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Effect of Biochemical and Physiological Response to Salt Stress in Camelina Sativa

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A**BSTRACT**

Camelina sativa is a promising low-input, high yielding biodiesel crop that can be suitable to grow on marginal lands and is a good source of omega-3 fatty acids. The objective of the study was to compare the rate of growth in different varieties of C. sativa in response to salt stress. Three biological replicates were collected from three varieties of C. sativa grown in vitro in a controlled growth room. The Blaine Creek, Cheyenne and Suneson varieties of C. sativa were analyzed for their physiological and biochemical response to salt stress using the following criteria: germination rates, measurement of length, dry weights, fresh weights, and stomatal size. The biochemical measurements focused on proline, chlorophyll, and flow cytometry analyses. Blaine Creek's growth during salt stress was overall the most affected, while Suneson and Cheyenne generated higher amounts of biomass, thus indicating that C. sativa can be grown on saline soils.

Keywords: Camelina sativa, Salt Stress, Proline, Germination, Chlorophyll

INTRODUCTION

Salinity is steadily affecting more of the world's land area each day and it is necessary to devise strategies to remediate lands for cultivation, or alternatively, to breed plants that can survive the osmotic and ionic stresses of increased salinity. Indeed, a global study of land use over the past 45 years found that 6% of the world's land under cultivation, 77 million out of 930 million, had become salinized (Munns et al. 2002). This problem extends to developed nations such as the United States. Massachusetts' study reports that sodium based compounds used to de-ice roads, caused distant dependent damage to plants along the roads as a function of salt concentration (Mills et al. 2001). Furthermore, it was found in California that poor surface irrigation water can also exacerbate the problem, bringing in 300 to 800 mg/L of total salts (Backlund et al. 1984).

In addition to sodium chloride (NaCl) induced osmotic and ionic stresses, it has been reported that the resistance to the diffusion of water vapor through a plant's stomata increases salinity that results in closure (Turan et al. 2009). Although closing of stomata prevents water loss, the stomata can no longer mediate gas exchange with atmospheric carbon dioxide (CO_2) efficiently, if at all. In consequence, Rubisco-mediated CO_2 fixation is compromised leading to a reduction in plant biomass and growth. Complementing these observations, it is reported that stomatal closure resulted in net decrease of photosynthesis in salt sensitive rice strains (Wang et al. 1988). It was concluded that the reduction in photosynthetic rate was due to damage of the

photosynthetic apparatus and diminished CO_2 diffusion (Wang et al. 1988).

Equally important, photosynthesis is also inhibited by the salt induced degradation of chlorophyll. It has been speculated that there is a strong correlation between decreased chlorophyll content, salt induced weakening of protein-pigment-lipid complex, and/or increased chlorophyllase enzyme activity (Turan et al. 2009). Since stomatal resistance has a profound effect on plant growth, complete characterization of stomatal development would be in order. Stomatal size has already been shown to be a concern as it relates to gas exchange (Doheny-Adams et al. 2012). Specifically, species that have high numbers of smaller stomata may serve as an efficient way of maximizing gas exchange and preventing CO_2 starvation, while smaller amounts of larger stomata in other species may express an alternative metabolic cost saving measure.

Any successful understanding that seeks to either prevent or remediate plant damage following salt stress must in part focus on those evolved defense responses necessary to maintain homeostasis (Sobhanian et al. 2010). Proline has been demonstrated an essential molecule necessary for cell life, following either biotic or abiotic stress (Szabados and Savouré, 2009). This amino acid has been shown to accumulate following exposure to high salinity, as well as high light ultra violet (UV) and drought, in addition to biotic stressors. Besides serving as an indicator of environmental stress, proline has also been proposed to serve as a stabilizing osmolyte (Woodward and Bennett, 2005). This amino acid has been shown to act as a scavenger of reactive oxygen species (ROS), thereby protecting plants from oxidative stress.

Salinity continues to be one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigor and crop production. Salinity has been a threat to the agricultural crops and continues to effect 20% of all cultivated land (Pitman and Läuchli, 2002). The poor water quality of irrigation channels continues to jeopardize crop yield leading to a substantial financial loss. As a result, strategies are currently being developed to improve crop yield by increasing salt tolerance in plants through the use of biotechnology. Salt-stress genes are being identified using molecular marker-based breeding strategies. Current research efforts are focused toward identifying salt tolerance determinants and the network effects on plant growth and development.

Nacl Causes both Ionic and Osmotic Stress

The apoplast in plants is the free diffusional space outside the plasma membrane including the cell wall, intracellular spaces, and the space within dead structures such as xylem and phloem. Apoplasts with high salt concentrations result with primary and secondary effects that can negatively impact the plant's survival, growth, and development. The primary effects presented by highly concentrated apoplasts include ionic toxicity, disequilibrium, and hyperosmolality. At high concentrations, Sodium (Na^+) and Chloride (Cl^-) can inhibit cytosolic and organellar activity, therefore proper concentrations are required for normal cellular activity (Jenks et al. 2005). As concentrations of Na^+ and Cl^- increase to 0.4 M or greater, enzymatic activities are inhibited due to change in the protein structure (Jenks et al. 2005). The structure of a protein is dependent on hydrophobic-electrostatic balance, which is disturbed at high concentrations of Na^+

and Cl^-, therefore appropriate Na^+ and Cl^- concentrations are essential. Additionally, at low concentrations of 0.1 M, Na^+ can be toxic to cells; this is an indication of its direct effect on physiological and biochemical activity (Jenks et al. 2005). In addition to ionic toxicity, high salt concentrations can lead to a decrease in water potential causing a low state of turgidity, also known as hyperosmotic shock. A reduction in turgor impedes cellular expansion. A significant decrease in apoplastic water potential leads to dehydration as water loss is increased with negative pressure.

Secondary Effects of Salt Stress

Fluctuation of NaCl introduces a manifold of secondary effects that include, but are not limited to, potassium (K^+) acquisition, membrane dysfunction, impairment of photosynthesis, generation of reactive oxygen species (ROS), and cell apoptosis (Jenks et al. 2005). Potassium is an essential plant nutrient and is required for plant growth and reproduction. Potassium plays a vital role in activation of enzymes, Adenosine triphosphate (ATP) production, osmo-regulation, protein synthesis, and photosynthesis by regulating the stomata. Sodium uptake is mediated by voltage-dependent channels and independent cation channels. Sodium competes with K^+ uptake through sodium-potassium co-transporters, particularly during imbalance ionic concentrations. At the appropriate concentration of sodium, a plant is functional and healthy at a molecular level, however, high sodium concentrations can lead to cell toxicity, which can be alleviated by Calcium (Ca^{2+}) via regulation of K^+/Na^+ selective accumulation (Guimarães et al. 2012). Additionally, hyperosmotic and ionic stresses can cause secondary metabolic effects that if left unaddressed, may impede plant growth and development. For example, salt stress causes

oxidative stress, one of many consequences, and increases ROS, such as superoxide radicals, hydrogen peroxide (H_2O_2), and hydroxyl radical, which are all produced in aerobic cellular processes during photorespiration (Esfandiari et al, 2007).

Plant Adaptations to Nacl Stress

Halophytic and glycophytic plants use various cellular processes to tolerate salt. Other plants that are not able to withstand salt stress develop adaptive morphological structures that allow them to avoid salt; some morphological structures include bladder on leaves that serve as salt sinks or reservoirs (Jenks et al. 2005). However, even plants that cannot tolerate salt proceed through conserved physiological processes upon exposure to salt conditions. Researchers are focused on understanding the mechanisms by which halophytes acquire in order to tolerate high saline environments. Studies have shown that in extreme halophytes, such as Salicornia and Sueada, many salt-adaptive features within the genes are responsible for stress tolerance (Jenks et al. 2005). Further understanding of salt-stress genes was attained from studies on wheat and tomato, and their halophytic relatives. Additionally, further research with glycophytes, such as tobacco and sorghum plants, proved that glycophytes have the capabilities to adapt to saline environments. Collective research concluded that all plants, halophytes and glycophytes, are able to tolerate and adapt to saline environments to an extent.

Camelina sativa is a hardy, herbaceous, and annual dicot C3 oilseed crop that has an efficient response to salt stress. It has been found to be grown best in northerly climates and can survive frost and freeze thaw cycles after emergence in late winter and early spring (Budin et al. 1995).

Moreover, C. sativa can be planted during fall to serve as a rotation crop over winter, such as winter wheat, helping the soil keep its nutrients (Putnam et al. 1993). In addition, Camelina is a short season crop that matures within 85 to 100 days, growing to a height ranging from 30 to 120 cm. Its seed production yields an average thousand seed mass (TSM) between 0.7 and 1.6 g, contingent on the variety and growing conditions (Putnam et al. 1993; Gehringer et al. 2006). Given its robust seed production and ability to grow on marginal lands, the objective of the present study is to investigate the physiological and biochemical responses of the three varieties of Camelina sativa to different intensities of salt stress.

MATERIALS AND METHODS

Plant Materials

The three varieties of C. sativa that were tested for salt stress are Blaine Creek, Cheyenne, and Suneson. All three strains were acquired from the University of Montana collection, and were chosen due to their high potential for biodiesel production. The ability of any of these Camelina strains to manage saline environments is of particular importance because its unique properties opens the possibility for its cultivation on marginal saline lands currently unsuitable for food production.

Media for Salt Stress

The basic medium consisted of Murashige and Skoog (Murashige and Skoog et al. 1962) with vitamins, supplemented with 3% sucrose and 0.7% agar. Prior to autoclaving, the media was adjusted to a final pH of 5.8. Salt stress was measured by the addition of NaCl delivered in increasing 50 mM increments. Six treatments with three replicates were tested ranging from 50 to 300 mM NaCl. The seventh treatment lacked salt and served as a control (Table 1). Each plate received 25 seeds and were used for estimation of proline and chlorophyll. The effect of salt concentration on morphology was performed at150 mM NaCl as the upper limit. Subsequently, the dry weights were measured at 24 and 48 hours (Table 2). The fresh weight and dry weight comparisons among the varieties under different salt stress conditions are shown in Table 2

Seed Sterilization and Germination

For each experiment, seeds of each variety were treated with tween-20 and sterilized sequentially with ethanol and bleach. The seeds were rinsed 10 to 15 minutes with water, and taken to a laminar flow hood (Labconco, Kansas City, MO). Under the hood, they were further treated with 70% ethanol for 5 min, subsequently rinsed three times with sterile deionized water (dH_2O), followed by a 10 min 25% bleach treatment, and finally rinsed 15 times with sterile dH_2O.

To initiate germination, the seeds were collected and transferred to media plates that were incubated in the dark at 28^{o}C for 48 hours. Following the incubation period, the plates were transferred and placed under fluorescent light for a 16/8 hour cycle. The seeds were kept for 3

weeks under these conditions while their germination progress was monitored.

Morphology Measurements

After 23 days, the fresh weight of the germinated plants was determined for each of the specified saline treatments. Each plant was then stripped of its seed coat and incubated at 37^{o}C for 48 hours, and its dry weight measured. Similarly after 23 days, the lengths of 20-30 seedlings were measured from the tip of the root to the tip of the furthest leaf.

Stomatal Measurements

Camelina sativa leaves were coated with a clear nail varnish on the abaxial side and were allowed to dry for approximately 15 minutes. Through a peeling process, the dried layer was isolated and placed onto a slide to measure the area of the 2 guard cells at 200x magnification. Stomata size was determined microscopically and measured using the ImageJ processing software. Nine to seventeen stomata were observed per leaf; two sets of measurements were taken for each saline treatment.

Chlorophyll Measurements

Chlorophyll measurements were performed according to the protocol established by Holden (1961) and were performed three times for each variety at each salt concentration.

Proline Measurements

The proline assay protocols, in addition to the preparation of reagents, are well known and are detailed in Bates (1973).

Statistical Analysis

A 2-way analysis of variance (ANOVA) test was performed on SPSS (Statistical Product and Service Solutions) using C. sativa variety, salt concentration, and a salt concentration variety interaction as independent variables and fresh weight, dry weight, length, or stomatal size as dependent variables. When interaction terms were significant, one-way ANOVAs were run to examine the effects of salt concentration on each dependent variable for each variety. A Tukey-Kramer test was also performed as a Post-Hoc test. P-values less than 0.05 were considered significant

RESULTS

Seed Germination

Germination was scored following 23 days on NaCl containing media, if either shoot or root development was observed. At concentrations of 200 mM NaCl and higher, the impact of salt stress on germination was profound (Figures 1A-1C). Irrespective of variety, shoot and root formation was virtually absent in 200 mM and higher salt concentrations. As indicated in Table 3, it may be concluded that Cheyenne expressed statistically significant reductions in

germination as compared to either Blaine Creek or Suneson at 250 mM and higher. In consequence, leaf material was harvested for the remaining experiments following germination at lower salt concentrations.

Morphology

For fresh weight, the 2-way ANOVA showed that variety (F_2=32.508), salt concentration (F_3=92.654), and interaction between variety and salt concentration (F_6=4.933) were significant for all variables (P<0.0001). Among the controls, Blaine Creek yielded significantly lower fresh weight mass than either Suneson or Cheyenne. Neither Suneson nor Cheyenne differed statistically from one another (Table 4a). In contrast, varietal fresh weight was significantly affected by salt concentration for Blaine Creek (F_3=44.514, P<0.0001), Cheyenne (F_3=37.331, P<0.0001), and Suneson (F_3=33.022, P<0.0001). Following the 50 mM salt stress, fresh weight increases were statistically significant, whereas at 100 mM and 150 mM concentrations resulted in significant decrease. Percent changes in plant dry weight as compared to fresh weight were evaluated for each variety following salt stress at 24 and 48 hours. Similarly, percent change in dry weight was calculated between control and NaCl concentrations.

Dry weight also differed across varieties (F_2=10.841, P<0.0001) and salt concentrations (F_3=5.845, P=0.001) and the variety salt concentration interaction was significant (F_6=3.444, P=0.003). The Blaine Creek variety had significantly lower dry weights as compared to the control; whereas the Cheyenne and Suneson varieties were not significantly different in their dry weights (Table 4b) when compared to the absence of salt stress. When individual analyses were conducted on each variety and salt concentration, it was found that Blaine Creek (F_3=31.928,

P<.0001) and Suneson (F_3=16.108, P<0.0001) dry weights were both impacted by salt concentrations. However, dry weights for Cheyenne did not differ across salt concentrations (F_3=2.297, P<0.081). For dry weight, Blaine Creek showed the same pattern as with fresh weight, whereas 50 mM concentration significantly increased dry weight by 36% relative to the control. Furthermore, 100 mM as well as 150 mM significantly decreased dry weight by 24-28% (Table 5), but dry weight at 100 mM and 150 mM were not statistically significant. For Suneson, the 50 mM NaCl treatment increased dry weight by 128% (Table 5), but the 100 mM and 150 mM treatments were not significantly different from the control, i.e. (100 mM, P=0.997, 150 mM, P=0.465 respectively).

Following a 24-hour treatment, Blaine Creek's dry weight dropped approximately 88.0 % at each salt concentration. This loss is comparable to what is observed with the control. No additional changes were observed at 48 hours. After 24 hours, the percent decrease of Cheyenne at 150 mM was found to be 67% and 74.4-83.6% at 0 mM, 50 mM, and 100 mM NaCl, respectively. Only Cheyenne in the control and at 50 mM NaCl treatment, expressed a statistically significant change at 48 hours, with each measuring an approximate 92% decrease in weight. Suneson articulated a 60-68% decrease in weight after 24 hours at 50 mM, 100 mM, and 150 mM NaCl, whereas a 76% decrease was observed in the control. After 48 hours, Suneson expressed 88-92% decrease in weight. The biggest decrease was observed between 24 and 48 hours at 50 mM NaCl. There was a 27- 42% increase in fresh weight for all three varieties at 50 mM NaCl and a 31-70% decrease at 100 mM and 150 mM when each treatment was compared to the control (Table 4c).

The length of the seedlings also varied across varieties (F_2=21.190, P<0.0001), salt concentrations (F_3=363.569, P<0.0001), and the variety salt concentration interaction was significant as well (F_6=10.412, P<0.0001). When the individual seed varieties were tested it was shown that the lengths of Blaine Creek (F_3=137.133, P<0.0001), Cheyenne (F_3=177.279, P<0.0001), and Suneson (F_3=89.029, P<0.0001) were all significantly reduced as a function of increased salt concentration (Table 5). In regards to length, only Suneson paralleled the pattern observed for fresh and dry weights, whereas Blaine Creek and Cheyenne steadily declined from reaching significance at 150 mM NaCl.

Stomatal Size

Overall, it was determined that stomatal size decreased with increasing salt stress for all three varieties. These results were consistent in independent trials (Figures 2A – 2F). Statistically significant changes in stomatal size are attributable to variety (F2=11.920, P<0.0001) and to salt concentration (F3=5.205, P=0.002), but not to a variety salt concentration interaction (F6=2.084, P=.058). Generally, Cheyenne maintained the largest average stomatal size followed by Blaine Creek and Suneson, respectively (Table 6). Following either 100 mM or 150 mM NaCl treatments, stomatal size was significantly reduced.

Chlorophyll Content

Although the three types of C. sativa differed with respect to varietal chlorophyll content, the amount of chlorophyll per dry weight of the leaves decreased with increasing salt concentration. The effect of NaCl on chlorophyll content was measured at 100 mM for Cheyenne and Suneson

and at 150 mM for Blaine Creek. The chlorophyll content of Cheyenne remained unchanged in response to 100 mM NaCl as compared to the control whereas both Blaine Creek and Suneson decreased significantly. When the molarity increased to 150 mM, chlorophyll content decreased significantly in Blaine Creek as compared to 100 mM (Table 7).

Proline Content

There was an overall increase in proline levels throughout all three varieties following the salt stress. Blaine Creek and Suneson exhibited greater increase in proline followed by Cheyenne, with Blaine Creek having the largest increase of all the varieties (Table 8). Three replications of proline content were performed using 150 mM NaCl on all plant Cheyenne at 150 mM where two were completed. At 50 mM NaCl, no significant increase in proline content was observed for either Cheyenne or Suneson over the control. In contrast, Blaine Creek expresses a substantial increase at 50 mM. No further significant changes occurred in proline content with respect to Blaine Creek following an increase of stress at 100 mM. At 150 mM, a profound increase in Blaine Creek was observed as compared to both 50 mM and 100 mM. These changes are even more remarkable when compared to the control. Blaine Creek proline levels at 150 mM are significantly increased as compared to Suneson, and each is amplified over the control. Indeed, under high salt stress, Suneson and Blaine Creek segregate themselves into defining populations with respect to proline content. Specifically, the amounts measured as μg/fresh weight are not only pointedly different from each other, but from the untreated population as well.

DISCUSSION

Changes in Growth due to Salt Stress

Morphological changes and inhibition of plant growth were observed with increasing salt stress. Although more Blaine Creek seedlings germinated, Cheyenne and Suneson had greater biomass and dry weight, indicating that these cultivars are more salt tolerant than Blaine Creek. Therefore, germination success did not necessarily correlate in our studies with the increase of plant growth and yield (see Figures 3A – 3C). It has been observed that β-conglycinin expression in salt stressed roots is higher when compared to non-stressed roots (Aghaei et al. 2009). Since β-conglycinin is degraded to provide nourishment for the soybean embryos and seedling, it is possible that salt stress interfered with the ability of Blaine Creek to use a similar protein or orthologs to acquire nourishment.

Camelina sativa is more salt tolerant than soybean when fresh weights among the cultivars are compared, and more tolerant than either maize or eucalyptus when dry weights are measured (See Tables 9a and 9b) (Woodward and Bennett et al. 2005; Turan et al. 2009). In contrast, the average length of all three varieties declined with increasing salt stress, with no significant difference between control and 50 mM NaCl treatments for Blaine Creek. There was a significant decline in the stomatal size at 100 mM and 150 mM NaCl treatments for all varieties (Figures 2A – 2F). At 50 mM NaCl, it is probable that Suneson would be the best variety to grow under saline conditions. Most notably, the seedlings had more overall fresh weight and biomass, and were longer at that concentration. It may be concluded that since multiple varieties

of C. sativa can grow on salinized land, these acreages are likely not suitable for either grains or legumes. Potential biofuel crops capable of growing on salt-stressed soil reduce the competition for land that should be in principle dedicated to food production.

Given C. sativa's high germination rates, fresh and dry weight changes should be compared to other candidate bioenergy crops for salt tolerance. Such comparisons would permit meaningful proteomic screenings that should, in principle, identify which proteins control germination and mediate biomass accretion, in addition to root and shoot elongation. Furthermore, protein profiling should delineate an understanding of how salt stress affects the expression of related growth proteins such as Proline Dehydrogenase (PDH), and Photosystem II type I chlorophyll a/b-binding protein.

Reductions in stomata size for each of the three C. sativa varieties, following the salt stress, are notable.

Specifically, it may be predicted that with a lessening of stomata size, there will be a concomitant cut in the rate of CO_2 intake, as well as a decrease in net carbon fixation with concomitant drops in nutrient and biomass accumulation (Turan et al. 2009). Overall, root growth decreased with increasing salt stress, paralleling what was observed with wild type wheat, which lacks the ability to compartmentalize Na^+ in specialized vacuoles (Xue et al. 2004). Indeed, root structures of transgenic wheat segregating sodium/hydrogen exchanger 1 Arabidopsis thaliana (AtNHX1) grow exceptionally well under salt stress because of efficient compartmentalization of Na^+ in the vacuole. AtNHX1 encodes vacuolar Sodium-hydrogen

(Na^+/H^+) antiporters and its overproduction allows for more efficient sequestration of Na^+ into the vacuoles (Xue et al. 2004).

Changes in Chlorophyll and Proline Levels

Chlorophyll levels decreased in all three C. sativa varieties. It is reported an approximate 8% chlorophyll loss, accompanied by decreases in CO_2 assimilation and transpiration rates, following the salt stress to maize cultivar Helix (Lohaus et al. 2000). It is also noted that a decrease in chlorophyll levels, in maize leaves and seedlings, is coincident with increasing salt concentration and limited osmotic potential in addition to structural damage to the chloroplasts (Cha-Um and Kirdmanee et al. 2009). Furthermore, the decline in chlorophyll content paralleled the decline of the net rate of photosynthesis in Z. Mays L. cv Ceratina, which is a salt tolerant maize cultivar (Cha-Um and Kirdmanee et al. 2009). Therefore, C. sativa's poorly developed chloroplasts, coupled to reduction in stomatal size and closure, resulted in an overall reduction in net photosynthesis and subsequent plant growth. Table 10 compares the chlorophyll levels between C. sativa and the maize cultivar Saccharata.

Conversely, while chlorophyll levels decreased, proline amounts increased with the increasing salt stress. Although Blaine Creek expressed the highest proline levels under salt stress, its growth was most negatively affected by salt stress. Similarly, fresh weight of the plant increased following an increase in proline levels in all of the three varieties tested at 50 mM NaCl. This indicates that proline may serve as an osmotic protector, albeit at limited concentrations. In excess of 50 mM NaCl, proline no longer serves as an osmotic stabilizer for C. sativa, but rather as a stress indicator since the levels of this amino acid decline (Bashir et al. 2014).

CONCLUSION

Camelina sativa has already shown great potential as a biodiesel crop that can grow on salt-stressed soils. Our studies indicated that Blaine Creek yielded the highest germination rates, while maintaining the largest biomass under salt stress. Suneson is also worth considering precisely because several individual seedlings proved hardy and were able to grow robustly under NaCl stress at 300 mM. This wide range of response associated with Suneson suggests that in segregating populations, lines of Suneson could be identified capable of sustaining vigorous growth under high salt stress. Cheyenne produced plants that had the largest amount of dry weight and stomatal size, whereas Blaine Creek expressed the lowest overall biomass. In order to harness C. sativa effectively for biofuel use, especially in light of increasing salinity and other degradation of once arable lands, C. sativa must be able to adapt. It must be able to grow and thrive in the new saline climates as while maintaining or bettering its oil content. Finally, targeted genetic manipulations of C. sativa may also allow it to boost its oil yield for biodiesel use.

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Table 1: Amount of Salt Added to Treatments (grams of NaCl per 500ml media)

Salt Concentration in Media	Salt Amount
0 mM (control)	0 g
50 mM	1.461 g
100 mM	2.922 g
150 mM	4.383 g
200 mM	5.844 g
250 mM	7.305 g
300 mM	8.766 g

Table 2: Percent difference of fresh weight as compared to dry weight after 24 and 48 hours.

Treatment	BC (24h)	BC (48h)	CH (24h)	CH (48h)	SU (24h)	SU (48h)
0 mM						
Control	89.92 %	90.11 %	83.62 %	91.73 %	76.14 %	91.59 %
50 mM	90.59 %	90.53%	81.87 %	92.14 %	60.79 %	86.26 %
100 mM	88.82 %	89.14 %	74.44 %	74.55 %	63.16 %	88.42 %
150 mM	89.42 %	89.66 %	67.90 %	67.91 %	67.10 %	90.27 %

Table 3: Percentage of geminated seeds after the 21 day incubation period

Treatment	BC	CH	SU
0 mM NaCl (control)	100 %	100 %	100 %
50 mM NaCl	100 %	96 %	100 %
100 mM NaCl	100 %	100 %	100 %
150 mM NaCl	96 %	92 %	100 %
200 mM NaCl	88 %	92 %	100 %
250 mM NaCl	88 %	36 %	100 %
300 mM NaCl	68 %	24 %	100 %

Table 4A: The average fresh weight of each of the three *Camelina sativa* varieties.

Treatments	**BC**	**CH**	**SU**
0 mM NaCl (control)	0.0421 g	0.0803 g	0.07092 g
50 mM NaCl	0.0599 g	0.1026 g	0.09514 g
100 mM NaCl	0.0288 g	0.0419 g	0.04676 g
150 mM NaCl	0.0248 g	0.0236 g	0.03592 g

Table 4B: The average dry weight of each of the three *Camelina sativa* varieties.

Treatments	**BC**	**CH**	**SU**
0 mM NaCl (control)	0.0042 g	0.0066 g	0.0057 g
50 mM NaCl	0.0056 g	0.0081 g	0.0131 g
100 mM NaCl	0.0031 g	0.0107 g	0.0054 g
150 mM NaCl	0.0026 g	0.0076 g	0.0035 g

Table 4C: Percent change in fresh weight (FW) and dry weight (DW) between control and NaCl treatments.

Treatment	50 mM FW	100 mM FW	150 mM FW	50 mM DW	100 mM DW	150 mM DW
BC	+42.05 %	-31.56 %	-41.05 %	+36.05 %	-24.86 %	-38.37 %
CH	+27.76 %	-47.79 %	-70.62 %	+21.39 %	+60.68 %	+13.99 %
SU	+34.14 %	-34.07 %	-49.34 %	+128.29 %	-5.41 %	-38.95 %

Table 5: The average lengths (cm) of each of the three *Camelina sativa* varieties.

Treatments	**BC**	**CH**	**SU**
0 mM NaCl (control)	12.287 cm	11.167 cm	9.070 cm
50 mM NaCl	13.187 cm	9.353 cm	12.220 cm
100 mM NaCl	5.803cm	4.703 cm	5.607 cm
150 mM NaCl	3.355 cm	2.550 cm	3.300 cm

Table 6: The average stomata size/ area (um^2) of each of the three *Camelina sativa* varieties.

Treatment	BC	CH	SU
0 mM NaCl (control)	27.858 um^2	41.888 um^2	18.909 um^2
50 mM NaCl	29.377 um^2	28.089 um^2	25.185 um^2
100 mM NaCl	23.753 um^2	27.127 um^2	15.645 um^2
150 mM NaCl	15.241 um^2	26.709 um^2	16.593 um^2

Table 7: The average total chlorophyll content (mg of chlorophyll /g of fresh weight) of each of the three *Camelina sativa* varieties.

Treatment	BC	CH	SU
0 mM NaCl (control)	1.451 mg/g	1.445 mg/g	1.085 mg/g
50 mM NaCl	0.973 mg/g	1.327 mg/g	1.065 mg/g
100 mM NaCl	1.1684 mg/g	1.298 mg/g	0.888 mg/g
150 mM NaCl	0.859 mg/g	0.836 mg/g	0.815 mg/g

Table 8: The average proline concentration (ug of proline/ 0.5g of fresh weight of plant) of each of the three *Camelina sativa* varieties.

Treatment	**BC**	**CH**	**SU**
0 mM NaCl (control)	51.732 ug/.5g	76.258 ug/.5g	39.826 ug/.5g
50 mM NaCl	139.475 ug/.5g	49.222 ug/.5g	86.356 ug/.5g
100 mM NaCl	171.699 ug/.5g	210.529 ug/.5g	94.632 ug/.5g
150 mM NaCl	335.143 ug/.5g	111.736 ug/.5g	217.086 ug/.5g

Table 9A: Fresh weight (FW) comparisons of each of the three *Camelina sativa* varieties to Maize (Saccharata) so soybean.

C. sativa **Species**	**FW control**	**FW 100mM NaCl**	**% FW Comparisons**
BC	0.0422 g	0.0289 g	-31.56 %
CH	0.0804 g	0.0420 g	-47.79 %
SU	0.0709 g	0.0468 g	-34.07 %
Soybean	0.1300 g	0.0630 g	-51.54 %

Table 9B: Dry weight (DW) comparisons of each of the three *Camelina sativa* varieties to Maize (Saccharata) to maize and eucalyptus.

C. sativa **Species**	**DW control**	**DW 100mM NaCl**	**% DW Comparisons**
BC	0.0043 g	0.0032 g	-24.86 %
CH	0.0132 g	0.0107 g	+60.68 %
SU	0.0169 g	0.0172 g	-5.41 %
Maize	25.3600 g	13.8300 g	-45.47 %
Eucalyptus [a]	0.0510 g	0.0350 g	-31.37 %

Table 10: Chlorophyll Comparisons (mg of chlorophyll /g of fresh weight) of each of the three *Camelina sativa* varieties to Maize (Saccharata).

***C. sativa* Species**	**Control**	**100 mM NaCl**
BC	1.45 mg/g	1.17 mg/g
CH	1.45 mg/g	1.30 mg/g
SU	1.08 mg/g	0.88 mg/g
Maize (Saccharata)	288.2 ug/g	208.4 ug/g

Figure 1A: Germination of *C. sativa* Blaine Creek (BC) under different salt concentrations (seeds germinated 21 days after sterilization seeds).

Figure 1B: Germination of *Camelina sativa* Cheyenne (CH) under different salt concentrations (seeds germinated 21 days after sterilization seeds).

Figure 1C: Germination of Camelina sativa Suneson (SU) under different salt concentrations (seeds germinated 21 days after sterilization seeds).

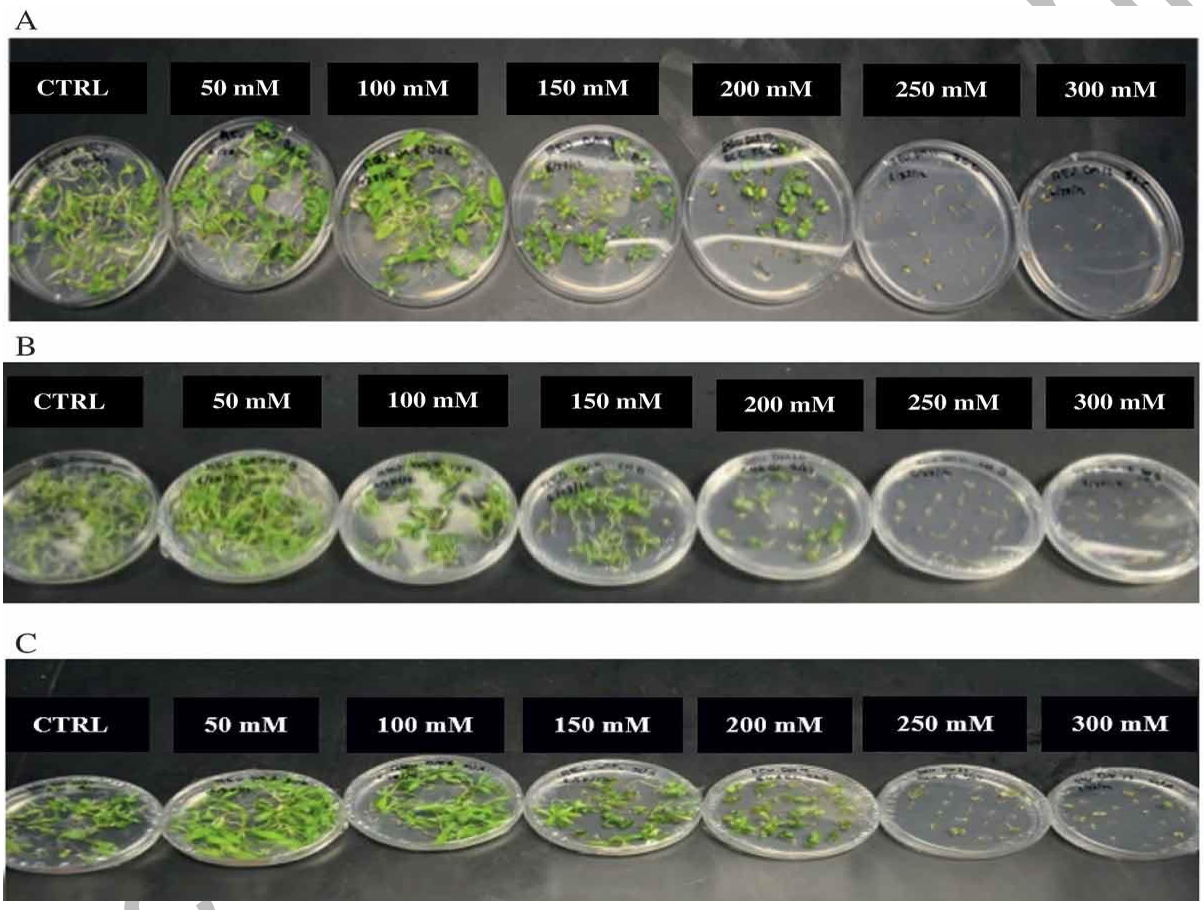


Figure 2A. Stomata of *C. sativa* Blaine Creek (BC) at 0 mM NaCL

Figure 2B. Stomata of *C. sativa* Blaine Creek (BC) at 150 mM NaCL

Figure 2C. Stomata of *C. sativa* Cheyenne (CH) at 0 mM NaCl

Figure 2D. Stomata of *C. sativa* Cheyenne (CH) at 150 mM NaCl

Figure 2E. Stomata of *C. sativa* Suneson (SU) at 0 mM NaCl

Figure 2F. Stomata of *C. sativa* Suneson (SU) at 150 mM NaCl

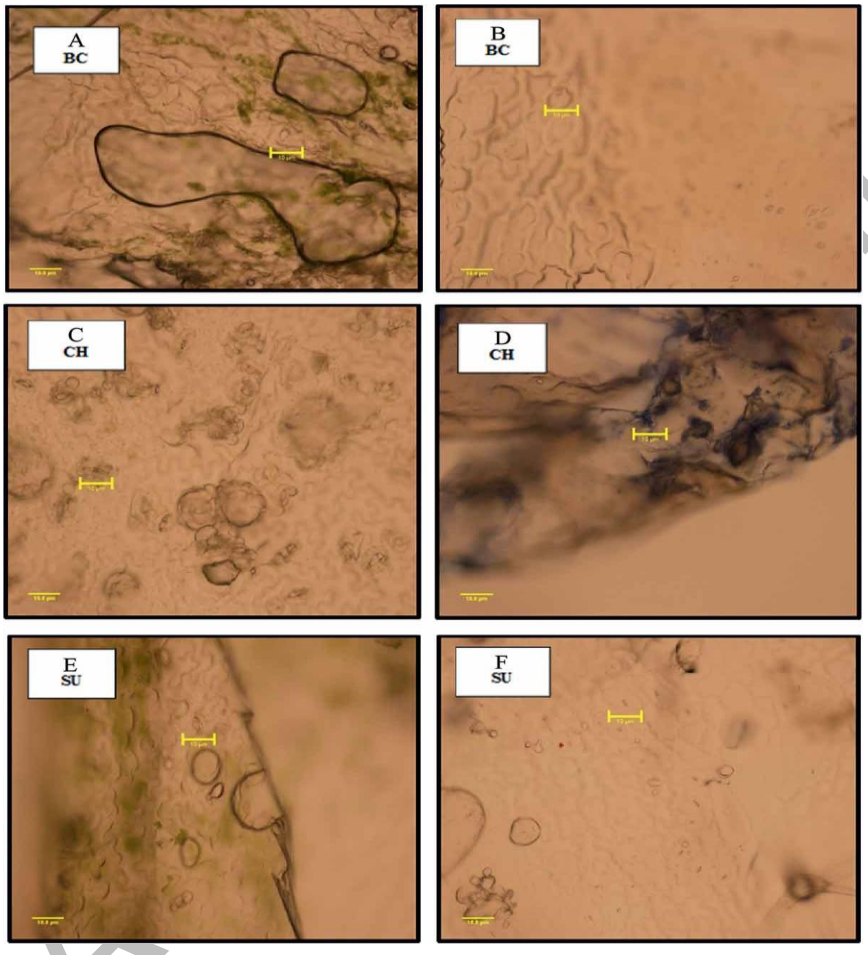
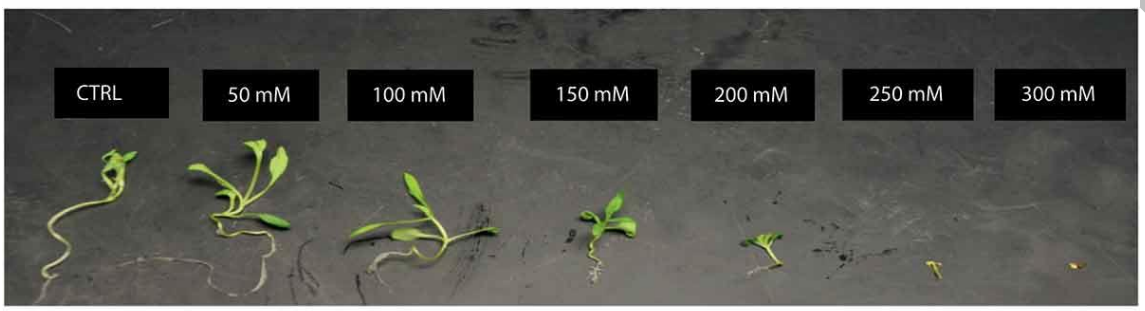


Figure 3A: Shown below are the individual shoots of *C. sativa* Blaine Creek (BC) under different NaCl concentrations varying from 0mM to 300 mM NaCl.

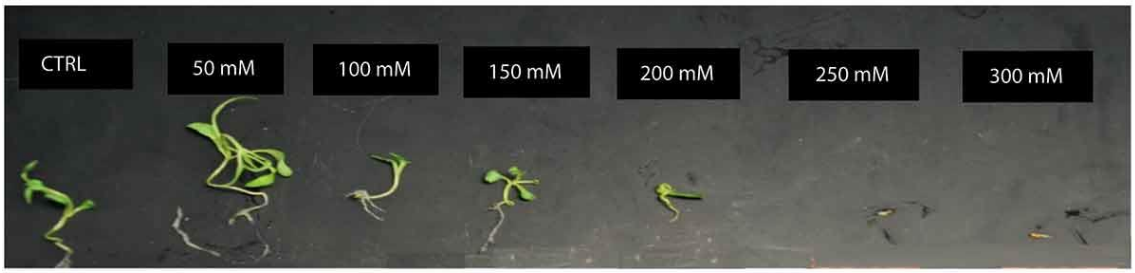
Figure 3B: Shown below are the individual shoots of *C. sativa* Cheyenne (CH) under different NaCl concentrations varying from 0mM to 300 mM NaCl.

Figure 3C: Shown below are the individual shoots of *C. sativa* Suneson (SU) under different NaCl concentrations varying from 0mM to 300 mM NaCl.

A



B



C