ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. XXXVIII — nr 1
1969

Growth response of excised and attached roots to external sucrose concentration

IV. The effect of exposure to air on growth and sugar requirement

A. SZWEYKOWSKA, U. PRUSIŃSKA AND W. RATAJCZAK

A relatively low sugar concentration has been found to be optimal for excised root culture (Bonner and Addicott 1937; White 1938; Street and McGregor 1952). In intact seedling axes, the optimal sugar concentration for the shoot growth is also low, however, that for the root growth is remarkably higher (Hoffmannowa 1964). This suggested that the growth of excised roots cultured in nutrient solutions is limited by some factors and that in appropriate conditions their growth and their requirement for sugar could be distinctly higher. In our previous paper (Szweykowska, Nowicka and Mossor 1964) we found that the growth of attached roots in pea reached its maximum at 8 per cent sucrose concentration in the medium, whereas that of excised roots at 3 per cent and was less vigorous in general. Searching for factors responsible for the difference between attached and excised roots in their growth rate and response to sugar concentration we considered several possibilities: 1) the osmotic factor of nutrient solutions, 2) nutritional or growth regulatory factors transported from or to epicotyl in intact seedling axes, 3) oxygen supply through partial exposure of plant organs to air.

MATERIAL AND METHODS

Seeds of *Pisum sativum* cultivar Victoria Łagiewnicka were used in the experiments. The methods of seed sterilization, isolation and cultivation of excised roots and of seedling axes (deprived of cotyledons) were described previously (Szweykowska, Nowicka and Mossor 1964). The nutrient solution consisted of mineral salts, sucrose and thiamine (Bonner and Devirian 1939, Szweykowska and Nowicka 1964). Unless otherwise stated, the cultures were grown in 100 ml Erlenmyaer flasks containing 20 ml of the medium, in the darkness and at about 25°C.

RESULTS AND DISCUSSION

The effect of osmotic concentration. In seedling axes, the positive growth response of roots to relatively high sucrose concentrations resembles a xeromorphic response of some plants to conditions of relatively high osmotic pressure in the root environment. Hoffmannowa (1964) found in lupine seedling axes cultured in vitro a distinct stimulation of root growth by NaCl or mannitol added to the nutrient medium to increase its osmotic concentration. In the present study a series of experiments was carried out in order to investigate the effect of the osmotic factors in the growth of pea roots. NaCl and mannitol were used to obtain various osmotic concentrations of the nutrient solutions. Both substances are not known to play any substantial role in the metabolism. Preliminary experiments showed that mannitol was not used by the pea organs for growth and in no extent could replace sucrose as a source of carbon. Media containing sucrose at 2 or 4 per cent were used (3 per cent being an optimal concentration for excised roots) to which NaCl at 1.8, 3.7, 5.7 and 7.8 g/l, or mannitol at 1, 2, 3 and 4 per cent were added (no additions in controls). In this way, media of various osmotic concentrations corresponding to 2—12 per cent concentration of sucrose were prepared (Walter 1936). With increasing osmotic concentration a gradual decrease in growth of both excised and attached roots was obtained. Attached roots showed a little more tolerance, but in no case a distinct stimulation of growth was obtained. The results indicated that the requirement of relatively high sucrose concentration of attached roots is not a requirement of a higher osmotic pressure in the medium, but rather of sucrose itself.

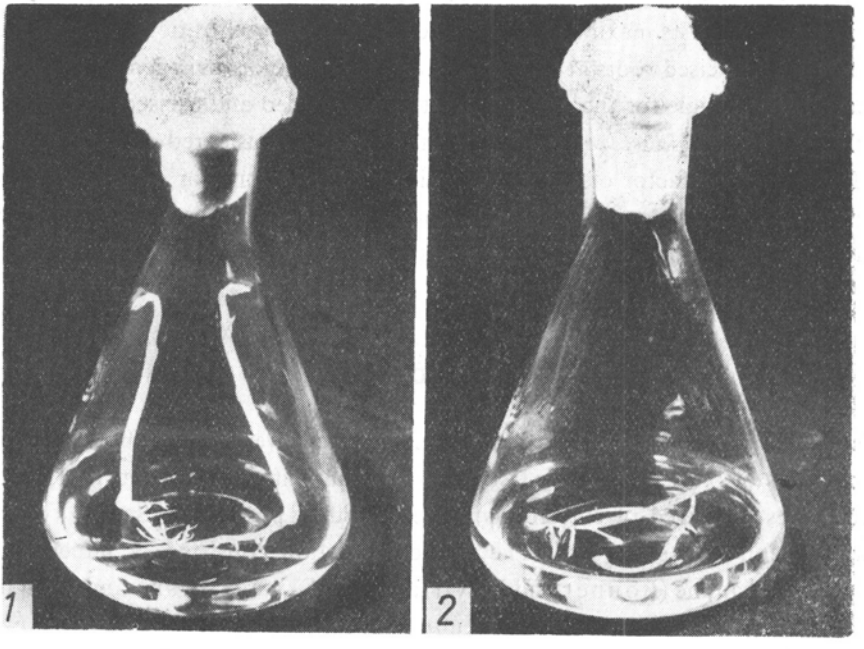

Fig. 1—2. Attached (*1*) and excised (*2*) roots in nutrient solutions

The effects of 3-indolylacetic acid (IAA), kinetin, casein hydrolysate and pea extracts. A factor synthesized in epicotyls and transported to the roots could be responsible for the higher growth rate and sucrose requirement in attached roots. Apart from products of photosynthesis, other factors elaborated in shoots are known to affect root development. Vitamins and auxins belong to the most active (Fries 1955; Richardson 1958; Torrey 1963). A promotion of growth of lupine seedling roots by kinetin has been described by Fries (1960), and Butcher and Street (1960) found an interaction between kinetin and sucrose concentration in the growth of excised tomato roots. In case of pea seedlings in which proteins are the main storage products, also amino acids could play some role in root development.

Searching for such additional factors, the effects of some growth regulators, amino acids and plant extracts on the growth of excised roots in media with high sucrose concentration (8 per cent) were investigated. No positive results were obtained with IAA or kinetin at concentrations from 0.01 µg/l to 1 mg/l and with caseine hydrolysate at concs. from 0.1 to 10 g/l. Extracts from shoot parts of seedling axes were prepared by a cold homogenization of epicotyls in water or a homogenization after boiling the epicotyls in water for 5 min. The extracts were added to the medium at a conc. of 2 per cent of an equivalent of fresh epicotyl weight either before autoclaving the medium or they were filter sterilized and added after the medium was autoclaved and cooled. The seed extracts were prepared by homogenization of swollen seeds after boiling them in water for 5 min. and added to the medium at 0.005—0.5 per cent of seed weight equivalent. No stimulation of excised root growth was obtained with these extracts added to the medium containing 8 per cent sucrose.

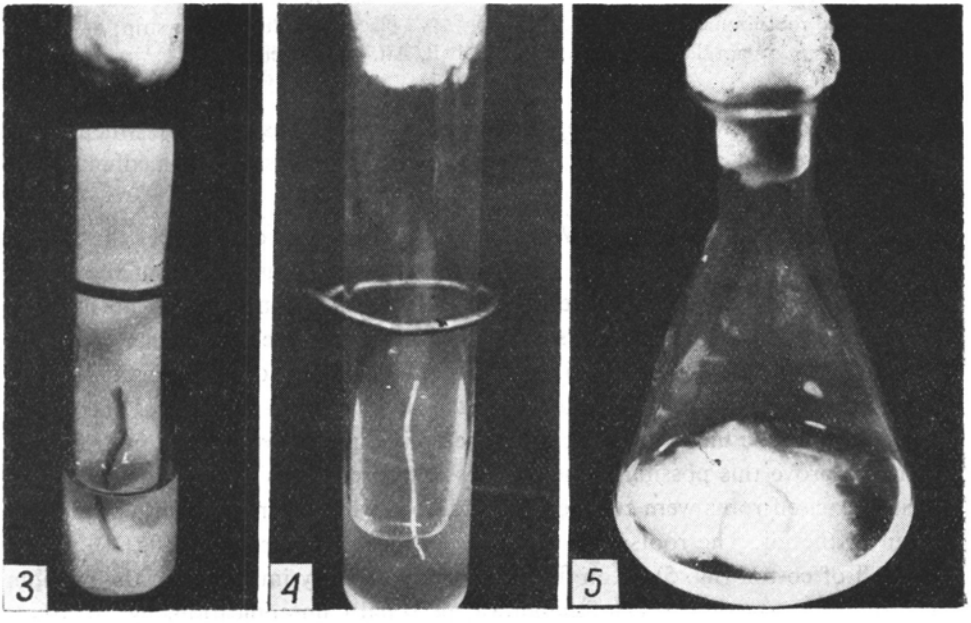

Fig. 3—5. Excised root culture on filter paper strip (*3*), agar slope (*4*) and cotton ball (*5*)

As a result of these experiments no positive conclusion could be drawn about substances coming from epicotyls and promoting the growth of pea roots and their sugar demand in the in vitro culture.

The effect of changing the medium. Another reason of the poor growth of excised roots could be an accumulation of inhibitors in the medium which in intact axes could be transported to the epicotyls. To prove this possibility, the excised roots cultured on 8 per cent sucrose were transferred to a fresh medium every second

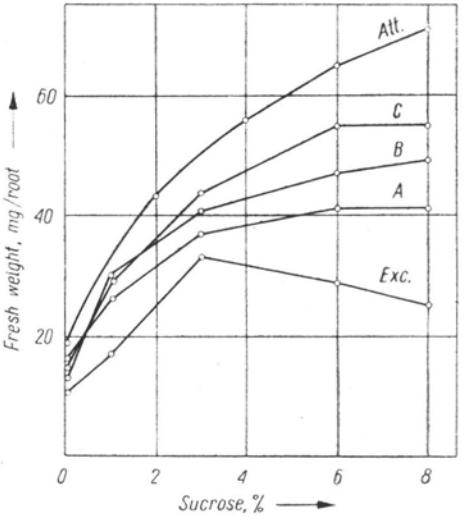

Fig. 6. The effect of culture conditions on the growth rate and response to sugar concentration. *Exc.* — excised roots immersed in nutrient solution; *A* — placed on a filter paper strip; *B* — on an agar slope; *C* — on a cotton ball, Att. — attached roots

day of culture. However, no improvement of growth was obtained when using this procedure. An accumulation of root growth inhibitors in the medium could thus be excluded.

The effect of air conditions. When culturing intact seedling axes, the shoots grow upwards above the medium level and in this way a great part of the axis is soon placed in the air. The excised roots, on the other hand, having no negative geotropic response, remain immersed in the medium for the whole time of culture (figs. 1—2). Royan-Subramaniam et al. (1967) observed that excised roots of *Phaseolus aureus* grew better on nutrient agar slants than in agar stabs. The supply of oxygen might be the limiting factor of the excised root growth in the nutrient solution. To prove this possibility the cultures of excised roots were aerated daily for 7 hrs. or excised roots were cultured in conditions which permitted parts of them to remain in the air. The roots were cultured on nutrient agar slopes (fig. 4), placed on a ball of cotton (fig. 5) or on filter paper strips partly immersed in the medium (according to a method of Hoffmannowa, personal communication, fig. 3). Aeration gave no positive results, but the remaining of parts of roots constantly in the

air improved their growth distinctly and also made their response to higher sucrose concentrations like that of attached roots (fig. 6). The oxygen supply seems thus to be the limiting factor of the growth and sucrose requirement of excised roots in liquid media. In conditions of a free access of air, the sucrose requirement of excised roots in an in vitro culture is relatively high, analogous to that of roots attached to the epicotyls in intact seedling axes.

SUMMARY

Excised pea roots in an in vitro culture show maximum growth at about 3 per cent sucrose concentration in the nutrient solution. Their growth is much improved and the requirement for sucrose is distinctly higher if culture conditions are modified in the way that parts of roots grow in the air. The optimal sucrose concentration amounts then up to 8 per cent and becomes similar to that required by attached roots of seedling axes.

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

(Entered: September 27, 1968)

LITERATURE

Bonner J. and F. T. Addicott, 1937, Bot. Gaz. 99: 144.
Bonner J. and P. S. Devirian, 1939, Amer. Jour. Bot. 26: 661.
Butcher D. N. and H. E. Street, 1960, Physiol. Plant. 13: 46.
Fries N., 1955, Experientia 11: 232.
Fries N., 1960, Physiol. Plant. 13: 468.
Hoffmannowa A., 1964, Acta Soc. Bot. Pol. 33: 193.
Richardson S. D., 1958, Physiol. Plant. 11: 698.
Royan-Subramaniam S., S. Subramaniam, G. Meenakshi and M. K. Subramaniam, 1967, Curr. Sci. 36: 184.
Street H. E., and S. M. McGregor, 1952, Ann. Bot. 16: 185.
Szweykowska A. and A. Nowicka, 1964, Acta Soc. Bot. Pol. 33: 719.
Szweykowska A., A. Nowicka and T. Mossor, 1964, Acta Soc. Bot. Pol. 33: 639.
Torrey J. G., 1963, Symp. Soc. Exper. Biol. 17: 285.
Walter H., 1936, Ber. d. D. Bot. Ges. 54: 328.
White P. R., 1938, Amer. Jour. Bot. 25: 348.

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

IV. Wpływ dostępu powietrza na wzrost i zapotrzebowanie na cukier

Streszczenie

Korzenie bezliścieniowych siewek hodowane in vitro wykazują maksimum wzrostu przy stosunkowo wysokich stężeniach sacharozy w pożywce, natomiast te same korzenie odcięte od reszty osi siewki i hodowane oddzielnie rosną słabiej i posiadają maksimum przy stężeniach znacznie niższych. W przypadku grochu te maksima wypadają odpowiednio przy 8 i 3 procentach sacharozy. Wykazano, że czynnikiem odpowiedzialnym za różną reakcję korzeni jest prawdopodobnie zaopatrzenie w tlen. W przypadku kultury całych osi siewek pędy wyrastają ponad płynną pożywkę, natomiast odcięte korzenie pozostają w płynnej pożywce całkowicie zanurzone. Gdy hodowlę izolowanych korzeni prowadzić w takich warunkach, że część korzenia rośnie w powietrzu, ich wzrost oraz reakcja na stężenie cukru stają się podobne do reakcji korzeni bezliścieniowych siewek.