Growth response of excised and attached roots to external sucrose concentration

III. The effect of some modifications in the experimental technique

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Working on the growth of the pea seedling root, Fries (1953, 1954) found that the roots of seedlings which were deprived of the cotyledons and cultivated in the dark in an organic medium grew very poorly not only when compared with normal unoperated seedlings, but also — strikingly enough — when compared with excised pea roots cultivated under identical conditions. The differences were interpreted as indicating that roots synthesize substances which are indispensable for root as well as for shoot development, the synthesizing capacity of root being sufficient for optimal growth of the root itself, but not for the root and the shoot together. As a result of shoot depleting root of these substances, attached roots developed weaker than excised ones growing without shoots as isolated organs. Evidence has been presented by Fries that arginine, glycine and adenine represent limiting factors in the growth of pea seedling root. These substances when added to the nutrient medium produced an almost normal growth rate of the attached seedling root.

In our investigations (Szweykowska, Nowicka and Mosor 1964) carried out with similar material and technique, we obtained results which were apparently opposite to those of Fries. In our experiments, attached seedling roots grew always better than excised ones. This was especially remarkable in media with higher sucrose concentration. The results brought us to a conclusion contrary to that of Fries. The shoot has been believed to supply the root with some factors which enabled it to grow vigorously even in relatively high sugar concentration. Because of striking differences between our and Fries's findings it seemed necessary to us to prove our results applying some modifications in our experimental technique which would make it more close to that of Fries.

MATERIAL AND METHODS

The experimental material consisted of decotylized embryos and excised roots of *Pisum sativum* Victoria, a variety which was also used in our previous study.

Unless otherwise stated, the procedures of seed sterilization and of embryo and root isolations were identical with those described earlier (Szweykowska, Nowicka and Mossor 1964). The isolated material was cultured in Erlenmayer flasks or tubes in a nutrient medium which was liquid or solidified by adding agar at 0.75%. Two basal nutrient media were employed: a simpler one of Bonner and Devirian (1939), used in our previous studies, and a more complex medium used by Fries (1954).

The compositions of the basal media were as follows:

a. Bonner and Devirian:

H ₂ O	1000	ml	KCl	61	mg
Ca(NO ₃) ₂ · 4H ₂ O	242	mg	KH ₂ PO ₄	20	mg
MgSO ₄ · 7H ₂ O	42	mg	Ferrictrate	1,8	mg
KNO ₃	85	mg	Thiamine	0,1	mg

b. Fries:

H ₂ O	1000	ml	CuSO ₄ · 5H ₂ O	0,008	mg
Ca(NO ₃) ₂ · 4H ₂ O	333,0	mg	H ₂ MoO ₄ · H ₂ O	0,009	mg
KCl	41,6	mg	Thiamine	50	μg
KNO ₃	83,2	mg	Pyridoxine	50	μg
KH ₂ PO ₄	83,2	mg	Nicotinamide	50	μg
MgSO ₄ · 7H ₂ O	83,2	mg	Riboflavin	200	μg
Ferrictrate (FeC ₆ H ₅ O ₇ · 3H ₂ O)	3,39	mg	Pantothenic acid	150	μg
Citric acid	3,39	mg	p-Aminobenzoic acid	200	μg
H ₃ BO ₃	0,286	mg	Biotin	25	μg
MnCl ₂ · 4H ₂ O	0,181	mg	Inositol	150	μg
ZnSO ₄ · 7H ₂ O	0,022	mg	Ascorbic acid	10	μg

To the basic media sucrose was added at various concentrations from 0 to 12%. The pH of the nutrient solutions was adjusted to 4,5. The cultures were kept in the dark and at a temp. of 24° C. After definite time of cultivation (7 or 10 days), the lengths and fresh weights of attached and excised roots were estimated.

EXPERIMENTAL RESULTS

The effect of incubation time

Fries found that the growth rate was about the same in attached and excised roots during the first five days of cultivation. Only after 10 days marked differences in growth appeared. Our previous experiments were carried out using cultures incubated for 7 days. It seemed possible that the time of incubation might affect to some extent the growth results. For this reason, the growth of attached and excised roots was estimated after several incubation times from 2 to 10 days, using the Bonner and Devirian basal medium with the addition of sucrose at 2%.

The growth of attached roots was higher at all incubation times, the differences being marked in all cases except after 2 days where the difference in root length was very small (Fig. 1).

Fig. 1. The effect of incubation time on the growth of attached and excised roots.

It has thus been shown that during the incubation times lasting from 4 to 10 days the growth differences between attached and excised roots are very distinct thus indicating that the incubation time in the range investigated does not modify experimental results.

The effect of isolation technique

Same differences between our and Fries's experiments concerned isolation technique of seedlings and roots. In our investigation, for seedling ("attached roots") cultures, decotylized embryos only 24 hours old were inoculated and roots were excised from 72 hours old seedlings in the way that only 10 mm long root tips were cut off and transferred to culture flasks. Fries used seedlings which were 66 hours old when decotylized and planted and in the case of excised roots they were

Table 1

The effect of isolation technique on the growth of attached and excised roots

Per cent sucrose	Isolation technique	Length in mm		Fresh weight in mg	
		attached	excised	attached	excised
2	Fries	67	63	54	45
	Szweykowska et al.	48	58	46	36
6	Fries	116	35	92	45
	Szweykowska et al.	78	42	67	26

separated from the rest of the seedlings by a cut just below the insertion point of the cotyledons. In this way, the seedlings and roots when planted were relatively large (the length of primary root being 2—3 cm at this time), presumably contained some notable nutrient materials drawn from cotyledons, and the excised roots possibly consisted also of the short pea hypocotyl. An experiment has been carried out to prove if these different isolation techniques might be responsible for differences between Fries's and our results. Seedlings and roots isolated in the two ways described were cultured for 10 days in Bonner and Devirian medium containing sucrose at 2 or 6 per cent. Material isolated by the method of Fries showed generally higher values of growth expressed in terms of length or fresh weight (Table 1). This fact was obviously connected with its more advanced development and higher size at the moment of inoculation, as was mentioned above. However, in both series, the growth of attached roots was higher than that of excised ones. The only exception concerned length of roots inoculated by our method and grown at 2 per cent sucrose. This was, however, not reflected in growth expressed as fresh weight.

The experiment has thus shown that the isolation techniques described above are of no importance for our previous results.

The effect of a complex medium

In our previous experiments, we found not only that the growth of attached roots was always better than that of excised ones but also that the growth response of attached and excised roots to increasing sucrose concentrations was very different, maximum growth occurring at 8 and 3 per cent sucrose resp. Thus the attached roots were shown to increase their rate of growth in conditions in which the growth of excised roots markedly decreased. It seemed interesting to prove if this difference in growth distribution on various sucrose concentrations would exist when using a more complex basal medium of Fries instead of a relatively simple medium of Bonner and Devirian used previously

in our experiments. The results presented in Fig. 2 show that in this complex medium and after 10 days of incubation, the growth of roots was affected by sucrose concentration in the same manner as stated

Fig. 2. The effect of sucrose concentration on length and fresh weight of attached and excised roots grown on Fries medium.

before, i.e. sucrose at above 3 per cent reduced the growth rate of excised roots whereas attached roots increased their growth up to 8 per cent sucrose.

The effect of solid and liquid media

All our experiments were carried out using liquid nutrient solutions. Since in experiments of Fries a solidified agar medium was used it also appeared necessary to compare the effects of solid and liquid media. The media contained basal nutrient solution of Fries, sucrose at 2% and the solid medium also agar at 0.75%.

In roots grown in the solidified medium, only length of the main root was estimated because we were not sure if delicate lateral roots had been all taken out of agar. With roots grown in liquid media, both length and fresh weight were estimated. The results are presented in

Table 2

The effect of solid and liquid media on the growth of attached and excised roots

Medium	Length in mm		Fresh weight in mg	
	attached	excised	attached	excised
Solid	55	43	-	-
Liquid	45	52	42	17

the table No. 2. In the agar medium, the attached roots were longer and possessed numerous and well formed lateral roots, whereas excised roots were shorter but with a small number of lateral roots.

The results confirmed thus our previous observations. In liquid medium, attached roots were shorter than excised ones but possessed a much higher fresh weight thus indicating for a more vigorous growth in general.

DISCUSSION

The application of several modifications of the culture method in our experiments on the growth of excised and attached pea roots confirmed our previous results that the growth of attached roots was always better and also that the optimal sucrose concentration for their growth was much higher when compared with excised roots. For the inconsistency of these results with the findings of Fries (1953, 1954), genetically controlled physiological differences between different pea varieties should probably be responsible. Fries used Torsdag II in his investigations and in our experiments Victoria Łagiewnicka cultivar was used. Intra-specific, intra-strain and even intra-clonal variations in morphology, growth rate or physiology of excised roots have been frequently observed (Street 1957). To some extent, the differences in our findings might also be caused by the fact that Fries used length of roots as the main growth criterion and rather low sucrose concentration (2%) in the nutrient medium. The more vigorous growth of attached roots was especially remarkable in media with higher sucrose concentration.

In our experiments, not only the growth but also organogenetic potencies of attached roots have been shown to be stronger. Lateral roots appeared always sooner and in greater number in attached than in excised roots which usually did not form laterals until about the seventh day of culture. The present paper confirms our previous conclusions that the root of Victoria pea is supplied by the shoot with factors indispensable for its vigorous growth, for lateral root initiation

and for continuing and increasing growth in higher sucrose concentrations. Similar conclusions have been drawn by Richardson (1958) who pointed out the role of shoot in the root development in *Acer saccharinum*. According to him, auxin produced in terminal shoot meristem controls root initiation; another factor, probably one of the B vitamins, formed in the leaves, determines root elongation. Torrey (1962) found that indoleacetic acid and adenine interact in lateral root initiation in pea and that also kinetin plays a role in this process. Mothes (1953) points out the interdependence of shoot and root in their synthetic and morphogenetic abilities. The growth of excised and attached roots of pea may be one more example of this phenomenon. The mechanism involved is still unknown and presents an interesting problem for future investigations.

SUMMARY

The experiments on the growth of excised and attached roots of *Pisum sativum* cultivar Victoria Łagiewnicka with the application of several technique modifications have confirmed our previous results that the growth of attached roots is always more vigorous and that the optimal sucrose concentration for root growth in the nutrient medium is much higher for attached roots.

The inconsistency of these results with the findings of Fries should be probably explained by genetically controlled physiological intra-specific variation in pea.

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

III. Wpływ modyfikacji w technice doświadczeń

Streszczenie

Dalsze doświadczenia nad wzrostem izolowanych i nie izolowanych korzeni *Pisum sativum* cultivar 'Victoria Łagiewnicka' z zastosowaniem szeregu modyfikacji w technice doświadczeń potwierdziły nasze poprzednie rezultaty, że wzrost korzeni nie izolowanych jest zawsze bujniejszy, oraz że optymalne dla wzrostu korzeni stężenie cukru w pożywce jest dla tych korzeni znacznie wyższe niż dla korzeni izolowanych.

Nie zgodność tych wyników z otrzymanymi przez Friesa należy prawdopodobnie wytłumaczyć kontrolowaną genetycznie wewnątrzgatunkową zmiennością fizjologiczną u grochu.