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Determination of fascicular, interfascicular and cork cambia in dicotyledonous plants

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RÉSUMÉ

RANA, M. & P. GAHAN (1983). Détermination fasciculaire, interfasciculaire et cambium cortical des plantes dicotylédones. *Saussurea* 14: 51-60. En anglais, résumé français.

Une étude cytochimique quantitative des activités naphtol AS-D estérase des racines, tiges et pétioles de diverses dicotylédones a montré la présence de niveaux élevés de carboxylestérases dans le cambium fasciculaire et une aptitude de la détermination du cambium fasciculaire à former des stiles. Ces niveaux élevés de carboxylestérases peuvent également servir d'indicateur d'une tendance semblable des cellules du parenchyme à former le cambium interfasciculaire. Cependant, des études du cambium cortical ont montré que l'activité naphtol AS-D estérase y est plus élevée que dans les cellules du parenchyme adjacent, mais plus basse que dans le cambium vasculaire. Les études d'inhibition indiquent une implication probable des carboxylestérases (50%), des arylestérases (40%) et des acétylestérases (10%) dans le cambium cortical alors que le cambium vasculaire a 90% de carboxylestérases et 10% d'acétylestérases.

ABSTRACT

RANA, M. & P. GAHAN (1983). Determination of fascicular, interfascicular and cork cambia in dicotyledonous plants. *Saussurea* 14: 51-60. In English, French abstract.

A quantitative cytochemical study of the naphthol AS-D esterase activities of roots, stems and petioles of a variety of dicotyledonous plants has indicated the presence of high levels of carboxylesterases in the fascicular cambium, a feature of determination of the fascicular cambium to form stelar elements. High carboxylesterase activities may be used also as a marker of similar

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commitment of the parenchymal cells to form the interfascicular cambium. However, studies on the cork cambium showed that naphthol AS-D esterase activity is higher than that of adjacent parenchyma cells, but lower than that of the vascular cambium. Inhibitor studies indicate a likely involvement of carboxylesterases (50%), arylesterases (40%) and acetylesterases (10%) in the cork cambium as opposed to the vascular cambium which has about 90% carboxylesterases and 10% acetylesterases.

High carboxylesterase activity has been shown to be associated with the determination of root apical meristem cells to form vascular tissue in *Vicia faba* and *Pisum sativum* (GAHAN, 1981; RANA & GAHAN, 1982) and in the vascular bundles of *Taraxacum officinale, Ranunculus bulbosus, Ranunculus ficaria, Spinacia oleracea, Lycopersicon esculentum, Mimosa pudica, Hypericum calycinum* and *Salix* sp. (GAHAN, 1981; RANA, 1982). The fascicular cambium from those species examined so far, and which is considered to arise from the primary meristem (FAHN, 1974; ESAU, 1977), has high carboxylesterase activity in the cambial zone (GAHAN, 1981) so indicating that the fascicular cambium is determined (FAHN, 1974). If the high carboxylesterase activity is peculiar to the determined fascicular cambium, then a secondary meristem of similar function e.g. interfascicular cambium, would be expected also to show high carboxylesterase activity, whilst meristems of differing functions e.g. cork cambium, would not be expected to produce the same level of carboxylesterase activity.

The present communication concerns (a) the examination of a broader spectrum of tissues and species to determine if high carboxylesterase activity is a general marker of determined fascicular cambia; (b) to see if a high carboxylesterase activity may also be used as an early marker of determination for cells forming the interfascicular cambium, and (c) to compare levels of naphthol AS-D esterase activities in the intrafascicular and cork cambia.

Materials and methods

Pieces of roots, stems and petioles from a number of species (Tabl. 1 and 2) were sectioned transversely either with a razor or by freeze-sectioning (GAHAN & al., 1967). The unfixed razor-cut or frozen sections were reacted cytochemically at 37°C for 1 hour for esterase activity at pH 6.5 (McLEAN & GAHAN, 1969), using naphthol AS-D acetate as substrate and fast blue BB as the diazonium salt (RANA & GAHAN, 1983). Sections from phenol-rich plants e.g. *Platanus acerifolia, Tilia europaea* and *Sambucus nigra,* were pretreated with 0.05M diazotized aniline at pH 4.7 (Onyia, in preparation) for 15 min prior to incubation

for the enzyme activity so as to prevent the reaction between endogenous phenols and the diazonium salt of the esterase reaction. The pretreated sections were then reacted for esterase activity as described above.

As controls, sections of tissues from all species were incubated in the absence of the substrate, or heat-inactivated sections were reacted in the full reaction medium. In addition, sections of *Trifolium repens, Pisum sativum, Ligustrum ovalifolium* and *Hedera helix,* were incubated for 1 hour at 37°C in the full reaction medium containing either 10^{-4}M eserine or 10^{-4}M CMPS (p-chloromercuriphenylsulphonic acid). Sections from these species were also incubated for 1 hour in 0.2 m tris-HCl buffer at pH 6.5 with or without the addition of 10^{-4}M DFP (diisopropylfluorophosphate) for 1 h at room temperature followed by incubation for 1 h at 37°C in the full reaction medium with or without the addition of 10^{-4}M DFP.

Esterase activity was quantified with a Vickers M86 integrating microdensitometer (Vickers Instruments, York, U. K.) at a wavelength of 640nm, using a × 20 objective, scan spot No. 2 and diaphragm A2 (RANA, 1982; RANA & GAHAN, 1983), the results being presented as absolute integrated optical density units per square micrometer of reacting cytoplasm (AIOD/μm^2).

Results

Fascicular cambium

The naphthol AS-D esterase activity in the fascicular cambium of petioles and stems of all plants examined as found to be about three times (or more) higher than that in the pith parenchyma with the exception of stems from *Antirrhinum orontium, Sonchus oleracea, Ranunculus bulbosus,* and *Plantago major* where it was only 1.3-2.5 times (Tabl. 1 and 2; Fig. 1, 2 and 5). An increased esterase activity was also observed in the root vascular cambium of the plants investigated (Tabl. 1 and 2; Fig. 3).

Interfascicular cambium

The regions between vascular bundles did not show high esterase activity in the petioles and young stems of *Ricinus communis, Trifolium repens, Arachis hypogea* and *Pisum sativum,* but in older stems of the same plants, esterase activity appeared to be initiated adjacent to the vascular bundles and extended into the interfascicular parenchyma which often appeared to be undergoing cell division (Fig. 2) to produce the interfascicular cambium.

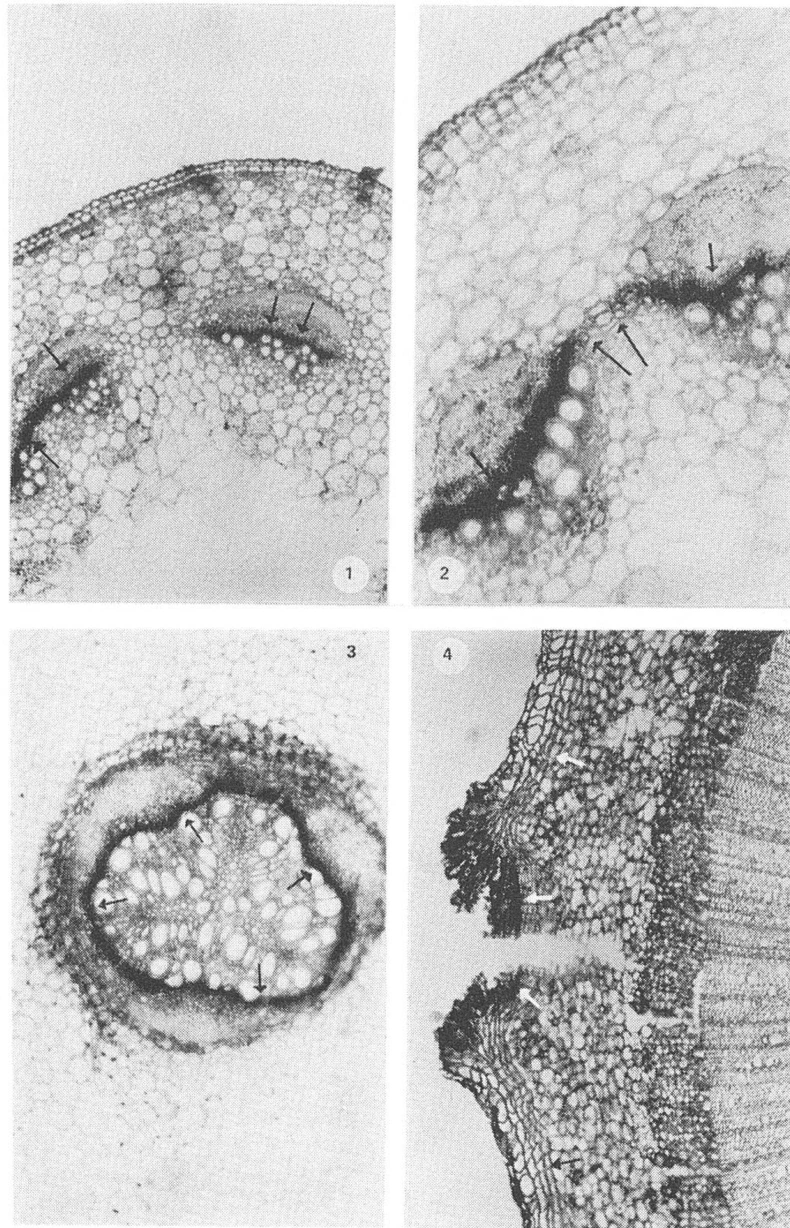

Fig. 1-4 are of unfixed, frozen, transverse sections incubated for 1 hour at 37°C for naphthol AS-D esterase activity.

1, stem from *Trifolium repens* showing high esterase activity (arrows) in the fascicular cambium. × 50. **2,** as in figure 1, but at a later stage of development showing high esterase activity in both fascicular (small arrows) and interfascicular (large arrows) cambia. × 100. **3,** twenty-one days old root of *Pisum sativum* showing high esterase activity (arrows) in cambium. × 60. **4,** three years old stem of *Ligustrum ovalifolium* showing esterase activity (arrows) associated with the cork cambium. × 50.

Plant species	*a*	*b*	*b/a*	*c*	*b/c*	*d*	*d/a*	*e*	*e/a*
Platanus acerifolia	0.09	0.35	4.0	0.13	2.6	0.25	2.8	–	–
Triplochiton scleroxylon	0.07	0.40	5.7	0.18	2.2	–	–	–	–
Tilia europae	0.10	0.51	5.1	0.33	1.5	–	–	–	–
Aesculus hippocastanum	0.08	0.35	4.3	–	–	–	–	–	–
Acer pseudoplatanus	0.06	0.25	4.1	0.11	2.2	–	–	–	–
Vicia faba	0.08	0.62	7.7	–	–	–	–	–	–
Pisum sativum	0.08	0.79	9.8	–	–	–	–	–	–
Hedera helix	0.07	0.25	3.5	0.17	1.4	–	–	0.23	3.2
Antirrhinum orontium	0.07	0.14	2.0	–	–	0.22	3.2	–	–
Lamium sp.	0.09	0.42	4.8	–	–	–	–	–	–
Sonchus oleraceae	0.05	0.14	2.5	–	–	–	–	–	–
Xanthium strumarium	0.08	0.37	4.6	–	–	0.17	2.1	0.28	3.5
Trifolium repens	0.07	0.51	7.3	–	–	–	–	–	–
Ligustrum ovalifolium	0.08	0.66	8.2	0.30	2.0	–	–	–	–

Table 1. – Esterase activity (AIOD/μm^2 = absolute optical density units per μm^2 of reacting cytoplasm) of secondary meristems in 15 μm frozen sections from stems, petioles and roots of various species.

a = esterase activity in stem pith parenchyma; *b* = esterase activity in stem fascicular cambium; *c* = esterase activity in cork cambium; *d* = esterase activity in root cambium; *e* = esterase activity in petiole fascicular cambium.

Plant species	*a*	*b*	*b/a*	*c*	*b/c*	*d*	*d/a*	*e*	*e/a*
Ranunculus spp.	0.19	0.25	1.3	–	–	–	–	–	–
Caltha palustris	0.21	0.96	4.5	–	–	–	–	0.62	7.7
Malva spp.	0.08	–	–	–	–	–	–	–	–
Chenopodium glaucum	0.06	0.32	5.3	–	–	–	–	–	–
Trifolium repens	0.08	0.94	11.75	–	–	–	–	–	–
Pisum sativum	–	–	–	–	–	0.81	10.1	–	–
Phaseolus vulgaris	0.06	0.44	7.3	–	–	0.59	9.8	–	–
Torilis spp.	0.17	0.89	5.2	–	–	–	–	–	–
Paeonia delrayi	0.11	0.97	8.8	–	–	–	–	0.84	7.6
Urtica dioica	0.12	0.93	8.4	–	–	–	–	–	–
Viola spp.	0.07	0.32	4.5	–	–	0.38	5.4	0.38	5.4
Ligustrum ovalifolium	0.11	0.52	4.7	0.17	3.0	–	–	–	–
Convolvulus spp.	0.16	0.88	5.5	–	–	–	–	–	–
Lycopersicon esculentum	0.07	0.53	7.5	–	–	–	–	0.69	9.8
Metha spp.	0.09	0.33	3.4	–	–	–	–	–	–
Plantago major	0.09	0.27	3.0	–	–	–	–	–	–
Sambucus nigra	0.08	0.84	10.5	–	–	–	–	0.68	8.5

Table 2. – Esterase activity (AIOD/μm^2) of secondary meristems in razor sections of various species.

a = esterase activity in stem pith parenchyma; *b* = esterase activity in stem fascicular cambium; *c* = esterase activity in cork cambium; *d* = esterase activity in root cambium; *e* = esterase activity in petiole fascicular cambium.

Species	Tissue	Total esterase (i)	B + esterase (ii)	10^{-4} M eserine (iii)	10^{-4} M CMPS (iv)	10^{-4} M DFP (v)
Trifolium repens	Pith Parench.	0.07±0.01	0.07±0.01	0.07±0.02	0.08±0.02	0.05±0.02
	Fascic. Cambium	0.51±0.14	0.56±0.12	0.45±0.13	0.56±0.11	0.07±0.01
Ligustrum ovalifolium	Pith Parench.	0.08±0.02	0.11±0.01	0.07±0.01	0.08±0.02	0.10±0.07
	Fascic. Cambium	0.66±0.11	0.58±0.14	0.51±0.03	0.51±0.05	0.18±0.01
	Cork	0.03±0.05	0.33±0.07	0.17±0.01	0.18±0.01	0.14±0.02
Hedera helix	Pith Parench.	0.09±0.02	0.08±0.03	0.08±0.01	0.08±0.01	0.08±0.02
	Fascic. Cambium	0.31±0.08	0.28±0.06	0.23±0.05	0.28±0.04	0.07±0.02
Pisum sativum	Pith Parench.	0.08±0.02	0.08±0.02	0.08±0.03	0.08±0.02	0.06±0.06
	Fascic. Cambium	0.37±0.05	0.38±0.03	0.31±0.05	0.32±0.06	0.07±0.06
	Interfasc. Cambium	0.25±0.07	0.24±0.05	0.23±0.05	0.22±0.03	0.06±0.06

Table 3. – Effects of 10^{-4} M eserine, CMPS and DFP on naphthol AS-D esterase activity. Activity of stem tissues expressed as AIOD/μm² ± standard deviation (mean of 10 measurements from sections of each of 4 specimens).

(i) Incubation for 1 h at 37°C in the full reaction medium. *(ii)* Pretreatment of sections with 0.02M tris-HCl buffer (pH 6.5) for 1 h at room temperature prior to incubation as in *(i)* above. *(iii)* Incubation for 1 h 37°C in full reaction medium containing 10^{-4} M eserine. *(iv)* As in *(iii)* but with 10^{-4} M CMPS. *(v)* As in *(iii)* but with 10^{-4} M DFP.

Cork cambium

The esterase activity in the cork cambium of the stems examined was found to be about two or more times less than that of the fascicular cambium, except in *Tilia europaea* and *Hedera helix* where it was about 1.5 times less (Tabl. 1 and 2; Fig. 4).

Controls

No reaction product was seen in sections incubated in the absence of substrate or in sections treated with boiling water prior to reacting in the complete reaction medium.

When DFP, eserine and CMPS were added to the reaction mixture, there was no measurable effect on the esterase activity of the pith parenchyma. In contrast, DFP gave 65-90% inhibition of the esterase

activity in the interfascicular cambium (Tabl. 3), whilst no inhibition of this activity was measurable with eserine and CMPS in this tissue in *Trifolium repens* and *Ligustrum ovalifolium.* A small (ca. 25%) inhibition of the fascicular cambial activity was given by eserine, but not by CMPS in *Pisum sativum,* whilst an almost complete inhibition of the interfascicular cambial esterase was observed with DFP, there being no effect measurable with both eserine and CMPS. The cork cambium of *Ligustrum ovalifolium* gave about a 50% inhibition with each of the inhibitors.

Discussion

The root vascular cambium and the fascicular cambium of petioles and stems showed high esterase activity in both frozen and razor-cut sections (Fig. 1 and 2; Tabl. 1 and 2). Although the razor-sectioning has the advantage of allowing the application of cytochemical tests immediately after removing the tissue from the plant in the field, variations of section thicknesses of both between and within sections do not permit accurate quantification of the reaction. However, razor-cut sections do give some information concerning the level of activity in adjacent groups of cells within a given section (Fig. 1 and 2).

Higher esterase activity was present in all cells between the mature xylem and phloem, such cells constituting a zone containing both cambium and differentiating stele elements. BANAN (1955) identified a single layer of cambial initial cells between xylem and phloem mother cells in *Thuja occidentalis* L. through their dividing less often than their derivatives whilst NEWMAN (1956) identified a single layer of cambial initial cells in *Pinus radiata* by their way of partitioning and wall thickness. However, CATESSON (1969) considered that individual initials could not be distinguished and that the cambium should be referred to as a cambial zone formed from several layers of similar cells, each endowed with the properties of initials with an equivalent power of multiplication. The presence of high esterase activity in all cells between the mature xylem and phloem would indicate that cambial initials (present as either a single layer or multi-layered) are committed cells. There appear to be no identifiable, uncommitted initials for the production of the secondary stele, all cells of the procambial cylinder showing a high carboxylesterase activity and hence are already determined (GAHAN, 1981; RANA, 1982).

This would confirm the idea that two programming steps, at least, are required for the production of stelar elements, namely:

a) a coarse or general programming initiated in the procambial tissue and passed on to the initials for secondary stele as they are formed, and identified by their high carboxylesterase activity;

b) a fine programming step (or steps) which indicate(s) the coarse-programmed initials of the secondary stele to make the elements of the secondary xylem and phloem.

FAHN (1974) and ESAU (1977) have suggested that the interfascicular cambium is not a continuation of the procambium, but develops from the interfascicular parenchyma. Thus, high esterase activity should appear in the interfascicular parenchymal cells only when they are about to make interfascicular cambium. SEIBERS (1971a, b; 1972) found activation of the interfascicular cambium and the differentiation of xylem to occur in interfascicular tissue isolated from *Ricinus communis* and cultured on a basal medium lacking auxin. He concluded that this meristem was determined at a very young stage during procambium development and was not dependent upon homogenetic induction from the fascicular cambium. In contrast, the results obtained in the present study on young and old stems of *Trifolium repens, Ricinus communis, Arachis hypogea* and *Pisum sativum* would indicate that high esterase activity was not present in the interfascicular parenchyma of young stems (Fig. 1), whilst in older stems, high esterase activity similar to that of the vascular cambium (Tabl. 3) was observed in the interfascicular parenchyma which was undergoing frequent cell divisions to produce the interfascicular cambium (Fig. 2). Thus, an increase in the esterase activity in the interfascicular parenchyma would seem to indicate commitment of these cells to form interfascicular cambium. These findings are in disagreement with the idea that commitment of this meristem occurs at a very early stage (SIEBERG, 1971a). However, it should be noted that cambial activity in young explants of *Ricinus communis* does require the addition of Kinetin to the culture for this activity to be triggered (SIEBERS, 1971b). The present results concur with those of FAHN (1974) and ESAU (1977) that interfascicular cambium develops from interfascicular parenchyma. Moreover, the signal to differentiate would seem to emanate from the adjacent fascicular cambium.

Cork cambium of the stems investigated showed 2-3 times less activity than the vascular cambium, except in *Hedera helix* and *Tilia europaea* where it was only 1.5 times less (Tabl. 1 and 2; Fig. 4). There was a different inhibitor response by esterases from the cork cambium and the vascular cambium with almost half of the cork cambium activity being sensitive to each inhibitor. In general, almost all cell types examined contained a basal level of acetylesterases i.e. about 10% of total esterases in the vascular cambium. These data may be interpreted such that if the DFP-sensitive enzyme is also resistant to eserine and CMPS, than half the observed activity will be due to carboxylesterases and the remaining 40% to arylesterases (HOLMES & MASTERS, 1967; GAHAN & al., 1983). The difference in the activities and responses to inhibitors by esterases in the cork cambium appeared to represent a functional difference in the tissue from which the cork cambium could originate

i.e. epidermis, collenchyma or parenchyma cells of the cortex, pericycle or phloem parenchyma (FAHN, 1974). Thus, the esterases present in the cork cambium probably included both the pre-existing esterases present in the mother cells together with esterases which may be formed to aid with new cell wall modifications (GAHAN, 1981). Equally, the presence of lower amounts of esterase activity in the cork cambium may be an expression of the functional differences between cork and vascular cambia.

The foregoing data would be in accord with the concept that the high level of activity of DFP-sensitive, eserine and CMPS resistant esterase may be a merker of cells which are commited to form elements of the stele.

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