ABIOTIC ANTIBIOTIC STRESS ON TRITICALE *IN VITRO* CULTURES

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Abstract

The research objective was to study the abiotic stress exerted by the two antibiotics or amoxicillin plus clavulanic acid (AMO-C) and tetracycline (TET) on germination of triticale in vitro culture (Triticosecale wittmack). On this line, after disinfection, the triticale kernels were placed to germinate on Murashige-Skoog culture medium with added of Gamborg vitamins (control) and supplemented with 2.2 mg / l amoxicillin plus clavulanic acid or 0.6 mg / l tetracycline. After 10 days of incubation, both antibiotics have led to inhibition of germination and of growth of the triticale plantlets, many tiny absorbent hairs on embryonic rootlets formation, as a react on additional stress generated in response to the presence of antibiotics in the culture media.

Key words: amoxicillin, tetracycline, micropropagation, germination, triticale

INTRODUCTION

Antibiotics are pharmaceuticals obtained, in particular, be microbial biotechnology, which have the property to kill or to inhibit, selectively, the development of pathogenic microorganisms and have been used extensively and effectively both in human medicine and animal medicine (Jurcoane et al., 2004). Benefits were seen in agriculture, aquaculture and beekeeping as a growth accelerator. Eliminating antibiotics or their metabolites (sometimes more toxic than the product itself) in the natural environment increases the level of the pollution, enters in to the food chain because they are absorbed more or less by plants, thus becoming toxic to these organisms or to the animals which are feed with it, either implicitly to the person who consume either animal or plant. Antibiotics can reach in to the soil either from manure used as fertilizer, or from the treated animals also from wastewater used in irrigation systems, because 30-80% of these antibiotics can pass through the gastrointestinal tract of animals. Understanding the antibiotics effects on plant anatomy and physiology activity is still limited, especially at their actual concentration in the environment (Opris et al., 2013; Petruş-Vancea et al., 2013). The adverse effects of these pollutants have been identified through effective monitoring and the decrease of pollution effect (Gothwal, Shashidhar, 2014). Since the beginning of the "antibiotics era" most of the effects that they have on plant were to inhibit the growth. These results have led to study the effects of antibiotics also to

in vitro seed germination, in standard laboratory conditions and in a sterile environment (Nickell, Finlay, 1954).

Meng et al. (2014) reported the effects of kanamycin, cefatoxym, carbenicillin and ampicillin on morphogenesis of Chinese cabbage (*Bassica rapa* L. Ssp. Pekinensis), cultivated on Murashige-Skoog medium (1962). Four antibiotics have a small effect on the induction of callus, but influenced the growth and differentiation of roots in varying degrees.

Cefatoxym, carbenicillin and kanamycin triggers various metabolic and enzymatic processes. It provides a possible mechanism for the inhibition or stimulation of growth of strawberry under the action of antibiotics (Qin et al., 2011).

The supplementation of carrot cultures with three types of beta-lactam antibiotics (cefatoxym, carbenicillin, timetin) in various concentrations from 100 to 500 mg/l in the culture medium led to a decrease in the plating efficiency when compared with control variant. However due to the antibiotics treatment, except carbenicillin, at a concentration of 400-500 mg/l occurred efficient regeneration of plants. Therefore it is believed that cefatoxym and temetym, with the analyzed concentrations, can be used to the carrot in complex *in vitro* procedures, as a prophylactic agent to prevent incidental contamination (Grzebelus, Skop, 2014).

Many experiments of the antibiotics effect on vegetal cultures have been reported (Costa et al., 2000; Danilova, Dolgikh, 2004; Petri et al., 2005; Tereso et al., 2006; Xia et al., 2006; Kang et al., 2010; Hamat-Mcbur et al., 2014).

Starting from fundamental research on the stress exerted by abiotic factors on plants cultivated *in vitro*, can continue work related to increase of plant resistance to such environmental factors, but these are of interest only to ornamental species and not on food plants because antibiotics can accumulate in plant bodies (Fodor et al., 2012; Sandor et al., 2012).

The aim of this study was to highlight the effect of antibiotics amoxicillin and tetracycline, in different concentrations, on the triticale species (*Triticosecale wittmack*) growth *in vitro* medium.

MATERIAL AND METHOD

Plant material consisted of triticale (*Triticosecale wittmack*) caryopses.

Antibiotics used in experiment were: *amoxicillin* plus clavulanic acid (AMO-C) and *tetracycline* (TET), which were dissolved in distilled water to prepare various concentrations. From the scientific literature (Hernández et al., 2003; Kim et al., 2005; Axuc-Opriș, 2012; Turdeanu, Petruș-Vancea, 2015) was found that two of the commonly used antibiotics in treating human and animal diseases occur predominantly as polluters of the

environment in certain concentrations. In this research was used these concentrations as a starting point: for amoxicillin was chose 2.2 mg/l concentration (variant V_1) and for tetracycline 0.06 mg/l concentration (variant V_2) (Table 1). In present research, antibiotics were not introduced into the culture medium in order to eliminate specific infections of culture initiations.

Table 1

Research protocol

Explant type	caryopses
Sterilization	Alcohol 70% - few seconds submersion Sodium hypochlorite 2% + Tween 80 - 20 minutes Clean with sterile water – 25 minutes
Culture medium	V_0 - MB-MS+G, Basal, solidified MB-MS+G, without growth regulators (*control*)
	V_1 - MB-MS+G plus 2.2 mg/l AMO-C V_2 – MB-MS+G plus 0.06 mg/l TET
Culture recipients	Uncolored glass recipients, with 11 cm height and 2 cm diameter
In vitro growth conditions	1700 lx, 16/24 h light, 22 – 24 ˚C
Culture period	10 days

Note: AMO-C – amoxicillin + clavulanic acid; TET – tetracycline; MB-MS – basal Murashige-Skoog (1962) medium; G – Gamborg et al. (1968) vitamins.

Research was done in September 2015 – April 2016 period. All data was mathematical and statistical processed. For statistical analysis it was used SPSS Statistic software.

RESULTS AND DISCUSSION

The first germinated caryopses were those belonging to lots containing added antibiotics (AMO-C or TET), which at three days after the release of germinated presented not only embryonic root but even coleoptile, which implies that the antibiotics stress led to intensification germination start.

At 10 days after the inoculation, the germination rate was between 76.4% and 84.2% (Fig. 1). There were no big differences between the experimental variants; however, as compared with reports in the literature on the *in vivo* triticale germination under the similar antibiotic concentrations, the differences are not large. Thus, Turdeanu and Petruş-Vancea (2015) reported that germination – at 7 days – was 75% and 84.5%, the highest rate being found to the lot with AMO-C addition.

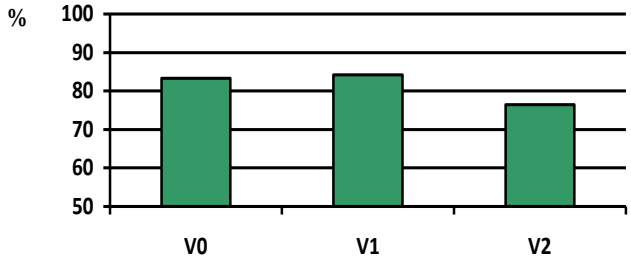

Fig. 1. Germination rate of triticale (*Triticosecale wittmack*) caryopses, at 10 days on different culture medium: V_0 - MB-MS + G (control – without antibiotics); V_1- MB-MS+G plus 2.2 mg/l AMO-C; V_2 – MB-MS+G plus 0.06 mg/l TET

In similar studies carried out recently, but on a dicotyledonous species, namely beetroot, Bot et al. (2016) reported that the percentage of germinated seeds was relatively low, even the control lot did not exceed 75% and at lots with antibiotics added it was drastically decreased, reaching 58% in the case of treatment with high concentrations of 2.2 mg/l AMO-C, but this species of dicot even *in vivo*, there is not a high germination (Turdeanu, Petruş-Vancea, 2015). Explanation of different germination percentage was found in a high number of seeds which do not germinate, and especially on the lot with AMO-C.

In this experiments with triticale, at 10 days from the germinated had registered only inhibitions (negative differences compared to the control), more or less statistically significant (Table 2).

Table 2

Statistical processing of data registered at triticale (*Triticosecale wittmack*) plantlets, at 10 days of germinations under sterile conditions of different culture medium: V_0 - MB-MS+ G (control - without antibiotics); V_1 - MB-MS+G plus 2.2 mg/l AMO-C; V_2 – MB-MS+G plus 0.06 mg/l TET

Measurements / **Variants**	**Rootles L. (cm)**	**Coleorhizae L. (cm)**	**Leaflets L. (cm)**	**Plantlets L. (cm)**
V_0 (*control*) X ± s	6.11±1.71	3.58±0.26	8.44±3.18	18.14±4.56
V_1-AMO-C X ± s	5.29±1.37	3.54±0.95	7.43±3.52	16.25±5.00
±d (cm)	-0.83	-0.05	-1.02	-1.89
P	*	ns	*	*
V_2 – TET X ± s	3.48±1.69	3.72±0.52	7.38±3.15	14.58±5.00
±d (cm)	-2.63	0.14	-1.06	-3.55
P	***	ns	*	***

Note: X ± s (average (cm) ± standard deviation), ±d (difference between control), p (significance of difference from control): p>0.5 – nonsignificant difference (ns), p<0.5 – significant difference (*), p<0.1 - distinct significant difference (**), p<0.01 – very significant difference (***).

These insignificant negative statistically differences in the case of coleorhizae average length, or significant in terms of the rootlets and leaflets average length, hence the size of entire seedling (calculated by adding rootles, coleorhizae and leaflets average lengths) can be seen in Figure 2.

AMO-C, but to a lesser extent TET have increased the roots absorbent hairs number of triticale seedlings (Fig. 3), this being due to additional abiotic stress exerted by them on seedlings. Even at this early stage, the embryonic root - with 0.4-0.5 cm length - presented abundant absorbents hair on media with the antibiotics addition, both AMO-C and TET.

A similar situation was reported by Bot et al. (2016) at beetroot, but here just TET induced increased of rootlets absorbent hairs number.

The effect of TET, unlike the AMO-C was much less negative in beetroot, as compared to triticale. This phenomenon appeared *in vitro* at triticale and beetroot was in contradiction with that observed *in vivo* which was performed with similar treatments from the same species and the same concentrations, by Turdeanu and Petruş-Vancea (2015). A possible explanation of this was that, by autoclaving at 121°C, at a pressure of 1 atm., antibiotics was transformed into other compounds, in the case of TET less toxic than the AMO-C.

Fig. 2. Triticale (*Triticosecale wittmack*) plantlets, at 10 day after caryopses making germinated under sterile conditions of different culture medium: V_0 - MB-MS+ G (control - without antibiotics); V_1 - MB-MS+G plus 2.2 mg/l AMO-C; V_2 – MB-MS+G plus 0.06 mg/l TET (bar means 1 cm)

Fig. 3. Aspect of radicular system of triticale (*Triticosecale wittmack*), at 10 day after caryopses making germinated under sterile conditions of culture medium with 2.2 mg/l AMO-C (bar means 1 cm)

Although the average plantlets fresh mass of control lot was highest of the three experimental variants, after dehydration, ashes, which in fact is the dry matter of seedling was most to variant with the AMO–C addition (Table 3), which implies a mineral higher accumulation in these seedlings, even if they were smaller in terms of growth indices measurements (Table 2).

Table 3

Fresh and dry mass of triticale (*Triticosecale wittmack*) plantlets, at 10 day after caryopses making germinated under sterile conditions of different culture medium: V_0 - MB-MS+ G (control - without antibiotics); V_1 - MB-MS+G plus 2.2 mg/l AMO-C; V_2 – MB-MS+G plus 0.06 mg/l TET

Mass	**V_0 (*control*)**	**V_1 – AMO - C 2.2**	**V_2 – TET**
***Fresh* (g)**	0.1879	0.1822	0.1415
***Dry* (g)**	0.0173	0.0189	0.0093

CONCLUSIONS

1. In monocots, namely triticale (*Triticosecale wittmack*), amoxicillin with clavulanic acid (except as regards the stimulation of the germination rate and the dry mass), and especially tetracycline determined inhibition of germination and plantlets growth.
2. Abiotic stress was highlighted as being most intense in cultures *in vitro* compared to the *in vivo* due to their specific features (particularly intense metabolism).
3. Amoxicillin with clavulanic acid and tetracycline induced the abundant absorbers hairs on rootles, assumed as a response to the plantlets in additional stress conditions.

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