Seed Germination and Physiological Response of Sunflower (*Helianthus annuus* L.) Cultivars under Saline Conditions

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Abstract

The purpose of the experiment was to highlight the germination of sunflower seeds affected by the presence of saline stress and the identification of tolerant genotypes. The biological material was represented by sunflower cvs. (*Helianthus annuus* L.): Coril, Select, Santiago and Fundulea-206. To simulate the saline conditions, germination solutions of sodium chloride (NaCl) were used with concentrations corresponding to the osmotic pressures -6 and -10 atm and the control seed hydration was performed with distilled water. Determination of seed germination, growth of seedling, percentage of plumules dry matter, chlorophyll content and free proline were performed. The experimental data obtained suppose the existence in the assimilation apparatus of sunflowers seedling subjected to stress a competitive chlorophyll/free proline biosynthesis processes. The experimental results regarding the effect of salinity on seed germination and seedling growth revealed important differences between genotypes. The radicle growth in the germination process were strongly affected by saline excess, with significant differences between cultivars. Saline stress results in significant reductions in the amount of chlorophyll, and high levels of free proline. It can be observed that with the increase of the stress level the percentage of the dry matter increases, indicating an accentuated water deficit.

Keywords: germination, chlorophyll, proline, salt stress

Introduction

The main challenges of 21st century agriculture are marked by: global water resource shortages, severe salinisation of soils and waters, pollution and climate changes. The reduction of cultivated areas associated with the increase in the number of inhabitants of the earth exerts an increasing pressure on different agricultural systems of food production.

Soil salinisation is a global problem that threatens land productivity and estimates show that 50% of all arable land will be affected by salinity by 2050. It is therefore important to have a global look at the soil's salinity response to minimize economic losses and food security (Butcher *et al.* 2016). Saline soils are defined

as having the electrical conductivity (EC) of the saturation extract (EC_e) in the root zone over 4 dS m⁻¹ (approximately 40 mM NaCl) at temperature 25 °C temperature and a 15% changeable sodium content. Productivity of crop plants is reduced to these values of electrical conductivity, although many cultivated species exhibit a reduction in yield at lower values (Munns, 2005, Jamil *et al.*, 2011). Estimates show that 20% of all cultivated crops and 33% of irrigated agricultural land are affected by high salinity. In addition, salinized areas grow at a rate of 10% annually for various reasons, including low precipitation, high surface evaporation, native rock influence, saline water irrigation and improper agricultural practices (Shrivastava and Kumar 2015).

To solve these problems, it is necessary to develop rapid techniques for testing new cultivars in order to identify genotypes capable of surviving in a certain type of stress. This can be accomplished by performing comparative, detailed studies of metabolic changes in sensitive or tolerant genotypes under abiotic stress conditions. The property of high oil content of seeds (33-56%) and the high food value of the oil (Hera, C. *et al.*, 1989) is the main attribute of sunflower, which gave it the right to move from ornamental plants to large areas cultivated plants.

The impact of saline soil on the productivity of different crops is disastrous (Tanji, 1990; Flowers *et al.*, 1995), which requires the identification and breeding of new genotypes of tolerant plants. At the genetic level, salinity tolerance is considered to be a qualitative element (Foolad and Jones, 1993, Jamil A. *et al.*, 2011).

In Romania there are more than 600,000 ha of saline soils, and an important part of these (over 250,000 ha) in two counties; Arad and Timis. The sunflower represents together with rape one of the most important oil species cultivated in Romania. In the literature there is very little data concerning the salinity tolerance of sunflower genotypes.

Therefore, it is necessary to achieve a balance between productivity and salinity tolerance, identifying and creating sunflower genotypes with a high relative productivity. Excess of salt in soil determine the inhibition of plant growth, reduction of assimilates translocation and intensity of photosynthesis process. Enzymatic activity is also affected and consequently disorders of biosynthetic processes in plants (Sumalan, 2009). The action of soil salinity on plants can occur at any stage of development, from germination throughout vegetative growth to flowering and fructification (Beinsan, C., 2008).

The aims of the work was to determine the influence of saline excess conditions on seed germination, radicles, plumules growth and free proline/chlorophyll biosynthesis from sunflower cotyledons seedlings.

Materials and methods

The biological material consisted of 4 sunflower cultivars (*Helianthus annuus* L.): Coril, Select, Santiago and Fundulea-206. To simulate saline stress conditions, germination solutions of sodium chloride (NaCl) were used with

concentrations corresponding to osmotic potential of 6 atm. (244 mM) and 10 atm (407 mM), and the control seed hydration was performed with distilled water. The experiment was carried out in three variants, as follows: - V1 (control) - distilled water was used as germinating solution; V2 - NaCl solution with OP (*Osmotic Potential*) - 6 atm.; V3 - NaCl solution with OP - 10 atm.

Osmotic potential of saline solutions were calculated with the formula:

$$\pi = nRT/V = MM RT$$

were: π - osmotic pressure (atm), R - the ideal gas constant (0.0821 L atm / mol K), T - the temperature in Kelvin, n - the number of moles of solute present, V - the volume of the solution (n/V is then the molar concentration of the solute), and MM - the molar mass of the solute.

The determinations were made in three and eight days after the beginning of the experiment. The first determination (3 days) consisted in measuring the length of radicle and plumule (cm) and germination energy (%) which is a measure of the speed of germination and hence, it is assumed, of the vigour of the seed and of the seedling which it produces. The second determination (8 days) consisted of: measuring the length of the radicle and the plumule (cm); determination of germination capacity (%); dry matter (%); the free proline determination (mg / g fw - *milligrams / grams of fresh weight*) and the content of chlorophyllin pigments in the plumule (mg/g fw).

The method for determination of germination : 50 seeds in three replicates were placed in Petri dishes (12 cm diameter) on filter paper (Wathman) at 22 ° C and 75% air humidity, in the growth chamber. Dry matter were determined by thermobalance (KERN MLS-300), the amount of free proline by the Bates method (Bates *et al.*, 1973), and the content of chlorophyll of the plumules were measured after acetone extraction by spectrophotometric *T 60 UV PG Instruments* according to Arnon (1949). The experiments were conducted in Plant Physiology laboratory of in november - december 2014. Analysis of variance (ANOVA) was conducted using Statistics SPSS 19.0.

Results and Discussion

Studying the phases of biosynthesis of proline and chlorophyll, it was found that there are common points in the synthesis of the two biomolecules, represented by common enzyme precursors on

Table 1. Estimated values for lenght of radicles and plumules after three days of germination

No.crt.	Cultivar	Variants	Radicles lenght (cm)		Plumules lenght (cm)	
			$\bar{x} \pm s_v$	S%	$\bar{x} \pm s_v$	S%
1	F - 206	V1	1.36 $\pm$ 0.05 a	23.25	2.60 $\pm$ 0.13 a	19.33
		V2	0.68 $\pm$ 0.08 b	19.31	0.23 $\pm$ 0.04 b	31.14
		V3	0.74 $\pm$ 0.05 b	11.21	0.28 $\pm$ 0.02 b	26.40
		Mean	0.93 $\pm$ 0.22 B	40.63	1.04 $\pm$ 0.39 C	65.31
2	Select	V1	3.30 $\pm$ 0.13 a	14.20	4.42 $\pm$ 0.21 a	24.90
		V2	2.50 $\pm$ 0.08 ab	18.33	1.26 $\pm$ 0.09 b	36.43
		V3	1.84 $\pm$ 0.06 b	15.04	1.33 $\pm$ 0.04 b	10.50
		Mean	2.55 $\pm$ 0.42 A	20.71	2.34 $\pm$ 0.52 AB	38.62
3	Santiago	V1	3.15 $\pm$ 0.13 a	14.02	4.95 $\pm$ 0.19 a	19.62
		V2	3.06 $\pm$ 0.32 ab	31.07	1.54 $\pm$ 0.05 b	15.35
		V3	2.96 $\pm$ 0.14 b	11.02	1.46 $\pm$ 0.27 b	30.12
		Mean	3.06 $\pm$ 0.05 A	3.11	2.65 $\pm$ 0.57 A	37.09
4	Coril	V1	1.96 $\pm$ 0.15 a	27.40	3.82 $\pm$ 0.11 a	22.62
		V2	0.97 $\pm$ 0.05 b	15.80	1.11 $\pm$ 0.03 b	13.08
		V3	0.59 $\pm$ 0.02 b	12.00	0.59 $\pm$ 0.01 b	10.81
		Mean	1.17 $\pm$ 0.41 B	60.28	1.84 $\pm$ 0.50 B	47.13

The means with the same letters do not differ at $P = 0.05$ based on Duncan's multiple range test

glutamate pathway (Sumalan, R., 1998). Due to this fact, we considered useful to carry out parallel quantitative determinations between chlorophyll and free proline from plumules of sunflower germinated seeds under salt stress.

In plants, proline is synthesized from glutamate in citosol and probably, also in chloroplasts, due to the action of delta-1-pyrroline-5-carboxylate synthetase (P5CS) and of P5C reductase (P5CR). P5CS produces glutamate semialdehyde, which is unstable and is immediately converted into pyrroline-5-carboxylate (P5C). P5CR reduces P5C to proline, reaction which takes place in the citosol and also in chloroplasts (Delauney and Verma, 1993; Hu *et al.*, 1992; Rayapati *et al.*, 1989; Strizhov *et al.*, 1997; Szabados and Savoure, 2010, 1993; Yoshida *et al.*, 1995, cited by Szabados *et al.*, 2011).

Another source for P5C is the pathway of mitochondrial decomposition of arginine, which first reaction is catalyzed by arginase, which forms ornithine and urea. In the second reaction, the ornithine-aminotransferase (OAT) produces P5C by desamination of ornithine in mitochondria (Xue *et al.*, 2009).

The amount of free proline accumulated under saline stress conditions may be a storage component for the formation of chlorophyll after removal of the stress factor. This allows sunflower genotypes, that accumulate significant amounts of proline in the foliar apparatus, to return on intense metabolism immediately after relieving the stress.

There are numerous plant species which have cultivars capable to accumulate large amounts of free proline in saline environments, these are more tolerant to the action of the stress factor.

The experimental results obtained regarding the effect of saline conditions, induced as osmotic and salt stress on germination and plant growth, show significant differences between tested cultivars. Thus, at a first determination, carried out three days after induction of germination (Tab. 1), the fastest rhythm growth rate was observed at the cv. Select in V1 (3.30 ± 0.13 cm) in which the seeds had been placed in distilled water. Under moderate saline stress (V2), the intense growth of the radicle occurred in the cv. Santiago (3.06 ± 0.32 cm) and Select (2.50 ± 0.08 cm).

Excessive salinity caused significant reductions in the intensity of radicles growth, ranging from 0.59 ± 0.02 cm to the cv. Coril, to 2.96 ± 0.24 cm at the Santiago. Therefore, the intensity of radicles growth in the germination process is strongly affected by saline excess, with differences between tested cultivars. In addition to the growth of the radicles, in the germination process the excess of salts also affects the growth of the plumules (Tab. 1).

There is a direct dependence on the growth of the two components in the germination process and stress conditions. The highest values of plumule lengths were recorded at Santiago 4.95 ± 0.19 cm for V1, 1.54 ± 0.05 in V2 and 1.46 ± 0.27 in V3.

Table 2. Estimated values for length of radicles and plumules after eight days of germination

No.crt.	Cultivars	Variants	Radicles length (cm)		Plumules length (cm)	
			$\bar{x} \pm s_x$	S%	$\bar{x} \pm s_x$	S%
1	F - 206	V1	3.45±0.11 a	11.02	4.35±0.20 a	17.93
		V2	0.68±0.04 b	7.71	2.47±0.15 b	22.60
		V3	0.72±0.05 b	6.13	1.65±0.08 b	18.29
		Mean	1.62±0.46 C	49.11	2.82±0.40 B	24.51
2	Select	V1	5.36±0.40 a	15.00	6.25±0.72 a	19.50
		V2	3.46±0.29 ab	13.42	4.35±0.31 ab	11.04
		V3	1.94±0.11 b	10.90	2.69±0.11 b	7.73
		Mean	3.59±0.49 B	23.89	4.43±0.51 A	20.11
3	Santiago	V1	5.85±0.62 a	17.30	6.15±0.81 a	29.66
		V2	4.85±0.31 ab	11.28	2.78±0.31 b	14.01
		V3	3.65±0.20 b	14.09	1.71±0.08 b	6.02
		Mean	4.78±0.32 A	11.51	3.55±0.67 AB	32.67
4	Coril	V1	3.46±0.31 a	11.04	4.46±0.50 a	18.82
		V2	1.46±0.15 ab	18.33	2.71±0.17 ab	12.00
		V3	0.74±0.09 b	10.14	0.73±0.02 b	4.12
		Mean	1.89±0.413 C	37.35	2.63±0.54 B	35.43

The means with the same letters do not differ at P = 0.05 based on Duncan's multiple range test

Table 3. Estimated values for the germination energy of sunflower seeds (three days after the start of the experiment)

No. crt.	Cultivar	Variants	Germinative energy (%)
1	F - 206	V1	94.17 a
		V2	16.63 b
		V3	6.05 b
		Mean	38.95±13.89 C
2	Select	V1	98.11 a
		V2	66.55 ab
		V3	48.00 b
		Mean	70.89±7.31 AB
3	Santiago	V1	100.00 a
		V2	100.00 a
		V3	64.62 b
		Mean	88.21±5.90 A
4	Coril	V1	98.75 a
		V2	70.70 a
		V3	4.14 b
		Mean	57.86±14.03BC

The means with the same letters do not differ at P = 0.05 based on Duncan's multiple range test

In F 206 cultivar, at three days after stress start (V2 and V3), both radicles and plumules lengths were significantly lower than control (V1). Stress intensity did not influence significantly the analyzed traits.

The genotype Select displayed a significant reduction in the value of the analyzed characters compared to the control. Against the background of moderate saline stress (V2), the differences from V1 and V3 were insignificant, showing a good tolerance to a moderate level of saline stress of 6 atm.

In the case of Santiago cv., medium (V2) and intense (V3) saline stress conditions led to strong

inhibition of radicle and plumules growth at three and eight days too. For cv. Coril, saline stress had a negative influence on the seedling growth at both determinations, but without significant differences between OP of -6 and -10 atm. Analyzing the effect of genotype on the radicle length, after three days on cv. Select and Santiago, the values were significantly superior to the other two cultivars. At cv. Santiago the plumules length was significantly higher compared with Coril and F-206.

Regarding the growth of the radicles, it was , under normal hydration conditions (V1), that the highest growth intensity had the cv. Santiago (5.85 ± 0.62 cm) and the lowest cv. F- 206 (3.45 ±

Table 4. Estimated values for the germination capacity of sunflower seeds (eight days after the start of the experiment)

No. crt.	Cultivar	Variants	Germinative capacity (%)
1	F - 206	V1	99.18 a
		V2	27.35 b
		V3	7.12 b
		Mean	44.55±13.97 B
2	Select	V1	99.52 a
		V2	69.40 ab
		V3	49.51 b
		Mean	72.81±7.27 AB
3	Santiago	V1	100.00 a
		V2	100.00 a
		V3	61.00 b
		Mean	87.01±6.50 A
4	Coril	V1	99.12 a
		V2	73.54 a
		V3	7.02 b
		Mean	59.89±13.72 AB

The means with the same letters do not differ at P = 0.05 based on Duncan's multiple range test

Figure 1. Estimated values for the percentage of plumules dry matter in tested genotypes of sunflower

0.11). The lowest growth intensity was recorded at F-206 (0.72 ± 0.05 cm) in V3 (Tab. 2).

Moderate saline stress conditions (-6 atm) determined a germinative energy to 16.63% for cv. F-206, 66.55% for cv. Select, and 70.70% for Coril without affecting the germination of the cv. Santiago.

The intense stress manifested by -10 atm OP, determined a reduction of germination to 4.14% for the Coril, 6.05% for the F-206, 48.0% for the Select and 64.62% for the Santiago cultivar (Tab. 3).

Regarding the germinative capacity after eight days, it was observed that under normal (V1) and moderate stress (V2) conditions the maximum germination percentage was recorded by Santiago, followed by Coril, and under intense stress conditions (V3) Santiago (61%). The lowest

germination capacity in V3 - Coril by 7.02% and F-206 by 7.12% (Tab. 4).

The results concerning the dry matter content of plumules (%) show that in normal conditions, the lowest values were recorded for the cv. Coril of 9.58% and the highest 14.81% for Santiago. In profound stress (V3), the biggest percent of dry matter was recorded at F-206 by 53.12%, and the lowest at Select (42.1%) (Fig. 1).

Our results are consistent with the other studies and shows that under saline conditions, the emergence time was delayed, and the emergence rate and seedling survival rate also decreased with increasing salinity (Liu, *et al.*, 2010, El Midaoui *et al.*, 2002, Ma *et al.*, 2016).

Under moderate stress conditions (V2) the free proline content begins to increase, ranging from 0.39 ± 0.03 mg / g fw for cv. Coril and 2.66

Figure 2. Estimated values for free proline content in tested genotypes of sunflower (mg / g fw)

Figure 3. Estimated values for chlorophyll content (mg/g fw)

± 0.31 mg / g fw at the Santiago cultivar. The experimental results show that under high salt stress conditions, the content of free proline increases significantly, so the highest level of 4.06 ± 0.28 mg / g fw was recorded in cv. Santiago (Fig. 2).

Therefore, our research shows that saline stress induced during the growth of sunflower plants immediately after germination, causes the accumulation of proline in the plumules in response to the stress factor. In this context, other studies have also shown that the accumulation of free proline in saline stress conditions is an osmotic control reaction of sunflower plants (Lehmann *et al.*, 2010; Madany and Khalil, 2017; Reddy *et al.*, 2004; Saleh and Madany, 2015).

The increase in saline stress led to a decrease in the amount of chlorophyll in all cultivars: for F-206 from 6.01 ± 0.45 mg / g fw as recorded in V1 decreased to 2.58 ± 0.13 in V3, and in the Santiago the amount of chlorophyll of 13.70 ± 0.56 mg / g fw in V1 to 8.23 ± 0.19 mg / g fw in V3, was recorded (Fig. 3). It has been observed that under normal hydration conditions the largest amount

of chlorophyll was achieved by the Santiago and Select cultivars.

The reducing of total chlorophyll and increasing of free proline content under salt stress conditions confirm our hypothesis of existence a proline/chlorophyll competitive biosynthesis in the plumules of sunflower under germination process.

Conclusions

The intensity of radicles and plumules growth in the process of sunflower germination was strongly affected by saline excess. Seed germination has been affected by saline stress, being inversely dependent on the intensity and duration of stress.

With increasing stress levels, the percentage of dry matter, indicating a hydric stress accentuated in the tissues.

Induction of saline stress led to increases in free proline content in parallel with the reduction in the amount of chlorophyll.

The experimental data obtained showed the existence in the assimilation apparatus of the

sunflower plants, subject to saline stress, of a competitive biosynthesis processes of chlorophyll and free proline.

Sunflower cultivars Santiago and Select had a good level of salinity tolerance.

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