PhD thesis

BIOLOGICAL EFFECT AND FORTIFICATION POSSIBILITIES OF INORGANIC SELENIUM FORMS IN HIGHER PLANTS

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1. Introduction and goal set

Selenium (Se) is a microelement that is found in rock, soil and water as well in the natural environment. However due to the result of the fast industrialization nowadays, a great amount of selenium gets in the environment as industrial by-product. In this way, there is a relatively big selenium resource on the Earth, however its distribution is very uneven. Why is this important? Selenium acts like a sword does with its two sides in the biological system. Its concentration range that is needed for the human and animal organism is rather tight and under this limit, the symptoms of lacking occur very fast, yet the toxic range can also be reached easily. Selenium is one of the most intensively researched microelement. A lot of information had been piled up by researches done at many scientific fields. By analytical-chemistry, it has become obvious that selenium may be present in the environment in different concentrations and species, depending on the physical and chemical factors. The form of Se greatly affects its intake to both Prokaryotes and Eukaryotes. In addition, in some living groups (bacteria, animals with higher order) for the normal metabolism selenium is vital. However it is not essential for other organisms (fungi and Higher plants) but it could be beneficial for their accumulating abilities.

Like every other researching fields directly or indirectly, selenium research is also focused towards the human being. Once it has been proven that selenium is vital for the human body, there has been a high interest towards it from the medical point of view. In literatures, experts have been listing the selenium containing enzymes and proteins. It has been demonstrated that it is essential for the normal functioning of the immune system, for the mobility of the spermatozoons, furthermore it seems that selenium inhibits the development of HIV into AIDS. It has also become obvious that there are some diseases that can be the result of lacking selenium, such as Keshan and Back-Keshan diseases. Given these results there is no doubt about the importance of sufficient selenium intake for the normal functioning of the human organism. The most important selenium resources used to be the cereals, vegetables, meet and animal products. However, nowadays in many countries there are significant fights against changing nutritional habits that result in poor selenium intake, as well as against such animal foods that contain very little selenium. Therefore appropriate selenium supplement is a great challenge. Chemical and medical industries have quickly reacted to this need and as per today, there are many selenium-containing products on the shelves of different shops and pharmacies. At the same time, another possible way is to increase the selenium content of foods directly or indirectly. The vegetables have special importance because of many of them are able to transform the inorganic selenium species to organic selenoaminoacid derivatives. The selenoaminoacid derivatives are beneficial for human. The research with vegetables and other plant is focused on the selenate and selenite as most common and bioavailable selenium forms. The selenide and elemental selenium are rare and almost unavailable for plants. However there is a less researched type of selenium, the red elemental nanoselenium, which has shown surprising results in animal experiments, but nobody has been examined in Higher plants.

To connect the unanswered questions which, based on plants and selenium interaction, with the possibilities of my department the work was built on three basic pillars.

My aim was to study the morphological, physiological and biochemical effects of red elemental nanoselenium on a tobacco model, in tissue culture system comparing with sodium-selenate.

Furthermore, my aim was to examine the biological effects of selenate, as the most often used and easily accessible inorganic selenium speciation on some vegetable species, in *in vitro.*

The final aim was to observe the biological effect of selenate in cultivars comparative experiments in greenhouse environment. Closed fortification system was applied which was able to produce organic selenium enriched functional spring onions. To use the fortificated spring onion was made food supplement from it as finished product, too.

2. Materials and method

2.1. Plant biological effect of inorganic selenium species *in vitro*

2.1. 1. Tobacco tissue culturing

Tobacco (*Nicotinia tabacum* L. cv. Ottawa Petit Havana) seeds were surface-sterilized and sow onto hormone-free MS medium (with macronutrients, micronutrients and vitamins according to basal MS medium composition) under aseptic conditions, shoot cultures were established and maintained. Both from sodium-selenate 0; 0.1; 1.0; 10; 50; 100 mg/l solution (Na_2SeO_4) and from red nanoSe 0; 0.1; 1.0; 10; 50; 100 mg/l suspension (Bionanoferm Ltf. Debrecen, Hungary) were prepared in solid MS medium. All culture media were supplemented with 2 mg/l zeatin; 0,01 mg/l NAA, 1,5% sucrose and 6 g/l Plant agar. Entire leaves were cut along the main vein into 7x7mm sections.

The explants were inoculated into the solid media combinations above, 10 pieces onto every Petri dish. The newly formed callus sectors regenerated micro shoots which were excised and cultured on hormone-free, solid MS medium in 100 ml Erlenmeyer flasks. Cultures were maintained under white fluorescent lamps (41 µmol m^{2-} s^{-1} photon flux density), at 23 C^o and 8/16 h photoperiod.

2.1.2. Measurements from tobacco tissue culture

During our investigation, three different kinds of *in vitro* explants were analyzed: callus pieces containing regenerated microshoots (0.5 - 2 cm of diameter, abbreviated as CAMS), shoot cuttings of rooted shoot cultures (3 - 15 cm high, abbreviated as RES) and elongated roots of the regenerated plantlets (see abbreviation as RRES).

Morphogenetic and weight measurements: fresh weight, dry weight, dry material content, root initiation index

Biochemical and analytical measurements:

- Total selenium content was determined by AFS (Atomic Fluorescence Spectroscopy) technique (*Cabanero et al., 2004)*
- Nicotinic acid, nicotinamide, trigonelline content were determined by HPLC (High Performance Liquid Chromatography) technique
- Chlorophyll A and B content were determined by spectrophotometric method *(Hendry és Price, 1993*)

2.2. *In vitro* fortification of vegetable seedlings used by sodium-selenate

2.2.1. *In vitro* experiments

Radish (*Raphanus sativus* L. cv. Tavaszi piros) and pepper *(Capsicum annuum* L. *cv.* Láva*)* sterilized seed sowing was performed in plastic boxes, 100-100 seeds/box.

The applied selenium forms and concentrations were 0, 2, 10, 50, 100, 200 mg/l sodium-selenate solution.

Cultures were maintained under white fluorescent lamps (41 µmol m^{2-} s^{-1} photon flux density), at 23 C^o and 8/16 h photoperiod.

2.2.2. Measurements from seedlings

Morphogenetic and weight measurements: germination index, fresh weight, dry weight, dry material content.

Biochemical and analytical measurements:

Total selenium content was determined by ICP-MS (Inductively Coupled Plasma Mass Spectrometry) technique.

2.3. Fortification of green onion used by sodium-selenate

2.3.1. Greenhouse experiments

Allium cepa cv. 'Makói bronz', and 'Makói lila' varietes were utilized for the experiments. The commercial-size onions were planted into perlite bed in propagating trays. All of them were covered with wood shavings.

The applied selenium forms and concentrations were 0, 2, 5, 10 mg/l sodium-selenate solution.

Cultures were maintained under natural photoperiod. To decrease the light intensity Raschel-net was used on the windows. After three weeks the new developed green onions were separated from the mother tubers.

2.3.2. Measurements from green onions

Morphogenetical and weight measurements: fresh weight, dry weight, dry material content, length (by COLIM program)

Biochemical and analytical measurements:

- Total selenium content was determined by AFS technique (*Cabanero et al., 2004)*
- Selenium speciations were determined by HPLC-ICP technique
- Antioxidant capacity of watersoluble components (ACW) were measured by Photochem technique (*Popov és Lewin, 1999*)
- Antioxidant capacity of lipidsoluble components (ACW) were measured by Photochem technique (*Popov és Lewin, 1999*)
- Chlorophyll A and B content were determined by spectrophotometric method *(Hendry és Price, 1993*)
- Glutathione-peroxidase activity was measured by spectrophotometric method in rate assay (*Chiu et al., 1976*)

3. Evaluation of results

3.1. Plantbiological effect of inorganic selenium species *in vitro*

To compare the two selenium forms, the most important morphological differences was in the organogenesis of tobacco. High concentrations of nanoSe (50 – 100 mg/l) didn't inhibit the callus (CAMS) formation, moreover the high concentration of nanoSe treatment caused well-differentiated micro shoots on the callus surface without any vitrification symptoms (Fig. 1/A-B). Contrary, the majority of CAMS explants were malformed and many of them vitrified on the selenium-free control culture medium under the same ventilation conditions (Fig. 1/C-D). The selenate in 50 – 100 mg/l inhibited the organogenesis (Fig. 1/E) however 1 mg/l selenate increased significantly the fresh weight of CAMS.

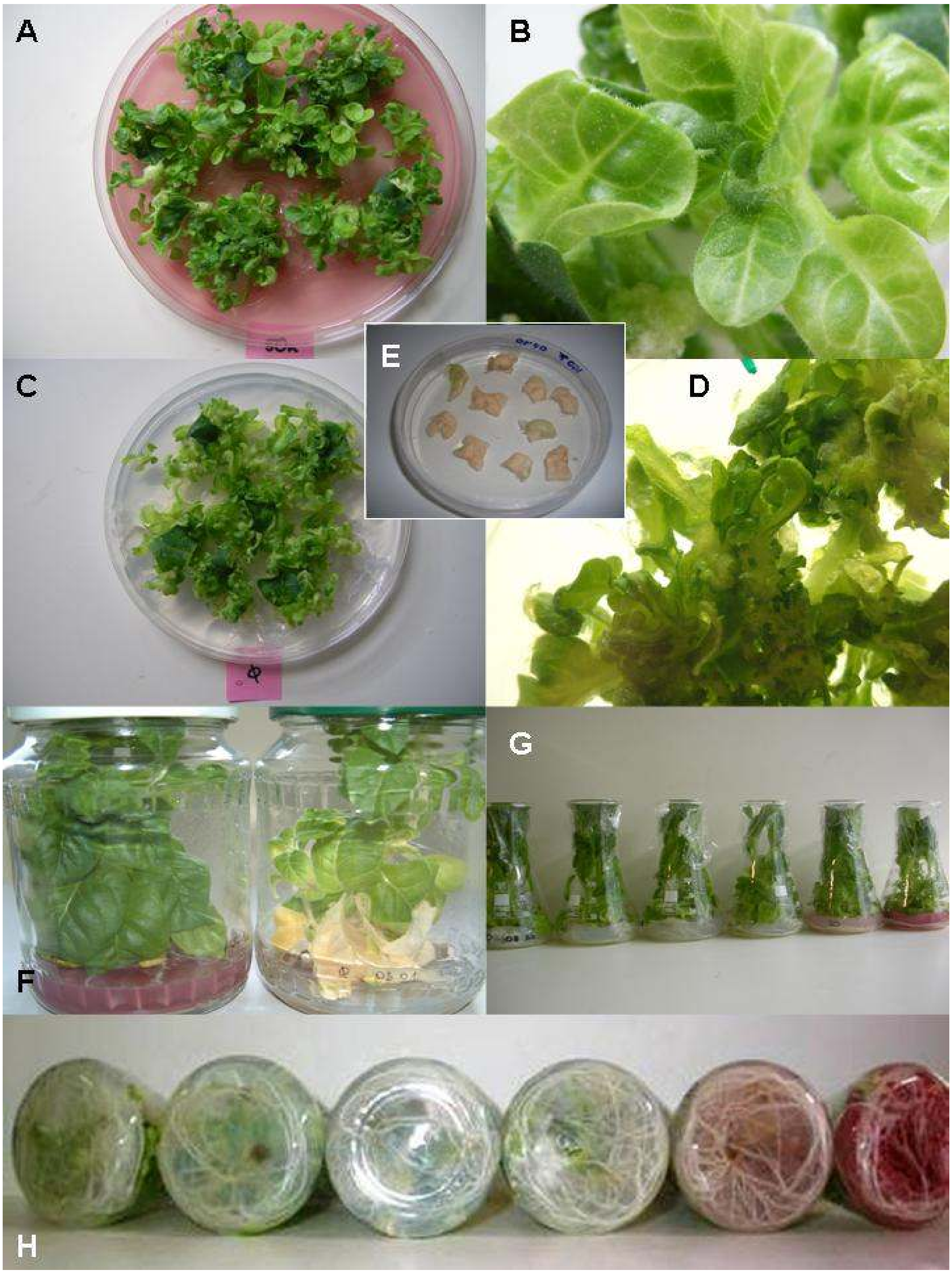

Fig.1. Callus formation and plant regeneration:

A-B: callus with microshoots (CAMS) on 100 mg/l nanoSe medium; C-D: callus with microshoots on control medium; E: lack of callus formation on 100 mg/l selenate medium; F: shoots of regenerated plants (RES) on control and 100 mg/l nanoSe medium; G: shoots of regenerated plants on medium which contained nanoSe in 0; 0,1; 1,0; 10; 50; 100 mg l^{-1} concentration; H: roots of regenerated plants (RRES) on medium which contained nanoSe in 0; 0,1; 1,0; 10; 50; 100 mg/l concentration.

The nanoSe didn't influence significantly the growth of shoot (RES) (Fig. 1/F-G), but ≥50 mg/l selenate inhibited it totally.

On the other hand the nanoSe stimulated significantly the root regeneration (RRES) in higher concentrations. The root was more extensive and dense used by 50 – 100 mg/l nanoSe (Fig. 1/H) and the fresh weight increased significantly, too. In contrast, the selenate inhibited the root formation totally in 50 - 100 mg/l concentration range.

The stimulating effect of nanoSe had already been detected during the rooting. The root formation of control plants reached 28% on the 8^{th} day, at the same time 40 – 73% of (10 -100 mg/l) nanoSe treated plants had got tiny roots (Fig. 2). On the 16^{th} day more than 90% of ≥10 mg/l nanoSe treated pants were rooted, however 70% of control plants developed root.

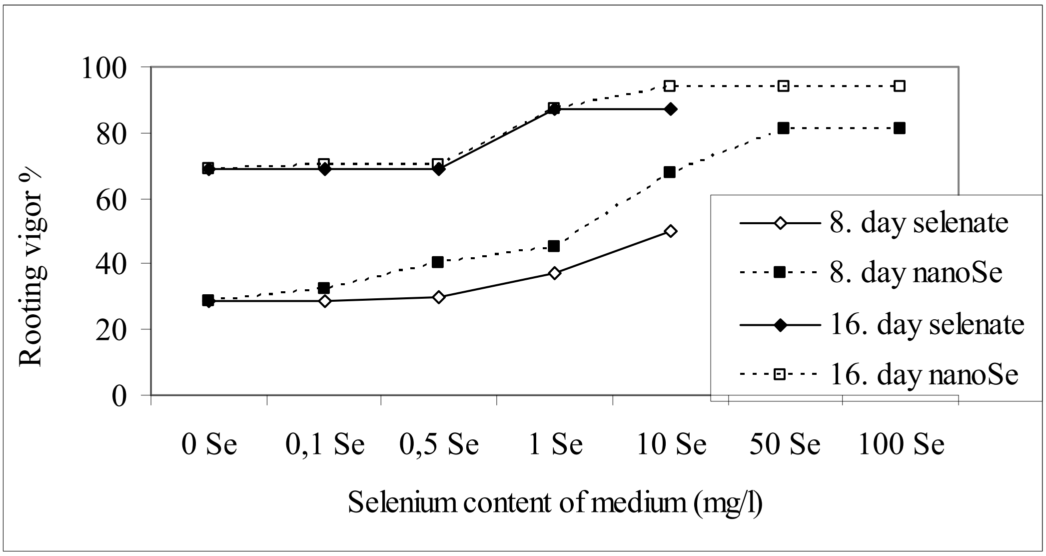

Fig. 2.: The rooting vigor of regenerated plants in two dates

Selenium accumulation was detected in both CAMS, RES and RRES treated with nanoSe although according to literature the elemental selenium is not phytoavailable (*White et al. 2004*). The nanoSe additions in medium significantly raised the total Se content in CAMS (Table 1). We assumed that nanoSe spheres as elemental form of selenium (Se^0) could diffuse into the plant tissue via the cutting area, epidermis and distributed in the intercellular space (apoplast) Inside of plant tissue in water soluble environment selenium ions can be released gradually from elemental nanoshperes. Thus the nanoSe could systematical provide a low steady state level of ionic forms to prove some benefit for the CAMS formation.

Total selenium values in CAMS were 2-3 times higher in the case of selenate than for nanoSe at the same treatment (Table 1). The selenate is ionic (Se^{6+}) form, which is a readily available selenium form. In excess is toxic and restricts development in higher plants *(Van Hoewyk et al., 2008*). Toxicity of Se is supposed to be due to its pro-oxidant ability to catalyze the oxidation of thiols and simultaneous generation of superoxide that can damage cellular components.

Table 1: Total selenium content of callus which contains micro shoots (CAMS), shoot of regenerated plants (RES), root of regenerated plants (RRES)

Treatment	**CAMS (mg/kg)**		**RES (mg/kg)**		**RRES (mg/kg)**	
	selenate	nanoSe	selenate	nanoSe	selenate	nanoSe
0 mg/l	0.23 ± 0.1	0.23 ± 0.1	0.07 ± 0.02	0.07± 0.02	0.02 ± 0.002	0.02 ± 0.002
0,1 mg/l	3.8 ± 0.32	0.71 ± 0.4	4.19 ± 0.4	1.05 ± 0.2	1.60 ± 0.07	4.83 ± 0.58
1,0 mg/l	16.0 ± 0.4	3.05 ± 0.3	53.2 ± 3.0	2.7 ± 0.3	15.3 ± 0.13	24.4 ± 4.0
10mg/l	300 ± 11	40.9± 1.9	318 ± 13	20.3 ± 2.2	74.8 ± 14.6	269 ± 11
50 mg/l		**164 ± 16**		**35.2± 3.8**		**1237 ± 42**
100mg/l		**391 ± 31**		**84,9± 9,8**		**2947 ± 99**

To observe the regenerated plants, it was found that the selenate was accumulated better in the RES than the nanoSe; meanwhile in RRES the accumulation of nanoSe was much higher than that of selenate. Extremely high total Se values (1237-2947 mg/kg) were measured in the root using 50 – 100 mg/l nanoSe treatment (Table 1). These results also confirmed our presumption that from the big amount of elemental selenium nanoparticles gradually released the ionic forms to systematical provide a low steady state levels. The very low steady ion concentration can cause direct and/or indirect way for stimulation of root regeneration and elongation. It is supposed that the released Se ions may interfere the plant hormones signal pathways especially the ethylene signal transduction pathway. The ethylene binding receptors have copper ions as cofactor (*Kendrick and Chang, 2008*) and the Se ion may change or interfere with copper to inhibit the ethylene pathway. This possibility has to be proved in details.

The nicotinamide and its metabolites such as nicotinic acid or trigonelline would participate in oxidative stress as transmitters (*Berglund et al., 1996)*. The nicotinamide, nicotinic acid and trigonelline could be separated in the same chromatographic system because of their chemical stuctures. The nicotinamide, nicotinic acid and trigonelline content increased in CAMS which treated with ≥50 mg/l nanoSe (Fig. 3/A-B-C). The selenate increased the nicotinamide and nicotinic acid content but didn't influence significantly the trigonelline content in CAMS. Based on these results it seems that the nanoSe in bigger concentration is also stress inducer like other selenium forms.

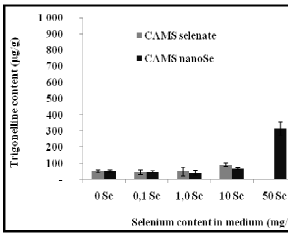

Fig. 3/A Trigonelline content in callus (CAMS)

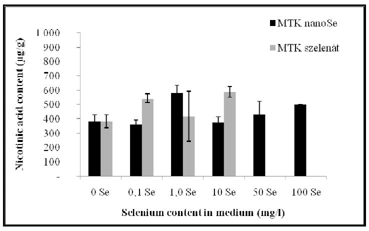

Fig. 3/B Nicotinic acid content in callus (CAMS)

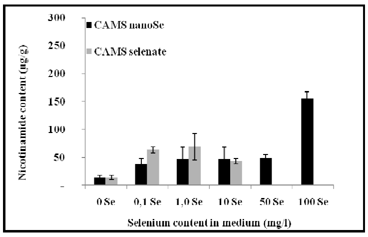

Fig. 3/C Nicotinamide content in (CAMS)

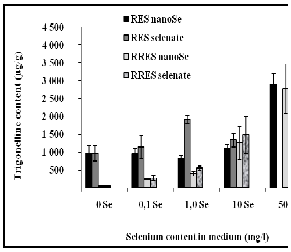

Fig. 4/A Trigonelline content in shoot and root of regenerated plant (RES and RRES)

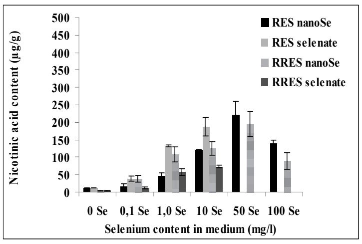

Fig. 4/B Nicotinic acid content in shoot and root of regenerated plant (RES and RRES)

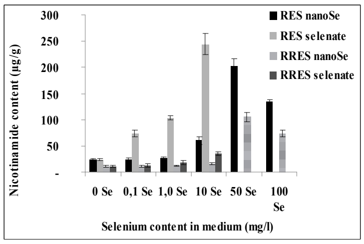

Fig. 4/C Nicotinamide content in shoot and root of regenerated plant (RES and RRES)

Both selenium species increased the contents of nicotinamide meabolites in regenerated plants although the effect of selenate was prevailed in lower concentration than the nanoSe (Fig. 4/A-B-C).

To compare the morphological and weight values with the nicotinamide, nicotinic acid and trigonelline results it seemed that both selenium species are stress inducer but there are differences in their biological effects. The nanoSe in higher concentrations (50 – 100 mg/l) induced eustress which was beneficial for the callus growth, vitrification inhibiton and the root formation. Contrarily to this the selenate caused so-called distress already in lower concentration (≥10 mg/l) which was harmful for the callus and root growth.

The selenate (≥10 mg/l) decreased significantly the concentration of chlorophyll A and B in CAMS (5/A), however the nanoSe didn't influence it significantly.

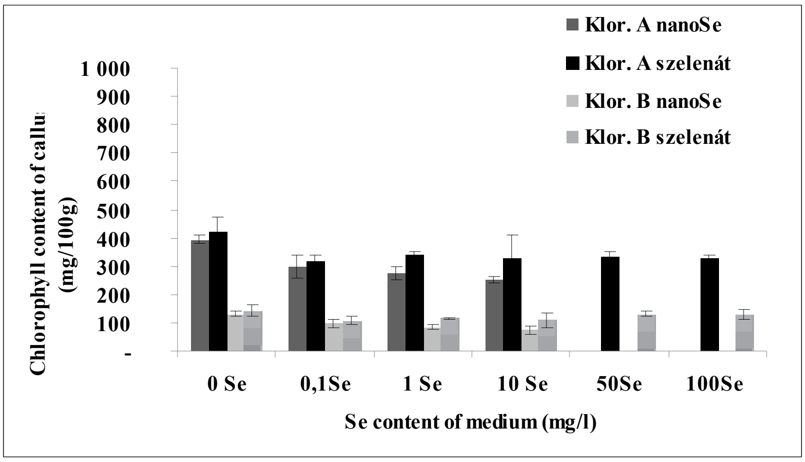

Fig. 5/A.: Chlorophyll A and B content in callus with microshoots (p<0,05)

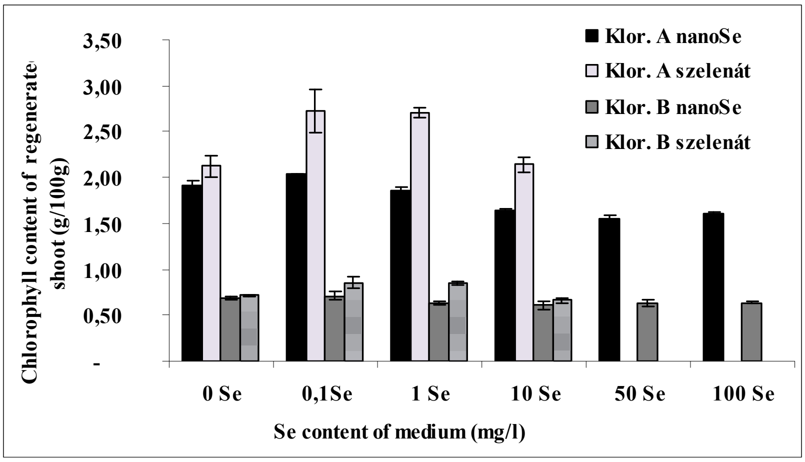

Fig. 5/B.: Chlorophyll A and B content in shoot of regenerated plants (p<0,05)

In case of RES, it was found that the nanoSe, ≥10 mg/l, decreased the chlorophyll A synthesis, but it couldn't be found significant difference in chlorophyll B content (Fig. 5/B). The selenate in 0,1 to 1,0 mg/l increased the chlorophyll A content.

3.2 Comparative experiments of sodium-selenate in selenium accumulator and non-accumulator vegetable seedlings

The rate of germination of the radish and pepper was different. The radish, as a shortseason species starts to germinate the day after seeds sowing. At the same time the pepper, as a long-season species has only started germinating on 6th day.

The selenium accumulating abilities of the two vegetable species were also visible during the germination.

In low concentration (2 mg/l selenate) the germination of both species had slightly been quickened. Despite of this, in 200 mg/l concentration, the germination of the pepper has been completely inhibited, while it made no change with the germination of the radish.

The selenate tolerance of the radish and pepper was also visible in the biomass production of the seedlings. 2 mg/l made no significant differences in the biomass production of the radish's shoot (7% weight increase per heads – Fig. 6/A). No concentrations were found in the examination of *Carlson et al. (1989)* either, which would have significantly increased the weight of the radish, cabbage, lettuce or wheat. The toxic effect was visible in 50 to 200 mg/l and was also statistically confirmed by the decreased weight results of the shoots. (Fig 6/A) The toxic effect of selenate was seemed at lower concentration in pepper than radish which caused the differences in selenium tolerance of them (*Dhillon and Dhillon, 2009).* The growth of the non-selenium accumulator pepper retained more compared to the radish seedlings, by the increasing selenate concentration (Fig. 6/A).

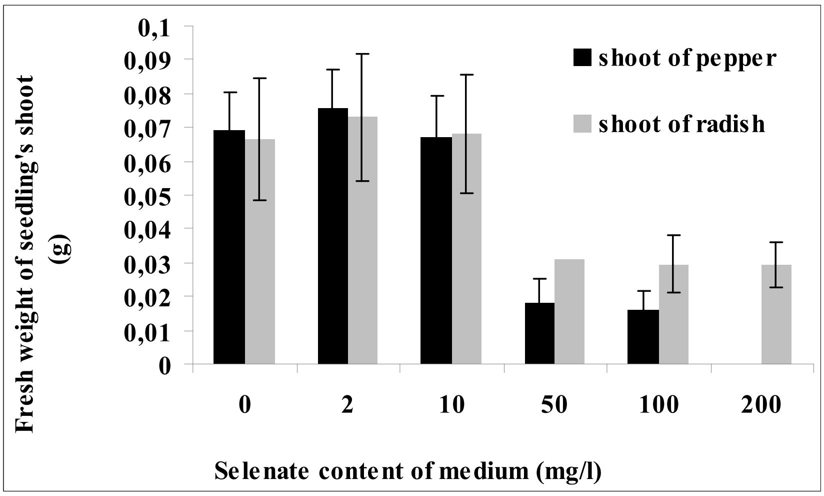

Fig.6/A. Average fresh weight of shoots of pepper and radish seedling on medium which contained selenate ($p<0,05$) (+/-SE)

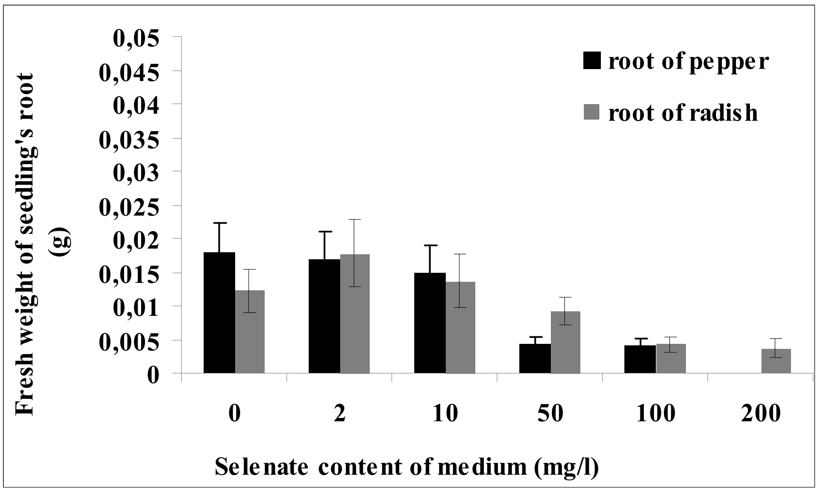

Fig. 6/B. Average fresh weight of roots of pepper and radish seedling on medium which contained selenate ($p<0,05$) (+/-SE)

There was a little difference in the change of weight of the roots compared to the shoots. In case of the radish, 2 mg/l selenate has significantly increased the root biomass production, unlike the pepper, where the same concentration had slightly reduced the fresh weight of roots. (Fig. 6/B). The concentration of 50 to 200 mg/l had significantly reduced the development of both radish and pepper roots.

Selenate is an easily available form of selenium for vegetables, which is proved by the results of full selenium content. At the same time, the selenium content of the seeds proved the selenium accumulating abilities for both two species. In selenium accumulator radish seed, the total selenium value (0,498 mg/kg) was an order of magnitude larger than in case of the pepper (0,015 mg/kg). The selenium content of the seedlings on control medium made further confirmation in the difference of the selenium accumulating abilities of the two vegetables, because the results here clearly showed how much selenium is freed from the seeds. The selenium content was larger in the radish than in the pepper by one fold, whether I considered the shoots (radish shoot: 0,0152g/kg ↔ paprika shoot: 0,0011g/kg) or the roots (radish roots: 0,123g/kg ↔ paprika roots: 0,0034g/kg) (Fig. 7/A-B). The vegetables in *Brassicacae* family, among them the radish and cabbage, is partly able to transform the admitted inorganic selenium into amino acid derivatives, which do not effect the metabolic process, therefore it is able to tolerate it in higher concentration (*Mounicou et al., 2006*). In contrast, in non-accumulator vegetables, a great part of the admitted selenium build into selenomethionine, to destroy many functional proteins, of which result the toxic symptoms appear at even lower selenate concentration. My experiment confirmed this, in case of 200mg/l the selenium fully inhibited the germination of the seed, while the radish seedlings developed moderately. To see the distribution of selenium content of different parts it was found that the roots accumulated much more higher than the shoots (Fig. 7/A-B) unlike literatures (*Li et al., 2008)*.

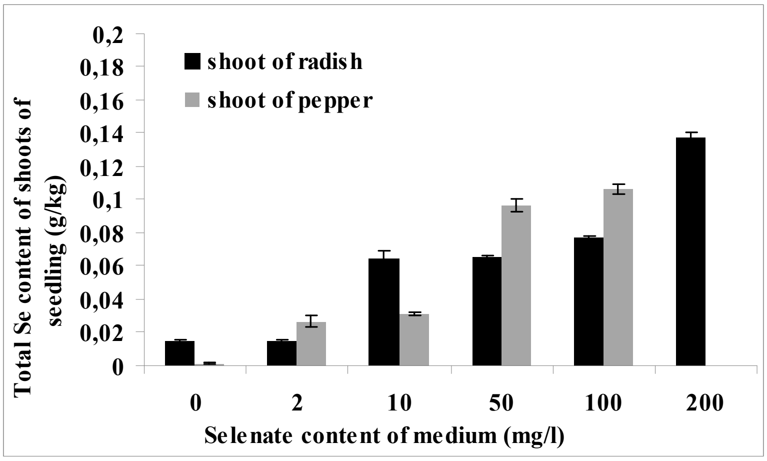

Fig. 7/A.: Total Se content of shoots of seedlings on medium which contained selenate ($p<0,05$) (+/-SE)

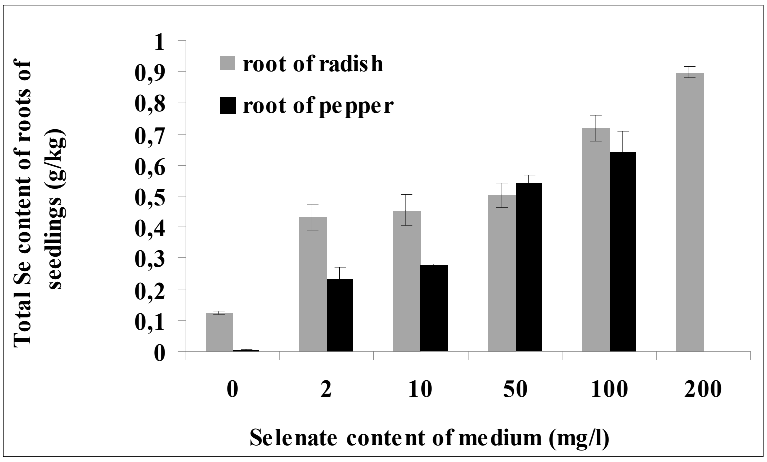

Fig. 7/B.: Total Se content roots of seedlings (g/kg) on medium which contained selenate (mg/l) ($p<0,05$) (+/-SE)

In their observation higher selenium accumulation was in the shoots than the roots. On the other hand, these results may contradict with my previous results in the tobacco tissue culture, where higher selenium values were measured in the shoot parts, compared to the root. However, there is a great difference between the two experiments: in case of the tobacco, cut surfaced microshoots were put onto the regenerating medium. A great amount of selenate was able to get in through the cutting surface by passive diffusion and by the time the root started developing (more than a week) much selenium had already accumulated in the shoot. In case of seedlings the selenate ion could only get in through the root from seeds.

3.3. Comparative experiments of sodium-selenate in Makói bronz and Makói lila spring onion cultivars

The cultivarss comparative trials of spring onions in greenhouse environment showed that the applied sodium-selenate (0 – 10 mg/l) did not influence the development of Makói lila to see the weight and length but the length of Makói bronz decreased significantly used by 10 mg/l selenate (Fig. 8/A-B). The obtained results were confirmed by the visual experience as well: the treated spring onions were shorter, but thicker compared to the control. (Fig. 8/B).

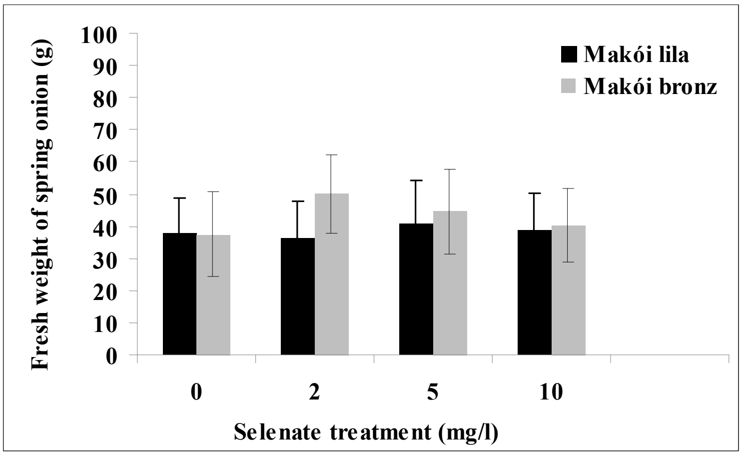

Fig. 8/A.: Fresh weight of Makói lila and Makói bronz varieties depending on selenate treatments ($p<0,05$) (+/-SE)

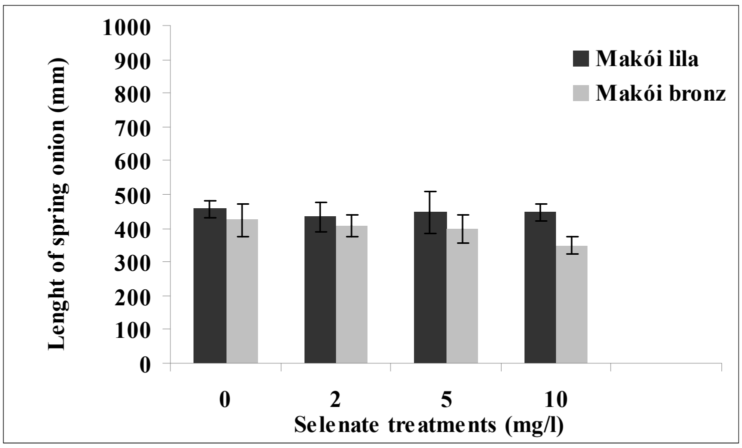

Fig. 8/B.: Lenght of Makói lila and Makói bronz varieties depending on selenate treatments ($p<0,05$) (+/-SE)

To see the dry materials it was found that the sodium-selenate did not effect significantly the dry material incorporation in the applied concentrations, the difference between cultivars was prevailed more (Fig.9). Makói bronz was measured a much higher value of dry materials (0,145 - 0,175 g/kg), than in case of Makói lila (0,087 – 0,101 g/kg).

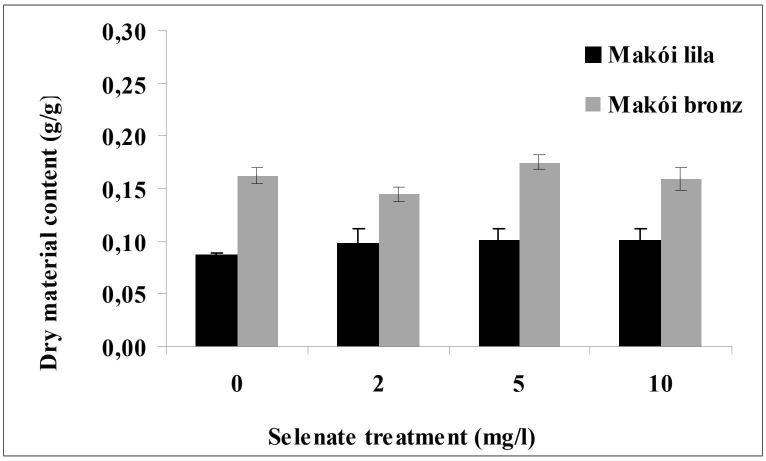

Fig. 9.: Dry materials of Makói bronz and Makói lila depending on selenate treatments ($p<0,05$) (+/-SE)

The total selenium content of spring onions increased proportionately with applied selenate concentration. To compare the two onion cultivars, no remarkable difference was showed between their selenium accumulating abilities.

Using speciation analytical method the inorganic selenate forms would be separated on the anion exchange column: the retention time was 5 min. in case of selenite (SeIV) and 20 min. of selenate (SeVI) (Fig. 10). By the available standards, only selenomethionine and selenocystine were identifiable of the organic selenium forms. On the column these two compounds were detectable with same retention time (3 minutes) therefore it can only be seen as an organic form (Fig.10).

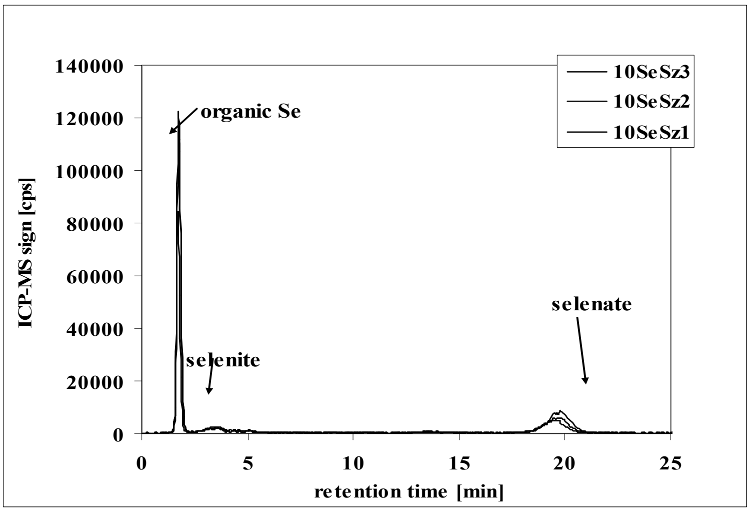

Fig. 10.: Chromatogram of selenium species from Makói lila onion in three repeats

To compare the relative concentrations of separated selenium forms it was found that Makói bronz transformed the admitted selenate into the organic forms in greater amount than Makói lila, taking the water soluble fraction into consideration. By increasing the doses of treatments in both cultivars' cases, the organic selenium forms increased more significantly. (Fig. 11/A-B)

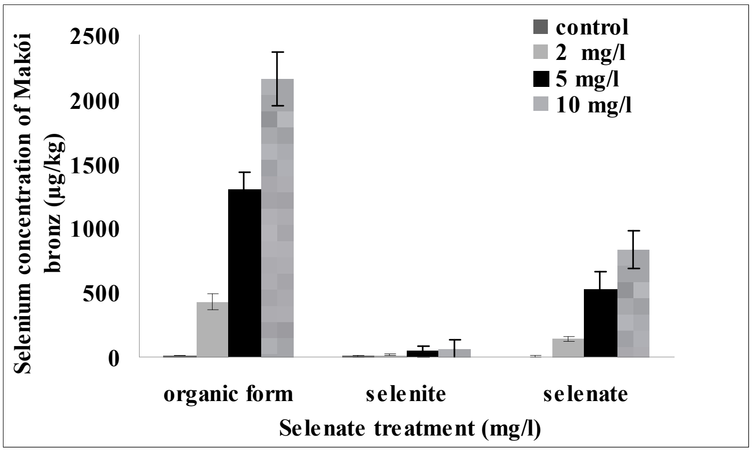

Fig.11/A.: Relative concentrations of selenium species in Makói bronz depending on selenate treatments ($p<0,05$) (+/-SE)

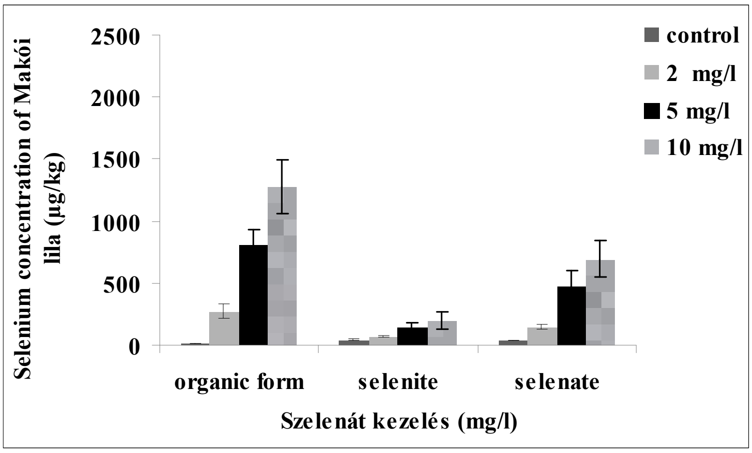

Fig.11/B.: Relative concentrations of selenium species in Makói lila depending on selenate treatments ($p<0,05$) (+/-SE)

To compare the cultivars with each other showed that the organic selenium content in Makó bronz was 1.5 fold than Makói lila (Fig. 11/A-B). The larger part of admitted selenate transformed into organic form, which is advantageous for human consumption. The selenoaminoacid derivatives are more available for human and more effective against of cancer than inorganic forms (*Ip et al., 2000; Spallholz et al., 2001*). The selenite was smallest amount in both cases of two spring onions. Specially low value was measured in Makói bronz and it was no big difference even with increasing the doses of the treatments. Selenite is a kind of intermediate in the transformation of selenate to selenoaminoacid. The result refers to the transformation was so quickly, so the concentration of the organic forms significantly increased, while the selenite's did not.

The total antioxidant capacity of lipid and the water-soluble antioxidant compounds were determined separately, by Photochem machine. The total antioxidant capacity is a parameter that depends on not just the species but inside of species there would be difference among the cultivars too as it was noticed. The water-soluble antioxidant capacity of Makói lila was much higher than the Makói bronz. The value of the Makói lila was between 4,4 – 5,2 µg/mg. while in case of the Makói bronz it was 2,66 – 3,15 µg/mg (Fig. 12/A-B).

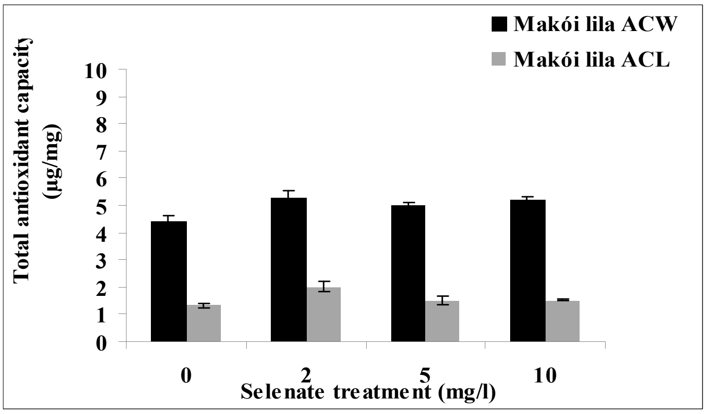

Fig. 12/A.: Total antioxidant capacity of Makói lila green onion depending on selenate treatments (p<0,05) (+/-SE)

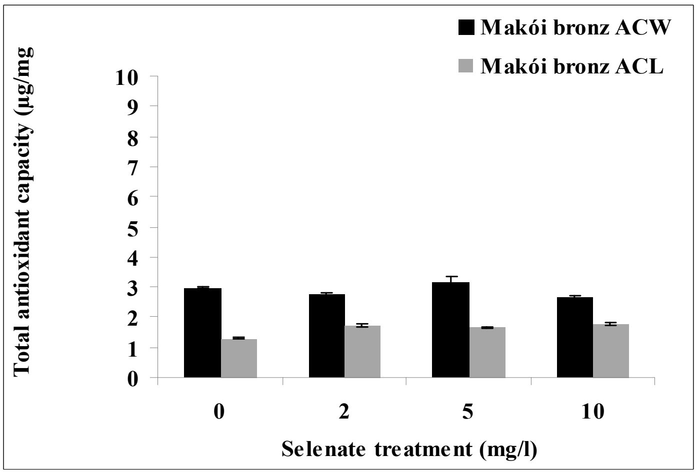

Fig. 12/B.: Total antioxidant capacity of Makói bronz green onion depending on selenate treatments (p<0,05) (+/-SE)

Its reason must be the high anthocyanin content of the Makói lila (which gives its colour) since this compound group has a very high antioxidant capacity while it is missing from the Makói bronz. Beside this, the amount of flavonoids that have also high antioxidant activity is substantially higher than it is in red onion (*Lugasi et al., 2003*). There was no significant difference in the amount of lipid soluble antioxidants comparing the two cultivars. The selenate treatments did not influence the ACW and ACL content of the Makói lila (Fig. 12/A-B). However, significantly lower ACW value could be measured in Makói bronz spring onion used by 10 mg/l concentration compared to the control. At the same time, the ACL values had significantly increased at 5-10 mg/l concentration in Makói bronz.

There was also significant difference in the chlorophyll A and B content between the two cultivars using selenate treatment (Fig. 13-14).

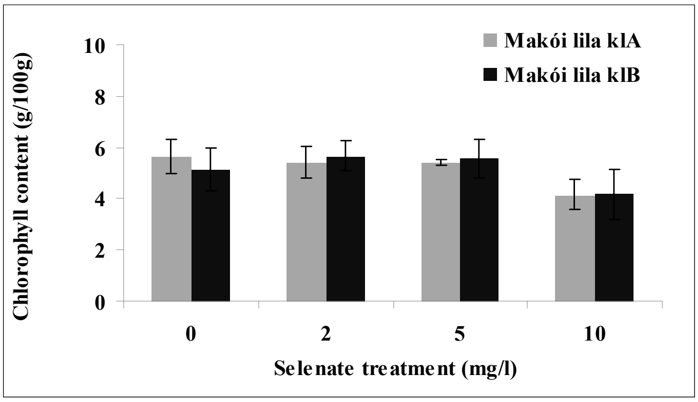

Fig. 13.: Chlorophyll A and B content of Makói lila (p<0,05) (+/-SE)

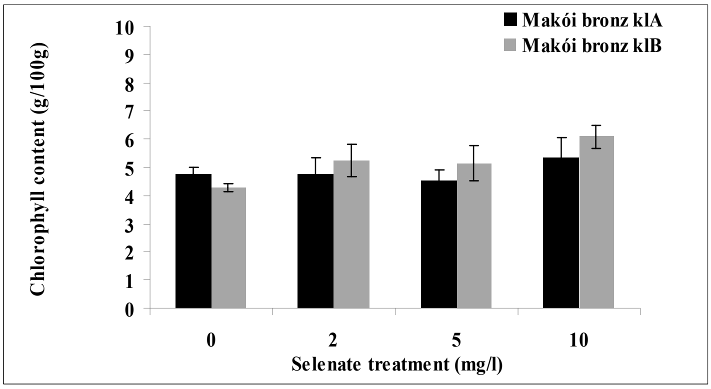

Fig. 14.: Chlorophyll A and B content of Makói bronz (g/100g) (p<0,05) (+/-SE)

Lower concentrations of selenate (2-5 mg/l) did not influence the chlorophyll synthesis of Makói bronz, while at 10 mg/l was realized significant decrease (Fig. 13). The tendency was the same in case of chlorophyll B, but there was no significant difference (Fig.13). The chlorophyll A and B content of Makói bronz increased used by selenate treatments, although statistically verifiable deviation occurred only at 10mg/l (Fig. 14).
Glutathione-peroxidase (GPx) as an antioxidant enzyme has great significance if a plant is stressed, so from the change of activity it could be infered to the changed state. No significant difference was found in glutathione-peroxidase activity in Makói lila used by selenate treatments in 2 – 10 mg/l range. However the GPx activity of Makói bronz was significantly decreased by the applied selenate concentrations. To compare the results of glutathione-peroxidase with the total antioxidant capacity and weight, it is conspicuous that Makói lila tolerates the selenate better than Makói bronz does. In case of the Makói lila there was no significant change neither in length nor in weight by the applied concentrations, together with it any biochemical stress could not be confirmed. The decrease was only shown in case of chlorophyll A at 10 mg/l selenate. At the same time,

the decrease experienced in Makói bronz's length growing seemed to be a sign of stress, which was confirmed by the changes observed in the capacity of ACW, ACL and glutathione-peroxidase activity.

4. New and novel scientific results

4.1. My experiment was the first which dealing with the biological effect of red elemental nanoselenium in Higher plant.

- It was proved that unlike selenate, the elemental nanoSe in higher concentration (50 – 100 mg/l) stimulated the organogenesis (callus initiation and root regeneration) however inhibited the virtification of tissue culture.
- The nanoSe similarly to selenate could generate some stress-inducible phenomena in tobacco tissue cultures as indicated by the decreased chlorophyll content in callus cultures and regenerated shoots. In addition, the nanoSe was able to modify the pyridine nucleotides-recycling pathway which was proven by the increased level of nicotinic acid, nicotinamide and trigonelline content both in callus cultures and in regenerated plants, too.

4.2. The examination of vegetable seedling *in vitro* confirmed the literature that both selenium accumulator and non-accumulator species are able to take in selenium, only the toxic symptoms appear at lower concentration in non-accumulator than it does at selenium accumulator species.

4.3. Closed system was applied to fortificate spring onion with selenate in cultivars comparative trials in green house environment.

- The comparative examination of Makói bronz and Makói lila spring onions was proved that there are difference in selenium tolerance not only between species, but also inside species, between the cultivars too. Makói bronz cultivar was more sensitive because 10 mg/l selenate inhibited the shoot elongation significantly, at the same time this concentration didn't cause any toxic symptoms in Makói lila. The ACW, ACL and glutathione-peroxidase activity measurement confirmed the morphological experiences, $\leq$10 mg/l selenate didn't influence these biochemical parameters of Makói lila. However, ACW and glutathione-peroxidase activity were decreased in case of Makói bronz and the ACL as well as chlorophyll content were influenced positively.
- The speciation analytics measurements also showed differences between onion cultivars. The rate of organic selenium forms was higher in Makói bronz than

Makói lila. Moreover, there were two unknown selenium species only in Makói bronz. It seemed these as characteristics of this cultivar.

5. Results for practical utilization

- Taking the organogenesis stimulation and vitrification inhibition effects into consideration, it seemed that nanoSe can be beneficial for tissue culture researchers for who in many cases had problems with the virtification of the cultures. In most of the cases, virtification is irreversible; therefore, the researcher has a clear loss. On the other hand, to add nanoSe into the medium can be beneficial for those plants which rooting difficultly *in vitro*.
- Both selenate and nanoSe also increased the nicotinic acid and nicotinamide's amount in tobacco callus and regenerated plants as well. These compounds are not just intermediates in NAD salvage cycle, but they are known as vitamin B3. Therefore, to produce selenium enriched vegetable using by nanoSe or selenate is good way to increase the vitamin B3 content as well.
- Based on experiments with vegetable seedlings it seemed that 2 mg/l selenate can be optimal concentration for producing selenium enriched vegetable seedlings both in case of selenium accumulator or non-accumulator species, at the same time this concentration doesn't influence negatively the development.
- Spring onion produced in a closed system enriched with selenium would be good source of organic selenium forms, specially in early spring which is the main season of forcing spring onion.
- From selenium enriched spring onion could also produce further functional produces. To continue the work in this direction, I have collected the fresh spring onions that were treated with 10mg/l selenate, after lyophilizing the dry materials were powdered. The powdered samples were capsuled by Dr Aliment Ltd. The product called SelenAl, "as an organic selenium containing food supplement made from spring onion" was authorized by the OÉTI with a notification number: 1859/2007.

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7. Publications in the field

Foreign language publications in international revised journal:

- **Domokos-Szabolcsy Éva -** Veres Zsuzsanna - Kovács Béla - Prokisch József - Holb Imre - Fári Miklós (2006): Impact of sodium-selenate on the growth of radish seedlings *in vitro*. Acta Horticulturae. ISSN: 0567-7572 725. 181-185.

- Mészáros Annamária - Kálai Krisztián - Halász Katalin - **Domokos-Szabolcsy Éva -** Fári Miklós (2006): An efficient micropropagation system: 3R Bioreactor. Acta Horticulturae. ISSN: 0567-7572 725. 621-624.

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- **Domokos-Szabolcsy Éva -** Holb Imre - Prokisch József - Kovács Béla - Veres Zsuzsanna - Fári Miklós (2005): Impact of sodium-selenate on the growth of radish (*Raphanus sativus* L.) seedlings *in vitro*. International Journal of Horticultural Science ISSN: 1585-0404. 11.2. 113-117.
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Foreign language publications in revised conference proceedings:

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- **Domokos-Szabolcsy Éva -** Miskei Márton - Remenyik Judit - Prokisch József - Fári Miklós (2009): Effect of nanoselenium in in vitro plant tissue culture 3. Trace elements in the food chain Congress Budapest, ISBN: 978 963 7067-198. 447-451.

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7. Innovations in the field

Product development:

- Organic enriched forcing spring onion
- SelenAl SelenAl, "as organic selenium containing food supplement made from spring onion" was authorized by the OÉTI with a notification number: 1859/2007.

Patent:

- Dr. Prokisch József, Dr. Fári Miklós, Dr. Győri Zoltán, **Domokos-Szabolcsy Éva**, Veres Zsuzsanna, Dr. Kovács Béla: Fruits with high biological value and process to produce P0501073 Hungarian Patent Office, 2005 Patent