

## COMPARATIVE ANALYSIS OF DROUGHT TOLERANCE OF *MEDICAGO L.* PLANTS UNDER STRESSED CONDITIONS

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### ABSTRACT

*As a perennial forage crop, alfalfa (Medicago L.) can be cultivated in marginal lands and has a high yield and good quality of high-protein content. Alfalfa has deep vertical roots; this species is able to absorb even depth waters about 5 m depth and more. This advantage saves plant's life in long term drought. The objective of this study was to compare the performance of drought 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. In order to expose the drought stresses in plant by polyethylene glycol (PEG 4000) with 0 (control), -0.3, -0.5 and -1.0 MPa for 2 weeks respectively. The results represented that Medicago sativa had higher seed germination percentage than other species and varieties. Water uptake, dry matter index are observed in M.sativa higher than others. The highest chlorophyll content was in Medicago varia Marthz var.Burgaltai. The highest result of root, shoot length and weight were observed in Medicago sativa. In contrast, the significantly lowest result of morphological parameters was in Medicago varia Martyn var.Nutag Belcheer-2. M.sativa is highly tolerant to drought stress among species. M.varia Marth var.Burgaltai was moderately tolerant to drought stress among varieties.*

**KEY WORDS:** *Medicago L., drought stress, PEG4000,*

### INTRODUCTION

Drought is the single most important weather – related natural disaster, which decreases crop productivity more than any other environmental stress [1, 2]. Drought depletes pasture, emaciate animals, and result in increased vulnerability to harsh winters [3]. Mongolia is vulnerable to extreme climate events, and is often affected by episodes of anomalously cold winters and droughts [4].

Mongolia's land area is categorized as 21.8% arid and 19.5% as semiarid [5].

As a perennial forage crop, alfalfa (*Medicago L.*) can be cultivated in marginal lands and has a high yield and good quality of high-protein content [6]. Alfalfa has deep vertical roots, which can extract moisture from depths of soil and can resist drought and heat for a longer time [7].

By comparing some perennial forage species, alfalfa morphological traits were more adapted to drought conditions [8]. *M. sativa* reduces atmospheric nitrogen association with the soil bacterium *Rhizobium meliloti* [14]. *M. falcata* is yellow flowered, has sickle-shaped pods, and tends to have a more decumbent growth habit and more winter hardiness than *M. sativa* or purple flowered alfalfa [15] and widely occurred in Russia, Mongolia, China and Scandinavia [16]. 9 Martyn var. Nutag Belcheer-2 is a hybrid between *M. sativa* and *M. falcata* and cultivated throughout China, escaping to fields and roadsides [17]. Firstly, Khaisan I who obtained *Medicago varia* Marthz var. Burgaltai by cross breeding *Medicago sativa* and *Medicago falcata* in 1986. 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 [19].

Ghasem Ali Dianati Tilake et al., (2009), Hamidi and Safarnejad (2010), Castroluna et al., (2014) are reported alfalfa seed germination under drought condition. Safarnejad (2008) is established about some morphological parameters of alfalfa plants. However, the effects of drought stress on alfalfa plants in laboratory conditions have not been established.

The aim of this study was to compare the morphological and physiological parameters of alfalfa plants under drought stress and selection of drought tolerant species and varieties for breeding programs.

## MATERIAL AND METHODS

### Plant materials

Mature seeds of 2 species and varieties of *Medicago L.* were used in this study including *M. falcata*, *M. sativa*, *M. varia* Marthz var. Burgaltai (Khaisan I. 1986), *M. 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 osmotic solution

The solutions were prepared based on Mongi Zekri methods by using polyethylene glycol (PEG4000)

with electrical conductivity of 0 (as control), -0.3, -0.5 and -1.0MPa osmotic potentials.

### Determining drought tolerance by seed germination experiment

All petri dishes and filter papers were disinfected in 121°C 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°C in darkness for eight days. Seed germination index is the Seed germination index is the percentage of seed which germinate at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> day of observation as indicates by nd2, nd4, nd6 and nd8.

percentage of seed which germinate at 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> day of observation. Percentage of seed germination drought stress tolerance index is determined as below:

$$\text{Germination index: } nd2 (1.00) + nd4 (0.75) + nd6 (0.50) + nd8 (0.25)$$

nd- day of seed germination

Percentage of seed germination stress tolerance index is determined as below:

$$\text{Germination index, \%} = \frac{\text{Germination index of stressed seeds}}{\text{Germination index of control seeds}} * 100$$

### Determining drought tolerance by water uptake

Water uptake was recorded after 2 weeks of watering by PEG4000. Water uptake percentage was calculated by the formula given.

$$\text{Water uptake, gr} = \frac{W2 - W1}{W1}$$

W1- initial weight of seed

W2 – weight of seed after absorbing water

### Determining drought 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

### Determining drought 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. After 2 weeks (2-3 fully expanded leaves) watered by 0, -0.3, -0.50 and -1.0MPa osmotic

### Statistical calculation and analysis

The data were analyzed using SPSS 21 analysis of variance, comparisons of mean for evaluated traits

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<sup>th</sup> day in stress. Triplicate reading using a SPAD-502 were taken around midpoint near the midrib of each leaf sample and averaged.

solutions for 2 weeks. Phenotypical 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 ( Sammar Raza M.A et al., 2012) formulas.

by least significant difference (Duncan) method at 0.05.

## RESULTS

### Seed germination index

The data indicated the significant variation among alfalfa species and varieties (Table 1, Figure 1). Significantly highest seed germination stress tolerance index was observed in *M.sativa* (64.1%),

followed by *M. falcata* (56%). The minimum seed germination rate observed in potential -1.0MPa in *M. varia* Martyn var.Nutag Belcheer-2 variety (48.0%).

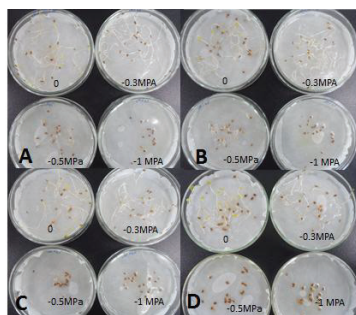


Figure 1. Seed germination of *Medicago* L. plants under stressed conditions after 8 days. A-*Medicago falcata*, B-*Medicago sativa*, C-*Medicago varia* Marthz var.Burgaltai D- *Medicago varia* Martyn var.Nutag Belcheer-2

Table 1

The mean of seed germination of *Medicago* L. plants in different osmotic solution levels

Plants	0	-0.3MPa	-0.5 MPa	-1.0 MPa	Mean
<i>Medicago falcata</i>	96.6	94.8	32.7	0.0	56.0 <sup>ab</sup>
<i>Medicago sativa</i>	96.6	92.3	67.4	0.0	64.1 <sup>a</sup>
<i>Medicago varia</i> Marthz	96.0	92.1	12.8	0.0	50.2 <sup>bc</sup>
<i>Medicago varia</i> Martyn	86.6	84.7	20.6	0.0	48.0 <sup>c</sup>
Mean	93.9 <sup>a</sup>	90.9 <sup>a</sup>	33.3 <sup>b</sup>	0.0 <sup>c</sup>	

The values are the mean of 3 replications  $\pm$ SE. The final seed germination percentage is illustrated at 8<sup>th</sup> day.

**Water uptake**

Result was observed between water uptake by leaf and increase of PEG4000 concentration up to -1.0MPa (Table 2). When PEG 4000 concentration was increased to -1.0MPa all plant's water uptake ability was declined in comparison to controls. For species *M. sativa*'s water uptake was decreased by 26.0% at -0.3MPa, 37.6% at -0.5 and 50.7% at -1.0MPa. In contrast, the lowest water uptake was in *M. varia* Martyn var.Nutag Belcheer -2 variety by

23.5% at -0.3MPa, 35.2% at -0.5MPa and 60.7% at -1.0 MPa.

The highest water uptake was recorded in *Medicago sativa*. Based on water uptake the *Medicago* L. plants can be arranged in the following order: *Medicago sativa*, *Medicago falcata*, *Medicago varia* Marthz var.Burgaltai, *Medicago varia* Martyn var.Nutag Belcheer-2.

Table 2

Effects of PEG 4000 concentration on water uptake by *Medicago* L. plants,g

Plants	0	-0.3MPa	-0.5MPa	-1.0MPa	Mean
<i>Medicago falcata</i>	0.58±0.03	0.49±0.01	0.47±0.02	0.34±0.03	0.47±0.09 <sup>a</sup>
<i>Medicago sativa</i>	0.69±0.03	0.51±0.05	0.43±0.12	0.34±0.05	0.49±0.14 <sup>a</sup>
<i>Medicago varia</i> Marthz	0.59±0.03	0.54±0.11	0.38±0.06	0.29±0.07	0.45±0.14 <sup>a</sup>
<i>Medicago varia</i> Martyn	0.51±0.05	0.39±0.07	0.33±0.02	0.20±0.06	0.36±0.12 <sup>b</sup>
Mean	0.59 <sup>a</sup>	0.48 <sup>b</sup>	0.40 <sup>c</sup>	0.29 <sup>d</sup>	

The values are the mean of 3 replications ±SE. The final seed germination percentage is illustrated at 8<sup>th</sup> day.

**Dry matter index**

Dry matter stress tolerance index of four *Medicago* L. plants differed slightly (Table 3). The significantly highest dry matter was recorded in *Medicago sativa*. It was higher than *Medicago falcata* and *Medicago*

*varia* Marthz var.Burgaltai by 5.1%. Significantly higher than *Medicago varia* Martyn var.Nutag Belcheer-2 by 17.9%.

Table 3

Dry matter index in *Medicago* L. plants under drought conditions, g

Plants	0	-0.3MPa	-0.5MPa	-1.0MPa	Mean
<i>Medicago falcata</i>	0.057±0.003	0.041±0.002	0.027±0.001	0.023±0.002	0.037±0.013 <sup>a</sup>
<i>Medicago sativa</i>	0.065±0.003	0.038±0.004	0.031±0.003	0.024±0.003	0.039±0.016 <sup>a</sup>
<i>Medicago varia</i> Marthz	0.061±0.022	0.037±0.003	0.028±0.002	0.024±0.003	0.037±0.018 <sup>ab</sup>
<i>Medicago varia</i> Martyn	0.045±0.006	0.030±0.05	0.028±0.005	0.024±0.004	0.032±0.009 <sup>ab</sup>
Mean	0.057 <sup>a</sup>	0.036 <sup>b</sup>	0.028 <sup>c</sup>	0.023 <sup>c</sup>	

The values are the mean of 3 replications ±SE. The final seed germination percentage is illustrated at 14<sup>th</sup> day.

### Chlorophyll content

Drought stress imposed at the vegetative, flowering stages and total chlorophyll content. Chlorophyll content varied among the accessions under well watered (control) conditions. Mild water stress (-0.3

MPa) had little effect on chlorophyll content of the *Medicago L.* plants. All *Medicago L.* plants showed the sharp decrease in chlorophyll content at 1.0MPa solution (Figure 2, Figure 3).

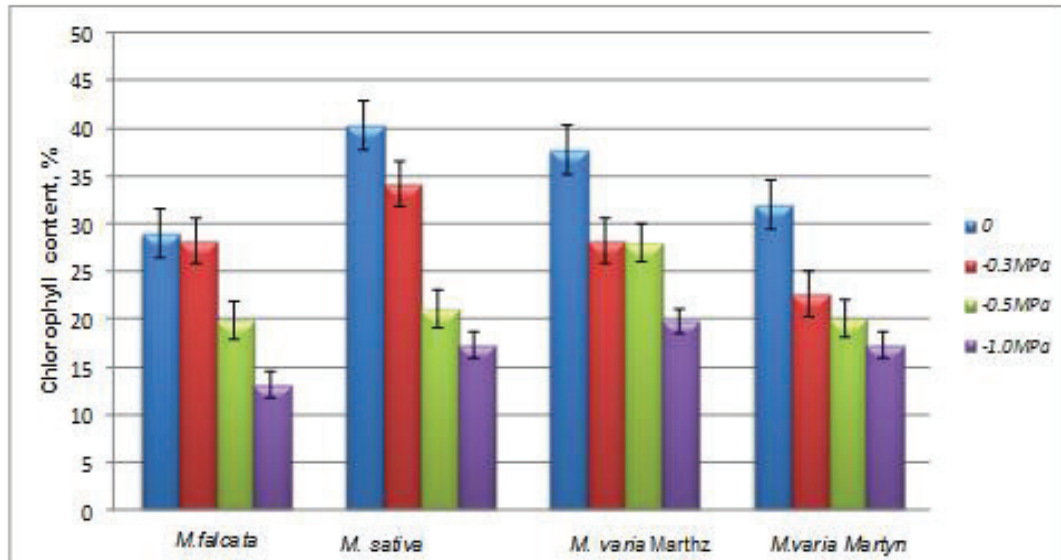


Figure 2. Chlorophyll content in four ecotypes of *Medicago L.* leaves. Chlorophyll content was measured after 14 days of growth at 0, -0.3, -0.5 and -1.0MPa external water potential. Data represent mean values of three replicates.

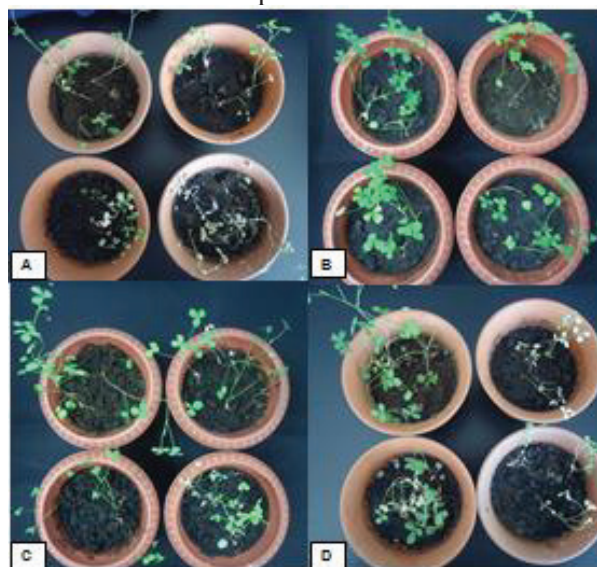


Figure 3. *Medicago L.* plants affected by PEG 4000 for 14 days. A- *Medicago falcata*, B- *Medicago sativa*, C-*Medicago varia* Marthz var. Burgaltai, D-*Medicago varia* Martyn var. Nutag Belcheer-2 (Top left-0, top right- 0.3MPa, bottom left-0.5MPa, bottom right-1.0MPa)

### Result of morphological parameters by measuring root, shoot length and weight

*Medicago L.* plant seedlings affected by PEG 4000 after 14 days their shoot, root length and weight were recorded. The highest shoot length was recorded in *Medicago sativa*. It was higher than *Medicago falcata* by 12.5%, higher than *Medicago varia* Marthz

var. Burgaltai by 6.7% and higher than *Medicago varia* Martyn var. Nutag Belcheer-2 by 20.8%. Based on shoot length alfalfa plants can be arranged in the following order: 1) *Medicago sativa*, 2) *Medicago varia* Marthz var. Burgaltai, 3) *Medicago falcata*, 4) *Medicago varia* Martyn var. Nutag Belcheer-2.



Table 4

The mean shoot length of <i>Medicago</i> L. plants under drought conditions, cm					
Plants	0	-0.3MPa	-0.5MPa	-1.0MPa	Mean
<i>Medicago falcata</i>	14.6±4.4	10.1±3.0	9.0±1.1	8.0±0.5	10.5±3.5 <sup>a</sup>
<i>Medicago sativa</i>	19.4±0.7	12.1±1.0	9.6±0.7	7.0±0.2	12.0±4.0 <sup>a</sup>
<i>Medicago varia</i> Marthz	18.4±2.5	10.8±0.5	8.2±0.5	7.2±1.5	11.2±4.7 <sup>ab</sup>
<i>Medicago varia</i> Martyn	16.2±3.9	8.1±0.2	7.7±0.6	5.7±1.1	9.5±4.5 <sup>b</sup>
Mean	17.2 <sup>a</sup>	10.3 <sup>b</sup>	8.6 <sup>c</sup>	6.9 <sup>d</sup>	

The values are the mean of 3 replications ±SE. The final seed germination percentage is illustrated at 14<sup>th</sup> day.

Increasing PEG 4000 concentrations adversely affected shoot weight although -0.3MPa gave the maximum value of 0.06 gr (Table 5). *M. sativa* showed the highest shoot weight (0.084 g) among *Medicago* L plants. The lowest shoot weight was

recorded in *Medicago varia* Martyn var.Nutag Belcheer-2 (0.065 g). The differences between *Medicago falcata* and *Medicago varia* Marthz var.Burgaltai were not- significant.

Table 5

The Mean shoot weight of <i>Medicago</i> L. plants affected by different levels of PEG 4000					
Plants	0	-0.3MPa	-0.5MPa	-1.0MPa	Mean
<i>Medicago falcata</i>	0.13±0.02	0.08±0.06	0.05±0.01	0.03±0.00 2	0.075±0.05 <sup>a</sup>
<i>Medicago sativa</i>	0.16±0.03	0.07±0.01	0.06±0.01	0.04±0.01	0.084±0.04 <sup>a</sup>
<i>Medicago varia</i> Marthz	0.15±0.01	0.06±0.02	0.04±0.01	0.03±0.01	0.076±0.05 <sup>a</sup>
<i>Medicago varia</i> Martyn	0.16±0.01	0.04±0.01	0.03±0.02	0.02±0.00 4	0.065±0.06 <sup>a</sup>
Mean	0.15 <sup>a</sup>	0.06 <sup>b</sup>	0.04 <sup>c</sup>	0.02 <sup>c</sup>	

The values are the mean of 3 replications ±SE. The final seed germination percentage is illustrated at 14<sup>th</sup> day

Control -0.3 0.5 -1.0MPa Control -0.3 -0.5 -1.0MPa

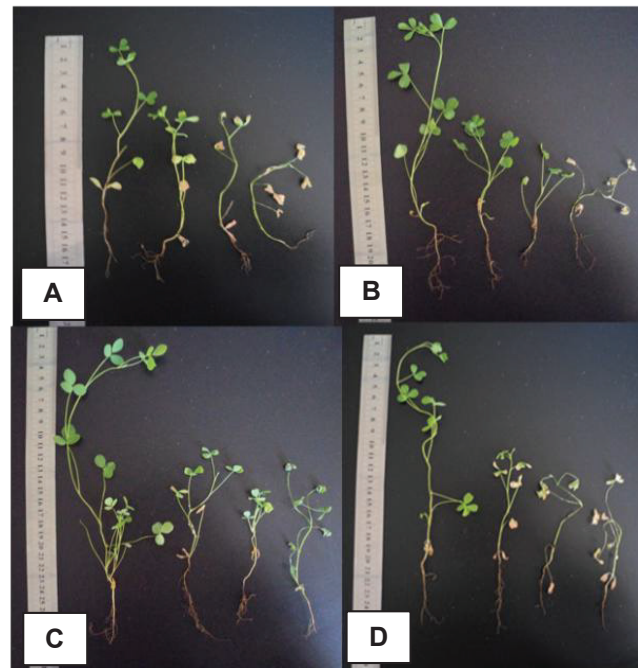


Figure 4. *Medicago* L. plant's growth affected by PEG 4000 after 14 days. A- *Medicago falcata*, B- *Medicago sativa*, C-*Medicago varia* Marthz var.Burgaltai, D-*Medicago varia* Martyn var.Nutag Belcheer

Difference in root length was not significant in different alfalfa plants (Table 6). Results represented that *Medicago sativa* (8.9 cm) and *Medicago falcata* (7.1cm) had more root length than *Medicago* L.

varieties. Among 2 varieties *Medicago varia* Marthz var.Burgaltai showed higher result than *Medicago varia* Martyn var.Nutag Belcheer-2.

Table 6

The mean root length of <i>Medicago</i> L. plants under drought conditions, cm					
Plants	0	-0.3MPa	-0.5MPa	-1.0MPa	Mean
<i>Medicago falcata</i>	10.3±0.4	7.2±0.4	5.7±0.3	5.0±0.7	7.1±2.1 <sup>a</sup>
<i>Medicago sativa</i>	11.4±0.7	9.1±1.0	8.1±0.8	7.2±0.6	8.9±1.7 <sup>b</sup>
<i>Medicago varia</i> Marthz	8.5±0.5	6.8±0.3	6.7±0.4	5.6±0.1	6.9±1.1 <sup>b</sup>
<i>Medicago varia</i> Martyn	7.1±0.6	6.3±0.5	5.9±0.5	5.6±0.8	6.2±0.8 <sup>c</sup>
Mean	9.3 <sup>a</sup>	7.4 <sup>b</sup>	6.6 <sup>c</sup>	5.8 <sup>d</sup>	

The values are the mean of 3 replications ±SE. The final seed germination percentage is illustrated at 14<sup>th</sup> day

Mean of root weight varied between 0.03-0.08 g (Table 7). The highest root weight was recorded in control of *Medicago sativa* and *Medicago falcata* (0.13g). Differences between 2 species of alfalfa was

not significant. In *Medicago varia* Martyn var.Nutag Belcheer-2 root weight was 0.03g which was lower than *Medicago varia* Marthz var.Burgaltai by 0.02 g

Table 7

The mean root weight of <i>Medicago</i> L. plants under drought stress, g					
Plants	0	-0.3MPa	-0.5MPa	-1.0MPa	Mean
<i>Medicago falcata</i>	0.13±0.01	0.07±0.06	0.06±0.01	0.01±0.01	0.07±0.05 <sup>a</sup>
<i>Medicago sativa</i>	0.13±0.01	0.11±0.01	0.06±0.01	0.03±0.01	0.08±0.04 <sup>a</sup>
<i>Medicago varia</i> Marthz	0.09±0.01	0.05±0.01	0.03±0.01	0.02±0.00 3	0.05±0.02 <sup>b</sup>
<i>Medicago varia</i> Martyn	0.05±0.03	0.03±0.002	0.02±0.03	0.02±0.03	0.03±0.01 <sup>c</sup>
Mean	0.1 <sup>a</sup>	0.06 <sup>b</sup>	0.04 <sup>c</sup>	0.02 <sup>d</sup>	

The values are the mean of 3 replications ±SE. The final seed germination percentage is illustrated at 14<sup>th</sup> day

## DISCUSSION

Ghasem Ali Dianati Tilake et al. (2009) reported that drought stress caused by PEG6000 decreased seed germination percentage in alfalfa especially at -0.8MPa osmotic potential solution obtained 0.0% of alfalfa [10]. Our data showed the same result on seed germination index by 0.0% at -1.0 MPa osmotic solutions.

Hamidi and Safarnejad (2010) concluded that under drought stress caused by PEG6000 on seed germination rate decreased by 72.2% at -0.9MPa osmotic solution comparing with control for 14 days [11]. Our experiment showed that seed germination rate declined by 66.1% at -0.5MPa osmotic solution at 8<sup>th</sup> day of treatment. Karimi (1990) reported that exposed to stress more than -20 bar, most seeds are not able to absorb enough water for embryo growth [12]. From our experiment un-germinated seeds were observed in 2 species and varieties of *Medicago* plants at -1.0MPa osmotic solution.

Safarnejad A (2008) reported that shoot and root lengths were reduced with an increase in PEG concentration. Root and shoot dry weights in Iranian (*Medicago Sativa*) species were also significantly reduced with increasing PEG concentration. For instance, shoot length decreased by 74.3%, root

length decreased by 56.0% comparing with control. Our experiment revealed that shoot length decreased by 63.9%, root length – by 36.8% comparing with control. Moreover, germination rate declined by 60.7%, while germination rate in our *Medicago* plants. decreased by 66.1%. Shoot and root dry weights were also significantly reduced with increasing PEG concentration. Shoot weight declined by 78.1% and root weight showed decrease by 71.4%. In our experiment shoot, root dry weight significantly dropped by 75-76.9%.

Castroluna A et al., (2014) had 0, 0.5 and -1.0MPa osmotic solution treatment for determining seed germination and seedling growth for 14 days [13]. Their result illustrated that seed germination index was 51.7%, root dry weight -0.03gr. Our results represented that seed germination index was 64.1%, root dry weight was 0.08 gr.

Casler M.D (2001) reported that selection for increased forage nutritional value is often associated with reductions in agricultural fitness traits, such as forage yield, disease and/or insects' resistance and stress tolerance [18]. Identically, chlorophyll content in *M.varia* Marthz was higher than other *Medicago* L. plants.

## CONCLUSION

1. Germination rate was determined under 0, -0.3, -0. And -1.0MPa osmotic solution. *M. sativa* showed the significantly highest result than others. *M.varia* Marthz var.Burgaltai seed germination rate was higher than *Medicago varia* Martyn var.Nutag Belcheer 2 by 4%.
2. *M. sativa* represented the maximum result on morphological parameters (root, shoot length and weight) than other three alfalfa plants. *M. varia* Martyn var.Nutag Belcheer was sensitive to drought stress than *M. varia* Marthz var.Burgaltai.



3. Maximum water uptake was observed in *Medicago sativa*. Water uptake by *M.varia* Marthz var.Burgaltai was higher than *M. varia* Martyn var.Nutag Belcheer -2 by 20%. The mean of dry weight index was significantly higher in *Medicago sativa*, *Medicago varia* Martyn var.Nutag Belcheer-2 showed the lowest weight.
4. *M.sativa* was highly tolerant to drought stress; *M.falcta* and *M.varia* Marthz var.Burgaltai were moderately tolerant to drought stress. *M.varia* Martyn var.Nutag Belcheer-2 was sensitive to drought stress among *Medicago L.* plants.

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