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Growth response of excised and attached roots to external sucrose concentration

I. *Linum usitatissimum*

A. SZWEYKOWSKA, D. MARCHLEWSKA

In numerous investigations, sucrose has been found to be a most utilizable carbon source for isolated roots cultivated aseptically in synthetic media (Street 1957). Its concentration in these media was rather low, 2—4% (White, 1938, Bonner and Addicott 1937). Thorough investigations carried out by Street and McGregor (1952) on the influence of sucrose concentration on the growth of tomato roots have shown that the optimum concentration is 1,5—4%, depending on the accepted growth measure (length, fresh or dry weight of roots).

On the other hand, in investigations on the influence of sugar on the development of isolated embryos of cabbage and lupin, a very strong root system development was observed in relatively high sugar concentrations amounting to 8—12% (Szweykowska 1959, Hoffmannowa 1963). These facts suggest, that the response of roots to sugar concentration in media may differ, depending on whether the root is excised and developed as an isolated system, or whether it grows attached to the shoot. To verify such a suggestion the influence of sucrose concentration on the growth of both excised and non-excised roots in one plant material had to be investigated.

After the initial tests, pea and flax were selected for investigations, since isolated pea and flax roots grow in cultures in comparatively simple media and embryos of these plants are easily isolated from seeds and grow well in *in vitro* culture. This paper presents results obtained for excised and attached roots of flax. Excised roots of flax belong to those of minimal growth requirements. They may be grown through many subcultures in a medium containing no vitamins, the growth, however, is vigorous in the presence of thiamin which is the only vitamin synthesized in these roots at a suboptimal rate (Bonner, Devirian 1939).

MATERIAL AND METHODS

Seeds of *Linum usitatissimum* „Lazur“ were sterilized in 96% ethanol for 1 minute and then in 0,1% mercuric chloride for 10 minutes. After thorough washing the seeds were put into a sterilized Petri dish with wet filter paper and allowed to germinate. After two days the cotyledonless embryos or 10 mm root tips were isolated and put into 100 ml Erlenmayer flasks containing 20 ml of nutrient medium. The culture medium was a modified solution of B o n n e r and D e v i r i a n (1939):

H_2O	1000 ml
$Ca(NO_3)_2 \cdot 4H_2O$	242 mg
$MgSO_4 \cdot 7H_2O$	42 mg
KNO_3	85 mg
KCl	61 mg
KH_2PO_4	20 mg
Fe-citrate	1,8 mg
Thiamine	0,1 mg

In addition, sucrose at various concentrations from 0 to 12% was added to the media. The cultures were kept in the dark and at a temp. of 25°C. After seven days of culture (Fig. 1), the roots and cotyledonless

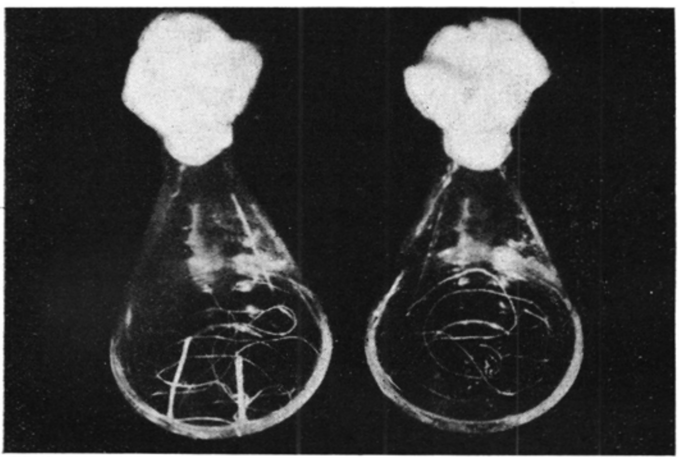

Fig. 1. Liquid cultures of cotyledonless seedlings and excised roots of flax

seedlings were taken out of the flasks, thoroughly washed and dried with a filter paper. The roots of the seedlings were cut off. The organs were then measured for length, weighed and used for analyses. A parallel experimental set consisted of 20—30 seedlings resp. excised roots.

Determination of protein nitrogen

The material for nitrogen analysis was kept in 80% ethanol. After removal from ethanol it was ground and placed for 15 hours in 10% trichloroacetic acid (TCA) at a temperature of 2°C. Next the precipitate was centrifuged, put into 5% TCA and heated for twenty minutes in water bath at a temperature of 90°C to remove nucleic acids (H o t t a and O s a w a 1958). After centrifuging and washing with 5% TCA, the precipitate was used as protein fraction for estimating protein nitrogen by Kjeldahl's micromethod, according to H u m p h r i e s (1956). The samples were transferred to test tubes in which they were digested after the addition of H_2SO_4 and catalyst mixture. The volumes of sulphuric acid added depended on the weights of fresh plant material used in analysis, and amounted to 0.5 ml, 0.75 ml, or 1.0 ml corresponding to 0—500 mg, 500—1000 mg or 1000—1500 mg of fresh weight. The catalyst mixture consisted of $CuSO_4$, K_2SO_4 and SeO_2 in a ratio 1 : 8 : 1. The samples were first boiled in an aluminium block at temperature of 150°C till the water evaporated, later at a temperature approximately 320°C until the digest cleared. The digest was then distilled in a Parnas-Wagner apparatus and the ammonia trapped into 5 ml of 2% boric acid, and next titrated with n/28 HCl in the presence of a mixture of 0.1% methyl red and 0.05% methylene blue as an indicator, till the green colour changed to violet. One ml of HCl corresponded to 0.5 mg of protein nitrogen.

Determination of soluble sugars

Fresh plant material was ground with hot 96% ethanol, covered with more hot ethanol and stored for analysis. Before starting analysis, the ethanol was removed in a water bath at 85°C and the residual heated with 20 ml of water in a boiling water bath for 15 minutes to extract soluble sugars. Next the soluble polysaccharids of the extract were hydrolized by means of HCl, and soluble sugar determinations were made by Hagedorn-Jensen modified method (S z w e y k o w s k a 1959)..

RESULTS

The effect of sucrose concentration in the medium on growth of excised roots in a 7-day culture is illustrated in Fig. 2. The roots show the best growth in 4% sucrose. The fresh weight of roots has its distinct maximum at this concentration, above and below of it the curve drops steeply.

The roots attached to seedlings respond differently to sucrose concentration. Maximum fresh weight of these roots is produced in 6% sucrose. Optimal sucrose concentration for fresh weight of shoots is 5%, but this maximum is not very distinct and the growth varies little in a range of 3—6% concentration.

Protein nitrogen. The growth response of roots to sucrose concentration in medium is thus variant depending on whether the root is excised and isolated from the rest of the seedling or whether it grows attached to the shoot.

With various sucrose concentrations the osmotic value of the medium changes and the amount of water absorbed by the tissues changes also.

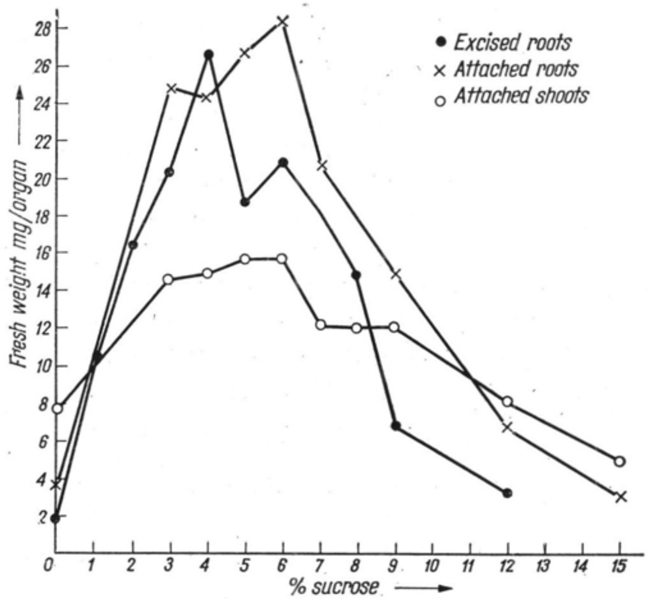

Fig. 2. Effect of external sucrose concentration on growth of flax seedling organs.

So the differences in fresh weight may — at least partly — depend on the differences in the amount of water in the tissues. This is why determinations were made in excised and attached roots of protein nitrogen, which is the objective measure of growth. Protein nitrogen was determined in roots growing on 0, 3, 4, 6, 9 and 12% sucrose. The results obtained are similar to those in which fresh weight was used as the measure of growth — i.e. the maximum growth for exised roots is in 4% and for attached roots in 6% sucrose (Fig. 3). Besides, attached roots generally show a greater tolerance for growth inhibiting effects of higher sugar concentrations in the medium.

Accumulation of sugar. Searching for an explanation of the differences in growth response between excised and attached roots, sugar accumulation in the organs was considered as the first possibility. It was assumed that one of the causes of growth rate inhibition in media with higher sucrose concentration is an excesive accumulation of sugar in the tissues. In whole seedlings, a part of the sugar absorbed by the roots is transferred to the shoot system and this may cause its smaller accumulation in the root. Investigation was made of soluble sugar concentration in excised and attached roots and shoots.

The amount of soluble sugars in organs grown in 0, 4, 6 and 8% sucrose was determined (Fig. 4). Soluble sugar accumulation in the

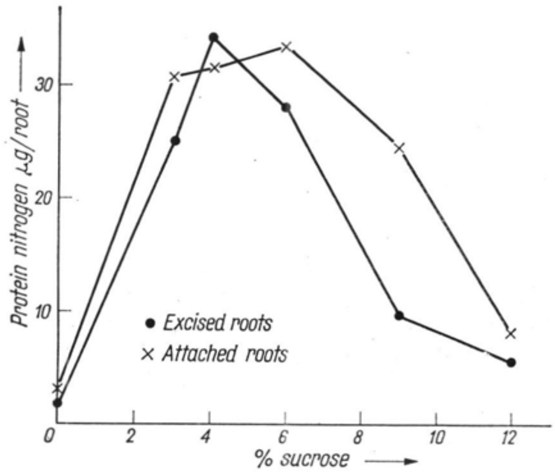

Fig. 3. Effect of external sucrose concentration on protein content of flax seedling organs.

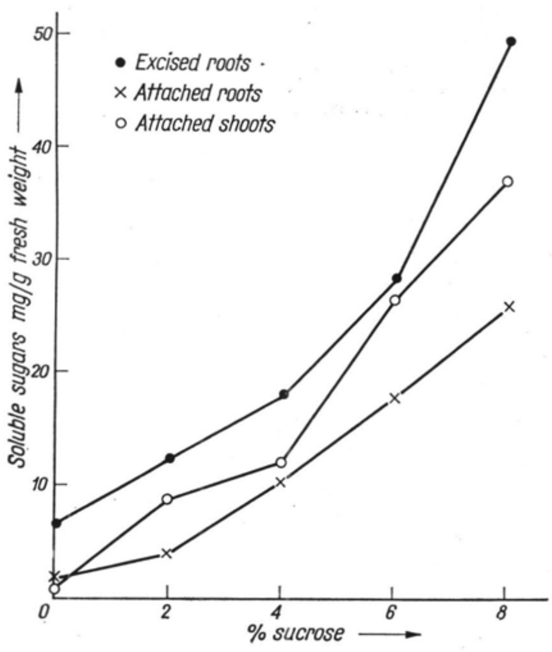

Fig. 4. Effect of external sucrose concentration on soluble sugar accumulation in flax seedling organs.

organs is proportional to its concentration in the medium and particulary intensive in higher concentrations. Differences occured in soluble sugar accumulation in excised and attached roots — in excised roots the accumulation is stronger. In seedlings growing in media with higher sucrose concentrations, the sugar content of shoots is higher than that of attached roots. This means that in whole seedlings occurs an active transportation of sugar material absorbed from the medium from the root to the shoot, as result of which the soluble sugar accumulation in shoots exceeds its accumulation in the roots. Excised roots do not have this ability of being depleted through transport to the shoot which is probably why the soluble sugar concentration is higher in these roots.

DISCUSSION

In agreement with the results of Bonner's (1940) investigations, the data obtained in this work show that 4% concentration of sucrose is optimal for growth of excised roots of flax. On the other hand, the growth response to sucrose concentration in attached roots is different. The data obtained here confirm the recent observations on lupin and cabbage that the maximum growth of attached roots occurs in higher sucrose concentrations. Hoffmannowa (1962) found the maximum growth of attached lupin roots in 8% and of cabbage roots in 12% sucrose. For attached flax roots the maximum growth occurs in 6% sucrose. It is characteristic that the positive growth response to relatively high sugar concentrations in the medium does not concern the whole embryo, but is limited only to the root, and the shoots show a maximum growth in lower concentrations.

It is well known that roots and shoots differ in their physiological and morphogenetic potencies. Experiments with excised organs showed that the root is heterotrophic not only in respect of organic carbon sucrose, but in the majority of cases also to ready made special organic substances, as vitamins and aminoacids (Street 1957), whereas shoots are completely autotrophic in this respect. In normal conditions the root is completely or partly supplied by the shoot with some ready made substances; in isolated conditions these substances must be supplied in the medium. On the other hand, some syntheses, especially of nitrogen compounds, for instance some alcaloids, occur chiefly of exclusively in the root, and the substances formed there are transported to the shoot (Mothes 1952).

The morphogenetic potencies of the root and shoot are also different. Excised shoots grown in sterile culture easily produce adventitious roots and in this way reproduce a whole plant. The potencies of differentiating shoot buds, however, are rare and weaker in excised roots.

In intact plant, there is an interaction between shoot and root. Physiological features of an excised root may therefore be different from those of a root *in situ*. Manos (1961) investigated the growth response in excised and attached pea roots to various growth substances and found differences between them, especially as regards indolylacetic acid (IAA). Growth was inhibited by IAA in excised roots, and in attached, after a temporary inhibition, it was reactivated and finally exceeded that of the control. The present study shows that different effects on growth occur when excised roots and roots attached to the shoot are subjected to various concentrations of external sugar. Stimulation of the growth of attached roots by high sugar concentrations in the medium may be an aspect of xeromorphic growth tendencies under the influence of a water deficit. This tendency however, is limited to attached roots only, excised roots show no sign of it. An interesting problem is the interacting mechanism enabling attached roots to grow vigorously in higher sugar concentrations in the medium. One of the causes may be the abstraction of excess sugar from the roots by the developing shoot. This fact as analyses showed may play a role in the case of flax roots, where in fact in media with the same sucrose concentration the sugar content was higher in excised than in attached roots.

SUMMARY

Investigations were made on the growth of excised and attached flax roots, in media with various sucrose concentrations. It was found that maximum growth occured in different sucrose concentrations depending on whether the root was detached or attached to the shoot. The optimal sucrose concentration for excised roots is 4%, for attached 6%.

Sugar accumulation in excised roots and in the organs of cotyledonless seedlings (attached roots and shoots) was also investigated. It was found that the sugar accumulation is stronger in excised roots and in attached shoots and much weaker in attached roots. So in flax the shoots seem to deplete the roots of excessive sugar, which may be one of the factors of the positive growth response of attached roots to higher sugar concentrations in the medium.

Department of General Botany
Adam Mickiewicz University
Al. Stalingradzka 14, Poznań, Poland

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Reakcje wzrostowe izolowanych i nie izolowanych korzeni na wzrastające stężenie cukru w pożywce

I. Linum usitatissimum

Streszczenie

Zbadano reakcję wzrostową izolowanych i nie izolowanych korzeni lnu na pożywkach z różną zawartością sacharozy. Stwierdzono różnice w reakcji wzrostowej na stężenie cukru między korzeniami izolowanymi i korzeniami połączonymi z systemem pędowym. Maksimum wzrostu dla korzeni izolowanych przypada na 4%, dla korzeni nie izolowanych na 6% sacharozy.

Zbadano także akumulację cukrów rozpuszczalnych w korzeniach izolowanych oraz organach bezliścieniowych siewek (korzenie i pędy). Stwierdzono silną akumulację w izolowanych korzeniach i nie izolowanych pędach oraz słabszą w nie izolowanych korzeniach.

Wydaje się że u siewek lnu następuje znaczne odciąganie nadmiaru pobranego cukru z korzeni do systemu pędowego. Może to być jednym z czynników pozytywnej reakcji wzrostowej nie izolowanych korzeni na wyższe stężenie cukru w pożywce.