

Influence of Salt and Osmotic Stress on Germination of Different Wheat Cultivars

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ABSTRACT

The aim of this research was to identify the cultivars of winter wheat which tolerate drought and increased salinity at the germination stage. The testing was carried out under controlled conditions with an aim to test reaction of 5 different cultivars of winter wheat to salinity and osmotic stress during the early stage of a seedling's growth. The test included examination of energy of germination and percentage of germination seeds. After being sterilized in 96% Ethanol and rinsed with distilled water, 50 uniform seeds were transferred to Petri dishes (divided per genotype and treatment) filled with a solution (Mannitol and salt) in which the water potential was of almost 0 (control), -0.3, -0.6, and -1.2MPa. Alcohol Mannitol was used to create water stress and NaCl to create salt stress. Seedlings were incubated for 7 days at 25°C. The results showed that the benchmark water potential (under the influence of Mannitol and salt), in which all varieties can germinate and have a good growth of seedlings, is of -0.3 MPa, i.e. of the low stress. Under the stronger stress (-0.6 MPa) all the varieties showed reduction in all measured parameters, while under the stress of -1.2 MPa all cultivars failed to germinate. Bosanka cultivar showed the best result germination and energy of germination under the influence of water and salt stress when compared to other cultivars. An exception was the cultivar Orion where stress conditions had inhibited energy of germination and germination, when compared to other cultivars. Reasons for stronger resistance, or sensitivity, of certain varieties may be numerous and further studies conducted on this or other varieties of wheat would contribute to understanding and explanation of these differences.

Key words: Germination, Cultivars, Mannitol, Wheat, Salt, Drought

INTRODUCTION

Stresses from the surrounding environment are limiting factor of agricultural production worldwide (Roy et al., 2011). Drought, increased salt concentration as well as other abiotic factors, adversely affecting the quality (Wang, 2011) and the amount (Dolferus et al., 2011) all produced grains, including wheat, which in the future could create very big problems since grains are important food most of the world population. Salinization of land can create external osmotic potential that can limit the absorption of water by the seed, or the sodium and chloride ions can accumulate in the seed that germinates, and has a toxic effect. The salinity of the soil affects the germination of both sides,

either as osmotic stress or a toxic effect of ions. To study the selected wheat as one of the most important and most cultivated plants, not only in our country but also in Europe and the world. In some parts of our country, it may appear soil water deficit, in the autumn, with winter wheat seedling stage. The selection of genotypes that tolerate drought and salt stress in this stage is therefore important, especially if the tolerance in the germination stage correlated with tolerance to drought and salt, with the period of grain filling. Therefore it is necessary through various surveys to better meet the physiological and biochemical processes that make the plant tolerant to drought.

MATERIALS AND METHODS

The tests included examination parameters of germination five different cultivars of winter wheat (Jelena, Kristina, Orion, Bosanka and Nova bosanka) under controlled conditions. The seeds have been selected in the Agricultural Institute of the Republic of Srpska. The experiment was set up in the laboratory of the Faculty of Agriculture in East Sarajevo. Left the only completely healthy and clean seeds for testing. Then the seeds were sterilized in 96 % alcohol for 30 seconds, then washed with distilled water several times, and 50 uniform, healthy seeds is transferred into sterile Petri dishes. The cups are with double layer of filter paper, soaked in saline certain concentration (mannitol and salt, Table 1.) , and it dissolves with water potential close to zero were controls, then, -0.3 , -0.6 and -1.2 MPa

(Braccini et al., 1996). Per 15 ml of solution was poured into each petri dish. Control petri dishes were with distilled water. Alcohol Mannitol is used to induce water stress and sodium chloride to induce stress of salt. Trial set with three replicates per each treatment and each variety separately. Seedlings were incubated in an incubator (Binder) 7 days, at a temperature of 25°C , and the appearance of the first true leaf . As the start of germination was recorded in one day when the primary roots have reached a length of 2 mm. During trial we measured energy germination, the 4th day of the trial , where we numbered all germinated seed to that period and expressed in percentage compared to the total number of seeds . On the seventh day of the trial, ie, the appearance of the first leaf we measured total number of germinated seeds in relation to the total number of seeds from one variants .

Table 1. Amounts of sodium chloride and mannitol used to obtain different levels of water deficit

Ψ_0 the level of MPa	NaCl (g/l distilled water)	Mannitol (g/l distilled water)
0	0	0
-0.3	4.20	22.29
-0.6	8.40	44.58
-1.2	16.81	89.17

All data were statistically processed using Statistically analysis of variance (ANOVA). Differences between treatments within each cultivar and differences between varieties within treatments were tested with the test of least significant difference (LSD).

RESULTS AND DISCUSSION

Alcohol Mannitol is used to induce water stress (Chen et al., 2010), as osmoticum in the germination test because it does not damage the embryo and causes the inhibition of seed germination by affecting the adoption of water energy. Test results of germination (Table

1,1a,1b), with different varieties of wheat, whose seeds were germinated on medium with various concentrations of Mannitol , show that the maximum value for all cultivars, were in control of the water potential 0MPa, except for cultivars Bosanka, whose maximum value was at a water potential of - 0.3MPa. With increasing water stress , at - 0.6MPa in all cultivars, decreasing the value of the energy of germination and it was highly significant in cultivar Orion, Jelena and Kristina. Cultivar Orion reduced germination of 95 % compared to the control. The results showed a significant difference between cultivars with different treatments. Changes in water

potential to more negative, cause the reduction in germination, proven Pratap and Sharma (2010) in studies on seed

Tab.1.Cultivars energy of germination

Cultivar	Average
Jelena	56
Orion	42
Kristina	54
Bosanka	66
Nova bosanka	47
SD _{5%}	1.80
SD _{1%}	2.40

Tab.1a.Energy of germination with different Mannitol concentrations

Mannitol(MPa)	Average
0	81
-0.3	73
-0.6	59
-1.2	0
SD _{5%}	1.60
SD _{1%}	2.16

Tab.1bInfluence of cultivar and Mannitol on energy of germination

Cultivar	Mannitol (MPa)	Average
Jelena	0	90
	-0.3	64
	-0.6	70
	-1.2	0
Orion	0	92
	-0.3	73
	-0.6	5
	-1.2	0
Kristina	0	75
	-0.3	73
	-0.6	69
	-1.2	0
Bosanka	0	90
	-0.3	89
	-0.6	86
	-1.2	0
Nova bosanka	0	60
	-0.3	66
	-0.6	63
	-1.2	0
	SD _{5%}	3.62
	SD _{1%}	4.83

Stronger deficit (Table 2,2a,2b) of water has led to highly significant reduction in the percentage of germination of all cultivars. In -0.6MPa worst result had

Vigna, where energy and germination percentage germination was inhibited by increasing the osmotic potential. cultivar Orion where there has been a reduction in measurement results by 69 % as compared to the control. A statistically significant difference in the percentage of germination was between individual varieties at different water resources. Cultivar Orion had significantly lower results in comparison to other tested cultivars, and 66 % lower score than cultivar Bosanka. In our experiment, at -1.2MPa all cultivars not germinate. According to Mayer and Poljakoff - Mayber (1989), these results may be due to lack of energy to start sprouting. Similar results were obtained by Braga et al., (1999), in seeds of bean where the potentials between -0.4 and -0.6MPa, led to a decline in the value of all parameters examined as germination percentage and germination. Also, seeds with low physiological quality showed greater decline parameters examined germination and at less negative water potential. Yang et al .,(2010) in tests on *Picea asperata* published that with increasing water potential and the degree of stress tends to fall germination percentage and germination index , especially at -0.6MPa . Also, with camomile , the percentage of germination and seedling growth were reduced with PEG - which included increased osmotic potential in the medium to germinate (Afza et al ., 2006). Demir et al ., (2008),examined the impact of drought and salt stress on seed germination of pepper . Their results showed that a high percentage of germination was in medium containing sodium chloride in relation to the PEG, at the same concentrations, indicating the detrimental effect of PEG on germination, that was stronger than the osmotic effect of the accumulation of specific ions. Similar results, in pepper, get the Chartzoularis and Klapaki (2000)

Tab.2. Cultivars germination

Cultivar	Average
Jelena	56
Orion	49
Kristina	54
Bosanka	66
Nova bosanka	56
SD _{5%}	1.19
SD _{1%}	1.59

Tab. 2a. Germination with different Manitol concentrations

Mannitol(MPa)	Average
0	86
-0.3	75
-0.6	67
-1.2	0
SD _{5%}	1.33
SD _{1%}	1.78

Tab.2b Influence of cultivar and Manitol on germination

Cultivar	Mannitol (MPa)	Average
Jelena	0	90
	-0.3	64
	-0.6	70
	-1.2	0
	0	95
Orion	-0.3	73
	-0.6	30
	-1.2	0
	0	75
	-0.3	73
Kristina	-0.6	70
	-1.2	0
	0	90
	-0.3	89
	-0.6	87
Bosanka	-1.2	0
	0	79
	-0.3	77
	-0.6	70
	-1.2	0
SD _{5%}	2.66	
SD _{1%}	3.56	

Table (Tab.3,3a,3b) shows results of germination different wheat cultivars whose seeds are germinated in the medium with different concentrations of salt. Maximum value of cultivar Jelena, Kristina and Orion had in control, but Bosanka and Nova bosanka bosanka at -0.3MPa. Lower stress in the cultivars

Jelena, Orion, Nova bosanka, caused a highly significant reduction in germination compared to the control. With increasing salt concentration in the medium for germination, the osmotic potential of -0.6MPa, in all varieties, caused a highly significant reduction in germination. In cv Orion germination decreased of 92 % compared to control. In the control test variant, the worst results showed cultivar Nova bosanka. Results indicate a highly significant difference in measurements between different varieties under different treatments. Variety Orion is at -0.6 MPa had a statistically significant decrease in germination when compared with other varieties and by 88 % compared to the variety Bosnian woman who showed the best result.

Of salt stress affects many physiological aspects of plant growth. Increasing the concentration of salt in the growth medium results in an increase in shoot and root respiration (Libnah et al ., 1996) . For winter crops, which includes winter wheat, land for sowing may contain more salt at the time of sowing due to high evapotranspiration during the previous summer, where salt migrates to the surface of the land , and as the seed sown at 10 cm depth that part and contains the most salt. To seed germinated in such medium, must have strong vigor, that he survive and passed through a layer of salt on the surface (Huang et al ., 2003) .

Tab.3. Cultivars energy of germination

Cultivar	Average
Jelena	55
Orion	43
Kristina	48
Bosanka	60
Nova bosanka	46
SD _{5%}	1.10
SD _{1%}	1.50

Tab.3a. Germination with different salt concentrations

NaCl (MPa)	Average
0	80
-0.3	76
-0.6	44
-1.2	0
SD _{5%}	0.97
SD _{1%}	1.30

Tab.3b Influence of cultivar and salt on energy of germination

Cultivar	NaCl (MPa)	Average
Jelena	0	92
	-0.3	77
	-0.6	50
	-1.2	0
Orion	0	97
	-0.3	66
	-0.6	8
	-1.2	0
Kristina	0	76
	-0.3	76
	-0.6	42
	-1.2	0
Bosanka	0	84
	-0.3	85
	-0.6	70
	-1.2	0
Nova bosanka	0	50
	-0.3	78
	-0.6	58
	-1.2	0
	SD _{5%}	2.22
	SD _{1%}	2.97

Similar results were also when we measured the percentage of germination (Tab.4,4a,4b), where a maximum of three cultivars was in control a cultivar Bosanka and Nova bosanka in -0.3MPa . In cultivar Jelena and Orion low- stress induced accumulation of Jelena and Orion there was a highly significant reduction in the percentage of germination compared to the control and the stronger stress in all varieties. In stronger stress, variety Orion had significantly lower test result, 69 % compared to control. When we comparing cultivars, in stronger the stress of salt the best result had cultivar Bosanka. A statistically significant

decrease in the percentage of germination had variety Orion compared to other varieties and by 57 % compared to the cultivar Bosanka who showed the best result. Our results are in agreement with published studies on the lens, where they treated with different concentrations of NaCl, and there was a reduction in the percentage of germination and growth of all tested parameters and the relative water content, with increasing concentration of the sodium chloride (Sidari et al ., 2007). Similar test results were obtained for the sugar beet, where the germination and growth of young seedlings was inhibited by NaCl (Wang et al ., 2011). De Lima and Torres (2009) reported similar results and concluded that the effect of salt stress depends on the plant species. Our results are in agreement with the Moezel and Bell (1987), where the influence of NaCl on germination, as the ionic effect, ie . when the ion reaches the cell toxic effect. Reji et al., (2010 found that the decline in the percentage of germination caused by the osmotic effect of salt as NaCl concentration that was used did not damage the embryo and not germinated seeds were able to germinate when the stress is removed and the salt that is in accordance with Rehman (1997) . At -1.2 MPa seeds not germinated.

Tab.4. Cultivars germination

Cultivar	Average
Jelena	56
Orion	48
Kristina	54
Bosanka	60
Nova bosanka	54
SD _{5%}	1.35
SD _{1%}	1.80

Tab.4a. Germination with different salt concentrations

NaCl (MPa)	Average
0	84
-0.3	76
-0.6	57
-1.2	0
SD _{5%}	1.21
SD _{1%}	1.62

Tab.4b Influence of cultivar and salt on germination

Cultivar	NaCl (MPa)	Average
Jelena	0	94
	-0.3	77
	-0.6	52
	-1.2	0
Orion	0	97
	-0.3	66
	-0.6	30
	-1.2	0
Kristina	0	76
	-0.3	76
	-0.6	66
	-1.2	0
Bosanka	0	84
	-0.3	87
	-0.6	70
	-1.2	0
Nova bosanka	0	70
	-0.3	78
	-0.6	69
	-1.2	0
	SD _{5%}	2.72
	SD _{1%}	3.65

CONCLUSION

Based on the examination of the effect of stressful conditions, different strengths of the osmotic potential and salinity, it can be concluded that all the varieties showed a reduction of all measured parameters of germination, in medium (-0.6MPa) and it could be an indication of their sensitivity to stressful conditions. Seedlings was small reduced with the water potential of -0.3MPa, more at -0.6MPa to at -1.2MP come to a complete inhibition of germination. Cultivar Bosanka showed significantly better result percentage of germination and

energy of germination in conditions of water deficit and salinity, as compared to other tested cultivars. It can be concluded that the osmotic stress and specific ions, both influence on germination energy and germination, although it is considered that mannitol and sodium chloride act through different mechanisms in seed and plant. We can conclude that the limit values of water resources when plant, under the influence of mannitol and sodium chloride, in all varieties can germinate quite well and have a good increase in seedling were at -0.3 MPa, ie in low stress.

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