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THE USE OF NANO-SILVER FOR DISINFECTION OF *Pennisetum alopecuroides* PLANT MATERIAL FOR TISSUE CULTURE

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ABSTRACT

Initiation of tissue culture of many plant species is a very difficult stage due to appearance of many contaminations. The other problem might be a choice of media for regeneration. Initiation of grass species tissue cultures are thought to be very difficult. Therefore, a research was undertaken to evaluate the use of nano-silver particles for plant material disinfection and to estimate a medium *Pennisetum alopecuroides*. The plant material were buds and nodal explants that were disinfected in 2% NaOCl for 30 min or 0.1% $HgCl_2$ for 1 min. Half of the explants disinfected with NaOCl were soaked in 50, 100 or 250 mg·dm^{-3} Ag NPs for 1 hour. Explants not soaked in nano-silver were placed on media with Ag NPs at concentrations of 4, 8 or 16 mg·dm^{-3}. An influence of growth regulators on *Pennisetum alopecuroides* was evaluated *in vitro*. Regenerated shoots were placed on MS media with: 3 mg·dm^{-3} BA + 0.3 mg·dm^{-3} IBA, 3 mg·dm^{-3} KIN + + 0.3 mg·dm^{-3} IAA, 1 mg·dm^{-3} BA + 0.1 mg·dm^{-3} IBA. It was observed that the use of nano-silver particles lowered the level of contamination. The best results were obtained when Ag NPs was used at concentration of 100–250 mg·dm^{-3} alone or as a supplementation of the media, at concentration of 4 mg·dm^{-3} for nodes and 16 mg·dm^{-3} for adventitious buds. The use of nodal explants allowed to obtain less contamination. Regeneration depended on a media content. The most regenerated shoots were obtained on the MS media supplemented with 1 mg·dm^{-3} BA and 0.1 mg·dm^{-3} IBA.

Key words: nano-particles, culture initiation, micropropagation, plant growth regulators, contaminations, regeneration

INTRODUCTION

Contamination, both fungal and bacterial, is a serious problem in tissue culture. It might decrease growth of plants, acclimatization and, as a consequence, lead to their dieback [Oyebanji et al. 2009]. Therefore, *in vitro* plants cultivation is usually carried out in sterile conditions to prevent cultures from being infected. However, the same sterile conditions might promote growth and development of contaminants [Yildiz and Er 2002]. Substances used for plant material disinfection the most often are sodium hypochlorite (NaOCl) or calcium hypochlorite (CaOCl), silver nitrate ($AgNO_3$), mercuric chloride ($HgCl_2$), antibiotics and alcoholic solutions [Kharrazi et al. 2011]. The above substances might lead to irreversible damage of explants, especially when used at inappropriate concentrations [Tymoszuk 2014]. They are not neutral for environment and human or animal health either [Shokori et al. 2014].

Nano-particles might be alternative substances for plant material disinfection, used in a form of a soaking solution or added to the growing media. Nano-particles are structures up to 100 nm size. They have only one dimension and a very large surface in relation to their weight, which increases their reactivity [Jo et al.

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2011]. A dynamic development of nano-biotechnology allows to use nano-particles, such as nano-silver (Ag NPs), for disinfection of explants for tissue culture initiation. The advantageous influence of silver nano-particles on reducing contaminations in plant tissue cultures of *Valeriana officinalis* [Abdi et al. 2008], *Gerbera jamessoni* [Fakhrfeshani et al. 2012], *Areucaria excelsa* [Sarmast et al. 2011], *Rosa hybrida* [Shokori et al. 2014] or *Chrysanthemum* × *grandiflorum* [Tymoszczuk 2014], has been confirmed.

In tissue cultures, nano-particles might be used as an addition to the media. According to many authors, presence of Ag NPs in the media reduces the microbiological contamination in tissue cultures of such plants as *Olea europea* 'Mission' [Rostami and Shahsavar 2012], *Iresine herbstii* [Nabell 2011], *Araucaria excelsa* [Sarmast et al. 2011] and almond × peach [Arab et al. 2014]. In *Araucaria excelsa*, the bacterial infection was reduced from 61.5% to 11.3% as a result of decontamination of explants with nano-silver compounds at concentration of 200 mg·dm^{-3} [Sarmast et. al 2011]. The addition of nano-silver to the media at concentration of 4 mg·dm^{-3} significantly improved the purity of *Olea europea* 'Mission' nodal segments cultures [Rostami and Shahsavar 2012]. However, nano-biotechnology gives rise to many doubts, as on one hand, nano-particles have antibacterial and antifungal properties [Rostami and Shahsavar 2012], but on the other hand, they might be deposited in tissues [Laure et al. 2014]. Laure et al., [2014] observed that nano-silver particles were transported and accumulated in leaves of *Lectuca sativa*.

The aim of presented research was to estimate the possibility to use the nano-silver particles for disinfection of *Pennisetum alopecuroides* explants for tissue culture initiation. It is an ornamental grass that belongs to the *Poacea* family, the new varieties of which appearing more and more often on the market, that may be propagated only vegetatively. Development of micropropagation techniques for *Pennisetum alopecuroides* propagation might be a good way for a fast and effective production of plants. Generally, grasses are concerned as difficult to disinfect, especially from adventitious bud explants or any pieces excised from underground part. Due to vast contamination and low rate of regeneration, during preliminary conducted research, it was observed that disinfection of nodal explants and adventitious buds with standard substances such as sodium hypochlorite (NaOCl), was not effective. Therefore, it was decided to use nano-silver particles, which might reduce microbiological contamination in tissue culture.

MATERIAL AND METHODS

The experiment was set up in the tissue culture laboratory of the Department of Ornamental Plants, Dendrology and Landscape Architecture of the University of Life Sciences in Lublin. The plant material were nodes (1 cm length) and adventitious buds (0.5–1 cm length) of *Pennisetum alopecuroides* excised from 5-year-old plants cultivated in a field, in the Felin Experimental Station of the University of Life Sciences in Lublin. The obtained pieces were disinfected in a following sequence: water with addition of a detergent (3 × 20 min), 70% ethanol (30 sec) and the solutions: 2% NaOCl (sodium hypochlorite) for 30 min or 0.1% $HgCl_2$ (mercuric chloride) for 1 min. Half of the explants disinfected with NaOCl were additionally soaked in nano-silver particles (Ag NPs) solution (Nano-BioTech, particles 6–12 nm) at concentration of 50, 100 or 250 mg·dm^{-3} for 1 h. The surface sterilized explants were then three times rinsed in a sterile deionized water.

Disinfected explants were placed individually in tubes on a solidified Murashige and Skoog (MS) media [1962], supplemented with vitamin B_1 – 0.1 mg·dm^{-3}, B_6 – 0.5 mg·dm^{-3}, PP – 0.5 mg·dm^{-3}, glycine – 2.0 mg·dm^{-3}, inositol – 100 mg·dm^{-3}, sucrose 30 g·dm^{-3} and growth regulators: benzyladenine (BA) – 1 mg·dm^{-3} and indolebutyric acid (IBA) – 0.3 mg·dm^{-3}. Explants that had not been soaked in nano-silver solutions were placed on the media supplemented with Ag NPs at concentrations of: 4, 8 or 16 mg·dm^{-3}. Explants soaked in 2% NaOCl solution only were considered a control. The media pH was established to 5.7 and steam sterilized for 21 min, in the temperature of 121°C and under pressure of 1 hPa. The tubes were placed in a growing room, in the temperature of 20°C during the day and 18°C at night, with 16-hour photoperiod. The source of light were fluorescent Fluora type lamps of the 30 µmol·m^{-2}·s^{-1} light intensity. Each treatment consisted of 25 replications (tubes).

The experiment lasted 12 weeks. The observations concerning contamination and regeneration were done once a week. At the end of the experiment, the following features were evaluated: number of explants without symptoms of contamination, number of regenerated plants (regardless of contamination), number of explants with symptoms of necrosis.

In the subsequent stage, an influence of growth regulators on development of *Pennisetum alopecuroides* was evaluated *in vitro*. Regenerated microshoots were placed on MS [1962] media supplemented with the following combinations of growth regulators: 3 mg·dm^{-3} BA (benzyladenine) + 0.3 mg·dm^{-3} IBA (indole-3-butyric acid), 3 mg·dm^{-3} KIN (kinetin) + 0.3 mg·dm^{-3} IAA (indole-3-acetic acid), 1 mg·dm^{-3} BA + 0.1 mg·dm^{-3} IBA. The combinations of cytokinins and auxins were chosen on the basis of the literature data studies. After 8 weeks of cultivation in tissue culture, the observations of the regenerated shoots were done.

The obtained data was statistically analyzed with the ANOVA analysis, for the two–factorial design with the use of Statistica 13 software (StatSoft). The significance of differences between the means was estimated with the use of Tukey's confidence intervals at the level of significance $\alpha = 0.05$. In order to compare percentage values, they were transformed into Bliss values and then retransformed into numerous values [Bliss 1938].

RESULTS AND DISCUSSION

The significant influence of the disinfection method and the type of explant on *Pennisetum alopecuroides* L. tissue culture initiation was proven in the presented studies. It was observed that the percentage of contamination free explants depended on both tested factors (Tab. 1).

Taking into consideration the method of disinfection, it was observed that the majority of explants without contamination symptoms were obtained when *Pennisetum alopecuroides* explants had been soaked in a solution of nano-silver particles at concentrations of 100 or 250 mg·dm^{-3}, without NaOCl (34%). It was proven that with the use of nano-silver at higher concentrations, the number of *Pennisetum alopecuroides* contamination free explants increased, both in case of nodes and adventitious buds. When nano-silver treatment followed NaOCl disinfection, the best results were obtained when Ag NPs was applied at concentration of 100 mg·dm^{-3} (18%). Use of higher concentrations of Ag NPs (250 mg·dm^{-3}) together with 2% of NaOCl gave worse results, as only 6% of explants

Table 1. Influence of the disinfection method and the type of explant on the percentage of contamination free explants after 12 weeks of experiment

Disinfection method	Type of explant		
	nodes	adventitious buds	mean A
70% ethanol + 2% NaOCl	8_{h}	0_{j}	4_{I}
70% ethanol + 2% NaOCl + Ag NPs 4 mg·dm^{-3} medium	40_{b}	0_{j}	20_{D}
70% ethanol + 2% NaOCl + Ag NPs 8 mg·dm^{-3} medium	12_{g}	0_{j}	6_{H}
70% ethanol + 2% NaOCl + Ag NPs 16 mg·dm^{-3} medium	8_{h}	20_{f}	14_{C}
70% ethanol + 2% NaOCl + Ag NPs 50 mg·dm^{-3} soaking	24_{e}	0_{j}	12_{F}
70% ethanol + 2% NaOCl + Ag NPs 100 mg·dm^{-3} soaking	36_{c}	0_{j}	18_{E}
70% ethanol + 2% NaOCl + Ag NPs 250 mg·dm^{-3} soaking	4_{I}	8_{h}	6_{G}
70% ethanol + Ag NPs 50 mg·dm^{-3} soaking	24_{e}	0_{j}	12_{F}
70% ethanol + Ag NPs 100 mg·dm^{-3} soaking	48_{a}	20_{f}	34_{A}
70% ethanol + Ag NPs 250 mg·dm^{-3} soaking	48_{a}	20_{f}	34_{A}
70% ethanol + 0,1% $HgCl_2$	28_{d}	36_{c}	32_{B}
Mean B	25_{A}	9_{B}	

Means followed by the same letter do not differ significantly at p = 0.05

were free of contamination. The least contamination free explants were obtained when they had been treated with NaOCl only (4%). Many authors proved that nano-silver particles positively influenced the effectiveness of disinfection. Diborov et al. [2002] stated that decrease in fungal and bacterial contamination after the nano-compounds treatment resulted from release of ions, e.g. silver ions, which might break membrane structures of the microorganisms and therefore penetrate them. Abdi et al. [2008] noted that nano-silver had great influence on reduction of contamination, mainly bacterial one, in *Valeriana officinalis* tissue culture. According to the literature reports, inhibition of infections in case of other species was observed when nano-silver particles were used in high concentration. Fakhrfeshani et al. [2012] obtained a high purity *Gerbera jamesonii* cultures when they used 200 $mg \cdot dm^{-3}$ of nano-silver particles for 15 min, while Sarmast et al. [2011] observed decrease of bacterial contamination in *Araucaria excelsa* cultures from 81.25% to 18.75%, when a Murashige and Skoog medium was supplemented with nano-silver particle solutions at high concentration of 400 $mg \cdot dm^{-3}$. Good results were also observed when mercuric chloride had been used for explants disinfection (32%), however it is not recommended as a life risky solution.

Considering the type of explant, it was observed, that nodes were much easier to disinfect than adventitious buds (25% and 9%, respectively). However, such results were expected, as adventitious buds are situated underground, therefore they are much more exposed to dirt and pathogens than nodes. Moreover, they are harder and hidden inside crown, hence it is much more difficult to clean them than soft tissues of nodes. According to Dar et al. [2012], disinfection of hidden parts of plants is difficult. Balilashaki et al. [2012] stated that an important factor influencing the effectiveness of disinfection is the age of donor plant, and the number of infections grows with the age. In the present experiment, explants were obtained from 5-year-old plants, which might have influenced the number of contamination events. The regenerated plants obtained from nodal explants are presented in Figure 1.

Taking into consideration disinfection of nodal explants, the best results were obtained when plant pieces were soaked at higher concentrations of Ag NPs (100 or 250 $mg \cdot dm^{-3}$), without NaOCl (48%). A supplementation of the media with low concentration of nano-silver (4 $mg \cdot dm^{-3}$) allowed to obtain 40% of contamination free explants. Good results were also obtained when plant pieces were treated first with NaOCl and then soaked in Ag NPs at concentration of 100 $mg \cdot dm^{-3}$ (36%). The least contamination free explants were noted when they had been soaked in 2% of NaOCl followed with Ag NPs at concentration of 250 $mg \cdot dm^{-3}$ (4%).

In case of adventitious buds, it was observed that the most contamination free explants, 36%, was obtained when 0.1% of $HgCl_2$ was used. The usefulness of this compound at concentration of 0.1% was confirmed in relation to reduction of microbiological contamination in the case of propagation of such species as *Pomegrante* cv. 'Gangesh' from tip explants [Singh et al. 2014] or paeonia 'Wu Long Peng Sheng' from lateral buds [Jia et al. 2011]. Disinfection was also possible with the use of Ag NPs, however at higher concentrations than for nodes – 100 or 250 $mg \cdot dm^{-3}$ used in a form of soaking and with the use of media supplemented with 16 $mg \cdot dm^{-3}$ of nano-silver particles. Higher concentrations of Ag NPs for disinfection of adventitious buds were needed as those types of explants are generally harder and more difficult to clean. The remaining treatments were unsuccessful as none of the objects was free from contamination.

Low concentration of nano-silver used for disinfection of explants (4 $mg \cdot dm^{-3}$) was proven effective in case of *Olea europea* 'Mission' propagated through nodal segments [Rostami and Shahsaver 2012] and *Iresine herbsti* [Nabell 2011]. In case of the latter species, the authors observed the increase of contamination free explants from 45 to 100%. On the other hand, in case of *Araucaria excelsa,* a positive effect was observed when nano-silver at concentration of 400 $mg \cdot dm^{-3}$ was added to the media [Sarmast et al. 2011]. In case of adventitious buds, the concentration of nano-silver should be increased to 16 $mg \cdot dm^{-3}$, which allowed to obtain 20% of contamination free explants.

It was observed that NaOCl used alone was ineffective for disinfection of *Pennisetum alopecuroides* explants. According to Yildiz and Er [2002], NaOCl is the most often used solution for disinfection of ex-

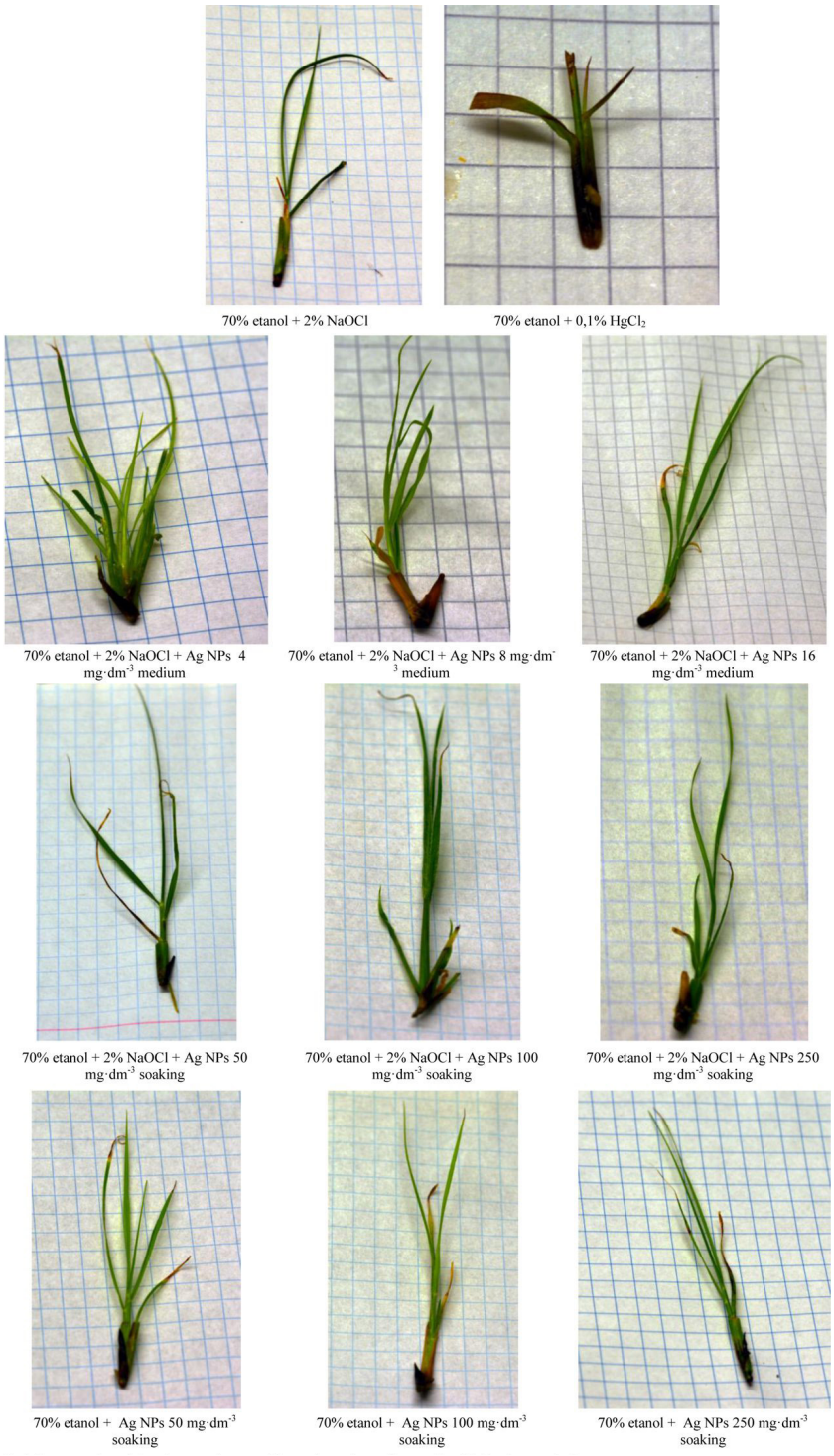

Fig. 1. Regeneration of *Pennisetum alopecuroides* explants depending on the disinfection method

plants in tissue culture of many species. Its effectiveness results from oxidation of biologically active substances, such as proteins and nucleic acids. However, the positive influence of NaOCl was not observed in the present work. Balilashaki et al. [2014] also noted that low concentration of sodium hypochlorite (3%) was not effective for disinfection of *Phalaenopsis amabilis* shoots. High concentration was ineffective as well, as 10% solution caused dying of the explants.

The method of disinfection has also influenced a number of regenerating and necrotic explants (Tab. 2).

It was noted that nodal explants almost twice better regenerated into shoots than adventitious buds (40% to 26%, respectively). Regeneration was also influenced by the disinfection method. The most regenerated shoots were observed when explants had been disinfected with NaOCl only (50%) or with additional soaking in Ag NPs solution at concentration of 50 mg·dm^{-3} (50%). Significantly less regenerated shoots were obtained when explants had been treated with NaOCl at higher concentrations of Ag NPs, 100 or 250 mg·dm^{-3} (39 and 38%, respectively). The addition of nano-silver particles into the media, use of Ag NPs without NaOCl and mercuric chloride inhibited regeneration of *Pennisetum alopecuroides* explants. On the other hand, Jha et al. [2009] and Arockiasamy et al. [2006] observed satisfactory regeneration rate of *Pennisetum glaucum* when the explants had been treated with 0.5% of $HgCl_2$ solution. On the basis of the obtained data, it could be stated that the use of nano-silver solution inhibits regeneration of explants. However, Taghizadeh and Solgi [2014] did not observe any negative influence of nano-silver at concentration of 200 mg·dm^{-3} on regeneration of *Cynodon dactylon* cv. 'Teefgreen'.

The use of 2% NaOCl solution alone, despite good results in relation to regeneration of explants, at the same time increased a number of necrotic explants (40%). The least explants had symptoms of necrosis when they had been treated with NaOCl followed by soaking in nano-silver solution at concentration of 50 mg·dm^{-3} (12%). Comparing the amount of necrotic explants between two types of explants, it was observed that nodes were almost twice more susceptible to necrosis than adventitious buds (40% and 16%, respectively), which could result from their more delicate tissues.

Table 2. Influence of the disinfection method and type of explant on a number of regenerating and necrotic explants (in percentage)

Disinfection method	Regenerating explants			Necrotic explants		
	nodes	buds	mean A	nodes	buds	mean A
70% ethanol + 2% NaOCl	44_{e}	56_{c}	$50_{\mathbf{A}}$	40_{e}	40_{e}	40_{A}
70% ethanol + 2% NaOCl + Ag NPs 4 mg·dm^{-3} medium	28_{h}	24_{i}	$26_{\mathbf{F}}$	44_{d}	8_{m}	26_{E}
70% ethanol + 2% NaOCl + Ag NPs 8 mg·dm^{-3} medium	28_{h}	22_{j}	$25_{\mathbf{G}}$	52_{b}	12_{l}	32_{B}
70% ethanol + 2% NaOCl + Ag NPs 16 mg·dm^{-3} medium	28_{h}	20_{k}	$24_{\mathbf{H}}$	44_{d}	16_{k}	30_{C}
70% ethanol + 2% NaOCl + Ag NPs 50 mg·dm^{-3} soaking	76_{a}	24_{i}	$50_{\mathbf{A}}$	16_{k}	8_{m}	12_{G}
70% ethanol + 2% NaOCl + Ag NPs 100 mg·dm^{-3} soaking	60_{b}	18_{l}	$39_{\mathbf{B}}$	36_{f}	20_{j}	28_{D}
70% ethanol + 2% NaOCl + Ag NPs 250 mg·dm^{-3} soaking	56_{c}	20_{k}	$38_{\mathbf{C}}$	28_{h}	16_{k}	22_{F}
70% ethanol + Ag NPs 50 mg·dm^{-3} soaking	$48_{\mathbf{d}}$	$24_{\mathbf{i}}$	$36_{\mathbf{D}}$	32_{g}	24_{i}	28_{D}
70% ethanol + Ag NPs 100 mg·dm^{-3} soaking	$32_{\mathbf{g}}$	$20_{\mathbf{k}}$	$26_{\mathbf{F}}$	48_{c}	16_{k}	32_{B}
70% ethanol + Ag NPs 250 mg·dm^{-3} soaking	$32_{\mathbf{g}}$	$40_{\mathbf{f}}$	$36_{\mathbf{I}}$	60_{a}	4_{n}	32_{B}
70% ethanol + 0,1% $HgCl_2$	$12_{\mathbf{m}}$	$20_{\mathbf{k}}$	$16_{\mathbf{E}}$	40_{e}	12_{l}	26_{E}
Mean B	40_{A}	26_{B}	–	40_{A}	16_{B}	–

Means followed by the same letter do not differ significantly at p = 0.05

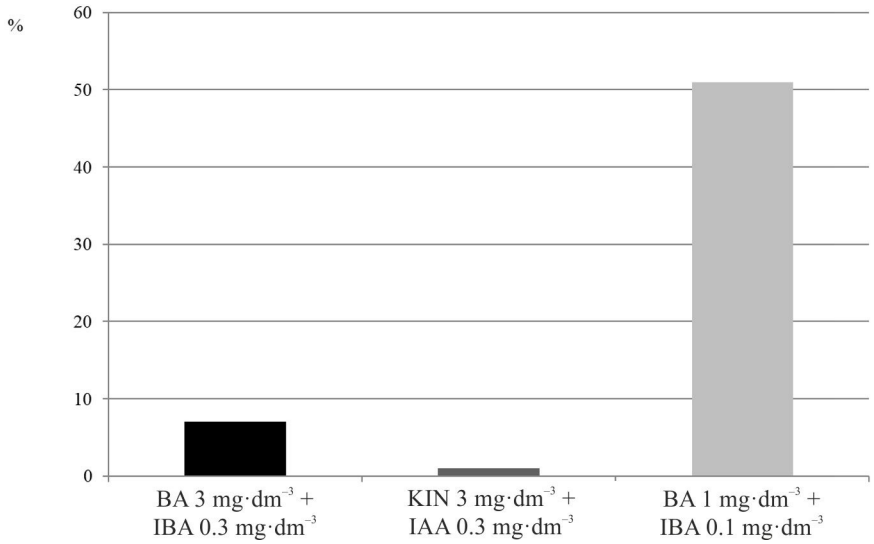

Fig. 2. The influence of the media composition on a number of regenerating explants of Pennisetum alopecuroides

Taking into consideration the influence of disinfection method on browning and dying of nodal explants, it was noted that the use of the highest concentration of Ag NPs alone or supplementation of the media with Ag NPs at concentration of 8 mg·dm^{-3} resulted in the most necrotic explants (60% and 52%, respectively). A large number of necrosis was also observed in case of 2% NaOCl disinfection and placing the explants on the media supplemented with Ag NPs at concentrations of 4 or 16 mg·dm^{-3} (44%) or soaking the explants at higher concentrations of Ag NPs solutions, 100 and 250 mg·dm^{-3} (48 and 60%, respectively). The least dead explants were obtained when they had been treated with 2% NaOCl followed by soaking in Ag NPs at concentration of 50 mg·dm^{-3} (16%).

In the case of adventitious buds, the most necrotic explants were noted when they had been disinfected with 2% NaOCl solution only (40%), while the best results were obtained with the use of Ag NPs at concentration of 250 mg·dm^{-3} (4%). A tendency was also observed, that the increase of concentration of Ag NPs solution caused that the number of necrotic explants decreased.

It should be noted that there are just few articles presenting data on the influence of nano-silver particles solutions on growth and development of plants. Arab at al. [2014] stated that supplementation of the media with Ag NPs at concentrations over 150 mg·dm^{-3} caused dying of explants, which was not confirmed in the presented research in case of adventitious buds. However, Kumari et al. [2009] proved that nano-silver inhibited the cell divisions in *Allium cepa* root tips. There are a few works available on the influence of other nano-particles compounds on development of plants. Nano-titanium oxide reduced transpiration and growth of corn leaves due to an interaction between the particles and cell walls, which inhibited water flow in plants [Asli and Neumann 2009]. Zhu et al. [2008] proved that nano-magnetite particles present in water were absorbed and accumulated in leaves or roots of *Cucurbita maxima* or *Lactuca sativa* [Zhu et al. 2008, Laure et al. 2014]. Lee et al. [2010] observed that *Arabidopsis thaliana* seeds germination was inhibited in the presence of hydrogen nanoparticles at concentration of 400 mg·dm^{-3}. Moreover, McGrath and Zhao [2003] suggested that such species as *Medicago sativa* and *Brassica juncea* might be hyper accumulators of nanoparticles. One of the hypothesis states that harmful activity of nanoparticles might result from producing reactive oxygen species (ROS), which might

damage cell organelles [Klaine 2008]. ROS might also bind with enzymes and therefore stop their activity and cause osmotic stress [Bhatt et al. 2011].

The regeneration of explants depends not only on a disinfection method but also on media composition, which was observed in the presented research (Fig. 2).

The media used for initiation of explants was MS media [1962] supplemented with growth regulators, which are considered the most important factors influencing development of plants in tissue culture. The largest number of explants regenerated into shoots when the media contained 1 mg·dm^{-3} BA + 0.1 mg·dm^{-3} IBA (51%). BA and IBA used at three times higher concentration decreased the regeneration of explants. The least growing shoots were obtained when explants were placed on the media supplemented with 3 mg·dm^{-3} KIN and 0.3 mg·dm^{-3} IAA (1%). Benzyladenine at concentration of 1 mg·dm^{-3} was confirmed by many authors to positively influence the growth and development of many grass species, such as *Pennisetum gaucum* [Aleksandrowa et al. 1996, Oldach et al. 2011] or *Shorghum bicolor* [Oldach et al. 2001], while Arockiasamy et al. [2006] used IBA at lower concentration of 0.1 mg·dm^{-3}. Jha et al. [2009] managed to obtain somatic embriogenesis of *Pennisetum glaucum* with the use of high concentrations of nano-silver particles – 3.97 mg·dm^{-3} and stated that higher concentrations decreased the regeneration.

CONCLUSIONS

Initiation of a tissue culture of *Pennisetum alopecuroides* is difficult due to a high level of contamination and low regeneration. It is easier to establish contamination free tissue culture with nodal explants than with the use of adventitious buds, as they are easier to disinfect and regenerate better. The use of nano-silver particles solution for disinfection lowers the extent of contamination. The best results are obtained when nano-silver is used at concentration of 100–250 mg·dm^{-3} alone or as a supplementation of the media at concentration of 4 mg·dm^{-3} for nodes and 16 mg·dm^{-3} for adventitious buds. Ag NPs used at high concentrations might cause necrosis of plants, therefore it is advised to use 100 mg·dm^{-3} solution. Regeneration depends on a media content. The medium recommended for *Pennisetum alopecuroides* tissue culture initiation is MS [1962] with addition of 1 mg·dm^{-3} BA and 0.1 mg·dm^{-3} IBA.

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