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THE EFFECT OF GROWTH REGULATORS ON THE SURVIVAL OF EXCISED PUMPKIN HYPOCOTYL-TISSUE

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Introduction

Chibnall (1939) has suggested that protein synthesis in leaves depends on some cooperation on the part of the root, and 1954 he proved that rooting of leaf cuttings prevents the yellowing of the blade. Mothes and Engelbrecht (1956) confirmed this finding and showed that root formation can in fact cause resumption of chlorophyll and protein formation. Those detached, rooted leaves can be kept alive much longer than if left on the plant (Mathuse 1906). Recently, it has been found possible to replace at least a part of the rooting effect by certain chemicals. Richmond and Lang (1957) showed that the chlorophyll and protein loss of detached *Xanthium pennsylvanicum* leaves was considerably slowed down, if the leaves were supplied with kinetin. Osborne (1959) observed similar effects of 2,4-D and 2,4,5-T on detached as well as attached leaves of *Prunus serrulata*.

The effect of rooted leaves as well as experiments with added chemicals indicated that growth regulators are undoubtedly important in the process of senescence.

After the investigations on the pumpkin-tissue (Jelaska 1972), similar effects of growth regulators on the survival of fragments of hypocotyls have been observed.

Materials and Methods

Experiments were made with the species *Cucurbita pepo L.* Seeds of pumpkin were sterilized with 3% calcium hypochloride and germinated in test tubes in distilled water under light for 10 days under

sterile conditions. Fragments of hypocotyls, 1 cm long, were cut and cultivated individually in test tubes (23 × 200 mm) on 25 ml of culture medium for one month. These fragments were implanted horizontally on the medium and finally the tubes were capped with thin aluminium foil. The basal medium was Heller's solution of mineral salts (Heller 1953) or Murashige-Skoog's solution (Murashige and Skoog 1962) + 3% glucose + 0,9% Difco Bacto agar + one of the growth substances or some combinations of them. Before autoclaving, the pH of the medium was 5,5—5,6. The media were autoclaved at 120 °C for 20 minutes. The cultures were maintained at 26 ± 1 °C under artificial light (485 ± 45 lux, fluorescent lamps IPR, 40 W, 220 V, 6500 °K, 16 h light and 8 h darkness daily). Each experiment was made with 20 explants.

Abbreviations: IAA — indoleacetic acid, 2,4-D — 2,4-dichlorophenoxyacetic acid, MS — Murashige and Skoog's medium.

Results

Systematic investigations (Jelaska 1972), which had been carried out on fragments of pumpkin hypocotyls (in vitro), have shown that Heller's basal medium without any addition of growth substances has no favourable influence to tissues. The fragments of hypocotyls hardly survived the culture period and decayed during the first month of culturing in nearly 100% of specimens. The combination of salts used by Murashige-Skoog, also without growth regulators, was much more suitable (Table 1). The tissue fragments survived on the MS-medium the culturing of one month, they retained their green colour and showed even the phenomenon of rhizogenesis, which was especially vigorous when the complete MS-medium was used.

By the addition of auxin and kinetin to Heller's medium the decay of the tissue was delayed and it showed even an increase of dry and fresh weight, which indicated that in spite of the unsuitable nutrient medium growth regulators activated the tissue. Table 2 shows the increase of the fresh and dry weight dependent on the concentrations of IAA and kinetin. This increase was more dependent on the increasing concentration of auxin than on that of kinetin (Table 2).

Table 3 shows the results obtained with 2,4-D and kinetin. One can easily see how the addition of 2,4-D causes, by its increase in concentration, the raise of the percentage of survived explants. The addition of kinetin made this numbers even higher. In this way 20% of explants survived on a medium with 2,4-D (10^{-8}). The addition of kinetin to this medium increased the survival to 40—50% of fragments, or to 70—75% when 2,4-D had been added in concentrations 10^{-7} or 10^{-6}. The addition of kinetin, thus, raised the percentage of survived explants still for about 20% in comparison to that of survived explants when cultivated only with 2,4-D.

Table 1. Survival of excised pumpkin hypocotyl-tissue and increase of fresh and dry weight after 1 month on Heller's and MS-medium.

Medium	Survival of cultures (%)	Fresh weight (mg)	Dry weight (mg)
Heller	20	90.5	5.5
Heller + 10^{-6} IAA + + 3×10^{-7} kinetin	70	188.1	15.5
MS- salts	70	140.2	7.2
MS- complete	85	207.8	17.5
MS- complete + 10^{-6} IAA + + 3×10^{-7} kinetin	90	259.0	21.6

Table 2. Effect of IAA and kinetin on fresh and dry weight of pumpkin hypocotyl-tissue after 1 month (Heller's medium).

IAA	Kinetin	Fresh weight (mg)	Dry weight (mg)
—	—	90.5	5.5
10^{-8}	10^{-7}	83.9	6.3
10^{-8}	3×10^{-7}	94.5	7.3
10^{-8}	10^{-6}	65.1	8.5
10^{-7}	10^{-7}	188.3	13.1
10^{-7}	3×10^{-7}	172.3	15.0
10^{-7}	10^{-6}	186.4	14.0
10^{-6}	10^{-7}	225.5	18.7
10^{-6}	3×10^{-7}	188.1	15.5
10^{-6}	10^{-6}	239.0	20.2

Table 3. Effect of 2,4-D and kinetin on fresh and dry weight and survival of excised pumpkin hypocotyl-tissue after 1 month (Heller's medium).

2,4-D	Kinetin	Survival of cultures (%)	Fresh weight (mg)	Dry weight (mg)
—	—	0	90.0	5.2
10^{-8}	—	20	103.5	8.2
10^{-7}	—	50	155.6	15.5
10^{-6}	—	50	208.6	18.6
2×10^{-6}	—	30	135.1	1.6
10^{-8}	10^{-7}	43	52.1	3.7
10^{-8}	3×10^{-7}	55	79.8	4.7
10^{-8}	10^{-6}	42	121.0	8.5
10^{-7}	10^{-7}	62	184.2	14.4
10^{-7}	3×10^{-7}	70	202.4	14.2
10^{-7}	10^{-6}	60	199.5	13.5
10^{-6}	10^{-7}	75	330.6	29.6
10^{-6}	3×10^{-7}	76	237.9	19.1
10^{-6}	10^{-6}	76	326.3	22.7

Discussion

Fragments of pumpkin hypocotyls, planted on Heller's medium with 3% glucose and 0,9% agar, were not able to survive the culturing period of one month. They lost they colour at a very high percentage in this period of one month and decayed without any considerable increase in weight. By adding growth regulators (2,4-D, IAA and kinetin) to Heller's medium, the senescence of the tissue was delayed for a longer time. The fragments showed even growth and cell divisions, which was evident from the increase of fresh and dry weight. Paupardin (1964a, b) has, however, proved by her anatomic investigations, that a dedifferentiation of tissues such as collenchyma, vessels and parenchyma, occurs under the influence of IAA and kinetin or 2,4-D. In the experiments presented here the addition of 2,4-D to Heller's medium induced even a weak differentiation of roots.

The senescence of plant tissue may be considered as a problem of phytopathology (Comfort 1956). It is certainly a result of a sequence of processes, which can be hardly analyzed separately, but which cause senescence and finally cell death. Auxins should also be responsible for that to a certain extent.

By investigating the quantities of growth hormones in young and senescent tissue (Went and Thimann 1937, Shoji et al. 1951, Galston and Dalberg 1954, Pilet and Galston 1955, Pilet 1956) it could be stated that the concentration of auxin is lowered in senescent cells. According to these findings the senescence of tissues could be partly explained by hypoauxiny, which would increase with progressing cell senescence. The experiments carried out by Osborne (1959) would confirm this statement. He treated leaves of *Prunus serrulata* with different auxin substances (2,4-D, 2,4,5-T) and got very clear symptoms of delayed senescence.

However, a whole sequence of other processes (Pilet 1959) complicates this relatively simple scheme of senescence stated for a certain tissue. The autor's experiments on pumpkin hypocotyls also confirm, to a certain extent, the influence of growth regulators on the processes of senescence.

Summary

Basic Heller's medium is not suitable for the growth and development of fragments of pumpkin hypocotyls. On this medium the fragments hardly survive a culture period of one month. The addition of growth regulators (IAA, 2,4-D or kinetin) to Heller's medium delays the senescence of the tissue and its decay. Moreover, it provokes cell divisions resulting in an increase of fresh and dry weight.

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SADRŽAJ

DJELOVANJE REGULATORA RASTA NA PREŽIVLJENJE FRAGMENATA HIPOKOTILA BUNDEVE

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Fragmenti hipokotila bundeve veličine 1 cm zasijani na Hellerovom mediju s 3% glukoze i 0,9% agara nisu mogli preživjeti period kultiviranja od mjesec dana. Oni su u vrlo velikom postotku, u tom vremenskom razdoblju propadali, izgubivši svoju zelenu boju i pokazujući neznatni prirast težine. Nasuprot tome oni su na MS-mediju u velikom broju preživjeli i znatno povećali svoju težinu (tabela 1).

Dodavanjem nekih regulatora rasta kao npr. IAA, 2,4-D i kinetina (tabela 2 i 3) Hellerovom mediju uočeno je da je postotak preživjelih kultura rastao s porastom koncentracija dodanih stimulatora. Na Hellerovom mediju s 2,4-D u koncentraciji 10^{-8} preživjelo je 20% eksplantata. Dodatak kinetina istoj koncentraciji 2,4-D povećao je preživljenje eksplantata na 40—50%. Kod koncentracije 10^{-7} i 10^{-6} 2,4-D preživjelo je 50% eksplantata, a dodatkom kinetina taj se postotak povisio još za daljnjih 20%. Dodatak stimulatora rastenja, osim na preživljenje eksplantata, djelovao je i na znatan porast svježe i suhe tvari.

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