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EFFECT OF SALINITY STRESS ON SOME PHISIOLOGICAL INDICES OF WHITE CLOVER (*TRIFOLIUM REPENS)*

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Abstract

Salinity is an factor which has a critical influence on seed germination and plant establishment. During the present study the effect of salinity on seeds germination, seedling growth and chlorophyll content of Trifolium repens was studied. Experiments were conducted in laboratory condition. The salinity was induced using three concentration of NaCl (0 mM NaCl, 40 mM and 80 mM). Determinations were recorded in three periods, after (3, 10 and 17 days) and were analysed percentage of germination, MDG and DGS, seedling growth and chlorophyll content. In normal condition Trifolium repens showed highest percentage of germination 79.33%, while in stress salt condition percentage of germination was reduced in proportion to the increase in saline concentration. In salt stress condition we observed that the mean daily germination (MDG) decreased, while daily germination speed (DGS) increased. The effect of the NaCl concentration on the growth in the clover seedlings they showed values between 2.372 cm on the V3 variant and 3.603 cm on the Vo variant. The application of treatments that induced saline stress led to a significant decrease in growth in proportion to the level of differences between treatments. Referring to the effect of the environment on the accumulation of chlorophyll, it was observed that the chlorophyll content decreased under conditions of saline stress (24.15 SPAD). Salt tolerance data may be used to select clover with the highest potential for agronomic production.

Key words*: clover, salt stress, germination, chlorophyll content.*

INTRODUCTION

One of the largest genera of the *Leguminosae* (Fabaceae) family is *Trifolium* with ca. 255 species (Zohary et al., 1987). All species are herbaceous perennials or annuals. Their habitats are temperate and, to a lesser extent, subtropic regions. White clover is very widespread in all ecological areas of our country. *Trifolium repens* L. is grown as a forage crop and is planted in various landscapes for soil conservation. In each year are numerous reports of failed white clover stands. A good understanding of the biology of white clover seed germination in relation to environmental factors is essential for the successful establishment of the crop. The successful establishment of any plant depends on its germination. Various factors such as temperature, water availability, light, salinity etc. can influence seed germination (Al-Ahmadi et al., 2007; Guma et al., 2009; Khan et al., 1984; Khan, 1987; Pérez-García et al., 2007; Ruano et al., 2009). Worldwide, almost 6% of the earth's total surface area is affected by salinity (Zoya Baig et al., 2015). 6.3 million are affected by salinity out of 20.36 million hectare of cultivable land (Qureshi, 1998). Globally, a major stress for plants is increasing soil salinity. In agricultural ecosystem plant production is directly affects by salinity. When plants are exposed to salinity according to Munns (1993), they immediately experience osmotic stress due to low water potential of the substrate. Soil salinity is considered to be one of the major environmental stresses affecting plant growth and productivity, leading to significant productivity losses (Azeem et al., 2011). Water potentials in the leaves of clovers (*Trifolium* sp.), length and dry mass of the stem is reduced by salinity, and are affected the length and conductivity of the root (Orak et al., 2005). For selecting salt tolerance in plants the most viable criteria used are germination and seedling characteristics. The most useable criteria for cultivar selection are germination

percentage, germination speed and seedling growth. Screening for seeds with a greater tolerance to salt stress aids in the development of salt tolerant cultivars. A critical point in seedling subsequent and establishment plant health and vigour is seed germination (Azeem et al., 2011). The main colour agent responsible for photosynthesis is chlorophyll. Under adverse circumstances, the chlorophyll level is a good indicator of the photosynthesis function (Xu et al., 2008). Salinity stress can cause severe disruptions in the plant morphology and physiology. The aim of this study was to explore salinity tolerance of *Trifolium repens,* variety Rivendel.

MATERIALS AND METHODS

The experiments were conducted at the Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timişoara. The biological material was represented by *Trifolium repens*, variety Rivendel. Seeds of *Trifolium repens*, with similar sizes were selected for each experiment and germinated under different salinity levels (40mM and 80mM) and version control without salt (0 mM NaCl). We used for germination tests Petri dishes with two layers of filter paper moistened with 6 ml of water or a NaCl solution. Three repetitions were used for each treatment and 100 seeds were placed in each Petri dish. The dishes were incubated in germinators at a constant temperature of 24ºC days/20ºC night. Germination was recorded at 3, 10 or 17 days intervals. Were considered germinated seeds those which presented emergence of radicle (Bewley et al., 1994). The data were transformed into germination percentages and were calculated means. The seeds were monitoring every day for 17 days, and the following parameters were analysed:

Mean daily germination (MDG) - This is an index of daily germination speed and calculated by:

MDG = FGP/d,

where: FGP: final germination percent; d: test period

Daily germination speed (DGS)

This index is converse of mean daily germination and calculated by:

DGS = 1/MDG (Niste et al., 2014).

The seedling growth was recordered after 7, 14, 21 days. The chlorophyll content were measured after 7, 14, and 21 days, using a clorophylmeter SPAD-502 (Konica Minolta), which measures the absorbance at 650 nm, (Barraclough et al., 2001). To process the experimental data were used analysis of variance and the "t" test [6]. Symbols (*, 0) were used to signify the differences between varieties (Ciulca, 2006).

RESULTS AND DISCUSSIONS

The effect of salinity (V1 - 0 mM NaCl to the control, V2 - 40 mM, V3 - 80 mM, NaCl) upon seeds germination at clover Rivendel variety was observed for 17 days.

Clover Rivendel seeds in laboratory conditions (Figure 1) started to germinate on 3nd day and in 17th day was obtained highest percentage of germination.

In control version (0 mM Nacl) the variety Rivendel registered a percentage of germination of 79.33%.

Compared to the control germination rate was affected by the first concentration of NaCl. At a concentration of 40 mM (V2) germination rate was 75.0% and in V3 (80 mM) was 73.33%.

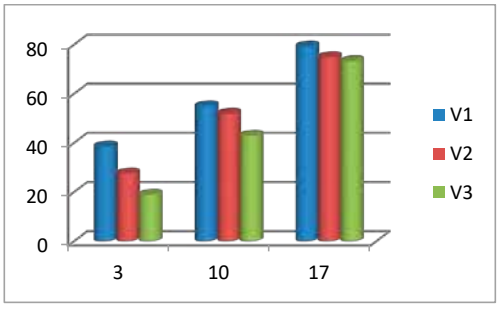

Figure 1. Percent of germination in clover variety Rivendel

On germination percentage it could be noticed that periods had a very significant influence, according to presented data in Table 1.

After a period of (17days) the average values of germination percentage shows an increasing in its value. The results obtained showed statistics assurance.

Table 1. The effect of period in percent of germination

Periods	Average (%)		Relative value (%)	Difference	Significance.
10 days-3 days	51.111	28.444	179.688	22.667	***
17 days-3 days	80.222	28.444	282.031	51.778	***
17 days-10 days	80.222	51.111	156.957	29.111	***
			LSD 5%	LSD 1%	LSD 0.1%
			4.756	6.551	9.019

Regarding the unilateral effect of NaCl concentration on germination percentage at clover seeds (Table 2), it can be observed that above mentioned treatment caused its significant decrease.

Germination percentage varied between 57.66%in variant V1 (0 mM NaCl) and 47.33 % in V3 variant (80 mM NaCl). Decreasing of germination due to salt stress has been reported in another research (Niste et al., 2015; Zoya et al., 2015).

Regarding some indices (Table 3) it can be observed that, MDG (4.31) decreased in stress condition while DGS (0.23) is increased.

Table 2. The effect of medium in percent of germination

Variants	Average		Relative value %	Difference	Signification
V2-V1	54.778	57.667	94.990	-2.889	
V3-V1	47.333	57.667	82.081	-10.333	oo
V3-V2	47.333	54.778	86.410	-7.444	o
			LSD 5%	LSD 1%	LSD 0.1%
			8.192	11.095	14.833

Table 3. Means comparison of seed germination and some indices

Variants	MDG	DGS
V1	4.666	0.21
V2	4.411	0.22
V3	4.313	0.23

The amount of water that the plant uses decreases due to the osmotic stress caused by the increase in the amount of salt in the soil and as a result physiological drought occurs.

Salinity affects seed germination by osmotic effect by preventing or delaying germination, toxicity or ions, causing unviable seed (Cokkizgin, 2012). Germination is one of the most critical periods in the life cycle of the plant according to (Debez, 2004). In plants, during different stages of development level of salinity tolerance is different from germination to mature plant (Khan et.al., 1998; Zoya et al., 2015).

It is also very important for plant establishment in varying geological and environmental regions around the world (Zoya et al., 2015).

Table 4. The effect of period in seedling growth of clover

Periods	Average (%)		Relative value %	Difference	Significance.
14 days - 7 days	3.164	2.239	141.326	0.925	**
21 days - 7 days	3.742	2.239	167.178	1.504	***
21 days - 14 days	3.742	3.164	118.293	0.579	*
			LSD 5%	LSD 1%	LSD 0.1%
			0.527	0.726	0.999

Regarding the effect of the determination period on the growth of the clover seedlings, it can be seen from Table 4 that the seedlings registered an average growth between 2.23 cm and 3.742 cm, 21 days after emergence. The analysis of the obtained results (Table 4) shows that the third determination period ensures a very significant increase in seedling growth

superior to the first determination period for the analysed genotype. Also, the second determination period achieves a significantly higher increase in seedling growth compared to the first determination period.

Table 5. The Efect of mediului upon seedling growth of clover

Variants	Average (cm)		Relative value %	Difference	Signification.
V2-V1	3.167873	3.603778	87.90423	-0.4359	-
V3-V1	2.372833	3.603778	65.84294	-1.23094	ooo
V3-V2	2.372833	3.167873	74.90304	-0.79504	oo
			LDS 5%	LDS 1%	LDS 0.1%
			0.490831	0.664766	0.888737

Considering the analysis of the effect of the culture medium on the growth of the seedlings in clover, a decrease of the growth on the medium with a saline concentration of 80mM NaCl is observed, registering an amplitude of variation of 1.231. Regarding the unilateral effect of the NaCl concentration on the growth in the clover seedlings (Table 5) these showed values between 2.372 cm on the V3 variant and 3.603 cm on the Vo variant (0 Bars H_2O). The application of treatments that induced water deficit led to a significant decrease in growth in proportion to the level of differences between treatments. Chlorophyll is one of the major components of chloroplasts, so the chlorophyll content is positively correlated with the rate of photosynthesis. Reduction of chlorophyll content in drought conditions is considered a typical symptom of oxidative stress that may be the result of photooxidation of pigments and degradation of chlorophyll (Anjum et al., 2011). In addition, a reduction in chlorophyll content in water-deficient conditions would be caused by the destruction of the chloroplast membrane, excessive swelling and denaturation of the vesicle lamellae, and the appearance of lipid droplets (Kaiser et al., 1981). A low concentration of photosynthetic pigments can lead to a direct reduction in photosynthetic potential and thus production. From the data in Table 6, it can be seen that the chlorophyll content accumulated in the study material showed values between 30.467 (SPAD) 7 days after stress induction and 29.9 SPAD after 21 days after water stress induction. Considering the analysis of the effects of seedling age on the chlorophyll content, it is observed that it does not have a significant influence.

Table 6. Effect of time on chlorophyll content (SPAD) in clover

Periods	Average (SPAD)		Relative value, %	Difference	Signification
14 days - 7 days	30.144	30.46783	98.9371	-0.32384	
21 days - 7 days	29.9	30.46783	98.13629	-0.56783	
21 days - 14 days	29.9	30.14399	99.19059	-0.24399	
		L DS5%	LDS 1%	LDS 0.1%	
		1.350535	1.860171	2.560921	

Regarding the effect of the environment on the accumulation of chlorophyll, it can be seen that under conditions of saline stress the chlorophyll content decreased (24.15 SPAD) (Table 7).

Table 7. Effect of the medium on chlorophyll content (SPAD)

Variants	Average (SPAD)		Relative value %	Difference	Signification.
V2-V1	30.55429	35.80554	85.33395	-5.25126	oo
V3-V1	24.15199	35.80554	67.45321	-11.6536	ooo
V3-V2	24.15199	30.55429	79.04616	-6.4023	ooo
		LDS 5%	LDS 1%	LDS 0.1%	
		3.248587	4.399785	5.88215	

CONCLUSIONS

Highest percentage of germination was obtained on 17th day. In control version (0 mM Nacl) the variety Rivendel registered a percentage of germination of 79.33%, while in salt conditions V3 (80 mM) germination rate was 73.33%.

The unilateral effect of NaCl concentration on the germination rate of clover seeds led to a significant decrease. Germination percentage varied between 57.66% in variant V1 (0 mM NaCl) and 47.33 % in V3 variant (80 mM NaCl). Mean daily germination (4.31) decreased in stress condition while daily germination speed (0.23) is increased.

The effect of the NaCl concentration on the growth in the clover seedlings they showed values between 2.372 cm on the V3 variant and 3.603 cm on the Vo variant. The application of treatments that induced saline stress led to a significant decrease in growth in proportion to the level of differences between treatments. Regarding the effect of the environment on the accumulation of chlorophyll, it was observed that under conditions of saline stress the chlorophyll content decreased (24.15 SPAD).

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