

## Effect of Seawater Irrigation on Germination Seed of *Ceratonia siliqua* L. and Their Treatment with Seaweed Extracts

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### ABSTRACT

The Libyan coasts provide a diverse environment with many seaweeds that are unstudied. The present study was conducted with the aim of evaluating the effect of irrigation with seawater at several concentrations (0, 10, 20, 30 and 40%) on the germination of seeds of *Ceratonia siliqua* L. (wild and cultivated), and treatment by pre-soaking for 24 h at a concentration 10% of three species of seaweed extracts (*Padina pavonica*, *Sargassum vulgare*, and *Posidonia oceanica*). The results showed that *C. siliqua* seeds were able to germinate under until a concentration of 30% by a germination percentage (18%). Generally, salinity stresses significantly decreased germination percentage, delayed its mean germination time, and reduced radical lengths. The statistical results suggest that there were highly significant differences in increasing the germination percentage, shortened mean germination time, and increased radical length and the seeds treated with seaweed extract could tolerate 40% salinity. *P. pavonica* was observed as more efficient in reducing the adverse effects of seawater salinity. The results showed no significant differences between wild and planted *C. siliqua* seeds for all studied traits. The study concluded that seaweeds could be used as a promising bio-stimulant in agricultural development fields.

**Keywords:** Germination; Seawater Irrigation; Seaweed extracts; *Ceratonia siliqua* L.

### تأثير الري بمياه البحر على إنبات بذور *Ceratonia siliqua* L. ومعالجتها بمستخلصات الأعشاب البحرية

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**المخلص:** تعد السواحل الليبية بيئة متنوعة تضم العديد من الأعشاب البحرية التي لم يتم استغلالها بشكل كاف في مجالات التنمية الزراعية. أجري الدراسة الحالية بهدف تقييم تأثير الري بمياه البحر بتركيزات (0، 10، 20، 30، 40%) على إنبات بذور الخروب (*Ceratonia siliqua* L البرية والمستزرعة)، ومعالجتها بالنقع المسبق لمدة 24 ساعة بتركيز 10% لثلاثة أنواع من مستخلصات الأعشاب البحرية (*Padina pavonica*, *Sargassum vulgare*, *Posidonia oceanica*). بينت النتائج أن بذور أشجار الخروب كانت قادرة على الإنبات تحت مستويات الري بمياه البحر حتى تركيز 30% بنسبة إنبات (18%). كما أدى الإجهاد الملحي إلى انخفاض معنوي في نسبة الإنبات، وتأخير متوسط زمن الإنبات، واختزال أطوال الجذور. أشارت النتائج إلى وجود فروق ذات دلالة إحصائية في زيادة نسبة الإنبات، وتقليل متوسط زمن الإنبات، وزيادة طول الجذور للبذور المعالجة بمستخلصات الطحالب البحرية تحت تراكيز الملوحة، مما حسن من أداء البذور لتصبح قادرة على تحمل الملوحة حتى تركيز 40%. كما لوحظ أن مستخلصات *P. pavonica* هي الأكثر كفاءة في الحد من التأثير السلبي للملوحة. كما أظهرت النتائج عدم وجود فروق معنوية بين بذور الخروب البرية والمستزرعة في جميع الصفات المدروسة. وخلصت الدراسة إلى إمكانية استخدام الأعشاب البحرية كمحفز حيوي واعد في مجالات التنمية الزراعية.

**الكلمات المفتاحية:** الإنبات، الري بمياه البحر، مستخلصات الأعشاب البحرية والخروب.



## 1. Introduction

The Carob tree, (*Ceratonia siliqua* L.) belongs to Caesalpinioideae subfamily of Fabaceae family [1,2], and is distributed widely in the Mediterranean region, especially in the El-Jabal El-Akhdar region of Libya [3], adapted to mild and dry areas with poor soils, environmentally it is used to prevent soil erosion and land degradation [4]. It is also used in many industries such as food, pharmaceuticals, and cosmetics [5]. The sea level rise along the Libyan coasts is considered a major environmental factor that affects soil characteristics and vegetation [6], due to saltwater intrusion in the water aquifers leading to coastal soil salinization [7]. Soil salinity reduces crop growth and productivity, and is therefore one of the main barriers to increasing global food production [8]. It causes a range of physiological and biochemical changes in plants, where excess  $\text{Na}^+$  ions cause endotoxicity, membrane dysfunction, ionic and nutrient imbalances, which hinders the germination of seeds and the formation of seedlings [9]. A study conducted in Algeria revealed that *C.siliqua* seeds were able to germinate in seawater up to a concentration of not more than 30% over a 15 day period [10]. Also [11] noted that leaf area and root traits have a progressive decrease with increasing salinity of *C.siliqua*. Seaweed biofertilizers can be used to improve the physiological and morphological performance of different plant species under abiotic stresses [12], due nutrients, hormones, vitamins, and secondary metabolites, inhibiting the accumulation of reactive (ROS), promoting plant growth, facilitating water retention, and soil aeration [13,14]. For example, seaweed treatments (*Sargassum vulgare*, *Colpomenia sinuosa*, and *Pandia pavonica*) mitigated the salt stress of *Pisum sativum* L. seedling [15]. Moreover, [16] demonstrated the possibility of using seaweed extracts of *Cystoseira barbata*, *Cystoseira granulata*, to improve germination rates of *Hordeum vulgare* L seeds.

The present work aimed to evaluate the effect of irrigation with seawater at a several concentrations (10, 20, 30 and 40%) on the germination of wild and cultivated seeds of *C.siliqua* and the effects of using three species of seaweed extract (*Padina pavonica*, *Sargassum vulgare*, and *Posidonia oceanica*).

## 2. Materials and methods

### 2.1 Seed collection and preparation

The laboratory study was conducted in Department of Biology, Faculty of Education, Omar Al-Mukhtar University Al-Bayda, Libya. The pods of wild and cultivated *Ceratonia siliqua* were collected in 2022 from mature growing trees in (Al-Hinya, alwasita) region in AlJabal Al-Akhdar, and were cleaned of impurities. Viability was tested by soaking in distilled water to eliminate empty seeds floating on the surface, then soaking the seeds in 1 % sodium hypochlorite solution for 8 minutes, and washing with distilled water. The seeds were mechanically scarified with nail clippers, to overcome seed coat dormancy which does prevent the imbibition of water and the exchange of gases [17].

### 2.2 Collection and preparation of seaweed samples

Fresh seaweed of three brown algae species from the Phaeophyceae (*Padina pavonica*, *Sargassum vulgare*, and *Posidonia oceanica*) were collected from the coastline of Al-Hamamah, north of Al-Bayda City / Al-Jabal Al-Akhdar / Libya, during summer season 2022. They were identified in the laboratory of the College of Natural Resources and Environmental Sciences, Tobruk University, by DR. Abdulraziq. A. Abdulraziq. They were washed and rinsed with distilled water in order to eliminate sand and plankton. After that, they were dried at room temperature, ground by an electric grinder and stored until use.

### 2.3 Seaweed extract preparation

The aqueous extract of seaweed was prepared by adding 10 g of dry powder to 100 ml distilled water, with continuous shaking for 24h, then the extracts were filtered through Whatman No.1 filter paper and kept at 4 °C until utilized. The obtained extract concentration was considered as (10%).

Treatment used:

The experiment seeds (wild and cultivated), were divided into culture four groups:

- Seeds were pre-soaked in distilled water for 24 hours (control).
- Seeds irrigated with seawater at a concentration (10, 20, 30 and 40%).
- Seeds pre-soaked in seaweed extracts for concentrations of 10% for 24 hours (control).
- Seeds pre-soaked in seaweed extracts for concentrations of 10% for 24 hours under concentrations of seawater (10, 20, 30 and 40%). A 10% seawater concentration was prepared by adding 10 ml of seawater to 9.0 ml of distilled water (v:v). All concentrations were prepared similarly.

### 2.4 Seed germination

Normally, ten seeds per Petri dish were placed on two Whatmans No.1 filter papers, and incubated at room temperature. Each treatment was repeated three times. Dishes were subjected to daily observation for 12 days, and follow-up of germination and water as needed [18]. The papers were changed once every 2 days to prevent salt accumulation. Germination was calculated by recording the number of germinated seeds in all treatments starting from second day when the first germination occurred. Germination criterion is the appearance of radicle outside seed cover. The papers were altered once after every 2 days to prevent salt accumulation [19]. At end of the experiment, following final results were obtained: Germination percentage (PG %) = number of germinated seeds / total number of seeds  $\times$  100 [20] (eq 1).

Mean germination time (MGT) = the total number of germinated seeds per day / total number of germinated seeds at end of the experiment [21] (eq 2).

Radical lengths (cm): The root lengths were taken using a graduated ruler, the averages were calculated by taking 5 seedlings from each plate.

### 3. Statistical Analysis

The study experiences were designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey's test at  $P < 0.05$  [22].

### 4. Results and Discussion

#### 4.1 Germination percentage

Table (1) depicts the effect of irrigation with sea water of concentrations (10, 20, 30 and 40%), on the germination percentage of wild and cultivated *C.siliqua* seeds and the effect of pre-soaking the seeds in seaweed extracts (*Padina pavonica*, *Sargassum vulgare*, and *Posidonia oceanica*) at a concentration of 10% for 24 hours. The results show showed that after 10 days of irrigation with seawater, a concentration of 10% has no effect on germination percentage for all seeds tested compared with control, Our results agree with [10], who indicated that seeds of *C.siliqua* can germinate well at low concentrations in seawater, while the germination percentage was reduced under conditions of seawater at a concentration of 20%. The results also showed highly significant differences as the concentration was increased to 30% seawater, which reduced germination to 18%, while a concentration of 40% caused complete inhibition of germination. These results are in agreement with those reported by [23,24], which showed a negative effect of salinity on plant growth of several plant species. Salt-induced inhibition of seed germination could be attributed to inducing ionic and osmotic stress (causing water deficit), by impairing various crucial metabolic functions, which directly impact plant growth and development [25]. Pre-soaking the seeds of *C.siliqua* in seaweed extracts, had

no significant effect compared with the controls and seawater concentration of 10%. However, the germination percentage under 20% seawater irrigation was increased by seaweed extract for untreated seeds from an average 88% to 100%. At 30%, pre-soaking the seeds with seaweed extract increased germination from 18% to 50% for *P.pavonica*, 48% for *S.vulgare*, and 28% for *P.oceanica*. The three seaweed extracts boosted the germination percentage of 40% seawater, which did not show any germination of untreated seeds *P.pavonica* showed the strongest effect. This result agrees with [26], who confirmed improved seedlings growth for two pepper varieties after treating with *P.pavonica* extract. The next most effective was *S.vulgare*. *S.vulgare* was reported to be effective in reducing the harmful effects of salinity on bean seed [27]. *P.oceanica* showed germination percentages (17%) in the 40% seawater. Our results are consistent with researchers [28], who confirmed that *P.oceanic* contains sufficient levels of carbon, nitrogen and phosphorus to be used as an agricultural fertilizer.

#### 4.2 Mean germination time

The results showed from Table (2) that seawater exposure delayed mean germination time for untreated *C.siliqua* seeds compared to control seeds. Seawater at 10, 20, and 30% of caused a delay in a mean germination time from 3.73 days for control to between 5.01-7.55 days. This result agrees with [29], who found that the mean germination time of *Bauhinia variegata* seeds was delayed under seawater irrigation. Our results also showed that pre-soaking *C.siliqua* seeds in the seaweed extracts resulted in a shortened mean germination time, compared with freshwater irrigation (control). Data demonstrated that there were highly significant differences in the germination time compared with seawater concentrations, where *P.pavonica* treatment produced the shortest germination time. This result is similar to what was confirmed by [30].

**Table 1.** Effect of seawater irrigation and treated with seaweed extract on germination percentage of *C. siliqua* seeds.

Treatment	<i>C. siliqua</i>	Seawater Concentration %					average
		Control	10%	20%	30%	40%	
untreated seeds	wild	100 a	100 a	90 ab	20 ef	0 d	62.0 c
	cultivated	100 a	100 a	86 b	16 f	0 d	60.4 c
	<b>average</b>	<b>100</b>	<b>100</b>	<b>88</b>	<b>18</b>	<b>0</b>	<b>61.2</b>
Soakin in <i>P.pavonica</i>	wild	100 a	100 a	100 a	50 a	30 a	76.0 a
	cultivated	100 a	100 a	100 a	50 a	33 a	76.6 a
	<b>average</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>50</b>	<b>31.5</b>	<b>76.3</b>
Soakin in <i>S. vulgare</i>	wild	100 a	100 a	100 a	40 b	20 b	72.0 ab
	cultivated	100 a	100 a	100 a	36 bc	21 b	71.4 ab
	<b>average</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>38</b>	<b>20.5</b>	<b>71.7</b>
Soakin in <i>P.oceanica</i>	wild	100 a	100 a	100 a	30 cd	16 c	69.2 b
	cultivated	100 a	100 a	100 a	26 de	18 bc	68.8 b
	<b>average</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>28</b>	<b>17</b>	<b>69.0</b>

**Table 2.** Effect of seawater irrigation and treated with seaweed extract on mean germination time of *C. siliqua* seeds (days).

Treatment	<i>C. siliqua</i>	Seawater Concentration %					average
		Control	10%	20%	30%	40%	
Untreated seeds	wild	3.70 b	4.93 b	5.60 a	7.50 a	0.00 d	4.34 f
	cultivated	3.76 a	5.10 a	5.43 b	7.60 a	0.00 d	4.34 f
	<b>average</b>	<b>3.73</b>	<b>5.01</b>	<b>5.51</b>	<b>7.55</b>	<b>0.00</b>	<b>4.34</b>
Soakin in <i>P. pavonica</i>	wild	2.66 g	3.30 h	4.00 f	5.44 b	7.43 c	4.56 e
	cultivated	2.68 g	3.43 g	4.00 f	5.53 b	7.60 b	4.64 d
	<b>average</b>	<b>2.67</b>	<b>3.36</b>	<b>4.00</b>	<b>5.48</b>	<b>7.51</b>	<b>4.60</b>
Soakin in <i>S. vulgare</i>	wild	2.93 d	3.70 d	4.45 d	5.53 b	7.88 a	4.89 b
	cultivated	3.09 c	3.90 c	4.70 c	5.45 b	7.92 a	5.01 a
	<b>average</b>	<b>3.01</b>	<b>3.80</b>	<b>4.57</b>	<b>5.49</b>	<b>7.90</b>	<b>4.95</b>
Soakin in <i>P. oceanica</i>	wild	2.80 f	3.63 e	4.20 e	5.44 b	7.60 b	4.73 c
	cultivated	2.87 e	3.50 f	4.00 f	5.50 b	7.83 a	4.74 c
	<b>average</b>	<b>2.83</b>	<b>3.56</b>	<b>4.10</b>	<b>5.47</b>	<b>7.71</b>	<b>4.73</b>

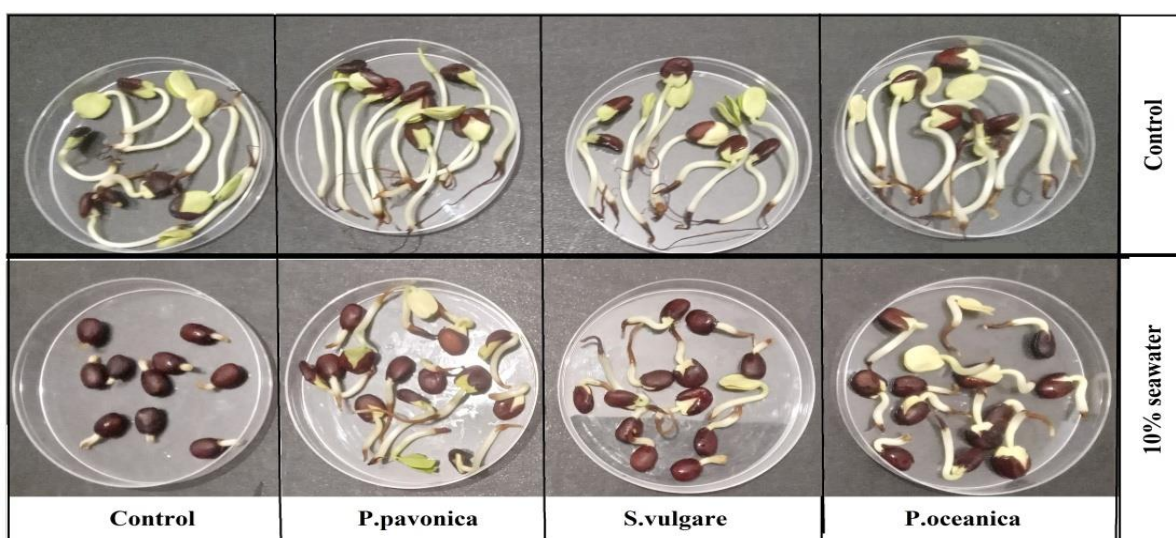
### 4.3 Radical length (cm)

The data recorded from Table 3 and Figure 1, show that the radical length was significantly affected by seawater levels compared to controls. Seawater irrigation at 10, 20, and 30% significantly decreased radical length by 2.4, 1.4, and 1.0cm respectively, compared to 5.4cm with fresh water. The three

seaweed extracts boosted radical length to 10.3, 6.5 and 7.7cm for *P.pavonica*, *S.vulgare* and *P.oceanica* respectively compared with fresh water irrigation(control). The increased radical lengths with seaweed extracts treatments may be due to the presence of auxins, gibberellins, cytokinins and amino acids [31].

**Table 3.** Effect of seawater irrigation and treated with seaweed extract on radical length of *C. siliqua* seeds.

Treatment	<i>C. siliqua</i>	Seawater Concentration %					average
		Control	10%	20%	30%	40%	
Untreated seeds	wild	5.3 d	2.7 bc	1.5 ab	1.0 b	0.0 b	2.1 c
	cultivated	5.5 d	2.2 c	1.3 b	1.0 b	0.0 b	2.0 c
	<b>average</b>	<b>5.4</b>	<b>2.4</b>	<b>1.4</b>	<b>1.0</b>	<b>0.0</b>	<b>2.0</b>
Soakin in <i>P. pavonica</i>	wild	10.5 a	4.5 a	3.0 a	2.5 a	1.8 a	4.4 a
	cultivated	10.2 a	4.3 a	3.0 a	2.3 ab	1.6 a	4.3 a
	<b>average</b>	<b>10.3</b>	<b>4.4</b>	<b>3.0</b>	<b>2.4</b>	<b>1.7</b>	<b>4.3</b>
Soakin in <i>S. vulgare</i>	wild	7.0 bc	3.4 b	2.0 ab	1.8 ab	1.0 ab	3.0 bc
	cultivated	6.0 cd	3.0 b	2.0 ab	1.5 ab	1.0 ab	2.7 bc
	<b>average</b>	<b>6.5</b>	<b>3.2</b>	<b>2.0</b>	<b>1.6</b>	<b>1.0</b>	<b>2.8</b>
Soakin in <i>P. oceanica</i>	wild	8.0 b	4.0 a	2.7 ab	2.3 ab	1.0 ab	3.6 ab
	cultivated	7.5 b	4.2 a	2.5 ab	2.0 ab	1.0 ab	3.4 ab
	<b>average</b>	<b>7.7</b>	<b>4.1</b>	<b>2.6</b>	<b>2.1</b>	<b>1.0</b>	<b>3.5</b>



**Figure 1.** Effect of seawater irrigation 10% and seaweed extract on germination rates of *C. siliqua* seeds.

#### 4. Conclusion

Seed germination is a process involving several phases, beginning with the imbibition of water and ending with the emergence of the radical, these stages in the plant life cycle are most vulnerable to salinity stressors, this was evident in the intolerance of *Ceratonia siliqua* L. seeds to seawater salinity above 30% concentration. The results showed no significant differences between wild and planted *C. siliqua* seeds for all studied traits under levels of salinity. This study showed that seed soaking in extracts of seaweeds was effective to mitigate stress due to diluted seawater irrigation, to improve seed germination to can be able to tolerance 40% salinity.

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#### Conflict of interest

The authors declare no conflict of interest.

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