JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

EFFECT OF DROUGHT STRESS INDUCED BY MANNITOL ON PHYSIOLOGICAL PARAMETERS OF MAIZE (*ZEA MAYS* L.) SEEDLINGS AND PLANTS

Katarzyna Możdżeń[1], Bartosz Bojarski[2], Grzegorz Rut[1], Grzegorz Migdałek[1], Peter Repka[3], Andrzej Rzepka[1]*

Address(es):
[1]Pedagogical University of Cracow, Faculty of Geography and Biology, Institute of Biology, Department of Plant Physiology, Podchorążych 2 Street, 30-084 Cracow, Poland, +48 12 662 67 20
[2]University of Agriculture in Cracow, Faculty of Animal Sciences, Institute of Veterinary Sciences, Department of Veterinary, Reproduction and Animal Welfare, Mickiewicza 24/28 Street, 30-059 Cracow, Poland, + 48 12 662 41 09
[3*]Faculty of Natural Sciences, Department of the Environment Sciences, Matej Bel University, Tajovského 40, SK-974 01 Banská Bystrica, Slovakia, +421 48 446 71 11

*Corresponding author: repka.peter@gmail.com

doi: 10.15414/jmbfs.2015.4.special2.86-91

ARTICLE INFO

Received 26. 11. 2014
Revised 1. 12. 2014
Accepted 9. 12. 2014
Published 2. 2. 2015

Regular article

OPEN ACCESS

ABSTRACT

Plants are exposed to various stress factors which might lead to structural damage and physiological function abnormalities. Drought is one of the environmental stress factors that reduce the productivity of plants. The aim of our study was to determine the influence of drought stress induced by mannitol (-0.5 and -1.5MPa) on selected physiological processes in *Z. mays* L. In the first stage we studied the effect of mannitol on the germination. In the second stage the effect of mannitol on the growth of plants germinated on distilled water and watered with mannitol in growth phase were measured. Mannitol, which decreased the water content in a concentration-dependent manner, had an inhibitory effect on germination and growth of seedlings and adult plants. Electrolyte leakage of cell membranes of the *Z. mays* seedlings showed high disturbances in the functioning of the membrane structures in the osmotic drought conditions. Similar results were obtained for maize roots, shoots and leaves in both treatment studies. Chlorophyll content showed only significant differences in plants from treated during the growth phase. Drought stress caused a decrease in chlorophyll content by almost a half compared to the control plants. Measurements of chlorophyll fluorescence of plant leaves from the second stage of experiments showed changes in fluorescence activity parameters F_v/F_m, NPQ, Rfd, qP, ect.; gas exchange measurements also showed changes in activity in each of the two phases.

Keywords: Mannitol, germination, electrolyte leakage, morphology, physiology, PSII, *Zea mays*

INTRODUCTION

Plants in natural conditions are constantly exposed to various stress factors. Stress is described all the environmental factors which act on the body and can lead to disturbances in the function and/or structure. The abiotic stressors may include e.g. excess or shortage of water in the environment, too low or too high ambient temperature, presence of toxic substances in the soil, lack of oxygen, gaseous pollutants, UV radiation and excessive salinity of the substrate (**Salisbury and Cleon, 1975**).
One type of the environmental stress, which influences plants living in natural conditions, is drought stress. Water scarcity is one of the main factors limiting the plants productivity. It causes reduction of chemical water potential and water transport inhibition, reduces hydrostatic pressure in the cells, impairs the transport of macromolecules, limits the proper functioning of cell membranes and disorganizes cooperation between cellular organelles (**Hsiao *et al.*, 1976**). Changes at the cellular level are reflected in the growth and plant development (**O'Neill, 1983; Tanguilig *et al.*, 1987; Yordanov *et al.*, 2003; Olszewska *et al.*, 2010**).
Osmotic adjustment is an indication of the plant adaptation to drought conditions, which consists in the solute accumulation in cells in response to changes in the water potential. In different stress conditions plants accumulate compatible solutes: sugars, amino acids, glycerol, mannitol etc. that are involved in the process. As a result of the osmotic potential reduction cells absorb water in order to maintain a proper turgor and conduct normal life functions (**Blum *et al.*, 1996**).
The aim of this study was to determine the influence of drought stress induced by mannitol (-0.5 and -1.5MPa) on selected physiological processes in *Z. mays* L. Mannitol is a polyhydric alcohol used as a causative agent of the drought osmotic stress in plants (**Soetaert *et al.*, 1999**). The influence of drought stress on germination, biometric parameters, water content, electrolytes leakage, chlorophyll content, chlorophyll fluorescence and intensity of gas exchange in maize (*Zea mays* L.) was examined.

MATERIAL AND METHODS

In the first part of the experiment, *Z. mays* grains were lined up on sterile Petri´s dishes with filter papers. Grains were then rinsed under the running tap water for 30 minutes, followed by deionized water. During the grain sorting on Petri´s dishes the gas burner sterilized tweezers were used. Grains were placed at equal distances from each other in order to ensure their uniform conditions for germination. Three groups were created: control (distilled water), and grains in two mannitol concentrations: -0.5MPa (0.2M) and -1.5MPa (0.6M). At the time of germination, the Petri´s dishes with *Z. mays* grains were placed in the dark, at a constant temperature conditions (22°C). Number of germinated grains was counted every 24 hours for 7 days.
After 72 hours since the start of the experiment 90 selected seedlings of *Z. mays* were planted in plastic cups filled with clean river sand (30 in control group; 30 in -0.5MPa of mannitol; 30 in -1.5MPa of mannitol).
Planted seedlings were transferred to the greenhouse, where the constant thermal conditions and natural light prevailed. Plants were watered with distilled water every 2 days and once a week with a standard culture medium (Steiner). After 21 days, the biometrics analysis and chlorophyll fluorescence imaging was performed, along with water content, electrolyte leakage, chlorophyll *a* and *b* content, of, as well as respiration and photosynthesis intensity measurements.
In the second stage of the research, *Z. mays* grains were washed under running tap water and placed in the same Petri's dishes, as in the previous part of the experiment. Germination conditions and method of calculating the germinated grains were the same. All grains were germinated only on the distilled water and watered with -0.5MPa and -1.5MPa mannitol solutions after implantation into plastic cups. A control group was watered with distilled water. Seedling growth conditions were the same as in the first part of the experiment. After 21 days, measurements of the physiological parameters were also analogical as in the first part of the study. Analysis was extended by the identification of chlorophyll fluorescence parameters in the dark adapted state (minimum fluorescence yield (F_0), maximum variable fluorescence yield (F_v), maximum fluorescence yield (F_m) and maximum fluorescence efficiency (F_v/F_m)).

In the case of biometric analysis the length of the 72 hours old seedlings, as well as underground and aboveground organs of *Z. mays* plants from two phases of experiment were measured with 0.5cm accuracy.
The effect of drought stress caused by mannitol on biomass was determined on the basis of changes in dry weight and water content of the seedlings and organs *Z. mays*. Fresh weight was determined in 10 replicates on electronic scale (*Ohaus Adventurer Pro Av 264C*, Melrose, USA). Material was frozen at -80°C and lyophilized in a freeze dryer (*Scanvac CoolSafeTM PRO 55-4*, Lynge, Denmark). The lyophilized *Z. mays* seedlings and organs were weighed to determine the dry weight and calculate the water content.
The electrolyte leakage from the cell membranes was determined using a multifunction device (*CX-701 Elmetron*, Zabrze, Poland). Plant material (roots, stems and leaves) was placed separately in tubes filled by 30ml of deionized water with a conductivity of $0.05 \mu S \cdot cm^{-1}$. The tubes were shaken on a shaker (*Labnet International Rocker*, New York, USA) for 3 hours and mixed using vortex (*Biomix BVX-10*, Blizne Jasinski, Poland). After measuring of the electrolyte leakage (L_Z) the plant material was frozen at -80°C in order to kill the cells. Then material was defrosted and subjected to the same procedure as above. Next, the total content of electrolytes in tissue (L_M) was checked. All measurements were performed in 10 replicates for each specimen. Electrolyte leakage percentage was calculated according to the formula: $EL = (L_Z/L_M)\ 100\%$.
Chlorophyll *a* and *b* content was determined according to **Barnes *et al.*, (1992)** with the wavelength: λ = 665 and 648 nm, using a spectrophotometer *Aquarius* 9500 (*Cecil Instruments*, Cambridge, UK). Chlorophyll concentration was converted into fresh weight with an accuracy of ± 0.01g.
Chlorophyll fluorescence imaging was performed using FluorCam- in FC 800MF Photon Systems Instruments, Czech Republic, according to Lichtenthaler *et al*., (2004). In order to quench the light phase reaction of photosynthesis the *Z. mays* plants of were adapted to darkness for 20min., and then the leaves were cut, put on filter paper with distilled water and inserted into the fluorimeter measuring chamber. We used to method quenching analysis which consists of three phases: measurement of dark adapted levels F_0, and F_m, measurement of the Kautsky effect and non-photochemical quenching in the light (with actinic and super light exposure). The results were analyzed using Fluorcam 7 and Microsoft Paint programs. Due to the destructive character of method used, the measurement of fluorescence was made each time on a different *Z. mays* leaf.
The chlorophyll fluorescence parameters (F_0, F_v, F_m, F_v/F_m) were determined using a fluorimeter (*FMS Fluorescence Monitoring System Hansatech*, Norfolk, United Kingdom), in the same environment conditions on *Z. mays* leaves from control and drought stress treated specimens. Dark-acclimated *Z. mays* leaves with the use of manufacture clips for 30min. were exposed to excitation light $1500 \mu mol \cdot m^{-2} \cdot s^{-1}$ duration of measurement for 1s.
Gas exchange measurements were made by using an infrared gas analyzer ADC-225 MK-3. The intensity of the luminous flux reaching to the leaves surface at the time of photosynthesis measurement was $100 \mu mol \cdot m^{-2} \cdot s^{-1}$. The temperature during the measurement was constant (25°C). The intensity of these processes was set out in the air containing 21% oxygen. The concentration of CO_2 was 300-$400 \mu mol \cdot mol^{-1}$ and air relative humidity of 75% in a closed system (**Rut *et al.*, 2010**). The intensity of photosynthesis and respiration of *Z. mays* leaves were expressed in CO_2 intake/output for plant dry mass (DM) [$\mu mol\ CO_2 \cdot g^{-1} \cdot h^{-1}$].

Statistical anaylsis

Statistical significance of the data was calculated by means of a one-way analysis of variance (ANOVA), followed when appropriate by Tukey's *post-hoc* test (HSD) for groups n=10, the values indicated by letters were significantly different at $p \leq 0.05$. Calculations were performed using Statistica for Windows 10.0.

RESULTS

Germination

After the first day, there was a small percentage of germinated grains on the Petri's dishes with distilled water (control). In the other two cases, there were no germinated *Z. mays* grains. After 48 hours, the share of germinated individuals were about 64% (control), 44% (-0.5MPa) and 12% (-1.5MPa). After another day results were as follows: 92%, 92% and 28% (the control group, -0.5MPa and -1.5MPa, respectively). After 96 hours, 100%, 100% and 68% of maize grains had germinated (Fig. 1).

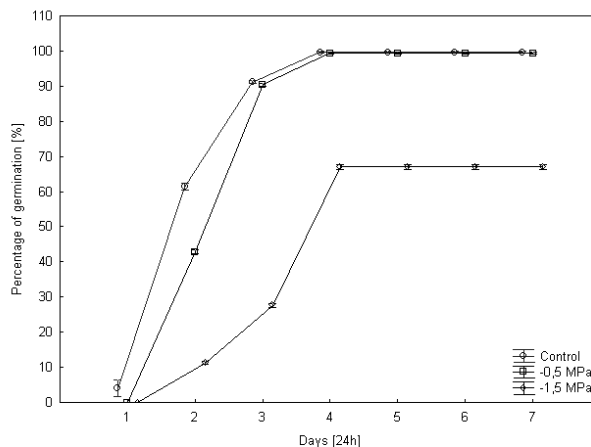


Figure 1 Percentage of germinated *Z. mays* grains in the Petri's dishes with mannitol concentrations -0.5MPa, -1.5MPa and on distilled water (control) for 7 days

Biometric anaylsis

Biometric analysis of maize seedlings performed after 72 hours treatment with lower (-0.5MPa) and higher concentration
(-1.5MPa) of mannitol showed an inhibitory effect of this stress factor.

Table 1 Length of *Z. mays* seedlings [cm] germinated in mannitol -0.5MPa and -1.5MPa concentrations

The length of seedlings [cm]	Treatment		
	Control	-0.5MPa	-1.5MPa
	4.20[a] ±0.18	2.88[b] ±0.11	1.42[c] ±0.03

Statistically significant differences in the seedling length were observed in both concentrations of mannitol (-0.5MPa and -1.5MPa) in relation to the seedlings growing on the Petri's dishes with distilled water (control). In the case of high concentration of mannitol, seedlings reached about 1.5cm, which accounted for approximately 34% of the length of maize seedlings from control. In low concentration, the length of the seedlings was about 60% of the control seedling length (Tab. 1).
Biometric analysis of *Z. mays*, growing from grains germinated on Petri's dishes with mannitol and watered with mannitol during growth, showed statistically significant differences in the length of the tested organs (Tab. 2).

Table 2 Length of *Z. mays* organs watered with mannitol (-0.5MPa. -1.5MPa) in the germination phase (A) and in the growth phase (B)

Organ	Treatment				
	Control	-0.5MPa		-1.5MPa	
		A	B	A	B
Root	19.03[a] ±1.96	9.53[b] ±1.12	6.13[b] ±0.76	17.03[a] ±1.47	8.17[b] ±0.35
Section from the root to the first leaf	3.47[ab] ±0.12	3.75[a] ±0.15	3.45[ab] ±0.05	3.23[ab] ±0.12	2.95[b] ±0.15
I leaf	3.8[c] ±0.17	4.23[bc] ±0.12	4.33[bc] ±0.03	3.9[b] ±0.06	5.2[a] ±0.1
II leaf	11.03[a] ±0.30	11.05[a] ±0.65	9.83[ab] ±0.15	10.2[ab] ±0.1	9.33[b] ±0.5
III leaf	21.72[a] ±1.59	16.20[a] ±3.21	17.75[a] ±0.15	16.93[a] ±2.31	11.4 ±0.5
IV leaf	29.56[a] ±3.99	22.57[a] ±0.30	30.4[b] ±1.83	6.13[a] ±0.66	2.63[b] ±1.81
V leaf	32.87[a] ±1.45	9.73[c] ±2.19	-	23.3[b] ±2.60	-
VI leaf	18.48[a] ±0.86	-	-	8.63[b] ±1.81	-
Remainder of shoot	8.84[a] ±0.76	5.07[ab] ±1.33	8.07[a] ±0.22	1.9[b] ±0.97	1.83[b] ±0.38

Plants watered with mannitol solution at a concentration of -0.5MPa during growth had the shortest roots (6.13cm) in comparison with control group (19.03cm). Significant differences in the length of section from the root to the first leaf was found only between *Z. mays* plants which had grown from grains germinating on dishes with -0.5MPa mannitol and plants watered with -1.5MPa mannitol about concentration in growth phase.

Generally, watering with increasing concentrations of mannitol in germination phase and growth phase showed stimulatory effect on the length of *Z. mays* 1st leaf with. Additionally, statistical analysis showed their inhibitory effects on the growth of the 2nd, 3rd and 4th *Z. mays* leaves . The highest concentrations of mannitol in both phases of growth completely inhibited the development of the 5th and 6th of the *Z. mays* leaves. Plants watered with mannitol during growth had the shortest remainder of shoot in comparison with control (Tab. 2).

Dry weight and water content [%]

According to the results of dry matter and water content in maize seedlings, it was indicated that mannitol-induced osmotic stress significantly affects water uptake by germinating maize grains.

Table 3 Dry weight [g] and water content [%] in *Z. mays* seedlings germinated on mannitol (-0.5MPa and -1.5MPa) and on distilled water (control)

	Treatment		
	Control	-0.5MPa	-1.5MPa
Dry weight [g]	3.14^{b} ±0.05	3.33^{a} ±0.03	3.44^{a} ±0.05
H_2O [%]	67.87^{a} ±0.59	59.11^{b} ±0.44	47.19^{c} ±0.45

The biggest difference in the percentage content of water in comparison with control (distilled water) was recorded for the seedlings in the highest concentration of mannitol (-1.5MPa). In the case of the dry weight, seedlings germinated on mannitol had slightly higher mass values (Tab. 3). Determination of dry matter of *Z. mays* organs showed statistically significant inhibition of weight gain in phases A and B after watering with mannitol (Tab. 4).

Table 4 Dry weight [g] of organs plants *Z. mays* treated with mannitol (-0.5MPa, -1.5MPa) and distilled water (control) in the germination phase (A) and in the growth phase (B)

Organ	Treatment				
	Control	-0.5MPa		-1.5MPa	
		A	B	A	B
Root	2.62^{b} ±0.015	0.89^{c} ±0.003	0.46^{d} ±0.016	3.55^{a} ±0.001	0.34^{d} ±0.147
Section from the root to the first leaf	0.20^{a} ±0.003	0.079^{c} ±0.001	0.06^{d} ±0.0001	0.10^{b} ±0.0007	0.03^{e} ±0.0008
I leaf	0.02^{a} ±0.0008	0.02^{a} ±0.001	0.02^{a} ±0.0003	0.02^{a} ±0.0003	0.02^{a} ±0.001
II leaf	0.14^{a} ±0.0003	0.06^{b} ±0.0006	0.03^{d} ±0.00008	0.03^{c} ±0.0002	0.03^{c} ±0.001
III leaf	0.68^{a} ±0.002	0.06^{b} ±0.002	0.05^{b} ±0.002	0.06^{b} ±0.002	0.02^{c} ±0.002
IV leaf	1.77^{a} ±0.01	0.10^{c} ±0.00008	0.001^{d} ±0.0001	0.17^{b} ±0.001	0.01^{d} ±0.0001
V leaf	0.28^{a} ±0.009	0.04^{c} ±0.0006	-	0.12^{b} ±0.0006	-
VI leaf	0.12^{a} ±0.0001	-	-	0.019^{b} ±0.0004	-
Remainder of shoot	0.38^{a} ±0.007	0.06^{b} ±0.0003	0.09^{b} ±0.04	0.03^{b} ±0.00005	0.01^{b} ±0.00008

Table 5 Water content [%] in organs of *Z. mays* plants watered with mannitol and distilled water (control) in the germination phase (A) and in the growth phase (B)

Organ	Treatment				
	Control	-0.5MPa		-1.5MPa	
		A	B	A	B
Root	79.00^{ab} ±0.12	80.65^{a} ±0.07	79.76^{ab} ±0.75	69.57^{c} ±0.04	75.62^{b} ±2.17
Section from the root to the first leaf	91.31^{a} ±0.17	91.33^{a} ±0.06	90.07^{b} ±0.04	91.60^{a} ±0.03	90.02^{b} ±0.31
I leaf	13.97^{a} ±8.69	27.69^{a} ±3.90	4.48^{a} ±2.51	2.04^{a} ±2.04	5.22^{a} ±2.03
II leaf	30.74^{e} ±1.14	84.88^{b} ±0.009	96.59^{a} ±0.033	76.42^{c} ±0.10	69.95^{d} ±0.90
III leaf	56.54^{d} ±0.15	88.10^{b} ±0.12	86.78^{c} ±0.33	91.00^{a} ±0.21	86.39^{c} ±0.30
IV leaf	40.26^{d} ±0.13	90.01^{b} ±0.006	99.04^{b} ±0.09	89.97^{a} ±0.11	65.30^{c} ±0.30
V leaf	87.10^{b} ±0.37	87.01^{b} ±0.07	-	89.99^{a} ±0.94	-
VI leaf	87.70^{b} ±0.14	-	-	89.20^{a} ±0.67	-
Remainder of shoot	91.79^{ab} ±0.15	93.27^{ab} ±0.03	90.34^{a} ±0.01	95.36^{b} ±1.98	91.58^{ab} ±0.09

Analysis of water content in the organs of *Z. mays* revealed a statistically significant reduction of water content in the root, the section from the root to the first leaf and the first leaves of plants watered with mannitol in the growth phase. The remaining organs of maize plants watered with mannitol in germination phase and in growth phase observed an increase of water content compared to the water content in the organs of control plants (Tab. 5).

Electrolyte leakage

The measurements of electrolyte leakage from germinating maize grains on Petri's dishes with mannitol showed disturbances in permeability of the cell membranes in comparison to the control (distilled water) (Tab. 6).

Table 6 The electrolyte leakage [%] from *Z. mays* seedlings germinated on mannitol (-0.5MPa and -1.5MPa) and distilled water (control)

	Treatment		
Electrolyte leakage from seedlings [%]	Control	-0.5MPa	-1.5MPa
	20.53^{b} ±1.50	27.65^{b} ±1.46	43.18^{a} ±3.11

The statistical analysis showed the greatest percentage of cell membrane disruption in a concentration of -1.5MPa in comparison with control seedlings. However, lower concentration showed no significant effect of mannitol on the cell membranes permeability (Tab. 6).

Measurement of electrolyte leakage from the cell membranes of *Z. mays* organs treated with mannitol in the germination phase and the growth phase, regardless of the concentration of mannitol (-0.5 and -1.5MPa), showed amount 50% increase in comparison with the electrolyte leakage from the organs of the control plants (Tab. 7,8).

Table 7 The electrolyte leakage [%] from the cell membranes in organs of *Z. mays* plants grown from grains watered with mannitol in germination phase

Organ	Treatment		
	Control	-0.5MPa	-1.5MPa
Root	20.37^{b} ±1.13	32.52^{a} ±0.75	35.52^{a} ±1.08
Section from the root to the first leaf	17.98^{b} ±1.54	49.47^{a} ±2.90	40.02^{a} ±1.29
Leaves	29.83^{b} ±2.28	32.70^{b} ±0.76	50.63^{a} ±0.43

Table 8 The electrolyte leakage [%] from the cell membranes organs *Z. mays* grown from seedlings watered of distilled water in germination phase and mannitol in growth phase

Organ	Treatment		
	Control	-0.5MPa	-1.5MPa
Root	20.12^{b} ±1.17	43.21^{a} ±0.51	41.80^{a} ±3.30
Section from the root to the first leaf	17.92^{b} ±1.49	51.26^{a} ±3.34	57.84^{a} ±3.96
Leaves	29.83^{b} ±2.28	38.92^{b} ±3.74	87.13^{a} ±1.37

The highest percentage of the cell membranes disorganization was found in the leaves of *Z. mays* plants grown from watered with mannitol at a concentration of -1.5MPa, and the lowest one for the leaves of plants grown from grains germinated on medium saturated with mannitol solution with a concentration of -0.5MPa (compared to control plants). Intermediate values were obtained for the roots and shoots of each of the test phases treatment (Tab. 8).

Chlorophyll

The table 9 below shows the changes in chlorophyll *a* and *b* content of *Z. mays* plant leaves treated with mannitol solutions of different concentrations in the two phases of the experiment.

Table 9 Chlorophyll content [mg/g FW] in *Z. mays* leaves treated with mannitol (-0.5; -1.5MPa) in the germination phase (A) and in the growth phase (B)

Treatment		[mg/g FW]		
		Chl *a*	Chl *a+b*	Chl *a*/Chl *b*
Control		0.75^{a} ± 0.07	0.92^{a} ± 0.09	3.82^{ab} ± 0.36
A	**0.5**	0.53^{a} ± 0.08	0.73^{a} ± 0.03	4.34^{a} ± 0.41
	1.5	0.52^{a} ±0.03	0.65^{a} ± 0.04	3.41^{ab} ± 0.43
B	**0.5**	0.20^{b} ± 0.05	0.31^{b} ±0.04	2.41^{bc} ± 0.45
	1.5	0.12^{b} ± 0.04	0.24^{b} ±0.07	1.07^{c} ± 0.21

The concentration of chlorophyll *a* and *b* in leaves of *Z. mays* plants grown from grains treated with mannitol in germination phase did not show the significant influence on the content of the green pigments. Statistically significant differences were obtained for the plants watered with mannitol solutions in growth phase. Generally, drought stress induced by mannitol has decreased the content of chlorophyll *a* and *b* in relation to the control objects. The chlorophyll *a* values ranged from 0.1 to 0.2 [mg/g FW], while the chlorophyll *b* content was two times lower. The plants watered with mannitol solution of -1.5MPa in concentration during the growth phase had the lowest amount of chlorophyll, compared to control plants (Tab. 9).

Chlorophyll fluorescence

Fluorimetric analysis of chlorophyll fluorescence parameters (F_0. F_m. F_v. F_v/F_m) confirmed the important effect of mannitol on PSII activity in plants *Z. mays* (Fig. 2).

Fluorescence measurements carried out after 14, 21 and 28 days on plants watered with mannitol in the second phase of experiment, showed a significant decrease in values of the studied parameters after 3 and 4 weeks in plants treated with the highest concentration (Fig. 2). The exception was parameter F_0 for which after 4 weeks, there was an increase of activity in both low and in a high concentrations of mannitol relative to the control plants (Fig. 2).

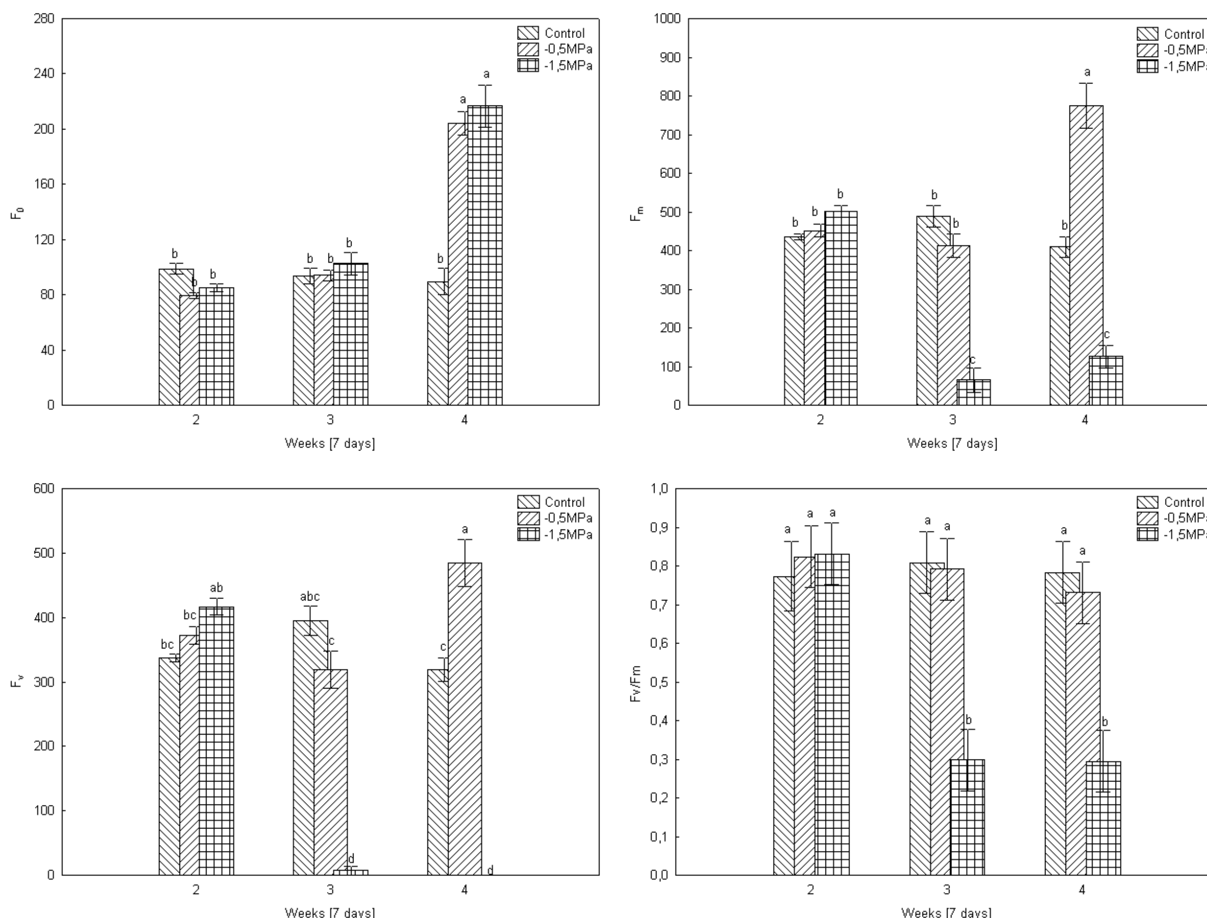
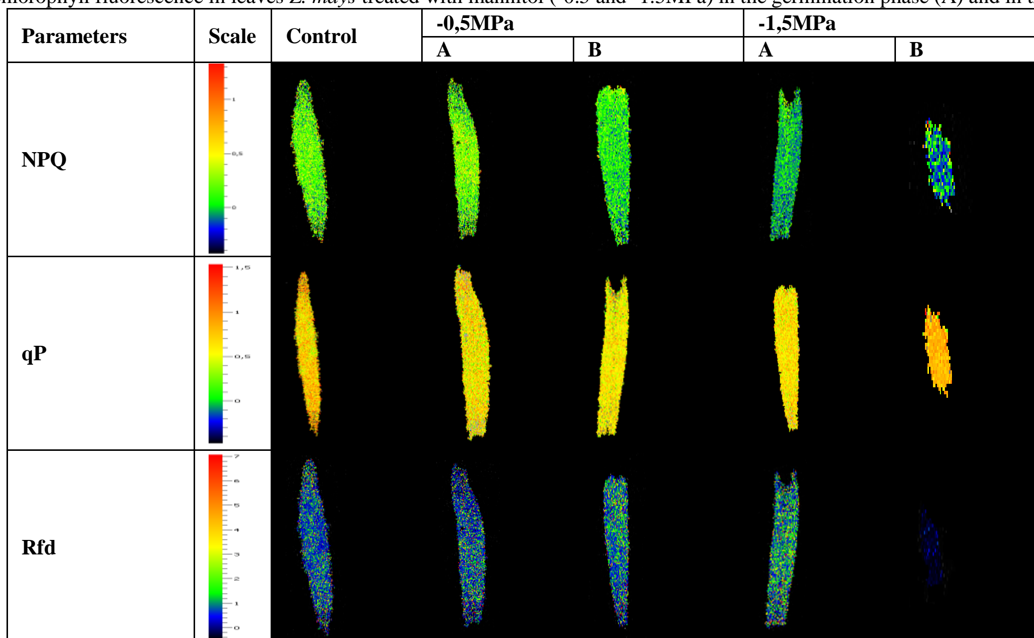


Figure 2 Chlorophyll fluorescence (F_0, F_m, F_v,F_v/F_m) measured after 14, 21 and 28 days in *Z. mays* plants watered with mannitol in the growth phase

The chlorophyll fluorescence imaging allowed us to distinguish the areas of maize leaves exposed to drought stress through the use of different concentrations of mannitol (Tab. 10).

Table 10 Imaging of chlorophyll fluorescence in leaves *Z. mays* treated with mannitol (-0.5 and -1.5MPa) in the germination phase (A) and in the growth phase (B)



Parameters	Scale	Control	-0,5MPa		-1,5MPa	
			A	B	A	B
NPQ						
qP						
Rfd						

Non photochemical quenching (NPQ) in leaves of control plants was higher compared to plants grown from grains treated with mannitol both in the germination and growth phases. The differences in vitality index (Rfd) were observed among the plants treated with mannitol in both low and high concentrations. Similarly, the photochemical quenching (qP) differences were visible between the control and plants of each used mannitol concentration. The values of fluorescence parameters decreased in the oldest parts of the leaves, especially on the edges and at ends. In the case of the plants watered with the highest concentration of mannitol almost totally destroyed PSII was observed. Based on the examined parameters the maize leaves virtually showed no fluorescence activity (Tab. 10).

Gas exchange

The intensity of net photosynthesis [µmol $CO_2 \cdot g^{-1} \cdot h^{-1}$] of *Z. mays* plants grown from the grains treated with mannitol in germination phase was (for dry and fresh weight, respectively): 65.61 and 434.60 (control), 49.63 and 324.91 (-0.5MPa), 61.00 and 394.25 (-1.5MPa) (Tab. 11). The rate of respiration [µmol $CO_2 \cdot g^{-1} \cdot h^{-1}$] in the control plants was: 16.04 and 107.06, in the group of low concentration of mannitol (-0.5MPa): 13.96 and 91.02, and at high concentration of mannitol (-1.5MPa): 15.31 and 100.26 (Tab. 11).

Table 11 The intensity of net photosynthesis (P_n) and respiration rate (R) [µmol $CO_2 \cdot g^{-1} \cdot h^{-1}$] based on the dry (DW) and fresh weight (FW) in *Z. mays* plants treated with mannitol in the germination phase (A) and in the growth phase (B)

[µmol $CO_2 \cdot g^{-1} \cdot h^{-1}$]		Treatment				
		Control	-0.5MPa		-1.5MPa	
			A	B	A	B
P_n	DW	65.61[a] ±0.31	49.63[c] ±0.32	16.43[d] ±0.22	61.00[b] ±0.01	15.80[d] ±0.008
	FW	434.60[a] ±4.03	324.91[c] ±0.16	95.10[d] ±0,15	394.25[b] ±0.63	37.12[e] ±0.06
R	DW	16.04[b] ±0.05	13.96[c] ±0.03	11.94[d] ±0.03	15.31[b] ±0.15	25.48[a] ±0.40
	FW	107.06[a] ±0.06	91.02[c] ±0.06	68.65[d] ±0.17	100.26[b] ±0.13	60.68[e] ±0.16

The intensity of net photosynthesis [µmol $CO_2 \cdot g^{-1} \cdot h^{-1}$] in *Z. mays* plants watered with mannitol in the growth phase was (for dry and fresh weight, respectively): 16.43 and 95.10 (-0.5 MPa) and 15.80 and 37.12 (-1.5MPa) (Tab. 11). The rate of respiration [µmol $CO_2 \cdot g^{-1} \cdot h^{-1}$] were: 11.94 and 68.55 (-0.5MPa) and 25.48 and 60.68 (-1.5MPa).

DISCUSSION

Water is the universal carrier of nutrients, a substrate in reactions of photosynthesis, a factor necessary for the proper growth and development of plants. Water stress is generally understood as an excess or lack of water which has a big impact on biochemical and physiological processes of plants. It makes its presence felt in the first stages of plant life reducing the germination of seeds (Liu *et al*., 2010). A similar phenomenon has been observed in the course of this work. High concentration of mannitol (-1.5MPa) reduced number of germinated grains of maize to 68% after 96 hours of germination as compared to the number of germinated *Z. mays* grains in control conditions (Fig. 1).

In the event of the long lasting water deficit in the soil, plants often undergo reduction of the ground parts, while their roots increase in size striving towards deeper soil layers that are more abundant in water. This phenomenon is observed in the case of wheat **(Volkman, 1997)**, rice (*Oryza sativa* L.) **(Lu and Neumann, 1999)**, *Populus kangdigensis* **(Chunying *et al*., 2005)** and strawberry **(Klamkowski and Treder, 2011)**. The results of this study showed a slight decrease in the length of the root of *Z. mays* in the group treated with mannitol in the germination phase and a significant decrease in the group watered with mannitol in the growth phase (Tab. 2). The length of the ground organs of *Z. mays* was reduced in each of the two phases. Statistically significant differences were demonstrated especially among the plants watered with solutions of mannitol in the growth phase (Tab. 2).

Water stress in plants reduces nutrient uptake and the weight gain, due to the limited transpiration and nutrient absorption as well as inhibited root transport mechanisms **(Tanguilig *et. al*., 1987)**. In the case of the *Z. mays* the significant inhibition of growth was observed through changes in dry mass for both aboveground and underground organs of plants treated with osmotic drought stress. The largest differences were observed in plants grown from grains germinated on distilled water and watered with mannitol in the growth phase (Tab. 4).

The membrane structures are one of the first elements of the cells exposed to abiotic stress factors. The membrane's destabilization causes a decrease in cell volume, increase of viscosity of the cytoplasmic components and the disorganization of metabolic processes **(Farooq *et al*., 2009)**. The results from our experiments showed increase in the electrolyte leakage from the cell membranes of both seedlings and plant organs of *Z. mays* (Tab. 6,7,8). The instability of the cell membranes was highest in seedlings germinated on mannitol at a concentration -1.5 MPa than in control group (Tab. 6). In the case of plants *Z. mays* largest percentage of the electrolyte leakage demonstrated in organs treated -1.5 MPa mannitol in the second phase of the experiment (Tab. 8).

Plant pigments, as one of the most important compounds in the plant, affect the rate of photosynthesis and biomass production. **Podleśny and Podleśna (2010)** showed a decrease in chlorophyll content in leaves of lupine and barley under drought conditions, and **Amuthavalli and Sivasankaramoorthy (2012)** observed a reduction of chlorophylls and carotenoids in *Cajanus cajan*. In the case of *Z. mays* the statistically significant decrease in the chlorophyll *a* and *b* content was observed in plants grown from seedlings on distilled water, and treated with mannitol (-0.5 and -1.5MPa) in the growth phase (Tab. 9). The obtained results may be due to increase degradation and inhibition of the pigments synthesis and mineral substance deficiency.

The study of chlorophyll fluorescence showed significant changes in the parameter values. Treatment with mannitol (-1.5MPa) inhibited the functioning of the PSII photosystem in *Z. mays* (Fig. 2). Changes in the minimum fluorescence yield (F_0) value suggest difficulties in the excitation energy transfer between molecules of pigments, while in the maximum fluorescence (F_m) and the variable fluorescence (F_v) may indicate disorders in the reduction of electrons and in scattering of the excitation energy as heat in the PSII (**Kalaji and Łoboda, 2010). Hura *et al*., (2007)** showed a decrease in the value of F_v/F_m in chlorophyll fluorescence of C_3 and C_4 plants, probably due to damage to PSII reaction centers or slow excitation energy quenching process induced by the effects of drought.
Chlorophyll fluorescence reflects the plants sensitivity to stress factors. On the basis of chlorophyll fluorescence imaging a decrease in the value of non-photochemical quenching (NPQ) and vitality index (Rfd) was shown as well as differences in the parameter value and photochemical quenching (qP) in *Z. mays* plants treated with mannitol during germination and growth phases (Tab. 10). According to **Kalaji and Łoboda, (2010)** changes in the activity parameters testify to the disturbances in the course of photochemical and enzymatic reactions in thylakoids and the chloroplast matrix, as well as to the amount of energy absorbed by PSII, and the losses of heat.
The photosynthesis process is associated with plant species **(Olszewska *et al*., 2010)** and is significantly modified by environmental factors. The rate of photosynthesis depends on the amount of water and its availability **(Stalmach *et al*., 2012)**. Under drought conditions, one of the defense reactions of plants is closing of the stomata, which leads to a reduction of transpiration and limits carbon dioxide intake **(Klamkowski and Treder, 2011)**. In the case of *Z. mays* plants grown from grains germinated on distilled water, and watered with both solutions of mannitol we observed an inhibition of the intensity of the gas exchange process (Tab. 11).

CONCLUSION

Drought tolerance requires changes throughout the plant, from the molecular and tissue level to the physiological processes. A typical plant response to water stress is manifested by yield reduction through decrease of the rate of photosynthesis, disruption of transport and distribution of assimilates. Drought stress simulated by mannitol has a negative effect on physiological processes in maize plants (*Z. mays*). The amount of the resulting changes depends on the stage of the plant life, the stressor concentration and exposure time. Studies showed that osmotic drought stress reduces germination, water uptake and inhibits the elongation growth of the *Z. mays* organs. It causes the disorganization of cell membranes in seedlings, underground and aboveground organs of *Z. mays*, regardless of the treatment phase. Osmotic drought stress has also impact on the parameters of chlorophyll fluorescence, chlorophyll content in leaves and changes in gas exchange in *Z. mays* plants.

REFERENCES

PODLEŚNY J., PODLEŚNA A. 2010. Effect drought stress on yield of a determinate cultivar of blue lupine grown in pure sowing and in mixture with barley. Acta Scientiarium Polonorum, Agricultura, 9(3):61-74.
RUT G., RZEPKA A., KRUPA J. 2010. The effect of hypoxia and post-hypoxia on the fluctuations in concentrations of malate and citrate, the activity of malic enzyme, and on the intensity of gas exchange in moss gametophores. Photosynthetica 48: 79–86. http://DOI: 10.1007 / s11099-010-0011-x.
RZEPKA A. 2008. Ecophysiological aspects of the response different species of mosses to abiotic stress factors. Monograph No. 501, Pedagogical Academy in Cracow (In Polish)
SOETAERT W., PETRA T. VANHOOREN P. T., VANDAMME E. J. 1999. The Production of Mannitol by Fermentation. Carbohydrate Biotechnology Protocols. Pp. 261-275. http://DOI: 10.1007/978-1-59259-261-6_21.
STALMACH K., BOREK M., ŚNIEGOWSKA K. 2012. Physiological aspects of responses spring barley (*Hordeum vulgare* L.) to drought stress. Episteme 14:395-408.
TANGUILIG V.C., YAMBAO E.B., O'TOOLE J.C., DE DATTA S.K. (1987). Water stress effects on leaf elongation, leaf water potential, transpiration, and nutrient uptake of rice, maize, and soybean. Plant and Soil 103:155-168. http://DOI: 10.1007/BF02370385.
VOLKMAN K. 1997. Water stress in roots of wheat: effects on leaf growth, Aust. J. Plant Physiol. 24:49-56. http://DOI: 10.1071/PP96063.
YORDANOV I., VELIKOVA V., TSONEV T. 2003. Plant responses to drought and stress tolerance Bulg. J. Plant Physiol. Pp. 187–206. http://DOI 10.1023/A:1007201411474
AMUTHAVALLI P., SIVASANKARAMOORTHY S. 2012. Effect of salt stress on the growth and photosynthetic pigments of pigeon pea (*Cajanus cajan*). Journal of Applied Pharmaceutical Science 2(11):131-133. http://DOI: 10.7324/JAPS.2012.21124
BARNES J.D., BALAGUER L., MANRIQUE E., ELVIRA S., DAVISON A.E. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. Environmental Experimental Botany 32:85-100.
BLUM A., MUNNS R., PASSIOURA J.B., TURNER N.C., SHARP R.E., BOYER J.S., NGUYEN H.T., HSIAO T.C. 1996. Letters to the editor Genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations? Plant Physiol. 110:1051–1053.
FAROOQ M., WAHID A., KOBAYASHI N., FUJITA D., BASRA S.M.A. 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29:185–212. http://DOI: 10.1051/agro.
HSIAO T.C., ACEVEDO E., FERERES E., HENDERSON D. W. 1976. Water Stress, Growth, and Osmotic Adjustment. Phil. Trans. R. Soc. Lond. *B* 273. http://DOI: 10.1098/rstb.1976.0026.
HURA T., HURA K., GRZESIAK M. RZEPKA A. 2007. Effect of long-term drought stress on leaf gas exchange and fluorescence parameters in C3 and C4 plants. Acta Physiol. Plant 29:103-113. http://DOI: 10.1007/s11738-006-0013-2
KALAJI M.H., LOBODA T. 2010. Chlorophyll fluorescence in the study of the physiological condition of plants. SGGW. (In Polish)
KLAMKOWSKI K., TREDER W. 2011. Effect of water deficit on gas exchange parameteres, growth and yield of two strawberry cultivars grown under greenhouse conditions. Infrasctructure and ecology of rural areas, Polish Academy of Science 5:105-113. (In Polish)
LICHTENTHALER H.K., BUSCHMANN C., KNAPP M. 2004. Measurement of chlorophyll fluorescence kinetics (Kautsky effect) and the chlorophyll fluorescence decrease ratio (RFd-values) with the PAM-Fluorometer. In: Analytical Methods in Plant Stress Biology (N. Filek, J. Biesaga-Koscielniak, I. Marcinska, eds.), The Franciszek Gorski Institute of Plant Physiology of the Polish Academy of Sciences, Cracow, 110:93-111.
LIU J., GUO W., SHI D. 2010. Seed germination, seedling survival, and physiological response of sunflowers under saline and alkaline conditions. Photosynthetica 48(2):278-286. http://DOI: 10.1007/s11099-010-0034-3.
LU Z., NEUMANN P.M. 1999. Water Stress Inhibits Hydraulic Conductance and Leaf Growth in Rice Seedlings but Not the Transport of Water via Mercury-Sensitive Water Channels in the Root. Plant Physiology 12:143–151. http://DOI:10.1104/pp.120.1.143.
OLSZEWSKA M., GRZEGORCZYK S., OLSZEWSKI J., BAŁUCH-MAŁECKA A. 2010. A comparison of the response of selected grass species to water stress. Grassland Science in Poland 13:127-136.
O'NEILL S. 1983. Role of Osmotic Potential Gradients during Water Stress and Leaf Senescence in *Fragaria virginiana.* Plant Physiol. 72:931-937. http://DOI: 10.1104/pp.72.4.931.