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SELENIUM BIOFORTIFICATION BIOTECHNOLOGIES OF WHEAT GRAIN IN SOUTH –EASTERN PART OF ROMANIA FOR A BETTER HUMAN HEALTH

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ABSTRACT. The low level of selenium in soils from different Romanian areas indicates a need for its supplementation. Biofortification through treatments applied to crops has the advantage of supplementation of food chain with controlled levels of selenium compounds, with high bioavailability. We present a biotechnological approach of biofortification, adapted to the conditions specific for the soils from South-Eastern part of Romania, which is based on the use of selected plant growth promoting rhizobacteria (PGPR). We evaluated, in controlled experiments, the ability of selected PGPR to increase, in a reference South-East Romania soil, the selenium bioavailability for wheat plant. We integrate this selenium biofortification biotechnology into a GIS-based decision support system. We are discussing Se biofortification in relation with its interferences with polyamines metabolism. We are evaluating the potential use of a new emerging biotechnological approach, based on plant tissue nitric oxide inductors, for a more equilibrated selenium compounds accumulation.

Keywords: selenium, biofortification, plant growth promoting bacteria, wheat, polyamines

INTRODUCTION

Selenium (Se) is an important element for human health. Selenium has a very narrow physiological window. It is a very small difference between the recommended daily human dietary intake for chronic diseases prevention and that producing pathophysiological effects. Statistical studies in humans have revealed a consistent trend of mortality risk response by chronic diseases in U shape, especially in cases of cancers (Rayman, 2012; Bleys *et al.,* 2008). This physiological response is determined by different expression of the main selenoproteins and selenium chemopreventive compounds, depending on dietary intake of selenium (Rocourt & Cheng, 2013).

Dietary intake is determined by the level of selenium in the soil, the global average regarded as being non-affected by deficits or excesses being 383 ± 255 mcg / kg (Kabata-Pendias & Pendias, 2001). In Romanian soils, selenium is at the deficit limit. Diseases caused by selenium deficiency have been reported in animals from different regions of Romania (Serdaru & Giurgiu, 2007; Serdaru *et al.,* 2003; Lăcătuşu *et al.,* 2002). Compared to the global value considered as being non-affected by deficit or excesses, selenium content is reduced by: 30% in Făgăraş Depression (Lăcătuşu *et al.,* 2012); 40% in Romanian Plain and 63% in Dobrogea (Lăcătuşu *et al.,* 2010). The deficit of selenium on Romanian Plain and Dobrogea soils is amplified by the low content of mobile selenium. Especially in Dobrogea soil the mobile selenium is very low, even in comparison with neighboring Romanian Plain. On the soils of Central and South Dobrogea 3.5 times lower average values of mobile selenium were registered (4 mcg/kg), as compared to the average mobile selenium content in the South-Eastern Romanian Plain soils (14 mcg/kg) (Lăcătuşu *et al.,* 2010).

The low level of selenium in the soils, and especially of bioavailable / mobile selenium causes a low intake of selenium in the diet, indicating a need to supplement it, in order to achieve the optimal level of selenium status, beneficial for reducing chronic disease risks (Mehdi *et al.,* 2013; Steinbrenner *et al.,* 2013). Biofortification of the food chain through treatments applied to plants (agronomic biofortification) have the advantage of introducing controlled levels of selenium compounds, with high bioavailability, widely accessible to different categories of people with risks of chronic diseases, including those with low incomes (Fageria *et al.,* 2012; White & Broadley, 2009).

Application of Se-fertilizer was used to increase the Se content of the food chains in various area of the world (Poblaciones *et al.,* 2014; White & Bradley, 2009; Lyons *et al.,* 2003). But inorganic selenium fertilization has several limitations. Selenium compounds are highly toxic and their use and application require special conditions and legal notifications. Selenite species are complexed in the inner sphere of soil (organo)mineral particles, thus became unavailable for plants (Gabos *et al.,* 2014). More mobile selenate species could be leached from soil (Tolu *et al.,* 2014) and could determine negative

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environmental side effects. On Finland large utilization of selenium fertilizers leads to a significant increase of the selenium content also in the aquatic systems (Alfthan, 2013).

The goal of our study was to develop a safer biofortification of food chain by a biotechnological approach. The main specific objectives related to this goal were as follows: (*i*) to select plant growth promoting rhizobacteria (PGPR), based on several *in vitro* characteristics related to the potential increase of selenium bioavailability, on conditions specific to South-East Romania soils, which are presenting deficits; (*ii*) to evaluate, in controlled experiments, the ability of bacteria to increase selenium mobility and bio-availability for wheat plants, in a reference soil from South-East Romania; (*iii*) to develop approaches for a precise application of selected PGPR inoculants, based on Geographical Information System (GIS) decision support, intended to exploit also the plant protection potential of such biotechnological Se biofortification; (*iv*) to establish a framework for a future development of biotechnological selenium agronomic fortification, which is balancing selenium and polyamine metabolism in Se biofortified plant tissues.

MATERIALS AND METHODS

Soil sampling.

We collected soil samples, from the upper horizon (0-20 cm) and from wheat plant rhizosphere, from the South-Eastern Part of Romanian Plains and from Central and Southern Dobrogea. In the Romanian Plain we collected samples from two areas, one bordered by the localities: Slobozia, Drajna, Călăraşi, Feteşti, Unirea, Ţăndărei, and Giurgeni, and the second one bordered by the localities: Slobozia, Ciochina, Lehliu, Valea Argovei, Mănăstirea, Călăraşi. In Central and Southern Dobrogea we collected soils and wheat rhizosphere samples from the area bordered by the localities: Vadu Oii, Hârşova, Saraiu, Rahmanu, Casimcea, Cheia, Sibioara, Ovidiu, Agigea, Amzacea, Comana, Negru Vodă, Cobadin, Adamclisi, Ion Corvin, Alimanu, Cochirleni. The sample soils were from the following class of soils: Typic Chernozem (Calcic Chernozem); Calcaric Kastanic Chernozem (Calcarocalcic Chernozem[2]); Kastanozem[1] (Kastanozem[2]); Regosol[1] (Regosol[2]); and Aluviosols[1] (Fluvisol[2]).

Isolation of bacterial strains.

We isolated bacterial strains from collected soil samples. Bacteria from Proteobacteria phylum were isolated on King B medium, according to method described by Laslo *et al.* (2012). Briefly, we prepared serial dilutions in axenic conditions from soils and wheat rhizosphere samples. Volumes of 0.1 ml from 10^4-10^6 dilutions were uniformly spread on Petri dishes using a Drigalski spatula. We isolated spore forming bacterial strains from sampled soils cultures on Nutrient Broth (NB) (Sicuia *et al.*, 2012). 1 gram of each soil sample was homogenized with 25 sterile NB and incubated for 24 h at 28°C and 150 rpm/min. We transferred 2 ml from such NB broth soil culture to a glass tube. Glass tubes with NB broth soil culture were kept for 15 min on boiling water. From this thermal treated sample we took 0.1 ml, which we spread on Petri dishes with Luria-Bertani agar medium. We incubated the inoculated Petri dishes for 48 h at 28°C. At 24 and 48 h we inspected inoculated Petri dishes and we noted the colonies morphology. Well individualized colonies were purified by growing on specific enrichment broth media (King B for Proteobacteria isolates and Luria-Bertani broth for spore forming PGPR). Based of morphological and physiological characteristics we selected 262 bacterial isolates, 140 from Proteobacteria phylum and 122 gram-positive spore forming bacilli.

Determination of soil characteristics

On collected soil samples we assayed the following characteristics: pH (by potentiometry, with combined glass / calomel electrode, in aqueous suspension, soil : water ratio 1:5); humus content (according to Walkley-Black method modified by Gogoaşă, by wet oxidation and titrimetric dosage); mobile forms of phosphorus in ammonium acetate-lactate (P_{AL}- Egnér, Riehm and Domingo, 1960).

Screening of PGPR strains and their identification

We initially screened the bacterial isolated for their characteristics related to enhancement of plant root growth and development: 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) activity; arginine decarboxylase activity (related to polyamines production ability); acetoin and 2,3 butanediole production (volatile plant growth promoting substances). ACC deaminase activity was determined by growing bacteria in Dworkin–Foster (DF) salts minimal medium containing ACC compounds as the sole N source (Glick, 2003). We screened bacteria for arginine decarboxylase activity by culturing bacterial isolates on specific agar media (5 g/l bacto-peptone, 5 g/l beef extract, 0.5 g/l glucose, 5 mg/l pyridoxine, 20 mg/l arginine monochlorhydrate, 20 mg/l phenol red, 15 g/l agar, final pH 6.0±0.2) at 25°C. Bacteria with arginine decarboxylase activity change the medium pH and the pH-indicator turns the color from yellow (in acid media) to red-fuchsia (in alkaline media). We used the Voges-Proskauer test for initial detection of acetoin producer strains. The bacteria were inoculated into 2 ml of Methyl Red-Voges-Proskauer Medium (MR-VP) individually, and incubated at 30°C for 48 hr. After incubation, 1 ml of bacterial culture, 3 ml of freshly prepared α-naphthol (5%) in absolute ethanol and 1 ml of 40% KOH were mixed and stirred vigorously. The formation of a red color indicated the presence of acetoin. We used the GC method described by Ryu *et al*. (2003) for further screening

among the acetoin producers, for identification bacterial isolates producing large amount of plant growth promoting volatiles (i.e. 2,3 butanediole).

We screened the isolates selected for their *in vitro* characteristics related to enhancement of plant root growth for their effects on wheat seedling. Briefly, surface-sterilized seeds of wheat, (*Triticum aestivum*, cv. Boema) were germinated on soft agar for 2 days and placed into plant growth pouches (Mega International, West St. Paul, MN), three seedlings per pouch. Growth pouches were amended with 15 ml of modified Knop plant nutrition solution (Keel *et al.*, 1989). We prepared bacterial suspensions containing 10^8 cells/ml and we added 1 ml of this suspension to each seedling. We incubated the inoculated wheat seedling for 10 days in a growth chamber, with 70% relative humidity and 12 h photoperiod (160 mcE/m^2/s) at 18°C. Six replications per each tested strain were made, and the experiment was repeated twice. We removed the wheat seedling from the growing pouch after a 10-day incubation period and we assessed the fresh weight and length of wheat seedling radicles.

We evaluated also the tri-calcium phosphate solubilization ability of the isolates selected for their *in vitro* traits related to root stimulation. We used Pikovskaya's agar (10 g/l glucose 10 g, 5 g/l $Ca_3(PO_4)_2$, 0.5 g/l yeast extract, 0,5 g/l $(NH_4)_2SO_4$, 0,2 g/l KCl, 0,1 g/l $MgSO_4$ *x* $7H_2O$, 0,1 mg/l $MnSO_4$ *x* $2H_2O$, 0.1 mg/l, $FeSO_4$ 0.1 mg, 15 g/l agar). Each tested bacterial isolate was spot-inoculated in the middle of the Petri dish. We inspected Petri dishes after incubation for 48 h at 28°C, a clear zone around the colony indicating inorganic phosphate solubilization (Malboobi *et al.*, 2009).

We used a polyphasic taxonomy approach for the identification of the isolates selected for the evaluation their ability to increase selenium mobility and bio-availability for wheat plants into a reference soil from South-East Romania. We determined the physiological characteristics by using a Biolog microbial identification system (Biolog, Hayward CA, USA). We use a simplified protocol for isolation of 16rDNA (Maciel *et al.*, 2009) and we sequenced isolated and specific amplified 16rDNA genes. We compared the resulted sequences with those existing on NCBI Gene Bank by using BLAST (Basic Local Alignment Search Tool) programme.

Determination of selenium

We determine total selenium contents in soil and plant samples after digestion with a mixture of concentrated mineral acids (nitric and perchloric) and peroxide (H_2O_2) (Lăcătuşu *et al.*, 2010). For assay of selenium in mineralized sample we use atomic absorption spectrometry, analyzing the hydrogen selenide formed after boron-hydride ($NaBH_4$) reducing procedure (HG-AAS). For determination of soil mobile selenium we extracted the samples in 1 N ammonium acetate (CH_3COONH_4) and 0.01 M ethylendiaminotetraacetic acid (EDTA-H_2) solution, at pH = 7,0 (Lăcătuşu *et al.*, 1987), and we measured the extracted selenium by HG-AAS method.

Greenhouse wheat inoculation assay

We surface sterilized wheat seeds (cv. Boema), by treatment with 70 % ethanol for 30 s and 20 % hypochlorite for 20 min, followed by three washes with sterile distilled water. We harvested bacterial cells by centrifugation, from overnight grown cultures on King N broth (proteobacteria) or NB broth (spore forming bacilli), and we repeatedly washed the bacterial sediment in the sterile saline (0.85% NaCl) solutions. We used for treatment one strain of proteobacteria, Ps33, and one strain of endospore forming gram-negative bacteria, B100. We inoculated wheat sterilized seed with selected bacterial strains, by mixing 100 g seeds with 3 ml bacterial suspension (10^7 ufc/ml in saline solution) and 2 ml 1% carboxymethylcellulose 100 cP solution. We transferred inoculated seeds to pots containing 500 g of soil from South Dobrogea (Amzacea, N 43º58.519' / EO 28º24.597', Typic Chernozem soil, pH 8,0, total selenium content 209 mcg/kg, mobile phosphorus content, P_{AL} 87.5 mg/kg, total humus content 3.4%). The seedlings were incubated under greenhouse conditions (20±2°C, 70 % relative humidity and 12 h photoperiod, supplemented with 160 mcE/m^2/s from halogen lamps, when sunlight intensity decreased bellow 500 mcE/m^2/s). All treatments were performed in five repetitions, each repetition consisting of 10 inoculated wheat seed. Controls without bacterial inoculation were also evaluated. After 28 days, wheat roots and leaves were harvested, washed with sterile distilled water and dried (65°C for 24 h). Se content in plant tissues was analysed by HG-AAS, as already described.

GIS based decision support development

We generated specific layer for main soil characteristics considered as determinant for soil selenium bio-availability in South-Eastern part of Romania (total selenium, pH, mobile phosphorus, total humus), by geostatistical interpolation of data obtained from analysis of geo-referenced soil samples. We established threshold for each of these characteristics, which we used for a binary decision value, 0 or 1. For example, for pH, for values between 6.0 and 8.5 the decision variable, V1 = 1, and for pH<6.0 or pH>8.5 the decision variable V1 = 0. From the intersection of the layers we obtained a composite layer, including 16 possible variables (2^4). The decision for bacterial inoculation of wheat seeds was considered positive when 3 of resulted 16 variables are equal to 1.

Statistical analysis

The data of greenhouse inoculation experiment were analysed by a one-way ANOVA. Comparisons were carried out for each pair with Tukey test using JMP software (version 11.0; SAS Institute, Cary, NC,

USA). All plant experiments were performed in five repetitions. The values are presented as means ± standard errors. Differences were considered to be significant when the P value was less than or equal to 0.05. The analytical data related to soil characteristics were statistically computed. We calculated the correlations of the mobile selenium content with different chemical soil characteristics.

RESULTS AND DISCUSSION

From the 262 bacterial isolates, 140 from Proteobacteria phylum and 122 gram-positive spore forming bacilli, we selected those with potential activity as plant growth promoting rhizobacteria, by using several *in vitro* characteristics. Bacteria presenting ACC-deaminase activity could enhance root growth, by lowering ethylene level (Glick *et al.*, 2007; Barret *et al.* 2011) and, consequently, limiting the growth inhibition effects induced by ethylene in plant roots.

Bacteria with high arginine decarboxylase activity are potential high producer of polyamines. Polyamines were found in soil, at a level which stimulates plant growth (Young & Chen, 1997). Exogenous applied polyamines were shown to induce symbiosis in legume inoculated with *Rhizobium* (Atici *et al.*, 2005) and to enhance mycorrhizal development on plants (Wu *et al.*, 2011) - thus an increased level of soil polyamines could promote the formation of beneficial plant symbiosis. Rhizobacteria producing polyamines are stimulating plant growth (Cassán *et al.*, 2009). Polyamines are known to have a function in plant resistance against abiotic stresses and diseases (Walters, 2003), being involved in NO signaling on plant defence (Bellin *et al.*, 2013).

Bacterial strains producing volatile components, 2,3-butanediol and acetoin, are stimulating plant growth (Ryu *et al.*, 2003), modulating plant root architecture (Gutiérrez-Luna *et al.*, 2010). These volatile components produced by inoculated PGPR were also involved on induction of systemic resistance (Ryu *et al.*, 2004) and in tolerance to drought (Cho *et al.*, 2008).

We tested 12 isolated for their effects on wheat radicle growth. Results are presented in bellow table 1.

Table 1

Effects of selected bacterial isolated on growth of the root system on wheat seedling (cv. Boema)

Treatment	Radicles mean length (mm)		Radicles mean fresh weight (mg)	
Control	148.2±2.7	b	44.2±5.3	c
B52	149.1±2.1	b	47.3±4.8	c
B66	147.5±2.9	b	46.6±5.2	c
Ps92	149.6±3.1	b	42.3±4.4	c
Ps33	160.6±2.2	a	68.4±6.3	a
B56.1	152.5±3.3	ab	52.2±4.8	b
Ps21	157.8±2.2	a	57.2±6.2	ab
Ps37.1	153.4± 2.6	ab	49.6±6,2	bc
B100	159.9±3.2	a	70.3±5.4	a
Ps28.1	147.8±3.1	b	43.8±6.3	c
B82	154.7±1.9	ab	45.9±5.8	c
B77.1	149.2±3.1	b	47,7±6.3	c
Ps5b	154.2±2.7	ab	52.3±5.2	bc

*Value followed by the same letter do not differ significantly for P<0.05

Three of the selected bacterial isolates (Ps33, Ps21, B100) were shown to significantly stimulate the development of root system of wheat seedling. We screened also the bacterial isolates for their phosphate solubilization ability. From the tested 262 isolate, 86 (almost one third) were able to mobilize calcium-phosphate. Among these isolated were included also Ps33 and B100 and these two strains were used in the greenhouse inoculation experiment. The results of this greenhouse inoculation experiments are presented in figure 1.

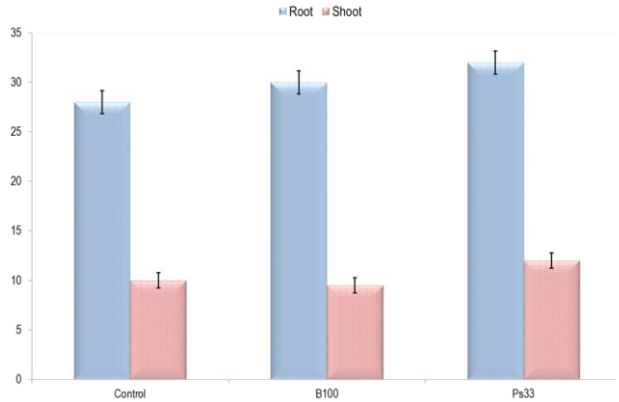

Fig. 1 Influence of seed treatment with bacterial inoculants on selenium accumulation on wheat (cv. Boema) roots and shoots

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Only Ps33 strain determine a statistical significant increase of selenium accumulation on wheat (cv. Boema) plant. By using polyphasic taxonomy Ps33 strain was identified. On the basis of the sequence of 1299 base pairs of 16S rDNA Ps33 strain presents 99.92% similarity with *Serratia plymuthica* G3 strain isolated from wheat rhizosphere, EU344964 access number, and respectively of 99.84% with *S. plymuthica* strain AY394724, high producer of biofilms (Van Houdt *et al*, 2005). Sequence of Ps33 strain 16S rDNA was deposited on GenBank, NCBI, under number EU1181134. Ps33 strain, identified as *S. plymuthica* was deposited as NCAIM (P) B0011366 and patented (Oancea *et al*. 2012).

PGPR as seed / plant rhizosphere inoculants were already reported to increase selenium uptake by wheat plant (Acuna *et al.*, 2012). The proposed mechanism was related to the translocation to the plant of the selenium acquired by PGPR / selenobacteria, including of the organic selenium species (selenomethionine, selenocisteine and their methylated forms). Co-inoculation of wheat seeds with these selenobacteria and with arbuscular mycorrhizal fungi (AMF) was shown to enhance the selenium content in the inoculated wheat plant (Duran *et al.*, 2013).

Increased soluble soil inorganic phosphate (P*i*) species, due to increased AMF activity on wheat roots, could be also considered as a mechanism for increased soil selenium bioavailability for wheat crops. Soluble phosphate ions dislocate selenate and the inner sphere complexed selenite from the soil (organo)mineral particle, making Se species more available to plants. Numerous studies found that the Se plant uptake is influenced by the amount of the soluble phosphate species (Altansuvd *et al.*, 2014; Lee *et al.*, 2011; Eich-Greatorex *et al.*, 2010; Nakamaru & Sekine, 2008), thus bacteria solubilizing soil phosphorus could enhance also selenium species bio-availability for plants.

Another mechanism could be involved into the observed effect of seed bacterial inoculation on wheat seedling selenium uptake. Inorganic phosphate solubilization by bacteria is a result of their ability to locally produce organic acid (Rodríguez & Fraga, 1999). In the soils from South-Eastern area of Romania the mobile selenium species are inverse correlated with soil pH- fig. 2. Bacteria inoculants producing acids and promoting root development could improve selenium bio-availability into these specific conditions also by locally decreasing soil pH into inoculated plant wheat rhizosphere.

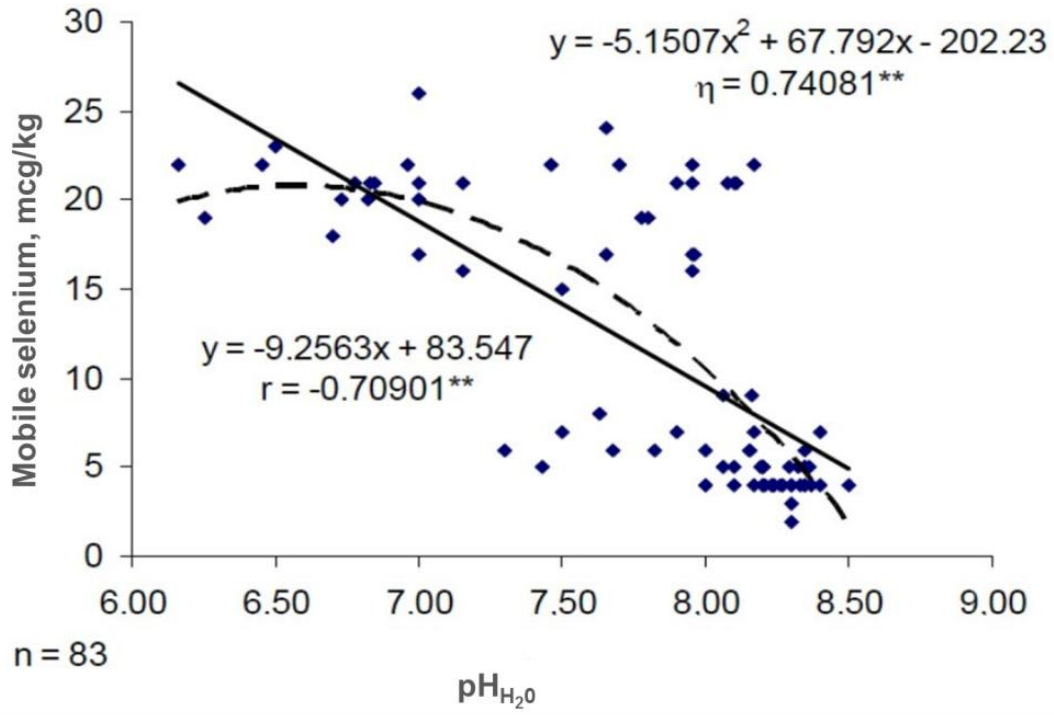

Fig. 2 The correlation between the soil (0-20 cm) mobile selenium content and its reaction in the South-Eastern part of Romania (according Lăcătușu *et al*., 2010)

In other areas of Romania the correlation between selenium mobility and soil pH present a different tendency (Lungu *et al.*, 2014), thus the proposed seed inoculation biotechnology is suitable mainly for the South-Eastern part of Romania. We used the developed GIS-based decision support in order to establish the specific area were the proposed selenium biofortification biotechnology, based on wheat seed inoculation, is the most appropriate. In fig. 3 we present the resulted composite GIS layer, illustrating the area where this microbial inoculant biofortification biotechnology is recommended, superposed over the map of South-Eastern part of Romania.

Fig. 3 Composite GIS-layer resulted from the use of decision support system, illustrating the area where this microbial inoculant biofortification biotechnology is recommended, superposed over the map of South-Eastern part of Romania

On the South-Eastern part of Romania mobile selenium in soil is directly correlated with aridity index (I_{ar}; after De Martonne) values (Lăcătuşu *et al.*, 2010). Increased selenium bio-availability could have also a protective effect on plants growing into this semi-arid area. Se biofortification improve the plants resistance to drought, when the hydric stress is combined with the oxidative one (Feng *et al.*, 2013; Yao *et al.*, 2009; Kuznetsov *et al.*, 2003). Studies in the past 15 years have shown that selenium, although it is not widely recognized as an essential micro-element for plant, stimulates plant growth (Hartikainen & Xue, 1999) and plays a significant role in plant protection against: insects and phytopathogenic agents attack (Hanson *et al.*, 2003), oxidative stress (Xue *et al.*, 2001), hydric stress (Wang *et al.*, 2011). The proposed biotechnological Se biofortification approach combines the protective effects of PGPR on plants with those of selenium.

For a more efficient protective selenium biofortification biotechnology it is necessary also to consider the interferences of selenium and polyamine (and sulfur) metabolism. Se assimilation by plant involves the metabolic pool of S-adenosylmethionine, a common compound for the metabolic pathways of selenium, polyamine and sulfur in plants (Matich *et al.*, 2005; Keck & Finley, 2004). Selenium treatment modifies polyamines level in plants, probably due to the interference with S-adenosyl-methionine pool (Turakainen *et al.*, 2008). The main biotechnological interventions which should be considered are related to the application of glycinebetaine, osmoprotectant acting as donor of methyl groups (for recycling methionine from homocysteine) and of nitric oxide tissue inductors, which should stimulate the production of nitric oxide, which activates Methionine-adenosyl-transferase (MAT) – fig. 4.

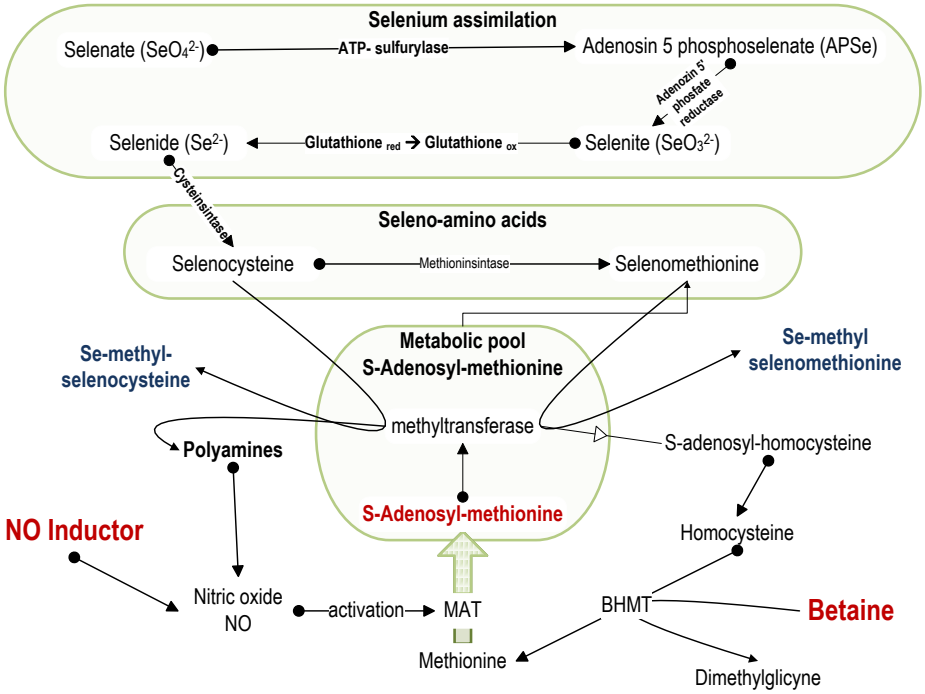

Fig. 4 Rebalancing the metabolic pool of S-adenosyl-methionine (SAM), overused in the metabolic reactions of selenium assimilation. SAM is donor of methyl groups necessary for the production of Se-methionine and methylated Se-amino acids. Application of exogenous betaine is intended to accelerate the formation of methionine from homocysteine. Nitric oxide produced by applying a stimulator of nitric oxide tissue production, activates MAT by nitrosylation. Information processed according to Besson-Bard *et al.*, 2008, Yu & Gu, 2013, Marget *et al.*, 2013.

The proposed biotechnological intervention means illustrated in figure 4 aim to: (*i*) maintain a sufficiently high level of S-adenosyl-methionine, which supports the formation of both methyl-Se-cysteine and methyl-Se-methionine (the main selenium chemopreventive compounds – Chen *et al.*, 2013; Sinha & El-Bayoumy, 2004; Medina *et al.*, 2001); (*ii*) support anabolism of sulfur compounds, including those involved in plant protection against biotic and abiotic stresses (Margret *et al.*, 2013) and (*iii*) potentiate selenium protective effects by polyamines formation (Margret *et al.*, 2013), polyamine having a role in plant protection against biotic and abiotic stress (Kusano *et al.*, 2008, Gupta *et al.*, 2013). The proposed approach should promote also the formation of Se-methionine, accumulated in wheat (Poblaciones *et al.*, 2014). Selenite and selenate are assimilated by the S-metabolizing enzymes of the plant because of the chemical similarities between Se and sulfur (S). Selenocysteine is incorporated nonspecifically into plant proteins, replacing cysteine, and this lead to phytotoxicity. Higher activity of methyltransferases, supported by a higher SAM metabolic pool, promote conversion of selenocysteine to selenomethionine (SeMet), Se Met is also can be incorporated mistakenly into proteins, but with generally less harmful effects (Neuhierl *et al.*, 1999).

Selenium role in plant nutrition was highlighted (Lăcătușu *et al.*, 2004) and even crop increase resulted when it was applied in the soil, on the plant or on the seed (Lăcătușu *et al*, 2004), thus biotechnologies for selenium biofortification should have also a beneficial effect related to wheat yield. The proposed protective Se biofortification technology is relevant especially for Dobrogea area of Romania. Into Dobrogea the level of mobile selenium and of the accumulation of Se in wheat grain is much lower than in neighboring Romania plain – fig. 5.

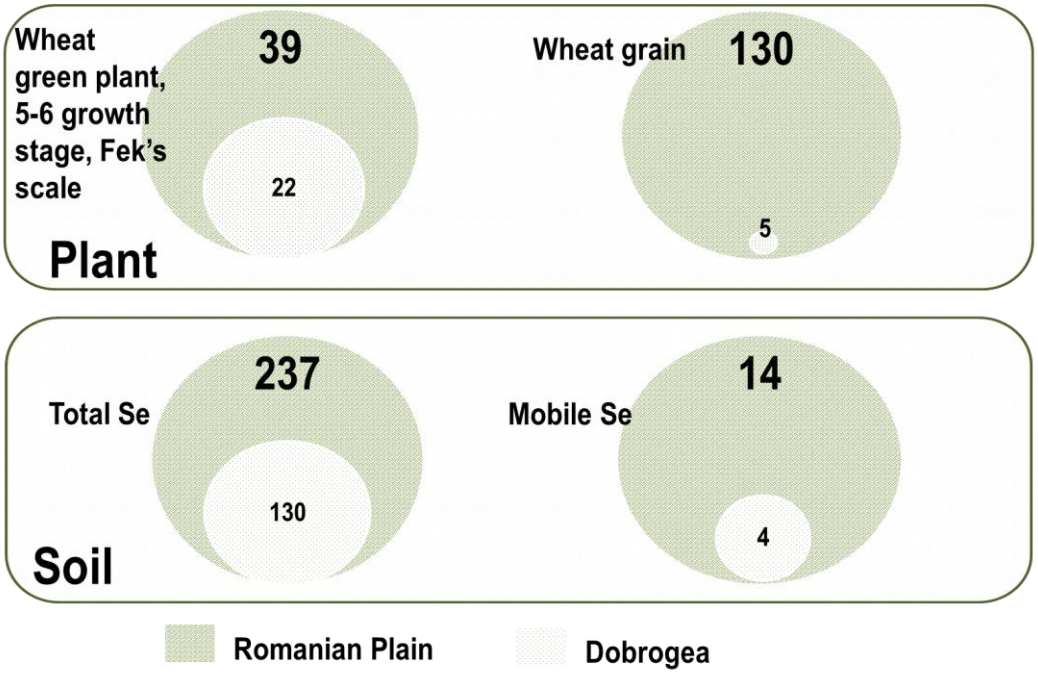

Fig. 5 Average Se level (mcg/kg) in wheat plant, wheat grain and total and mobile Se level in soils, in Romanian plain and in Dobrogea

The resulted protective Se biofortification biotechnology will generate also direct advantages for the farmers and not only benefits for the consumers of Se biofortified crops. Because of this potential direct interest, such Se protective biofortification biotechnology should have a greater adoption potential by the farmers.

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