

Biostimulant priming for germination and seedling quality of carrot seeds under drought, salt and high temperature stress conditions

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Abstract

Abiotic stresses are serious problems that hinder crop production. Seed germination and seedling development are stages which are sensitive to abiotic stress. Seed priming improves the performance of seeds/seedlings and provides faster and synchronized emergence under stress conditions. The present study aimed to investigate the effect of priming with biostimulants, vermicompost (5%), karrikinolide (10^{-7} M) and seaweed (5%) using the solid matrix method (5 days, dark, 15 °C, 2:1:3, seed:vermiculite:organic solution, w:w:w) on germination and seedling quality of carrot seeds under abiotic stress conditions. Biostimulants were used alone and in double and triple combinations. Drought stress was simulated by PEG-6000 (-0.3 MPa), salinity by using NaCl at 100mM, and high temperature by 30 °C. Dry control and distilled water treated were used as controls. Priming treatment with biostimulants improved performance of seeds and seedlings, though not always significantly ($p=0.05$). Seaweed alone and its combination with karrikinolide showed the best performance for all the parameters. The germination percentage for dry control of carrot seeds were 37, 63 and 72% in salt, drought and high temperature stresses while distilled water treated seeds had values of 74, 79 and 77%, respectively. Seeds treated with seaweed+ karrikinolide and seaweed alone had 80 and 89% germination. The same treatments stimulated seedling emergence from 57% to 84-88%, 25 to 69-76%, 71 to 85-87% under drought, salt and high temperature stress, respectively. Seedling criteria, seedling height, fresh weight, dry weight and root fresh weight were also higher with these treatments in all stress conditions. Catalase activity of treated seeds was higher for seaweed (0.400 EUg⁻¹seed) and seaweed karrikinolide (0.411 EUg⁻¹seed) treated seeds than for both controls (non-primed: 0.299, distilled water: 0.239 EUg⁻¹seed). Biostimulants have potential as seed priming agents to enhance seed quality in carrots.

Keywords: Abiotic stresses, Karrikinolide, Seaweed, Vermicompost, Catalase activity

Introduction

Crops encounter environmental stresses which are both abiotic and biotic. Various analyses have suggested that abiotic stresses, mainly drought, salinity and extreme temperatures, are the major factors that obstruct crops from realizing their full yield potential (Wang et al., 2003; Fetri et al., 2014). According

to Wang et al. (2003), drought and salinity are becoming the prevalent problems in many regions, and will account for serious salinization problems in more than 50% of all arable lands by the year 2050. Abiotic stress affects all stages of crop growth and development. However, seed germination, early growth of seedlings and flowering stages are the most sensitive

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stages (Yadav et al., 2011). Under abiotic stress conditions, it's difficult for a seed to be able to germinate and produce a good crop stand in the field. Hence, seed treatments are a useful tool for seeds to cope with the existing abiotic stress (Paparella et al., 2015).

Seed priming is a simple, low cost practice, which is used to overcome the problems of abiotic stress in crop production. Several methods of seed priming were developed in order to enhance the quality of seeds and minimize the risk of environmental stresses (Lutts et al., 2016). The effect of priming was attributed to metabolic repair and activation of seeds during imbibition (Basra et al., 2005). Solid matrix priming in which water uptake by seeds is controlled, was developed as a solution to overcome the problem of aeration in osmo-priming and the high cost of osmotic agents (Paparella et al., 2015). During matrix conditioning, seeds are mixed and incubated with a wet solid (water carrier) for a certain period of time so as to control the uptake of water by the seeds. Afterward, seeds are separated from the carrier, rinsed, and back-dried to the level of their original moisture content. The use of solid medium allows seeds to hydrate slowly and simulates the natural imbibition process occurring in the soil (McDonald, 2000). Different types of osmotica and chemicals are used for priming purposes. However, the issue of environmental problems is common due to intensive use of chemicals. The use of organic farming is receiving due consideration. Among such treatments, we considered seaweed (Balakrishnan et al., 2007, Muhie et al., 2020), vermicompost (Muhie et al., 2020) and smoke extracts (Demir et al., 2018). Such organic biostimulants were found to enhance seed quality in various crop seeds such as *Lupinus angustifolius* (Plazek et al., 2018), *Capsicum annuum* (Rekha et al., 2018) and onion (Muhie et al., 2020). Furthermore, there is not much research on priming combined (single, double and triple) with organic extracts (biostimulants) for carrot seeds, especially under abiotic stress conditions. We presume that they may synergistically affect seed quality under stressful conditions. Thus, the objective of this research is to investigate the effect of biostimulants (seaweed, vermicompost and smoke derived, karrikinolide) alone or in combination by using matrix priming on performance of carrot seed germination and seedling quality under drought, salinity and high temperature stress conditions.

Materials and Methods

Seed and biostimulants

Carrot (*Daucus carota* L. cv. Maestro F1 hybrid) seeds were obtained from a commercial company. The germination percentage of the lot was 85% and seed moisture content was 6.6%.

Karrikinolide (Kar) was provided by Prof. Van Staden from South Africa, University of Kwazulu Natal. 10^{-7} M karrikinolide (Mavi et al., 2010) was used in this experiment. Seaweed (Sw, green algae, *Ulva lactuca*) was collected from Marmara Sea in Turkey (northwestern part). The seaweed extract was prepared using the physical integration method. The particle size was gradually reduced and diluted with water to a ratio of 1:3. There was no heat, acid or any alkaline hydrolysis used for extraction (Demir et al., 2006). Then the diluted seaweed was filtered

through a muslin cloth. The filtrate was considered 100% seaweed extract (Demir et al., 2006). The seaweed extract was stored at 4 °C for further applications. Balakrishnan et al. (2007) reported that best seedling performance was observed at 5% concentration. So, we used the same concentration of seaweed extract. Liquid vermicompost (V) was obtained from a compost producing company in Turkey (Aybasol Ltd. Polatlı/Ankara). Liquid vermicompost was diluted with distilled water to make 5% vermicompost extracts which displayed best seedling performance (Arancon et al., 2012) in our preliminary experiments. The extract was stored at 4 °C for further applications.

Priming procedure

Solid matrix priming was done at the ratio of 2:1:3 (Seed:vermiculite:organic solution (w:w:w)) for five days at 15 °C. Priming was done with single solutions of Sw (Seaweed), V (Vermicompost), Kar (Karrikinolide) and double combinations Sw+V, Sw+Kar, V+Kar and the triple combination of Sw+V+Kar. All combination treatments were done under dark conditions. Seeds were also treated with distilled water (Dw) and untreated dry seeds (Np) were considered as controls. So, 7 treatments and two controls were allocated. After treatments, seeds were dried on the laboratory bench until the initial seed weight was reached. Then, the seeds were kept at 5 °C to be used for germination and emergence tests.

Seed germination under abiotic stresses

Drought stress condition was simulated using Polyethylene Glycol-6000 (PEG) at 10%, -0.30 MPa (Muscolo et al., 2014). Primed and control carrot seeds (four replicates of 50 seeds) were placed on filter paper in 9 cm Petri dishes containing 3 cm³ of -0.3 MPa 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 14 days in the dark. Seeds were considered germinated when the radicle emerged by at least 2 mm and is expressed as percentage. In the salt stress experiment, NaCl at concentration of 100mM was used to simulate salinity stress. The same germination procedure was followed.

Primed and control carrot seeds were allowed to germinate at 30 °C. Four replicates of 50 seeds were used for germination tests. Monitoring and recording of data were as described under earlier stress conditions.

Seedling emergence and quality tests

The seedling emergence test were carried out in three replicates of 25 seeds each under moisture, salinity and temperature stress conditions. For this purpose, plastic germination trays were used. The seeds were sown to a depth of 2 cm in peat moss and the appearance of the cotyledon leaves above the peat moss was considered as the emergence criterion. Trays were kept at 20 ± 2 °C. Control and stress levels were induced as described above. Trays were watered with an appropriate solution according to the treatment design (-0.3 MPa PEG, 100mM NaCl, tap water) during the emergence period. Seedling emergence percentage (EP) was calculated based on percentages of seedlings appearing on the surface of the peat. After 21 days of sowing, five seedlings in each

replicate were destructively taken and shoot height (SH, cm/plant), shoot fresh (SFW, mg/plant) and dry weight (SDW, mg/plant), and root fresh weight (RFW, mg/plant) were calculated. Seedling dry weight was calculated after keeping seedlings at 80 °C for 24 hours. Vigor index was recorded according to Abdul-Baki and Anderson (1973).

Catalase analysis

The two treatments that gave best results for germination and emergence tests and two controls (distilled water primed and non-primed) were subjected to catalase enzyme analysis. An amount of 0.5 grams of three replicates in each treatment were used. CAT (Catalase) activity was analyzed based on the rate of hydrogen peroxide decomposition according to the method and is expressed as per g seed, and one unit represents 1 µmol of substrate undergoing reaction per g per seed (Abedi and Pakniyat, 2010).

Mineral analysis

Mineral nutrient analysis was completed only for the three biostimulants for macro and micro nutrients at the soil science laboratory of Ankara University. Analysis was done using an inductively coupled plasma spectrophotometer (Mertens, 2005).

Table 1. Germination percentages of carrot seeds treated with different biostimulants at optimum condition, drought (-0.3 MPa, PEG), salt (100 mM NaCl) and high temperature (30°C) stresses. The values in a column with the same letter are not significantly different (P<0.05).

Priming agent	Stresses				Mean
	Optimum (20 °C/ Dw)	Drought	Salt	High temperature	
Np	85 ^a	63 ^a	37 ^a	72 ^a	64
V+Kar	85 ^a	70 ^b	73 ^b	76 ^{ab}	76
Sw+V+Kar	87 ^{ab}	78 ^c	74 ^b	77 ^{ab}	79
Dw	87 ^{ab}	79 ^c	74 ^b	77 ^{ab}	79
V	88 ^{ab}	82 ^{cd}	74 ^b	82 ^{bc}	81
Sw+V	87 ^{ab}	83 ^{cd}	75 ^b	84 ^{cd}	83
Kar	89 ^{ab}	85 ^d	79 ^{bc}	85 ^{cd}	85
Sw+Kar	89 ^{ab}	86 ^{ab}	87 ^c	88 ^{cd}	88
Sw	92 ^b	89 ^c	80 ^{bc}	89 ^d	88

NP:None primed (Control), Dw:Distilled water, Sw:Seaweed, Kar:Karrikinolide, V:Vermicompost

Effect of priming on seedling emergence and quality

The effect of drought stress was observed in the seedling emergence test at -0.30 MPa. Maximum seedling emergence of 89% was observed from seeds primed with seaweed extracts (Table 1). A significant difference (P<0.05) was observed against NP (none primed) in terms of EP (emergence percentage). Under drought stress the maximum EP was recorded from Sw (%88) and Sw+Kar (%84) treatments while the minimum was from the control (%57) (Table 2). Seedling parameters like seedling fresh weight, seedling dry weight, root fresh weight and vigor index were also statistically significantly different between the treatments. The maximum emergence of 76% was observed in SW and Sw+Kar at 100 mM salt stress (Table 3). Seedling height showed statistically significant difference among treatments The maximum seedling height was recorded in SW (4.9 cm) for low quality and for high quality carrot seeds. All other parameters like SFW, SDW and

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 among treatment means.

Results and Discussion

Effect of priming on seed germination

Germination percentages for carrot seeds under optimum conditions varied from 85% in Np to 92% in Sw treated. The other treatments varied between these values and significance also changed (Table 1). Drought, salt and high temperature stresses affected seed germination at various levels. The greatest impact was seen in Np seeds in which seed germination declined to as low as 37% in salt, 63% in drought and 72% in high temperature conditions, respectively. Under stress and optimum germination conditions, Sw treatment provided the highest germination percentages between 89 and 92% except for salt stress which had 80%. Biostimulant priming advanced carrot germination 10% in drought, 13% in salt and 12% in high temperature stress when the best treatment (Sw, Sw+Kar) was subtracted from Dw (distilled water) treatment. This difference was not significant except for high temperature germination (p<0.05).

RFW also showed statistical significance differences at various levels among treatments (Table 3). Seedling emergence tests all showed more positive responses for all the parameters by using Sw and other biostimulants under high temperature stress. Np seeds had 71% emergence (EP) which went up to 83% when Dw was used and the Sw treatment increased this to 87% (Table 4). All seedling criteria were found to be higher in Sw-treated carrot seeds. But it was not significantly separated from the other treatments in all cases.

Catalase enzyme analysis was done on the best treatments according to germination and seedling emergence test results. From the nine treatments, Sw and Sw+Kar gave the best results for all the parameters studied in relation to germination and emergence. DW (Control 2) and non-primed (control 1) were also considered. These treatments were tested for the activity of enzymes via Catalase (CAT), which was maximum in Sw treatment. The activity of CAT was maximum in Sw+Kar

treatment but the difference was not significant compared to Sw (Table 5).

Mineral nutrient content of biostimulants

The results for mineral nutrient content are presented in Table 6. The amount recorded in Kar was lower than that of seaweed and vermicompost. Maximum amounts of Ca (12.30 mgKg⁻¹), Mg (4.22 mgKg⁻¹) and S (10.19 mgKg⁻¹) were recorded from seaweed extract. Vermicompost also showed maximum results for minerals such as Fe (0.25 mgKg⁻¹), P (1.19 mgKg⁻¹), and K (175 mgKg⁻¹) (Table 6).

The present work indicates that biostimulant priming treatments increased tolerance to drought, salt and temperature stress in carrot seeds. Especially seaweed and the seaweed and karrikinolide combination provided the highest germination and seedling emergence. Seed germination, emergence, and seedling establishment are the most vulnerable growth processes and can be affected by an increase in abiotic stress conditions (Atia et al. 2006, Akbari et al., 2007; Nascimento and

Pereira, 2007). Such abiotic conditions adversely affect seed germination which leads to slow and non-uniform emergence in the field or modules in transplant production (Pereira et al., 2009). Therefore, pre-sowing seed treatments are used to obtain faster and synchronized germination particularly under stress conditions. Biostimulants foster plant growth and development throughout the life cycle of crops from seed germination to plant maturity in a number of ways. One of which is priming of seeds with biostimulants because biostimulants include a variety of plant promoting substances such as hormones, humic substances, micronutrients, manure, seaweed extracts and/or smoke-derived karrikinolides (Kulkarni et al., 2011, Paparella et al., 2015, Muhie et al. 2020). Biostimulants are also valuable in organic farming systems and were proposed as a solution to environmental issues and to encourage the use of organic materials starting from seed treatment.

Table 2. Effect of priming with different biostimulants on seedling emergence and quality of carrot under drought stress at -0.30 MPa of PEG. The values in a column with the same letter are not significantly different (P<0.05).

Priming agent	EP (%)	SH (cm/p)	SFW (mg/p)	SDW (mg/p)	RFW (mg/p)	Vigour Index
Np	57 ^a	4.9 ^a	25.3 ^a	2.3 ^a	3.0 ^a	316 ^a
V+Kar	61 ^{bc}	5.3 ^{ab}	27.0 ^{ab}	2.7 ^{ab}	3.3 ^{ab}	321 ^a
Sw+V+Kar	65 ^{bc}	5.7 ^{abc}	28.0 ^{ab}	3.0 ^{abc}	3.7 ^c	349 ^a
Dw	69 ^{abc}	5.9 ^{abc}	29.3 ^{ab}	3.0 ^{abc}	3.7 ^c	355 ^a
V	74 ^{a-d}	5.9 ^{abc}	29.7 ^{ab}	3.0 ^{abc}	3.7 ^c	357 ^a
Sw+V	76 ^{a-d}	6.0 ^{abc}	29.7 ^{ab}	3.0 ^{abc}	3.7 ^c	389 ^a
Kar	80 ^{bcd}	6.3 ^{abc}	33.7 ^{abc}	3.3 ^{abc}	4.2 ^{cd}	419 ^{ab}
Sw+Kar	84 ^{cd}	6.4 ^{bc}	35.67 ^c	3.7 ^{bc}	4.6 ^{de}	515 ^b
Sw	88 ^d	6.6 ^c	41.3 ^c	4.0 ^c	5.0 ^e	519 ^b

NP:None primed (Control), DW:Distilled water, SW:Seaweed, Kar:Karrikinolide, V:Vermicompost, EP:Emergence percentage (%), SH:Seedling height (cm/p), SFW:Seedling fresh weight (mg/p), SDW:Seedling dry weight (mg/p), RFW:Root fresh weight (mg/p).

Table 3. Effect of priming with different biostimulants on seedling emergence and quality of carrot under saline condition at 100mM NaCl. The values in a column with the same letter are not significantly different (P<0.05).

Priming agent	EP (%)	SH (cm/p)	SFW (mg/p)	SDW (mg/p)	RFW (mg/p)	Vigour Index
Np	25 ^a	3.5 ^a	14.0 ^a	1.3 ^a	2.0 ^a	91 ^a
V+Kar	26 ^{ab}	3.8 ^a	15.7 ^{ab}	1.5 ^a	2.3 ^{ab}	99 ^a
Sw+V+Kar	36 ^{abc}	4.2 ^{ab}	17.3 ^{ab}	1.7 ^{ab}	2.3 ^{ab}	149 ^{ab}
Dw	48 ^{abc}	4.4 ^{ab}	19.7 ^{abc}	2.0 ^{ab}	2.7 ^{bc}	200 ^{ab}
V	53 ^{abc}	4.5 ^{ab}	20.3 ^{bc}	2.0 ^{abc}	3.0 ^c	255 ^{ab}
Sw+V	57 ^{abc}	4.5 ^{ab}	20.7 ^{bc}	2.0 ^{abc}	3.0 ^c	269 ^{ab}
Kar	65 ^{abc}	4.7 ^b	21.5 ^{bc}	2.3 ^{bc}	3.7 ^d	271 ^{ab}
Sw+Kar	69 ^{bc}	4.8 ^b	23.7 ^{cd}	2.4 ^{bc}	3.7 ^d	311 ^b
Sw	76 ^c	4.9 ^b	26.0 ^d	2.7 ^c	3.7 ^d	323 ^b

Np:None primed (Control), Dw:Distilled water, Sw:Seaweed, Kar:Karrikinolide, V:Vermicompost, EP:Emergence percentage(%), SH:Seedling height (cm/p), SFW:Seedling fresh weight (mg/p), SDW:Seedling dry weight (mg/p), RFW:Root fresh weight (mg/p).

Table 4. Effect of priming with biostimulants on seedling emergence and quality of carrot at high temperature of 30°C. The values in a column with the same letter are not significantly different ($P < 0.05$).

Priming agent	EP (%)	SH (cm/p)	SFW (mg/p)	SDW (mg/p)	RFW (mg/p)	Vigour Index
Np	71 ^a	6.3 ^a	33.3 ^a	3.0 ^a	1.7 ^a	513 ^a
V+Kar	75 ^b	7.0 ^b	35.3 ^{ab}	3.3 ^a	1.7 ^a	549 ^{ab}
Sw+V+Kar	79 ^{bc}	7.0 ^b	37.3 ^{ab}	3.7 ^a	1.8 ^a	554 ^{ab}
Dw	83 ^{cd}	7.3 ^{bc}	38.3 ^{ab}	3.7 ^a	1.8 ^{ab}	579 ^{abc}
V	84 ^{cd}	7.3 ^{bc}	39.0 ^{ab}	3.7 ^a	2.4 ^{ab}	584 ^{abc}
Sw+V	84 ^{cd}	7.7 ^c	43.0 ^b	4.0 ^a	3.0 ^{bc}	601 ^{abc}
Kar	85 ^{cd}	8.0 ^c	44.0 ^{bc}	4.0 ^a	3.0 ^{bc}	672 ^{bcd}
Sw+Kar	85 ^{cd}	8.7 ^d	52.3 ^{cd}	5.0 ^b	3.7 ^{cd}	715 ^{cd}
Sw	87 ^d	9.0 ^e	59.6 ^d	5.7 ^b	4.1 ^d	792 ^d

Np:None primed (Control), Dw:Distilled water, Sw:Seaweed, Kar:Karrikinolide, V:Vermicompost, EP:Emergence percentage, SH:Seedling height (cm/p), SFW:Seedling fresh weight (mg/p), SDW:Seedling dry weight (mg/p), RFW:Root fresh weight (mg/p).

Table 5. Changes in catalase (CAT). activity of none primed (Np). distilled water (Dw). seaweed (Sw). seaweed and Kar (Sw+Kar) treated carrot seeds. The values in a column with the same letter are not significantly different ($P < 0.05$).

CAT	EUg ⁻¹ seed
Np	0.299 ^a
Dw	0.239 ^a
Sw	0.411 ^b
Sw+Kar	0.401 ^b

Table 6. Results of mineral analysis from the three biostimulants (mg kg⁻¹)

Extracts	P	K	Ca	Mg	S	Fe	Zn	Cu	Mn	B
V	1.19	175.20	9.93	3.07	5.86	0.25	0.17	0.11	0.31	0.18
Sw	0.26	5.37	12.30	4.22	10.19	0.05	0.14	0.11	0.29	0.06
Kar	0.11	0.82	0.41	0.22	0.52	0.02	0.13	0.11	0.28	0.25

V:Vermicompost, Sw: Seaweed and Kar: Karrikinolide

Seedling characteristics such as EP, SH, SFW, SDW, RFW and vigor index were affected at 100mM (Table 3). The inhibitory effect of salinity on germination and emergence of carrot was previously studied (Elena and Lagunovschi, 2015). Slow germination, or inability to germinate, in carrot at 100mM or drought might be due to insufficient osmotic potential to hinder the uptake of threshold water. Moreover, high salinity has an inhibitory effect on cell division and enlargement. Sayar et al. (2010) mentioned that water uptake by a seed can be limited by a decrease in the osmotic potential of soil solution due to the accumulation of soluble salts in the growth medium, which in turn results in a decrease in the activity of physiological process such as germination, growth and development. Salt (NaCl) and PEG at the same osmotic potential were used to simulate salinity and moisture stress. But, the inhibitory effect of PEG was greater than salinity at same osmotic potential. In a similar study on wild mustard, both NaCl and PEG treatments decreased final germination percentage and seedling growth characteristics, but the effects of NaCl

were lower on germination compared to PEG (Kayacetin et al., 2018). Demir and Mavi (2008) reported the effects of salt and osmotic stresses on the germination of pepper seeds of different maturation stages, and stated that the inhibition of germination at the same water potential of NaCl and PEG resulted from the osmotic effect rather than the salt toxicity. Seed priming with different biostimulants exerted a positive effect on carrot seeds. Sw and Sw+Kar were more effective in improving the performance of carrot seeds (Table 3) and seedlings under salinity stress conditions. Sw improved not only germination but also seedling characteristics such as EP, SH, SFW, SDW, RFW and vigor index. In previous reports, seaweed extract caused positive responses in different crops when used as priming agent (Kalaivanan and Venkatesalu, 2012; Shahbazi et al., 2015). A promotional effect on germination and seedling characteristics was also observed using 5% seaweed extract during priming of *Cyamopsis tetragonola* (Balakrishnan et al., 2007) as we used the same concentration. This is in line with the findings of the present study. The promotional effect of

priming with seaweed (SW) in carrot seeds might be attributed to the presence of enzymes, phytohormones, minerals and other growth-promoting substances (Godlewska et al., 2016; Masondo et al., 2018).

Carrot is a cool season crop and vulnerable to loss from thermal stress during early stages of development. Most commercial carrot cultivars have reduced and erratic germination at high temperatures (Nascimento and Pereira, 2007). The metabolic activation of the embryo is triggered by a favorable temperature preparatory to the start of germination. High temperatures hinder activation of enzyme compliments within the cells, changes in nucleic acids in the nuclei, mobilization of stored energy, and synthesis of new materials during the early stages of germination. All these changes prepare the radicle for elongation and the transition to normal seedling growth and development (Kozarewa et al., 2006). The indirect injuries include inactivation of enzymes, inhibition of protein synthesis, protein degradation and membrane integrity (Goraya et al., 2017). Under mild temperature stress (30°C), matrix priming of seeds with seaweed extracts and seaweed plus karrikinolide combinations was effective in minimizing the problems related to temperature stress and improving the germination/emergence of carrot seeds and seedling characteristics such as seedling height, seedling fresh and dry weight, and vigor index. Effects of seed priming on germination stress were observed in muskmelon (Nascimento and Aragao, 2004), watermelon (Demir and Oztokat, 2003) and asparagus (Bittencourt et al., 2004). The positive effect of Sw as a biostimulant for seed priming was also observed in many crops by different researchers (Balakrishnan et al., 2007; Kalaivanan and Venkatesalu, 2012; Shahbazi et al., 2015).

One of the original aspects of the present work was to test the double and triple combinations of three different biostimulants in order to accelerate the priming effect. However, combinations appeared not to be more effective than treatments alone. This indicates that there is no integrated effect since combinations did not provide higher seed germination than single treatments. Seaweed alone and its combination with karrikinolide were most effective in improving the quality of carrots seeds both under normal and all stress conditions. Interestingly the positive effect of ranking in all treatments was the same under all stress conditions with Sw being the best and Sw+Kar being the second-best treatment (Tables 2, 3, and 4). The positive effect on germination under abiotic stress conditions was also reported in our earlier work in onion seeds (Muhie et al., 2020). Distilled water (humidifying, soaking etc.) is commonly used in priming. The promotional effect of priming with seaweed (Sw) in carrot might be attributed to the presence of enzymes, phytohormones, minerals and other growth-promoting substances (Sivasankari et al., 2006 Godlewska et al., 2016; Masondo et al., 2018, Muhie et al., 2020). In the present study, maximum amount of CAT was observed in seeds treated with seaweed and Sw+Kar which might contribute to the metabolic activity of seeds and enhanced seed germination. High sulfur in Sw co-relates to the production of enzymes, since S is the precursor of methionine and other amino acids (Svozil and Baerenfaller, 2017). Sulfur containing compounds

play a critical role in the response of plants to abiotic stress factors including drought and salinity (Cao et al., 2014). Thus, rich mineral contents may contribute to the beneficial effect of seaweed. Researchers also revealed the presence of various sources of growth-promoting substance/hormones in seaweed (Masondo et al., 2018).

Conclusion

Solid matrix priming of carrot seeds with seaweed extract alone and its combination with karrikinolide showed the best results for carrot seed germination and seedling characteristics. Further research should be done to identify the best biostimulant priming for each crop because there is no single best suited method for priming of every crop.

Compliance with Ethical Standards

Conflict of interest

Authors declare no potential conflicts of interest with respect to the publication of the article.

Author contribution

Seid Hussen developed the theoretical idea of implementing the experiment. Ibrahim Demir modified the research idea and its methodologies. Seid Hussen carried out the experiment and wrote the manuscript which was proofread by Ibrahim Demir. Nurcan Memis and Cihat Ozdamar conducted the experimental applications. Zeynep Gokdas created figures and tables and searched the literature.

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Data availability

Not applicable

Consent for publication

Not applicable

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References

- Abdulbak.Aa, & Anderson, J. D. (1973). Vigor Determination in Soybean Seed by Multiple Criteria. *Crop Science*, 13(6), 630-633. Doi: <https://doi.org/10.2135/cropsci1973.0011183x001300060013x>
- Abedi, T., & Pakniyat, H. (2010). Antioxidant Enzyme Changes in Response to Drought Stress in Ten Cultivars of Oilseed Rape (*Brassica napus* L.). *Czech Journal of Genetics and Plant Breeding*, 46(1), 27-34. Doi: <https://doi.org/10.17221/67/2009-Cjgpb>
- Akbari, G., Sanavy, S., & Yousefzadeh, S. (2007). Effect of Auxin and salt stress (NaCl) on seed germination of wheat cultivars (*Triticum aestivum*). *Pakistan Journal of Botany*, 10 (15), 2557-2561. Doi: <https://doi.org/10.3923/pjbs.2007.2557.2561>
- Arancon, N. Q., Pant, A., Radovich, T., Hue, N. V., Potter, J. K., & Converse, C. E. (2012). Seed Germination and

- Seedling Growth of Tomato and Lettuce as Affected by Vermicompost Water Extracts (Teas). *Hortscience*, 47(12), 1722-1728. Doi: <https://doi.org/10.21273/Hortsci.47.12.1722>
- Atia, A., Debez, A., Rabhi, M., Athar, H. U. R., & Abdelly, C. (2006). Alleviation of salt-induced seed dormancy in the perennial halophyte *Crithmum maritimum* L. (Apiaceae). *Pakistan Journal of Botany*, 38(5), 1367-1372. Retrieved from <http://www.pakbs.org/pjbot/PDFs/38%285%29/PJB38%285%291367.pdf>
- Balakrishnan, C. P., Kumar, V., Mohan, V. R., & Athiperumalsami, T. (2007). Study on the effect of crude seaweed extracts on seedling growth and biochemical parameters in *Cyamopsis tetragonoloba* (L.) taub. *Plant Archives*, 7(2), 563-567. Retrieved from <https://www.researchgate.net/publication/295726991>
- Basra, S. M. A., Farooq, M., Tabassam, R., & Ahmad, N. (2005). Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). *Seed Science and Technology*, 33(3), 623-628. Doi: <https://doi.org/10.15258/sst.2005.33.3.09>
- Bittencourt, M. L. C., Dias, D. C. F. S., Dias, L. A. S., & Araujo, E. F. (2004). Effects of priming on asparagus seed germination and vigour under water and temperature stress. *Seed Science and Technology*, 32(2), 607-616. Doi: <https://doi.org/10.15258/sst.2004.32.2.29>
- Cao, M. J., Wang, Z., Zhao, Q., Mao, J. L., Speiser, A., Wirtz, M., . . . Xiang, C. B. (2014). Sulfate availability affects ABA levels and germination response to ABA and salt stress in *Arabidopsis thaliana*. *Plant Journal*, 77(4), 604-615. Doi: <https://doi.org/10.1111/tbj.12407>
- Demir, I., & Mavi, K. (2008). Effect of Salt and Osmotic Stresses on the Germination of Pepper Seeds of Different Maturation Stages. *Brazilian Archives of Biology and Technology*, 51(5), 897-902. Doi: <https://doi.org/10.1590/S1516-89132008000500004>
- Demir, I., Ozden, E., Yildirim, K. C., Sahin, O., & Van Staden, J. (2018). Priming with smoke-derived karrikinolide enhances germination and transplant quality of immature and mature pepper seed lots. *South African Journal of Botany*, 115, 264-268. Doi: <https://doi.org/10.1016/j.sajb.2017.07.001>
- Demir, N., Dural, B., Yildirim, Y. (2006). Effect of seaweed suspension on seed germination of tomato, pepper and aubergine. *Journal of Biological Science*, 6 (6), 1130-1133. Doi: <https://doi.org/10.3923/jbs.2006.1130.1133>
- Demir, I., & Oztokat, C. (2003). Effect of salt priming on germination and seedling growth at low temperatures in watermelon seeds during development. *Seed Science and Technology*, 31(3), 765-770. Doi: <https://doi.org/10.15258/sst.2003.31.3.26>
- Elena, D., & Lagunovschi, V.L. (2015). Germination and vigour of primed *Daucus carota* L. seeds under saline stress conditions. *Romanian Biotechnological Letters*, 20 (5). Retrieved from <https://www.researchgate.net/profile/Lagunovschi-Luchian-Viorica/publication/313900032>
- Fetri, M., Ahmad, D., & Rajabi, M. (2014). Effect of drought and salinity tensions on germination and seedling growth of Common Yarrow (*Achillea millefolium* L.) in laboratory conditions. *International Journal of Advance Biological and Biomedical Research*, 2(2), 383-391. Retrieved from http://www.ijabbr.com/index.php/article_7092.html
- Godlewska, K., Michalak, I., Tuhy, L., & Chojnacka, K. (2016). Plant Growth Biostimulants Based on Different Methods of Seaweed Extraction with Water. *Biomed Research International*, 2016. Doi: <https://doi.org/Artn597376010.1155/2016/5973760>
- Goraya, G. K., Kaur, B., Asthir, B., Bala, S., Kaur, G., & Farooq, M. (2017). Rapid Injuries of High Temperature in Plants. *Journal of Plant Biology*, 60(4), 298-305. Doi: <https://doi.org/10.1007/s12374-016-0365-0>
- Kalaivanan, C., & Venkatesalu, V. (2012). Utilization of seaweed *Sargassum myriocystum* extracts as a stimulant of seedlings of *Vigna mungo* (L.) Hepper. *Spanish Journal of Agricultural Research*, 10(2), 466-470. Doi: <https://doi.org/10.5424/sjar/2012102-507-10>
- Kayacetin, F., Efeoglu, B., & Alizadeh, B. (2018). Effect of NaCl and PEG-induced osmotic stress on germination and seedling growth properties in wild mustard (*Sinapis arvensis* L.). *Anadolu Journal of Aegean Agriculture Research Institute*, 28 (1), 62 – 68. Retrieved from <https://arastirma.tarimorman.gov.tr/etae/Belgeler/AnadoluDergisi/2018/1/11%20mak%2062-68.pdf>
- Kozarewa, I., Cantliffe, D. J., Nagata, R. T., & Stoffella, P. J. (2006). High maturation temperature of lettuce seeds during development increased ethylene production and germination at elevated temperatures. *Journal of the American Society for Horticultural Science*, 131(4), 564-570. Doi: <https://doi.org/10.21273/Jashs.131.4.564>
- Kulkarni, M. G., Light, M. E., & Van Staden, J. (2011). Plant-derived smoke: Old technology with possibilities for economic applications in agriculture and horticulture. *South African Journal of Botany*, 77(4), 972-979. Doi: <https://doi.org/10.1016/j.sajb.2011.08.006>
- Lutts, S., Benincasa, P., Wojtyla L., Kubala S.S., Pace R., Lechowska K., Quinet M., & Garnczarska M. (2016). Seed Priming: New Comprehensive Approaches for an Old Empirical Technique, New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology, Susana Araujo and Alma Balestrazzi, IntechOpen, Doi: <https://doi.org/10.5772/64420>
- Masondo, N. A., Kulkarni, M. G., Finnie, J. F., & Van Staden, J. (2018). Influence of biostimulants-seed-priming on *Ceratotheca triloba* germination and seedling growth under low temperatures, low osmotic potential and salinity stress. *Ecotoxicology and Environmental Safety*, 147, 43-48. Doi: <https://doi.org/10.1016/j.ecoenv.2017.08.017>
- Mavi, K., Light, M. E., Demir, I., van Staden, J., & Yasar, F. (2010). Positive effect of smoke-derived butenolide priming on melon seedling emergence and growth. *New Zealand Journal of Crop and Horticultural Science*, 38(2), 147-155. Doi: <https://doi.org/10.1080/01140671.2010.482967>
- Mc Donald, M.B. (2000). Seed priming. In: Black M, Bewley J.D, editors. *Seed Technology and its Biological Basis*. Sheffield, Sheffield Academic Press, UK.
- Mertens D. (2005). AOAC official method 922.02. In: Horwitz, W., Latimer, G.W. (Eds.), *Plants Preparation of Laboratory Sample. Official Methods of Analysis*, 18th ed. AOAC-International Suite, Gaithersburg, MD, USA.
- Muhie, S. H., Yildirim, E., Memis, N., & Demir, I. (2020). Vermicompost priming stimulated germination and seedling emergence of onion seeds against abiotic

- stresses. *Seed Science and Technology*, 48(2), 153-157. Doi: <https://doi.org/10.15258/sst.2020.48.2.02>
- Muhie, S., Özdamar, C., Gökdaş, Z., Njie, E., Memiş, N., & Demir, İ. (2020). Effect of Solid Matrix Priming With Seaweed Extract on Germination and Seedling Performance of Onion Seeds under Abiotic Stress Conditions. *Black Sea Journal of Agriculture*, 3 (4), 233-238. Retrieved from <https://dergipark.org.tr/tr/pub/bsagriculture/issue/56447/685251>
- Muscolo, A., Sidari, M., Anastasi, U., Santonoceto, C., & Maggio, A. (2014). Effect of PEG-induced drought stress on seed germination of four lentil genotypes. *Journal of Plant Interactions*, 9(1), 354-363. Doi: <https://doi.org/10.1080/17429145.2013.835880>
- Nascimento, W.M., & Aragao, F.A.S. (2004). Muskmelon seed priming in relation to seed vigor. *Scientia Agricola*, 61, 114-117. Doi: <http://dx.doi.org/10.1590/S0103-90162004000100019>
- Nascimento, W. M., & Pereira, R. S. (2007). Preventing thermo-inhibition in carrot by seed priming. *Seed Science and Technology*, 35(2), 504-507. Doi: <https://doi.org/10.15258/sst.2007.35.2.25>
- Paparella, S., Araujo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. *Plant Cell Reports*, 34(8), 1281-1293. Doi: <https://doi.org/10.1007/s00299-015-1784-y>
- Pereira, M. D., Dias, D. C. F. D., Dias, L. A. D., & Araujo, E. F. (2009). Primed Carrot Seeds Performance under Water and Temperature Stress. *Scientia Agricola*, 66(2), 174-179. Doi: <https://doi.org/10.1590/S0103-90162009000200005>
- Plazek, A., Dubert, F., Kopec, P., Dziurka, M., Kalandyk, A., Pastuszak, J., & Wolko, B. (2018). Seed Hydropriming and Smoke Water Significantly Improve Low-Temperature Germination of *Lupinus angustifolius* L. *International Journal of Molecular Sciences*, 19(4). Doi: <https://doi.org/ARTN 99210.3390/ijms19040992>
- Rekha, G. S., Kaleena, P. K., Elumalai, D., Srikumaran, M. P., & Maheswari, V. N. (2018). Effects of vermicompost and plant growth enhancers on the exo-morphological features of *Capsicum annum* (Linn.) Hepper. *International Journal of Recycling of Organic Waste in Agriculture*, 7(1), 83-88. Doi: <https://doi.org/10.1007/s40093-017-0191-5>
- Sayar, R., Bchini, H., Mosbahi, M., & Ezzine, M. (2010). Effects of salt and drought stresses on germination, emergence and seedling growth of Durum wheat (*Triticum durum* Desf.). *African Journal of Agricultural Research*, 5(15), 2008-2016. Doi: <https://doi.org/10.5897/AJAR.9000265>
- Shahbazi, F., Seyyed nejad, M., Salimi, A., & Gilani, A. (2015). Effect of seaweed extracts on the growth and biochemical constituents of wheat. *International Journal of Agricultural Crop Science*, 8 (3), 283-287.
- Sivasankari, S., Venkatesalu, V., Anantharaj, M., & Chandrasekaran, M. (2006). Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Bioresource Technology*, 97(14), 1745-1751. Doi: <https://doi.org/10.1016/j.biortech.2005.06.016>
- Svozil, J., & Baerenfaller, K. (2017). Proteomic in biology. *Methods in enzymology*, Edited by Arun K. Shukla. 585, 113-114
- Wang, W. X., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, 218(1), 1-14. Doi: <https://doi.org/10.1007/s00425-003-1105-5>
- Yadav, P.Y., Kumari, M., & Ahmed, Z. (2011). Chemical seed priming as a simple technique to impart cold and salt stress tolerance in capsicum. *Journal of Crop Improvement*, 25 (5), 497-503. Doi: <https://doi.org/10.1080/15427528.2011.587139>