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Effects of some treatments on seed dormancy in branched asphodel (*Asphodelus ramosus* L.)

Bazı uygulamaların çirişağusu (*Asphodelus ramosus* L.) dormansisi üzerine etkisi

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ABSTRACT

Branched asphodel (*Asphodelus ramosus* L.) is an unpalatable geophyte that has been increasing spreading in the pastures of Aegean region of Türkiye. To formulate effective management strategies at various growth phases, it is necessary to initiate the germination of dormant seeds. Preliminary experiments indicated that branched asphodel seeds germinated better in darkness than in light. Several dormancy-release techniques were tested to develop a rapid, uniform and better germination protocol for branched asphodel seeds including cold stratification at +4 and -18 °C, mechanical scarification with sandpaper, chemical scarification with sulfuric acid, ethanol or hydrogen peroxide and application of chemicals, i.e., gibberellic acid or potassium nitrate. Chemical scarification with 95% sulfuric acid (for 1 min) or 20 mM or 40 mM hydrogen peroxide (for 24 hours) resulted in the highest germination percentages (over 81.3%) and adequately reduced Mean Germination Time (MGT). While chemical scarification with sulfuric acid for 5, 15, and 30 min also reduced MGT, extending scarification duration beyond one-minute decreased germination rates. Lower germination rates with gibberellic acid treatment and increased germination with scarification methods suggest that the seeds have physical dormancy rather than physiological dormancy. Manual scarification with sandpaper achieved germination rates over 75% and reduced MGT by more than 57%. In conclusion, manual sandpaper application considered to be preferable for avoiding the adverse effects of chemical treatments while providing an acceptable germination rate.

INTRODUCTION

Branched asphodel (*Asphodelus ramosus* L.) is a member of Asphodelaceae family and has perennial tuberous roots, which protect the plant from extreme heat and drought by enabling its survival under adverse effects (Anonymous 2022). This plant that is the subject of various studies due to the presence of numerous beneficial compounds (Apaydin and Arabaci 2017, Reynaud et al. 1997). However, the increasing density of this species in the pastures is causing significant problems, as both large and small ruminants tend to avoid it.

Unpalatable geophytes, such *Asphodelus*, are predominant life forms in the areas of the Mediterranean region that have been degraded by overgrazing and fire (Noy-Meir and Oron 2001, Terzi 2023). *Asphodelus* species are widely distributed across the Aegean, Marmara, Mediterranean, and Southeastern Anatolia regions of Türkiye, where Mediterranean climate prevails (Alatürk and Gökkuş 2019).

It has been reported that the density of *Asphodelus* in the pastures of the Aegean region can reach up to 10 units/m^2 (Eltez 1995). This species is commonly found in meadow and pasture soils, along roadsides, and especially in the hilly areas between agricultural fields in the Aydin district of the Aegean region. Its persistence is associated with the development of secondary chemical compounds and it is lower dependence on seed production compared to annual species (Sternberg et al. 2000).

Summer drought is one of the environmental constraints faced by branched asphodel in the western part of Türkiye, as is the case with summer asphodel (*Asphodelus aestivus* Brot.) in the Mediterranean region. Both species withstands summer drought by drying out their aboveground parts (Pantis et al. 1994). While biomass reduction is observed in the tuberous roots during the prolonged dry periods, as in *A. aestivus* (Sawidis et al. 2005), branched asphodel can still spread by its usually dormant seeds upon dispersal.

Branched asphodel is becoming one of the most troublesome weeds in pastures in Aydin province, Türkiye. Although mowing, with or without the removal of cut material, has shown promising results in restoring grassland biodiversity in *Asphodel*-dominant communities (Tesei et al. 2018), this method is limited due to the lack of funds and labor and the difficulty using equipment on sloping pastures in the region. A study conducted in Aydin province found that the lowest population of *A. aestivus* was achieved through paraquat application and hand weeding (Sürmen and Kara 2022). However, these methods are not applicable for branched asphodel recently, since paraquat (in addition to not having systemic effect in plants) is banned in Türkiye and hand weeding requires a lot of labor and financial resources.

No chemical control methods are currently being implemented in the pastures of Aydin province, Türkiye, which has contributed to the increasing density of branched asphodel. Due to the varying sizes of its tuberous roots, it has been difficult to assess the efficacy of chemical management strategies for this plant in trials. Initiating the germination process of dormant seeds is essential for formulating management strategies for the plant's various growth phases. Seed dormancy in branched asphodel may result either from an inhibitory action of the seed coat or from the characteristics of the embryo itself. This study aims to break seed dormancy and improve the germination of branched asphodel using different methods. The results of this study may also benefit researchers seeking to extract various chemicals from this plant.

MATERIALS AND METHODS

Seed collection and photoperiod treatments

Mature seeds of branched asphodel were collected from the pastures in Cine (37°37' N, 27° 57' E, 148 m above sea level), Efeler (37°47' N, 27° 56' E, 122 m above sea level), and Kocarli (37°45' N, 27°45' E, 44 m above sea level) districts of Aydin province, Türkiye, during June 2021. The seed collection sites characterized by a dry climate, with a mean temperature of 17.7 °C and mean annual rainfall of 659.9 mm. Seeds were stored in paper bags at room temperature (approximately 25 °C) in darkness until the experiments began in July 2021.

Seed dormancy was tested before the experiments using 20 seeds in four replicates with two repeats. Seeds were surface-sterilized with a 5% sodium hypochlorite solution for 15 minutes and then rinsed three times with sterile distilled water for 5 minutes each. The seeds were placed in glass Petri dishes containing sterile filter paper and moistened with 5 ml sterile distilled water. Potential seed germination was tested under optimal conditions: a temperature of 15 °C and 50% humidity. The photoperiod treatments were tested under 8 h light (10.000 lux)/16 h dark and complete darkness in climatic test chamber (Mikrotest/MIT-600).

Seeds were considered germinated when the radicle emerged to length of at least 2 mm. The experiments were conducted over 28 days, and seed germination was checked on day's 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 10^{th}, 14^{th}, 21^{st}, and 28^{th} day after the initiation of the experiments. All germinated seeds were removed from Petri dishes upon observation.

Experiments for breaking seed dormancy

Seeds of branched asphodel were exposed to incubation in low temperatures, mechanical scarification (with sandpaper), and chemical scarifications [with sulfuric acid (H_2SO_4), ethanol or hydrogen peroxide (H_2O_2)] as well as chemical treatments [with gibberellic acid (GA_3) or potassium nitrate (KNO_3)]. Sterile distilled water applied (5 ml in each Petri dish) was applied to seeds evaluated as controls. All seeds were surface sterilized as described in the pretreatment section before applications, except for seeds in

cold storage (seed surfaces were sterilized after incubation at low temperatures).

Cold stratification

To evaluate the effect of low temperatures on seed dormancy, seeds were placed in paper bags covered with aluminum foil, stored in refrigerator at 4 °C and -18 °C, and kept for 30 days. Afterwards, they were surface sterilized before starting the experiments.

Mechanical scarification

Mechanical scarification was investigated in two different ways. Seeds were placed in sandpaper-coated plastic containers and shaken at 800 rpm for two hours in an orbital shaker (Miprolab MLS 3535). In other way, seeds were gently and manually rubbed between two sheets of sandpaper for 10 seconds until the seeds coats were thinned.

Chemical scarification

Different chemicals at different concentrations or at different durations were used for chemical scarification. Dry seeds were soaked in 95% H_2SO_4 (Tekkim Chemistry) for 1, 5, 15, 30, 60, and 120 minutes; in 95% ethanol for 30 and 60 minutes; or in H_2O_2 (30%, Tekkim Chemistry) at concentrations of 20 and 40 mM for 24 hours. All seeds were washed with sterile distilled water three times for 5 minutes each before being placed them in Petri dishes.

Treatments with gibberellic acid and potassium nitrate

To evaluate effectiveness of chemical treatments on seed dormancy, two different concentrations (500 and 1000 ppm) of GA_3 (Aldrich Chemistry) and two concentrations of KNO_3 (0.1% and 0.2%; Sigma-Aldrich) were applied at 5 ml to the Petri dishes containing intact seeds.

Germination tests

Twenty seeds were placed in each glass Petri dish (90 mm), and all the experiments were conducted with four replications and two experimental runs. Petri dishes were placed in a climatic test chamber adjusted to 15 °C, 50% relative humidity, under complete darkness. Seed germination was monitored on the 1st, 3rd, 5th, 7th, 10th, 12th, 14th, 17th, 19th, 21st, 24th, 26th, and 28th day after the initiation of the experiments. Germinated seeds (>2 mm radicle) were removed from Petri dishes.

Statistical analysis

For all the experiments, the cumulative germination percentage (mean ± standard error) and mean germination time (MGT, mean in days ± standard error) were calculated. The seed germination percentage was calculated by multiplying the ratio of germinated seeds to the total number of viable seeds in a single Petri dish. MGT represented the average length of time in days it took seeds to germinate and was calculated by the following formula:

MGT: $\Sigma DN/\Sigma N$

Where D is the number of days counted from the date of sowing, and N is the number of seeds germinated on day D.

The data from the experimental runs for the light:dark experiment were combined for analysis since the interaction between runs was found non-significant in pretreatments. Then two different photoperiods were compared by independent t-test (P=0.05).

To compare the effects of dormancy-release treatments on germination percentage (%), the data were analyzed using General Linear Model/Univariate procedure and the treatment means were separated Tukey's HSD test at P≤0.05. Since H2SO4 applications at 60 and 120 minutes caused losses in seed viability, the results obtained from these applications were eliminated from statistical analysis. To satisfy normality and homogeneity assumptions, Lg10 transformed data of MGT were used. Statistical analysis was performed using IBM SPSS Statistics 21.

RESULTS

Effects of photoperiod on seed germination

Seed germination percentage varied among photoperiod conditions, while MGT remained unaffected (Table 1).

As seen in Figure 1 cumulative germination percentage was higher in darkness (45.63%) than 8-h photoperiod (40.63%). Therefore, the treatments for breaking dormancy were carried out in darkness.

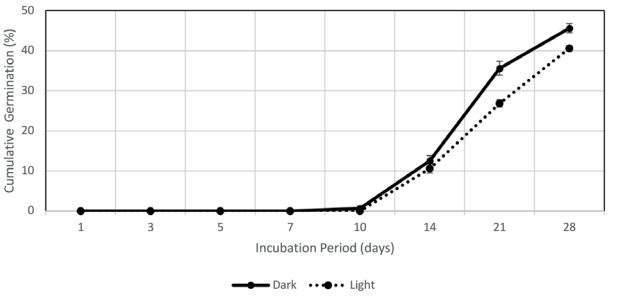

Figure 1. Cumulative germination percentage of branched asphodel seeds incubated under dark or light (8 h light/16 h dark) conditions (Vertical lines indicate the standard error.)

Table 1. Independent T-test results of the germination percentage and mean germination time (MGT) of branched asphodel seeds incubated in darkness or 8-h light conditions

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Germination percentage	3.864	14	.002	5.00000	2.22492	7.77508
MGT[a]	-2.046	14	.060	-.95375	-1.95365	.04615

[a]Abbreviation: MGT, mean germination time

Effects of treatments for breaking dormancy

Germination percentage and MGT varied significantly among 17 different treatments. Differences in germination percentage (%) and MGT in the first experimental run, tested by Tukey's HSD test, are shown in Table 2.

As seen in Table 2, treatments in the first repeat significantly increased germination percentage compared to the control (38.8%) were H_2SO_4 (1 min) and H_2O_2 applications at 20 mM and 40 mM concentrations. These same treatments also shortened the MGT, along with other H_2SO_4 durations and manual sandpaper applications.

Tukey's HSD test results of germination percentage and MGT of the second experimental run are shown in Table 3.

The treatments that significantly increased germination percentages of branched asphodel seeds in the second repeat were H_2SO_4 (1 and 5 min), 20 and 40 mM H_2O_2 and manual sandpaper. Addition to these treatments, other H_2SO_4 durations and sandpaper usage at 800 RPM for two hours significantly decreased MGT.

Table 2. Germination percentages and mean germination time (MGT) of branched asphodel seeds subjected to different treatments under darkness (1st experimental run)

Treatments	Germination (%)		MGT (days)	
H_2SO_4[a] (1 min)	86.3 ± 5.5[b]	ef	8.5 ± 0.1	bc
H_2SO_4 (5 min)	62.5 ± 8.3	cdef	7.4 ± 0.2	ab
H_2SO_4 (15 min)	53.8 ± 5.5	cdef	6.3 ± 0.6	a
H_2SO_4 (30 min)	12.5 ± 2.5	ab	7.9 ± 0.4	ab
0.1% KNO_3	51.3 ± 11.3	cde	20.2 ± 1.1	de
0.2% KNO_3	35.0 ± 10.8	abc	19.1 ± 0.9	de
500 ppm GA_3	61.3 ± 9.4	cdef	18.0 ± 0.5	d
1000 ppm GA_3	50.0 ± 6.1	bcde	18.5 ± 0.4	de
20 mM H_2O_2	90.0 ± 3.5	f	10.4 ± 0.4	c
40 mM H_20_2	81.3 ± 9.4	ef	8.9 ± 0.9	bc
+ 4 C° (1 month)	62.5 ± 6.0	cdef	18.7 ± 0.7	de
-18 C° (1 month)	53.8 ± 13.9	cdef	19.9 ± 1.3	de
95% ethanol (30 min.)	55.0 ± 2.9	cdef	18.3 ± 0.7	d
95% ethanol (60 min.)	27.5 ± 2.5	abc	17.0 ± 1.1	d
Sandpaper (800 RPM/2 hours)	7.5 ± 2.5	a	24.0 ± 1.1	e
Manual sandpaper	75.0 ± 6.1	def	7.4 ± 0.4	ab
Distilled water (Control)	38.8 ± 3.8	abcd	20.9 ± 0.9	ab

[a]Abbreviations: H_2SO_4, sulfuric acid; KNO_3, potassium nitrate; GA_3, gibberellic acid; H_2O_2, hydrogen peroxide; RPM, round per minute; MGT, mean germination time

[b]Values are represented by mean ± standard error. Different lowercase letters indicate significant differences among treatments in the same column

Table 3. Germination percentages and mean germination time (MGT) of branched asphodel seeds subjected to different treatments under darkness (2nd experimental run)

Treatments	Germination (%)		MGT (days)	
H_2SO_4[a] (1 min)	88.8 ± 4.7[b]	f	8.2 ± 0.3	c
H_2SO_4 (5 min)	77.5 ± 5.2	cdef	6.8 ± 0.2	b
H_2SO_4 (15 min)	37.5 ± 4.8	ab	6.4 ± 0.1	b
H_2SO_4 (30 min)	20.0 ± 3.5	a	8.0 ± 0.3	c
0.1% KNO_3	62.5 ± 8.3	bcdef	20.0 ± 0.5	fgh
0.2% KNO_3	57.5 ± 2.5	bcde	19.5 ± 0.3	fgh
500 ppm GA_3	62.5 ± 6.3	bcdef	18.3 ± 0.4	fg
1000 ppm GA_3	53.8 ± 8.3	bcd	17.6 ± 0.1	f
20 mM H_2O_2	87.5 ± 6.6	ef	9.6 ± 0.4	d
40 mM H_20_2	91.3 ± 5.5	f	5.2 ± 0.1	a
+ 4 C° (1 month)	66.3 ± 6.6	bcdef	19.3 ± 0.4	fgh
-18 C° (1 month)	53.8 ± 9.0	bcd	17.4 ± 0.5	f
95% ethanol (30 min.)	51.3 ± 8.0	bc	21.1 ± 0.2	gh
95% ethanol (60 min.)	37.5 ± 1.4	ab	21.4 ± 0.7	h
Sandpaper (800 RPM/2 hours)	61.3 ± 4.7	bcdef	14.2 ± 0.2	e
Manual sandpaper	82.5 ± 6.6	def	7.9 ± 0.3	c
Distilled water (Control)	45.0 ± 2.0	ab	18.6 ± 0.8	fg

[a]Abbreviations: H_2SO_4, sulfuric acid; KNO_3, potassium nitrate; GA_3, gibberellic acid; H_2O_2, hydrogen peroxide; RPM, round per minute; MGT, mean germination time

[b]Values are represented by mean ± standard error. Different lowercase letters indicate significant differences among treatments in the same column

DISCUSSION

The branched asphodel seeds germinated better in darkness during the current study, in contrast to germination studies of onionweed (*Asphodelus tenuifolius* Cav.), which were conducted in darkness and a 10-h photoperiod (Tanveer et al. 2014). Özturk and Pirdal (1986) also reported summer asphodel (*A. aestivus* Brot.) light sensitive based on studies carried out in different photoperiods and darkness conditions. Although we found a significant difference in light requirements, germination percentages (45.63% in darkness, 40.63% in photoperiod) were close to each other, indicating that both dark and light periods can be used for germination studies of branched asphodel. Previous research shows that light dependence declines with increasing seed size (Milberg et al. 2000), so the light independence for germination of branched asphodel seeds can be attributed to that.

Sulfuric acid treatments are usually preferred to break dormancy in seeds, especially those with a hard seed coat (Wang et al. 2007). Based on hardness of seed coat, the time required for soaking in H_2SO_4 solutions varies. In our study, as the treatment time with H_2SO_4 exceeded one minute, the germination rate decreased, and no germination was observed in some cases of H_2SO_4 applications. The seed coat destroyed in H_2SO_4 applications at 60 and 120 min (results were not shown and not included in the statistical analysis) and the seeds lost their viability. However, the H_2SO_4 (1 min) application gave good results for increasing germination and shortening MGT.

At low concentrations, potassium nitrate can stimulate seed germination in a variety of plant species, whereas high levels decrease seedling growth (Hernandez et al. 2022). Seed treatment with potassium nitrate (KNO_3) has been reported in many studies to break dormancy and improve germination (Anosheh et al. 2011) by modulating ABA metabolism or ABA signaling in developing seeds (Chahtane et al. 2017, Matakiadis et al. 2009) However, nitrate stimulation of seed germination is often associated with plant species whose seeds require light for germination and seed age (Footitt et al. 2013, Henson 1970). Therefore,

the fact that potassium nitrate treatments (0.1 and 0.2%) did not increase the germination percentage and germination speed significantly was attributed to the fact that the young seeds of branched asphodel not needing light to germinate and the thickness of seed coat in our study.

Gibberellins (GAs) play an important role in the stimulation of seed germination (Bewley 1997), and GA_3 is a well-known germination stimulator that can fully or partially replace light, after-ripening, and cold requirements (da Silva et al. 2005). Contrary to many reports on the stimulatory effect of GA_3 during seed germination, GA_3 can inhibit radicle protrusion in some species (Olvera-Carrillo et al. 2003) as well. The addition of GA_3 (500 and 1000 ppm) did not enhance seed germination of branched asphodel seeds (<65%) significantly in our study, suggesting that the seeds do not have physiological dormancy. GA_3 applications also did not shorten MGT.

With the enhancement of H_2O_2 in the embryo, seeds lose their dormancy, and the imbibitions of seeds with hydrogen peroxide (H_2O_2) increases the germination as well as seedling growth in many studies (Debska et al. 2013). H_2O_2 acts as a signaling molecule in the beginning of seed germination, involving specific changes at proteomic, transcriptomic and hormonal levels (Barba-Espín et al. 2012). In some cases, seed coat-imposed dormancy can be alleviated with oxidants such as H_2O_2, which can oxidize the phenolic compounds present in the seed envelopes and may allow improved oxygenation of the embryo during seed imbibitions (Ogawa and Iwabushi 2001). The imbibition of seed for 24 hours at two different concentrations of H_2O_2 (20 and 40 mM) improved seed germination and significantly shortened the MGT in this study.

In most weed species investigates, the main factors in the natural environment that lead to release from dormancy are light, two attributes of temperature—chilling (or stratification) and alternating temperature—and nitrate ions (Vincent and Roberts 1977). The effects of temperature on seed dormancy during chilling differ depending on whether the seed is wet or dry (Roberts and Totterdell 1981), and pre-chilling time is also important for including morphological changes in seed coat and increasing germination (Jordan and Jordan 1982). The effect of chilling on germination differs among species (Rezvani et al. 2014) There was an increase in germination percentage of branched asphodel seeds under dry cold storage (better in +4 °C than -18 °C), but it was not significant, and the MGT of seed under these treatments was similar to the untreated control. Increasing the cold storage period may contribute to raising the germination percentage. Wet pre-chilling can also be studied to improve germination and shorten the MGT.

Ethanol promotes germination in some species, such as oat and rice (Adkins et al. 1984, Miyoshi and Sato 1997) by inducing gibberellin, increasing oxygen uptake, or perturbing membranes (Taylorson and Hendricks 1979). It may play a role in breaking dormancy by promoting the Krebs cycle and/or glycolysis and may also stimulate germination as a respiratory substrate (Adkins et al. 1984, Corbineau et al. 1991). Germination percentages and MGT results obtained from ethanol treatments of branched asphodel seeds were similar to or below the control in this study. Therefore, it was thought that ethanol concentrations used in experiments were too high to promote germination. Since ethanol concentrations and the duration of ethanol effect the stimulation of germination, and failure to reduce ethanol concentration inhibits germination (Taylorson and Hendricks 1979), lower concentrations and durations should be studied in further.

Mechanical scarification methods are successful in stimulating germination of seeds, especially those with seed-coat imposed dormancy (Eisvand et al. 2006). It is thought that dormancy in branched asphodel seeds is based on seed coat permeability, so overcoming dormancy in these seeds was expected with mechanical scarification methods. However, placing the seeds in sandpaper-coated container and shaking them for two hours at 800 rpm in an orbital shaker did not provide sufficient scouring of the seed coat, while rubbing them between sandpaper for 10 seconds increased seed germination and shorted MGT.

When all the results are evaluated, scarification treatments of H_2O_2 (20 mM and 40 mM) and H_2SO_4 (1 min) significantly increased the germination percentage of branched asphodel seeds in both experiments. The increase in germination percentage observed with H_2SO_4 (5 min) and manual sandpaper applications in one repeat was not significant in another. Since increased H_2SO_4 duration reduced germination rate and seed viability, the one-minute duration in H_2SO_4 is preferable for breaking dormancy. Manual sandpaper application also showed promise for increasing germination percentage, with values exceeding 75% in both experiments.

As with increasing germination percentage, H_2O_2 (20 mM and 40 mM) and H_2SO_4 (1 min) applications significantly shortened the MGT compared to control. Other H_2SO_4 durations and manual sandpaper also decreased MGT significantly in both repeats, but they did not significantly increase germination in some cases.

In conclusion, H_2O_2 (20 mM and 40 mM) and H_2SO_4 (1 min) applications were found sufficient to germinate branched asphodel seeds and accelerated the germination process. Although it was not significant in one repeat, manual sandpaper application also resulted in a germination percentage of over 75% and shortened MGT by over 57%. Considering the possible negative effects of H_2O_2 and H_2SO_4 on seedling growth, rubbing the seeds between sandpaper for 10 seconds was found to be more reasonable method for germinating branched asphodel seeds in further studies.

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Author's Contributions

Authors declare that each author's contribution is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Çirişağusu (*Asphodelus ramosus* L.), Ege bölgesi meralarında giderek artan, yenmeyen bir geofittir. Farklı büyüme aşamalarında kontrol stratejileri geliştirebilmek için dormant tohumlarının çimlendirilmesine ihtiyaç duyulmuştur. Ön deneylerde çirişağusu tohumlarının karanlıkta ışıktan daha iyi çimlendiği görülmüştür. Tohumlarında hızlı, tekdüze ve daha iyi çimlenme sağlamak için, çeşitli dormansi kırma yöntemleri (+4 ve -18 °C'de soğutma, zımpara kağıdı, sülfürik asit, etanol veya hidrojen peroksit ile aşındırma ve gibberellik asit veya potasyum nitrat ile işlemler) uygulanmıştır. Tohumların %95 sülfürik asitte (1 dakika boyunca) veya 20 mM veya 40 mM hidrojen peroksitte (24 saat boyunca) bekletilmesi, en yüksek çimlenme yüzdelerini (%81.3'ün üzerinde) sağlamış ve ortalama çimlenme zamanını (OÇZ) kısaltmıştır. Sülfürik asit diğer sürelerde de (5, 15 ve 30 dakika) OÇZ'yi kısaltmış, ancak sürenin bir dakikadan fazla arttırılması çimlenme oranını azaltmıştır. Gibberellik asite duyarsızlık ve aşındırma yöntemleriyle çimlenmenin artması, tohumların fizyolojik bir dormansi yerine fiziksel dormanside olduğunu göstermiştir. Sonuç olarak, çimlenmeyi %75'in üzerinde sağlayan ve OÇZ'yi >%57 kısaltan zımpara kağıdı ile mekanik aşındırmanın, kimyasalların olumsuz etkilerinden kaçınmak ve kabul edilebilir çimlenmeyi sağlamak açısından tercih edilebilir olduğu düşünülmektedir.

Anahtar kelimeler: kimyasal işlemler, dormansi kırma, çimlenme, düşük sıcaklıklar, aşındırma

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