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THE EFFECT OF GROWTH REGULATORS AND CULTURE CONDITIONS ON THE CALLUS INDUCTION IN TOMATO *Lycopersicon Sp.*

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Abstract. Effect of auxins: (NAA, IAA), cytokinin BAP and culture conditions (light, darkness) on callus induction in cotyledons of tomato cultivar 'Maskotka' and the wild form of *Lycopersicon peruvianum* was investigated. Callus was obtained in all experimental combinations, except of the culture with *L. peruvianum* on the medium with 1.0 mg·dm^{-3} of IAA. Callus weight, colour and structure depended on the tomato genotypes and experimental conditions. Best medium for the culture of tomato cultivar 'Maskotka' and the wild form *L. peruvianum* proved to be MS medium supplemented with 2.0 mg·dm^{-3} of IAA and 1.0mg·dm^{-3} of BAP.

Key words: tomato, cotyledons, callus, growth regulators, light, darkness

INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.) is an important subject of studies for geneticists and breeders. For geneticists it became a model plant due to the well investigated and relatively small genome (0.7–1.0 pg). On the other hand breeders, due to its considerable economic value, continuously search for new genomes determining a higher quality as well as resistance to diseases and unfavourable environmental conditions. With this aim, callus cultures are often used, as they are commonly considered an important source of genetic variability [Gamborg et al., 1974; Ling et al., 1998; Meyers and Simon 1999; Soniya et al., 2001].

The initiation of callus cultures and their use in future organogenesis or embryogenesis depends on numerous factors [Brown 1990; Brown and Charlwood 1990; Malik an d Bach 2004; Monnier 1990; Nabiałkowska et al., 2004; Skucińska 2001; Szybka-Hrynkiewicz and Janeczko 2004]. Thus each time they need to be determined experimentally.

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The present studies aimed at determining the effect of the tomato genotype, type of original explant and hormonal composition of the medium on the induction and stability of the callus tissue obtained.

MATERIAL AND METHODS

The experiment was conducted on a cultivated variety of tomato *L. esculentum* cultivar 'Maskotka' and its natural form – *L. peruvianum*. The seeds were obtained from the Wiesław Legutko Seed Breeding Plant ('Maskotka') and the Tomato Genetics Resource Centre, Department of Vegetable Crops, University of California, Davis, USA (*L. peruvianum*). Dry seeds were sterilized for 10 minutes in 7% sodium hypochlorite, twice rinsed in sterile, distilled water and placed in twist-top jars (10 in each) with 30 ml of medium MS [Murashige and Skoog 1962] with 3% sucrose and 0.6% agar. After 14 days two types of explants were taken from the seedlings: apical meristems (30 explants each form) and cotyledon fragments, 0.5 cm^2 in area (50 explants each form). The callus was induced on a MS medium containing only auxin IAA (indolilo-acetic acid) or NAA (naphtyl-1-acetic acid) in concentration: 1.0 and 2.0 $mg \cdot dm^{-3}$ or together with cytokinin BAP (6-benzylaminopurine) in concentrations 1.0 $mg \cdot dm^{-3}$ (1:1 or 2:1 ratio, tab.1). The pH of the media was adjusted to 5.7 prior to autoclaving at 121°C for 20 min.The initial cultures were placed in a fitotron, in darkness, at a temperature of 25°C or in a light of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature of 25°C, a 16 hour photoperiod and humidity of 70–80%.

The percent of explants creating a callus, its structure, weight, colour and the number of shoots and roots was determined 4–6 weeks after starting the cultures. The meristem cultures proved of no value for the induction of the tomato callus (no induction or a rapid decay). For this reason only the callus cultures induced on cotyledon fragments are described further.

The green coloured callus, obtained in the presence of light, was moved onto a fresh medium containing only 1.0 $mg \cdot dm^{-3}$ of auxin NAA or IBA (indolilo-3-butyric acid) or the same auxins together with cytokinin BAP in a 1:0.5 ratio. Two weeks later the weight and colour of the callus was determined. Each type of medium was represented by 100 fragments of the primary callus weighing 0.05 g.

RESULTS

Forming of the callus was induced on cotyledon fragments of both tomato forms after 4–6 weeks. In case of the cultures of 'Maskotka' cultivar maintained in light, the forming of the callus was observed on 28–60% of the explants , while in cultures maintained in darkness on 15–38%. In the case of *L. peruvianum,* these values were 5–75% and 15–35%, respectively (tab. 1).

The effectiveness of the callus induction depended on the type of growth regulators used and conditions in which the cultures were maintained. Light stimulated the development of the callus. In the case of *L. peruvianum,* thegreatest number of callus creat-

Table 1. Influence of different growth regulators proportion and culture condition (light – darkness) on callus formation from cotyledons explants of tomato *Lycopersicon peruvianum*

Tabela 1. Wpływ różnych proporcji regulatorów wzrostu i warunków kultury (światło – ciemność) na formowanie się kalusa na fragmentach liścieni odmiany pomidora 'Maskotka' i formy naturalnej *Lycopersicon peruvianum*

Growth regulators Regulatory wzrostu (mg · dm^{-3})	'Maskotka' cv.				L. peruvianum			
	light – światło		darkness – ciemność		light - światło		darkness – ciemność	
	explants forming callus (%) eksplantaty formujące kalus	callus structure struktura kalusa	explants forming callus eksplantaty formujące kalus, %	callus structure struktura kalusa	explants forming callus eksplantaty formujące kalus, %	callus structure struktura kalusa	explants forming callus eksplantaty formujące kalus, %	callus structure struktura kalusa
Control – MS without growth regulators Kontrola – MS bez regulatorów	20	loose – luźny	8	loose – luźny	40	loose – luźny	28	loose – luźny
IAA+BAP (1:1)	53[1]	compact – zbity	20	compact – zbity	53	compact – zbity	35	compact – zbity
NAA+BAP (1:1)	53	loose – luźny	38	compact – zbity	63	loose – luźny	30	loose – luźny
IAA+BAP (2:1)	60	compact – zbity	38	compact – zbity	75	loose – luźny	35	loose – luźny
NAA+BAP (2:1)	53	loose – luźny	15	loose – luźny	45	loose – luźny	35	loose – luźny
IAA (1:0)	35	compact – zbity	23	compact – zbity	0	–	25	compact – zbity
NAA (1:0)	53	compact – zbity	15	loose – luźny	45	compact – zbity	23	loose – luźny
IAA (2:0)	28	compact – zbity	23	compact – zbity	5	compact – zbity	15	compact – zbity
NAA (2:0)	45	loose – luźny	23	loose – luźny	45	loose – luźny	23	loose – luźny

[1] – percent in comparison of all explants, procent w stosunku do wyłożonych eksplantatów

Table 2. Comparison of callus derived from cotyledons of *Lycopersicon. peruvianum* on the light and darkness in respect of some its traits
Tabela 2. Porównanie kalusa inicjowanego z liścieni *Lycopersicon peruvianum* na świetle i w ciemności pod względem niektórych jego cech

Regulatory wzrostu Growth regulators mg · dm^{-3}	Callus weight (g) Masa kalusa			Shoots number Liczba pędów			Shoots length (cm) Długość pędów			Roots number Liczba korzeni			Roots length (cm) Długość korzeni		
	ś	c	d	ś	c	d	ś	c	d	ś	c	d	ś	c	d
Kontrol Control MS	0.08	0.02	0.06	1	0	1	3.20	0.00	3.20**	13	8	5	5.24	1.76	3.48**
IAA+BAP (1:1)	1.06	0.78	0.28	194	75	61**	1.14	0.75	0.39**	18	0	18	1.99	0.00	1.99**
NAA+BAP (1:1)	1.53	0.92	0.61**	26	0	26	1.70	0.00	1.70**	488	107	381**	3.18	1.67	1.51**
IAA+BAP (2:1)	0.72	1.02	-0.30	306	105	201**	0.84	1.52	0.32**	32	0	32	2.43	0.00	2.43**
NAA+BAP (2:1)	1.59	1.11	0.48**	20	0	20	0.89	0.00	0.89**	477	67	410**	2.09	1.33	0.76**
IAA (1)	0.00	0.44	-0.44**	0	5	-5	0.00	0.40	-0.40**	0	82	-82*	0.00	2.76	-2.76**
NAA (1)	0.69	0.84	-0.15	5	0	5	0.64	0.00	0.64**	277	80	197**	1.77	1.59	0.18**
IAA (2)	1.46	0.73	0.73**	8	5	3	2.19	0.94	1.25**	20	85	65*	3.93	1.09	2.14**
NAA (2)	0.93	1.45	-0.52**	0	0	0	0.00	0.00	0.00	112	131	-19	1.29	1.99	-0.70**

ś – light - światło; c – darkness - ciemność; d – differences – różnice
* Differences significant at p = 0.05; ** – at p = 0.01
* Różnice istotne przy p = 0,05; ** - przy p = 0,01

Table 3. Comparison of callus derived from cotyledons of tomato 'Maskotka' cv. on the light and darkness in respect of some its traits
Tabela 3. Porównanie kalusa inicjowanego z liścieni odmiany pomidora 'Maskotka' na świetle i w ciemności pod względem niektórych jego cech

Growth regulators Regulatory wzrostu mg · dm^{-3}	Callus weight Masa kalusa g			Shoots number Liczba pędów			Shoots length Długość pędów cm			Roots number Liczba korzeni			Roots length Długość korzeni cm		
	ś	c	d	ś	c	d	ś	c	d	ś	c	d	ś	c	d
Kontrola Control MS	0.19	0.23	- 0.08	0	0	0	0.00	0.00	0.00	4	0	4	7.80	0.00	7.80**
IAA+BAP (1:1)	0.58	0.18	0.40**	65	6	59**	1.23	0.95	0.28**	4	1	3	0.88	0.20	0.68**
NAA+BAP (1:1)	1.04	0.42	0.62**	5	0	5	0.62	0.00	0.62**	128	12	116**	2.07	1.98	0.09
IAA+BAP (2:1)	0.87	0.14	0.73**	80	19	61**	1.19	0.25	0.94**	14	0	14	1.99	0.00	1.99**
NAA+BAP (2:1)	0.97	0.38	0.59**	0	0	0	0.00	0.00	0.00	103	1	102**	1.63	0.40	1.23**
IAA (1)	0.27	0.16	0.11	0	0	0	0.00	0.00	0.00	129	33	96**	4.00	2.66	1.34**
NAA (1)	0.15	0.18	-0.03	0	0	0	0.00	0.00	0.00	18	0	18*	1.39	0.00	1.39**
IAA (2)	0.08	0.15	-0.07	0	0	0	0.00	0.00	0.00	17	37	-20*	5.40	1.15	4.25**
NAA (2)	0.38	0.22	0.16**	0	0	0	0.00	0.00	0.00	27	0	27**	0.99	0.00	0.99**

ś – light – światło; c – darkness - ciemność; d – differences – różnice
* Differences significant at p = 0.05; ** - at p = 0.01
* Różnice istotne przy p = 0,05; ** - przy p = 0,01

Table 4. Comparison of callus derived from cotyledons of 'Maskotka' cv. (L. e.) and *L. peruvianum* (L. p.) on the light and darkness in respect of some its traits (in % of control – mean from *L. peruvianum* i 'Maskotka')

Tabela 4. Porównanie kalusa inicjowanego z liścieni *Lycopersicon esculentum* odmiany 'Maskotka' (L. e.) i *Lycopersicon. peruvianum* (L. p.) na świetle i w ciemności pod względem niektórych jego cech (w % kontroli – średnia dla *L. peruvianum* i 'Mascotka')

Growth regulators Regulatory wzrostu mg · dm^{-3}	Callus weight Masa kalusa		Shoots number Liczba pędów		Shoots length Długość pędów		Roots number Liczba korzeni		Roots length Długość korzeni			
	light – światło		light – światło		light – światło		light – światło		light – światło		darkness – ciemność	
	L. e.	L. p.	L. e.	L. p.	L. e.	L. p.	L. e.	L. p.	L. e.	L. p.	L. e.	L. p.
Kontrola – Control MS	0,14g = 100%		0,51 = 100%		1,60 cm = 100%		8,50 = 100%		6,52cm = 100%		0,88cm = 100%	
IAA+BAP (1:1)	414	757**	35	153**	77	71	47	212	13	31**	23	0
NAA+BAP (1:1)	743**	109	82	180**	39	106**	1506	5741**	32	49**	225**	190
IAA+BAP (2:1)	621	514	27	200**	74*	53	165	376	31	37	0	0
NAA+BAP (2:1)	693	1136**	75	218**	0	56	1212	5612**	25	32	45	151**
IAA (1)	193	0	31	86**	0	0	1518	0	61	0	302	314
NAA (1)	107	493**	35	165**	0	40	212	3259**	21	27	0	181
IAA (2)	57	1043**	29	143**	0	137	200	235	83**	60	131	124
NAA (2)	271	664**	43	284**	0	0	318	1318**	15	20	0	226
Mean – Średnia	336	674	45	179	63	77	647	2393	35	37	145	198

* Differences significant at p = 0.05; ** – at p = 0.01

* Różnice istotne przy p = 0,05; ** - przy p = 0,01

ing explants (75%) was observed in light and on a medium containing auxin IAA and cytokinin BAP in a 2:1 ratio. A similar effect (60%) was obtained in the case of cultures of the 'Maskotka' cultivar maintained in light.

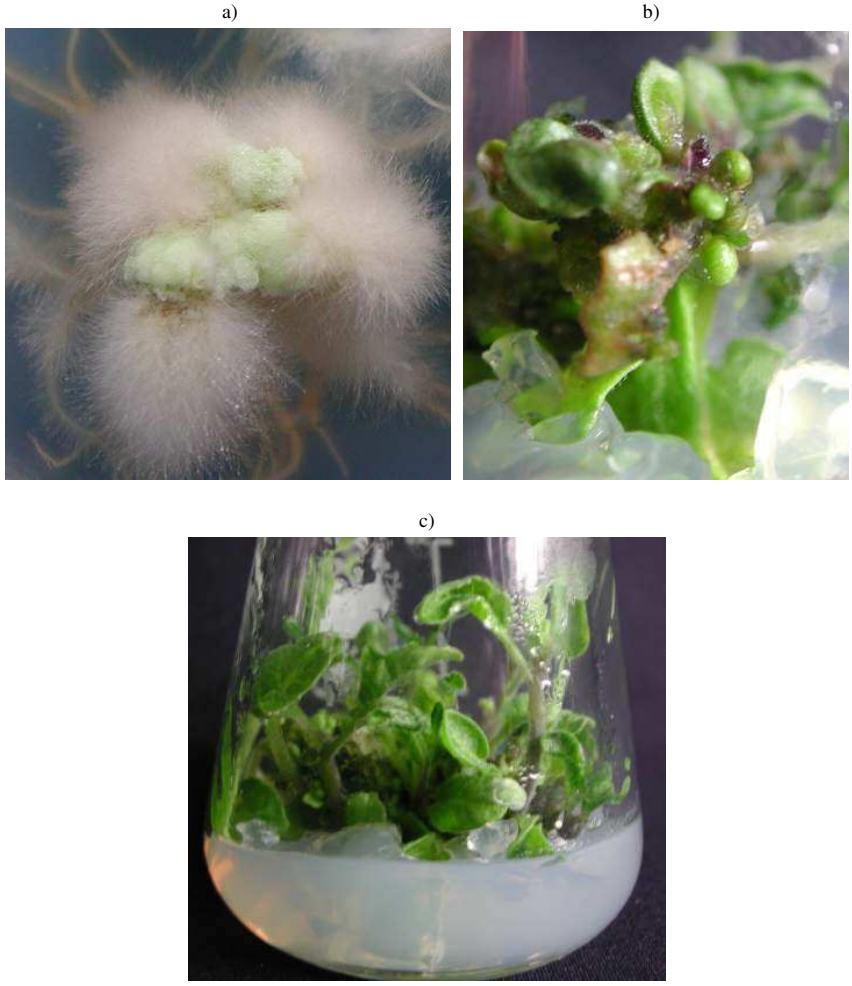
In the case of the 'Maskotka' cultivar, the callus created only individual dense nodules, white through white-brown and brown in colour, which tended to decay; the callus of the *L. peruvianum* was larger, often loose (friable), green in the light and from white to brown in darkness (phot. 1)

Phot. 1. Callus originated from cotyledons of *Lycopersicon peruvianum* (a – green, b – white, c – brown)

Fot. 1. Uformowany na liścieniach kalus *Lycopersicon peruvianum* (a – zielony, b – biały, c – brązowy)

The weight of the *L. peruvianum* callus induced in light ranged from 0.69 to 1.59 g and from 0.44 to 1.45 g, when induced in darkness (tab. 2). The greatest weight of the callus in cultures maintained in light were obtained on madia containing auxin NAA

and cytokinin BAP, in both proportions examined. In the case of cultures maintained in darkness, a callus of the highest weight was obtained on a medium containing 2.0 mg·dm^{-3} of auxin NAA (1.45 g) and NAA together with cytokinin in a 2:1 ratio (1.11 g). When the culture was maintained in light or in darkness, auxin NAA and cytokinin BAP in a 1:1 ratio had a stimulating effect on the forming of the 'Maskotka' callus (tab. 3).



Phot. 2. Callus with regenerated roots *of Lycopersicon peruvianum* (a), shoots (b) and plants (c).
Fot. 2. Zregenerowane z tkanki kalusowej *Lycopersicon peruvianum*: korzenie (a), pędy (b) i rośliny (c).

Table 5. Tomato callus multiplication under influence of different growth regulators proportion
Tabela 5. Namnażanie kalusa u pomidora pod wpływem różnych proporcji regulatorów wzrostu

No. Lp.	Species Gatunek	Growth regulators Regulatory wzrostu $mg \cdot dm^{-3}$	Callus weight after 2 weeks propagation Masa kalusa po 2 tygodniach namnażania g	Callus colour Barwa kalusa
1	L*ycopersicum. esculentum* cv. 'Maskotka'	NAA (1)	0,053 b	dark yellow – ciemnożółty
		IBA (1)	0,132 a	light yellow – jasnożółty
		NAA+BAP (1 : 0.5)	0,121 a	dark yellow – ciemnożółty
		IBA+BAP (1 : 0.5)	0,145 a	light yellow – jasnożółty
Mean Średnia			0,110 b	
2	Lycopersicum peruvianum	NAA (1)	0,203 c	light green – jasnozielony
		IBA (1)	0,756 a	light yellow – jasnożółty
		NAA+BAP (1 : 0.5)	0,305 bc	green cream – zielono-kremowy
		IBA+BAP (1 : 0.5)	0,456 b	yellow – żółty
Mean Średnia			0,450 a	

$LSD_{0.05}$ = 0.056; means followed by the same letter do not differ significantly
$NIR_{0,05}$ = 0,056; a, b, c– średnie oznaczone tymi samymi literami alfabetu nie różnią się istotnie

The callus formed on tomato cotyledons was of a morphogenic character. It included roots, shoots or rooted multi-plantlets (phot. 2, tab. 2 and 3). In both the tomato cultivars examined, the progress of morphogenesis depended on the culture conditions and growth regulators used (tab. 2, 3). Light stimulated the development of shoots and roots. In the case of *L. peruvianum* the greatest number of regenerated shoots was observed in a callus induced on a medium containing IAA and BAP in a 2:1 ratio (in light) or containing 2.0 mg dm^{-3} NAA (in darkness). Medium long shoots (3.20 cm) and longest roots (5.24 cm) were observed for the control combination cultures maintained in light. In the case of the 'Maskotka' cultivar, differences between shoots length obtained in light and in darkness were observed only in three experimental combinations with auxin IAA and cytokinin BAP in a 1:1 and 2:1 ratio and with NAA and BAP in a 1:1 ratio (tab. 3). The greatest number of shoots was observed in cultures maintained in light, when the medium contained IAA and BAP. A similar relation was observed for the length of shoots.

Light was also favourable for the induction of roots in tomato 'Maskotka' callus cultures. In such conditions the greatest number was formed on media containing 1.0 mg dm^{-3} of auxin IAA or NAA and BAP in both the proportions tested. In light the longest roots were obtained on a MS medium containing no growth regulators (7.8 cm) or containing auxin IAA irrespective of the dose used (in light), while in the darkness on a medium containing 1.0 mg dm^{-3} IAA.

The results obtained indicate that the efficiency of the callus induction depended on the genotype of the plant (tab. 4 and 5). On *L. peruvianum* explants the callus was in most cases green in different shades, while on the 'Maskotka' cultivar – most often brown with a tendency to decay. The *L. peruvianum* callus was as a rule characterised by a higher weight than in the case of 'Maskotka' and by better parameters for the shoots and roots obtained as result of a spontaneous organogenesis.

Taking into onsideration the efficiency of callus initiation and the progress of morphogenesis, one may accept that the combination of auxin NAA and cytokinin in a 2:1 ratio is optimum for its forming on *L. peruvianum* cotyledons, while the NAA and BAP combination in a 1:1 ratio – for the *L. esculentum cv*. 'Maskotka' (tab. 2, 3).

The explant genotype also had a significant effect on the ability of the callus to multiply as measured on the basis of the weight increase two weeks after placing it on the medium (tab. 5). In the case of *L. peruvianum*, the highest increase in the weight of the callus was obtained on a medium containing 1 mg dm^{-3} of auxin IBA, while for "Maskotka" – on a medium containing auxin IBA together with cytokinin BAP in a 1:0.5 ratio (tab. 5).

DISCUSSION

Callus cultures constitute currently an important source of genetic variability, which is of considerable importance in the breeding of many species of cultivated plants. Their induction depends on numerous factors – different for different cultivars and species [Brown 1990].

Auxins typically used for the callus induction are IAA, 2,4-D and NAA, cytokinins – kinetin, BAP and zeatin in various quantities and combinations, which must be each time determined experimentally. Among the important factors is also the choice of the basic medium and culture conditions (light or darkness). Also, the morphology of the callus can be determined by manipulating the growth regulators. Most often it depends on the auxin to cytokinin ratio in the medium [Brown 1990; Monnier 1990; Skucińska 2001].

Germinated seeds and young seedlings are an ideal material for callus induction. As explants can be used fragments of cotyledons, embryo roots and hypocotyls [Ahmed et al., 2001; Brown 1990]. In the case of beans for the callus induction on root fragments medium B5 was used [Ahmed et al., 2001; Gamborg et al. 1974], while on hypocotyl and apical meristems – medium MS with 1.0 mg·dm^{-3} of kinetin and 2.0 mg·dm^{-3} 2,4-D. The greatest quantity of callus formed on explants from roots and hypocotyl, the least – on shoot apex [Ahmed et al., 2001]. In studies conducted by Rzepka-Plevneš [2004] good results were obtained when cotyledon fragmentswere used, what corresponds with the results of studies conducted by Ling et al. [1998] on tomatoes and by Giovinazzo et al. [1993] on beans. The results reported by Ahmed et al. [2001] showed that in the case of beans, 1.0 mg·dm^{-3} of kinetin and 2.0 mg·dm^{-3} of auxin 2,4-D were the best hormones for inducing the callus.

In the present experiments the induction of the callus was performed using auxins IAA and NAA separately or together with cytokinin BAP in a 1:1 or 2:1 ratio. Two culture conditions were tested – light and darkness. The greatest quantity of the callus was obtained on fragments of *L. peruvianum* cotyledons on the MS medium supplemented with NAA and BAP in a 2:1 ratio or with only 2 mg·dm^{-3} of IAA. The *L. peruvianum* explants were characterised by a higher than in the case of *L. esculentum* ability to regenerate shoots and roots, the regeneration of shoots being observed only on media containing both auxin (IAA or NAA) and cytokinin BAP. The greatest number of roots in both species examined were obtained on media containing NAA and BAP in 1:1 or

2:1 ratios and on media containing only auxin NAA (1 or 2 mg·dm^{-3}). Relations were observed between the regeneration of roots and the ratio between auxins and cytokinins [Brown and Charlwood 1990].

Light proved indispensable for the initiation of organogenesis in the tomato callus. Meyers and Simon [1999], working on the regeneration of garlic through a callus induced in darkness on a modified B_5 media, observed a lowered ability of this species to regenerate on media containing no growth regulators. Similar results were obtained in the present experiment on tomato. On the control, MS medium maintained in light, no regenerated shoots were observed and only a small number of roots. In both tomato species light and the lack of growth regulators in the medium stimulated the elongonal growth of roots.

The callus texture is controlled genetically and often one may find it difficult to correctly determine the distribution of cells as caused by different conditions [Brown 1990; Skucińska 2001]. As a rule however, it is possible to improve the cell distribution by manipulating the composition of media in subcultures. In the present experiment, the ability to form a loose, tubercular and often green callus was observed on cultures of *L peruvianum*. At the multiplication stage, the green callus with good physiological characteristics was obtained on *L. peruvianum* explants on media MS supplemented with NAA (1.0 mg dm^{-3}) and NAA + BAP (1 : 0.5 mg dm^{-3}). The greatest increase of the weight of a light yellow callus was obtained on a medium containing 1.0 mg·dm^{-3} auxin IBA.

CONCLUSIONS

1. The effectiveness of callus culture induction depended on the type of growth regulators used and the culture conditions. In a majority of cases, its forming was stimulated by light and the combination of auxins and cytokinine BAP.

2. The characteristics of the callus (weight, colour, texture, ability to start organogenesis) depended on the genotype of the tomato, the culture conditions and medium composition.

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WPŁYW ROŚLINNYCH REGULATORÓW WZROSTU I WARUNKÓW PROWADZENIA KULTURY NA INDUKCJĘ KALUSA U *Lycopersicon peruvianum*

Streszczenie. W pracy określono wpływ auksyn (NAA i IAA) i cytokininy BAP oraz warunków kultury (światło, ciemność) na tworzenie się kalusa na liścieniach odmiany uprawnej pomidora 'Maskotka' oraz formy dzikiej *Lycopersicon peruvianum*. Indukcję kalusa obserwowano u obu badanych form niezależnie od składu hormonalnego pożywki, za wyjątkiem kombinacji, z auksyną IAA w ilości 1.0 $mg \cdot dm^{-3}$. Masa, zabarwienie i struktura otrzymanego kalusa zależały od genotypu badanych form pomidora oraz od warunków prowadzenia kultury. Inicjacja kalusa pomidora odmiany 'Maskotka' i *L. peruvianum* powinna odbywać się na pożywce MS uzupełnionej 2,0 mg dm^{-3} IAA i 1,0 mg dm^{-3} BAP.

Słowa kluczowe: pomidor, liścienie, kalus, regulatory wzrostu, światło, ciemność

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