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Full Length Research Paper

Germination responses of two cultivars of M. sativa (Yazdi and Hamedani) to drought stress caused by PEG-6000 at the different potentials

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Drought and high salinity are important environmental factors that cause osmotic stress and as a result reduce plant growth and crop productivity. Alfalfa or lucerne (*Medicago sativa* L.) is a valuable forage crop, which is grown in areas of limited rainfall and high temperatures and where the land is often salt affected. In this paper, we studied the germination responses of two cultivars of *M. sativa* (Yazdi and Hamedani) to drought stress caused by PEG-6000 at the different potentials: 0.0 (distilled water or control), -0.2, -0.4, -0.6, -0.8, and -1.0 Mega Pascal (MPa) and tolerance to salt (NaCl) in different salinity levels including: distilled water (control), 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 mmol. The objective of this study is to determine factors responsible for germination and early seedling growth due to drought and salinity and to optimize the best priming treatment for these stress conditions. The results indicated that salinity and drought levels effects were significant (P < 0.05) for seed germination percentage, seed germination velocity, mean time to germination, length of the tigella and radicle, and seed vigor. Seed germination decreased significantly by increasing salinity and drought levels. Totally, the results showed that *M. sativa* cul. Yazdi is more tolerant than *M. sativa* cul. Hamedani against drought and salt stresses.

Key words: Drought stress, salinity stress, PEG, NaCl, germination, *Medicago sativa* cul. Yazdi, *Medicago sativa* cul. Hamedani.

INTRODUCTION

Plant tolerance to drought results from both morpho-logical adaptation and responses at the biochemical and genetic levels (Levitt, 1980). In arid and semi-arid areas due to lack of water, starting of germination has delaying and slow amount of germination (Young et al., 1983). Water stress has no equal effect on total processes of plant growth. Some stages are very sensitive to increasing water stress, while other stages are affected little under water stress (Safarnejad, 1996). Drought stress is considered to be one of the most important abiotic factors limiting plant growth and yield in many areas (Kramer and Boyer, 1997).

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Salt stress causes decrease in plant growth and productivity by disrupting physiological processes, especially photosynthesis (Sudhir and Murthy, 2004). Saline soils contain soluble salts in quantities that affect plant growth at various stages and create yield differences between crops and differences in the ion composition of crops at maturity (Sharma, 1997).

Salinity imposes serious environmental problems that affect grassland cover and the availability of animal feed in arid and semi-arid regions (El-Kharbotly et al., 2003). Salt stress was unfavorably affected by plant growth and productivity during all development stages. For example, Epstein et al. (1980) reported that salinity decreases seed germination, retards plant development and reduces crop yield. Salt stress negatively affects seed germination, either osmotically through reduced water absorption or ionically through the accumulation of Na

and Cl causing imbalance in nutrient uptake and toxicity. Saline soils contain multiple types of soluble salt components, each of which has a different effect on the initial growth of plants and the composition of soluble salts in saline soils differ greatly among locations (Tobe et al., 2002).

Any effect that drought might have should be most considerable under salt stressed conditions, because salinity can affect germination and seedling growth either by creating an osmotic pressure (OP) that prevents water uptake or by toxic effects of sodium and chloride ions on the germinating seeds (Bewley and Black,1982).

In arid and semi-arid regions, where growth is limited by water shortage or by water of poor quality, the use of saline water for crop production is often unavoidable. Most crops tolerate salinity to a threshold level above which yields decrease as salinity increases (Maas, 1986). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri et al., 2001).

Alfalfa is one of the most important forage plants that have up to 60 different species, in which two thirds are annual and one third are perennials (Safarnejad, 1996). Different *Medicago sativa* cultivars are planted in different areas of Iran but few are known about their priority in the view point of drought and salinity resistance. The current research focused on finding the resistant cultivar under stress in germination stage.

MATERIALS AND METHODS

Study species

Alfalfa is an herbaceous perennial that can produce large amounts of nutritious forage material. It is one of the leading forage crops. It was first originated in Asia before 700 BC. It was thought to have been cultivated first in Iran.

Alfalfa lives from three to twelve years, depending on variety and climate. It is a cool season perennial legume, sometimes growing to a height of 1 m. It also has a deep root system sometimes stretching to 4.5 m. This makes it more resilient, especially to droughts. This plant has a wide range of adaptation and can be grown from very cold northern plains to high mountain valleys, from rich temperate agricultural regions to Mediterranean climates and searing hot deserts (Milius, 2007).

Alfalfa, which has the ability to go dormant during extended dry periods, is one of the few crops that can recover once adequate precipitation or irrigation occurs. It is considered the Queen or Cadillac of forages. The alfalfa crop can be used as a pasture, hay, or silage crop. It can also be cut and dehydrated to make protein rich meal or pellets for livestock.

It is vitally important to maintain a viable plant root system (must be white, moist and pliable) to enable alfalfa plant to survive. Under drought conditions, the alfalfa plant reduces stem number, stem elongation and yield while increasing leaf to stem ratio. Photosynthesis is maintained during the early phase of drought but slows as the drought continues and as the stomata close. Nitrogen fixation and nodule formation is reduced but more nutrients accumulate in the roots compared to the foliage (Winter and Martens, 2007).

Therefore, with these values of *Medicago*, we studied two cultivars of it in Iran to make a comparison between them in the view point of their germination under two dominant environmental stresses.

Effect of drought and salinity on germination

To evaluate drought and salinity stresses during germination, two experiments were carried out in the laboratory. Alfalfa seeds were taken from Pakan-Bazr Corporation in Isfahan. The experiments were performed in a Completely Randomized Design (CRD) with 4 replicates in the seed laboratory, Natural Resources Faculty, University of Tehran.

Experiment 1: Drought stress

Drought stress according to Michel and Kaufmann (1973) method was supplied in potentials: 0.0, -0.2, -0.4, -0.6, -0.8 and -1.0 Mpa with PEG-6000 (Poly Ethylen Glycol), and distilled water was used for 0.0 potential. At first, the observed seeds were antisepticized by Hypochloride sodium (10%) for 15 min, then washed for several times with distilled water and placed on filter paper in 9 cm Petri dishes which had been stirred for 20 min in 121°C temperature and 1 bar pressure in autoclave set. For each experimental treatment, in each frequency, 4 Petri-dishes with 25 seeds in it were used. For determining the different potentials of PEG solution, Michel and Kaufman (1973) formula was used. Based on that, we considered 25°C for temperature, because this temperature is average temperature in arid and semi-arid areas of Iran (Beheshti et al., 1996). In each Petri-dish, 10 ml of each solution was poured. In order to avoid solution evaporation, we closed the Petri-dishes with paraphilm.

Experiment 2: Salt stress

To evaluate salt tolerance during germination, 25 seeds of each cultivar were placed on filter paper in 9 cm Petri-dishes and submerged in 5 ml of each solution of NaCl at concentrations of 0 (control), 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 mmol. The water level was adjusted at 2-d intervals with distilled water to avoid changes in salinity due to evaporation.

In two experiments, germination counts were made daily, and the seeds were considered to have germinated when the radicle emerged. At the end of the germination period, the germination percentage, germination velocity, the mean time to germination, and length of the tigella

and radicle under salinity and drought condition were calculated. Germination percentage was calculated using the equation:

$$\text{Final germination percentage} = \frac{\text{number of germinated seeds}}{\text{total number of seeds planted}} \times 100$$

Also, germination velocity and the mean time to germination were calculated using the following equations:

$$\text{Germination velocity} = \sum \frac{\text{number of germinated seeds}}{\text{day of count}}$$ (Maguire, 1962).

Mean time to germination = $(\sum ni \times di) / N$, where n is the number of seeds germinated at day i, d is the incubation period in days, and N is the total number of seeds germinated in the treatment (Brenchley and Proberet, 1998). Also, the seed vigour was obtained using the following equation (Abdul-baki and Anderson, 1973):

$$\text{Seed vigour} = \frac{(\text{length of stem} + \text{length of root}) \times \text{germination percentage}}{100}$$

Statistical analysis

A multrivariate ANOVA was used to evaluate the effects of salinity and drought on seed germination. Data were analysed using SPSS 13 for windows (SPSS Inc., 1999). When significant main effects existed, differences were tested by a multiple comparison Tukey test at 95% confidence. Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance.

RESULTS

Germination percentage, mean time to germination and germination velocity

We found that germination percentage under water deficiency conditions was significantly different between two cultivars (Table 1). In Hamedani cultivar, germination percentage in the control treatment was less than all treatments except the -1.0 MPa. Germination in all treatments (from control to -0.8 MPa, except -1.0 MPa) showed increasing trend.

The germination percentage in 3 treatments (-0.4, -0.6, -0.8 MPa) were similar. According to Table 1, -1.0 MPa has the least germination percentage. In the view point of germination percentage, Yazdi cultivar shows higher level when compared with Hamedani. Yazdi cultivar which has approximately equal germination in all different solutions of PEG.

The mean time to germination in the two cultivars was similar, and they had approximately equal mean time to germination. In both cultivars, the -1.0 MPa treatment had the highest mean time to germination (Table 1).

For germinating velocity, the two cultivars of *M. sativa* show similar behaviors in all of the treatments except -0.4 and -1.0 MPa. In Hamedani cultivar, the -1.0 MPa PEG solution has significant difference from others. In this cultivar, at first with increase in PEG solutions (increasing in drought stress), the velocity increases, so that in -0.4 MPa treatment we have the most average of germination velocity while after that a decrease in velocity of germinating happens, then in -1.0 MPa we have the lowest germination velocity.

In Yazdi cultivar, we observed that like Hamedani, -1.0 MPa treatment had the lowest velocity of germination (results of this level of stress are significantly different from those of the other treatments), while the other treatments' effects are similar. In the second experiment (salt stress effects on germination properties by NaCl solution), two cultivars showed significantly different germination percentages (Table 2).

In 9 treatments, we observed that for Yazdi cultivar in 0, 30 and 60 mmol solutions, germination percentage was similar. For all treatments, the trend was negative with increase in the salinity. In 210, 240, 270 and 300 mmol solutions, the germination percentage was approximately equal with each other. In Hamedani cultivar, the germination was similar in 0, 30, 60, 90 and 120 mmol solutions. Also, in 210, 240, 270 and 300 mmol solutions, the germination percentage was approximately the same (Table 2).

The results of mean time to germination showed that in Yazdi and Hamedani cultivars, different treatments were affected. Similarly, only in 3 treatments (30, 270, and 300 mmol), the results were different between two cultivars. After 210 mmol, the mean time has an increasing trend while in treatments before that, mean time is similar and low. It was found that the mean time to germination increased with increase in NaCl salinity solutions, except for 300 mmol solution in Yazdi cultivar that showed no germination (Table 2).

It was observed that the germination velocity was approximately similar in the two studied cultivars of *M. sativa* except for 120 mM solution of NaCl. Furthermore, it was observed that with an increase in salinity, the velocity of germinating showed remarkable decrease. So, from the control treatment to the end, the germination velocity had a decreasing trend (Table 2).

Length of the tigella and radicle

Length of the tigella in the first experiment (drought stress) in two cultivars of *M. sativa* was approximately similar except treatments of -0.2 and -0.8 MPa solutions

Table 1. Germination percentage, germination velocity, and time to germination of Hamedani and Yazdi cultivars seeds under drought stress by PEG solutions.

PEG solutions (MPa)	Germination percentage		Mean time to germination		Germination velocity	
	Hamedani	Yazdi	Hamedani	Yazdi	Hamedani	Yazdi
0	79 ± 5.03 cA	92 ± 0.0 aB	1.25 ± 0.25 bcA	1.32 ± 0.17 bcA	18.77 ± 0.57 cA	21.64 ± 0.79 aA
-0.2	82 ± 10.58 bcA	90 ± 2.31 aA	1 ± 0.23 cA	1.12 ± 0.14 cA	20.5 ± 0 bcA	21.84 ± 0.35 aA
-0.4	96 ± 0 aA	83 ± 6.83 aB	1 ± 0.27 cA	1.22 ± 0.10 cA	24 ± 0 aA	19.65 ± 0.65 aB
-0.6	94 ± 2.31 abA	94 ± 4 aA	1.17 ± 0.36 cA	1.43 ± 18 bcA	22.88 ±0.3 abA	21.60 ± 1.02 aA
-0.8	96 ± 3.27 aA	96 ± 4.62 aA	1.96 ± 0.41 bA	1.84 ± 0.047 bA	19.66 ± 2.98 bcA	20.60 ± 1.61 aA
-1.0	61 ± 5.03 dA	86 ± 12.44 aB	2.95 ± 0.39 aA	2.44 ± 0.36 aA	9.03 ± 3.44 dA	14.94 ± 3.79 bB

Values are mean ± S.D. Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letters are significantly different from each other (Tukey test; $p< 0.05$).

Table 2. Germination percentage, germination velocity, and time to germination of *Medicage sativa*, Hamedani and Yazdi cultivar seeds in salinity stress by NaCl solutions.

NaCl solutions	Germination percentage		Germination velocity		Mean time to germination	
	Yazdi	Hamedani	Yazdi	Hamedani	Yazdi	Hamedani
0	99.0 ± 2.0 aA	92 ± 8.6 aA	21.7 ± 0.3 aA	20.4± 0.1 abA	1.3 ± 0.0 bA	1.4 ± 0.1 cA
30	97.0 ± 2.0 aA	92 ± 5.7 aA	21.6 ± 0.2 aA	19.8± 0.1 abA	1.4 ± 0.1 bA	1.6 ± 0.1 cB
60	91 ± 6.8 aA	97 ± 2 aA	19.5± 0.1 abA	21.3 ± 0.1 aA	1.5 ± 0.0 bA	1.6 ± 0.1 cA
90	72.0 ± 8.0 bA	78 ± 7.7 aA	17.2 ± 0.1 bcA	17.5 ± 0.1 bA	1.2 ± 0.0 bA	1.3 ± 0.1 cA
120	58 ± 14.8 bA	79 ± 8.2 aB	13.5 ± 0.1 cdA	17.4 ± 0.1 bB	1.3 ± 0.1 bA	1.3 ± 0.1 cA
150	32 ± 5.7 cA	47 ± 13.6 bB	12.2 ± 5.7 dA	10.6 ± 0.1 cA	1.4 ± 0.1 bA	1.4 ± 0.1 cA
180	24 ± 11.3 cdA	30 ± 12.4 bcA	5.2± 0.1 eA	6.6± 0.1 dA	1.6 ± 0.1 bA	1.5 ± 0.1 cA
210	3 ± 2 eA	11.0 ± 5.0 cdA	0.3 ± 0.1 fA	1.5± 0.1 eA	3.7 ± 0.1 aA	2.6 ± 0.1 bcA
240	10 ± 6.9 deA	9 ± 6.8 dA	0.0 ± 0.1 efA	2.2 ± 0.1eA	3.4 ± 0.1 aA	2.6 ± 0.1 bcA
270	5 ± 7.6 eA	1 ± 2 dA	0.4 ± 0.1 fA	0.0± 0.0 eA	4.4 ± 0.1 aA	6.1 ± 0.1 aB
300	0 ± 0 eA	2 ± 4 dA	0.1 ± 0.0 fA	0.1± 0.0 eA	0 ± 0 bA	4.0 ± 0.1 bB

Values are mean ± S.D. Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $p< 0.05$).

of PEG. Results showed that with increasing drought condition for the two cultivar seeds, average of length of the tigella had decreased. Also, we had the maximum length of the tigella in two cultivars in distilled water (control treatment). According to Table 3, for length of the radicle, two cultivars were different only in two PEG solutions (-0.8 and -1.0 MPa). The maximum length of the radicle was related to -0.2 PEG solution and the minimum to -1.0 MPa.

In salinity stress, the average of length of the tigella was similar in all solutions of NaCl in two cultivars. In Yazdi cultivar, according to Table 4, it is seen that in 150, 180, 210, 240, 270 and 300 mmol solutions of NaCl, and in Hamedani cultivar in the last 5 treatments like Yazdi cultivar with the exception of 150 mM, the length of the tigella was low and similar to each other. Just as we saw in the other observed factors, the length of the radicle in two cultivars was alike except for 90 and 150 mmol solutions. Also, a very small length of the radicle was seen in the 6 last treatments. With increase in the salinity, the length of the radicle decreases (Table 4).

Seed vigour

In the last experiment for determining the seed vigour, we found that the seed vigour in drought stress in Hamedani cultivar increased with increase in PEG solutions, then in -0.6, -0.8, and -1.0 MPa, the seed vigour decreased. In Yazdi cultivar, a similar trend like Hamedani cultivar was observed. In the two studied cultivars, except for -0.2, -0.8 and -1.0 treatments, the seed vigour was equal with each other (Table 3).

Seed vigour is one of the important factors that we studied in the second experiment. Once more there is similarity between the two cultivars in all treatments except 90, 120 and 150 mmol solutions. However, the maximum seed vigour was observed in 30 mmol solution of NaCl (Table 4).

Table 3. Length of tigella, length of radicle, and seed viguority of Hamedani and Yazdi cultivar seeds in drought stress by PEG solutions .

PEG solutions (MPa)	length of the tigella		length of the radicle		seed vigor	
	Hamedani	Yazdi	Hamedani	Yazdi	Hamedani	Yazdi
0	18.1 ± 6.08 aA	18.9 ± 3.51 aA	56.8± 19.3 aA	46.7 ± 16.01 abA	56.88± 13.01 aA	58.72 ± 16.93 aA
-0.2	11.7 ± 3.97 aA	18.7 ± 4.76 aB	68.10 ± 16.8 aA	68.3± 12.98 aA	64.30± 13.78 aA	77.49 ± 13.31 aB
-0.4	15.4 ± 3.92 aA	13.4± 4.22 abA	65.7± 29.9 aA	62.7± 24.55 aA	77.85± 27.87 aA	61.37 ± 20.64 aA
-0.6	12.6 ± 4.58 aA	13.0± 1.83 abA	58.60± 24.01 aA	47.8 ± 11.29 abA	65.36± 20.82 aA	54.52 ± 10.17 aA
-0.8	3.0 ± 2.16 bA	8.3 ± 2.63 bcB	5.71± 2.36 bA	21.1± 7.46 bcB	7.47± 1.52 bA	26.02 ± 9.03 bB
-1.0	1.0 ± 1.05 bA	1.6 ± 1.58 cA	1.5 ± 1.84 bA	3.6± 2.37 cB	1.17± 0.88 bA	3.77 ± 2.21 bB

Values are mean ± S.D. Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; p< 0.05).

Table 4. Length of tigella, length of radicle, and seed viguority of *Medicage sativa*, Hamedani and Yazdi cultivar seeds in salinity stress by NaCl solutions.

NaCl solutions	length of the tigella		length of the radicle		Seed vigour	
(mM)	Yazdi	Hamedani	Yazdi	Hamedani	Yazdi	Hamedani
0	12.4 ± 3.1 aA	14.9 ± 6 aA	22.4 ± 18.2 aA	27.4 ± 14.2 aA	34.1 ± 18.5 aA	38.7± 18.0 aA
30	10.6 ± 1.6 abA	12.1± 3.5 abA	32.6 ± 14.3 aA	30.9 ± 15.9 aA	41.4 ± 14.2 aA	39.9± 15.0 aA
60	9.2 ± 3.9 bcA	8.3 ± 2.2 bcA	23.3 ± 13.2 aA	24.7 ± 6.9 aA	29.6 ± 12.6 aA	31.7 ± 7.0 aA
90	4.8 ± 2.7 dA	8.8 ± 3.1 bcB	4.0 ± 2.3 bA	13.2 ± 2.3 bB	6.3 ± 2.6 bA	17.3 ± 2.1 bB
120	6.9 ± 1.8 cdA	8.4 ± 3.1 bcA	2.7 ± 1.6 bA	7.2 ± 6.0 bcA	5.2 ± 1.5 bA	12.1± 5.9 bcB
150	1.7 ± 1.8 eA	6.6± 2.5 cB	0.8 ± 1.0 bA	4.6 ± 2.4 bcB	0.8 ± 0.6 bA	5.3 ± 2.0 cdB
180	0.6 ± 1.1 eA	1.3± 1.4 dA	0.8 ± 1.3 bA	0.6 ± 0.7 cA	0.4 ± 0.6 bA	0.5± 0.6 dA
210	0.5 ± 1.1 eA	0.5± 0.7 dA	0.5 ± 1.1 bA	0.2 ± 0.4 cA	0.0 ± 0.1 bA	0.1± 0.1 dA
240	0.4 ± 0.8 eA	0.3± 0.5 dA	0.5 ± 1.3 bA	0.3 ± 0.5 cA	0.1 ± 0.1 bA	0.0± 0.1 dA
270	0.4 ± 1.0 eA	0.2 ± 4 dA	0.3 ± 0.7 bA	0.2 ± 0.4 cA	0.1 ± 0.2 bA	0.0± 0.0 dA
300	0.0 ± 0.0 eA	0.2 ± 4 dA	0.0 ± 0.0 bA	0.2 ± 0.4 cA	0.0 ± 0.0 bA	0.0± 0.0 dA

Values are mean ± S.D. Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; p< 0.05).

In Yazdi cultivar, all treatments except 0, 30, and 60 mmol solutions had similar seed vigour to each other, and their seed vigour was very low. In Hamedani cultivar after 30 mmol solution, there was a gradual decrease in seed vigour.

Significant differences were obtained for all the considered factors (P<0.05).

Conclusion

In this study, a decreased level was observed in germination and length of the tigella and radicle for *M. sativa* L. cultivars Yazdi and Hamedani with increasing drought levels. Zeid and Shedeed (2005) reported that decrease in water potential of the PEG solution reduced germination rate, germination percentage, and growth criteria. Also, Finch et al. (2001) in their study observed that maximum amount of germination was observed in 0 MPa treatment with increase in osmotic potential (increasing in drought stress) and amount of germination, though the length of the tigella and radicle had declined. Akhondi et al. (2004) showed that with increasing drought stress, percentage and rate of germination was decreased. They proved that Yazdi alfalfa was the most tolerant and Ranger was the most sensitive cultivar in response to osmotic stress among the studied Alfalfa cultivars. In this study, we found such a result when comparing Yazdi and Hamedani.

Radhouane (2007) stated that with osmotic stress, the rate of germination is correlated positively with the shoot length. While in this study no correlation was noted between germination and root length in the presence of osmotic stress.

We found that salinity has the negative effect on length of the tigella so that in the control condition, the length of the tigella is the maximum. For length of the radicle, the 30 mmol solution was the best. Perhaps, the existence of beneficial salts in this situation is an evidence of the

nutrient requirement for growth in *Medicago*. For salinity stress, the best condition is the control or without salinity and in 30, 60 and 90 mmol, the best condition is the growth and germination, while in salinity levels higher than 90 mmol, germinating and growth was very low or ceased.

Germination delayed in both solutions (NaCl and PEG), having variable germination with different priming treatments. NaCl had less inhibitor effect on seedling growth than the germination. It was concluded that inhibition of germination at the same water potential of NaCl and PEG resulted from osmotic effect rather than salt toxicity (Kaya et al., 2006). Also, in this research, we observed delay in germination with increasing PEG and NaCl solutions.

For Yazdi cultivar under drought stress, it was seen that in the control and -0.2 treatments, roots are pale and tall, tigellas are short and inclined, fresh, green and with two leaves. In -0.4 MPa treatment, a decrease was observed in the length of the tigella and shoot with fresh leaves. This was less than that observed for the control treatment. Apart from the fact that the radicle was brown from white and being abnormal, in some of them, the green color of the leaves changes from yellow to faint green. In -0.6 treatment, germination was high and seedlings were relatively fresh in the first case but were not able to produce two leaves. In -0.8 and -1.0 treatments, length of the tigella and radicle was very few and the germination was very little.

For Hamedani cultivar, in the control treatment, germination was very high (about 95%) and seedlings were very fresh but for the other treatments, total trend was approximately like that of Yazdi cultivar but the occurred decreases in the understudy variables were larger. This shows that Hamedani is sensitive against environmental stresses when compared to Yazdi cultivar. This result will be of benefit in selecting an adoptable alfalfa cultivar for planting in hard conditions.

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