A physiological study of adventitious bud formation in potato

Agricultural Research Reports 787

P. Miedema

Foundation for Agricultural Plant Breeding, Wageningen

A physiological study of adventitious bud formation in potato

Centre for Agricultural Publishing and Documentation

Wageningen – 1973

ISBN 9022004244

Dr Miedema received his degree on 9 February 1973 at the University of Groningen, the Netherlands, after defending a thesis with the same title and contents.

Abstract

MIEDEMA, P. (1973) A physiological study of adventitious bud formation in potato. Agric. Res. Repts (Versl. landbouwk. Onderz.) 787. ISBN 90 220 0424 4, pp. (viii) + 66, 16 tabs, 19 figs. Eng. and Dutch summaries.
Also: Thesis, Groningen.

Formation of adventitious buds was studied on (1) rooted tuber-slices with the roots grown in soil or nutrient solution, and (2) tuber pieces without roots, kept in moist sand or cultured aseptically on nutrient media. Tuber pieces with roots produced many more buds than those without roots. The bud-promotive influence of the roots depended on mineral nutrition. In root extracts cytokinin activity was found. But in non-rooted tuber-tissue, buds could not be initiated by minerals, nor by cytokinins or other growth regulators. In one variety, adventitious buds were formed spontaneously on non-rooted tuber-tissue. Evidence was obtained that tuber tissue of this variety was less sensitive to auxin than tuber tissue of a variety without spontaneous bud formation.

Contents

Abbreviations and names of chemicals

B-9	succinic acid 2,2-dimethylhydrazide
BA	6-benzylaminopurine
CCC	2-chloroethyl trimethylammonium chloride
2,4-D	2,4-dichlorophenoxyacetic acid
DCP	2,4-dichlorophenol
GA	gibberellic acid (GA used in experiments was from British Drug Houses Ltd; it contained about 90% GA_3)
IAA	indoleacetic acid
IBA	indolebutyric acid
kinetin	6-furfurylaminopurine
M	mole/litre
NAA	naphtaleneacetic acid
PCIB	*p*-chlorophenoxyisobutyric acid
TIBA	2,3,5-triiodobenzoic acid
zeatin	6-(4-hydroxy-3-methyl-2-butenylamino)purine

1 General introduction

1.1 Shoot formation

One of the most obvious characters of vascular plants is the differentiation into shoot and roots. At the earliest stage of embryo development polarization occurs, determining which cells form the root and which cells the shoot. During later stages of plant development, new root and shoot meristems can be formed in different parts of the plant body. These phenomena have been described by several authors so that a rather complete picture of the morphology and anatomy is available. However, our knowledge of what regulates these developmental processes is rather fragmentary. (For reviews see Sinnot, 1960; von Guttenberg, 1960; Wardlaw, 1968).

The present study concerns the problem of shoot formation, with special attention to shoot initiation. Three types of shoot formation can be distinguished:

1. Shoot formation at embryogenesis.
2. Formation of axillary buds and shoots.
3. Adventitious shoot formation. This type includes all kinds of shoot formation other than (1) and (2). Adventitious shoots can originate from stems, leaves, roots, or from parts of these organs. Also shoots from callus tissue must be regarded as adventitious shoots.

We may assume that both the initiation of normal shoots and the initiation of adventitious shoots are regulated by the same physiological factors. The inception of normal buds proceeds in a regular pattern that is hardly influenced from outside. Initiation of adventitious buds, however, mostly requires special conditions, like removal of existing buds and application of growth regulators. Therefore factors regulating shoot initiation are most easily determined studying the requirements for adventitious bud formation.

1.2 Scope of the investigations

In 1966, I developed a new method to initiate adventitious buds in potatoes (Miedema, 1967). This method is based on the phenomenon that pieces of potato tuber, from which the eyes are removed, very easily form adventitious buds if they have been previously rooted. Two phases can be distinguished: (1) rooting of tuber-slices by auxin treatment, (2) adventitious bud formation on rooted slices. A scheme of the method is presented in Fig. 1.

Originally the method was developed to produce non-chimerical potato mutants

for plant breeding. Mutants can easily be produced by mutagenic treatment of tuber buds. A consequence of this procedure, however, is formation of chimeras, which are caused by the multicellular plant origination. Assuming that in the formation of adventitious buds a comparatively small number of cells is involved, I tried to solve the chimera problem by producing adventitious buds from previously mutated tissue. This subject, however, lies outside the scope of this study. Results will be published elsewhere.

The present study deals with physiological aspects of adventitious bud formation in potato. Some of the investigations concerned bud formation on isolated tuber tissue. Most attention was paid to bud formation on rooted tuber-slices. Since root formation was a prerequisite here, some work on this subject was necessary. I investigated the most suitable conditions for bud formation, which could also be applied to the production of non-chimerical potato mutants. The main objective, however, was to acquire some insight into the mechanism of shoot induction. I tried to answer the following questions:

1. Which conditions and treatments are required for optimum root formation? (Chapter 4)
2. Which environmental conditions are optimum for adventitious bud formation? (Chapter 5)
3. Are there relationships between varietal differences in formation of callus, adventitious roots, and adventitious buds? (Chapter 6)
4. What are the effects of growth regulator treatments on adventitious bud formation? (Chapter 7)
5. What is the influence of mineral nutrition? (Chapters 8 and 10)
6. What is the role of endogenous cytokinins in the process of bud formation? (Chapter 9).

2 Literature on adventitious bud formation in potato

Many studies on adventitious bud formation in potatoes are associated with plant breeding problems, viz. the production of graft chimeras (Simmonds, 1964), the production of polyploids from callus tissue (Krenke, 1933a, b), the investigation of mutants on periclinal chimerism (Asseyeva, 1927), and the production of non-chimerical mutants (Miedema, 1967). For the production of adventitious buds mostly tubers are used, though other parts of the potato plant can also form adventitious buds.

2.1 Adventitious buds from tubers

Rechinger (1894) observed that pieces of potato tuber, from which all eyes had been removed, developed callus and adventitious buds on their cut surfaces. The tuber pieces had to be stored in moist sand at room temperature. The buds differentiated directly from the tuber tissue or from callus protuberances. According to Rechinger a wound stimulus initiates cell division in cambium cells which results in the formation of callus or shoots.

Other studies on bud regeneration of potato tuber tissue were published by Kupfer (1907), Snell (1921), Krenke (1933b), Dubrowitzkaja (1933), Lauer & Krantz (1957), Simmonds (1964) and Claver (1967). The technique used was similar to that of Rechinger: de-eyed pieces of potato tuber were stored in moist conditions. The main results were as follows. Callus formation was a general phenomenon, adventitious buds occurred at a comparatively low frequency. Callus and adventitious buds only developed at cut surfaces, mostly on the vascular ring. Transversely cut tuber-slices only formed callus and adventitious buds on their heel directed cut faces (basipetal polarity) or in eye-holes. Varietal differences were observed in production of callus and in the frequency of bud formation.

2.2 Adventitious buds from stems, roots, and leaves

Klopfer (1965) obtained adventitious buds on stem sections. Internodes from etiolated sprouts were planted in soil. Shoots differentiated from callus tissue both on the apical and the basal end. Krenke (1933a, b) obtained adventitious shoots from apical cut surfaces of decapitated sprouts on tubers. Lauer & Krantz (1957), using potato plants with all growing points removed, observed basipetal callus formation in stem incisions. These calluses produced new buds or tubers. Great differences in adventitious shoot production were observed between genetically different clones (Lauer, 1967).

Shoot regeneration on roots of decapitated sprouts on tubers was reported by Dubrowitzkaja (1933). Light-exposed roots of potato plants developed shoots when all apical meristems of the stem were removed (Lauer & Krantz, 1957). Howard (1964) reported on a similar technique for the production of root shoots. In my method, where adventitious shoots are obtained from rooted tuber-slices, a part of the buds originated from the roots (Miedema, 1967).

Shoot formation on leaf cuttings is very common in many dicotyledonous species (Broertjes et al., 1968). In potato, leaf cuttings easily form roots, shoots however are formed very seldom (Kupfer, 1970; van Harten, 1972) or not at all (Simmonds, 1964).

2.3 Physiological factors affecting adventitious bud formation

Adventitious bud formation on decapitated sprouts was increased after infection with *Agrobacterium tumefaciens* (Krenke, 1933a, b). Belskaja (1933) reported shoot formation in crown gall on petioles. The cause of the difference in regeneration ability between tumorous and non-tumorous tissue is not known.

The influence of environmental factors was investigated by Lauer (1963, 1967) using disbudded potato plants. Long photoperiods appeared to promote adventitious bud formation. Temperatures above 27 °C were detrimental. A root medium with a high nitrogen, a high phosphorus and a low potassium level favoured adventitious bud formation.

Removal of all organized buds seems to be a general prerequisite for the formation of adventitious buds. Li et al. (1968) found that disbudding and the consequent growth arrest of potato plants caused a rise in the concentration of sugars, starch, acid-soluble phosphate, inorganic phosphate, and nitrogen fractions in the leaves. A comparison of two varieties differing in their shoot regenerating ability, showed a higher concentration of total nitrogen, protein nitrogen, starch and inorganic phosphate, and a lower sugar concentration in the variety with high adventitious bud producing capability. It may be interesting to investigate the significance of such differences for the bud-induction process.

2.4 Adventitious bud formation in sterile culture

Sterile cultures of potato plants, organs, tissues, and single cells have been used by several scientists. This technique deserves attention because of its feasibility for testing different chemical factors. From the extensive literature it is obvious that, in some species, buds can be initiated by low auxin and high cytokinin levels (see Section 7.1).

In potato, growth of callus tissue can easily be obtained (Steward & Caplin, 1951; Chapman, 1955; Lingappa, 1957; Bajaj & Dionne, 1967). Only one report is known on bud formation in isolated potato callus (Svobodová, 1964). Trials with cell suspension cultures of potato showed that, in contrast to species like *Daucus carota*, embryogenesis did not occur (Bajaj & Dionne, 1967).

Sterile cultures of tuber explants can produce buds (Wurm, 1960; Fellenberg, 1963;

Okazawa et al., 1967), although sometimes no bud formation was obtained (Simmonds, 1964; Claver, 1967). The different results may be due to varietal differences of the plant material (Fellenberg, 1963). Conclusive results on growth regulator requirements were not presented. It can not be excluded that adventitious bud formation on tuber explants in sterile culture is basically similar to adventitious bud formation on tuber pieces kept in moist sand. Then the formation of shoots is independent of external chemical factors.

In sterile cultures of potato roots Bajaj & Dionne (1968) observed the formation of nodule-like structures, differentiation however into shoots did not occur.

It may be concluded that the potato, compared with other plant species, is relatively recalcitrant to adventitious shoot formation. The same holds true for embryo formation in cell cultures (Steward et al., 1970). One of the challenging problems is to account for these genotypically determined differences in physiological response.

3 Material and methods

3.1 Plant material

Potato tubers, used in most of the experiments, were sound seed potatoes obtained commercially, usually with a minimum diameter of 45 to 55 mm. The tubers were stored before use at 3 to 5 °C. Sprouting later in the season, was inhibited by treatment with the fungicide' AArdisan', an organic mercury compound; this treatment did not noticeably influence regeneration behaviour. The variety Bintje was used, unless stated otherwise.

In the variety trials, mentioned in Section 4.5 and Chapter 6, tubers were used that were grown in the same field. In other variety trials the tubers were commercially obtained.

The above mentioned potato material was harvested immature (seed potatoes). In the dormancy experiments, mentioned in Section 4.4, self-grown mature tubers were used.

3.2 Rooting of tuber-slices

Rooted tuber-slices were used in most of the bud-formation experiments. A scheme of the whole procedure of root induction and the consequent bud formation has been presented in Fig. 1.

The method of root initiation is largely based on experimental results from Chapter 4. Non-dormant tubers, from which the eyes were removed, were cut into transverse slices 15 mm thick. Mostly two slices per tuber could be obtained. Freshly cut slices were washed with tap water and treated with auxin to induce root formation. For routine production of rooted slices 1% IAA in talc powder was applied at the basal cut faces. In some of the root formation experiments the auxin was applied as an aqueous solution in which the slices were soaked for 1 h. To increase the solubility of the auxins a certain amount of potassium hydroxide was usually added to the solution. Slices treated with auxin solution were washed with tap water afterwards; in the material treated with talc the auxin-talc mixture remained on the slices during the period of rooting.

The auxin-treated slices were buried at a depth of 5 cm, with the basal surface down, in perlite which was moistened with tap water. After about 14 days the first roots appeared. All roots developed at the basal surface, most of them at the vascular ring (Fig. 2). After 5 to 6 weeks the roots were long enough to use the slices in bud formation experiments.

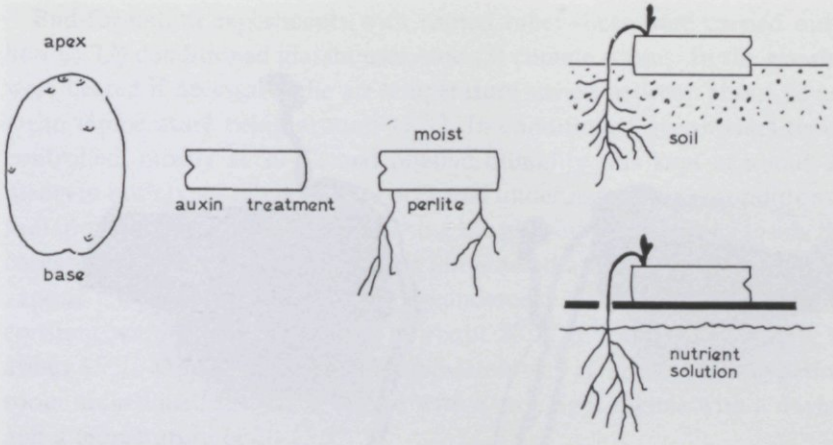


Fig. 1. Scheme of the method.

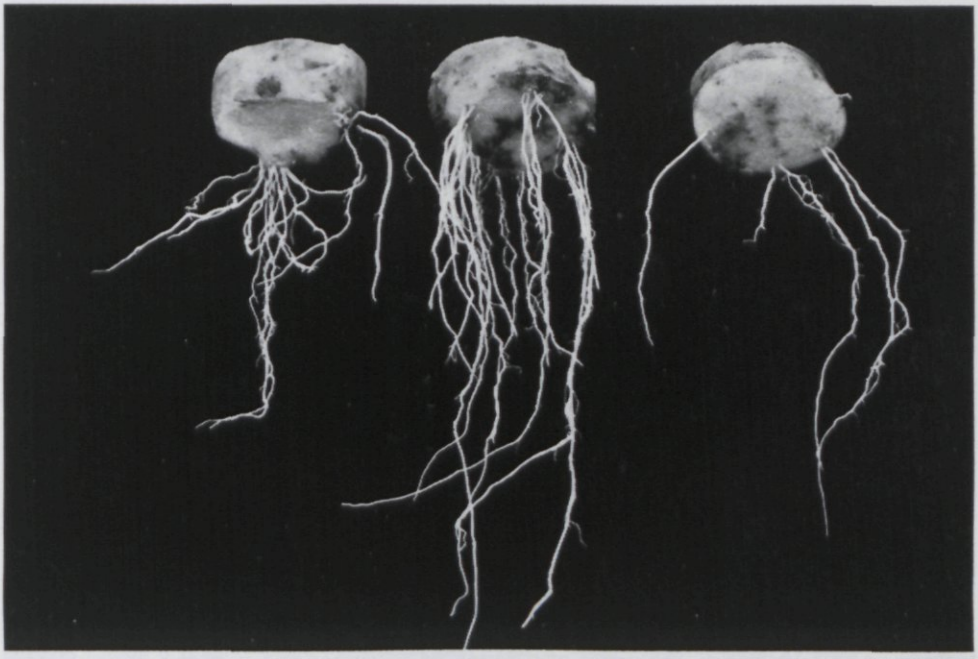


Fig. 2. Root formation on tuber-slices, 5 weeks after application of 1 % IAA in talc.

3.3 Bud formation on rooted tuber-slices

Rooted slices were washed, to remove perlite, talc, and auxin. To uniform the material and to simplify the handling of it, the roots were shortened and the number of roots was reduced to a small group; damaged tissue was cut away. The rooted slices were planted in soil with the apical side down (Fig. 1). The basal surface and the upper

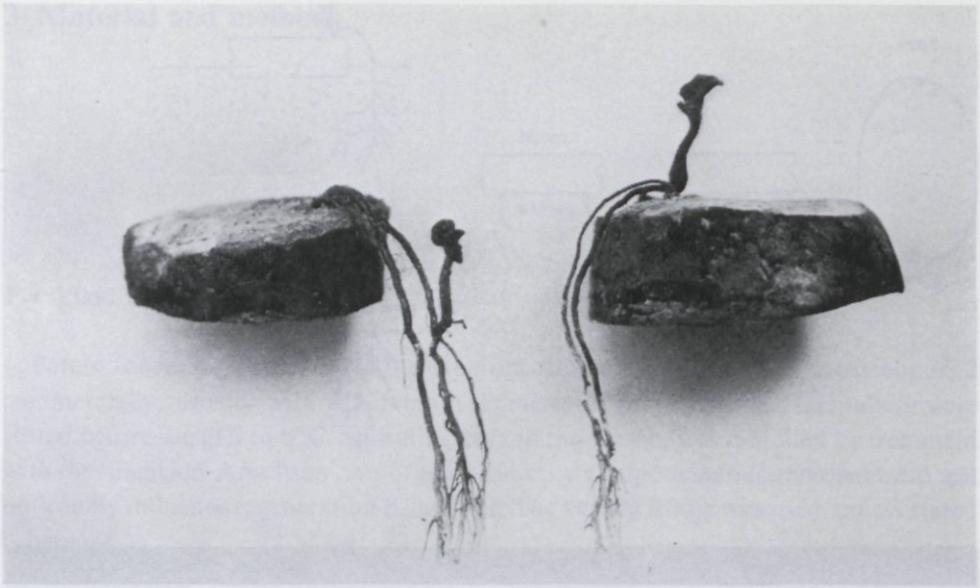


Fig. 3. Adventitious shoot formation on rooted tuber-slices, which were grown in nutrient solution. The left shoot arose just above the water level.

parts of the roots were exposed to the air. In a number of experiments the water culture technique was used (Chapter 8).

The roots started growing soon after planting of the slices. The tuber-slices, when kept in the light, turned green, as did the light-exposed parts of the roots.

About two to three weeks after planting the first bud primordia appeared. Bud formation could go on for at least three months. Most buds developed at the root base, some buds nearby this place on the tuber tissue, or on the air-exposed parts of the root (Fig. 3). The apical surface of the tuber-slice and the non-exposed parts of the roots never produced buds. The formation of a bud was often accompanied by lateral buds. Besides buds callus was frequently formed on the tuber-slices, especially close to the root base.

Adventitious shoots can be separated from the rooted slices and planted in soil. They form roots and develop into normal looking tuber-bearing plants.

During rooting, when the slices were buried in moist perlite, adventitious bud formation was never observed. The cause of this will be discussed in Chapter 8.

3.4 Environmental conditions

Rooting of tuber-slices for bud-formation experiments was done in a glasshouse in large perlite-filled benches. The perlite was covered with transparant plastic foil to prevent drying out. I tried to keep the temperature in the perlite between 15 and 20 °C; at higher temperatures the slices easily rotted. Investigations on root formation were carried out in the dark in a similar way (Chapter 4).

Bud-formation experiments with rooted tuber-slices were carried out in (1) glasshouses, (2) conditioned glasshouses, and (3) climate rooms. In the glasshouses, which were heated if necessary, the air temperature varied between about 12 and 30 °C, the mean temperature being around 18 °C. In conditioned glasshouses temperature was controlled, mostly at 20 °C, and relative humidity was kept at about 85%. Experiments in both types of glasshouse occurred under natural light conditions. In summer, radiation was dimmed by whitewashing the glasses or by shading inside the room with cheesecloth. The climate rooms were illuminated artificially by high pressure mercury vapour lamps, supplemented with incandescent lamps. The light intensity was kept constant within each experiment at about 20 W/m^2; relative humidity amounted to about 85%. Mostly continuous illumination was given; in some experiments lack of room necessitated the use of rooms with a day/night regime with a daylength of 14 h and a temperature of 20/15 °C.

Experiments with de-eyed tuber pieces kept in moist sand, to study callus development and bud formation, were carried out at about 18 °C.

3.5 Design of experiments, assessment of results, and statistical analysis

Experiments were usually done in 3 to 5 replicates, which were started simultaneously.

In root-formation trials 10 tuber-slices per replicate were used.

Fig. 4. Rooted tuber-slices planted in soil.

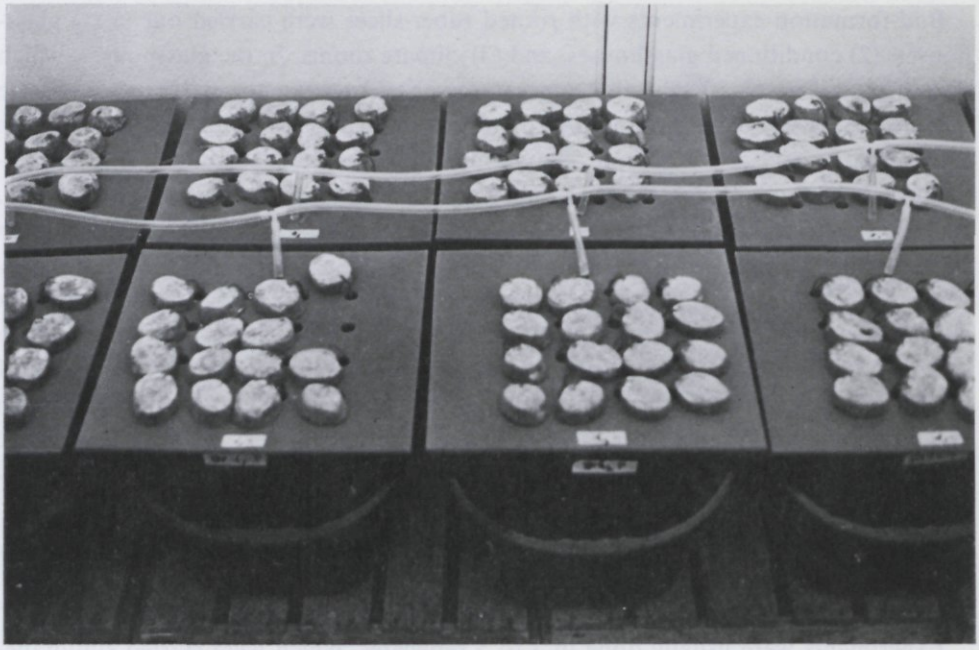


Fig. 5. Rooted tuber-slices in water culture.

In bud-formation experiments on rooted tuber-slices, planted in soil, 10 slices per replicate were used; Fig. 4 shows the procedure: the transverse rows are replicates of different series. In experiments with rooted slices on water culture, buckets with 12 to 20 slices were taken as replicates (Fig. 5; see also Section 8.2).

The degree of root formation and bud formation was evaluated as the percentage of tuber pieces that had produced one or more organs at a given time. Roots were easily distinguished with the naked eye. In bud-formation trials a magnifying glass was used, if necessary, to distinguish real bud primordia from not yet differentiated nodule-like structures. Observations were made weekly. In bud-formation experiments with rooted slices, data were mostly reported when the budding percentage in the control series was about 50% or more.

Because of differences in environmental conditions and in the tuber material the response of the plant material varied from experiment to experiment (see Section 5.3). Thus the results of different experiments are not fully comparable.

All weight data of tubers, roots, and callus, are given in fresh weight. Roots were blotted with tissue paper before weighing.

Experimental data were statistically analysed by Mr J. Post Jr (Foundation for Agricultural Plant Breeding). Analysis of variance was carried out. To improve normality of distribution, data on root and bud formation were transformed according to Freeman-Tukey's arc-sine transformation as described by Mosteller & Youtz (1961). The significance of difference between means was assessed by Student's t test. In graphs and tables, data designated by the same letter did not differ significantly from each other at $P = 0.05$.

4 Rooting of tuber-slices

4.1 Introduction

Spontaneous root formation on potato tuber tissue is a very rare phenomenon (Rechinger, 1894; Guthrie, 1939). Guthrie (1938, 1939) discovered that root formation on cut surfaces of tuber pieces is induced by treatments with the auxins IAA and NAA. Tuber explants in sterile culture reacted similarly to a certain auxin level in the medium (Wurm, 1960; Fellenberg, 1963; Okazawa et al., 1967). These data indicate that root formation in potato tuber tissue is dependent on exogenous auxin.

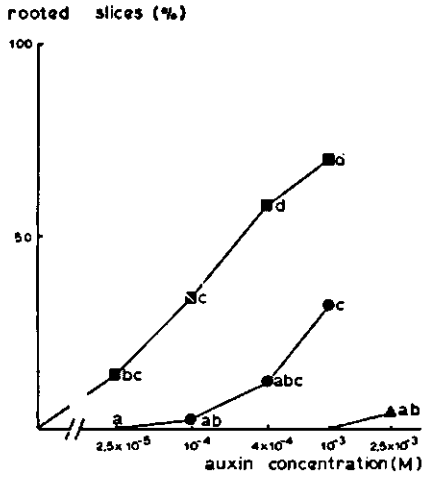
In my experiments on adventitious bud formation, large numbers of rooted tuber-slices were needed. Therefore it was useful to know the optimum conditions for root formation. In horticulture rooting of auxin-requiring cuttings is performed by treatment with IAA, IBA, or NAA in talc mixtures, or in solution (see Hartmann & Kester, 1968). In a similar way I tried to find a suitable treatment for potato tuber-slices. I also studied the effect of temperature, the influence of tuber dormancy, and varietal differences.

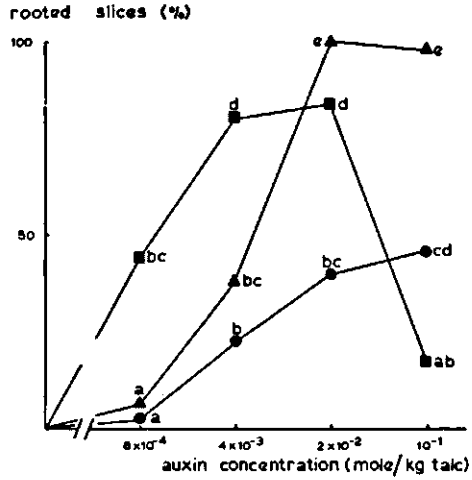
4.2 Auxin treatment

Tuber-slices were treated with IAA, IBA, and NAA in various concentrations. The auxins were applied in aqueous solutions or in talc mixtures. The auxin-treated slices were set in moist perlite in a greenhouse bench. The temperature in the perlite ranged from 7 to 30 °C, the mean being about 20 °C.

The results of the experiments with aqueous solutions are presented in Fig. 6. NAA appears to give the best rooting; the effect of IAA is negligible. No optima were found within the concentration used, although in the highest NAA concentration brown colouring of the tissue, a symptom of toxicity, was observed. Although the whole slice was soaked in the auxin solution, roots only appeared at the basal surface. This basipetal polarity, a general feature in rooting of stem sections, may be ascribed to polar transport of the auxin (see Leopold, 1961).

The effect of auxins applied in talc mixtures is demonstrated in Fig. 7. Here IAA was more effective than in the previous experiment, probably because it remained on the cut surface longer. After a short term treatment of IAA in solution, the auxin may be inactivated in the tissue before it can initiate root formation. Compared with NAA and IBA, IAA is rapidly oxidized in vivo (see Hare, 1964). High NAA concentration (2×10^{-2} to 10^{-1} mol/kg talc) resulted in severe damage of the basal tissue.





Figs. 6 (left) and 7 (right). Root formation, 5 weeks after treatment with different concentrations of IAA (▲), IBA (●) and NAA (■). Each point represents 5 × 10 slices; data with the same letter are not significantly different from each other at P = 0.05. Fig. 6: auxin applied as a 1 h soak. Fig. 7: auxin in talc mixtures applied on the basal surfaces.

When both root production and toxic effects were considered, the most suitable auxin treatments were, in order of effectiveness: 1% IAA in talc, 0.1% NAA in talc, 20 to 40 mg/l NAA solution, 1% IBA in talc, and 200 mg/l IBA solution. For practical purposes the concentrations are given in weight units.

Roots induced by IAA were thin, long and numerous; NAA-induced roots were thick, short and less numerous; IBA-induced roots were intermediate between the IAA and NAA type.

The auxin treatments increased callus formation at the basal surfaces. Only after soaking in NAA solutions of high concentration did some apical callus occur.

4.3 Temperature

Auxin-treated tuber-slices were kept at temperatures ranging from 11 to 28 °C. The auxins IAA and NAA were applied in concentrations which were supposed to be sub-optimum (see figs 6 and 7).

The results are presented in Fig. 8. Rooting response in this experiment was better than in Section 4.2; the cause of the difference is not known. The NAA concentration appeared to be too high to demonstrate the optimum temperature when root formation was expressed as the percentage of rooted slices. Therefore a second curve, representing the mean numbers of roots per slice, is given. It appears that the optimum temperature is about 17 °C for both IAA and NAA. At 11 °C root development and root growth were very slow. Above 20 °C some decay occurred; a part of the lower rooting response at higher temperatures may be caused by this decay.

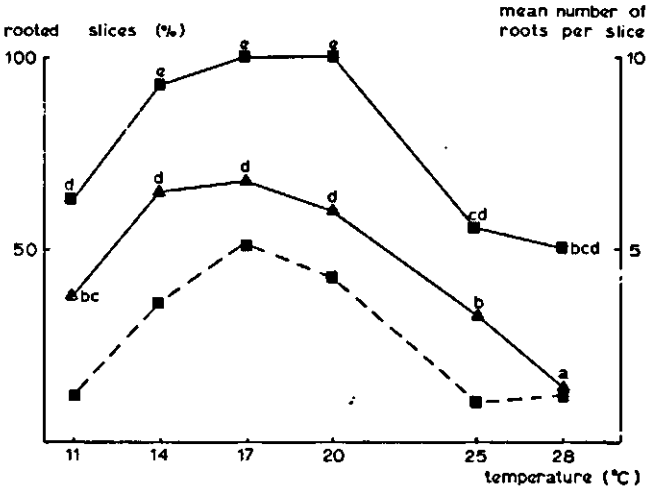


Fig. 8. The effect of temperature on root formation, induced by IAA (▲) and NAA (■); — percentage of slices with roots, ---- number of roots per slice. Each point represents 4 × 10 slices; data with the same letter are not significantly different from each other at $P = 0.05$.

4.4 Dormancy

Dormancy has been defined in different ways. In this study the dormant period has been considered as the time after harvest when the buds were not growing under the given conditions (see Burton, 1963). Guthrie (1939) observed already that auxin-induced root formation on tuber pieces is inhibited during the period of tuber dormancy. By pretreatment of the tubers with the dormancy breaking substance ethylene chlorohydrine, the rooting was increased. In order to obtain more detailed information on the rooting capacity during the dormancy period, an experiment was carried out to test the rooting ability at different times after harvest, without and with application of dormancy breaking substances. In potato tubers bud dormancy can be broken in different ways. I used potassium thiocyanate (KSCN) (Denny, 1926) and gibberellic acid (Brian et al., 1955; Bruinsma et al., 1967).

Mature tubers were harvested on 28 August 1967. The tubers, which were not treated with AArdisan, were stored at 20 °C. Rooting tests were carried out at intervals of 5 weeks, starting 1 week after harvest. In each rooting test tuber-slices underwent the following treatments:

a. Control, soaking 1 h in water, followed by 1 h in 20 mg/l NAA
b. KSCN, soaking 1 h in 1 % KSCN, followed by 1 h in 20 mg/l NAA
c. GA, soaking 1 h in 1 mg/l GA, followed by 1 h in 20 mg/l NAA

The treated tuber-slices were set in moist perlite at 20 °C. Root formation was determined 5 weeks after treatment. The dormancy state of the tuber stock is indicated by the percentage of tubers with sprouts at least 2 mm long.

The results, given in Fig. 9, show that during the 'dormancy period' and some time after that, root formation was fully inhibited. With KSCN rooting was somewhat earlier, GA had no effect. In parallel experiments with tuber pieces with a single eye, however, I found GA to be more effective than KSCN in breaking bud dormancy.

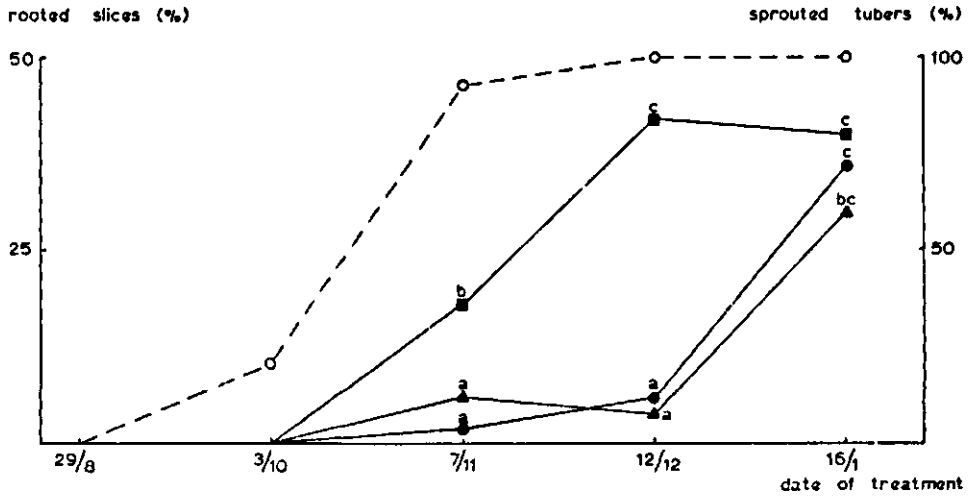


Fig. 9. The influence of tuber dormancy on rooting capacity. Pretreatments: water (●), KSCN (■), and GA (▲). Each point represents 4 × 10 tuber slices; data with the same letter are not significantly different from each other at P = 0.05. Sprouting percentages (○) were determined in a sample of 100 tubers.

This differential response may be of interest for physiological research of the dormancy problem. For practical purposes, I only concluded that rooting of tuber slices could be done with non-dormant tubers.

I have learned from experience that slices from cool stored seed-potatoes, harvested in July, can be rooted from about December until more than one year after harvest.

4.5 Varietal differences

Preliminary experiments revealed the existence of great varietal differences in auxin-induced root formation. This led to an experiment in which 40 potato varieties were tested on their rooting capacity. Amongst them a selected number were genetically related, in order to obtain data on the heredity of rooting capacity. From each variety 50 tuber-slices were treated with 1 % IAA in talc.

Table 1. Varietal differences in root formation on auxin-treated tuber-slices.

Slices with roots (%)	Number of varieties
0– 20	12
20– 40	8
40– 60	5
60– 80	5
80–100	10

The results of an observation, 25 days after treatment, are given in Table 1. Two varieties did not root at all, the variety Bintje and four other varieties showed 100% rooting. Amongst the poor rooting varieties some were genetically related. A simple inheritance of the rooting capacity may be excluded since many varieties were intermediate.

In Chapter 6 experiments are described to relate the above varietal differences with the variety-determined differences in wound callus and adventitious bud formation.

5 Bud formation on rooted tuber-slices: Influence of environmental factors and age of tubers

Before studying the role of well defined physiological factors, it was useful to know the effects of environmental factors. My first experiments showed that rooted tuber-slices, planted in soil and exposed to light, produce adventitious buds on the exposed parts of the roots (Miedema, 1967). This result suggests that light is essential. Experiments, described in this chapter, demonstrate that under dark conditions bud formation also occurs. Other investigations described here concern the effect of different temperatures and the influence of the age of the tubers.

5.1 Temperature

In order to find the most suitable temperature for bud formation, the following experiment was carried out. Rooted tuber-slices, planted in soil, were placed in conditioned glasshouses with mean air temperatures of 12, 15, 19, 22 and 27 °C; temperatures fluctuated ± 2 °C. Higher temperatures in the tissue by solar heating could not be excluded since no shading was applied.

The results are presented in Fig. 10. A temperature ranging from 19 to 27 °C was beneficial for bud formation. An exact estimate of the optimum was impossible because of the inaccuracies of the conditions. At temperatures up to at least 12 °C no bud formation occurred in the first five weeks. A rough observation of root development showed, that better root growth occurred at lower temperatures. In this experiment both slice and root temperatures were varied. The question arises whether

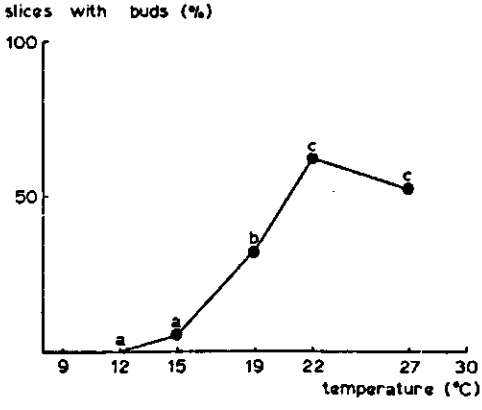


Fig. 10. Bud formation after 5 weeks at different temperatures. Each point represents 4 × 10 slices; data with the same letter are not significantly different from each other at P = 0.05.

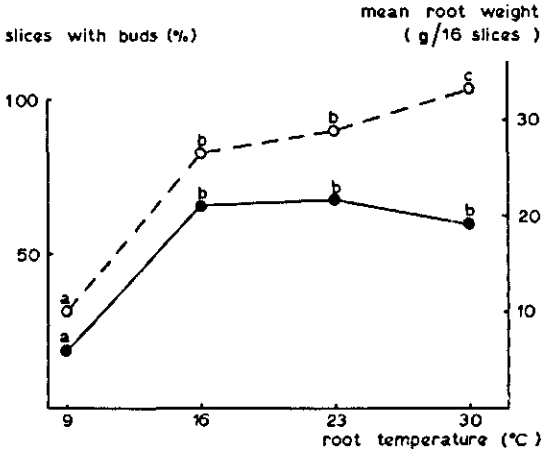


Fig. 11. Bud formation (●) and root growth (○) after 5 weeks at different root temperatures. Each point represents 4 × 16 slices; data with the same letter are not significantly different from each other at P = 0.05.

the differences in bud formation should be ascribed to temperature effects in the roots or in the slices.

In a second experiment I tried to vary only the temperature in the root medium. This was possible by using the water culture technique (Section 8.2). The buckets with Hoagland's solution of 15% of the normal strength were hung in large tanks filled with water of a constant temperature. The whole experiment was conducted in a growth chamber: air temperature 23 °C, relative humidity 80 to 90%, continuous fluorescent light of 20 W/m^2. Four different root temperatures were applied: 9, 16, 23 and 30 °C. I tried to keep the slice temperature at 23 °C. Unfortunately, insufficient isolation of the root media and to some extent of the lamps, resulted in deviating temperatures. I measured 21, 23, 24.5 and 25.5 °C respectively. These temperatures are within the range suitable for bud formation, as estimated in the first experiment.

The results, presented in Fig. 11, showed no great differences in root growth and bud formation between 16 and 30 °C. The differences in bud formation in the same temperature range in the first experiment were probably due to temperature effects in the tuber-slices, or in the bud forming tissue only. A root temperature of 9 °C strongly inhibited bud formation. At this temperature also the development of the root system was inhibited.

5.2 Light

A few experiments were conducted to investigate the effects of light intensity and daylength. Only the main results of these experiments will be given here. Daylength effects could not be demonstrated. In some experiments bud formation was increased at high light intensities (up to 50 W/m^2). In others there was hardly any difference between material grown in the light and that grown in the dark (see Section 5.3). On account of the variable response it was decided not to investigate the physiological significance of the light effects. In experiments on bud formation, light conditions were kept equal within each experiment.

5.3 Age of the tubers

Experiments on bud formation in rooted tuber-slices can be started in January or February, when the end of the dormancy period allows root initiation (Section 4.4). It is well known that during storage physiological and chemical changes occur in potato tubers (see Burton, 1966). It was useful to know whether such changes influenced bud-formation capacity, in order to determine the period of the year suitable for experiments. Therefore I carried out bud-formation trials at different times of the year.

The tuber stock was stored at 3 to5 °C; no 'AArdisan' treatment was applied. At intervals of 10 weeks, samples were taken, which were rooted with 1 % IAA at 20 °C. Five weeks after auxin treatment, rooted slices were planted in soil. One part was kept in a dark growth chamber, the other part in a light chamber. Four bud-formation trials were done, the first was planted on 24 January.

It appeared that with increasing age of the tubers, root forming capacity decreased and the susceptibility for decay increased. This susceptibility was due to flaccidity of the tubers which was caused by sprouting. If sprouting was inhibited by Aardisan or by storage at 2 °C, no such difficulties were observed.

Table 2. Bud formation (%) in light and dark on rooted slices from tubers of different age. Conditions in the growth chambers: temperature 18 to 20 °C; in the light chamber: daylength 16 h light intensity 30 W/m². The values in brackets are referring to experiments with a night temperature of 14 °C. All data were recorded 6 weeks after planting date. Figures designated by the same letter are not significantly different at P = 0.05

	Planting date			
	24/1	3/4	12/6	21/8
Light room	68 cd	75 d	(63) cd	(50) bc
Dark room	60 bcd	60 bcd	38 b	5 a

The data on bud formation are presented in Table 2. In 16 h of light, bud formation occurred at a rather constant frequency. In the dark, however, the rooted slices showed a diminishing capacity for bud formation with increasing age of the tubers. The cause of this phenomenon has not been investigated.

From the results of this experiment, supplemented by experience in many other trials in different years, I concluded that bud formation on rooted tuber-slices can be done from January until September, with tubers harvested in the previous year. However, one has to be careful in comparing results obtained from tuber material of different age.

6 Varietal differences in the formation of roots, buds, and wound callus

6.1 Introduction

The ability to regenerate roots and shoots depends upon the genotype. This can be concluded from differences between species, and from differences between varieties within a species.

In potato, varietal differences were found in shoot regeneration on disbudded tuber pieces (Dubrowitzkaja, 1933; Simmonds, 1964), and in shoot regeneration on plants from which all growing points were removed (Lauer, 1967). In a preliminary experiment I observed varietal differences in bud formation on rooted tuber-slices. Experiments described in Section 4.5 demonstrate that potato varieties differ widely in their ability to initiate roots in auxin-treated tuber tissue. Snell (1924) suggested varietal differences in wound-callus formation on disbudded tuber pieces.

The question arises whether there are relationships between the different types of regeneration. Correlations, positive or negative, would indicate common causal factors. In the present chapter investigations are described in which a number of varieties were tested on their ability to form (1) wound callus on tuber-slices, (2) adventitious buds on disbudded tuber pieces, (3) adventitious buds on rooted tuber-slices, and (4) adventitious roots on tuber-slices, treated with auxin. A first experiment concerned the phenomena (1) and (2), a second one (2), (3), and (4).

6.2 Relationship between wound-callus formation and bud formation

In the following experiment six potato varieties were used. From each variety 120 de-eyed tuber-slices were buried in moist sand with the apical side down. Observations on adventitious bud formation were made for three months. After this time callus weight of 60 slices of each variety was determined.

The results are presented in Table 3. Great varietal differences in callus production (Fig. 12) and adventitious bud formation were established. The majority of the buds were formed in eye-holes, some buds developed at the basal cut faces. Callus formation occurred at the basal faces, occasionally some callus developed in eye-holes. At the apical faces no callus and no buds were observed.

A relationship between callus production and bud formation could not be found. Also other varietal characteristics like earliness and dry matter content of the tubers did not correlate with the regeneration phenomena.

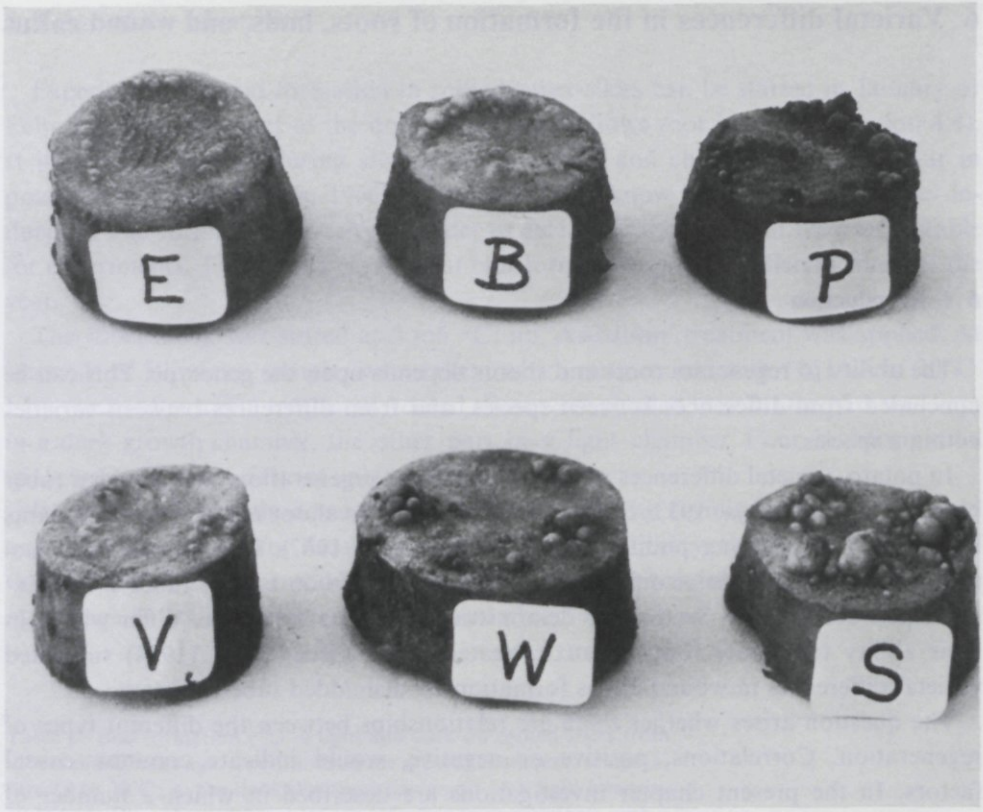


Fig. 12. Varietal differences in wound callus formation. The letters designate the varieties listed in Table 3.

Table 3. Callus production and adventitious bud formation on tuber-slices of six varieties.

	Callus production (g callus/kg tuber-slice)	Slices with buds (%)
Eigenheimer	3.8	11.7
Voran	6.1	2.5
Bintje	12.7	0
Woudster	15.2	0
Pimpernel	30.2	0.8
Sirtema	43.9	14.4

6.3 Relationship between bud formation and auxin-induced root formation

Nine potato varieties were tested for their capacity for adventitious bud formation on rootless and rooted tuber pieces, and their ability for root formation on auxin-treated tuber-slices. Some of the varieties were the same as in the previous experiment.

Table 4. Varietal differences in adventitious bud formation on tuber halves and on rooted tuber-slices, and in auxin-induced root formation of tuber-slices. Observations after 3 months, 6 weeks, and 4 weeks respectively.

	Tuber halves with buds (%)	Rooted slices with buds (%)	Slices with roots (%)
Bintje	0	88	98
Civa	0	22	90
Pimpernel	0	·	0
Voran	1.0	12	68
Alpha	1.6	21	66
Woudster	2.0	·	0
Sirtema	13	51	22
Allerfr. Gelbe	27	·	37
Multa	68	41	30

From each variety 100 tuber halves cut lengthwise, were disbudded and buried in moist sand. This technique, also used by Asseyeva (1927), was chosen because the higher number of eye-holes might result in more adventitious buds. Indeed most buds developed in eye-holes, also some at the longitudinal cut faces (Fig. 13).

In six varieties adventitious bud formation on rooted tuber-slices was investigated; 30 to 50 slices per variety were planted in soil.

Auxin-induced root formation was determined by treating 50 tuber-slices of each variety with 1% IAA in talc.

The bud and root formation frequencies are presented in Table 4. Within each type of regeneration great varietal differences occurred, interrelationships, however, could not be shown.

6.4 Discussion

No relationships could be demonstrated between the varietal differences in callus formation, the two types of bud formation, and root initiation. This suggests a certain degree of independence of the underlying processes. Nevertheless there are common characters, viz. the association with the vascular tissue and the basipetal polarity, indicating the involvement of vascular transport as a general prerequisite. The end result, however, appears to be dependent upon different factors. These may be promoting factors indispensable in the complex regeneration process, or factors which actively inhibit the process involved. The key role of growth hormones in regeneration processes has been well established (see Dore, 1965). Whether the above varietal differences are caused by variety-determined differences in endogenous hormone levels has to be investigated.

Insufficient adventitious root formation, even after appropriate auxin treatments, is hindering the propagation by cutting of many species and varieties. Investigations, devoted to this problem, demonstrated the presence of root-forming substances other

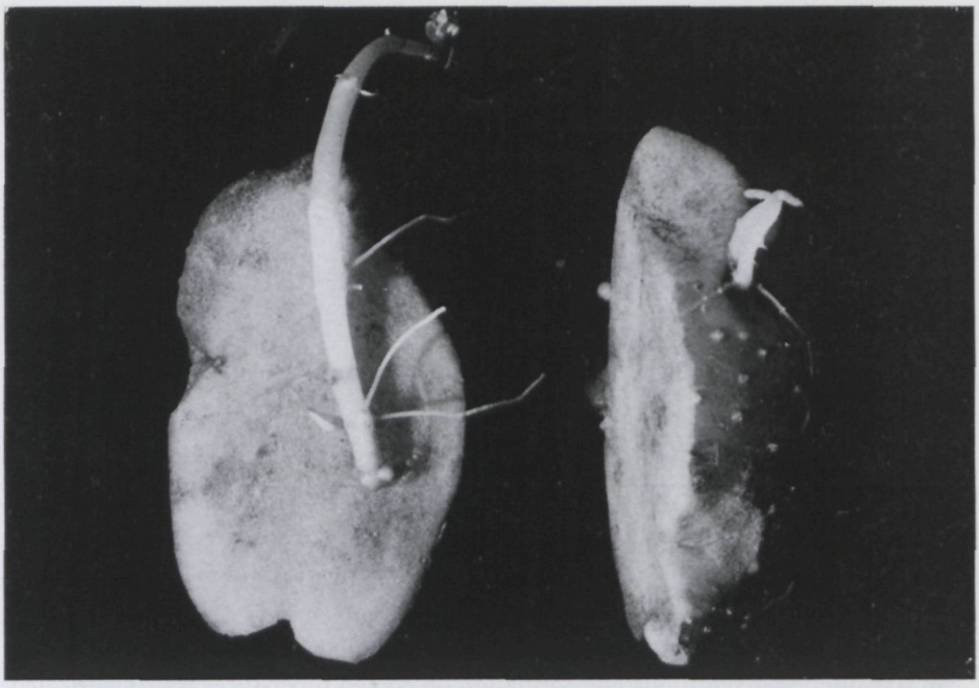


Fig. 13. Adventitious sprouts on tuber halves of the variety Multa.

than auxin, which occur in higher amounts in easy-to-root genotypes (Richards, 1964; Hess, 1964; Fadl & Hartmann, 1967). Also some evidence for root inhibitors was found (Richards, 1964; Paton et al. 1970). The varietal differences in root formation of potato tubers might be ascribed to similar substances. It would be very interesting to investigate this subject, especially as large quantities of tuber tissue for extraction are easily obtained. The physiology of root initiation, however, is beyond the scope of my study.

The above investigations have shed some new light on the problem of adventitious bud formation. There are distinct differences between the two types of bud formation. Adventitious bud formation on rootless tuber pieces is usually poor, except in the variety Multa. From rooted tuber-slices many more adventitious buds can be obtained; the varietal differences are smaller. The absence of any correlation between the two types of bud formation indicates that the root system brings about a profound change in the physiology. Presumably the roots are providing some bud-inducing factor. Three phenomena have to be accounted for: (1) the varietal differences in bud formation on rootless tuber pieces, (2) the varietal differences in bud formation on rooted tuber-slices, and (3) the difference in bud formation between rootless and rooted tuber pieces. Investigations into these problems are presented in the following chapters.

The main conclusion of this chapter is that the different regeneration phenomena are regulated by different factors. A generalization in the physiological backgrounds is not yet possible.

7 Effects of applied growth regulators on bud formation

7.1 Introduction

Bud formation appears to be regulated mainly by two classes of hormones: cytokinins and auxins. A comparatively high cytokinin level and a low auxin level is prerequisite for bud initiation. This has been found by Skoog & Tsui (1948) and Skoog & Miller (1957) with tobacco tissue cultures. Many investigations show that the findings of Skoog and his co-workers apply to many plant species and to different types of tissue; reviews were published by Dore (1965). Torrey (1966), and Halperin (1969). It appears however, that bud formation is not always brought about. Three reaction types can be distinguished: (1) spontaneous bud formation, which is presumably induced by endogenous hormone levels, (2) bud formation, artificially induced by growth-regulator applications, and (3) no bud formation, either spontaneously, or after growth-regulator treatments.

From literature (Section 2.4) it appears that isolated tissues and organs of potato mostly behave according to type (3). This might suggest some mechanism other than hormones, or in addition to hormones, by which bud formation is inhibited. However, before any conclusions could be made, the effects of different growth-regulator treatments had to be investigated more extensively. Thus attempts were made to induce bud formation on rootless tuber-slices of the potato variety Bintje, which normally do not form adventitious buds (see Chapter 6). The results presented in Section 7.3 show that the growth-regulator treatments applied, did not alter the situation. Then the effect of growth regulators on bud formation in rooted slices was studied. I tried to find out what treatments promoted and what treatments inhibited spontaneous bud formation in rooted slices, as the results might give some indication of how endogenous hormones function in bud formation.

Bud formation may be limited by shortage of hormones; to investigate this possibility, auxins, cytokinins, and gibberellic acid were applied. On the other hand bud formation may be inhibited by an excess of hormones, especially auxin. So I tried to influence the action of endogenous auxin by treatments with 2,3,5-triiodobenzoic acid (TIBA), a substance inhibiting polar auxin transport (Niedergang-Kamien & Skoog, 1956; Greenwood & Goldsmith, 1970), and *p*-chlorophenoxyisobutyric acid (PCIB), which may counteract the auxin effects by competitive inhibition (McRae & Bonner, 1953; Fransson, 1958). Endogenous auxin levels may be dependent on the activity of IAA-oxidase (see Hare, 1964). Cofactors of this enzyme system are certain monophenolic compounds e.g. *p*-coumaric acid, *p*-hydroxybenzoic acid, and 2, 4-

dichlorophenol (DCP). These substances increase IAA degradation (Zenk & Müller, 1963); *p*-hydroxybenzoic acid has been shown to promote bud formation in tobacco tissue cultures (Lee & Skoog, 1965). Contrary to monophenols, polyphenolic compounds like caffeic acid and chlorogenic acid are supposed to inhibit IAA degradation. In my research DCP and caffeic acid were tested for their influence on bud formation, without, however, determining their effect on endogenous auxin levels. In addition, the effects of the growth retardants 2-chloroethyltrimethylammonium chloride (CCC) and succinic acid 2,2-dimethylhydrazide (B-9) were investigated. CCC has been assumed to inhibit the biosynthesis of gibberellins (see Lang, 1970). B-9 may exert its action by inhibition of auxin biosynthesis (Reed et al., 1965), but reduction of endogenous gibberellin levels has also been reported (Younis & El-Tigani, 1970).

7.2 Material and methods

In experiments with rootless tuber-slices, growth regulators were applied as aqueous solutions or as talc mixtures. Aqueous solutions were applied by soaking de-eyed tuber-slices for 1 h. Talc mixtures were administered to the basal cut faces of tuber-slices (see Section 3.2). The treated slices were set in moist perlite with the apical side down; about 30 slices per treatment were used. Observations were made at regular intervals for 2 to 3 months after starting the experiment.

Rooted slices were treated after they were planted in soil. Growth regulators were applied as aqueous solutions to the tuber-slice. Small pieces of filter paper were put on the slices to absorb the solution, to wet the upper surface of the slice, and to prevent losses into the root medium. In each treatment 0.5 ml per slice was given. The treatments were repeated 3 to 5 times at intervals of 7 to 5 days. The treatments were stopped as soon as the first adventitious buds appeared in the control series.

The experiments were carried out in a glasshouse.

7.3 Experiments with non-rooted tuber-slices

Tuber-slices of the variety Bintje were treated with the cytokinins adenine, kinetin and benzyladenine at concentrations of 0.001, 0.01, 0.1 and 1% in talc. Although adenine is not considered a real cytokinin it has been proved to promote bud formation in tobacco-stem explants (Skoog & Tsui, 1948). Treatments with auxins have been described in Chapter 4. Gibberellic acid was applied in aqueous solutions of 0.01, 0.1, 1, 10 and 100 mg/l. In experiments with TIBA, slices were soaked in solutions of 1, 5, 20 and 80 mg/l.

None of the above growth-regulator treatments resulted in bud formation. Also in the control series no bud formation was observed. Auxins induced root formation and increased callus growth (Chapter 4). Cytokinins slightly promoted callus growth; sometimes gibberellic acid was also found to increase callus development. TIBA influenced the polarity of callus formation. At concentrations of 20 to 80 mg/l some callus at the apical surfaces was observed. In later experiments it was found that the

apical callus formation was increased if the treatment with TIBA was combined with a treatment with NAA. This result demonstrates the role of basipetal auxin transport in the polarity of callus formation.

7.4 Experiments with rooted tuber-slices

Rooted tuber-slices of the variety Bintje were treated with cytokinins, auxins, GA, TIBA, PCIB, B-9, CCC, and with the phenolic compounds DCP and caffeic acid. The results are presented in Table 5. A comparison of the controls of Exp. 1 and Exp. 2 showed that the rate of bud formation was higher in Exp. 2. This was presumably due to a higher glasshouse temperature during the second experiment.

Kinetin and PCIB significantly increased bud formation. Gibberellic acid and auxin, especially NAA at high concentration, inhibited it.

Additional effects of high concentrations of NAA were increased callus formation and thickening of the roots.

The mechanism of inhibition of bud formation by gibberellic acid was probably different from the mechanism of inhibition by auxins. In the GA-treated series, the buds remained much longer in a smooth nodule-like stage. When buds grew out, they were usually long and thin, and less differentiated than normal adventitious shoots. Possibly GA supressed bud formation by inhibiting differentiation of the meristems, whereas auxins inhibited in an earlier stage.

TIBA and caffeic acid inhibited bud formation significantly at 10^{-5} M; remarkably, however, the inhibiting effect seemed to be less at higher concentrations.

B-9, CCC, and DCP did not significantly influence bud formation.

Table 5. The effect of growth regulator treatments on bud formation in rooted tuber-slices of the variety Bintje. The figures represent percentages of slices with buds, obtained in 5 replicates of 10 slices. The data of Exp. 1 are from an observation 7 weeks after planting, of Exp. 2 5 weeks after planting. * and ** designate significantly different from control at $P = 0.05$ and $P = 0.01$, respectively.

	Growth-regulator	Concentration (M)					
		0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	2.5×10^{-4}
Exp. 1	Control	32					
	BA			32	34		26
	Kinetin			40	54*		42
	IAA			40	30	18	
	NAA			34	26	2**	
	B-9			44	32	40	
	CCC			34	42	30	
	PCIB			26	40	54*	
Exp. 2	Control	48					
	GA			32	14**	6**	
	TIBA		42	24*	36		
	DCP			38	30	38	
	Caffeic acid			24*	44	48	

Table 6. The effect of growth regulator treatments on bud formation in rooted tuber-slices of the variety Alpha. The data are from an observation 7 weeks after planting; each treatment comprised 5 replicates of 10 rooted slices.

Growth regulator treatment	Slices with buds (%)
Control	26
Kinetin, 10^{-4} M	38
B-9, 10^{-3} M	20
CCC, 10^{-3} M	30
PCIB, 10^{-3} M	24

7.5 Experiments with rooted tuber-slices of the variety Alpha

Rooted tuber-slices of the variety Alpha show a low frequency of bud formation compared with Bintje (Table 4). If this poor bud formation was due to a high auxin level, a high gibberellin level, or a low cytokinin level, it might be possible to improve bud formation by appropriate growth-regulator treatments. I tried to increase bud formation by application of kinetin, PCIB, B-9 and CCC. The results, presented in Table 6, show no statistically significant differences between treated and non-treated series. Kinetin tended to promote bud formation as in the Bintje variety, but PCIB treatment had no effect.

The results of these treatments did not provide any explanation for the differences between the varieties Alpha and Bintje.

7.6 Discussion

The experiments with non-rooted tuber-slieces of the variety Bintje have shown that the inability to form buds remained after growth regulators had been applied. Presumably this inability is not merely caused by simple imbalance of endogenous hormones. To prove this assumption, however, a very extensive study on growth-regulator effects is needed, which has to be combined with investigations of endogenous hormone levels. In this study another approach was chosen (chapters 8 and 9). Instead of studying the inability, I tried to account for the ability to form buds which has been found in rooted tuber-slices and in non-rooted tuber-pieces of some varieties.

The results of the growth-regulator treatments of rooted slices confirmed the widely accepted view that bud formation is inhibited by auxins and promoted by cytokinins. Slightly increased bud formation could be obtained by application of kinetin or the anti-auxin PCIB. Considerable promotion of bud formation was not observed, which might indicate that the endogenous hormone levels are optimum or near optimum. Strong inhibition of bud formation was only obtained by high concentrations of NAA and GA. The inhibitory effect of GA is in a agreement with the effects observed in

Begonia leaf cuttings (Heide, 1969) and in tobacco callus cultures (Murashige, 1964).

The main objective of the experiments described in this chapter was to gain information about the effects of growth regulators on the end result of bud formation, without going into detail about the mechanisms of action of the substances. Summarizing the results it may be stated that, with regard to the formation of adventitious buds, a high degree of autonomy exists in the potato. In the non-rooted tuber-slices this autonomy was expressed by the inability to form buds and by the ineffectiveness of growth-regulator treatments, whereas in the rooted slices the autonomy was brought about by a rather constant rate of spontaneous bud formation, which was inhibited by growth-regulator treatments only when high concentrations were used.

8 Influence of mineral nutrition on bud formation in rooted tuber-slices

8.1 Introduction

One of the main problems in the present study concerns the role of the root system in the initiation of adventitious buds. In an intact plant the roots provide the plant with water and minerals. Moreover the synthesis of specific organic substances, indispensable for the above-ground parts, occurs in the roots (see Chapter 9). In rooted tuber-slices the root system may have similar functions. At first sight an influence of mineral nutrient supply seems unlikely in this case, since the tuber tissue itself is rich enough in minerals to support the development of normal sprouts. It will be shown, however, that, if the roots are deprived of external mineral nutrients, bud formation does not occur.

This chapter describes experiments on the effect of the concentration of the nutrient solution, the effects of macronutrient deficiencies, and the influence of mineral nutrition in different stages of bud formation.

8.2 Material and methods

In the mineral nutrition experiments the water culture technique was used (Fig. 1 and Fig. 5). Buckets of black polyethylene were filled with nutrient solution, and covered with plates of polyvinylchloride. Rooted tuber-slices were set on the plates, in which a number of holes were made to let through the root bundles. Each bucket contained 13.5 l of nutrient solution, which was aerated by means of a glass tube. On each plate 12 to 20 slices were placed; each trial series mostly comprised three replicates.

In the first experiments (sections 5.1 and 8.3) a Hoagland's solution was used, containing KNO_3 5 mM, $Ca(NO_3)_2 \cdot 4H_2O$ 5 mM, KH_2PO_4 1 mM, and $MgSO_4 \cdot 7H_2O$ 1 mM. Later a nutrient solution composed by Steiner (1970) was applied. In routine experiments this solution was used in a dilution of 15% of its original strength (see Table 7: complete). Solutions deficient in macronutrients were prepared according to a recipe of Steiner (pers. commun.). This recipe was composed in such a way that the osmotic values of the deficient solutions and the complete solution were equal (Table 7). The initial pH of the deficient solutions was 6.2 ± 0.1. Mineral uptake by the roots only slightly changed the pH values, except in the solution deficient in nitrogen, which showed a considerable acidification, presumably due

Table 7. Composition of macronutrient deficient solutions.

Element omitted	Macronutrient ions (meq/l)					
	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	$H_2PO_4^-$	SO_4^{2-}
None(complete)	1.01	1.27	0.57	1.68	0.14	0.98
K		2.24	1.01	1.91	0.14	1.12
Ca	1.66		0.93	1.52	0.14	0.89
Mg	1.23	1.54		1.62	0.14	0.95
N	1.27	1.60	0.72		0.14	3.34
P	1.01	1.26	0.57	1.79		1.05
S	0.91	1.14	0.51	2.36	0.14	

to predominant cation uptake (see Hewitt, 1966). The pH in the N-deficient solution was adjusted at regular intervals by adding an appropriate amount of calcium hydroxide; in later nitrogen-deficiency experiments addition of an excess of calcium carbonate was found to be adequate.

In all nutrient solutions iron was supplied as ferric citrate at 12 mg/l. The other micronutrients were according to the A-Z solutions of Hoagland & Snyder (1933). When the concentration of the macronutrients was varied the concentration of the micronutrients was changed in the same proportion.

The nutrient solutions were not renewed during an experiment, unless stated otherwise.

8.3 Concentration

To investigate the effect of the strength of the nutrient solution different concentrations of a Hoagland's solution were tested. A pilot experiment showed that relatively low concentrations were sufficient for bud formation. In the present investigation concentrations ranging from 0 to 2/5 of the original strength were used. Contrary to the general method described before, the groups of slices were set on shallow vessels with 5.5 l of nutrient solution. Three replicates of 15 slices were used per concentration. The experiment was done in a glasshouse.

After six weeks the experiment was stopped, the percentage of slices with buds and the fresh weight of the roots were determined. The results presented in Fig. 14, show a clear relationship between the strength of the nutrient solution and both bud formation and root growth. In demineralized water, root growth was very poor and no bud formation occurred. At a concentration of 1/80 adventitious buds appeared. With increasing concentrations root growth and bud formation were promoted; a plateau was already reached at a concentration of 1/10. The parallelism in the behaviour of root growth and bud formation suggests that both processes have similar nutrient requirements. However other experiments, not reported in detail here, showed that relatively good root growth could be obtained in tap water or in demineralized water enriched with $CaCO_3$, while no bud formation was observed.

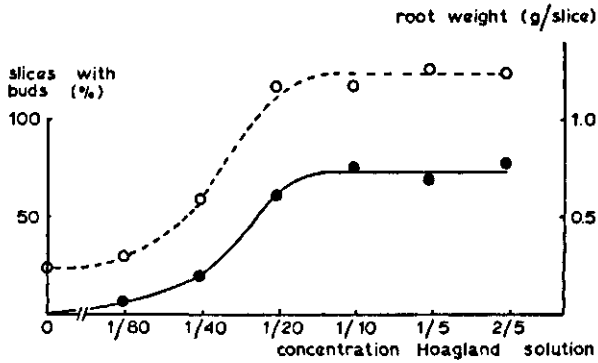


Fig. 14. Bud formation (—) and root growth (-----) at different concentrations of a nutrient solution.

In all series the rooted slices remained turgescent. When the roots were removed, the slices became flaccid within some days. This effect indicates that water evaporates out of the slices. In the intact rooted slices, the evaporated water will be replenished by the roots through some kind of transpiration stream. Whether mineral nutrients are transported by the roots to the tuber-slice has not been investigated.

8.4 Macronutrient deficiencies

The nutrient solution used in the previous experiment contained all elements which are supposed to be essential for plant growth. The question arises whether identical mineral requirements exist for the formation of adventitious buds. I tried to investigate this problem using deficient solutions. For practical reasons the investigation was confined to macronutrients only.

Roots of rooted tuber-slices were grown in solutions deficient in one of the macronutrients K, Ca, Mg, N, P, or S. The composition of the solutions has been described in Section 8.2. The experiment was carried out in a conditioned glasshouse at 20°C. After 5 weeks the experiment was stopped. The effects of mineral deficiencies on bud formation and root growth can be seen in Table 8.

It appears that mineral deficiencies can affect root growth as well as bud formation. Root growth was inhibited most strongly with Ca deficiency. Here the roots remained very short; they were brittle and mostly their growing points died. Also with N deficiency poor root growth occurred; in later experiments (Section 9.3), when the pH control was achieved by $CaCO_3$, instead of $Ca(OH)_2$, root growth was less inhibited. This result indicates that a part of the growth depression was caused by pH fluctuations. Slight inhibition of root growth occurred with P and S deficiency.

Bud formation did not occur with N deficiency, and was very poor with solutions deficient in K or Ca. A moderate inhibition was found with P deficiency; with solutions lacking Mg or S, bud formation was equal to the control. The inhibitive effects of solutions lacking K, Ca, or N were highly reproducible, whereas with P deficiency in later experiments nearly normal bud formation was found.

Table 8. The influence of macronutrient deficiencies on bud formation and root growth of rooted tuber-slices. Data 5 weeks after starting the treatment. Each figure represents 3 replicates of 20 slices; * and ** designate significantly different from complete at $P = 0.05$ and $P = 0.01$, respectively.

Element omitted	Slices with buds (%)	Fresh wt roots (g/20 slices)
None (complete)	58	55.9
K	8*	48.0
Ca	1.7**	13.7**
Mg	57	60.7
N	0**	13.8**
P	27*	43.7
S	57	42.7*

An apparent coincidence of inhibition of both root growth and bud formation only occurred with Ca deficiency and, in this experiment, with N deficiency. The effects of K deficiency showed that nearly normal root growth can coincide with inhibited bud formation.

To interpret the variation in root growth with different mineral deficiencies, two sources of minerals have to be taken into account: the root medium and the tuber-slice. For root growth a normal complement of nutrient elements will be required (Butcher & Street, 1964). That means that, if root growth is normal or nearly normal as in solutions lacking K, Mg, P, or S, the missing elements are transported from the tuber-slice to the roots. This transport is probably insufficient for Ca. It is well known that redistribution of Ca in the plant tissue is usually slight (Bollard & Butler, 1966). Moreover the response of the roots in the present experiment corresponded to the symptoms of Ca deficiency in roots of other plants (Baumeister, 1958).

To explain the effects of nutrient deficiencies on bud formation is rather difficult, since a decreased bud formation may be caused directly by diminished availability of the nutrient involved, or by the disturbance of some root function necessary for bud formation. The latter case will be more interesting, because it may provide some clue to specific bud-inducing processes. In that respect the effects of Ca deficiency are less attractive, as it has been demonstrated that Ca deficiency influences a wide range of processes including the transport of other ions (see Mengel, 1969). Most interesting for a further study are the effects of K deficiency, since here bud formation was suppressed without concomitant reduction of root growth (see sections 8.6 and 8.7).

The fact that rooted tuber-slices need minerals for their adventitious bud formation was established in experiments with the potato variety Bintje. It seems worthwhile to know whether these mineral requirements apply to other varieties. Of special interest are those varieties whose tuber pieces can form buds without preceding root formation. From Table 4, the Multa variety was chosen as an outstanding example of this group. Experiments to test the mineral requirements of rooted tuber-slices of Multa are described in Section 8.8.

8.5 The influence of mineral nutrition on bud initiation and bud extension

Rooted tuber-slices with the roots grown in soil or in nutrient solution develop visible buds two to three weeks after planting. In the period preceding the bud emergence, the initiation of a microscopic bud meristem must have taken place. This process of initiation is followed by a process of extension of the meristem to a visible bud, which in turn gives rise to a foliate shoot. Thus three phases can be distinguished (1) bud initiation, (2) bud extension, and (3) shoot growth. With regards to the influence of nutrients it is of interest to know whether the minerals are required for bud initiation, for bud extension, or for both phases of bud formation. I tried to investigate this question by giving mineral nutrients or demineralized water in different parts of the growth period. The growth period was divided into intervals of 2 weeks; the following treatments were used:

	0–2 wk	2–4 wk	4–6 wk	6–8 wk	8–10 wk
a.	minerals	minerals	minerals	minerals	minerals
b.	minerals	H_2O	H_2O	H_2O	H_2O
c.	H_2O	minerals	H_2O	H_2O	H_2O
d.	minerals	roots removed			

In series (a) the nutrient solution was renewed every 2 weeks. In series (b) and (c) the demineralized water was renewed every 2 weeks during the treatment with water. Preliminary trials had shown that in treatment (b) buds develop, when the roots are grown in water only. To investigate whether the presence of roots was necessary during bud extension, a series, (d) was added in which the roots were cut off at a distance of 1 cm from the slice tissue; to prevent drying out after root removal, the slices were set on moist sand in a humid atmosphere. The entire experiment was carried out in a growth chamber at 20 °C and a light intensity of 40 W/m^2.

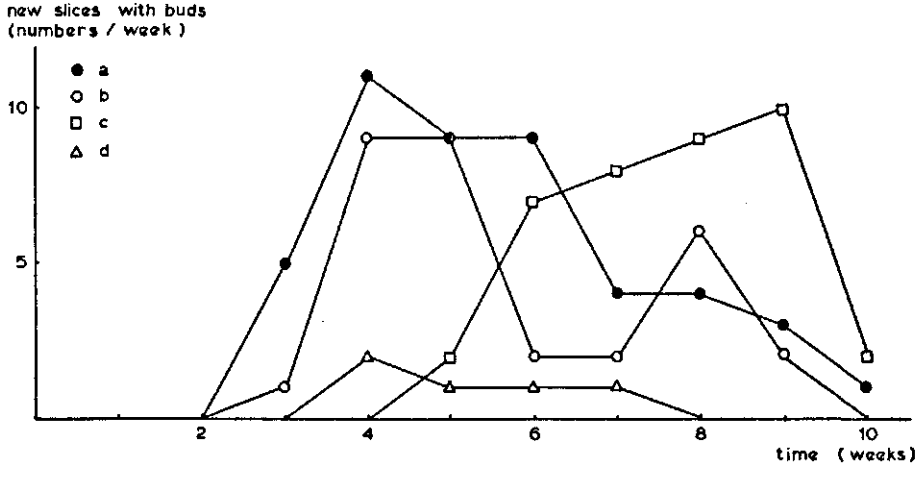


Fig. 15. Weekly bud formation of 3 × 16 slices, submitted to various root treatments. Explanation in the text.

The results of the experiment are given in Fig. 15, in which the numbers of newly developed buds during each week are plotted against time. A comparison of the curves of (b) and (c) shows that a pretreatment with water of two weeks, followed by mineral nutrition causes a delay of bud emergence of two weeks. Presumably bud initiation does not start when the roots are deprived of mineral nutrients from outside. The emergence of buds in (b) and (c) during the water treatment demonstrates that no absorption of minerals has to take place during bud extension. The well developed root system may have a reserve of mineral nutrients, which can influence growing out of the buds. When the results of (b) and (d) are compared, it can be seen that removal of the roots results in a slight delay of bud emergence and a considerable inhibition of total bud production. The presence of roots is not strictly required, but nevertheless is highly beneficial during bud extension. A mineral reserve in the roots may lengthen the period of bud initiation. This reserve may explain why bud formation continues for a long time after the mineral nutrient supply to the roots has stopped.

In later stages shoot growth is inhibited in series (b) and (c), and fully suppressed in series (d). This phenomenon, however, is outside the scope of this study.

To summarize, this experiment has demonstrated that the presence of mineral nutrients in the root medium is essential during bud initiation. After initiation, buds can grow out independent of external root nutrition.

8.6 The influence of potassium deficiency on bud initiation and bud extension

In the experiments on mineral deficiency (Section 8.4), the roots of the tuber-slices remained in the deficient solutions during the entire growth period. The previous experiment showed that the presence of mineral nutrients during the first two weeks was sufficient. From this result it seemed logical to conclude that the essential elements K, Ca, N, and P have to be present in this first period and that they play a role in bud initiation. However it is possible that a deficient solution inhibits bud extension, while water does not do so. In the case of potassium deficiency, metabolic disturbances may have taken place similar to those reported in literature. Sinclair (1969) demonstrated accumulation of putrescine in potassium-deficient barley plants. This substance, which appears to be very toxic, has also been found in other plant species suffering from potassium deficiency (Baumeister, 1958). High potassium levels have been shown to release axillary buds from inhibition in *Solanum sisymbrifolium* (Wakhloo, 1970). In view of these data it seemed worthwhile to investigate whether potassium deficiency affected the initiation or the extension of buds.

Rooted tuber-slices were grown for 2 weeks in either a K-free solution or a complete solution, and were transferred to a complete solution, to a K-free solution, or to demineralized water. The design of the experiment and the end results are presented in Table 9. The experiment was done in a conditioned glasshouse at 20 °C. The results of treatments (a) and (b) clearly showed that after an initiation phase with a complete solution, a K-free solution did not inhibit the extension of buds. If during the initiation phase roots were grown in a K-free solution, (c), (d) and (e), bud formation was inhibited,

Table 9. Effect of potassium deficiency on initiation and extension of buds. In each treatment 3 replicates of 13 slices were used.

	Mineral nutrition		Slices with buds (%)
	1st period, 2wk.	2nd period, 3wk.	
a.	Complete	Complete	41
b.	Complete	K-free	46
c.	K-free	K-free	8
d.	K-free	H_2O	5
e.	K-free	Complete	10

practically irrespective of the mineral nutrition afterwards.

From the above results I concluded that potassium has to be present in the root medium during bud initiation. Growing out into visible buds is not hindered by the absence of potassium in the root medium.

8.7 Site of action of potassium

Potassium in the root medium appears to influence the initiation of buds. The question arises whether this influence is a direct effect of the presence of K^+ at the site of bud formation, or an indirect effect of some potassium-requiring process in the roots. The adventitious buds mostly develop on or near the root base, that means very close to, or on the tuber-slice. It is well known that potato tubers are very rich in potassium (Schwimmer & Burr, 1967) and therefore it seemed unlikely that supply of potassium from the roots was directly involved in bud formation. In the following experiments I tried to trace the site of action of potassium.

Rooted tuber-slices, cultured in a K-free solution, were treated in four different ways (see Table 10). KCl solution was administered either to the tuber-slice at the site of bud formation (c), or to the nutrient solution (b). In the control series (a) no potassium was given; in a second control (d) NaCl solution was administered to the tuber-slice to test the influence of chloride at the site of bud formation. Treatments were given six times, at intervals of 2 to 3 days, during the first two weeks. Volumes and concentrations were chosen so that the total amount of potassium applied was approximately the amount usually present in the nutrient solutions. In each treatment 0.5 ml of water, KCl 200 meq/l, or NaCl 200 meq/l was administered per slice; the nutrient solution of series (b) received 8 ml KCl (200 meq/l) per treatment. In the slices a small hole was made in the central tissue adjacent to the root base. In these holes the solutions were dropped; a plug of glass-wool was put in each hole to maintain the solution for some time. To the water and the solutions administered to the slices 0.02% Tween-20 was added.

In a first experiment, carried out in a conditioned glasshouse at 20°C, only bud formation was studied. Each trial series consisted of 3 replicates of 16 rooted slices.

Table 10. The effects of KCl administered to the tuber-slice or to the nutrient solution. Bud formation data designated by the same letter are not significantly different at $P = 0.05$

Treatments		Exp. 1	Exp. 2	
tuber-slice	root medium	Slices with buds (%)	K^+ content (meq/kg)	
			nutrient solution	root tissue
a. H_2O	—	6 a	<0.01	29
b. H_2O	KCl	71 c	0.52	63
c. KCl	—	58 bc	<0.01	40
d. NaCl	—	21 ab	<0.01	30

Bud formation data, obtained after four weeks, are presented in Table 10. Bud formation was low at complete potassium deficiency (a), and high when potassium chloride was supplied to the root medium. However, considerable bud formation was also observed when the tuber-slices were treated with potassium chloride. Sodium chloride applied in the same way had only a small promotive effect. In later stages of bud development shoot growth was suppressed on the potassium deficient root media of series (a), (c) and (d) (see also Section 8.5).

The high frequency of bud formation, after administration of potassium chloride to the tuber-slices, suggests a direct effect of potassium at the site of bud formation. Before drawing this conclusion it is necessary to investigate any possible translocation to the roots. In a second experiment potassium levels in the roots of the four treatments were compared.

Experiment 2, a single replicate of experiment 1, was carried out in a growth chamber at 20 °C and continuous light of 22 W/m^2. After two weeks the roots were harvested and analysed for potassium content. The tissue was digested with nitric acid and perchloric acid. The amount of potassium was determined with an atomic absorption spectrometer. The nutrient solutions were analysed to investigate whether leakage of potassium from the roots had taken place.

The results, presented in Table 10, showed that the deficient solutions were practically free from potassium. Analysis of the roots showed that in a deficient solution the potassium content was about half of the control level. The somewhat higher amount in the roots of series (c) may be attributed to translocation of potassium applied to the slice. This result complicates the picture, since it seems difficult now to distinguish between potassium at the site of bud formation and potassium in the roots.

The above experiments have shown that: (1) potassium deficiency in the root medium leads to a low potassium content in the roots; (2) potassium in the root medium can largely be substituted by potassium applied to the tuber-slice at the root base; (3) bud formation is presumably stimulated directly by the potassium supplied.

8.8 Mineral requirements of the variety Multa

Rooted tuber-slices of the variety Bintje readily form adventitious buds whereas bud formation on rootless tuber pieces has not been observed up to now. In the Multa variety, however, non-rooted tuber pieces show a high bud regeneration capacity (Table 4). Possibly the roots of Bintje provide substances which are already present in tubers of Multa. Then it should be possible to initiate adventitious buds on rooted slices of the Multa variety independent of the root function. Previous experiments (sections 8.3 and 8.4) suggest that the root function in this respect largely depends on mineral nutrition from outside. In this section experiments are described to test the response of rooted tuber-slices of Multa to nutrient solutions without K, N, or P, and to demineralized water.

Table 11. Bud formation of rooted tuber-slices of the variety Multa, cultured in different mineral solutions.

	Mineral nutrition	Number of slices	Slices with buds (%)
Exp. 1.	Complete	3×12	33
	K-free	3×12	11
	N-free	3×12	0
Exp. 2.	Complete	2×8	75
	P-free	2×9	67
Exp. 3.	Complete	15	47
	H_2O	15	0

Tuber-slices were rooted with 1% IAA in talc. Root formation appeared to be very poor compared with the variety Bintje (see also Table 4). Consequently only small numbers of rooted slices were available and more experiments had to be done (Table 11). Experiment 1 was carried out in a conditioned glasshouse, the experiments 2 and 3 in a growth chamber at a 20/15 °C day-night regime, a daylength of 14 h, and a light intensity of 20 W/m^2.

The data of the observations after 5 weeks are presented in Table 11. The results show a general similarity to the results of experiments with the Bintje variety. It can be concluded, therefore, that bud formation on rooted slices of both varieties Bintje and Multa is dependent on mineral nutrients in the root medium.

8.9 Discussion

The experiments described in this chapter have shown that bud formation on rooted tuber-slices depends on mineral nutrients in the root medium. These nutrients have to be present during the first two weeks. A relatively low nutrient concentration is suffi-

cient. Macronutrients essential for bud formation are K, Ca, N and to some degree P; Mg and S can be omitted in the nutrient solution. The influence of micronutrients has not been investigated.

Mineral nutrition affected root development. Root growth was very poor in demineralized water and in very low nutrient concentrations. Also in calcium deficient solutions root growth was inhibited. During the rooting phase of tuber-slices in moist perlite usually good root development was observed. The perlite was moistened with tap water which contained mainly calcium, magnesium, and sulphate. In water-culture experiments rather good root growth was observed on a solution of $CaCO_3$ and also on tap water. These data indicate that the poor root growth on demineralized water is primarily caused by lack of calcium.

What is the relationship between root growth and adventitious bud production? A positive correlation was found with respect to the effect of the nutrient concentrations and calcium deficiency. On the other hand with potassium deficiency and, in some experiments, with nitrogen deficiency good root growth was found together with suppressed bud formation. Bud formation was never observed in experiments with tap water or $CaCO_3$ solution, or during the rooting phase in moist perlite. Obviously no simple quantitative relationship exists between root growth and bud production. A well developed root system may be prerequisite for bud formation but it is apparently not the only factor.

The precise role of mineral nutrition factors in the bud formation process cannot yet be determined, since insufficient experimental data are available. Whether the mineral nutrients are directly necessary at the site of bud formation or for some intermediary process in the roots is not yet known. Some evidence for a direct influence of potassium was obtained. Other elements like nitrogen and phosphorus may also work in a direct way at the site of bud formation. It may be assumed that certain nutritive requirements exist in the tissue where the bud meristems are formed. Deficiency in the root medium can lead to decreased availability of mineral nutrients at the site of bud formation by: (1) insufficient supply of minerals *from* the roots, and/or (2) export of minerals *to* the roots. The latter situation may be of importance when in a mineral deficient solution root development is not hindered. Mineral nutrients will be necessary to support root growth. Nutrients not present in the root medium will be delivered by the tuber-slice; they may partly be withdrawn from the tissue near the root base, which is the site where most adventitious buds are formed.

Both possibilities mentioned above concern a direct influence of mineral nutrition. In both cases a too low nutritional level is considered to limit bud formation. It seems unlikely that the mineral salts function as bud inducing factors. This fact may be deduced from the negative response of tuber explants cultured aseptically on media containing mineral nutrients (cf. Claver, 1967) Mineral nutrients may have an indirect influence, for example, if bud induction depends on some specific process in the roots which is disturbed by certain mineral deficiencies. In this respect synthesis of cytokinins in the root system deserves attention (see Chapter 9).

From the results of the experiments described in this chapter no conclusive inter-

pretations could be made about the role of mineral nutrients in bud formation. The system of rooted tuber-slices is too complicated, since it is very difficult to distinguish mineral effects in the root system from mineral effects in the bud-forming tissue. A better method of studying the influence of mineral nutrition on bod formation was found when it was discovered that aseptically cultured tuber explants of the variety Multa could regenerate buds quite easily. Studies of the mineral requirements of bud formation with this method are described in Chapter 10.

9 Relationship between bud formation and endogenous cytokinins

9.1 Introduction

In Chapter 8 the biogenesis of cytokinins was mentioned as a possible contribution of the root system to the initiation of buds. The existance of cytokinin-like substances was already suggested by Went (1938) and by Chibnall (1939). Went's experiments with etiolated pea seedlings showed that a root system was indispensible for shoot growth and for the release of axillary buds. Went assumed, without direct proof however, the involvement of some specific substance from the roots. This substance was called 'caulocaline', denoting that it is a requisite for stem growth. In the same paper Went (1938) considered caulocaline to play a role in adventitious bud formation on leaf cuttings; this view was based on an extensive study of Hagemann (1932), who showed that in almost all plant species whose leaves were able to regenerate buds, bud formation was preceded by root development. Chibnall's (1939) work concerned protein metabolism in leaves. Leaves which were detached from the parent plants showed a sharp increase in breakdown of protein and chlorophyll. Chibnall (1939) suggested that protein metabolism was regulated by some hormonal influence from the roots. This suggestion was supported by the observation that protein breakdown was retarded or stopped when the isolated leaves developed roots (Chibnall, 1954; Mothes, 1956).

New evidence for the root-hormone hypotheses was obtained after the discovery of a new group of growth regulators: the cytokinins (see review articles by Miller, 1961; Mothes, 1966; Conrad, 1967; Letham, 1967; Skoog & Armstrong, 1970). The research on cytokinins started with the discovery of kinetin, a synthetic compound which appeared to be very active in different plant physiological processes. The supposed properties of the hypothetical root hormone were largely covered by activities of kinetin and related compounds (cf. Miller, 1961). Richmond & Lang (1957) demonstrated that yellowing and protein degradation in detached leaves without roots could be delayed if the petioles were inserted in a solution of kinetin. Similar kinetin effects were shown in extensive studies by Mothes and co-workers (see Mothes, 1960). Kulaeva (1962) demonstrated that yellowing of detached tobacco leaves was retarded by root exudate from tobacco plants; kinetin at a concentration of 0.1 to 0.5 mg/l produced a similar effect. Cytokinin activity appeared to occur in root exudates and root extracts of various plant species e.g. *Helianthus annuus* (Kende, 1964; 1965), *Vitis vinifera* (Loeffler & Van Overbeek, 1964), *Phaseolus vulgaris* (Seth & Wareing, 1965), *Oryza sativa* (Yoshida et al. 1971) and other species (see Conrad,

1967). Within the root, the root tips appeared to contain much more cytokinin than the older parts (Weiss & Vaadia, 1965; Short & Torrey, 1972). The root tip, therefore, may be the site of cytokinin synthesis. Roots of decapitated plants produced cytokinin-containing sap for at least four days (Kende, 1965) which indicates that cytokinin synthesis in the root is largely independent of the other parts of the plant. The root system, however, has not been proven to be the only source of cytokinin; high activities in coconut milk, developing fruits, immature and germinating seeds (Letham, 1967; Conrad, 1967) may suggest synthesis in other tissues as well.

Most demonstrations of natural cytokinins involve the detection of unknown substances which exhibit kinetin-like activity in bioassays. In recent years purification and identification of cytokinins from immature maize kernels has been successful (Letham, 1967; Skoog & Armstrong, 1970). The main compounds were identified as 6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purine, (which was called zeatin), its riboside and its ribotide. Chromatographic data and other characteristics of fractions with cytokinin activity in root exudates indicate the presence of at least zeatin and its derivatives (Klämbt, 1968; Yoshida et al. 1971).

It must be emphasized that, in addition to the synthesis of cytokinins the roots may play a role in the synthesis or conversion of gibberellins (cf. Crozier & Reid, 1971) and in the synthesis of auxin (see Åberg, 1957). Also synthesis in the roots of non-hormonal substances like vitamins (see Torrey, 1965) and amino acids (Ivanko, 1971) may have some significance for the induction of buds. In this chapter, attention is confined to cytokinins because of the requirement of high levels of this growth regulator in the process of bud formation. In rooted tuber-slices one can imagine that cytokinins move upward in the roots by the transpiration stream (see Section 8.3) and induce bud formation in the upper parts of the roots, at the root base, or on tuber tissue near the root base.

Cytokinin activity in root extracts was estimated with the soya callus assay. Preliminary experiments showed positive results. I tried to find out whether cytokinin levels varied with capacity for bud formation by comparing the two varieties Bintje and Alpha, that differed in bud formation on rooted slices, and by comparing groups of rooted slices with different mineral nutrition.

9.2 Material and Methods

The significance of cytokinins from the roots should preferably be investigated by determining cytokinin activity in root exudates. Roots excised from tuber-slices produced little exudate, especially if the roots had certain mineral deficiencies. Therefore root extractions were made to obtain cytokinins.

Roots to be extracted were obtained from rooted slices grown in water culture. Mostly the roots were harvested about 14 days after placing them in the nutrient solution; that was before the emergence of the first adventitious buds. The roots were cut off at 1 cm from the tuber slice, washed, blotted with tissue paper, weighed, and stored at —20 °C until extraction.

The extraction and purification procedures were similar to the methods described by Letham (1963) and Dekhuijzen & Staples (1968). Tissue of a fresh weight of for instance 100 g was blended with 400 ml 96% ethanol. The resulting brei was stirred at room temperature for 1 h, and filtered. The residue was extracted in 200 ml 96% ethanol at 4 °C for 24 h; after filtration the filter was washed with 200 ml 96% ethanol. The filtrates were combined and the ethanol evaporated in a rotary film evaporator *in vacuo* in a water bath at 40 °C. The remaining aqueous solution was made up to 50 ml. To remove chlorophyll and other non-soluble substances, the solution was centrifuged at 4 °C with 34000 × *g* for 30 min. The supernatant was made up to 100 ml with water. It contained the ethanol extractable substances which were soluble in water and was called fraction W. This fraction could be examined directly in the cytokinin bioassay. Mostly, however, a further purification was necessary. The fraction W was extracted at pH 3 with ethyl acetate (3 × 100 ml) to remove possible inhibitors (Letham, 1963), gibberellins and auxins. The acid ethyl acetate fraction was discarded, the remaining fraction was extracted at pH 8 with *n*-butanol (6 × 50 ml). The basic butanol soluble fraction (B) was supposed to contain the cytokinins. The butanol was evaporated in a rotary film evaporator *in vacuo* in a water bath at 50 °C. If the fraction B was used directly in the soya callus assay butanol remnants in the residue had to be removed thoroughly since concentrations of *n*-butanol from as low as 0.01% inhibited callus growth. For this purpose the residue was dissolved in 50% ethanol which was evaporated together with the butanol remnants. Then the entire residue was dissolved in water, which was acidified to pH 4 if necessary to dissolve insoluble substances.

Fractions W or B were purified further by paper chromatography. A solution equivalent to a certain amount of tissue was streaked on Whatman 3MM paper, and developed in *n*-butanol/ammonia 25% (4:1, v/v). The chromatograms were dried and slightly sprayed with water to remove remaining butanol. The paper was divided into transverse strips of 0.1 Rf each. These strips were cut into longitudinal pieces equivalent to a certain amount of tissue. The chromatogram pieces were added directly into the callus culture medium before autoclaving.

Cytokinin activity was determined with the soya callus assay as described by Miller (1963). The assay medium contained mineral salts (according to Miller, 1965), 30 g sucrose, 10 g agar 100 mg *meso*-inositol, 0.5 mg nicotinic acid, 0.1 mg pyridoxine · HCl, 0.1 mg thiamine · HCl, and 2 mg NAA per litre. Stock cultures were grown on this medium, supplemented with 0.5 mg kinetin per litre. The media were sterilized by autoclaving at 120 °C for 15 min. Callus was cultured in tubes containing 10 ml, or in Erlenmeyer flasks containing 40 ml of medium. One piece of callus was planted per tube, 3 pieces were planted per flask. The cultures were maintained at 27 °C under continuous fluorescent light of about 0.5 W/m^2.

Different strains of soya callus were used. One strain (A) was several years old. Callus of this strain was soft and deprived of chlorophyll; it exhibited no growth on media without cytokinin and moderate growth on kinetin containing media. Strain A appeared to be relatively sensative to impurities in the medium. It showed growth

inhibition on media containing fractions W or B of plant extracts (cf. Dekhuijzen & Staples, 1968). It was used therefore only for cytokinin determination of paper chromatogram fractions. To test cytokinin activity in fractions W and B new callus strains (B, C) were started from explants of cotyledons of germinated soya (Miller, 1963). Callus from young strains usually showed good growth on media containing extract fractions W and B. Although young callus appeared to be more sensitive to kinetin, it also showed some growth on media without cytokinin. In the beginning the new cultures formed hard irregular green callus. After ten to twenty transfers the new strains lost most of their characteristics and became similar to Strain A.

9.3 Cytokinin activity in root extracts of the varieties Alpha and Bintje

Varietal differences in bud-formation capacity of rooted slices (see Table 4) may be caused by differences in cytokinin production in the roots. To investigate this possibility, two varieties Alpha and Bintje were chosen, which have shown low and high bud-formation capacity respectively.

Rooted slices of the two varieties were grown in water culture in a conditioned glasshouse at 20 °C. Seven replicates of 16 slices were used per variety. After 16 days three replicates were harvested for cytokinin assay, the rest was maintained to determine bud-formation frequencies.

The results are presented in Table 12. Bud formation frequency of Bintje was twice that of Alpha. Cytokinin activity could be detected in root extracts of both varieties. The varietal differences were very small and not significant ($P = 0.05$). Cytokinin activity in root extracts of Bintje may be higher than in Alpha, but the amount of root tissue in Alpha was greater.

It can be concluded that the above varietal differences in bud formation are not associated with differential levels in cytokinin activity in extracts of the roots.

Table 12. Bud formation, root growth, and cytokinin activity in roots of the varieties Alpha and Bintje. Cytokinin activity was determined with callus strain B, using fraction B of the ethanolic root extracts at a concentration equivalent to 8 gram tissue per flask (40 ml medium); each value is the mean of six replicates. Data designated by the same letter are not significantly different at $P = 0.05$.

	Slices with buds (%), after 5 wk.	Roots harvested after 16 days	
		root weight (g/16 slices)	soya callus growth (mg/40 ml medium)
Alpha	33	62a	510b
Bintje	67	34b	595b
Kinetin 0 M			97a
Kinetin 10^{-7} M			864c

9.4 The effect of mineral nutrition on cytokinin activity in the roots

Cytokinin production in roots can be influenced by mineral nutrition factors. Experiments by Wagner (1970) and Wagner & Michael (1969, 1971) demonstrated that nitrogen deficiency in *Helianthus annuus* resulted in a decrease of the cytokinin level in root extracts and root exudates. In experiments described in Chapter 8 some types of mineral deficiency decreased bud formation on rooted tuber-slices. It seemed worthwhile to investigate whether the minerals acted through an effect on cytokinin production in the roots.

Rooted slices were grown in various mineral solutions in a growth chamber at 20 °C and under continuous light of 20 W/m^2. The roots were harvested and extracted according to the standard procedure. First, I studied the influence of macronutrient deficient solutions, similar to those used in Section 8.4. Two replicates of 16 rooted slices each were grown in most of the solutions, in Ca-free and N-free solutions four replicates were grown. The roots were harvested after 14 days of culture. Secondly, the influence of a total lack of nutrients was investigated. Root development in demineralized water is very poor (Fig. 14). In order to obtain enough root tissue rooted slices were grown first in a complete nutrient solution for 2 weeks. Then one group was transferred to a new complete solution, another group to demineralized water. Each group consisted of three replicates of 16 slices. The roots were harvested after a total culture period of 3 weeks. Cytokinin activity was tested in both fractions W and

Table 13. Cytokinin activity in extracts of roots grown in various mineral solutions. Soya callus media were enriched with an amount of extract equivalent to 0.1 gram tissue per tube (10 ml medium). Callus strain C was used in this trial. Each value is the mean of 4 tubes for the extract assays, and 6 tubes for the kinetin assays; * denotes significantly different ($P = 0.05$) from complete within the fraction. Other statistical data are mentioned in the text.

Mineral nutrition of the roots	Root weight (g) per 16 slices	Soya callus weight (mg/10 ml medium)	
		fraction W	fraction B
Complete	28.5	227	178
Without K	22.3	185	141
Without Ca	8.0	88*	137
Without Mg	25.0	152*	150
Without N	20.9	112*	155
Without P	22.8	155	181
Without S	21.8	161	212
Compl. → Compl.	34.1	192	250
Compl. → H_2O	26.4	167	165
Kinetin 0 M		117	
Kinetin 10^{-8} M		172	
Kinetin 10^{-7} M		609	

B of the ethanolic root extracts.

The results are presented in Table 13. Before discussing the data it must be noted that the soya callus assay was not reliable enough to prove small differences in cytokinin content. This unreliability is mainly caused by variability in growth response of different callus explants. It was also found that the variation in series containing plant extract was higher than in the kinetin series. When the results of the different mineral treatments were considered the soya callus weight was found to be higher on media containing root extract than on the control medium (i.e. kinetin 0 M). Only on the W fractions of the Ca-free and N-free treatments were lower callus weights observed, which were concomitant with a darker colour of the tissues. Callus growth on the B fractions of Ca-free and N-free treatments was significantly ($P = 0.05$) better, which may indicate that inhibiting substances are removed by purification of the fraction W. The data suggest that cytokinin activity is present in root extracts of all series, although statistical analysis showed that the values of Ca-free and Mg-free treatments were not significantly higher than the control at $P = 0.05$. The main purpose of this experiment was to trace possible differences in cytokinin level between non-deficient roots and deficient roots. A comparison of the callus weights showed that only within the W fractions did significant differences exist between the treatment with the complete solution and those treatments with solutions lacking Ca, Mg, and N. In the B fractions no significant differences could be detected.

Since possible differences in cytokinin activity may be masked by the presence of inhibiting substances further purification by paper chromatography was done. The most promising series for further research are probably the N-free treatment and the water treatment, since here bud formation was inhibited most severely. Paper chromatograms were made of the W fractions of the two series and of their respective controls which had received a complete solution. The fraction W was used and not the butanol fraction since a part of the cytokinin activity may be represented in the nucleotide form which is less soluble in butanol than in water (Miller, 1965).

Callus weights obtained on the chromatogram fractions are presented in Fig. 16. The differences between treatments with N-free and complete solutions were more pronounced than in tests of the crude extracts. The peak of complete at Rf 0.5–0.6 is significantly different ($P = 0.05$) from the corresponding peak of the extract of roots grown in N-free solution. The lower cytokinin activity in nitrogen deficient roots is in agreement with the results of Wagner (1970) and Wagner & Michael (1969, 1971). A comparison of treatment with a complete solution and water treatment shows significantly ($P = 0.05$) higher activity in the extract of the non-deficient roots at Rf 0.6–0.7. The histograms of N-free and water treatments show about the same low cytokinin activity.

Although it was outside the scope of the investigations to identify the substances with cytokinin activity, Rf values may yield some indications. The activity at Rf 0.5–0.7 is presumably due to zeatin, to its riboside, or to both substances; the peak at Rf 0.1–0.2 (Fig. 16 B) may be due to zeatin ribotide (cf. Miller, 1965; Dekhuijzen & Overeem, 1971).

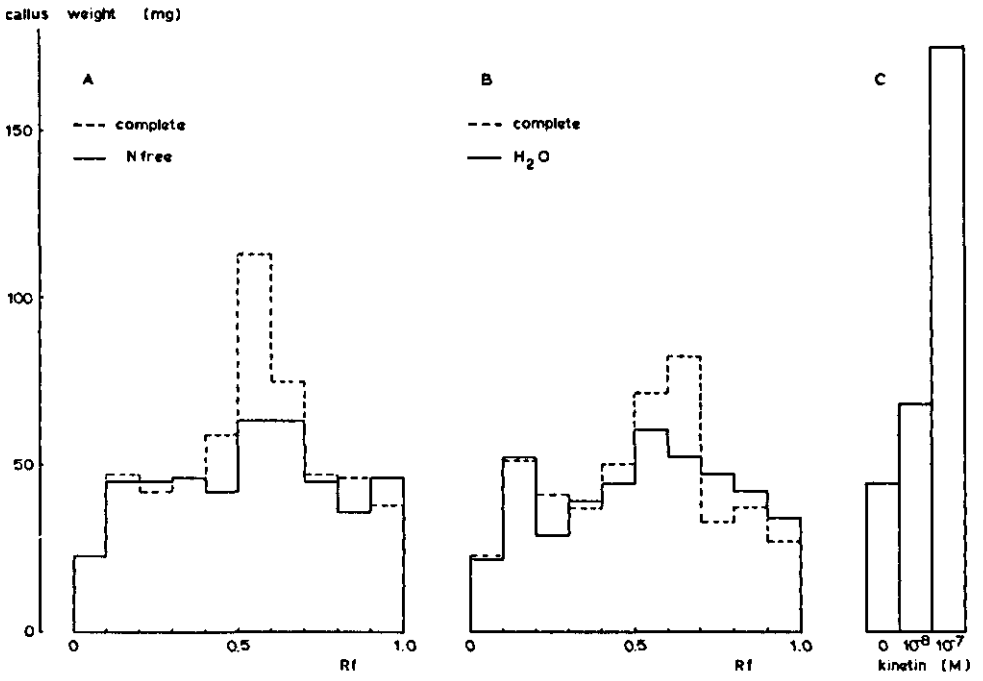


Fig. 16. Cytokinin activity in chromatogram fractions of different root extracts. Paper chromatograms were developed with *n*-butanol/ammonia 25% (4:1, v/v). The bioassay was carried out in 4 replicates. Each callus weight represents 2.5 grams of root tissue.

In summary, it can be concluded that cytokinin activity could be demonstrated qualitatively in extracts of roots from different mineral solutions. Quantitative differences which correlated with bud-formation capacity were hard to trace, when crude extracts were examined. After chromatography, it was possible to detect decreased cytokinin activity at nitrogen deficiency, and to some extent at total mineral deficiency.

9.5 Discussion

The role of cytokinins in the process of bud initiation has been well established (literature see Section 7.1). In the natural situation cytokinins seem to be produced especially by the roots of a plant. These two facts, added to the observation that the presence of a root system facilitates the formation of adventitious buds in potato tuber-slices, led to the hypothesis that bud formation on this material was induced by root-born cytokinins. Attempts to test this hypothesis by looking for a relationship between cytokinin levels in extracts of the roots and potential bud production did not yield conclusive results. First, cytokinin activity could not be shown to be responsible for differences in bud-formation frequency of the two varieties Alpha and Bintje. Secondly, in investigations of roots from different mineral treatments cytokinin activity was detected in all treatments even with nitrogen deficiency

and with deficiencies of potassium and calcium. Thus the presence of cytokinins does not imply that bud formation will take place. Differences in bud-formation frequency may be caused by differences in cytokinin concentration. It seemed rather difficult, however, to obtain reliable quantitative data. The soya callus assay exhibited great variability. Moreover impurities in the test solutions increased the variability, and appeared to inhibit callus growth, which masked to some extent the actual cytokinin activity (cf. Dekhuijzen & Staples, 1968). Cytokinin tests of paper-chromatogram fractions, which were applied to a small number of samples, yielded better results than testing crude extracts or butanol fractions. It could be demonstrated that, especially with nitrogen deficiency, cytokinin activity was decreased when compared with a complete nutrient solution. Whether the lower cytokinin level is the primary cause of the absence of adventitious buds in this case remains an open question. Common nutritive factors may limit bud formation (see discussion 8.9). Also the action of inhibiting substances deserves attention. In ethanolic extracts of roots from a Ca-free solution and a N-free solution the presence of factors inhibiting soya callus growth could be demonstrated. Whether such inhibiting substances play a role in bud formation is not known.

The investigations of the role of cytokinin should be improved by using better techniques for isolation and determination. Such experiments were not undertaken, since a more direct approach to the problem of root functioning proved to yield more conclusive results (Chapter 10).

10 Bud formation on tuber tissue in sterile culture

10.1 Introduction

Some essential questions about the influence of the root system on bud formation are still unsolved. First, the precise role of mineral nutrients is not yet clear. Secondly, an influence of cytokinins from the roots could not be proved. A basic difficulty in the investigations was the complexity of the subject i.e. the rooted tuber-slices. It was impossible to separate the different root functions; also influences of the tuber-slice and influences of the roots could not be analysed separately. Less complicated subjects would be sterile cultures of isolated roots or isolated tuber tissue. Up to now, however, attempts to induce bud formation in potato roots in sterile culture have been unsuccessful (Bajaj & Dionne, 1968). Tuber explants are more promising subjects, although bud formation, so far reported, occurred at low frequency (for literature see Section 2.4). In this chapter it will be seen that the potentiality for bud formation of tuber tissue in sterile culture depends strongly on variety. Tuber tissue of the variety Bintje did not produce buds, whereas tuber explants of the variety Multa developed buds very easily These fiindings provided a new approach to the questions mentioned above.

Experiments with tissue explants of Multa tubers were performed to study the influence of mineral nutrients directly supplied by the culture medium. In addition the influence of carbohydrate nutrition was investigated.

Experiments with Bintje tuber tissues were undertaken with the purpose of initiating buds by adding possible missing factors to the culture medium. The effect of growth regulators was tested. When these substances failed, attempts were made to introduce unknown bud-forming substances which may be present in roots of rooted slices or in tuber tissue of the Multa variety.

Finally, I tried to relate varietal differences in bud-regeneration capacity to differences in auxin sensitivity.

10.2 Material and methods

The experiments were carried out with common sterile culture techniques (see Gautheret, 1959; White, 1963; Butenko, 1968).

Preliminary experiments demonstrated that bud formation was inhibited in tissues from dormant tubers. Explants including tissue from the vascular ring showed better bud regeneration than explants from the central medulla tissue. The explants had to be placed with the apical side in the culture medium; in the reversed position hardly

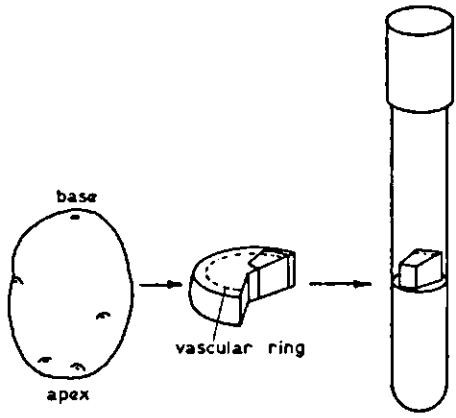


Fig. 17. Scheme of the tissue-culture technique.

any buds were formed. These results, combined with data from literature led to the following standard procedure.

Non-dormant tubers were surface sterilized by placing them in 80% ethanol for 2 min, followed by an immersion in a solution containing 2% active chlorine, for 1 h. According to the scheme presented in Fig. 17, a transverse slice, 1 cm thick, was cut out. From this slice the peel was removed, which reduced the chance of infection. Tissue fragments, including a part of the vascular ring and weighing about 0.8 g, were excised. Care was taken that no tissue from the region of the eyes was included in order to obtain anatomically uniform material. The explants were transferred to culture tubes and placed with the apical cut face into the medium. Contaminations amounted to about 10% of the cultures; most infections originated from the tissues. From one tuber more tissue explants could be excised; within each treatment, however, no more than one piece from the same tuber was used.

The culture tubes contained 10 ml nutrient solution. The standard composition was (mg/l): KCl, 380; KH_2PO_4, 122; NH_4NO_3, 480; $CaSO_4 \cdot 2H_2O$, 689; $MgSO_4 \cdot 7H_2O$, 483; NaFeEDTA, 25; $MnSO_4 \cdot 4H_2O$, 6.5; $ZnSO_4 \cdot 7H_2O$, 2.7; H_3BO_3, 1.6; KI, 0.75; nicotinic acid 0.5; pyridoxine · HCl, 0.5; thiamine · HCl. 0.1; *meso*-inositol, 100; sucrose, 20000; and agar, 10000. The amounts of macronutrient elements were approximately those concentrations recommended for water cultures by Steiner (1970). The salts were chosen so that solutions deficient in potassium, nitrogen, or phosphate could be made easily without important changes in the concentrations of the remaining essential elements. A potassium deficient solution was made by omitting KCl, and replacing KH_2PO_4 by an equivalent amount of $NH_4H_2PO_4$, causing only a negligible shift in the NH_4^+ concentration. A nitrogen deficient solution was obtained simply by omitting NH_4NO_3. Phosphate deficiency was accomplished by replacing KH_2PO_4 by an equivalent amount of KCl. Concentration of the elements K, N, and P could be varied by similar measures. The media were autoclaved at 120°C for 15 min. The pH was adjusted to 5.8 before autoclaving. Growth regulators and plant extracts were added to the media before autoclaving.

Trial series comprised 24 or 36 tubes. During the culture period the numbers could be lowered by contaminations. The tissue cultures were kept in a dark room at 20 °C. Weekly observations were made in daylight. Bud formation was evaluated as the percentage of explants with one or more buds.

10.3 Varietal differences

The experiments of Chapter 6 have clearly shown great varietal differences in the bud-regeneration capacity of de-eyed tuber pieces which were kept in moist sand. In the following experiment regeneration of tuber tissue was examined in sterile conditions on a nutrient medium.

Tissue explants of tubers of the varieties Bintje and Multa were cultured on standard medium. After 4 weeks the first buds appeared on the Multa tissues. Bud formation data after 7 weeks, presented in Table 14, showed that most Multa tissues produced buds while Bintje explants did not (see also Fig. 18). In both varieties callus development was observed at the morphologically basal surfaces. In Bintje explants, callus

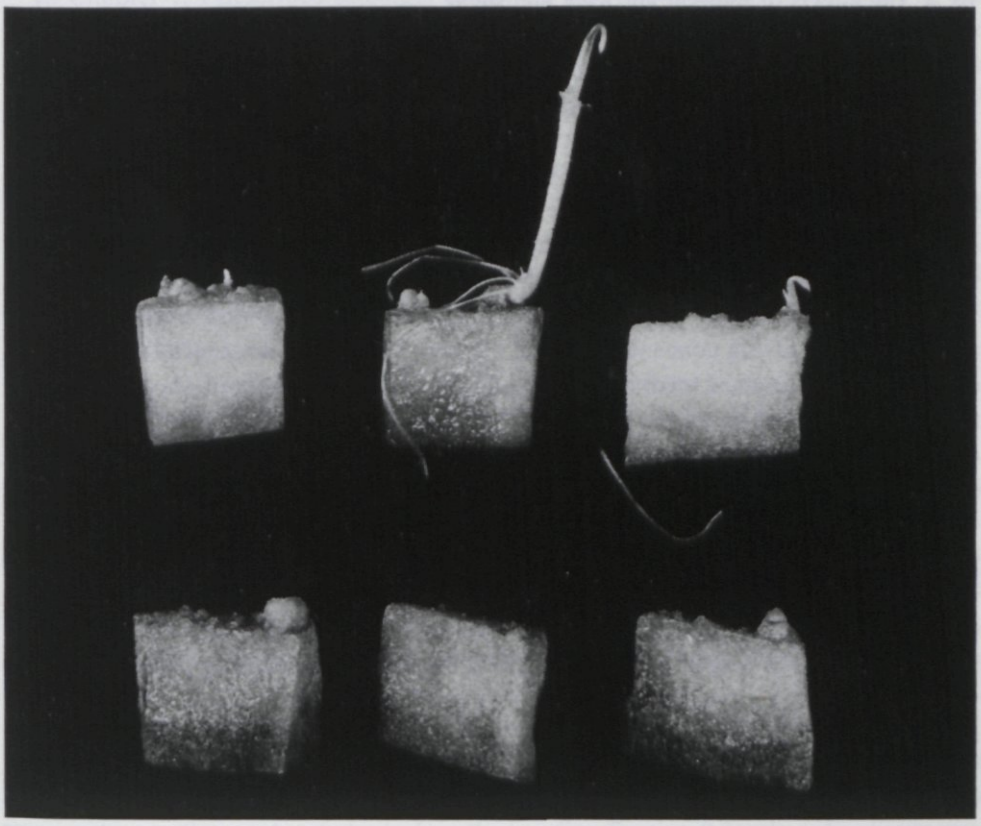


Fig. 18. Tuber explants of Multa (upper row) and Bintje (lower row) showing formation of callus and adventitious buds.

Table 14. Bud formation on tuber explants of the Bintje and Multa variety.

	Number of explants	Explants with buds (%)
Bintje	30	0
Multa	31	72

was situated mainly on the vacular ring, while in tuber pieces of Multa callus proliferations were distributed all over the surface. Also nearly all buds appeared at the basal surfaces. The buds frequently arose from callus proliferations and were also spread all over the surface.

When the basipetal polarity in the formation of buds and callus, and the varietal differences in bud formation are taken into account, the above data are consistent with the phenomena observed on de-eyed tuber pieces in moist sand.

10.4 The effects of mineral nutrition and sugar

Rooted slices of Bintje and Multa tubers only form adventitious buds when the roots are supplied with mineral nutrients (Chapter 8). It is not yet known whether the minerals exert their action in a direct way at the site of bud formation or in an indirect way by influencing the synthesis of bud-initiating substances in the roots. Rootless tuber-pieces of Multa, cultured in sterile conditions, easily form buds on a medium containing minerals, sucrose, and minor organic additives. The influence of mineral nutrition on this type of bud formation was studied in the following experiments.

In a first experiment the influence of potassium, nitrogen, and phosphorus was investigated by giving concentrations of 1/3 and 3 times the concentrations of these elements in the standard medium. The results, not presented in detail, showed that bud formation occurred on all media at a high frequency; 81 to 94% of the explants produced buds after seven weeks. Thus at concentrations of 1/3 the media contained enough of the elements to inititate buds.

In a second experiment solutions completely deficient in potassium, nitrogen, or phosphorus were tested. To examine the effect of carbohydrate supply, one trial series was added in which sucrose was omitted in the medium. The results of two observation times are presented in Table 15. Potassium deficiency did not impede bud formation. With nitrogen or phosphorus deficiency the percentage of regenerating tissues tended to be lower; the differences with the complete medium were relatively small however.

Sucrose deficiency also slightly inhibited bud formation.

From these data it can be concluded that bud formation on isolated tuber explants of the variety Multa is independent of potassium supply and largely independent of the supply of nitrogen, phosphorus and sucrose.

Table 15. Bud formation on Multa tuber explants grown on media of various deficiencies.

Medium	Number of explants	Expl. with buds (%)	
		5 wk.	8 wk.
Complete	31	35	72
Without K	34	56	73
Without N	36	11	58
Without P	34	35	50
Without sucrose	32	25	56

It was also noticed that after some time sprouts growing on tuber explants developed stolons and tubers. This tuberization, however, was only observed in the K and N deficient treatments.

10.5 The effects of applied growth regulators

In Chapter 7, I described attempts to initiate bud formation in tuber tissue of the variety Bintje by the application of growth regulators. These attempts failed. The sterile culture technique, however, may provide a better method. In pilot experiments the effects of growth regulators were tested on the spontaneous bud formation of Multa explants. This enabled an approximation of the required concentrations.

Experiments with Bintje tuber explants were carried out with three growth regulators at various concentrations, namely NAA, 10^{-7} and 10^{-6} M; zeatin, 10^{-7} and 10^{-6} M; a combination of NAA 10^{-7} M and zeatin 10^{-6} M; GA, 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M. Each trial series consisted of 24 tubes. The tissue explants were cultured for up to three months. No buds were observed; only at 10^{-6} M NAA did some root formation occur.

10.6 The influence of extracts from tubers and roots

Bintje tuber tissues, up to now, have failed to regenerate buds, even after various growth-regulator treatments. This inability may be caused by lack of substances, different from the known hormonal and nutritive factors. Such bud-forming factors may be present in tubers of the Multa variety and also in roots of rooted slices. On the other hand, the inability to form buds may be caused by specific inhibiting substances in tubers of Bintje. The following experiments were carried out to examine the above possibilities.

Tuber explants of Bintje were cultured on a standard medium and on standard media enriched with extracts from roots of rooted tuber-slices of Bintje and extracts from tubers of the Multa variety. Both extracts were fractions W of ethanolic extracts prepared according to the methods described earlier (Section 9.2); the tuber tissue was lyophilized before extraction. The concentrations utilized were equivalent to 100 g

Table 16. Bud formation on tuber explants of Bintje and Multa, cultured on media with plant extracts.

Variety	Medium	Expl. with buds (%)			Buds per explant 8 wk.
		5 wk.	6 wk.	8 wk.	
Bintje	Standard	0	0	0	0
Bintje	Root extract	0	0	0	0
Bintje	Multa tuber extr.	0	0	0	0
Multa	Standard	26	71	83	2.0
Multa	Root extract	35	87	91	3.3
Multa	Bintje tuber extr.	5	58	100	2.2

tissue per litre medium. Tuber explants of Multa were cultured on a medium with root extract to test its effect on spontaneous bud formation. Another series of Multa explants was grown on a medium with extract of Bintje tubers to examine whether it contained bud-inhibiting substances.

The results, presented in Table 16, showed that again Bintje tissues did not form buds.

The responses of Multa tissue to the extracts were the following. Tuber extract of Bintje did not prevent bud formation, although some inhibition in the beginning was observed. Tubers of Bintje may contain inhibiting substances; whether such substances are active in the living tissue is not known. Unfortunately, no data on the effect of Multa tuber extracts on Multa tissues are available. Root extracts seem to promote bud-formation capacity, which was demonstrated by initial bud-formation frequencies and by the numbers of buds per explants. Roots seem to contain substances, nutritional or hormonal, which are beneficial for bud formation.

10.7 Varietal differences in auxin sensitivity

Experiments, described in sections 7.3, 10.5 and 10.6 demonstrated that the application of growth regulators or plant extracts was insufficient for bud initiation in tuber tissues of the Bintje variety. However, treatments with auxin resulted in luxurious root formation (Chapter 4). This root formation was much better in Bintje than in Multa (sections 6.3 and 8.8). This may be caused by varietal differences in sensitivity to auxin of the tuber tissue. Such differences in sensitivity may also apply to other effects of auxin, e.g. its role in bud initiation, its requirement in cell division and cell enlargement. In the following tissue-culture experiments callus growth, a result of cell division and cell enlargement, was investigated in the varieties Bintje and Multa.

Callus of potato can grow on a medium of minerals and sugar, supplemented with 2,4-D and coconut milk (Steward & Caplin, 1951). Preliminary experiments showed that callus tissue obtained from tuber explants grew well on a medium containing: mineral salts according to Murashige & Skoog (1962); vitamins and inositol (see

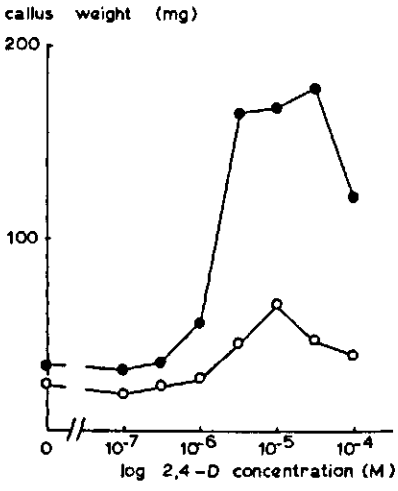


Fig. 19. Callus growth of Bintje (●) and Multa (○) at different concentrations of 2,4-D. Each point represents the mean weight of 6 tissues.

Section 10.2); agar, 10 g/l; sucrose, 20 g/l; 10% (v/v) coconut milk and 2,4-D, 10^{-5} M. In a first experiment it turned out that callus on tuber explants of Bintje grew much better than callus of Multa. This could be due to a different sensitivity to auxin at the concentration involved, or to a different optimum concentration of auxin. Therefore, a second experiment was carried out in which tissues of the two varieties were submitted to a concentration range of 2,4-D. First tuber explants of the two varieties were grown on the above medium with 10^{-5} M 2,4-D, for 4 weeks. These explants developed callus from which equal pieces were transferred to the test media. After 19 days of culture the experiment was stopped. The results of this experiment are presented in Fig. 19. The optimum auxin concentration was about 10^{-5} M for both varieties. Within the whole concentration range Bintje outyielded Multa. Whether the varietal differences in callus growth were caused by differences in cell volume, cell number, or both has not yet been investigated.

Nevertheless, callus tissue derived from Bintje tubers seems to be more sensitive to auxin than callus from Multa tubers. This fact provides a new possibility to explain differences in regeneration behaviour of the two varieties (see Section 11.3).

10.8 Summary

This chapter has yielded a number of new data which necessitate a reconsideration of earlier results. A comprehensive discussion will be given in the next chapter. Here only the main findings are summarized:

1. Tuber explants of the variety Multa produce adventitious buds very easily, whereas explants of the variety Bintje do not form buds at all.
2. Bud formation in Multa tuber explants is not impeded when K is omitted in the medium; with a deficiency of N, P, or sucrose bud formation is somewhat decreased.
3. Bud formation in Bintje tuber explants was not obtained, either by various growth-

regulator treatments, or by feeding the tissues with extracts from roots of rooted slices or extracts from tubers of the Multa variety.

4. Striking differences in response of tuber tissue to auxin were found between Bintje and Multa. Callus derived from Bintje tubers showed considerably better growth than callus from Multa tubers, whereas the optimum auxin concentration was the same.

11 General discussion

11.1 Introduction

The formation of a bud (or root) is the result of a complex program of cell division, cell arrangement and cell differentiation. According to Gorter (1968) the following sequential phases can be distinguished: (1) dedifferentiation, if the organ arises from mature tissue, (2) meristem formation, (3) determination of the organ and (4) growth. In spite of the complexity of the development as a whole, the onset can be brought about simply by the level of certain hormones. Several investigations have demonstrated that buds are initiated by a high level of cytokinin combined with a low level of auxin. In numerous plant species and types of tissue, however, attempts to initiate bud formation by growth regulator treatments were unsuccessful. It is very difficult to explain these differences in response as the precise mode of action of the hormones is largely unknown.

The present study deals with adventitious bud formation in *Solanum tuberosum*, a species with a relatively low bud regenerative capacity (for literature see Chapter 2). Two instances of adventitious bud formation at a relatively high frequency were found, viz. bud formation on rooted tuber-slices and bud formation on tuber tissue of the variety Multa. I tried to find out why bud formation was much better in these cases than in others. Attention was paid to the influence of nutrients and the role of hormones. In the following sections these subjects will be discussed in detail.

11.2 Nutritional factors

Mineral nutrients appeared to be required for bud formation in rooted tuber-slices (Chapter 8). Omission in the root medium of K, Ca, N, and to some degree, P resulted in decreased bud formation. Bud formation on tuber pieces in sterile culture was not inhibited on a K-deficient medium; on media deficient in N or P a slight inhibition was observed (Section 10.4). The tuber tissue itself, therefore, seems to be rich enough in minerals for considerable bud initiation. For rooted tuber-slices the minerals are probably required to sustain root growth and to prevent withdrawal of mineral elements from the sites where the adventitious buds arise. This withdrawal will especially apply to deficiency of K and N in the root medium since then substantial root growth occurs. With a Ca deficiency root growth is almost nil; here the inhibition of bud formation may be caused by other factors.

Literature data indicate that the formation of adventitious buds is especially de-

pendent on phosphorus and nitrogen. Lauer (1963, 1967), studying bud formation on disbudded potato plants, found high N and high P levels to be beneficial for bud formation. In tissue cultures of tobacco, bud promotion could be promoted by increasing the phosphate level in the medium (Skoog & Tsui, 1948). High nitrogen levels increased bud formation in tissue cultures of cauliflower (Margara, 1969). Harris & Hart (1964), investigating regeneration behaviour of excised leaf squares of *Peperomia sandersii*, demonstrated that both bud and root formation were inhibited when the stock plants suffered from deficiency of nitrogen or phosphorus; the inhibition could be overcome by direct supply of mineral nutrients to the leaf squares.

My experiments on mineral nutrient effects in rooted potato-slices indicate that mineral deficiency can block the formation of buds at a very early stage (sections 8.5 and 8.6). The initiation of a bud meristem seems to be more critical to deficiency than the extension growth.

Carbohydrate nutrition may also play a role. Bud formation on aseptically cultured tuber explants of the potato variety Multa tended to be lower on a medium without sugar (Section 10.4). Thorpe & Murashige (1970) showed that bud formation in callus cultures of tobacco was preceded by starch accumulation in the cells that would form the bud meristems. Carbohydrate supply has also been found to be required in early stages of root formation (Gautheret, 1969).

The initiation of a bud requires a relatively high level of different nutrients (nitrogen, phosphorus, carbohydrate). Since in literature similar requirements were reported for root initiation, we may conclude that lack of the above nutrients inhibits the formation of meristems and not specifically the formation of buds.

11.3 Regulatory role of hormones

Up to now all attempts to initiate bud formation in rootless tuber pieces of the potato variety Bintje have been unsuccessful. Application of various growth regulators seemed to be insufficient (chapters 7 and 10). Also in literature no conclusive reports are known about bud formation in potato induced by growth regulators. What is the cause of this inability? Lack of nutritional factors seems unlikely, since pieces of tuber tissue can form callus (Fig. 12) and roots (Fig. 2) without a nutrient supply from outside. Moreover, the inability to form buds remained when tuber pieces were cultured on media containing various nutrients (Chapter 10; literature Section 2.4). These data led to the suggestion that in tuber tissue of the variety Bintje some regulatory system exists which specifically inhibits formation of buds. Tuber tissue of the variety Multa, on the other hand, forms buds very easily. A comparison of the characteristics of tuber tissue of the two varieties may provide a clue to the underlying mechanism.

The following data seem to be of interest: (1) Bintje forms many more roots than Multa after treatment with IAA (sections 6.3 and 8.8). (2) Sterile cultures of callus tissue obtained from Bintje tubers grew much better than that of Multa tubers on media with 2,4-D (Section 10.7). These data strongly suggest that tuber tissue of the two varieties differs in sensitivity to auxin. This differential auxin sensitivity may also cause the

differences in adventitious bud formation. If we postulate that the endogenous auxin levels are the same for both varieties, we can ascribe the inability of Bintje to form buds merely to a blocking imposed by the tissue. An alternative hypothesis, based on the same experimental data, may be that tuber tissue of the two varieties differs in sensitivity to cytokinins. Experiments to test the validity of the above assumptions are in progress. First of all tuber tissue of Bintje and Multa will be analysed for endogenous levels of auxin and cytokinin[1].

The hypothesis of differential sensitivity to hormones emphasizes the possible regulatory significance of the site of hormone reception. In studies of organogenesis very little attention has been paid to this aspect.

Recently Yamada et al. (1972) obtained evidence on a receptor for cytokinins specifically associated with bud formation. They tested the effect of optically active isomers of two synthetic N^6 substituted adenines in tobacco callus cultures. Callus growth was promoted by both enantiomers of the two substances, whereas shoots were only initiated by the levorotatory isomers. The differential effects can be explained by different characteristics of cytokinin receptors.

Evidence about hormone receptors was also obtained from investigations on the effect of hormones in nucleic acid metabolism. Many physiological effects of auxins, cytokinins and gibberellins are associated with increased RNA synthesis (see Key, 1969). These hormones can stimulate RNA synthesis in an *in vitro* system, consisting of isolated nuclei or chromatin, RNA precursors and RNA polymerase, which may indicate a direct influence on gene action. The hormonal effect on RNA synthesis, however, appeared to depend on the presence of specific proteins that bind the hormones to the chromatin. This has been demonstrated for 2,4-D (Matthysse & Phillips, 1969; Matthysse, 1970) and for cytokinins (Matthysse & Abrams, 1970); the experiments with 2,4-D revealed that the activity of auxin-reactive protein was specific for the plant species and, to some degree, for the organ type within a plant species.

Whether hormone-reactive proteins have a regulatory function in morphogenetic processes has to be investigated. It might be that genotypically determined differences in regeneration behaviour are caused by unequal hormone-reactive proteins.

11.4 Role of the root system

One of the major problems of the present study was to account for the high bud-regenerative capacity of rooted tuber-slices. Undoubtedly the root system has some specific influence on the initiation of shoots.

In some instances a relationship was found between root growth and bud-formation capacity. At low root temperatures (Fig. 11) and with a Ca deficiency (Chapter 8) both root growth and bud formation were inhibited. In other cases however, viz. deficiency of K and N (Chapter 8; Table 13), nearly normal root growth coincided with inhibited

1. Recently I found that the cytokinin levels were equal for both varieties.

bud formation. Moreover a comparison of the varieties Alpha and Bintje showed that a negative correlation between root development and bud production can occur (Table 12). Apparently no simple relationship between root growth and bud formation exists. Up to now, with very poor root growth no or very little bud formation has been observed.

One of the most important functions of the root system in an intact plant is the uptake of mineral nutrients. In rooted tuber-slices bud formation was inhibited considerably by mineral deficiencies (Chapter 8). However, it was impossible to demonstrate specific bud-initiating effects of minerals (see discussion sections 8.9 and 11.2). It seemed more likely that bud formation in rooted tuber-slices is initiated by cytokinins that are produced by the root system. To investigate this possibility cytokinin analyses were made on materials which differed in bud-forming capacity (Chapter 9). Cytokinin activity was demonstrated in root extracts and in some instances a relationship between the cytokinin level and bud production was found. When, however, I tried to substitute for the root system by directly supplying cytokinins to the tuber tissue (sections 7.3 and 10.5), or by culturing tuber pieces on media containing ethanolic root extracts (Section 10.6), no bud formation was observed.

From the available experimental data no conclusive interpretation can be made about bud formation in rooted tuber-slices. A non-limiting level of nutrients and probably a certain concentration of cytokinins will be required but are not the only factors. Other possibilities and additional factors will be mentioned below.

Up to now, bud-formation ability of rooted tuber-slices was ascribed to the deliverance by the root systems of bud-forming substances. Alternatively the root system may absorb bud-inhibiting substances from the tuber-slice. Hoad et al. (1971) demonstrated that in willow cuttings adventitious roots can function as a sink for auxin. Similarly in rooted tuber-slices endogenous auxin may be transported to the roots. The activity of IAA-oxidase is usually higher in root tissue than in other parts of the plant (Hare, 1964) so that the root system may function as a site of auxin destruction.

A factor largely neglected in studies of regeneration is the influence of water relations. In rooted slices a bud was never formed below the level of the soil or the nutrient solution, which may indicate that evaporation is necessary at the site of bud formation. Evaporation causes a transport stream of water, which may lead to accumulation of required substances. The significance of this aspect has to be investigated, especially because of the inability of bud formation in potato roots in liquid culture (Bajaj & Dionne, 1968).

Another point not investigated in this study concerns the histological origination of the bud meristems in rooted tuber-slices. Many buds arise at the root base, some at the upper parts of the roots. Buds also develop on the tuber tissue near the root; however, these buds may originate from remnants of removed roots. If all buds originate from root tissue, a search for the underlying mechanism can be directed to specific characteristics of this tissue. In many plant species adventitious buds are formed more easily in roots than in other parts of the plant (Beijerinck, 1886; Priestly & Swingle, 1929).

Summary

The purpose of this study was to investigate physiological factors regulating the initiation of adventitious buds in potato (*Solanum tuberosum*).

Adventitious bud formation was studied on de-eyed tuber pieces kept in moist sand. In most varieties the ability of this material to form buds was small or even absent. Attempts to initiate buds by growth regulators were unsuccessful. Tuber-slices treated with auxin formed adventitious roots in most varieties.

Rooted tuber-slices easily formed buds when they were planted in soil. I tried to account for the bud-promotive influence of the roots. Water-culture experiments showed that rooted slices did not produce buds when the roots were deprived of mineral nutrients; especially potassium and nitrogen seemed to be required. No supply of these minerals, however, was necessary to initiate buds in non-rooted tuber pieces in sterile culture. This result suggested that the roots do not promote bud formation by their uptake of minerals. Attempts were made to relate bud formation on rooted tuber-slices to cytokinin synthesis in the roots. Cytokinin activity was demonstrated in root extracts but a causal relationship with bud formation could not be proved. Experiments with tuber explants in sterile culture demonstrated that the bud-promotive influence of the root system could not be substituted by application of root extract or cytokinin. Further research will be necessary to trace the cause of bud formation on rooted tuber-slices. Since nearly all buds originate on or near the roots, specific characteristics of the living root tissue may be involved.

A different approach of the bud formation problem was made possible by the genotypic variation in regeneration behaviour of non-rooted tuber pieces in sterile culture. Tuber tissue of the variety Multa formed buds very easily, whereas in the variety Bintje no bud formation occurred. Trials to initiate bud formation in Bintje by application of Multa tuber extract were unsuccessful. A comparative investigation of tuber tissue of the two varieties showed a considerably higher sensitivity to auxin in Bintje than in Multa. It was hypothesized that differences in bud regenerative ability are caused by differences in hormone sensitivity of the tissue.

Samenvatting

Het doel van het onderzoek was na te gaan welke fysiologische factoren een rol spelen bij het ontstaan van adventieve spruiten bij de aardappel (*Solanum tuberosum*).

Adventieve spruitvorming werd bestudeerd aan ontoogde knolstukken bewaard in vochtig zand. Bij de meeste aardappelrassen werd op deze wijze geen of weinig spruitvorming verkregen. Pogingen spruitvorming te induceren door groeiregulatoren leidden niet tot succes. Door knolschijven met auxine te behandelen kon bij vele rassen wortelvorming worden geïnduceerd.

Wanneer bewortelde knolschijven in grond werden geplant ontstonden gemakkelijk adventieve spruiten. Onderzocht werd wat de oorzaak was van de spruitvorming bevorderende werking der wortels. Proeven met watercultuur toonden aan dat bewortelde knolschijven geen spruiten vormden wanneer de wortels geen minerale voeding kregen; vooral K en N leken nodig te zijn. Toevoer van deze mineralen bleek echter niet noodzakelijk voor spruitvorming aan wortelloze knolstukjes in steriele cultuur. Dit wijst erop dat de invloed van wortels op de spruitvorming niet berust op de opname van mineralen. Daarna werd getracht na te gaan of er een verband was tussen spruitvorming aan bewortelde knolschijven en cytokininesynthese in het wortelstelsel. In extracten van wortels werd cytokinine-activiteit gevonden, een causaal verband met de spruitvorming kon echter niet worden aangetoond. Proeven met knolexplantaten in steriele cultuur wezen uit dat de spruitvorming bevorderende werking der wortels niet kon worden vervangen door toediening van wortelextract of van cytokinine. Verder onderzoek zal nodig zijn om de oorzaak van de spruitvorming aan bewortelde knolschijven op te sporen. Aangezien bijna alle spruiten op of nabij de wortels ontstaan, zouden specifieke eigenschappen van het levende wortelweefsel een rol kunnen spelen.

Een andere benadering van het spruitvormingsprobleem werd mogelijk gemaakt door rasverschillen in het regeneratievermogen van steriel gekweekte knolstukjes. Het bleek dat knolweefsel van het ras Multa zeer gemakkelijk spruiten vormde, terwijl bij het ras Bintje geen spruitvorming werd verkregen. Pogingen bij Bintje spruitvorming te induceren door toediening van extract van Multa knollen hadden geen resultaat. Een vergelijkend onderzoek van de twee rassen wees uit dat knolweefsel van Bintje aanzienlijk gevoeliger was voor auxine dan dat van Multa. Dit leidde tot de hypothese dat verschillen in spruitregeneratie worden veroorzaakt door verschillen in hormoongevoeligheid van het weefsel.

Acknowledgments

I am greatly indebted to my promotor, Professor Dr M. H. van Raalte, for his interest and encouragement during the research and for his useful comments on the manuscript.

I am also indebted to:

- Professor Dr R. Brouwer for criticism of the manuscript.
- Dr H. Lamberts, Director, and Dr F. E. Nijdam, former Director, of the Foundation for Agricultural Plant Breeding, Wageningen, for permission to undertake this study and to publish the results as a thesis.
- Dr P. Gaastra, Director, and Dr Iz. de Haan, former Director, of the Centre for Plant Physiological Research, Wageningen, for providing laboratory facilities.
- Dr H. M. Dekhuijzen for advice on isolation and assay of cytokinins; Mr A. A. Steiner for advice and discussions on mineral nutrition; and Mr J. Post Jr for statistical analysis.
- Mr P. J. Groot for skilfull technical assistance; and Mr C. F. Krechting for assistance with tissue cultures.
- Mr J. S. de Block and Mrs E. Brouns-Murray for correcting the English; Mr C. G. L. Dirks for editing; Mrs J. H. M. Hoksbergen-Modderkolk for typing the manuscript; Mr G. Post for preparing the figures; and Mr J. Post Sr and Mr H. W. Sengers for the photographs.

This work was subsidized by a grant from the Euratom /ITAL Association.

References

Åberg, B., 1957. Auxin relations in roots. A. Rev. Pl. Physiol. 8: 153–180.

Asseyeva, T., 1927. Bud mutations in the potato and their chimerical structure. J. Genet. 19: 1–26.

Bajaj, Y. P. S. & L. A. Dionne, 1967. Growth and development of potato callus in suspension cultures. Can. J. Bot. 45: 1927–1931.

Bajaj, Y. P. S. & L. A. Dionne, 1968. The continuous culture of potato roots. N. Z. J. Bot. 6: 386–394.

Baumeister, W., 1958. Haushalt der Mineralstoffe. B. Die einzelnen Elemente. a. Hauptnährstoffe. In: W. Ruhland (Ed.): Handbuch der Pflanzenphysiologie, Vol. 4, p. 482–557. Springer-Verlag, Berlin.

Beijerinck, M. W., 1886. Beobachtungen und Betrachtungen über Wurzelknospen und Nebenwurzeln. Verh. K. Akad. Wet. 25C: 1–150.

Belskaja, T. N., 1933. Experimentelle Adventivsprosse bei zwei Kartoffelsorten ('Narodnij' und 'Jubel') (Russian with German summary). In: N. P. Krenke (Ed.): Phänogenetische Variabilität, Vol. 2, p. 223–255. Timiriaseff Institute, Moscow.

Bollard, E. G. & G. W. Butler, 1966. Mineral nutrition of plants. A. Rev. Pl. Physiol. 17: 77–112.

Brian, P. W., H. G. Hemming & M. Radley, 1955. A physiological comparison of gibberellic acid with some auxins. Physiologia Pl. 8: 899–912.

Broertjes, C., B. Haccius & S. Weidlich, 1968. Adventitious bud formation on isolated leaves and its significance for mutation breeding. Euphytica 17: 321–344.

Bruinsma, J., A. Sinnema, D. Bakker & J. Swart, 1967. The use of gibberellic acid (GA) and N-dimethylsuccinamic acid (B9) in testing of seed potatoes for virus infection. Eur. Potato J. 10: 136–152.

Burton, W. G., 1963. Concepts and mechanism of dormancy. In: J. D. Ivins & F. L. Milthorpe (Eds): The Growth of the Potato, p. 17–41. Butterworths, London.

Burton, W. G., 1966. The Potato, 2nd edition. H. Veenman & Zonen N.V., Wageningen, The Netherlands.

Butcher, D. N. & H. E. Street, 1964. Excised root culture. Bot. Rev. 30: 513–586.

Butenko, R. G., 1968. Plant Tissue Culture and Plant Morphogenesis. Israel Program for Scientific Translations, Jerusalem.

Chapman, H. W., 1955. Potato tissue cultures. Am. Potato J. 32: 207–210.

Chibnall, A. C., 1939. Protein Metabolism in the Plant. Yale University Press, New Haven.

Chibnall, A. C., 1954. Protein metabolism in rooted runner bean leaves. New Phytol. 53: 31–37.

Claver, F. K., 1967. Formacion de yemas adventicias en tejidos de papa (*Solanum tuberosum*). Revta Fac. Agron. La Plata 43: 45–54.

Conrad, K., 1967. Native Cytokinine. Biol. Rundschau 5: 253–267.

Crozier A. & D. M. Reid, 1971. Do roots synthesize gibberellins? Can. J. Bot. 49: 967–975.

Dekhuijzen, H. M. & J. C. Overeem, 1971. The role of cytokinins in clubroot formation. Physiol. Pl. Path. 1: 151–162.

Dekhuijzen, H. M. & R. C. Staples, 1968. Mobilization factors in uredospores and bean leaves infected with bean rust fungus. Contr. Boyce Thompson Inst. 24: 39–51.

Denny, F. E., 1926. Hastening the sprouting of dormant potato tubers. Am. J. Bot. 13: 118–125.

Dore, J., 1965. Physiology of regeneration in cormophytes. In: W. Ruhland (Ed.): Handbuch der Pflanzenphysiologie, Vol. 15(2), p. 1–91. Springer-Verlag, Berlin.

Dubrowitzkaja, N., 1933. Experimentelle Adventivsprosse bei zwei Kartoffelsorten: 'Mindalnij' und 'Silesia' (Russian with German summary). In: N. P. Krenke (Ed.): Phänogenetische Variabilität, Vol. 2, p. 257–268. Timiriaseff Institute, Moscow.

Fadl, M. F. & H. T. Hartmann, 1967. Isolation, purification and characterization of an endogenous root-promoting factor obtained from basal sections of pear hardwood cuttings. Pl. Physiol. 42: 541–549.

Fellenberg, G., 1963. Über die Organbildung an *in vitro* kultiviertem Knollengewebe von *Solanum tuberosum*. Z. Bot. 51: 113–141.
Fransson, P., 1958. Studies on the interaction of antiauxin and native auxin in wheat roots. Physiologia Pl. 11: 644–645.
Gautheret, R. J., 1959. La Culture des Tissues Végétaux. Masson & Cie, Paris.
Gautheret, R. J., 1969. Investigations on the root formation in the tissues of *Helianthus tuberosus* cultured *in vitro*. Am. J. Bot. 56: 702–717.
Gorter, C. J., 1968. Hormone translocation and rooting. In: Y. Vardar (Ed.): The Transport of Plant Hormones, p. 293–308. North-Holland Publishing Company, Amsterdam.
Greenwood, M. S. & M. H. M. Goldsmith, 1970. Polar transport and accumulation of indole-3-acetic acid during root regeneration by *Pinus lambertiana* embryos. Planta 95: 297–313.
Guthrie, J. D., 1938. Effect of ethylene thiocyanohydrin, ethyl carbylamine, and indoleacetic acid on the sprouting of potato tubers. Contr. Boyce Thompson Inst. 9: 265–272.
Guthrie, J. D., 1939. Control of bud growth and initiation of roots at the cut surface of potato tubers with growth-regulating substances. Contr. Boyce Thompson Inst. 11: 29–53.
Guttenberg, H. von, 1960. Grundzüge der Histogenese höherer Pflanzen. I. Die Angiospermen. Handbuch der Pflanzenanatomie 8 (3). Bornträger, Berlin.
Hagemann, A., 1932. Untersuchungen an Blattstecklingen. Gartenbauwissenschaft 6: 69–195.
Halperin, W., 1969. Morphogenesis in cell cultures. A. Rev. Pl. Physiol. 20: 395–418.
Hare, R. C., 1964. Indoleacetic acid oxidase. Bot. Rev. 30: 129–165.
Harris, G. P. & E. M. H. Hart, 1964. Regeneration from leaf squares of *Peperomia sandersii* A. DC.: a relationship between rooting and budding. An. Bot. N. S. 28: 509–526.
Harten, A. M. van, 1972. A suggested method for investigating L_1 constitution in periclinal potato chimeras. Potato Res. 15: 73–75.
Hartmann, H. T. & D. E. Kester, 1968. Plant Propagation, 2nd edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
Hess, C. E., 1964. Naturally occurring substances which stimulate root initiation. In: J. P. Nitsch (Ed.): Régulateurs Naturels de la Croissance Végétale, p. 517–527. Centre National de la Recherche Scientifique, Paris.
Heide, O. M., 1969. Non-reversibility of gibberellin-induced inhibition of regeneration in *Begonia* leaves. Physiologia Pl. 22: 671–679.
Hewitt, E. J., 1966. Sand and water culture methods in the study of plant nutrition, revised 2nd edition, p. 205. Commonwealth Agricultural Bureaux, Farnham Royal, England.
Hoad, G. V., S. K. Hillamn & P. F. Wareing, 1971. Studies on the movement of indole auxins in willow. Planta 99: 73–88.
Hoagland, D. R. & W. C. Snyder, 1933. Nutrition of strawberry plant under controlled conditions: (a) Effect of deficiencies of boron and certain other elements; (b) Susceptibility to injury from sodium salts. Proc. Am. Soc. hort. Sci. 33: 288–295.
Howard, H. W., 1964. Experimental production of buds on the roots of potatoes. Nature, Lond. 203: 1303–1304.
Ivanko, S., 1971. Metabolic pathways of nitrogen assimilation in plant tissue when ^{15}N is used as a tracer. In: Nitrogen-15 in soil-plant studies, p. 119–156. IAEA, Vienna.
Kende, H., 1964. Preservation of chlorophyll in leaf sections by substances obtained from root exudate. Science, N.Y. 145: 1066–1067.
Kende, H., 1965. Kinetinlike factors in the root exudate of sunflowers. Proc. natn. Acad. Sci. U.S.A. 53: 1302–1307.
Key, J. L., 1969. Hormones and nucleic acid metabolism. A. Rev. Pl. Physiol. 20: 449–474.
Klämbt, D., 1968. Cytokinine aus *Helianthus annuus*. Planta 82: 170–178.
Klopfer, K., 1965. Erfolgreiche experimentelle Entmischungen und Umlagerungen periclinalchimärischer Kartoffelklone. Züchter 35: 201–214.
Krenke, N. P., 1933a. Wundkompensation, Transplantation und Chimären bei Pflanzen (translated from Russian into German). Springer-Verlag, Berlin.
Krenke, N., 1933b. Die Methode der Auslösung von Adventivsprossen bei der Kartoffel zwecks Bildung polyploider Sorten und Chimären (Russian with German summary). In: N. P. Krenke (Ed.): Phänogenetische Variabilität, Vol. 2, p. 173–222. Timiriaseff Institute, Moscow.
Kulaeva, O. N., 1962. The effect of roots on leaf metabolism in relation to the action of kinetin on leaves. Soviet Pl. Physiol. (English translation) 9: 182–189.

Kupfer, E., 1907. Studies in plant regeneration. Mem. Torrey bot. Club 12: 195–241.
Lang, A., 1970. Gibberellins: structure and metabolism. A. Rev. Pl. Physiol. 21: 537–570.
Lauer, F. I., 1963. Influence of high and low levels of N and K on adventitious bud formation in the potato. Am. Potato J. 40: 302–307.
Lauer, F. I., 1967. Factors affecting adventitious bud formation in the potato. Am. Potato J. 44: 87–94.
Lauer, F. I. & F. A. Krantz, 1957. Formation of buds from callus tissue in the potato. Am. Potato J. 34: 158–164.
Lee T. T. & F. Skoog, 1965. Effects of substituted phenols on bud formation and growth of tobacco tissue cultures. Physiologia Pl. 18: 386–402.
Leopold, A. C., 1961. The transport of auxin. In: W. Ruhland (Ed.): Handbuch der Pflanzenphysiologie, Vol. 14, p. 671–682. Springer-Verlag, Berlin.
Letham, D. S., 1963. Regulators of cell division in plant tissues. I. Inhibitors and stimulants of cell division in developing fruits: their properties and activity in relation to cell division period. N. Z. J. Bot. 1: 336–350.
Letham, D. S., 1967. Chemistry and physiology of kinetin-like compounds. A. Rev. Pl. Physiol. 18: 349–364.
Li, P. H., F. I. Lauer & C. J. Weiser, 1967. The influence of disbudding on the metabolite composition of potato leaves. Proc. Am. Soc. hort. Sci. 91: 436–442.
Lingappa, Y., 1957. Tissue culture of *Solanum tuberosum* and *Ipomoea pandurata*. Am. J. Bot. 44: 419–423.
Loeffler, J. E. & J. van Overbeek, 1964. Kinin activity in coconut mulk. In: J. P. Nitsch (Ed.): Régulateurs Naturels de la Croissance Végétale, p. 77–82. Centre National de la Recherche Scientifique, Paris.
McRae, D. H. & J. Bonner, 1953. Chemical structure and antiauxin activity. Physiologia Pl. 6: 485–510.
Margara, J., 1969. Étude des facteurs de la néoformation de bourgeons en culture *in vitro* chez le chou-fleur (*Brassica oleracea* L., var. *Botrytis*). Annls. Physiol. vég. 11: 95–112.
Matthysse, A. G., 1970. Organ specificity of hormone-receptor-chromatin interactions. Biochim. biophys. Acta 199: 519–521.
Matthysse, A. G. & M. Abrams, 1970. A factor mediating interaction of kinins with the genetic material. Biochim. biophys. Acta 199: 511–518.
Matthysse, A. G. & C. Phillips, 1969. A protein intermediary in the interaction of a hormone with the genome. Proc. natn. Acad. Sci. U.S.A. 63: 897–903.
Mengel, K., 1969. Der Eintritt der Nährstoffe in den Stoffwechsel und ihre weitere Verarbeitung. A. Makronährstoffe. g. Erdalkalimentalle. In: H. Linser (Ed.): Handbuch der Pflanzenernährung und Düngung, Vol. 1(1), p. 440–456. Springer-Verlag, Vienna and New York.
Miedema, P., 1967. Induction of adventitious buds on roots of the potato. Euphytica 16: 163–166.
Miller, C. O., 1961. Kinetin and related compounds in plant growth. A. Rev. Pl. Physiol. 12: 395–408.
Miller, C. O., 1963. Kinetin and kinetin-like compounds. In: H. F. Linskens & M. V. Tracey (Eds.): Modern Methods of Plant Analysis, Vol. 6, p. 194–202. Springer-Verlag, Berlin.
Miller, C. O., 1965. Evidence for the natural occurrence of zeatin and derivatives: compounds from maize which promote cell division. Proc. natn. Acad. Sci. U.S.A. 54: 1054–1058.
Mosteller, F. & C. Youtz, 1961. Tables of the Freeman-Tukey transformations for the binomial and Poisson distributions. Biometrika 48: 433–440.
Mothes, K., 1956. Stoffliche Beziehungen zwischen Wurzel und Spross. Angew. Bot. 30: 125–128.
Mothes, K., 1960. Über das Altern der Blätter und die Möglichkeit ihrer Wiederverjüngung. Naturwissenschaften 47: 337–351.
Mothes, K., 1966. Zur Problematik der Phytokininwirkung. Biol. Rundschau 4: 211–224.
Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Pl. 15: 473–497.
Murashige, T., 1964. Analysis of the inhibition of organ formation in tobacco tissue cultures by gibberellin. Physiologia Pl. 17: 636–643.
Niedergang-Kamien, E. & F. Skoog, 1956. Studies on polarity and auxin transport in plants: I. Modification of polarity and auxin transport by triiodobenzoic acid. Physiologia Pl. 9: 60–73.
Okazawa, Y., N. Katsura, & T. Tagawa. 1967. Effects of auxin and kinetin on the development and differentiation of potato tissue cultured *in vitro*. Physiologia Pl. 20: 862–869.

Paton, D. M., R. R. Willing, W. Nicholls & L. D. Prior, 1970. Rooting of stem cuttings of *Eucalyptus*: a rooting inhibitor in adult tissue. Aust. J. Bot. 18: 175–183.
Priestly, J. H. & C. F. Swingle, 1929. Vegetative propagation from the standpoint of plant anatomy. Tech. Bull. U.S. Dep. Agric. 151: 1–98.
Rechinger, C., 1894. Untersuchungen über die Grenzen der Theilbarkeit im Pflanzenreich. Verh. zool.-bot. Ges. Wien 43: 310–334.
Reed, D. J., T. C. Moore & J. D. Anderson, 1965. Plant growth retardant B995: A possible mode of action. Science, N.Y. 148: 1469–1471.
Richards, M., 1964. Root formation on cuttings of *Camellia reticulata* var. Capt. Rawes. Nature, Lond. 204: 601–602.
Richmond, A. E. & A. Lang, 1957. Effect of kinetin on protein content and survival of detached *Xanthium* leaves. Science, N.Y. 125: 650–651.
Schwimmer, S. & H. K. Burr, 1967. Structure and chemical composition of the potato tuber. In: W. F. Talburt & O. Smith (Eds.): Potato Processing, 2nd edition, p. 12–43. The Avi Publishing Company, Inc., Westport, Connecticut.
Seth, A. & P. F. Wareing, 1965. Isolation of a kinin-like root-factor in *Phaseolus vulgaris*. Life Sci. 4: 2275–2280.
Short, K. C. & J. G. Torrey, 1972. Cytokinins in seedling roots of pea. Pl. Physiol. 49: 155–160.
Simmonds, N. W., 1964. Observations on potato callus and adventitious shoot formation. Am. Potato J. 41: 129–136.
Sinclair, C., 1969. The level and distribution of amines in barley as affected by potassium nutrition, arginine level, temperature fluctuation and mildew infection. Pl. Soil 30: 423–438.
Sinnot, E. W., 1960. Plant Morphogenesis. McGraw-Hill Book Comp., Inc., New York, Toronto, and London.
Skoog, F. & D. J. Armstrong, 1970. Cytokinins. A. Rev. Pl. Physiol. 21: 359–384.
Skoog, F. & C. O. Miller, 1957. Chemical relation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. exp. Biol. 11: 118–131.
Skoog, F. & C. Tsui, 1948. Chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro*. Am. J. Bot. 35: 782–785.
Snell, K., 1921. Versuche über die Fähigkeit zu Neubildungen dei der Kartoffel. Mitt. biol. Reichsanst. Ld.- u. Forstw. 21: 240–242.
Steiner, A. A., 1970. Soilless culture. In: Proceedings of the 6th Colloquium of the International Potash Institute, p. 324–341. International Potash Institute, Berne, Switzerland.
Steward, F. C., P. V. Ammirato & M. O. Mapes, 1970. Growth and development of totipotent cells. Ann. Bot. 34: 761–787.
Steward, F. C. & S. M. Caplin, 1951. A tissue culture from potato tuber: The synergistic action of 2,4-D and coconut milk. Science, N.Y. 113: 518–520.
Svobodová, J., 1964. Freeing potato plants from virus. In: Abstracts of the 10th International Botanical Congress, p. 485–486. Edinburgh.
Thorpe, T. A. & T. Murashige, 1970. Some histochemical changes underlying shoot initiation in tobacco callus cultures. Can. J. Bot. 48: 277–285.
Torrey, J. G., 1965. Physiological bases of organization in the root. In: W. Ruhland (Ed.): Handbuch der Pflanzenphysiologie, Vol. 15 (1), p. 1256–1327. Springer-Verlag, Berlin.
Torrey, J. G., 1966. The initiation of organized development in plants. Adv. Morphogenesis 5: 39–91.
Wagner, H., 1970. Einfluss von unterschiedlicher Ernährung auf die Cytokininbildung in der Pflanzenwurzel. Thesis, University Hohenheim.
Wagner, H. & G. Michael, 1969. Cytokinin-Bildung in Wurzeln von Sonnenblumen. Naturwissenschaften 56: 379.
Wagner, H. & G. Michael, 1971. Der Einfluss unterschiedlicher Stickstoffversorgung auf die Cytokininbildung in Wurzeln von Sonnenblumenpflanzen. Biochem. Physiol. Pfl. 162: 147–158.
Wakhloo, J. L., 1970. Role of mineral nutrients and growth regulators in the apical dominance in *Solanum sisymbrifolium*. Planta 91: 190–194.
Wardlaw, C. W., 1968. Morphogenesis in Plants. A Contemporary Study. Methuen & Co., London.
Weiss, C. & Y. Vaadia, 1965. Kinetin-like activity in root apices of sunflower plants. Life Sci. 4: 1323–1326.
Went, F. W., 1938. Specific factors other than auxin affecting growth and root formation. Pl. Physiol. 13: 55–80.

White, P. R., 1963. The Cultivation of Animal and Plant Cells, 2nd edition. The Ronald Press Company, New York.
Wurm, G., 1960. Vergleichende Untersuchungen über Wachstum und Organbildung an Segmenten Pflanzlicher Speicherorgane bei Kultur *in vitro*. Flora 149: 41–76.
Yamada, Y., J. Sekiya & K. Koshimizu, 1972. Cytokinin-induced shoot formation. Phytochemistry 11: 1019–1021.
Yoshida, R., T. Oritani & A. Nishi, 1971. Kinetin-like factors in the root exudate of rice plants. Pl. Cell Physiol. 12: 89–94.
Younis, M. E. & S. El-Tigani, 1970. The role of N-dimethylaminosuccinamic acid in the control of vegetative development of tomato plants. Z. Pfl. Physiol. 63: 225–237.
Zenk, M. H. & G. Müller, 1963. *In vitro* destruction of exogenously applied indolyl-3-acetic acid as influenced by naturally occurring phenolic acids. Nature, Lond. 200: 761–763.