

Effect of Seaweed Liquid Extract of *Sargassum vulgare* on Growth of Durum Wheat Seedlings (*Triticum durum* 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. In order to improve crop tolerance to this abiotic stress, many research studies have the importance of seaweed extract (SWE) in alleviating stress damage to plants. Seaweed extracts are used as nutrient supplements or biofertilizers in agriculture to increase plant growth and yield. In this study, we examined the effect of liquid seaweed extracts made from *Sargassum vulgare* on the germination and growth of durum wheat (*Durum triticum* L) (cv Karim) under salt stress in laboratory and greenhouse conditions using foliar applications. We assessed SWE at different concentrations (0.2, 0.5, 25 and 50 %) on germination parameters (percentage, mean time) and growth parameters (radicle length, shoot length, shoot fresh and dry weights) of durum seedlings. Our results indicate that seeds treated with SWE of *Sargassum vulgare* at lower concentrations (0.2 %) showed enhanced germination (better response in germination rate associated with lower mean germination time and consequently greater seedling vigor and greater radicle length). Furthermore, *Sargassum vulgare* was found to be more successful and better candidates for developing effective biostimulants to improve the growth of wheat plants under salt stress. This study provides important information on the identification and utilization of Tunisian seaweed resources for agriculture and is the first study to report on the uses of these seaweeds as a source of liquid extracts as biostimulants in agriculture.

KEYWORDS: Seaweed extract, wheat, seed germination, seedling growth, salt stress, *Sargassum vulgare*.

1 INTRODUCTION

Salinity is a major limiting factor in agriculture production affecting different plant growth stages. Germination is one of the most critical periods in life cycle of crop [1]. Salt stress affects germination parameters (total germination, germination rate and seedling growth) in different ways depending on plant species [2]. It was reported that maximum germination percentage was reduced with a high NaCl concentrations [1]. Excess salinity with the plant root zone has a deleterious effect on plant growth [3]. To limit the negative effect of salinity on plant growth, many methods have been used. A lot of research studies have shown the importance of liquid extracts obtained from seaweeds as foliar sprays for several crops to improve growth under adverse factors [4], [5]. SWE contains growth promoting hormones (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, Ni), vitamins and amino acids [6].

Wheat is a major cereal crop in many parts of the world, especially in both Tunisia and Morocco, and is commonly known as king of cereals. Globally, wheat is the second most produced food among the cereal crops after maize and rice. It is a moderately salt-tolerant crop and its yield is substantially reduced as the soil salinity level rises to 100 mM NaCl [7]. It is

cultivated over a wide range of environments because of wide adaptation to diverse environmental conditions. As a result, it is a promising crop for cultivation in moderate salty soils of semi arid regions [8]. To produce satisfactorily wheat under saline conditions, seeds must germinate and seedlings must dynamically pass through the salty layer of the soil and survive [9]. Under such condition, vigorous seedling growth is very important for crop establishment. Rapid and uniform seed germination under saline condition not only increases early seedling but also has the advantage of higher salt tolerance [10].

Therefore the aim of the present study were to assess the impact of salt stress on germination and seedling growth parameters of wheat under laboratory and greenhouse conditions and to screen out the impact of seaweed extract of *Sargassum vulgare* application on wheat (cv Karim) tolerance to salt stress.

2 MATERIALS AND METHODS

2.1 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.

2.2 PREPARATION OF LIQUID SEaweED EXTRACT

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 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 [11]. The resulting supernatant was taken as 100% seaweed liquid extracts. Seaweed liquid extracts were prepared with different doses: control (0%), 0.2%, 0.5%, 25% and 50%.

2.3 SEEDS TREATMENT

Ten Seeds of wheat 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) separately at four different doses (S1: 0.2%, S2: 0.5%, S3: 25%, S4: 50%). All sets were labeled as control (non-treated seeds with seaweeds extract of *Sargassum vulgare*); S1 (Seeds supplemented with 0.2% of SWE); S2 (Seeds supplemented with 0.5% SWE); S3 (Seeds supplemented with 25% SWE); S4 (Seeds supplemented with 50% SWE).

Seeds were placed on top of the filter paper wetted with 5 ml of each different concentrations of SWE 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 7 after the start of the experiment. After fourteen days, seedlings were taken for the observations. Parameters measured in this experiment were:

Total seed germination (TG) measured in the sixth day using the formula $TG (\%) = (\text{total number of germinated seeds} / \text{total seeds}) \times 100$.

Mean germination time (MGT) calculated according the formula of Ellis and Roberts [12]. $MGT = \sum (ni/di)$. With ni: number of germinated seeds and di: day of counting.

2.4 GREENHOUSE GROWTH BIOASSAY

Wheat 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 $\sim 25 \pm 2$ °C, in 85 % relative humidity.

The experiment comprised of five treatments, (control, water spray), 0.2; 0.5; 25 and 50%; (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, root length, total height, fresh weight, and dry weight were measured.

3 RESULTS AND DISCUSSION

3.1 LABORATORY CONDITIONS

3.1.1 TOTAL GERMINATION

Germination percentage was reduced in wheat seeds (*Triticum durum* L) in response to increasing concentration of NaCl in Petri dishes. When the treated seeds were supplemented with SWE (0.2; 0.5; 25 and 50%) along NaCl salt, the germination percentage was increased (Table 1). Total germination from both treated and non-treated seeds with SWE of *Sargassum vulgare* decreased significantly with increasing salt stress. 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 NaCl concentration from 0 to 4 g/l. Results indicated that application of seaweeds extract significantly increase germination in wheat seeds using respectively SWE S1 (0.2%) and S2 (0.5%) when compared to control seeds. For instance, total germination (TG) was optimal in S1 (0.2%) concentration of SWE in all NaCl concentration. In other concentrations such as S3 (25%) and S4 (50%) total germination decreased in comparison to control seeds. This may be due to the toxic effect of mineral contained in seaweed extract.

In general, increasing salinity causes a decrease in wheat germination; this may be due to the toxic effects of Na⁺ and Cl⁻ in the process of germination [13]. It seems also that salinity stress affects seed germination via the limitation of seed water absorption [14], excessive use of nutrient pool [15] and creation of disorders in protein synthesis. Many authors have shown the positive effects of seaweed extracts on germination of various crops [16], [17]. This positive effect is due to many growth regulating substances contained in seaweed extracts like ethylene, kinetin and gibberellic acid which are involved on reversal of induced dormancy in seeds [18]. The ameliorating effect of SWE may also be due to the growth hormones available which would have triggered de novo the synthesis of hydrolytic enzymes [19]. Speer and Tupper [20] have indicated that seaweeds extracts induce leakage of inhibitors possibly abscisic acid from the seeds which improve germination percentage. This present study is in accordance with the earlier results of Mohan et al. [21] and Johnsi Christobel [22].

3.1.2 MEAN GERMINATION TIME

Results showed that salinity significantly increase mean germination time (MGT) for both treated and non-treated wheat seeds with seaweed extract. However, treated seeds with *Sargassum vulgare* extract have lower MGT compared to control seeds. Data in Table 1 indicated that increasing salinity significantly delayed mean germination time of 1.21 days. However, application of SWE significantly shortened MGT when compared to control seeds (Table 1). According to Reinhardt and Rost [23], most plants are more sensitive to salinity during germination and seedling growth. This is in agreement with our study. 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 [24]. Furthermore, seaweed extracts contain various betaines and betaine-like compounds [25]. 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 [26]. All that information supports our results about seed germination and improved mean germination time with SWE.

Tableau 1. Effect of *Sargassum vulgare* extract on germination behavior of wheat (cv Karim) under salt stress.

	0 g/l					2 g/l					4 g/l				
	Control	S1	S2	S3	S4	Control	S1	S2	S3	S4	Control	S1	S2	S3	S4
TG	99.8 ± 0.64	100 ± 0.84	98.6 ± 0.71	97.3 ± 0.69	96.4 ± 0.79	88.5 ± 0.77	91.7 ± 0.56	89.4 ± 0.42	86.7 ± 0.32	85.3 ± 0.61	63.4 ± 0.96	65.8 ± 0.66	64.3 ± 0.81	60.8 ± 0.73	58.1 ± 0.53
MGT	2.27 ± 0.53	1.86 ± 0.14	2.10 ± 0.11	2.68 ± 0.84	2.79 ± 0.75	3.16 ± 0.92	2.92 ± 0.61	3.07 ± 0.48	3.32 ± 0.95	3.58 ± 0.98	3.97 ± 0.82	3.76 ± 0.86	3.84 ± 0.46	4.13 ± 0.34	4.37 ± 0.62

3.2 GREENHOUSE CONDITIONS

3.2.1 RADICLE LENGTH

Radicle length in wheat seedling decreased significantly with increasing salinity for both treated and un-treated seeds (Table 2). However, this inhibitory effect was significantly less pronounced in seedlings from treated seed with *Sargassum vulgare* extract in comparison with control ones of wheat. The maximum length of radicle was observed in S1 seaweed treatment (0.2%) of *Sargassum vulgare* extract for all NaCl treatments. However, it was decreased in S3 and S4 seaweed concentrations treatments. Plants treated with S4 seaweed treatment showed very less value of radicle length in all salt treatments (Table 2). The application of SWE improved notably radicle growth. 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 [6].

3.2.2 SHOOT LENGTH

The results presented in Table 2 indicated that great reduction of shoot growth occurred with NaCl treatments. Decrease in length of shoot was more pronounced in all NaCl salt treatments in wheat. The shoot length showed that the maximum length was noticed in S1 concentration of SWE of *Sargassum vulgare* for all salt treatments, while the control plants showed the lowest plants shoot length. In other concentrations of SWE, better shoot length was noted in S2 and S3 treatments (Table 2).

According to He and Cramer [27], growth analysis is fundamental to the characterization of plant's response to an environmental stress. Bauci et al. [28] and Khosravinejad et al. [29] have noticed a significant decrease in shoot elongation in barley genotypes with increasing NaCl treatment. From our results, it is very clear that shoot length wheat was increased by 13.17% at 100 mM NaCl concentration while it was adversely affected by higher doses of salinity (Table 1). 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 [30] where there was an increase in vegetative growth by the application of seaweed extract.

The negative responses of increased concentration of seaweed extracts on vegetative growth plant can be attributed to presence of regulator hormones or high levels of minerals.

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 [31], [32].

3.2.3 SHOOT FRESH AND DRY WEIGHTS

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 [33]. 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 [34], 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 [35].

Tableau 2. Effect of *Sargassum vulgare* extract on seedling growth of wheat (cv Karim) under salt stress.

	0 g/l					2 g/l					4 g/l				
	Control	S1	S2	S3	S4	Control	S1	S2	S3	S4	Control	S1	S2	S3	S4
RL	7.76	8.23	7.84	6.52	5.87	7.05	7.38	7.12	5.93	5.12	6.12	7.04	6.32	5.32	4.61
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.23	0.63	1.03	1.14	0.84	0.97	0.72	0.51	0.89	0.91	1.01	1.21	0.53	1.03	1.11
SL	69.3	76.1	74.7	72.5	70.1	61.6	74.4	69.1	68.4	67.3	54.1	69.3	66.5	61.3	60.2
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.19	0.93	1.23	1.53	0.94	1.05	1.08	1.16	1.19	1.31	1.27	1.37	1.41	1.21	1.03
SFW	5.78	6.22	5.93	5.41	5.01	5.21	5.76	5.51	5.09	4.37	4.81	5.17	4.97	4.62	4.09
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.29	1.08	1.28	1.37	1.19	1.14	1.34	1.13	1.27	1.33	1.43	1.04	1.45	1.26	1.54
SDW	2.31	2.52	2.47	2.28	2.17	2.02	2.23	2.18	1.97	1.86	1.83	1.96	1.91	1.76	1.69
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.33	0.38	0.39	0.78	0.27	0.17	0.29	0.77	0.58	0.19	0.48	0.52	0.47	0.45	0.44

4 CONCLUSION

We have observed in the present study that S1 (0.2%) and S2 (0.5%) concentrations of *Sargassum vulgare* extract applied at wheat plants showed higher germination and vegetative growth. The extract of this specie showed better result when they were applied at lower concentrations than more concentrated extracts (25%) and 50%). This shows that only a small amount of seaweed extract can be used to enhance plant growth under salt stress conditions.

The same result showed that SWE treatment reduced the water deficit effect which protected the plant against peroxidation imposed by salt stress. Consequently, the present findings encourage the application of such seaweeds as natural fertilizer in agricultural sector. As perspective, we opt to carry out more research including isolation, characterization and identifications of growth hormones and antioxidant substances in seaweeds extract.

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