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ANALYSIS OF SALT TOLERANCE OF *MEDICAGO* L. PLANTS

A. Molor, E. Vanjildorj

Adiyasuren Molor
M. Sc., Department of Biotechnology and Breeding,
School of Animal Science and Biotechnology,
Mongolian University of Life Sciences
Zaisan 17024, Ulaanbaatar, Mongolia
E-mail:adya.molor@gmail.com

Enkhchimeg Vanjildorj
PhD, Department of Biotechnology and Breeding, School of Animal Science and Biotechnology, Mongolian University of Life Sciences
Zaisan 17024, Ulaanbaatar, Mongolia
E-mail: enkchimeg.v@muls.edu.mn

Salinity becomes a concern when an 'excessive' amount or concentration of soluble salts occurs in the soil, either naturally or as a result of mismanaged irrigation water. Alfalfa (*Medicago* L.) acts as a most important legume forage crop and is widely cultivated in various environments. The objective of this study was to compare the performance of salt stress during germination and growth stage of 2 species (*Medicago sativa, Medicago falcata*) and 2 varieties (Mongolian *Medicago varia* Marthz var. Burgaltai and Inner Mongolian *Medicago varia* Martyn var.Nutag Belcheer-2) in laboratory condition. Salt stress in plants was induced by NaCl (0-as a control, 50, 100 and 200 mM concentration) for 2 weeks. The results represented that *Medicago sativa* had higher seed germination percentage than other species and varieties. Root length and weight, dry matter index are observed in *M.sativa* higher than others. *M.falcata* showed the maximum result of shoot weight and length. *M. varia Martyn* var.Nutag Belcheer-2 illustrated higher chlorophyll content and water uptake than *M.varia Marthz* var.Burgaltai. In conclusion, *M.sativa* and *falcata* are tolerant to salt stress. *M.varia* Martyn var.Nutag Belcheer-2 is moderately tolerant to salt stress. *M.varia* Marthz is sensitive to salt stress than other *Medicago* L. plants.
Keywords: salt stress, *Medicago* L., germination index, morphological and physiological parameters, chlorophyll content.

Introduction

Salinity is one of the abiotic stresses limiting crop production in arid and semiarid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching [Neumann, 1995; Saboora, 2006]. Irrigation systems are particularly prone to salinization; about half the current irrigation systems in world are influenced by salinization, alkalization or waterlogging [Munns, 2002]. There are 54.1-thousand-hectare yield is available for irrigation crop land in Mongolia, consequently, 40.4-thousand-hectare yield is irrigated for plant crop.

Five species of *Medicago* L. grow naturally in Mongolia, and one introduced variety of alfalfa has been released in Mongolia. *Medicago* L. is the third most important forage species in the germplasm collections behind Elymus and Agropyron [Jigjidsuren and Johnson, 2003].

There is comparative analysis of salt tolerance between *Medicago falcata* and *Medicago truncatula*. Min Lui et al., [2014] demonstrated that the *Medicago falcata* is more tolerant to salt stress than annual legume *Medicago truncatula.*

In 1986, Khaisan I, who evolved variety Burgaltai by cross pollinating *Medicago sativa* and *Medicago falcata*. Geographically Burgaltai variety distributed in steppe, forest steppe zones and Mongolian — Dahurian mountains in Mongolia. From field experiment Burgaltai variety showed that it's tolerant to drought, cold and insects [RIAH, 2014]. Because of the dry character of the country, especially in the Gobi and steppe zones, a reliable harvest vegetables or other crops is possible only using irrigation, rain fed crop production is limited [FAO, 2003].

However, the effects of salinity stress on var.Burlgatai and var. Nutag Belcheer -2 has not been established.

Material and Methods

Plant materials

Mature seeds of 2 species and varieties of *Medicago L*. were used in this study including *Medicago falcata, M. sativa*, *Medicago varia* Marthz var.Burgaltai [Khaisan I.1986] and *Medicago varia* Martyn var. Nutag Belcheer-2. The seeds were obtained from Gene bank of Research Institute of Animal Husbandry. Before cultivation, seeds were sterilized in 2% sodium hypochlorite solution for 3 min, then were rinsed with distilled water for 3 times.

Preparation of NaCl solution

The solutions were prepared with electrical conductivity of 0 (control), 50 mM (moderate level), 100mM (high level), 200 mM (extremely high level).

Determining salt tolerance by seed germination experiment

All petri dishes and filter papers were disinfected in 121^0C for 25 minutes in autoclave. The experiment was carried out in 3 replicates where 20 seeds from each species and variety were separately germinated on sheet of Whatman No.1 filter paper in Petri dishes. Priority, 10 ml from one respective test solution was poured into the plate. The plates were placed into an incubator at 25 ± 2^0C in darkness for eight days. Seed germination index is the percentage of seed which germinate at 2^{nd}, 4^{th}, 6^{th} and 8^{th} day of observation. Percentage of seed germination drought stress tolerance index is determined as below:

Germination index: nd2 (1.00) + nd4 (0.75) + nd6 (0.50) + nd8 (0.25),

nd ― day of seed germination

Seed germination index is the percentage of seed which germinate at 2^{nd} 4^{th}, 6^{th} and 8th day of observation as indicates by nd2, nd4, nd6 and nd8. Percentage of seed germination stress tolerance index is determined as below:

Germination index, %= (germination index of stressed seeds/germination index of control seeds)*10.

Determining salt tolerance by water uptake

Water uptake was recorded after 2 weeks of watering by salt solutions. Water uptake percentage was calculated by the formula given.

Water uptake, gr = (W2-W1)/W1

W1 — initial weight of seed

W2 — weight of seed after absorbing water

Determining salt stress tolerance by dry matter index

After drying stressed and control plant in oven at 70^0C for 24 hours, the dry matter stress tolerance index was recorded as follows:

dry matter stress tolerance index, gr = dry matter of stressed plants/dry matter of control plants

Determining salt stress by chlorophyll measurement

The Minolta SPAD-502 (Konica Minolta sensing, Japan) is a hand-held light meter used to measure the relative greenness leaves in a rapid manner. The latest model, SPAD -502 determines the relative amount of chlorophyll present by measuring the transmittance of the leaf in two wave bands (600-700 and 400-500 nm). Single fully expanded leaf samples from each species and varieties were obtained at the 14^{th} day in stress. Triplicate reading using a SPAD-502 were taken around midpoint near the mid-rib of each leaf sample and averaged.

Determining salt tolerance by morphological parameters

The experiment was carried out in pots. Eight seeds from each cultivar were separately sown per pot at the depth of 3 cm. Plants were (2-3 fully expanded leaves) watered by 0, 50, 100 and 200mM salt solutions for 2 weeks. Phenotypically observation was done daily. The data for the shoot length (cm), root length (cm), weight of root (g) and weight of shoot (g) were measured and recorded as Akbarimoghaddam et al., [2011] formulas.

Statistical calculation and analysis

The data were analyzed using SPSS 21 analysis of variance, comparisons of mean for evaluated traits by least significant difference (Duncan) method at 0.05.

Results

The seed germination under salt stress

Soil salinity blocks the water uptake, consequently, it negatively affects the plant growth and seed germination. Seed germination *M. sativa* at 50 mM was lower than control by 0.8 %, *M. falcata* by 6.9%, *M. varia* Marthz — 7.3% and *M.varia* Martyn var.Nutag Belcheer-2 — 28.2% than control. When the concentration increased until 100 mM seed germination decreased in all alfalfa plants. Seed germination observed 0.0% at 200 mM (Table 1, Figure 2).

Table 1

The mean seed germination of *Medicago* L.
plants affected by different levels of NaCl, %

Plants	0	50 мМ	100 мМ	200 мМ
Medicago falcata	$96.6^{(a)}$	$89.9^{(a,b)}$	$63.3^{(c,d)}$	$0.0^{(f)}$
Medicago sativa	$93.3^{(a,b)}$	$92.5^{(a,b)}$	$67.6^{(c,d)}$	$0.0^{(f)}$
Medicago varia Marthz	$88.3^{(a,b)}$	$63.4^{(c,d)}$	$22.8^{(e)}$	$0.0^{(f)}$
Medicago varia Martyn	$79.1^{(a,b,c)}$	$73.3^{(b,c,d)}$	$56.8^{(d,e)}$	$0.0^{(f)}$
$P_{variety\ (A)}$	**	**	**	
$P_{NaCl\ (B)}$	**	**	**	
P_{A*B}	*	*	*	

Means of 3 replicates. The seed germination is illustrated at 14th day. Statistically significant differences are observed between the control and stress variants (a,b,c,d,e,f). P value < 0.05 according to Duncan test.

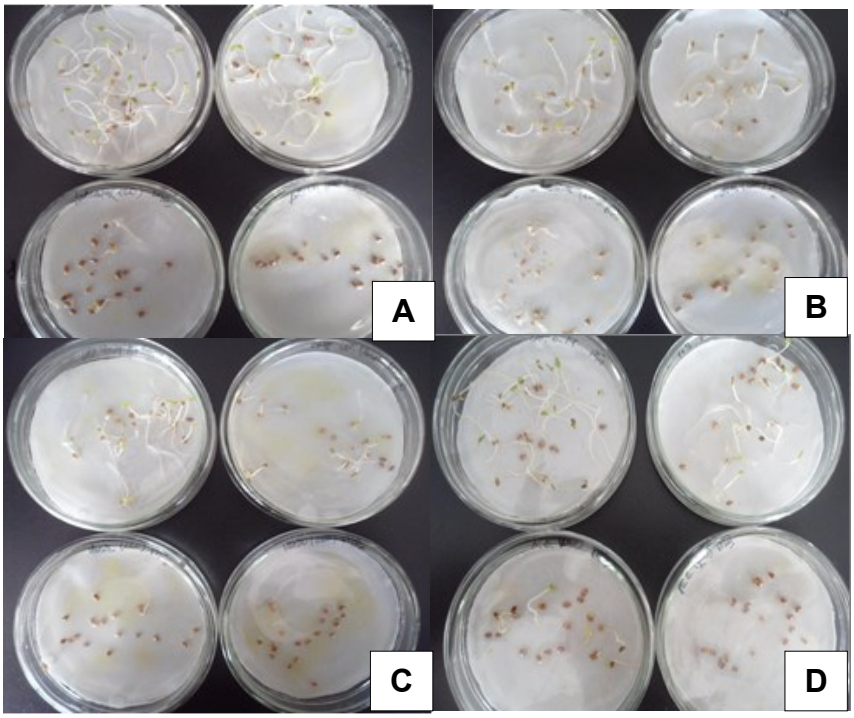

Figure 1. Seed germination of *Medicago* L. plants affected by different levels of NaCl. A-*M.falcata,* B-*M.sativa,* C-*M.varia* Marthz var.Burgaltai*,* D-*M.varia* Martyn var.Nutag Belcheer-2 (Top left-0, top right- 50 mM, bottom left-100 mM, bottom right-200mM)

Result of relative water content

Effect of increasing NaCl levels on water uptake is shown in Table 2. Water uptake ability is decreased when concentration was escalating in comparison to controls. Water uptake in *M.falcata* at 50 mM was declined by 20.6%, at 100 mM 21.4%, at 200 mM by 25.6% lower than control. *M. sativa* also showed a decrease by increasing salt concentration. Under salt condition water uptake by *M. varia* Martyn var.Nutag Belcheer-2 was higher than *M. varia* Marthz var.Burgaltai by 8.5% respectively (Table 2).

Table 2

Water uptake by alfalfa plants affecting by salt stress, gr

Plants	0	50 мМ	100 мМ	200 мМ	Mean (A)
Medicago falcata	1.21±0.1	0.96±0.1	0.95±0.2	0.90±0.3	1.04±0.2d
Medicago sativa	1.66±0.1	1.26±0.3	0.96±0.2	0.81±0.1	1.17±0.3bc
Medicago varia Marthz	1.54±0.2	1.11±0.2	1.08±0.3	1.05±0.3	1.19±0.3b

Medicago varia Martyn	1.72±0.2	1.41±0.2	1.33±0.1	1.11±0.3	1.30±0.3a
Mean of solutions (B)	1.5a	1.2b	1.1bc	0.9d	
$P_{\text{variety (A)}}$	**	**	**		
$P_{\text{NaCl (B)}}$	**	**	**		
P_{A*B}	Ns	ns	ns		

Means of 3 replicates. The water uptake is illustrated at 14th day. Statistically significant differences are observed between the control and stress variants (a, b, c). P value < 0.05 according to Duncan test.

Result of chlorophyll content

The chlorophyll content was declined because of induction of chlorophyllase enzyme. Additionally, accumulation of minerals in chloroplast is one of the reasons of decreasing chlorophyll under salt stress condition. Based on chlorophyll content alfalfa plants can be arranged in the following order: 1) *M. varia* Martyn, 2) *M. sativa*, 3) *M. falcata*, 4) *M. varia* Marthz. Chlorophyll content in *M.falcata* was lower than *M. sativa* by 8.1 %. Among varieties var.Nutag Belcheer -2 was higher than var.Burgaltai by 2.1% respectively (Figure 2).

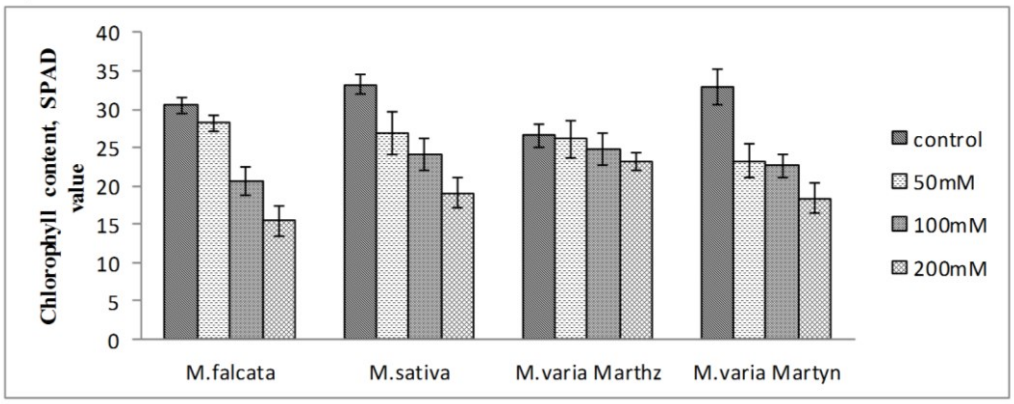

Figure 2. The effect of different NaCl concentration on chlorophyll content after 14 days of salt stress. The columns indicate the mean of 3 replications±SD

Result of dry matter index

Difference in dry weight was significant in alfalfa plants. However, it adversely affected by different NaCl levels (Figure 3).

As a result of mean dry matter index, the heaviest dry matter index was in *M. sativa*. It was higher than *M.falcata* by 10.5 %, var.Burgaltai — 31.5%, var. Nutag Belcheer-2 by 42.1 %.

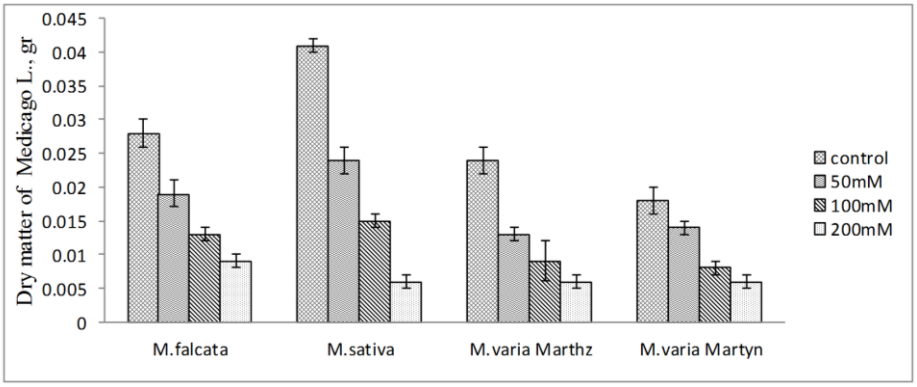

Figure 3. The effect of different NaCl concentration on dry matter index after 14 days of salt stress. The columns indicate the mean of 3 replications±SD

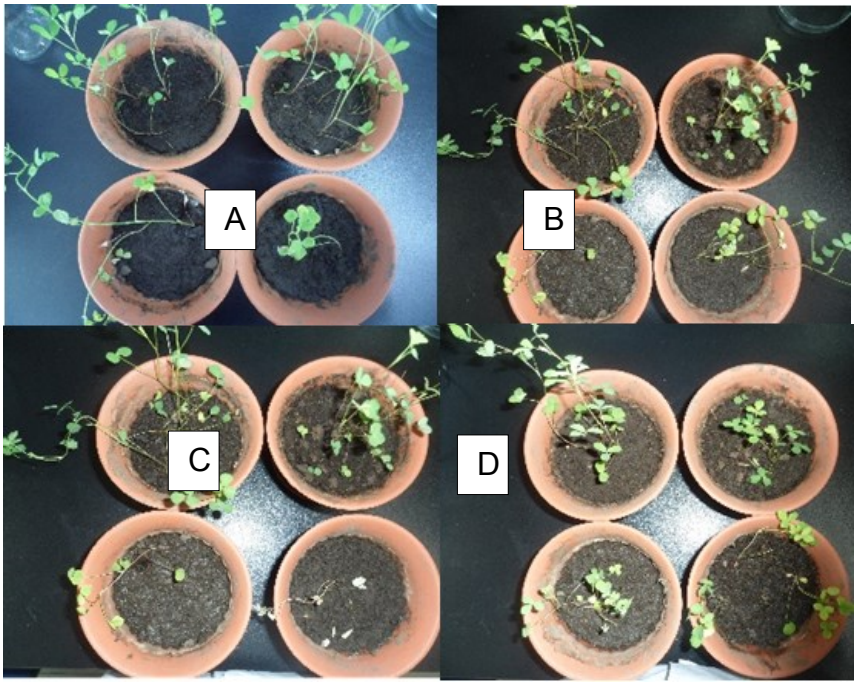

Figure 4. *Medicago* L. seedlings affected by salt for 14 days. A- *Medicago falcata,* B- *Medicago sativa*, C-*Medicago varia* Marthz var.Burgaltai, D-*Medicago varia* Martyn var.Nutag Belcheer-2 (Top left-0, top right- 50mM, bottom left-100mM, bottom right-200mM)

Result of morphological parameters measuring root, shoot length and weight

After 14 days of irrigating different concentration of NaCl shoot, root length and weight were measured (Table 3). Mean of shoot length varied between 8.8 — 11.6 cm. The longest shoot length observed in var.Nutag Belcheer -2 and *Medicago falata*. *Medicago varia* Marthz showed the lowest result among other plants. Shoot length in *Medicago varia* Martyn was longer than *Medicago varia* Marthz by 24.1%.

Table 3

The shoot length of *Medicago* L. plants affected by different level of NaCl, cm

Plants	0	50 мМ	100 мМ	200 мМ	Mean
Medicago falcata	14.6±3.4	11.8±2.7	10.8±2.9	8.4±1.2	11.4±2.5
Medicago sativa	14.5±2.2	9.0±1.6	8.2±0.3	6.9±2.2	9.6±1.9
Medicago varia Marthz	13.6±4.6	9.7±2.6	6.7±0.5	5.2±0.9	8.8±2.0
Medicago varia Martyn	17.7±3.0	11.1±3.5	8.9±3.6	7.6±2.4	11.6±3.3
P value	ns	ns	ns	ns	

The values are the mean of 3 replications. The final shoot length is measured at 14 th day of salt stress. ns-not significant.

Table 4

The shoot weight of alfalfa plants affected by different level of NaCl, gr

Plants	0	50мМ	100мМ	200 мМ	Mean
Medicago falcata	0.19±0.1	0.15±0.1	0.11±0.6	0.06±0.3	0.11±0.7
Medicago sativa	0.21±0.1	0.12±0.7	0.10±0.9	0.09±0.04	0.10±0.6
Medicago varia Marthz	0.15±0.2	0.10±0.1	0.08±0.02	0.07±0.6	0.07±0.7
Medicago varia Martyn	0.13±0.3	0.13±0.1	0.11±0.1	0.11±0.1	0.12±0.9
P value	ns	ns	ns	ns	

The values are the mean of 3 replications. The shoot weight is illustrated at 14 th day. ns-not significant.

As a result of comparing by shoot weight var.Burgaltai was lower than *Medicago falcata* — 9.1%, var.Nutag Belcheer -2 — 41.6 %. The significantly lowest shoot weight observed in var.Burgaltai.

Table 5

The root length of alfalfa plants affected by different level of NaCl, cm

Plants	0	50мМ	100мМ	200 мМ	Mean
Medicago falcata	5.6±0.6	5.3±0.6	4.6±0.7	3.8±1.0	4.8±0.8
Medicago sativa	7.4±0.7	6.2±1.2	4.7±1.2	4.5±2.1	5.7±1.3
Medicago varia Marthz	5.4±1.4	4.7±1.0	4.1±1.0	3.7±0.7	3.4±0.7
Medicago varia Martyn	6.5±1.0	4.9±0.4	4.4±1.0	4.3±1.1	5.0±1.0
P value	ns	ns	ns	ns	

The values are the mean of 3 replications. The percentage is illustrated at 14 th day. ns-not significant.

While increasing NaCl concentration root length of alfalfa is declined. Root length of *M. varia* Martyn was decreased by 24.6 % at 50 mM, at 100 mM — 32.3%, 200 mM — 33.8 % in comparison to control plant. *M. varia* Marthz showed the declining root length by 12.9% at 50mM, 24.0 % at 100 mM, 31.4 % at 200 mM than control. Among species *M.sativa* had longer root length than *M. falcata* (Table 5).

Table 6

The root weight of *Medicago* L. plants affected by different level of NaCl, gr

Plants	0	50мМ	100мМ	200 мМ	Mean
Medicago falcate	0.032±0.01	0.023±0.1	0.014±0.1	0.014±0.1	0.021±0.008
Medicago sativa	0.032±0.03	0.031±0.02	0.031±0.03	0.024±0.01	0.030±0.003
Medicago varia Marthz	0.014±0.06	0.013±0.06	0.009±0.02	0.008±0.05	0.011±0.003
Medicago varia Martyn	0.027±0.01	0.019±0.01	0.018±0.08	0.011±0.08	0.018±0.005
P value	ns	ns	ns	ns	

The values are the mean of 3 replications ±SE. The final seed germination percentage is illustrated at 14 th day. ns-not significant.

Based on root weight, *Medicago sativa* showed highest than *Medicago falcata* by 30 %, var.Nutag Belcheer -2 by 40 %, var.Burgaltai by 63.3% (Table 6). Relating to salt concentration root weight was gradually decreased in all plants.

Discussion

According to Castroluna [2014] research of salt stress tolerance in alfalfa showed declining result in germination and vegetative stage. *M. sativa* seed germination index was by 7% at 200 mM and 54% at 100 mM compare to control. From our research result revealed that *M. sativa* seed germination was 67.6% at 100 mM and 0.0% at 200 mM of NaCl. We can conclude that the NaCl is one the reason for inhibiting the *Medicago* L. plants seed germination.

Morphological and physiological properties were decreased under salt stress. Taiz and Ziger [2002] stated Na^+ is a harmful ion responsible for a majority of agricultural losses whereas K^+ essential ion to plant growth. Pen Gua et al., [2016] exposed salt stress in laboratory condition to *M. sativa* with 250 mM solution. Root length shortened by 68.9%. In our research, M. *sativa* root length was decreased by 70% compare to control even *M. sativa* was selected as the best salt tolerant species. Cordovilla [1999] reported that in legumes, salt stress from 50 to 200 mM NaCl significantly limits productivity by interfering with plant growth.

Emam et al., [2009] concluded that a glycophyte crop such as alfalfa, Na and Cl content were increased, whereas K and Ca were decreased consistently with the progressive increase in salt level of the growth medium.

Rahdari et al., [2012] reported that chlorophyll is one of the major chloroplast components for photosynthesis. Chlorophyll content was declining in all alfalfa plants, especially sharply decreased at 200 mM NaCl. According to Rao and Rao [1981], NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: chlorophyllase, inducing the destruction of the chloroplast structure.

Salt experiment showed that both morphological and physiological properties of 2 varieties and species is degraded with increase of salt concentration.

Conclusion

According to the above experiments it can be concluded that due to high salt concentration *Medicago* L. morphological and physiological feature is degraded. *Medica-*

go sativa was the salt tolerant species compare to *Medicago falcata*. *Medicago varia* Marthz var.Burgaltai was sensitive to salt stress than *Medicago varia* Martyn var.Nutag Belcheer -2.

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АНАЛИЗ СОЛЕУСТОЙЧИВОСТИ ЛЮЦЕРНЫ (*MEDICAGO L.*)

А. Молор, В. Энхчимэг

Адьясурен Молор
магистр,
Монгольский университет общественных наук
Монголия, 17024, г. Улан-Батор, Зайсан
E-mail: adya.molor@gmail.com

Ванчиндорж Энхчимэг
кандидат технических наук,
Монгольский университет общественных наук
Монголия, 17024, Улан-Батор, Зайсан
E-mail: enkchimeg.v@muls.edu.mn

Проблема засоления почв является следствием чрезмерного внесения удобрений, когда концентрация растворимых солей в почве повышается либо естественным путем, либо в результате неправильного орошения водой. Люцерна (*Medicago* L.) является важнейшей кормовой бобовой культурой и выращивается в разных условиях. Цель данного исследования заключается в сравнении показателей солевого стресса двух видов (*Medicago sativa, Medicago falcata*) и двух сортов (Mongolian *Medicago varia* Marthz var.Burgaltai и Inner Mongolian *Medicago vatia* Martyn *var*. Nutag Belcheer-2) на этапах прорастания и роста в лабораторных условиях.

Солевой стресс растений индуцировался NaCl (0 — контрольная концентрация, 50, 100 и 200 мм) в течение двух недель. Результаты показали, что *Medicago sativa* имеет более высокий процент прорастания семян, чем другие виды и сорта. Длина и масса корней, индекс сухого вещества у *M. sativa* выше, чем у другого вида. *M. falcata* показал максимальный результат по массе и длине побега. Сорт *M. varia* Martyn var. Nutag Belcheer-2 характеризуется более высоким содержанием хлорофилла и водопоглощением, чем сорт Mongolian *Medicago varia* Marthz var. Burgaltai.

Таким образом, *M. sativa* и *M. falcata* устойчивы к солевому стрессу, сорт *M. varia* Martyn var. Nutag Belcheer-2 — умеренно устойчив, а Mongolian *Medicago varia* Marthz var. Burgaltai более чувствителен к солевому стрессу, чем другие растения рода *Medicago* L.

Ключевые слова: солевой стресс; Medicago L.; индекс всхожести; морфологические и физиологические параметры люцерны; содержание хлорофилла в люцерне.