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Effect of Ceftriaxone and Timentin antibiotics on morphogensis in the *in vitro* culture of bread wheat *Triticum aestivum* L.

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Aim. To study the influence of antibiotics Ceftriaxone and Timentin on the morphogenic calli formation shoots regeneration and roots formation in two bread wheat varieties *Triticum aestivum* L., to study the effect of Ceftriaxone on the growth dynamics of plants. **Methods.** *In vitro* plant tissue culture, statistical methods: analysis of variance and correlation and regression analysis. **Results.** The influence of β-lactam antibiotics Ceftriaxone and Timentin on morphogenesis (morphogenic calli formation, shoots regeneration and rhizogenesis), was studied in apical 18-day-old wheat calli. Two wheat genotypes of with a different growth pattern, winter and winter-spring, were used. **Conclusions.** Timentin and Ceftri-axone affected morphogenesis in the bread wheat apical calli by stimulating morphogenic calli formation; Ceftriaxone additionally accelerated the appearance of the meristematic zones in the initial calli. The roots formation depended primarily on the wheat genotype, independently of an antibiotic applied. The presence of Ceftriaxone in the culture medium stimulated rooting and growth of regenerating plants as well as their biomass increment.

Keywords: Timentin, Ceftriaxone, morphogenic callus, shoots regeneration, root formation, *Triticum aestivum* L.

Introduction

Plant tissue and organ culture is a powerful instrument of modern biotechnology. Biotechnology of bread wheat, which is one of the main sources of nutrients in the diet of mankind, develops intensely during the last few decades. The wheat *in vitro* culture is still at a stage of the improvement of existing techniques and development of new methods. The morphogenetic processes and, in particular, regeneration, are here under special attention since regeneration in wheat like in many grasses is complicated.

Since the inception of plant cell, tissue and organ culture, antibiotics are widely used either as selective markers for genetic transformation (Kanamycin, Paromomycin, Hygromycin, *etc.*) or to eliminate any bacterial contamination (in particular during *in vitro Agrobacterium*-mediated transformation – Cefotaxime, Carbenicillin, *etc.*). Recently new generation antibiotics of the β-lactam group, such as Timentin and Ceftriaxone are used more often [1–11].

All researchers, which apply antibiotics when working with plant culture, report their effects on the morphogenetic processes. In some cases the antibiotics Timentin and Ceftriaxone inhibit the plant regeneration [3–5], in others – vice versa, increase it [6–13]. Accordingly, the objective of our study was to find out how the antibiotics Timentin and Ceftriaxone affect the morphogenic callus development, shoot regeneration and root development in the bread wheat, *Triticum* *aestivum* L. The effect of the antibiotic Ceftriaxone on the morphogenetic processes in wheat was studied for the first time, and the effect of Timentin has not been studied yet in bread wheat.

The plant sensitivity to antibiotics is not only species-specific, but also genotype-dependent; therefore in our study we used two wheat genotypes of different growth habit, winter wheat and winter-spring wheat. Although the β-lactam antibiotics are considered to be non-toxic to plants [12], many researchers have noted that their effect on the regeneration and morphogenesis may vary significantly depending on the plant species and the antibiotic concentration [14, 15]. Hence, in order to establish the pattern of action of the new generation antibiotics of β-lactam group on the morphogenetic processes (morphogenic callus development, shoot regeneration and rhizogenesis) in wheat, we used two antibiotics – Timentin and Ceftriaxone.

Materials and Methods

The effect of Timentin and Ceftriaxone on the morphogenic callus development (designated in tables and figures as "morphogenesis"), shoot regeneration and root development (development of the roots on the callus) was studied in the 18-day-old wheat calli of apical origin, obtained according to [16].

We used calli of two wheat genotypes – varieties Podolyanka (winter) and Zymoyarka (winter-spring), the seeds of which were kindly provided by the Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Kiev.

Previously, it was found that the highest regeneration frequency in the wheat callus of apical origin was observed on the medium MSR4 [16]: up to 36 % (average – 30.7±0.6 %) for Zymoyarka, and up to 33 % (average – 30.8±0.4 %) for Podolyanka. This medium has become the basic and simultaneously the control medium in our research.

For studying the effect of different concentrations of antibiotics, initial calli (18-day old) were placed (50 samples per Petri dish) on the regeneration agar medium MSR4 which contained: MS salts [17], Gamborg's vitamins [18], 20 mg/l sucrose, 10 mg/l $AgNO_3$, 0.5 mg/l BAP and 0.15 mg/l picloram [16]. Timentin or Ceftriaxone was added to the MSR4 medium at a concentration of 25 to 500 mg/l, in increments 25 mg/l. The calli were cultured at 24 °C and 16-hour photoperiod during 30 days, every 14 days the calli were transferred to the fresh medium. The experiments were conducted in three replicates.

The effect of antibiotics was assessed by three indicators (parameters):

– frequency of the morphogenic callus development, as a ratio of the number of callus explants with meristematic zones on 10^{th}–15^{th} day of cultivation, to the total number of initial callus explants,

– shoot regeneration frequency, as a ratio of the number of callus explants that formed shoots from 15^{th} to 30^{th} day of cultivation (counting was performed every 5 days), to the total number of initial callus explants,

– frequency of root development, as a ratio of the number of callus explants that formed roots on 15^{th}–30^{th} day of cultivation, to the total number of initial callus explants.

In addition to the study of the antibiotics effect on the morphogenetic processes in the initial callus, the effect of Ceftriaxone on the plant growth dynamics after the start of rooting was also studied. To study the growth dynamics, the shoots, obtained on the MSR4 medium supplemented with 400 mg/l of Ceftriaxone, were transferred to a rooting medium which contained: MS salt, Gamborg's vitamins, 10 g/l glucose, 20 g/l maltose and 0.15 g/l asparagine monohydrate, [19]. The control shoots, obtained on the MSR4 medium without adding the antibiotic, were transferred to the same medium. The antibiotics were not added in the rooting medium. Only those plants that had at least one root on 3^{rd} – 4^{th} day after transfer to the rooting medium were used in the experiment. That is, the zero-day is the day when a shoot formed the first root. Every five days after the appearance of the first root and up to 30 days, 5 control and 5 test plants were collected. The raw mass of shoot (the ground part) of a regenerated plant and its separated roots were measured. Also the length of shoot and roots was measured. Before weighing, the plants were washed from the agar medium and dried with a filter paper. After weighing, the plant material was placed in plastic bags with labels and dried by freeze-drying for 3–4 days (until constant weight) and re-weighted.

Relative increment was calculated for the wet and dry weights of plants and roots shoot length, root length by the formula

$\boldsymbol{RI=(x-x_0)/x_0}$, where $\boldsymbol{x}$ – the average growth rate value (root or plant dry or wet weight, root length, plant height), $\boldsymbol{x_0}$ – the average value of the index at the beginning of the experiment, or on the day of rooting.

When creating scripts for the statistical data analysis and graphical plots, the standard functions in a programming language R [20] of the version 3.0.2. were used. For values, expressed as a percentage, previous logit transformation was applied.

Results and Discussion

The results on the influence of transformation the antibiotics Timentin and Ceftriaxone on the morphogenetic processes (morphogenic callus development, shoot regeneration and root development) in two wheat cultivars compared to the control are shown in Fig. 1 and Tables 1 and 2 (Additional materials).

Morphogenic callus formation

On the MSR4 medium without antibiotics (control) the meristematic zones in the initial callus appeared on the 15th day of cultivation (DoC), with the morphogenic callus development frequency 55,8 ± 1,8 % for the cv Podolyanka and 76.6 ± 2, 1 % for the cv Zymoyarka (Fig. 1 A, B) (Table 1, 2 – Additional materials). If by the 15th DoC meristematic zones were not formed in the initial calli, it remained non-morphogenic until the end of the experiment (30 days).

When Timentin was added to the medium, meristematic zone in the initial callus also appeared on the 15th DoC, while with Ceftriaxone added – on the 10th DoC. The average frequency of the morphogenic callus development on the media supplemented with Timentin or Ceftriaxone shows significant increase, as compared to the control medium, in both cultivars (especially in the cv Podolyanka) (Fig. 1 A, B). The increased morphogenic callus development frequency is observed even at minimum concentrations of both antibiotics (25 mg/l), and starting with 100 mg/l of antibiotics, morphogenic callus development frequency reaches the value of > 90 % and remains as high irrespective of further increase of antibiotics concentrations.

Thus, we have shown that antibiotics Ceftriaxone and Timentin stimulate the morphogenic callus development, and Ceftriaxone additionally accelerates the appearance of the meristematic zones in the initial callus.

Shoot regeneration

The shoot regeneration dynamics were studied during the time interval from 15th to 30th DoC (Fig. 3, 4 – Additional materials). The first regenerated shoots appeared at the 21st DoC in the control medium, at the 18th – 20th DoC - when Timentin was added to the medium, and at the 15th DoC – when Ceftriaxone was added.

The regeneration frequency on the MSR4 medium without antibiotics averaged for cv Podolyanka 25,5 ± 2,4 %, and for cv Zymoyarka 26,5 ± 1,6 % (Fig. 1 C, D). (See also Table 1, 2 – Additional materials).

The average regeneration frequencies of both wheat cultivars on the medium supplemented with Timentin showed no significant changes, except for the slight tendency for decreased regeneration frequency on the medium with the antibiotic, regardless of its concentration.

Adding Ceftriaxone to the medium negatively affects the shoot development in the cv Podolyanka in general. However, in the cv Zymoyarka Ceftriaxone in concentrations of 25–300 mg/l leads to an increase of the shoot development frequency to 40 %–50 %.

So, unlike Timentin, Ceftriaxone has obvious genotype-dependent influence on the shoot regeneration in wheat (see also Fig. 5, 6 – Additional materials). The same concentrations of Ceftriaxone and Timentin have the opposite effect in cv Zymoyarka. For example, at a concentration of 75 mg/l, Timentin suppresses regeneration and Ceftriaxone – increases.

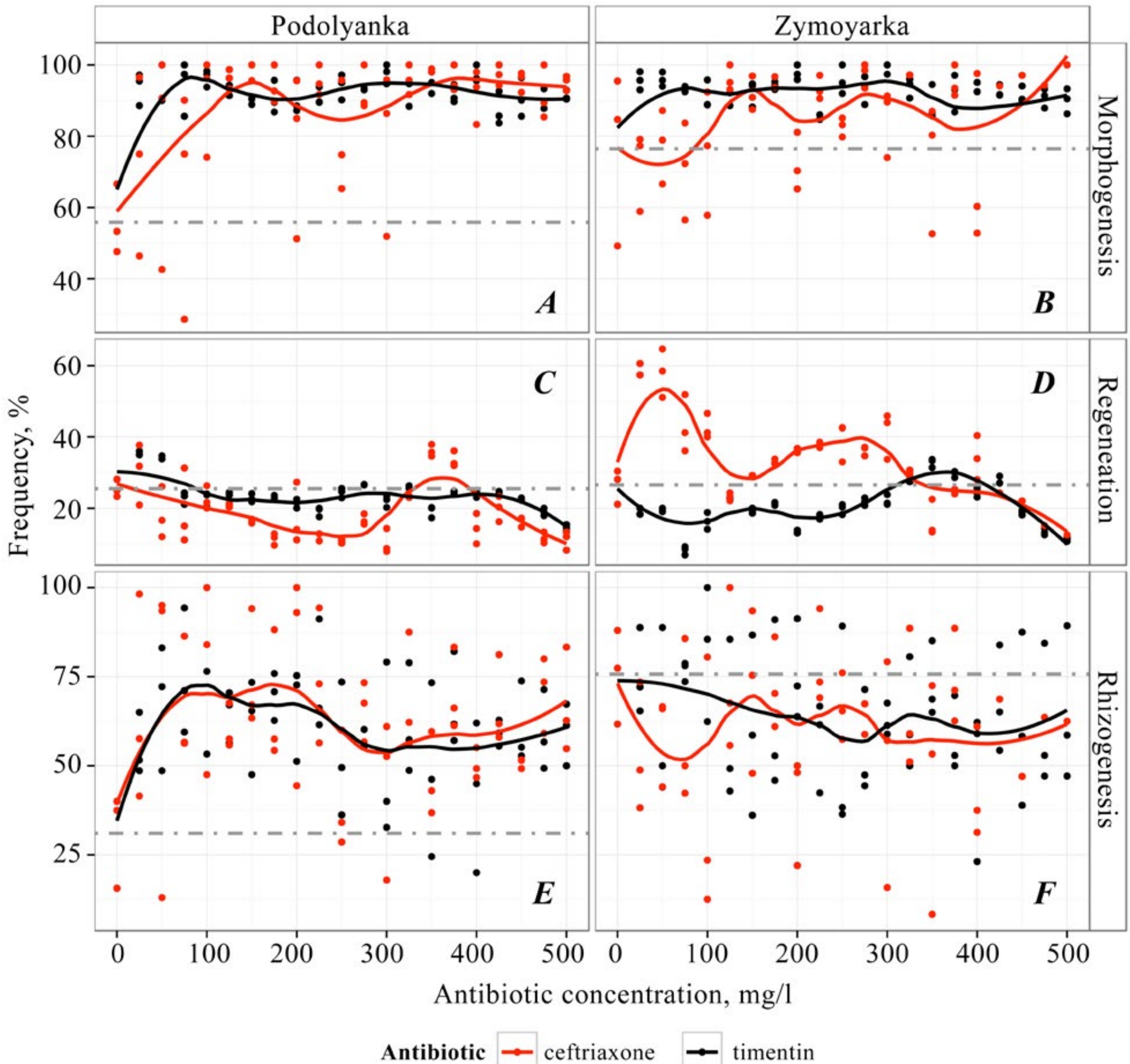

Fig. 1. Effect of the antibiotics Ceftriaxone and Timentin on the frequency of meristematic islands appearance (“morphogenesis”) in the initial callus, shoot regeneration (“regeneration”) and root development in two bread wheat cultivars, Podolyanka (A, C, E) and Zymoyarka (B, D, F).

A, *B* – “morphogenesis” frequency at 10th–15th days of cultivation (DoC) in MSR4 medium, C, *D* – shoot regeneration frequency at 15th–30th DoC, E, F - rhizogenesis frequency at 15th–30th DoC.

Legend: point – the experimental data; dash-dotted line - mean value obtained for the antibiotic-free medium; continuous line – smoothed conditional mean for morphogenic callus, shoots and roots development from callus.

The analysis of Variance confirmed the dependence of the regeneration frequency on several factors: the plant genotype ($p < 0.05$), the presence of the antibiotic in the medium, and in case of Ceftriaxone – the antibiotic concentration ($p < 0.001$).

Thus, to achieve a higher shoot regeneration frequencies, for every wheat genotype the specific β-lactam antibiotic and its concentration must be individually selected, but all tested concentrations of Timentin and Ceftriaxone (25–500 mg/l) in the medium accelerated shoot regeneration.

Rhizogenesis

The root development frequency (development of the roots from the original callus) on the the MSR4 medium without antibiotics averaged for cv Podolyanka 31,0 ± 1,6 %, and for cv Zymoyarka 75,7±2,7 % (Fig. 1 E, F). It should be noted that the root development frequency in the control was very high for cv Zymoyarka. (Table 1, 2 – Additional materials).

The analysis of averaged root development frequencies on the media supplemented with Timentin or Ceftriaxone showed that plant calli of cv Podolyanka and cv Zymoyarka reacted differently to the addition of antibiotics.

Adding Timentin to the medium significantly increases the frequency of rhizogenesis in cv Podolyanka, achieving 75 % at concentrations of 75–225 mg/l, and then decreasing to 55 % at concentrations of 250 mg/l and higher. In cv Zymoyarka all tested concentrations of Timentin steadily lower the frequency of rhizogenesis, as compared to the control medium, to a value of 55–60 %.

The analysis of the average frequencies of root development on the medium supplemented with Ceftriaxone showed that this antibiotic significantly increases the root development frequency in cv Podolyanka, and reduces – in cv Zymoyarka, almost regardless of its concentration.

The analysis of variance showed the dependence of the root development frequency on the antibiotic concentration in the medium ($p < 0.005$). The influence of other factors, and combinations thereof, on the rhizogenesis were within statistical error (Fig. 7, 8 – Supplementary materials).

Thus, the root development frequency depends primarily on the genotype – on the control medium it is high in cv Zymoyarka and low in cv Podolyanka. The antibiotics of β-lactam group, Timentin and Ceftriaxone, have the opposite effect on the root development frequency in two wheat cultivars.

It should be noted that under prolonged culture of the calli with developed shoots on the MSR4 medium supplemented with Ceftriaxone, the rooting process begins. While first roots appeared at 21st DoC on the control MSR4 medium, adding the antibiotics to the medium shifted the first roots development to the 20th day (350 mg/l of Timentin) or to the 15th–18th day (25–500 mg/l of Ceftriaxone).

Therefore, we can conclude that the antibiotic Ceftriaxone has a stimulating effect on the shoot regeneration and the plants regeneration in two wheat genotypes. It is possible for each genotype to select the concentration of the antibiotic that increases the regeneration frequency.

Growth dynamics

We observed that on the media supplemented with Ceftriaxone, regenerated shoots and plants grew faster than the control plants. They were larger, and were better adapted to the non-sterile growing conditions (Fig. 9 – Supplementary materials).

To confirm these observations, we measured a relative increase in the plant biomass, plant height, and root biomass and root length of regenerated plants. The results are presented in Tables 3 and 4 (Additional materials). The statistically processed data are presented in Figures 2 and 3 and in Tables 5–7 (Additional materials).

The graph of the relative increase in wet biomass (Fig. 2 A) can be divided into three stages: 1) exponential - from the beginning of the experiment until the fifth day after rooting, 2) linear - from the fifth to 20th–25th day, 3) exponential again - from the 20th–25th day until the 30th. During the 5th–25th day (Table 5 – Additional materials), there is a strong correlation (0.96-0.97) between the time of cultivation and the average relative increase of the wet plant biomass. The angle of slope of the plot to the horizontal axis in the area of the 5th–25th day is corresponding to an average relative growth rate (RGR) of wet weight. RGR for the media containing Ceftriaxone is higher than in antibiotic-free media for Podolyanka (0.24 *vs.*

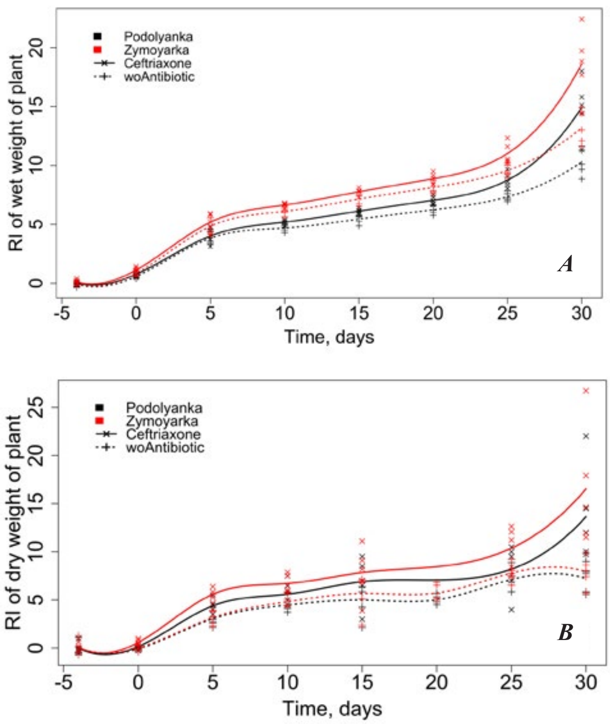

Fig. 2. The average relative increase (RI) of raw (A) and dry (B) mass of the shoots (ground part) of regenerated plants of two bread wheat cultivars, Podolyanka (black) and Zymoyarka (red), obtained on the media either with 400 mg/l Ceftriaxone (solid line and the mark "x") or without the antibiotic (cross-hatching line and the mark "+"), depending on the culture period. The experimental data of individual measurements and smoothed line through the averaged frequency values are shown.

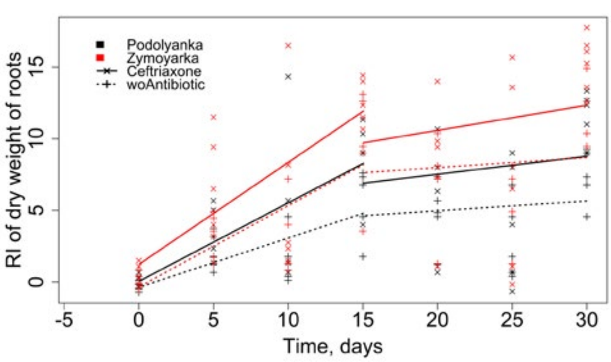

Fig. 3. Relative increment of the roots dry weight of regenerated plants in two wheat cultivars, Podolyanka (black) and Zymoyarka (red), obtained on the media either supplemented with 400 mg/l Ceftriaxone (solid line and the mark "x"), or without it (cross-hatching line and the mark "+"), depending on the culture period. The experimental data of the individual measurements and results of the linear approximation by the method of least squares are shown.

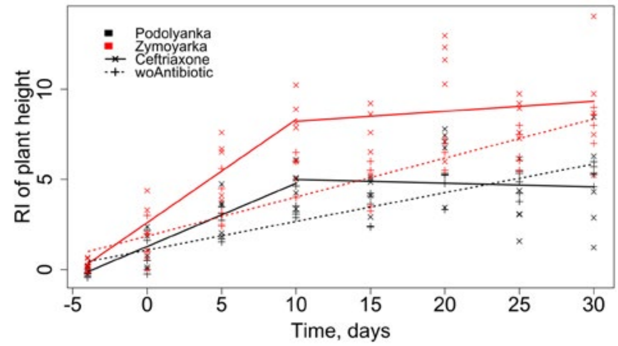

Fig. 4. The relative increment of regenerated plants height in two wheat cultivars, Podolyanka (black) and Zymoyarka (red), obtained in the media either supplemented with 400 mg/l Ceftriaxone (solid line and the mark "x"), or without the antibiotic (cross-hatching line and the mark "+"), depending on the culture period. The experimental data of individual measurements and linear approximation results by the method of least squares are shown.

0.17 day^{-1}), and for Zymoyarka (0.29 vs. 0.23 day^{-1}). The same is true for the dry biomass as well (Fig. 2 B), RGR for Podolyanka is 0.19 vs. 0.17 day^{-1} and for Zymoyarka – 0.24 *vs*. 0.20 day^{-1}, although the correlation coefficients are somewhat lower (from 0.61 to 0.79)

The root system in both cultivars was developed within 15 days after the first signs of rooting, regardless of the presence of the antibiotic in medium. The aver-

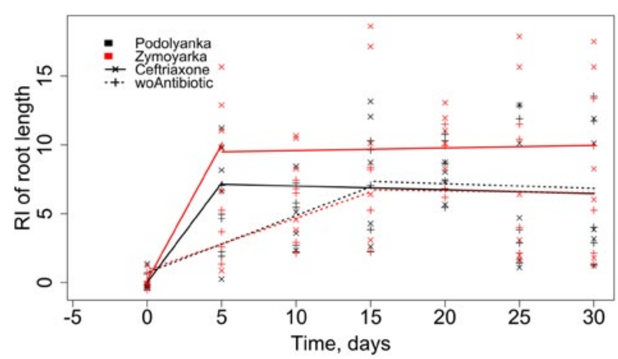

Fig. 5. The relative increment of the regenerated plants root length in two wheat cultivars, Podolyanka (black) and Zymoyarka (red), obtained in the media either supplemented with 400 mg/l Ceftriaxone (solid line and the mark "x"), or without the antibiotic (cross-hatching line and the mark "+"), depending on the culture period. The experimental data of individual measurements and linear approximation results by the method of least squares are shown.

age relative increment of the root system dry weight during the period was higher in the presence of Ceftriaxone for both Podolyanka (0.55 day^{-1}) and Zymoyarka (0.71 day^{-1}); on the antibiotic-free medium it was from 0.34 to 0.57 day^{-1}, respectively (correlation coefficient R = 0,70–0,77). Importantly, the growth termination of the root system dry mass was noted either in the presence of the antibiotic, or without it, which led to the development of more advanced root system on the medium containing Ceftriaxone. During the period from the 15th to the 30th day, the average weight of roots on the antibiotic-containing medium was 2.675 mg versus 2.225 mg for cultivar Podolyanka, and 2.875 mg versus 2.025 mg – for cultivar Zymoyarka. A similar pattern was observed for the wet roots weight.

Also the heights of plants grown on the medium without antibiotic and supplemented with Ceftriaxone were measured (data are presented in Tables 3 and 4 (Additional materials). The processed data of the regenerated plants height relative increment are shown in Figure 4 and Table 8 (Additional materials).

The plant height increase on the antibiotic-free medium was linear throughout the whole experiment (Fig. 4), as evidenced by the high correlation coefficients (0.90 and 0.91), despite the lower RGR: 0.16 vs. 0.35 for Podolyanka and 0.22 vs. 0.57 for Zymoyarka. Due to a prolonged growth period, the plants shoots height on the antibiotic-free medium and on the antibiotic-containing medium was almost similar at the end of the experiment.

The fastest shoot growth on the media containing Ceftriaxone was observed between the time of transfer to the rooting medium and up to the 10th day of cultivation (Fig. 4), after which the growth slowed down or suspended. The negative value of the average relative growth rate (RGR) of cultivar Podolyanka shoot length during the period from the 10th to the 30th day is due to the fact that the measurements were done not for the same plant during the experiment, but for randomly selected groups, and as from the 10th day shoot growth stopped, a plant having a lower height could get in the group, measured later.

A similar pattern was observed for the root length growth (Fig. 5) Table 9 (Additional materials). During the first 5 days, a rapid growth of roots from shoots grown on the media supplemented with antibiotic. The roots of shoots, grown on the medium without the antibiotic, grew slightly slower. Subsequently, the roots of shoots, which were grown on the antibiotic-free medium, grew at a slower rate.

Thus, it can be concluded that the presence of antibiotic Ceftriaxone in the medium for shoots development, stimulates both the growth of the regenerated plants and their biomass increase.

The antibiotics Timentin and Ceftriaxone, belonging to the β-lactams, are used to treat a range of infectious diseases in humans. These antibiotics are active *in vitro* against most gram-negative and gram-positive microorganisms. It was found that the β-lactam antibiotics cause lysis of bacteria by inhibiting such enzymes as transpeptidase and carboxypeptidase, catalyzing the synthesis of peptidoglycan [1].

A target for β-lactams in the plant body is not established yet. Most probably, the effect of antibiotics Timentin and Ceftriaxone on plants is due to their similar chemical structure to auxin [12]. It is considered that Ceftriaxone is able to induce the formation of phenylacetic acid, a weak natural auxin [15]. Thus, we can assume that the antibiotics Timentin and Ceftriaxone can imitate the plant growth regulators and act as weak auxins.

It is not possible to exclude the interaction of antibiotics and their degradation products with the endogenous hormones of plant cells [15, 21–23], or with the components of culture medium (BAP or picloram in this case).

Timentin is used in the plant *in vitro* culture for a long time. The first publications on the application of Timentin date back to 1997 [10]. Ceftriaxone became common in the *Agrobacterium*-mediated plant transformation rather recently [1, 2, 4, 5].

There is discordant evidence about the dependence of the effect of Timentin and Ceftriaxone on the morphogenetic processes in different plant species on their concentration.

It is known that the β-lactam antibiotics adversely affect the regeneration of explants cultured *in vitro*: Ceftriaxone at a concentration of 400 mg/l completely inhibits the blueberry shoots growth [4], 200 mg/l – suppresses the leaves, petioles and stems regeneration of ox knee (*Achyranthes bidentata*) [5]. The high concentrations of Timentin (500–1000 mg/l) in the culture medium, as well as all β-lactams, decrease the somatic embryoids regeneration in walnut [3].

In some cases Timentin (250 mg/l) has no negative effect on the callus growth, for example, in rice (*Oryza sativa* L.) [6]. There are reports that Timentin stimulates the morphogenetic processes in plants [7–13]. It increases organogenesis from leaf explants of tobacco *Nicotiana tabacum* (150 mg/l) [10] and the cotyledon of tomato explants (300 mg/l) [11], callus formation of mature wheat germ (50 mg/l) [9], shoots regeneration from strawberry leaf explants (300 mg/l) [13] and the regeneration of white ash (*Fraxinus americana*) (500 mg/l) [7], the efficient plant regeneration from protoplasts of three carrots cultivars (400–500 mg/l) [12] and the shoot induction in tomato (400 mg/l) [8].

It is established that the influence of Timentin on the regeneration processes depends on its concentration in culture medium [14, 15]: during the shoots regeneration of London plane tree (*Platanus acerifolia* Willd) from leaf explants, Timentin in concentration of 100 and 500 mg/l adversely affect the plant regeneration, and in concentration of 300 mg/l – significantly increased the shoot regeneration frequency [14]; during the hypocotyls and cotyledons regeneration of teak (*Tectona grandis*) Timentin in concentration of 100–300 mg/l stimulated the shoots regeneration, and in concentration 500 mg/l induced the excessive calli formation, but inhibited regeneration [15]. These data confirm our observation that Timentin in high concentrations inhibits the regenerative processes, and in average – can stimulate them.

Si-Nae Han [9] found that in the durum wheat cultivars Keumkangmil, Alchanmil and Bobwhite, Timentin influence was manifested as follows: the highest callus development frequency was observed at concentration of 50 mg/l, and the regeneration frequency – at 500 mg/l. Moreover, Timentin in high concentrations (500 mg/l) accelerated the shoots formation from mature wheat germ [9]. These data are in conflict with ours – Timentin in high concentrations (500 mg/l) in the culture medium inhibits the regeneration processes in bread wheat.

In two tomato cultivars, the rooting of shoots that were regenerated on the Timentin-supplemented medium was observed. Moreover, the rooting occurred on both Timentin-supplemented medium and Timentin-free medium [11]. We observed a similar phenomenon for the rooting of wheat on Ceftriaxone-supplemented medium – antibiotics induce a mechanism of roots formation, and their presence in a medium was no longer necessary.

Although Ceftriaxone is already being used to eliminate *A. tumefaciens* during the genetic transformation of wheat germ [1] and lettuce cotyledons *Lactuca sativa* [2], its impact on plants is not studied. We did not find any information about the positive effect of Ceftriaxone on the morphogenetic processes in plants.

In our research comprehensive and consistent investigation of the Timentin and Ceftriaxone effect on the morphogenetic processes in two bread wheat genotypes was carried out for the first time. We have established the regeneration frequency dependence on three factors: the antibiotic and its concentration in culture medium, and the plant genotype. Considering our results, it is clear why the data of other authors look contradictory. We can confirm that all of the researchers, who claimed that Timentin raises or lowers the regeneration of different plant species, were right, because the antibiotic effect obviously depends on the plant species and plant genotype characters.

Conclusions

The antibiotics Timentin and Ceftriaxone are able to affect the morphogenetic processes in the bread wheat apical calli. However, although both antibiotics belong to the β-lactams, they have different influence. Both antibiotics stimulate the morphogenic calli development and Ceftriaxone also accelerates the meristematic zones appearance in the initial calli. Moreover, a) to stimulate the morphogenic calli development higher concentrations of Ceftriaxone than of Timentin are required; b) to increase the morphogenic calli development frequency, the Timentin supplementation to the nutrient medium is important, and not its concentration.

The influence on the shoot regeneration frequency depends on the antibiotic (Timentin or Ceftriaxone), and in the case of Ceftriaxone – its concentration matters. Unlike Timentin, Ceftriaxone shows an obvious genotype-dependent influence on the shoot regeneration in wheat. Timentin and Ceftriaxone in the same concentrations exert opposite effects on the studied wheat genotypes: the first one increases regeneration frequency, while the second – decreases. Thus, to achieve a higher shoot development frequency for each wheat genotype a β-lactam antibiotic and its concentration must be individually selected, but the adding of Timentin or Ceftriaxone in all tested concentrations (25–500 mg/l) accelerates the shoots regeneration.

The root development frequency depends primarily on the wheat genotype – in one cultivar (Zymoyarka) it is initially high, in the other (Podolyanka) – low. The root development frequency decreases in Zymoyarka and increases in Podolyanka when adding the β-lactam antibiotics.

The presence of Ceftriaxone in culture medium stimulates the rooting, growth of regenerated plants and their biomass growth. Thus, Ceftriaxone is a promising antibiotic for the wheat *in vitro* culture.

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Вплив антибіотиків цефтриаксону і тиментину на морфогенетичні процеси в культурі *in vitro* м'якої пшениці *Triticum aestivum* L.

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Мета. Вивчити вплив антибіотиків тиментину та цефтріаксону на утворення морфогенного калюсу, регенерацію пагонів і ризогенез у двох сортів м'якої пшениці Triticum aestivum L., вивчити вплив цефтріаксону на динаміку росту рослин. **Методи.** Культура тканин рослин in vitro, дисперсійний та кореляційно-регресійний аналізи. **Результати.** Вивчено вплив антибіотиків бета-лактамів цефтриаксону і тиментину на утворення морфогенного калюсу, регенерацію пагонів і ризогенез, що відбуваються в 18-добовому калюсі м'якої пшениці апікального походження. Використані два генотипи пшениці різного типу розвитку – озима та озимо-яра. **Висновки.** Тиментин і цефтриаксон стимулюють утворення морфогенного калюсу апікального походження м'якої пшениці, а цефтриаксон також прискорює появу меристематичних зон в калюсі. Характер впливу на частоту регенерації пагонів залежить від антибіотика (тиментин або цефтріаксон). Вплив цефтриасона на регенерацію пагонів залежить від генотипу рослини та від концентрації антибіотика. Частота ризогенезу залежить в першу чергу від генотипу м'якої пшениці незалежно від використаного антибіотика. Наявність цефтриаксону в культуральному середовищі, на якому були отримані пагони, стимулює укорінення і ріст рослин-регенерантів, а також приріст їх біомаси.

Ключові слова: тиментин, цефтриаксон, морфогенний калюс, регенерація пагонів, ризогенез, *Triticum aestivum* L.

Влияние антибиотиков цефтриаксона и тиментина на морфогенетические процессы в культуре *in vitro* мягкой пшеницы *Triticum aestivum* L

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Цель. Изучить влияние антибиотиков тиментина и цефтриаксона на образование морфогенного каллуса, регенерацию побегов и ризогенез у двух сортов мягкой пшеницы Triticum aestivum L., изучить влияние цефтриаксона на динамику роста растений. **Методы.** Культура тканей растений in vitro, дисперсионный и корреляционно-регрессионный анализы. **Результаты.** Изучено влияние антибиотиков бета-лактамов цефтриаксона и тиментина на морфогенетические процессы (образование морфогенного каллуса, регенерацию побегов и ризогенез), происходящие в 18-суточном каллусе мягкой пшеницы апикального происхождения. Использованы два генотипа пшеницы разного типа развития разного направления – озимая и озимо-яровая. **Выводы.** Тиментин и цефтриаксон стимулируют образование морфогенного каллуса апикального происхождения мягкой пшеницы, а цефтриаксон также ускоряет появление меристематических зон в каллусе. Характер влияния на частоту регенерации побегов зависит от антибиотика (тиментин или цефтриаксон). Влияние цефтриаксона на регенерацию побегов зависит от генотипа растения и от концентрации антибиотика. Частота ризогенеза зависит в первую очередь от генотипа мягкой пшеницы независимо от использованного антибиотика. Наличие цефтриаксона в культуральной среде, на которой были получены побеги, стимулирует укоренение и рост растений-регенерантов, а также прирост их биомассы.

Ключевые слова: тиментин, цефтриаксон, морфогенный каллус, регенерация побегов, ризогенез, *Triticum aestivum* L.

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