BIOLOGIA PLANTARUM (PRAHA)

30 (6) : 409–413, 1988

Nutritional Changes Induce Xylogenesis in Callus of *Haplopappus gracilis* (NUTT.) A. GRAY

WIESŁAWA KUTERNOZIŃSKA*, LUCYNA WAJDA*, and W. KOROHODA**

Laboratory of Cytophysiology, Department of Plant Physiology, Polish Academy of Sciences, 31-130 Cracow, Kremerowska 2*, and Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, 31–120 Cracow, Aleje Mickiewicza 3**, Poland

Abstract. Xylogenesis was induced in cultures of calli of *Haplopappus gracilis* (NUTT.) A. GRAY by metabolic shocks brought about by changes in nutrition. Xylogenesis was observed to occur in response to three changes in callus nutrition, caused by the following modifications of Eriksson's nutrient medium and culture conditions: i) omission of sucrose in the medium for 3 to 6 days and then transfer of the calli onto complete Eriksson's medium; ii) replacement of sucrose by 0.36 mol l^{-1} xylose for the whole culture period; iii) omission of nitrogen sources (*i.e.* glycine and NH_4NO_3 omission and replacement of KNO_3 with equimolar KCl) for the whole culture period.

The induction of xylogenesis in response to nutritional stress in *H. gracilis* can be used as a suitable model system for research concerning plant cell differentiation.

Plant cell differentiation has been studied in various experimental systems: *in vitro* in callus or cell suspension cultures or in explants as well as *in vivo* during morphogenesis of plant organs, or wound healing (SHININGER 1979, ALONI 1980, FUKUDA and KOMAMINE 1980, PHILLIPS 1980). Attention has been paid mainly to conditions permitting and/or inducing morphological differentiation of cells and, more recently, to some biochemical aspects of cytodifferentiation (KOMAMINE and FUKUDA 1982, ROBERTS *et al.* 1982). Further study, however, will require more precise determination of the enetic aspects associated with the regulation of gene expression (GOLDBERG 1986).

Among higher plants, *Haplopappus gracilis* (NUTT.) A. GRAY seems to be a suitable model organism for the study of cell differentiation because of its very low, 2n=4, chromosome number (FAROOK and VIG 1980). The only serious limitation for a broader usage of this species is that so far differentiation and organogenesis *in vitro* have been achieved only from shoot-tip cultures (TANAKA and IKEDA 1983) and from young leaves and stem internodes (RAMULU and DIJKHUIS 1984).

The aim of the preliminary experiments described in this paper was to induce xylogenesis in cultures of *H. gracilis* calli propagated *in vitro* for over 7 years in our laboratory.

Received May 10, 1988; accepted June 17, 1988

MATERIAL AND METHODS

The seeds of *Haplopappus gracilis* (NUTT.) A. GRAY (*Compositae*) were generously provided by Dr P. A. Th. J. Werry (I.T.A.L. Wageningen, the Netherlands) to one of the authors (L. Wajda).

The callus tissue was obtained from plants grown from these seeds in 1980. It was grown on Eriksson's nutrient medium (ERIKSSON 1965) with 0.2 mg l^{-1} of kinetin, 1.0 mg l^{-1} of indol-3-ylacetic acid and 0.4 mg l^{-1} of thiamine in the dark. The experiments described below were carried out in the dark to prevent greening of the callus and the synthesis of sucrose.

The formation of tracheary elements in calli grown on control and modified media was followed with a light microscope (contrast phase or bright field). In some experiments the number of tracheary elements (TE) was determined in callus pieces derived from the control and experimentally treated calli and then calculated per 100 mg of fresh mass.

For the experiments the control Eriksson's medium was modified by: i) the omission of sucrose; ii) replacement of sucrose by 0.36 mol l^{-1} of xylose or 2 % glycerol; and iii) the omission of glycine and NH_4NO_3 and replacement of 18.8 mmol l^{-1} of KNO_3 by 18.8 mmol l^{-1} of KCl.

RESULTS

The callus of *H. gracilis* which was cultured on Eriksson's nutrient medium did not differentiate and showed no signs of morphogenesis. Only in very old cultures might a few tracheary elements sometimes be found. It was observed that after 5 to 10 days of culture on Eriksson's medium in which sucrose was omitted numerous noduli of tracheary elements appeared in the callus (Fig. 1a,b). Differentiation of parenchymal cells to tracheary elements was also observed if the callus was incubated for 3 or 6 days on the sucrose-free Eriksson's medium and then transferred onto the complete Eriksson's

TABLE 1

Induction of tracheary elements (TE) in calli of *H. gracilis* starved for sucrose for 3 or 6 days and then transferred onto the full medium. Numbers of TE were estimated in samples of 100 mg of fresh mass 21 days after the return transfer of the calli onto the full medium. Restoration of growth after the return transfer is expressed as the ratio of fresh mass at the end of the experiment minus the fresh mass of the callus returned onto the full medium to the fresh mass of the returned callus. Average data of 4—6 replicates

Pretreatment	Number of TE per 100 mg of fresh mass	Growth of callus
Control without starvation	4	4.79 ± 1.33
3 days of sucrose starvation	82	3.78 ± 1.22
6 days of sucrose starvation	90	1.23 ± 0.71

TABLE 2

Induction of tracheary elements (TE) by nutritional stress in calli of *Haplopappus gracilis* grown on modified Eriksson's medium EM. TE were observed after 14 days of calli incubation on the control or modified media. Denotations: less than 1 TE in the microscopic field of view = 0; 1–2 TE = +; 3–6 TE = ++; more than 6 = +++. On average 50 to 100 cells were observed in one microscopic field of view, magnification 150×; 20 fields were observed

Medium	Number of TE per microscopic field of view
Control-complete EM	0
EM without sucrose	++
EM in which sucrose was replaced by 2 % glycerol	+
EM in which sucrose was replaced by 0.36 mol l^{-1} xylose	+++
EM without nitrogen-containing compounds	+++

medium. Twenty-one days after the return transfer numerous tracheary elements were found (Table 1). They were usually chaotically distributed throughout the calli as groups of several differentiated cells clumped together. No parallel alignment of tracheary elements could be detected. In several repetitions of these two experiments it was observed that the numbers of tracheary elements with a well-developed secondary cell wall pattern, usually of the reticulate type, exceeded in permanently or 3–6 days sugar-starved cultures 20 to 100 times those found in control, well nourished cultures.

In the next series of experiments, in which sucrose in the medium was replaced by 0.36 mol l^{-1} xylose, groups of tracheary elements were found in callus incubated for 14 days on this medium (Table 2). The calli survived much better on the medium in which sucrose was replaced by xylose than on that in which sucrose was simply omitted.

It was also observed that the callus of *H. gracilis* survived much longer on Eriksson's medium without nitrogen than without sugar. After 25 days of culture on such a medium the fresh mass was still increasing (Fig. 2). When the samples of calli grown on the nitrogen-free Eriksson's medium were analyzed abundant tracheary elements were found after 6 to 14 days of culture. The number of tracheary elements per 100 mg of fresh mass of callus always exceeded several times that in controls and in transplanted calli at zero time of the experiment. In some samples the numbers of tracheary elements reached over 250 in 100 mg of callus but usually 60 to 80 tracheary elements per 100 mg of callus were found. The absence of nitrogen sources induced the same response in the callus of *H. gracilis*, *i.e.* xylogenesis, as did sugar starvation or replacement of sucrose with xylose.

DISCUSSION

The results reported here are in agreement with those of other investigators, who carried out their research on different plant species (WETMORE *et al.*

1964, RIER and BESLOW 1967, ROBERTS and BABA 1982, SILVENTE and TRIPPI 1986, SUGIYAMA *et al.* 1986). Nevertheless, in our experiments some new problems emerged. It was observed that in the same callus three different modifications in cell nutrition evoked the same cell response, *i.e.* xylogenesis. In the above-cited papers attention was paid to concentrations of sugar or

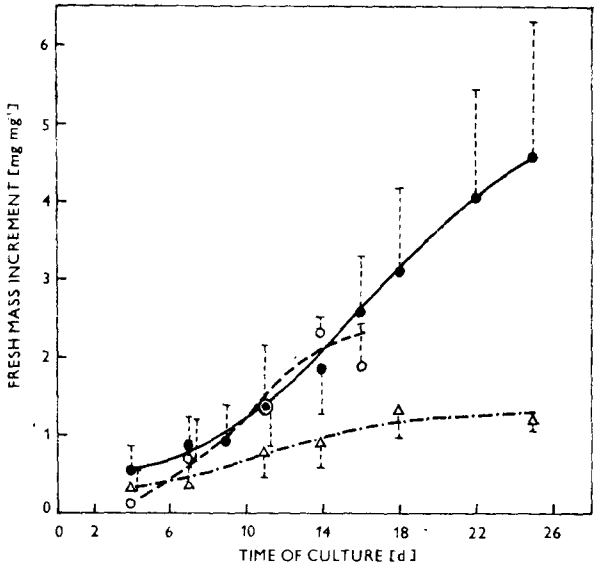

Fig. 2. Effect of nutritional stress upon growth of the callus of *H. gracilis*. — The complete Eriksson's medium (control ●); Eriksson's medium modified by replacement of sucrose with 2 % glycerol (○); Eriksson's medium modified by nitrogen sources omission (△).

No growth was observed when sucrose was omitted in this medium. Fresh mass increment (FMI) was expressed as

$$(FM_c - FM_b)/FM_b$$

where: FM_c — fresh mass of callus cultured for a number of days shown in the Figure; FM_b — fresh mass of transplanted callus at the beginning of the experiment.

of nitrogen-containing compounds and there was no agreement as to the most suitable concentrations of particular compounds for xylogenesis. Our results seem to suggest that processes of cytodifferentiation are induced as the cell response to nutritional stress. This conclusion corresponds with all previous results as well as with the literature data concerning the effects of various stress-inducing conditions upon the regulation of plant gene expression. As pointed out by MATTERS and SCANDALIOS (1986), changes in gene expression elicited during periods of environmentally induced stress provide models for the study of gene regulation in differentiated cells. The function of such stress-induced genes is unknown. Nutritional stress, on the other hand, has been shown to induce processes of differentiation in such dissimilar organisms as acellular and cellular slime mould plasmodia or animal cells (for example KONIJN and VAN HAASTERT 1984, SCARPA *et al.* 1985, SCHAAP *et al.* 1985). It remains to be seen whether it is only an incidental similarity of responses to nutritional stress in various eukaryotic organisms or if there is a similar molecular mechanism underlying the undifferentiated

cell reaction. The callus of *H. gracilis*, a plant with the low chromosomal number, 2n=4, seems to be a suitable model system for the study of cyto-differentiation of plant cells.

Acknowledgement

This work was supported by a grant from the Polish Academy of Sciences: C.P.B.P.-05.02.4.20.

REFREENCES

ALONI, R.: Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. — Planta **150** : 255—263, 1980.

ERIKSSON, T.: Studies on the growth requirements and growth measurements of cell cultures of *Haplopappus gracilis*. — Physiol. Plant. **18** : 976—993, 1965.

FAROOK, S. A. F., VIG, B. K.: Sequence of centromere separation: analysis of mitotic chromosomes of *Crepis capillaris* and *Haplopappus gracilis*. — Biol. Zentralbl. **99** : 675—682, 1980.

FUKUDA, H., KOMAMINE, A.: Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from the mesophyll of *Zinnia elegans*. — Plant Physiol. **65** : 57—60, 1980.

GOLDBERG, R. B.: Regulation of plant gene expression. — Phil. Trans. roy. Soc. London B **314**: 343—353, 1986.

KOMAMINE, A., FUKUDA, H.: Biochemical mechanism of cytodifferentiation. — In: Proc. 5th Int. Cong. Plant Tissue and Cell Culture. Pp. 91—92. 1982.

KONIJN, T. M., VAN HAASTERT, P. J. M.: Cell interactions in the cellular slime moulds. — In: PIRSON, A., ZIMMERMANN, M. H. (ed.): Encyclopedia of Plant Physiology, N. S., Vol. 17. Pp. 309—332. Springer—Verlag, Berlin—Heidelberg—New York—Tokyo 1984.

MATTERS, G. L., SCANDALIOS, J. G.: Changes in plant gene expression during stress. — Develop. Genet. **7** : 167—175, 1986.

RAMULU, K. S., DIJKHUIS, P.: Growth and differentiation of callus derived from leaf and stem internodes of *Haplopappus gracilis* (NUTT.) GRAY. — J. Plant Physiol. **115** : 245—251, 1984.

RIER, J. P., BESLOW, D. T.: Sucrose concentration and the differentiation of xylem in callus. — Bot. Gaz. **128** : 73—77, 1967.

ROBERTS, L. W., BABA, S.: Glycerol and myo-inositol as carbon sources for the induction of xylogenesis in explants of *Lactuca*. — Can. J. Bot. **60** : 1204—1206, 1982.

ROBERTS, L. W., BABA, S., SHIRAISHI, T., MILLER, A. R.: Progress in cytodifferentiation under *in vitro* conditions. — In: Proc. 5th Int. Cong. Plant and Cell Culture. Pp. 87—90. 1982.

SCARPA, S., UHLENDORF, B. W., CANTONI, G. L.: The differentiation of 5/A10 myoblast cell line a subclone of L5 line is controlled by changes of culture conditions. — Cell Different. **17** : 105—114, 1985.

SCHAAP, P., PINAS, J. E., WANG, M.: Patterns of cell differentiation in several cellular slime mould species. — Develop. Biol. **111** : 51—61, 1985.

SHININGER, T. L.: The control of vascular development. — Annu. Rev. Plant Physiol. **30** : 313—337, 1979.

SILVENTE, S. T., TRIPPI, V. S.: Sucrose-modulated morphogenesis in *Anagallis arvensis* L. — Plant Cell Physiol. **27** : 349—354, 1986.

SUGIYAMA, M., FUKUDA, H., KOMAMINE, A.: Effects of nutrient limitation and γ-irradiation on tracheary element differentiation and cell division in single mesophyll cells of *Zinnia elegans*. — Plant Cell Physiol. **27** : 601—606, 1986.

TANAKA, R., IKEDA, H.: Perennial maintenance of annual *Haplopappus gracilis* (2n=4) by shoot-tip cloning. — Jap. J. Genet. **58** : 65—70, 1983.

WETMORE, R. H., DE MAGGIO, A. E., RIER, J. P.: Contemporary outlook on the differentiation of vascular tissues. — Phytomorphology **14** : 203—217, 1964.

Fig. 1 at the end of the issue.