ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLIII, nr 1
1974

Effects of auxins and cytokinins on tomato callus from anthers

JANINA H. ROGOZIŃSKA and LEOKADIA SKUTNIK

Agricultural Academy Poznań, Branch at Bydgoszcz

(Received: July 13, 1973.)

Abstract

An investigation was carried out on growth substance requirements of tomato callus derived from anthers for culture *in vitro*. Linsmaier and Skoog (1965) medium was used with various levels of auxins (IAA and NAA) and cytokinins (K and BAP).
The results show that cytokinin is an absolute requirement for callus growth irrespective of the auxin level. The optimum concentration of auxin in combination with cytokinin was found to be 5 μM of NAA or 25 μM of IAA, with 5 μM of K or BAP. Callus growth on media with NAA and cytokinin was superior to that on IAA, amounting to 6.05 g per piece on medium with 5 μM of NAA and BAP. Tissues grown on this medium have the highest water content. At the onset of culture the tissue is characterized by weak growth and attains its maximal increase in fresh weight after 6 weeks.

INTRODUCTION

Among plants obtained in tissue culture, the tomato has not been dealt extensively. With this plant, White (1934) succeeded in the obtaining first continuous root culture. Norton and Boll (1954) reported on differentiation of *Lycopersicon peruvianum* Mill. roots into callus and shoots and subsequently into flowering plants. Using very complex media, Nysterakis (1961 a, b) obtained good callus growth and seedlings from tomato fruit fragments. Fukami and Mackinney (1967) induced abundant callus formation in hypocotyls of some tomato mutants by supplementing the medium (containing growth regulators and vitamins) with pea extract (Rogozińska et al., 1965). The addition of pea extract was also required in media with varied mineral composition and growth substances, when shoots and fruits were used as initial material (Ulrich and Mackinney, 1969).

Gresshoff and Doy (1972), investigating 43 tomato races, obtained callus from three only, using anthers as the initial material. The basal medium, besides mineral salts, contained vitamins and growth substances. The main aspect here was differentiation of haploid callus for which an adequate photoperiod is required. The callus differentiated into shoots, roots and pseudo-fruits. Similar results were obtained by Krzyśko and Rogozińska (1974) using cv. Canadian beefsteak.

Various attempts to produce callus and haploid plants from anthers of numerous plants failed. It seemed important to study the conditions necessary for success. As follows from a study of the literature, the details of growth substance requirements for tomato tissue from anthers have not been investigated in detail so far. This was the stimulus to undertake the present work.

MATERIAL AND METHODS

Tomato tissue used in this investigation was isolated in July of 1971. Flower buds (at a length of about 2—3 mm) of raspberry tomato, *Lycopersicon esculentum* Mill. cv. Canadian beefsteak, and *L. pimpinellifolium* Mill., were sterilized by dipping into chlorous water and rinsed several times with distilled water. The anthers (with pollen mother cells in the stage of tetrad or microspores, were then isolated and transferred to Linsmaier and Skoog (1965) medium with 2 mg/l of indole-3-acetic acid (IAA) and 4 mg/l of kinetin (K).

Callus appeared on the anthers of *L. esculentum* only. It was subcultured at five week intervals and maintained during one year on this medium. After the first three transfers, IAA was replaced by 1 mg/l of β-naphthaleneacetic acid (NAA) and K by 4 mg/l of benzylaminopurine (BAP). Before use in the present experiments, the level of BAP was lowered to 2 mg/l. This resulted in much better growth. At that time the tissue had various ploidy.

Callus pieces of a fresh weight of 40 mg ± 2.5 were transferred onto media with individual growth substances. Auxins (IAA or NAA) were used at 1, 5 and 25 μM levels in combination with cytokinins (K or BAP) at concentration of 0.0016, 0.008, 0.04, 0.2, 1, 5 and 25 μM. Each series comprised 10 tissues cultured in separate test tubes on 20 ml medium solidified with agar (0.9%).

The cultures grew at 25° ± 1 under continuous white fluorescent light of about 1500 lux. After 6 weeks, the fresh and dry weight of the tissues were determined. All experiments were repeated twice with analogous results. Standard error of the mean did not exceed 0.06 g.

RESULTS

Influence of auxins and cytokinins on callus growth

The growth of the tissues cultivated on media with various growth substances is plotted in diagrams which illustrate the fresh and dry weight of the tissue after 6 weeks of growth. Auxins and cytokinins were found to have interacting effects in the growth of tomato tissue.

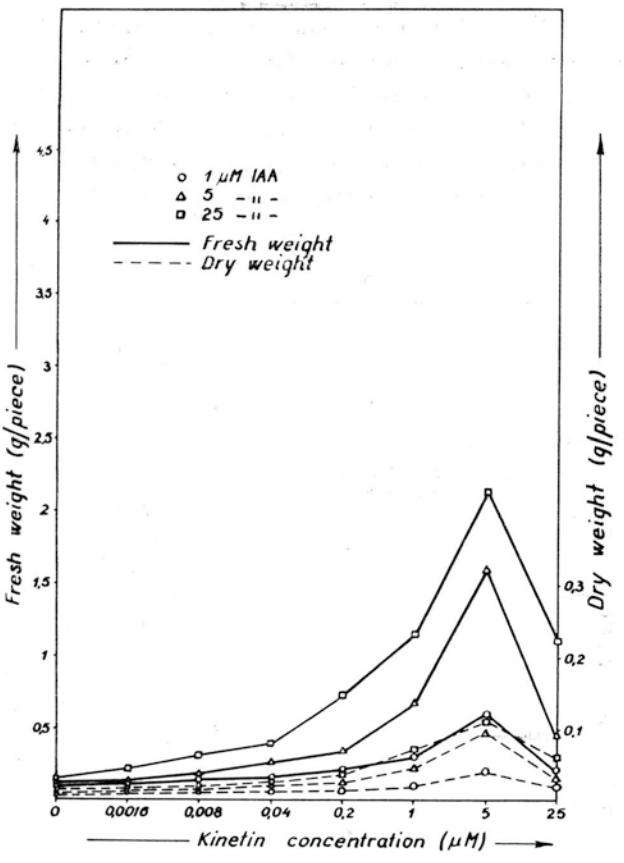


Fig. 1. Influence of IAA and K on the growth of tomato callus

Interaction of IAA with K. Growth of the tissue on media with IAA and K was rather slow. The results of one of the experimental replicates at various IAA concentration (1, 5 and 25 µM) in combination with K, ranging from 0.0016 — 25 µM, are shown in Fig. 1.

Growth of the tissue occurs at the weakest conc. of K applied, and is proportional to this concentration attaining a distinct optimum at

5 μM, upwards of which there appears a marked suppresion of fresh and dry weights at 25 μM of K. The fresh and dry weight dependence on IAA concentration exhibits the highest growth promotion at 25 μM. At the optimal conc. of 5 μM K and 25 μM IAA, the tissue yield amounted to 2.15 g.

Interaction of IAA with BAP. In experiments in which K was replaced by BAP the yield of fresh and dry weight increases up to 5 μM BAP (Fig. 2), attaining a maximum. At this concentration of BAP with 5 μM of IAA, the tissue weight amounted to 3.89 g and, at 5 times higher concentration of BAP, to only 34% of this weight. The best growth of the tissue was obtained on a medium containing 5 μM of BAP and 25 μM of IAA on which the fresh weight of the tissue reached 4.37 g. Thus, on medium containing BAP and IAA, callus growth was superior to that on the medium with K.

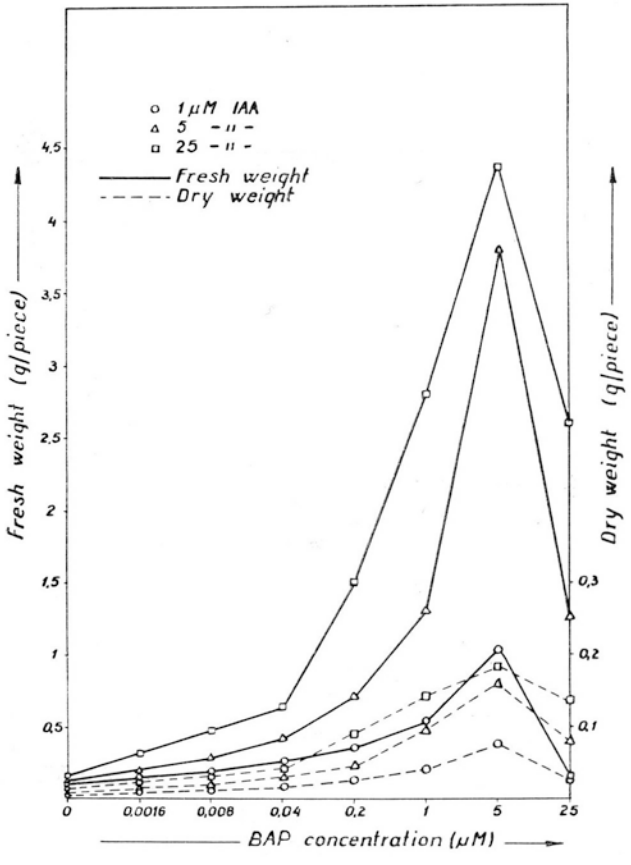


Fig. 2. Influence of IAA and BAP on the growth of tomato callus

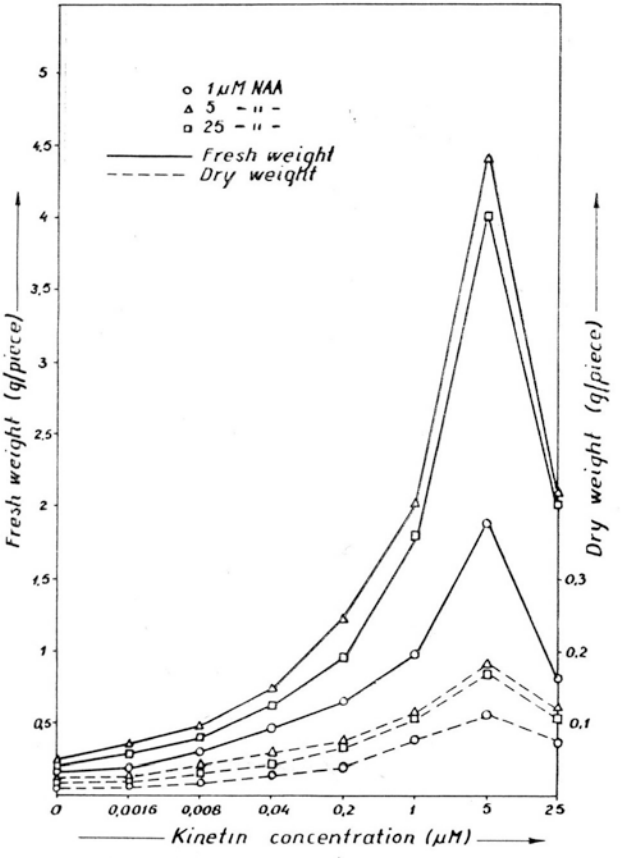
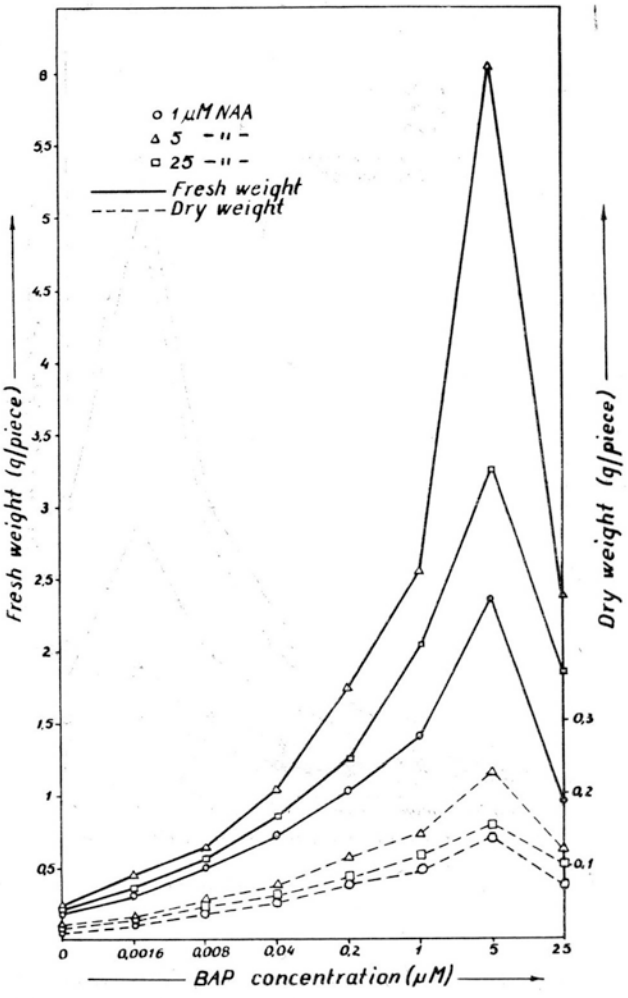


Fig. 3. Influence of NAA and K on the growth of tomato callus

Interaction of NAA with K. The influence of various NAA concentrations in combination with variable K concentration is shown in Fig. 3. The increase in fresh and dry weight of the tissue is proportional to the K concentration attaining a maximum also at 5 μM, with distinct suppression at 25 μM. The best growth occurred here with 5 μM of NAA and 5 μM of K, and the fresh weight per piece of tissue amounted to 4.52 g. This is somewhat higher than on the optimal concentration of IAA in combination with BAP or K.

Interaction of NAA with BAP. Growth interaction of NAA with BAP is shown in Fig. 4. Here, too, the fresh and dry weight gradually increases, reaching a maximum at 5 μM of BAP. Five times higher concentration of BAP resulted in distinct suppression of the fresh and dry weight. In NAA conc. ranges tested in combination with BAP, optimal appears to be the medium containg 5 μM of BAP with 5 μM of NAA, on which the tissue attained a weight of 6.05 g.



Fig, 4. Influence of NAA and BAP on the growth on tomato callus

Thus, of all the investigated auxins and cytokinins and their concentration tested, this one yielded the best results.

The tissue yields increased in parallel, attaining maxima at 5 μM of cytokinins with 5 μM of NAA or 25 μM of IAA, i.e. at a ratio of 1:1 or 1:5 respectively. Strict correlation between cytokinin concentration and tomato tissue growth suggests its use as yet another material for cytokinin biotests (Rogozińska and Skutnik 1973).

Dry weight percentages of the tissues

In order to characterize more precisely the influence of individual auxins and cytokinins at 1:1 ratio (5 μM), the percentage of dry weight of the tissue grown on the individual media was determined.

Table 1

Influence of growth substances (5 μM) on dry weight (in %%).

Medium	Fresh weight g/piece	Dry weight g/piece	% of dry weight content
Control	0.051	0.004	8.1
K	0.086	0.006	7.5
BAP	0.093	0.007	7.4
IAA	0.136	0.010	7.2
NAA	0.230	0.016	6.9
K+IAA	1.596	0.090	5.6
BAP+IAA	3.858	0.162	4.2
K+NAA	4.555	0.186	4.0
BAP+NAA	6.110	0.221	3.6

Initial fresh weight of a piece was 40 mg ±2.5 in all cases.

As indicated by the results of Table 1 and Fig. 5, tissues grown without growth substances increased their weight but insignificantly. On medium containing 5 μM of K or BAP, the tissue showed very poor growth which, however, was somewhat better on medium with BAP. Auxins stimulated tissue growth slightly more than cytokinins, and NAA was more effective than IAA.

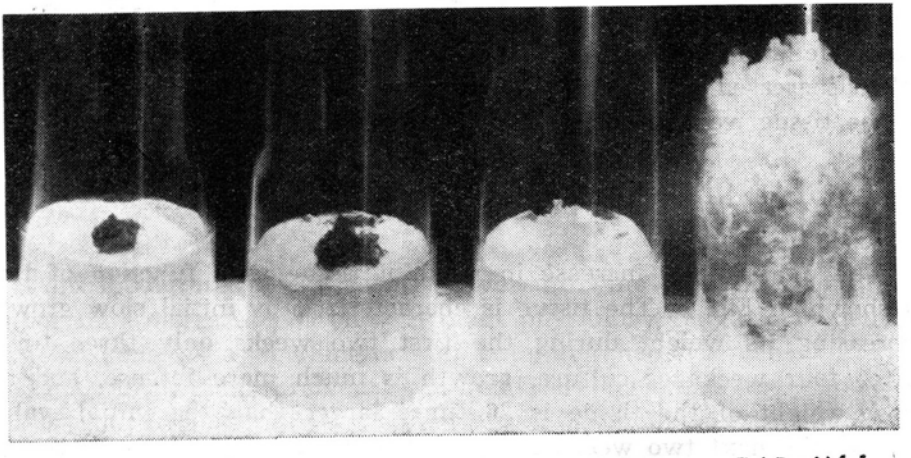


Fig. 5. Synergistic interaction of NAA (5 μM) with BAP (5 μM) in the growth of tomato callus

Addition of both an auxin and a cytokinin to the medium leads to callus formation the rate of which depends on the growth substances tested. They showed synergism in their interactions. The lowest growth was obtained as previously on medium with IAA and K, and twice larger growth on medium containing IAA and BAP. The yield of fresh weight on medium with NAA and K, was 3 times higher than that on media with IAA and K. The best tissue growth was obtained with NAA and BAP. The weight of the tissues grown on this medium was almost four times higher than that on IAA and K. Hence, for optimal callus growth, auxins as well as cytokinins are necessary.

The percentage of dry weight of the tissue depends on the growth substances tested. The highest dry weight content was found in tissue grown on control medium (8.1%), the lowest on medium with optimal concentration of BAP and NAA (3.6%). Thus, the water content is proportional to the growth intensity of the tissues.

Morphological description of the tissues

Fig. 5 reflects some of the differences in the exterior appearance of the tissue depending on the medium used. On control medium or on medium containing cytokinins only, the weight of the tissue increased slightly and this was accompanied by its browning. However, on medium with an addition of auxine alone, on which minimal growth occurred, the tissue was lighter and brown patches were not numerous.

The characteristical appearance of the tissues, depending on cytokinin concentration, is apparent at all auxin concentration. On medium containing from 0.0016 — 0.2 μM of cytokinin, the tissue was pale green, showing only a few brown spots. In the range from 1 — 5 μM, the tissue was pale green with a yellowish undertone. The intensity of green colour increased with cytokinin concentration up to 25 μM. The structure of the tissue was irregular with soft and fragile consistence.

Growth dynamics of the tissue

The quantitative increase in yield of tissue as a function of time is shown in Fig. 6. The tissue is characterized by initial slow growth, increasing its weight during the first two weeks only three times. After four weeks of culture, growth is much more intense, and the fresh weight of the tissue is 26 times larger than the initial value. During the next two weeks the period of maximal fresh and maximal dry weight increase, the weight increases 160 times amounting to 6.10 g. During the last two weeks of culture a slackening in growth intensity takes place. The dry weight, however, still increases slightly (by 4%)

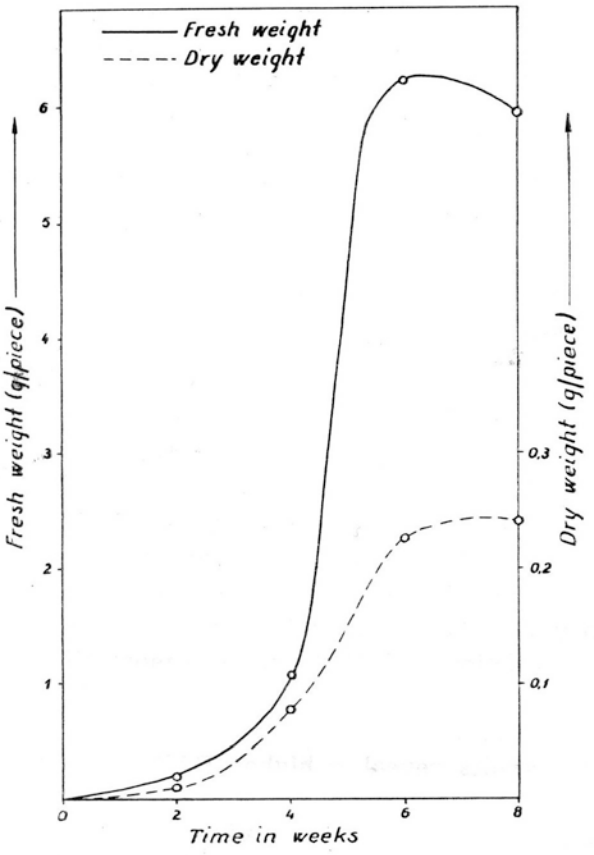


Fig. 6. Fresh and dry weight yields of tomato callus cultures as a function of time

in comparison with the value obtained after 6 weeks, but the fresh weight begins to decrease (by 3%). This can indicate the onset of senescence.

DISCUSSION

Anther cultures are presently given growing attention, and their importance increases not only in plant physiology but also in genetics and breeding as a new method of obtaining haploid plants (Zenkteler, 1972). This problem is a relatively new one and papers on callus and haploid plants derived from anthers are available for several species only.

Using Linsmaier and Skoog (1965) medium, suitable for tissue culture of many plants, growth substance requirements were established for optimal growth of tomato callus derived from anthers.

When auxins or cytokinins were applied separately with the nutrients, very little growth of the callus occurred. If however cytokinin was incorporated into the agar medium along with auxin, large callus mass was obtained. The growth rate of the tomato tissue is slow at the beginning, attaining after four weeks only 20% of its maximal growth achieved after six weeks. Tobacco tissue, for example, after four weeks of growth attains already 80% of its maximal growth, achieved after five weeks (Rogozińska, 1966).

Numerous data are known concerning the interaction of auxins and cytokinins in various plant tissues grown in vitro (Steward and Krikorian, 1971; Wareing and Phillips, 1970). It is well established that the cytokinin : auxin ratio is decisive with regard to the type of growth. A high cytokinin to auxin ratio favours bud formation, whereas high auxin to cytokonin ratio favour root formation, or promote unorganized callus growth. Best callus growth was obtained on medium having 1:5 ratio of cytokinin and IAA, or a 1 to 1 ratio of cytokinin and NAA, indicating the optimal level for cytokinesis. IAA alone does not cause proliferation of the tissue whereas its interaction with BAP is better than with K. NAA is a more active auxin and its interaction with BAP was also more effective than with K. Thus, if the proportion of auxins and cytokinins was varied, the pattern of growth was altered.

Our present results reveal a higher BAP activity than that of K in growth promotion of tomato tissue, in agreement with the data obtained in earlier bioassays (Rogozińska, 1969). The reason for this higher BAP activity as compared to K is as yet unclear from the physiological and biochemical point of view. It may well be due to the benzene ring of BAP as compared to the furfuryl ring of K, obviously having a different pattern of metabolism. It has been suggested that natural cytokinins become attached to specific transfer RNA's and are thus active in RNA metabolism. Among auxins, NAA proved to be more active than IAA; this is also related with the specific structure of its molecule and, consequently, with the mechanism of its action in the cell. Some hypotheses are able to explain the relative activities of various auxins as apparently due to effects of substitution in the ring on the position and size of the positive charge of the auxin molecule. Neither the nature, nor the location within the cell, of the receptor molecule is presently known (Wareing and Phillips, 1970). Thus, various auxins and cytokinins modify the metabolism of the cell in different ways affecting the balance between cell division and cell enlargement.

The addition of pea extract to modified White's medium required for good callus growth was reported by Fukami and Mackinney (1967) and Ulrich and Mackinney (1969). Our present results

indicate that, by using well defined minimal media, good callus growth can be achieved. This suggests that there is apparently a considerable diversity in the nutritional requirements of tissues from different tomato varieties or even from different locations within the plant.

Acknowledgement: The assistance of mgr A. Wdowińska in isolation of anthers is gratefully aknowledged.

REFERENCES

Fukami T. and Mackinney G., 1967. Culture of tomato callus tissue. Nature 213: 944—945.

Gresshoff P. M. and Doy C. H., 1972. Development and differentiation of haploid *Lycopersicon esculentum* (Tomato), Planta 107: 161—170.

Krzyśko K. and Rogozińska J. H., 1974. Różnicowanie pylników pomidora w hodowli in vitro. In press.

Linsmaier E. and Skoog F., 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18: 100—127.

Norton J. P. and Boll W. G., 1954. Callus and shoot formation from tomato roots *in vitro*. Science 119: 220—221.

Nysterakis M. F., 1961. Les fragments de fruits de tomates, de tous âges mais encore vertes, croissant *in vitro* dans des milieux très simples. C. R. Acad. sci. 252: 1653—1655.

Nysterakis M. F., 1961. Formation de plantules au sein des tissues de tomates cultivés *in vitro*. C. R. Acad. sci. 253: 1006—1008.

Rogozińska J. H., Helgeson J. P., Skoog F., Lipton S. H. and Strong F. M., 1965. Partial purification of a cell-division factor from peas. Plant Physiol. 40: 469—476.

Rogozińska J. H., 1966. Biotest ilościowego oznaczania kinetyny i jej analogów w zakresie stężeń minimalnych. Acta Soc. Bot. Pol. 35: 425—435.

Rogozińska J. H., 1969. Struktura a aktywność cytokinin. Post. Biochem. 15: 435—445.

Rogozińska J. H. and Skutnik L., 1973. Interaction of auxins and cytokinins in the growth of tomato callus. The 8th International Conference on Plant Growth Substances, Tokyo, collected abstracts, 140.

Steward F. C., and Krikorian A. D., 1971. Plants, chemicals and growth. Academic Press, New York and London. 232 pp.

Ulrich J. M. and Mackinney G., 1969. Callus cultures of tomato mutants. Physiol. Plantarum 22: 1282—1287.

Wareing P. E. and Phillips I. D. J., 1970. The control of growth and differentiation in plants. Pergamon Press, Oxford. 303 pp.

Zenkteler M., 1972, Hodowla *in vitro* pylników nową metodą uzyskiwania roślin haploidalnych. Postępy Nauk Roln. 5: 17—26.

Author's address:
Dr Janina H. Rogozińska
Mgr Leokadia Skutnik
Agricultural Academy,
ul. Bernardyńska 6,
85-029 Bydgoszcz, Poland

Wpływ auksyn i cytokinin na wzrost kalusa uzyskanego z pylników pomidora

Streszczenie

Przebadano współdziałanie substancji wzrostowych celem uzyskania optymalnego wzrostu kalusa z pylników pomidora. Stosowano pożywkę Linsmaier i Skooga (1965) z różnymi stężeniami auksyn (IAA i NAA) oraz cytokin (K i BAP).

Wykazano, że cytokinina jest niezbędna dla wzrostu kalusa bez względu na poziom stosowanej auksyny. Optymalne stężenie auksyny w kombinacji z cytokininą wynosi 5 μM NAA lub 25 μM IAA, a cytokininy 5 μM zarówno dla K jak i BAP. Wzrost na pożywkach z NAA i cytokininą był lepszy niż na IAA z cytokiną i wynosił ponad sześć gramów na tkankę, przy 5 μM NAA i BAP. Tkanka hodowana na tej pożywce charakteryzuje się najwyższym uwodnieniem. W początkowym okresie hodowli wykazuje ona słaby wzrost, osiągając maksymalny przyrost świeżej masy dopiero po 6 tygodniach hodowli.

Instytut Przyrodniczych Podstaw Rolnictwa
Akademia Rolnicza w Poznaniu, Filia w Bydgoszczy