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COMPARATIVE EFFECT OF SELENIUM AND SELENIUM TOLERANT MICROBES ON *BRACHIARIA REPTANS* L. GROWTH

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Abstract

Brachiaria reptans L. is an annual grass. It is good fodder grass which is distributed in tropical Asia, Kenya, Pakistan and India and introduced throughout the tropics. In present study we determine the growth correlation among *B. reptans*, selenium (Se) and two Se tolerant bacteria (*Bacillus licheniformis*–YAP7 and *Bacillus cereus*-YAP6). Plants treated with Se showed a significant decrease in shoot length (33%) and fresh biomass (41%) compared to control. When plants were co-cultivated in the presence of bacteria or Se and bacteria both the shoot length increased (16-34%) significantly compared to control. *B. reptans* plants treated with Se have shown a significant decrease in peroxidase contents (59%) compared to control. However, bacterial inoculation of Se treated plants resulted in significant increase in peroxidase contents (113-171%). Selenium treatment caused increase in leaf soluble protein contents compared to control. In conclusion, bacteria can enhance *B. reptans* growth under Se stress.

Key words: *Brachiaria reptans*, Selenium, *Bacillus* sp., Peroxidase, Proteins.

Introduction

Selenium (Se) is an important essential micronutrient for humans and animals with a narrow range between toxicity and dietary essentiality. This element is frequently described as double edged sword. The status of Se in the humans and animals populations generally depends upon the diet, which is considered primary source of dietary Se intake (Rodrigo, 2013). According to European recommended dietary allowance (RDA) humans can consume 55 µg Se/ day (Elmadfa, 2009). However, in several clinical trials the regular oral dose of 200 µg / day of Se is being used to test the effectiveness of Se against some health related disorders e.g. cardiomyopathy, certain types of cancers, free radical induced diseases and protection against HIV (Arthur, 2003; Reid *et al*., 2008). Low dietary intake of Se by humans can results in many health related disorders, including high risk of cancer, reduced immune function and fertility, and oxidative stress related disorders (Reid *et al*., 2008). Recent laboratory and fields studies showed that Se could reduce mercury (Hg) toxicity in plants, fish, humans and other organisms (Nascimento-Pinheiro *et al*., 2009; Sørmo *et al*., 2011; Zhang *et al*., 2012). Selenium deficiency in livestock is a common problem that can results in various animal diseases such as hepatosis dietetica in pigs, white-muscle disease in sheep and cattle and pancreatic fibrosis (Hawkesford & Zhao, 2007).

B. reptans is a good fodder grass which is distributed in tropical Asia, Kenya, Pakistan, India and throughout the tropics. Its grains have nutritional values and used as food during famine periods. The concentration of Se in crops and forage can be controlled by its bio-available types and level of Se in the soil (Fordyce, 2005; Zhao *et al*., 2005). In soil Se can be present in the form of selenate, selenite and various organic forms (SeMet and SeCys) and elemental Se^0 (Terry *et al*., 2000). In plants selenate is transported across the plasma membrane through sulfate transporters and selenite is transported through phosphate transporters (White & Broadley, 2009) or may enter in plants cells passively (Terry *et al*., 2000). In general, the exchangeable and water soluble Se is available for plants uptake by roots but the Se which is bounded with organic matter (OM-Se) can be a potential source of Se for plants also (Qin *et al*., 2012; Wang *et al*., 2012). So the various chemical forms of Se in the soil are very important for assessing the Se concentration in forage, crops, animals and humans. All plants have the potential to uptake and metabolize Se but the question that Se is essential for vascular plants or not remained unsolved even after 70 years of investigation. The rhizosphere of plants is a very complex and active microenvironment where microbes are important rhizospheric companions of plants which can play an important role in bioavailability and mobility of Se. Microorganisms have the potential to stimulate the plant growth under stressed conditions and to enhance the phytoremediation process of various toxic chemicals in the environment by mobilizing metals for uptake by plants roots (Lebeau *et al*., 2008; Whiting *et al*., 2001).

The purpose of this study is to find the impact of Se-treatment of soil and inoculation with Se- tolerant bacteria (*Bacillus licheniformis*–YAP7 and *Bacillus cereus*-YAP6) on *B. reptans plants* growth. Moreover, to our knowledge no specific information is available in literature concerning the effect of Se on *B. reptans* plant growth and its various biochemical components, and it can be a new study.

Material and Methods

Soil sample descriptions and biological materials: Sodium selenate (Uni-Chem) is used as a source of Se supply to *B. reptans* plants. The experiment was conducted in triplicate sets of pots and every pot was filled with 10 kg of field soil. Three mL of individual pure bacterial cultures of *Bacillus licheniformis*–YAP7 and *Bacillus cereus*-YAP6 were added in the soil, respectively. These Se resistant bacteria were isolated from soil.

Plants growth experiment design and co-cultivation: This study was conducted in a wire hose of University of the Punjab Lahore (Latitude: 31° 35' North, Longitude: 74° 18' East) under natural rain fed conditions. Six months before starting this experiment, the test soil was pre-inoculated with 3 mL of each bacterial culture and pre-treated with 3mg Se kg^{-1} soil. For bacterial inoculation of soil purified bacterial strains were cultured over night at 37°C in L-broth medium at 150 rpm shaking speed. To get equal number of bacterial cells the optical density of the cultures was adjusted at 1 with sterilized distilled H_2O_2.Then 3 mL bacterial culture was taken and diluted to 100 mL with sterilized distilled water and given to the respective pots. For Se treatment weighed amount of sodium selenate (3mg Se kg^{-1} soil) was dissolved in 1000 mL distilled H_2O_2 and given to the respective pots. The *B. reptans* plants were allowed to grow in monsoon season under totally natural and undisturbed conditions for a period of 2 months (August-September, 2014). Lahore has a semi-arid climate with long extremely hot and rainy summer season. Average rail fall in Lahore is 470 mm (18.51 inch) and in monsoon 2012 total 576 mm rainfall was recorded in Lahore from August - September, 2012 (http://www.pmd.gov.pk/FFD/index_files/ daily/ rainfalljul12.htm). At maturity the plants were harvested and their fresh and dry bio mass were measured.

Peroxidase contents estimation: For quantitative analysis of peroxidase content Davids & Murray (1965) method was used. One gram of frozen plant material was crushed in 4 mL phosphate buffer (0.1M, pH 7) with Heidolph SilentCrusher-M at 16000 rpm in an ice bath. After centrifugation at 10,000 rpm at 4°C the supernatant was use for estimation of peroxidase content. The absorbance for peroxidase contents was taken at 470 nm with help of Cecil Aquarius CE7200 double beam spectrophotometer. The formula used for peroxidase content estimation was:

$$\text{Peroxidase content (unit g}^{-1}) = \frac{\text{O.D of Test - O.D of Control}}{\text{O.D of Control}} \text{ x Weight of plant material (g)}$$

The method describe by Lowry *et al*., (1951) was used for soluble protein content estimation.

Statistical analysis: For statistical data analysis SPSS-V20 software was used. To compare multiple means Analysis of variance followed by post hoc Tukey test were used.

Results

Two Se tolerant bacterial strains *Bacillus licheniformis*-YAP7and *Bacillus cereus*-YAP6 which could tolerate >20 mg Na_2SeO_3 / mL of L-broth medium were used in this study. The strains characteristics and accession numbers are given in Table 1. It was observed that *B. reptans* is a good selenium tolerant plant and it showed remarkable growth in a soil with high Se content when inoculated with Se resistant bacterial strains (Fig. 1) and the results were significantly different at the level of 0.05 (Table 2). Bacterial inoculation as well as Se treatment showed significant effect over plant shoot length (Fig. 1). It was found that high concentration of Se in the soil showed a negative impact over *B. reptans* growth. Under only Se-treatment, shoot length, fresh and dry biomass of the plants were significantly decreased compared to un-treated control (Table 2). In Se-treated plants (Br-Se) the shoot length was reduced (33%) significantly compared to control. However, in bacteria inoculated plants (Br-YAP6) and inoculated Se-treated plants (Br-YAP6-Se and Br-YAP7-Se) the shoot length increased (25-34%) significantly compared to control, except Br-YAP7 which did not show remarabale increase (16%) in shoot length compared to control.

Selenium treated plants (Br-Se) show a significant decrease in fresh biomass (41%) compared to control (Tabel-2). When selenium treated plants (Br-YAP6-Se and Br-YAP7-Se) were inoculated with bacteria the fresh biomass significantly increased compared to un inoculated Se-treated plants (Br-Se). Inoculated plants (Br-YAP6-Se) showed a significant increase in fresh biomass compared to control. Selenium treated plants (Br-Se) showed a significant decrease in dry biomass compared to control (Tabel-2). Inoculated Se-treated plants (Br-YAP6-Se) showed a significant increase in dry biomass compared to control and Br-Se plants.

Peroxidase enzymes have antioxidant ability. Plants treated with Se and inoculated with bacteria exhibited a significant difference in peroxidase content (Tabel-2). *B. reptans* plants when treated with Se showed a significant decrease in peroxidase content compared to control. However, inoculation of Se treated plants resulted in significant increase in peroxidase content (Tabel-2). The Se-treated inoculated plants (Br-YAP6-Se and Br-YAP7-Se) showed a significant increase in peroxidase contents (113-171%) compared to Br-Se plants.

The soluble protein content were increased in Se-treated plants (Br-Se) compared to control (Table 2). However, inoculation in Se-treated plants caused decrease in soluble protein content compared to un-inoculated Br-Se plants. While Se treated inoculated plants (Br-YAP7-Se) showed no difference in soluble protein content compared to control.

Table 1. Characteristics and accession numbers of bacteria.

Strains code	Closest species	Homology with closet species	Accession number	Source	Locality	Na_2SeO_3 tolerance mg mL^{-1}
YAP7	*Bacillus licheniformis*	99%	JX203255	Soil	Lahore	20
YAP6	*Bacillus cereus*	99%	JX203254	Soil	Lahore	20

Table 2. Comparison of Mean ± SE (n=3) of plants fresh/dry biomass (per pot), soluble proteins and peroxidase contents. Lower case letters above values in the same column indicate statistically significant differences among

Tests plants	Fresh biomass/ pot (g)	Dry biomass/ pot (g)	Peroxidase contents (unit g^{-1})	Soluble proteins content (mg g^{-1} fresh biomass)
Control	95.00 ± 2.0 ^{b}	23.49 ± 0.99 ^{b}	87 ± 1.2 ^{bc}	21.4 ± 0.4 ^{b}
Br-Se	56.00 ± 2.0 ^{a}	18.10 ± 0.60 ^{a}	36 ± 1.8 ^{a}	26 ± 0.44 ^{c}
Br-YAP6-Se	116.5 ± 3.5 ^{c}	31.00 ± 1.00 ^{c}	77 ± 2.4 ^{b}	20.3 ± 0.37 ^{b}
Br-YAP7-Se	93.50 ± 2.5 ^{b}	24.90 ± 1.76 ^{b}	98 ± 0.6 ^{c}	17.4 ± 0.55 ^{a}

ANOVA with post hoc Tukey analysis $p<0.05$

Br-Se: *B. reptans* plants treated with Se.

Br-YAP6-Se: *B. reptans* plants treated with Se and inoculated with *Bacillus cereus*- YAP6.

Br-YAP7-Se: *B. reptans* plants treated with Se and inoculated with *Bacillus licheniformis*-YAP7.

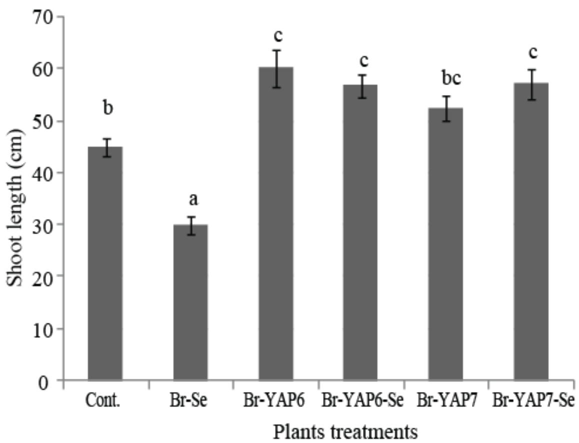

Fig. 1. Comparison of mean ± SE (n=6) of plants shoot length. Lower case letters above columns indicate statistically significant differences among plants (ANOVA with post hoc Tukey analysis $p<0.05$).

Discussion

Selenium is an important micronutrient for animals and humans (Hartikainen, 2005; Lobanov *et al*., 2008), and plant based foods are important dietary sources of Se supply for humans and livestock to fulfill their daily requirements for Se. Selenium is not important for growth of higher plants but at low concentration it can stimulate growth of some plant species and at high concentration in soil it may cause toxic effects on growth of some plants species. However, unicellular algae required Se for their optimal growth (Novoselov *et al*., 2002). Selenium hyper accumulator plants could grow in high Se content soils (seleniferous soils) without exhibiting any symptoms of Se toxicity, while Se-tolerant plants (e.g some grasses) could exhibit symptoms of Se toxicity at higher levels of Se in soil. *B. reptans* is a good fodder grass and its grains were also used as food during famine periods. In this study the impact of bacterial inoculation was tested on the growth of *B. reptans* plant grown in soil with and without high Se content. It was observed that Se treatment caused a decrease in *B. reptans* growth. When plants were treated with Se (3 mg Se/kg soil) the shoot length, fresh and dry biomass of the plants were significantly reduced compared to control (Table 2). In a previous study Sharma *et al*., (2010) have also reported that rapeseed plants treated with 4mg Se/ kg soil exhibited decrease in dry biomass of plants compared to control. However, inoculation with Se resistant microbes stimulated plant growth under Se supplemented conditions and *B. reptans* plants exhibited a remarkable growth in soil with high Se content. These results indicated that the microbial communities in rhizosphere of plants grown in high Se areas can play an important role in growth stimulation of *B. reptans* plants and potentially for other plants. However, un-treated inoculated plants (Br-YAP6 and Br-YAP7) did not show any significant difference in shoot length compared to Se treated inoculated plants (Br-YAP6-Se and Br-YAP7-Se). Hence it indicates that the Se resistant bacterial strains YAP6 and YAP7 can stimulate *B. reptans* plants growth in the absence or presence of high Se content in soil. Both of these strains showed auxins production (results not shown here) which may enhanced the shoot growth in *B. reptans* plants.

Peroxidases are antioxidant enzymes which produced during oxidative stress response and could potentially reduce H_2O_2 in to non-toxic form (H_2O). H_2O_2 causes the oxidation of bio-molecules which have damaging effects on living organisms. It was found that plants treated with Se exhibited low peroxidase contents compared to control. Both pro-oxidant and antioxidant roles have been reported for Se which depends upon the quantity of Se in the plants and as well as in the media. Gomes-Junior *et al*., (2007) has reported that the indication of Se induced oxidative stress and Se induced LPO was obtained with the increased amount of TBARS in the coffee cells when plants were treated with 0.05 and 0.5 mM selenite. But co-cultivation of plants and bacteria in Se treated soil showed increase in peroxidase contents. Probably, bacteria can play an important role in reducing the oxidative stress induced by high concentration on heacy metals in the soil. Selenium treatment caused little increase in soluble proteins content in plants (Table 2) but when Se-treated plants were inoculated with bacteria the soluble protein content reduced significantly. We don't know exactly about the mechanism but might be Se resistant bacteria could stimulate the production of some Se containing amino acids which might cause decrease in leaf soluble protein content. Moreover, White *et al*., (2004) has reported that in plants Se toxicity is may be because of non-specific exchange of sulfur by Se in some protein molecules and other Sulfur containing compounds.

Conclusion

From our findings we can conclude that inoculated *B. reptans* plants grown in soils with high Se content with increased biomass and peroxidase contents can be a good fodder crop for grazing animals. *B. licheniformis*-YAP7 and *B. cereus*-YAP6 have the potential to enhance the growth *B. reptans plants* and potentially of other grasses in the pastures. So these Se resistant bacteria can be potentially used for Se-biofortification of grasses.

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