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Opportunities for Regulation of Sugar Beet Storage Root Growth

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Abstract. The percentage of sucrose in sugar beet storage root fresh and dry matter is closely related to root structure. It has been suggested that the sucrose content might be increased by using plant growth regulators to modify storage root structure through control of cambial development, cell division and cell expansion. During storage root development correlations were found between the changing phytohormone profiles and the formation of secondary cambia and their subsequent cell division and expansion. Sugar beet root derived cell suspension cultures were used for detailed studies of the roles of endogenous phytohormones. The gibberellin synthesis inhibitor paclobutrazol was tested in cell cultures and whole plants. The observations provide a basis for development of plant growth regulator regimes to optimise sucrose yield from sugar beet.

During the twentieth century there has been a dramatic increase in the rate of growth of world demand for food. Agronomists have responded by an unprecedented flood of improvements in mechanisation, pesticides, fertilizers and plant breeding. The technological advances have revolutionised agriculture in the developed world and are now making a significant impact in developing countries.

In the developed world most of the technology has already been optimised so that, for example, further modifications of fertilizer and pesticide regimes would not yield appreciable benefits and would certainly not be cost effective. Since the need for additional food production is still increasing some new approach is needed.

The agrochemical industry which has been so successful in developing herbicides and fertilizers has been less successful in developing ways positively to modify crop growth to man's advantage. This is mainly due to the more dificult nature of the problems. The authors believe that the next wave of change in agriculture will be made possible only by the development of a sophisticated understanding of the endogenous regulatory systems which control growth and cropping. Once such knowledge is available relatively fine tuning of developmental processes may well produce the new wave of technological advances in agriculture which the world so desperately needs.

Sugar beet provides 40% of the world's total production of sugar (FICK *et al.* 1975). Selective breeding since the 1890's and improved agricultural

practices since the 1940's have increased the fresh mass concentration of sucrose in beet from 4 to 18% (MILFORD *et al.* 1980). Continued selection may increase concentrations further but progress in combining high dry matter yield with high sucrose concentration is likely to be slow because sugar concentration and root dry mass (DM) are strongly negatively cor-

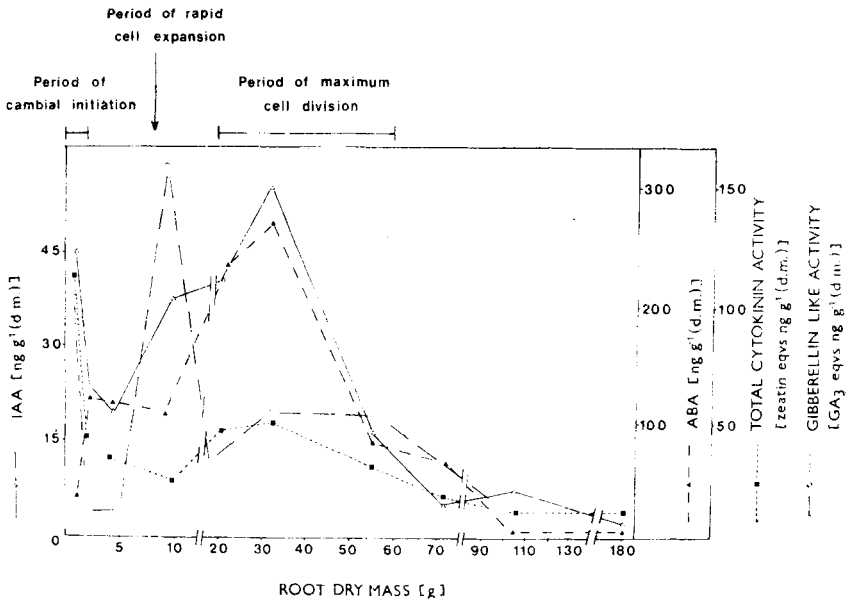

Fig. 1. Changes in phytohormone concentration in the sugar beet storage root through the growing season related to phases of root development. Error bars have been omitted for clarity. The largest standard errors (which were at the maximum concentrations) were ±17 ng (cytokinin), ±10 ng (IAA), ±17 ng (ABA) and ±25 ng (gibberellins).

related. MILFORD (1973) and WYSE (1979) argued that higher sugar yields and higher sugar concentrations would be achievable in roots containing more cambial rings so that the diffusion paths between the phloem and storage cells were shortened. MILFORD and LENTON (1978) suggested that plant growth regulators could be used to cause such changes in root structure.

In earlier papers (ELLIOTT *et al.* 1983, HOSFORD *et al.* 1984) we have described our comparative studies with three subspecies of *Beta vulgaris* L. (Sugar beet itself, mangold and Swiss Chard). IAA, cytokinin, gibberellin and abscisic acid concentrations in the storage roots were measured throughout the growing season and the changes were related to stages in storage root development viz 1) initiation of cambia, 2) production and differentiation of derivatives, 3) divisions in the parenchyma beyond the cambium and 4) expansion of parenchyma. In spite of the crudity of the approach (dictated by the predicted need of the agrochemical industry to keep required treatments as simple as possible) the results proved to be instructive and provided a basis for further consideration of growth regulator applications which will produce sugar beet storage roots of narrow ring width and high sugar storage capacity.

For sugar beet itself (Fig. 1) it was noted that high concentrations of IAA, gibberellins and cytokinins were found during the period of cambial initiation when the level of ABA was low. At the end of this developmental period IAA, gibberellins and cytokinins sharply declined while ABA started to increase. This last may simply have reflected the water status of the field grown plants but its possible functional significance was not overlooked (HOSFORD *et al.* 1984).

Immediately before the period of rapid cell expansion there was a dramatic increase in gibberellin levels and a slower increase in IAA concentration. Cytokinin levels remained relatively low during this period but begin to increase again as the root approaches the stage of rapid cell division in the regions beyond the cambia. During this phase IAA, cytokinins and ABA remain high but slowly decline.

Although there appeared to be significant correlations between phytohormone status and developmental characteristics of the three sub-species the data would be of value to the agrochemist only if the changing phytohormone patterns determine the changes in development. Clearly a more sophisticated plant system was required if further information on this point was to be readily interpretable. For these further studies root-derived sugar beet cell suspension cultures were used.

MATERIALS AND METHODS

Cell Cultures

The sugar beet cell suspension cultures were established from sugar beet seedling root tissue (cv. Bush Mono G) and have been maintained for four years in HOOKER and NABORS (1977) medium without added cytokinin. All experimental cultures were incubated in the light (500 cd sr m^{-2}) at 25 °C on orbital flat bed shakers operating at 120 rev. min^{-1}.

When paclobutrazol was added to the cultures it was dissolved in propylene glycol at such a concentration that when 1 cm^3 was added to 70 cm^3 of autoclaved culture medium (after filter sterilisation through a 0.45 μm Millipore filter), the required concentration of paclobutrazol was obtained. Although propylene glycol did not affect culture growth all control treatments contained 1 cm^3 propylene glycol. Replicate flasks were prepared for each treatment. Flasks were inoculated (at a cell density of 170×10^3 cells cm^{-3}) with sugar beet cells from twenty one day stock cultures. Culture parameters were monitored as described previously (HENSHAW *et al.* 1966).

Procedures for phytohormone and macromolecule assays have been described previously (ELLIOTT *et al.* 1983, HOSFORD *et al.* 1984).

RESULTS AND DISCUSSION

Fig. 2 shows that cell number changes during batch culture of sugar beet cells reveal the usual sigmoid culture growth pattern. Cytokinin levels in the cells increase rapidly during the short lag period and reach a maximum during the early stages of most active cell division. After this time the cytokinin levels in the cells decline and the lowest cellular cytokinin levels are found during the stationary phase. Similar trends of association between changing cytokinin levels and cell division in suspension cultures have been

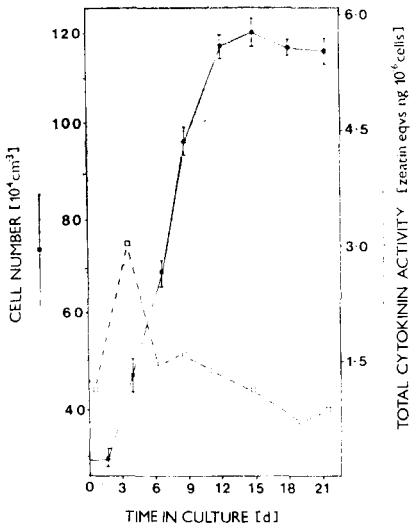

Fig. 2. Changes in cell number and total cytokinin activity throughout the growth cycle of a sugar beet cell suspension culture.

reported by other workers for cells of other plant species (MACKENZIE and STREET 1972). These studies on batch cultures do not give sufficiently precise information on the relative timing of the increase in cytokinin levels and the onset of cell division for clear implications of cause and effect to appear. We have, on the other hand, succeeded in producing cell cultures in which divisions are highly synchronised (ROBINSON *et al.* 1984). Fig. 3 shows data for such a culture of sugar beet cells. Cell numbers, total cytokinin, DNA and protein levels were monitored (ELLIOTT *et al.* 1983). The cellular cytokinin level rises to a maximum and falls again during the period from

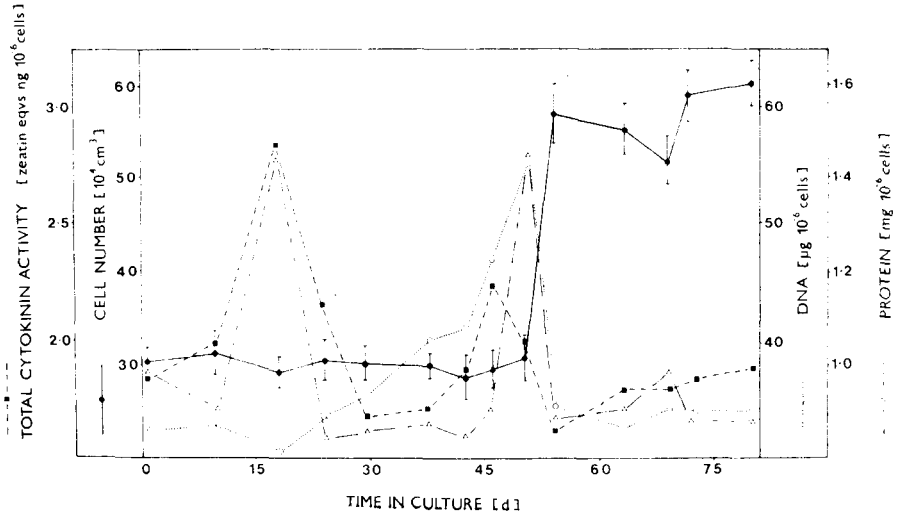

Fig. 3. Changes in cell number, total cytokinin activity, total protein and DNA during the first division period of a sugar beet cell suspension culture.

10—30 h after inoculation. The total cellular protein shows a parallel rise and fall in line with LALOUE'S (1978) emphasis on the role of cytokinins in controlling rates of protein synthesis and with the observations of other workers (VERMA and MARCUS 1974, BEVAN and NORTHCOTE 1981). The stimulation has been attributed to an increase in polyribosome content (SHORT *et al.* 1974, FOSKET *et al.* 1977, TEPFER and FOSKET 1978).

Shortly before cytokinesis, but clearly preceding it, there is a further peak of cellular cytokinin. There is also another peak of protein and DNA doubles. The relationship between changes in cytokinin status of the cells and the onset of cell division is such as to indicate that the former may be involved in controlling the latter (NISHINARI and SYONO 1980, ROBINSON *et al.* 1984). The relationship between cytokinins and mitosis has been partly explained in terms of a requirement for cytokinin induced synthesis of specific proteins required for the functioning of the mitotic apparatus (FOSKET and SHORT 1973, JOUANNEAU 1975, FOSKET 1977, PÉAUD-LENOËL 1977, NISHINARI and SYONO 1980).

Clearly these studies leave open the possibility that the changes in cytokinin status of the storage root were causal of the changes in cell division activity. Thus we must consider the possibility that agrochemical modification of the cytokinin profiles may stimulate activity of those secondary cambia which do not, at present, make significant contributions to storage root growth (HOSFORD *et al.* 1984).

We have noted elsewhere (ELLIOTT *et al.* 1983) that there is an apparent association between increases in the levels of gibberellin-like substances in the sugar beet storage root and the onset of the period of rapid cell expansion. It is interesting to find that during the period of most active cell div-

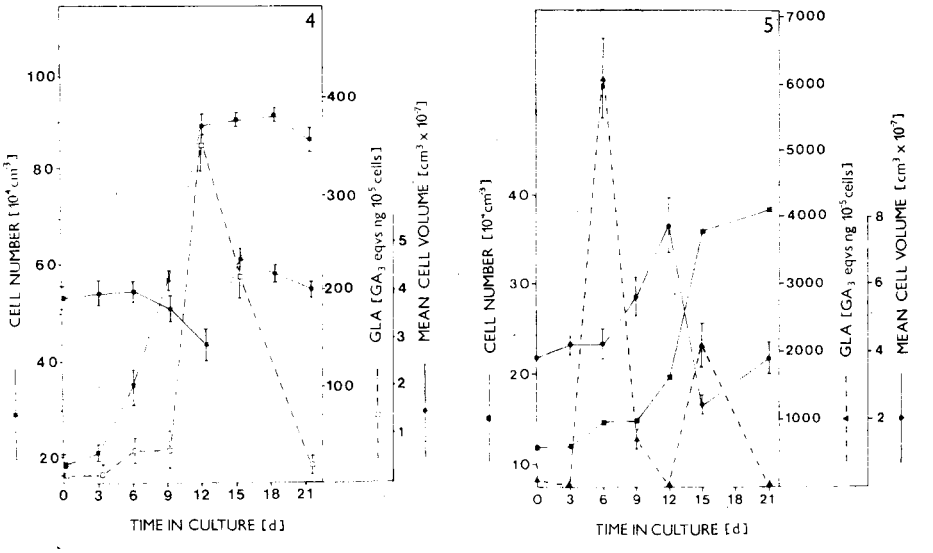

Fig. 4. Changes in cell number, mean cell volume and gibberellin-like activity throughout the growth cycle of a sugar beet cell suspension culture.

Fig. 5. Changes in cell number, mean cell volume and gibberellin-like activity through the growth cycle of a sugar beet cell suspension culture treated with 4×10^{-5} mol dm^{-3} paclobutrazole.

ision in the sugar beet cell suspension cultures mean cell volume declines (Fig. 4). The cessation of rapid cell division and commencement of the stationary phase is marked by a rapid increase in mean cell volume and this increase is preceded by a dramatic increase in cellular gibberellin-like substances. This reinforcement of the suggestion that gibberellin levels may at least partially control cell enlargement caused us to consider the possibility that gibberellin synthesis inhibitors would be useful agents in sugar beet crop management.

Paclobutrazol, (2*RS*,3*RS*,)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pentan-3-ol, is a relatively new broad spectrum growth retardant whose activity is not accompanied by phytotoxicity or scorch even when applied at high rates. GOLDSMITH *et al.* (1983) showed that in cultures of *Gibberella fujikuroi* paclobutrazol inhibited gibberellin biosynthesis at the *ent*-kaurene to *ent*-kaurenoic acid step in the biosynthetic pathway. DALZIEL and LAWRENCE (1984) noted that in higher plants the activity of kaurene oxidase was inhibited. In our studies paclobutrazol caused a concentration dependent reduction in final cell numbers in cultures treated with concentrations in the range 4.0×10^{-7} mol dm^{-3} to 4.0×10^{-5} mol dm^{-3}. At the higher concentrations the lag phase was extended and the total number of cell divisions was reduced. At the lowest concentration there was no effect on the length of the lag phase but the final cell number was slightly reduced. Cell volume increases were not prevented by the inhibitor and, in fact, the inhibited cultures had greatly enlarged cells. BAYLISS (reported by DALZIEL and LAWRENCE 1984) made similar observations with Paul's Scarlet Rose cultures.

The effect of the retardant could be reduced by the inclusion of 10^{-4} mol dm^{-3} GA_3 in the culture medium. This result is consistent with the view that the retardant inhibits endogenous GA synthesis but the physiological effect is precisely the opposite of that which might be predicted from the whole plant data. Accordingly we determined changes in cell number, mean cell volume and cellular gibberellin-like substances in cell cultures to which 4×10^{-5} mol dm^{-3} paclobutrazol was added. Fig. 5 shows (when compared with Fig. 4) that cell number in the culture is reduced and mean cell volume is increased by the inhibitor. However, rather surprisingly, the gibberellin-like substances in the cells are greatly increased and this at a time which preceded the dramatic cell enlargement. It must be noted that the precise physico-chemical characterisation of the gibberellin-like substances has not yet been attempted but there are reports in the literature of levels of gibberellin-like substances being increased after treatment of the plant material with supposed inhibitors of gibberellin biosynthesis (REID and CROZIER 1970). The continuing association of high levels of gibberellin-like substances with cell enlargement, although surprising in this context, made it appropriate to consider the effects of paclobutrazol on whole sugar beet plants. In seedling plants shoot: root ratios are reduced by application (at 3.4×10^{-3} mol dm^{-3}) of a foliar spray to plants at the four-leaf stage (LAWRENCE and RIDLEY 1984) and the thickness is doubled due to the increase in the length and volume of the mesophyll cells (DALZIEL and LAWRENCE 1984). In contrast to the effect of the inhibitor in causing enlargement of these leaf cells and of root-derived cells in suspension culture there is the desired dramatic effect in reducing cell enlargement in roots of whole plants sprayed at the

four-leaf stage with a 13.6×10^{-3} mol dm^{-3} solution of paclobutrazol. One might speculate that this means that the root cells *in situ* are less able to overcome the gibberellin synthesis block. This is, at worst, a testable hypothesis and does not ignore the many alternative possibilities.

These studies may imply that it is indeed possible to devise plant growth regulator application regimes for producing sugar beet storage roots of narrow ring width and high sugar storage capacity.

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