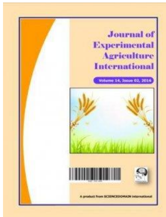

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Effect of Nitric Oxide on Seed Germination and Seedlings Development of Carrot under Water Deficit

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Authors' contributions

This work was carried out in collaboration between all authors. Author GFVG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PTC and CAF managed the analyses of the study. Author DTP managed the literature searches. Author IGP worked translating Portuguese to English. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Seed germination is strongly influenced by water deficit. Reduction of the osmotic potential and, consequently, of the water availability decreases the percentage of normal seedlings. It has been reported that nitric oxide (NO) is efficient in stimulating germination under both normal and stress conditions. The present work aims to evaluate the effect of exogenous application of NO, through a donor (SNP), on seed germination and on the development of carrot seedlings under water stress. Seeds of carrot cv. "Brasília" were submitted to water stress by PEG 6000, -0.3 MPa, and to SNP applications in the following concentrations: 100, 200 and 300 µM. The germinative assay was conducted at 20°C in germinator for 14 days. The first germination count (FGC), total germination (G), germination speed index (GSI), and dry matter of normal seedlings were evaluated. The SNP positively affected the germination and development of carrot seedlings under water stress. The most efficient concentration was observed at 100 µM.

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1. INTRODUCTION

Abiotic stresses are classified as tensions caused by environmental conditions such as light, drought, radiation, heavy metals, hypoxia, temperature, and salinity [1]. It is estimated that abiotic stresses currently affect plant development as well as it decreases world agricultural production by up to 70% [2].

Germination is described as a three-phase process, initiated by seed imbibition (phase 1), activation of metabolic processes (phase 2), and root protrusion (phase 3) [3]. Thus, water is the most important factor regarding the germinative process, being responsible for rehydration of the tissues, intensification of respiration, and several metabolic processes that provide energy to the embryo [4].

Water availability decreases as the osmotic potential of the medium reduce, which causes negative impacts to both percentage and speed of germination [5]. Some osmotic agents exhibit similar behavior to that exerted by colloidal soil particles and can be used to simulate effects of water deficit on seeds [6]. For being chemically inert and non-toxic, polyethylene glycol (PEG 6000) is currently the most used solute to simulate water deficit once it keeps the seeds undamaged [7].

Aiming to recover the germination of seeds under water stress, the application of nitric oxide (NO) has been highlighted. NO is a gas considered as an outstanding mediator of intracellular and extracellular activities, regulating different physiological processes which attenuate effects of stresses, such as germination, antioxidative enzymes activity, breakage of dormancy, maturation, senescence, flowering, gravitropism, among others [8,9,10,11,12].

Sodium nitroprusside (SNP) is the most often NO donor used in germination studies due to its capacity to release this molecule when it is induced by the presence of light [13].

Due to the fact that it is usual the seedlings root system bifurcation in the soil, which makes them unfeasible, carrot plant (*Daucus carota* L.) is produced through direct seeding [14]. Moreover, it is considered as a high sensitive crop regarding drought conditions [15] and, therefore, studies related to water deficit at seed phase and also the mechanisms involved in this process are of great importance.

Few reports are found regarding the action of NO on the recovery of carrot germination under such conditions. In this sense, this study's objective was to evaluate the effect of exogenous application of nitric oxide through sodium nitroprusside on seed germination and on the development of carrot seedlings under water deficit condition.

2. MATERIALS AND METHODS

2.1 Location and Germination under Water Stress and Dry Matter of Seedlings

The experiment was carried out at the Laboratory of Seeds Research, Plant Science Department, Universidade Federal de Viçosa, Brazil. Seeds of carrot (cultivar ''Brasilia''), produced by ISLA Ltda, was used in all tests.

Initially, preliminary germination tests were conducted on different PEG 6000 osmotic potentials, which were calculated according to the methodology of Villela et al. [7]. Based on such results, the potential of -0.3 MPa was selected in order to induce water stress in carrot seeds.

Seeds were placed on paper towel adhered to plastic "gerbox" boxes, moistened with PEG 6000 solutions at the osmotic potential of -0.3 MPa. The control was only treated with distilled water (0.0 MPa). The volume of solution was set as it corresponded to 2.5 times the dry paper weight. The gerbox boxes were kept in a germinator at 20°C. Results were expressed in percentage of normal seedlings obtained at the 14th day after sowing, as determined in the Rules for Seed Analysis [16]. First germination count (FGC) was performed alongside with the germination test, which consisted of the percentage of normal seedlings obtained at the 7th day after sowing [16].

The germination speed index (GSI) was performed through daily counts of normal seedlings, according to Maguire [17] (Equation 1).

Equation 1. Germination speed index (GSI)

$$GSI = (\frac{G1}{N1} + \frac{G2}{N2} + \cdots + \frac{Gn}{Nn})$$

Where:

GSI = germination speed index;
G = number of normal seedlings counted;
N = number of days from sowing to stabilization.

In regard to the evaluation of dry matter of seedlings, normal seedlings obtained from the germination tests were kept in paper bags and placed in a forced air-circulating oven at 75ºC for 48 hours. Subsequently, seedlings were weighed in a precision scale and the mean results expressed in grams.seedling^{-1}.

2.2 Effect of NO on Germination

Besides the germination evaluated at 0 and -0.3 MPa, 3 treatments under stress (-0.3 MPa) were conducted containing SNP at concentrations of 100, 200 and 300 µM. Germination, GSI, and dry matter of seedlings were evaluated as above-mentioned.

2.3 Experimental Design and Statistical Analysis

The experiment was conducted in a completely randomized design with 5 treatments (control; stress; stress + 100 µM SNP; stress + 200 µM SNP; stress + 300 µM SNP) and four replications. Each experimental unit was composed of 50 seeds. Data were submitted to ANOVA and, subsequently, means were compared by the F test at 1% probability. Means of the different doses of SNP were adjusted to regression equations as well as their coefficients evaluated by the t-test at 1% and 5% probability in the SAS software.

3. RESULTS AND DISCUSSION

The analysis of variance showed significant effect for all variables. Thus, it is possible to infer that the water stress simulated by PEG 6000 solution caused negative effects on the germination of carrot seeds as well as on the development of seedlings. On the other hand, nitric oxide through SNP was able to partially recover both of them. By comparing results obtained by the control with the treatment submitted to water deficit, it is possible to state certain sensitivity of the carrot to water stress. Results of stress-only treatment were lower for all evaluations. As for the first count test, germination under the potential of -0.3 MPa was zero. According to Barroso [18], in unfavorable conditions, the germination time tends to increase until the seeds are able to develop adaptation mechanisms or until it is totally inhibited.

Carrot seeds regarding the control (0 MPa) and under water deficit (-0.3 MPa) presented germination rates of 82 and 22%, respectively. Such results show the drastic difference between the two treatments in regard to the percentage of normal seedlings (Fig. 1A).

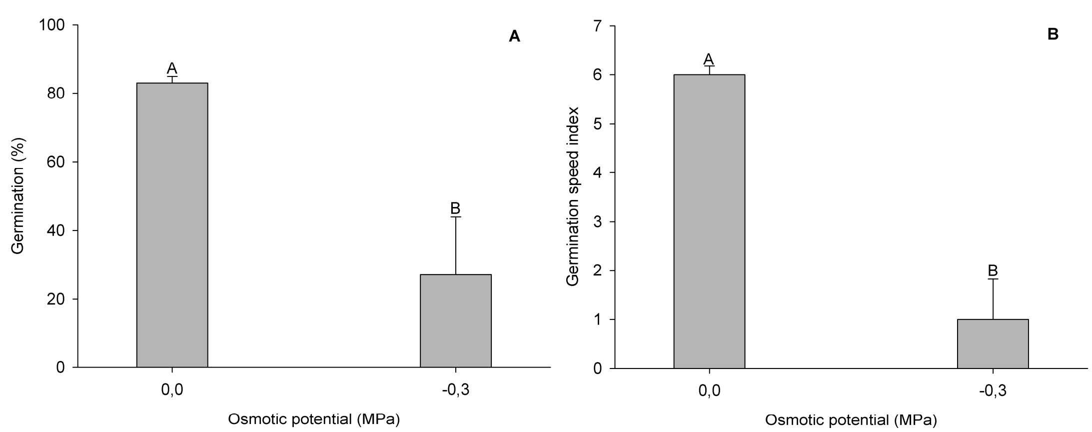

Fig. 1. Germination (A) and germination speed index (GSI) (B) for carrot seeds under water stress
Means followed by different letters differ statistically by F test at 1% probability (P≤0.01)

The predicted sensitivity to water stress condition is confirmed by Rodrigues et al. [19], who verified a reduction of carrot seed germination rates, also at the osmotic potential of -0.4 MPa, induced by PEG 6000 solution.

By the decrease of GSI, it is safe to say that the seeds germination speed was also affected by the osmotic potential of -0.3 MPa (Fig. 1B). Similarly, Silva et al. [20] observed a reduction in carrot seeds vigor at the potential of -0.3 MPa, which negatively impacted the GSI and germination speed.

Besides affecting imbibition, germination rates, and germination speed, water deficit also interferes with the seedlings growth. The stress condition can alter root meristem cells as well as the plant shoot system, interrupting division and cellular expansion along with cells turgescence. Such effects also harm seedlings development [21]. This fact explains the reduced dry matter observed in the stress-treated plants as compared to control (Fig. 2).

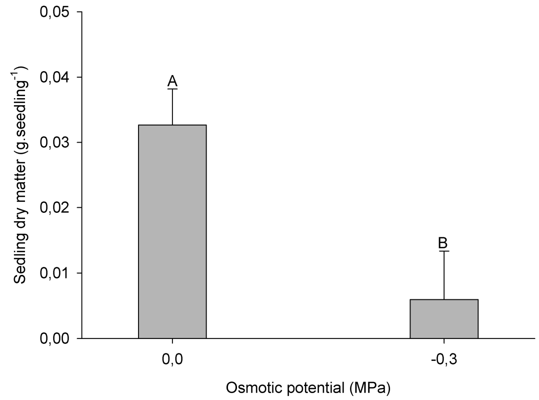

Fig. 2. Dry matter of carrot seedlings under water stress

Means followed by different letters differ statistically by F test at 1% probability (P≤0.01).

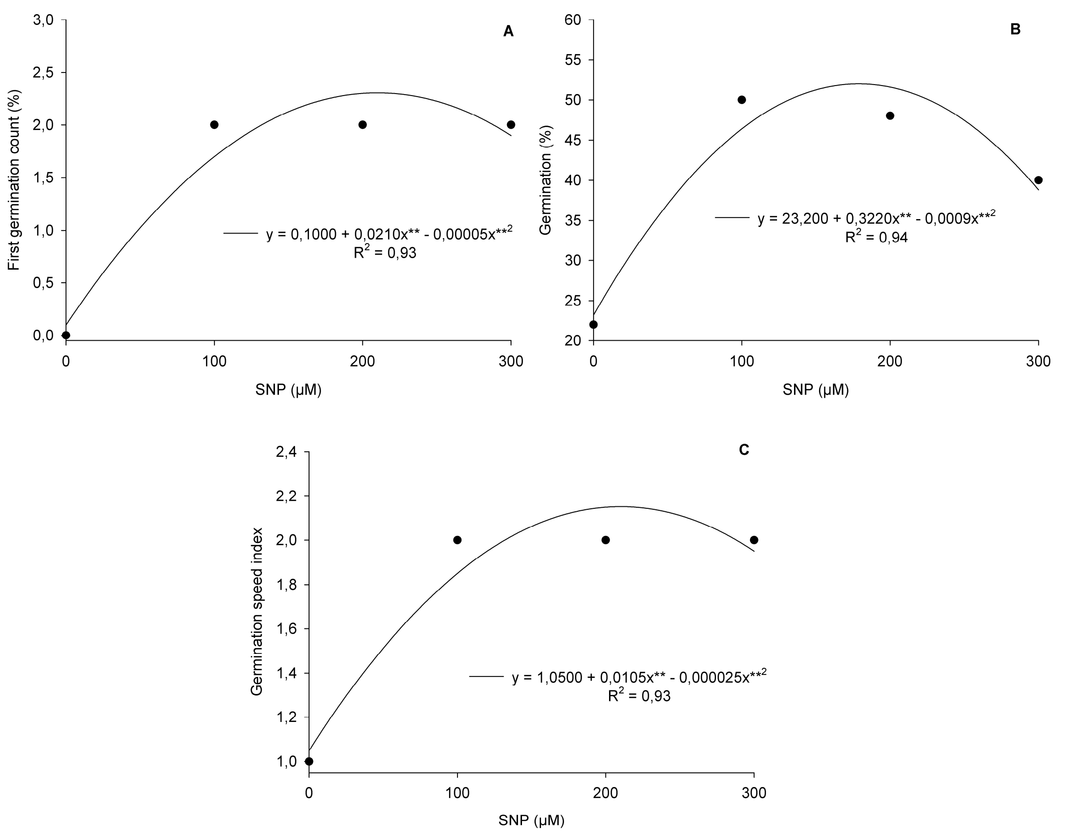

Fig. 3. First germination count (A), germination (B), and germination speed index (GSI) (C) of carrot seeds under water stress treated with SNP

***Equations are significant by F test at 1% probability (P≤0.01)*

Application of NO through the SNP donor increased first count and germination rates in relation to seeds submitted only to water stress (Fig. 3A and 3B). Several authors stated positive effects of NO on seed germination performance [22,23]. The causes may be related to the role of NO in the mobilization of β-amylase enzyme in seeds during germination [24].

In general, different concentrations of SNP slightly affected first germination count (Fig. 3A). Differently, as for germination rates, the concentration of 100 µM of SNP was the most efficient (Fig. 3B). Li et al. [25] found different results, when evaluating the effect of nitric oxide on parsley seeds under salt stress, pointed out an increase in germination rates at the concentration of 200 µM.

Seeds treated with SNP showed higher GSI values than those submitted only to stress (Fig. 3C). This fact supports the hypothesis that NO is efficient on recovering seed germination. However, there was no significant difference among the concentrations. Sarath et al. [23] and Liu et al. [26] state that the application of NO donors stimulates the seeds as they increase germination rates and also germination speed. It can be explained by the increase of α-amylase [27] and β-amylase activities [24], which promotes a greater and faster conversion of starch to soluble sugars [28].

Dry matter was positively influenced by the presence of SNP. The highest values were observed at the 100, 200 and 300 µM concentrations, respectively (Fig. 4).

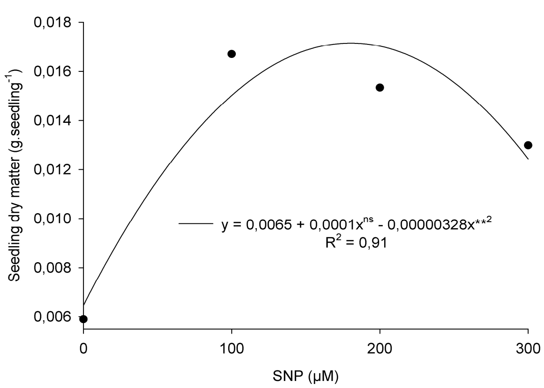

Fig. 4. Dry matter of carrot seedlings under water stress at different SNP concentrations
***Equation is significant by F test at 1% probability (P≤0.01)*

As stated by Sarath et al. [23], NO targets proteins that act on elongation (radicle emergence) and cell growth (extension of coleoptiles) during the germination process. In a complementary way, Correa-Aragunde et al. [29] explain that SNP at low concentrations stimulates root growth, while high concentrations promote an inhibitory effect.

In general, the application of NO favored both germination and dry matter for carrot as compared to the stress treatment at -0.3 MPa. Such results corroborate with Pereira et al. [30], while in a study performed with *Plathymenia reticulata*, observed in pre-soaked seeds with SNP (100 µM) increases up to 155% in GSI. Furthermore, Kopyra & Gwóźdź [31] verified that *Lupinus luteus* seeds treated with NO increased germination rates by 20-40% after 18 and 24 hours.

4. CONCLUSION

Application of SNP showed to be able of reduce physiological damage of carrot seeds under water stress. The highest values of germination and dry matter of seedlings were observed at the concentration of 100 µM.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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