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EFFECT OF SOLID MATRIX PRIMING WITH SEAWEED EXTRACT ON GERMINATION AND SEEDLING PERFORMANCE OF ONION SEEDS UNDER ABIOTIC STRESS CONDITIONS

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Abstract

This work was carried out to test the effect of seaweed treatment on germination and seedling emergence in two onion seed lots. Distilled water containing 5% of seaweed was applied through solid matrix priming at a ratio of 2:1:3 Seed: vermiculite: seaweed extract (w: w: w) over two days at 15º C in the dark. Seeds of seaweed, distilled water treated and dry control were tested at 10 and 15 % PEG (drought), 100 and 150 mM NaCl (salt), and 30 and 35ºC (temperature) stresses, respectively. Seedling emergence, seedling height, fresh weight and dry weight were tested at 30ºC, 10% PEG and 100 mM NaCl. Seaweed treatment enhanced germination and seedling emergence compared not only to the dry control, but also to the distilled water treatment. The difference in germination was up to 18% compared with the dry control and 6% compared with the distilled water treatment. Moreover, seedling height was increased by the seaweed treatment. Results showed that seaweed extract priming may have a potential to enhance the resistance of onion seeds under abiotic stress conditions.

Keywords: Drought, Temperature, Salt stress, Solid matrix priming

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1. Introduction

Modern horticulture is looking for new technologies that would allow for a reduction in the use of chemical inputs without affecting crop yield or farmers' income. Inorganic fertilizers are being replaced by organic forms. The use of natural seaweed as a fertilizer has allowed for the partial replacement of conventional synthetic fertilizer (Zodape et al., 2010). Many commercial seaweed extract products are available for use in horticulture and can be used as liquid extracts applied as foliar spray, soil drench, or in granular/powder form as soil conditioners and manure (Thirumaran et al., 2009). These extracts are marketed as liquid biostimulants because a chemical analysis of seaweeds and their extracts have revealed the presence of a wide variety of plant growth-promoting substances such as auxins, cytokinins and betaines (Khan et al., 2009). These substances can influence shoot and root system development (Stirk et al., 2004). Seaweed extracts also contain macronutrients and micronutrients which can promote the growth of various horticultural crops (Moller and Mith, 1998; Demirkaya 2010; Sivritepe et al., 2015). Other researchers have also reported many beneficial effects of seaweed extracts such as improved germination, root development, leaf quality, plant vigor and resistance to biotic and abiotic stresses (Khan et al., 2009).

The most critical stage in the life of crops is seed germination, which is most influenced by environmental factors. Drought and salinity are key environmental factors that affect seed germination (Llanes et al., 2015). Many horticultural crops such as onions and carrots are seriously affected by abiotic stress. The problem is more serious at the germination and seedling development stages. Furthermore, the problem of abiotic stress on seedling emergence is more pronounced in low quality seeds (Jisha et al., 2012). The seeds of horticultural crops such as onions have low storage potential and lose their viability due to ageing. Onion (Allium cepa L.) seeds are naturally less viable and more hygroscopic than seeds of most other common vegetables (Thirusendura and Saraswathy, 2017). Seed viability and vigor decrease with ageing and improper storage conditions, leading to increased lipid peroxidation and decreased activities of several free radical and peroxide scavenging enzymes (Kibinza et al., 2011). Therefore, there must be some mechanism of seed quality enhancement, especially for low quality seeds which have less potential to perform under stress conditions.

Seed priming is a seed quality enhancement mechanism which can improve the germination and seedling performance of crops under both normal and stress conditions (Jisha et al., 2012). Seed priming has been successful in improving the seed vigor of many vegetable crops, leading to rapid and uniform germination and seedling emergence (Saranya et al., 2017; Wu et al., 2019; Sharma et al., 2016). Among the different priming techniques, solid matrix priming is important in repairing ageing and improving seed quality (Ermiş et al., 2016). The use of seaweed extracts in minimizing the problem of abiotic stress is well understood and recognized (Sharma et al., 2014). Hence, the aim of the present study was to investigate the effect of solid matrix priming with seaweed extracts on the performance of onion seeds and seedlings under different abiotic stress conditions.

2. Material and Method

2.1. Material

Two seed lots (high and low quality) of onions (Allium cepa L. cv. Metan) were used in the present study. The germination percentage of these high (Lot 1) and low-quality (Lot 2) seeds was 97.5 and 88.5% respectively. The high-quality seed had a seed moisture content of 11.9%, whereas the seed moisture content of the low-quality seed was 9.6%.

2.2. Preparation of Seaweed Liquid Extract (SW) and Priming

Green seaweed (Ulva lactuca), which is also known as sea lettuce, was collected from Marmara Sea in the north west of Turkey. The seaweed extract was prepared using the physical integration method. The particle size was gradually reduced and diluted with water in a ratio of 1:3. No heat, acid or alkaline hydrolysis was used for extraction (Demir et al., 2006). The diluted seaweed was filtered through muslin cloth, and the filtrate was considered as 100% seaweed extract (Demir et al., 2006). The seaweed extract was stored at 5°C for further applications. Balakrishnan et al. (2007) reported that the best seedling performance was observed at 5% concentration. Therefore, 5% concentrations of seaweed extract were used in the present study.

Solid matrix priming was performed at 15°C for two days, at a ratio of 2:1:3 (Seed: vermiculite: seaweed extract (w: w: w) SW). Distilled water (DW) was used instead of seaweed as the same rate. DW and dry seeds (NP) were considered as controls. After treatment, seeds were dried on the laboratory bench until the initial seed moisture content was reached.

2.3. Germination under Abiotic Stresses

Drought stress conditions were simulated using Polyethylene Glycol-6000 (PEG) at different concentrations of 10 and 15%. The osmotic potentials for the 10 and 15% concentrations were 0.30 and 0.51MPa respectively. The primed and control onion seeds (four replicates of 50 seeds) were placed on a filter paper in 9cm Petri dishes containing 3 cm3 of the different concentrations (10 and 15% PEG). The Petri dishes were sealed with stretch film to prevent evaporation and kept according to a completely randomized design in a growth chamber. Germination was carried out at 20°C for 12 days according to the principle of ISTA (2009). Seeds were considered germinated when the radicle emerged by at least 2 mm, and this was expressed as a percentage. In the salt stress experiment, NaCl at 50 and 100mM was used to simulate salinity stress. The germination procedure was followed as described. Control and treated onion seeds were allowed to germinate at 30 and 35°C for temperature stress.

2.4. Seedling Emergence Tests under Stress

The seedling emergence test was carried out in three replicates of 25 seeds each, under drought, salinity and temperature stress conditions in plastic germination trays (32 x 16 x 6 cm, length x width x depth). Non-primed, distilled water and seaweed treated seeds of two lots were sown at a depth of 2 cm in peat moss and watered with 10% PEG, 100mM NaCl and distilled water and kept at 20±2°C with 80% RH and a light intensity of 72 μmol m-2s-1 for 16 hours a day, for drought and salinity experiments, whereas for temperature stress experiment the trays were kept at 30°C. The appearance of the cotyledon leaves above the peat moss was considered as the emergence criterion. Trays were watered with the appropriate solution during the emergence period.

Twenty-one days after sowing, five seedlings in each replicate were destructively taken, and shoot length (mm/plant), shoot fresh weight (mg/plant) and dry weight (mg/plant) and root fresh weight (mg/plant) were calculated. Dry weights were calculated after keeping seedlings at 80°C for 24 hours. The seaweed extract (5%) was analyzed by spectrophotometry (plasma, optima 2100DV, Perkin-Elmer, Shelton CT) according to Mertens (2005). Vigor indices were recorded according to Yan (2015) formula as SVI = GP x Seedling dry weight.

2.5. Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using SPSS v. 20, and Duncan's Multiple Range Test was applied to compare the differences (5%) among treatment means.

3. Results

Under high temperature stress (35 °C), priming with seaweed extract improved the germination performance by 20% and 31% as compared with the dry control (NP) for first and second onion seed lots respectively. SW also significantly ($P<0.05$) improved germination under salinity and moisture stress compared to dry control seeds (Table 1). In all cases SW was significantly effective on germination under stress conditions than dry control. When SW was compared to DW treatment the differences (between 1 and 5 %) in favour of SW in all cases but were not significantly higher ($P<0.05$).

Table 1. The effect of seaweed extract (SW) and distilled water (DW) treatment on germination of onion seeds under abiotic stress (temperature, salt and drought stress) conditions. Dry seeds (NP) was considered as control

Seed lot	Treatment	30°C	35°C	50mM NaCl	100mM NaCl	10% PEG	15% PEG
Lot 1	SW	95^{a}	83^{a}	94^{a}	93^{a}	97^{a}	94^{a}
	DW	93^{ab}	78^{a}	94^{a}	92^{a}	94^{ab}	93^{a}
	NP	91^{b}	63^{b}	91^{a}	83^{b}	92^{b}	70^{b}
Lot 2	SW	93^{a}	81^{a}	91^{a}	90^{a}	90^{a}	84^{a}
	DW	91^{a}	79^{ab}	90^{a}	88^{a}	88^{a}	81^{a}
	NP	85^{b}	50^{b}	85^{b}	81^{b}	80^{b}	64^{b}

Means with different letters in the same criterion are significantly different, P < 0.05.
Lot 1= high quality seeds, Lot 2 = low quality seeds

The performance of onion seedling criteria treated with seaweed extract under temperature stress (30 °C) were better ($P<0.05$) than the dry control (NP) except one case in lot 1. It improved seedling emergence percentages by 8% for lot 1 and by 10% for lot 2. The highest seedling height, seedling fresh weight, seedling dry weight and vigor index were recorded from the SW treatment in both lots (Table 2). For example, the vigor index was improved from 231 to 306 for lot 1 and from 190 to 262 for lot 2 (Table 2).

Table 2. The effect of seaweed extract (SW) and distilled water (DW) treatment on emergence and seedling quality of onion seeds under temperature stress (30ºC). Dry seeds (NP) was considered as control.

Seed lot	Treatment	Emergence (%)	SH (cm)	SFW (mg/plant)	SDW (mg/plant)	Vigor Index
Lot 1	SW	84^{a}	12.1^{a}	71.6^{a}	3.7^{a}	306^{a}
	DW	78^{a}	11.8^{ab}	68.3^{a}	3.4^{a}	282^{ab}
	NP	76^{a}	10.8^{b}	57.3^{b}	3.0^{b}	231^{b}
Lot 2	SW	78^{a}	11.6^{a}	62.5^{a}	3.2^{a}	262^{a}
	DW	72^{a}	11.3^{a}	60.8^{a}	3.0^{ab}	214^{a}
	NP	68^{b}	7.2^{b}	49.2^{b}	2.5^{b}	190^{b}

Means with different letters in the same criterion are significantly different, P < 0.05.
Lot 1= high quality seeds, Lot 2 = low quality seeds, SH= seedling height; SFW= seedling fresh weight; SDW= seedling dry weight

In a similar way, priming of onion seed lots with seaweed extract greatly improved seedling characteristics under salinity stress. Seedling emergence was improved from 62 to 74% in lot 1, from 48 to 62 % in lot 2. Differences between SW and NP were reported as 2 and 5 % which are not significantly different (P>0.05). Seedling criteria were higher in SW treated seeds than those of DW in both lots but non-significant (P>0.05). Vigor index was found the highest in SW treated ones but not significantly different from those of DW and NP in lot 1. It was significantly different from NP but not DW in lot 2 (Table 3).

Priming of onion seed lots with seaweed extract also stimulated their growth and performance under moisture/drought stress conditions. Seedling emergence percentages were improved by 18% and 11% for lot 1 and 2 respectively as compared to the dry control (NP). Likewise, SH was improved by 1.4cm and 0.8cm for lot 1 and 2 respectively. Seedling vigor index and the fresh and dry weight of seedlings also significantly (P<0.05) improved as compared to the dry control (Table 4). The seaweed extract contained high Ca and S, and a reasonable amount of the other macro and micronutrients (Table 5).

Table 3. The effect of seaweed extract (SW) and distilled water (DW) treatment on seedling quality of onion lots under salinity stress (100mM). Dry seeds (NP) was considered as control

Seed lot	Treatment	Emergence (%)	SH (cm)	SFW (mg/plant)	SDW (mg/plant)	Vigor Index
Lot 1	SW	74^{a}	9.1^{a}	48.2^{a}	2.7^{a}	196^{a}
	DW	72^{a}	8.8^{a}	45.9^{a}	2.5^{a}	181^{a}
	NP	62^{b}	7.9^{b}	40.1^{b}	2.2^{a}	153^{a}
Lot 2	SW	62^{a}	8.9^{a}	44.8^{a}	2.4^{a}	145^{a}
	DW	57^{a}	8.6^{ab}	42.8^{a}	2.3^{a}	135^{a}
	NP	48^{b}	7.8^{b}	35.4^{b}	2.0^{b}	97^{b}

Means with different letters in the same criterion are significantly different, P < 0.05.
Lot 1= high quality seeds, Lot 2 = low quality seeds, SH= seedling height; SFW= seedling fresh weight; SDW= seedling dry weight

Table 4. The effect of seaweed extract (SW) and distilled water (DW) treatment on seedling quality of onion seed lots under drought stress (10% PEG). Dry seeds (NP) was considered as control

Seed lot	Treatment	Emergence (%)	SH (cm)	SFW (mg/plant)	SDW (mg/plant)	Vigor Index
Lot 1	SW	74^{a}	9.8^{a}	55.3^{a}	3.3^{a}	245^{a}
	DW	70^{b}	9.3^{a}	46.0^{b}	2.8^{b}	187^{ab}
	NP	56^{ab}	8.4^{b}	42.6^{b}	2.6^{b}	152^{b}
Lot 2	SW	65^{a}	9.5^{a}	46.6^{a}	2.6^{a}	173^{a}
	DW	60^{a}	8.9^{b}	42.3^{ab}	2.4^{ab}	143^{b}
	NP	54^{b}	8.7^{b}	39.8^{b}	2.2^{b}	138^{c}

Means with different letters in the same criterion are significantly different, P < 0.05.
Lot 1= high quality seeds, Lot 2 = low quality seeds, SH= seedling height; SFW= seedling fresh weight; SDW= seedling dry weight

Table 5. Mineral composition (mg/kg) of the seaweed (5%) extract used in the study

Macronutrients					Micronutrients				
P	K	Ca	Mg	S	Fe	Zn	Cu	Mn	B
0.265	5.373	12.34	4.22	10.19	0.254	0.146	0.117	0.295	0.068

4. Discussion

Results of the present study showed that seaweed extract enhanced seed germination and seedling quality of two onion seed lots under salt, drought and temperature stresses. The difference was significant not only between seaweed extract treated and dry seeds but also with distilled water treated ones in many cases.

Drought, salinity and temperature extremes are major agricultural constraints that prevent crops from performing to their full potential (Llanes et al., 2015; Yan 2015). These abiotic factors affect crops most seriously at the germination and early growth stages. Onion is a vegetable crop which can be affected by extreme abiotic factors (Thirusendura and Saraswathy 2017). In order to minimize the serious problems exerted on crops at an early stage of growth, a number of mechanisms have been developed. One of the techniques used is priming. Solid matrix priming of onion seeds with seaweed extract in our study showed the best result on onion seed germination, seedling emergence and seedling quality. Many researchers have also demonstrated enhanced seed germination and seedling characteristics with the application of seaweed treatments (Khan et al., 2009; Craigie 2011; Mattner et al., 2013).

The stimulated effect of priming with seaweed (SW) might be attributed to the presence of enzymes, phytohormones, minerals and low molecular weight compounds present in extracts (Stirk and Van Staden 1997). Some reports indicate that polysaccharides and polyphenols may also be effective as biostimulants, and this may enhance resistance to stress conditions (Zhang et al., 2006; Gonzalez et al., 2013). Cytokinins and cytokinin-like compounds are the most widely reported in seaweed extracts, followed by auxins and auxin-like compounds, and it has been speculated these may be responsible for the plant growth enhancing effects of the extracts (Stirk et al., 2004; Khan et al., 2009). Our analyses of seaweed showed that seaweed extract contains high levels of Ca, Mg, S, Zn and Cu, at 12.3, 4.22, 10.19, 10.146 and 0.116 mg/kg respectively. The high content of sulfur in SW might contribute to the production of enzymes, because S is the precursor of methionine and other amino acids that may initiate faster germination and emergence (Jahan et al., 2015). Again, the positive effect of seaweed might have come directly from mineral nutrients. S, Mg and Ca were observed in relatively higher amounts in seaweed extract than other nutrients. Sulfur (S), along with calcium and magnesium, is one of the three secondary nutrients required by plants for normal, healthy growth. Sulfur-containing compounds play a critical role in the response of plants to abiotic stress factors including drought and salinity (Cao et al., 2014; Jahan et al., 2015). Other minerals such as K, P, Fe, Mn and Cu were also found in seaweed extract (Table 5). Even though it is lack of sodium content, it has an extensive content of solution. Thus, the beneficial effect of seaweed might have also come from these minerals.

In general, commercial seaweed extracts are made from brown seaweeds such as Ascopyllum nodosum and Fucus, Laminaria, Sargassum and Turbinaria spp. (Sharma et al., 2014). In this work, we used green seaweed (Ulva lactuca) which was collected from Sea of Marmara during summer season. Seaweed extracts are complex structures with a mixture of components that may vary according to the seaweed source, collection site, season of collection, and extraction procedure. Sharma et al. (2014) reported that there were significant differences in the concentrations of the minerals Fe, I, K, Mg and S among five seaweed species collected from the same region. The same authors found that not only the mineral content but also the IAA content varied between 3 and 47 ng/g among the seaweed species. This can be expected from samples collected at different times of the year, but interestingly, variations in mineral contents and compositional structure were also observed in commercial formulations produced from A. nodosum (Shekhar et al., 2012). This conclusion shows that variation in composition is inevitable.

Seaweed extract enhanced the formation, length and volume of roots in crop seeds (Kenanoğlu, 2016). Such enhancement in the root system of the plant may be a key point in the resistance to abiotic stresses (Sharma et al., 2014) because a developed root structure may initiate a higher rate of water uptake and tolerance to stressful conditions.

5. Conclusion

In conclusion, priming of seeds with seaweed extracts has a superior potential to get better germination, emergence and seedling performance in onions. Moreover, the more influential effect of seaweed extract on the seed quality of the lots in relation to distilled water (DW) treatment in abiotic stress environment shows that it has a potential to enhance germination at sub-optimum sowing conditions (i.e early spring).

Conflict of interest

The authors declare that there is no conflict of interest.

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