maturation Of all the enzymes studied, only the glucosyltransferase showed increasing activity during maturation, it may be considered, along with the glucosylated derivatives, as a biochemical marker of maturation in tomato

INTRODUCTION

The distribution of phenolic compounds varies greatly between the various parts of a plant, at the organ, tissue and cellular levels Hydroxycinnamic acids (HCA) are frequently more abundant in the external regions of organs, particularly in fruits [1, 2], where they may have a role in resistance to fungal infections [3] or in wounding [4] and also as a filter against UV light [5]. However, the literature concerning the distribution of HCA derivatives in fruits is incomplete These compounds are often studied after chemical breakdown instead of in their original state [6] Furthermore, studies of the enzymatic activities implied in their metabolism are either limited to a single enzyme [7, 8] or to a single part of the fruit [9]

In cherry tomato, HCA are present as quinic acid or glucose derivatives [10] In the latter case, two types of conjugation have been identified, either with the carboxylic group (GE) or with one of the phenolic hydroxyl groups (GI) of HCA [11] The variations of these components during fruit life have been reported previously [10] but this work did not distinguish between the different parts of the fruit Recent studies of respiratory and photosynthetic metabolisms, of chloroplasts and of pigments for the same variety of tomato, have shown a structural, physiological and metabolic opposition between fruit pulp and pericarp [12] It seemed important, therefore, to investigate whether similar observations could be found at the level of phenolic metabolism For this purpose, the distribution of HCA derivatives and of some enzymes responsible for their biosynthesis was surveyed in the pulp and the pericarp of the developing tomato fruit Among the several enzymes of phenolic synthesis the following were studied phenylalanine ammonia-lyase (PAL, EC 4 3 1 5) the key enzyme of phenolic metabolism [13], hydroxycinnamate CoA ligase (CL, EC 6 2 1 12) which permits the activation of free acids before their esterification with quinic acid [14], *O*-methyltransferase (OMT, EC 2 1 1 6), which assures methylation [15] and glucosyltransferase (GT) which is responsible, in cherry tomato, for the biosynthesis of GE and GI [16] As the spatial distribution may vary with the physiological state, the results given concern several stages of fruit development, the characteristics of which, i e fr and dry wts, and proteins, have been shown previously [17]

RESULTS

Compartmentation of HCA derivatives

Both qualitative and quantitative variations were observed (Table 1) Some compounds were only present in one part of the fruit, such as neochlorogenic acid and caffeoylglucose which appeared at the end of growth and could only be detected in the pulp In contrast, feruloylglucose and sinapoylglucose were exclusively located in the pericarp All the other derivatives (chlorogenic and *p*-coumaroylquinic acids, *p*-coumaroylglucose and glucosides of *p*-coumaric and caffeic acids) were present in both parts

The amount of each of these derivatives may vary greatly according to the part of the fruit and its physiological stage In contrast to the evolution of chlorogenic acid, the overall level of glucose derivatives (GE plus GI) rose in both parts of the fruit, over the whole growth period Such a result is characteristic of red fruits (Table 1) in pericarp, the amount of glucose derivatives exceeds that of quinic esters whereas they remain the major compounds in pulp

Compartmentation of PAL, CL, OMT and GT activities

The level of PAL activity was constantly higher in pericarp than in pulp It increased in both parts of the fruit

Table 1 Amounts (nmol/g dry wt) of hydroxycinnamic acid derivatives in pulp and pericarp during growth (G_{12} and G_{16} stages) and maturation (b and R stages) of tomatoes

Hydroxycinnamic derivatives	Pericarp				Pulp			
	Growth		Maturation		Growth		Maturation	
	G_{12}	G_{16}	b	R	G_{12}	G_{16}	b	R
Hydroxycinnamic derivatives	5363	4772	5091	3343	11690	10097	5081	3070
Quinic esters*	4116	3360	2850	1524	10535	8245	3333	1782
Glucose derivatives*	1247	1412	2241	1819	1155	1852	1746	1288
Quinic esters								
Chlorogenic acid	4086	3279	2718	1352	10511	8064	2992	1365
Neochlorogenic acid	—	—	—	—	—	81	193	387
p-Coumaroylquinic acid	23	50	66	100	8	100	148	30
Feruloylquinic acid	7	31	66	72	16	—	—	—
Glucose esters*	268	413	514	877	68	50	96	326
Caffeoylglucose	—	—	—	—	—	+†	54	296
p-Coumaroylglucose	75	73	72	70	68	50	42	30
Feruloylglucose	193	300	395	721	—	—	—	—
Sinapoylglucose	—	40	47	86	—	—	—	—
Glucosides*	979	999	1727	942	1087	1802	1650	962
Caffeic acid	389	761	1226	726	408	1403	1102	512
p-Coumaric acid	490	238	501	216	679	399	548	450
Ferulic acid	100	—	—	—	—	—	—	—

In every case the coefficient of variation, estimated from 10 different experiments, was less than 10% of the total

*Total amount of hydroxycinnamic acid derivative

†Trace component

over the entire growth period and reached a maximum at the G_{16} stage (Fig 1)

The OMT activity rose sharply in pulp during growth, yet varied little in pericarp In contrast, it decreased strongly in pulp and slightly in pericarp during maturation Thus, the activities in the two compartments were very different at the G_{16} stage but virtually identical in the red fruit

No CL activity could be discerned with respect to sinapic acid either in pulp or pericarp regardless of the stage studied For the other substrates (*p*-coumaric, caffeic and ferulic acids), CL activity was identical at the G_{10} stage in the two parts of the fruit but its subsequent evolution was very different In the pericarp, this activity diminished from the G_{10} to the G_{12} stages then remained essentially constant However, in the pulp, the activity increased strongly, reached a maximum at the G_{14} stage and then decreased at the end of growth and during maturation

The overall GT activity evolved very differently in the two parts of the fruit, the activity strongly increasing in pericarp, especially during maturation, whereas it varied little in pulp Thus, GT was the only enzyme whose activity increased during maturation These results have been confirmed by studying the biosynthesis of GE (Fig 2A) and GI (Fig 2B) separately

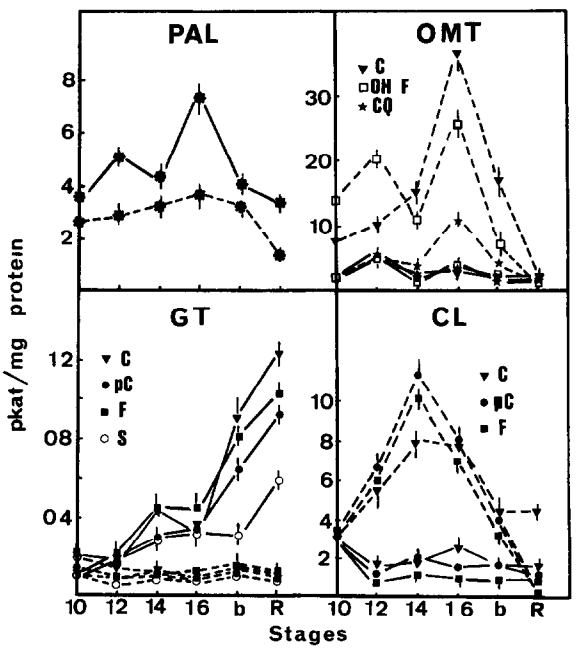

Fig 1 Evolution of activities of PAL, OMT, GT and CL in pulp (– – – –) and pericarp (——) during growth (G_{10}–G_{16} stages) and maturation (b and R stages) of tomatoes Substrates caffeic (C), *p*-coumaric (pC), ferulic (F), 5-hydroxyferulic (OHF), sinapic (S) and chlorogenic (CQ) acids Vertical bars indicate ± s e for five different experiments No CL activity was detected with sinapic acid

DISCUSSION

Pulp and pericarp of tomato fruit can ensure the biosynthesis of each hydroxycinnamic derivative which accumulates, therefore, the fruit cannot be just considered as a storage organ for these compounds possibly formed

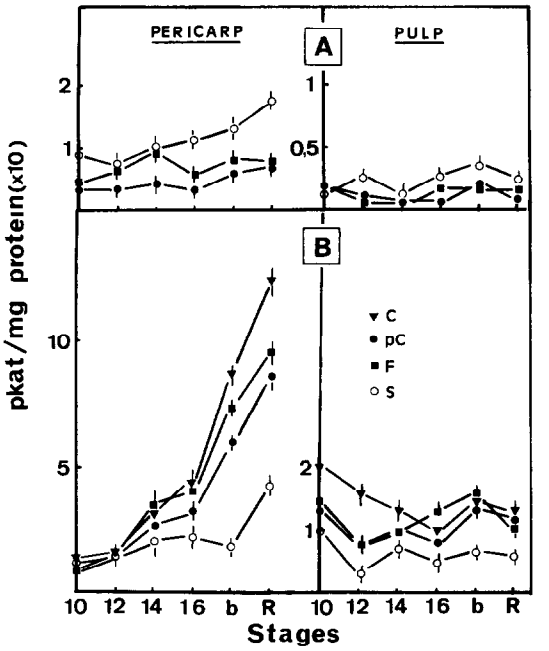

Fig 2 Variations of GT activity in pulp and pericarp during growth (G_{10}–G_{16} stages) and maturation (b and R stages) of tomatoes (A) Formation of esters, (B) formation of glucosides Substrates caffeic (C), *p*-coumaric (pC), ferulic (F) and sinapic (S) acids Vertical bars indicate ± s e for five different experiments

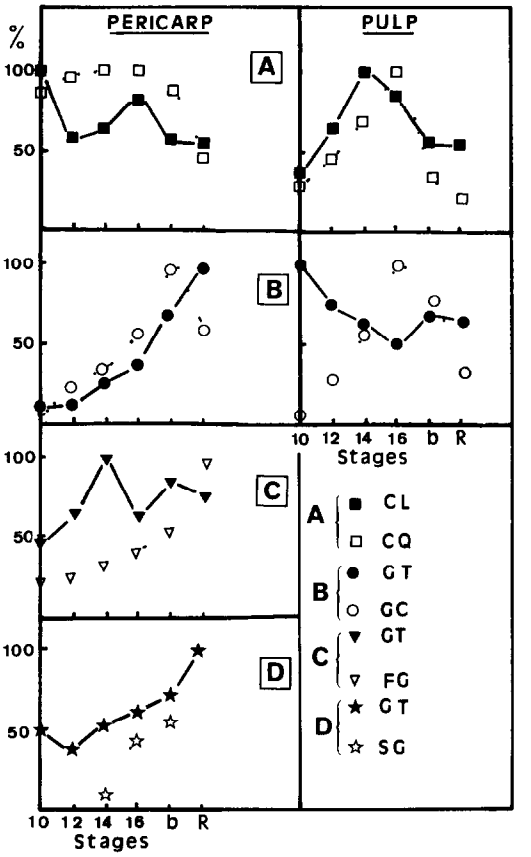

Fig 3 Comparative evolution of CL (A) and GT (B–D) activities and of the quantities of chlorogenic acid (CQ), caffeic acid glucoside (GC), feruloylglucose (FG) and sinapoylglucose (SG) during growth (G_{10}–G_{16} stages) and maturation (b and R stages) in pericarp and pulp of tomatoes For each parameter, results are given as percentages of the maximal value calculated from Figs 1 and 2 and ref [17] Substrates of enzymes caffeoyl CoA (A), or caffeic (B), ferulic (C) and sinapic (D) acids

in other parts of the plant Except for glucose derivatives, during maturation and in contrast to other fruits [2, 18], the overall amounts of these compounds in cherry tomato are greater in pulp than in the external tissues during growth

The results reported indicate a very clear difference in the expression of HCA derivative metabolism between the two parts of tomato fruits Maturation is characterized by a diversification whereas some new derivatives of caffeic acid (neochlorogenic acid and caffeoylglucose) appear in pulp, the derivatives of methylated acids (feruloylglucose, feruloylquinic acid, ferulic acid glucoside, sinapoylglucose) are strictly localized in the pericarp at this stage of fruit development The metabolism of HCA derivatives is compartmentalized with time (growth and maturation) and also with space (pulp, pericarp) it is orientated towards the formation and accumulation of quinic acid derivatives during growth, particularly in pulp, and towards those of glucose derivatives during maturation, especially in pericarp During the whole life of the fruit, PAL activity is greatest in pericarp Consequently, in tomato fruit, it cannot be the limiting factor in the accumulation of HCA derivatives which are more abundant in pulp during growth On the contrary, several examples show a good correlation between the variations of CL and GT activities and the quantities of quinic and glucose derivatives in the two parts of the fruit (Fig 3) This is clearly established by comparing the enzymatic activities and the quantities of HCA derivatives accumulated per fruit with respect to the physiological stages For this purpose, the results were first expressed per fruit (either for pulp or for pericarp) and then as a percentage of the maximum, according to an expression frequently used [19, 20] The evolution of CL, OMT and GT activities is very different in the two parts of the fruit Taking into account the different origins of pulp and pericarp it is possible that the enzymatic activities measured correspond to several isoenzymatic forms, as has been shown in other materials for CL [21] and OMT [22]

The results obtained for tomato fruit compare with those reported for diverse enzymes in apple cell suspensions [23, 24] and other plants [19, 25] PAL and CL activities are greatest at the beginning of growth and glucosylation always appears as a later stage Due of these very characteristic variations, the metabolism of HCA derivatives may be considered as a biochemical marker for maturation The metabolism of glucose derivatives in particular is entirely unique because GT is the only one among the enzymes studied whose activity increases with maturation This variation leads to an accumulation of glucose derivatives and is observed only in the pericarp which is the first part of tomato fruit to ripen The same evolution is observed when maturation is precociously stimulated by wounding the fruits [26]

The knowledge of spatial and temporal distribution of phenolic metabolism is an important element in the study of the role of these compounds in fruit physiology It is possible that the differences in the maturation rate between pulp and pericarp may originate from the characteristic duality observed at the phenolic metabolism level between the two parts of the fruit, either by direct action of these compounds on the biosynthesis of ethylene [6, 27] or indirectly by their role in the regulation of auxin metabolism [28]

EXPERIMENTAL

Plant material Tomato fruits (*L esculentum* Mill var *cerasiforme*) were collected from plants cultivated under natural conditions and selected according to colour and diameter Six physiological stages were studied four stages of growth (10, 12, 14 and 16 mm diameter green fruits G_{10}, G_{12}, G_{14}, G_{16}), and two stages of maturation (breaker and red fruits b, R) Each group consisted of 50 or 100 fruits which were divided into two parts, pulp and pericarp Pericarp is defined as the internal and external walls of the fruit, including placenta, while pulp is used to describe the seeds and jelly-like tissue which surrounds them After lyophilization, each part was ground in liquid N_2, followed by two types of extraction from the homogeneous powder, one for phenolic compounds and one for enzymatic proteins

Extraction, separation and estimation of HCA derivatives The 80% EtOH extraction, chromatographic separation and spectrophotometric determination of compounds has already been described [10, 11] The results are given in nmol/g dry wt

Measurement of enzymatic activities The extraction and purification of enzymes were identical to the procedures previously described [26] The PAL and CL activities were determined spectrophotometrically [11] and GT and OMT activities by isotopic assays [16, 26] As far as GT was concerned, three different results were obtained global activity and activities in relation to GE or GI formation These last two measurements were obtained after chromatographic separation of GE and GI [16] All results are given in pkat/mg protein and are expressed as mean ± s e for five independent expts Proteins were estimated according to ref [29]

REFERENCES

1 Van Buren, J (1970) in *The Biochemistry of Fruits and Their Products* (Hulme, A C, ed) Vol 1, p 229 Academic Press, London
2 Macheix, J J and Delaporte, N (1973) *Lebensm-Wiss Technol* **6**, 19
3 Hulme, A C and Edney, K L (1960) in *Phenolics in Plants in Health and Disease* (Pridham, J B, ed) p 87 Pergamon Press, New York
4 Fleuriet, A and Deloire, A (1982) *Z Pflanzenphysiol* **107**, 259
5 Tronchet, J (1968) *C R Acad Sci, Paris, Ser D* **267**, 495
6 Wardale, D A (1973) *Phytochemistry* **12**, 1523
7 Maier, V P and Hasegawa, S (1970) *Phytochemistry* **9**, 139
8 Melin, C, Moulet, A M, Dupin, J F and Hartman, C (1977) *Phytochemistry* **16**, 75
9 Rhodes, M J C and Wooltorton, L S C (1977) *Phytochemistry* **16**, 655
10 Fleuriet, A and Macheix, J J (1981) *Phytochemistry* **20**, 667
11 Fleuriet, A and Macheix, J J (1980) *Phytochemistry* **19**, 1955
12 Laval-Martin, D (1974) *Protoplasma* **82**, 33
13 Hanson, K R and Havir, E A (1981) in *The Biochemistry of Plants* (Conn, E E, ed) Vol 7, p 577 Academic Press, New York
14 Zenk, M H (1979) in *Biochemistry of Plant Phenolics* (Swain, T, Harborne, J B and Van Sumere, C F, eds) p 139 Plenum Press, New York
15 Poulton, J E (1981) in *The Biochemistry of Plants* (Conn, E E, ed) Vol 7, p 668 Academic Press, New York
16 Fleuriet, A, Macheix, J J, Suen, R and Ibrahim, R K (1980) *Z Naturforsch* **35C**, 967
17 Fleuriet, A (1982) Thèse Doct (Etat) Sciences, Montpellier, France
18 Hulme, A C (1958) *Bull Soc Fr Physiol Veg* **4**, 43
19 Hahlbrock, K, Knobloch, K H, Kreuzaler, F K, Potts, J R M and Wellmann, E (1976) *Eur J Biochem* **61**, 199
20 Wiermann, R (1981) in *The Biochemistry of Plants* (Conn, E E, ed) Vol 7, p 85 Academic Press, New York
21 Ranjeva, R, Faggion, R and Boudet, A M (1975) *Physiol Veg* **13**, 725
22 Fritig, B, Hirth, L and Ourisson, G (1970) *Phytochemistry* **9**, 1963
23 Koumba-Koumba, D and Macheix, J J (1982) *Physiol Veg* **20**, 137
24 Macheix, J J, Suen, R and Ibrahim, R K (1981) *Biochem Physiol Pflanzen* **176**, 195
25 Poulton, J E and Kauer, M (1977) *Planta* **136**, 53
26 Fleuriet, A and Macheix, J J (1984) *Physiol Plant* **61**, 64
27 Yang, S F (1967) *Arch Biochem Biophys* **122**, 481
28 Lee, T T, Starratt, A N and Jevnikar, J J (1982) *Phytochemistry* **21**, 517
29 Lowry, O H, Rosebrough, N J, Farr, A L and Randall, R F (1951) *J Biol Chem* **193**, 265