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Effect of Drought Stress on Alfalfa Cultivars (*Medicago sativa* L.) in Germination Stage

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Abstract: Drought is one of the most important natural phenomenon which affects on plant growth. Alfalfa (*Medicago sativa* L.) is an essential fodder product which has extensive and depth roots system, this advantage enables the plant to tolerate long term drought without any damage to its regrowth process. In order to measure germination percentage and rate, root and shoot growth of 6 alfalfa cultivars (each cultivar has few years old) under various osmotic potential, an experimental design was carried out complete randomized *in vitro* condition in four replications. To expose drought stress over plant polyethylene glycol (PEG6000) with 0 (control), -3, -6, -9 bar was used. Those cultivars under experiment include Yazdi, Hamedani, Qareh Yonjeh, Maupa, Simmer Chenskaya and CUF101. The results indicated that by reducing osmotic potential of culture environment higher drought stress, a significant decrease in germination percentage and rate, radicle and plumule length and seed vigority index were observed, while the ratio of radicle to plumule was increased. Correlation coefficients of studied plant traits also showed that germination percentage and rate have significant and positive correlations with plumule length, but significant and negative correlation with the ratio of radicle to plumule. Among cultivars, Yazdi and Maupa had higher germination percentage and rate under sever stresses, therefore their tolerance against drought stress during germination stage is more than other cultivars.

Key words: Alfalfa · Drought stress · Germination · *Medicago sativa* · Tolerance

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is an essential hay product which grow in arid and semi arid regions. Because of its depth and straight 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. Also alfalfa plant which goes to stagnation status in drought and cold conditions, in good weather conditions again returns to its normal life and growth [1]. One barrier against this plant in growth period is water stress which is the result of salty and dry environment [2]. Drought is the most important natural factors which influences on plant growth and water shortage is another important factor which limits production [3].

Drought stress influences on plants and results in morphological, physiological, molecular and growth mutations [4].Water stress affects on plant in different levels ranging from a cell to a plant colony [5]. Germination is the first stage in plant life. Germination is an amphiboly process, in another words a metabolism process [6]. Water, oxygen and temperature are three main controlling factors for germination process and in some plants, light is another environmental factor which controls germination process [7].

Proper environmental conditions for germination of a plant in normal status include enough water, oxygen, light and proper temperature without any external different matter like mineral salts and organic materials which cause germination initiate. By decreasing water potential, absorption of water by plant is affected. However, to decrease germination percentage, water potential must fall below certain amount [8].

Germination stage for those plants which reproduce through their seeds is very important because of its indirect influence on plants concentration. Drought stress usually delay germination process and decreased germination percentage and rate and growth rate [9-11].

Jalali Honarmand [6] reported that germination percentage in wheat seed was decreased under drought

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conditions in which various cultivars 15 species had significant differences in this case. Drought stress in young plants results in metabolic mutations which based on their genotype and perimeter environment the degree of mutation diverse [12]. Other elements inside plant influenced by drought are the length of radicle, plumule and their ratio [13]. Several experiments showed that radicle length mutates under stress and by increasing stress, its growth rate decreased as well. Of course, plumule is more influenced by stress than radicle [14]. While normal stress, perhaps roots length stay intact and even increase in some cases to resist water shortage and this is probably due to essential role of roots in plant function and life [14].

Lack of moisture brings about severe decease in plumule length [15]. Other studies indicated that plumule length is more sensitive to drought stress than radicle [16]. Also other studies indicated that stem length is more sensitive to drought stress than root length [16, 17].

Drought stress in young plants results in some metabolic and morphologic changes which varies based on their genotype, drought stress and duration [15]. Alfalfa growth analysis in drought conditions shows that as stress increases, the length of root and stem decreases. Also as water stress increased the length of mid-node and decreased leaf size within 14 days after stress in alfalfa plant [14].

Drought stresses resulting from (PEG6000) in alfalfa leads to reduction in stem and root growth, this reduction in root and stem length might be the result of roughening in cell wall. The results of this study confirm this fact that stem growth often is more affected by water stress than root that another reason is that roots ability to osmotic regulation is more than branches [15].

Moisture stress affects unequally on all stages in plant growth. Some growth stages are more sensitive to moisture stress, while other stages are less affected by water moisture [15]. For example the crisis stage in alfalfa life is when the plant lives in plantlet stage, blooms and once produces seed [7].

The most optimum way with drought is accommodation to condition, that is applying agricultural operations in a way that water storage increases or culture more resistant cultivars to drought conditions. Therefore, those cultivars which are optimum in moisture conditions are in attention by physiologists [18].

The objective of this study is to investigate the effects of drought stress during germination growth stage of some alfalfa cultivars (*Medicago sativa* L.).

MATERIALS AND METHODS

In order to determining the germination percentage and rate, radicle and plumule and vigority index as well as seed vigority index, 6 alfalfa cultivars were used under various osmotic potential by using factorial experiment in complet randomize design with four replications. These cultivars include theree local cultivars namely Yazdi, Hamedani, Qareh Younjeh and Maupa, Simmer Chenskaya and CUF101 introduced from abroad. Distilled water used as a control (0 bar) and osmotic potentials -3, -6 and -9 bar were prepared adding polyethylene glycol (Sigma Chemicals) to distilled water according to Michel and Kaufmann's equation [19]. All Petri dishes and filter papers were disinfected in 120°C for two hours post culture. After a surface scratch by a soft file, seeds were disinfected by mercury chloride 2% solution for 3 minutes and washed 3 times in distilled water. For each petri dish $10cm^3$ of solution was added which contains various drought stresses. After culture, all dishes were kept inside germination in 20°C for 14 days.

Germination rate was calculated by using $R=\Sigma\ n/\Sigma\ (Dn)$ formula. Where, n is the number of germinated seeds, D is the number of spent days from beginning and R is germination rate mean. The vigour index (VI) of the seed was estimated as suggested by Abdul-Baki and Anderson [20] as follow:

Vigour index =
[germination percentage × mean (radicle length + plumule lenght] / 100

Statistical Analysis: All statistical tests were carried out using the Statistical Analysis System SAS Institute [21]. Duncan Multiple Range Test (DMRT) at p=0.05 for comparison of means was used.

RESULTS AND DISCUSSION

In this study, some alfalfa cultivars in terms of germination percentage and rate, plumule length, the ratio of radicle to plumule and vigority index of seed indicated that there is a significant difference ($p< 0.01$) (Table 1).

There is discrepancy among cultivars in terms of germination rate reduction. Notable the reduction in germination percentage among cultivars begins from potential -9 bar, so that the amount of germination reduction in this density is less in Yazdi and Maupa in comparison with other cultivars (Table 2).

Table 1: Analysis results of statistics variance and measured values for various traits in germination stage

(S.O.V)	df	Mean Squares(MS) Germination percentage	Germination rate (Day)	Radicle Length (mm)	Plumule Length (mm)	Radicle: plumule ratio	Vigour index
Treatments	23	1644.972**	3.664**	1197.818**	575.063**	2.271**	3621.034**
Cultivar	5	3320.263**	6.763**	2331.918**	507.919**	1.688**	36900.937**
Osmotic Potential Cultivar*	3	6266.03**	14.967**	4035.804**	3503.345**	12.598**	38599.975**
Osmotic Potential	15	162.331*	0.371**	252.187*	11.788n.s	0.400**	7782.86**
Error	72	75.529	0.0999	118.773	20.918	0.152	177.992
Coefficient Variation % (C.V)		15.67	16.64	25.87	14.62	25.88	25.62

* Significant at p ≤ 0.05; ** Significant at p ≤ 0.01; NS – non significant

Table 2: Measured traits mean in germination stage

Cultivar	Osmotic potential (bar)	Germination %	Germination rate (day)	Radicle Length (mm)	Plumule Length (mm)	Radicle: plumule ratio	Vigor index
Yazdi	0	93abc	2.86abcd	30.25efghij	45.25a	0.65gh	70.9bcd
	-3	91abcd	2.84abcd	68.38a	45.48a	1.51cde	103.02a
	-6	79cdef	2.36def	69.98a	28.85d	2.42ab	77.84bc
	-9	61fg	1.51i	43.48bcdef	17.95e	2.57a	36.13efg
Hamedani	0	86bcd	2.57bcde	22.83hij	42.9ab	0.53h	56.94cde
	-3	85bcde	2.55cde	45.08bcdef	40.4ab	1.14efgh	72.31bcd
	-6	75def	2.14efg	69.05a	27.45d	2.55a	72.79bcd
	-9	28h	0.64jk	38.15cdefgh	13.68e	2.8a	15.11ghi
Maupa	0	93ab	2.97abc	32.1defghij	46a	0.7fgh	73.73bcd
	-3	91abcd	2.92abc	54.95abc	41.43ab	1.32cdef	87.35ab
	-6	85bcd	2.56cde	71.05a	31.88cd	2.25ab	87.16ab
	-9	34h	0.92j	49.33bcd	19e	2.61a	23.33ghi
Qareh yunjeh	0	97a	3.14a	30.28efghij	42.53ab	0.71fgh	70.68bcd
	-3	96ab	3.08ab	47.23bcde	37.63bc	1.25cdefg	81.36b
	-6	91abcd	2.7abcd	55.23abc	30.93cd	1.81bcd	79.06b
	-9	27h	0.7jk	40.45cdefgh	18.2e	2.32ab	16.5ghi
CUF101	0	55g	1.51i	20.08ij	29.93d	0.69fgh	28.15fgh
	-3	31h	0.83j	25.03ghij	27.78d	0.89efgh	16.19ghi
	-6	21hi	0.54jk	18.65ij	19.15e	1.05efgh	7.51hi
	-9	10i	0.22k	14.38j	4.05f	1.2defg	3.04i
Simmer chenskaya	0	66efg	2.03fgh	27.48fghij	44.6ab	0.62gh	47.43ef
	-3	62fg	1.83ghi	41.6cdefg	43.55ab	0.96efgh	52.79de
	-6	60fg	1.63hi	59.8ab	33.03cd	1.8bcd	56.73cde
	-9	23hi	0.58jk	36.08efghi	19.4e	1.84bc	13.83hi

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test

Yunis *et al*. [18], Zarif Ketabi [5] and Pahlevanpoor and Khalberin [22] reported tha at various osmotic potential germination percentage in alfalfa was reduced. Similar results were also reported by Abdul-baki and Anderson [20] and Hamidi [9] and Zhang [11]. The reduction in germination percentage and plantlets growth under water stress conditions might be the result of slower decomposition in endosperm materials or transmission of decomposed materials into plantlets. In corn plant, water stress affects on protein metabolism and nucleic acids and through this plantlet's growth decreases [8].

Safarnejad [8] and Pahlavanpoor and Khaldberin [22] reported that drought stress caused by (PEG 6000) decreased germination percentage in alfalfa. These results are in agreement with those obtained by

Mir Hosseini [23] on sainfoin and Safarnejad [8] on alfalfa. Drought effect on alfalfa seeds and their germination is not exceptional to other plants. In other examination, drought effect on alfalfa seeds and germination of 8 alfalfa cultivars was measured in which germination percentage and rate decreases [11]. Also as alfalfa seeds are exposed to sever drought, their later growth steps is affected due to damages to embryo textures and plumule or radicle growth disorders [3].

Yadvi *et al*. [14] concluded that germination percentage was decreased as osmotic potential goes to negative under drought stress caused by (PEG6000) on 6 barely cultivars.

The highest reduction in germination rate happens in potential -9 bar in CUF101 cultivars and less reduction is related to Yazdi in control potential 0 bar (Table 2).

The reduction in germination rate as a result of drought stress has been reported by Abdul-baki and Anderson [20], Michel and Kaufman [19] and Zhang [11]. Drought stress reduced reduction in germination percentage, while germination rate was als reduced under sever drought stress [3, 8]. Also, Karimi [24] reported that exposed to stresses more than -20 bar, most seeds are not able to absorb enough water for embryo growth.

Environmental water potential which surrounds the seed has direct effect on water absorption rate by seed and existing salts solved in water solution have notable effect in this process as well. In addition, poisoning by ions might result in reduction of germination and growth rate [24].

Data in Table 2 indicated that by decreasing negative water potential the radicle length was increased,which the increase in length continues up to potential -6 bar and after that the length of radicle was decreased.

Other studies, Abdul-baki and Anderson [20] and Busso and Fernandez and Fedorenko [3] reported that the radicle growth was increased under drought stress,while Hamidi [9] and Koucheki *et al*. [10] reported that the radicle length was reduced under water stress. These differences might be due to tolerance genes in chromosome structure and high moisture absorption by resistant cultivars [3, 9]. De and Kar [13] pointed that expose soybean cultivars to drought stress from 0- -16 bar reduced the osmotic potential, germination percentage reached to 81%, radicle and plumule lengths were decreased and their appearance was effected. By decreasing water potential the length of plumule in studied cultivars was reduced (Table 2). Under drought condition, plumule length was more affected than radicle length [9]. The reduction in plumule length as a result of drought stress is due to plant breathing disorders in initial germination and photosynthesis in bifoliate stages. These results are in accordance with the findings of Abdul-baki and Anderson [20] Hamidi [9] Koucheki and Rashed and Nasiri and Sadr Abadi [10]. Data in Table 2 indicated that the ratio of radicle to plumule length aws increased by increasing drought stress.The ratio of radicle length to plumule length is a flexibility trait which it was increased as the result of drought stress [9, 2].

Sadrabadi [4] in study on alfalfa plant argues that in this plant dedication of carbon hydrates to root and leave is in precedence than stem. Also leavs and roots have priority in absorbing water and photosynthesis process for plant and that they are more protected than stem, therefore the increase in ratio of radicle length to plumule length was decreasd in growth of aerial organs [8]. Data also in Table showed that the vigour index of seed cultivars was decreased by increasing at water potential -9 bar.This reduction in Yazdi, Hamedani, Maupa, Qareh Yunjeh, CUF101 and Simmer Chenskaya were 49.04, 73.46, 68.38, 76.65, 89.2 and 70.84% respectively (Table 2).

There is a positive and significant correlation between germination percentage, rate germination and plumule length. Also there is a negative and significant correlation between ratio of radicle to plumule length with plumule length. There is a positive and significant correlation between germination rate and percentage with radicle length and vigour index in 5% level. The results indicated that Yazdi and Maupa cultivars that possess higher germination percentage and rate germination, plumule length and vigour index, have more tolerance under sever drought stress during germination stage than other alfalfa cultivars.

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