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COMPARISON OF DROUGHT TOLERANCE OF CULTIVATED ALFALFA (*MEDICAGO SATIVA* L.) VARIETIES

Oyunbolor Altangadas, Nominkhishig Khishigdelger, Gurbazar Damdinsuren, Enkhchimeg Vanjildorj

© Oyunbolor Altangadas
M. Sc.,
Department of Biotechnology and Breeding
School of Animal Science and Biotechnology
Mongolian University of Life Sciences
Zaisan 17024, Ulaanbaatar, Mongolia
bolorboogii@muls.edu.mn

© Nominkhishig Khishigdelger
Researcher,
Department of Biotechnology and Breeding
School of Animal Science and Biotechnology
Mongolian University of Life Sciences
Zaisan 17024, Ulaanbaatar, Mongolia
2217003@muls.edu.mn

© Enkhchimeg Vanjildorj
Ph.D., A/Prof.,
Department of Biotechnology and Breeding
School of Animal Science and Biotechnology
Mongolian University of Life Sciences
Zaisan 17024, Ulaanbaatar, Mongolia
enkhchimeg.v@muls.edu.mn

© Gurbazar Damdinsuren
Ph.D.,
Department of Pasture, Nutrition and Chemistry
Mongolian University of Life Sciences
Zaisan 17024, Ulaanbaatar, Mongolia
gurbazar@muls.edu.mn

Abstract. Alfalfa is a perennial fodder plant with high nutritional value, efficient grass yield and high protein content [Erdenejav, 2015]. Drought stress is a major environmental factor that affects the germination and growth stages of plants. In this study, the drought tolerance of Turkish alfalfa variety, Gozlu-1 and Mongolian alfalfa variety, Burgaltai was compared using polyethylene glycol (PEG 20 000) and mannitol both *in vitro* and *ex vitro*. According to the results of the study, the germination rate of Gozlu-1 variety in drought was 4.4–20.2%

better than that of Burgaltai variety. When comparing the alfalfa varieties according to some morphological parameters that indicates the resistance of artificial drought, Gozlu-1 variety showed 8.4% more resistance than Burgaltai variety, and Gozlu-1 variety has 17.5–28% more water absorption intensity than Burgaltai variety.
Keywords: blue flowered alfalfa Gozlu-1 variety, yellow flowered alfalfa Burgaltai variety, drought stress tolerance, morphological and physiological parameters.

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Introduction

Due to global climate change, the average annual air temperature in Mongolia has increased by 1.56 degrees over the last 60 years. According to research, droughts occur once every 4–5 years in 25 percent of the country's territory, and the number of days with dust storms in the steppe and Gobi regions tends to increase 3–4 times today compared to 1960[1]. A United Nations study estimates that 90% of Mongolia's pastureland is at risk of desertification. Seventy six percent of them believe that desertification has already started. Therefore, it is necessary to sustain the relatively fresh pastures without losing their basic condition, to support the recovery of degraded perennial grasses, to reduce the proportion of inedible and useless plants, and to introduce a use that can reduce soil erosion in a short time[2].

The biggest challenge to the sustainability of Mongolia's livestock production is the deterioration of pasture condition, quality or pasture productivity and the associated environmental degradation, which is the main source of fodder and habitat. There is a need for cultivation for perennial forage plants for sustainable use of natural pastures, improvement of hayfields by irrigation and fertilization, increase of fodder production, and replacement of natural fodder due to the need to reduce the impact of natural disasters caused by global warming.

Our country produces most of its fodder from forest-steppe, high mountain and agricultural areas. As of 2017, the area under fodder crops was 26.2 thousand hectares which is 5 times less than the previous period and the harvest was 10 times lower.Within the framework of the Intensive Livestock Support Program, it is planned to increase the area of fodder crops to 100.0 thousand hectares, in 2021 we are working to supply green fodder seeds to local communities at a discounted price and increase sowing[3] .

[1] Desertification and Mongolia. Available at: https://news.mn/r/635416/ (accessed 17.06.2013)

[2] Mongolian National Pastureland Status Report 2015. Available at: https://www.eda.admin.ch/dam/countries/countries-content/mongolia/en/Mongolia-Rangeland-Health-Report_MN.pdf. P. 5.

[3] Report of the Ministry of Food, Agriculture and Light Industry 2022. Available at: http://www.msrm.mn/index.php?option=com_content&view=category&layout=blog&id=83&Itemid=468&limitstart=5 (accessed 17.06.2013)

Alfalfa, a forage plant, is a valuable perennial crop that regenerates degraded pastures and degraded crops, grows in natural pastures. Therefore, we studied alfalfa varieties *in vitro* and *ex vitro* conditions to compare the drought tolerance of Turkish blue-flowered alfalfa Gozlu-1 with the results of Mongolian yellow flowered Burgaltai variety, and to determine the ability of alfalfa to grow in drought conditions with high productivity.

Ghasem et al. [2009], Hamidi and Safarnejad [2010], Castroluna et al. [2014], Adiyasuren et al. [2016] are reported alfalfa seed germination ability under drought condition. In addition, Safarnejad [2008] established some morphological parameters of alfalfa plants and Adiyasuren et al. [2016] presented some result of drought tolerance of *M. sativa*, *M. falcata*, *M. varia* Marthz, *M. varia* Martyn. Jansaya Yerbolat [2019] reported comparative analysis of drought tolerance in alfalfa varieties of *M. sativa,* M. Uuganzaya [2017] determined drought tolerance on some alfalfa species by some morphological and physiological parameters.

Materials and methods

Plant materials

Seeds of Gozlu-1 blue flowered alfalfa variety of Turkey and Burgaltai alfalfa variety of Mongolian were used as a plant materials.

To determine *the effect of drought on the stress tolerance index of seed germination* seeds of alfalfa varieties were sterilized and placed in 20 petri dishes with filter paper 3–5 times. The following formula was used to measure the effect of drought on the seed germination index on the 2nd, 4th, 6th and 8th days after the start of the experiment by irrigating with 10 ml of different osmotic pressure solution daily.

Drought-affected seed germination = nd2 (1.00) + nd4 (0.75) + nd6 (0.50) + nd8 (0.25), where *nd* — the day of seed germination.

The seed germination drought resistance index is calculated by the following formula:

$$\text{Seed germination index (\%)} = (\text{drought-affected seed germination}) / (\text{controlled seed germination}) \times 100$$

Methodology for determination of drought stress in vitro. Drought stress of alfalfa Gozlu-1 and Burgaltai varieties was determined *in vitro* under the method of Kadota [2001] with 3-5 replications. Sterilized seeds were transplanted for 1 month on mannitol-containing media at concentrations of 50, 100, 200, 300, 400, and 500 mM. Plant viability was determined by seed germination rate, plant height and root length.

Determining the effect of drought on the shoot and root length stress resistance index of alfalfa varieties. Phenotypical observations of alfalfa varieties were performed daily for 30 days until the plants were saturated with solutions of different concentrations of mannitol (0–500 mM). When there is a phenotypic difference, the stress resistance index of shoots and roots is calculated according to the method of Sammar Raza [2012] using the following formula.The experiment was performed with 3 replications.

$$\text{Shoot length stress resistance index (\%)} = (\text{drought-affected plant shoot length}) / (\text{control plant shoot length}) \times 100$$

Root length stress resistance index (%) = (root length of drought-affected plants) / (root length of control plants) × 100

Determining the effect of drought on the dry weight index of plants. The leaves of the drought-stressed and control plants are cut, dried in an oven at 70 °C for 24 h, and weighed to determine the dry weight.

Dry weight index (%) = (drought-affected plant weight) / (control plant weight) × 100

Plant shoot weight index (%) = (drought-affected plant shoot weight) / (control plant shoot weight) × 100

Data calculation and analysis

Each experiment was performed with 3 replications, and the values of the mean and standard deviations of the experiment were calculated using Microsoft Excel.

Result and discussion

The effect of drought on the stress tolerance index of seed germination

Polyethylene glycol 20 000 (PEG) — a seed germination index for alfalfa varieties based on a method that restricts plant growth and seed germination by increasing the osmotic pressure and limiting water absorption by forming a high-molecular-weight synthetic drought.At a pressure of -0.3 MPa, the Turkish Gozlu-1 variety decreased by 3.1% while the Burgaltai variety decreased by 23.2% compared to control plants.

In a solution with a pressure of -0.6 MPa, the Gozlu-1 variety decreased by 5.2% while the Burgaltai variety decreased by 32.9% compared to control plants.

In a solution with a pressure of -0.9 MPa, the Gozlu-1 variety decreased by 34.3% whereas the Burgaltai variety decreased by 40.2% compared to control plants.

In a solution with a pressure of -1.2 MPa, the Gozlu-1 variety decreased by 60.4% whereas the Burgaltai variety decreased by 64.6% compared to control (Table 1, Figure 1).

Table 1

The seed germination of alfalfa plants in different osmotic solution level, %

Plants	0	-0.3MPa	-0.6 MPa	-0.9 MPa	-1.2 MPa	Mean
Burgaltai	82±0.1	63±0.1	55±0.5	49±0.2	29±0.4	55.6
Gozlu-1	96±0.2	93±0.3	91±0.1	63±0.3	38±0.1	76.2

Means of three replicates ±SD after 15 days of drought stress test

Determination of drought stress in vitro condition

Water scarcity or dehydration is one of the factors limiting plant growth, which can lead to significant changes in plant physiology. In order to test the drought stress resistance *in vitro*, the sterilized seeds were transplanted and tested for 21 days after preparation of mannitol-containing medium with concentrations of 50, 100, 200, 300, 400, 500 mM according to the Kadota [2001] method. Drought stress was artificially generated with mannitol and grown in a nutrient medium. Although controlled plant growth was normal, seed growth rates were slowed in drought-tolerated variants.

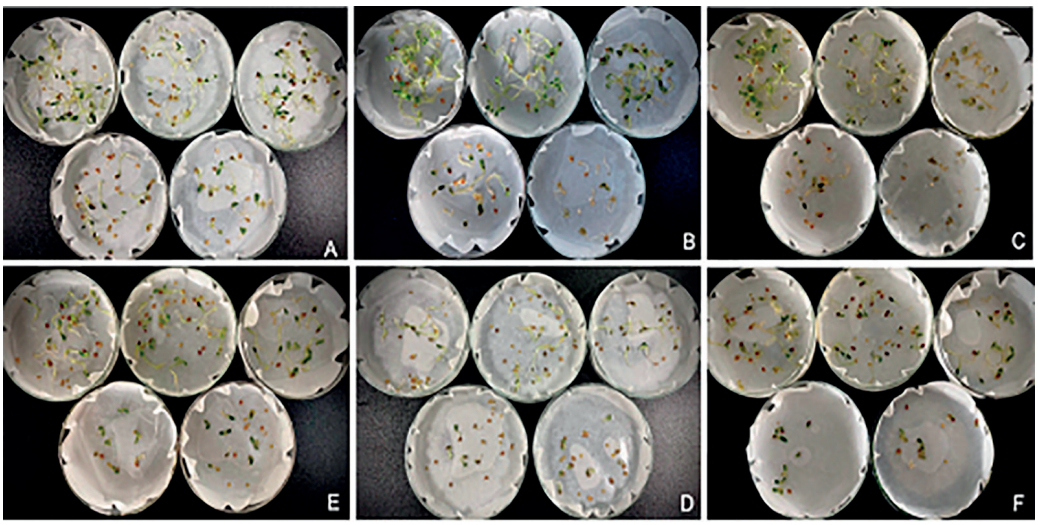

Figure 1. Seed germination of alfalfa plants affected by different levels of osmotic solution (A, B, C: Gozlu-1; D, E, F: Burgaltai; left hand side PEG 0, -0.3, -0.6, -0.9, -1.2 MPa; 5, 10, 15 days)

Based on this methodology, in determining the germination index of alfalfa varieties, Gozlu-1 variety was reduced by 3.3% and Burgaltai variety by 19.2% in the medium containing 50 mM mannitol solution. At 100 mM, the Gozlu-1 variety decreased by 10% compared to control and the Burgaltai variety by 30.8%, at 200 mM, the Gozlu-1 variety was reduced by 15% and the Burgaltai variety by 35%, at 300 mM, the Gozlu-1 variety decreased by 18.4% and the Burgaltai variety decreased by 68.6%, at 400 mM, the Gozlu-1 variety decreased by 26.7% and the Burgaltai variety decreased by 100%, at 500 mM, the Gozlu-1 variety lost 35% of its control, while the Burgaltai variety did not germinate at all (Figures 2, 3).

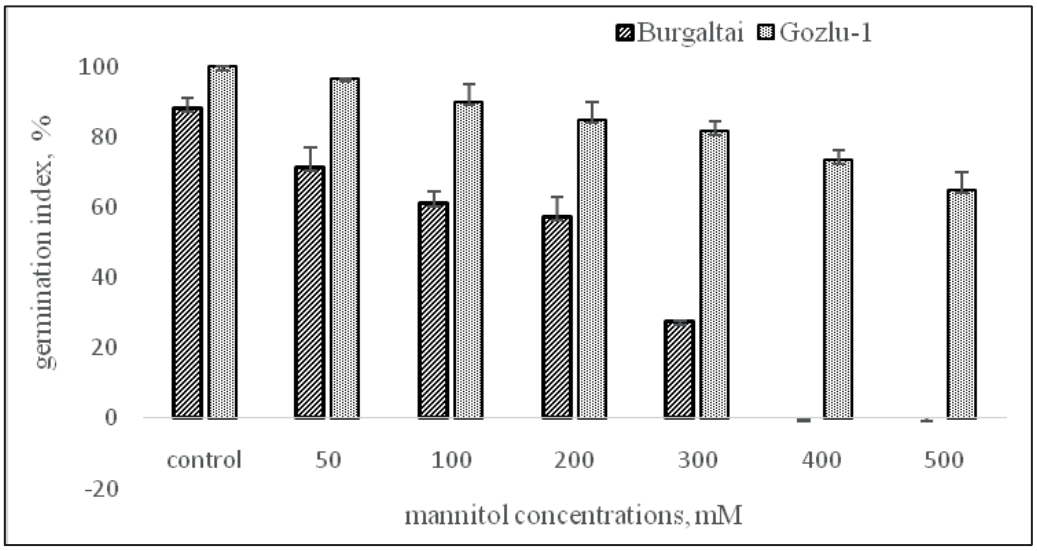

Figure 2. Germination index of alfalfa varieties treated with mannitol, %.
The value of mean ±standard deviation of three replications

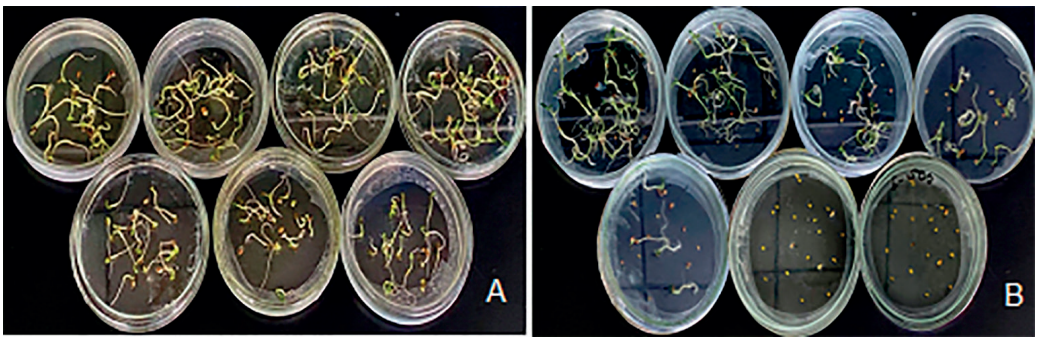

Figure 3. Seed germination of alfalfa varieties grown in mannitol-containing media (A: Gozlu-1, B: Burgaltai)

Effect of drought on shoot and root length stress resistance index

When the irrigation was stopped for seven or 14 days, the shoot length index of Burgaltai variety, which was affected by drought for 7 days, decreased by 5.8%, and that of Gozlu-1 variety decreased by 14.2%. The shoot length index of Burgaltai variety, which was affected by drought for 14 days, decreased by 29.4%, and that of Gozlu-1 variety decreased by 24.4% (Figure 4).

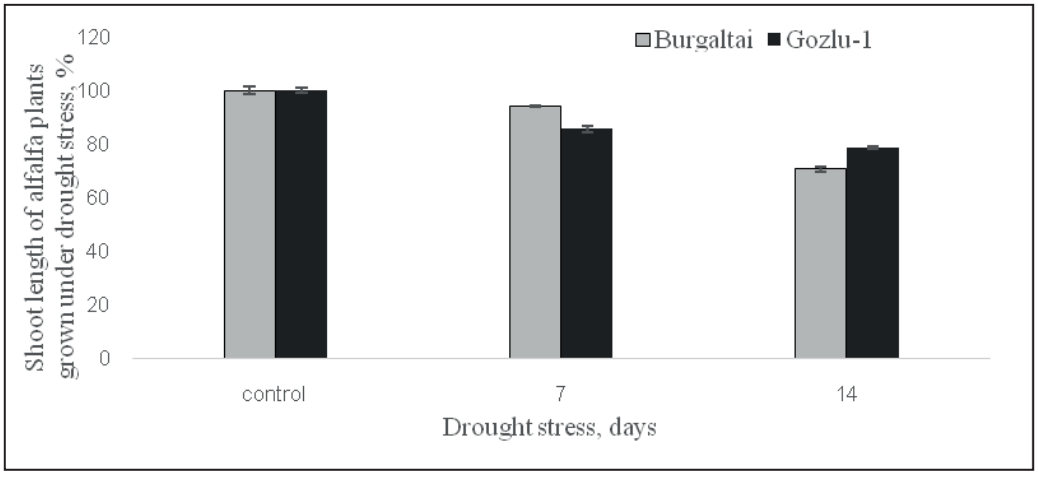

Figure 4. Shoot length index of drought-affected alfalfa varieties, %. The value of mean ± standard deviation of three replications

When the irrigation was stopped for 7 or 14 days, the root length index of Burgaltai variety, which had been affected by drought for a week, decreased by 20%, and that of Gozlu-1 decreased by 16.6%. However, the root length index of Burgaltai variety, which was affected by drought for 14 days, decreased by 35%, and that of Gozlu-1 variety decreased by 33.3% (Figure 5, 6).

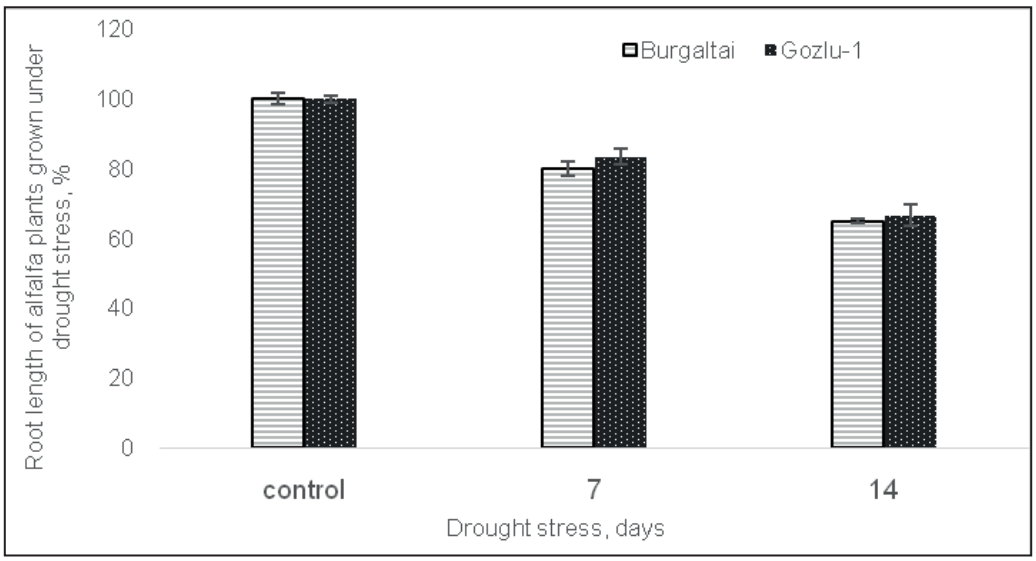

Figure 5. Root length index of drought-affected alfalfa varieties, %.
The value of mean ± standard deviation of three replications

Figure 6. Phenotypic status of drought-affected alfalfa varieties
(A: Gozlu-1, B: Curly varieties; Left-handed control, after 7 and 14 days)

Effect of drought on shoot and root weight stress resistance index

When the irrigation was stopped for seven or 14 days, the weight index of the shoots of Burgaltai variety, which had been affected by drought for a week, decreased by 35%, and that of Gozlu-1 decreased by 74.2%. However, the shoot weight index of Burgaltai variety, which was affected by drought for 14 days, decreased by 95%, and that of Gozlu-1 variety decreased by 95.4% (Figure 7).

The root weight index of Burgaltai variety, which was affected by drought for a week, decreased by 63.6%, and that of Gozlu-1 variety decreased by 33.3% after a seven- and 14-day irrigation shutdown. The root weight index of Burgaltai variety, which was affected by drought for 14 days, decreased by 78.4%, Gozlu-1 variety by 66.6%, and Gozlu-1 variety by 11.8-30% (Figure 8).

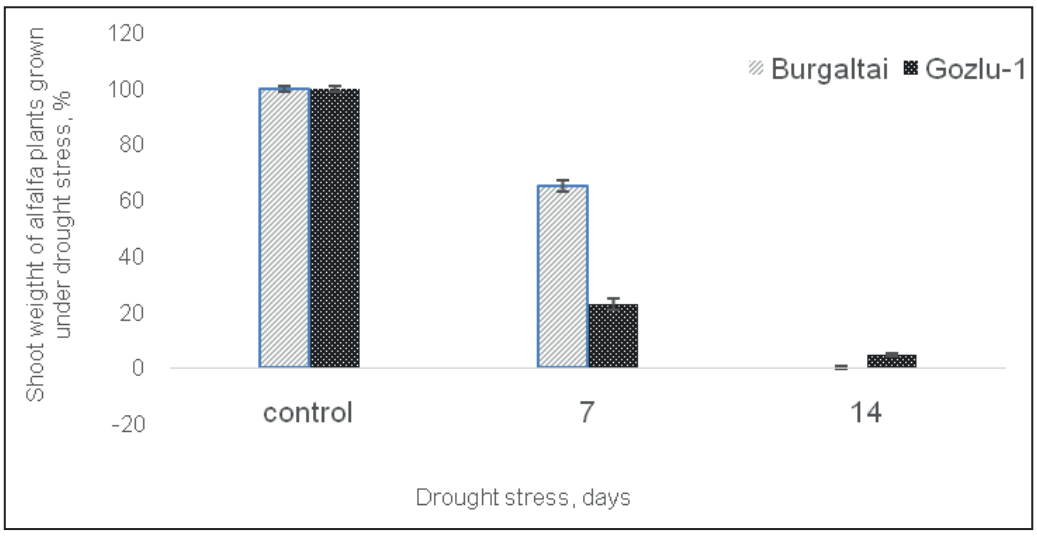

Figure 7. Shoot weight index of drought-affected alfalfa varieties, %.
The value of mean ± standard deviation of three replications

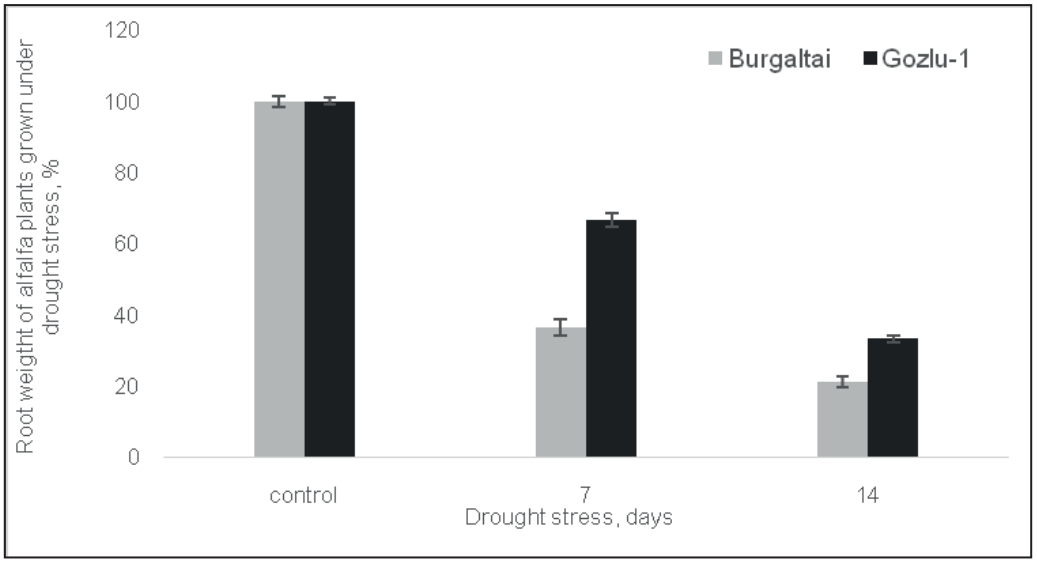

Figure 8. Root weight index of drought-affected alfalfa varieties, %.
The value of mean ± standard deviation of three replications

Effect of drought on the stress resistance index of dry weight

When Gozlu-1 was used in solutions with pressures of -0.3, -0.6, -0.9 and 1.2 MPa, the dry weight index was 30.3% higher than in the solution with a pressure of -0.3 MPa, 51.1% in 0.6 MPa and 63.3% in 0.9 MPa. iar, decreased by 70.6% at 1.2 MPa, and the dry weight index decreased by 90.5% when subjected to drought stress without any irrigation for 21 days. From this, we can assume that the dry weight index decreases as the concentration of the osmotic pressure solution increases (Figure 9).

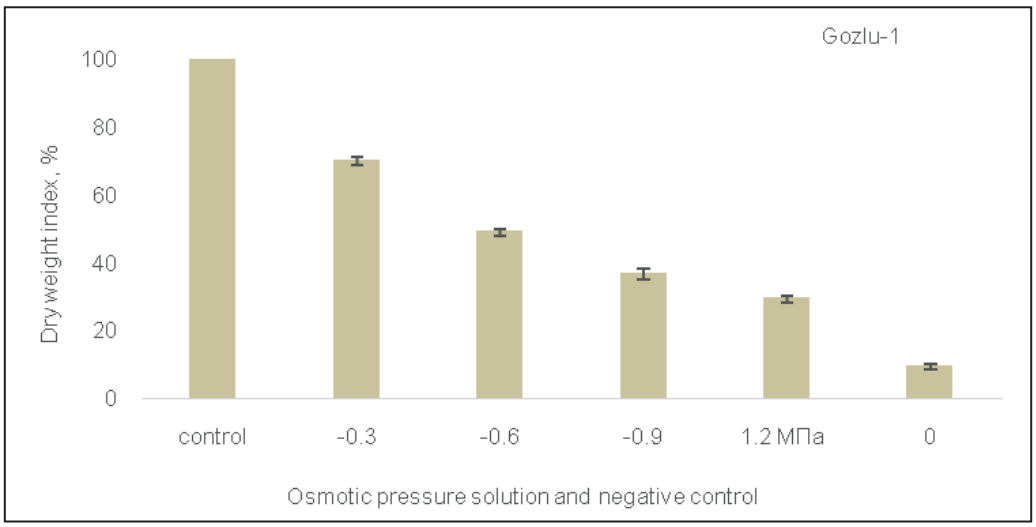

Figure 9. Dry weight index of Gozlu-1 cultivar treated with osmotic pressure solution, %. The value of mean ± standard deviation of three replications

Effect of drought on total chlorophyll content

The chlorophyll content of the leaves of Gozlu-1 and Burgaltai varieties of alfalfa exposed to drought under laboratory conditions was compared with a spectrophotometer.

The chlorophyll content of Gozlu-1, which was not subjected to drought stress or in the control variant, was higher than that of Burgaltai.

The chlorophyll content was measured 17.2% of the "Burgaltai" variety after the drought stress without watering for a week. "Gozlu-1" variety decreased by 44.3%, while "Burgaltai" variety, which was exposed to drought stress for 14 days, decreased by 49.5%. The Gozlu-1 variety decreased by 55.7 percent. Chlorophyll content after stress. Gozlu-1 variety is better than Burgaltai variety (Figure 10).

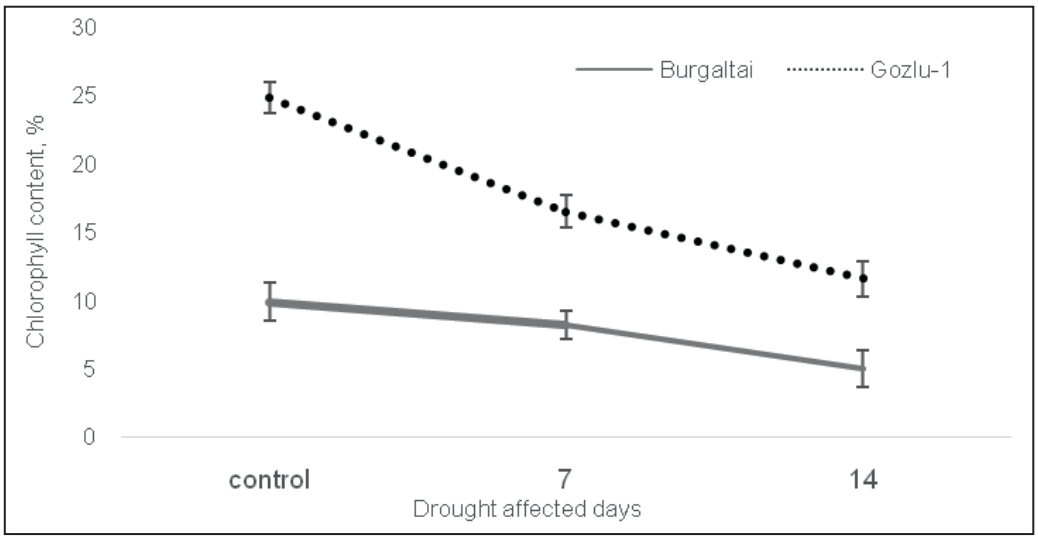

Figure 10. Chlorophyll content of leaves of alfalfa varieties affected by drought stress. The value of mean ± standard deviation of three replications

When measuring the chlorophyll content of Gozlu-1 alfalfa plants under stress by applying osmotic solutions of -0.3, -0.6, -0.9 and -1.2 MPa, the Gozlu-1 variety was reduced by 64-84% from the control variant (Figure 11).

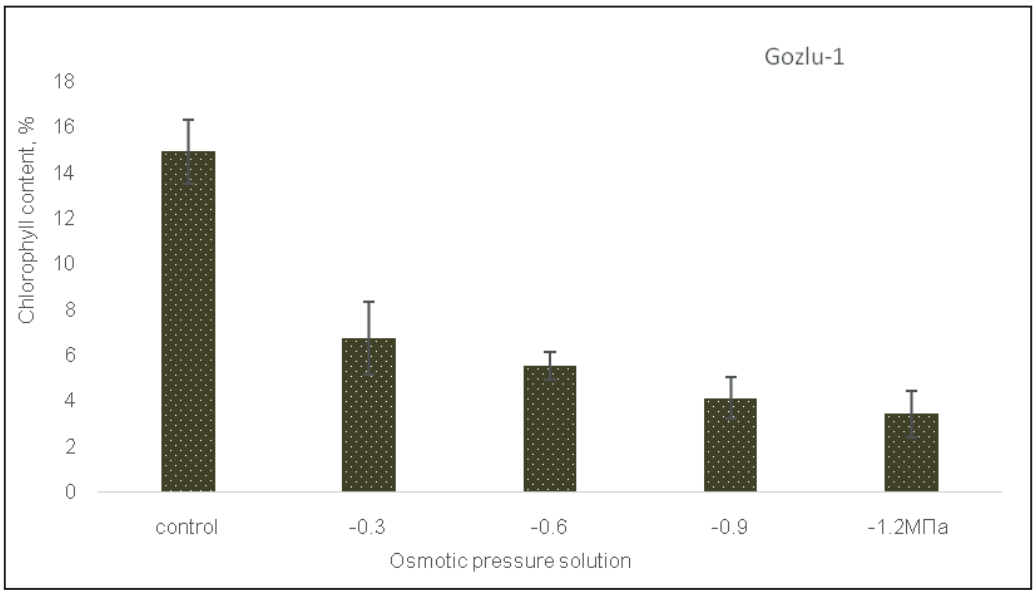

Figure 11. Chlorophyll content of alfalfa of Gozlu-1 variety subjected to drought stress by osmotic pressure solution. The value of mean ± standard deviation of three replications

Discussion

Drought is one of the natural phenomena that limits plant growth. The alfalfa national syshoot is well developed and drought tolerant, but long-term drought limits its growth and development [Safarmejad, 2008]. Compared to the drought tolerance intensity, morphology and physiology of alfalfa varieties, Gozlu-1 variety is better than Burgaltai variety in Mongolia. The germination of alfalfa seeds is inversely related to the pressure of the solution, ie the rate of seed germination decreases with increasing osmotic pressure of the artificial drought-forming solution [Ghasem et al., 2009].

Morphologically, the shoot length of Gozlu-1 (*Medicago sativa* L.) was 8–9 cm at -0.3 and 0.6 MPa, and 5–6 cm at -0.9 and 1.2 MPa, and Gozlu-1 (*Medicago sativa* L.) decreased by 36.8% from control.According to a previous study [Uuganzaya, 2017], the length of shoot control of drought-stressed alfalfa (*Medicago falcata* L.) was 45.2%, *Medicago varia* Marthz decreased by 60.8% and *Medicago varia* Martyn decreased by 64.8%.Compared to the alfalfa species studied by the previous researcher, Gozlu-1 variety is 8.4–28% more drought tolerant than *Medicago falcata* L., *Medicago varia* Marthz, and *Medicago varia* Martyn (*Medicago varia* Martyn) [Uuganzaya, 2017]. Determination of seed germination index of alfalfa varieties based on the method of limiting plant growth and seed germination by increasing the osmotic pressure and restricting water absorption by artificial drying with high molecular weight compound of polyethylene glycol 20 000.

Burgaltai variety by 23.2%, Zhansaya researcher's study [2019] of Taezhnaya variety of Russia by 12.5%, Vega 87 varieties decreased by 78.6%, In -0.5 MPa pressure solution, Taejnaya variety decreased by 88.2% from control Vega 87 did not germinate, while in -0.6 MPa pressure solution, Gozlu-1 variety decreased by 5.2% from control and Burgaltai variety decreased by 32.9% from control, Seed germination of Gozlu-1 variety was reduced by 34.3% and Burgaltai variety by 40.2% in -0.9 MPa pressure solution, Taejnaya variety and Vega 87 variety in -1.0 MPa pressure solution.

However, in -1.2 MPa pressure solution, Gozlu-1 variety decreased by 60.4% from control and Burgaltai variety decreased by 64.6% from control. This shows that the germination index of Gozlu variety is 20.1% higher in -0.3 MPa solution than in Burgaltai variety, 9.4% higher than Taejnaya variety studied by Zhansaya researcher, and 75.5% higher than Vega 87 variety.

However, in the solution with a pressure of -0.6 MPa, the germination index of Gozlu-1 variety is 27.7 higher than that of Burgaltai variety, 83% higher than that of Taezhnaya variety, and 100% higher than that of Vega 87 variety. Seed germination index of Gozlu-1 variety in -1.2 MPa pressure solution is 4.2% higher than Burgaltai variety, 100% higher than Taejnaya variety and 100% higher than Vega 87 variety.

Seed germination rates of alfalfa varieties were determined in vitro by a medium containing 50 mM mannitol solution, which reduced the Turkish Gozlu-1 variety by 3.3% and the Burgaltai variety by 19.2%, Gozlu-1 variety decreased by 10% from 100 mM, Burgaltai variety decreased by 30.8%, Gozlu-1 variety decreased by 15% from 200 mM, Burgaltai variety decreased by 35%, Gozlu-1 variety decreased by 18.4% from 300 mM , Burgaltai variety decreased by 68.6%, Gozlu-1 variety decreased by 26.7% from 400 mM, Burgaltai variety decreased by 100%, Gozlu-1 variety decreased by 35% from 500 mM, and Burgaltai variety decreased by 100% .

This shows that the germination rate of Gozlu-1 variety in medium containing 50 mM mannitol solution is 15.9% higher than that of Burgaltai variety, 20.8% at 100 mM, 16.6% at 200 mM, and 50.2% at 300 mM. Germination rates were 47.3% higher at 400 mM and 70% higher at 500 mM than at Burgaltai.

Conclusion

1. To determine the drought tolerance of alfalfa varieties by seed germination intensity and physiological parameters, Gozlu-1 variety is 23.2-100% more resistant than Burgaltai variety.
2. Gozlu-1 variety germinated better than Burgaltai variety in terms of drought tolerance of alfalfa varieties.
3. The chlorophyll content in the leaves of alfalfa plants affected by drought showed that as the concentration increased, the amount of chlorophyll in the plant decreased and the morphological changes were stronger than in the control plants.
4. Gozlu-1 variety is considered to be suitable for semi-irrigated and non-irrigated conditions due to its early drought tolerance and drought tolerance of Burgaltai variety according to some morphological parameters.

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СРАВНЕНИЕ ЗАСУХОУСТОЙЧИВОСТИ СОРТОВ КУЛЬТУРНОЙ ЛЮЦЕРНЫ (*MEDICAGO SATIVA* L.)

Оюнболор Алтангадас, Номинхишиг Хишигделгер,
Гурбазар Дамдинсурэн, Энхчимег Ванджилдорж

Оюнболор Альтангадас
магистр,
кафедра биотехнологии и селекции,
аакультет зоотехники и биотехнологии,
Монгольский университет естественных наук
Зайсан 17024, г. Улан-Батор, Монголия
bolorboogii@muls.edu.mn

Номинхишиг Хишигдельгер
научный сотрудник,
кафедра биотехнологии и селекции,

факультет зоотехники и биотехнологии,
Монгольский университет естественных наук
Зайсан 17024, г. Улан-Батор, Монголия
2217003@muls.edu.mn

Энхчимег Ванжилдорж
кандидат технических наук, доцент,
кафедра биотехнологии и селекции,
факультет зоотехники и биотехнологии,
Монгольский университет естественных наук
Зайсан 17024, г. Улан-Батор, Монголия
enkhchimeg.v@muls.edu.mn

Гурбазар Дамдинсурэн
кандидат технических наук,
кафедра пастбищ, питания и химии
Монгольский университет естественных наук
Зайсан 17024, г. Улан-Батор, Монголия
gurbazar@muls.edu.mn

Аннотация. Люцерна является многолетним кормовым растением с высокой питательной ценностью, эффективным урожаем и высоким содержанием белка [Erdenejav, 2015]. Засуха является основным экологическим фактором, влияющим на стадии прорастания семян растений. Исходя из этого, наша задача — сравнить засухоустойчивость люцерны Гозлу-1 (Турция) и Бургалтай (Монголия) с использованием полиэтиленгликоля и маннита в среде *in vitro* и *ex vitro*.
Полученные результаты показывают, что всхожесть сорта Гозлу-1 в условиях засухи была на 4,4–20,2 % лучше, чем сорт Бургалтай. При сравнении устойчивости к искусственной засухе по некоторым морфологическим показателям, сорт Гозлу-1 показал на 8,4% большую устойчивость, чем сорт Бургалтай. Интенсивность водопоглощения сорта Гозлу-1 на 17,5–28% выше, чем сорта Бургалтай.
Ключевые слова: люцерна, Medicago sativa L., засухоустойчивость, сорт Гозлу-1, сорт Бургалтай.

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