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The effect of high dilutions of *Pulsatilla nigricans* on the vigour of soybean seeds subjected to accelerated aging

Hingrid Ariane da Silva[1], Angela Valderrama Parizotto[2], Flavia Carolina Moreira[1], Rosimar Maria Marques[3], Bruno Reis[1] and Carlos Moacir Bonato[4*]

*[1]Programa de Pós-graduação em Agronomia, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [2]Programa de Pós-graduação em Biologia Celular, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [3]Programa de Pós-graduação em Biologia Comparada, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [4]Departamento de Biologia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: cmbonato@uem.br*

ABSTRACT. The aim of this study was to evaluate the effect of high dilutions of *Pulsatilla nigricans* in dinamisations 6, 12, 18, 24 and 30 CH on the vigour of soybean seeds subjected to accelerated aging. The experiment was conducted according to a randomised design with 6 treatments and 10 replicates. The treatments consisted of dinamisations 6, 12, 18, 24 and 30 CH and a distilled-water control. After the treatments, the seeds were subjected to accelerated aging (48h at 42ºC) in a growth chamber (25 ± 2ºC). The study evaluated the germination, the length of primary roots and shoots, the fresh weight of roots and shoots and the enzymatic activity of peroxidase (POX-EC1.11.1.7). The variables were analysed by ANOVA, and the means were compared using the Scott-Knott test (p = 0.05). The germination and the fresh weight of roots and shoots of the seedlings treated with *Pulsatilla nigricans* were higher than the water control, except that CH 30 did not significantly increase the fresh weight of shoots. The dinamisations 6, 24 and 30 CH produced a lower primary root length compared with the control. The dinamisations 12, 18 and 30 CH yielded a greater length of shoots. The total length of seedlings was reduced by the high dilutions 6 and 24 CH.

Keywords: dinamisation, accelerated aging, germination, growth, homeopathy, *Glycine max.*

O efeito de altas diluições de *Pulsatilla nigricans* no vigor de sementes de soja submetidas ao envelhecimento acelerado

RESUMO. O objetivo do presente trabalho foi de avaliar o efeito de altas diluições de *Pulsatilla nigricans* nas dinamizações 6, 12, 18, 24 e 30 CH, no vigor de sementes de soja submetidas ao envelhecimento acelerado. O experimento foi conduzido em delineamento inteiramente casualizado com 6 tratamentos e 10 repetições. Os tratamentos foram constituídos das dinamizações 6, 12, 18, 24 e 30 CH e testemunha com água destilada. Após aplicação dos tratamentos, as sementes foram submetidas ao teste de envelhecimento acelerado (48h a 42ºC), em câmara de crescimento (25 ± 2ºC). Avaliou-se a germinação, o comprimento da raiz primária e da parte aérea, massa fresca da raiz e da parte aérea e atividade enzimática da peroxidase (POX- EC1.11 .1.7). As variáveis foram analisadas por ANOVA e as médias comparadas pelo teste Scott-Knott a 5%. A germinação e a massa fresca da raiz e da parte aérea das plântulas tratadas com *Pulsatilla nigricans* foram superiores ao controle com água, exceto na dinamização 30 CH para massa fresca da parte aérea. As dinamizações 6, 24 e 30 CH tiveram menor comprimento da raiz primaria comparadas ao controle. As dinamizações 12, 18 e 30 CH, apresentaram maior comprimento de parte aérea. O comprimento total de plântulas foi reduzido com as diluições 6 e 24 CH.

Palavras-chave: dinamização, envelhecimento acelerado, germinação, crescimento, homeopatia, *Glycine max*.

Introduction

High-dilution preparations based on the science of homeopathy are being used successfully in modern agriculture to control pests and diseases, to increase the medicinal properties of herbs, to detoxify plants contaminated by metals, and to improve seed germination (ALMEIDA et al., 2003; BETTI et al., 2007; CASALI et al., 2006; CAVALCA et al., 2010; FONTES, 2005). Homeopathy considers diseases or physiological disturbances as consequences of a loss of self-regulation by the organism.

The loss of self-regulation may produce changes in the phyto-pathological plant state (BONATO, 2007). Homeopathy uses the organism's reaction capacity to stimulate its defences against pathogenic agents and to help it recover its dynamic equilibrium.

During germination, a sequence of biochemical reactions occurs. The reserve substances are broken

down, transported and resynthesised in the embryonic axis. After soaking, the respiratory rate increases. Respiratory and hydrolytic enzymes are activated. Seeds with low vigour can be affected by these events (BEWLEY; BLACK, 1994). These processes lead to higher rates of seedling growth because the mobilisation of the reserve supply is reflected in increased production capacity (DAN et al., 1987). Andreoli et al. (2002), have observed a reduction of 21 to 25% of corn yield in a low stand of plants produced by the use of seed lots of low physiological quality. This result demonstrated that a plant population is significantly affected by the quality of the seeds used. Seed quality is characterised by germination and vigour.

One of the most important issues in seed conservation is the problem of accelerated aging. Accelerated aging intensifies the deterioration processes by exposing the seeds to high temperature and humidity. If the seeds are subjected to high temperature and relative humidity, the rate of deterioration increases considerably (MARCOS FILHO, 1999) because these conditions affect the biochemical, physiological, and genetic processes in plants (MARCOS FILHO, 2005). Studies done by Bonato (2007) have demonstrated that plant stress triggers a wide range of changes from genes to cell metabolism and yields biomass. The reaction of a plant to stress depends on the plant's capacity to tolerate the effects caused by stressing factors. Following the similia principle of homeopathy, we may argue that *Pulsatilla nigricans* can help seeds withstand the effects of accelerated aging because this organism is described in the medical literature to exhibit symptoms similar to those reported during the process of aging.

The objective of this study was to evaluate the effect of high-dilution preparations of *Pulsatilla nigricans* on the vigour of soybean seeds by using dilutions of 6, 12, 18, 24 and 30 CH (hahnemannian order).

Material and methods

This study was conducted at the Laboratory of Physiology and Homeopathy of the Universidade Estadual de Maringá (UEM), Maringá. Soybean seeds (*Glycine max* (L.) Merrill) (var. BRS 184) were obtained from COCAMAR®, an agro-industrial cooperative enterprise. The homeopathic matrix *P. nigricans* 2 CH and the subsequent high-dilution treatments were prepared in the laboratory according to the Brazilian Homeopathic Pharmacopeia (BRASIL, 1997).

For each level of high dilution in the dinamisation process, one part of the dilution obtained at the previous level was added to 99 parts of distilled water (1/99) and sucussioned 100 times in a mechanical dinamiser (Denise 50-Autic). The high-dilution treatment solutions of *P. nigricans* were 6, 12, 18, 24 and 30 CH. Distilled water was used as a control. The experiment was totally randomised with 4 repetitions.

A total of 200 soybean seeds (var. BRS 184) were analysed in the accelerated-aging bioassay using a treatment/gerbox. The seeds were distributed uniformly on the surface of a metallic screen suspended in a plastic box. Forty millilitres of the *P. nigricans* treatments (6, 12, 18, 24 and 30 CH) and of the distilled water control were added to the internal base of each gerbox used to administer the treatment. The seeds placed on the screen were maintained at 100% relative humidity in a BOD-type chamber at 42°C for 48 hours (AOSA, 2002). Following this period of aging, the seeds were left to germinate.

Ten replicates of 20 seeds each were used for each experimental unit, a total of 200 seeds (BRASIL, 2009). The seeds were distributed longitudinally on germination paper previously wetted in distilled water so that the resulting weight was 2.5 times the original weight of the paper. To conduct the germination test used to evaluate the effects of the treatments and accelerated aging, rolls of germitest paper were made and placed in separate beakers for treatment. A total of 600 mL of distilled water (1/3 of their capacity) was added to the beakers. Beakers were then placed in a BOD chamber at 25 ± 2ºC for seven days (NAKAGAWA, 1994; MARCOS FILHO et al., 2000). The experiment was conducted according to a double-blind procedure, in which treatments were coded and their identification hidden.

Peroxidase enzyme activity was determined using soybean root apices (0.2 g). The root apices were split in a mortar. They were combined with polyvinylpyrrolidine and 67 mM potassium phosphate buffer. The homogenised substance was then centrifuged for 15 minutes at 4000 g. The supernatant was used for the enzymatic evaluation and in administering the protein dosage. The processes were all conducted at 4ºC. Enzyme activity was determined by adding 25 μL of the crude enzyme extract to a reaction mixture containing 25 mM potassium phosphate buffer at pH 6.8. The mixture also contained 100 μL 2.58 mM guaiacol and 50 μL 10 mM H_2O_2. The absorbance of the solution was 470 nm. Peroxidase activity was determined through the use of tetraguaiacol according to molar extinction coefficient calculations of 25.5 mM^{-1} cm^{-1}. The method of Lowry et al. (1951) was employed for protein

dosage. The variables evaluated were the percentage of germination (GP), shoot length (SL), primary root length (PRL), total shoot length (TSL), relationship between shoot and root system (S/RS), root fresh mass (RFM), shoot fresh mass (SFM) and peroxidase enzyme activity (POX). Variance analysis (ANOVA) was applied to the data. Treatment means were compared using the Scott-Knott test ($p = 0.05$).

Results and discussion

The potential germination of soybean seeds that were submitted to accelerated aging increased significantly in seeds treated with high-dilution preparations of *Pulsatilla nigricans* (Figure 1A). *P. nigricans* at 6 and 24 CH exhibited the highest percentage of germination. However, *P. nigricans* at 12 and 18 CH showed a slightly decreased percentage of germination. The literature indicates that the intensity of the response to stress differs among seeds of different species. The results obtained by Bonfim et al. (2010) contrast to our findings. These authors did not find any influence of *Calcarea carbonic* and *Alumina* at high dilutions of 6 and 12 CH on the germination of seeds of *Lactuca sativa* L.

The primary root length (PRL) was decreased by 6, 24 and 30 CH relative to the control (Figure 1.B). In contrast, Bonfim et al. (2008) have reported that *Arnica montana* at 3 and 6 CH increased the root length of *Rosmarinus officinalis* L. and *Lippia alba* (Mill). Bonfim et al. (2010) have also observed an increase of the root length of lettuce seedlings treated with *Calcarea carbonic* or *Alumina*, 6 and 12 CH.

The shoot length of soybean was increased by dilutions 12, 18 and 30 CH. The values of shoot length for dilutions 6 and 24 CH did not differ from the control (Figure 2A). Marques et al. (2008) have found increases of shoot length in *Sida rhombifolia* with 3, 6, 12 and 30 CH of *Cymbopogon winterianus* J. (citronella), confirming the action of high-dilution substances in both crops and weeds. Cell expansion usually begins with changes in cell turgor pressure. These changes trigger cell elongation and produce increased growth, less susceptibility to environmental stress and anticipation in the shoot establishment (TAIZ; ZEIGER, 2010). It can be inferred that the greatest growth of seedlings treated with these dinamisations occurred owing to increased turgor in the cells.

Pulsatilla nigricans at 6 and 24 CH decreased the total shoot length relative to the control (Figure 2B). According to Bonato (2007), stressed plants alter their gene expression, cell metabolism, growth rate and yield. Possibly, in the current experiment *P. nigricans* at 6 and 24 CH interfered with the water uptake of the soybean plants, triggered changes in the cell turgescence, and decreased the cell length. Treatment 30 CH increased TSL, and the 9, 12 and 18 CH dinamisations caused intermediate levels of inhibition.

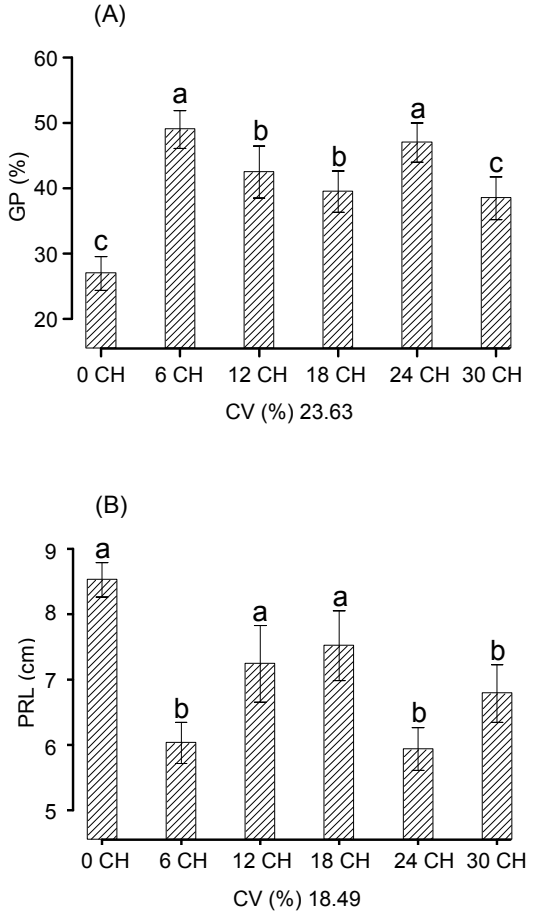

Figure 1. Effects of the homeopathic remedy *Pulsatilla nigricans* on soybean subjected to accelerated aging. (A) percentage of germination (PG). (B) primary root length (PRL). Standard deviations are shown by bars. Means followed by the same letter do not differ significantly (Scott-Knott test, $p = 0.05$).

Evidently, high-dilution *P. nigricans* at 12, 18, 24 and 30 CH dinamisation resulted in the transfer of smaller amounts of carbon from the shoot to the root system. This finding can be supported by the smaller rate associated with the shoot/root system relationship (S/RS) (Figure 3A). During the initial growth phase of the soybean shoot, the cotyledon reserves are the sole source of nutrients (HARRIS et al., 1986). According to Ferreira et al. (2004), seeds undergoing accelerated aging show decreased hydrolytic enzyme activity. In turn, this decrease causes the mobilisation of the cotyledon reserves.

The shoot fresh biomass was increased by the 6, 12, 18 and 24 CH dinamisations (Figure 3B). The production of root system fresh biomass was higher for all dinamisations (Figure 4A).

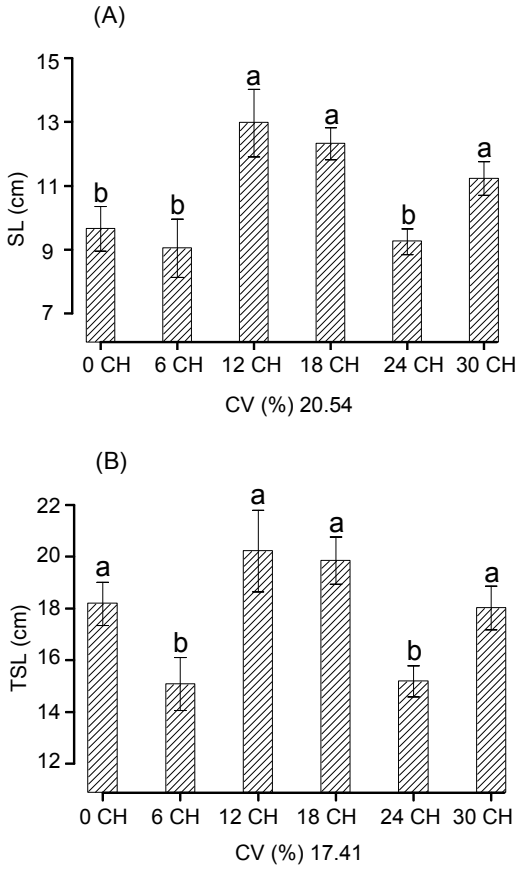

Figure 2. Effect of the homeopathic remedy *Pulsatilla nigricans* on soybean subjected to accelerated aging. (A) shoot length (SL). (B) total shoot length (TSL). Standard deviations are shown by bars. Means followed by the same letter do not differ significantly (Scott-Knott test, p = 0.05).

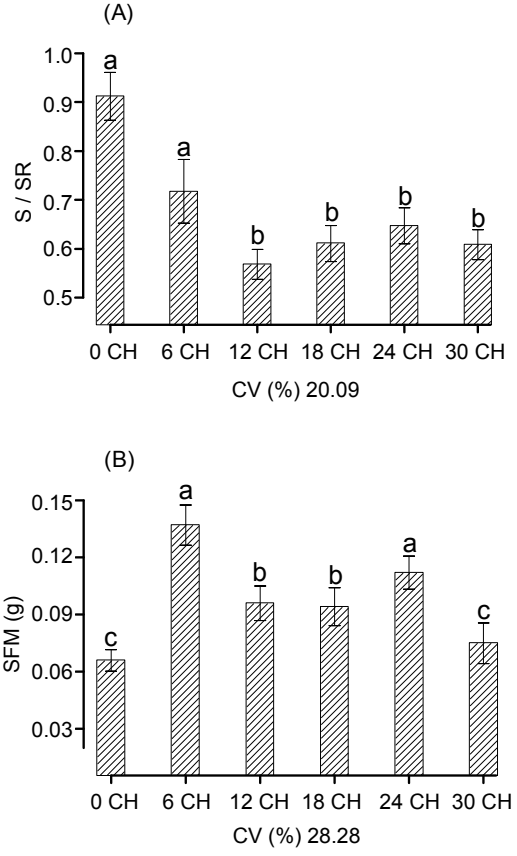

Figure 3. Effect of the homeopathic remedy *Pulsatilla nigricans* on soybean subjected to accelerated aging. (A) relationship between shoot and root system (S/RS). (B) shoot fresh mass (SFM). Standard deviations are shown by bars. Means followed by the same letter do not differ (Scott-Knott test, p = 0.05).

Also, Marques et al. (2008) have observed an increase in total fresh weight for dinamisations 6, 12, 24 and 30 CH with the homeopathic preparation *C. winterianus*. It is therefore possible to conclude that high-dilution preparations serve to increase biomass accumulation and water.

These results show that the metabolic efficiency of the seedlings increased.

The peroxidase enzyme activity in the experimental treatments was less than the value found for the control (Figure 4B).

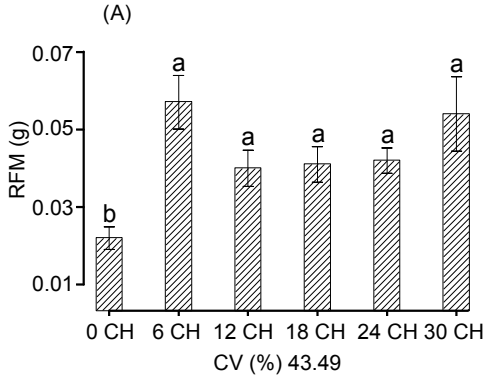

Figure 4. Effect of homeopathic medicine *Pulsatilla nigricans* on soybean subjected to accelerated aging. (A) root fresh mass (RFM). (B) in peroxidase enzyme activity (POX). Standard deviations are shown by bars. Means followed by the same letter do not differ (Scott-Knott test, p = 0.05).

These results suggest that plants treated with *P. nigricans* during early aging had a lower production of oxygen-reactive species. These species can greatly damage cell homeostasis.

Conclusion

At 6 and 24 CH, *P. nigricans* had a positive effect on the germination percentage. The primary root length was decreased by dinamisations 6, 24 and 30 CH. The shoot length was increased by dinamisations 12, 18 and 30 CH. Dinamisations 6 and 24 CH decreased the total shoot length. Treatments 12, 18, 24 and 30 CH produced the least carbon allocation from the shoot to the root system. The production of shoot fresh biomass increased with the dinamisation 6 and 24 CH.

A high dilution of *Pulsatilla nigricans* decreased the activity of peroxidase enzyme dinamisation in soybean. The study first found that most of the high dilutions tested minimised the strength of the effects of accelerated aging caused by temperature in the seeds tested.

The results of the current investigation suggest that homeopathic medicines cause important physiological and positive changes in seeds and shoots subsequent to temperature and humidity stress.

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