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Response of Onobrychis Genotypes to PEG 10000 Induced Osmotic Stress

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Abstract: In order to evaluation of drought stress tolerance of seven onobrychis genotype *in vitro* condition, was done in Ardabil, Iran in 2009. This experiment was performed by use of factorial design on the basis of completely randomized in three replications. A factor includes five osmotic potential levels (0, -3, -6, -9 and -12 bar) and B factor includes seven onobrychis genotypes (Syntetic, Mako Shoot, Osko Asfanjan, Khosro Shahr Tazekand, Osko Askandaran, Ardabil Garjan and Ardabil Hasanbarogh). For making the different osmotic potentials were used the PEG 10000 and distilled water as control. The variance analysis results showed that there is significant difference between drought levels, genotypes and their interaction as attributes such as coleoptile length, germination uniformity and percent, between drought different levels as attribute germination speed. The Synthetic genotype had the most coleoptile length, germination uniformity and germination percentage in -3 bar and control in compare of the other genotypes. Synthetic genotype showed the most tolerance to drought stress in comparison of the other genotypes and selected the tolerant genotype.

Key words: Drought, PEG, *in vitro*, onobrychis

INTRODUCTION

The environmental stress is the most important limited factor of natural sources and agriculture productions. One of the most important factors is the drought stress. Ardabil plain that has area as amount of 85,000 hectares, is the fertile and potentially place that it located in North West of Iran. It is one part of fields that created of Khazar sea watershed rivers sediment. Regarding to the immethodical using of the agriculture pit and climatic changes in later years that caused to the rainfall dispersion change, rivers debi and pit debi change. So, Ardabil plain underground waters fell down quickly (In some places nearly 20 m or more than this). Nearly 40,000 hectares of Ardabil plain areas face to the intensive water stress and every year increase the areas that have water deficiency.

Onobrychis are Eurasian perennial herbs of the, legume family (Fabaceae). The Flora Europaea lists 23 species of Onobrychis; the main centre of diversity extends from Central Asia to Iran, with 56 species 27 of which are endemic in the latter country alone. Onobrychis, typically have a deep taproot and so are very drought resistant, but do not recover well from overgrazing.

Water is one of the main active germination factors. Water deficiency is one of the important factors to prevent the seed germination under field condition (Misra *et al*., 2002). Because of the stress the speed and percent of germination decreases or retards germination (Sadeghian and Yavari, 2004; Misra, 1994). Water deficiency causes to delay the plant establishment (Misra *et al*., 1996, 1997a, b, 2002; Prisco *et al*., 1992).

Water existence is necessary for germination and plant settlement. Germination uniformity mean is the time that the germination receives from 10% until 90% (D10-D90). Germination uniformity is a negative number that its low amounts shows the less uniformity and its excess amounts means more germination uniformity (Soltani *et al*., 2002).

Lagrorof showed in 1961 that PEG can be used to the nutrient culture solutions osmotic potential change and to make the water deficiency in plant in compare to the controlled condition. During the 1970 and 1980 decades PEG with 4000 and 8000 molecular mass was used to make drought stress under controlled condition in nutrient solution culture in physiologic experiments (Blum, 2008; Misra *et al*., 2002). In 1989 was predicted the standard calibration by Money (1989). Bermingham *et al*. (2006), Nasirzadeh and Shookoh (2006) and Kaboli and Sadeghi (2002) evaluated the effect of water stress on Onobrychis species by use polyethylene glycol (PEG) and introduced Onobrychis species for water stress condition.

To make the stress condition in lab was used of PEG. This material has more application because of the conditions similar to the natural environmental stress conditions (Hardgree and Emmerich, 1990). The PEG with excess molecular weight is sufficient to make the drought stress in compare of the molecules that have low

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weight (Blum, 2008). These study researches was performed to evaluation of the drought stress effects on the germination components and plantlets growth of seven onobrychis genotypes and determine drought stress tolerance of seven onobrychis genotype *in vitro* condition.

MATERIALS AND METHODS

In order to evaluation of drought stress tolerance of seven onobrychis genotype *in vitro* condition, was done in Ardabil Agriculture and Natural Sources Research Station laboratory, Iran in 2009. In this experiment was performed by use of factorial design on the basis of completely randomized in three replications. A factor includes five osmotic potential levels (0, -3, -6, -9 and -12 bar) and B factor includes seven Onobrychis genotypes (Synthetic, Mako Shoot, Osko Asfanjan, Khosro Shahr Tazekand, Osko Askandaran, Ardabil Garjan and Ardabil Hasanbarogh). For making the different osmotic potentials were used the PEG 10000 and distilled water as control. The Petri dishes and filter papers were disinfected into the incubator with 120°C during 90 min and the grains were sterilized by hypocoloryte sodium solution during 30 sec (Farzaneh *et al*., 2008). After this, was washed by distilled water that distilled twice and planted in Petri dishes. In every Petri dish was planted 25 seeds and was added 5 mL PEG solution. The laboratory temperature was 25±2°C during experiment doing. The whole of experiment stages was done under completely sterile conditions. After the seeds culture end, was located the planted Petri dishes in dark places 10 days. The attributes such as primary roots length, coleoptile length, the germinating speed and uniformity was measured. PEG concentration that needs to every osmotic potential was accounted by Michel and Kaufmann (1973). The seeds accounting was this case: germinated seeds with 2 mL length. Every seed with this property was accounted. Accounting continued till that the germinated seeds number became steady during 3 consecutive days. To design the germination component was designed the collective germination percent developing curve in front of the time (h), since the experiment start. Then by using these curves was accounted the experiment start time until receiving the 10% of germination (D10), 50% of germination (D50) and 90% of germination (D90) (Soltani *et al*., 2002).

The start time until germination, germination uniformity and germination speed was determined like follow:

- **D10:** Start time until germination (h)
- **D10-D90:** Germination uniformity (h)
- **1/D50:** Germination speed (h)

The variance analysis and mean comparison was accounted by LSD test and MSTATC software.

RESULTS AND DISCUSSION

The variance analysis results showed that there is significant difference between drought levels, genotypes and their interaction as attributes such as coleoptile length, germination uniformity and percent, between drought different levels as attribute such as germination speed (Table 1).

Osmotic potential decrease caused to coleoptile length significant decrease. Coleoptile length became zero in -6, -9 and -12 bar. The germination percent changed from 46.09% in control treatment to 1.91% in drought stress in -3 bar. Although, the drought stress increased but the germination speed decreased and this decrease amount was 40% in -3 bar in comparison of the control. The control treatment and -3 bar had the more germination uniformity in comparison of the other treatments (Table 2). Santetic, Osko Shahr, Ardabil Garjan genotypes and Osko Skandaran and Mako Shoot had the most and the least coleoptile length, respectively. Between the evaluated genotypes, the least germination uniformity and germination percentage was related to the Osko Skandaran and the most was related to the Synthetic. The Synthetic, Garjan and Hasanbarogh had the most germination speed and Khosro Shahr, Osko Skandaran and Mako Shoot had the least germination speed (Table 3). There were significant and positive correlations between coleoptile length, germinating speed, germinating uniform and germinating percent (Table 4). The Synthetic

Table 1: ANOVA traits mean of treatment for Onobrychis genotypes in osmotic potential different levels

		MS			
SOV	df	Coleoptile length	Germinating speed	Germinating uniformity	Germinating (%)
Osmotic potential (A)	4	11.25**	0.001**	1015132.38**	8754.89**
Genotype (B)	6	0.46**	0.0001	12184.58**	234.2*
AB	24	0.29**	0.0001	14623.78**	221.21*
E	70	0.06	0.0001	3674.45	3.96

* and **: Significant at 5 and 1% level of probability, respectively

Table 2: Mean of measured traits for osmotic potential different levels

Osmotic potential	Coleoptile length (mm)	Germinating speed (h)	Germinating uniformity (h)	Germinating (%)
0 bar	16.7a+	0.45a	-502.0a	46.49a
-3 bar	1.6b	0.05b	-57.6b	1.910b
-6 bar	0.0c	0.00c	0.0c	0.0c
-9 bar	0.0c	0.00c	0.0c	0.0c
-12 bar	0.0c	0.00c	0.0c	0.0c

+Mean with the same letters in each column does not have significant difference at the 5% level of probability to according to value of LSD

Table 3: Mean of measured traits for Onobrychis genotypes

Genotypes	Coleoptile length (mm)	Germinating speed (h)	Germinating uniformity (h)	Germinating (%)
Synthetic	0.965a+	16a	-176.40a	18.76a
Mako Shoot	0.223cd	8a	-96.87b	10.93b
Osko Asfanjan	0.330bc	9a	-100.07b	11.47b
Khosro Shahr	0.450b	8a	-102.0a	7.20c
Osko Askandaran	0.140d	11a	-99.33b	4.00d
Ardabil Garjan	0.410b	11a	-104.67b	7.50c
Ardabil Hasanbarogh	0.310bcd	11a	-104.0b	7.50c

+Mean with the same letters in each column does not have significant difference at the 5% level of probability to according to value of LSD

Table 4: Linear correlation coefficients between different traits for Onobrychis genotypes in osmotic potential different levels

Correlation coficient	Coleoptile length	Germinating speed	Germinating uniform	Germinating (%)
Coleoptile length	-			
Germinating speed	0.99**	-		
Germinating uniform	0.89**	0.94**	-	
Germinating %	0.92**	0.87**	0.94**	-

**: Significant at 5 and 1% level of probability, respectively

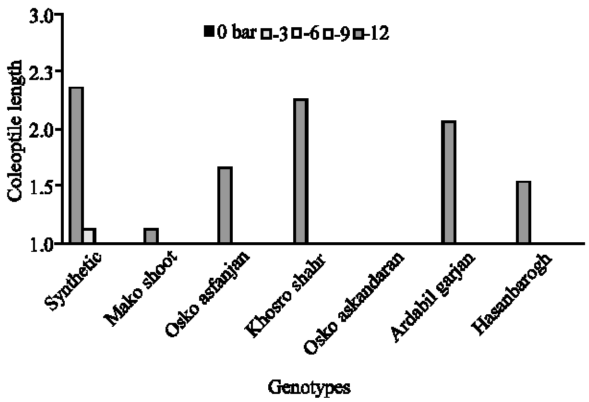

Fig. 1: Mean of coleoptile length for Onobrychis genotypes in osmotic potential different levels

genotype had the most coleoptile length (Fig. 1), germination uniformity (Fig. 3) and germination percentage (Fig. 4) in -3 bar and control in compare of the other genotypes. Also, only the Synthetic genotype had the seed germination in -3 bar osmotic potential (Fig. 2).

Synthetic showed the most tolerance to drought stress in compare of the other genotypes and selected the tolerant genotype.

The results showed that drought stress influenced on the germination component (percent, speed and uniformity) and coleoptitle length. The negative effect of osmotic potential decrease was reported on the germination speed and percent in some research on the agronomy plant (Clarke *et al*., 1993; De and Kar, 1995; Misra, 1994; Misra *et al*., 1996, 1997a,b).

In this study moisture stress caused the germination speed, percent and uniformity decrease in the whole of genotypes. But the germination component reaction

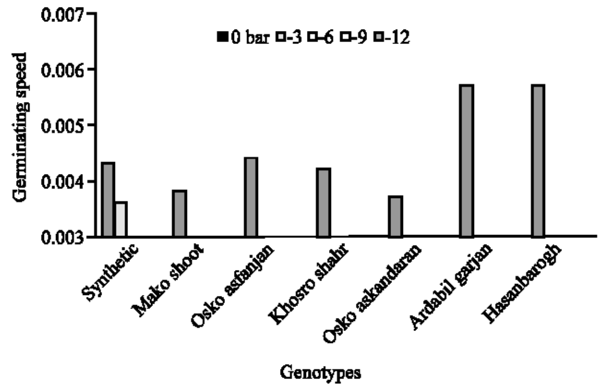

Fig. 2: Mean of germinating speed for Onobrychis genotypes in osmotic potential different levels

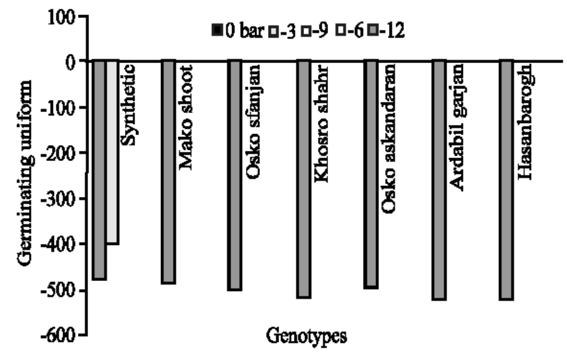

Fig. 3: Mean of germinating uniformity for Onobrychis genotypes in osmotic potential different levels

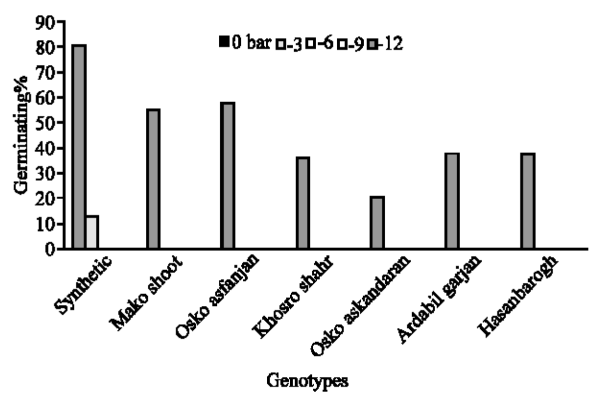

Fig. 4: Mean of germinating percent for Onobrychis genotypes in osmotic potential different levels

to the drought stress was not similar. It means that the decrease amount was different for the genotypes.

Synthetic genotype had the least decrease for the germination speed and percent and evaluated as the tolerant genotype to draught stress. This genotype had the most coleoptile length in compare of the other genotypes. The similar result was reported to the other agronomy plants such as wheat (Singh, 2001) and bean (Prisco *et al*., 1992).

The drought stress influences on the germinating by the limiting the water absorption by the seed, by the effect on the move and transfer of seed reserves and protein synthesis in germ (Dodd and Donvan, 1999; Misra, 1994; Misra *et al*., 2002).

PEG causes the seed reserves materials hydrolysis decrease and finally the germination percentage decrease (Fisher and Maurer, 1978). The different reaction of the onobrychis genotypes final germination percent to the drought stress related to the different factors such as the water absorption decrease in sensitive genotypes and germination decrease. The seed hardship is effective to determine the tolerant genotype. Because having a hardship level on the seed causes to increase the necessary moisture for seed germination in compare of the other plant as amount of twice (Farzaneh *et al*., 2008).

The germination speed of the tolerant genotypes to the drought stress was more than the sensitive genotypes germination speed like the germination percent.

Germination speed is one of the indexes to evaluate to the drought tolerant. The genotypes that included more germination speed than others under drought stress condition had more probably for greening (Farzaneh *et al*., 2008; Misra *et al*., 1997a,b, 2002).

The germination uniformity can have important to manage the field as the result of the greening synchronous especially in stress periods. The coleoptile decreased when the osmotic potential decreased. The root length decreasing reported under drought condition (Malik *et al*., 2004; Misra *et al*., 1996, 1997a,b, 2002).

Under drought stress condition, there are genial or physiological mechanisms in plant that by becoming active some special works, maintain the tissue need water (Blum, 1996). The seeds germinating become complete when the root comes out of grain skin and comes in the rhizosphere. The germinating happens when the germ cells length increase and cellular division and roots growth start after this, are not part of germinating stage (Baraldi *et al*., 1991; Bewley and Black, 1985). Allen *et al*. (1986) showed that creation of the seedling and coleoptile length have relationship and the varieties that have more coleoptile length become green more early than the varieties that have short coleoptile length and they resulted in their own experiment that the varieties have short coleoptile length, create their seedling slowly and the production green percentage is less. In this study, the genotypes that had excess germination percentage had the excess coleoptile length too. The seeds that had good root and caulicle growth under drought condition could have excess settlement rapidly and had high yield under insufficient environmental condition. Synthetic and Ardabil Garjan Onobrychis genotypes had the driest and wet fodder yield under field conditions for two years (2007-2008).

CONCLUSION

The Synthetic genotype had the most coleoptile length, germination uniformity and germination percentage in compare of the other genotypes. Synthetic genotype showed the most tolerance to drought stress in compare of the other genotypes and selected the tolerant genotype. This genotype had the most dry and wet fodder yield in field evaluation.

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