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THE LOSS OF MORPHOGENETIC POTENTIAL AND INDUCTION OF PHENYLALANINE AMMONIA-LYASE IN SUSPENSION CULTURES OF *PHASEOLUS VULGARIS*

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SUMMARY

The loss of morphogenetic potential in bean suspension cultures has been investigated by measuring the amounts of phenylalanine ammonia-lyase activity induced in the cells when they are transferred from a medium in which they are grown and maintained to an induction medium.

The tissue has been grown in 2 types of medium: (1) supplemented with 2,4-dichlorophenoxyacetic acid as the only growth hormone, and (2) supplemented with 2,4-dichlorophenoxyacetic acid and coconut milk. When cells were grown in medium with only 2,4-dichlorophenoxyacetic acid for a period of 5–10 subcultures and samples were transferred to the induction medium at intervals during the subcultures, the amounts of phenylalanine ammonia-lyase activity and the number of xylem elements induced progressively declined. Cells grown in the presence of coconut milk did not lose the ability to induce phenylalanine ammonia-lyase or xylem elements.

Cells grown in the presence of coconut milk were cloned and clones capable of producing different amounts of phenylalanine ammonia-lyase when transferred to induction medium were obtained. However, clones producing low amounts of activity did not grow faster in the medium lacking coconut milk and no evidence was obtained to show that selective growth of non-inducible cells was responsible for the loss of morphogenetic potential.

In addition to the induction brought about by the presence of naphthylacetic acid and kinetin in the induction medium the cells could also be stimulated to produce phenylalanine ammonia-lyase activity by dilution at subculture.

This increase in activity occurred within 10 h of the dilution, whereas that produced by the hormones in the induction medium occurred after 120 h. The induction produced by dilution also occurred in these cells which had lost their ability to respond to the hormonal induction. Thus the mechanism that produced the increase in phenylalanine ammonia-lyase activity was intact but had lost its ability to respond to the hormones of the induction medium. The loss of inducibility was therefore probably not due to a genetic change in the cells bought about by continuous growth in a medium lacking coconut milk, but to reversible changes in the hormonal requirements of the cells necessary for induction.

INTRODUCTION

Haddon & Northcote (1975, 1976*a*) showed that the ability of *Phaseolus vulgaris* callus to form roots when transferred to an induction medium (IM) declined during continued subculture (approx. 7 subcultures) on maintenance medium (MM) that contained 2,4-dichlorophenoxyacetic acid (2,4-D) as the only growth regulator. This decline was correlated with a decrease in induced levels of phenylalanine ammonia-

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lyase (PAL E.C. 4.3.1.5) and *O*-methyl transferase (E.C. 2.1.1.6) activity. Growth of *Phaseolus* callus was also maintained on a medium (CMD) containing 2,4-D and coconut milk. Under these conditions the callus retained for at least 15 subcultures, and probably much longer (Jeffs & Northcote, 1966, 1967), the ability to form roots and meristematic nodules together with increased amounts of PAL and methyl transferase activities when it was subcultured on to the induction medium.

Attempts have been made to correlate the loss of morphogenetic potential with changes in ploidy of the cells that occurred during their growth in the absence of coconut milk. Haddon & Northcote (1976*b*) found that growth of *Phaseolus* callus in maintenance medium led to an increase in the ploidy of the callus from a predominantly diploid tissue up to one that was octoploid. The connexion between ploidy changes and morphogenetic potential was, however, unclear. Callus isolated from *Phaseolus* anthers had a wide range of ploidy after it was grown in maintenance medium, but it was still capable of morphogenesis when transferred to induction medium. These results indicated that there may be no direct connexion between ploidy and morphogenetic potential. This conclusion is further supported by the work of Gould (1978) who showed that the morphogenetic potential of *Brachycome* callus was lost without any change in the karyotype of the cells. It has also been shown that plants were able to be differentiated from tobacco callus, the cells of which had remarkably large variations in karyotype (Sacristan & Melchers, 1969).

The correlation between xylogenesis and induced amounts of PAL activity has been shown for sycamore xylem (Rubery & Northcote, 1968), swede roots (Rhodes & Wooltorton, 1973), soybean callus (Hahlbrock, 1974) and tobacco callus (Kuboi & Yamada, 1978). In addition, the activities of cinnamic acid hydroxylase, *O*-methyltransferase, and 4-coumarate-CoA ligase are increased during lignin deposition in soybean and tobacco callus, and in swede root. Thus there is evidence that an increased flux through the phenylpropanoid pathway is necessary to provide the monomers for lignin synthesis. The flux of phenylpropanoid intermediates in callus which cannot increase its PAL activity is probably only sufficient to account for a basal level of phenolic synthesis. The strict correlation that has been observed between xylogenesis and inducibility of increased PAL activity in many differentiating tissues implies that cells which cannot be made to increase the activity of PAL cannot undergo xylogenesis.

The purpose of the present investigation has been to study, by measuring the loss of inducibility of PAL activity, the processes which cause cultures grown on maintenance medium to lose their ability to differentiate.

Since only a small proportion of cells of a *Phaseolus* callus undergo xylogenesis when grown in an inductive medium, it is possible that only a small sub-population of cells can be induced to increase their PAL activity. This inducible sub-population of cells could be diluted out by the differential growth of less inducible cells in maintenance medium (MM). The growth of these 2 populations of cells on medium supplemented with coconut milk (CMD) could occur at the same rate so that selection does not occur, and hence this would be the reason for the retention of morphogenetic potential on CMD.

Another cause of the loss of the inducible increase in PAL activity could be the conversion of inducible cells to non-inducible cells. This change could be in the genetic material of the cells, and involve the loss or inactivation of the PAL gene, or other genes necessary for the proper induction of PAL. The increased aneuploidy and ploidy observed in cells grown in maintenance medium may be associated with chromosomal breakages or chromosome elimination. However the conversion of inducible cells to non-inducible cells could also be due to a change in their ability to respond to the hormonal stimulus which involves no permanent genetic change.

MATERIALS AND METHODS

Materials

Analytical grade chemicals and twice-distilled water were used wherever possible. L-[U-^{14}C]phenylalanine (> 16·6 GBq/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks; kinetin (furfurylaminopurine) from Sigma Ltd., Poole, Dorset; 1-naphthylacetic acid (NAA) and *trans*-cinnamic acid from Koch-Light Laboratories, Colnbrook, Bucks; 2,4-dichlorophenoxyacetic acid (2,4-D) from B.D.H. Ltd, Poole, Dorset; N.Z. Amine Type A, from Sheffield Chemicals Co., Norwich, New York; Special Agar Noble, from Difco Laboratories, Detroit, Michigan; sterile containers and Petri dishes from Sterilin Ltd., Teddington, Middlesex; and Silica gel plates, 0·25-mm plastic backed, from Machery-Nagel, Duren, Federal German Republic.

Media

The medium of Gamborg, Miller & Ojima (1968) was used. Tissue cultures were routinely grown on medium supplemented with 9×10^{-6} mol dm^{-3} 2,4-D, 20 % (v/v) deproteinized coconut milk, and 2 % sucrose (CMD). Some cultures were grown in medium supplemented with 9×10^{-6} mol dm^{-3} 2,4-D and 2 % sucrose (maintenance medium, MM). These lose their PAL inducibility after 5–8 subcultures (Haddon & Northcote, 1976*a*). Medium supplemented with $5{\cdot}4 \times 10^{-6}$ mol dm^{-3} NAA, $9{\cdot}3 \times 10^{-7}$ mol dm^{-3} kinetin and 3 % sucrose (induction medium, IM) was used for the induction of PAL activity (Haddon & Northcote, 1975). Media were solidified by the addition of 1 % agar before autoclaving at 103·4 kN m^{-2} for 30 min. Suspension cultures were grown in 100 cm^3 of medium in 500-cm^3 conical flasks.

Isolation and growth of tissue cultures

Two types of suspension culture were obtained: a fast- and a slow-growing tissue. Callus was initiated from sterile hypocotyl sections of *Phaseolus vulgaris* var. Canadian Wonder that had been germinated in the dark for 6 days. Pieces of hypocotyl were placed on CMD solidified with agar. The initiated callus was routinely subcultured twice on solid CMD before the initiation of suspension cultures. Approximately 10 g of solid callus were chopped coarsely with a spatula and inoculated into 100 cm^3 of CMD. After shaking at 80 rev/min for 2 weeks in the dark at 26 °C the resultant suspension of cells was used to inoculate another flask. These slow-growing suspension cultures were subcultured to fresh medium every 15 days. They were maintained as slow-growing cultures for approximately 3 months. After approximately 30 subcultures these cells were dividing rapidly and grew in clumps of 10–50 cells. The fast-growing cell cultures were subcultured to fresh CMD every 3–4 days.

Since the number of transfers of the cultures and the type of media used are in some cases necessarily complicated we have used a code to indicate the growth history of the tissue. The number of transfers to each medium is indicated by a prefix, thus the code 4 MM, 1 IM indicates that the culture has been transferred 4 times to fresh maintenance medium and finally to induction medium.

Cloning

This method was adapted from that of Meins & Binns (1977). Suspension cultures were filtered successively through 1000 μm and 200-μm stainless steel mesh and the suspension of single cells was sedimented at 50 g for 2 min and resuspended in CMD to give a final concentration of 5–10 × 10^3 cells cm^{-3}. These cells were plated in 10 cm^3 CMD containing 0·6 % agar and 0·1 % N.Z. Amine to give a final concentration of 500–1000 cells cm^{-3} at 35 °C. The Petri dishes were sealed with parafilm and incubated at 26 °C in the dark until the clones were approximately 2 mm in diameter (4–5 weeks) when they were picked out with a small spatula and transferred to individual pots containing a 10 cm^3 CMD solidified with 1 % agar. The cloning efficiency was approximately 25 % for fast-growing cells and 10 % for slow-growing cells.

PAL assay

The enzyme was extracted from filtered samples of cells (1 g fresh weight) by grinding them with a little quartz sand in 1 cm^3 100 mM Tris-HCl containing 0·1 % mercaptoethanol at 4 °C, pH 8·8. The homogenate was centrifuged at 10000 g for 1 min and the supernatant was used for the enzyme assay. The assay was adapted from that of Dixon & Fuller (1976). Each assay contained 76·9 μmol Tris-HCl, pH 8·8, 7·69 μmol L-phenylalanine (700 dpm/nmol), and 10–20 μg protein in a total volume of 50 mm^3. The assay was started by incubation at 38 °C, and stopped by drying the entire assay mixture onto a silica gel thin-layer plate. A solution of 1 % cinnamic acid in ethanol was streaked through the origin, and the chromatogram was developed in toluene:ethyl formate:formic acid 70:20:10 v/v/v. Cinnamic acid has an R_f of 0·6 in this solvent, while phenylalanine remains at the origin. The cinnamic acid was visualized under ultraviolet light, scraped into a scintillation vial and mixed with 2 cm^3 of scintillation fluid (4 g dm^{-3} 2,5-diphenyloxazole in sulphur-free toluene) and counted in a Searle Mk III liquid scintillation spectrophotometer. The efficiency of counting for ^{14}C was approximately 96 %. PAL activity was expressed as nmol of cinnamic acid produced min^{-1} mg^{-1} protein.

Protein estimation

Protein samples in a total volume of 0·1 cm^3 were added to 3 cm^3 of Coomassie blue G reagent, and the absorption (595 nm) of the blue protein-dye complex was measured (Spector, 1978).

Dry weight determination

Samples (2·5 cm^3) of cells were removed aseptically from suspension cultures using a wide-bore automatic pipette, and filtered onto pre-weighed glass fibre disks. These were dried *in vacuo* at 60 °C overnight. All values given were the means of triplicate samples. The cumulative growth (no. of cell generations) and doubling constant (the reciprocal of the mean generation time) of cultures were calculated.

RESULTS

The induction of PAL in suspension-cultured cells

The amount of PAL activity present at various times in slow-growing cells grown on CMD and then transferred to IM (6 CMD, 1 IM) or CMD (7 CMD) is shown in Fig. 1. Prolific root and xylem formation was observed after 7–9 days growth on induction medium. These results are similar to those of Haddon & Northcote (1975) who used solid callus. The increase in PAL activity and the rate of PAL induction occurred 2–3 days earlier in suspension-cultured cells compared with the solid callus. The amount of PAL activity present at various times in fast-growing cells grown for

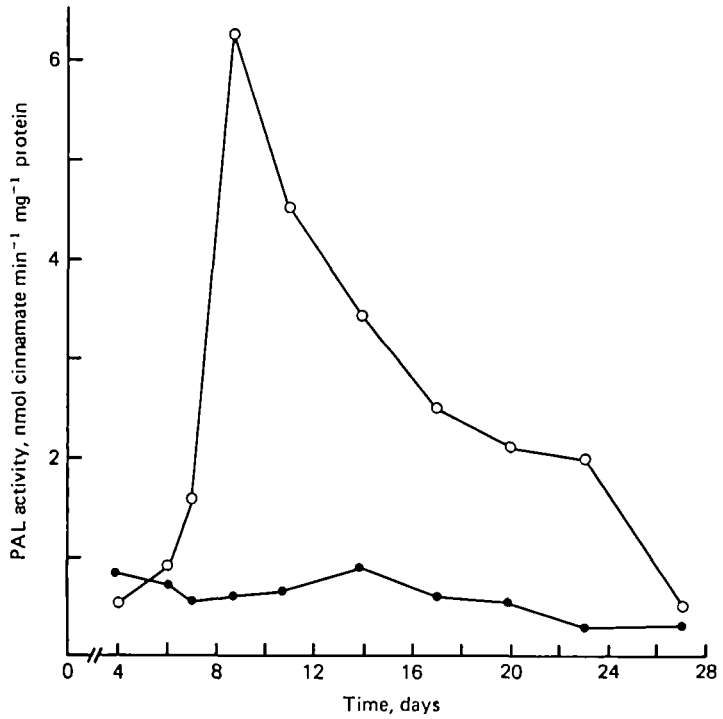

Fig. 1. The induction of PAL in slow-growing suspension cultures. Cells were subcultured into 200 cm^3 induction medium (O) and 200 cm^3 medium containing coconut milk (●).

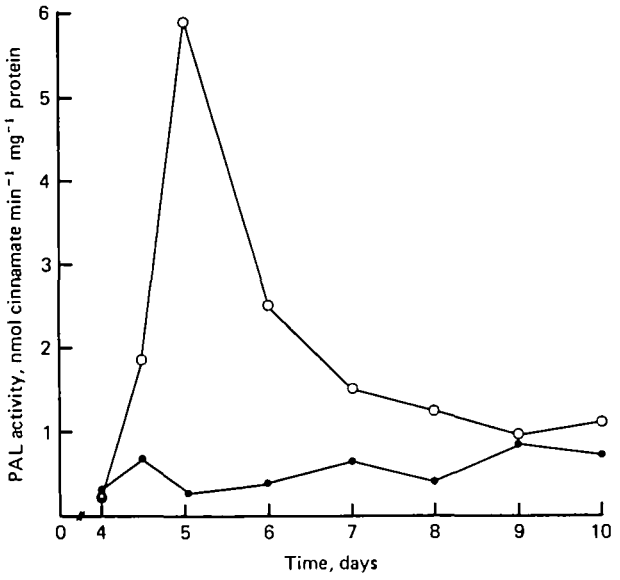

Fig. 2. The induction of PAL in fast-growing suspension cultures. Cells were subcultured into 200 cm^3 induction medium (O) and 200 cm^3 medium containing coconut milk (●).

60–70 subcultures on CMD, transferred to induction medium (60–70 CMD, 1 IM) or CMD (61–71 CMD) is shown in Fig. 2. In these fast-growing suspension culture cells, although PAL activity was induced, no root or xylem differentiation was ever observed.

The loss of PAL inducibility

Slow-growing suspension-cultured cells that had been subcultured twice on CMD were transferred to induction medium (200 cm^3 cells in 200 cm^3 medium) and the amount of PAL induced was measured over a period of 28 days (Fig. 3). The total amount of PAL induced between the fourth and twenty-first days was calculated from the area under the curve (Fig. 3). Another sample of these cells grown on CMD medium was subcultured into 200 cm^3 of maintenance medium and the initial and

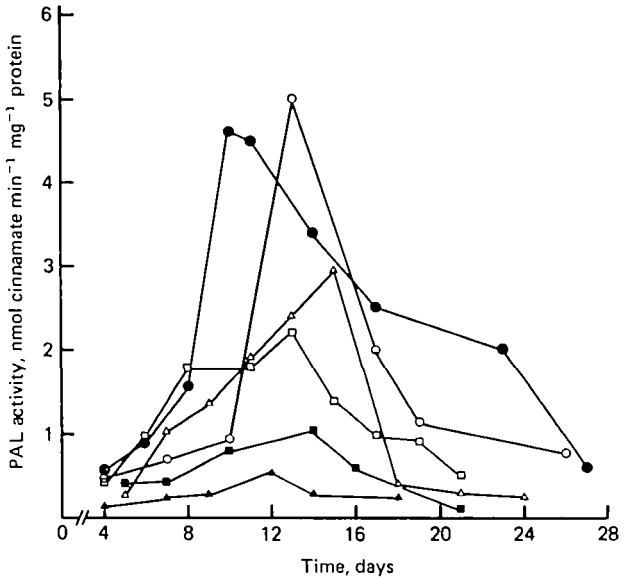

Fig. 3. The loss of PAL inducibility in slow-growing suspension cultures. Cells grown for 2 subcultures on medium containing coconut milk were subcultured to induction medium and another sample of these cells grown in the presence of coconut milk was transferred to medium lacking coconut milk. After each subculture to fresh medium lacking coconut milk a sample of cells was transferred to induction medium. ●, 2 CMD 1 IM; ○, 2 CMD 1 MM 1 IM; △, 2 CMD 2 MM 1 IM; □, 2 CMD 3 MM 1 IM; ■, 2 CMD 4 MM 1 IM; ▲, 2 CMD 5 MM 1 IM.

final dry weights of the cells were determined after 12–15 days growth. After the first subculture in maintenance medium, a 20-cm^3 sample of cells was transferred to induction medium (2 CMD, 1 MM, 1 IM), and another sample of cells was transferred to maintenance medium (2 CMD, 2 MM). This procedure was repeated for up to 11 subcultures into maintenance medium (approximately 165 days) (2 CMD, 1–11 MM, 1 IM). By calculation of the areas under the curves this experiment showed the progress of the loss of PAL inducibility during continued growth (up to 11 subcultures) in the maintenance medium (Fig. 4). An identical experiment was conducted using fast-growing cells (Fig. 5).

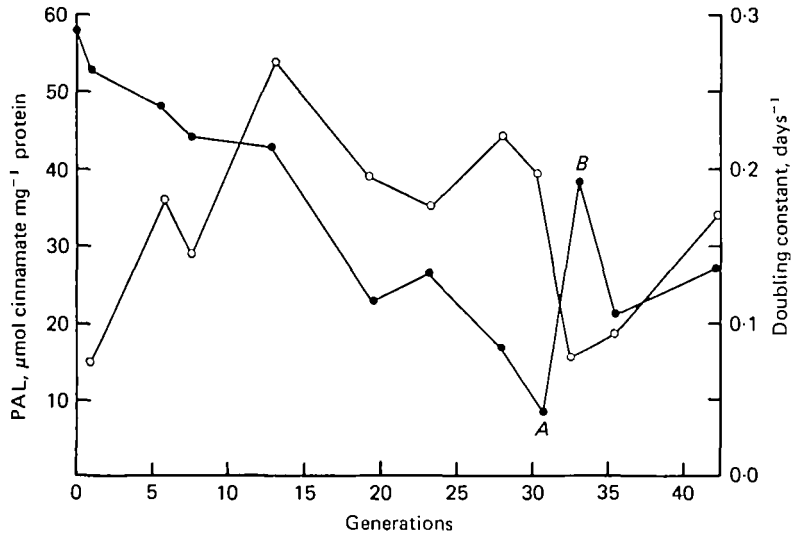

Fig. 4. The loss of PAL inducibility in slow-growing cells. The amount of PAL induced in cells grown for various times in the absence of coconut milk and then transferred to induction medium was calculated by measuring the area under the curve of induced PAL activity on induction medium from 4 to 21 days. Dry weight determinations before and after each subculture in the absence of coconut milk were used to calculate the number of cell generations undergone in the absence of coconut milk, and also the growth rate of cells during growth in the absence of coconut milk. ●, integrated PAL activity; O, doubling constant of the cells. Data at points *A* and *B* have been used in Fig. 6.

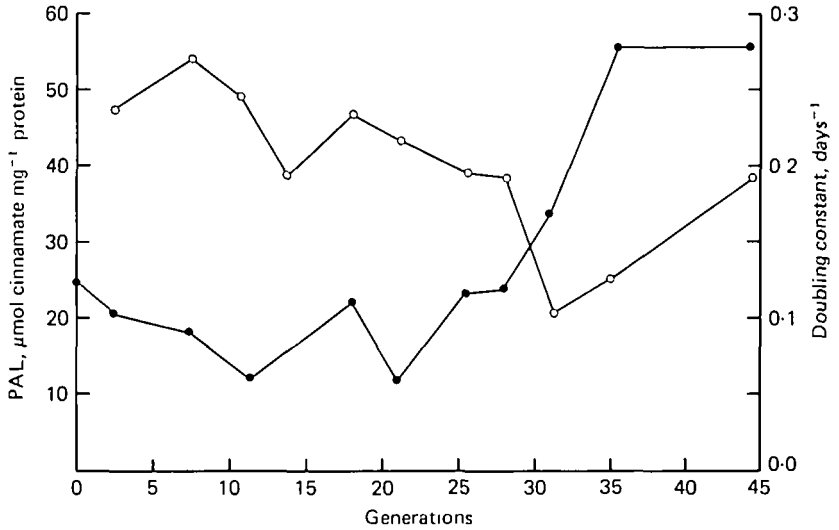

Fig. 5. The loss of PAL inducibility in fast-growing cells. The amount of PAL induced in cells grown for various times in the absence of coconut milk and then transferred to induction medium was calculated by measuring the area under the curve of induced PAL activity on induction medium from 4 to 21 days. Dry weight determinations before and after each subculture in the absence of coconut milk were used to calculate the number of cell generations undergone in the absence of coconut milk, and also the growth rate of the cells in the absence of coconut milk. ●, integrated PAL activity; O, doubling constant of the cells.

As can be seen in Fig. 3 there is a gradual decline in the amounts of PAL induced over a period of 4 subcultures on maintenance medium until after the fifth subculture the amounts of PAL induced in cells transferred to induction medium did not rise above a basal level. The extent of root formation decreased rapidly (slow-growing cells), and was not observed after the second subculture in maintenance medium (2 CMD, 2 MM, 1 IM). Similarly, the extent of xylem formation declined steadily until it was not observed after the third subculture in maintenance medium (2 CMD, 3 MM, 1 IM). Root and xylem formation occurred in slow-growing cultures grown

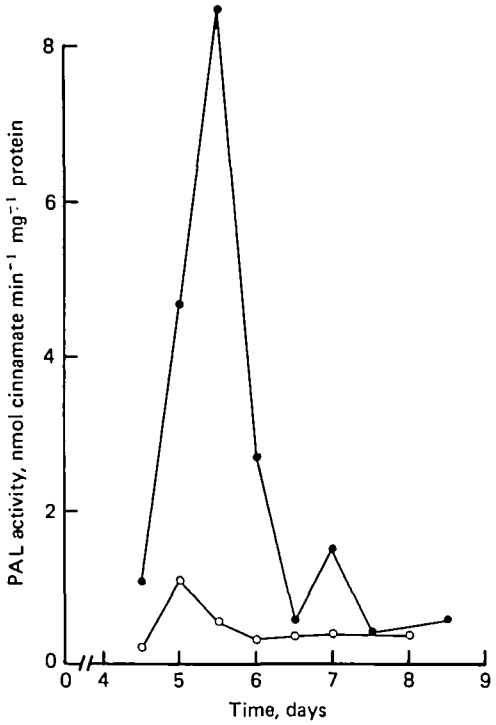

Fig. 6. The recovery of PAL inducibility. Time course for data shown at points *A* and *B* in Fig. 4. Cells grown for 8 subcultures in the absence of coconut milk were transferred to induction medium (point *A*, Fig. 4) and to medium lacking coconut milk for a further subculture (point *B*, Fig. 4). After 15 days they were transferred to induction medium. O, 2 CMD 8 MM 1 IM; ●, 2 CMD 9 MM 1 IM.

in CMD for up to 20 subcultures, when they were transferred to induction medium (20 CMD, 1 IM). Fig. 4 shows the decline in inducibility of PAL caused by growth of the cells in maintenance medium. After 30 generations (2 CMD, 8 MM), the PAL activity did not increase above a basal level when the cells were transferred to induction medium. However, after the next subculture into maintenance medium (2 CMD, 9 MM), there is a sudden recovery of inducibility which is coincident with a rapid decrease in the growth rate of the cells (Fig. 4). The magnitude of this recovery is apparent from Fig. 6 which shows the time course of the PAL inducibility of cells subcultured 8 and 9 times on maintenance media. As can be seen from Fig. 6 the total amount of PAL inducibility (area under the curve) that is recovered after 30

generations on maintenance medium is made up of a rapid increase in PAL after 4–5 days in the induction medium followed by a rapid decrease after 6–7 days. This can be contrasted with the initial PAL inducibility shown in Fig. 3. This recovery of inducibility would not have been detected by Haddon & Northcote (1976*a*) since they estimated inducibility by a single measurement, 21 days after transfer to induction medium. The recovery of inducibility occurs too rapidly (1 subculture in MM) to be caused by the selective growth of an inducible population of cells. The loss of PAL inducibility in slow-growing cultures is shown in Fig. 7 to be linear with respect to the number of cell generations on maintenance medium.

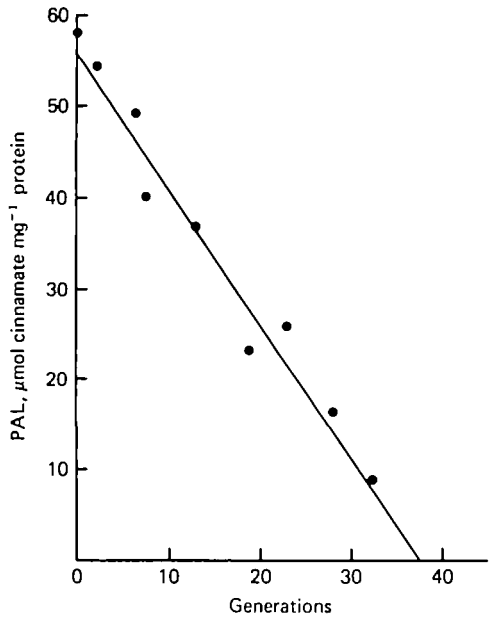

Fig. 7. The linear rate of loss of PAL inducibility. Data were derived from the experiment described by Fig. 4 and plotted to calculate parameters of the loss. Slope, −1·453; correlation coefficient, −0·98.

Fast-growing cells subcultured into maintenance medium did not show the decline in PAL inducibility (Fig. 5). The already low inducibility of these cultures did not change appreciably over a period of 27 generations (60–70 CMD, 7 MM), until after 27 generations the inducibility suddenly increased, as it did in slow-growing cells. This increase in inducibility was also coincident with a decrease in the growth rate of the cells (Fig. 5).

Cloning of inducible cells

An inducible suspension culture of slow-growing cells grown on CMD for 2 subcultures was cloned. Portions of the isolated clones were assayed for inducibility 21 days after transfer to induction medium solidified with 1% agar. The growth rates of these clones on CMD and on maintenance medium was measured. Fig. 8 shows the

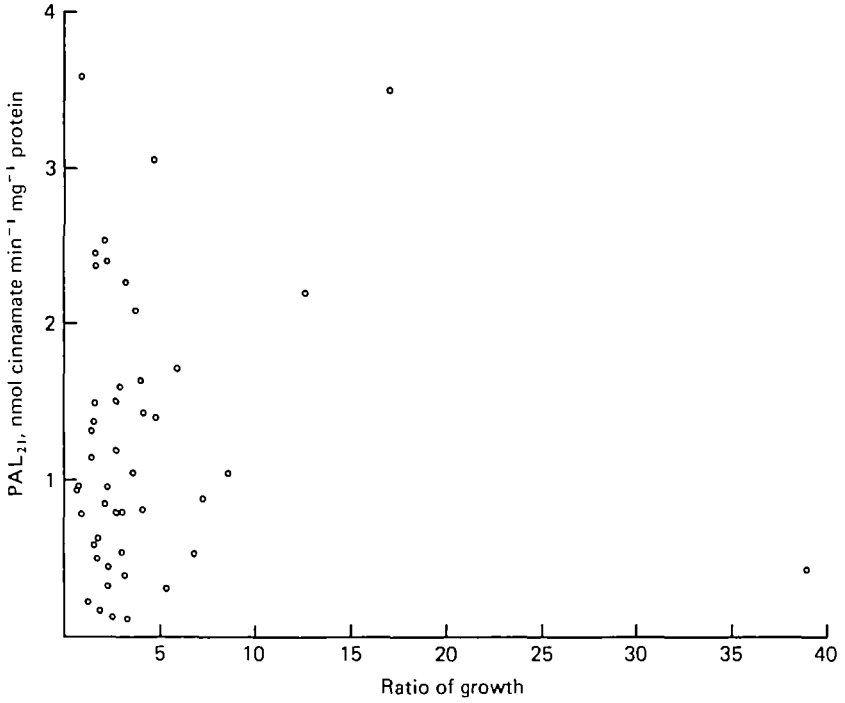

Fig. 8. The clonal variation of PAL inducibility in slow-growing cells. The levels of PAL induced after 21 days in induction medium are plotted against the ratio of growth in the presence and absence of coconut milk.

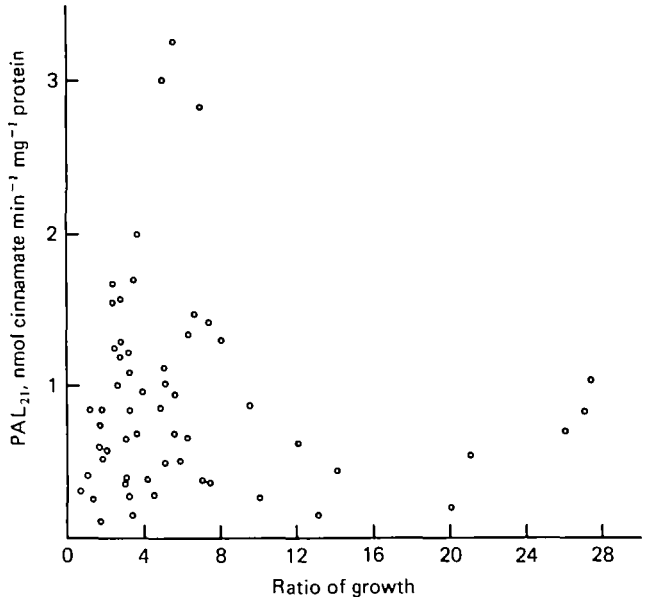

Fig. 9. The clonal variation of PAL inducibility in fast-growing cells. The levels of PAL induced 21 days after transfer to induction medium are plotted against the ratio of growth in the presence and absence of coconut milk. 63 clones were assayed.

relationship between PAL inducibility and the relative growth rates of 45 clones on media that allowed for the retention (CMD) and loss (MM) of PAL inducibility. There was a wide clonal variation in PAL inducibility which clustered about that of the original suspension from which they were derived (PAL activity, 1·5 nmol min^{-1} mg^{-1} protein). The growth rates of the clones on CMD clustered about the growth rate of the original suspension. This indicated that the clones assayed were derived from a representative sample of cells in the original culture.

No relationship between relative growth rates and inducibility was found (Fig. 8). The majority of both highly inducible and less inducible clones had growth rates on CMD between 1·5 and 4 times greater than on maintenance media. Similar results were found for clones derived from fast-growing cultures (60–70 CMD) (Fig. 9).

Dilution induction of PAL

Hahlbrock & Wellmann (1973) showed that the subculture of parsley suspension-cultured cells into a large volume of medium, or water (Hahlbrock & Schroder, 1975), caused a rapid increase in the specific activity of PAL. When fast-growing *Phaseolus* cells (60–70 CMD) were subcultured at low initial density (20 cm^3 cells into 2 dm^3 of

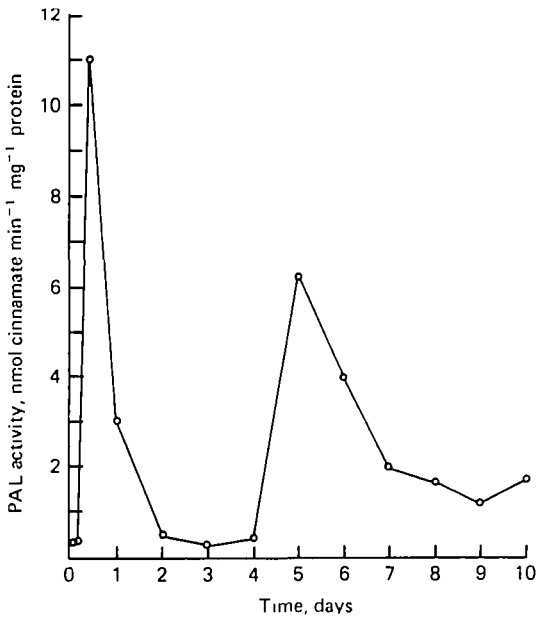

Fig. 10. Dilution induction and hormonal induction of PAL in fast-growing suspension cultures. Cells were transferred to 2 dm^3 of induction medium.

medium) into induction medium 2 peaks of PAL activity occurred, one 10–12 h after subculture, and the other 4–5 days after subculture (Fig. 10). The earlier peak started to appear 2·5 h after subculture; this is the characteristic lag period noted by Hahlbrock & Schroder (1975) and the maximum activity also occurred at the same time as that of parsley cells. The later peak of activity was that induced by NAA and kinetin,

and was not observed when cells were diluted into CMD rather than induction medium. When slow-growing cells that had lost their PAL inducibility due to continued growth in maintenance medium were subcultured at high dilution into induction medium, there was only one peak of PAL activity, which was due to dilution stimulus (Fig. 11). Thus the mechanism of PAL induction remained intact even when cells could no longer respond to concentrations of NAA and kinetin that brought about normal induction.

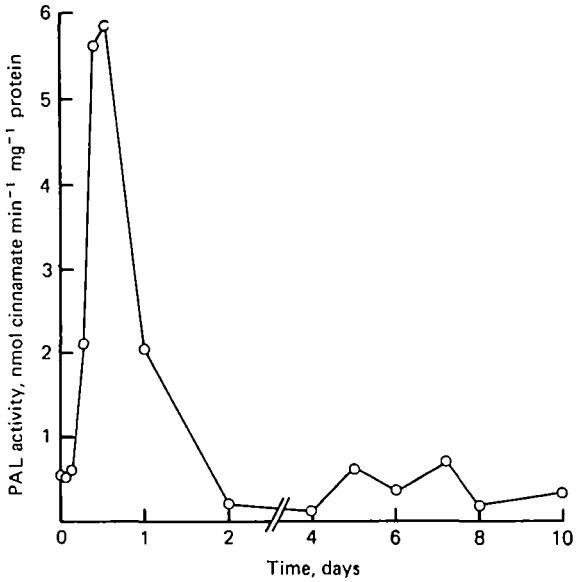

Fig. 11. Dilution induction in cells that have lost their ability to induce PAL in response to growth in the absence of coconut milk. Cells were taken from culture which had minimal PAL inducibility (2 CMD 8 MM) and subcultured into 2 dm^3 of induction medium.

DISCUSSION

The particular problem that we have investigated in this work is the mechanism whereby a suspension culture loses its ability to differentiate into xylem elements as it is grown on a maintenance medium but retains its ability when it is grown on a medium containing coconut milk.

The evidence against selection of a particular cell population being the cause of the cultures losing their ability to induce PAL activity when transferred to induction medium is 3-fold. Firstly, the decline in the ability to induce PAL was found to be linear when the cultures were grown on maintenance medium. An exponential rate of loss would result if the number of inducible cells decreased due to either a difference in the growth rate of inducible and non-inducible cells, or to any other type of competition between cell types such as those described in carrot cultures (Bayliss 1977*a*, *b*).

Secondly, the cloning experiments indicated that although a clonal variation in PAL inducibility existed, the relative growth rates of these clones in the presence (CMD) and absence (MM) of coconut milk would not give rise to an increase in the number

of less-inducible cells. The majority of the clones, irrespective of inducibility, grew 1·5–4 times more slowly on maintenance medium compared to CMD. Thus during continued growth on maintenance medium there would be no selection, by differences in growth rate, of the cells with low inducibility.

The possibility remained that a particular type of cell which had a high inducibility and whose growth rate was much greater in the presence of coconut milk than in its absence, was present in very low numbers in the inducible cultures. In these circumstances they may not have been detected in the sample of clones taken from the original culture. But because they occurred in low numbers the relative proportion of these cells would not change sufficiently during the first 2 subcultures in the absence of coconut milk to bring about the magnitude of the loss of inducibility that was observed initially. The rate of loss of the inducibility is linear over 8 subcultures with no lag period.

Thirdly, it has been shown that fast-growing cell lines do not lose their inducibility during growth in the absence of coconut milk (MM). These cells have a similar clonal variation of PAL inducibility to the variation found in slow-growing cells, and if selection occurred in these cells, due to different growth rates, a rapid decline in inducibility would be expected. This does not happen.

We have shown in addition that reverse of the loss of inducibility, its recovery, occurred during one subculture and was too rapid to be accounted for by a selection mechanism.

Since selection of particular cells was not the cause of the loss of inducibility in the cultures, it was possible that a genetic change occurred in the cells such that they lost the ability to synthesize or activate PAL. However, this was not the case because cells in which PAL activity could not be induced by the application of the hormones in the induction medium, did synthesize or activate PAL in response to a dilution stimulus. Hahlbrock and his colleagues (Schroder, Betz & Hahlbrock, 1976, 1977; Schroder, 1977) have shown that the response to dilution involves an increase in the availability of PAL-mRNA for translation. In addition we have shown that the response to the hormones was reversible and after some 30 generations the tissue responded again to the induction medium by an increased amount of PAL activity.

If the loss of inducibility was not due to cell selection or to genetic changes it would seem to be connected with a reversible change in the cells' response to the applied hormones in the induction medium. One way by which this might occur, since the cells produce endogenous growth factors, is that the amounts of these and their proportions could vary during continued growth and with different types of media. Thus the amounts of auxin and kinin within the cell may determine PAL induction and these amounts depend on both exogenous and endogenous supplies. Therefore it is possible that the cells which have lost their inducibility for this reason would respond to different exogenous concentrations of auxin and cytokinin. However, other changes in the cell could be envisaged whereby they no longer respond to any concentrations of exogenous growth hormones.

Dudley & Northcote (1978) showed that the transfer of *Phaseolus* cells from CMD medium into induction medium caused a change in the *in vitro* translation products

of isolated mRNA. But it remains to be seen if there is a change in the amounts of PAL-mRNA caused by NAA and kinetin.

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