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Effect of different dormancy-breaking methods on seed germination and vigour of *Atraphaxis spinosa*

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Abstract

Goat's wheat manna (*Atraphaxis spinosa* L.), which naturally spreads in arid and unproductive areas (Iğdır-Aralık Province) in the Northeast of Türkiye, is an important species in erosion control and feeding of grazing animals. For this reason, it is of great importance to know the seed viability and dormancy-breaking methods of *A. spinosa* to bring into production the marginal areas where very few species grow. *A. spinosa* seeds collected at the wind erosion site were germinated for 28 days at 10, 15, 20, 25, 20/10, 20/15 25/10, and 25/15 °C in dark conditions, and the mean germination time of the seeds, total and normal germination rates have been determined. As a result of the experiment, the highest normal (21%) and total (27%) germination rates were determined at 25/10°C and 25/15°C, respectively, and these results showed a high dormancy of seeds. To eliminate the germination problem, 34 different pre-treatments were applied at different application times and levels of 12 dormancy-breaking methods: (1) matrix-priming, (2) hydro-priming, (3) gibberellic acid (GA_3), (4) potassium nitrate (KNO_3), (5) chemical (sulphuric acid, H_2SO_4) scarification, (6) mechanical scarification, (7) warm stratification, (8) cold stratification, (9) warm + cold stratification, (10) cold + warm stratification, (11) soaking in cold water, and (12) soaking in hot water. Afterwards, seed germination characteristics were determined at temperatures where the highest germination (25/10°C and 25/15°C) was achieved. The highest normal (94.6%) and total (100%) germination rates and the fastest germination time (1.0–4.1 days) were obtained from the seeds incubated at 25/15°C after cold stratification (4 weeks) and cold + warm stratification (3 weeks cold + 1 week warm).

Keywords: Goat's wheat manna shrub, dormancy, mean germination time, temperature.

Introduction

Although the environmental conditions are suitable, healthy seeds cannot show their optimum germination without any stimulus of external intervention due to their morphological, anatomical, and physiological characteristics (Finch-Savage, Footitt, 2017). This negative linear relationship between germination and seed dormancy is undesirable because it causes an inhomogeneous emergence and a low yield in cultivated species (Quintero et al., 2018). However, seed dormancy is an important physiological event due to the continuation of the generations of species grown in nature and their adaptation to the environment, and this is a desirable situation (Baroux, Grossniklaus, 2019). Seed dormancy has been seen as an important problem in increasing the cultivation opportunities and densities of these species, whose usage areas and economic value have increased in recent years. For this purpose, scientists have shown that by determining the common dormancy types in seed plants suitable dormancy-breaking methods can be applied and seed germination rates can be improved (Li et al., 2010a; Mirmazloum et al., 2020).

There are 36 species in the *Atraphaxis* (Linnaeus, 1753) genus (Polygonaceae), which has adapted to the steppe and desert climate of the world. One of these species is *A. spinosa*, which spreads in the Eastern and South-eastern Anatolia regions of Türkiye and follows the C_3 photosynthetic pathway (Sanchez et al., 2011). This plant, which has strong root system and spreads over a wide area on the soil surface, is of great importance in the conservation and continuity of natural resources (water and soil) (Karakuş, Keskin, 2017). The thin shoots and leaves of *A. spinosa*, which maintain their greenery for a long time in summer and autumn periods when feed material is scarce, are an important feed resource for ruminants (Karakuş, Keskin, 2018). However, exposure of *A. spinosa* to intense grazing and its use as fuel by removing it significantly reduce the frequency of the plant per unit area. As a result, when the soil surface becomes bare, the risk of erosion increases, and the animals grazing in the region cannot be fed adequately. Therefore, it is of great importance to reveal suitable germination temperatures and dormancy-breaking methods to *A. spinosa* seeds due to planting marginal areas and bringing them into production.

Li et al. (2010b) subjected the *A. spinosa* seeds collected in two periods to the germination test at 15/6,

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20/10, 25/15, and 30/20 °C and reported that the seeds collected in September had a higher seed dormancy compared to June. In the current study, the highest and lowest germination rates were obtained at 30/20°C and 15/6°C, respectively. In the study of Li et al. (2010a), it was revealed that *A. spinosa* seeds exposed only to cold stratification for 8 weeks had a much higher germination rate than seeds without cold stratification. However, Jankju-Borzelabad and Tavakkoli (2008) reported that 9 dormancy-breaking methods applied to *A. spinosa* seeds did not cause any increase in seed germination rates.

A. spinosa, which grows naturally in extreme environmental (climatic and soil) conditions, is an important species for such geographies due to erosion control and providing forage material for grazing animals. However, due to unconscious usage (removal, overgrazing, etc.), the danger of erosion (water and wind) has increased in the region where it grows, and the plant has lost its productivity. For this, it is important to increase the density of the plant per unit area. However, since *A. spinosa* has dormant seeds, it will take many years to wait for its density to increase spontaneously in the habitat where it grows. For this reason, this species should be spread by human beings in marginal areas. For this, suitable germination temperatures of seeds and dormancy-breaking methods should be determined. For this purpose, an experiment was planned, and mean germination rates and mean germination times of seeds at different temperatures (4 constant and 4 fluctuating) were tested.

Afterwards, to improve the germination rate of seeds, 12 different dormancy-breaking methods were tested at the two temperature values where the highest germination rate was obtained.

Material and methods

Seeds belonging to Goat's wheat manna (*Atraphaxis spinosa* L.) shrub were collected at the country's second largest (13,542 ha) wind erosion field in Iğdır-Aralık Province (mean temperature, relative humidity, and total precipitation according to the long-term average are 12.5°C, 54.8%, and 264.0 mm, respectively) located in the Northeast of Türkiye in September 2019. The collected seed samples were stored at 5°C in airtight packages until the experiment was established. Seed viability and dormancy-breaking treatments were carried out in the Department of Field Crops, Faculty of Agriculture, Iğdır University, Türkiye in 2020.

To determine the optimum germination temperature, seeds were subjected to the germination test in an incubator in the dark for 28 days at 4 constant (10, 15, 20, and 25 °C) and 4 fluctuating (12/12 h) temperatures (20/10°C, 20/15°C, 25/10°C, and 25/15°C). The experiment was set up in a completely randomised design with three replications, and 75 (3 × 25) seeds were used for each temperature application. Before the germination test, the seeds were kept in 5% NaClO (sodium hypochlorite) for 1 min for surface sterilisation and then washed with distilled water. Then the seeds were germinated between 20 × 20 cm germination papers imbrued with 2 ml of distilled water. Humidity control was made during the germination period and when needed, 10 ml of distilled water prepared by adding 0.2% Pomarsol was given for each Petri dish. Counts were made daily for 28 days, and the seeds with a radicle length of 2 mm were considered as germinated. At the last count, seedlings were classified as normal or abnormal (ISTA, 2017).

Normal seedling rates at the last count were accepted as normal germination. The mean germination time (MGT) was determined by the equation $\sum n \times t / N$, where t is the number of days from the beginning of the test, n is the number of seeds germinated on day t, and N is the total number of germinated seeds.

To improve the germination rate of seeds, 34 different pre-treatments were applied at different application times and levels of 12 different dormancy-breaking methods (ISTA, 2017). Afterwards, the seeds were subjected to the germination test again for 28 days based on the temperature values of 25/10°C and 25/15°C where the highest germination rate was obtained. At this stage, the methods applied at the seed germination test were followed, and the average germination time, total and normal germination rates were determined. When the highest germination percentage was obtained at the 8 initial temperature values, the 25/15°C temperature application was taken as a control and compared with the highest germination rates obtained by each dormancy-breaking method.

Matrix-priming (1). Seeds left in opaque containers at a ratio of 2: 1: 3 (seed: vermiculite: water) were kept at 15°C for 24, 36, and 48 h and then dried at 25°C in the dark to their initial weight.

Hydro-priming (2). The seeds were kept in 40 ml of water for 5 h at 20°C, and then the surface was dried. After the surface drying process, the seeds were left on a wire tray where the seeds would not come into contact with the water in aging pods filled with 40 ml of water, and the cups were covered with a stretch film. The seeds kept in aging pots for 60, 72, and 96 h at 20°C were dried at room temperature until they reached their initial weight.

Gibberellic acid (GA_3) (3). The seeds were kept in the dark for 24 h in 250, 500, and 1000 ppm GA_3 solution until they were completely submerged and then dried.

Potassium nitrate (KNO_3) (4). The seeds were kept in the dark for 6 h in 2% and 4% KNO_3 solution until they were completely submerged and then dried.

Chemical (sulphuric acid, H_2SO_4) scarification (5). The seeds were kept in 96% H_2SO_4 for 10, 20, and 30 s and then the seeds were washed with distilled water and left to dry on blotting paper.

Mechanical scarification (6). The seeds were scarified for 5, 10, and 15 min using 10 grit sandpaper.

Warm stratification (7). The seeds were kept for 1 and 2 weeks at 20°C between completely moistened coarse filter papers and then surface drying was applied to the seeds.

Cold stratification (8). After the seeds were placed between the completely moistened coarse filter papers, they were placed in a shirred tulle pouch, and the applications were stored at 5°C for 3 and 4 weeks.

Warm + cold stratification (9). The seeds were kept between the completely moistened coarse filter papers at 20°C for 1 and 2 weeks, kept at room temperature for 24 h, and then kept at 5°C for 3 and 4 weeks and cold stratified. Afterwards, surface drying was done on the seeds.

Cold + warm stratification (10). The seeds were kept between the completely moistened coarse filter papers at 5°C for 3 and 4 weeks followed by 24 h at room temperature for 1 and 2 weeks at 20°C, and then the surface was dried.

Soaking in cold water (11). The seeds were placed in the shirred tulle pouch, immersed in water so

that they were completely submerged and kept at 5°C for 1, 2, and 4 weeks, and then the surface was dried.

Soaking in hot water (12). The seeds were placed in the shirred tulle pouch and kept in boiling (100°C) water for 2 and 4 min, and then the surface was dried.

Statistical analysis. The data obtained from the dormancy-breaking treatments were subjected to analysis of variance (ANOVA) according to a factorial experiment based on the completely randomised design with three replicates with the software JMP, version 13 (SAS Institute Inc., USA). Differences between the means were compared by the LSD test at $p \leq 0.05$. Other data were analysed by using one-way ANOVA with SPSS for Windows, version 21.0, and the means were separated by the Duncan's multiple range test at $p \leq 0.05$.

Results and discussion

Determination of optimum seed germination temperature. The results showed that *A. spinosa* seeds had a better germination rate and time at variable temperatures than at constant ones (Table 1). The fastest germination time and the highest total and normal germination rates were determined at varying temperatures of 25/10°C and 25/15°C.

The results showed that *A. spinosa* seeds had a low germination rate and thus a high dormancy state. Regarding the subject, Li et al. (2010b) reported that the highest germination rate (21.88%) of *A. spinosa* seeds was determined at 30/20°C and the lowest one (4.29%) at 15/6°C. Also, seeds of *Paliurus spina-christi* and *Punica granatum* were found to have higher germination rates at variable (25/20°C) temperature than at the constant one (Tilki, Kebeşoğlu, 2009). These findings are in parallel with the results of the current experiment. This is thought to be since species, which follows the C_3 photosynthetic pathway, responded better to variable temperatures due to the interaction of temperature and light.

Matrix-priming (1). According to Table 2, the normal and total germination rate of seeds increased as the solid matrix-priming application time increased. The highest germination rate was obtained at the 48-h matrix-priming application. Due to the literature studies, matrix-priming application was not tested in *A. spinosa* species. However, it has been reported that the germination rate of *Astragalus gummifer* seeds exposed to matrix-priming application at different times was not the same, and the highest germination rate was obtained at the 36-h matrix-priming application (Gürel et al., 2022). It was found that matrix-priming application increased the seed germination rate of different species compared to the control (Pandita et al., 2010). Due to temperature, the seeds had a higher germination (normal and total) rate at 25/10°C. This may be because *A. spinosa* is a cool climate (C_3) plant, as cool climate plants can generally show a better germination performance in lower temperature conditions (Fenner, Thompson, 2005). The matrix-priming application may have reduced the temperature required for the seed germination, and this may have enabled seeds to germinate at low variable temperatures (Elkoca et al., 2007). Due to the temperature and matrix-priming interaction, while an increase was found from the seeds kept at 25/15°C after 36 h of the matrix-priming application in the normal germination rate, it decreased in 24-h and 48-h matrix-priming applications, which may have caused the bilateral interaction to be significant (Figure).

Table 1. The effect of different temperatures on the vigour of *Atraphaxis spinosa* seeds

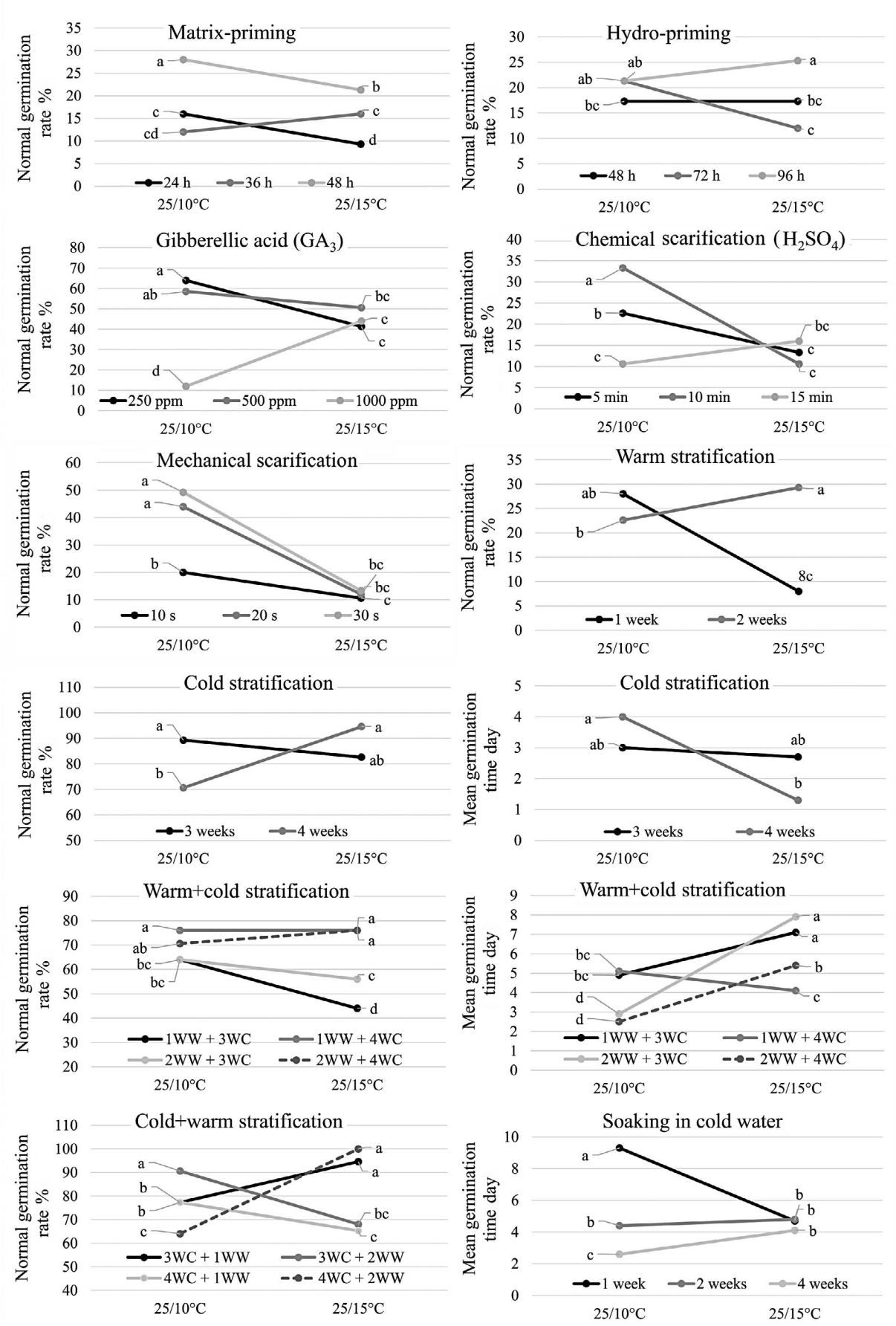
Characteristics	10°C	15°C	20°C	25°C	20/10°C	20/15°C	25/10°C	25/15°C
Total germination rate %	8.0 c	10.6 bc	10.6 bc	12.0 bc	9.3 c	14.6 b	25.3 a	28.0 a
Normal germination rate %	0.0 b	6.6 b	6.6 b	7.6 b	2.6 b	6.6 b	20.0 a	22.6 a
Mean germination time, days	20.1 a	16.0 a–d	18.2 ab	13.0 b–d	17.2 a–c	12.7 cd	11.9 d	11.6 d

Note. a, b, c – the means represented by different letters in the same row differ statistically.

Hydro-priming (2). Only the normal germination rate was found to be important for hydro-priming application, and the highest rate (23.3%) was measured from 96 h hydro-priming application (Table 2). Due to the literature review, hydro-priming application on viability of *A. spinosa* seeds was not tested. However, Elkoca et al. (2007) stated that the seed germination rate increased as the hydro-priming application time increased in chickpea seeds, and these results support findings of our current study. However, Gürel et al. (2022) revealed that hydro-priming application did not cause any increase in the seed germination rate of *A. gummifer*.

Gibberellic acid (GA_3) (3). GA_3 is a hormone that stimulates or promotes germination by reducing the activity of chemical substances that prevent germination of seeds with physiological dormancy. During the experiment, the GA_3 application increased the germination of *A. spinosa* seeds. The highest normal and total germination rates were obtained at the 250 and 500 ppm GA_3 application, which are in the same statistical group, and the fastest germination time was obtained at the 500 ppm GA_3 application (Table 2). Although Jankju-Borzelabad and Tavakkoli (2008) stated that GA_3 application had no effect on the germination rate of *A. spinosa* seeds increasing GA_3 doses up to a certain dose in different seed types increased seed germination rates (Aliloo, Darabinejad, 2013), and the highest germination rates were obtained at the 250–500 ppm GA_3 application (Keshtkar et al., 2008). In the present study, the highest total germination rate and germination time were determined at 25/10°C. This may be because the temperature requirements for the seed germination were reduced after GA_3 application. Because GA_3 disrupts the endosperm cell walls surrounding the embryo, they can enable seeds to germinate at a higher rate and speed (Elkoca et al., 2007). Due to the temperature and application interaction, the highest normal germination was obtained from the seeds kept at 25/10°C after the 250 ppm GA_3 application (Figure).

Potassium nitrate (KNO_3) (4). There was no effect of KNO_3 application on the total and normal germination rates of *A. spinosa* seeds (Table 2), and this result was found to be consistent with the findings of Jankju-Borzelabad and Tavakkoli (2008). However, it has been determined that KNO_3 increases the germination rate in different seed species (Gao et al., 2011; Ertem, Adak, 2022). The effect of temperature on seed viability was found to be significant and the highest total (28.6%) and normal (18.6%) germination rates were determined at 25/10°C. Previously, there was no research demonstrating the viability of *A. spinosa* seeds at different temperatures



WC – week cold, WW – week warm

Figure. The effect of dormancy-breaking methods interaction on the mean germination time and normal germination rate of *Atraphaxis spinosa* seeds

Table 2. The effect of matrix-priming, hydro-priming, gibberellic acid (GA_3), potassium nitrate (KNO_3), chemical (sulphuric acid, H_2SO_4) scarification and mechanical scarification methods in different temperatures on the mean germination time, total and normal germination rates of *Atraphaxis spinosa* seeds

Treatment	Total germination rate %			Normal germination rate %			Mean germination time, days		
	25/10°C	25/15°C	mean	25/10°C	25/15°C	mean	25/10°C	25/15°C	mean
Matrix-priming (1)									
24 h	18.6	12.0	15.3 c	16.0	9.3	12.6 b	–[1]	–	–
36 h	17.3	21.3	19.3 b	12.0	16.0	14.0 b	–	–	–
48 h	40.0	24.0	32.0 a	28.0	21.3	24.6 a	–	–	–
Temp. mean	25.3 a	19.1 b		18.6 a	15.5 b		–	–	
Hydro-priming (2)									
48 h	26.6	17.3	22.0	17.3	17.3	17.3 b	–	–	–
72 h	30.6	21.3	26.0	21.3	12.0	16.6 b	–	–	–
96 h	21.3	36.0	28.6	21.3	25.3	23.3 a	–	–	–
Temp. mean	26.2	24.8		20.0	18.2		–	–	
Gibberellic acid (GA_3) (3)									
250 ppm	77.3	46.6	62.0 a	64.0	41.3	52.6 a	7.8	9.2	8.5 b
500 ppm	69.3	50.6	60.0 a	58.6	50.6	54.6 a	4.3	8.5	6.4 c
1000 ppm	40.0	53.3	46.6 b	12.0	44.0	28.0 b	8.7	13.1	10.9 a
Temp. mean	62.2 a	50.2 b		44.8	45.3		6.9 b	10.2 a	
Potassium nitrate (KNO_3) (4)									
2%	28.0	9.3	18.6	18.6	8.0	13.3	–	–	–
4%	29.3	16.0	22.6	18.6	10.6	14.6	–	–	–
Temp. mean	28.6 a	12.6 b		18.6 a	9.3 b		–	–	
Chemical (sulphuric acid, H_2SO_4) scarification (5)									
10 s	30.6	20.0	25.3 b	20.0	10.6	15.3 b	8.9	8.7	8.8
20 s	54.6	14.6	34.6 a	44.0	12.0	18.0 a	8.2	7.2	7.7
30 s	61.3	16.6	40.0 a	49.3	13.3	31.3 a	6.1	9.6	7.9
Temp. mean	48.8 a	17.7 b		37.7 a	12.0 b		7.7	8.5	
Mechanical scarification (6)									
5 min	32.0	18.6	25.3 a	22.6	13.3	18.0 ab	–	–	–
10 min	44.0	16.0	30.0 a	33.3	10.6	22.0 a	–	–	–
15 min	14.6	22.6	18.6 b	10.6	16.0	13.3 b	–	–	–
Temp. mean	30.2 a	19.1 b		22.2 a	13.0 b		–	–	

Note. a, b, c – values represented by the same letters in the same row and column do not differ statistically; [1] – since the total germination rate was below 50%, the mean germination time was not calculated.

after KNO_3 application. However, it has been reported that the seed germination rates kept at different temperatures after KNO_3 application in *Sorbus pohuashanesis* differ, and the highest germination rate was obtained at 25°C (Bian et al., 2013).

Chemical (sulphuric acid, H_2SO_4) scarification (5). It was found that the highest total and normal germination rates were obtained from the seeds exposed to H_2SO_4 for 20 and 30 s (Table 2). This may be because the seed coat was better eroded by the longer treatment of the seeds with H_2SO_4. In studies conducted with different species (Keshtkar et al., 2008; Pipinis et al., 2011), the germination rate of seeds increased as the duration of treatment with H_2SO_4 increased. Due to temperature, the highest total and normal germination rates were determined at 25/10°C. This may have resulted in damage to the seed coat causing dormancy after H_2SO_4 application resulting in a better seed germination at lower variable temperature. Tilki and Kebeşoğlu (2009) reported that H_2SO_4 application shortens the germination period by eliminating the dormancy problem caused by the seed coat and allows the seed to germinate at lower variable temperatures. Due to the temperature and application interaction, the highest normal germination rate was obtained from the seeds kept at 25/10°C after H_2SO_4 application for 20 and 30 s (Figure).

Mechanical scarification (6). According to Table 2, the highest normal germination rate (22.0%) was obtained for 10 min, and the total germination rates (25.3–30.0%) were obtained at scarification for 5 and 10 min. Similar results were also demonstrated in different seed species, and it was reported that the highest germination rate was obtained at scarification performed in a shorter time (5–10 min) (Kitiş, Aktaş Kaya, 2018; Gürel et al., 2022). The results obtained in the present study showed that the dormancy of *A. spinosa* was due to internal factors not the seed coat. Due to temperature, the highest normal (22.2%) and total germination (30.2%) rates were obtained from the seeds kept at 25/10°C. This may be because *A. spinosa* is a cool season plant and reduces the temperature requirement for germination after mechanical scarification. Üstüner (2003) stated that the most germination in dormancy-breaking treatments was obtained at variable temperature applications. According to the Figure, the normal seed germination rate was decreased at 25/15°C compared to 25/10°C after scarification for 5 and 10 min, while there was no significant difference in scarification for 15 min, which may have caused the bilateral interaction to be significant.

Warm stratification (7). Germination rates showed significant differences due to application, and the highest values were obtained from the seeds exposed to 2 weeks of warm stratification. Similarly, warm stratification increased the seed germination rates of *A. gummifer* seeds (Gürel et al., 2022). Due to temperature, it was found that a higher total and normal germination rates were obtained at 25/10°C (Table 3). This may be due to the high response of *A. spinosa* to low variable temperatures in germination, as it is a cool season plant. Due to the warm stratification and temperature interaction, the highest normal germination rate was obtained from the seeds treated with warm stratification for 2 weeks and kept at 25/15°C (Figure).

Cold stratification (8). The total germination rate and average germination time were found to be important due to temperature applications, and the highest total germination rate and germination time were determined at 25/15°C (Table 3). Li et al. (2010a) reported that the germination rate of *A. spinosa* seeds, which were subjected to the germination test at varying temperatures of 15/6, 20/10, 25/15, and 30/20 °C for 12/12 h daily after cold stratification at 5°C for 0–8 weeks, increased as the temperature increased, and these results show parallelism with our findings. During the experiment conducted with *Cercis siliquastrum* seeds, the dormancy was strongly broken, and the germination rate increased from the seeds kept at 20–25°C after cold stratification (Pipinis et al., 2011). Due to the cold stratification and temperature interaction, the highest normal germination rate was obtained from the seeds kept at 25/10°C and 25/15°C after 3 and 4 weeks, and the highest mean germination time was obtained from the seeds kept at 25/15°C after 4 weeks of cold stratification (Figure). These results showed that the dormancy of *A. spinosa* seeds was embryo-induced. Similarly, Li et al. (2010a) reported that the highest germination rate was obtained from *A. spinosa* seeds kept at 30/20°C following 8 weeks of cold stratification. This may be due to the fragmentation of chemical substances that prevent germination of seeds in low temperature and humid conditions encouraging seed germination (Vandelook et al., 2009).

Warm + cold stratification (9). The effect of warm + cold stratification on germination characteristics was found to be significant, and the highest germination rate was obtained at 1 week warm + 4 weeks cold and 2 weeks warm + 4 weeks cold stratification in the same statistical group (Table 3). The average germination time of the seeds was shortened with the increase of the germination rate. These results showed that the effect of the increase of the cold stratification times following the warm stratification on the germination rate was more significant and that the germination barrier was caused by the embryo. In most seed species, warm + cold stratification has been suggested as one of the methods used to eliminate the germination barrier caused by the embryo and has been reported to increase germination rates compared to the control (Tilki, Çiçek, 2005; Gürel et al., 2022).

Table 3. The effect of warm stratification, cold stratification, warm + cold stratification, cold + warm stratification, soaking in cold water and soaking in hot water methods in different temperatures on the mean germination time, total and normal germination rates of *Atraphaxis spinosa* seeds

Treatment	Total germination rate %			Normal germination rate %			Mean germination time, day		
	25/10°C	25/15°C	mean	25/10°C	25/15°C	mean	25/10°C	25/15°C	mean
Warm stratification (7)									
1 week	40.0	17.3	28.6 b	28.0	8.0	18.0 b	–[1]	–	–
2 weeks	33.3	37.3	35.3 a	22.6	29.3	26.0 a	–	–	–
Temp. mean	36.6 a	27.3 b		25.3 a	18.6		–	–	
Cold stratification (8)									
3 weeks	89.3	92.0	90.6	89.3	82.6	86.0	3.0	2.7	2.8
4 weeks	79.0	100.0	88.0	70.6	94.6	82.0	4.0	1.3	2.7
Temp. mean	82.6 b	96.0 a		80.0	88.6		3.5 a	2.0 b	
Warm + cold stratification (9)									
1WW + 3WC	69.3	49.3	59.3 b	64.0	44.0	54.0 b	4.9	7.1	6.0 a
1WW + 4WC	81.3	76.0	78.6 a	76.0	76.0	76.0 a	5.1	4.1	4.6 bc
2WW + 3WC	70.6	61.3	66.0 b	64.0	56.0	60.0 b	2.9	7.9	5.4 ab
2WW + 4WC	77.3	84.0	80.6 a	70.6	76.0	73.3 a	2.5	5.4	3.9 c
Temp. mean	74.6 a	67.6 b		68.6 a	63.0 b		3.8 b	6.1 a	
Cold + warm stratification (10)									
3WC + 1WW	86.6	100.0	93.3 a	77.3	94.6	86.0 a	1.1	1.0	1.1 c
3WC + 2WW	100.0	78.6	89.3 a	90.6	68.0	79.3 a	2.6	3.1	2.8 ab
4WC + 1WW	82.6	73.3	78.0 b	77.3	65.3	71.3 b	2.7	3.9	3.3 a
4WC + 2WW	78.6	100.0	89.3 a	64.0	100.0	82.0 a	2.7	2.4	2.6 b
Temp. mean	87.0	88.0		77.3	82.0		2.3	2.6	
Soaking in cold water (11)									
1 week	73.3	61.3	67.3 c	87.3	58.6	58.0 b	9.3	4.7	7.0 a
2 weeks	81.3	72.0	76.6 b	81.3	69.3	75.3 a	4.4	4.8	4.6 b
4 weeks	86.6	77.3	82.0 a	81.3	72.0	76.6 a	2.6	4.1	3.3 c
Temp. mean	80.4 a	70.2 b		73.3	66.6		5.4 a	4.5 b	
Soaking in hot water (12)									
2 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Temp. mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Note. WW – week warm, WC – week cold; a, b, c – values represented by the same letters in the same row and column do not differ statistically; [1] – since the total germination rate is below 50%, the mean germination time was not calculated.

Due to temperature, the highest germination rate was obtained at t 25/10°C, and the seeds were germinated in a shorter time at this temperature (Table 3). Similarly, Tilki and Çiçek (2005) revealed that the germination temperatures (30/20°C, 20°C, and 25/4°C) after warm + cold stratification had a significant effect on the germination performance of *Fraxinus angustifolia* subsp. *oxycarpa* seeds, and the highest germination rate was obtained at 25/4°C. The effect of the temperature and application interaction on the investigated parameters was found to be significant (Figure). The germination rate was generally higher from the seeds kept at 25/15°C after 1 and 2 weeks warm + 4 weeks cold stratification. However, the germination time was faster from the seeds kept at 25/15°C after 1 week warm + 3 weeks cold stratification and 2 weeks warm + 4 weeks cold one.

Cold + warm stratification (10). According to Table 3, the highest total and normal germination rates were obtained at 3 WC + 1 WW, 3 WC + 2 WW and 4 WC + 2 WW doubling conditions, and the fastest germination time was obtained at the 3 WC + 1 WW application. Similar results were obtained from the *Corylopsis* seeds exposed to warm stratification after cold stratification (Kim et al., 2016). However, cold + warm

stratification application time did not have any effect on the germination rate of *A. gummifer* seeds (Gürel et al., 2022). Due to the cold + warm stratification application and temperature interaction, while the normal germination rate of seeds increased at 3WC + 1WW and 4WC + 2 WW doubling conditions compared to 25/10°C and 25/15°C, it decreased at other applications (Figure). This may have caused the bilateral interaction to be important.

Soaking in cold water (11). The results showed that germination rates increased and germination times decreased as the cold-water soaking time increased (Table 3). Similarly, it has been reported that the seed germination rate and germination time of *Terminalia belerica* seeds increased with the increase of cold-water soaking time (Hossain et al., 2014). It has been reported that germination rates differ according to the cold-water soaking times in studies conducted with different species. This method increases germination rates compared to the control and it is an effective dormancy-breaking method (Odoi et al., 2019; Gürel et al., 2022). Due to temperature, the highest total germination rate was obtained at 25/10°C, and the fastest germination time was obtained from the seeds kept at 25/15°C (Table 3). This may have been because *A. spinosa* is a cool season plant, and lower variable temperatures encourage seed germination. Cool climate plants vary according to the species, but they can achieve the optimum germination at 15–20°C (Fenner, Thompson, 2005). The response to low temperature may differ significantly between species (Baskin, Baskin, 2000). Only the mean germination time was found to be important due to the cold water soaking and temperature interaction, and the fastest germination time was determined from the seeds kept at 25/10°C after 1 week of cold-water application (Figure).

Soaking in hot water (12). According to Table 3, the application of soaking in hot water did not ensure the germination of *A. spinosa* seeds and lost the viability of the seeds. This may be because the embryo lost its viability with the application of hot water. Indeed, Keshtkar et al. (2008) stated that the germination rate of *Astragalus cyclophyllon* seeds, which were kept in water at 100°C for 10 min, decreased compared to the control. However, it has been stated that soaking in hot water increases the germination rate of some species with hard seed coats and is a good dormancy method for these species (Tung, Serrano, 2011; Mohammadi et al., 2012; Abdulazeez, 2016; Gürel et al., 2022).

Comparison of different dormancy-breaking treatments compared to the control. According to Table 4, the highest total (100%) and normal (94.6%) germination rates compared to the control (22.6%) were cold stratified (4WC, 25/15°C) and cold + warm stratified (3WC + 1WW, 25/15°C). The fastest average germination time (1.0 days) was obtained at cold + warm stratification (3WC + 1WW, 25/15°C). These results showed that 78.4–80.0% of ungerminated *A. spinosa* seeds were in dormancy due to internal factors, i.e., embryo-induced dormancy. Similarly, Li et al. (2010a) stated that 100% germination rate was achieved from the seeds kept at 30/20°C following cold stratification, and the seeds had physiological dormancy characteristics that were not deep. It has been reported that the chemical substances that prevent germination of the seed (from the embryo) decompose at low temperature and humid conditions and promote seed germination (Vandelook et al., 2009). However, other dormancy-breaking methods tested during the current experiment increased the total germination and normal germination (4% KNO_3, 25/10°C excluded)

Table 4. Comparison of different dormancy-breaking methods showing the highest viability of *Atraphaxis spinosa* seeds

Treatment	Total germination rate %	Normal germination rate %	Mean germination rate, days
Control, 25/15°C	28.0 f	22.6 fg	11.6 ab
(1) Matrix-priming, 48 h, 25/10°C	40.0 e	28.0 ef	10.9 abc
(2) Hydro-priming, 96 h, 25/15°C	36.0 ef	25.3 fg	12.6 a
(3) Gibberellic acid (GA_3), 250 ppm, 25/10°C	77.3 c	64.0 c	9.2 c
(4) Potassium nitrate (KNO_3), 4%, 25/10°C	29.3 f	10.6 g	9.8 bc
(5) Chemical (H_2SO_4) scarification, 30 s, 25/10°C	61.3 d	49.3 d	6.1 d
(6) Mechanical scarification, 10 min, 25/10°C	44.0 e	33.3 e	9.9 bc
(7) Warm stratification, 1 week, 25/10°C	40.0 e	28.0 ef	10.8 abc
(8) Cold stratification, 4 weeks, 25/15°C	100.0 a	94.6 a	4.1 de
(9) Warm + cold stratification, 2 weeks + 4 weeks, 25/15°C	84.0 bc	76.0 b	2.9 ef
(10) Cold + warm stratification, 3 weeks + 1 week, 25/15°C	100.0 a	94.6 a	1.0 fg
(11) Soaking in cold water, 4 weeks, 25/10°C	96.6 b	91.3 b	2.6 ef
(12) Soaking in hot water, 2 min, 25/10°C	0.0 g	0.0 h	0.0 g
Significance	**	**	**

Note. ** – $p < 0.01$; a, b, c – values represented by the same letters do not differ statistically in the same column.

rates and average germination time (96 h hydro-priming, 25/15°C excluded) of the seeds compared to the control.

Previous studies (Keshtkar et al., 2008; Pipinis et al., 2011; Tung, Serrano, 2011; Aliloo, Darabinejad, 2013; Kheloufi et al., 2018; Kitiş, Aktaş Kaya, 2018) have also shown that different dormancy-breaking methods increase the seed germination rate compared to the control and differ according to species and cultivars. However, Jankju-Borzelabad and Tavakkoli (2008) stated that 9 different dormancy-breaking methods applied to *A. spinosa* seeds had no effect on the seed germination rate.

Conclusion

In the present study, where different (4 constant and 4 variables) temperatures were tested, the total and normal germination rates of Goat's wheat manna (*Atraphaxis spinosa* L.) seeds varied between 8.0–28.0% and 0.0–22.6%, respectively, and the seeds had a higher germination rate and germination rate at variable temperatures (except 25/10°C). To increase the germination rate and shorten the emergence time, 12 different dormancy-breaking methods were tested, and it has been revealed that the most suitable dormancy-breaking methods are cold stratification (waiting at 25/15°C after 4 weeks cold stratification), cold + warm stratification (waiting at 25/15°C after 3 weeks cold + 1 week warm stratification), and soaking in cold water (waiting at 25/10°C after 4 weeks cold water application). There was a high dormancy status of *A. spinosa* seeds. The average germination time (1.0–4.1 days) of seeds were shortened, while the total (96.6–100%) and

normal (91.3–4.6%) germination rates increased in the dormancy-breaking methods determined compared to the control (25/15°C).

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