Uluslararası Gıda, Tarım ve Hayvan Bilimleri Dergisi (IJFAA) 2024, 4(1), 1-9
International Journal of Food, Agriculture and Animal Sciences (IJFAA)

OPEN ACCESS

BİNGÖL ÜNİVERSİTESİ

Uluslararası Gıda, Tarım ve Hayvan Bilimleri Dergisi
International Journal of Food, Agriculture and Animal Sciences (IJFAA)
e-ISSN : 2791-8807
https://dergipark.org.tr/tr/pub/ijfaa

Screening the Polyethylene Glycol 6000 Induced Upland Cotton *(Gossypium hirsutum* L.*)* Cultivars Drought Response at The Germination Stage

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Article info
Received: 19.02.2024
Accepted: 07.03.2024

Article type: Research

Keywords:
Drought, cotton, PEG_{6000}, germination, tolerance

Abstract
There are about 20 biotic and abiotic stresses that limit the growth and production of cotton. Drought is one of the most destructive abiotic stresses in all cotton-growing areas, therefore a multifaceted approach is necessary to cope with drought. This experiment was conducted at the University of Bingöl, Genç Vocational School, Agriculture Biotechnology laboratory, in May 2023. The objective of the research was to explore upland cotton cultivar's reaction to drought conditions induced by osmotic pressure stresses of 0.0 MPa (control), -0.4 MPa, -0.6 MPa, -0.8 MPa, and -1 MPa using PEG_{6000} chemical on Ten upland cotton cultivars at the germination stage. Germination percentage (GP, %) of cultivars was measured. In conclusion, May 344 and May 455 cultivars display notably superior tolerance to Osmotic Stress in contrast to other varieties, with Beren and SG-125 also showing relatively favorable tolerance levels. These discoveries offer valuable perspectives into the germination characteristics of these cultivars across diverse osmotic environments. Cultivars exhibiting consistently high germination percentages (GP) should be sown in uncontrolled field conditions to evaluate their germination and emergence potential. Moreover, cultivars demonstrating a fast germination rate and emergence may have genes associated with the earliness traits in cotton.

Citation: Çelik, S. (2022). Polyethylene Glycol 6000 Induced Upland Cotton *(Gossypium hirsutum* L.*)* Cultivars Drought Response at The Germination Stage. *International Journal of Food, Agriculture and Animal Sciences*, 4 (1), 1-9.

Polietilen Glikol 6000 uygulanmış Upland Pamuk (*Gossypium hirsutum* L.) Çeşitlerinin Çimlenme Döneminde Kuraklık Stresine Karşı Reaksiyonlarının Gözlemlenmesi

Makale bilgileri
Geliş Tarihi: 19.02.2024
Kabul Tarihi: 07.03.2024

Makale türü: Araştırma

Anahtar kelimeler
Kuraklık, pamuk, PEG6000, çimlenme, tolerans

Öz
Pamuğun büyümesini ve üretimini sınırlayan yaklaşık 20 kadar biyotik ve abiyotik stres faktörleri vardır. Kuraklık, pamuk yetiştirilen tüm alanlarda en yıkıcı abiyotik streslerden biridir, bu nedenle kuraklıkla başa çıkmak için çok yönlü bir yaklaşım gereklidir. Bu deney Mayıs 2023'te Bingöl Üniversitesi Genç Meslek Yüksekokulu Tarımsal Biyoteknoloji laboratuvarında gerçekleştirilmiştir. Araştırmanın amacı, on tane upland pamuğu üzerinde PEG_{6000} kimyasalı kullanılarak 0.0 MPa (kontrol), -0.4 MPa, -0.6 MPa, -0.8 MPa ve -1.0 MPa ozmotik basınç stresinin neden olduğu kuraklık koşullarına upland pamuk çeşitlerinin çimlenme döneminde reaksiyonlarını ölçmektir. Bu çalışmada çeşitlerin çimlenme yüzdesi (GP, %) ölçülmüştür. Sonuç olarak, May 344 ve May 455 çeşitleri diğer çeşitlerin aksine Ozmotik Strese karşı oldukça üstün tolerans sergilerken, Beren ve SG-125 de nispeten daha az tolerans seviyeleri göstermişlerdir. Bu bulgular, bu çeşitlerin çeşitli ozmotik ortamlarda çimlenme özelliklerine ilişkin değerli bakış açıları sunmaktadır. Sürekli olarak yüksek çimlenme yüzdesi (GP) sergileyen çeşitler, çimlenme ve ortaya çıkma potansiyellerini değerlendirmek için kontrolsüz tarla koşullarında ekilmesi önerilmektedir. Ayrıca, hızlı çimlenme oranı ve çıkış gösteren çeşitler, pamukta erkencilik özellikleriyle ilişkili genlere sahip olabileceği düşünülmektedir.

Atıf: Çelik, S. (2024). Polietilen Glikol 6000 Uygulanmış Upland Pamuk (*Gossypium hirsutum* L.) Çeşitlerinin Çimlenme Döneminde Kuraklık Stresine Karşı Reaksiyonlarının Gözlemlenmesi, *Uluslararası Gıda, Tarım ve Hayvan Bilimleri Dergisi*, 4 (1), 1-9.

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Introduction

Upland cotton (*Gossypium hirsutum* L.) species belongs to the *Gossypium spp*. genus (Khadi *et al*., 2010) is the most widely cultivated species worldwide contributes significantly in the economies of developing countries, and serves as a livelihood for millions of rural small-scale farmers worldwide (FAO, 2024). Normally, cotton plant is classified as drought-tolerance plants but under prolonged drought stress cotton plants are adversely affected. Drought, a condition where plants experience prolonged water scarcity (Tian *et al*., 2024; Abdelraheem *et al*., 2019), is exacerbated by global warming and climate change, exerting detrimental effects on cotton production (Saranga *et al*., 2009; Li *et al*., 2009). Drought, characterized as a harsh environmental stressor, poses a significant challenge to the growth and development of plants (Seleiman *et al*., 2021). Cotton is accepted as the best natural fiber source used in the textile industry by providing %81 natural fiber source (FAO, 2021; Christopher and Wendel., 2023), but due to the wide cultivation of cotton, it faces many abiotic and biotic stress factors such as drought, salinity, low and high temperatures, which reduce yield, and quality and restricts adaptation (Zhang *et al*., 2021). Abiotic stress causes 73% of world cotton production reduced (Saranga *et al*., 2009). Drought and salinity cause a 45% reduction in cotton production worldwide (Abhinandan *et al*., 2018).

Assessing seed germination potential is a widely employed technique to determine plant tolerance levels against abiotic stresses (Larcher *et al*., 2000). The formation and development of plants begins with the seed and includes the germination of the seed, the seedling period, and vegetative and generative stages (Faiza *et al*., 2019; Quanmber *et al*., 2019). In order to form a new plant and ensure the continuation of its generation, seed formation in the generative period is possible by germination of the seed (Donohue *et al*., 2010; Ali *et al*., 2022). Germination can be defined as the dormant seed swelling by taking in water and the radicle, which is a part of the embryo, coming out (Kucera *et al*., 2005, Ali *et al*., 2022). External factor signal catchers of the seed receive the signal from the outside and factors such as calcium ion and reactive oxygen species become active to break the dormancy within the seed and initiate germination (Shu *et al*., 2018). In general, extending the radicle 2-3 mm beyond the seed coat is a sufficient condition for germination to occur (Côme, 1982). Germination is the most important stage of the plant, depending on both internal and external factors. External factors such as contamination, air, humidity, water, light, soil, nutrients, temperature, coldness, and coating of the seeds with a thick membrane after the delintation process affect dormancy and germination (Yang *et al*., 2017).

Early germination of the seed and early development and strengthening of the plant enables the plant to gain resistance against abiotic stress conditions, Broomrape (*Orobanche spp*.), which is a completely parasitic weed, and other biotic stress factors. For this purpose, in the seed priming process, the seeds are processed with various plant extracts and chemicals, hot water, etc. It is aimed to break dormancy, to achieve germination, and to ensure rapid germination by exposing it to treatments. Chemicals applied during the germination period, one of the most sensitive periods of plants, can reveal some of the abilities of plants. Polyethylene glycol (PEG) chemical is an important chemical used to create artificial drought during the germination period (Muscolo *et al*., 2014). Polyethylene glycol is a water-soluble non-ionic natural polymer molecule (Ranjbarfordoei *et al*., 2000). The pores in the cell membrane do not allow the toxic chemical PEG_{6000} to pass through because its molecules are large. However, molecules of PEG chemicals with a molecular weight of less than 6000 enter the cell and poison the cell, disrupting its functioning (Zerrouk *et al*., 2001).

Polyethylene glycol is employed to regulate water potential in seeds during both the seedling and germination phases, serving as a method to evaluate the drought resistance of various cotton varieties (Heikal *et al*., 1982; Dodd and Donovan, 1999). Ashraf *et al*. (1996) reported that: The application of polyethylene glycol induces osmotic stress in plants, serving as a recognized method for simulating artificial drought conditions. In a study employing polyethylene glycol 6000 to induce artificial drought conditions, twelve upland cotton cultivars were utilized to assess drought tolerance. Among these, the cotton cultivars ARB-970, GIHV218, CNH-120MB, and BS-279 were identified as exhibiting resilience to elevated osmotic potential levels (Babu *et al*., 2014).

In this study, the effects of different doses of PEG_{6000} chemical on the germination of ten Upland cotton (*Gossypium hirsutum* L.) cultivars were investigated.

Material and Method

Plant materials

All plant materials used in the experiment belong to the allotetraploid (2n=4X=52) *Gossypium hirsutum* L. cotton species with an AD genome group (Wendel and Cronn, 2003). Cotton genetic materials are provided by Bingol University, Genç Vocational School, and Agriculture biotechnology laboratory.

Method

The trial was conducted at the Agricultural Biotechnology Laboratory within Bingöl University's Genç Vocational School, situated in Bingol, Turkey, with coordinates of 38° 44' 58" N and 40° 32' 11" E. The experiment took place in May 2023, in a controlled laboratory room. The climate chamber was set to maintain temperatures ranging from 28°C to 38°C and humidity levels between 57% and 67%, following the guidelines established by Zahid *et al*. (2021).

The investigation was conducted using a factorial experimental design with a completely randomized design (CRD), comprising three replications (3x5). Before the experiment, all cotton seeds were delinted. All cotton cultivars were exposed to a 10 mL^{-1} Sulphuric acid solution (Merck, Darmstadt, Germany) for 3 minutes. Subsequently, the seeds were transferred into a lime water mixture and then rinsed thoroughly with clean water (Medeiros *et al*., 2006), Lopes *et al*.,2006). Subsequently, they underwent a 5-minute treatment with a 1% Sodium Hypochlorite (NaOCl) solution to eliminate any potential diseases or pests.

During the germination stage, autoclaved Petri dishes measuring 100 x 20 mm were prepared with two layers of sterile paper napkins lining the bottom. Each Petri dish accommodated 10 cotton seeds, with a total of 150 seeds used for each genotype. After sowing, an additional sterile paper napkin consisting of two layers was placed over the seeds. The Petri dishes were then sealed and stored at room temperature. In a controlled chamber trial, ten commonly cultivated cultivars of Upland cotton with AD genome group (ADN 811, BA-525, Beren, DP-396, DP-499, May 344, May 455, Beyaz altın 119 (BA 119), Nihal, SG-125) were induced to Polyethylene glycol 6000 (PEG_{6000}) and determined the tolerance levels of genotypes. PEG_{6000} solutions were prepared to achieve osmotic potentials of 0.0 MPa, -0.4 MPa, -0.6 MPa, -0.8 MPa, and -1.0 MPa. Osmotic Potential (OP, bar) values were calculated using the formula outlined by Michel and Kaufmann (1973) based on the PEG_{6000} concentrations used in the solutions. A volume of 10 m L^{-1} of PEG_{6000} solution was applied to each petri dish at three-day intervals, as per the protocol described by Babu *et al*. (2014). It's worth noting that seedling emergence and germination are pivotal stages in the growth cycle, as highlighted by Lamichhane *et al*. (2019).

Osmotic Potential (OP)= (-1.18 x 10-2) x C-(1.18 x 10-4) x C+ (2.67 X 10-4) x CxT + (8.39 x l0-7) x C2T (bar).

where **C**=PEG concentration (1m L^{-1} PEG concentration in water, W/V%), **T**=Temperature (°C). The seed germination rate was assessed on the 12^{th} and 15^{th} days after sowing (DAS) using the methodology outlined by Abdul-Baki and Anderson (1973). Coton cultivar's seed germination percentages were screened. The germination percentage (%) was calculated using the following formula:

$$\text{The Germination percentage (GP)} = \frac{n}{\text{N}} x100$$

The variable "**n**" represents the count of germinated seeds, while "**N**" signifies the total number of seeds, as defined by Belcher and Miller (1974).

Data Analysis
Analysis of variance (ANOVA) was conducted using JMP 17.0 ver. software (SAS Institute Inc., Cary, NC, 1989–2021). The means of 0.0 (control) MPa, -0.4 MPa, -0.6 MPa, -0.8 MPa, and -1.0 MPa stress levels were compared, and Significance among trait means was assessed at a 5% level of probability ($p<0.05$), using Student' t-test. Frequency charts were generated using Microsoft Office Excel 2016.

Results and Discussion

Ten cotton cultivars belonging to the Upland cotton (*Gossypium hirsutum* L.) species, which is the most produced and has the widest adaptability in the world, were used. Different doses of PEG_{6000} chemical 0.0 MPa (Control), -0.4 MPa, -0.6 MPa, -0.8 MPa, and -1.0 MPa) were applied during the germination period and their germination abilities under osmotic stress were examined. As a result of the analysis of variance, the differences between the mean germination percentages of the varieties under -1.0 MPa drought stress were not found to be statistically significant. At 0.0 MPa (Control), -0.4 MPa, -0.6 MPa, and -0.8 MPa stress levels, the differences between the mean of germination percentages of the varieties were found to be statistically significant ($P<0.05$).

Table 1. Germination percentage means of PEG_{6000} seed priming application

Germination Percentage (GP,%) Means						
Cultivars	**0.0 Mpa (Control)**	**-0.4 MPa**	**-0.6 MPa**	**-0.8 MPa**	**-1.0 MPa**	**Means**
ADN 811	73 c	47 b	47 ab	27 b	17 bc	42
BA-525	90 ab	73 a	47 b	33 ab	20 bc	53
Beren	97 a	63 ab	47 ab	40 ab	10 c	51
DP-396	83 bc	47 b	50 a	40 ab	20 bc	48
DP-499	90 ab	67 ab	47 ab	40 ab	17 bc	45
May 344	97 a	80 a	60a	40 ab	37 a	63
May 455	93 ab	83 a	60 a	40 ab	37 ab	63
BA 119	87 ab	80 a	47 b	50 a	27 ab	58
Nihal	90 ab	70 a	47 b	27 b	17 bc	50
SG-125	97 a	67 ab	50 a	37 ab	17 bc	54
Means	89,7	67,7	50,2	33,8	21,9	

Abbreviations: Germination percentage (GP, %)

At 0.0 MPa: Beren, May 344, May 455, and SG-125 cultivars show the highest germination percentages, with means ranging from 93 to 97%. The mean of 0 MPa is 89.7 and almost all cultivar's germination percentages are higher than the cultivar's mean (Table 1, Figure 1). As a result of our previous study, it was considered that this drought stress (-0.2 MPa) should not be included in the experiment, since the germination percentages of cotton plants were nearly the same as those of the control group.

At -0.4 MPa Osmotic Potential drought stress level May 455 also shows notable germination percentages (83%a) and was followed by May 344, and Beyaz altın 119 with the 80% germination percentage. In drought stress conditions (-0.4 MPa), where the overall germination mean is 67.7%, half of the cultivars recorded a GP above the mean, while the other half showed a GP below the general mean (Table 1, Figure 1). At -0.6 MPa stress level the GP mean was 50.2%. May 344 and May 455 cultivars showed the highest germination percentage (60%) followed by Deltapine-499 (DP-499) and Suregrow-125 (SG-125). Compared to the -0.4 MPa, the germination rate of cultivar AND811 was not changed, the cultivar DP-499 germination rate was increased at -0.4 MPa, but remained the cultivar's GP significantly decreased (Table 1, Figure 1). At -0.8 MPa most cultivars have germination percentages above the general mean. While the Beyaz Altın 119 cultivar has the highest germination percentage (50a) and ADN 811 and Nihal cultivars have the lowest (27b). BA-525 and SG-125 cultivar's means are close to the mean, while the rest are above it.

At -1 MPa osmotic stress, Cultivars May 344 and May 455 have germination percentages significantly above the mean. Cultivar Beren has the lowest GP and is significantly below the mean. The rest of the cultivars are below or close to the mean, with varying levels of significance. Meneses *et al*. (2011) stated that germination potential decreased as osmotic potential increased in PEG_{6000} solutions, and germination was inhibited at -1.0 MPa. In contrast to our study, at -1.0 MPa where germination completely stopped suggests that this situation may be due to the absence of genes responsible for drought tolerance or their masking in the genotypes used. Similar to our findings, Souza Filho (2006) reported *Leucaena leucocephala* germination in all PEG_{6000} 0.0, -0.3, -0.6, -0.9 and -1.2 MPa. Under five different PEG_{6000} stress in *Pisum sativum* L., by increasing the PEG_{6000} concentration, a decreased germination percentage was screened (Okçu *et al*., 2005). Wang (2011), Bai *et al*. (2020), Tsaliki *et al*. (2019), Bokobana *et al*. (2023), and Saleh *et al*. (2021) reported that a similar approach to increasing the PEG_{6000} concentration is inversely proportional and decreasing the germination rate. In general, germination decreases with increasing PEG_{6000} concentration, but in articles where the same amount of PEG_{6000} chemical is used, germination stops at -1.0 MPa, which may be due to the cotton varieties used being extremely sensitive to drought or trial error.

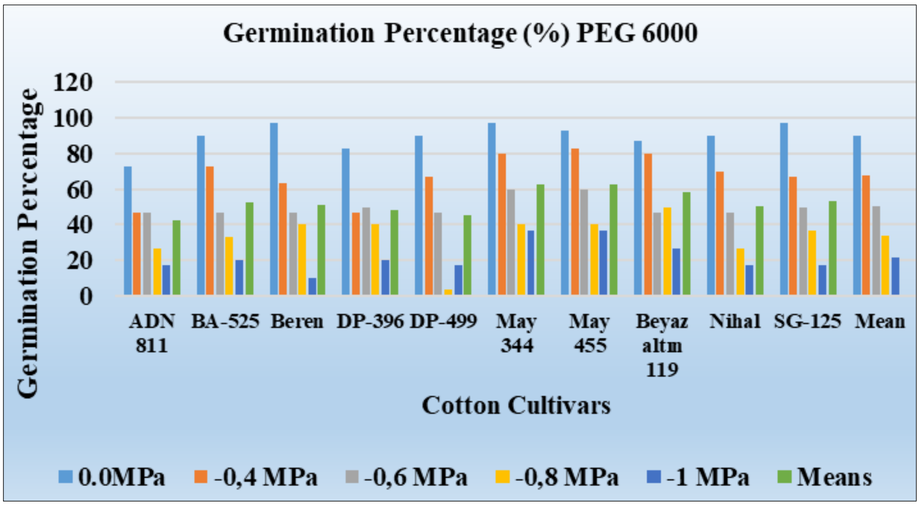

Figure 1. Germination percentage (GP) of ten Upland cotton cultivars under different osmotic stress of PEG_{600} chemical. Each color represents the osmotic stress and control group. Each Bar of the graph represents the germination percentage of a cotton variety under osmotic stress. The Y-axis shows the germination percentage, while the X-axis represents the genotypes.

Both germination and seedling stages directly effect the quantity and quality of Field crops (Subedi and Ma, 2005). In this study, it has been observed that as the amount of PEG_{6000} increases, the overall germination rates of cotton genotypes tend to decrease. Similarly, Pawar and Veena (2020) stated that with the increasing PEG_{6000} amount, GP was decreased. However, in the case of the Beyaz altın 119 119, cultivar, the germination rate at -0.8 MPa was higher compared to the lower osmotic stress of -0.6 MPa. This situation could be attributed to the genetic mechanisms of seeds in response to abiotic stresses. Despite cotton being a self-pollinating plant, the possibility of gene transfer from a different cotton plant through cross-pollination allows for seeds obtained from different bolls within the same cotton plant to exhibit different responses.

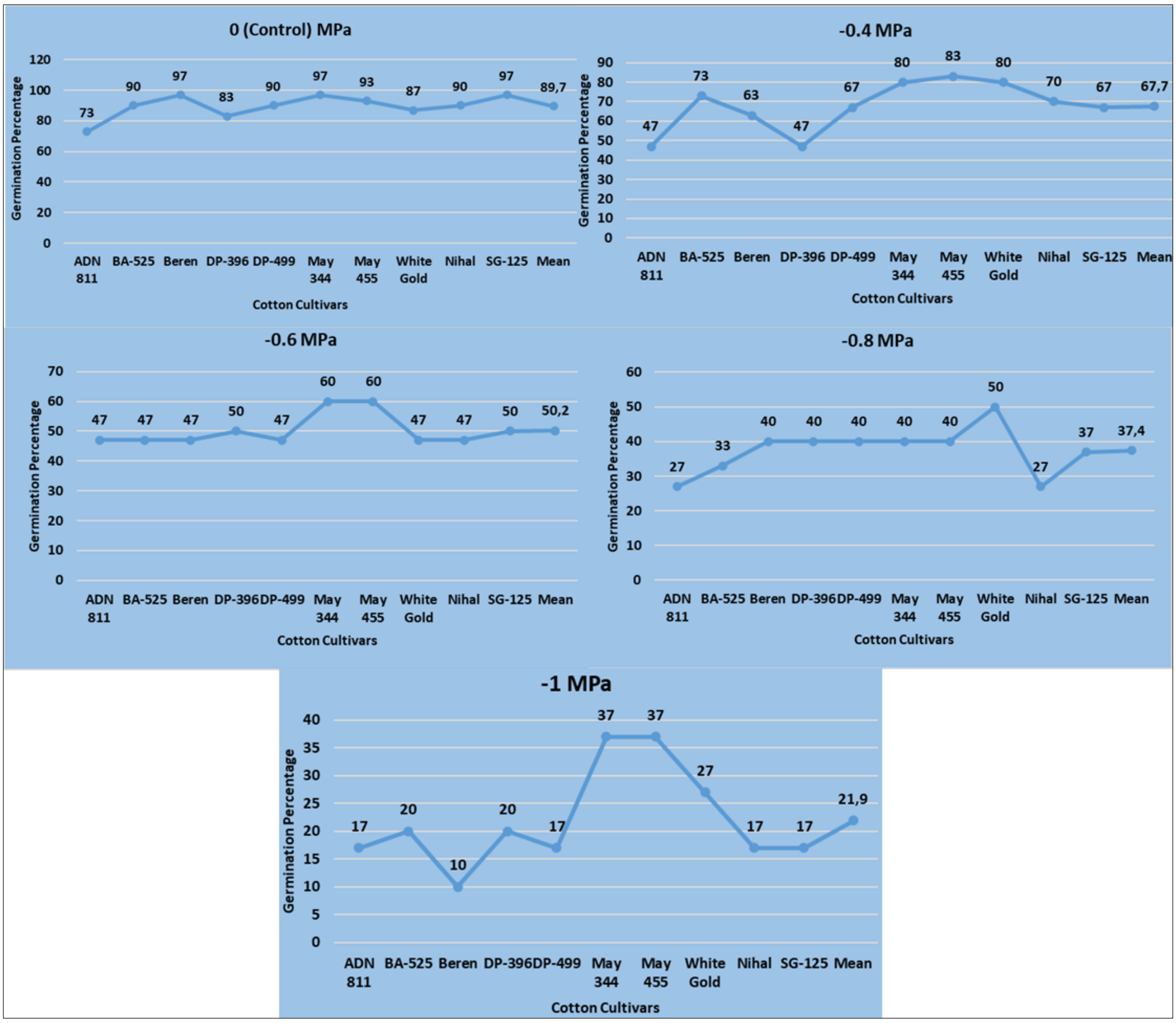

Figure 2. The cultivar's germination behaviors under the PEG_{6000} seed priming application. Each breaking point in the graph represents the germination percentages of the varieties on the X-axis, respectively.

The cultivars May 344 and May 455 consistently exhibit high germination rates across most osmotic potential levels. Cultivars Beren and SG-125 also show relatively high germination percentages across different osmotic potentials. The Landraces ADN 811 and Nihal generally exhibit lower germination rates compared to others. May 344 and May 455 appear to be more tolerant to osmotic stress, showing higher germination rates across various levels. Cultivars Beren and SG-125 (Suregrow-125) also demonstrate relatively good tolerance to osmotic stress. ADN 811 and Nihal seem to be more sensitive to osmotic stress, showing lower germination rates overall. As osmotic potential increases, the germination rates generally decline across all cultivars. At each osmotic potential level, Cultivar May 344 consistently maintains high germination rates compared to other cultivars (Table 1, Figure 1).

Conclusions

In conclusion, Cultivars May 344 and May 455 exhibit remarkably higher tolerance to Osmotic Stress (OP) compared to other cultivars, while cultivars Beren and SG-125 also demonstrate relatively good tolerance. These findings provide valuable insights into the germination behavior of these cultivars under varying osmotic conditions. The cultivars that have generally high germination percentage (GP) should be sown in uncontrolled field conditions to screen their germination and emergence capability. The cultivars that have fast germination and emergence may have genes associated with the earliness trait in cotton.

Funding Statement: The author (s) received no specific funding for this study.

Conflicts of Interest: The authors declare they have no conflicts of interest to report regarding the present study.”

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