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Effects of eustress induced by low concentrations of salinity on broccoli (*Brassica oleracea*) and purslane (*Portulaca oleracea*) microgreens

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Abstract

Most controlled environments utilize municipal water for crop irrigation. Many of these sources exceed the EPA guidelines of < 500 mg·L^{-1} total dissolved salts. Issues can arise when tap water with the above limit salt concentrations is used for irrigation. Eustress is defined as the use of slight stress (from stressors such as salinity, temperature, or light) to induce positive effects without distress. While eustress is commonly used on mature plants, the effects on early growth stages of plants, such as microgreens, are not well documented. As microgreens are typically more stress sensitive, the concentrations of salinity to induce eustress may be lower than for mature plants. To identify how eustress affects microgreens, salinity concentrations commonly found in tap water were used in these experiments. *Brassica oleracae* (moderately salt tolerant) and *Portulaca oleracea* (highly salt tolerant) microgreens were evaluated. Both species of microgreens were cultivated using salinity irrigation treatments ranging from 0 dS·m^{-1} to 1.5 dS·m^{-1}. Plants were analyzed for microgreen yield (fresh weight and dry weight), percent moisture content (% MC), percent dry matter (% DM), vitamin C (T-AsA, AsA) and proline concentrations. The results indicate that yields of both variety remained unaffected by the salinity treatments. However, %MC and proline significantly increased under 1 and 1.5 dS·m^{-1} NaCl in broccoli. Vitamin C also decreased as salinity increased in broccoli microgreens. Purslane microgreen vitamin C and proline remained unaffected by salinity. In conclusion, while low salinity levels had no negative impacts on microgreen yields, there were varied impacts on the phytochemistry between each variety.

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INTRODUCTION

Controlled environment systems are unique in that many utilize public tap water or well water to irrigate plants. This is done for ease of use, and to ensure the highest quality irrigation water possible resulting in high quality and value crops[1]. However, according to Dieter et al.[2], some states, such as Texas, California, Florida, and Virginia are withdrawing from saline groundwater for public supply use. Highly saline water is typically processed and regulated by water treatment plants prior to municipal use. However most water treatment plants still provide water with total dissolved solids (TDS) levels ranging between 250-1,000 ppm based on state and federal regulations[3]. For example, Phoenix, AZ (USA), reported TDS as high as 766 ppm in 2021[4]. Similarly, Salt Lake City, UT (USA), reported TDS as high as 808 ppm in some water wells in 2021[5]. Both cities regulate maximum TDS levels based on the US Environmental Protection Agency (20 (EPA)) secondary standards of 500 mg·L^{-1} TDS. The Texas Commission of Environmental Quality however, allows for an upper threshold of 1,000 mg·L^{-1} of TDS[6]. According to water quality reports in Lubbock and Abilene, TX (USA), TDS averaged between 512 and 671 ppm respectively with sodium (Na) being one of the major contributors[7,8]. While these salts fluctuate across season and location, there are consistently measurable quantities of salts delivered by municipal sources. These salts can lead to unfavorable growing conditions for plants, such as salinity accumulation in the rootzone, which can further lead to stress responses that reduce plant growth and decrease nutritional value. While saline water from municipal systems is usually not high enough to damage plants possessing moderate salinity tolerance, plant responses to this low level of salinity stress increase phytochemical content important to human health[9]. The process of inducing positive responses in plants through controlled stress is referred to as eustress[10]. Since water conditions can be changed in a controlled environment, eustress induced by salinity could be achieved in these production systems. For example, Rouphael & Kyriacou[9] found that tomatoes, peppers, and cauliflower grown in a controlled environment, benefitted from salinity eustress levels varying from 2 to 9 dS·m^{-1} depending on variety and stage of growth.

Eustress in younger plants, such as microgreens, could be detrimental as salinity can negatively impact germination and growth; however, this is highly dependent upon plant species[11,12]. Microgreens are young, cotyledonary, nutrient dense plants usually harvested between one to two weeks after germination. Microgreens are growing in popularity as they contain many of the same nutrients as their mature counterparts but require significantly less care and space due to their short growth period[13,14]. However, since microgreens are harvested young, the plant cells do not have enough time to

build tolerances to stressful environments. This makes microgreens more sensitive and susceptible to stress than mature plants. Most vegetable crops can be grown as microgreens, for example, kale, arugula, basil, broccoli, and purslane microgreens are popular for their flavor and color[15]. Many of these varieties have vastly different tolerances to salinity, particularly at the germination and seedling stages. Purslane has a high tolerance for salt and is considered a halophyte as a mature plant[16]. Broccoli is less salt tolerant compared to purslane and is classified as having slight tolerance as a mature plant[17]. However, salt tolerance has not been determined for the seedling stages of these plants. The salt tolerances of mature broccoli and purslane may be too strong to achieve eustress with salinity levels found in tap water. However, eustress could be achieved at these low concentrations because microgreens do not have the ability to build up tolerance to chloride salt.

Stress in plants can be measured through physiological and phytochemical parameters. In microgreens, physiological measurements of plant stress are more difficult to quantify due to small plant size and tenderness of plant tissues. Phytochemically, many compounds can be measured to determine the plant response to stress, such as proline, vitamin C, antioxidant activity, reactive oxygen species (ROS), etc. Proline and vitamin C concentrations can indicate osmotic stress, and antioxidant induced stress, respectively. Vitamin C is known to interact with ROS by eliminating harmful free radicals in plant cells[18]. It is also an essential nutrient for human health to prevent deficiencies that can cause immunity issues[19]. In this experiment, Total Ascorbic acid (T-AsA) and Ascorbic Acid (AsA) will be analyzed to determine the full effect of salinity eustress on vitamin C. Proline is also used for stress defense in plants, but with a different mechanism than vitamin C. As an osmolyte, the mechanism of proline-protein interactions induced by water/saline stress act to mitigate water loss in plant cells[20]. Unlike vitamin C, proline is a nonessential nutrient, but is beneficial as an amino acid and for maintaining glutamate homeostasis[21]. Therefore, the objectives of this study were to determine if salinity eustress at different rates could increase phytochemical composition of purslane and broccoli microgreens without affecting yield.

MATERIALS AND METHODS

Experimental setup

This experiment on broccoli and purslane microgreens was conducted in two separate trials under laboratory conditions at Texas Tech University (USA) in September and October 2021. A simple 5-tiered shelf (36 in × 16 in × 72 in) (HDX, Atlanta, GA, USA) was placed in a cool dry corner of the room. Red and blue spectrum LED light fixtures (47 in × 1.5 in) (Barrina LED Grow Light, Paris, France) were attached to the bottom of each shelf at 38 cm above the germination zone. Grow light photon flux densities (PFD) emitted approximately 142 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at a distance of 28 cm. Light blocking materials were placed under the microgreen trays to prevent light penetration through shelves. Microgreen trays were organized in a complete block design within the shelves.

Growth environment

Each trial lasted 14 d before plants were harvested. During the experiments, the room temperature averaged 21.7 ± 1.2 °C in trial 1 and 19.1 ± 4.3 °C in trial 2. Temperature was collected using a temperature and humidity sensor (tempi.fi, Woburn, MA, USA) suspended in air between two shelves. The room temperature was preset by university facilities and sensor readings were collected each minute throughout the trials. We noted some environmental differences in each trial that were attributed to seasonal changes.

Seed germination

Broccoli (*Brassica oleracea*) and purslane (*Portulaca oleracea*) seeds were purchased from Johnny's Select Seeds (Fairfield, ME, USA). Microgreen trays were set up by adding 50 mL of DI water to a plastic clamshell container containing a microgreen growing pad (4 in × 4 in) (Micromat, Salt Lake City, UT, USA). Then, 0.75 g of purslane and 1.5 g of broccoli seeds were added to their respective trays. The trays remained closed under natural light for 4 d to reduce water loss and facilitate germination. After the germination period, the trays were opened, and the lights were scheduled for the pre-selected cycle as mentioned above.

Salinity treatments and irrigation

Each tray was irrigated with the treatments as needed. In each trial, there were a total of four treatments, each containing five replicates. Irrigation treatments were created by dissolving NaCl (VWR BDH Chemicals ACS NaCl, Solon, OH, USA) in DI water to achieve four different concentrations: 0 $dS \cdot m^{-1}$ (0 mg of NaCl in 250 mL of water, control), 0.5 $dS \cdot m^{-1}$ (80 mg of NaCl in 250 mL of water), 1.0 $dS \cdot m^{-1}$ (160 mg of NaCl in 250 mL of water), and 1.5 $dS \cdot m^{-1}$ (240 mg of NaCl in 250 mL of water). Treatment EC was calculated by converting $dS \cdot m^{-1}$ to mmol followed by a conversion to grams of NaCl per liter of water. The specific treatments were chosen to represent levels of salinity found in tap water that also fall into ranges that may not detrimentally affect microgreens. The volume of treatments added is presented in Table 1.

Microgreen harvest

Microgreens were harvested by cutting the stems about 1 mm above the growing pad with sterilized scissors. The samples were weighed and stored at −80 °C. The samples were then freeze dried in a Freeze Dryer (Harvest Right, Salt Lake City, UT, USA), weighed, and ground into a powder in the presence of liquid nitrogen to facilitate analysis. The samples were then stored at −80 °C until further use.

Chemical analysis

Vitamin C

Vitamin C was analyzed using methods adapted from Kathi et al.[22], Stevens et al.[23], and Sérino et al.[24]. Briefly, 100 mg of dry sample was weighed and placed in 2 mL microtubes. Then, 1 mL of ice cold 6% Trichloroacetic Acid (TCA) was added to the microtubes and vortexed to create a homogenous solution. The samples were then left on ice for 15 min to quench the metabolism and then centrifuged. The standard curve was prepared by adding 1 mg/mL ascorbic acid in 6% TCA with additional 6% TCA to create concentrations of 0, 500, 1,000, 1,500, 3,000, 5,000, 7,500, and 10,000 µmol. The samples and standards were added to the microplate to be read along with along with dithiothreitol (for T-AsA) and phosphate buffer (for AsA) followed by N-ethyl maleimide and then color reagents. The plates after the specified reaction time were read at 550 nm using microplate reader (SpectraMax iD3, San Jose, CA, USA).

Table 1. Irrigation treatments of broccoli and purslane microgreens for trials 1 and 2.

Treatment	Trial 1				Trial 2			
	Broccoli		Purslane		Broccoli		Purslane	
	TIV	TS	TIV	TS	TIV	TS	TIV	TS
0 dS·m^{-1}	203.3	0	190.1	0	196.0	0	173.8	0
0.5 dS·m^{-1}	205.6	0.066	194.9	0.062	194.4	0.062	161.4	0.051
1 dS·m^{-1}	214.7	0.137	187.8	0.12	196.4	0.126	174.4	0.112
1.5 dS·m^{-1}	192.0	0.184	184.9	0.178	198.4	0.190	172.0	0.165

TIV = Total Irrigated Volume (mL); TS = Total NaCl (g).

Proline

Proline was analyzed using methods modified from Lee et al.[25]. For the extraction, 0.03 g of dried plant samples were added to 2 mL microtubes. Along with 1 mL of 1% sulfosalicylic acid the tubes were vortexed to make a homogenous solution. The microtubes were then centrifuged for 10 min at 5,055 rpm. The standard curve was prepared by mixing a 150 μg·mL^{-1} proline stock solution with 1% sulfosalicylic acid, glacial acetic acid, and ninhydrin solution (1.25% ninhydrin in 80% glacial acetic acid) to create concentrations of 0, 3.125, 6.25, 12.5, 25, 50, 75, 100, and 150 μg·mL^{-1}. This was added to a microplate along with the plant samples.

Proline was measured by adding 66 μL of plant extract supernatant to the microplate. To this, 132 μL of ninhydrin solution was added and the plate was incubated at 100 °C for 60 min. The reaction was stopped by placing the microplate in an ice bath for 10 min. Absorbance for proline was read at 510 nm using a microplate reader (SpectraMax iD3, San Jose, CA, USA).

Statistical analysis

Statistical analysis was performed using JMP 16.0.0 (SAS, Cary, NC, USA). Salinity trials occurred during two different times and were analyzed separately. Due to the biomass requirements of chemical analyses, one container of microgreens was considered one replication for a standard least squares factorial analysis and ANOVA. In total, 40 samples/replicates (i.e. five samples per treatment, four treatments, two plant species) of each trial were analyzed for each parameter to test for interaction effects. Significant differences were determined at $p \leq 0.05$. Where statistical differences occurred, a student's t test was used to determine mean separation.

RESULTS

Physical analysis

The fresh and dry weight of broccoli microgreens for trial 1 was not significantly affected by salinity treatments. However, the fresh weight of trial 2 broccoli microgreens was significantly different ($p = 0.043$) with the highest recorded weight under the 1 dS·m^{-1} NaCl treatment (Table 2). Additionally, broccoli microgreens % DM and % MC were not significantly different among treatments in trial 1 but differed significantly in trial 2. In the second trial, % DM of broccoli microgreens were significantly greater ($p = 0.008$) in the control treatment (0 dS·m^{-1} NaCl). As well, % MC was significantly greater ($p = 0.008$) in the 1 dS·m^{-1} NaCl treatment followed by 1.5 dS·m^{-1}, 0.5 dS m^{-1}, and then the control treatment (Table 2). Alternatively, purslane microgreen fresh weight, % DM, and % MC were not affected by treatment in both trials. The dry weight of purslane microgreens was significantly different ($p = 0.04$) in trial 1, but this difference was not seen in trial 2 (Table 2).

Chemical analysis

In trial 1, AsA and T-AsA of broccoli microgreens differed significantly ($p < 0.0001$, $p < 0.0001$) per treatment with the greatest concentrations in the control treatment (0 dS·m^{-1}). However, broccoli AsA in trial 2 was recorded as highest in the 1.5 dS·m^{-1} NaCl treatment ($p = 0.009$). Broccoli microgreens had the lowest concentration of T-AsA in the 1.5 dS·m^{-1} NaCl treatment in both trials (Table 3). In addition, broccoli microgreens had a significantly increased ($p = 0.074$, $p = 0.012$) proline concentration in the highest salinity treatment (1.5 dS·m^{-1} NaCl) in both trials (Table 3). The experimental results show that the salinity treatments had no significant effect on T-

Table 2. Average fresh weight (g), dry weight (g), dry matter (%), and moisture content (%) of broccoli and purslane microgreens for trials 1 and 2. Table data represents the mean of the five containers of samples in each treatment.

Treatments	Broccoli				Purslane			
	Fresh weight (g)	Dry weight (g)	% DM	% MC	Fresh weight (g)	Dry weight (g)	% DM	% MC
Trial 1								
0 dS·m^{-1}	4.91	0.638	13.0	87.0	1.93	0.219 B	11.3	88.7
0.5 dS·m^{-1}	5.32	0.646	12.4	87.6	2.15	0.244 B	11.4	88.6
1.0 dS·m^{-1}	6.37	0.709	11.2	88.8	2.59	0.306 A	12.3	87.7
1.5 dS·m^{-1}	5.65	0.697	12.6	87.4	2.08	0.237 A B	11.2	88.8
p value	0.238	0.524	0.213	0.303	0.185	*0.0395*	0.859	0.895
Trial 2								
0 dS·m^{-1}	6.11 B	0.794	13.2 A	86.8 C	3.57	0.306	9.2	0.908
0.5 dS·m^{-1}	6.22 B	0.760	12.2 A B	87.8 B C	3.04	0.262	8.9	0.911
1.0 dS·m^{-1}	8.84 A	0.935	10.6 C	89.4 A	2.93	0.360	13.2	0.868
1.5 dS·m^{-1}	7.54 AB	0.821	11.1 B C	88.9 A B	2.57	0.298	13.7	0.863
p value	*0.043*	0.314	*0.0077*	*0.0077*	0.847	0.773	0.152	0.152

Values followed by the same letter are not significantly different at the $p < 0.05$ level. p treatments were identified using a student's t test.

Table 3. Results for vitamin C and proline concentration in broccoli and purslane microgreens for trials 1 and 2.

Treatments	Broccoli			Purslane		
	T-AsA (mg/100 g FM)	AsA (mg/100 g FM)	Proline (umol/g DW)	T-AsA (mg/100 g FM)	AsA (mg/100 g FM)	Proline (umol/g DW)
Trial 1						
0 dS·m^{-1}	53.6 A	40.1 A	19.0 B	29.8	17.6	6.44
0.5 dS·m^{-1}	21.6 B	20.7 C	22.0 A B	26.9	17.9	7.97
1.0 dS·m^{-1}	22.6 B	24.4 C	18.4 B	28.2	18.8	7.47
1.5 dS·m^{-1}	27.2 B	28.7 B	28.9 A	27.9	20.6	7.29
p value	*<0.0001*	*<0.0001*	*0.0740*	0.642	0.165	0.560
Trial 2						
0 dS·m^{-1}	45.0 A	28.0 A B	9.57 B	20.5	12.2	6.47
0.5 dS·m^{-1}	54.4 A	19.2 B	13.2 B	18.9	11.6	5.84
1.0 dS·m^{-1}	41.9 A	19.9 B	15.9 A B	23.3	12.1	6.27
1.5 dS·m^{-1}	19.0 B	36.8 A	21.8 A	24.5	14.7	6.79
p value	*0.0006*	*0.0093*	*0.0119*	0.109	0.287	0.887

Values followed by the same letter are not significantly different at the $p < 0.05$ level. p treatments were determined using a student's t test.

AsA, AsA, and proline concentrations of purslane in either trial (Table 3).

Data correlation analysis

As expected, broccoli weight and moisture content were highly correlated (Table 4). Interestingly, the relationship of proline and T-AsA to broccoli moisture and biomass varied with treatments. In the broccoli control, proline concentrations decreased as dry weight increased, yet increased as AsA increased (Table 4). In the 1.5 dS·m^{-1} treatments, proline was negatively correlated to fresh weight, dry weight, and % MC. However, proline concentrations were positively correlated to % DM. Broccoli T-AsA showed an inverse relationship to % DM in 1 dS·m^{-1} treatments, and positively relationship to % DM in 1.5 dS·m^{-1} treatments. Additionally, in the highest salinity level given to the broccoli microgreens (1.5 dS·m^{-1}), T-AsA decreased as fresh weight and % MC increased (Table 4). Alternatively, broccoli AsA was negatively related to fresh weight and positively related to %DM only under 1 dS m^{-1} NaCl (Table 4).

In purslane, as AsA increased, so did T-AsA in every salinity treatment. Purslane AsA also decreased as fresh weight and dry weight increased under 1.5 dS·m^{-1} NaCl (Table 5). Proline was positively correlated to purslane T-AsA and AsA under 0.5 dS·m^{-1} NaCl (Table 5).

DISCUSSION

Microgreens are a relatively newly explored crop in horticultural research. So, little is known about production factors that affect yields and physiology of microgreens. For example, most studies report yields and phytochemical findings based on different light exposures while few report on different salinity treatments and nutrient fortification[26]. For example, Mlinarić et al.[27] found that antioxidant and bioactive compounds in chia sprouts (*Salvia hispanica* L.) were positively affected by 48 h of continuous light exposure. This demonstrates that phytochemical concentrations in young seedlings can be manipulated by external environments. While not as common, nutrient and phytochemical fortification has also been a topic of study for microgreens. Li et al.[28] fertilized 10 species of microgreens with a general fertilizer (20-20-20, NPK) and reported that the addition of fertilizer was successful in increasing nitrogen (N), phosphorus (P), and potassium (K) in Red Garnet amarant. Additionally, Kathi et al.[22] found that exogenous application of ascorbic acid could increase internal concentrations of vitamin C in arugula microgreens. In a different study regarding salinity, Islam et al.[29] reported that wheat microgreen extract contained higher concentrations of β-carotene, flavonoids, vitamin C, and phenolic acid when applied with 12.5 mM of NaCl. These studies highlight the various practices that can affect phytochemicals in these young plants.

Broccoli

If eustress is to be achieved at different salinity levels (ranging from 0–1.5 dS·m^{-1}), then yield, vitamin C, and proline increases should be seen in the microgreens. This is due to eustress being a strategy to increase plant nutrients and phytochemicals[9].

Conflicting results were observed in broccoli microgreen yield. In trial 1, yield was not affected by any of the salinity treatments. However, trial 2 broccoli microgreens had the highest fresh weight when irrigated with 1 dS·m^{-1} NaCl. This indicates that low levels of salinity stress may positively impact broccoli microgreen yields. Wang et al.[30] showed similar results when broccoli sprouts had increased yields at 40 and 80 mM of NaCl but declined at higher salinities (160, 200 mM). However, while there was an increase in fresh weight in trial 2, there was no effect in overall dry biomass. The increase in fresh weight of broccoli microgreens in trial 2 is most likely due to the increase in % MC also seen under the same salinity treatments. This implies that salinity eustress may slightly increase water uptake by microgreens, increasing biomass and therefore yields. The increase in salt concentration could have either directly caused the increase in moisture content, or it could have triggered a chemical response, such as the production of proline[31]. The gathered results agree most with the second theory on the effects of salinity on proline (Table 3). Stress produced by the slightly saline environment (eustress) affected the osmotic balance between cells and the plant environment causing an inflow of water to the cells.

When considering the other phytochemical results, broccoli microgreens contained the lowest T-AsA concentrations under the highest salinity treatment (1.5 dS·m^{-1}). However, AsA concentrations differed between trials. Salinity induced eustress affected internal AsA concentrations along with other factors, such as environmental stressors[32]. Some factors such as room

Table 4. Data correlation between all variables measured for broccoli microgreens.

Treatments		Broccoli						
		Fresh weight	Dry weight	% MC	% DM	T-AsA	AsA	Proline
0 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.9312***	1					
	%MC	0.5300	0.1926	1				
	% DM	−0.5296	−0.1921	−1.000***	1			
	T-AsA	0.0962	0.2844	−0.3143	0.3144	1		
	AsA	−0.2944	−0.3595	0.1050	−0.1048	0.4336	1	
	Proline	0.6026	−0.6530*	−0.0169	0.0167	0.2401	0.7005*	1
0.5 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.7505***	1					
	%MC	0.6142	−0.5220	1				
	%DM	−0.6129	0.0527	−1.000***	1			
	T-AsA	0.3352	0.5327	−0.0941	0.0946	1		
	AsA	0.0968	0.2995	−0.2846	0.2845	−0.1707	1	
	Proline	−0.7628	−0.5170	−0.5597	0.5587	−0.4473	0.2567	1
1.0 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.9664***	1					
	%MC	0.6151	0.4100	1				
	%DM	−0.6434*	−0.4299	−0.9414***	1			
	T-AsA	0.2723	0.2824	0.2447	−0.1560	1		
	AsA	−0.6928*	−0.5800	−0.6211	0.6436*	−0.1336	1	
	Proline	−0.5280	−0.4962	−0.3846	0.2497	0.0678	0.5033	1
1.5 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.09629***	1					
	%MC	0.8306**	0.6659*	1				
	%DM	−0.8295**	−0.6646*	−1.000*	1			
	T-AsA	−0.6654*	−0.5550	−0.7671**	0.7664**	1		
	AsA	−0.0967	−0.1354	0.0557	−0.0545	−0.2588	1	
	Proline	−0.8163**	−0.7476*	−0.7696**	0.7690**	0.5210	−0.0442	1

Order of significance is represented by *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$.

Table 5. Data correlation between all variables measured for purslane microgreens.

Treatments		Purslane						
		Fresh weight	Dry weight	% MC	% DM	T-AsA	AsA	Proline
0 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.9215**	1					
	%MC	0.7837**	0.5110	1				
	%DM	−0.7836**	−0.5108	−1.000***	1			
	T-AsA	−0.3355	−0.1582	−0.5585	0.5588	1		
	AsA	−0.3718	−0.2186	−0.4611	0.4615	0.9348***	1	
	Proline	−0.1510	−0.0997	−0.1580	0.1586	−0.0779	−0.1119	1
0.5 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.9692***	1					
	%MC	0.8837**	0.7677*	1				
	%DM	−0.8838**	−0.7697*	−1.000***	1			
	T-AsA	−0.3889	−0.2668	−0.4450	0.4445	1		
	AsA	−0.4437	−0.3489	−0.5353	0.5348	0.9474**	1	
	Proline	−0.4920	−0.4995	−0.2918	0.2915	0.7421*	0.6802*	1
1.0 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.6296	1					
	%MC	0.8086**	0.0943	1				
	%DM	−0.8165**	−0.1016	−0.9984***	1			
	T-AsA	−0.1020	−0.0028	−0.0815	0.1096	1		
	AsA	−0.2123	−0.3741	0.0182	0.0049	0.8671**	1	
	Proline	−0.3062	−0.5166	0.0397	−0.0360	−0.0458	0.3514	1
1.5 dS·m^{-1}	Fresh weight	1						
	Dry weight	0.6449	1					
	%MC	0.6686	−0.0763	1				
	%DM	−0.6685	0.0765	−1.000***	1			
	T-AsA	−0.8680	−0.8333	−0.3945	0.3944	1		
	AsA	−0.7501*	−0.7763*	−0.1968	0.1966	0.7527*	1	
	Proline	−0.3970	0.0382	−0.5932	0.5931	0.1183	0.3700	1

Order of significance is represented by *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$.

temperature and light could have been attributed to differences in vitamin C between trials. Due to seasonal changes, there was likely an effect of PAR (photosynthetically active radiation) and temperature on plants. The effects of PAR and temperature on the germination of seeds largely depend on the variety of the seed. For example, Motsa et al.[33] found that the best temperature for germinating amaranth, non-heading Chinese cabbage, and pumpkin was between 29 °C and 32 °C. However, this temperature increased for cowpea to 36 °C. In addition, amaranth, Jew's mellow, and cowpea did not respond positively when germinated with light. Furthermore Kalisz et al.[34] found that chilling broccoli seedlings after sprouting for one to two weeks at temperatures of 6, 10, 14, and 18 °C caused a decrease in L-ascorbic acid at all temperature levels. In conclusion that temperature effects vitamin C content in seedlings.

Furthermore, T-AsA positively correlated with % DM. This relationship could be partially explained through the biosynthesis and function of ascorbic acid in the form of ascorbate oxidase located in the wall of a plant cell. Smirnoff & Wheeler[35] have reported that high cell wall ascorbate oxidase activity is correlated to high cell expansion which resulted in increased biomass.

In conclusion, broccoli microgreens, eustress was induced by increased salinity treatments. As a result, there was an increase in proline.

Purslane

Purslane is often cultivated in saline environments as it is considered to be a halophyte[36]. Salts have been known to stimulate halophyte seedling germination, seedling growth, and cause increases in plant dry mass[37]. Eustress, however, was not experienced in purslane microgreens at the concentrations tested in this study, as there were no significant impacts on biomass, vitamin C, or proline when saline water was applied. The only significant impacts of salinity on purslane was seen in the 1.0 $dS \cdot m^{-1}$ treatment in trial 1, where dry weight of microgreens was increased illustrating the beneficial impacts of low levels of salinity[37]. Teixeira & Carvalho[38] also reported minimal effects of low salt concentrations on tissue production in mature purslane. Moreover, purslane biomass only began to decrease due to salinity in treatments at or above 6.8 $dS \cdot m^{-1}$ NaCl.

Additionally, the highest salinity treatment (1.5 $dS \cdot m^{-1}$ NaCl) AsA negatively correlated with fresh weight and dry biomass (Table 5). Salinity is a known stressor to crops as it decreases growth and plant productivity[39]. A likely explanation for the correlation above is that the stress caused by salinity affected plant productivity which then induced a salinity tolerance[40,41]. However, the stress experienced due to the salinity treatments was not enough to cause any significant changes in purslane microgreens. In comparison to the results shown in broccoli microgreens, for eustress to be achieved, purslane will likely require higher salinity levels then the treatments applied.

In purslane microgreens, eustress was not induced by increased salinity treatments. There was no effect on fresh weight, vitamin C, or proline.

CONCLUSIONS

While mild salinity stress can increase proline concentrations in broccoli microgreens, yields and vitamin C concentrations varied with treatments. Similarly, mild salinity did not induce eustress in purslane microgreens, which is likely due to its halophytic nature. Future research needs to be conducted to determine the lowest salinity tolerance possible for microgreen eustress under mildly saline conditions.

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Conflict of interest

Catherine Simpson is the Editorial Board member of journal *Technology in Horticulture*. She was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and her research group.

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