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The role of temperature on the germination activity of leguminous crops exposed to saline conditions

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Abstract— Germination is an important starting point of plant life. Abiotic stresses during the germination stage in seeds can threaten the development process of a plant species. Abiotic factors such as temperature and salt concentration influence the germination process of various crop seeds, including leguminous species. The aim of this study is to determine the germination rate and seedling growth of leguminous cover crops under two different temperatures and four levels of salt stress. Alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), and chickpea (*Cicer arietinum*) were studied in this in vitro trial. The study results showed that the increase in sodium chloride (NaCl) concentration suppressed the growth of the germinated seedlings. At the same time, the increase in temperature reduced the germination rate of red clover and chickpea at higher salt concentrations. The data also showed a significant relationship between salt concentration and temperature on shoot and radicle growth in all three leguminous species. These data may benefit farmers and growers trying to cultivate these crops in unfavorable conditions.

Key-words: temperature stress, salt stress, germination, seedlings development, leguminous crops.

1. Introduction

Seed germination is a biological process influenced by biotic and abiotic factors, including water, oxygen, and temperature, to achieve successful germination. A combination of conducive environmental factors and various cellular processes will allow physiological and morphological changes within the seed, resulting in the activation of the embryo. Under optimal temperature, germination starts as the seed absorbs water (seed imbibition), resulting in expansion of the seed and elongation of the embryo (*Tina* and *Leubner-Metzger*, 2017). During germination, starch stored in the endosperm of the seeds is converted to soluble sugars such as sucrose through combined actions of enzymes such as α-amylase, β-amylase, and α-glucosidase. Favorable growing conditions will allow adenosine triphosphate (ATP) regeneration hence, allowing activation of hormones and enzymes responsible for germination, such as abscisic acid (ABA), gibberellins, ethylene, and auxin (IAA) (*Joshi*, 2018). Germination ends when the radicle has grown out of the seed's coating layers and the emergence of the coleoptile protrusion (*Miransari* and *Smith*, 2014).

Abundant studies show that abiotic stresses can affect plant growth at all stages, with stresses during plants' reproductive stage greatly influencing the yield produced. *Gyuricza et al*., (2012) showed that extreme precipitation levels could affect the yield value, protein content, and starch content in wheat and maize grains. At the point of limited water availability for the plant roots, the transpiration rate started to decrease, decreasing biomass produced and simultaneously affecting the yield produced (*Nagy* and *Ján*, 2006). However, stresses inflicted in the early development stage, including germination and seedling development, will significantly affect crop yield. Under abiotic stress conditions, seeds will undergo secondary dormancy, which will prevent seed germination from occurring in unfavorable conditions. This protective mechanism acts as a survival strategy to prevent the emerging seedling from dying or failing to develop further until the reproductive stage (*Miransari* and *Smith*, 2014). Once favorable soil condition is achieved, secondary dormancy will be terminated, and the germination process starts.

According to *Kaymakanova* (2009), high salinity in soil solutions will result in high osmotic pressure that restricts the seed imbibition by preventing water absorption and entry into the seed. In Hungary, about 10% of the soil is classified as strong salinity and alkalinity. The salt contributes to the formation of a cemented, impermeable soil surface which limits the water infiltration into a deeper soil layer (*Várallyay,* 2008). The inability to absorb water due to high salt concentration will also prevent the mobilization of essential nutrients needed for germination. Besides that, a high saline condition during the early growth stage also caused sodium ions (Na^+) and chloride ion (Cl^-) toxicity to the embryo and young seedlings resulting in stunted development of the plants (*Khajeh-Hosseini et al*., 2003; *Kaymakanova*, 2009).

Salt stress caused by NaCl also decreases the content of essential hormones for germination, such as gibberellins, while increasing the ABA levels (*Atia et al.,* 2009). *Shu, et al.,* (2017) also observed the same effect in soybean seed germination, which is delayed by the reduced GA/ABA ratio. Furthermore, a study also shows that salt stress reduces the germination rate by negatively affecting the seeds' nitrogen content, thus reducing the amino acids and protein biosynthesis. Hence, nitrogen compound application in areas with salt stress is suggested to alleviate the side effects of high salinity to the crop (*Atia et al.,* 2009). Another study also suggests fluoride as a potential plant growth regulator that works by inhibiting ABA biogenesis and stimulating soybean seeds' germination under high saline soil (*Shu et al*., 2017).

Climate change is one of the main concerns in the agriculture industry nowadays. Temperature rises around the globe affects food production all around the world. Analysis at the Carpathian Region in Central and Eastern Europe shows an increased temperature pattern in large part of this region within the 49 years of the study conducted. The increase in the yearly frequency of warm nights caused the growing season to start earlier in the majority area of this region (*Lakatos et al.,* 2016). Meanwhile, studies show that temperature elevation can cause thermoinhibition in seed germination. A study on Nigeria's main crops, including maize, rice, and sorghum, shows the impact of increases in temperature on seed germination and seedling development. The high temperature also caused a significant reduction in the length of sorghum stem and leaf length reduction in rice (*Iloh et al*., 2014). In *Arabidopsis thaliana*, high temperature causes B3-domain transcription factor FUSCA3 (FUS3) to accumulate during seed imbibition. Overexpression of FUS3 protein generates a seed that is hypersensitive to a higher optimal temperature, thus preventing germination through de novo synthesis of ABA. Consequently, this will cause the seed to maintain or lead to secondary seed dormancy hence, inhibiting germination and vegetative development (*Chiu et al*., 2012).

It is common for one agricultural land to handle more than one abiotic stress simultaneously. A combination of two or more abiotic stresses will aggravate the impact of each stress, and farmers may need to spend more input to remove or lessen the impacts. High temperature, simultaneously with salt stress, is a common condition, especially in semiarid and arid regions in the world (*Shahid et al*., 2018). High temperatures caused more damaging effects on germination under salinity stress compared to at optimum temperatures. It was observed that the combination of these stresses reduced the germination rate, shoot length, and dry weight of wheat seedlings compared to the effects of one stress alone. The combined stresses also reduced the photosynthetic rate in wheat crops due to a decline in pigmentation (*Neelambari et al*., 2018). *Luan et al.,* (2014) show that a higher rate of germination occurs in sunflower seeds at lower alternating temperatures (10–20 °C) than at higher alternating temperatures (20–30 °C) under the same salinity conditions induced by NaCl. The high temperature may have increased the moisture evaporation causing salt content elevation by capillary movement and slowing down the activation of metabolic processes, thus reducing the activity of different enzymes responsible for germination (*Luan et al*., 2014).

Therefore, this study was conducted to determine the effect of different salt concentrations and temperatures on the germination and growth of various legume species. The relationship between these two stresses on the germination and development of these crops may benefit farmers and further research.

2. Materials and methods

The trial was conducted in the laboratory of the Crop Production Institute of the Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary. For this trial, three species of leguminous crops; alfalfa, chickpeas, and red clover were chosen. The seeds were germinated at four different saline treatments and two different temperatures. Sodium chloride (NaCl) was diluted with distilled water to produce 0.5%, 1%, 1.5%, and 2% salt solution. 0% solution consisting of distilled water was used as the control. Seeds were surface sterilized with 5% Hypo solution for 5 minutes and rinsed with distilled water three times. All seeds were germinated on 13.5 cm petri dishes containing single layer Whatman filter paper. Memmert type climatic chamber at 70% moisture was used to control the growing temperature at 10 °C and 20 °C.

Each treatment was repeated four (4) times with each petri dish was filled with 20 seeds except for chickpeas with only 10 seeds per petri dish. All seeds were allowed to germinate at each treatment for 10 days. The number of seeds germinated was counted, and the length of plumule and radicles developed were measured using a ruler on the 10th day after the treatment started. The data collected were analyzed using Microsoft Excel 2010 for charts, while IBM SPSS Statistics 27 was used for the analysis of variance (ANOVA).

3. Results

3.1. Germination percentage at different temperatures and salinity levels

In general, the germination rate of all three cover crop species decreases as the saline concentration increases in both temperature conditions. Moreover, the increase of temperature from 10 °C to 20 °C improves the germination of all three species germinated in 0.5% and 0% saline solutions. However, in 1% saline solution, red clover and chickpea seeds showed a 10.1% and 15% higher germination rate at 10 °C than at 20 °C (*Figs. 1* and *2*). *Fig. 3* showed that only alfalfa seeds germinated at 1.5% NaCl at 20 °C, and all three crops failed to germinate at 2% NaCl. Based on the germination rate, it can be concluded that alfalfa had the highest salt tolerance, followed by red clover, and chickpeas had the lowest tolerance as the salt concentration increased. The data also showed no significant difference between the germination rate at 0% and 0.5% NaCl for both red clover and chickpeas.

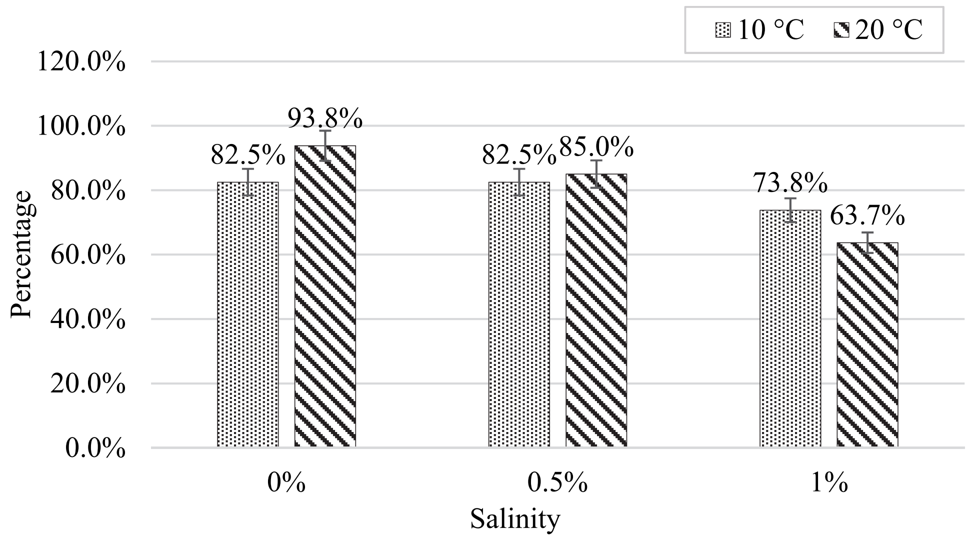

Fig. 1. Germination rate of red clover at 10 °C and 20 °C.

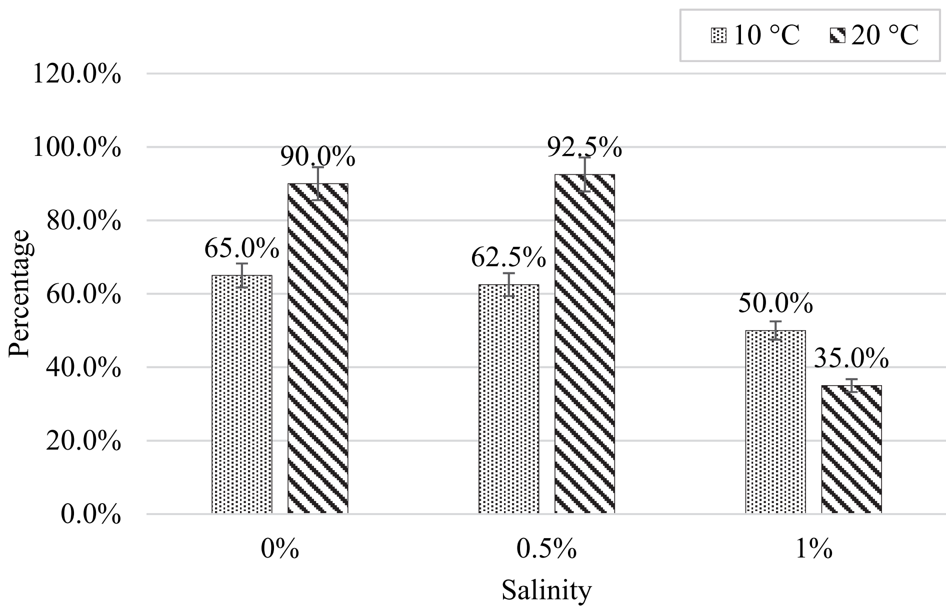

Fig. 2. Germination rate of chickpeas at 10 °C and 20 °C.

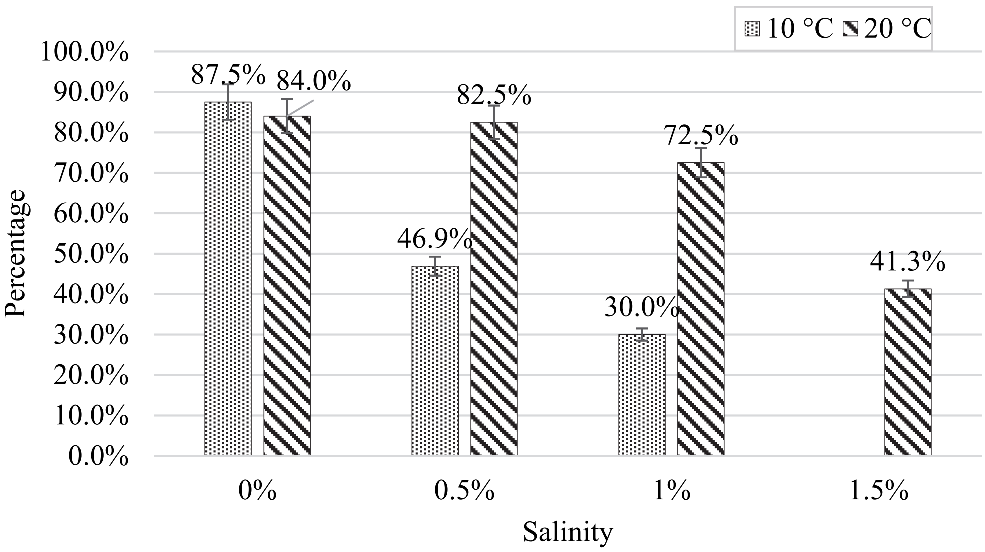

Fig. 3. Germination rate of alfalfa at 10 °C and 20 °C.

Analysis of variance in *Table 1* showed that there was a highly significant difference in the germination rate between groups of cover crop species $F(2, 1279) = 19.084$, at $p<0.001$. *Table 2* shows a significant difference in germination rate between red clover and chickpeas and between alfalfa and red clover with $p<0.001$. In contrast, there was no significant difference in germination rate between chickpeas and alfalfa with $p=0.784$.

Table 1. Analysis of variance for germination rate of seeds between crop species

	Sum of squares	**df**	**Mean square**	**F**	**Sig.**
Between groups	7.771	2	3.886	19.084	<0.001
Within groups	260.404	1279	0.204		
Total	268.175	1281			

Table 2. Post hoc test of germination rate between species

	(I) Cover crop	**(J) Cover crop**	**Mean difference (I-J)**	**Std. error**	**Sig.**
Tukey HSD**	Red clover	Chickpeas	0.144*	0.036	<0.001
		Alfalfa	0.167*	0.028	<0.001
	Chickpeas	Red clover	-0.144*	0.036	<0.001
		Alfalfa	0.023	0.035	0.784
	Alfalfa	Red clover	-0.167*	0.028	<0.001
		Chickpeas	-0.023	0.035	0.784

* The mean difference is significant at the 0.05 level

** Turkey's honest significance test

3.2. *Radicle and shoot lengths in different salt concentrations*

The radicle and shoot lengths were measured and recorded after 10 days. *Figs. 4* and *5* showed that the increase in salinity suppressed both radicle and shoot growths in all cover crop species. Alfalfa at 20 °C produced longer radicles and shoots average at 0.5%, 1.0%, and 1.5% NaCl concentration. In contrast, chickpeas produced the shortest radicles and shoots average in both temperatures compared to the other crops. Analysis of variance (*Table 3*) showed a highly significant difference of both radicle length $F(3, 1278) = 63.296$ and shoot length $F(3, 1278) = 170.707$ developed under the different salt concentrations with $p<0.001$.

However, there was no significant difference in radicle length developed between 1.0% and 1.5% NaCl. The data was supported by the post-hoc test carried out, as shown in *Table 4* with $p=0.285$. *Fig. 5* showed the same decreasing pattern on shoot length as salt concentration increased. There was significant difference in shoot development in all salt concentrations except between 1% and 1.5% NaCl, which was proved by the post-hoc test with $p=0.704$ (*Table 4*).

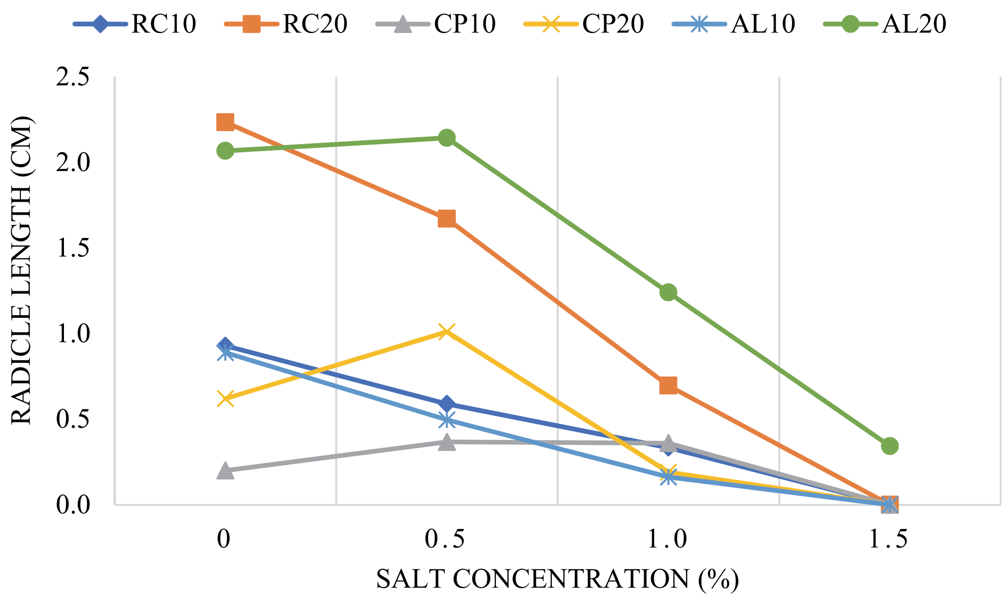

Fig. 4. Mean of radicle length developed after 10 days. RC: Red clover, CP: Chickpeas AL: Alfalfa, 10: 10 °C and 20: 20 °C.

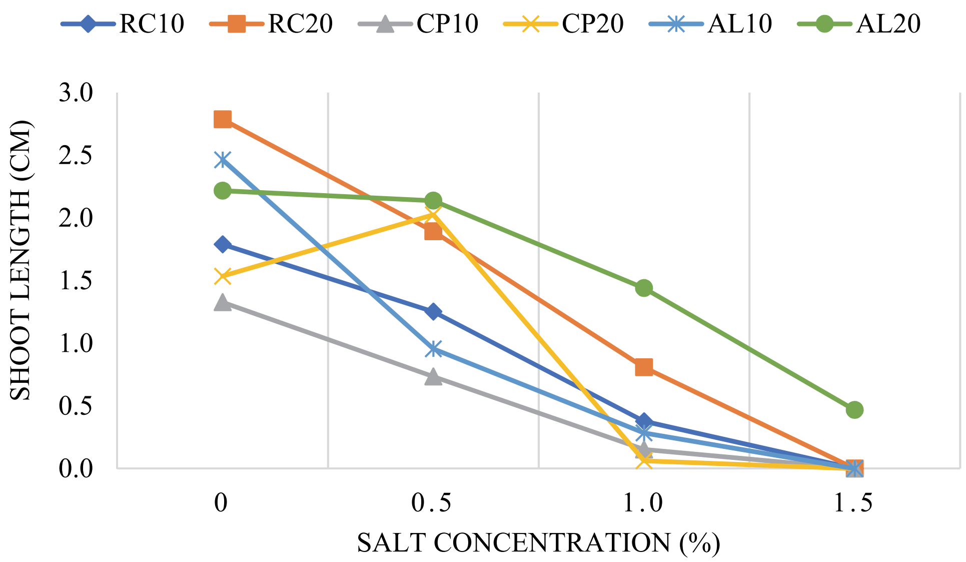

Fig. 5. Mean of shoot length developed after 10 days. RC: Red clover, CP: Chickpeas AL: Alfalfa, 10: 10 °C and 20: 20 °C.

Table 3. Analysis of variance of radicle and shoot lengths at different salt concentrations

		Sum of squares	df	Mean square	F	Sig.
Radicle length	Between groups	158.592	3	52.864	63.294	<.001
	Within groups	1067.411	1278	.835		
	Total	1226.003	1281			
Shoot length	Between groups	545.688	3	181.896	170.707	<0.001
	Within groups	1361.764	1278	1.066		
	Total	1907.453	1281			

Table 4. Post-hoc test (Tukey's honest significance test)

Dependent Variable	(I) Salinity	(J) Salinity	Mean difference (I-J)	Std. error	Sig.
Radicle length	0%	0.5%	0.19152*	0.06454	0.016
		1.0%	0.76608*	0.06458	<0.001
		1.5%	0.96498*	0.11191	<0.001
	0.5%	0%	-0.19152*	0.06454	0.016
		1.0%	0.57456*	0.06458	<0.001
		1.5%	0.77346*	0.11191	<0.001
	1.0%	0%	-0.76608*	0.06458	<0.001
		0.5%	-0.57456*	0.06458	<0.001
		1.5%	0.19890	0.11193	0.285
	1.5%	0%	-0.96498*	0.11191	<0.001
		0.5%	-0.77346*	0.11191	<0.001
		1.0%	-0.19890	0.11193	0.285
Shoot length	0%	0.5%	0.6155*	0.0729	<0.001
		1.0%	1.5342*	0.0729	<0.001
		1.5%	1.6702*	0.1264	<0.001
	0.5%	0%	-0.6155*	0.0729	<0.001
		1.0%	0.9187*	0.0729	<0.001
		1.5%	1.0547*	0.1264	<.001
	1.0%	0%	-1.5342*	0.0729	<0.001
		0.5%	-0.9187*	0.0729	<0.001
		1.5%	0.1360	0.1264	0.704
	1.5%	0%	-1.6702*	0.1264	<0.001
		0.5%	-1.0547*	0.1264	<0.001
		1.0%	-0.1360	0.1264	0.704

* The mean difference is significant at the 0.05 level

3.3. *Interaction between variables on radicle and shoot lengths*

A three-way analysis of variance was conducted to compare the main effects of cover crop species, temperature, and salinity as well as their interaction on the radicle length (*Table 5*). The cover crop species, temperature and salinity effects were statistically significant at $p<0.001$. The main effect of cover crop species yielded an effect size of 0.124, indicating that 12.4% of the variance in the radicle length was explained by cover crop species ($F(2,1263)=89.242$, $p<0.001$). The main effect of temperature yielded an effect of 0.237, indicating that 23.7% of the variance in the radicle length was explained by temperature ($F(1, 1263)=392.305$, $p<0.001$). Lastly, the main effect of salinity yielded an effect size of 0.265, indicating that 26.5% of the variance in radicle length was explained by salinity level ($F(3,1263)=151.828$, $p<0.001$). The interaction with all three effects was significant ($F(4,1263)=4.294$, $p=0.002$), showing that there was a combined effect of cover crop species, temperature, and salinity.

Table 5. Analysis of variances on radicle length

Tests of between-subjects effects

Dependent Variable: Radicle length

Source	Type III sum of squares	df	Mean square	F	Sig.	Partial eta squared
Corrected model	614.688[a]	18	34.149	70.554	<0.001	.501
Intercept	555.438	1	555.438	1147.556	<0.001	.476
Covercrop	86.390	2	43.195	89.242	<0.001	.124
Temperature	189.883	1	189.883	392.305	<0.001	.237
Salinity	220.462	3	73.487	151.828	<0.001	.265
Covercrop * Temperature	40.483	2	20.242	41.820	<0.001	.062
Covercrop * Salinity	25.871	4	6.468	13.363	<0.001	.041
Temperature * Salinity	24.362	2	12.181	25.166	<0.001	.038
Covercrop * Temperature * Salinity	8.313	4	2.078	4.294	0.002	.013
Error	611.315	1263	.484			
Total	2378.946	1282				
Corrected total	1226.003	1281				

a. R Squared = .501 (Adjusted R squared = 0.494)

A three-way analysis of variance was also conducted to compare the main effects of cover crop species, temperature, and salinity, as well as their interaction with the shoot length (*Table 6*). The cover crop species, temperature, and salinity effects were statistically significant at $p<.0001$. The main effect of cover crop species yielded an effect size of 0.055, indicating that 5.5% of the variance in the shoot length was explained by cover crop species (F (2,1263)=37.078, p<0.001). The main effect of temperature yielded an effect of 0.088, indicating that 8.8% of the variance in the shoot length was explained by temperature (F(1, 1263)=122.085, p<0.001). Lastly, the main effect of salinity yielded an effect size of 0.338, indicating that 33.8% of the variance in shoot length was explained by salinity level (F (3, 1263)=215.149, p<0.001). The interaction with all three effects was significant (F (4,1263)=17.790, p<0.001), showing that there was a combined effect of cover crop species, temperature, and salinity.

Table 6. Analysis of variances on shoot length

Tests of between-subjects effects

Dependent Variable: Shoot length

Source	**Type III sum of squares**	**df**	**Mean square**	**F**	**Sig.**	**Partial eta squared**
Corrected model	838.220[a]	18	46.568	55.007	<0.001	0.439
Intercept	1407.578	1	1407.578	1662.661	<0.001	0.568
Covercrop	62.779	2	31.389	37.078	<0.001	0.055
Temperature	103.355	1	103.355	122.085	<0.001	0.088
Salinity	546.424	3	182.141	215.149	<0.001	0.338
Covercrop * Temperature	2.409	2	1.205	1.423	0.241	0.002
Covercrop * Salinity	19.941	4	4.985	5.889	<0.001	0.018
Temperature * Salinity	25.168	2	12.584	14.865	<0.001	0.023
Covercrop * Temperature * Salinity	60.244	4	15.061	17.790	<0.001	0.053
Error	1069.233	1263	0.847			
Total	4286.480	1282				
Corrected total	1907.453	1281				

a. R Squared = .439 (Adjusted R squared = 0.431)

4. Conclusions

In conclusion, based on the investigation, increased salt stress negatively affects the germination and growth of all three crops species in both growing temperatures. A similar observation was also discovered in other plant species by other studies such as *Laghmouchi et al*. (2017) and *Sharma et al.* (2014). In this trial, elevation in salt concentration reduces the germination rate and inhibits radicle and shoot elongation on all three legume species. A study on other different legume species shows that salt stress inhibits embryonic axis growth in legume seeds. This defence mechanism against salt stress caused delay and stunted growth in the plumule and radicle of the seedlings (*Tlahig et al*., 2021). *Nadeem et al.,* 2019 also mentioned that leguminous species are more sensitive to salt stress at seedling growth stage than at the germination stage.

Furthermore, the germination data shows, that alfalfa had higher salt stress tolerance during germination at 20 °C than at 10 °C. However, for red clover and chickpeas, the increase in temperature amplifies the salt stress by inhibiting the seeds from germinating, thus affecting the germination rate at higher salt concentrations. Identical results were also presented in other studies on different crop species (*Gulzar et al*., 2001; *Zhang et al*., 2012). In a study on other forage legumes, a higher accumulation of chloride (Cl) ions was found in the seed embryo at a higher temperature. This chloride ion caused toxicity in the embryo, leading to germination suppression (*Humphrey*, 1995).

This study may be extended to other crop species and may be carried out on open field trials in the future. Due to climate change and soil quality degradation nowadays, studies on any abiotic stress impact on every crop are essential for farmers and growers. These data can also be the starting point to discovering the best mitigation action on the adverse effect of abiotic stress on crop plants.

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