

sprouts (*Brassica oleracea* L. var. *gemmifera* DC.), which were treated with kinetin (6-furfurylamino-purine; KN). The base of freshly picked buttons was dipped in talcum powder containing 0, 0.01, 0.1 or 1.0% KN, after which the buttons were planted in a glasshouse at about 20° C. After 12 days observations were made on the rooting and the development of the axillary buds just above the base of the buttons (Table).

Table. The effects of kinetin on rooting and development of axillary buds of buttons of Brussels sprouts

| | % of buttons with roots | | | | buttons with swollen buds | | | |
|--------|-------------------------|-------|------|-------|---------------------------|-------|------|-------|
| | 0% | 0.01% | 0.1% | 1% KN | 0% | 0.01% | 0.1% | 1% KN |
| Exp. 1 | 53 | 52 | 35 | 14 | <0.5 | 1 | 5 | 16 |
| Exp. 2 | 97 | 96 | 91 | 34 | <0.5 | 1 | 3 | 18 |

The formation of roots was inhibited by KN. This is in agreement with the results of other research workers [1]. The base of the buttons treated with KN showed a distinct formation of callus.

KN caused a very remarkable thickening of the axillary buds at the base (Fig. 1). Sometimes the buttons were completely surrounded by a crown of small swollen buds. As the concentration of KN became higher the percentage of buttons with swollen buds increased.

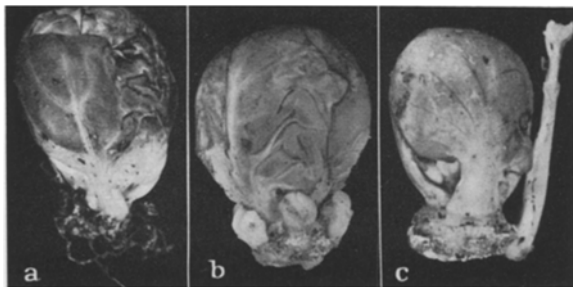


Fig. 1a—c. Buttons of Brussels sprouts; a: control; b: swelling of axillary buds induced by kinetin 1%; c: outgrowth of axillary buds induced by kinetin 1% + gibberellic acid 1%

From the results of WICKSON and THIMANN [2] a disappearance of the apical dominance by application of KN is concluded. In our trials buds did not show further outgrowth. This could be achieved by application of gibberellic acid, simultaneously with KN (Fig. 1). Buttons treated only with gibberellic acid did not respond at all. These observations are in accordance with those of SACHS and THIMANN [3] with Alaska pea plants. One of the characteristic features of Brussels sprouts is the swelling of the axillary buds on the main stem. The above experiments demonstrate that a similar phenomenon can be imitated in excised buttons by applied KN. This suggests that the development of buttons of Brussels sprouts may also be controlled by kinins. This process might be conceived as follows. In the resting axillary buds on the main stem kinins accumulate, the buds may even be production centres of kinins. As a consequence transport of compounds necessary for growth into the buds take place, which leads to thickening and growth of the buds. This agrees with the results of other investigators [4, 5], who observed a transport of aminoacids and other compounds towards organs treated with KN and a promotion of protein synthesis by this compound. I thank Dr. O. BANGA, Mr. J. P. BRAAK and Mr. L. SMEETS for their advice.

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In Vitro Induction of Proliferation in Female Gametophytic Tissue of *Ephedra Foliata* Boiss

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Although cytologically apart, the female gametophytic tissue in gymnosperms and endosperm in angiosperms appear functionally alike in being nurse tissues "subservient to the devel-

oping embryo" [1]. Because of the unique genetic constitution many attempts have been made to culture, grow and induce differentiation in these tissues. However, the knowledge relating to the nutritional as well as the hormonal requirements of these tissues are still far from complete [1, 2]. The following report concerns the *in vitro* proliferation of the excised female gametophytic tissue of *Ephedra foliata*.

The experimental material was obtained from the developing ovules containing almost mature embryos. The ovules were washed twice in chlorine water and the "integuments" were removed with the help of a sterile scalpel. A longitudinal cut was then made on the female gametophytic tissue and the embryos contained therein were scooped out. The embryos were preserved for other experiments while the female gametophytic tissue thus obtained was inoculated aseptically on White's modified medium (WBM) containing 4% sucrose [3], traces of vitamins (riboflavin, thiamine, niacin and inositol) and 0.8% Difco bactoagar. Simultaneously, a large number

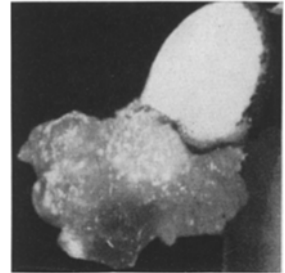


Fig. 1. Excised female gametophytic tissue showing proliferation on media

of pieces of the gametophytic material prepared for inoculation as above were also inoculated on the media noted below: 1. WBM + coconut milk (20%) + yeast extract (250 ppm); 2. WBM + 2,4-dichlorophenoxy acetic acid (5 ppm) + kinetin (1 ppm) + coconut milk (20%) + inositol (100 ppm). The cultures were incubated at about 26° C and were exposed to diffuse laboratory light.

In basal medium as well as media 1. the female gametophytic tissue did not indicate any proliferation. Within two weeks after inoculation the tissue turned pale and ultimately became dark brown and shrivelled. However, the other culture slants (those reared on media 2.) proved of interest. In a few of these within one week after inoculation a mass of tissue started protruding out from the inner side of the parental tissue (Fig. 1). This tissue mass simulated very much the shape of a "sand castle" seen emerging out from the soil when an ant colony is established or of earthworm moulds generally seen in garden soils. In the initial stage the calli thus produced were almost pure white but as the growth proceeded a distinct greenish yellow tinge became clearly visible. The tissue appeared more green if the concentration of 2,4-dichlorophenoxy acetic acid was lowered to 1 ppm. Presumably the green color of the tissue was due to the development of certain pigments. Recently TULECKE [4] has also reported on the haploid tissue obtained from the female gametophytic tissue of *Ginkgo*. This tissue was chlorophyllous and even indicated differentiation into vascular elements. TULECKE had isolated the tissue from an ovule before fertilization. But in the present investigation the material used was obtained from older ovules that contained an almost mature embryo. Details of certain physiological and biochemical experiments carried out on this tissue shall be reported elsewhere.

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Presumed Aerotropic Growth of Roots of Certain Species

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Since MOLISCH [1], who was the first to describe aerotropic growth of roots, only few investigators have conducted further research. The results of BENNETT [2] are negative, while SAMMET [3] gives corroborative evidence. In the field the

growth of tree roots back towards the surface has also often been observed under wet conditions and aerotropic behaviour might be suspected.

In our own investigations concerning utilization of the subsoil normal root behaviour was observed for apple, oats, broad bean, tomato and wheat. Upward growth towards better aerated layers was noted for *Brassica napus*, potato and onion. This difference in geotropic response between species has also been observed by DE ROO [5].

The conditions in which this behaviour was observed for *Brassica* and potato were as follows [6]. Asbestos pipes, diameter ± 12 cm and 100–40 cm long, filled with soil, were

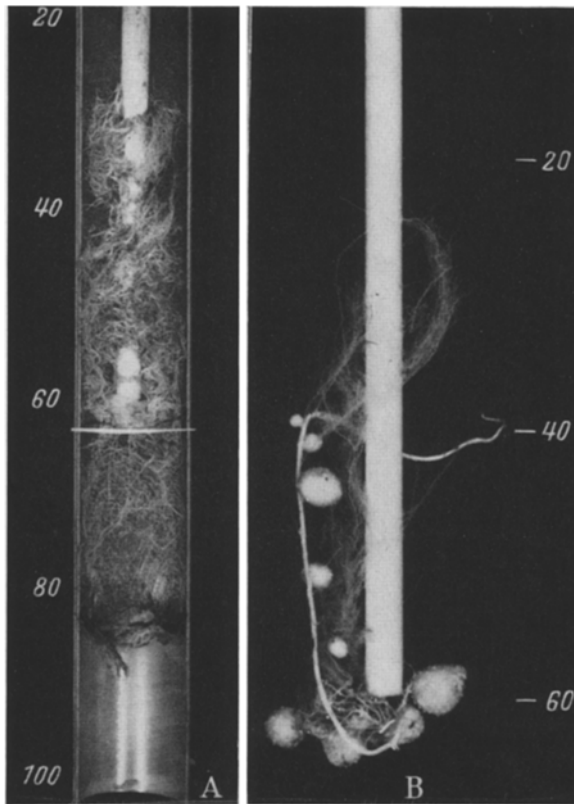


Fig. 1. A, *Brassica* plant with roots growing downwards to about 15 cm above watertable and part of the root system growing upwards. — B Potato plant with roots released at a depth of near critical aeration and growing towards better aerated layers

placed in a shallow trough with water. This resulted in an increasing moisture content with depth and a decreasing aeration. The oxygen diffusion rate measurements [7] varied from $30 \mu\text{A}$ at 10 cm depth to $9 \mu\text{A}$ at the bottom of the tube for potato. No roots grew into layers with less than a $13 \mu\text{A}$ reading. By means of plastic tubes the root system was released at varying depths (DE ROO and WIERSUM [4]) (Fig. 1). Upward root growth was only observed if the depth of release exceeded 40 cm and no root growth upwards was noticed if the O. D. R. reading was more than $25 \mu\text{A}$ at the level of release.

As there is no fertility gradient in the experiment and upward growth is only manifested when the roots are released into the poorly aerated layers the conclusion that they have grown towards better aerated layers seems warranted.

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CO₂-Gaswechsel, Wasserpotential und Sättigungsdefizit bei der Antrocknung epidermisfreier Blattscheiben von *Valerianella*

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Untersuchungen über den Einfluß eines angespannten Wasserhaushaltes auf den CO₂-Gaswechsel höherer, homoiohydrer Pflanzen stoßen auf große experimentelle Schwierigkeiten. Die als variable Diffusionswiderstände wirkenden Stomata greifen schon bei geringen Wasserdefiziten regulierend in den Gaswechsel ein. Man kann daher nicht sicher trennen, inwieweit bei Hydraturänderungen die Stoffwechselleistung beeinflusst wurde oder der CO₂-Diffusionsweg. Bei *Valerianella locusta* (L.) BERCKE var. *oleracea* (SCHLECHT.) BREISTR. läßt sich die untere Epidermis der Blätter ohne wesentliche Beschädigung des Mesophylls abziehen. Epidermisfreie Blattscheiben dieser Art sind geeignete Objekte, um den Einfluß von Hydraturänderungen auf die Stoffwechselleistungen Photosynthese und Atmung zu untersuchen.

Mit dem URAS wurde der CO₂-Gaswechsel in temperierten Küvetten gemessen (20°C ; $0,10 \text{ cal cm}^{-2} \text{ min}^{-1}$, HQL 400 W). Bei Begasung mit Luft von 90% rel. Feuchte trocknen die Blattscheiben im Laufe von 12 Std im Licht-Dunkelwechsel aus. Tabelle 1 zeigt die Abhängigkeit des CO₂-Gaswechsels

Tabelle 1. Abhängigkeit von CO₂-Gaswechsel (% der Werte bei Wassersättigung) und Sättigungsdefizit (%)

| Sättigungsdefizit | 0% | 20% | 40% | 60% | 80% | 90% |
|---|-----|-----|-----|-----|-----|-----|
| reelle CO ₂ -Aufnahme (%) | 100 | 92 | 81 | 65 | 40 | 22 |
| apparente CO ₂ -Aufnahme (%) | 100 | 92 | 79 | 59 | 30 | 10 |
| Dunkelatmung (%) | 100 | 98 | 93 | 88 | 80 | 68 |

der Blattscheiben vom Sättigungsdefizit. Mit zunehmendem Sättigungsdefizit geht die Photosyntheseleistung stärker zurück als die Atmung; auch bei einem Sättigungsdefizit von 90% ist noch apparente CO₂-Aufnahme nachweisbar.

Parallel dazu wurde in Dampfkammern bekannten Dampfdrucks die Abhängigkeit von Sättigungsdefizit und Wasserpotential epidermisfreier Blattscheiben gemessen. Die Ergebnisse (Tabelle 2) zeigen, daß der Photosyntheseapparat auch

Tabelle 2. Abhängigkeit von reeller CO₂-Aufnahme (% der Werte bei Wassersättigung) und Wasserpotential (bar)

| reelle CO ₂ -Aufnahme | 100% | 50% | 10% |
|----------------------------------|------|-----|-----|
| Wasserpotential | | | |
| <i>Valerianella locusta</i> | 0 | 69 | 190 |
| <i>Evernia prunastri</i> [1] | 0 | 62 | 203 |

bei hohem Wasserpotential noch aktiv bleibt. Die gemessenen Werte für epidermisfreie Blattscheiben liegen in der für niedere, poikilohydre Pflanzen nachgewiesenen Größenordnung [1]. Die Grenze nachweisbarer CO₂-Aufnahme höherer Pflanzen mit Epidermis — nach bisherigen Untersuchungen bei einem Wasserpotential von etwa 15 bar [1, 2] — wird durch hydraturbedingten Spaltenschluß verursacht.

Es erscheint demnach fraglich, ob die Photosyntheseleistung ein Maß für den "water stress" höherer Pflanzen sein kann. Es wäre zu prüfen, auf welche Weise der Wasserhaushalt in die Spaltöffnungsregulation eingreift [3]. Der Photosyntheseapparat der homoiohydrer Pflanzen ist offenbar sehr austrocknungsfähig [vgl. auch 4] und zeigt damit noch durchaus „poikilohydre“ Eigenschaften.

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