

Short Term Cultivation of Isolated Barley Roots and their Mitotic Activity

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Abstract. Roots were excised from barley embryos cultivated in the complete liquid nutrient solution and cultivated in the same nutrient solution separately. The excised roots continued their growth but a progressive decrease in the growth rate was observed. There was a considerable short-term drop of the mitotic activity immediately after excision, which was followed by a compensatory increase and then equilibrium was reached 12 h after excision. During the next at least three days, the mitotic index of isolated barley roots varied between 5—6.5%, which is slightly lower than the mitotic index of the root meristems of isolated barley embryos under identical conditions. The mitotic cycle index of isolated barley roots and the size of the root meristem later decreased gradually.

Isolated roots of some plant species cultivated *in vitro* like tomato (STREET and MCGONAGLE 1953) or wheat (FERGUSON 1963) are capable of unlimited growth. It is, of course, not the original meristem but the succeeding branches that can grow for unlimited time (PETRŮ 1965, STREET 1967). The *in vitro* growth of roots of some other plant species like barley and oats (ALMENSTRAND 1957) is only transient. Isolated plant roots cultivated *in vitro* can be useful in some kinds of cytogenetic experiments. The cultivation *in vitro* of detached roots offers a good control over the mitotic cycle kinetics. Pea root cultures were already used for the determination of the replicon size and distribution of replicons under different nutritional conditions (VAN'T HOF 1976a, b, VAN'T HOF and BJERKNES 1977).

The growth of isolated barley embryos was tested in our previous experiments (GICHNER *et al.* 1976, LANDOVÁ and ONDŘEJ 1979). In the presented paper, the growth and mitotic activity of isolated barley roots were tested.

Material and Methods

Spring barley (*Hordeum vulgare* L., cv. Ametyst), fraction with seed diameter 2.5—2.8 mm was used throughout the experiments. As there is no reliable method of sterilization of whole seeds without reducing the vitality of seedlings, roots were excised from embryo cultures established as already described (GICHNER *et al.* 1976, LANDOVÁ and ONDŘEJ 1979). Embryos were

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cultivated in the liquid aerated medium according to MIFLIN (1969), where casein hydrolysate served as a source of nitrogen and medium was diluted 1 : 1. Embryos were in vials with 2 cm diameter; 10 embryos and 4 ml of medium were put into each vial. Vials were placed on the dish of the gyratory shaker RT 60 and shaken with 140 rpm, with the diameter of rotation 4 cm. After 4 days of cultivation, roots were cut off aseptically. If not given otherwise, 3–4 roots 1 cm in length were cut off from each embryo and 5 roots were put into each vial. Roots were measured after different periods of cultivation. Thirty roots were used in each experimental variant. In the experiments with long-term cultivation (4 weeks), 20 roots and 70 ml of medium were put into each 250 ml Ehrlenmayer flask and put on the dish of the gyratory shaker, with 100 rpm. Temperature was kept at 26 °C, in the dark.

For the microscopic technique, roots and embryos were fixed in ethanol-acetic mixture 3 : 1, stained by the Feulgen's method and squashes were made. For autoradiography, [³H]thymidine with the activity 37 kBq (= 1 µCi) ml⁻¹ and specific activity 2.96 MBq µg⁻¹ was applied the last 1 h before fixation. After making Feulgen stained squashes, coverslips were removed by the dry ice method and slides were covered by nuclear emulsion Ilford K 2, exposed in 4 °C in the dark for 4 weeks and developed. MI and LI were evaluated on the basis of scoring nuclei on 5 slides, 1 000 nuclei on each one. Means and confidence intervals for 5% probability were calculated.

Results

The Growth of Isolated Roots

The purpose of the first experiment was to find out whether the growth of isolated roots depends on their initial length. Roots of the initial lengths 5 mm, 10 mm and 20 mm were cultivated and Fig. 1 shows that the growth rate was independent of the initial length. In comparison with the growth of unexcised roots of the embryos cultivated in identical conditions (LANDOVÁ and ONDŘEJ 1979) the growth rate was much lower.

The influence of the velocity of shaking was tested in the next experiment, together with a more detailed study of the effect of the number of roots per vial. In this experiment, different numbers of roots were put into vials containing 4 ml of the medium, namely 2, 5, 10 and 16 roots and shaken by different velocities, namely 60, 100 and 140 rpm. Roots were measured the fourth day of cultivation. As seen in Fig. 2, practically no growth was observed with speeds 60 rpm and 100 rpm. With 140 rpm, a considerable degree of growth was observed. The average length of roots increased with increasing the number of roots per vial. The differences between the length of roots per vial were statistically significant.

Long-term cultures were also established. The roots survived for at least 4 weeks, but they did not show any considerable growth activity.

Abbreviations used: MI — mitotic index, *i.e.* percentage of nuclei that are in mitosis; LI — labelling index, *i.e.* percentage of nuclei that are labelled by [³H] thymidine.

Fig. 1.

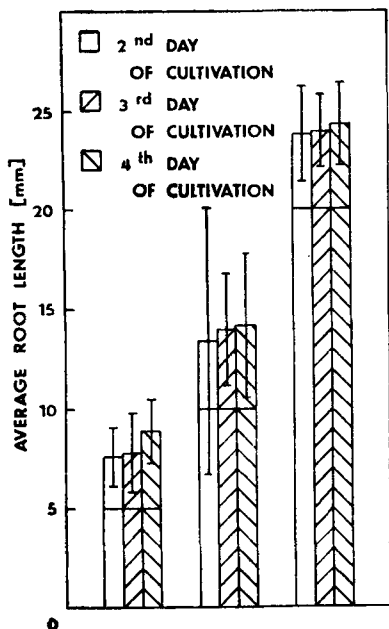


Fig. 2.

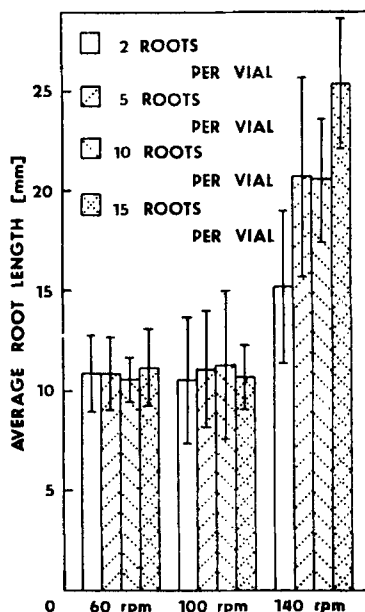


Fig. 1. The influence of the initial length of excised roots on their growth. Average lengths and confidence intervals are given. The bars on columns denote the initial root lengths.

Fig. 2. The effect of velocity of shaking and number of roots per vial on the growth of isolated barley roots.

Mitotic Activity of the Meristems of Isolated Roots

The MI and LI values were measured in excised roots with the aim to gain some information on the applicability of barley root cultures in cytogenetic experiments. Fig. 3 shows a sharp drop of LI immediately after excision but 24 h later a small rise. MI shows a small steady decrease after excision.

To test the effect of excision on MI in detail, another experiment was established in which MI of root meristems of isolated embryo roots and MI of excised roots were compared. Fig. 4 shows a considerable drop of MI after excision followed by a compensatory increase above the control value. Similar drop was also found after excision of pea roots by VAN'T HOF (1965). An equilibrium of MI values was reached approximately 12 h after excision. For the next at least three days, MI varied in rather narrow limits. It showed a small but permanent decrease in comparison with MI values of embryo root tip meristems. After 10 days of cultivation of embryos the medium was exhausted. After one week of cultivation of roots the size of the root tip meristems was considerably reduced and this reduction obviously is the primary cause of the cessation of the growth of the barley excised roots. After 2 weeks of cultivation root meristems were extremely small and only a few (usually less than ten) mitoses were observed.

To summarize, MI after the initial decrease (starting from the first 12 h) is reasonably high and constant for at least three days of cultivation. This period is quite sufficient for practically all cytogenetic experiments.

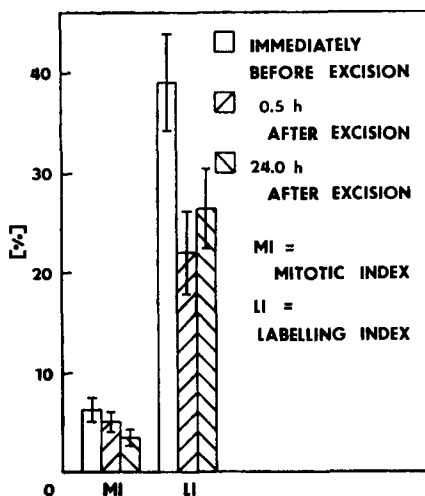


Fig. 3. Mitotic index and labelling index in root meristems.

Discussion

During our experiments on the cultivation of excised barley root tips we got the experience that the isolated barley roots are able to grow on the artificial medium for a limited time, but that their growth rate gradually decreases. The cause of the cessation of the growth is the continuous decrease of the size of the root meristem. ALMENSTRAND (1957) came to the same conclusion and he showed that, among cereals, this pattern is a specific response of barley roots.

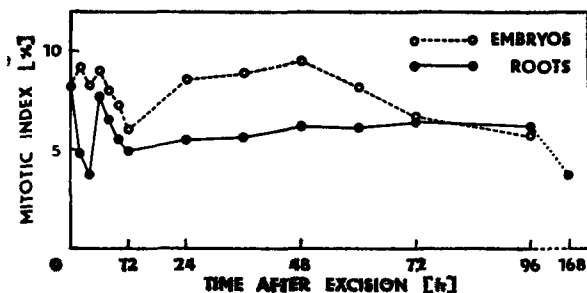


Fig. 4. Mitotic index values of isolated barley roots in different intervals after excision as compared to that of unexcised roots of cultivated embryos.

Under our experimental conditions, the most important factor of the growth of roots was sufficient aeration of the nutrient solution. The growth was very slow with shaking velocities under 140 rpm. It is in contrast to the experience of the authors who used liquid media without any aeration (ALMENSTRAND 1957, FERGUSON 1963). Only VAN'T HOF (1965) used a shaker for his pea root cultures. In our experiments, roots were excised from isolated embryos cultivated in the complete liquid nutrient medium. According to our experience (LANDOVÁ and ONDŘEJ 1979) aeration is a necessary condition for the good growth of barley embryo cultures. Perhaps roots excised from such embryos are adapted to a high level of oxygen in the medium and the lowering of this level causes a sharp decrease in growth.

The next factor which was found to influence the growth rate was the number of roots per vial; the more roots the better is the growth. The differences were statistically significant in one experiment only, but a similar tendency was found in other experiments with isolated roots, but not with cultivated embryos (LANDOVÁ and ONDŘEJ 1979). In isolated roots, the supply of nutrients was not the limiting factor of the growth. The roots probably changed the nutrient solution by releasing endogenous substances like hormones into the medium, by changing pH and concentration of nutrients or by still other mechanisms, and some of the changes impaired the growth of isolated roots.

The growth rate was not influenced by the initial length of roots in the verge 5 mm—20 mm. Originally we supposed that with increasing the initial length the growth would increase because of possible storage of some substances in the longitudinal growth zone, but this supposition was not confirmed.

Lateral roots appeared after 5—10 days of cultivation of the excised roots. Their meristems were small and their growth ceased soon. Their appearance is a normal feature of the development of roots. These lateral roots appear approximately at the same time as in unexcised roots of the embryo cultures and they are the consequence of the continuing normal differentiation of excised roots.

MI of isolated barley roots was slightly lower than that in embryo root tips cultivated in identical conditions, but it was on an almost constant level for at least three days, which is a quite sufficient period for most of cytogenetic experiments. A small decrease of MI is compensated by other advantages connected, for instance, with the experiments with exogenous DNA. Excised barley roots have already been utilized in such experiments (ONDŘEJ *et al.* 1979) and they showed a new type of autoradiographic distribution of the label from [^3H] DNA, which was not observed in root tips of excised embryos cultivated in similar conditions.

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BOOK REVIEW

Sussman, M.: *Molekularbiologie und Entwicklung*. — Paul Parey, Berlin–Hamburg 1978. 264 pp. DM 29,—. (Translation from English original: *Developmental Biology*. — Prentice Hall, Inc., Englewood Cliffs, New Jersey 1973.)

There is a special reason for a student in plant development to recommend Sussman's book. Time has come to check whether the developmental concepts elaborated using zoological models are also feasible for plants. I bear in mind such regulatory mechanisms as *e.g.* morphogenetic fields, differential gene activity *etc.* Plant physiologists working in the field of development are still biased by complicated ecological regulation to be found in plants. It is from this point of view that this book provides a first hand survey of ideas and approaches which form, a framework of contemporary developmental biology.

Apart from this, the work serves its primary destination: to be a textbook on development (it appears in series: Pareys Studentexte). It embodies all the important features of a good textbook, such as clear style, logical set-up and instructive illustrations of high quality. Five chapters cover a wide range of developmental subjects, starting with explanation of basic dogmas of molecular biology and focussing mainly on regulation of morphogenesis in vertebrata. The book may be used equally well in introductory courses for botanists, zoologists and microbiologists as in special lectures for students in related fields.

This book is a real alphabet of modern developmental biology and like any other alphabet even this molecular one is simple enough to be understood and used as part of our general knowledge.

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