

## Screening drought tolerance of alfalfa in early growing stages<sup>1</sup>

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### Abstract

**Introduction:** For nomadic herders like Mongolians, the continental climate of the country brings the biggest challenge, particularly during winter or spring when the temperature reaches -30°C or when the livestock deliver its off-spring. During this time, nutritious hay requirements become the key to survival. In semi-arid climate conditions like Mongolia, cultivating drought-tolerant crops for hay has becomes important.

**Materials and Methods:** Nowadays, one of the recommended crops is alfalfa (*Medicago F.*). Alfalfa has deep vertical roots; this species is able to absorb waters from about 5m in depth and, more importantly, it is a great source of protein. Thus, it is aimed to investigate the alfalfa's drought tolerance in early growing stages. In order to differentiate levels of drought tolerance on alfalfa varieties, two experiments were conducted to establish the screening method under drought stress and compare different drought resistance among alfalfa varieties grown from different places. Alfalfa's drought tolerance was tested in the growing stage in its box by withholding irrigation. In the second stage, drought stress is stimulated by different concentrations of PEG6000 to determine alfalfa's drought tolerance in the seedling stage.

**Results:** It revealed that in the box test, even after the irrigation was stopped, the alfalfa kept growing while only some nodes dried out. During the drought period, the proline content increased significantly in all varieties. In the first measurement, it fluctuated between 0.1 to 0.4 but, seven days later in the second measurement, it was between 0.7 and

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0.9. Based on the proline content result, it can be said that varieties “Nutag Belcheer” and “Burgaltai” are best during long-term drought stress. Finally, the PEG (Polyethylene Glycol) was used as irrigation in different concentrations and applied twice at 3-day intervals. After seven days of second PEG treatment, varieties “Middle East” and “Burgaltai” remained alive in all treatments. Variety “Known You Alfalfa” in 10% PEG died just after the first time PEG was applied and “Nutag Belcheer” in 30% died after the second PEG was applied. Proline content was also measured and 20% PEG treatment had the highest proline content. In terms of varieties, “Known You Alfalfa” had the highest result, and both “Nutag Belcheer” and “Burgaltai” had the same result as each other, which was also the lowest.

**Conclusions:** In conclusion, in long-term drought stress, varieties “Burgaltai” and “Nutag Belcheer” were better than the other two varieties. Furthermore, in the PEG drought simulation, 20% PEG treatment could be the best concentration to test the drought tolerance of plants. In comparing PEG simulated drought and box tests, the box test which gives the opportunity to screen drought longer is preferred. Based on these results, it can be said that the four varieties had different drought tolerance depending on the growth stage. In the early growth seedling stage, varieties “Middle East” and “Known You Alfalfa” were the most drought-tolerant, whereas varieties “Nutag Belcheer” and “Burgaltai” were better in long-term drought during the later growth stage. It also revealed that 20% PEG treatment could be the best concentration to simulate drought and test drought tolerance of plants.

Among the methods we have used to screen drought, the box-screening method is preferred to the PEG-simulated method because the box-screening method could be more similar to naturally rainless conditions and results collected from this method may be used in irrigation management in alfalfa fields. Moreover, the box test gives a chance to test the drought tolerance of plants at each growth stage but, in terms of PEG, it continues only up to a period of ten days.

**Keywords:** Alfalf; Drought, PEG, Proline, Withholding irrigation, Mongolia.



## 1. Introduction

Mongolia is a landlocked country in East Asia which is known as a nomadic nation and one of the few truly pastoral countries where the economy depends almost entirely on livestock, with little crops, forestry or industry. The grassland and arid grazing cover 1,210,000 km<sup>2</sup> (80%) of the land area; therefore, this area is extensive grazing exploited by traditional pastoral methods and pasture grass is the main food resource for livestock (Probst & Smith, 2011). However, herders have to prepare hay for winter and early springtime. During wintertime, the heavy snow covers the pasture and cold temperatures (-25°C to -45°C) become limitations. The key to overcoming these challenges is nutritious hay. Nowadays, one of the recommended crops is alfalfa (*Medicago F.*) which is also known as the “Queen of Forages” and as a great source of protein. According to Mongolian field researchers, alfalfa should be harvested from the second year of its harvest. In the second year, from a 5 hectare alfalfa field, it can harvest a 40 ton yield which can feed 750 sheep from the 1<sup>st</sup> of December to the next spring, around May, without going to pasture (Probst & Smith, 2011).

Meanwhile, drought stress is the major environmental factor that constrains the productivity and stability of plants (Eludoyin *et al.*, 2017; Tuncok & Eslamian, 2017). Worldwide losses in crop yields from drought stress probably exceed the losses from all other abiotic stresses combined (Soni *et al.*, 2014; Pati & Eslamian, 2017). The Mongolian precipitation rate is determined by Siberian anticyclones which result in low temperatures in winter and low precipitation. Furthermore, high mountain ranges isolate the country from the influence of the Atlantic and Pacific climates (Suttie, n.d.).

Accordingly, it is aimed to study the alfalfa drought tolerance in the early growing stages. In order to differentiate levels of drought tolerance on alfalfa varieties, two experiments were conducted to establish screening methods under drought stress and compare different drought resistance among alfalfa varieties from different places.

## 2. Materials and methods

In this study, four varieties of alfalfa were chosen as a plant material: Mongolia-originated varieties such as Burgaltai and Nutag Belcheer and Taiwan-based varieties such as “Known-You Alfalfa” and “Middle East” were selected to screen drought tolerance in nurseries of National Pingtung University of Science and Technology (NPUST), Taiwan, located at 22°38.28.26., longitude north, 120° 36.07.69, latitude east, and 49m above sea level.

A box-growing experiment was conducted to screen for tolerance and observe shoot and root development under drought stress. As the second part of the experiment, we used Polyethylene Glycol (PEG) 6000 to stimulate drought tolerance and screened the effect of drought in the seedling growth stage.

During 4 months (from April to July, 2016), alfalfa with 3 replications from each variety was grown in plastic boxes ( $60 \times 15 \times 70$  cm) with a ratio of sand to perlite of 1:1 medium (figure 1) to evaluate shoot and root responses under drought stress. Alfalfa emerged from the germination trays and, when the plant reached about 5cm in height, it was transplanted to the box. For a period of one month, the crop was irrigated every day and fertilizer Peters N-P2O-K2O 20:20:20 was applied once a week. After a month, irrigation was stopped and plant height, root length and node numbers were observed in the drought-stressed condition.

In the second experiment, four varieties of alfalfa emerged from the germination trays and, after two weeks, seedlings were transplanted to 3 pots with 3 plants each and they were irrigated once every two days, with fertilizer being applied three times. After a week

of transplanting, regular irrigation was stopped and PEG in 4 different concentrations (zero (control), 10%, 20% and 30% (0 MPa, -0.6 MPa, -1.2 MPa and -1.8 MPa)) were used as an irrigation source twice a week. After the second irrigation with PEG 6000, alfalfa was harvested and height, node number, root and shoot weight, and proline content were measured. Additionally, proline was analyzed based on the Bates *et al.* (1973) method.

Data were analyzed using SAS 9.4 with an analysis of variance (ANOVA). The least-significant-differences (LSD at  $p \leq 0.05\%$ ) test was used to define differences among treatment means.



**Figure 1.** The plastic box with sand: perlite (1:1)

### 3. Results and Discussions

#### 3-1. Box test for evaluation plant shoot and root responses under drought stress

Alfalfa continued to grow despite more than twenty days of withholding irrigation. One month after irrigation was stopped, the plants were harvested for further investigation of shoot height and weight, root length and weight and node numbers.

The heaviest shoot belonged to variety “Middle East” (0.7g) and the other varieties had the same results as each other (0.3 g) (Table 1).

The shoot weight of other the varieties in different boxes were similar to each other. The root of variety “Middle East” was heavier than the shoot weight.

Variety “Nutag Belcheer” had the tallest shoot (25cm) and the shortest varieties were “Middle East” and “Burgaltai” (19cm). Variety “Nutag Belcheer” had the longest root (14cm) and other varieties’ root length fluctuated between 11 to 12 centimeters. In terms of node number, varieties “Burgaltai” and “Middle East” had many more nodes (5 nodes) than the other two varieties (Table 1).

Proline content was measured twice with a seven-day interval: on the day of harvest and a week before harvest. The proline content of each variety is shown in Table 2. From this, it can be seen that all varieties had increased proline concentration during the 7 days: variety “Middle East” by 0.7 mg/g, variety “Burgaltai” by 0.5 mg/g, variety “Nutag Belcheer” by 0.5 mg/g and variety “Known You Alfalfa” by 0.8 mg/g. Overall, varieties “Nutag Belcheer” and “Burgaltai” had the same proline content as each other, as did “Known You Alfalfa” and “Middle East”.

**Table 1.** Plant character responses of tested variety

Variety	Shoot weight	Root weight	Shoot height	Root length	Node
	G		cm		
Burgaltai	0.3±0.1 b	0.4±0.2 b	19±4.0 a	11.1±1.13 a	4±2.2 a
Nutag Belcheer	0.3±0.01 b	0.4±0.19 b	25±4.0 a	14±1.4 a	5±1.09 a
Known You Alfalfa	0.3±0.06b	0.3±0.05 b	22.5±3.1 a	12.4±0.4 a	3.5±0.2 a
Middle East	0.7±0.2 a*	0.65±0.2 a	19.5±6.2 a	12.4±0.3 a	5.7±3.6 a

\*-Values are means ± standard error. Means followed by different letters within each column showed significantly different according to LSD multiple comparisons

**Table 2.** Proline content in four varieties in first and second measurement

Varieties	First*	Second
Burgaltai	0.263±0.14 a**	0.785±0.003 b
Nutag Belcheer	0.202±0.002 a	0.781±0.01 b
Known You Alfalfa	0.17±0.05 a	0.944±0.03 a
Middle East	0.1805±0.004 a	0.89±0.06 ab

\* First – first measurement

Second- last measurement

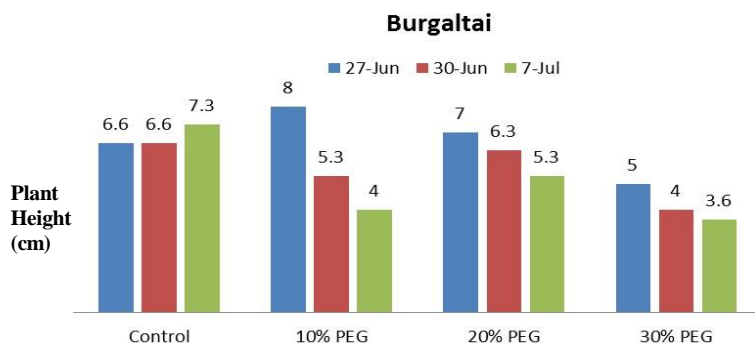
\*\* Values are means ± standard error. Means followed by different letters within each column showed significantly different according to LSD multiple comparisons.

In addition, the box test was a good screening method for screening drought tolerance due to its similarity to natural conditions; in other words, the result of the test can be a reference for irrigation management.

### 3-2. Alfalfa's drought (PEG-simulated) tolerance in the seedling growing stage

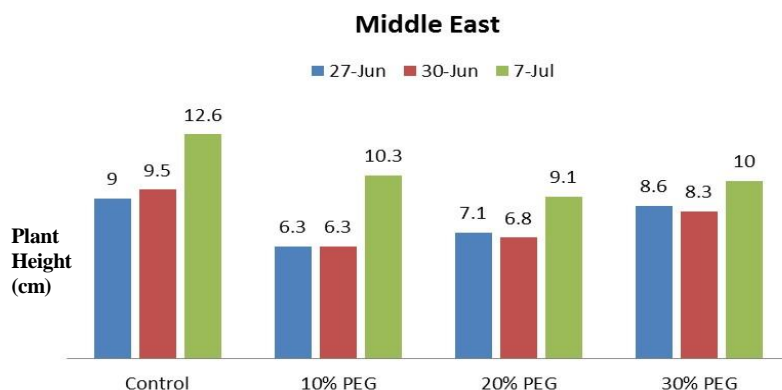
Alfalfa plant height was measured three times: before applying the first PEG treatment, before applying the second PEG treatment, and before harvesting.

In the control group, the height of “Burgaltai” tend to increase throughout the experimentation period. On the other hand, treatments of 10, 20 and 30 percent of PEG resulted in a decrease of height. At 30% PEG, it was the shortest (3.6 cm) at the end of experiment (Figure 2). Generally, the 20% PEG treatment showed medium performance compared to the other treatments throughout the experiment.



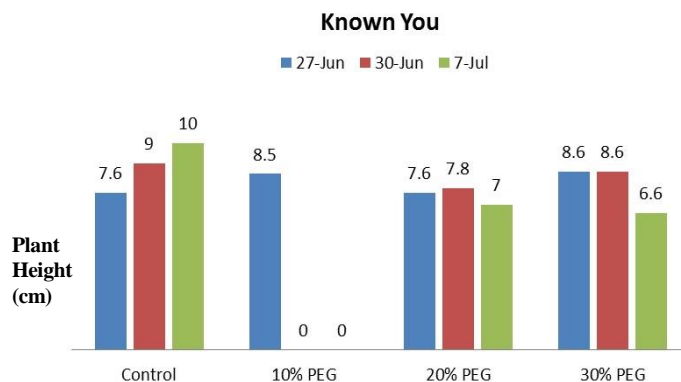
**Figure 2.** The plant height of variety “Burgaltai” (cm) in all treatments during whole experiment period

The height of variety “Middle East” increased by 2-3 centimeters in all four treatments (Figure 3). The tallest one belonged to the control treatment and, at the end of the experiment, it reached 12.6 centimeters, while the second-tallest belonged to the 30% PEG treatment.



**Figure 3.** The plant height of variety “Middle East” (cm) in all treatments during whole experiment period

Variety “Known You Alfalfa” increased in the control group and in 20% PEG; the growth stabilized during the experimentation period (Figure 4). On the other hand, at the end of the experiment in 30% PEG, the height decreased by two centimeters, while the plants given 10% PEG treatment all died.

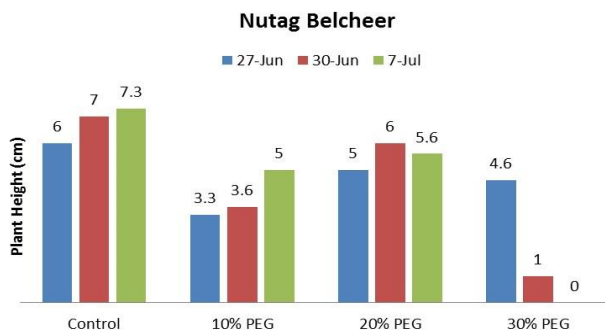


**Figure 4.** The plant height of variety “Known You Alfalfa” (cm) in all treatments during whole experiment period

\*0 represents tht all plants in a pot died.

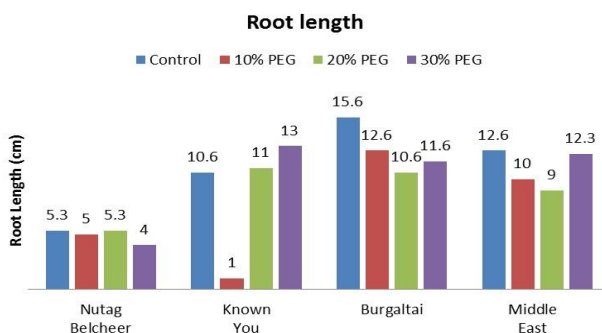
In the case of variety “Nutag Belcheer”, the height in the control group,

10% and 20% PEG treatments remained stable after first-time application of PEG; however, 10% PEG had grown by around 1 centimeter at the harvested date (figure 5). On the other hand, in 30% PEG the alfalfa started to die after the first PEG was applied and, by the time of harvesting, the plants had all died.



**Figure 5.** The plant height of variety “Nutag Belcheer” (cm) in all treatments during whole experiment period \*0 represents that all plants in a pot died

Seven days after applying the second PEG treatment, all alfalfa were harvested and root length, weight and shoot weight were measured. Overall, variety “Nutag Belcheer” had the shorter root in all treatments compared to the other varieties, with the exception of “Known You Alfalfa” in 10% PEG, which had the shortest route at just one centimeter (figure 6). The longest belonged to Burgaltai (16 cm) in the control and 10% PEG groups. In 20% PEG treatment, the variety “Burgaltai” and “Known You Alfalfa” had almost the same result (10.6 and 11 centimeters) and the longest compared to the other two varieties. Also, varieties “Known You Alfalfa” (13 cm) and “Middle East” (12.3) had the longest root in 30% PEG.



**Figure 6.** The root length of four varieties at the time of harvesting





Relating to root length, the weight had a similar result (Table 3). The variety “Nutag Belcheer” had shortest route and was also the lightest. The weight of this variety ranged from 0.03 to 0.06 gram in all treatments. Variety “Burgaltai” in 10% PEG treatment had the heaviest root.

**Table 3.** Root weight of four varieties in all treatments at the time of harvesting

	Root weight (g)			
	Nutag Belcheer	KnowYou	Burgaltai	Middle East
Control	0.03±0.01	0.11±0.04	0.17±0.01 b*	0.18±0.06
10% PEG	0.05±0.02	0.06±0.01	0.5±0.1 a	0.38±0.25
20% PEG	0.06±0.02	0.26±0.16	0.16±0.06 b	0.12±0.03
30% PEG	0.03±0.01	0.26±0.1	0.13±0.03 b	0.16±0.03

\*Means followed by different letter showed significant differences among treatments (LSD  $p < 0.05$ )

In terms of shoot weight, variety “Burgaltai” in 10% PEG also had the heaviest shoot. The other three varieties in all treatments did not have significant difference with each other, ranging from 0.02 to 0.09 grams (Table 4).

**Table 4.** Shoot weight of four varieties in all treatments at the time of harvesting

	Shoot weight (g)			
	Nutag Belcheer	Known You	Burgaltai	Middle East
Control	0.04±0.02	0.07±0.03	0.09±0.01 ab*	0.09±0.01
10% PEG	0.07±0.01	0.07±0.01	0.12±0.03 a	0.08±0.02
20% PEG	0.02±0.005	0.06±0.01	0.02±0.003 b	0.08±0.008
30% PEG	0.03±0.01	0.07±0.01	0.06±0.008 ab	0.07±0.02

\*Means followed by different letter showed significant differences among treatments (LSD  $p < 0.05$ )

Proline content was measured by different treatments and varieties. Variety “Known You Alfalfa” had the highest content in 20% PEG (2.415 mg/g), followed by control (1.613 mg/g) and 10% PEG (1.382 mg/g).

Variety “Nutag Belcheer” in 30% PEG showed the lowest result (0.466 mg/g) (Table 5).

**Table 5.** Proline content of varieties for each treatment

	Control	PEG 10%	PEG 20%	PEG 30%
Burgaltai	0.094 j*	0.798 f	0.366 h	1.023 e
Nutag Belcheer	0.189 ij	0.270 i	1.383 c	0.466 j
Known You Alfalfa	1.613 b	1.382 c	2.416 a	1.053 e
Middle East	0.497 g	0.176 j	1.124 je	1.303 cd

\*-Values are means  $\pm$  standard error. Means followed by different letters within each column are significantly different according to LSD multiple comparisons.

In PEG irrigated condition the variety “Middle East” was better than the other three varieties as it kept growing in all PEG concentrations and had the heaviest root and shoot. However, proline content was a little bit higher (0.775 mg/g) than 2 of the varieties (Burgaltai and Nutag Belcheer at 0.57 mg/g).

Plant deaths were observed in the 10% and 30% PEG treatments, so 20% PEG could be a better PEG treatment concentration than the others.

**Conclusions**

The drought tolerance response of alfalfa by two different methods are evaluated: withholding irrigation and stimulating drought tolerance by PEG. In the box test, even after the irrigation was stopped, the alfalfa continued growing and only some nodes dried out. During the drought period, the proline content increased significantly in all varieties. In the first measurement, it fluctuated between 0.1 and 0.4 but, seven days later, it increased to 0.7-0.9. Based on the proline content result, it can be said that varieties “Nutag Belchee” and “Burgaltai” are best in long-term drought stress. PEG was applied to test the germination rate of four varieties. In 30% PEG treatment, none of alfalfa germinated, while the control and 10% PEG groups had similar (highest) germination results to each other. 20% PEG had a lower germination percentage. At the end of the experiment, plant height was up to 1.8cm and root length was up to 4.2cm, while variety “Burgaltai” was the tallest (10% PEG - 1.7cm; 20% PEG - 0.8cm). In terms of root variety, “Middle East” had the longest root (4.2cm) in 10% PEG, variety “Burgaltai” in 20% PEG (2.9cm). Finally, the PEG was used as irrigation in different concentrations and applied twice with 3-day interval. Seven days after the second PEG treatment, varieties “Middle East” and “Burgaltai” were still alive in all treatments. Variety “Known You Alfalfa” in 10% PEG died just after the first time that the PEG treatment was applied and “Nutag Belcheer” in 30% died after the second PEG treatment was applied. Proline content was also



measured and 20% PEG treatment had the highest proline content. In terms of varieties, “Known You Alfalfa” had the highest result, while “Nutag Blecheer” and “Burgaltai” had the same result, which was also the lowest.

Based on these results, it can be said that the four varieties had different drought tolerance depending on the growth stage. In the early growth seedling stage, varieties “Middle East” and “Known You Alfalfa” were the most drought-tolerant, whereas varieties “Nutag Belcheer” and “Burgaltai” were better in long-term drought during the later growth stage. It also revealed that 20% PEG treatment could be the best concentration to simulate drought and test drought tolerance of plants.

Among the methods, screen drought has been used, the box-screening method is preferred to the PEG-simulated method because the box-screening method could be more similar to the naturally rainless conditions and results collected from this method may be used in irrigation management in alfalfa fields. Moreover, the box test gives a chance to test the drought tolerance of plants at each growth stage but, in terms of PEG, it continues only up to a period of ten days.

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