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Role of Auxin and Sucrose in the Differentiation of Sieve and Tracheary Elements in Plant Tissue Cultures

Roni Aloni
Department of Botany, Tel Aviv University, The George S. Wise Faculty of Life Sciences, Tel Aviv 69978, Israel

Abstract. The differentiation of sieve and tracheary elements was studied in callus culture of *Daucus carota* L., *Syringa vulgaris* L., *Glycine max* (L.) Merr., *Helianthus annuus* L., *Hibiscus cannabinus* L. and *Pisum sativum* L. By the lacmoid clearing technique it was found that development of the phloem commenced before that of the xylem. In not one of the calluses was differentiation of tracheary elements observed in the absence of sieve elements. The influence of indole-3-acetic acid (IAA) and sucrose was evaluated quantitatively in callus of *Syringa, Daucus* and *Glycine*. Low IAA levels resulted in the differentiation of sieve elements with no tracheary cells. High levels resulted in that of both phloem and xylem. IAA thus controlled the number of sieve and tracheary elements, increase in auxin concentration boosting the number of both cell types. Changes in sucrose concentration, while the IAA concentration was kept constant, did not have a specific effect on either sieve element differentiation, or on the ratio between phloem and xylem. Sucrose did, however, affect the quantity of callose deposited on the sieve plates, because increase in the sucrose concentration resulted in an increase in the amount of callose. It is proposed that phloem is formed in response to auxin, while xylem is formed in response to auxin together with some added factor which reaches it from the phloem.

Key words: Auxin – Callus culture – Phloem differentiation – Sucrose – Xylem differentiation.

Introduction

It is widely believed that auxin and sugar are vital for controlling the differentiation of xylem and phloem (see books by Esau 1965, pp. 103–107 and Cutter 1978, pp. 156–169 and pp. 191–194). However, the evidence in the literature from in vivo and in vitro studies is rather confusing. In *Coleus* stems, Jacobs (1952) showed that the auxin indole-3-acetic acid (IAA), formed in young leaves, was the normal limiting factor for xylem regeneration. Subsequently La-Motte and Jacobs (1963) found that IAA was the limiting factor in phloem regeneration and that sucrose did not affect phloem regeneration in *Coleus* internodes. Thompson and Jacobs (1966) confirmed the earlier findings indicating that IAA was the common controlling factor in both phloem and xylem regeneration.

In tissue culture, Wetmore and Rier (1963) reported that the differentiation of phloem and xylem induced by buds grafted into the callus of *Syringa* was fully replaceable by sugar plus auxin. They were unable to observe the correlation between auxin concentration and the quantity of vascular tissue, which had been described for *Coleus* stems. With auxin concentration kept constant, 1.5–2.5% sucrose sufficed to induce strong xylem differentiation with little or no accompanying phloem differentiation, whereas differentiation of phloem with little or no xylem differentiation was obtained with 3–4% sucrose. Intermediate sucrose concentrations (2.5–3.5%) favoured the production of xylem and phloem, usually with cambium in-between. Experiments with fern prothalli showed that at low sugar concentrations (1.5–3%) xylem was formed, while at higher concentrations (4.5–5%) phloem was differentiated (Wetmore et al. 1964). However, the results of Rier and Beslow (1967) with callus of *Parthenocissus* appear more contradictory than supportive of these findings, for the number of xylem elements was directly proportional to the sucrose concentration in the medium up to 8%. These latter results were confirmed quantitatively in excised *Coleus* internodes (Beslow and Rier 1969) and in cultured tuber tissue of *Helianthus* (Minocha and Halperin 1974). Unfortunately, in none of these studies

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was attention paid to phloem differentiation and its possible relevance for the sugar-auxin hypothesis. In *Phaseolus,* the most advanced stage of differentiation of both phloem and xylem was obtained with 2% sucrose (Jeffs and Northcote 1966). Judging from the results of Wetmore and associates, this sugar concentration is rather low and one would expect it to favour xylem formation only. Cronshaw and Anderson (1971) were unable to induce the differentiation of sieve elements with 4% sucrose without also inducing the formation of xylem cells in stem-pith cultures of *Nicotiana.*

Jeffs and Northcote (1967) found that maltose, trehalose and sucrose, but not various other sugars induced nodules in *Phaseolus* which contained both phloem and xylem, and suggested that the three α-glucosyl disaccharides just mentioned exerted a specific activity on vascular differentiation in addition to their value as a carbon source. Minocha and Halperin (1974) reported a similar effect of these disaccharides on the differentiation of xylem elements in callus of *Helianthus,* adding also glucose, a monosaccharide, to this category; comparing growth to differentiation they found that the effect of the three disaccharides was not specific for vascular differentiation.

While it is possible that differences in the results of different authors stem from the use of different systems or species, nevertheless there are some doubts as to whether the role of sugar as a controlling factor in vascular differentiation is invariably applicable. On the other hand, auxin has been shown to be a stimulus and a limiting factor of vascular differentiation in the intact plant, but its role in the differentiation of phloem per se or of phloem in relation to xylem has not been thoroughly investigated in tissue culture.

A major problem when studying the differentiation of phloem in tissue culture is the difficulty of its detection. Jacobs (1970, pp. 265) in his review on phloem differentiation, emphasized this fact by noting that "We should remember that there are special difficulties in trying to study sievetube differentiation in callus culture: not only are the cells apt to be very small, but they do not form such predictable locations as in regenerating internodes, nor do they usually differentiate as a strand or sievetube elements. The difficulty of searching through a whole callus for a few cells that might show sieve plates or slime plugs undoubtedly explains why the work published so far has not included actual counts of sieve elements, nor shown a photograph of recognizable sieve elements induced by chemicals." To overcome this technical difficulty and to accomplish a quantitative study of phloem differentiation, I have made use of the lacmoid clearing technique (Aloni and Sachs 1973), which permits a quick search of both phloem and xylem throughout the whole callus. A thin-section method was employed in addition.

The work reported here was designed to elucidate quantitatively the role of IAA in the differentiation of phloem and xylem in tissue culture and to re-investigate the role of sucrose in this process. A preliminary account on some of the results has been published previously (Aloni 1979 b).

Materials and Methods

Plant Material and Preparation of Explants. Callus tissues of *Daucus carota* L., *Syringa vulgaris* L., *Glycine max* (L.) Merr., *Helianthus annuus* L., *Hibiscus cannabinus* L. and *Pisum sativum* L. were used in this study. The callus of *Daucus* was obtained from the cambial region of the root, that of *Syringa* from the cambial region of the stem and those of *Glycine, Helianthus, Hibiscus* and *Pisum* from the first internode of sterilized seedlings. The explants and the seeds were sterilized with 5% sodium hypochlorite for 15 min and were rinsed repeatedly with sterile distilled water. The explants were placed in culture tubes (25 mm diameter, 75 mm long) containing 8 ml of Murashige and Skoog's (1962) medium.

Culture Conditions. The callus tissues were maintained at a temperature of $25 \pm 1°$ C, either in darkness throughout or exposed for 12 h daily to 2.5 W m^{-2} of light from F15 T12-CW cool-white fluorescent lamps (Sylvania, Seneca Falls, N.Y., USA). All experiments on the effect of IAA (Merck, Darmstadt, Germany) or sucrose (BDH Chemicals, Poole, U.K.; No. 10274) were conducted entirely in the dark in order to prevent possible change in sugar level by photosynthesis and a destruction of IAA by light.

Experimental Techniques. After the callus tissues were well established, they were subcultured every three or six weeks, unless otherwise specified. Callus tissues were used for experiments after a subculture on 0.05 mg/l IAA and 2% (w/v) sucrose in darkness, in order to obtain a homogeneous parenchyma tissue. In the experiments small uniform blocks of tissue, averaging 9–12 mg fresh weight, were cut from the surface of the callus. The cultured tissues were harvested at various intervals during the experiment or at the end of the 21-d culture period, unless otherwise stated.

In each experiment five callus tissues were used per treatment. All major experiments were repeated three to five times with each of the different species. All six species listed above were used to study the development and pattern of phloem and xylem differentiation. The experiments on the effect of IAA and sucrose on vascular formation were performed with *Glycine, Syringa* and *Daucus.*

Preparation of Specimens for Microscopic Study. Two techniques were used regularly for studying the differentiation of the vascular elements. The callus tissue was fixed with FAA (formalin-alcohol-acetic acid) fixation fluid. Half of the callus was used to prepare thick longitudinal hand sections (at 1–3 mm). These were cleared in pure lactic acid at 50° C for 5–20 min. The sections were allowed to cool at least 1 h, stained at room temperature with a 0.2% solution of lacmoid in lactic acid for about 0.5 h, then rinsed in water until the tissue (which was red in the acid) became blue. (The lacmoid was obtained from Eastman Kodak Co., Rochester, N.Y., USA; the solutions were prepared by prolonged stirring and were kept only one month at a temperature of 4° C). At this point, the sections were transferred to 60% sodium lactate for observations (modified from Aloni and Sachs, 1973). From the second half of the callus tissue, thin longitudinal hand sections were prepared. The sections were stained with 2% lacmoid in

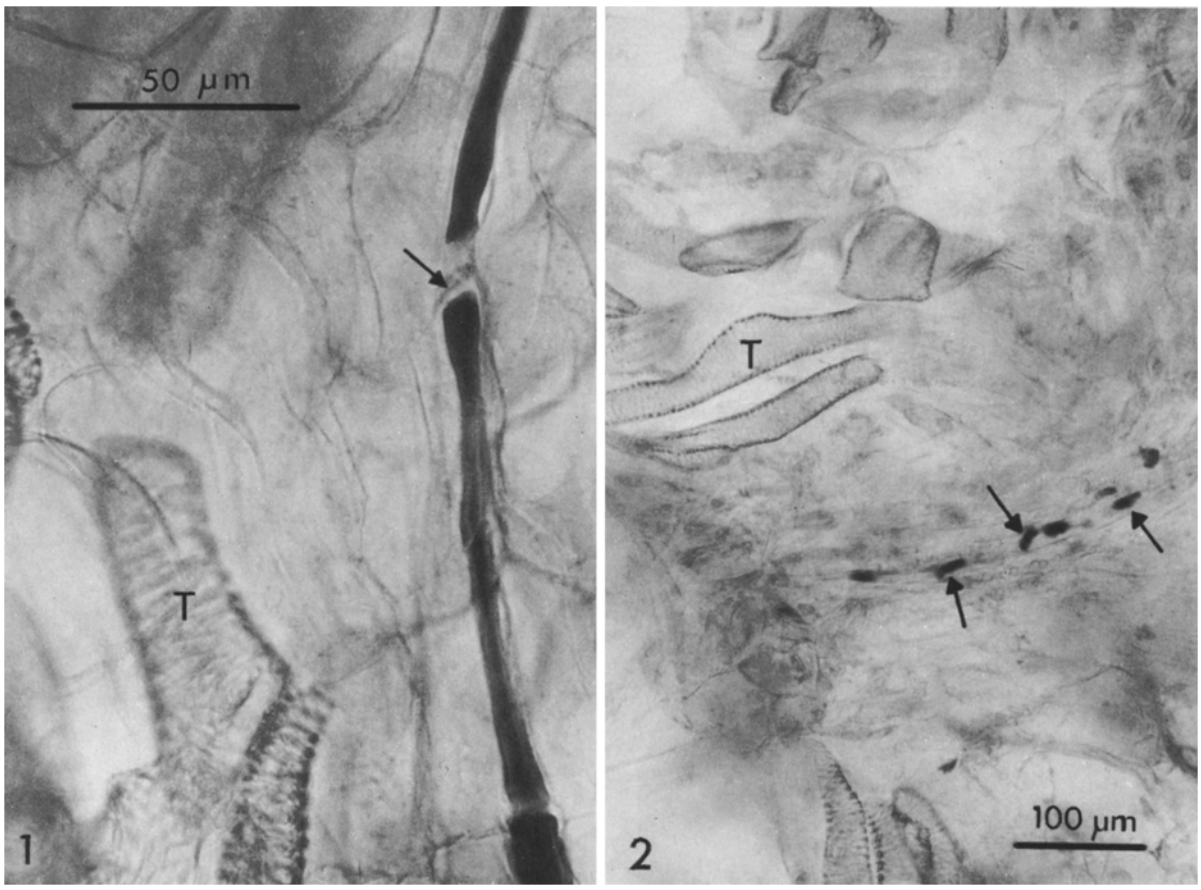
96% ethanol for 2–5 s, washed with tap water, and then transferred to 60% sodium lactate for observation (Aloni 1976).

Measurements. The sieve and tracheary elements were counted under a light microscope. The area to be counted was defined as the field of vision at 100-fold magnification. In each callus an average of the number of sieve and tracheary elements or the type of callose was calculated from three random counting areas. The amount of callose was categorized according to Peterson and Rauser (1979) as follows: (a) Interporous-evidence of callose is limited to the pores of the sieve plates (Fig. 1), (b) thin-callose evident within the sieve plate pores and also as a thin covering over the plate surfaces (Fig. 6), (c) medium-callose within the sieve plate pores and also as a thick layer distributed over the plate surfaces (Fig. 7), (d) thick-callose evident as a thick dome-shaped deposit over the sieve plate surfaces (Fig. 7). All counts and measurements were made from coded slides to eliminate possible subconscious bias (Aloni 1979a).

Statistical analysis and test of significance were by Student's t-test for the differentiation of sieve and tracheary elements (Tables 1, 3), and by the G-test of independence according to Sokal and Rohlf (1969) for the deposition of callose on the sieve plates (Table 2).

Results

Development and Pattern of Vascular Differentiation in Callus. Callus tissues which were growing on medium containing low auxin concentrations (0.03 or 0.05 mg/l) exhibited very slow growth and consisted of homogeneous parenchyma cells without vascular differentiation, in all six species examined. When such callus was transferred to a medium with 1.0 mg/l IAA, random centers of mitotic activity started appearing in the tissue. In the periphery of the callus, large parenchyma cells developed from cells at the surface of the tissue, expanding the callus by additional cell divisions. Differentiating vascular elements could be detected in the callus 5 d after the transfer. The first vascular elements to differentiate were sieve elements, recognizable either by the presence of callose in their sieve plates, or by blue-stained cytoplasm, probably involving a reaction of the P-protein (Fig. 1; see also



Figs. 1 and 2. Photographs of vascular elements in thick, cleared and stained tissue culture preparation of 21-d old callus

Fig. 1. *Syringa vulgaris.* Longitudinal view of a continuous file of sieve elements (right) and tracheary cells (left). The cytoplasm of the sieve elements was deeply stained. The arrow marks one of the sieve plates with relatively small amount of callose, classified as interporous. The tissue developed on 1 mg/l IAA and 1% sucrose, T, tracheary element. ×600

Fig. 2. *Daucus carota.* Pattern of differentiation of sieve and tracheary elements. The sieve elements are recognizable by their plates bearing relatively large amounts of callose (arrows mark some of these sieve plates), the tracheary elements by their reticulated secondary wall thickening. The tissue developed on 0.5 mg/l IAA and 4% sucrose. ×170

Aloni and Sachs 1973, Figs. 7 and 8). A day or two later, tracheary cells developed in some of the nodules of the phloem, identifiable by their reticulate secondary wall thickenings (Figs. 1, 2). It should be emphasized that in none of the calluses did tracheary elements differentiate in the absence of sieve elements. This was invariably true in more than a total of one thousand callus tissue samples of the six species grown on various combinations of auxin and sugar, in darkness as well as in light.

In a longitudinal section through a callus grown at a high auxin level (1.0 mg/l IAA) two concentric zones could be distinguished: a peripheral zone comprised of newly developed nodules of sieve elements with no tracheary elements, or of nodules of phloem with little xylem; and a central zone made up of nodules or short strands of well-developed phloem and xylem (Fig. 3). Closed rings of either sieve or tracheary elements were also discerned. The vascular nodules and strands were haphazard in arrangement and orientation, but in some of the fast-growing callus tissues the vascular elements had a longitudinal orientation parallel to the general axis of the main growth. Xylem was characteristically oriented towards the

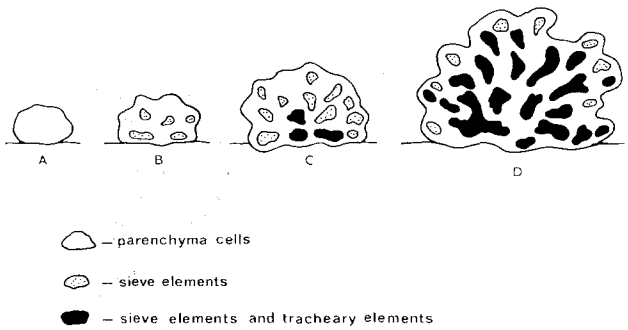


Fig. 3A–D. Diagrams illustrating stages in the pattern of differentiation of phloem and xylem in tissue culture. **A** A callus tissue consisting of homogeneous parenchyma cells with no vascular elements, grown on low-auxin medium (0.03 or 0.05 mg/l IAA). **B** Nodules of sieve element with no tracheary cells developed either on medium containing a low auxin level (0.1 mg/l IAA), or at an early stage of vascular differentiation after transfer of homogeneous parenchyma to a high-auxin medium (0.1 mg/l IAA). **C** Intermediate stage in which tracheary elements start to appear in some of the nodules of phloem located in the center of the callus. **D** Typical pattern of callus tissue grown at high auxin level (0.5 or 1.0 mg/l IAA). In the periphery of the callus there are either newly developed nodules of phloem with no xylem, or nodules of phloem with little xylem. The center of the callus comprises nodules or short strands of well-developed phloem and xylem

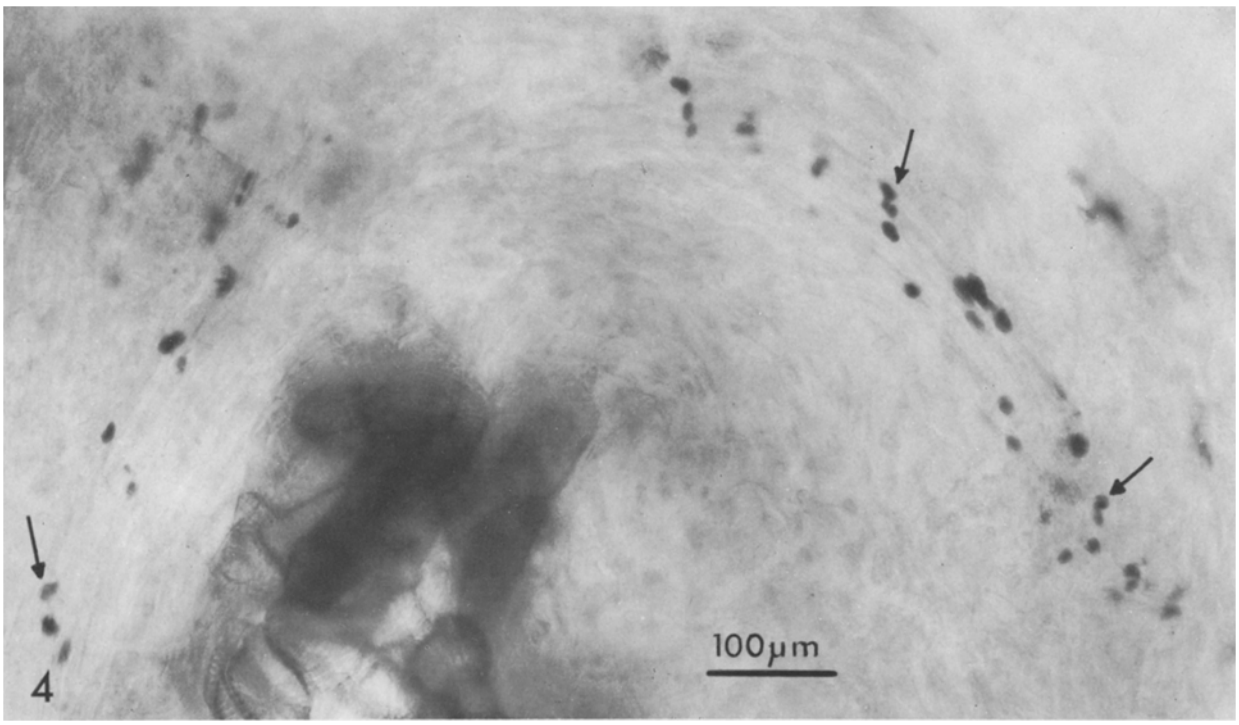


Fig. 4. Photograph of thick, cleared and stained tissue culture of *Glycine max* after 21 d on 1 mg/l IAA and 6% sucrose. The figure shows a typical pattern of differentiation in a nodule. The tracheary elements are in the centre of the nodule (lower centre) surrounded by the sieve elements. Arrows mark some groups of sieve plates. × 170

Table 1. Effect of IAA on the differentiation of sieve and tracheary elements in tissue culture of *Syringa vulgaris* and *Daucus carota*. Values are mean ± standard error of the number of vascular elements in the counted area (100-fold magnification). Sample size was five in all cases. The concentration of sucrose in the two experiments was 3% (w/v)

Treatment		A	B	C	D	E
IAA concentration (mg/l)		0.05	0.1	0.25	0.5	1.0
Syringa[a]	No. of sieve elements	0±0	18.4±3.9	26.6±4.0	30.6±3.5	37.4±3.2
	No. of tracheary elements	0±0	0.4±0.4	14.8±3.9	21.0±3.4	29.6±5.0
Daucus[b]	No. of sieve elements	0±0	26.8±3.6	31.4±3.4	43.2±5.0	46.6±4.9
	No. of tracheary elements	0±0	0±0	24.8±3.1	35.2±3.8	50.8±4.2

[a] In *Syringa*, the difference between the number of sieve elements in treatments B and D was significant ($P < 0.05$), and that between treatments B and E highly significant ($P < 0.01$). Likewise, the difference between the number of tracheary elements in treatments C and E was significant ($P < 0.05$), and that between C and B highly significant ($P < 0.01$)

[b] In *Daucus*, the difference between the number of sieve elements in treatments B and D was significant ($P < 0.05$), and that between B and E highly significant ($P < 0.01$). The difference between the number of tracheary elements between treatments C and E was highly significant ($P < 0.01$), and that between D and E significant ($P < 0.05$)

center of the callus or the center of the nodules, whereas the phloem was oriented towards the periphery and usually surrounded the xylem (Fig. 4).

The Effect of Indoleacetic Acid. IAA was found to stimulate the differentiation of both sieve and tracheary elements. Figure 5 and Table 1 ilustrate the response of the tissue to varying concentrations of auxin. At 0.1 mg/l IAA, many fully differentiated sieve elements developed with usually no tracheary cells. In *Syringa* and *Glycine*, more than half of these callus tissues continued to develop only sieve elements for about three months when transferred (every four weeks) to the same medium (0.1 mg/l IAA). A few tracheary elements differentiated as well in the rest of the calluses which were generally larger than those with only phloem. At 0.25 mg/l IAA and at higher auxin concentrations xylem elements developed. Increase in auxin concentration boosted the number of both cell types.

Auxin also exerted an effect on the growth of the callus of *Syringa, Glycine* and *Daucus*, increase in IAA concentration usually resulting in an increase in the final size of the callus. The biggest callus tissues were also the hardest probably because of stronger cell adhesion.

The Effect of Sucrose. Sucrose was found to influence the callose formation on the sieve plates (Figs. 6, 7). Sucrose at 1% produced the lowest amounts of callose. In three of the nine experiments examined (two in *Glycine* and one in *Daucus)*, the amount of callose formed on the sieve plates at the low sucrose concentrations was not different from the quantity deposited at the high sucrose concentrations. In the remaining six experiments, increase in the sugar level within the concentration range of 1–3% usually resulted in an increase in the amount of callose (Table 2). At

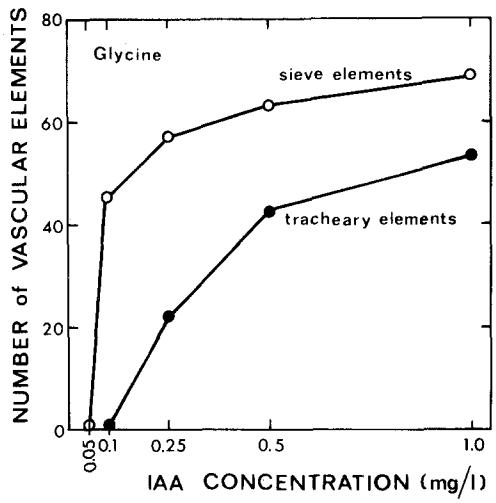
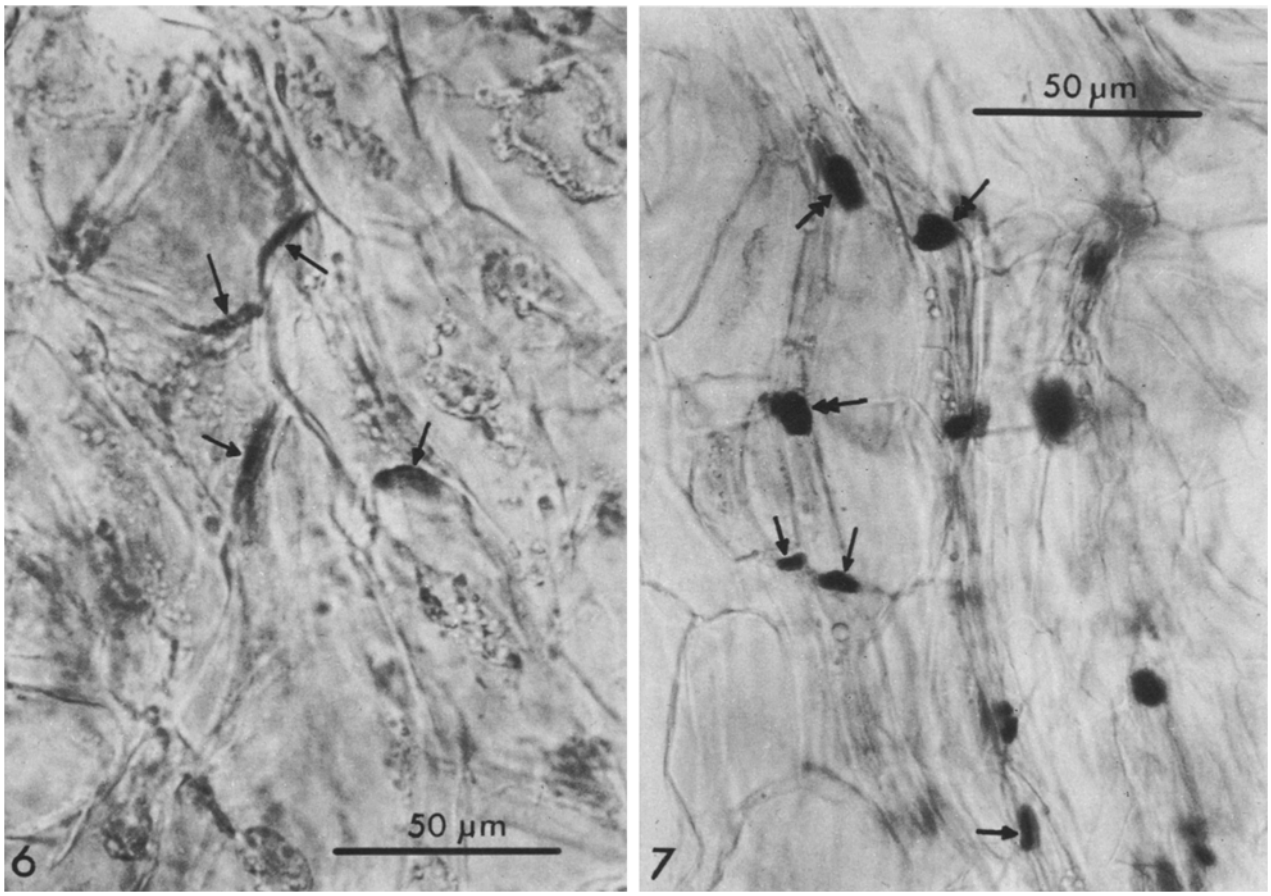


Fig. 5. Effect of IAA on the differentiation of sieve and tracheary elements in tissue culture of *Glycine max* after 21 d. Sucrose concentration was 3%. Each point represents an average of three replicates from five callus tissues

1% sucrose, the sieve plates in the peripheral zone of the callus were difficult to detect because of the paucity of callose. However, nodules with relatively high amounts of callose on the sieve plates were found in some of the callus tissues grown at the low sugar concentrations. These nodules were located in the center of the callus and probably differentiated from the original piece of tissue which gave rise to the callus. In callus tissues grown on high sugar concentrations relatively large amounts of callose were deposited on the sieve plates both in the periphery as well as in the center of the callus (Fig. 7).

Changes in the sucrose concentration, while auxin concentration was kept constant, had no effect on the ratio between phloem and xylem (Table 3). Sucrose at 1% produced the lowest number of both



Figs. 6 and 7. Photographs of sieve elements in thick, cleared and stained tissue cultures of *Daucus carota* after 21 d of growth at different sucrose concentrations (IAA concentration, 1 mg/l). Arrows mark some of the sieve plates

Fig. 6. Small amounts of callose deposited on the sieve plates at 2% sucrose. Callose deposition classified as thin. ×600

Fig. 7. Larger amounts of callose formed on the sieve plates at 6% sucrose. Callose deposition classified as medium is marked by a single-headed arrow, and callose deposition classified as thick is marked by a double-headed arrow. ×600

sieve and tracheary elements. In *Syringa,* there were no significant differences in the number of either sieve or tracheary elements under any of the sucrose concentrations used (Table 3). However, in *Daucus* and *Glycine,* increase in sucrose concentration yielded an increase in the number of both cell types. In the latter species the high averages of sieve or tracheary elements in 4 and 6% sucrose were not significantly different by the t-test from the appropriate 2% sucrose averages (Table 3).

In *Glycine* and *Daucus,* the size of the callus was roughly related to the sugar concentration in the medium. Within a concentration range of 1–4%, increase in the sucrose level yielded increase in the dimensions of the callus. Histological examination of the callus tissues grown at the various sucrose concentrations demonstrated a larger overall number of vascular elements in the big calluses than in the small ones. Within the range of sucrose concentrations studied, no changes were noted in the proportions of phloem and xylem when the auxin concentration was kept constant.

Discussion

The quantitative results of the present study are in contradiction to the hypothesis of Wetmore and Rier (1963) that low sugar concentrations favour xylem formation, whereas high sugar concentrations favour phloem differentiation. It must be stated that the Wetmore and Rier's hypothesis was based on subjective estimations using only thin sections. My study shows that in tissue cultures auxin controls the differentiation of both phloem and xylem. However, an IAA-sucrose interaction cannot be ruled out as sucrose

Table 2. Effect of sucrose on the deposition of callose on the sieve plates in tissue culture of *Syringa vulgaris, Daucus carota* and *Glycine max*. The incidence of each type of callose is reported as a percentage of the total number of sieve plates examined in five callus tissues per treatment. The concentration of IAA was 1 mg/l throughout

Species	Callose deposition[a]	Treatment (sucrose concentration, % w/v)				
		A (1.0)	B (2.0)	C (3.0)	D (4.0)	E (6.0)
Syringa	Interporous	92.7	86.9	72.1	71.7	70.5
	Thin	7.3	12.3	24.4	25.1	23.9
	Medium	0	0.6	3.5	2.4	5.2
	Thick	0	0.2	0	0.8	0.4
	(No. of sieve plates examined)	(479)	(493)	(515)	(488)	(532)
Daucus	Interporous	78.1	71.1	65.7	66.8	61.5
	Thin	21.6	26.5	29.1	27.3	29.7
	Medium	0.3	2.4	2.9	4.1	6.4
	Thick	0	0	2.3	1.8	2.4
	(No. of sieve plates examined)	(557)	(582)	(653)	(612)	(624)
Glycine	Interporous	86.8	81.0	76.9	77.0	78.9
	Thin	11.4	15.6	17.5	19.3	15.2
	Medium	1.8	3.2	1.9	2.5	3.6
	Thick	0	0.2	3.7	1.2	2.3
	(No. of sieve plates examined)	(746)	(804)	(785)	(789)	(830)

[a] In *Syringa, Daucus* and *Glycine*, the difference between the amount of callose in treatment A and that in treatments B, C, D and E was highly significant ($P < 0.01$). This was also true for the difference between the amount of callose under treatment B and that in treatments C, D and E ($P < 0.01$)

Table 3. Effect of sucrose on the differentiation of sieve and tracheary elements in tissue culture of *Syringa vulgaris, Daucus carota* and *Glycine max*. Values are mean number ± standard error of vascular elements in the counted area (100-fold magnification). Sample size was five throughout. The concentration of IAA in these experiments was 1 mg/l. All the cultures were harvested after 21 d

Species		Treatment (suxrose concentration, % w/v)				
		A (1.0)	B (2.0)	C (3.0)	D (4.0)	E (6.0)
Syringa[a]	No. of sieve elements	27.8 ± 3.5	37.2 ± 4.3	33.2 ± 5.2	31.2 ± 4.8	30.8 ± 3.7
	No. of tracheary elements	23.4 ± 3.0	35.8 ± 4.7	25.2 ± 2.8	34.6 ± 4.9	26.0 ± 4.2
Daucus[b]	No. of sieve elements	31.8 ± 5.8	39.0 ± 4.9	49.6 ± 3.7	52.4 ± 5.9	54.6 ± 6.7
	No. of tracheary elements	32.6 ± 6.4	46.2 ± 4.4	53.6 ± 5.6	50.8 ± 4.9	55.8 ± 6.0
Glycine[c]	No. of sieve elements	60.8 ± 4.4	70.2 ± 8.7	64.0 ± 6.5	75.6 ± 9.4	81.6 ± 6.7
	No. of tracheary elements	53.5 ± 3.6	59.4 ± 3.4	63.8 ± 5.4	72.6 ± 8.8	93.2 ± 16.4

[a] There were no significant differences in the numbers of sieve elements and of tracheary elements under any of the five treatments in *Syringa*

[b] In *Daucus*, the difference between the number of sieve elements in treatment A and those in treatments C, D and E was significant ($P < 0.05$), and this was also true with regard to tracheary elements between treatment A and treatments C and E

[c] In *Glycine*, the difference between the number of sieve elements under treatment A and treatment E was significant ($P < 0.05$), and this was also true for the difference between the number of tracheary elements under treatments A and E

was present in all media. Furthermore, my results show a positive correlation between sucrose level and the number of sieve elements formed in callus of *Glycine* and *Daucus*, similar to that which was found for the xylem. These findings indicate that, within the range of sucrose concentrations used, sucrose does not determine the type of vascular element formed in callus cultures.

My results are the first to illustrate that IAA controls sieve element differentiation in tissue culture, showing a positive correlation between the concentration of auxin in the medium and the number of sieve elements formed in the tissue. The present results, obtained with callus of *Syringa, Daucus* and *Glycine*, disagree with those of Wetmore and Rier (1963) who were unable to observe any correlation between auxin concentration and the quantity of sieve elements induced in callus of *Syringa*. My results show that low IAA levels favour the differentiation of phloem with no xylem. To induce xylem differentiation there is a need for relatively high auxin concentration; an increase in auxin concentration increased the number

of tracheary elements proportionally. These results confirm the results of Minocha and Halperin (1974) that auxin controls quantitatively the differentiation of xylem elements in cultured tuber slices of *Helianthus*, and those of Jacobs' laboratory (Jacobs 1952; LaMotte and Jacobs 1963; Thompson and Jacobs 1966) which show a positive correlation between auxin concentration and the quantity of vascular tissue in stem internodes of *Coleus*. They provide parallel evidence for the role of IAA as the limiting and controlling factor in phloem and xylem differentiation in both in vitro and in vivo systems.

The present study demonstrates that sucrose concentration influences the amount of callose deposited on the sieve plates. As sucrose is known to be the chief source of callose (Eschrich 1961; see also review by Clark and Stone 1963), this result is to be expected. In tissue culture the pattern of vascular differentiation is not predictable and consequently the callose becomes a major indicator of sieve elements in any light-microscope study. In view of the finding that low sucrose concentrations yielded low quantities of callose in tissue culture, it would seem that when low sugar concentrations were reported to induce the differentiation of xylem with no phloem in callus of *Syringa* (Wetmore and Rier 1963), this was because of a failure to detect the callose-poor sieve elements. It is clear from my results that phloem (including that of *Syringa*) does differentiate at low sucrose concentrations and that in agar-grown callus tissue tracheary cells are always accompanied by sieve elements.

The technical difficulty in detectung the initiation of the sieve elements in tissue culture is probably the reason for divergent reports on the early stages of phloem and xylem development. When very thin sections of *Streptanthus* were used in an electron-microscope study, it was not "possible to predict either the site or the time of their formation. As a result the earliest stages of the differentiation process have not been observed" (Sjolund 1968, pp. 123). When thicker preparations were used in light-microscope investigations of growing aggregates of cells of *Daucus*, derived from a single cell, xylem formation was observed prior to phloem differentiation (Steward et al. 1958). But when vascular differentiation in cultured tissue was observed, the process was found to "begin by the differentiation of islets of phloem" (review by Gautheret 1966, pp. 67). The present data, based on a study of thick, cleared preparations of six species, support the latter concept and show that in tissue culture there is the same pattern of development as in intact plants, where phloem has been reported to differentiate before xylem both in stem (Esau 1945; Larson 1975) and root (Heimsch 1951; Miller 1958).

In view of the evidence that low levels of auxin induce the differentiation of phloem with no xylem, that auxin acts as a common stimulus for the differentiation of both phloem and xylem, and that xylem always accompanies phloem, it is proposed that phloem is formed in response to auxin only, while the xylem is formed in response to both auxin and some lateral inductor which reaches it via the phloem. This hypothesis is consistent with the fact that in the plant body, phloem can differentiate without xylem, as for example in the case of phloem anastomoses (Aloni and Sachs 1973; Aloni and Jacobs 1977), normal and regenerative phloem with no xylem (Aloni and Jacobs 1977; Houck and LaMotte 1977), and differentiation of secondary phloem which may precede that of secondary xylem by several weeks (Evert 1963; Alfieri and Evert 1965). In contrast, no instances have been found in nature where xylem differentiates in the absence of phloem. It is further suggested, in conclusion, that the occurrence of sieve tubes without xylem in members of the brown algae and also the differentiation of strands of phloem with no xylem in angiosperms are both the consequence of low levels of auxin rather than of sugar/auxin ratios.

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