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PERFORMANCE OF PEPPER SEEDLINGS DEVELOPED FROM SEEDS PRETREATED WITH ALGAL AQUEOUS EXTRACTS

Imen RINEZ[1]*, Ines SAAD[2], Asma RINEZ[1], Nadia GHEZAL[3], Rabiaa HAOUALA[2]

*E-mail: imen.rinez@yahoo.fr

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ABSTRACT. This study aims to evaluate the effect of seed priming for two pepper varieties by extracts of two algae thalli on the performance of growth at vegetative stage.The seeds were soaked in thalli aqueous extracts (at 20, 40, 60, 80 and 100 g/L), for 24 h at 28±2°C, rinsed thoroughly with distilled water and then dried to their initial weight as a first batch (SD), a second batch of seeds was considered, corresponding to seeds placed in Petri dishes to germinate directly after washing (SND). Untreated seeds were the control. The results showed than the priming has improved seedlings growth for both varieties. Seed priming with algae aqueous extracts has improved roots and shoots length. The best stimulations were 220% and 70%, respectively, recorded for seedlings of Baklouti and Chargui varieties, following the priming with the extract of *Padina pavonica* at 40 g/L concentration. These improvements appear to be related to a better photosynthetic intensity, an increase in the number of leaves and the water content, which were noted in plants from pretreated seeds. Finally, the results indicate that, generally, the plantlets from SND were more vigorous. The results of this work show that algae could be an inexhaustible resource of substances, that can be used as an alternative to chemicals used in the pretreatment of seeds, reducing the risk of pollution and preserving the biological material.

Keywords: *Capsicum;* priming; aqueous extracts; algae; growth.

INTRODUCTION

The seeds constitute the main vector of plant propagation and they

[1] Department of Biology, Faculty of Sciences Bizerte, University of Carthage, Jarzouna, Tunisia
[2] Department of Biological Sciences and Plant and Environment Protection, Higher Institute of Agronomy of Chott Meriem, University of Sousse, Chott Meriem, Tunisia
[3] Departement of Biology, Higher Institute of Biotechnology of Monastir, Monastir, Tunisia

represent a critical development stage with many specificities. Seeds possess a wide range of systems (protection, detoxification, repair), allowing them to survive in the dry state and to preserve a high ability of germination (Rajjou and Debeaujon, 2008). Seed germination is one of the most important phases in the plant life cycle and is highly responsive to existing environment (Kuriakose and Prasad, 2007).

Seed germination of several species is extremely slow, even under favorable environmental conditions, and many tests and techniques are used to counteract this problem. Several techniques are used such the physical pretreatments, which are illustrated as scarification, cold, magnetic field, etc, or the chemical pretreatments, which are known with the soaking in dilute solutions of hydrogen peroxide, sulfuric acid, potassium salts, etc.

Seed priming is a successful method, that has been proven to improve seed germination and emergence of seedlings. It is a controlled hydration treatment at low water potential, that allows pregerminative metabolism to proceed, but it prevents radicle emergence (Bradford, 1986). Seed priming improves germination and emergence of seeds for many vegetable, under normal, as well as stress condition and many substances are used as osmotic, which lower the water potential, such as PEG, NaCl, KNO_3, $ZnSO_4$ and $CaCl_2$ (Sivritepe *et al.,* 2003; Barasa *et al.,* 2005; Ehsanfar *et al.,* 2006; Esmaielpour *et al.,* 2006; Neamatollahi *et al.,* 2009). In fact, Demir and Mavi (2004) found watermelon seed priming with KNO_3 solution, effectively, improved germination and seedling growth of the seeds under salinity, compared to non-primed seeds.

Pepper is widely cultivated for its fruits, which have a recognized nutritional value. In fact, they are an excellent source of various antioxidant compounds, like flavonoids, carotenoids and vitamin C (Chuah *et al.,* 2008). This later protects human body against oxidative damage and prevents various diseases, such as cancer and cardiovascular diseases (Oboth and Rocha, 2007). In Tunisia, pepper is the major cultivated plant and its fruits are mainly consumed either fresh cooked. It is cultivated on open field and under greenhouse.

It has also been reported that pepper seed germination and emergence is slow and non-uniform, under normal as well as stressed conditions (Demir and Okcu, 2004). For example, under high or low temperature it can take up to one month for emergence. Khan *et al.* (2009) reported improvement of the germination of pepper by priming with water and NaCl.

Indeed, macro algae have awoke the attention of many works and are an immense reservoir of active substances, particularly in three areas: the food, agriculture and health. In addition, this biological material could be an alternative, inexpensive

and environmentally friendly, to chemical products used for the same purpose, but which have proved very formidable results.

View that the use of chemicals is often criticized, the aim of this study is to evaluate the effects of pretreatment of seeds, by aqueous extracts of two marine algae (*Jania rubens* and *Padina pavonica*) at two peppers varieties (Baklouti and Chargui) on plant growth during the vegetative stage.

MATERIALS AND METHODS

Biological material

Two species of algae were chosen: a red (*Jania rubens*) and a brown algae (*Padina pavonica*). The thalli were collected from the Tunisian littoral (Monastir, Bekalta), in July 2012, at a depth of about 1 m. The identification was made based on color and morphological characters of the thallus (Taylor, 1960). Their aqueous extracts were used for the pretreatment of pepper (*Capsicum annuum* L.) seeds of two varieties Baklouti and Chargui**.**

Preparation of aqueous extracts

The algae thalli were washed thoroughly with tap water, to remove all epiphytes and attached debris, air dried, for 15 days, at laboratory temperature and then ground into a fine powder using a mortar. The aqueous extracts were prepared at five concentrations: 20, 40, 60, 80 and 100 g/L. Algal powders were soaked in distilled water for 24 h, at room temperature and darkness (Khan *et al.,* 2005). The mixture was filtered through a filter paper several times to obtain a stock solution, which stored at 4°C, in the dark for further use.

Seed priming and germination test

Seeds were soaked in the aqueous extracts of algae (at 20, 40, 60, 80 and 100 g/L) with a ratio of seed mass weight to solution volume of 1:5 (g ml^{-1}) (Farooq *et al.,* 2006) for 24 h at 28 ± 2°C, then washed thoroughly with distilled water. Untreated dry seeds were taken for comparison (Farooq *et al.,* 2005). Two batches were considered *i)* seeds were dried (SD), closer to original moisture level, in forced air at 28 ± 3°C, sealed in craft bags, and stored at 4°C, until use *ii)* seeds were placed in Petri dishes to germinate directly, after pretreatment and washing, seeds not dried (SND).

Effects of priming on seedling growth

After priming, the seeds were germinated in Petri dishes and pepper seedlings of both varieties were planted in pots with a height of 7 cm and which diameters, upper and lower, were 9 and 6 cm, respectively. The pots were filled with 150 g of a mixture of soil and peat (2/3, 1/3) and the seedlings were irrigated every other day. Untreated seeds were used as controls and five repetitions were performed for each treatment. The experiment was conducted under uncontrolled greenhouse. To estimate the aqueous extracts priming effect on the both varieties seedling growth, the length of roots and aerial parts was measured 60 days after transplanting and biomass production was evaluated by determining the masses of fresh matter and dry matter.

The effect of pretreatment on the hydration of the seedlings was assessed by calculating the water content (TE), according to the following formula: TE = [(PF-PS)/PS] * 100, where, PF = mass fresh material; PS = weight of the dry material obtained after drying for three days at 60°C.

The results were expressed as percent of control using the formula (Chung *et al.,* 2001): Length / weight % of control = [(Extract-control) / control] * 100, where, Extract = measured parameter for the pretreated seeds extracts of algae; Control = parameter measured for untreated seeds.

Measuring photosynthesis (P), stomatal conductance and transpiration in the leaves

Measurements of photosynthesis, transpiration and leaf conductance were carried out by an IC 301 CO2 Analyzer apparatus. Pepper sheet was well spread out and then placed in the receiving cell of light. For each pot five replicates were performed. The intensity of photosynthetic radiation active (PAR) is also given by the device expressed in µmol/m^2/s. The photosynthetic activity (P), stomatal conductance and transpiration are expressed, respectively, in µmol/m^2/s and mMol/m^2/s.

Statistical analysis

The experiment was laid out in completely randomized design in factorial arrangement with five replications. The data were subjected to statistical analysis using SPSS 13.0 for Windows. Least significant difference (LSD) test was applied to compare the treatment means ($p< 0.05$) and graphical presentation of data was carried out using Microsoft Excel program.

RESULTS

Effect of the priming of seeds on the seedling growth

Effect on root growth

Seedlings of the variety Baklouti were significantly improved in both cases of seeds pretreatment (SD and SND) by *J. rubens* extracts. Thus, the stimulations reached 86% and 189% for the roots from SD and SND pretreated by extracts from *J. rubens*. The best results were recorded from 40 g/L in the case of roots from SND and up to 80 g/L for those of SND (*Fig. 1*).

For variety Chargui, improved root growth was registered only when seeds were primed with 80g/L, where respective stimulations of 37 and 16% for SD and SND were registered (*Fig. 1*).

Priming by the *P. pavonica* extracts significantly improved root growth of the variety Baklouti and the stimulations were an average of 99% and 116% for SD and SND, respectively, recorded at 40 g/L. Concerning Chargui variety, priming with extracts of *P. pavonica*, did not touch the root growth (*Figs.1 and 2*).

Effect on the aerial part

The stimulation of seedling length Baklouti, from SND, varied between 99 and 389% for different concentrations of *J. rubens* extracts, and between 80 and 324% for *P. pavonica* extracts. In both cases, the maximum was recorded at 40 g/L.

For seedling Chargui from SD, pretreated with *P. pavonica* extracts, the aerial parts showed a better growth, compared to roots, with similar behavior at all concentrations, the average stimulation was 87% (*Figs.1 - 4*).

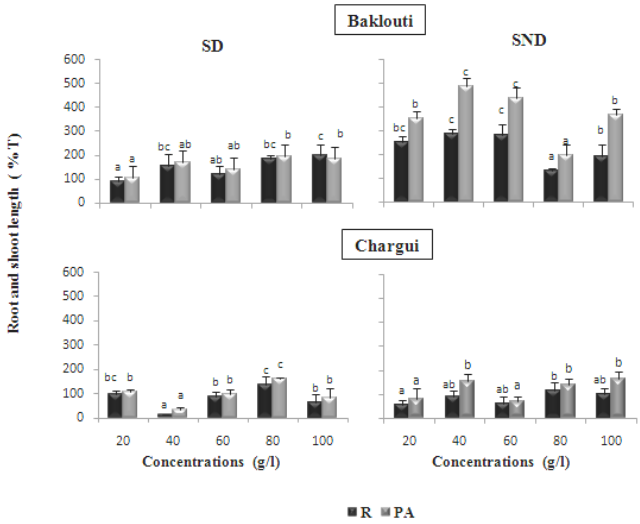

Figure 1 - Root (a) and shoot length (b) expressed in percent of control (%T) of varieties Chargui and Baklouti pepper seedlings, produced from pretreated dried (SD) and not dried (SND) seeds, by aqueous extracts (20, 40, 60, 80 and 100 g/l) of *Jania rubens* thalli. The bars on each column show standard error. Values (N=5±S.E.). Different letters on columns indicate significant differences among treatments at $p< 0.05$.

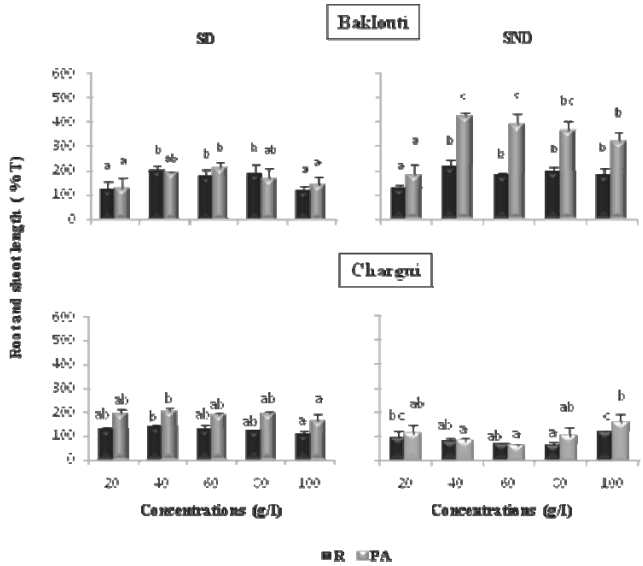

Figure 2 - Root (a) and shoot length (b) expressed in percent of control (%T) of varieties Chargui and Baklouti pepper seedlings, produced from pretreated dried (SD) and not dried (SND) seeds, by aqueous extracts (20, 40, 60, 80 and 100 g/l) of *P. pavonica* thalli. The bars on each column show standard error. Values (N=5±S.E.). Different letters on columns indicate significant differences among treatments at $p< 0.05$.

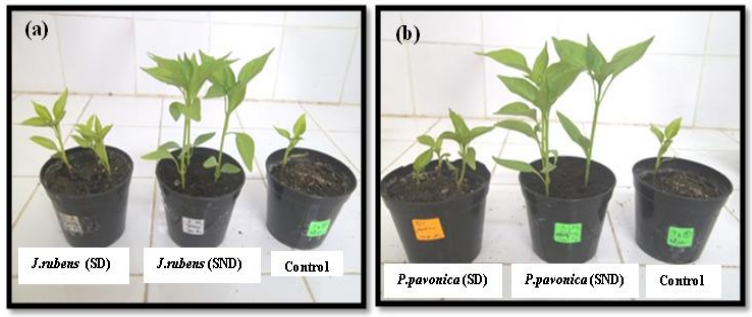

Figure 3 - Seedlings of the variety Baklouti, from untreated seeds (control) and after pretreatment and drying (SD) and not drying (SND) of the seeds by the aqueous extract of *J. rubens* (a) and *P. pavonica* (b) at 100 g/Lconcentration.

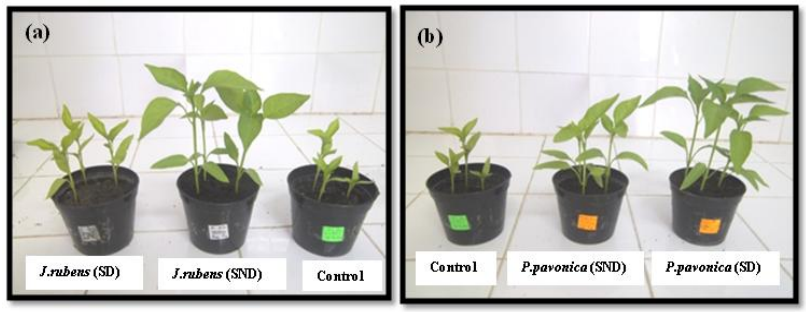

Figure 4 - Seedlings of the variety Chargui, from untreated seeds (control) and after pretreatment and drying (SD) and not drying (SND) of the seeds by the aqueous extract of *J. rubens* and *P. pavonica* (b) at 100 g/Lconcentration.

Effect on seedlings hydration

For variety Baklouti, hydration seedling was close to the control or slightly improved (15%) for SD treated by aqueous extract of *J. rubens*, at all concentrations. For those from SND, a mean decrease (24%) of the water content has been recorded at 40 and 80 g/L. Concerning variety Chargui, the water content of its seedlings is negatively affected by the two types of priming of their seeds, thus average reductions of 39% and 23% were recorded, respectively, for the SD and SND. The main reduction was observed at 40 g/L for SD (*Fig. 5*).

Priming by the extracts of *P. pavonica* has improved hydration of seedling variety Baklouti, and this for SD at concentrations up to 60 g/L, where an average stimulation of 42%, compared to the control, was noted. Beyond, the effect was negative and the water content was only 60% of the control. In the case of SND, no significant effect was recorded. For variety Chargui, hydration of seedling was not significantly affected, with respective inhibitions of 19% and 7%, recorded in the case of SD and SND (*Fig. 6*).

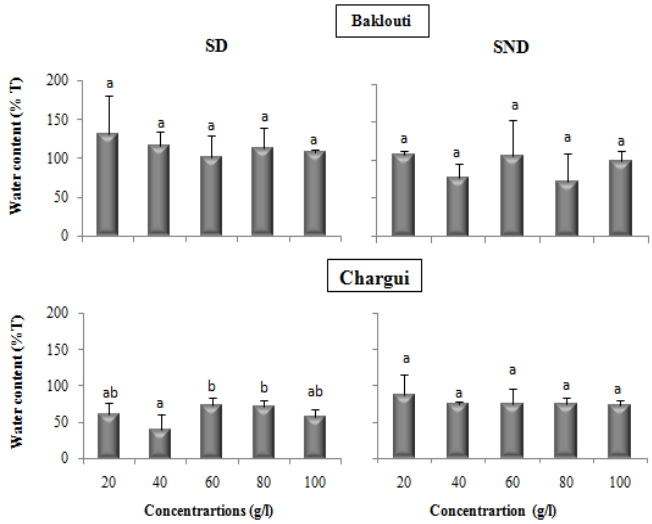

Figure 5 - Water content, expressed in percentage of control of seedlings of both pepper varieties (Baklouti and Chargui) after pretreatment and drying (SD) and not drying (SND) of their seeds by aqueous extract of *J. rubens* at different concentrations. The bars on each column show standard error. Values (N=5±S.E.). Different letters on columns indicate significant differences among treatments at $p< 0.05$.

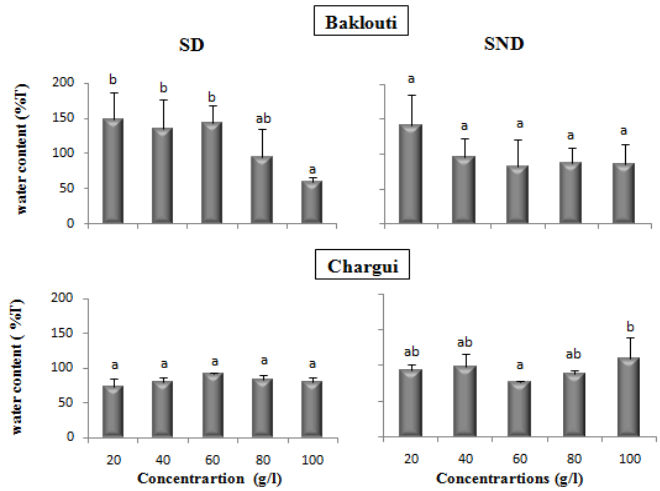

Figure 6 - Water content, expressed in percentage of control of seedlings of both pepper varieties (Baklouti and Chargui) after pretreatment and drying (SD) and not drying (SND) of their seeds by aqueous extract of *P. pavonica* at different concentrations. The bars on each column show standard error. Values (N=5±S.E.). Different letters on columns indicate significant differences among treatments at $p< 0.05$

Effect on the number of seedling leaves

The two types of priming by the aqueous extract of *J. rubens* were favorable on the leaves number for Baklouti variety (*Table 1*). For plants from SD, the best result was recorded at 80 g/L, where the number of leaves was an average of 9.8 per seedling. For concentrations up to 60 g/L, the number was reduced to 6.4 leaves and in the other cases the values were similar to the control (8.4 leaves). Concerning the seedlings from SND, the number of their leaves has increased in a manner proportional to the concentration, averaging 10.5 leaves at 100 g/L (*Table 1*).

Similarly, the number of seedlings leaves of the variety Chargui has increased thereafter to both types of pretreatments. For the control, this number was an average of 5.8 leaves and reached a maximum of 8.1 for the seedlings from SD, and 8.86 for those from SND, when seeds were primed by *J. rubens* aqueous extract at 80 g/L and 40 g/L, respectively (*Table 1*).

For priming by *P. pavonica* aqueous extract, the seedlings number leaves of Baklouti variety from SD was close to the control (7.63 leaves) at all concentrations, except at 40 g/L, where a reduction of 1.7 leaves was recorded. As for the seedlings from SND, the number of leaves increased from 8.3, for the control, to an average of 9.48, following the priming with this extract at 20, 60 and 80 g/L. For the other concentrations, the number was close to the control (*Table 1*).

Table 1 - Number of leaves of seedlings, aged 2 months, of two pepper varieties (Baklouti and Chargui) developed from non-pretreated (control) and pretreated dried (SD) and not dried (SND) seeds, by aqueous extracts of *J. rubens* and *P. pavonica* thalli at different concentrations.

Treated	Cc (g/l)	*J. rubens*		*P. pavonica*	
		Baklouti	Chargui	Baklouti	Chargui
		Number of leaves		Number of leaves	
SD	Control	8.3a	5.8b	8.3a	5.8a
	20	6.3a	7c	7.75a	8.8b
	40	6.5a	4.6a	6.6a	9.5b
	60	8.5a	5.8	8a	8.5b
	80	9.8a	8.1ab	7.6a	8.8b
	100	8.6a	7.8ab	7.2a	9.1b
SND	Control	8.3a	5.8a	8.3a	5.8ab
	20	8.1a	6.8ab	9.3a	6ab
	40	9.2a	8.8c	7.7a	5.2a
	60	9.3a	7.4bc	9.5a	5.3a
	80	9.6a	7.4abc	9.6a	7.4b
	100	10.5a	7.8bc	8.6a	7.5b

For the variety Chargui from SD pretreated with extracts of *P. pavonica*, the number of leaves increased at all concentrations, reaching a maximum of 9.55 at 60 g/L, 5.8 leaves were noted for the control. As, for the seedlings from the SND, this number was an average of 7.5 leaves for pretreatments at concentrations above 60 g/L, and similar to the control (5.7 leaves) for to the other concentrations (*Table 1*).

Effect on photosynthesis (P)

The photosynthesis of Baklouti variety was significantly improved, in both cases of pretreatments (SD and SND) by extracts *J. rubens*. In the first case, the maximum value of P (10.43 µmol/m^2/s, against 2.19 µmol/m^2/s in the control) was obtained at 40 g/L, for the other concentrations stimulation were lower and P values ranged between 6 and 9.86 µmol/m^2/s, for the control, P was 2.19 µmol/ m^2/s. For seedling developed from SND, the best result was recorded at 60 g/L (8 µmol/m^2/s) (*Fig. 7*).

Regarding Chargui variety, improving P was smaller for the two types of pretreatment, compared to the first variety. The maximum stimulation was obtained at 60 g/L (4.75 µmol/m^2/s) and 80 g/L (5.63 µmol/m^2/s) for seedlings from SD and SND, respectively, P of the control was 1.53 µmol/m^2/s. For other concentrations, the values were more or less comparable to the control (*Fig. 7*).

The priming by the aqueous extract of *P. pavonica* has benefited leaf photosynthesis of variety Baklouti for two pretreatments. The best results of 8.93 and 7.78 µmol/m^2/s, respectively, for the SD and SND, were recorded at 20 g/L. In other cases, the values were similar to the control for the seedling SD, while stimulation was obtained at 80 g/L for those of SND (*Fig. 7*).

The photosynthetic intensity of the leaves of Chargui variety from SND has suffered no improvement following the pretreatments by extracts of *P. pavonica*, with a reduction noted at 20 g/L. However SD seedlings, their photosynthesis have been improved and the best result was recorded at 20 g/L, 6.42 µmol/m^2/s, against 1.54 42 µmol/m^2/s in control (*Fig. 7*).

Effect on the transpiration

The leaf transpiration of plants from SD of the variety Baklouti has not changed due to pretreatment with the extract of *J. rubens*, and the values were similar to the control (0.28 mMol/m^2/s). For SND, the leaf transpiration was increased at all concentrations, reaching two maxima of 0.96 and 1.18 mMol/m^2/s, at 20 and 60 g/L, respectively. For the variety Chargui, the leaf transpiration has increased in the leaves of both types of seedlings, from 0.3 has to maxima of 1.1 and 1.4 mMol/m^2/s at concentrations, respectively, of 20 and 60 g/L for to those from the SD and a maximum of 1.54 mMol/m^2/s, to 80 g/L for those SND (*Fig. 8*).

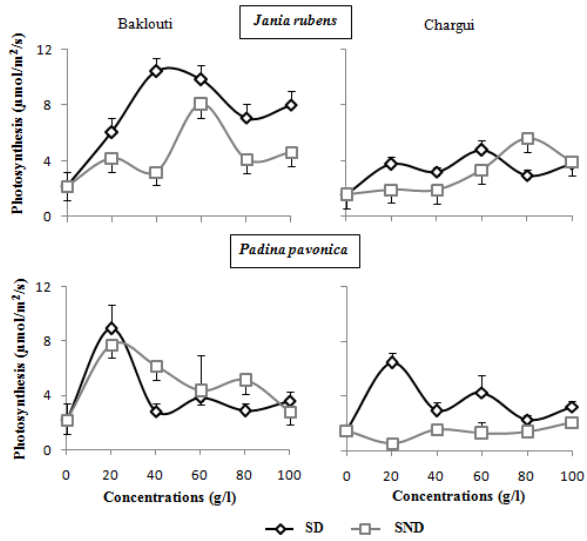

Figure 7 - Photosynthesis, expressed in µmol/m^2/s of seedlings of both pepper varieties (Baklouti and Chargui) developed from untreated seeds (control) treated and after pretreatment and dried (SD) and not dried (SND) of their seeds by aqueous extracts of *P. pavonica* and *J. rubens* at different concentrations.

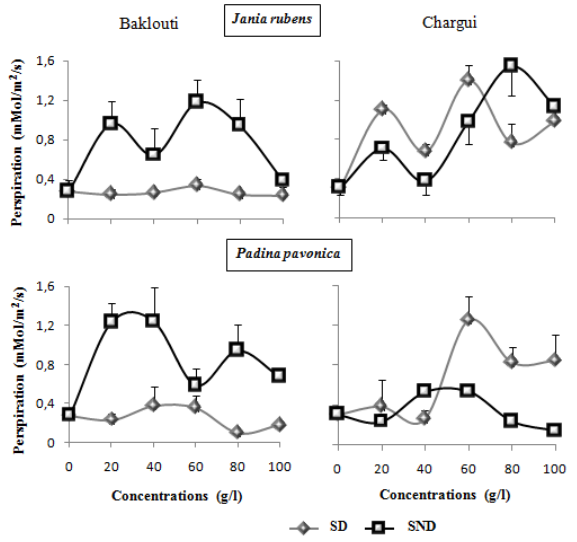

Figure 8 - Transpiration, expressed in mMol/m^2/s of seedlings of both pepper varieties (Baklouti and Chargui) developed from untreated (control), treated and dried (SD) and not dried (SND) seeds by aqueous extracts of *P. pavonica* and *J. rubens* at different concentrations.

The aqueous extract of *P. pavonica* at all concentrations has not affected significantly on the leaf transpiration of Baklouti variety, from SD. However, for those of the SND, sweating was multiplied by a factor of 4.5 times, at 20 and 40 g/L, and has been, almost, doubled or tripled in the other case (*Fig. 8*). In contrary, to the first variety, the transpiration of the variety Chargui was not affected for the SND plants, for concentrations less than 80 g/L and was reduced to 100 g/L. However, this extract has increased significantly her transpiration, in the case of plants grown from SD, inducing sweating of 1.25 mMol/m^2/s, to 60 g/L, while this value was 0.3 mMol/m^2/s in controls (*Fig. 8*).

Effect on stomatal conductance

The priming by the aqueous extract of *J. rubens* improved stomatal conductance of variety Baklouti indeed, the values of the control (10.23 mMol/ m^2/s) were multiplied by four and five times for plants grown, respectively, from SD and SND, treated at 100 and 60 g/L. Similarly, for the variety Chargui, which to accused stimulation greater, thus the respective growth factors were seven and 10 times, at 60 and 80 g/l (*Fig. 9*).

The extract of *P. pavonica* was favorable for this parameter, inducing an increase of two and six times for plants grown from SD and SND, respectively, and 20 g/L for Baklouti variety. Improving stomatal conductance of variety Chargui was noted at 60 and 100 g/L, respectively, for SD and SND (*Fig. 9*).

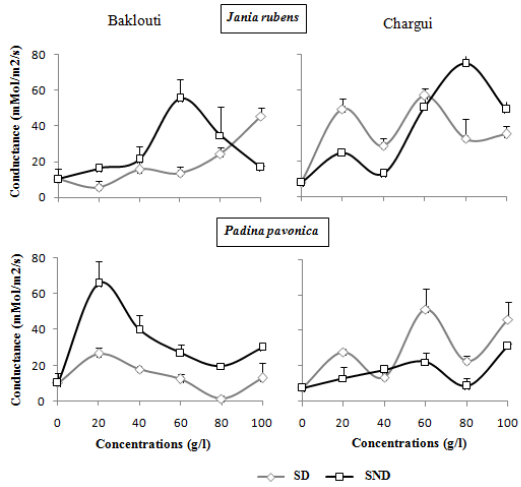

Figure 9 - Stomatal conductance expressed in mMol/m^2/s of seedlings of both pepper varieties (Baklouti and Chargui) developed from untreated (control) treated and dried (SD) and not dried (SND) seeds by aqueous extracts of *P. pavonica* and *J. rubens* at different concentrations.

DISCUSSION

The results achieved show that, overall, the priming has improved significantly the plants growth of both varieties; however, differences were recorded related to the pretreatment type (SD and SND), both pepper varieties, the original extracts and their concentrations.

The variety Baklouti presented the best results, considering the effect of priming by aqueous extracts of the algae on the growth of plants.

For Chargui variety, the best results were recorded at 80 g/L, for the two pretreatments, by the *J. rubens* extract. Following the pretreatment with *P. pavonica* extract, the growth of roots and aerial parts has been improved for the plantlets developed from SD. In previous studies, the different seed pretreatments, before sowing, were successfully integrated to improve plant vigor (Taylor *et al.,* 1988). Indeed, Farooq *et al.* (2008) found that pretreatment techniques led to improved rice seed vigor. The pretreated seeds had higher levels of vigor, which result in an earlier and uniform emergence, resulting in improved plant height and the rate of crop growth. In the present work, improved root length was obtained essentially for Baklouti variety, following pretreatment with both algae extracts. The improvement of the length of the root and shoot and the number of secondary roots can be explained by an increase in the rate of cell division in the root tips (Bose and Mishra, 1992). An improvement in the aerial part was observed at the two varieties. This appears to be the result of an improvement in photosynthesis. Indeed, the photosynthetic intensity was significantly improved in both cases of pretreatments (SD and SND). For variety Baklouti, it increased in leaves from plantlets SD 2.19 to 10.43 $\mu mol/m^2/s$. This is in line with the growth of shoots. The high values of the photosynthesis could be attributed to the increase of the water content in plants. Indeed, in this work, pretreatment with aqueous extracts of algae had a significant effect on the water content of the seedlings. According to Hopkins (2003), plants consist mainly of water, and lose a lot of water through evapotranspiration. Yamauchi and Winn (1996) suggest that the improvement in the growth speed may be the result of a start-up vigorous and energetic of pretreated plants, which leads to the improvement of the leaf area index. A positive correlation between seed vigor and field performance was also found.

Another manifestation of the effect of priming of seeds pepper by aqueous extracts of algae was observed level leaves. Indeed, the number of leaves of seedlings of both varieties, Baklouti and Chargui, was increased following both types of priming. Leaf area of variety Chargui has been improved, especially in seeds pretreated with the aqueous extract of *P. pavonica.* The improvement of the growth from of pretreated seeds could be due to better

efficiency of the plant in the production and distribution of photosynthesis (Farooq *et al.,* 2006).

Thus, the improvement of the growth was confirmed by a number of parameters. The number of leaves, contents of water and photosynthesis, improved compared to the control, in the majority of cases, and this improvement depends on the origin of the extract, its concentration, plant variety and type of pretreatment.

CONCLUSION

Seed priming of two pepper varieties, Baklouti and Chargui, by aqueous extracts of thalli of *J. rubens* and *P. pavonica* has improved significantly their growth. These improvements have varied with the origin of biological material, the concentration of the extract and pepper variety. The improvement concerned both the length of roots and aerial parts as the number of leaves per plant. This improvement would be related to a stimulation of photosynthesis, transpiration and stomatal conductance

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