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GERMINATION PERFORMANCE OF PISTACHIO (*PISTACIA VERA* L.) UNDER SALCYLIC ACID AND SALT STRESS EFFECT

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Abstract
Description of the subject: Salicylic acid (SA) is considered to be a potent plant hormone in preventing germination seeds in many plants.
Objective: The present study was to compare the effect of different concentration of salicylic acid (SA) on the germination vigour and rooting ability of two varieties of *Pistacia vera* L. (Bayadhi and Achouri) subjected to salt stress.
Methods: Seeds were pretreated with four concentrations of salicylic acid (0.25–0.5–0.75 and 1 mM), and then sprayed with H_2O and NaCl solution. The untreated seeds were used as control.
The following parameters were evaluated; germination speed, germination percent and radicle elongation.
Results: The obtained results showed a reduction in germination parameters of the untreated seeds (control), especially in presence of 100 mM NaCl compared to 50 mM NaCl. However, the SA treated seeds performed better than the untreated seeds. Significant difference between the treatments effect on germination were observed at $p \leq 0.05$. The doses of 0.25 and 0.5mM SA had a positive effect on the speed, the rate of germination and the radical elongation than 0.75mM SA, even when seeds were sprayed with water or solution of NaCl. On the other hand, the dose of 1mM SA produced a significant reduction of germination and root elongation, in the presence or the absence of salt stress.
Conclusion : It can be concluded that the lowest doses of salicylic acid increased significantly the germination performance compared to the highest doses depending on the dose used and the variety..
Keywords : *Pistacia vera L.*, Salicylic acid, Germination kinetics, Salt stress.

EFFET DE L'ACIDE SALICYLIQUE SUR LA GERMINATION DE DEUX VARIETES DE *PISTACIA VERA L.* (BAYADHI AND ACHOURI)

Résumé
Description du sujet : L'acide salicylique est considéré comme étant une hormone essentielle qui est impliqué dans l'amélioration de la germination de plusieurs plantes.
Objectifs : Cette présente étude a pour objectives de comparer l'effet de différentes concentrations de l'acide salicylique sur la germination et l'élongation d la radicule de deux variétés de *Pistacia vera* L. (Bayadhi et Achouri), soumises à l'eau distillé et un stress salin.
Méthodes : Les **g**raines des deux variétés ont été pré-traitées avec quatre doses d'acide salicylique (0,25–0,5 -0,75 et 1 mM), puis arrosées avec H_2Od soit une solution de NaCl. Les **g**raines non traitées et arrosées avec H_2O ont été utilisées comme témoins. La cinétique de germination et le taux final ont été évalués, ainsi que la longueur de la radicule.
Résultats : Les résultats obtenues ont démontré une réduction de la germination des graines non traitées et soumises au stress salin, spécialement les raines arroses avec 100 Mm NaCl. Par contre, les graines traitées avec l'AS ont enregistré une bonne germination en comparaison avec celles non traitées. Des différences significatives de la germination ont été observes entre doses appliquées à $p \leq 0,05$. Les doses 0,25 et 0,5mM AS ont enregistrée un effet positif sur la vitesse et le taux de germination ainsi que la longueur de la radicule par rapport à 0,75mM AS, soit quand les raines ont été arrosées avec l'H_2Od soit sous l'arrosage par 100 Mm NaCl. La dose la plus forte d'AS (1mM) a réduit significativement la germination et a donné les radicules les plus courts sous toutes les conditions de l'expérimentation.
Conclusion : Suite à ces données, il a été constaté que les doses les plus faibles de l'acide salicylique ont augmenté considérablement les paramètres de la germination des deux variétés du pistachier. On peut suggérer que l'effet de l'acide salicylique dépend des doses appliquées et de la variété.
Mots clés : *Pistacia vera L.*, Acide salicylique, Cinétique de germination, Stress salin, H_2Od.

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INTRODUCTION

For a long time, bio productivity of plants has been one of the main topics of agricultural sciences research, with a view on the interest to the farmer and the consumer. Some plants have opted to defend themselves by synthesis metabolites that are used in plant protection and growth regulation. In this category are cited natural defence stimulators (NDS) as a scientific and agronomic interesting solution. According to Swiss law, stimulators have to be registered as a plant protection substance or as fertilizers regulators. Most of these compounds are based on inorganic or organic substances or contain microorganism. Many of these substances are applied in plant protection, yet with varying success [1]. Salicylic acid (SA) is considered to be a potent plant hormone [2], because of its role in regulation of physiological phenomena such as photosynthesis, flowering, membrane permeability, heat production, plant growth, development regulator and plant-pathogen interactions, and its constitute an interesting avenue for understanding interactions in plant breeding for several studies [3, 2, 4, 5]. Research about the effects of SA on plants germination is limited, it was reported in a study exploring the effects of SA that long-term applications were more effective than short-term applications in preventing germination of cabbage (*Brassica oleracea L.*), tomatoes *(Lycopersicon esculentum L.)*, and cucumber (*Cucumis sativus L.*) seeds [6, 7]. It was reported that SA concentrations increased the leaf area and root development, its affect the plants in relation with plant type and life period of application. The pistachio (Anacardiaceae) is a fruit species and is a vital source of protein, fibber, and micronutrients that support good health, but they are rich in phytosterols, antioxidants, unsaturated fat (the good stuff), carotenoids, vitamins and minerals, fibber and had a high forage value. However, the plant is exposed to different biotic and abiotic factors [8, 9]. In Algeria, four species are present, *Pistacialentiscus, Pistacia terebinthus, Pistacia atlantica* and *Pistacia vera*, pistachio is mostly found in Tlemcen, Saida, Sidi Bel Abbés, Batna, Khenchela, Sétif and Tébessa [10]. The seeds of *Pistacia.sp* species are surrounded by a hard sclerotic endocarp, the germination rate in these species is low [11]. Various chemical solutions are used to stimulate seed germination. Under these conditions, it is also possible to use pre-treatments such as osmotic agents [12], gibberellic acid (GA) [13], scarification and cold stratification [14]. In this fact we tend to investigate the effect of salicylic acid (SA) on seeds germination of two variety of *Pistacia vera* L., and to determine the appropriate concentrations of SA used to ameliorate germination vigour against salt condition.

MATÉRIEL ET MÉTHODES

1. Plant material

The experiment was conducted in the faculty of Natural and Life Sciences of Mustapha Stambouli of Mascara University, Algeria. This study focused on two varieties of *Pistacia vera* L. (Bayadhi and Achouri). These seeds orchard at the experimental farm and from adult trees in September 2020 stored away from moisture in paper bags and selected according to their size, shape, and color. Seeds previously disinfected using sodium hypochlorite (2%) for 3 minutes and distilled water (Fig. 1).

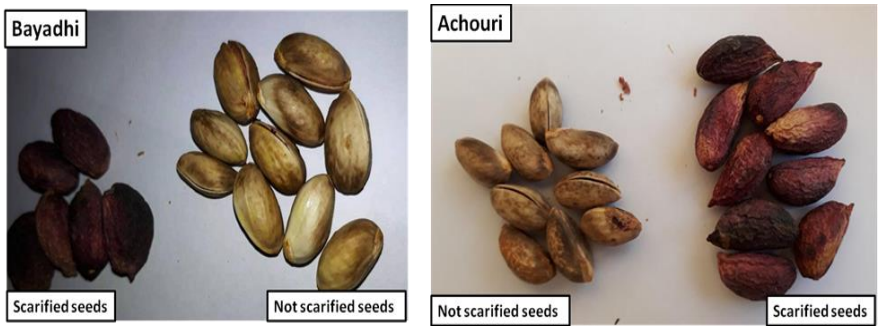

Figure 1. Seeds of *Pistacia vera* L. (Bayadhi and Achouri)

2. Preparation of salicylic acid (SA)

Salicylic acid (2-hydroxybenzoic acid) was initially dissolved in 1 ml ethanol and diluted with distilled water. The concentrations of 0.25, 0.50, 0.75 and 1mM were prepared with H_2O (pH 6.0 – 6.5) [15]. Seeds were pretreated with 50ml of distilled water (control), or 50ml of the different salicylic acid solutions for 24 h until the solution was completely absorbed.

3. Treatment of seeds

Seeds of Bayadhi and Achouri varieties were divided into four lots:

Lot 1- The untreated seeds (control) were divided in three lots; one lot was soaked with water to test the process of germination potential for each variety, and the second and third lots were soaked with two concentration of sodium chloride (50 mM and 100 mM NaCl) respectively, in order to test the influence of salinity on the germinability of the two varieties.

Lot 2-The treated seeds with each concentration was divided in two lots. One lot was soaked with tap water, and the other lots of treated seeds were sprayed with solutions of sodium chloride (100 Mm NaCl).

4. Germination essay

All seeds were placed on double sterilized filter paper in a Petri dish (9 cm in diameter and 1.3 cm thick), each dish contains 50 seeds was kept in the heat chamber thermo-regulated (LIEBHERR), at (22°C ±1°C) with 70-80% relative humidity. Data were based on the calculation of kinetic of germination every week. The final rate of germinated seeds is expressed according to the following formula: ***Germination rate (%)=(Ni/Nt)×100.*** Where : ***Ni:*** total number of germinated seeds, ***Nt:*** total initial number of seeds used. The rooting was calculated based on the radicle elongation (cm).

5. Statistical analysis

Each treatment was analyzed with four replicates and a standard deviation (SD) was calculated and data were expresses as ± SD. The germination model parameter between control and treatment was obtained using ANOVA one factor, (XLSTAT Microsoft 2020).

RESULTS

Rapid and uniform germination represent an important phase for improved seedling emergence and crop production. The evaluation of the effect of salicylic acid on *P. vera* seeds presented significant variations in both varieties according to the concentration applied.

1. Effect of NaCl on the kinetics germination of untreated seeds

The germination of untreated seeds of the two varieties of *P.vera* were began since the second week, either for the seeds sprayed with H_2O or the seeds subjected to 50 mM and 100 mM NaCl. Seeds of Bayadhi variety showed a germination rate of 10% since the first week (78.5%, on the fourth week) (Fig. 1A). The time of germination for Achouri is rather long than Bayadhi (30% the second week and 62% in the fourth week) (Fig. 1B). The concentration of 50 mM NaCl had a lowest effect on the kinetic seed germination of both varieties compared to 100 mM NaCl. The solution of 100 mM NaCl had a significant effect by reducing the speed of seed germination of Bayadhi and Achouri, and resulting in a very low percentage germinated seeds since the second weeks to the last week for both varieties (Fig. 1A and Fig.1B).

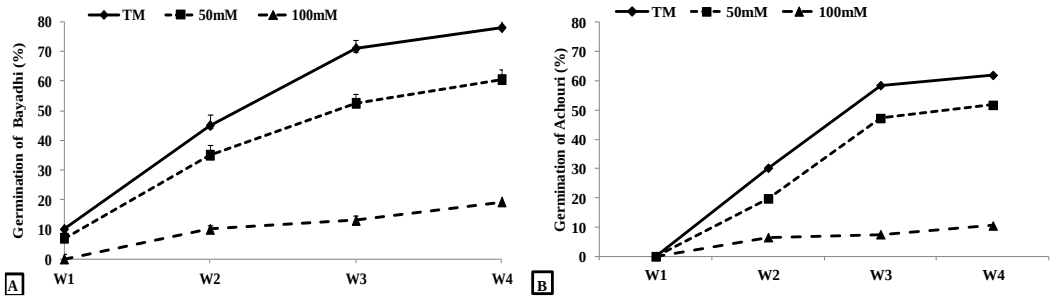

Figure 1: Effect of different levels of Sodium hypochlorite (NaCl 50 and 100 mM) on the kinetic of the germination of untreated seeds of A) Bayadhi and B) Achouri (Mean of 4 repetitions are calculated at $p \leq 0.05$)

2. Effect of SA on the kinetics germination of treated seeds sprayed with H_2O

Comparative results of SA concentrations on the kinetic germination seeds of the both varieties, showed a highest seed germination since the first week with 0.25 mM followed by 0.5 and 0.75 mM SA, either in Bayadhi and Achouri (Fig. 2). There is a significant difference in the germination parameters between treated seeds and untreated seeds of Bayadhi (R_2=79%) and Achouri (R_2=88%). The kinetic germination was linearly activated by 0.25 and 0.5 Mm SA in both Bayadhi and Achouri on the following weeks. The analysis of the variance of the treatment revealed a very highly significant on germination for the Bayadhi ($p<0.01$), and significant for Achouri ($p<0.05$) (Table 1). In contrast, the lowest kinetic germination seed was registered with 1mM SA treatment in both varieties since the first week until the last week.

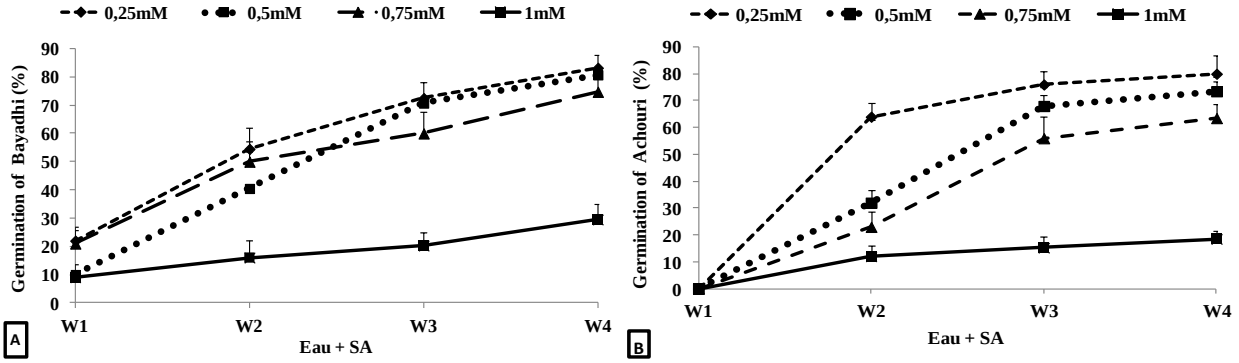

Figure 2: Germination speed of *Pistacia vera L* seeds: A) Bayadhi and B) Achouri treated with salicylic acid (0.25 - 0.5 – 0.75 and 1 Mm) and sprayed with H_2O, during 4 weeks (W).
Average of 4 repetitions is calculated at $p \leq 0.05$.

3. Effect of SA on the kinetics germination of treated seeds sprayed with NaCl

The data in Figures A3 and B3 showed the variability in germination speed depending on the variety and on the application dose of SA. Among SA concentrations, the highest germination speed was accomplished by 0.5 mM followed by 0.25 mM SA in Byadhi and Achouri. The concentration of 0.75 generated a lowest kinetic germination along the four weeks. On the other hand, 1mM SA increased the time and reduced the level of the germination in both varieties.

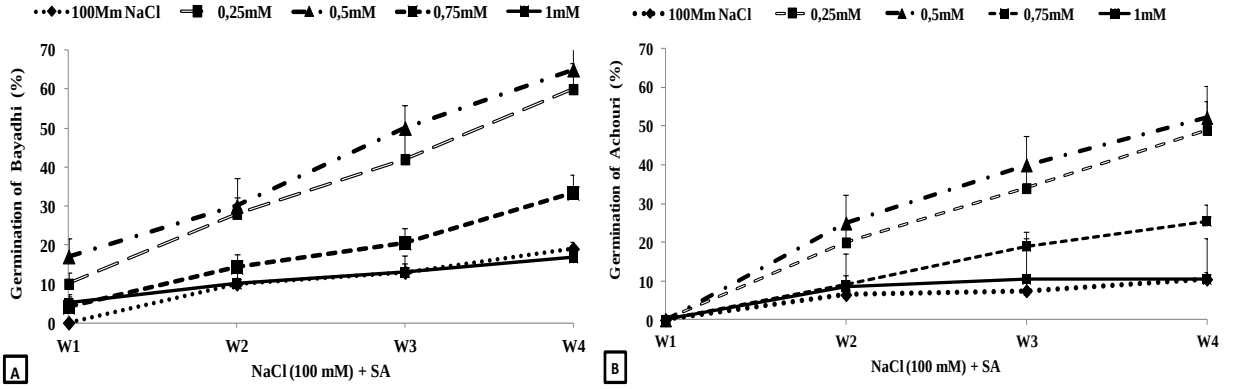

Figure 3: Kinetics germination seeds of *Pistacia vera L* (%): A) Bayadhi and B) Achouri treated with salicylic acid (0.25 - 0.5 – 0.75 and 1 Mm) and sprayed with NaCl 100 mM during 4 weeks (W).
Average of 4 repetitions is calculated at $p \leq 0.05$.

4. Effect of SA on the germination rate of Pistacia vera L

In both Bayadi and Achouri, a significant effect was showed between the doses of salicylic acid applied on the rate of germinated seeds under all conditions (Table 1, Table 2). The germination rate of pistachio seeds decrease with the increasing salinity. The application of the low concentrations of NaCl (50 mM) reduced the level of the germinate seeds in Bayadhi and Achouri (-10%) while the application of high concentration of NaCl (100 mM) reduced significantly the germination rate (-55.50%) in untreated seeds (Table 2). Based on the the affinity of *P. vera* to 100 Mm NaCl, we had study the effect of 100 mM NaCl on the germination of treated seeds with different treatments of SA. Among the different treatment on the final rate of treated seeds of the two varieties, it was noted that the highest germination rate was obtained with treatment 0.25 and 0.5 mM SA (p<0.01) compared to control, which improved a moderate germination when the seeds were sprayed with H_20 (Tab 1). The treatment with 1 mM SA caused a significant reduction in the germination rate when seeds were sprayed with H_2O (-46.25%) (Tab 1).

Table 1: Calculation of the variations in reduction (-) and increase (+) of the germination percentage of seeds treated with control and the different concentration of salicylic acid and sprayed with H_2O. (T-test for two paired samples / Two-tailed test at *p≤0.05* ANOVA one factor, XLSTAT Microsoft 2020).

SA (mM) / H_2O	0,25-C (**)	0, 5-C (**)	0,75-C (ns)	1-C (**)
Difference (%)	11.37	11.88	1.87	-46.25
t (Value observed)	4.307	6.188	0.521	-25.74
p-value (bilateral)	0.023	0.008	0.639	0

(ns insignificant, **significant)

On the other hand, the application of SA treatment when the seeds were subjected to NaCl caused significant augmentation of germinated seeds with 0.25 and 0.5 Mm SA to (+39.75 and +43.87%), respectively (Tab 2), while, 0.75 Mm caused a moderate increase (+14. 87%). There was an insignificant difference in the rate of germination between the untreated seeds and treated seeds with 1 Mm SA, when seeds were sprayed with 100 mM NaCl (-1.12%) (Tab 2).

Table 2: Calculation of the variations in reduction (-) and increase (+) of the global germination percentage of seeds treated with control and the different concentration of salicylic acid and sprayed with 100 Mm NaCl. (T-test for two paired samples / Two-tailed test at *p≤0.05* ANOVA one factor, XLSTAT Microsoft 2020).

SA (Mm)/ NaCl	Control -NaCl (**)	0,25 –NaCl (**)	0,5 -NaCl (**)	0,75 -NaCl (**)	1- NaCl (ns)
Difference (%)	-55,5	39,75	43,87	14,87	-1,125
t (Value observed)	22,13	4,455	5,147	0,371	0,762
p-value (bilateral)	0,023	0,008	0,639	0	0,846

(ns insignificant, *significant)

The calculation of the difference between the concentrations of SA applied revealed that there was an insignificant effect between 0.25 and 0.5 mM SA on the germination rate of *P.vera (*Tab 3). However, there was a significant difference on the germination rate between 0.75 mM SA and 0.25 and 0.5 mM. The treatment with 1 mM gave a considerable variation on the germination level in comparison with the other treatments in all conditions of the experiment (*p*≤0.01**)**.

Table 3: Calculation of the variations in reduction (-) and increase (+) of the germination percentage of seeds treated with the different concentration of salicylic acid and sprayed with H_2O or 100 Mm NaCl. (T-test for two paired samples / Two-tailed test at *p≤0.05* ANOVA one factor, XLSTAT Microsoft 2020).

	H_2O						NaCl					
SA (Mm)	*0,25-0,5 (ns)*	*0,25-0,75 (**)*	*0,25-1 (**)*	*0,5-0,75 (**)*	*0,5-1 (**)*	0,75-1 (**)	*0,25-0,5 (ns)*	*0,25-0,75 (**)*	*0,25-1 (**)*	*0,5-0,75 (**)*	*0,5-1 (**)*	0,75-1 (**)
Difference (%)	-0,5	9,5	+57,6	10	58,1	48,1	-4,13	249	40,9	29	45	16
t (Value observed	-0,21	8,79	44,99	3,45	48,6	23,2	-1,23	7,57	11,9	8,19	18,7	11,9
p-value (bilateral))	0,85	0,003	<0,0001	0,04	<0,0001	0	0,003	<0,0001	0,04	<0,0001	0	0,02

(ns insignificant, *significant)

5. Effect of salicylic acid on the elongation of the radicle

The average measurements made on the root lengths of sprouted seeds showed differences between both varieties of *Pistacia vera.* Measurements of root elongation show that Bayadhi gives longer roots than Achouri. The most rate of root elongation reached 1.2 cm in control seeds (70%). Treatment with SA depending on the doses applied enhanced rooting between 0.4 cm and 1.5 cm in Bayadhi. The doses of 0.25 and 0.5 mM SA showed the longest rootlets (40%) and (55%), respectively. The dose of 0.75 Mm SA resulted in an elongation of 1 cm (56%). While 1mM SA gave a short length of 0.4 cm for all germinated seeds (100%) (Table 3). However, a minor rooting was showed in Achouri between (0.2 and 0.4). The elongation was 0.4 cm for (70 and 50%), respectively, with 0.25 and 0.5 mM SA. The dose of 0.75 and 1 mM SA elongation of roots did not surpassed 0.2 cm (60 and 100%), respectively (Table 3).

Table 4. Measurements of root lengths of germinated seeds of Bayadhi and Achouri in the presence of different salicylic acid concentrations

Treatment / variety	Bayadhi				Achouri	
Length of radicles (cm)	0.4	1	1.2	1.5	0.2	0.4
TM	15	15	70	0	75	25
0.25 mM AS	11	23	40	26	30	70
0.5 mM AS	21	20	55	19	50	50
0.75 mM AS	30	56	9	5	60	40
1 mM AS	100	0	0	0	100	0

DISCUSSION

Rapid and uniform germination and seedling emergence represent the main important phase for improved crop production. The salt stress affects differently the germination level and speed of both Bayadhi and Achouri. The highest dose of NaCl decreases the germination parameters [16]. The decrease in the final germination rate corresponds either to an increase in external osmotic pressure, which affects the absorption of water by the seeds and the emergence of the radical, or the accumulation of Na^+ and $C1^-$ ions led to the alteration of the germination and in the extreme case to the death of the embryo [17, 18, 19].

Although, salicylic acid gave a signifiant variation according to the doses of the treatment applied. Results showed that with low concentration of SA, the rate and the speed of germination increases in Bayadhi and Achouri either in the appplication of H_20 or the salt solution (Table 1). These results are consistent with those of Hamada & Al-Hakimi [20], Szepesi et *al.* [21], Brueggeman et *al.* [18], Shakirova [22], Saavedra & Land Rodolfo [23], Baghizadeh et *al.* [24] on beans and commelina, Muhammad et *al.* [25] and Boukraâ et *al.* [26] on wheat, Boukraâ et *al.* [27], Sakhabutdinova et *al.* [28] and Eastmond & Graham [29] on *Atriplex halimus* and chickpea. Among all applied seeds treatments, the low doses raised the speed, the level of germination seeds and rooting elongation [16, 30].

Based on these results, treatment of seeds with SA reduced the depressant effect of salt on germination of *Pistachio vera L.* germination has been remarkably enhanced in treated seeds compared to untreated seeds. Szepesi et *al.* [21], demonstrated that the pre-treatment with SA improves germination vigour by increasing the moisture content in seeds under saline stress conditions. Boukraâ et *al.* [26] and Boukraâ et *al.* [27] found that SA treatments reduce salinity damage by increasing some biochemical and mineral molecules as K^+, proteins, polyphenols as well as the antioxidant activity and antioxidant enzymes activity like Catalase and SOD in chickpea seeds during germination and growth.

On the other hand, 1 mM SA affected the germination parameters by reducing significantly the speeds and the rate of germinated seeds, either in the absence or presence of NaCl [26, 27, 28]. Eastmond & Graham [29] and Rajjou et *al.* [30], indicated that high concentrations of salicylic acid altered the germination by inhibiting the synthesisof ethylene or by causing oxidative stress. The emergence of the radicle represents the third phase of the germination process [31]. The doses of 0.25 and 0.5 mM SA showed the longest rootlets. These results were showed by Waseem et *al.* [32] under drought stress and Boukraâ et *al.* [33] under salt stress. According to Ling [34] and Sakhabutdinova et *al.* [28], salicylic acid is involved in regulating cell division and root elongation in synergy with auxin.

CONCLUSION

In conclusion, first, it appears from the recorded results that the germination parameters of Bayadhi variety are better than Achouri. Furthermore, it can be observed that salicylic acid at low concentrations had significant effect for enhancing the germination speed and rate. It was resulting in an improvement of the germination parameters either in favourable condition or beside salt stress. On the other hand, high dose of SA should be excluded in the pre-treatment of *Pistacia vera* L. seeds during germination phase.

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