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FIBER FLAX GERMINATION AT DIFFERENT TEMPERATURES AND SALINITY STRESS CONDITIONS

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SUMMARY

In this study the fiber flax (*Linum usitassimum* L.) cultivar Lirina was tested to seed germination under salinity of NaCl and two different temperatures. The experiment was set up in controlled conditions in fitotron (Aralab). There were two temperatures (10°C and 20°C) and different NaCl water solution used for salinity stress: 0 mM, 20 mM, 40 mM, 60 mM, 80 mM and 100 mM. There were 100 seeds sown in 4 replications. The germination energy (%) and germination rate (%) were determined on 3rd and 7th day, respectively. At the lower temperature (10°C) the seeds were not sprouted on 3rd day, whereas at higher temperature (20°C) mean germination energy was 31%. Germination rate (7th day) was quite similar at both temperatures (58% at 10°C and 59% at 20°C). The higher salinity stress of 80 and 100 mM had negative influence on germination energy as well germination rate. With increased salinity, the total seedlings length was also decreased. With increased salinity (over 20 mM), the total seedlings length was also decreased. In out study, low salinity stress of 20 mM even increased the germination rate and germination energy and resulted with the longest seedlings of the fiber flax cultivar Lirina.

Key words: fiber flax, NaCl, temperature, germination, seedlings

INTRODUCTION

Fibre flax (*Linum usitatissimum* L.) can be used either for the fiber extraction or for the seed production (linseed). Their seeds are rich in oil and protein, so they are used for production of linseed oil (Laza and Georgeta, 2012). According to historical records, the earliest example of preserved linen appears to be a needle-netted linen headpiece from Nahal Hemar Cave in Israel 8500 years ago, and Swiss Lake Dwellers used a native flax to make cloth 5000–6000 years ago (Debnath, 2017). Flax fibers are soft with fineness and flexibility. Flax is grown in Western Europe, Eastern Europe, China, and Egypt (Kozlowski et al., 2005). The main production area is Western

Europe, and particularly France. In the Republic of Croatia, the fiber flax production is almost gone and it is grown on small areas, about 15 to 30 ha (Butorac et al., 2010; Pospišil, 2013; Butorac et al., 2017).

The germination process incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley, 1997; Bukvić et al., 2015). Soil salinity is a common problem over the world, especially in areas with water deficit and irrigation water supply for field crops. NaCl may be inhibitory to the activities of some enzymesthat may play critical roles in seed germination (Katembe et al., 1998). In recent decades the scientists over the world made considerable improvements in crop species by conventional selection and breeding techniques (Aflaki, et al. 2017; Markulj Kulundžić et al., 2016). According to Mahajan and Tuteja (2005), understanding the mechanism of stress tolerance along with a plethora of genes involved in stress signalling network is important for crop improvement. Studies with seeds and early seedlings have great importance in evaluating the early growth of the plants under stress conditions (Song et al., 2008; Kaymakanova, 2009; Nimac et al., 2018; Buranji et al., 2019). The studies on halophytes are also very interesting, due to their salt tolerance (Tobe et al., 2004; Jamil et al. 2006; Panuccio et al., 2014; Orlovsky et al., 2016).

The objective of the present study was to analyse fiber flax seed germination in salt stress conditions (0 to 100 mM NaCl) and seedlings morphologyat two temperatures (10 and 20°C).

MATERIAL AND METHODS

Fiber flax cultivar Lirina (RWA) was used in this study. The study was set up in the Laboratory of plant analysis at the Faculty of Agrobiotechnical Sciences in Osijek, Republic of Croatia. The germination test of salt stress and temperatures was set up in the controlled conditions in the growth chamber (Fitoclima, Aralab) at 24 h dark conditions. The germination test was done according to the International Seed Testing Association – ISTA (ISTA, 2006). Before seting up the experiment, flax seeds were prechilled at temperature 8° for 7 days. There were different temperatures tested (10º C and 20°C) and salinity stress levels (0, 20, 40, 60, 80 and 100 mM NaCl water solutions). The granulated NaCl was used for the water solutions of different molarity. There were 55 ml of saline water solution added on the filter paper (Munktell, 580 x 580 mm, 80 g/qm) before the sowing. The seeds were sown on thewet filter paper (within the filter paper) in 4 replications.The filter paper was then rolled, marked and put into clean plastic bag. Every replication consisted 100 seeds. After 3 days the germination energy (%) was determined by counting the seeds which had started to germinate. Then, after 7 days the final germination rate (%) was determined, by counting all the germinated seeds.

After germination energy and germination rate determination 25 seedlings per replication were chosen randomly in order to measure the length of the seedlings root, stem and total seedlings length (cm). At the temperature of 10°C there was only total length of the seedlings determined since the seedlings were small and there was no clear difference between root and stem.

The results were transformed into Microsoft Office Excel programme and the statistical analysis was done using SAS Enterprise Guide 7.1.The grouping means was done by the Fisher LSD method at 95% confidence.

RESULTS AND DISCUSSION

At 10°C on the 3rd day no seeds geminated, but on the 7th day germination rate was 58% (Table 1). The mean germination energy in our study at 20°C was 31%, while the germination rate was similar at both temperatures tested.

Table 1 Germination energy (%) and germination rate (%) with regard to temperature and salinity
Tablica 1. Energija klijanja (%) i ukupna klijavost (%) ovisno o temperaturi i salinitetu

	Germination energy, 3rd day (%)		Germination rate, 7th day (%)	
NaCl	10°C	20°C	10°C	20°C
0 mM		31	61	60
20 mM		35	66	65
40 mM	0	33	61	61
60 mM		34	64	59
80 mM		27	43	53
100 mM		28	50	58
Mean	0	31	58	59

Even though from the previous study on pH and temperature influence on germination of fiber flax cultivar Lirina (B u r a n j i et al., 2019) it was determined that the period of 7 days was not enough to develop the seedlings with clear root and stem limit at 10°C. The salinity experiment was conducted at 10°C temperature, due to fiber flax time of sowing, which is in Republic of Croatia from 15th March to 15th April, when the temperatures are not higher. K u r t and Bozkurt (2006) tested 5 temperatures (10, 15, 20, 25 and 30°C) and they also found that total percentage of seedling emergence and seedling emergence rate of varieties were significantly affected by the temperature, but the accelerated seedling emergence rate was fastest at 30 and slowest at 10°C. On the contrary, in pots experiment, O' c o n n o r and Gusta (1994) found that temperature had no effect on flax germination, so the time for 50% of the seeds to germinateat 5"C was on average 160 h compared with 30 h at 15"C. K u r t (2012) reported that the optimum temperatures of flax cultivars Antares and Bionda were found to be 22.10 and and 22.05 °C. High temperatures have a negative influence on flax germinatios.

In our study the salinity had significant ($p<0.05$) influence on seedlings elongation (Table 2). With increasing salinity, the total seedlings length was decreased on both temperatures tested. The average for all salinity levels, the seedlings length after 7 days on 10°C was 0.4 cm, whereas at 20°C it was 10.7 cm. The seedlings length at 20°C varied from 9.8 cm (100 mM) to 12.7 cm (20 mM). Increasing the NaCl salinity (40 – 100 mM), the total length was decreased. But, it was interesting to find that small salinity (20 mM NaCl) at 20°C significantly ($p<0.05$) increased the total length of the seedlings as compared to the control.

Table 2 The length (cm) of the fiber flax seedlings at different NaCl water solution
Tablica 2. Dužina klijanaca predivog lana ovisno o zaslanjenosti vodene otopine s NaCl

NaCl	Total seedling length (10°C)	Total seedling lenght (20°C)	Root length (20°C)	Stem length (20°C)
0 mM	0.6 ^{a}	11.3 ^{c}	6.1 ^{b}	5.1 ^{b}
20 mM	0.5 ^{b}	12.7 ^{a}	7.1 ^{a}	5.6 ^{a}
40 mM	0.4 ^{c}	10.3 ^{c}	5.4 ^{c}	4.9 ^{b}
60 mM	0.4 ^{c}	10.4 ^{c}	5.4 ^{c}	4.9 ^{b}
80 mM	0.3 ^{d}	9.9 ^{c}	5.8 ^{bc}	4.1 ^{c}
100 mM	0.2 ^{d}	9.8 ^{b}	5.6 ^{bc}	4.2 ^{c}
Mean	**0.4**	**10.7**	**5.9**	**4.8**

The differences marked with letter $^{(a, b, c)}$ in the column at 95% confidence

The histograms (Figure 1) show that in the control at 10°C most of the seedlings were between 0.7 and 0.8 cm (32) long, whereas in the control at 20°C most of the seedlings were in the range of 11.7 and 14.1 cm long (27). At the highest salinity, up to 100 mM, the most common length of fiber flax seedlings at 10°C was in the range of 0.2 to 0.3 cm (40) and at 20°C the most common seedlings length was in the range of 9.5 and 10.6 cm (28).

Under salt stress conditions elongation rate of the coleoptiles may decrease by low soil water potential (F r a n c o is et al., 1986). According to K a t e m b e et al. (1998.) in some cases, NaCl in the cytoplasm can result in toxic accumulation of a particular ion or decreased availability of some essential nutrients, but the authors also stated that ion toxicity of the $Na^{+}$and Cl^{-}ions in the cells may induce changes in protein activity because ions affect the structure of the hydration water which surrounds the protein molecule. For the salt tolerant flax varieties, Q a y y u m et al. (2019) stated that they had high potential to check Na^{+} entry to the upper parts of plant and bind more Na^{+} in their roots in comparison of salt sensitive genotypes, but also proved themselves as the high accumulator to K^{+} in stems. In Canada, B e k e and Volkmar (1995) found that cation concentration (Na^{+}, K^{+}, Ca^{+}, Mg^{2+}) in plant flax tops from saline soil decreased with physiological age and that flax tops on saline soil had higher Mg^{2+} and Na^{+} concentrations.

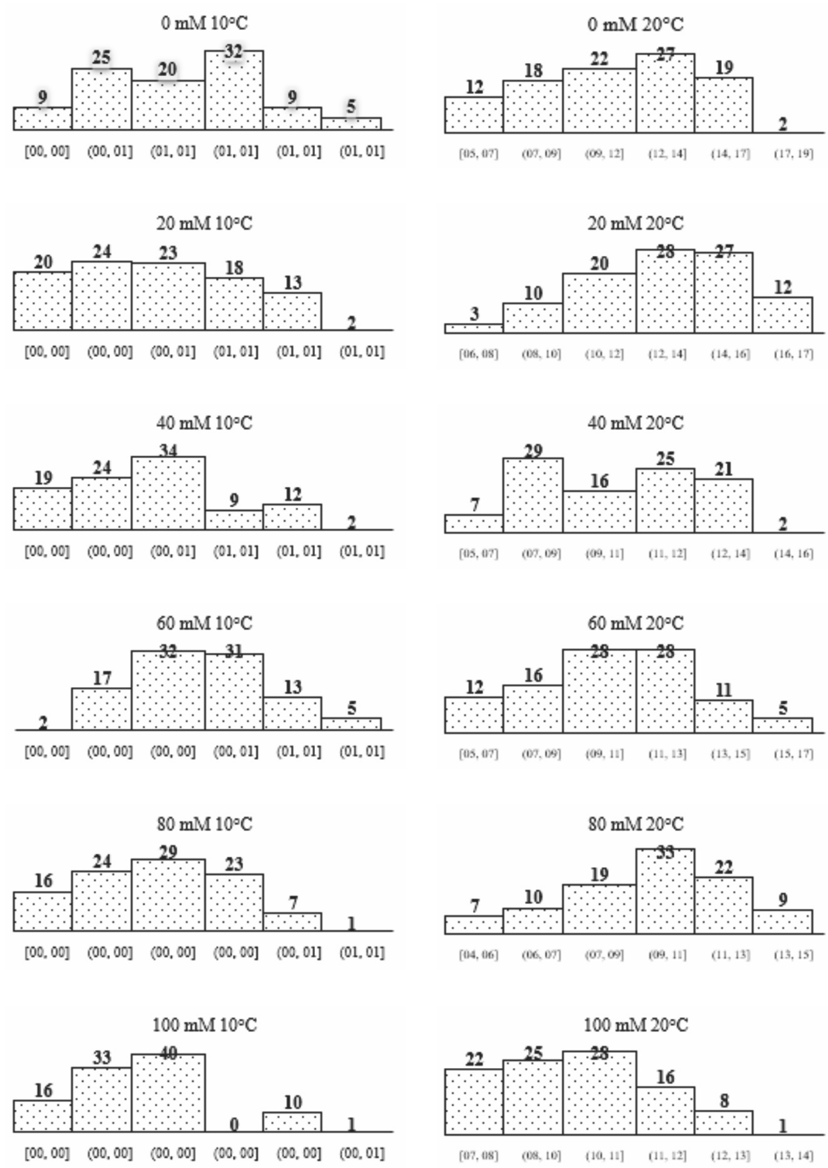

Figure 1 The histograms of total flax seedlings length (cm) regard to salinity and temperature
Grafikon 1. Histogrami ukupne dužine klijanaca predivog lana ovisno o salinitetu i temperaturi

Moghaddam et al. (2018) used six kinds of salts with concentrations of 0, 50, 100 and 200 mM. The author found that flax seeds were able to germinate even in 200 mM NaCl and that germination at various salts was in the order of NaCl > $CaCl_2$>KCl> Na_2CO_3> Na_2SO_4> $CaCO_3$. Sabei et al. (2007) found that for physiological parameters the sodium chloride inhibited germination via osmotic and toxic effects, so that seed viability was altered, especially in flax cultivar(H52). Moreover, authors found that at 200 mM NaCl, lipid mobilization was delayed in the earliest germination phases.

Atwa and Elgazzar (2013) examine the tolerance of seven flax varieties in Egypt (Sakha 1, Sakha 2, Sakha 101, Sakha 102, Eclena, Elona and Esclena) on soil salinity. The authors found that under salinity stress conditions, the flax straw and seed yield decrease, but also the technical length (cm) of the stem and stem radius (mm) was also decreased.

CONCLUSION

In this study the salinity stress conditions were examined in order to above their influence on fiber flax cultivar Lirina germination energy, germination rate and seedlings morphology. Increasing salinity with NaCl up to 80 and 100 mM negatively effects the germination energy and germination rate. Low level of salinity, of 20 mM had a positive effect on germination energy (35% on 20°C), germination rate (66% at 10°C and 65% at 20°C) and seedings elongation. Moreover, at 20 mM seedlings developed the longest root (7.1 cm) and stem (5.6 cm). This cultivar could be grown even on the soils with salinity problem, but for the further conclusions, the agronomic value and fiber yields lists should also be conducted.

KLIJAVOST PREDIVOG LANA PRI RAZLIČITIM TEMPERATURAMA U UVJETIMA SOLNOG STRESA

SAŽETAK

Cilj istraživanja bio je utvrditi energiju klijanja (%), ukupnu klijavost (%) te dužinu klijanaca u uvjetima solnog stresa (0 mM, 20 mM, 40 mM, 60 mM, 80 mMi 100 mM) na dvije temperature (10 i 20 °C). Eksperiment je postavljen u kontroliranim uvjetima u fitotronu (Aralab). U istraživanje je bila uključena sorta predivog lana (*Linum usitassimum* L.) Lirina. Posijano je 100 sjemenki u 4 ponavljanja. Energija klijanja (%) i klijavost (%) određeni su trećeg, odnosno sedmog dana. Na nižoj temperaturi (10 °C) sjeme nije proklijalo 3. dan, dok je pri višoj temperaturi (20 °C) prosječna energija klijanja bila 31%. Ukupna klijavost (7. dan) bila je podjednaka za obje temperature (58% na 10 °C i 59% na 20 °C). Općenito je veći solni stres od

80 i 100 mM smanjio energiju klijanja i ukupnu klijavost. S povećanjem zaslanjenosti preko 20 mM, ukupna duljina klijanaca predivog lana je također smanjena. Pri tretmanu 20°C uz 20 mM, čak je povećana ukupna klijavost, energija klijanja i dužina klijanaca, u odnosu na kontrolu i ostale razine zaslanjenosti za sortu Lirina.

Ključne riječi: predivi lan, NaCl, temperatura, klijavost, klijanci

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