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Original Article

Influence of Selected Antibiotics on the Tomato Regeneration in *In Vitro* Cultures

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Abstract

Generally, antimicrobial agents are frequently used in micropropagation techniques to obtain free elite clones or after genetic transformation to select putative transformants. Their successful application minimizes bacterial contamination however; they may be phytotoxic and may diversely affect the regeneration ability in plant tissue cultures. The objective of the current study was to estimate the effects of four antibiotics i.e. ampicillin, carbenicillin, cefotaxime and kanamycin on morphogenesis of three Polish tomato cultivars, cultured on MS medium with phytohormones. In this experiment the ability of tomato cotyledon explants to regenerate entire plants *via* indirect organogenesis was tested. Among four antibiotics tested, kanamycin was most harmful for the explants. This antibiotic, even at low doses (10-20 mg/L), inhibited tomato morphogenesis. On the other hand, the current study revealed significant influence of different concentrations of ampicillin, carbenicillin, cefotaxime on the frequency of bud formation. While the addition of cefotaxime at low concentration (100-200 mg/L) stimulated the bud formation, its increasing concentration adversely affected the organogenesis of tomato. The results clearly pointed out that carbenicillin and ampicillin at low concentrations (100-400 mg/L) were not only non-toxic, but they promoted bud regeneration. The obtained results show the crucial role not only of use of efficient antibiotics, but also of their proper doses in obtaining successful transformation and regeneration of tomato.

Keywords: aminoglycosides; β-lactam antibiotics; *Solanum lycopersicum*; tissue culture
Abbreviations: AMP: ampicillin; BAP-6: benzylaminopurine; CARB: carbenicillin;CEF: cefotaxime; IAA: indole-3-acetic acid; KM: kanamycin**;** MS: Murashige and Skoog medium; PAA: phenylacetic acid

Introduction

Efficient regeneration protocol is a crucial for successful genetic manipulation aiming at plant improvement. Techniques routinely used (agroinfection) to introduce foreign genes into a plant genome require the application of specific antibiotics (e.g. selectable or germicidal ones) (Kazemi *et al*., 2014; Sun *et al*., 2015). The vulnerability of plant tissue to antibiotics depends on a broad range of factors including species, genotype, explant type, culture conditions, so it is indispensable to verify the impact of given antibiotics on plant tissue, before the transformation procedure (Mamidala and Swamy Nanna, 2009; Farzaneh *et al*., 2013; Gerszberg *et al*., 2015b; Sun *et al*., 2015).

Antibiotics belonging to the β-lactam antibiotic group (e.g. ampicillin, carbenicillin, cefotaxime) and aminoglycosides (e.g. kanamycin) are most frequently exploited in plant transformation research (Kazemi *et al*., 2014; Sun *et al*., 2015). However, these antimicrobial agents can be not only toxic but they may also influence the regeneration response of a plant tissue (Sharma *et al*., 2012; Kazemi *et al*., 2014; Mahadev *et al*., 2014). Some literature data pointed out that antibiotics positively affected explant growth and/or shoot and root differentiation (Grzebelus and Skop, 2014; Meng *et al*., 2014). This phenomenon could be explained by the auxin-related structure of the β-lactam antibiotics which allows them to imitate the action of plant hormones (Grewal *et al*., 2006; Mahadev *et al*., 2014). This large class of antibiotics, which is chemically heterologous, is known to be active against both Gram-positive and Gram-negative bacteria. The influence of β-lactam antibiotics on *in vitro* cultures of different plant species (eg. *Oryza sativa,* hybrid aspens*, Centella asiatica* (L.), *Daucus carota*, *Brassica rapa* ssp. *pekinensis* (L.), *Tectona*

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grandis (L.)) (Grewal *et al*., 2006; Bosela, 2009; Grzebelus and Skop, 2014; Meng *et al*., 2014; Panathula *et al*., 2014; Tambarussi *et al*., 2015) *Cucumis melo* (L.) was investigated.

To the best of our knowledge, there is little information on the impact some of the aforementioned antibiotics on the tomato regeneration (Sharma *et al*., 2012). Moreover, most of the research considered only antibiotics at a low concentration range. Thus, the current study focused on the influence of the selected antibiotics used at a wide range of concentrations on organogenesis of different tomato cultivars.

Materials and Methods

Plant material

In the present study three commercially available cultivars ('Jokato', 'Frodo' and 'Poranek') of tomato (*Solanum lycopersicum* L.) were used. The chosen cultivars are popular and widely cultivated in Poland. The seeds purchased from PlantiCo Ltd. (Zielonki, Poland) were surface sterilized with 70% (v/v) ethanol for 2 minutes and next treated with 15% commercial bleach for 10 minutes. Subsequently, they were rinsed 4 times in sterile distilled water. The sterilized seeds were placed on ½ MS medium (1% sucrose, 0.8% agar, pH=5.8) (Murashige and Skoog, 1962) and cultured for 10 days.

Origin of explants and culture conditions

In the present investigation, cotyledons were tested as explants because they gave better morphogenic response in comparison to hypocotyls as it was described in our previous work (Gerszberg *et al*., 2016). Explants originating from cotyledons excised in a sterile way from 10-day-old seedlings and placed on MS basal medium (3% sucrose, 0.8% agar, pH= 5.8) supplemented with BAP (6-benzylaminopurine (2 mg/L) and IAA (indole-3-acetic acid) (0.2 mg/L) as well as on MS medium with the aforementioned phytohormones and the tested antibiotics, ampicillin (AMP), carbenicillin (CARB), cefotaxime (CEF) (each of them in concentrations: 100, 200, 300, 400, 500, 600, 800, 1000 mg/L) and kanamycin (KM) (0, 10, 20, 30, 40, 50 mg/L). The pH of all media was adjusted before autoclaving. The antibiotics as well as IAA were appended after the media were autoclaved when their temperature was approximately 55-60 °C. The cultures were placed under controlled conditions: at 23 °C under a 16/8 h (light/dark) photoperiod regime (3000 lux, approx.).

Data analysis

The data were recorded every four weeks while the regeneration characteristics were collected on a continuous basis. Each variant of treatment was performed in three replications. For one replication (in the case of KM) 72 explants (12 cotyledons per each variant of medium), were used. In the case of CEF, CARB and AMP – for one replication 108 explants were used. The frequency of shoot regeneration was evaluated for cotyledons that had been cultured for 30 days, as described in our previous work (Gerszberg *et al*., 2015b; Gerszberg *et al*., 2016).

Statistical analysis

All the values in this study were expressed as mean ± standard error (SE). The statistical analysis was performed with Statistica 10.0 software (Statsoft Poland) for repeated measurements. The statistically significant differences were also assessed by applying the Kruscal-Wallis test ($p < 0.05$).

Results

Control variants

In the control variants (explants grown on MS media +2 mg/L BAP + 0.2 mg/L IAA) callus induction was observed on the surface of all three tomato cultivars tested. The explants began swelling after 3-4-day culture and callus initiation started after 10-12 days at the cut edges. The visible callus was cream-yellow and green. Shortly thereafter bud formation on the obtained callus tissue was observed. Among the tested cultivars slight differences in bud frequency formation were recorded; the shoot regeneration efficiency varied from 92% for variety 'Poranek' to 94% for 'Frodo' (Tables 1-3) (no statistical differences between cultivars). The explants formed on average 1.4 to 1.8 buds, depending on the variety.

Influence of ampicillin on shoot regeneration and callus induction

Ampicillin added to MS medium with PGRs, regardless of its concentration, triggered callus formation in all cultivars (86%-100%) (no statistical differences between varieties). Moreover, the presence of ampicillin in the medium contributed to the accelerated formation of buds. They began to appear after 12-14 days compared to ~16 days for the control. However, the high rate of callus formation at all ampicillin concentrations used did not translate into an equally high ability to regenerate the shoots. For all three varieties, after treatment with the lowest ampicillin concentration (100 mg/L) shoot formation dynamics and the number of obtained shoots were similar to the control (Tables 1-3). With increasing concentration of this antibiotic gradual decrease in these parameters was observed. And at its highest concentration (1000 mg/L), organogenesis was found only on about 50% of explants (Tables 1-3).

Influence of carbenicillin on shoot regeneration and callus induction

Similarly to ampicillin, in case of carbenicillin frequency of callus formation was very high. Among three tested cultivars callus induction ranged from 92% to 100% and only slight differences in callus initiation connected with antibiotic concentration was found (for cultivar 'Poranek' and 'Jokato', no statistical difference between various carbenicillin content were found) (Tables 1-3). Moreover, carbenicillin supplementation positively influenced the process of bud formation, which began to appear after 13-14 days in comparison to ~16 days for the control variants. At these antibiotic concentrations from 100 mg/L to 400 mg/L, the organogenesis efficiency for all varieties was similar to that obtained in the control (no statistical

differences). For 'Frodo' and 'Jokato' varieties, the numbers of shoots obtained under these conditions were also similar to those in the controls. Only in the case of 'Poranek' cultivar, even slightly increasing concentration of carbenicillin (up to 200 mg/L) resulted in a decrease in the number of buds obtained. Further increase in the antibiotic concentration caused gradual decrease in bud formation frequency. The lowest efficiency (about 50%) was observed in the presence of 1000 mg/L carbenicillin, it was twice lower than in the (medium without antibiotic).

Influence of cefotaxime on shoot regeneration and callus induction

In contrast to these two abovementioned antibiotics, the concentration of cefotaxime in the medium influenced the percentage of callus formation. This was particularly evident in the case of 'Poranek' variety, where the induction of callus tissue fluctuated in the range of 49.6-100%. However, the observed differences between treatments were usually not statistically significant due to a large discrepancy between the results within one concentration (Tables 1-3). Additionally, supplementation of culture media with cefotaxime contributed to delaying callus formation by 2-3 days with respect to the control, and on the media with cefotaxime at the concentrations above 400 mg/L the callus culture began to brown with time. The highest response in bud regeneration frequency was observed at the lowest concentrations of this antibiotic (100-200 mg/L). However, the observed percentage of regeneration (60-70%) (depending on the cultivar) was significantly lower in the presence of cefotaxime compared to the control. Regardless of the variety, the increasing concentration of cefotaxime caused significant decrease in bud differentiation; starting from 600 mg/L, no buds were noticed on the explants (Tables 1-3).

Influence of kanamycin on shoot regeneration and callus induction

The addition of kanamycin had an adverse effect on the formation and differentiation of the callus tissue on tomato explants (Tables 1-3). In all media supplemented with the antibiotic, callus initiation was delayed by about 3 days, in comparison with the control. This antibiotic only at its two (for 'Poranek' and 'Jokato') or three (for 'Frodo') lowest

Table 1. The effect of different concentration of antibiotics on *Solanum lycopersicum* cv. 'Frodo', regeneration

Type and concentration of the antibiotics [mg/L]	Percentage of explants capable to organogenesis	Mean No. of buds per explants	Callus induction (%)
Control	94.17 ± 1.78ab	1.63 ± 0.065ab	99.31 ± 0.69ab
AMP 100	94.44 ± 5.56ab	1.41 ± 0.087bc	91.67 ± 4.82bc
AMP 200	86.11 ± 2.78b	1.35 ± 0.089bc	97.22 ± 2.78ab
AMP 300	91.67 ± 4.82ab	1.12 ± 0.059cd	97.22 ± 2.78ab
AMP 400	91.67 ± 4.82ab	1.15 ± 0.064cd	100 ± 0.0a
AMP 500	80.55 ± 2.78b	1.17 ± 0.073cd	97.22 ± 2.78ab
AMP 600	77.78 ± 10.03bcd	1.14 ± 0.069cd	94.45 ± 2.78abc
AMP 800	66.66 ± 12.74cd	1.17 ± 0.079cd	94.45 ± 2.78abc
AMP 1000	55.56 ± 7.36d	1.25 ± 0.102bcd	97.22 ± 2.78ab
CARB 100	88.89 ± 2.78b	1.59 ± 0.15abc	100 ±0.0a
CARB 200	94.44 ± 5.56ab	1.50 ± 0.14abc	97.22 ± 2.78ab
CARB 300	97.22 ± 2.78a	1.20 ± 0.08bc	94.44 ± 5.56abc
CARB 400	94.45 ± 2.78ab	1.44 ± 0.12abc	91.67 ± 4.82bc
CARB 500	86.11 ± 2.78b	1.03 ± 0.033cd	91.67 ± 0.0c
CARB 600	83.33 ± 4.82b	1.00 ± 0.0d	97.22 ± 2.78ab
CARB 800	63.89 ± 2.78cd	1.09 ± 0.061cd	94.44 ± 5.56abc
CARB 1000	55.55 ± 2.78d	1.05 ± 0.051cd	94.45 ± 2.78abc
CEF 100	66.67 ± 12.74cd	1.75 ± 0.19ab	100 ± 0.0a
CEF 200	66.67 ± 4.82cd	1.54 ± 0.16ab	97.22 ± 2.78ab
CEF 300	50.0 ± 14.45d	1.83 ± 0.19ab	91.67 ± 4.82bc
CEF 400	30.55 ± 2.78e	1.36 ± 0.15bc	91.67 ± 4.82bc
CEF 500	25.0 ± 4.82e	1.11 ± 0.11cd	94.45 ± 2.78abc
CEF 600	0.0 ± 0.0	0.0 ± 0.0	91.67 ± 4.82bc
CEF 800	0.0 ± 0.0	0.0 ± 0.0	83.33 ± 4.82c
CEF 1000	0.0 ± 0.0	0.0 ± 0.0	83.3 ± 4.82c
KM 10	52.78 ± 2.78d	1.47 ± 0.14abc	88.89 ± 7.36bc
KM 20	19.45 ± 2.78e	1.29 ± 0.18bcd	61.11 ± 10.03d
KM 30	2.78 ± 2.78f	2.00 ± 0.0a	11.11 ± 2.78e
KM 40	0.0 ± 0.0	0.0 ± 0.0	5.55 ± 2.78e
KM 50	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Results are the mean of three determinations ± SE. The means marked with the same letter do not differ significantly according to the Kruskal-Wallis, at 5% significance level.

Table 2. The effect of different concentration of antibiotics on *Solanum lycopersicum* cv. 'Jokato' , regeneration

Type and concentration of the antibiotics [mg/L]	Percentage of explants capable to organogenesis	Mean No. of buds per explants	Callus induction (%)
Control	93.06 ± 2.72ab	1.75 ± 0.07a	98.61 ± 1.39a
AMP 100	88.89 ± 5.56abc	1.78 ± 0.16a	91.67± 4.82ab
AMP 200	91.67 ± 4.82ab	1.36 ± 0.086ab	91.67 ± 4.82ab
AMP 300	88.89 ± 5.56abc	1.32 ± 0.087ab	97.22 ± 2.78a
AMP 400	83.33 ± 9.63abc	1.17 ± 0.07b	94.44 ± 5.56ab
AMP 500	75.0 ± 4.82bcd	1.11 ± 0.063b	94.44 ± 5.56ab
AMP 600	69.0 ± 5.56bcd	1.12 ± 0.068b	100.0 ± 0.0a
AMP 800	55.55 ± 14.71cdef	1.25 ± 0.102b	97.22 ± 2.78a
AMP 1000	52.78 ± 15.48cdef	1.21 ± 0.099b	91.67 ± 4.82ab
CARB 100	88.89 ± 5.56abc	1.91 ± 0.16a	97.22 ± 2.78a
CARB 200	97.22 ± 2.78a	1.66 ± 0.13ab	100.0 ± 0.0a
CARB 300	91.67 ± 8.34abc	1.64 ± 0.13ab	94.44 ± 5.56ab
CARB 400	86.11 ± 2.78bc	1.52 ± 0.11ab	94.45 ± 2.78ab
CARB 500	69.44 ± 5.56bcd	1.52 ± 0.10ab	97.22 ± 2.78a
CARB 600	55.56 ± 7.35de	1.4 ± 0.11ab	91.67 ± 4.82ab
CARB 800	52.78 ± 16.92cdef	1.21 ± 0.099b	91.67 ± 4.82ab
CARB 1000	52.78 ± 10.03cdef	1.15 ± 0.088b	94.44 ± 5.56ab
CEF 100	72.22 ± 2.78d	1.52 ± 0.02ab	94.44 ± 5.56ab
CEF 200	61.11 ± 5.56de	1.59 ± 0.17ab	83.33 ± 9.63abc
CEF 300	44.44 ± 7.36ef	1.69 ± 0.20ab	72.22 ± 12.12bc
CEF 400	27.78 ± 5.56f	1.2 ± 0.14b	72.22 ± 7.36bc
CEF 500	16.67 ± 0.0g	1.0 ± 0.0b	72.22 ± 10.03bc
CEF 600	0.0 ± 0.0	0.0 ± 0.0	72.22 ± 7.36bc
CEF 800	0.0 ± 0.0	0.0 ± 0.0	69.45 ± 14.72bcd
CEF1000	0.0 ± 0.0	0.0 ± 0.0	66.67 ± 17.47abcd
KM 10	50.0 ± 4.82e	1.39 ± 0.17ab	100.0 ± 0a
KM 20	38.89 ± 2.78ef	1.29 ± 0.13ab	55.55 ± 2.78c
KM 30	0.0 ± 0.0	0.0 ± 0.0	50.0 ± 8.34cd
KM 40	0.0 ± 0.0	0.0 ± 0.0	38.89 ± 2.78d
KM 50	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Results are the mean of three determinations ± SE. The means marked with the same letter do not differ significantly according to the Kruskal-Wallis, at 5% significance level.

concentrations triggered callus formation and organogenesis. It was the only one among the antibiotics used in our study which markedly inhibited callus formation. Prolonged treatment with high kanamycin concentrations (more than 20 mg/L) was lethal for explants. Kanamycin at the concentration of 50 mg/L (and in the case of 'Poranek' variety even of 30 mg/L) completely inhibited the formation of callus on explants. As it can be seen in the Tables 1-3, even the use of kanamycin at the optimal concentration (10 mg/L) decreased frequency of bud formation in comparison to the control variants. In presence of kanamycin at the concentration of 30 mg/L, only 2.78% bud frequency was noticed in the case of cv. 'Frodo', while the two other cultivars did not give any morphogenic response.

The results of regeneration (% of shoot regeneration, % of callus induction, and number of buds on explant) obtained for all cultivars were also correlated with the antibiotic type and concentration (Table 4). In all cases, very high negative correlations were observed between capability for organogenesis for all cultivars and antibiotics concentration (r between -0.86 and -0.97); the organogenesis ability of explants decreased with the increase antibiotics concentrations. Lower correlation coefficients were found for number of buds obtained on the explants in the case of all cultivars for ampicillin, in the case of 'Poranek' cultivar for carbenicillin and in the case of 'Frodo' cultivar for kanamycin. The greatest differences connected with the type of antibiotic and the variety used, concerned the ability to form callus. For example, lack of relationships were observed between % frequency of callus formation and ampicillin concentration for 'Frodo' and 'Jokato' cultivars (respectively, 0.1 and 0.03), while for the 'Poranek' cultivar, the percentage of callus induction was highly correlated with the ampicillin content (-0.87).

Discussion

A wide range of antibiotics is routinely used in culture media for plant tissue after genetic transformation to eliminate *Agrobacterium* or as a selective agent (Gerszberg, 2018). Since it was evidenced that antibiotics supplemented to culture media could have a positive or negative effect on morphogenetic processes in *in vitro* cultures (Grzebelus and Skop, 2014; Meng *et al*., 2014; Saporta *et al*., 2014; Naderi *et al*., 2016), accurate assessment of their effects on plant recovery is fully justified. Not only, must the effectiveness of an antibiotic in eliminating bacteria, but also its impact on

Table 3. The effect of different concentration of antibiotics on *Solanum lycopersicum* cv. 'Poranek', regeneration

Type and concentration of the antibiotics [mg/L]	Percentage of explants capable to organogenesis	Mean No. of buds per explants	Callus induction (%)
Control	92.42 ± 2.49a	1.39 ± 0.053ab	97.22 ± 1.88a
AMP 100	88.89 ± 2.78ab	1.28 ± 0.082ab	94.44 ± 5.56ab
AMP 200	91.67 ± 4.82ab	1.21 ± 0.073b	91.67 ± 4.82ab
AMP 300	88.89 ± 5.56ab	1.19 ± 0.071bc	88.89 ± 5.56ab
AMP 400	83.33 ± 4.82b	1.17 ± 0.07bc	88.89 ± 2.78b
AMP 500	72.30 ± 9.98bc	1.11 ± 0.063bc	86.11 ± 2.78b
AMP 600	63.89 ± 7.36cd	1.22 ± 0.09b	88.89 ± 2.78b
AMP 800	50.0 ± 4.82cd	1.24 ± 0.109abc	83.33 ± 9.63ab
AMP 1000	50.0 ± 12.74cd	1.11 ± 0.078bc	86.11 ± 2.78b
CARB 100	86.11 ± 2.78ab	1.39 ± 0.11abc	97.22 ± 2.78a
CARB 200	97.22 ± 2.78a	1.09 ± 0.049c	100 ± 0.0a
CARB 300	91.67 ± 4.82ab	1.06 ± 0.043c	94.44 ± 5.56ab
CARB 400	83.33 ± 9.63abc	1.06 ± 0.048c	97.22 ± 2.78a
CARB 500	75.0 ± 8.34bc	1.04 ± 0.038c	94.45 ± 2.78ab
CARB 600	72.22 ± 7.36bc	1.08 ± 0.054c	91.67 ± 4.82ab
CARB 800	58.33 ± 9.63cd	1.10 ± 0.067bc	91.67 ± 4.82ab
CARB 1000	52.78 ± 14.72cde	1.11 ± 0.074bc	94.44 ± 5.56ab
CEF 100	69.44 ± 19.47bcd	1.48 ± 0.104ab	94.44 ± 5.56ab
CEF 200	69.45 ± 14.72bcd	1.16 ± 0.076bc	83.33 ± 9.63ab
CEF 300	41.66 ± 8.34de	1.67 ± 0.19a	72.22 ± 12.12b
CEF 400	25.0 ± 4.82ef	1.22 ± 0.156bc	49.55 ± 22.49b
CEF 500	11.14 ± 2.82f	1.25 ± 0.25abc	75.0 ± 12.74b
CEF 600	0.0 ± 0.0	0.0 ± 0.0	72.22 ± 7.36b
CEF 800	0.0 ± 0.0	0.0 ± 0.0	69.45 ± 14.72b
CEF1000	0.0 ± 0.0	0.0 ± 0.0	69.44 ± 19.47ab
KM 10	41.67 ± 12.74cdef	1.67 ± 0.19a	72.22 ± 12.12b
KM 20	11.11 ± 5.56f	1.0 ± 0.0	5.55 ± 2.78c
KM 30	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
KM 40	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
KM 50	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Results are the mean of three determinations ± SE. The means marked with the same letter do not differ significantly according to the Kruskal-Wallis, at 5% significance level.

Table 4. Correlation coefficients (r) between antibiotics concentrations and different regeneration parameter for three cultivars of tomato

Parameter	Cultivar	AMP	CARB	CEF	KM
% explants capable to organogenesis	'Frodo'	**-0.94**	**-0.88**	**-0.94**	**-0.91**
	'Jokato'	**-0.97**	**-0.91**	**-0.93**	**-0.92**
	'Poranek'	**-0.96**	**-0.94**	**-0.91**	**-0.86**
Number of buds	'Frodo'	-0.62	**-0.84**	**-0.89**	-0.74
	'Jokato'	-0.72	**-0.96**	**-0.91**	**-0.92**
	'Poranek'	-0.65	-0.59	**-0.85**	**-0.90**
% callus induction	'Frodo'	-0.10	-0.53	**-0.93**	**-0.96**
	'Jokato'	-0.03	-0.69	**-0.85**	**-0.96**
	'Poranek'	**-0.87**	-0.69	-0.60	**-0.86**

Bold- strong correlation, red- lack of correlation

plant tissues be investigated. However, establishment of an efficient plant regeneration system in the presence of antibiotics, which is the first step to obtain modified plants, is still far from routine. This study presents the report evaluating the effect of four antibiotics (ampicillin, carbenicillin, cefotaxime and kanamycin) at different concentrations on bud regeneration in tomato cultures.

Kanamycin is one of the antibiotics most commonly used as selective agents in plant transformation process (Saporta *et al*., 2014; Sun *et al*., 2015), although a wide range of species is extremely sensitive to it (Zhang *et al*., 2001; Sharma *et al*., 2012; Mahadev *et al*., 2014; Meng *et al*., 2014; Gambhir *et al*., 2017). Our research revealed that among the antibiotics studied, kanamycin was most harmful for the explants. Even at the lowest concentrations, it significantly reduced callus and shoot formation of all tomato cultivars in comparison to the control. The explant response was completely inhibited for all cultivars by kanamycin at the concentrations of 50 mg/L. Similar results were obtained in lettuce (Ahmed *et al*., 2007), potato

(Khokan *et al*., 2009) or grapevine (Saporta *et al*., 2014). It is not surprising as that kanamycin belongs to aminoglycoside antibiotics, antibacterial agents that inhibit protein biosynthesis. For this reason, it could also decrease plant growth and regeneration ability (Katayama *et al*., 2003).

The other antibiotics tested (except cefotaxime in the case of 'Poranek' cultivar) strongly affected explant dedifferentiation but no significant concentration-related differences concerning callus frequency formation were observed. Similar results were obtained for Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) (Meng *et al*., 2014) and melon (*Cucumis melo* var. 'Gorgab') (Naderi *et al*., 2016). β-Lactams do not inhibit plant growth, because their mechanism of antibacterial activity is based on blocking the biosynthesis of peptidoglycan, one of the components of a bacterial cell wall, which is not present in plant cells (Shaikh *et al*., 2015). On the other hand, the present study showed significant influence of the used antibiotics and their concentration on the frequency of bud formation. This remains in line with the results of other authors showing that the presence of β-lactam antibiotics in the medium could be important for plant morphogenesis (Bosela 2009; Panathula *et al*., 2014; Tambarussi *et al*., 2015). In the present investigation, cefotaxime added to the culture medium strongly stimulated callus formation, however, its influence on tomato organogenesis was unfavourable, and with increasing concentration it inhibited bud formation frequency. Similar results were noticed in *Solanum viarum* ('Dunal') (Mahadev *et al*., 2014), *Centella asiatica* L. (Panathula *et al*., 2014) and Chinese cabbage (Meng *et al*., 2014). Moreover, correspondingly to our results, Meng *et al*. (2014) and Naing *et al*. (2014) showed phytotoxicity of cefotaxime, especially at high concentrations to *S. lycopersicum* explants. On the other hand, Gambhir *et al*. (2017) observed no significant influence of cefotaxime concentrations (0-500 mg/L) on cabbage (cv. 'Pride of India') regeneration potential. In contrast to our findings, the positive effect of cefotaxime on plant morphogenesis was shown for indica rice (Grewal *et al*., 2006) or melon (Naderi *et al*., 2016). However, Grewal *et al*. (2006) tested this antibiotic only at a very low concentration (100 mg/L). Additionally, Tambarussi *et al*. (2015) observed that cefotaxime, but also only at lower concentrations 100 and 300 mg/L stimulated morphogenesis of *Tectona grandis*.

In the present study, carbenicillin, which was the most useful antibiotic for *Solanum lycopersicum* organogenesis, revealed a double nature of action depending on its concentration. The positive effect of its lower concentrations (100-400 mg/L) on tomato morphogenesis was noticed. Similar findings were recorded by Grzebelus and Skop (2014) in carrot protoplast culture and by Mamidala and Swamy Nanna (2009) in tomato. The stimulating properties of carbenicillin, as well as of other β-lactam antibiotics, result from its chemical structure. Carbenicillin belongs to penicillin G group antibiotics and is degraded to phenylacetic acid (PAA), a natural auxin (Meng *et al*., 2014). Several studies indicated that auxins were required to induce callus formation and morphogenesis (Wei *et al*., 2003; Bosela, 2009). High callus induction ability was observed in our study for all concentrations of carbenicillin, and other antibiotics of this group. However, the higher concentrations of carbenicillin (800-1000 mg/L) decreased tomato capability of organogenesis. Bosela (2009) and Tambarussi *et al*. (2015) connected this with carbenicillin degradation model and the fact that the excess of its auxin-derivative degradation products could inhibit plant organogenesis. According to Tambarussi *et al*. (2015), β-lactam antibiotics strongly induce callus formation, but at high concentrations negatively affect shoot regeneration.

In the present investigation, ampicillin semisynthetic penicillin) exerted a similar effect on tomato morphogenesis as carbenicillin. Moreover, this antibiotic, especially at lower concentrations, was beneficial for bud regeneration of all used *S. lycopersicum* cultivars. Similar biological activities of these two antibiotics are due to their similar benzene ring-shaped structure. Our findings are coherent with Haddadi *et al*. (2015) results. However, Bosela (2009) and Meng *et al*. (2014) obtained different results concerning the morphological changes caused by these antibiotics in *Populus* sp. and *Brassica rapa*. On the other hand, it is known that the stimulatory effects of the tested β-lactam antibiotics may not only be attributed to their chemical properties which simulate plant PGRs action, but may also depend on the tested plant species and their genotypes. It could be connected with the difference in endogenous hormone levels among different species/cultivars and their different sensitivity to plant growth regulators.

Conclusions

Based on the literature data, it appears that the effect of antibiotics on regeneration largely depends on plant species and their cultivars. Moreover, kind of an antibiotic selected and its concentration are significant factors, because the presence of antibiotics in the medium could inhibit the regeneration of a plant. Our studies confirm the fact that the cultivar and the type of antibiotic as well as its concentration affect tomato regeneration. The obtained results showed that carbenicillin and ampicillin at low concentrations could be used as selection agents in *S. lycopersicum* cultures to control *Agrobacterium* growth. These antibiotics at the 100-400 mg/L concentrations were non-toxic to tomato cells and promoted bud regeneration. Additionally the compounds, especially carbenicillin, had a broad spectrum of activity and effectively eliminated most bacteria strains. On the other hand, the results clearly pointed to the unfavourable effect of high concentrations of ampicillin and carbenicillin on the tomato regeneration process. Therefore, determination of their right dose is crucial for future transformation and regeneration of this plant.

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