EFFECTS OF HYDROPRIMING AND CHEMICAL PRETREATMENTS OF *Trigonella foenum-greacum* (L.) SEEDS ON GERMINATION, ANTIOXIDANT ACTIVITIES AND GROWTH

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Abstract: Seed treatment is often used for improving germination performance, growth parameters and tolerance to abiotic stresses. Thus, the seeds can undergo chemical pretreatments by imbibition in the presence of certain molecules such as sodium nitroprusside (nitrogen monoxide donor) and calcium chloride (Ca^{2+} being involved in signaling). Hydropriming is a pregermination treatment which helps in stopping imbibition before the end of the reversible phase of germination, i.e before the start of the lengthening of the radicle. Our results showed an improvement in the germination performance and growth of fenugreek (*Trigonella foenum-greacum L.*) seedlings when treated in the same manner. These improvements were accompanied by the formation of reactive oxygen species (hydrogen peroxide and superoxide). Measurements of the antioxidative activities of catalase, ascorbate peroxidase, superoxide dismutase and guaiacol peroxidase showed strong stimulation in the radicles of pre-treated or hydroprimed seeds. This activity was stronger in hydroprimed seeds compared to the control and chemical treatment. Thus the improvement in germination performance and growth seems to be linked to the oxidative stress observed during germination.

Key words: *Trigonella foenum-greacum*; seed priming; germination; pretreatment; sodium nitroprusside, calcium chloride.

INTRODUCTION

Plant productivity, as a capital economic criterion in world food security, is closely dependent on environmental conditions, the nature of the soil as well as the quality of seeds and germination.

Seeds are important sources of plant and industrial raw materials which are often used directly as a food resource and / or as seeds. Seeds are of great socio-economic and agronomic importance as they significantly influence crop production. They play an important and essential role in the development of agriculture, particularly in improving productivity.

A good seed is essential for productive agriculture thanks to its germination capacity, the rapidity of seedling emergence and the capacity to establish vigorous and productive plants even under conditions of environmental constraints. Indeed, plants are constantly confronted during their life cycle with environmental variations which have a negative impact on crop yield and product quality, thus altering plant productivity. Thus, it is necessary to use plants capable of tolerating these stresses such as drought.

For this purpose, pre-treatments and pre-germination treatments of the seeds are used to improve the quality of the seeds and to obtain satisfactory germination. In this field, priming is largely studied and widely used pregermination treatment allowing to obtain better germination performances, synchronous and homogeneous germination and plants more tolerant to abiotic stress [4, 8, 16, 32, 38, 52, 53, 75].

Seed priming is a presowing technique in which seeds are moderately hydrated to the point where pregermination metabolic processes begin without actual germination. Seeds are then redried to near their actual weight for normal handling [36]. This technique is based on the fact that germination can be divided into several stages and that it can be interrupted just before the radicle protrusion, i.e. during the reversible phase of germination, during which the seed can return to its initial state without damage [9]. Hydropriming is an inexpensive priming technique in which the seeds are soaked, then re-dehydrated before the radicle emerges and then returned to germinate. This is a common method which increases the rate, percentage and uniformity of germination as well as drought tolerance the plants from which they come [16, 27].

Thus, various treatments may be applied during the reversible phase of germination. Seeds can be soaked in tap water (hydropriming), low-water potential solutions of polyethylene glycol or salt solutions as KNO_3, KCl, NaCl, or $MgSO_4$ (osmopriming), plant growth regulators, polyamines (hormonal priming), plant growth-promoting bacteria (biopriming), macro or micronutrients (nutripriming) or some plant-based natural extracts [4, 63, 81]. Since nitric oxide (NO) and calcium (Ca^{2+}) are molecules which play a key role in the activation of certain signaling pathways, sodium nitroprusside (SNP), as a nitric oxide donor, and calcium chloride ($CaCl_2$) are used in chimiopriming to improve germination performance, growth, yield and especially tolerance to abiotic stresses [10, 33, 73, 81, 82].

The reactive oxygen species (ROS) produced during germination are also involved in signaling. They act as a positive signal capable of breaking seed dormancy by facilitating the transition from a dormant state to an active state [62].

When there is an excess of this production, there is activation of the enzymatic antioxidant systems (superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase) and non-enzymatic (phenolic

compounds, tocopherols, ascorbate and glutathione) in order to restore physiological balance [23, 28, 31].

This study will focus on fenugreek seeds (*Trigonella foenum-greacum* L.) known for their numerous therapeutic properties: stimulation of appetite and lactation, antidiabetics, antimicrobials, antiparasites, hypocholesterolaemiants [57]. Fenugreek seeds will undergo pregermination treatment (hydropriming) and chemical pretreatments with sodium nitroprusside (SNP) and calcium chloride ($CaCl_2$). Sodium nitroprusside is a donor of nitrogen monoxide (NO) which involved in signaling in the same way as calcium (Ca^{2+}) [11, 39, 46, 65, 71, 77, 79]. The effects will be assessed through germination performance, growth parameters as well as ROS production and antioxidant activities.

MATERIAL AND METHODS

Plant material

Trigonella foenum-greacum L. seeds are from a local variety (Halba) from eastern Algeria. Fenugreek seeds were selected and subjected to surface sterilization with 5% hypochlorite solution for 5 min then washed with distilled water.

Imbibition kinetics

In order to identify the reversible phase of germination of *Trigonella foenum greacum* L. during which treatments can be applied. Twenty five fenugreek seeds were germinated on absorbent paper in a 90 mm Petri dishes and soaked in distilled water at 26° C. These seeds were weighed every 2 h for 24 h. The imbibition kinetics curve was determined by measuring the percentage of cumulative absorbed water [44]:

% cumulative water at time (t) = (Weight of imbibed seed (t) - weight of dry seed) * 100 / weight of dry seed

Pregermination treatment and seed pretreatments

Pregermination treatment or Hydropriming

One batch of seeds was imbibed for 6 h (reversible phase) in 15 mL of distilled water, followed by redehydration in an oven at 26 ° C for 48 h, to return to their initial moisture content.

Chemical Pretreatments

Two batches of seeds were soaked respectively in a 50 µM sodium nitroprusside (SNP) solution and a 5 mM $CaCl_2$ solution. After soaking for 6 hours in this solutions, the seeds were washed several times with distilled water and then germinated. The concentrations of these solutions, as well as the duration of imbibition (6 h), were determined following several preliminary tests. We considered a control batch for which the seeds were soaked in distilled water for 6 hours before germination.

Germination

The seeds thus treated and those untreated were germinated for 5 days at 25 ± 1° C in total darkness on absorbent paper in germination trays (20 cm x 20 cm). A seed was considered germinated when the radicle protrudes 2 mm from the seed coat.

Potting

The five-day-old plantlets, resulting from the germination of seeds having undergone the various treatments, were transferred into pots containing potting soil.

Parameters measured

Germination performance

After 5 days of incubation, the germination percentage, the average germination time, the vigor index and the length of the roots were determined using the following formulas:

- ***The germination percentage*:** According to Mazliak [50], the germination capacity is the maximum germination percentage obtained under experimental conditions. Germination percentage is the ratio of number of germinated seeds to total number of seeds.
- ***The mean germination time (GMT):*** was calculated using the following Kotowski formula [43]:

$$TMG = (N_1T_1 + N_2T_2 + N_3T_3\ldots. + N_nT_n) \times 100 / (N_1 + N_2 + N_3\ldots. + N_n).$$

with N_n equal to the number of seeds germinated between time T_{n-1} and time T_n.

- ***Vigor index*:** The vigor index value was calculated using the following formula suggested by Abdul-Baki and Anderson [1] and expressed as a whole number.

Vigor index = Germination (%) x Seedling length (cm).

Biometric measurements

Growth of radicle

The growth in length of the radicles was followed by measuring the length of seedlings roots obtained from the treated and untreated seeds after 5 days of germination. Each value represented the average of ten measurements. It was expressed in cm. A kinetic of the growth in length of the radicle was then carried out.

Growth of aerial parts

The growth in length of treated and untreated seedlings was followed by measuring the length of the aerial parts during 21 days of growth in the pots. Each value represented the average of ten measurements.

Cytochemical detection of ROS

Cytochemical detection of hydrogen peroxide (H_2O_2)

Hydrogen peroxide was detected by a cytochemical method using 3.3'-diaminobenzidine (DAB). Hydrogen peroxide causes redox with polymerization of the DAB molecule resulting in a very stable brown precipitate at the site of the reaction. The radicles was immersed in a 1 $mg \cdot mL^{-1}$ solution of DAB in water for 24 hours at room temperature with stirring and in the dark. The DAB solution was prepared before each experiment in order to avoid any auto-oxidation [69].

Cytochemical detection of superoxide anion (O^{2-})

The superoxide anion was detected by a cytochemical method using tetrazolium nitroblue (NBT) (N6876, Sigma-Aldrich) [59]. The superoxide radicals present reduce the NBT to stable formazan blue of blue-indigo color [15]. The radicles was immersed in a solution of NBT at 0.5 mg·mL^{-1} in sodium phosphate buffer at pH 7.6 for two hours, at room temperature, in the dark. The radicles was then rinsed with 50% ethanol for 10 minutes and then stored in a glycerol-ethanol solution (1/4, v/v) until the photographs are taken under a binocular magnifying glass.

Biochemical analyses

Total water soluble protein content

The soluble proteins was assayed according to Bradford method [20]. For their extraction, 100 mg of radicles are cold ground in 1 mL of extraction buffer (Tris-HCl pH 8.1). Centrifugation is then carried out at 12000 rpm for 20 min at 4° C. The supernatant is analyzed to measure the protein content by a colorimetry technique using coomassie blue or bradford's reagent.

Enzymatic antioxidant activities

The enzymatic activities were measured by spectrophotometry. For Catalase, APX and SOD, the activities are measured on the same extracts of total proteins.

Catalase (CAT): The activity of CAT is determined by following the decomposition of H_2O_2 at 240 nm (extinction coefficient ε = 36 M^{-1}cm^{-1}) [25]. The reaction medium consists of 2 mL of potassium phosphate buffer (KH_2PO_4 / K_2HPO_4) at 100 mM pH 7 and 100 µL of protein extract containing the enzyme. The reaction is initiated by adding 10 mM H_2O_2. The activity is expressed in nmoles of H_2O_2 degraded per minute and per mg of protein.

Ascorbate peroxidase (APX): The ascorbate peroxidase (APX) activity is measured according to the method of Nakano and Asada [55] by following the oxidation of ascorbate by hydrogen peroxide at the wavelength of 290.The reaction medium consists of 100 mM potassium phosphate buffer, pH 7, containing 0.1 mM Na_4EDTA, 0.5 mM ascorbic acid and 100 µL of the extract. The reaction is initiated by adding 0.1 mM H_2O_2. Enzymatic activity is expressed in mmol of oxidized ascorbate.min^{-1} mg^{-1} of protein. This activity was calculated using the ascorbate extinction coefficient which is 2.8 mM^{-1} cm^{-1}.

Superoxide dismutase (SOD): The determination of SOD was carried out according to the method of Marklund and Marklund [48]. The autooxidation of pyrogallol in the presence of EDTA was inhibited by SOD. The evaluation of pyrogallol autoxidation was carried out by differential measurement between a control and a test. The control cuvette contains 2 mL of 50 mM Tris-HCl buffer pH 8.2, 1 mM EDTA. The reaction was initiated by adding 100 µl of 1 mM pyrogallol (prepared in 0.01N HCl). The increase in absorbance at 420 nm was due to the autooxidation of pyrogallol, the change in absorbance is measured every 30 seconds for four minutes. The test cuvette contains 2 mL 50 mM Tris-HCl buffer pH 8.2, 1 mM EDTA and 100 µM of the plant extract. The reaction was initiated by adding 100 µl of 1 mM pyrogallol. The change in absorbance is measured a second time. The % of inhibition during one minute was calculated according to the following equation:

$$(\Delta A_{control} - \Delta A_{test}) / \Delta A_{control} \times 100$$

An enzymatic unit was defined as the quantity of enzyme capable of inhibiting 50% of the auto-oxidation of pyrogallol under the conditions of the assay. The activity of SOD was expressed in units of SOD per minute and per mg of protein.

Guaiacol peroxidase (GPOX): The activity of guaiacol peroxidase was determinated according to the method of MacAdam et al. (1992) [47] modified by Boucelha et al. (2019) [17]. The enzyme was extracted from 100 mg of radicles, ground in 1 mL of potassium phosphate buffer (KH_2PO_4 / K_2HPO_4) at 0.1 M pH 6.5 in cold conditions. After centrifugation for 20 min at 12000 rpm at 4° C, the supernatant (the enzyme extract) was collected and kept cold until use. The activity was measured on 100 µl of the enzymatic extract to which are added 2 mL of the same phosphate buffer used for the extraction, 36 mM of guaiacol and 10 mM of H_2O_2. The reaction begins as soon as 100 µl are added. H_2O_2 to the reaction mixture. The activity was monitored as a function of time and expressed in µmoles of oxidized guaiacol per minute and per mg of protein, using the tetragaiacol molar extinction coefficient (ε = 26.6 mM^{-1} cm^{-1}).

Total non-enzymatic antioxidant capacity (TAC)

The total non-enzymatic antioxidant capacity was estimated by the method of Prieto et al. (1999) [58]. A simple of 200 mg of radicles was ground in 1 mL of pure methanol. Then, the extracts were macerated at 4° C for 24 h, with stirring. For the assay, 300 µL of sample extract was mixed with 3 mL of ammonium molybdate reagent. The tubes were incubated in a water bath at 95° C for 90 min. After cooling the mixture to ambient temperature, the absorbance of the solution was measured at 695 nm. TAC is expressed in mg equivalents of ascorbic acid per g of FW. For this purpose, a standard curve was prepared using 0-300 µg mL^{-1} of ascorbic acid .

Variation percentage

The percentage change was calculated for each parameter studied according to the following relation: variation percentage = (Assay- Control) * 100 / Control

Statistical Test

The statistical analysis of the results obtained is carried out by the STATISTICA software (version 6). The results obtained are subjected to analysis of

variance (ANOVA) at a probability level of 5%. The means are compared by Tukey's test in order to determine the groups homogeneous at p = 0.05.

RESULTS

Kinetics of imbibition

From the curve obtained, we could deduce that during the germination of fenugreek seeds, water absorption was characterized by a three-phases pattern (fig. 1) which was similar to the configuration of most seeds [14]. The reversible phase of fenugreek seed germination ends after 20 hours of imbibition. So pretreatment and priming must be applied within this time interval *i.e* before the elongation of the radicle.

Germination performances

Germination was evaluated through the capacity, the speed and the vigor index.

The germination capacity was reached after 18 hours of germination for hydropriming and after 24 hours for pretreatment with SNP and $CaCl_2$, while for the control batch, this germination capacity is only reached after 48 hours (fig. 2).

The mean germination time (GMT) represents an assessment of germination speed. Statistical analysis revealed a significant difference between the treatments and the control ($p < 0.05$). Indeed, all treatments induced an increase of this speed. This difference was pronounced between the control batch (≈ 20 hours) and the tests (≈ 14 h to 15 h) (fig. 3).

Hydropriming and germination pretreatments significantly affected the germination vigor of fenugreek seeds. Indeed, seeds that have undergone pretreatment with SNP shown better vigor with an increase of 75% of the index compared to the control. For batches that have undergone hydropriming and pretreatment with $CaCl_2$, this increase was approximately 65% (Fig. 4).

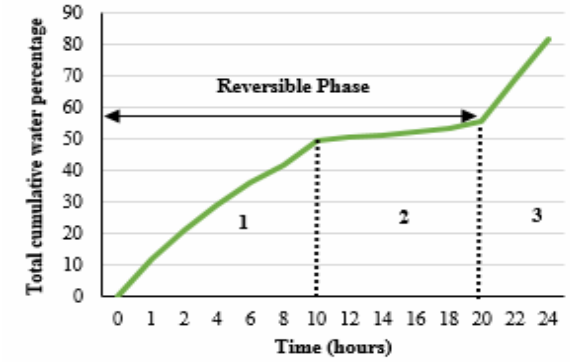

Figure 1. Kinetics of seed imbibition of *Trigonella foenum greacum* L. (Fenugreek); 1: imbibition phase; 2: sensu strict germination ; 3: growth phase (irreversible phase).

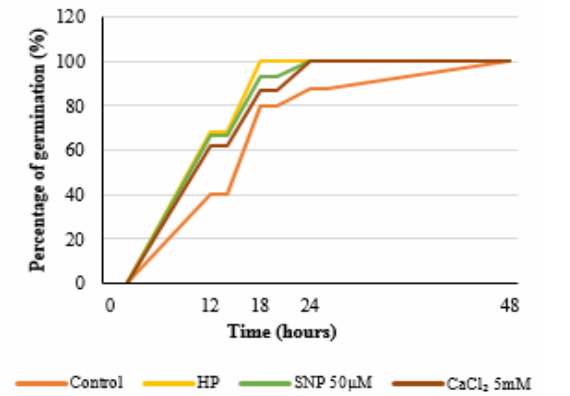

Figure 2. Germination capacity of fenugreek seeds according to the different treatments; HP= Hydropriming; SNP= Sodium nitroprusside

Biometric measurements

Growth in length was studied by monitoring the length of the radicles over a period of 5 days (fig. 5).

Growth of radicle length

Compared to the control, a greater growth was observed for the radicles of the batches which had undergone hydropriming or pretreatments with SNP or $CaCl_2$ .This elongation varied according to the type of treatment. Thus, it was recorded an increase of 46% for hydropriming and 53% for pretreatment with $CaCl_2$. The best root growth was observed with SNP pretreatment (60%) (Fig. 6).

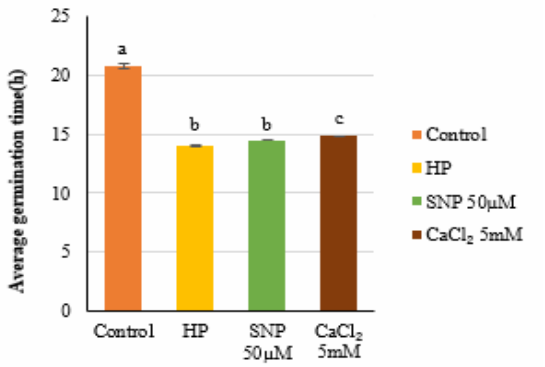

Figure 3. Germination speed of fenugreek seeds according to the different treatments; HP= Hydropriming; SNP= Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p<0.05$).

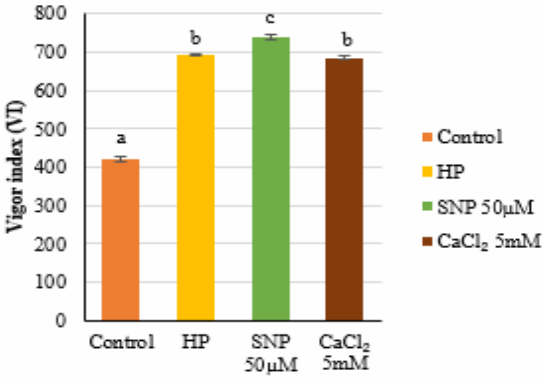

Figure 4. Index of vigor of fenugreek seeds according to the different treatments; HP= Hydropriming; SNP= Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p<0.05$).

The growth in length of the aerial parts

Growth in length was studied by monitoring the stem length over a period of 21 days. Greater growth was observed in plants from treated seeds compared to untreated seeds. Thus, pretreatment with SNP resulted in an increase in growth of approximately 36% compared to the control. While for hydropriming and $CaCl_2$ pretreatment, the increase was only about 20% (fig. 7).

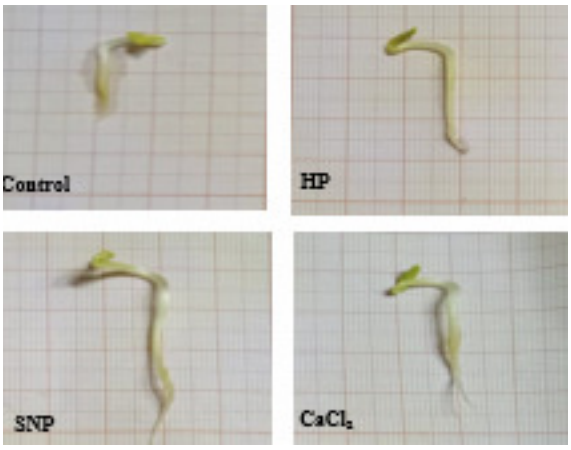

Figure 5. Photographies showing fenugreek seedlings 5 days old according to the different treatments; HP= Hydropriming; SNP= Sodium nitroprusside

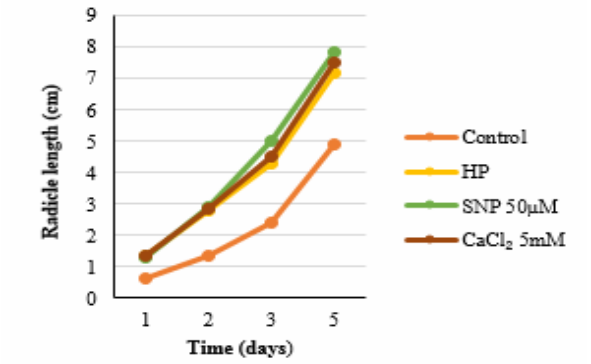

Figure 6. Kinetics of radicles growth according to the different treatments; HP=Hydropriming; SNP=Sodium nitroprusside

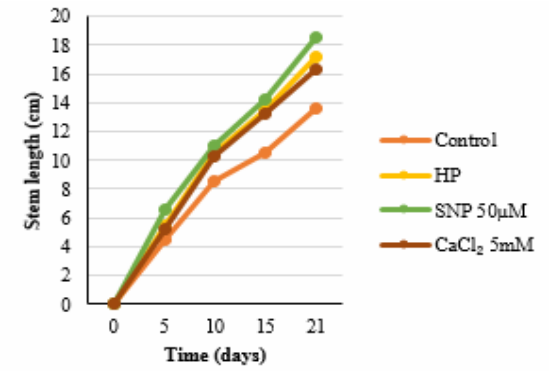

Figure 7. Linear growth kinetics of aerial parts according to the different treatments; HP= Hydropriming; SNP= Sodium nitroprusside

ROS production

Detection by DAB shown a low accumulation of hydrogen peroxide (H_2O_2) in the radicles from untreated seeds. However, the radicles from the pretreated and hydroprimed seeds shown a more accumulation observed at the level of the cap and the region of radicle elongation. The intensity of this accumulation differed depending on the type of treatment applied (fig. 8).

The NBT test shown that the radicles from the control seeds were characterized by a strong accumulation of superoxide anion (O^{2-}) in the hypocotyl. While in the radicles from pretreated seeds, clear areas were observed (fig. 9).

Total soluble protein content

The results shown that the germination pretreatments and hydropriming of fenugreek seeds induced a high protein content in the radicles. This increase depended on the type of treatment. Indeed, it was observed that $CaCl_2$ pretreatment had a more

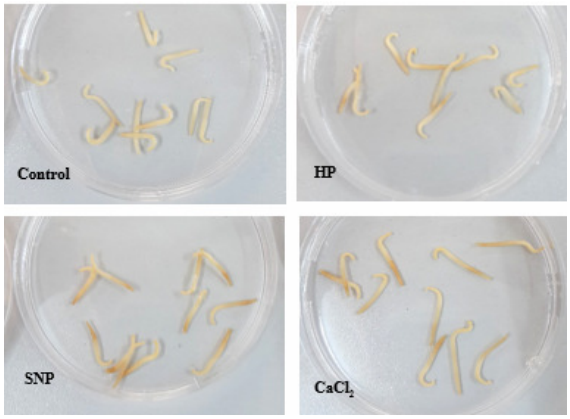

Figure 8. Photographies showing the detection of hydrogen peroxide (H_2O_2) by DAB in the radicles after 48 hours of germination; HP= Hydropriming; SNP= Sodium nitroprusside; The presence of hydrogen peroxide (H_2O_2) is indicated by browning due to polymerization of diaminobenzidine (DAB).

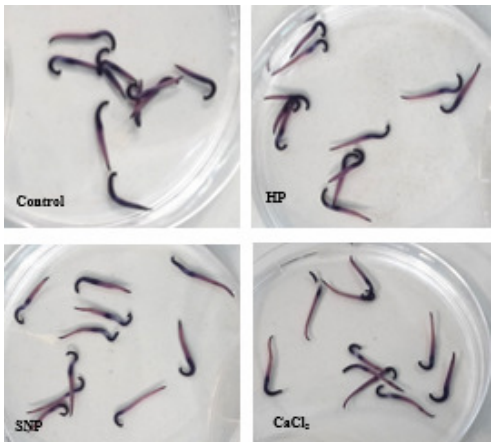

Figure 9. Photographies showing the detection of the superoxide anion (O_2^-) by NBT at the level of the radicles after 48 h of germination; HP = Hydropriming; SNP = Sodium nitroprusside; The presence of the superoxide anion (O_2^-) is indicated by an indigo-blue coloration following the transformation of tetrazolium nitroblue into formazan.

significant effect on the increase in protein content compared to the radicles obtained from untreated seeds (fig. 10).

Antioxidant activities

Catalase

The antioxidant enzymatic activity of catalase in the radicles indicated a significant increase in the treated batches. The rate of this increase depended on the type of treatment. Thus, the radicles obtained from hydroprimed seeds were characterized by the highest activity compared to control (95% of increase). The chemical pretreatment with SNP and $CaCl_2$ induced a respective increase in this activity of 50% and 29% compared to the control.

Guaiacol peroxidase (GPOX)

Pregermination treatments of fenugreek seeds shown a significant increase in the activity of guaiacol peroxidase in the radicles. This increase was greater for hydropriming and SNP pretreatment compared to the control (+61%). While for $CaCl_2$ pretreatment, this increase was only about 42% compared to the control (fig. 12).

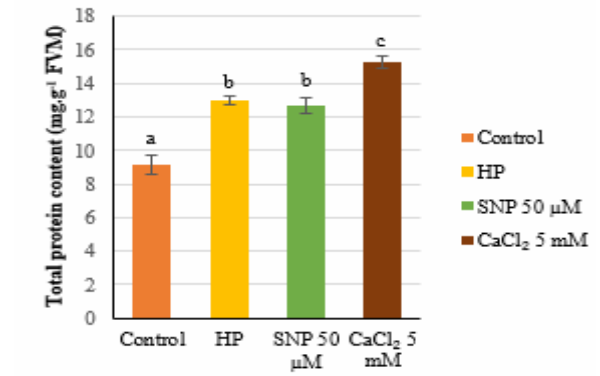

Figure 10. Total protein content of the radicles according to the different treatments; FVM = Fresh vegetable matter; HP =Hydropriming; SNP=Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p < 0.05$).

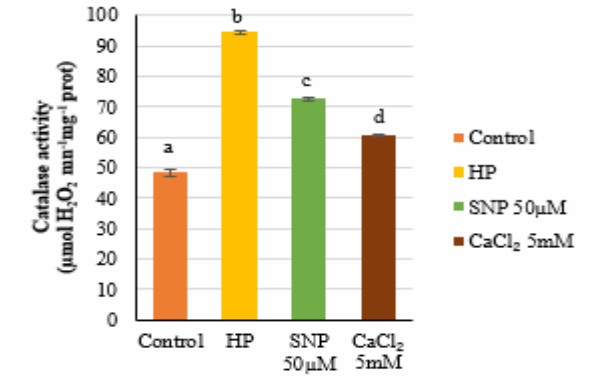

Figure 11. Activity of catalase in the radicles according to the different treatments; HP = Hydropriming; SNP = Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p < 0.05$).

Ascorbate peroxidase (APX)

Following of the type of treatment, it was a significant stimulation of total APX activity in the radicles. The intensity of this increase was depending on the type of treatment applied. Indeed, hydropriming and pretreatment with SNP induced respectively an increase in this activity of 49.74% and 40.68% compared to the control. While, the $CaCl_2$ pretreatment, this increase was only 15.78% (fig. 13).

Superoxide Dismutase (SOD)

The measurement of the SOD activity in radicle revealed that hydropriming and SNP pretreatment caused a significant increase (about 68%). While, for the $CaCl_2$ pretreatment, this increase is only 43% compared to the control (fig. 14).

Total non-enzymatic antioxidant capacity (TAC)

Pretreatment with $CaCl_2$ induced a significant increase in TAC (+30%), compared to the control. While, for hydropriming and SNP pretreatment, the induced increase was only 9% compared to control (fig. 15).

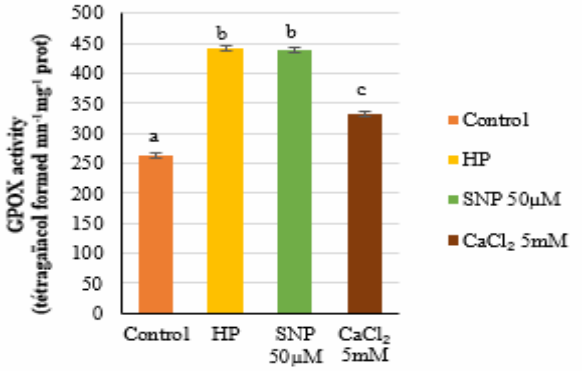

Figure 12. Guaiacol peroxidase activity of the radicles according to the different treatments; HP = Hydropriming; SNP = Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p < 0.05$).

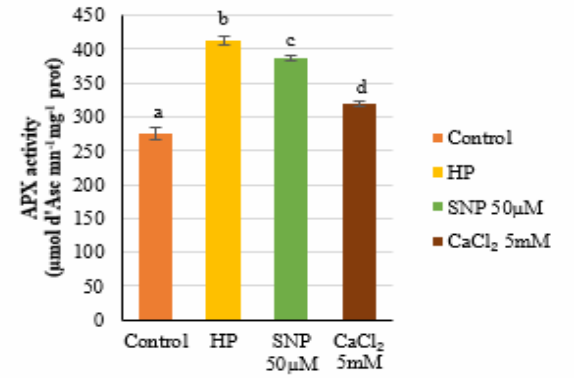

Figure 13. Activity of ascorbate peroxidase in radicles according to the different treatments; HP = Hydropriming; SNP = Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p < 0.05$).

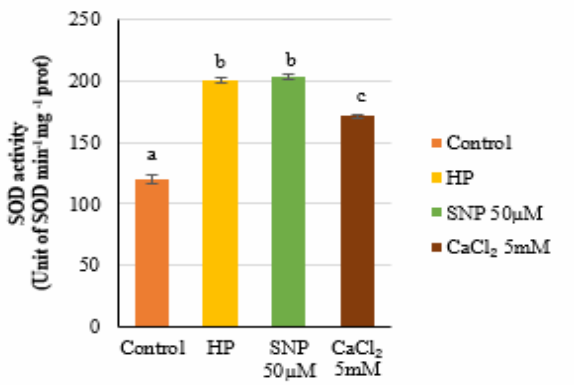

Figure 14. Activity of the superoxide dismutase of the radicles according to the different treatments; HP = Hydropriming; SNP = Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p < 0.05$).

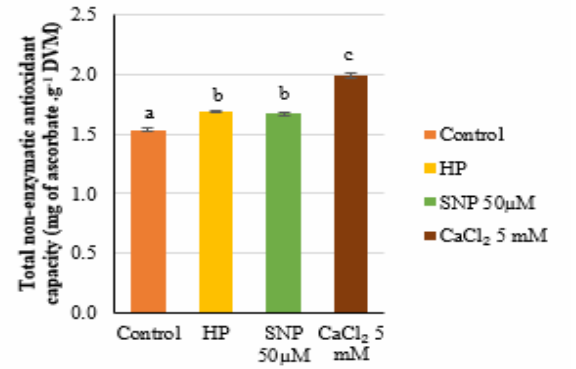

Figure 15. Total non-enzymatic antioxidant capacity of radicles from untreated treated plants; DVM = dry vegetable matter ; HP = Hydropriming; SNP = Sodium nitroprusside; Different alphabetical letters indicate a significant difference ($p < 0.05$).

DISCUSSION

Our present work aimed to study the effect of seed pretreatments (hydropriming and chemical pretreatments) on germination performance, growth parameters as well as ROS production and antioxidant activities of fenugreek seeds (*Trigonella foenum-greacum* L.).

The results showed that these pretreatments led significantly ($p < 0.05$) to a noticeable improvement in germination performance and growth as well as antioxidant activities at the level of the radicle. However, the intensity of this improvement varied depending on the type of treatment.

Effect of pregermination treatments on germination performance

According to our results, hydropriming significantly improves the germination performance of fenugreek seeds. Our results are in agreement with many authors who have shown in different species of field crops such as beans, lentils, wheat, corn, rice, watermelon, melon, tomato, carrot and pigweed, that seed priming allows the acceleration and synchronization of germination [36, 52], as well as better growth, earlier flowering, greater tolerance to stress and higher yield [8, 32, 53]. As a result, several authors have explained this rapid and synchronized germination by an activation of pregermination processes which induce quantitative and qualitative biochemical modifications at the level of seeds [49, 72] such as repair membranes [40], a strong synthesis and activation of enzymes involved in the degradation and mobilization of reserves [72, 74], an activation of endo-β-mannase which is the enzyme responsible for the synthesis of ethylene (hormone which allows the degradation of albumen for the breaking of dormancy) [72] and an activation of certain genes tolerance to dehydration, which stimulates the synthesis of osmolytes playing a role in osmotic adjustment thereby improving abiotic stress tolerance. We speculate that the expression of these tolerance genes would be under epigenetic control. Epigenetics can explain "information storage" which has led some authors to refer to "epigenetic memorization" [21, 68].

The chemical pretreatment with sodium nitroprusside (SNP) at 50 µM also caused a marked improvement in germination performance as well as the growth and development of radicles and aerial parts of fenugreek seeds. Our results are in agreement with those reported by many authors [11, 33, 39, 46, 63, 65, 71, 77, 79] who also observed that chemical seed treatment improved germination performance, vigor and growth of several species crops such as wheat, barley, tomato and rice. We could explain these beneficial effects by the involvement of nitric oxide (NO), a bioactive signaling molecule that plays a central role in many physiological processes of plants, such as regulation of growth and development and induction of germination [34, 78]. Different NO donors have been known to stimulate seed germination such as some woody plants [29], lettuce [10] and Arabidopsis [45]. It has also been shown that NO breaks dormancy controlled by ABA by increasing the activity of enzymes degrading this hormone [12] with the initiation of germination stimulated by gibberellic acid [13]. As for treatment with $CaCl_2$, the improvement in germination performance was due to the involvement of Ca^{2+} in the activation of cell signaling pathways. The observations presented are evidence to suggest a second messenger role for exogenous Ca^{2+} during sandalwood somatic embryogenesis [3]. Calcium is an intracellular secondary messenger involved in the transduction of a large number of stimuli [18]. Calcium transmits the signals received at the cell surface towards the interior of the cell thanks to the spatio-temporal concentration changes decoded by a set of Ca^{2+} sensors [60, 61, 70, 83]. The signalling mode depends on a rapid and transient ion channel-mediated increase in cytosolic Ca^{2+} [67], referred to as a Ca^{2+} signal. This signal is essential for decoding internal and external stimuli, transducing them into physiological and gene expression responses [19, 24, 26, 35, 65]. Beyond its role in plant nutrition as a macroelement and its involvement in the structural and functional integrity of the membrane and other cellular and

histological structures, calcium is known to modulate many aspects of development and growth [41, 51, 60].

The results lead to the conclusion that it was the SNP treatment that produced the better germination performance.

Effect of pregermination treatments on the production of reactive oxygen species

Biochemical analyzes revealed that fenugreek seed pretreatments enhanced the activities of antioxidant enzymes and promoted protein biosynthesis at the radicle level. Soeda et al. (2005) and Varier et al. (2010) [66, 72] have already shown that the treatment of seeds promotes the synthesis of proteins involved in cell metabolism by improving the machinery for their synthesis. in the other hand, cytochemical tests revealed a production of ROS, more particularly hydrogen peroxide (H_2O_2), which is more intense in the pre-treatments at the level of the radicles and more precisely at the level of the meristematic areas of the elongation zone which reflects the strong activation of cell division during treatment. Numerous studies have shown that the change from a quiescent seed to a metabolically active organism upon imbibition is associated with the production of ROS. For example, the production of hydrogen peroxide has been demonstrated from the start of the seeds imbibition of soybean [27], radish [64], corn [37], sunflower [6] wheat [22], peas [76] and tomato [54]. ROS play an ambivalent role in seeds as well as in the whole plant. They are necessary for breaking dormancy and germination, but when they accumulate, they induce cellular damage which can lead to the death of the seeds [30, 56]. This is why Bailly et al. (2008) [7] proposed the oxidative window theory which refers to a critical level of ROS necessary for germination and breaking dormancy. This critical level is between two low and high limits. Such an effect could result from the interaction between ROS and hormonal signaling pathways, thus leading to changes in gene expression or cellular redox state. When the ROS content is low in seeds, it does not allow dormancy or germination to break down. When the ROS content is high, the seeds do not germinate but age. They undergo a lot of cellular damage at the level of lipids, proteins and nucleic acids. Antioxidant systems are overwhelmed which leads to a strong build-up of ROS which can lead to cell death [7]. In order to maintain the redox balance, there will be an activation of the antioxidant systems and more particularly of the enzymatic systems (catalase, guaiacol peroxidase, ascorbate peroxidase, superoxide dismutase) for hydropriming and treatment with SNP and not enzymatic for treatment with $CaCl_2$.

Indeed, SNP enhanced the activities of antioxidant enzymes (catalase, guaiacol peroxidase, ascorbate peroxidase as well as superoxide dismutase) in fenugreek radicles. Similar results have been reported in wheat roots [73] with the application of NO, *Perilla frutescens* [82] as well as Ataide et al. (2016) [5] found that seeds soaked in SNP increased catalase activity. In addition, it has been suggested that NO acts as an antioxidant and limits ROS formation [42, 80, 83].The same is true for treatment with $CaCl_2$, Zhang CP et al. (2010) [81] observed that the treatment of the seeds of Perilla frutescens with Ca^{2+} increased the enzymatic activities (CAT, SOD, peroxidase POD).

Hydropriming stimulated the activation of antioxidant enzymes in the radicles. These results are in agreement with several studies which have clearly demonstrated that the treated seeds of several cultivated species are characterized by very strong antioxidant enzymatic activities [2, 72]. Likewise, several authors have explained the improvement of germination in treated seeds by the increase of these antioxidant enzymatic activities which allow the elimination of free radicals synthesized in response to oxidative stress to avoid cell damage [2, 72].

Aknowledgements. The present study is part of the research funded by PHC Maghreb project (41482RL / 19MAG41) and supported by the Ministry of High Education and Scientific Research of Algeria.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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Received: May 10, 2021
Accepted: November 3, 2021
Published Online: November 5, 2021
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House