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Investigation of Tolerance Amount to Salinity of *Dodonea viscose* in *In vitro* Condition

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Abstract: Environmental stresses such as salinity which is result of land use changes are caused to change the vegetation cover of an area. These stresses are caused eliminating and the gradual adaptation of plant species with different climatic conditions during the time. Investigation of Response of plants to salinity is one of research projects that can help to introduce plants for environmental hard conditions. In the research first was provided medium L_2 (culture environment). Then the treated seeds of *Dodonea viscose* were established in medium L2 where the concentration of its ions including K^+, Na^+, Ca^{++} and mixed K^+ and Na^+ had been increased to 100, 200 and 300 mmol. Then effects of this concentration increase was evaluated on seeds by investigation of the germination of seeds and germination indicators such as germination rate (GR), mean daily germination (MDG) and etc. and then was compared with control. Statistical analysis was carried out in the factorial design in SAS software. After diagnosis of significant treatments, averages comparison was accomplished by LSD test method at 1 percent statistical level. The results showed that the effect of salt concentration on germination indicators of *Dodonea viscose* as amount of these indicators was significantly high in the control treatment rather than other salt concentrations and by increasing the salinity decreases germination and all of germination indicators. The plant response to combined K^+ and Na^+ concentration was negative among of investigated salts as germination was observed even in 100 mmol concentration of this salt. The effect of salt type was also Significant on the germination and germination rate and the effect of these treatments was not significant on final germination.

Key words: Resistance · Salinity · *Dodonea viscose* · Calcium · Sodium · Potassium

INTRODUCTION

Environmental stresses are one of the most important limiting factors for agricultural products in the world [1]. Salinity stress is one of the main factors that caused the destruction of species in Arid and semi-arid area [2]. Considering to approximately 12.5 percent of soils In Iran are saline and alkaline, soil salinity phenomenon is a serious problem and it is increasing day by day [3]. Soil salinity affects on plants in terms of many aspects such as metabolic, anatomical and morphological. These changes are often as adaptations that increases the tolerance of plant to salinity [4-7].

Results of the performed researches show that plants show the morphological and physiological changes (reduction in leaf area, leaf abscission, root development, etc.) with salinity increasing that the result is reduction in performance and biomass [4, 6, 8-12]. The plants adaptation with salt stress by different ways such as reduction of roots or shoots (Ghassemi Firoozabadi, 1999, [7, 9, 13, 14]. In Addition the salt type has a significant effect on plant production and sodium and chloride ions have a greater adverse effect on growth reduction and yield efficiency rather than other cations and anions [1, 4, 6, 8, 9, 10,12]. Mass (1993) [15] analyzed the Plant growth response to salinity stress in the laboratory environment and concluded that the production rate (biomass) of roots and stems in sodium sulfate treatment is greater than sodium chloride treatment. Many plants have growth reduction in the saline environments that it can be attributed to concentrations of toxic ions such as sodium and chloride in plant tissues. [4, 8, 9, 11, 16].

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According to opinions of some researchers, the effect of salinity on plant is due to osmotic pressure and the specific effects of various ions in the root environment [17]. In fact the Energy consumption during osmotic adjustment with salinity is a major cause of reduction in root growth in plant [18].

Amiri and Fahimi (2004) [19] investigated the effect of different concentrations of Nacl and Kcl on tissue culture of bean plant (*Phaseolus vulgaris* L.). The results showed that increase of Nacl and Kcl concentration has the adverse effects on plant growth. So the fresh weight and dry weight, ratio of wet weight to dry weight and the amount Of Total protein decreases by increasing of Kcl and Nacl in culture medium. Results of This study also Showed that adverse effects of Kcl on growth is more than Nacl in the equal concentrations.

Katembe *et al*. [20] studied the Effect of salinity stress on seed germination and rootlet length of the halophyte and concluded that increasing the salt concentration decreases the germination percentage, germination rate and rootlet length. Effect of Sodium and potassium salts on the germination of *Atriplex prostrate* also showed that germination percentage decrease by increasing of these salts. In addition the initial effects of salt on germination reduction are due to the disruption in osmotic pressure [21]. Karimi *et al*. [22] investigated the effect of salinity stress on the germination of *Atriplex verrucifera* in the different treatments of 0, 100, 200, 300 and 400 mMolar Nacl and concluded that germination decreased by increasing of salt concentration. In addition maximum germination has been occurred in the control treatment. In addition stemlet and rootlet length increased from 0 to 200 mMolar Nacl concentration but rootlet length decreased by further increasing of salt. The Fresh and dry weight of seedling also was higher in low concentrations of salt.

Since salinity and soil salt are the most important factors in the distribution of plant species [10, 17, 23, 24] and evaluation of the plants tolerance to various environmental factors help to determine their resistance to the environmental stress threshold, we investigated the effects of the salinity stress due to salts of $Na^{+,}$ $K^{+,}$ K^{+} with Na^{+} and Ca^{++} in different concentrations of 100, 200 and 300 mmol on the *Dodonea viscose* under invitro to determine the adaptation range of this species to different amounts of salt and salinity in this research.

MATERIALS AND METHODS

This Research was performed in Biotechnology Laboratory of Sari Agriculture and Natural Resources University (SANRU). Objective of this research was Evaluation of Resistance to salinity of *(Dodonea viscose)* Species in Different Salinity conditions under Invitro condition. Therefore the Collected Seeds were set under the Germination Treatment. For this purpose seeds were set in hot water for 5 minutes. Then seeds were set in 98% sulfuric acid for 45 minutes. Then we prepared the L_2 medium containing the high-Consumption and Low -Consumption ions, Vitamins and Sugar. Then Ions Concentration of Ca^{++}, K^{+}, Na^{+}, Na^{+} and K^{+} composition were increased to 100, 200 and 300 mmol in L_2 medium. Effects of Concentration increasing was investigated by placing of treated seeds in the Glass Dishes with given Concentration. 10 seeds were placed in each Glass Dishes and 5 glass dishes were considered for Each Treatment. 50 Seeds were placed in L_2 medium For Each Treatment totally. Then Effects of these Treatments were investigated on Germination rate of *Dodonea viscose* Seeds. Situation of the Seeds Germination were investigated by calculating of germinated seeds weekly. Seedlings were extracted of L_2 medium After 56 days and other measurements were done on them. Statistical Analysis was performed at Factorial Plan Format in SAS software. LSD mean Test in statistical Level of 1% was performed after the determination of Significance of Treatments.

RESULTS

Investigation of Situation of Seeds Germination Based on Treatments Separation: Results Showed That the Witness (Control) Treatment With The mean average of 9.75 Of 10 Cultured seeds in L_2 medium has the highest germination among the investigated treatments. on the other hand K^{+} Na^{+} treatment in Three Levels of salt concentration (100, 200, 300 mmol) and Ca300 treatment with Zero germination have had The lowest germination. In addition K100 treatment among other Treatments has had the highest germination after Witness (Control) Treatment with The mean germination of 9. Germination rate of seeds in Other Treatments Also has been shown in Figure 1.

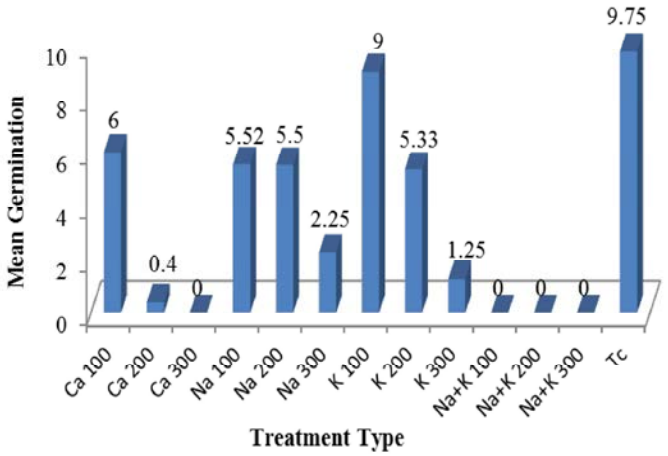

Fig. 1: Mean germination of seeds in medium L2 based on Treatments Separation (Source: research findings)

Table 1: Variance analysis of effect of salt type, salinity and the reciprocal effect of salt type and salinity on the investigated factors (Source: research findings)

	Salt Type				Salt concentration				Salt Type* salinity			
Changes resources	df	SS	MS	F-value	df	SS	MS	F-value	df	SS	MS	F-value
Germination Index (GI)	2	784.8	0.39	5.08*	2	1476.1	738.01	9.55***	2	123.52	61.76	0.8^{ns}
Final germination (FG)	2	1077.6	0.83	1.49^{ns}	2	7236.6	3618.32	9.99***	2	0.01	848.51	2.34^{ns}
Mean daily germination (MDG)	2	0.34	0.17	1.49^{ns}	2	1.31	1.15	9.99***	2	0.54	0.27	2.34^{ns}
Germination rate (GR)	2	2.47	1.23	4.89*	2	3.69	1.84	7.30***	2	0.18	0.09	0.35^{ns}
Seed stamina (SS)	2	4282.4	0.2	16.57***	2	12538.4	6269.23	48.52***	2	690.3	345.15	2.67^{ns}

Table 2: Mean Comparison of Germination Parameters in 100 mmol Concentration using LSD method (Source: research findings)

Salt type	Germination Index (GI)	Final germination (FG)	Mean daily germination (MDG)	Germination rate (GR)	Seed stamina (SS)
Calcium	28^{bc}	60^{b}	1/071^{b}	1/413^{b}	17/353^{b}
Sodium	12/66^{c}	43/33^{b}	0/774^{b}	0/543^{b}	17/40^{b}
Potassium	28/31^{b}	63/33^{b}	1/131^{b}	1/392^{b}	37/706^{b}
Witness (control)	54/43^{a}	97/50^{a}	1/741^{a}	3/748^{a}	126/668^{a}

Variance Analysis of Effect of Treatments on Germination Factors: Results of Variance analysis showed that the effect of salt type on Germination Index (GI) and Germination rate (GR) was significant in the 5% level and effect of salt type on Seed stamina (SS) was significant in the 1% level. On the other hand effect of salt type on Mean daily germination (MDG) and Final germination (FG) was not significant (Table 1). Furthermore, the Salinity effect on germination Factors Including Germination Index, Final germination, Mean daily germination, Germination rate and Seed stamina was significant in the 1% level. Results Also Showed that the reciprocal effect of salt type and salinity on Mentioned germination Factors was not Significant (Table 1).

Comparison of Means of Treatment Effect of Salt Type on Germination Factors: Comparison of means Show that difference between Values of Witness (Control) Treatment with other Treatments is noticeable in germination factors such as Final germination (FG), Mean daily germination (MDG), Germination rate (GR) and Seed stamina (SS) except Germination Index (GI) and the Numerical Values of these factors in Witness Treatment is more than Other Treatments significantly. Numerical Values of potassium salt are more than calcium salt and Numerical Values calcium salt are more than sodium among Numerical Values of germination factors. But values of these three salts statistically are significant despite of difference (Table 2). The means values are Different in germination Index (GI) factor with other factors. As Difference between the Witness value with values of all the salts in 100 mmol Mole is significant. In addition Numerical Values mean of sodium salt is significant with Numerical Values of potassium salt with 12.66 values. But Difference Between Values of Calcium Salts With Sodium salts And Values of Calcium With potassium is not significant and have Approximately near Numerical Values.

DISCUSSION AND CONCLUSION

The first determinant Parameter in Relevant Studies To Treatments Effect Salinity With Different Concentrations That it was caused the stress in Plant is Germination of Plant. Results of this research Showed That the Witness (Control) Treatment With The mean average of 9.75 Of 10 Cultured seeds in L_2 medium has the highest germination among the investigated treatments because of Normal Limit of salts. In fact only about 0.25 of seeds in this treatment have not germination Ability. On the other hand germination percent were decreased by increasing in salts Concentration in Other Treatments except K 100 treatment that has high mean germination after Witness (Control) treatment.

Results of Other Research also showed that Germination was decreased by increasing of Concentration of Sodium and potassium Salts and the initial effects of salt on germination reduction are due to the disruption in osmotic pressure [21]. Katembe *et al*. [20] also concluded that increasing the salt concentration decreases the germination percentage. In addition investigation of the salinity stress effect on the germination of Atriplex verrucifera in the different treatments of 0, 100, 200, 300 and 400 mMolar Nacl showed that germination was decreased by increasing of salt concentration and maximum germination has been occurred in the control treatment [22].

Notable Point in this research is that germination was observed in Na, K and Ca salts at least in 100 mmol Concentration and germination was decreased by increasing of salts Concentration. But germination wasn't observed in Na^+ K^+ salt even in 100 mmol Concentration. Reciprocal effect of these two salts may be caused the limitation of growth and prevention of seeds germination of this species.

Comparison of means Showed that difference between Values of Witness (Control) Treatment with other Treatments was noticeable in germination factors such as Final germination (FG), Mean daily germination (MDG), Germination rate (GR) and Seed stamina (SS) except Germination Index (GI) and the Numerical Values of these factors in Witness Treatment was more than Other Treatments significantly. As the results of Other Researches Also showed that the rootlet length of halophytes decreases by increasing the salt concentration and effects of salinity stress [20].

Finally we recommend that the tolerance amount to salinity of other halophytes in Iran will be investigated at invitro condition in the future researches to determine the range of their adaptation to different salts types and different salinity amounts and to evaluate their resistance amounts in hard conditions.

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