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# EFFECT OF SEAWEED EXTRACT OF *SARGASSUM VULGARE* ON GERMINATION BEHAVIOR OF TWO TOMATOES CULTIVARS (*SOLANUM LYCOPERSICUM* L) UNDER SALT STRESS

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**Abstract:** Salt stress is a major adverse factor that can lower seed germination and seedlings growth, leading to reduced plant growth and ultimately lower crop productivity in arid and semi arid regions of the world. Tomato (*Solanum lycopersicum* L.) is an important crop occupying a large area in both Morocco and Tunisia, where salt stress is the most limiting factor. In the present investigation, an attempt has been made to study the effect of seaweed liquid extract (SWE) from *Sargassum vulgare* at different concentrations on seed germination and seedling growth of two tomatoes cultivars: cv Agatha and cv Nemadore under salt stress.

**Keywords:** Salt stress; Seaweed extract; Seed germination; Seedling growth; Tomato

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## INTRODUCTION

Salinity is one of the most important problems in irrigated soils of the arid and semi-arid areas in the world. Currently, there is about 275 million hectares of irrigated land of which about 20% is salt affected (Ghassemi et al., 1995). On the other hand, the ever growing world population causes great pressure on marginal lands to be brought into cultivation in the developing and under developing countries, which were previously not cropped due to their high degree of natural salinity (Flowers and Yeo, 1995). Salinity affects seed germination either by creating an osmotic stress thus preventing the seed from water uptake or through the toxic effects of sodium and chlorides ions on the germinating seed (Hosseini et al., 2003). Seeds and seedlings are particularly vulnerable to increasing salinity because at that stage plants have not yet developed the physiological mechanisms to tolerate rising salinity concentrations (Adam, 1990). This has led to concentrate research efforts on salt tolerance of plants, in order to improve crop yield (Zhu, 2001). Many research studies have shown the beneficial effect of seaweeds extracts in stimulating growth of plants (Blunden, 1991; Washington et al., 1999). They contain all major and minor plant nutrients including bio-control properties; they also contain organic compounds such as auxins, gibberellins and precursors of ethylene and betaine that impact plant growth (Wu et al., 1997). Beneficial effects from the use of seaweed extracts as natural regulators have induced increased crop yield and plant vigor to withstand adverse environmental effects (Featonby-Smith and Van Staden, 1983).

Tomato (*Solanum lycopersicum* L.) is a major legume in many countries. However, this crop is moderately tolerant to salinity like many other crops (Ashraf and Waheed, 1993) and is typically cultivated in regions that are exposed to soil salinization (Cuartero and Fernandez, 1999). Many crop plants including tomato are susceptible to cell damage from high salinity and

can survive only with decreased yields. That's why; this study was undertaken to test the effect of seaweed liquid fertilizer extract of *Sargassum vulgare* on germination behavior of two tomatoes cultivars under salt stress.

## EXPERIMENTAL

**Experimental material** Seeds of two tomatoes (*Solanum lycopersicum* L.) cultivars were included in this study. The first one is Agatha and the second is Nemadore.

**Seed material** Germination experiment was conducted in the laboratory of High Institute of Agriculture Chott Mariem, Tunisia to analyze the effect of sodium chloride salinity added with seaweed liquid extract or not on germination behavior and seedling growth of tomato. Seeds used in this study were *Solanum lycopersicum* L. This plant belongs to the family of *Solanaceae.* It is moderately sensitive to salt stress.

**Collection of seaweeds** Seaweeds *Sargassum vulgare* (*Phaeophyceae*) used in the present study were collected from the coastal area of Chott Mariem, Tunisia (35.8° N and 10.6° E). Morphologically distinct thallus of algae were placed in polythene bags and transported to the laboratory. Samples were washed thoroughly using tap water to remove the salt.

**Seaweeds treatment** Seaweeds were shade dried for four days, followed by oven dry for 12h at 60°C. Then the materials were hand crushed and made as coarse powder, was added with distilled water in a ratio of 1:20 (w/v) and boiled at 121°C for 30 minutes. The hot extracts were filtered through a double-layered cheese cloth and allowed to cool at room temperature (Rama Rao, 1990). The resulting supernatant was taken as 100% seaweed liquid extracts. Seaweed liquid extracts were prepared with different doses: control (0%), 0.2% and 0.5%.

**Seed Treatment** Ten Seeds of tomato were placed in 9 cm Petri dishes on a two layers of filter paper (Whatman # 41). Salt stress was induced by sodium chloride (NaCl). Three sets were treated with 0, 2 and 4 g/l of NaCl and were considered as control as they don't receive extract of *Sargassum vulgare.* Seeds under study were treated with salt at various concentrations (0, 2 and 4 g/l of NaCl) and each concentration was supplemented with seaweed extracts (SWE) of *Sargassum vulgare* separately at two different doses (S1: 0.2% and S2: 0.5%). All sets were labeled as control (non-treated seeds with seaweeds extract of *Sargassum vulgare*); S1 (Seeds supplemented with 0.2% of *Sargassum vulgare* extract); S2 (seeds supplemented with 0.5% of *Sargassum vulgare* extract). Seeds were placed on top of the filter paper wetted with 5 ml of each different concentrations of seaweed extracts in the Petri dishes and were kept under photoperiod for 14 days. The culture room temperature was maintained at 25°C. Seed germination was recorded daily up to day 14 after the start of the experiment. After fourteen days, seedlings were taken for the observations. Parameters measured in this experiment were:

Total seed germination rate (TG) measured in the sixth day using the formula TG (%) = (total number of germinated seeds/ total seed) x 100.

Mean germination time (MGT) calculated according the formula of Ellis and Roberts (Ellis and Roberts, 1981). MGT= Σ (ni/di). With ni: number of germinated seeds and di: day of counting.

Seedling fresh, dry weight and radicle length were measured. Seedlings kept at 60± 5°C for 48 hours and weighted for determination of their dry weights.

**Greenhouse growth bioassay** Tomato plants were grown under greenhouse under 16-h light regime at 25 °C and 8-h dark regime at 18 °C. Seeds were sown in pots at a depth of 0.5 cm below the soil level and were allowed to germinate. After germination in each pot, five healthy plants were retained and other plants were removed. The experiment was made in triplicates. Plants were grown into plastic pots containing sand and peat (50% - 50%). They were also irrigated separately with saline water which was added with sodium chloride (NaCl) at different concentrations (0, 2 and 4 g/l) every third day. Potted plants were grown for 7 weeks in a greenhouse at ~25±2 °C, in 85% relative humidity.

The experiment comprised of three treatments, (control, water spray), 0.2 and 0.5% (volume/volume; v/v) of seaweed extract in water. Sprays of *Sargassum vulgare*-derived extract were applied, two times a week, at the

seedling stage (30 days after sowing) and for 7 weeks. Morphological characteristics such as shoot length (SL), shoot fresh weight (SFW) and shoot dry weight (SDW) were measured.

**Statistical Analysis** Data were analyzed statistically for standard deviation using SPSS 13.0. All measurements were performed with triplicates and the mean values were presented.

## RESULTS AND DISCUSSION

**Total Germination** The percentage of germination was reduced in both tomato seeds genotypes as response to salinity stress. The decrease in the total germination (TG) was found to be more with the increasing concentration of NaCl. When the treated seeds were supplemented with SWE (0.2 and 0.5%) along NaCl salt, the germination percentage was increased (Table 1 and 2). Total germination from both treated and non-treated seeds with SWE of *Sargassum vulgare* decreased significantly with increasing NaCl salinity. However, this reduction in total germination was significantly higher for non-treated seeds, compared to treated ones. Data suggested a reduction of about 10% on total germination due to an increase in salinity from 0 to 4 g/l. Results indicated that application of seaweeds extract increase germination by 2% and 5% in both tomatoes cultivars using respectively SWE S1 (0.2%) and S2 (0.5%) when compared to control seeds (Tables 1 and 2).

In general, increasing salinity causes a decrease in tomato germination; this may be due to the toxic effects of $Na^+$ and $Cl^-$ in the process of germination (Khajeh-Hosseini et al. 2003). It seems also that salinity stress affects seed germination via the limitation of seed water absorption (Dodd and Donovan, 1999), excessive use of nutrient pool (Bouaziz and Hicks, 1990) and creation of disorders in protein synthesis. The effects of seaweed extracts on germination of various crops have been shown by many authors (Taylor et al. 1990; Moller and Smith, 1998). It was indicated that seaweeds extracts induce leakage of inhibitors possibly absisic acid from the seeds which improve germination percentage (Speer and Tupper, 1975). The involvement of growth regulating substances in seaweed extracts like ethylene, kinetin and gibberellic acid were effective on reversal of induced dormancy in seeds (Dunlap and Morgan, 1977). The ameliorating effect of SWE may be due to the growth hormones available which would have triggered *de novo* the synthesis of hydrolytic enzymes. This present study is in accordance with the earlier results of (Venkataraman, 1993; Johnsi Christobel, 2008). It may be due to the presence of growth-promoting substance in SWE (Mohan et al., 1994).

**Mean germination time (MGT)** Results showed that salinity significantly increase mean germination time (MGT) for both treated and non-treated tomato seeds with seaweed extract. However, treated seeds with *Sargassum vulagre* extract have lower MGT compared to control seeds. Data in Table 1 and 2 indicated that increasing salinity significantly delayed mean germination time of 1.218 days. However, application of SWE significantly shortened MGT when compared to control seeds. Data show also that rising concentration of NaCl delayed significantly MGT in both tomato genotypes; this delay was less pronounced in treated seeds with SWE. According to Reinhardt and Rost (1995), most plants are more sensitive to salinity during germination and seedling growth. This is in agreement with our study whereby a lower mean germination time was found with treatments of *Sargassum vulgare.* The probable reason for early emergence of the seaweed extract treated seeds maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the SWE treated seeds germinated soon after planting compared with untreated ones (Ozbingol et al., 1999). Furthermore, seaweed extracts contain various betaines and betaine-like compounds (Blunden et al., 1986; Ghoul et al., 1995). In plants, betaines serve as a compatible solute that alleviates osmotic stress induced by salinity stress. Those compatibles solutes have been shown to play a part in successful formation of somatic embryos from cotyledonary tissues and mature seeds of tea (Akula et al., 2000). All that information supports our results about seed germination and improved mean germination time with SWE.

**Seedling fresh and dry weight (sFW, sDW)** Increasing salinity decreased tomato seedlings fresh and dry weight for both treated and un-treated seeds. In fact, the increase in salt concentration in culture medium brought down

seedlings fresh weight from 1.284 g at 0 g/l to reach 0.873 g at 4 g/l (Table 1). However, seeds supplemented with SWE showed better performance than non-supplemented seeds. Data in Table 1 shows that seeds supplemented with 0.2 % and 0.5 % of SWE enhanced tomato seedling fresh (sFW) and dry weight (sDW) as compared to control. Reduction in seedlings growth (seedlings fresh and dry weights) was visible in salt stressed seeds with increasing concentration of NaCl. However, the application of SWE improved seedlings growth which was visible in seeds supplemented with SWE of *Sargassum vulgare*.

Reduction in seedling growth as a result of salt stress has been reported in several others species (Achakzai et al., 2010; Akram et al., 2010). Salinity has both osmotic and specific ionic effects on seedlings growth (Dioniso-Sese and Tobita, 2000). Similarly, toxic ion accumulation ($Na^+$ and $Cl^-$) negatively affect plant metabolism (Grieve and Fujiyama, 1987). It has also been reported that salinity suppresses the uptake of essential nutrients like P and K (Nasim et al. 2008), which could adversely affect seedlings growth. (Cicek and Cakirlar, 2002) have reported that salinity reduced fresh and dry weight of maize seedlings.

Seaweed include macro and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid that affect cellular metabolism in treated seeds, leading to enhanced seedlings growth (Crouch and Van Staden, 1993; Stirk et al., 2004). In addition, seaweeds contain precursors of elicitor compounds that promote germination (Stephenson, 1974).

Another possibility is the presence of polysaccharides in SWE, as sugars that are known to improve seedling growth in a similar way to hormones (Rolland et al., 2002). Zeatin is another candidate for induction of rooting in plants by seaweed (Finnie and Van Staden, 1985). Furthermore, seaweed extracts contain various betaines and betaine-like compounds (Blunden and Gorden, 1986; Ghoul et al., 1995). In plants, betaines serve as a compatible solute that alleviates osmotic stress induced by salinity stress.

**Radicle length (rL)** Salinity had an inhibitory effect on radicle length (rL) for both treated and un-treated seeds (Table 1 and 2). However, this effect was significantly less pronounced in seedlings from treated seed with *Saragasum vulgare* extract in comparison with control seeds. The application of SWE improved the growth of the radicle significantly. The increased in radicle length may be due to presence of some growth promoting substances such as IAA and IBA, Gibberellins, Cytokinins, micronutrients and amino acids (Challen and Hemingway, 1966).

**Tableau 1. Effect of seaweed extract of *Sargassum vulgare* on seedling growth of tomato (cv. Agatha) under salt stress**

| | | TG (%) | MGT (days) | sFW (g) | sDW (g) | rL (cm) |
|---|---|---|---|---|---|---|
| 0 g/l | Control | 98.32 ± 1.543 | 4.421 ± 0.154 | 1.284 ± 0.091 | 0.071 ± 0.048 | 6.314 ± 1.258 |
| | S1 | 99.45 ± 1.642 | 4.127 ± 0.103 | 1.489 ± 0.121 | 0.084 ± 0.088 | 6.675 ± 0.621 |
| | S2 | 100 ± 1.754 | 3.987 ± 0.238 | 1.664 ± 0.404 | 0.098 ± 0.129 | 6.967 ± 0.532 |
| 2 g/l | Control | 91.65 ± 1.394 | 4.859 ± 0.731 | 1.047 ± 0.088 | 0.064 ± 0.027 | 5.423 ± 0.533 |
| | S1 | 93.48 ± 1.219 | 4.625 ± 0.278 | 1.182 ± 0.376 | 0.071 ± 0.067 | 5.714 ± 0.532 |
| | S2 | 95.93 ± 1.295 | 4.377 ± 0.309 | 1.261 ± 0.526 | 0.084 ± 0.076 | 5.978 ± 0.221 |
| 4 g/l | Control | 83.28 ± 1.632 | 5.059 ± 0.481 | 0.873 ± 0.376 | 0.052 ± 0.142 | 4.434 ± 0.747 |
| | S1 | 85.39 ± 1.725 | 5.156 ± 0.521 | 0.959 ± 0.212 | 0.059 ± 0.057 | 4.716 ± 0.546 |
| | S2 | 87.43 ± 1.731 | 5.345 ± 0.635 | 0.982 ± 0.202 | 0.077 ± 0.048 | 4.837 ± 0.680 |

*Means with standards errors

**Tableau 2. Effect of seaweed extract of *Sargassum vulgare* on seedling growth of tomato (cv. Nemadore) under salt stress**

| | | TG (%) | MGT (days) | sFW (g) | sDW (g) | rL (cm) |
|---|---|---|---|---|---|---|
| 0 g/l | Control | 98.72 ± 1.592 | 4.623 ± 0.454 | 1.196 ± 0.091 | 0.068 ± 0.048 | 6.352 ± 1.328 |
| | S1 | 99.75 ± 1.821 | 4.429 ± 0.803 | 1.259 ± 0.121 | 0.072 ± 0.078 | 6.627 ± 0.651 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **S2** | 100 ± 1.729 | 4.167 ± 0.338 | 1.384 ± 0.404 | 0.081 ± 0.149 | 6.871 ± 0.432 |
| **2 g/l** | **Control** | 90.75 ± 1.642 | 4.879 ± 0.731 | 1.027 ± 0.088 | 0.064 ± 0.067 | 5.632 ± 0.633 |
| | **S1** | 92.48 ± 1.419 | 4.615 ± 0.378 | 1.291 ± 0.376 | 0.069 ± 0.057 | 5.852 ± 0.212 |
| | **S2** | 94.83 ± 1.729 | 4.347 ± 0.349 | 1.351 ± 0.526 | 0.077 ± 0.026 | 5.926 ± 0.211 |
| **4 g/l** | **Control** | 82.28 ± 1.754 | 5.349 ± 0.381 | 0.992 ± 0.376 | 0.061 ± 0.207 | 4.618 ± 0.847 |
| | **S1** | 84.69 ± 1.721 | 5.116 ± 0.421 | 1.039 ± 0.212 | 0.063 ± 0.037 | 4.732 ± 0.326 |
| | **S2** | 86.73 ± 1.394 | 5.035 ± 0.645 | 1.112 ± 0.202 | 0.077 ± 0.018 | 4.842 ± 0.320 |

*Means with standards errors

**Shoot length (SL)** Results presented in Table 3 and 4 indicated that great reduction of shoot growth occurred with NaCl treatments. Decrease in shoot length was more pronounced in all NaCl salt treatments in tomato. The shoot length showed that the maximum length was noticed in S2 concentration of SWE of *Sargassum vulgare* for all salt treatments, while the control plants showed the lowest plants shoot length (Table 3 and 4).

According to He and Cramer (1993), growth analysis is fundamental to the characterization of plant's response to an environmental stress. Bauci et al. (2003) and Khosravinejad et al. (2009) have noticed a significant decrease in shoot elongation in barley genotypes with increasing NaCl treatment. From our results, it is very clear that tomato shoot length was increased by increasing concentrations of SWE while it was adversely affected by higher doses of salinity (Table 3 and 4). All the results obtained were statistically significant. From the present results, it can be seen that the shoot length of the grass species was stimulated at lower levels of salinity and it appears that the grass species studied exhibit a moderate salinity tolerance as far as linear growth is concerned.

Our findings are in accordance with earlier studies carried out on soybean (Chaudhary et al. 2009) where there was an increase in vegetative growth by the application of seaweed extract. The enhancing of vegetative growth can be related to seaweed components such as macro- and microelement nutrients, amino acid, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances affecting cellular metabolism in plants leading to enhanced growth (Durand et al. 2003; Stirk, 2003).

**Shoot fresh and dry weights (SFW, SDW)** Results in Table 3 and 4 indicated that great reduction of shoot fresh and dry weights occurred with NaCl treatments. This decrease was more pronounced in all NaCl salt treatments in tomato. Maximum SFW and SDW were noticed in S2 concentration of SWE of *Sargassum vulgare* for all salt treatments, while the control plants showed the lowest ones (Table 3 and 4).

The increase in shoots characteristics might be due to the Auxins content in the seaweed extracts which have an effective role in cell division and enlargement. This leads to an increase in shoot growth and plant fresh and dry weights (Gollan and Wright, 2006). This positive effect might be due to the minerals Zn, Cu and B content in the seaweed extracts, which have a great role in cell division and enlargement (Lopez et al. 2008), or might also due to the macronutrient content in seaweed extracts, which have a great role in plant nutrition like nitrogen, potassium and phosphorous, very essential for the growth and development of the plant (Attememe, 2009).

**Tableau 3. Effect of seaweed extract of *Sargassum vulgare* on seedling growth of tomato (cv. Agatha) under salt stress**

| | | SL (cm) | SFW (g) | SDW (g) |
|---|---|---|---|---|
| **0 g/l** | **Control** | 48,34 ± 1,043 | 53.365 ± 1.691 | 13.048 ± 1.068 |
| | **S1** | 52,19 ± 1,045 | 55.187 ± 1.021 | 16.481 ± 1.088 |
| | **S2** | 57,87 ± 0,976 | 58.176 ± 1.504 | 18.072 ± 1.649 |
| **2 g/l** | **Control** | 46,86 ± 0,654 | 50.927 ± 1.098 | 11.964 ± 1.767 |
| | **S1** | 50,43 ± 1,321 | 52.871 ± 1.676 | 13.427 ± 1.857 |
| | **S2** | 55,65 ± 1,643 | 56.592 ± 1.526 | 15.129 ± 1.526 |
| **4 g/l** | **Control** | 43,87 ± 1,532 | 48.732 ± 1.776 | 9.241 ± 1.607 |
| | **S1** | 47,98 ± 0,986 | 49.792 ± 1.812 | 10.977 ± 1.637 |
| | **S2** | 53,76 ± | 54.789 ± | 12.763 ± |

| | | | | |
|---|---|---|---|---|
| | | 0,737 | 1.902 | 1.518 |

*Means with standards errors

**Tableau 4. Effect of seaweed extract of *Sargassum vulgare* on seedling growth of tomato (cv. Nemadore) under salt stress**

| | | SL (cm) | SFW (g) | SDW (g) |
|---|---|---|---|---|
| **0 g/l** | **Control** | 47,74 ± 1,343 | 52.735 ± 1.491 | 12.538 ± 1.248 |
| | **S1** | 51,39 ± 1,069 | 54.987 ± 1.331 | 15.381 ± 1.068 |
| | **S2** | 56,96 ± 0,686 | 55.456 ± 1.404 | 17.942 ± 1.429 |
| **2 g/l** | **Control** | 45,76 ± 0,954 | 49.727 ± 1.358 | 10.364 ± 1.587 |
| | **S1** | 49,48 ± 1,741 | 51.631 ± 1.536 | 12.447 ± 1.257 |
| | **S2** | 54,95 ± 1,843 | 55.652 ± 1.656 | 14.729 ± 1.466 |
| **4 g/l** | **Control** | 42,55 ± 1,652 | 47.922 ± 1.876 | 8.281 ± 1.957 |
| | **S1** | 46,76 ± 0,846 | 48.922 ± 1.742 | 9.967 ± 1.927 |
| | **S2** | 52,68 ± 0,747 | 53.059 ± 1.352 | 11.763 ± 1.948 |

*Means with standards errors

## CONCLUSION

It is clear from the current study that seaweeds extract of *Sargassum vulgare* have an ameliorating effect on tomato seeds under salt stress because of the presence of growth hormones, nutrients and other important physiochemical compounds. So, the supplementation of SWE could be used as a biological amendment in soil reclamation technique which can boost food production not only in cultivated lands but also in barren soils accumulated with salt. Further study will be needed to test the influence of SWE on later growth and yield of tomato cultivated in salt stress.

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