SEED PRIMING WITH SELENIUM IMPROVES DROUGHT STRESS TOLERANCE IN RADISH SEEDLING

PRÉ-TRATAMENTO COM SELÊNIO EM SEMENTES DE RABANETE AUMENTA TOLERÂNCIA DE PLÂNTULAS SOB ESTRESSE HÍDRICO

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ABSTRACT

Abiotic stress is a major environmental concern that affects large areas worldwide and plants have intriguing defense mechanisms to avoid or minimize damages. Brassicaceae is a medium-sized and economically important family of flowering plants and classified as a primary accumulator and are able to absorb Se through the seeds. The aim of this study was to verify if seed priming with selenium (Se) could improve germination rate, decrease in lipid peroxidation and hydrogen peroxide contents to drought-stressful condition in radish seedlings. Our results indicated alterations in germination rate and lipid peroxidation, according to Na_4Se concentration and time of seed priming, which indicated that Se could improve defense responses to stress condition. These data will be useful to optimize the use of water in agriculture and contribute in biofortification strategies with Se.

Key words: Seed Priming. Radish. Selenium. Abiotic stress.

RESUMO

O estresse abiótico é uma grande preocupação ambiental que afeta grandes áreas em todo o mundo e as plantas têm intrigantes mecanismos de defesa para evitar ou minimizar danos. Brassicaceae é uma família de tamanho médio e economicamente importante de plantas com flores classificadas como um acumulador primário: capazes de absorver Se através das sementes. O objetivo deste estudo foi verificar se o tratamento de sementes com selênio (Se) poderia melhorar a taxa de germinação, diminuição da peroxidação lipídica e conteúdo de peróxido de hidrogênio em plântulas de rabanete sob estresse hídrico. Nossos resultados indicaram alterações na taxa de germinação e peroxidação lipídica de acordo com a concentração de Na_4Se , sugerindo que o Se pode melhorar as respostas de defesa à condição de estresse. Estes dados serão úteis na otimização do uso da água na agricultura, além de contribuir para estratégias de biofortificação com Se.

Palavras-chaves: Pré-tratamento de sementes. Rabanete. Selênio. Estresse Abiótico.

Plants are constantly exposed to stressful conditions throughout their growth and development. Drought stress affects agricultural areas around the world and cause losses in crop production. During a stress condition, as drought, may occur an over production of reactive oxygen species (ROS), such as H_2O_2, OH^- and $O_2^{-\bullet}$. ROS can cause lipid peroxidation, trigger damages to cell balance and may be detoxified by complex enzymatic and non-enzymatic mechanisms to maintain the cell redox state [1].

Several techniques have been developed to avoid stress in plants and improve the agriculture yield. Seed priming is a recent strategy which can be used to acquire tolerance in plants against various abiotic stresses. In this context, seeds exposed to natural or synthetic compounds that lead a particular physiologic state, can improve the plant to respond more quickly or aggressively to the stress condition. There are many priming methods and agents, which varies with different crops and stresses [2.3].

Se is not considered an essential element for higher plants, although in low concentration it can bring benefits in stress tolerance by enhancing their antioxidant system [4] , decreasing lipid peroxidation levels [5] and improving stress tolerance [6]. Furthermore, current literature has showed that Se can increase shoot growth [7] and total respiratory activity in leaves and flowers, which may improve a higher seed production in Brassicaceae [8]. Moreover, the use of Se in agriculture is an interesting tool to supply world nutritional deficiency in humans [9]. For instance, Se decreases risks of heart diseases, hypothyroidism, weakened immune system and enhanced susceptibility to infections and cancer [10].

Se is available to higher plants in selenite and selenate ionic forms. Higher plants are capable to uptake Se because it has a chemical similarity with sulfur metabolism. Selenate is easier to transport than selenite, for this reason, selenite is generally found in root, whereas selenate moves to shoot [11]. Plants are able to accumulate or tolerate various Se levels being classified such as non-accumulator, accumulator and hyperaccumulator groups. Brassicaceae are classified as a primary accumulator, because they have a high potential to accumulate more than thousands of milligrams of Se Kg [12].

Priming with selenium is a novel technique that combines the positive effects of seed priming with an improved of Se supply, which may enhance plant tolerance against environmental adversities [3]. By this way, we used this strategy to investigate if seed priming with Se could improve germination rate, decrease in lipid peroxidation and hydrogen peroxide contents to drought-stressful condition in radish seeds.

Seeds of radish (*Raphanus sativus* L.) cv Cometo were sterilized with sodium hypochlorite during 3 minutes and afterwards was applied the following treatments: 120 minutes in a solution content with 0, 10, 40 or 70 μM L^{-1} of sodium selenite. Afterwards, seeds was dried and then, fifth seeds was germinated each identified germibox contain 20 ml of distillate water or polietilenoglicerol solution (PEG- PA 6000, LS Chemicals ®) at -0,1 MPa to induced physiological drought in seeds by inducing dehydration though water potential reduction.

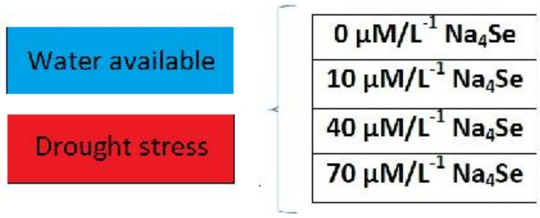

Figure 1 Experimental design

The rate of germination was evaluated each 12 hours and the seedlings were collected after seven days to analyzed lipid peroxidation and. H_20_2. Lipid peroxidation was measured by estimating the content of thiobarbituric acid reactive substances (TBARS) as described by [13]. The concentration of malondialdehyde (MDA) equivalents was determined spectrophotometrically between 535 and 600 nm; data were calculated using an extinction coefficient of 1.55 x 10-5 mol-1 cm-1 [14]. Malondialdehyde (MDA) is a reactive specie resultant of lipid peroxidation, with indicates the level of oxidative stress. H_2O_2 content was estimated following the method of [15]. Seedlings tissues were homogenized in thiobarbituric acid (0.1%) and centrifuged at 10,000x g for 10 min. The supernatant was added to100 mM potassium phosphate buffer (pH 7.50) and 1 M potassium iodide solution. This solution was incubated on ice for one hour, the absorbance was read at 390 nm, and the H_2O_2 content was determined using a known H_2O_2 concentration curve as a standard [16].

The date was performed to statistical analysis using the Assistat software 7.7 Beta ®. A multiple comparison between means using Duncan test was followed by an individual ANOVA for each character at a 0.05 level of significance.

Our results indicate that seed priming with selenium altered some responses under drought stress. When germination percentages was concerned, we observed that in 0 µM/L-[1] Se + PEG treatment occurred the lowest germination percentages, but it was not enough to exhibit statistic difference (Table 1). Although no priming seeds exhibited low mean of germination time (MGT) under drought stress, there was no statistically significant difference among treatments.

Table 1. Germination percentage and mean of germination time (MGT) in seeds of radish treated with different Se concentrations.

Treatment	% Germination				MGT			
0 µM/L-[1] Se + Water	97,8	±	2,22	a	8,962	±	0,16	a
10 µM/L-[1] Se + Water	95,6	±	2,14	a	10,362	±	1,32	a
40 µM/L-[1] Se + Water	93,3	±	6,67	a	8,339	±	0,74	a
70 µM/L-[1] Se + Water	97,8	±	2,45	a	8,173	±	1,18	a
No Priming Water	97,8	±	2,39	a	6,54	±	0,28	a
0 µM/L-[1] Se + PEG	88,9	±	2,66	a	7,678	±	0,33	a
10 µM/L-[1] Se + PEG	91,1	±	2,11	a	7,81	±	0,07	a
40 µM/L-[1] Se + PEG	91,1	±	2,31	a	7,17	±	1,83	a
70 µM/L-[1] Se + PEG	91,1	±	2,57	a	6,103	±	0,62	a
No Priming PEG	91,1	±	4,44	a	5,88	±	1,37	a

Averages followed by the same letter in the column do not differ at 5% probability by Duncan test.

We observed that all treatments increased MDA content under drought stress in radish seedlings (Fig 2A). However, in this case, the seed priming with Se was not effective to decrease MDA content and avoid damages to cell membrane. On the other hand, seed priming with Se reduced MDA content in rice (2). H_2O_2 analysis clearly indicates the highest content in seedling submitted to seed priming with 0 µM/L-1 Na_4Se concentration (35,58 µM/g $^{-1}$ FW) under drought stress (Fig 2B). Whereas, seeds treated with Se reduced H_2O_2 content in seedlings under stress and exhibited a similar content when compared with seedling that germinated in a non-stressed condition. It can suggest that seed priming with Se can mitigate drought stress by alleviating H_2O_2 content, which contributes to improve defense responses to drought stress in radish seedlings.

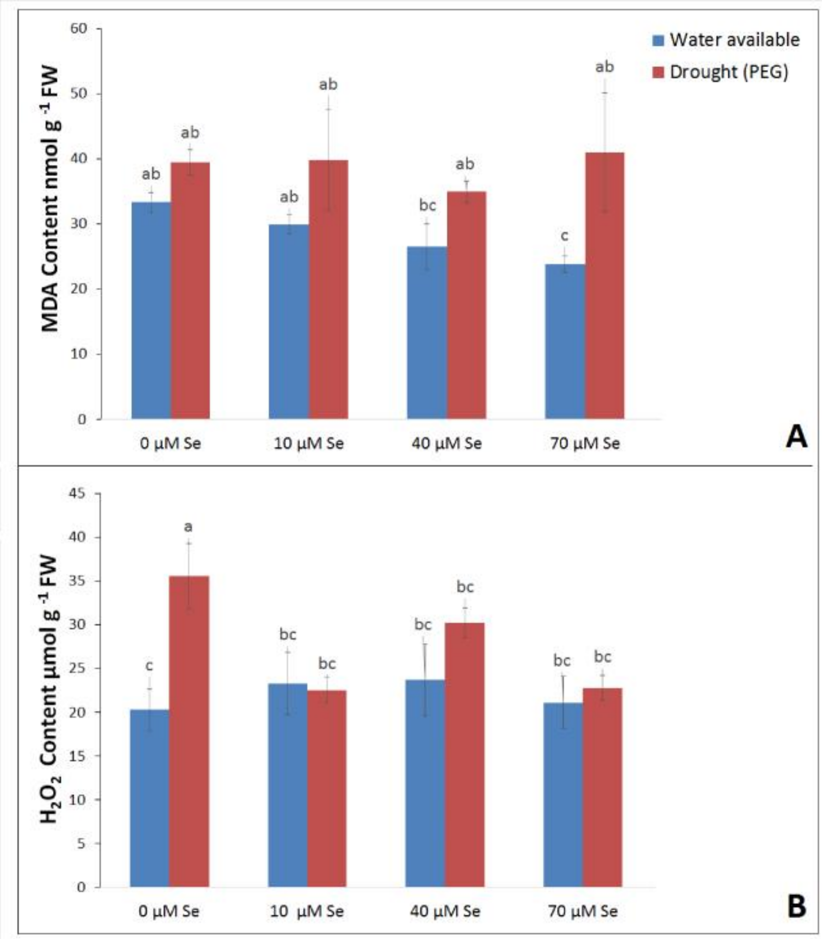

Figure 2. (**A**) Lipid peroxidation measured as MDA content (nmol g^{-1} FW) and (**B**) hydrogen peroxide content (µmol g^{-1} FW) in 7 days seedlings.

In conclusion, seed priming with Se in radish was able to decrease hydrogen peroxide content to drought-stressful condition. However, this response can vary according to Se concentration and time of exposure to seed priming. Thus, to improve these results is necessary evaluate further Se concentrations, times of priming and other responses related to stress tolerance, such antioxidant systems. There is a lack in literature about the complex correlation between drought stress in bioaccumulations plants and elements to improve defense mechanism. Moreover, the application of Se in bioacumulators plants can be a strategy to biofortification studies associated with nutrition politics, which reinforce the importance to develop studies in this area.

REFERÊNCIAS:

(1) GRATÃO, P.L., MONTEIRO, C.C., TEZOTTO, T., CARVALHO, R.F., ALVES, L.R., PETERS, L.J., AZEVEDO R.A. Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. **BioMetals**, Netherlands, v.28, n.5, p. 803-816. 2015

(2) KHALIQ, A., ASLAM, F., MATLOOB, A. HUSSAIN, S., GENG, M., WAHID, A., HEHMAN, H. Seed Priming with Selenium: Consequences for Emergence, Seedling Growth, and Biochemical Attributes of Rice. **Biological Trace Element Research**, United States of America, v.166, n.2, p.236. 2015

(3) JISHA, K. C., VIJAYAKUMARI K., PUTHUR J. T. Seed priming for abiotic stress tolerance: an overview. **Acta Physiologiae Plantarum**, Poland, v.35, n.5, p.1381–1396. 2013

(4) HASANUZZAMAN, M., FUJITA, M. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. **Biological Trace Element Research,** United States of America, v.143, n.3, p.1758-1776. 2011

(5) CARTES, P., GIANFREDA, L., MORA M.L. Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. **Plant Soil,** Netherlands, v. 276, n. 1(2), p.359–36. 2005

(6) RÍOS, B. B., CERVILLA, L.M., ROSALES, M.A., E. SANCHEZ-RODRIGUEZ, ROMERO, L., RUIZ, J.M. Production and detoxification of H_2O_2 in lettuce plants exposed to selenium. **Annals of Applied Biology**, United Kingdom, v. 154, n.1, p. 107-116. 2008

(7) RAMOS, S. J., FAQUIN, V., GUILHERME, L. R. G., CASTRO, E. M., ÁVILA, F. W., CARVALHO, G. S., BASTOS, C.E.A., OLIVEIRA, C. Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. **Plant Soil Environment**, Czech Republic, v.56, n.12, p. 584-588. 2010

(8) LYONS, G.H., GENC, Y., SOOLE K., STANGOULIS, LIU, J. C. R. F., GRAHAM, R. D. Selenium increases seed production in Brassica. **Plant Soil,** Netherlands**,** v. 318, n. 1, p. 73-80. 2009

(9) MALAGONI, M, SCHIAVON M., DALL'ACQUA, S., PILON-SMITH EAH. Effects of selenium biofortification on crop nutricional quality. **Frontiers in Plant Science**, Switzerland , v.6, p. 280. 2015

(10) ROMAN, M., JITARU P., BARBANTE C. Selenium biochemistry and its role for human health. **Metallomics,** United Kingdom**,** v. 6, n.1, p. 25–54. 2014

(11) ARVY, M.P. Selenate and selenite uptake and translocation in bean plants (Phaseolus vulgaris). **Journal of Experimental Botany**, United Kingdom, v. 44, n.6, p. 1083–87. 1993

(12) TERRY, N. A., ZAYED, M., SOUZA, M. P. de, TARUN, A. S. Selenium in higher plants. **Plant Physiology and Plant Molecular Biology**, India, v. 51, p. 401-432. 2000

(13) HEATH, R.L., PACKER, L. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. **Archives in Biochemistry Biophysics,** United States of America, v.125, n. 1, p. 189-198. 1968

(14) GRATÃO, P.L., MONTEIRO, C.C., CARVALHO, R.F., TEZOTTO, T., PIOTTO, F.A., PERES, L.E.P., AZEVEDO, R.A. Biochemical dissection of diageotropica and Never ripe tomato mutants to Cd-stressful conditions. **Plant Physiology and Biochemistry**, Netherlands, v. 56, p. 78-96. 2012

(15) ALEXIEVA, V., SERGIEV, I., MAPELLI, S., KARANOV, E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat**. Plant, Cell and Environment**, United Kingdom, v.24, n. 12, p.1337–1344. 2001

(16) ALVES, L.R., MONTEIRO, C.C., CARVALHO, R.F., RIBEIRO, P.C., TEZOTTO, T., AZEVEDO, R.A., GRATÃO, P.L. Cadmium stress related to root-to-shoot communication depends on ethylene and auxinin in tomato plants. **Environmental and Experimental Botany**, Netherlands, v.28, n.5, p. 102-115. 2017