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In vitro comparative study of two *Arundo donax* L. ecotypes' selenium tolerance

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Summary: Selenium tolerance of two somatic embryo-derived *Arundo donax* L. ecotypes (Blossom, 20SZ) were compared in *in vitro* culture. Sodium-selenate (1 – 100 mg L^{-1}) as known the most phytoavailable selenium form and the less studied red elemental nanoselenium (100 mg L^{-1}) were applied as selenium treatments. Basis on the results Blossom ecotype seemed to be more sensitive to the sodium-selenate than 20SZ. Inhibiting effect of selenate was effectuated above 10 mg L^{-1} in case of Blossom, which was manifested in decreased survival rate and growing parameters. Contrast to this 20SZ could tolerate the selenate ≤ 20 mg L^{-1} without any toxic symptoms. Lower selenate tolerance of Blossom could be explained with higher selenium accumulation. Both of two ecotypes could also uptake and accumulate the red elemental nanoselenium however in much less extent compared to selenate.

Keywords: *Arundo donax* L., ecotype, sodium-selenate, red elemental nanoselenium, survival rate

Introduction

Selenium (Se) status in the environment is unique phenomenom. Hence Se is essential for human and animals in low concentration however it can easily become toxic with increasing concentration (El-Ramady et al., 2014b). On the other hand no clear evidence is exist for selenium essentiality in Higher plants but they can take up it from environment (Pilon-Smits et al., 2009; El-Ramady et al., 2013; El-Ramady et al., 2014a). Selenium uptaking and transforming abilities of plant can contribute to alleviate selenium deficiency which is an emerging problem worldwide, especially in developing countries (Zhao & Mcgrath, 2009). At the same time the selenium polluted areas are also increasing o other districts of world as a consequence of anthropogenic impacts (Dhillon & Dhillon, 1991, El-Ramady et al., 2014c). Selenium polluted areas are known in India, China, USA and other American countries (Haug et al., 2007). Conventional methods (such as chemical, physical) used for reclamation of contaminated soils are usually costly to install and facilitate (Pilon-Smits & Freeman, 2006). However recently Higher plants usage to remove pollutants from soils or sediments is a promising strategy with increasing scientific substantiation. Critical point of phytoremediation is the characteristics and ability of plants. The most important requirements of phytoremediator plants are high tolerance against pollutants, fast growing with high yield, possible economical impact and they can remove, reduce, degrade or immobilize environmental pollutants in high extent (Pilon-Smits, 2005). Besides energy purposes biomass plants (Antal et al., 2014; Kurucz et al., 2014; Kurucz and Fári, 2013; Kurucz et al., 2012) have been moving to the forefront of interest using in phytoremediation. Generally these plants are not able to accumulate as much pollutants, than real hyperaccumulator species but considering their higher yield ultimately they can present great potential.

Giant reed *(Arundo donax* L.) is one of the good candidate for energy production and paper industry (Elhawat et al., 2014; Borin et al. 2013). At the same time some kind of heavy metals, other abiotic stress tolerance, remediation potential of it has also been confirmed (Alshaal et al., 2013; Mirza et al., 2010; Papazoglu, 2007; Sabeen et al., 2013; Shabana et al., 2012). However no information was found about selenium tolerance of giant reed.

Basic on these knowledge the main objective of present work was to compare selenium tolerance and accumulation ability of two, biotechnologically propagated *Arundo donax* ecotypes in *in vitro* culture.

Materials and methods

Selenium uptake and tolerance of giant reed were observed in semisolid culture, using sterile uniform germinated somatic embryo-derived clusters of two ecotypes

(Blossom-American and 20SZ-Hungarian). Plant materials of Arundo were propagated by somatic embryogenesis in the Ottó Orsós Laboratory, Department of Plant Biotechnology as described Márton and Czakó (2004; 2007).

Hormon free, semisolid media were prepared with selenium as sodium-selenate (Na_2SeO_4) 0, 1, 10, 20, 50, 100 mg L^{-1} Se concentration range and selenium treatment was also used as red elemental selenium (nanoSe) suspension in nano-size scale (100 mg L^{-1} Se). Cultures were maintained under white fluorescent lamps (41 µmol m^{2-} s^{-1} photon flux density), at 24°C and 8/16 h photoperiod. The average relative humidity was recorded to be 75%.

Selenium tolerance of somatic embryo-derived Arundo donax ecotypes in germinated cluster phase were comparised in *in vitro* culture considering the survival rate and growth parameters of them. Total selenium concentration of clusters were determined by hydride generation atom fluorescence spectrometry (HG-AFS) with wet acid digestion (AFS, Millennium System, P.S. Analytical Ltd., England). The AFS method was based on that described by Dernovics et al., *(2002)*; Cabanero et al. *(2004)* with some modification.

Data analysis (mean values and standard deviation) was fulfilled using Microsoft Excel 2007 program. Statistical analysis was conducted using one-way analysis of variance (ANOVA) at 95% confidence interval in SPSS version 13.0 [SPSS, Inc. Chicago, IL]. Significantly different means between treatments were separated using the Games-Howell test.

Results and discussion

Selenium tolerance of embryo-derived plants were observed in *in vitro* culture using germinated clusters. It was found no negative impact of sodium-selenate between 1 – 20 mg L^{-1} considering the survival of 20SZ ecotype. More than 90% of total clusters were still remained alive in this concentration range similarly to the control (Fig. 1). The 20SZ clusters grew well and almost half part of them started to rooting in this concentration range (Fig. 2). The inhibiting effect of selenate was expressed from 50 mg L^{-1} where ~50 % of 20SZ clusters died. The growth of clusters were diminished, most of them remained small as at the beginning of the experiment without any root formation (Fig. 2). The clusters treated with 100 mg L^{-1} sodium-selenate vegetated for a while and then all of them died (Fig. 1).

Basis on the results the Blossom ecotype seemed to be more sensitive for the sodium-selenate then 20SZ. The 10 mg L^{-1} concentration didn't influence the survivals of clusters comparing to control. However the negative effect of sodium - selenate in 20 - 50 mg L^{-1} concentration range was more noticeable in case of Blossom ecotype than in 20SZ (Fig. 1; Fig. 2). Plant growth inhibiting effect of selenate was already detected from 8 ppm concentration in barley seedling and ≥ 10m g L^{-1} in case of tobacco (Akbulut & Cakir, 2010, Domokos-Szabolcsy, 2011). All Blossom clusters died using 100 mg L^{-1} sodium - selenate similarly to 20SZ (Fig. 1).

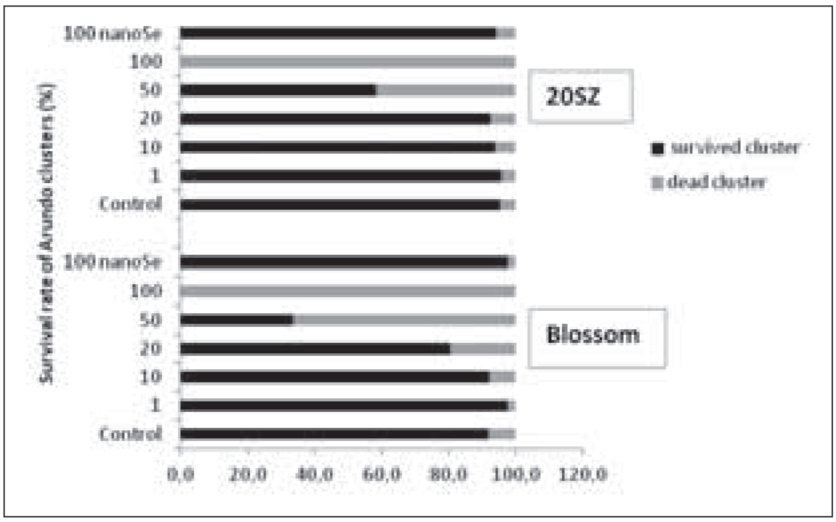

Fig. 1: Survival rate of Blossom and 20SZ Arundo donax embryo-derived germinated clusters treated with 0; 1; 10; 20; 50; 100 mg/L^{-1} sodium-selenate or red elemental nanoselenium suspension (100 mg/L^{-1})

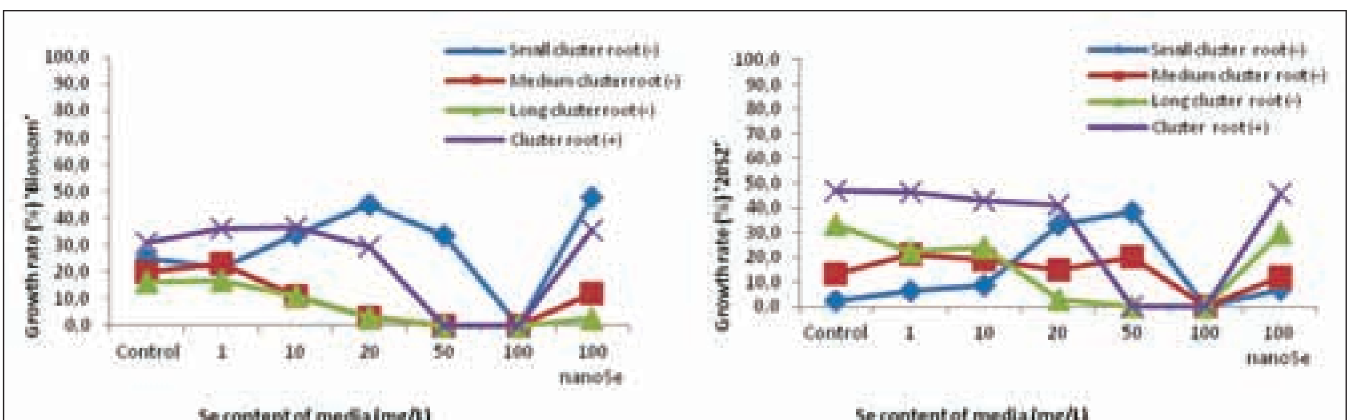

Fig. 2: Growth rate of Blossom and 20SZ *Arundo donax* L. embryo-derived germinated clusters treated with 0; 1; 10; 20; 50; 100 mg L^{-1} sodium-selenate or red elemental nanoselenium suspension (100 mg L^{-1})

Fig. 3: *In vitro* Blossom and 20SZ *Arundo donax* L. ecotypes treated with 0; 1; 10; 20; 50; 100 mg L^{-1} sodium-selenate or red elemental nanoselenium suspension (100 mg L^{-1})

At the same time big difference was achieved comparing the two chemical forms of selenium. In contrast to selenate, the red elemental nanoselenium treatment, even 100 mg L^{-1} was not toxic for neither Arundo ecotypes (Fig. 1; Fig. 3). Both ecotypes developed like as controls with intensive root formation (Fig. 2).

According to the literature the selenate is the most phytoavailable selenium form (White et al., 2004). Our results confirmed this statement hence accumulation of selenium in both of two Arundo clusters significantly increased with the increasing selenate content of media (Fig. 4). Comparing the two ecotypes we observed differences considering selenium accumulation. Higher total selenium content was realized in the Blossom ecotype comparing 20SZ. For instance the Blossom clusters could accumulate 920 mg/kg Se (DW) used 20 mg L^{-1} sodium-selenate their media. On the other hand 20SZ clusters weren't able to accumulate as much amount (896 mg/kg Se) even they were treated with 50 mg L^{-1} selenate.

Both Arundo ecotypes could uptake and accumulate the red nanoSe however in lower concentration comparing to the selenate (Fig. 4). *In vitro* tissue culture tobacco also expressed lower uptake from nanoSe than from selenate (Domokos-Szabolcsy et al., 2012). Unlike selenate, higher concentration of nanoSe was accumulated in 20SZ (two-times higher) than in Blossom.

In summary there are difference in selenium tolerance of Hungarian and American giant reed ecotypes. The Blossom could tolerate less (higher survival rate and diminished growth parameters), toxic symptoms were occured from 20 mg L^{-1}. The Se tolerance difference between two ecotypes correlates with the Se accumulation difference of two ecotypes. Hence Blossom accumulated the selenium in much higher extent than 20SZ beside increasing selenate treatment.

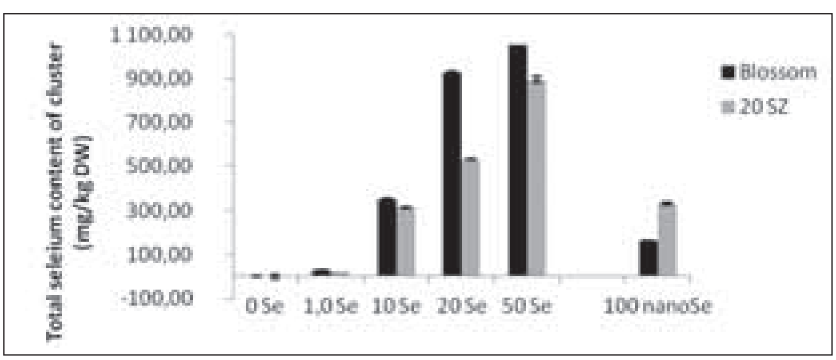

Fig. 4: Total selenium content of Blossom and 20SZ *Arundo donax* L. ecotypes treated with 0; 1; 10; 20; 50 mg L^{-1} sodium-selenate or red elemental nanoselenium suspension (100 mg L^{-1})

Results also shown that the elemental selenium of red allotrope in nano-size scale are available for Arundo clusters however they can accumulate it in much lower amount than selenate.

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