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Cell Expansion and Tracheary Element Differentiation Are Regulated by Extracellular pH in Mesophyll Cultures of *Zinnia elegans* L.[1]

Alison W. Roberts* and Candace H. Haigler

Botany Department, University of Rhode Island, Kingston, Rhode Island 02881 (A.W.R.); and Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409 (C.H.H.)

The effects of medium pH on cell expansion and tracheary element (TE) differentiation were investigated in differentiating mesophyll suspension cultures of *Zinnia elegans* L. In unbuffered cultures initially adjusted to pH 5.5, the medium pH fluctuated reproducibly, decreasing about 1 unit prior to the onset of TE differentiation and then increasing when the initiation of new TEs was complete. Elimination of large pH fluctuations by buffering the culture medium with 20 mM 2-(*N*-morpholino)ethanesulfonic acid altered both cell expansion and TE differentiation, whereas altering the starting pH of unbuffered culture medium had no effect on either process. Cell expansion in buffered cultures was pH dependent with an optimum of 5.5 to 6.0. The direction of cell expansion was also pH dependent in buffered cultures. Cells elongated at pH 5.5 to 6.0, whereas isodiametric cell expansion was predominant at pH 6.5 to 7.0. The onset of TE differentiation was delayed when the pH was buffered higher or lower than 5.0. However, TEs eventually appeared in cultures buffered at pH 6.5 to 7.0, indicating that a decrease in pH to 5.0 is not necessary for differentiation. Very large TEs with secondary cell wall thickenings resembling metaxylem differentiated in cultures buffered at pH 5.5 to 6.0, which also showed the greatest cell expansion. The correlation between cell expansion and delayed differentiation of large, metaxylem-like TEs may indicate a link between the regulatory mechanisms controlling cell expansion and TE differentiation.

The acid growth hypothesis (Cleland, 1971; Hager et al., 1971) has served as a working model for the regulation of cell expansion for two decades and the predictions of this model have been demonstrated in a variety of plant tissues (Taiz, 1984; Rayle and Cleland, 1992). However, in some cell cultures the pH optimum for cell expansion has been shown to be higher than predicted by the acid growth hypothesis (Nesius and Fletcher, 1973; Smith and Krikorian, 1992). It has also been shown that long-term auxin-induced growth in coleoptiles has a pH optimum higher than predicted by the acid growth hypothesis (Cleland, 1992). These observations indicate that factors other than acid-induced cell wall loosening may control cell expansion under some circumstances.

Medium pH has been reported to influence morphogenesis as well as cell expansion in a variety of cell cultures (Minocha, 1987). For example, embryogenic carrot cultures can be maintained as preglobular stage proembryos at pH 4.0 but form mature embryos at pH 5.8 (Smith and Krikorian, 1990a, 1990b, 1992). In tobacco thin-layer cultures, high pH increased the ratio of flower bud to vegetative bud formation (Cousson et al., 1989, 1992; Pasqua et al., 1991). Shoot proliferation was promoted at pH 5.4, whereas shoot elongation was promoted at pH 4.8 in *Euphorbia fulgens* cultures (Zhang and Stoltz, 1989). In cultured citrus fruit vesicles, TE differentiation had a pH optimum of 5.0 to 6.0 (Khan et al., 1986). With a few exceptions (Cousson et al., 1992; Smith and Krikorian, 1992), these studies were carried out in unbuffered medium. In one case (Pasqua et al., 1991), reported pH changes of buffered cultures were nearly identical with those of unbuffered cultures.

Few systematic studies have been carried out to characterize these effects, considering the potential influence of extracellular pH on the growth and differentiation of cultured plant cells (for review, see Minocha, 1987). In many of the studies in which pH-dependent effects on growth and development were noted, cultures were not buffered and pH was not monitored throughout the culture period (Banthorpe and Brown, 1990). It is difficult to draw conclusions from these studies, since it is clear that pH can fluctuate dramatically in unbuffered plant cell cultures. Suspension cultures of mesophyll cells isolated from the leaves of *Zinnia elegans* L. have been used extensively to investigate cellular processes involved in the differentiation of TEs (Fukuda and Komamine, 1985; Fukuda, 1989, 1992; Seagull and Falconer, 1991). Here we report the influence of extracellular pH on cell expansion, as well as the timing of differentiation and the size and secondary cell wall pattern of resulting TEs.

MATERIALS AND METHODS

Isolated mesophyll cells from *Zinnia elegans* L. var Envy were cultured in shell vials as described previously (Roberts and Haigler, 1990). Growth regulators in the inductive medium included 0.5 μM NAA and 0.9 μM BA. For most experiments, buffered media were prepared with 20 mM Mes-free acid titrated with KOH or sodium salt titrated with HCl.

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* Corresponding author; fax 1-401-792-5974.

Abbreviations: NAA, α-naphthyleneacetic acid; TE, tracheary element.

Some cultures were buffered with one of the following at 20 mM: sodium potassium tartrate/HCl, sodium acetate/acetic acid, sodium citrate/citric acid, maleic acid/NaOH, potassium biphthalate/NaOH, glycyl-glycine/piperazine, succinic acid/NaOH, or sodium potassium phosphate. The pH was adjusted before autoclaving and was checked afterward.

Culture medium pH was measured at daily intervals using an Accumet model 925 H^+/ion meter (Fisher Scientific, Pittsburgh, PA) and Sure-Flow semi-microelectrode (Orion Research, Inc., Boston, MA). Osmolarity was determined by vapor pressure osmometry (Wescor, Logan, UT).

The secondary cell wall thickenings characteristic of TEs were detected by fluorescence microscopy using an epifluorescence microscope (Olympus BH-2) and UV filter pack after glutaraldehyde-fixed cell suspensions were stained with 0.01% (w/v) Tinopal LPW in 0.05 M phosphate buffer (pH 7.2). Differentiation was scored as described previously (Roberts and Haigler, 1990). Points on graphs indicate the means of three to four replicates from a single representative experiment, and error bars indicate the SD of the sample. All experiments were repeated at least twice with similar results.

Cell areas were measured using a computer image analysis system consisting of an Olympus Vanox microscope, Hitachi video camera, IBM PCAT computer, digitizing tablet, and Microcomp planar morphometry software (Southern Micro Instruments, Inc., Atlanta, GA). Fifty cells were measured from each of three replicate samples for each treatment. The relationships among TE length, width, and secondary cell wall pattern were investigated by classifying each TE according to cell wall pattern and measuring its length and width by computer image analysis. For irregularly shaped cells, widths were always measured at the widest point and perpendicular to the length. Secondary cell wall patterns were classified as helical, helical-reticulate, reticulate, or pitted according to the method of Bierhorst (1960).

Photomicrographs were produced using Tmax 100 film and developer (Kodak, Rochester, NY) and a Zeiss Universal microscope with bright-field optics.

RESULTS

Changes in the pH of the Culture Medium during TE Differentiation

Unbuffered culture medium was adjusted to pH 5.5 before autoclaving and measured 5.3 to 5.7 after autoclaving. As described previously (Roberts et al., 1992, and refs. therein), TE differentiation in *Zinnia* mesophyll suspension cultures was first detectable with fluorescence microscopy at 48 to 60 h in culture and reached a maximum of 50 to 70% of living cells about 20 h later (Fig. 1). A comparison of the time courses of TE appearance and pH fluctuation shows an initial increase in pH during the first 24 h, followed by a decrease in pH prior to the onset of TE differentiation (Fig. 1). The pH remained below 5.0 during the initiation of new TEs (45–76 h). When new TEs ceased to appear (79 h) the pH increased (Fig. 1) and eventually stabilized at about pH 5.5 (data not shown). Although the time of first TE appearance varied among experiments, the correlation between TE formation and the decrease in pH was highly reproducible, being consistent in at least 18 separate trials. When differentiation was deliberately delayed by reducing the mannitol concentration of the culture medium to 0.1 M (Roberts et al., 1992), the decrease in pH was also delayed and again corresponded with the onset of differentiation (data not shown).

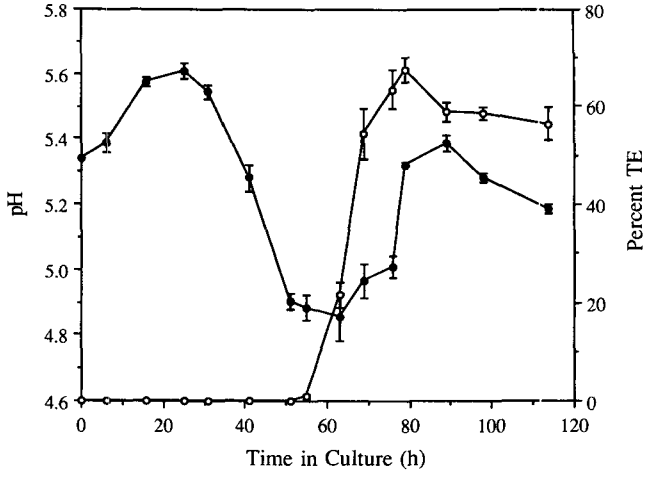

Figure 1. Time courses of differentiation (O) and change in extracellular pH (●) in suspension cultures of *Zinnia* mesophyll cells. Percentage differentiation was determined by fluorescence microscopy with Tinopal LPW staining.

The normal fluctuations in medium pH were prevented by buffering the medium with 20 mM Mes at starting pH 4.5, 5.0, 5.5, 6.0, 6.5, or 7.0. The change in pH under these conditions was <0.5 pH units throughout the 8-d culture period for all but one treatment (data not shown). Mes-buffered medium initially adjusted to pH 4.5 increased to 4.7 during autoclaving and to 5.2 after 8 d in culture. This is not unexpected since the pK_a of Mes is 6.1. Cells were also cultured in unbuffered medium adjusted to pH 4.0, 5.5, or 7.0 before autoclaving. Autoclaving altered the pH of these media slightly. The pH of the three media converged during the first 24 h after inoculation and then decreased below pH 5.0 during TE initiation (data not shown).

Media were buffered with 20 mM acetate, citrate, maleate, phthalate, glycyl-glycine/piperazine, succinate, tartrate, or phosphate in an attempt to mimic the effects of buffering with Mes. However, cells were not viable in media buffered with acetate, citrate, maleate, and phthalate. Glycyl-glycine/piperazine reduced cell viability, especially above pH 5.0. Phosphate buffer did not maintain the culture pH. Succinate and tartrate induced cell expansion, but only a few large TE differentiated in media containing these buffers.

Effect of pH on Cell Expansion

The expansion of *Zinnia* mesophyll cells was pH dependent in Mes-buffered culture medium. During the first 3 d in culture the mean projected cell area as measured by image analysis increased more rapidly in media buffered at pH 5.5 to 6.5 compared to unbuffered medium or media buffered at pH 4.5 to 5.0 and pH 7.0 (Fig. 2). Cells became difficult to measure after 60 h because of extensive cell division.

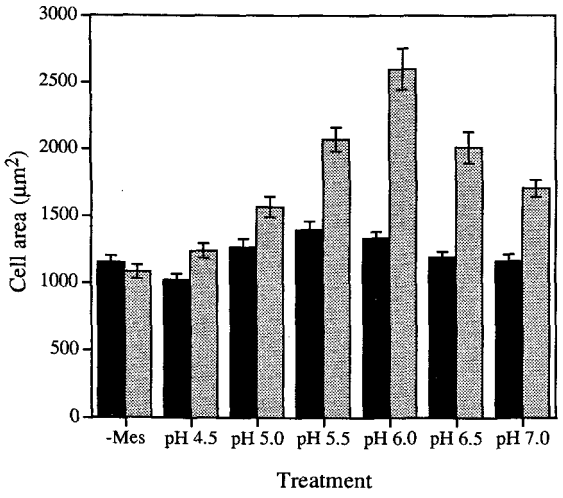

Figure 2. Mean projected cell area at 25 h in culture (solid bars) and 59 h in culture (shaded bars) of *Zinnia* mesophyll cells incubated in unbuffered medium and buffered medium adjusted to various pHs.

Differences in the orientation of cell enlargement were apparent by 96 h (Fig. 3). Cells cultured in medium buffered at pH 4.5 to 5.0 (Fig. 3, C and D) were similar in appearance to cells cultured in unbuffered medium throughout the culture period (Fig. 3B). When the culture medium was buffered at pH 5.5 to 6.0, cells became strikingly elongated (Fig. 3, E and F). In contrast, cells expanded isodiametrically when culture medium was buffered at pH 6.5 to 7.0 (Fig. 3, G and H).

Effect of pH on TE Differentiation

Changes in the pH of Mes-buffered culture medium altered the timing, synchrony, and final percentage of TE differentiation (Table I). The time of appearance of TEs as detected by Tinopal LPW fluorescence in unbuffered and Mes-buffered culture medium (pH 4.5–7.0) is shown in Table I. The first TEs appeared at about the same time in unbuffered medium and medium buffered at pH 5.0. In some experiments differentiation was detected slightly earlier in pH 5.0 buffered medium compared to unbuffered medium, but the increase in percentage of differentiation was always less rapid in buffered medium. The delay in differentiation increased with increasing pH above the optimum of pH 5.0, with no differentiation detected in medium buffered at pH 7.0 until d 8 or 9 (data not shown). Differentiation was also less synchronous in buffered cultures, in which new TEs continued to appear for 5 d or more. In unbuffered cultures, new TEs stopped appearing 20 h after the first ones were observed, except for a second asynchronous "burst" of differentiation beginning after about 6 d (Falconer and Seagull, 1988). The final percentage of differentiation in cultures buffered at pH 4.5 to 6.0 approached the percentages measured in unbuffered cultures. In cultures buffered at pH 6.5 to 7.0 the percentage of differentiation was never above 2%, even after 9 d (data not shown).

Medium pH also altered the size, shape, and secondary cell wall patterns of TEs as illustrated in Figure 4. These differences were quantified by measuring the lengths and widths of TEs from 132-h cultures (unbuffered and buffered at pH 4.5–6.0) and 197-h cultures (buffered at pH 6.5–7.0) by image analysis. TEs in these cultures were also scored for secondary cell wall pattern based on the following criteria (Bierhorst, 1960): helical patterns were defined as having transverse bands with no apparent connecting bands (Fig. 4A), helically reticulate patterns had generally helical patterns with forks and anastomoses (Fig. 4B), reticulate patterns had a netted thickening pattern (Fig. 4C), and pitted cells had secondary wall material covering the entire cell except for small, nearly round pits (Fig. 4D). To avoid classifying TEs on the basis of immature cell wall patterns that could change because of continued cell wall deposition (Falconer and Seagull, 1988), only autolyzed TEs were counted. When the secondary cell wall pattern varied over the cell surface, the cell was categorized according to the most complex pattern. Unbuffered cultures were scored before the second burst of differentiation, during which very large TEs with predominantly reticulate or pitted secondary cell wall patterns form (Falconer and Seagull, 1988).

The mean lengths and widths of TEs from various buffered media are summarized in Figure 5A. The length of TEs peaked at pH 6.0, whereas width increased with pH to 7.0. The only exception to this pattern was the occurrence of longer and wider cells at pH 4.5 than at pH 5.0. It should be noted that the delay of differentiation was also greater at pH 4.5 than at pH 5.0. The secondary cell walls of the larger TEs that differentiated at high pH were predominantly "metaxylem-like," with a greater proportion of reticulate or pitted patterns, rather than the "protoxylem-like" helical or helical-reticulate patterns most common in unbuffered medium (Fig. 5B). Cultures buffered at pH 4.5 to 5.5 had nearly equal proportions of metaxylem-like and protoxylem-like TEs.

Based on the results summarized in Figure 5, we surmised that secondary cell wall pattern had a greater dependence on cell width than on cell length. When the lengths and widths of individual TEs from all treatments were plotted, cells with different secondary cell wall patterns formed clusters that tended to separate along the axis describing cell width (Fig. 6). A plot of the mean lengths and widths of TEs with different cell wall patterns also reflected a relationship between cell geometry and wall pattern (Fig. 6, inset).

In contrast to results obtained with buffered cultures, altering the initial pH of unbuffered culture medium (pH 4.0–7.0) had no effect on cell expansion or differentiation. Furthermore, no difference in the time course of differentiation or in the appearance of TEs or undifferentiated cells could be detected (data not shown).

DISCUSSION

The pH 5.5 to 6.0 optimum for cell expansion in Mes-buffered *Zinnia* suspension cultures is higher than the pH 4.0 to 4.5 optimum predicted by the acid growth hypothesis (Rayle and Cleland, 1977, 1992; Taiz, 1984). However, higher pH optima for expansion of cultured cells have been noted previously. For example in carrot cell cultures, cell expansion was inhibited when the medium was buffered at pH 4.0 but

stimulated when cells were transferred to medium buffered at pH 5.8 with or without auxin (Smith and Krikorian, 1992). Cultured rose cells had a pH optimum for growth (measured as increase in fresh weight) of 5.2 to 5.4 during the cell division phase and an optimum of pH 5.8 to 6.0 during the cell expansion phase (Nesius and Fletcher, 1973). Recently it has been shown that the pH optimum for long-term auxin-induced growth in *Avena* coleoptiles is higher than 5.0 (Cleland, 1992). In this tissue the pH optimum for growth was <5.0 during the first 1 to 2 h after auxin treatment but increased to 5.5 to 6.0 after 2 h. It was proposed that the initial auxin-induced growth phase was regulated by extracellular acidification but that prolonged growth was controlled by a different auxin-mediated process with a less acidic pH optimum. The higher pH optimum for cell expansion in *Zinnia* cultures is consistent with a growth control mechanism similar to long-term auxin-induced growth in intact organs. It is interesting that buffering influences the direction as well as the magnitude of cell expansion, indicating that pH effects are not solely due to general cell wall

Table I. *Time of first appearance of TEs and final percentage differentiation in unbuffered cultures and cultures buffered with 20 mM Mes at pH 4.5 to 7.0*

Treatment	Time of TE Appearance	Final Percentage Differentiation (at 197 h in culture)
	h in culture	
−Mes[a]	59	47.3 (52.5)[b]
pH 4.5	72	47.4
pH 5.0	59	43.3
pH 5.5	72	39.2
pH 6.0	96	53.6
pH 6.5	184	<1
pH 7.0	197	<1

[a] Initial pH was 5.5. [b] In this treatment the percentage differentiation declined after reaching a peak of 52.5 at 72 h. The decline was due to division of nondifferentiated cells.

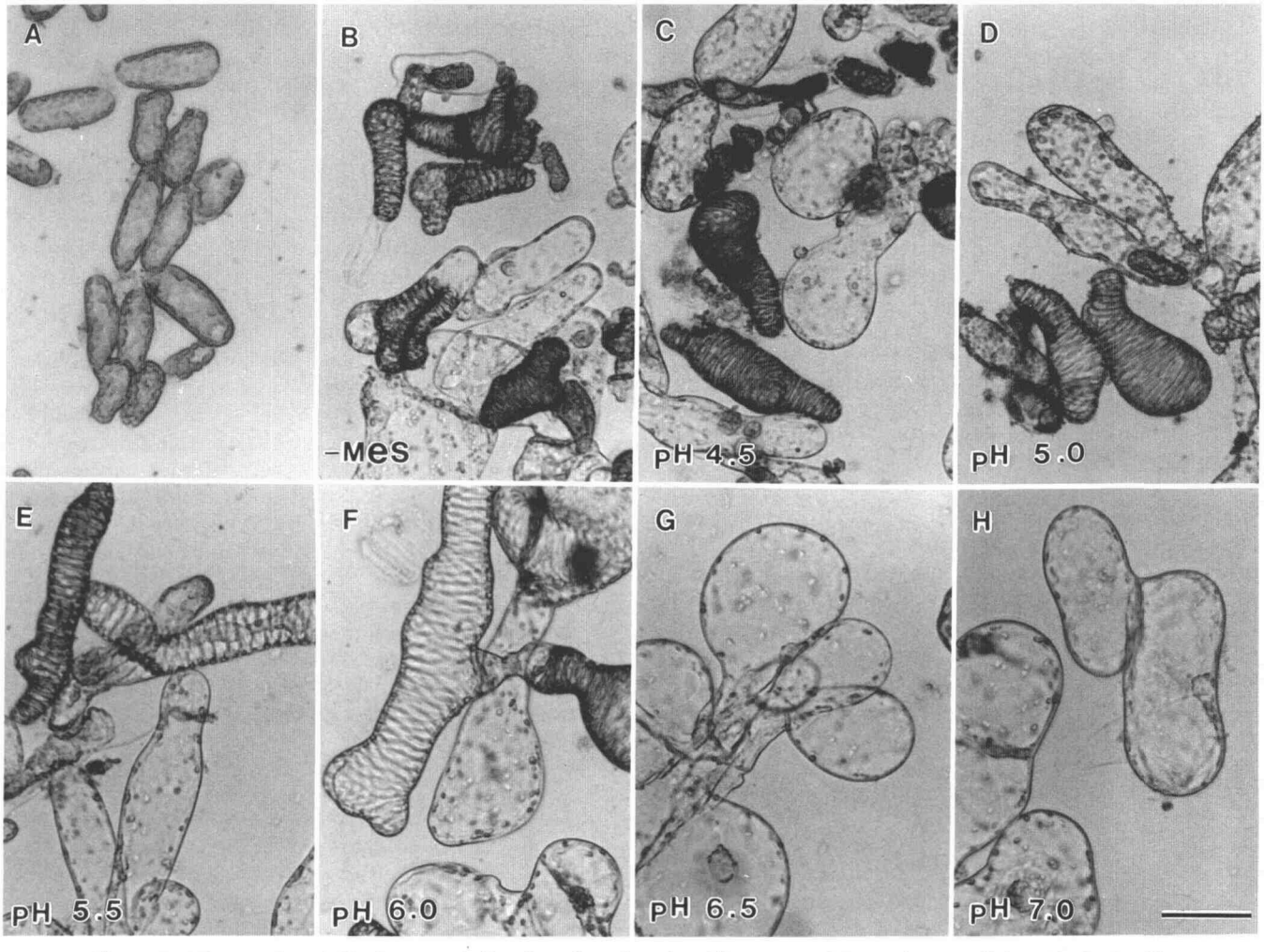

Figure 3. Micrographs of *Zinnia* mesophyll cells cultured under different conditions of extracellular pH. A, Freshly isolated cells. B, Cells cultured for 96 h in unbuffered medium. C to H, Cells cultured for 96 h in medium buffered with 20 mM Mes initially adjusted to the pH shown in the lower left of each micrograph. Bar = 50 μm.

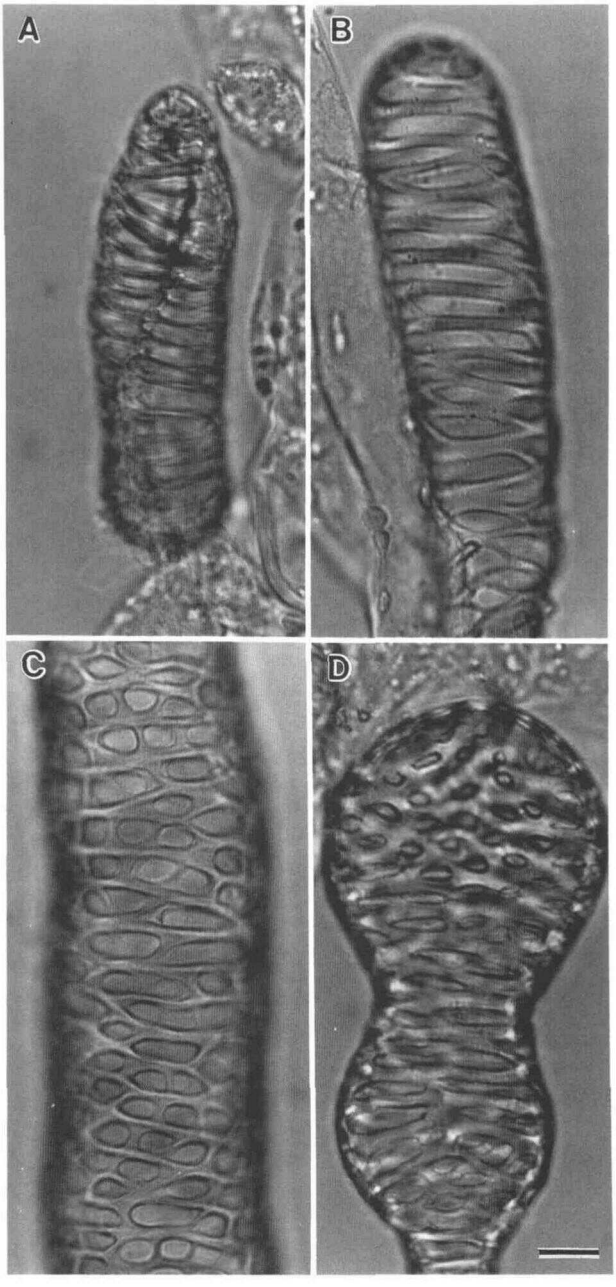

Figure 4. TEs representing each of the four classes of secondary cell wall patterns: A, helical; B, helical reticulate; C, reticulate; D, pitted. Bar = 10 μm.

relaxation, which would be expected to alter only the magnitude of cell expansion. Because auxins are weak acids, their uptake is influenced by extracellular pH (Raven, 1975). For example, the uptake of IAA is reduced by about 30% at pH 6.0 compared to 5.0 and by an additional 10% at pH 7.0 compared to pH 6.0 (Raven, 1975). However, all buffered cultures in this study contained 0.5 μM NAA even though *Zinnia* cells expand maximally with 0.038 to 0.5 μM NAA (Roberts et al., 1993). Therefore, altered NAA uptake is not likely the primary explanation for the effect of extracellular pH on cell expansion.

The coincidence of medium acidification with the onset of differentiation, along with the observation that cultures buffered at pH 5.0 produce TEs earlier than any other buffered cultures, indicates a pH optimum for differentiation of 5.0. A pH optimum of 5.0 to 6.0 has also been noted for TE differentiation in citrus vesicle cultures (Khan et al., 1986). Extracellular pH fluctuations observed previously in unbuffered plant cell cultures are species specific and may include acidification or alkalinization (Minocha, 1987). In most cases the processes leading to these fluctuations are unknown. However, use of ammonia rather than nitrate as a nitrogen source may result in medium acidification (Veliky and Rose, 1973; Martin et al., 1977). In differentiating *Zinnia* cultures, the decrease in pH below 5.0 in unbuffered cultures is closely correlated with the period of secondary cell wall deposition, even when differentiation is delayed. Therefore, it is likely

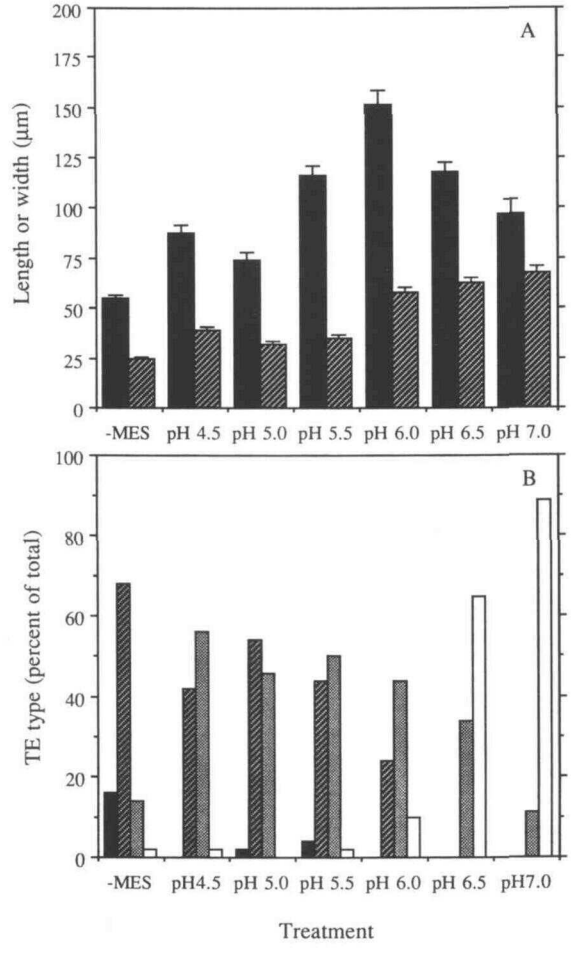

Figure 5. Characteristics of TEs formed under different conditions of extracellular pH. Measurements were made at 132 h for unbuffered cultures and cultures buffered at pH 4.5 to 6.0 and at 197 h for cultures buffered at pH 6.5 to 7.0. Fifty TEs from each treatment were measured. A, Mean length (solid bars) and width (shaded bars) of TEs as measured by image analysis. B, Percentage of TEs with secondary cell wall patterns of each of the four types: helical (solid bars), helical-reticulate (striped bars), reticulate (shaded bars), and pitted (open bars).

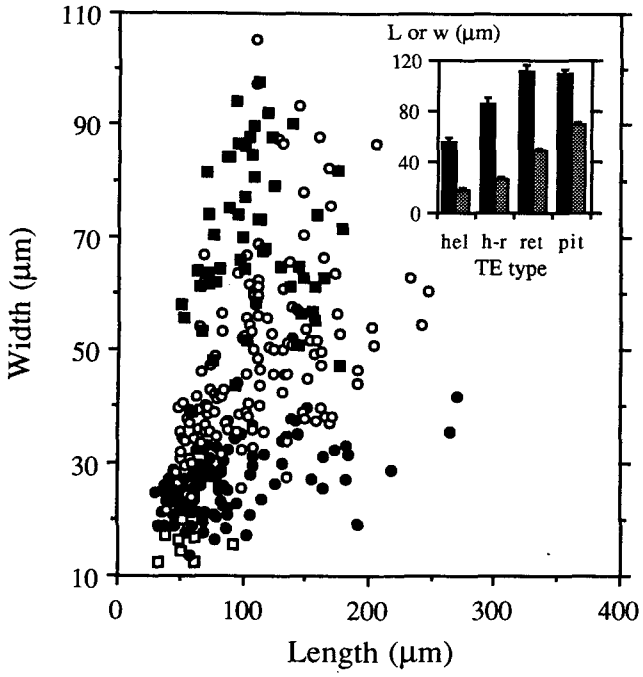

Figure 6. Scatter plot illustrating clustering of helical (□, hel), helical reticulate (●, h-r), reticulate (○, ret), and pitted (■, pit) TEs according to length and width. Data were pooled from all treatments represented in Figure 5. Inset, Mean length (solid bars) and width (shaded bars) of TEs of four types.

that the decrease in pH is related to differentiation rather than other factors such as nutritional status. The pH decrease is also not correlated with the onset of cell division, which begins much earlier (Roberts et al., 1992). However, pH 5.0 is not necessary for differentiation, since TEs develop abundantly in buffered medium in which the pH never decreases below 5.8. Although differentiation was delayed in most Mes-buffered cultures, the magnitude of the delay was pH dependent and TEs formed in pH 5.0 Mes-buffered cultures at about the same time as in unbuffered cultures, indicating that the delay could not be attributed to Mes alone. Reduced NAA uptake is not a likely explanation for delayed differentiation, since reduction in the auxin concentration has been shown to inhibit, but not delay, differentiation in these cultures (Church and Galston, 1988). However, it is possible that reduced NAA uptake plays a role in the inhibition of differentiation at pH 6.5 to 7.0.

The larger TEs that develop in buffered cultures could result directly from either pH-dependent stimulation of cell expansion or pH-dependent delay in differentiation. In the first case, higher pH directly stimulates cell expansion and indirectly causes a delay in differentiation, which cannot begin until expansion ceases. In the second case, the higher pH directly delays the onset of differentiation so that cells have a longer period of time to expand before the secondary wall is deposited. The second case is less likely, since cells in cultures buffered at pH 5.5 to 6.0 have already expanded more than cells in unbuffered cultures long before TE differentiation starts.

Cultured *Zinnia* mesophyll cells express polar development despite the apolarity of a swirling cell suspension. For example, it has been noted that secondary cell wall thickenings are always deposited perpendicularly to the longitudinal axis of the differentiating cell, except when cultures are treated with cytoskeleton-disrupting drugs such as taxol (Falconer and Seagull, 1985). This indicates that the in situ polarity of the mesophyll cells is retained in culture and that the cytoskeleton may play a role in maintaining this polarity. Extracellular pH modifies the expression of cell polarity in *Zinnia* cultures, and again the cytoskeleton is implicated. Much evidence supports the role of microtubules in controlling cellulose microfibril orientation and thus the direction of cell expansion (Shibaoka, 1991). Furthermore, a relationship between extracellular pH and the orientation of cortical microtubules has been established in the giant cells of the alga *Chara* (Wasteneys and Williamson, 1992), which exhibit banded patterns of net proton efflux (acid bands) and net proton influx (alkaline bands). The acid bands of maturing cells contain parallel microtubules, whereas microtubules in the alkaline bands are not ordered. The cell elongation that occurs at pH 5.5 to 6.0 in *Zinnia* cultures would be consistent with ordered arrays of microtubules as seen in the acid bands of *Chara*, whereas isodiametric growth that occurs at more alkaline pH would be consistent with less ordered microtubule arrays. We are now using immunofluorescence microscopy to investigate the effect of extracellular pH on microtubule orientation in *Zinnia* suspensions.

The pH-dependent alteration in cell size and shape in *Zinnia* cultures correlates with changes in the secondary cell wall, with larger diameter cells having metaxylem-like reticulate or pitted patterns. Previous observations have indicated that the secondary cell wall pattern is influenced by cell shape and the "prebundling" microtubule array present at the onset of rearrangement for secondary cell wall deposition. Falconer and Seagull (1986) showed that, when cell shape in *Zinnia* cultures was changed from elongated to spherical with microtubule-disrupting drugs, microtubule arrays reformed in random arrays after drug removal. Spherical cells with random microtubule arrays formed secondary cell walls in "webbed" patterns, which resembled those associated with metaxylem. However, since microtubule arrays and cell shape could not be altered independently, it was not possible to determine whether the prebundling microtubule arrays were the primary determinant of secondary cell wall pattern, or whether cell shape exerted primary control over the reforming microtubule arrays, which in turn influenced the secondary cell wall pattern (Seagull and Falconer, 1991).

When cell shape is altered by changing the extracellular pH, the resulting TEs have 2- to 3-fold larger diameters than those in unbuffered cultures, but unlike the spherical cells generated by treatment with microtubule-disrupting drugs, these elongating cells would be expected to have transversely arranged microtubules. If this is true, the microtubule patterns that mediate the deposition of the metaxylem-like wall patterns in these large-diameter cells would differ from those that give rise to similar webbed patterns in that they develop from parallel rather than random microtubule arrays. The lateral association model previously invoked to explain the formation of webbed patterns in drug-treated cells (Falconer

and Seagull, 1986; Seagull and Falconer, 1991) can also explain the formation of reticulate and pitted patterns in large-diameter cells. In small-diameter cells, transverse microtubules associate with one another to form single bundles around the cell circumference. The process can be visualized as beginning with associations formed at single sites and proceeding in a zipper-like fashion. In cells of larger circumference, transverse microtubules bundled together at a single site have more opportunity to associate with microtubules in forming bundles above and below that site. This could form the bifurcations and interconnecting strands characteristic of metaxylem-like secondary cell wall patterns.

Cell expansion has been correlated with delayed differentiation not only in buffered cultures but also in cultures to which conditioned medium has been added (Roberts, 1992) and cultures with reduced osmotic concentrations (Roberts et al., 1992). These results are consistent with the hypothesis that the regulatory mechanisms controlling cell differentiation and cell expansion are linked (Fry, 1990). Further evidence for this link has been summarized by Torrey et al. (1971), who pointed out that TEs differentiate abundantly in the compact callus that forms when water availability is restricted by growth on agar or in medium with a high osmotic concentration. The pattern of secondary cell wall deposition in TEs has also been related to cell expansion (Brower and Hepler, 1976; O'Brien, 1981).

In another well-characterized differentiating culture system, embryogenic carrot cell suspensions, higher pH promotes cell expansion but inhibits differentiation of somatic embryos (Smith and Krikorian, 1992). Further evidence that cell expansion is incompatible with embryogenesis comes from experiments with tunicamycin-treated carrot cultures (van Engelen and de Vries, 1992). Tunicamycin promotes cell expansion and blocks embryo development, whereas a secreted peroxidase inhibits tunicamycin-induced cell expansion and restores embryogenesis. The microtubule cytoskeleton is thought to play an important role in both cell expansion (reviewed by Shibaoka, 1991) and TE differentiation (reviewed by Seagull and Falconer, 1991). Therefore, microtubules may mediate the transition from expansion to differentiation within the cell. In expanding cells, the arrays associated with cell expansion could be stable, delaying differentiation until cortical microtubules can assume the bundled arrays associated with secondary cell wall deposition. Cell expansion, through its influence on the timing of differentiation, may determine the functional characteristics of TEs by altering their size, shape, and secondary cell wall pattern (Roberts, 1976). Thus, the regulatory mechanisms controlling these processes merit further investigation.

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