MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Effect of Marine Algae Extracts on Seed Germination of some Plants

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Received:26/6/2020
Accepted:24/8/2020

Abstract: This study focuses on evaluating the impact of marine algae extracts prepared from three Egyptian seaweeds on seed germination of *Vigna sinensis*, *Pisum sativum* and *Zea mays*. Seeds of the three plants were primed with seaweed liquid extracts (SLEs) of *Ulva fasciata* (ULE), *Cystoseira compressa* (CLE) and *Laurancia obtusa* (LLE) in 2.0% extract strength. LLE induced the maximum positive magnitude of response in the following five germination parameters: germination percentage (GP), relative seed germination (RSG), germination index (GI), seedling vigor index (SVI) and relative root elongation (RRE). LLE produced the maximum increases in all seedling morphological parameters with percent of promoting response 87.3% for hypocotyl growth of *V. sinensis*, 80% epicotyl growth for *P. sativum* and 86.96% coleoptile elongation of *Z. mays*. Concerning germination metabolism, the maximum amylase activity was recorded to LLE priming (80.36, 59.11 and 61.44 mg soluble sugars min^{-1} g^{-1} FW) for germinating seeds of *V. sinensis*, *P. sativum* and *Z. mays*, respectively. The same pattern of response was documented with protease activity giving 89.59, 90.42 and 98.84 µg amino acids min^{-1}g^{-1} FW for germinating seeds of *V. sinensis*, *P. sativum* and *Z. mays*, respectively. This study demonstrates the effectiveness of seaweed liquid extracts (ULE, CLE and LLE) in functioning as plant biostimulant enhancing seed germination of *V. sinensis*, *P. sativum* and *Z. mays*.

keywords: Seaweed Liquid Extract (SLE), germination indices, growth promotion, amylase, protease

1.Introduction

Conventional agricultural activities are mainly based on the usage of different chemical fertilizers for supplying crops with mineral nutrients. Meanwhile, prolonged administration of this type of fertilization have negative impact on soil fertility and micro biota leading to reduction in fertilizer efficiency and accumulation of its residues in soil [1, 2]. Modern agricultural practices tend to look for alternative biotechnology for reducing usage of chemical fertilizers without influencing crop yield and income with economic feasibility [3, 4]. So, according to the increasing demand for organic food as well as growing environmental awareness, biostimulants market is rabidly upward all over the world [5] Seaweed liquid extracts represent key category of biostimulants which used for promoting plant growth and absorption of nutrients uptake. Usage of natural seaweed extracts for fertilizing crops enable the partial replacement of chemical synthetic fertilizers. Marine algae are an ecofriendly natural resource commonly used as an organic fertilizer since these polysaccharide-enriched seaweed extracts represent significant group of biostimulants that improve seed germination, plant growth and development [6]. Many previous studies have been documented for the improvement of plant seed germination after treatment with seaweed extracts [7, 8]. The positive functioning roles of SLEs may be ascribed to the plant growth regulators (auxins, cytokinin, gebrilines) that may be found as well as some polyamines and brasinosteroides. In addition to some components which enhance growth activity as polyphenols and polysaccharides (fucoidan, alginate isolated from phaeoyphyta; laminaran and carrageeenans isolate from rhodophyta; ulvans from green algae) or their derived oligosaccharides that contribute to plant growth enhancing functions [3, 4, 9]. Different ways of seaweed liquid extracts supplementation demonstrate many positive responses including germination promotion, root developing system, pigmentation, photosynthetic area, fruit quality as well as resistance to pathogens [10-14].

Many studies indicated the improvement of seed germination percentage following the foliar treatment with crud macroalgae polysaccharides extracts and their fractions as oligosaccharides as well as enhancement of root growth and harvest crop quality in addition to induction of stress resistance in different crops [15, 16]. This promotion effect may be interpreted on basis of increasing in overall photosynthesis, carbon and nitrogen assimilatory pathways, activation of basal metabolism enzymes [5, 17].

This study aimed to elucidate the enhancing potentiality of Ulva fasciata, Cystoseira compressa and Laurancia obtuse liquid extracts on seed germination of Vigna sinensis, Pisum sativum and Zea mays

2. Materials and methods

seedling development was assessed as demonstrated in Seedling Evaluation Handbook [19]. Throughout the early stage of germination (4 days), radical length as well as hypocotyl in *V. sinensis* and epicotyl in *p. sativum* and coleoptile in *Z. mays* seedlings were measured, in addition to calculation of the following germination parameters. Germination percentage (GP) = (number of germinated seeds/total number of seeds) ×100. Germination index (GI) was computed as defined by the Association of Official Seed Analysts [20], using the equation: $GI=\Sigma$ (Gt/Tt), where Gt is the number of seeds germinated on day t and Tt is the number of days. Relative seed germination (RSG) = (number of seeds germinated in test solution / number of seeds germinated in control) × 100, as described by Teaca and Bodirlau [21]. After 7 days of germination, seedling vigor index (SVI) = (seedling length (cm) × germination percentage) was computed according to Orchard [22] as well as Relative root elongation (RRE) = (mean root length in test solution / mean root length in control) × 100 [21].

Assay of enzymatic activities in germinating seeds

Germination metabolism was investigated through estimating of both amylase and protease activities after 4 days of germination. Amylase activity is estimated according to Dubois, Gilles [23] whereas, Protease activity is assayed according to Salmia, NYMAN [24] and Haroun and Hussein [25].

3. Results and Discussion

Bioassays for seed germination under the influence of different SLEs

Seed coat rapture and emergence of radical was initiated after 24 hours in all treatments. The different priming extracts induced significant stimulation response on all estimated germination indices (GP, GI, RSG, SVI and RRE) for the three studied crop plants (table1). Seeds of both *V. sinensis* and *P. sativum* primed with either of ULE, CLE or LLE responded positively for germination percentage with magnitude of response 7.59%, 11.33% and 13.25% for germinating seeds of *V. sinensis* and 7.61%, 11.53% and 15.34% for germinating seeds of *P. sativum* relative to control. The same pattern of response was documented for germinating seeds of *Z. mays* giving the following increases 9.76%, 11.76% and 15.64% compared with control, whereas, the maximum frequency of GP was recorded to LLE priming. LLE priming induced the highest magnitude of positive responses in the following germination indices GI, RSG, SIV and RRE in *V. sinensis*, *P. sativum* and *Z. mays* as following GI 13.33, 12.83, 12.75, RSG 113.52, 115.97, 116.07, SIV 1880.0, 1040.0, 1625.2 and RRE 146.28, 174.81, 126.39, respectively. *Vigna sinensis* seed germination characterized by rabid growth of hypocotyl designating epigeal typeof

germination which induced positively to priming with the different SLEs used recording the maximum percent of response (87.14%) to LLE. Priming of *P. sativum* seeds with different SLEs resulted in marked increases in epicotyl growth giving the highest percent of increase (80%) with LLE as well as in *Z. mays* in which the magnitude of response in coleoptile elongation was 85.71%. Maximum radicle length was induced by LLE priming giving the following promotion percent 68.77%, 43.93% and 28.55% for *V. sinensis*, *P. sativum* and *Z. mays*, respectively.

Regarding the onset of germination, the reserves in cotyledons (polysaccharides and proteins) are degraded under the effect of amylases and proteases giving up respiratory substrates. Generally, amylase and protease activities demonstrate significant positive responses under the different SLEs treatments relative to control. The maximum amylase activity was recorded to LLE priming (80.36, 59.11 and 61.44 mg soluble sugars min^{-1} g^{-1} FW) for *V. sinensis*, *P. sativum* and *Z. mays*, respectively. Similar positive response was achieved with protease activity giving 89.59, 90.42 and 98.84 µg amino acids min^{-1}g^{-1} FW for *V. sinensis*, *P. sativum* and *Z. mays*, respectively.

Table (1) Seed germination parameters of *Vigna sinensis*, *Pisum sativum* and *Zea mays* seeds: Germination percentage (GP), Germination index (GI), Relative seed germination (RSG), seedling vigor index (SVI) and Relative root elongation (RRE) as affected by different SLEs treatments.

	SLEs treatments	GP%	GI	RSG	RRE	SVI
V. sinensis	Control	88.3 ± 3.33^{b}	9.58 ± 0.38^{a}	-	-	1159.67 ± 28.08^{a}
	ULE	95.0 ± 2.89^{ab}	12.42 ± 1.46a	107.64 ± 2.07	121.81	1488.33 ± 32.14^{b}
	CLE	98.3 ± 1.67^{a}	13.08 ± 1.63^{a}	111.56 ± 3.58	132.98	1677.65 ± 39.86^{c}
	LLE	100.0 ± 0.00^{a}	13.33 ± 1.67^{a}	113.52 ± 4.13	146.28	1880.00 ± 49.33^{d}
P. sativum	Control	86.7 ± 4.41^{a}	9.17 ± 0.25^{b}	-	-	517.31 ± 24.07^{c}
	ULE	93.3 ± 4.41^{a}	11.50 ± 1.08^{b}	108.01 ± 3.82	127.41	715.30 ± 49.76^{b}
	CLE	96.7 ± 1.67^{a}	12.00 ± 1.17^{b}	112.13 ± 3.49	144.44	831.62 ± 39.08^{b}
	LLE	100.0 ± 0.00 ^{a}	12.83 ± 1.42^{b}	115.97 ± 2.18	174.81	1040.00 ± 35.12^{a}
Z. mays	Control	85.0 ± 2.89^{b}	8.92 ± 0.21^{c}	-	-	1039.83 ± 29.58^{c}
	ULE	93.3 ± 1.67^{ab}	11.50 ± 1.08^{c}	110.17 ± 5.47	111.46	1296.87 ± 37.71^{b}
	CLE	95.0 ± 2.89^{ab}	11.92 ± 1.21^{c}	111.91 ± 3.73	118.75	1428.17 ±38.91^{ab}
	LLE	98.3 ± 1.67^{a}	12.75 ± 1.46^{c}	116.07 ± 5.67	126.39	1625.23 ±38.63^{a}

- Treatment: Ulva liquid extract (ULE), Cystoseira liquid extract (CLE) and Laurencia liquid extract (LLE)

Table (2) Effect of seed priming with different seaweed liquid extracts (SLEs) on hypocotyl length in *V. sinensis*, epicotyl length in *p. sativum*, coleoptile length in *Z. mays* and radical length

	V. sinensis		P. sativum		Z. mays	
	Hypocotyllength	Radicallengh	Epicotyl length	Radical length	Coleoptile length	Radical length
Control	2.10 ± 0.208	3.97 ± 1.027	0.50 ± 0.058	2.80 ± 0.200	0.77 ± 0.088	6.90 ± 0.115
ULE	2.90 ± 0.321	4.60 ± 0.702	0.70 ± 0.115	3.30 ± 0.208	1.03 ± 0.240	7.63 ± 0.589
CLE	3.37 ± 0.578	5.20 ± 0.115	0.83 ± 0.088	3.57 ± 0.328	1.10 ± 0.208	8.20 ± 0.529
LLE	3.93 ± 0.504	6.70 ± 0.929	0.90 ± 0.115	4.03 ± 0.296	1.43 ± 0.318	8.87 ± 1.132
LSD 0.05	1.271	2.703	0.303	1.025	0.751	2.527

- Ulva liquid extract (ULE), Cystoseira liquid extract (CLE) and Laurencia liquid extract (LLE)

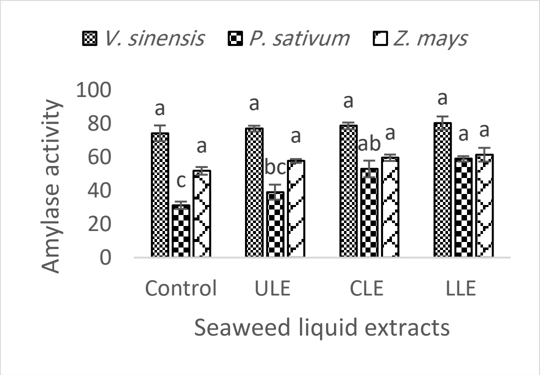

Fig. (1) Amylase activity (mg soluble sugars min^{-1} g^{-1} fresh weight) of *Vigna sinensis, Pisum sativum* and *Zea mays* seedlings affected by priming with different seaweed liquid extracts (SLEs)

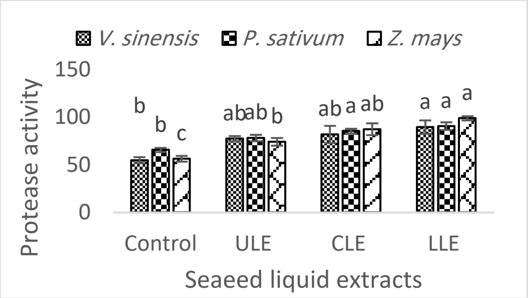

Fig. (2) Protease activity (µg amino acids. min^{-1}. g^{-1}. fresh weight) of *Vigna sinensis, Pisum sativum* and *Zea mays* seedlings by priming with different seaweed liquid extracts (SLEs)

Encouragement of seed germination in addition to seedling growth in many crop plants as a result of priming with different SLEs has been documented in several studies. Divya, Roja [26] reported that introducing of *Ulva lactuca* as a biofertilizer commonly increasing plant crop productivity as a result of high contents of nitrogenous fractions, nutrients as well as phytohormones.

The present results are in coincidence with the findings of Castellanos-Barriga, Santacruz-Ruvalcaba [8] who documented high level of both germination percentage (97 to 100%) and germination index (25 to 27) as well as improved seed vigor index (1543 to 1800) after the treatment of acidic *Ulva lactuca* extract, in addition to significant stimulated responses in both plumule and radical length in *mung bean* plants. *Codium tomentosum* supplementation, exhibited significant enhancement in GP of pepper seeds (68%) [7] and the same response in barley under the effect of *Ascophyllum nodosum* and *Laminaria hyperborean* extracts [27]. *Chaetomorpha antennina* extract enhanced the early emergence of radical in tomato seeds as germination percentage, mean germination time, germination energy and seed vigor index which quantified to three times better than that of control [28].

The promotion of SVI was documented by Patel, Pandya [29] who treated the seeds of brinjal, tomato and chilli with *Ulva lactuca*, *Ulva reticulata*, *padina pavonica* and *Sargassum johnstonii* extracts. Moreover the induced increments in seed germination and seedling vigor by priming with SLEs were documented in many studies as Mohan, Venkataraman Kumar [30] on *Cajanus cajan*, Rajkumar and Subramanian [31] on *Zea mays*, Venkataraman and Mohan [32] on *Vigna radiate* and Sivasankari, Venkatesalu [33] on *Vigna sinensis* whereas, their findings were interpreted on basis of the presence of plant growth regulating componants (auxins, gibberellins and phenyl acetic acid) and the physiologic stimulatory effect of micro-nutrients content of SLEs as well [34].

In cereals, growth and development of plants subsequent germination mostly depending on the mobilization of nutrients previously accumulated in endosperms which establish nourishment to meristem tissues and support embryo growth [35, 36].

Nitrogen and carbohydrate status (storage reserves) are the major players in mediating growth of seedlings, mobilization and photosynthetic gene expression as explained by Martin, Oswald [37]. Starch mobilization including degradation of polymeric polysaccharides into soluble sugars by hydrolases. Alpha amylase is an essential enzyme, that with α-glucosidase and starch phosphorylase are the initiator enzymes for hydrolysis of starch in the germinating seeds [38, 39], where α-glucosidase affects the α-(1-4) ponds in random fashion ending with formation of various low molecular weight products. The resulted sugars provided the necessary energy for cellular metabolism and adjusted osmosis during growing embryo [40, 41]. Previous studies indicated that alpha-amylase mediates the process of starch hydrolysis generating energy in order to provide a sustainable resource of carbon skeletons in anabolic pathways for biosynthesis of the different building blocks for embryos growth and development [4, 42, 43]. Ashraf, Azmi [44] demonstrated that life cycle of plants are regulated by enzymes as proteases which take a significant role during seed germination in metabolizing proteins into small peptides and free amino acids that used synthesizing proteins and enzymes essential for seedling growth. Seaweeds provide polysaccharide-enriched extracts may be functioned successfully as biostimulants for plant growth as suggested by Mzibra, Aasfar [5]. The present results may attribute the enhancement of seed germination, amylase and protease activities and the metabolic changes during germination to SLEs composition that may take a principal role in stimulating of protein biosynthesis, promotion of enzyme activity and/or membrane permeability [25]

Conclusion

The current study demonstrates the effectiveness of seaweed liquid extracts of *Ulva fasciata*, *Cystoseira compressa* and *Laurancia obtuse* in functioning as plant biostimulant enhancing seed germination of *V. sinensis*, *P. sativum* and *Z. mays*. These

SLEs are potential candidates for preparing efficient natural plant biostimulants in order to limit chemical fertilizers utilization for organic agriculture.

4. References

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