Germination of Industrial Hemp (*Cannabis sativa* L.) at Different Level of Sodium Chloride and Temperatures

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Summary

In this study the morphological characteristic of the industrial hemp (*Cannabis sativa* L.) seedlings were observed at different salinity level (0, 50, 100 and 150 mM NaCl) and temperatures (10°C, 15°C and 20°C) in the controlled conditions. In this study, on the 3rd day the average germination energy was 41%. On the 8th day, the germination rate was on average 48%, with the highest germination rate of 55% (150 mM 10°C) and the lowest was 43% (50 mM 20°C). The total length of the *C. sativa* seedlings was the highest at 50 mM (10.4 cm) and decreased by 2.8% with 150 mM NaCl. From this study it is recommended to use higher seeds rates for sowing on saline soils.

Key words

salinity, industrial hemp, seedlings, stem, root, temperatures

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Introduction

There is growing interest for industrial hemp (*Cannabis sativa* L.) cultivation in many regions of the world. This is because of their multi-purpose uses. Thus, it can be used for numerous products, eg. fibre from straw, seed in human consumption, meal for animal feed, oil, fibres in textile industry, biomass, etc. The seeds are also used for oil extraction and inflorescence is used for phytocannabinoids extraction. Except for humans use, the seeds, oil, cake and meal can be used for animal fed (Klir et al. 2019).

In the Republic of Croatia there was a long tradition of industrial hemp production during 18th, 19th and 20th centuries But, in the recent history, the industrial hemp production was gone because of the closing of the factories for stem processing and fibre production. In recent years there was a law on seed production, which actually permitted cultivation of industrial hemp for the purpose of food and feed production purposes (OG 80/13) and the hemp stalk should be destroyed. It is important to grow hemp varieties listed in the EU Database of registered plant varieties, containing 0.2% THC or less (OG 39/19). Since 2019 there has no longer been restriction for stem use, so the industrial hemp definitely has a bright future in Republic of Croatia (Markus Klarić et al. 2020).

There are several abiotic stresses like salinity, heat, water excess or drought that are the main constraints of field crop production over the world (Parihar et al. 2015; Gao et al. 2018; Desheva et al., 2020), which can reduce the yields somewhere between 20% and 50% (Shrivastava and Kumar 2015). Salts can come from both natural and anthropogenic sources. In natural environment soil may be rich in salts because parent rock from which it was formed contains salts, but in dry areas it is very common to irrigate the soils with water containing some salts (FAO 1985). Soil salinities limit the productivity of field crops, because most of the field crops are very sensitive to high concentration of salts. A saline soil is generally defined as one in which the electrical conductivity (EC) of the saturation extract (ECe) in the root zone exceeds 4 dS m^{-1} at 25°C, which is approximately 40 mM NaCl (Shrivastava and Kumar 2015; Shahid et al. 2018). Soil salinity in most cases has negative influence on plant vegetative growth and productivity. This is because the salt in the plants causes cell dehydration (alteration of water transport), the generation of reactive oxygen species (ROS), osmotic stress and lack of K^+ due to Na^+ competition (Liu et al. 2016). Evelin et al. (2019) explains that due to similar physico-chemical nature, Na^+ and K^+ compete at the transport sites for entry into the symplast and therefore, in saline soils where concentration of Na^+ in rhizosphere is very high, K^+ uptake faces a stiff competition from Na^+.

The industrial hemp can be also sown as a phytoremediation plant, but only if it is used for textile industry, or their biomass (not seed for humans or animal use). Furthermore, it is not extremely sensitive to salinity (Javadi et al. 2014). It is normal that around 60% of sown seeds do not survive until the harvest. Abiotic factors as temperature, light, salt stress and pH are the most studied limiting factors for seed germination and seed growth (Laghmouchi et al. 2017; Nimac et al., 2018).

The germination represents the most vulnerable stage of life cycle of the plants. Thus, the aim of this study was to determine the effect of constant temperatures (10°C, 15°C and 20°C) and different concentrations of NaCl dissolved in water: 0 mM (control), 50 mM, 100 mM and 150 mM, on industrial hemp (*Cannabis sativa* L.).

Materials and Methods

The germination test was done according to the International Seed Testing Association – ISTA (2006). Different concentrations of NaCl (0 mM (control), 50 mM, 100 mM and 150 mM) dissolved in deionozed water and constant temperatures (10°C, 15°C and 20°C) were applied. In this study the seeds of industrial hemp from market, all produced in the Republic of Croatia, were used.

One hundred of industrial hemp seeds were sown in 4 replicates on the filter paper. The filter paper (Munktell, 80 g/qm) was moistened with 50 ml water solution. Afterwards each filter paper was rolled and put separately into a marked plastic bag . The study was conducted in controlled conditions in the plant growth chamber (Fitoclima, Aralab). The germination test was done in 24 h dark conditions and at three different temperatures: 10, 15 and 20°C for 8 days.

The germination energy (%) was determined on the 3th day and total germination rate (%) on the 8th day. Also, after 8 days the shares of normal, abnormal and non-sprouted seeds were recorded. Afterwards, the length of the root, stem and total length (cm) of 25 normally developed seedling, were measured.

The ANOVA was performed using SAS 9.4 Software. The LSD test was used to calculate the differences between the means at $P < 0.05$ and $P < 0.01$.

Results and Discussion

Soil salinization is increasing problem all around the world (Jing et al. 2018). In this study, on the 3rd day the average germination was 41% (Figure 1.), while the lowest germination energy was at 10°C and 100 mM NaCl (18%) and the highest at 20°C with 50 and 100 mM NaCl (54%). The germination rate was on average 48%, with the highest germination rate of 55% (150 mM 10°C) and the lowest was 43% (50 mM 20°C). In this study the average share of non-sprouted seeds was 52%, of abnormal seedlings 6% and of normal seedlings 42% (Figure 2).

Hu et al. (2018) found that low concentrations of salt (50 mM neutral salt) stimulated *C. sativa* germination and seedlings had longer radicles and hypocotyls than the control, but the authors highlighted that salt stress to hemp seed germination varied with the salt type (NaCl, Na_2SO_4, Na_2CO_3, $NaHCO_3$) and salt concentration (0 – 300 mM).

The salinity level, temperature and their interaction had the very significant ($p<0.01$) influence on the *C. sativa* seedlings root, stem and total length (Table 1). The analysis of the morphological characteristics of the seedlings showed that the average seedlings length was 8.8 cm. Regarding salinity, the average length was decreased from 10.4 cm (50 mM) to 6.7 cm at the highest salinity (150 mM). At the lowest temperature 10°C the seedlings were the shortest (4.6 cm), while the highest temperature (20°C) stimulated seedlings elongation and they were on average up to 11.4 cm. The highest germination energy (55%) was found at 50 mM and 20°C, where the longest seedlings were developed (14.0 cm).

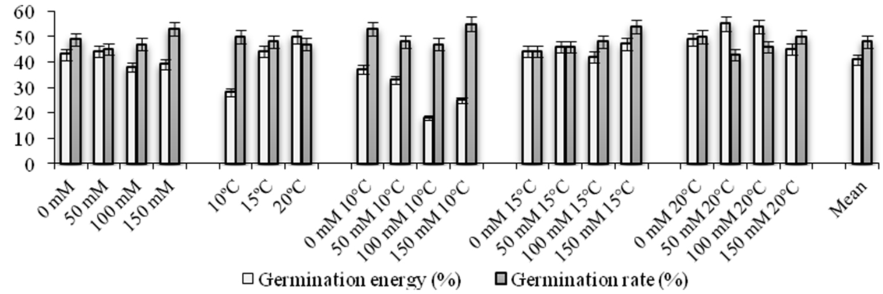

Figure 1. Germination energy (%) and final germination rate (%) of the industrial hemp in the presence of different concentrations of NaCl and at different temperatures (error bars show 95% confidence intervals for differences)

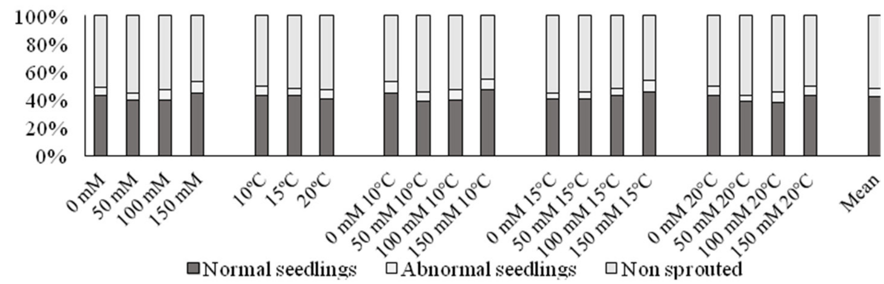

Figure 2. The share of normal, abnormal seedlings and non-sprouted seeds of industrial hemp after germination in the presence of different concentrations of NaCl and at different temperatures

In compost sand (1:1 w:w) mixture, Guerriero et al. (2017) found that the diameter of salt stressed hemp plants aged 15 days was statistically significantly reduced on the hypocotyl thickness.

In comparison of four medicinal plants, *Nigella sativa* L., *Cannabis sativa* L., *Trigonella foenum graecum* and *Cynara scolymus* L., Javadi et al. (2014) found that increasing salinity (0 to 20 dS m^{-1}) significantly reduced germination rate of the *C. sativa*, but also the shoot root and seedling length. Liu et al. (2016) investigated differential expression of genes and related pathways of two *C. sativa* varieties and found that salinity (500 mM NaCl for 2, 4 or 6 days in 3-4 leaves phase) resulted in proline content increase after 2 days and the authors concluded that industrial hemp might have developed variety-specific adaptive mechanisms to salinity in different habitats.

There are not many of studies of hemp's response to growth and fibre quality on saline soils. For other fibre crops as cotton (*Gossypium hirsutum* L.), which is one of the major fibre crops of the world, more studies have been conducted (Razzouk and Whittington 1991; Dilnur et al. 2019). Although it is classified as a salt tolerant crop, Soares et al. (2018) reported that cotton is more sensitive to irrigation water salinity in the flowering and boll development stages and the fibre length was greater under no saline stress application. Worldwide, flax (*Linum usitatissimum* L.) is an economically important crop confined to barren high-salinity plots and there is need to find the varieties of the flax with high salt stress tolerance (Wu et al. 2018; 2019). Nasri et al. (2017) found that mean germination time of *Linum usitatissimum* seeds at 200 mM was higher than the control. For the kenaf (*Hibiscus cannabinus* L.), Kashif et al. (2020) found that salt levels 0 to 250 mM had less damage regard to genotype (P3A and P3B). Fot the Agave species grown at various salinity, Bergsten et al. (2016), found the highest mortality (*A. utahensis* ssp. *utahensis*) of the seedlings in the 9.0 dS·m^{-1} treatment where it was 25%, 50% and 75% higher than in the 6.0, 3.0, and 0.6 dS·m^{-1} treatment, respectively. For the Egyptian flax cultivars, Abido and Zsombik (2019) found that Giza 11 was the most tolerant to salinity stress, so it can be observed for breeding salt tolerant genotypes.

The optimum temperature for industrial hemp germination is 20 °C (ISTA, 2006). The minimum temperature for germination in field conditions is from 1 °C to 2 °C, but for normal germination the soil temperature should be 7 °C and the air-temperature 12 °C (Pospišil, 2013). Qin et al. (2014) found that the fire hemp seeds germinated from the minimum temperature 5 °C to the maximum temperature 35 °C, but the maximum germination percentage (95%), was at 20 °C. Lisson et al. (2000) measured temperatures between 10 and 40 °C and found that industrial hemp (Kompolti) seedlings elongation was relatively consistent at optimum temperature of 28.6 °C and maximum temperature of 40.7 °C. Except for the temperature, Small and Brookes (2012) emphasize the storage maintenance on germination of *Cannabis sativa* seeds. Our study confirms that the optimum temperature for the hemp germination is 20 °C. It is normal that from the total sown seeds of industrial hemp, around 60% of the seeds germinate and develop a whole plant until the harvest. In our study, it was confirmed that ratio of non-sprouted, abnormal seedlings and sprouted seeds was on average 52:6:42. For the field sowing it is recommended to use more seeds than regular rate, especially if it is grown on saline soils.

Table 1. Morphological characteristic of industrial hemp seedlings after germination in the presence of different concentrations of NaCl and at different temperatures

Total length (cm)

Temperature	NaCl (A)				
(B)	0 mM	50 mM	100 mM	150 mM	Average
10°C	5.6	5.3	3.8	3.5	**4.6**
15°C	11.3	11.8	9.7	7.6	**10.1**
20°C	11.5	14.0	11.1	8.9	**11.4**
Average	**9.44**	**10.4**	**8.2**	**6.7**	***8.8***
	$LSD_{0.05}$ (A) = 0.62		$LSD_{0.05}$ (B) = 0.40		$LSD_{0.05}$ (AxB) = 0.68
	$LSD_{0.01}$ (A) = 0.82		$LSD_{0.01}$ (B) = 0.52		$LSD_{0.01}$ (AxB) = 0.89

Root length (cm)

Temperature	NaCl (A)				
(B)	0 mM	50 mM	100 mM	150 mM	Average
10°C	3.8	3.6	2.9	2.8	**3.3**
15°C	5.9	6.3	5.6	5.1	**5.7**
20°C	6.1	6.3	5.8	4.9	**5.8**
Average	**5.3**	**5.4**	**4.8**	**4.2**	***4.9***
	$LSD_{0.05}$ (A) = 0.34		$LSD_{0.05}$ (B) = 0.26		$LSD_{0.05}$ (AxB) = 0.49
	$LSD_{0.01}$ (A) = 0.45		$LSD_{0.01}$ (B) = 0.34		$LSD_{0.01}$ (AxB) = 0.65

Stem length (cm)

Temperature	NaCl (A)				
(B)	0 mM	50 mM	100 mM	150 mM	Average
10°C	1.8	1.7	0.9	0.8	**1.3**
15°C	5.4	5.5	4.1	2.5	**4.4**
20°C	5.4	7.7	5.3	4.0	**5.6**
Average	**4.2**	**5.0**	**3.5**	**2.4**	***3.8***
	$LSD_{0.05}$ (A) = 0.36		$LSD_{0.05}$ (B) = 0.23		$LSD_{0.05}$ (AxB) = 0.34
	$LSD_{0.01}$ (A) = 0.47		$LSD_{0.01}$ (B) = 0.30		$LSD_{0.01}$ (AxB) = 0.45

Fresh mass (g seedling^{-1})

Temperature	NaCl (A)				
(B)	0 mM	50 mM	100 mM	150 mM	Average
10°C	0.13	0.09	0.09	0.06	**0.09**
15°C	0.14	0.14	0.12	0.13	**0.13**
20°C	0.13	0.16	0.12	0.13	**0.14**
Average	**0.13**	**0.13**	**0.11**	**0.10**	***0.12***
	$LSD_{0.05}$ (A) = n.s.		$LSD_{0.05}$ (B) = 0.018		$LSD_{0.05}$ (AxB) = 0.028
	$LSD_{0.01}$ (A) = n.s.		$LSD_{0.01}$ (B) = 0.024		$LSD_{0.01}$ (AxB) = 0.037

Dry mass (g seedling^{-1})

Temperature	NaCl (A)				
(B)	0 mM	50 mM	100 mM	150 mM	Average
10°C	0.05	0.04	0.04	0.04	**0.04**
15°C	0.04	0.04	0.04	0.04	**0.04**
20°C	0.01	0.04	0.04	0.04	**0.03**
Average	**0.03**	**0.04**	**0.04**	**0.04**	***0.04***
	$LSD_{0.05}$ (A) = n.s.		$LSD_{0.05}$ (B) = 0.005		$LSD_{0.05}$ (AxB) = 0.004
	$LSD_{0.01}$ (A) = n.s.		$LSD_{0.01}$ (B) = 0.007		$LSD_{0.01}$ (AxB) = 0.006

Conclusion

The common problem with high-salinity soils exists in areas that require large water inputs to sustain crop production. This study evaluates the industrial hemp seed germination and seedlings' morphological characteristic at various NaCl salinity (0 – 150 mM) and temperatures (10, 15 and 20°C). With the increasing of salinity level, the seedlings develop smaller root and stem. Even though there are only morphological characteristics shown, this study provides useful insights into salt tolerance of industrial hemp, especially if it is grown on potentially saline soils.

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