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Article

Evaluation of different methods for breaking cypsela dormancy in two local populations of Scotch thistle (*Onopordum acanthium* L.) collected from the west and northwest of Iran

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Abstract

Scotch thistle (*Onopordum acanthium* L.) is a weed species on farmlands and pastures in parts of the west and northwest of Iran. Cypselas can remain in the soil seedbank over a prolonged period due to varying degrees of dormancy. This study examined different methods for breaking cypsela dormancy in two local populations of Scotch thistle at the research laboratory of the Faculty of Agriculture of Azarbaijan Shahid Madani University in 2020. In these experiments, the effects of sulfuric acid, wet and dry prechilling, potassium nitrate, and gibberellic acid were evaluated on the breaking of dormancy. All the experiments were conducted as a completely randomized design with four replications and two runs. The response of two local populations of Scotch thistle were similar, and no significant difference was observed. The experiment treatments significantly increased cypsela germination compared with the control. The maximum germination percentages were observed in cypselas soaked in 98% sulfuric acid for 10 min (60.60%), KNO3 for 96 h (69.36%), GA3 for 48 h (88.43%), and wet prechilling for 72 h (34.61%). Although prechilling increased germination, it was suggested that germination could be more than the recorded values if the duration of wet prechilling was increased. The best cypsela germination rate, mean germination time, T_{50}, and radicle and plumule length were observed after soaking in GA_3 for 48 h and KNO_3 for 96 h. It seems that water-soluble inhibitors in the embryo and probably the cypsela coat cause seed dormancy in this weed species. These findings could help develop effective management strategies associated with the dormancy of cypselas for this species.

Key words: cypselas, cold and chemical stratification, physiological seed dormancy, chemical scarification

Résumé

L'onoporde acanthe est une adventice qui peuple les terres arables et les pâturages de l'ouest et du nord-ouest de l'Iran. Ses graines peuvent rester longtemps en dormance à un degré variable dans le sol. En 2020, les auteurs ont examiné plusieurs méthodes pour tirer les graines de deux populations locales de leur dormance, au laboratoire de la faculté d'agriculture de l'Université Shahid Madani en Azerbaïdjan. Lors de ces expériences, ils ont évalué l'efficacité de l'acide sulfurique, de la réfrigération préalable en présence ou en l'absence d'eau, du nitrate de potassium et de l'acide gibberelique pour sortir les graines de leur dormance. Les expériences ont toutes suivi un modèle entièrement aléatoire, reproduit quatre fois à deux reprises. Les deux populations locales d'onoporde ont réagi de la même façon et les auteurs n'ont relevé aucune variation digne de mention. Les traitements augmentent sensiblement la germination, comparativement au témoin. Les taux de germination maximaux observés étaient les suivants : 60,60 % pour la scarification avec de l'acide sulfurique à 98 % pendant 10 minutes, 69,36 % pour le trempage dans du KNO3 pendant 96 heures, 88,43 % pour le trempage dans du GA3 pendant 48 heures et 34,61 % pour la réfrigération préalable en présence d'eau pendant 72 heures. Bien que la dernière méthode améliore la germination, celle-ci pourrait sans doute être plus importante que celle observée si on prolongeait la période de réfrigération. Les meilleurs résultats pour le taux de germination, le temps de levée moyen, la T50, la longueur des radicules et celle de la plumule ont

été relevés après trempage pendant 48 heures dans du GA3 et pendant 96 heures dans du KNO3. Apparemment, chez cette espèce, la dormance résulte d'inhibiteurs hydrosolubles présents dans l'embryon et, vraisemblablement, dans les téguments de la graine. Ces observations pourraient contribuer à l'élaboration de stratégies efficaces pour combattre la dormance des semences de cette espèce. [Traduit par la Rédaction]

Mots-clés : graine, stratification par le froid, stratification chimique, dormance physiologique des graines, scarification chimique

Introduction

Scotch thistle (*Onopordum acanthium* L.) (Asteraceae) has a Eurasian origin, and it is a native of southern Europe, western and central Asia, and Asia Minor (Young and Evans 1969; Cavers et al. 2011). It represents an invasive weed in the western United States, Australia, Argentina, parts of Canada, and New Zealand, and it is a serious problem in pastures, along roadsides, rangeland, gravelly riverbanks, and well-drained sandy or gravelly soils (Harizanova et al. 2010; Cavers et al. 2011). Also, it has been found in agricultural fields, so its infestation causes annual losses to ranchers of US$16.60/ha in wheatgrass stands in northern California, USA (Hooper et al. 1970; Qaderi 1998; Smith et al. 1999). The plant is commonly a monocarpic winter annual, biennial, or short-lived perennial, propagated by cypsela (a dry, single-seeded fruit) production (Qaderi et al. 2005). It flowers from late June to October and ovule fertilization occurs by self-or cross-pollination, which can be accomplished by wind and (or) insects. Depending on size, a single Scotch thistle plant can produce as few as 100 to as many as 50 000 cypselas. Cypselas are spatula-like, with four to five angled surfaces, without hairs, and mottled brown to nearly black (Qaderi 1998; Schuster and Prather 2003).

Scotch thistle has a unique pattern of population dynamics, so some cypselas may germinate shortly after dispersal (in fall). In contrast, others may remain dormant but viable for at least 40 years in the soil (Toole and Brown 1946; Qaderi and Cavers 2002). Control of Scotch thistle is difficult due to the varying degrees of cypsela dormancy and prolonged persistence in soil seedbanks. Cavers et al. (1995) reported that in the *Onopordum* genus cypselas varying greatly in dormancy were found in the same population, and Meier (1995) found that cypselas from a single plant can differ significantly in seed dormancy. Roberts and Chancellor (1979) declared that the level of innate dormancy in their sample of Scotch thistle appeared to be somewhat more significant than that in the other species they tested. Variation in germination pattern within local populations of Scotch thistle is beneficial for the survival of this species (Qaderi and Cavers 2002). Scifres and McCarty (1969) reported that cypselas of Scotch thistle contain water-soluble germination inhibitors and are sensitive to differences in light quality. Young and Evans (1972) declared that this sensitivity-to-light quality is a symptom of the regulation of germination by phytochromes, so both the soluble inhibitors and the sensitivity-to-light quality function in the embryo, not in the cypsela coat.

Various authors have reported germination responses in Scotch thistle to high temperatures, gibberellic acid (GA_3), increased soil nitrate levels, scarification, stratification, leaching, exposure to red light, and other factors as stimulating germination (Scifres and McCarty 1969; Young and Evans 1972; Perez-Garcia 1993). Techniques such as mechanical scarification with sandpaper (Ali et al. 2011), chemical scarification with acids (H_2SO_4, HNO_3, and HCl), soaking of seeds (or fruits) in hot water for a short time (Olmez et al. 2008), and perforation of the seed/fruit coat (Aliero 2004) are the most commonly used methods for breaking the physical dormancy of seeds/fruits. Sulfuric acid treatment damages the surface of the seed/fruit coat or splits the palisade layer of the micropylar, thereby facilitating the diffusion of oxygen and water and reducing the mechanical resistance to radical emergence (Schelin et al. 2003). Contreras and Ruter (2009) have observed the positive effect of sulfuric acid scarification (15 to 30 min soaking in concentrated sulfuric acid) on the germination of American beautyberry (*Callicarpa americana* L.) seeds (located in the fruit berry-like drupe with a fleshy exocarp and hard endocarp separated into four pyrenes, each containing a single seed at ripening).

A period of prechilling (stratification) also relieves the dormancy of many species across many plant families (Conner 2008; Tang et al. 2008). Furthermore, the importance of cold stratification has been confirmed in many species of the Asteraceae family. For example, cold stratification breaks cypsela dormancy in narrow-leaved purple coneflower (*Echinacea angustifolia* DC.) (Baskin et al. 1992), whiteflower leafcup (*Polymnia canadensis* L.) (Bender et al. 2003), *Guizotia scabra* (Vis.) Chiov., Santa Maria feverfew (*Parthenium hysterophorus* L.), golden crownbeard [*Verbesina encelioides* (Cav.) Benth. & Hook. f. ex A. Gray] (Karlsson et al. 2008), as well as false mayweed [*Tripleurospermum maritimum* (L.) W.D.J. Koch] (Bochenek et al. 2010), while a moderate thermal stratification has a positive influence on cypsela germination in Canada thistle [*Cirsium arvense* (L.) Scop.] (Bochenek et al. 2009). Cold stratification simulates cold winter conditions for seeds/fruits with internal dormancy (Karlsson et al. 2006). A prechilling treatment changes the inhibitor and promoter balance in seeds/fruits (Ren and Guan 2008). Conner (2008) and Kucera et al. (2005) reported that a prechilling treatment increased GA_3 synthesis in the embryo and thus was a germination promoter.

Potassium nitrate (KNO_3) is the most common chemical that was used for breaking seed dormancy and promoting seed germination (Gashi et al. 2012). KNO_3 has been used for many years, with positive studies beginning in the 1980s, but it often increased the germination of photodormant seeds (Shanmugavalli et al. 2007). The most significant germination of long-headed poppy (*Papaver dubium* L.) and common poppy (*Papaver rhoeas* L.) seeds in a light/dark regime was observed at concentrations of 0.5 g L^{-1} KNO_3, by 40.8% and 44.2%, respectively (Golmohammadzadeh et al. 2015). GA_3 also is a successful chemical in breaking dormancy and promoting germination in seeds (or fruits) (Bewley and Black 1982). For germi-

nation, seeds or fruits need GA_3 to offset the action of abscisic acid (ABA), a dormancy regulator (Hilhorst and Karssen 1992) that is not resynthesized in mature seeds (or fruits) (Karssen 1995). Qaderi and Cavers (2000) observed that GA_3, even at a very low concentration, causes most viable cypselas of Scotch thistle to germinate. Perez-Garcia and Duran (1990) have also shown that the addition of 2 mmol L^{-1} GA_3 enhances the germination of cypselas of Moor's cotton thistle (*Onopordum nervosum* Boiss.), and the germination percentage (GP) increases as the GA_3 concentration increases. However, these chemical compounds do not promote germination in all seeds/fruits, and depending on the method and dosage, they can even inhibit germination. Thus, for each species the appropriate means of treatment, concentration, and other conditions need to be investigated (Baskin and Baskin 2014).

Several authors have studied cypsela dormancy of Sotch thistle in the invaded areas (Scifres and McCarty 1969; Young and Evans 1972; Qaderi and Cavers 2003; Qaderi et al. 2003, 2012), but there is currently little information on cypsela germination patterns in biotypes of Scotch thistle to be studied from a native area. We chose to work with Scotch thistle because it germinates intermittently and can be a good model system for the investigation of dry storage in the dormancy state. Information from this type of study will be helpful for researchers who store seeds or fruits under dry conditions for extended periods. Based on this information, this study aimed to determine an effective method(s) for breaking cypsela dormancy in two local populations of Scotch thistle collected from Kermanshah and Tabriz, Iran. To address this aim, we examined the GP, germination rate (GR), mean germination time (MGT), T_{50}, and radicle and plumule length of Scotch thistle cypselas by applying sulfuric acid as a chemical scarification, dry and wet prechilling, and GA_3 and KNO_3 pretreatments as a cold and chemical stratification. Our work may help to clarify which of the dormancy-breaking methods can mitigate the dormancy in Scotch thistle cypselas and promote germination.

Materials and methods

Plant materials

Three Petri dish experiments in two runs were conducted at the research laboratory of the Faculty of Agriculture of Azarbaijan Shahid Madani University of Tabriz, Iran (37°48′49″N lat.; 45°56′01″E long.; alt. 1318.8 m) in 2020. In these experiments, breaking Scotch thistle cypsela dormancy was evaluated using concentrated sulfuric acid (98%, H_2SO_4, Merck, Germany), dry and wet prechilling, KNO_3 (Sigma-Aldrich 7757-79-1, Inc.), and GA_3 (Sigma-Aldrich G7645, Inc.). Scotch thistle cypselas were collected from at least five randomly selected capitula from 30 plants in each of two local populations of Kermanshah (34°19′21″N lat.; 47°06′03″E long.; alt. 1372 m above sea level) on 6 August 2019 and Tabriz (37°48′49″N lat.; 45°56′01″E long.; alt. 1318.8 m above sea level) on 13 September 2019. The collected cypselas from the two local populations of Kermanshah and Tabriz were pooled separately and were stored at 4 °C and 40% relative humidity for 12 months prior to the start of the experiments. Thirty cypselas were placed in 9 cm diameter glass Petri dishes with a single layer of Whatman (Whatman, England) No. 1 filter paper. The filter paper was moistened with 6 mL of distilled water. All the Petri dishes were sealed with parafilm to inhibit evaporation and water loss. The Petri dishes were placed in a seed germinator at 25–15 °C and 60% relative humidity with an 8 h photoperiod to germinate the cypselas (Young and Evans 1972). Fluorescent lamps provided the light to produce a light intensity with an amount of illumination of 112 μmoles m^{-2} s^{-1} (Young and Evans 1972). Before germination tests, cypselas were sterilized with 1% sodium hypochlorite (NaOCl) for 10 min and subsequently rinsed with distilled water several times. Cypsela germination was monitored for 39 days after incubation, and the experiments were terminated after a 5 day period with no germination (according to Qaderi and Cavers 2000). The cypselas were germinated when the radicle and plumule lengths reached 2 mm. After incubation for 20 days, five seedlings of similar size were selected from each Petri dish to determine radicle and plumule lengths. The viability of nongerminated cypselas was determined using a 0.1% tetrazolium chloride (T8877, Sigma Chemical Co., St. Louis, MO, USA) solution (Peters 2000). Treatments on the cypselas of the two local populations of Scotch thistle were carried out in three experiments, including the following.

Sulfuric acid treatments

Concentrated sulfuric acid was used to soak cypselas for 10, 20, 30, 40, and 50 min. The cypselas were then washed in running water for 10 min to remove any trace of acid before being tested for germination. Next, Petri dishes containing cypselas were placed in a seed germinator, and their solution was replaced every 4 days to eliminate water-soluble inhibitors in the embryo or cypsela coat.

Dry and wet prechilling treatments

The cypselas were placed between either two layers of dry paper (dry prechilling) or moistened with distilled water (wet prechilling) and placed in plastic bags. The samples were stored in a refrigerator at a temperature of 4 °C for 24, 48, and 72 h. When treatment was applied longer than 24 h, distilled water was renewed daily. Then, cypselas in the Petri dish were placed in a seed germinator.

KNO_3 and GA_3 treatments

The cypselas were soaked in 1 g L^{-1} solutions of KNO_3 and GA_3. The cypselas were soaked in KNO_3 for 24, 48, and 96 h and in GA_3 for 12, 24, and 48 h, at room temperature (25 ± 2 °C), before germination tests. The duration of the KNO_3 and GA_3 treatments was determined based on the pretest.

Data analysis

The germination status of the cypselas was inspected at 2–3 day intervals after seeding. The GP was calculated using the

following formula:

$$GP = \left(\frac{n}{N}\right) \times 100$$

where n is the number of germinated cypselas, and N is the number of cypselas used at the beginning of the experiment. The GR was calculated according to the following formula (Maguire 1962):

$$GR = \sum_{i=1}^{n} \left(\frac{S_i}{D_i}\right)$$

where S_i is the number of germinated cypselas on day i on which a count was made and seedlings removed and D_i is the number of days from the start of the experiment. The following equation was used to calculate the MGT (Ellis and Roberts 1981; Kwon et al. 2020):

$$MGT = \sum \left(\frac{d \times n}{N}\right)$$

where d is the number of days from the beginning of the test, n is the number of germinated cypselas between scoring intervals, and N is the total number of germinated cypselas in the treatment at the end of the experiment. The time from seeding to reaching 50% of the final GP (T_{50}) was calculated according to the following equation (Coolbear et al. 1984; Farooq et al. 2005; Kwon et al. 2020):

$$T_{50} = T_i + \left\{[(N/2) - n_i]\left(T_j - T_i\right) / \left(n_j - n_i\right)\right\}$$

where N is the total number of germinated cypselas and n_i and n_j are the cumulative germination numbers in sequential counts at times (days) T_i and T_j, when $n_i < N/2 < n_j$. The lengths of the radicle and plumule were measured at the end of the experiments.

All the experiments were conducted as a completely randomized design with four replications. Each experiment was repeated two times (two runs). All the data were subjected to analysis of variance (ANOVA) using PROC GLM or PROC MIXED in SAS version 9.2.0 statistical software. Treatments of breaking dormancy were assumed as fixed factors; the population and repetition of the experiments were random factors. The assumption of the variance analysis was tested by ensuring that the residuals were random, homogeneous, and with a normal distribution about a mean of zero using residual plots and the Anderson–Darling test. Final GPs were normalized by the arcsine square root of the percentage where it was required and means were compared on the transformed scale and were converted back to the original scale for presentation of results. Means were separated using Tukey's mean comparison test at a 0.05 significance threshold.

Results and discussion

Scotch thistle cypselas were retrieved on day 39 of incubation from the seed germinator and then the germination characteristics of the two local populations were calculated using sulfuric acid as a chemical scarification, dry and wet prechilling, and GA_3 and KNO_3 pretreatments as a cold and chemical stratification (according to Kwon et al. 2020).

Sulfuric acid treatments

The GP, GR, MGT, and T_{50} of Scotch thistle cypselas were affected by sulfuric acid ($p \leq 0.01$) (Table 1) and there was a significant difference between cypselas soaked in sulfuric acid based on the time duration compared with the control. Among sulfuric acid treatments, the highest percentage (60.6%) and rate (2.99) were found in sulfuric acid treatment for 10 min. Also, the lowest MGT (10.42 days) and T_{50} (7.87 days) were found in sulfuric acid treatment for 10 min (Table 2). The radicle length of Scotch thistle cypselas was significantly influenced under sulfuric acid use ($p \leq 0.01$) (Table 1), such that the greatest radicle length was observed when cypselas were soaked in sulfuric acid for 10 min (1.90 cm) (Table 2). In addition, cypselas soaking in sulfuric acid were effective on the plumule length compared with the control ($p \leq 0.01$) (Table 1), such that the longest plumule was observed when cypselas were soaked in sulfuric acid for 10 min. Despite the radicle and plumule lengths reducing on increasing the duration of soaking cypselas in sulfuric acid, the 20 min treatment was not different from all the other treatments that had an exposure time of greater than 20 min (Table 2). Our experiments demonstrated that soaking Scotch thistle cypselas in 98% sulfuric acid for 10–20 min significantly improved the GP, GR, and radicle and plumule lengths, and reduced the MGT and T_{50}, and it was the most effective at dissolving the cypsela coat compared with the control. Previous studies indicated that soaking seeds in 98% sulfuric acid for 15 min reduces seed hardness in Russian fenugreek [*Medicago ruthenica* (L.) Ledebour] erect ecotypes (Xu and De 1996). It seems that pretreating by sulfuric acid led to disrupting the cypsela coat and thereby imbibition of water into the embryo. This likely led to water-soluble inhibitors being discharged in the water solution and their concentration being alleviated in the cypsela coat and embryo. Schuster and Prather (2003) stated that Scotch thistle cypselas contain water-soluble germination inhibitors and require moisture to break dormancy. Earlier studies also showed that water-soluble compounds such as chlorogenic acid and *para*-substituted benzamide bound to the cypsela wall have an inhibitory effect on cypsela germination and are involved in the dormancy of fresh cypselas of Scotch thistle (Qaderi et al. 2003; Qaderi et al. 2012).

However, germination characteristics such as GP, GR, and radicle and plumule lengths were reduced in the two local populations of Scotch thistle on increasing the duration of soaking cypselas in 98% sulfuric acid. In contrast, MGT and T_{50} were increased on increasing the duration of soaking Scotch thistle cypselas in 98% sulfuric acid. These findings indicated that soaking cypselas in 98% sulfuric acid for a long time might cause injury in storage parts or the embryo. Aliero (2004) reported that 98% concentrated sulfuric acid gave the highest percentage of germination within the shortest period compared with 90%, 70%, and 50% concentrations, respectively.

Table 1. ANOVA (p value) of the germination characteristics of Scotch thistle cypselas on using sulfuric acid as a physical seed dormancy-breaking mechanism.

Source of variance	Df	GP	GR	MGT	T_{50}	Radicle length	Plumule length
Run	1	NS	NS	NS	NS	NS	NS
Population (P)	1	NS	NS	NS	NS	NS	NS
Treatment (T)	5	<0.001	<0.001	<0.005	<0.0036	<0.001	<0.01
$P \times T$	5	NS	NS	NS	NS	NS	NS

Note: NS, not significant.

Table 2. The response of germination characteristics of Scotch thistle cypselas following treatment with sulfuric acid as a physical seed dormancy-breaking mechanism.

Treatment	GP (%)	GR	MGT	T_{50}	Radicle length (cm)	Plumule length (cm)
Control	6.78c	0.17d	13.39a	12.32a	0.51c	0.53b
10 min	60.60a	2.99a	10.42b	7.87c	1.90a	2.38a
20 min	59.96a	2.78ab	10.55b	9.10bc	1.70ab	2.24a
30 min	49.49ab	2.40abc	10.77b	8.87bc	1.50ab	2.18a
40 min	40.92b	2.28bc	11.01b	9.71b	1.44b	2.04a
50 min	40.93b	2.09c	11.59b	10.23b	1.38b	1.94a

Note: Within a column, means followed by the same letter are not significantly different at the 0.05 probability level according to Tukey's multiple comparison test.

Dry and wet prechilling treatments

The GP and GR were affected by the prechilling treatments ($p \leq 0.01$) (Table 3), such that the highest GPs of 34.61% and 29.59% occurred in the 72 h wet and dry prechilling , respectively. Nevertheless, the maximum GR of 1.73 cypselas per day was recorded for the 72 h wet prechilling. In contrast, the 24 h wet and dry prechilling caused a reduction in the GP and GR compared with the 72 h prechilling (Table 4). Also, there was a significant difference among treatments using dry and wet prechilling in the MGT and T_{50} of Scotch thistle cypselas compared with the control ($p \leq 0.01$) (Table 3), such that the lowest (9.54 and 7.15 days) were related to cypselas soaked in wet prechilling for 72 h (Table 4). The radicle and plumule lengths were significantly affected on using prechilling treatments compared with the control ($p \leq 0.01$) (Table 3), such that the greatest radicle and plumule lengths (1.55 and 2.11 cm) were recorded when cypselas were soaked with 72 h wet prechilling. However, there was no significant difference between the 72 h wet and dry prechilling in radicle and plumule lengths. The lowest radicle and plumule lengths, 0.56 and 0.93 cm, respectively, occurred when cypselas were treated with 24 h dry prechilling (Table 4). Overall, the cypsela GP, GR, and radicle and plumule lengths in the two local populations were improved on increasing the duration of prechilling. In addition, wet prechilling was more effective than dry prechilling in breaking dormancy in Scotch thistle cypselas. Schutte et al. (2012) reported that giant ragweed (*Ambrosia trifida* L.) embryos excised from nearly fresh cypselas (i.e., stored dry at 4 °C for 45 days) were still highly dormant and that a period of stratification (i.e., moist storage at 4 °C) was an absolute requirement for dormancy loss. Giant ragweed cypselas required a minimum of 6 weeks of stratification to alleviate dormancy, and up to 80% of viable cypselas germinated (Page and Nurse 2015). Golmohammadzadeh et al. (2015) indicated that 45 days of wet prechilling was sufficient to break the seed dormancy in long-headed and common poppy, and as a reaction to the prechilling treatment, common poppy showed a 48% higher level of germination than long-headed poppy. Increasing the moist prechilling duration from 15 to 30 days enhanced the seed germination of downy woundwort [*Stachys germanica* (L.) subsp. *bithynica* (Boiss.) R. Bhattacharjee] from 68% to 95%, respectively (Güleryüz et al. 2011). Therefore, it can be concluded that stratification, especially wet prechilling, could effectively break the physiological dormancy of Scotch thistle cypselas if this pretreatment is used for a long time. Kambizi et al. (2006) stated that cytokinin and gibberellin levels increased in the embryo of ashwagandha [*Withania somnifera* (L.) Dunal] during prechilling. In contrast, the ABA levels in the seeds that received a moist prechilling treatment declined during the imbibition regimes of the cold treatment, because of leaching. Also, Nkomo and Kambizi (2009) and Gashi et al. (2012) stated that moist prechilling acts as a priming treatment to provide the seeds' moisture requirement to activate the gibberellin-synthesizing mechanism and the hydraulic enzymes.

KNO_3 and GA_3 treatments

KNO_3 and GA_3 concentrations were effective in breaking Scotch thistle cypsela dormancy ($p \leq 0.01$) (Table 5), such that the GP and GR increased as the KNO_3 and GA_3 concentrations increased. The highest GP and GR were observed when cypselas were soaked in GA_3 for 48 h (88.43% and 4.96 cypselas per day) and KNO_3 for 96 h (69.36% and 3.30 cypselas per day) (Table 6). KNO_3 and GA_3 were significantly effec-

Table 3. ANOVA (p value) of the germination characteristics of Scotch thistle cypselas on using dry and wet prechilling as a physiological seed dormancy-breaking mechanism.

Source of variance	Df	GP	GR	MGT	T_{50}	Radicle length	Plumule length
Run	1	NS	NS	NS	NS	NS	NS
Population (P)	1	NS	NS	NS	NS	NS	NS
Treatment (T)	6	<0.001	<0.003	<0.005	<0.0001	<0.002	<0.001
$P \times T$	6	NS	NS	NS	NS	NS	NS

Note: NS, not significant.

Table 4. The response of germination characteristics of Scotch thistle cypselas following treatment with dry and wet prechilling as a physiological seed dormancy-breaking mechanism.

Treatment	GP (%)	GR	MGT	T_{50}	Radicle length (cm)	Plumule length (cm)
Control	6.83d	0.17c	13.50a	12.39a	0.52d	0.54d
Dry prechilling, 24 h	12.65cd	0.62c	9.95bc	8.33bc	0.56d	0.93c
Dry prechilling, 48 h	28.76b	1.12b	11.18abc	9.90ab	1.34ab	1.78a
Dry prechilling, 72 h	29.59ab	1.26b	10.21bc	7.96bc	1.51a	2.04a
Wet prechilling 24 h	14.44c	0.45c	12.13ab	10.41ab	0.83c	1.32b
Wet prechilling, 48 h	28.36ab	1.54ab	9.86bc	7.34c	1.13b	1.84a
Wet prechilling, 72 h	34.61a	1.73a	9.54c	7.15c	1.55a	2.11a

Note: Within a column, means followed by the same letter are not significantly different at the 0.05 probability level according to Tukey's multiple comparison test.

Table 5. ANOVA (p value) of the germination characteristics of Scotch thistle cypselas on using KNO_3 and GA_3 as a physiological seed dormancy-breaking mechanism.

Source of variance	Df	GP	GR	MGT	T_{50}	Radicle length	Plumule length
Run	1	NS	NS	NS	NS	NS	NS
Population (P)	1	NS	NS	NS	NS	NS	NS
Treatment (T)	6	<0.0011	<0.0051	<0.01	<0.001	<0.007	<0.0041
$P \times T$	6	NS	NS	NS	NS	NS	NS

Note: NS, not significant.

tive in reducing the MGT of Scotch thistle cypselas ($p \leq 0.01$) (Table 5), such that the lowest MGTs were 9.47 and 9.34 days for cypselas soaked in GA_3 for 48 h and KNO_3 for 96 h, respectively (Table 6). The T_{50} of Scotch thistle cypselas was significantly affected by the KNO_3 and GA_3 pretreatments ($p \leq 0.01$) (Table 5). The lowest values of T_{50} were observed when cypselas were treated with GA_3 for 48 h (7.10 days) and KNO_3 for 96 h (6.86 days) (Table 6). The radicle and plumule lengths were also significantly affected on using KNO_3 and GA_3 ($p \leq 0.01$) (Table 5), such that the greatest radicle and plumule lengths (2.96 and 3.57 cm) among treatments were recorded when cypselas were soaked in GA_3 for 48 h. Furthermore, the longest radicle (2.07 cm) and plumule (2.46 cm) lengths were observed when cypselas were treated with KNO_3 for 96 h (Table 6). The current research revealed that exogenous KNO_3 and GA_3 applications induced higher GP, GR, and radicle and plumule lengths, and lowered the MGT and T_{50}, in cypselas of the two local populations of Scotch thistle compared with the control. KNO_3 effectively broke dormancy in the Scotch thistle when cypselas were soaked in this solution for 96 h. Kwon et al. (2020) reported that KNO_3 at 0.20 g L^{-1} improved the germination characteristics of *Maesa japonica* (Thunb.) Moritzi & Zoll. seeds (such as MGT and T_{50}) and increased the GP to 74% at 30 days. Priming with 0.2% or 0.5% solution of KNO_3 for 72 h is a recommended method (GP greater than 74.4%) that can be practically applied for increasing the germination of seashore paspalum (*Paspalum vaginatum* Swartz) under an alternating-temperature (25/35 °C) condition (Shim et al. 2008). The positive effect of KNO_3 could be related to its role in balancing seed hormones that reduce germination inhibitors, such as ABA (Gashi et al. 2012). Duermeyer et al. (2018) suggested that a gene encoding the ABA catabolic enzyme CYP707A2 is directly regulated by the NIN-like protein 8 transcription factor (which acts downstream of nitrate signaling), and this regulation triggers a nitrate-induced ABA decrease that permits seed germination. However, it seems that a higher concentration of KNO_3 or soaking for a long time is required to stimulate metabolic activity and to lower the ABA concentration in the embryo and cypselas coat. Maximum germination was observed when cypselas were soaked in GA_3 for 48 h, in agreement with Kwon et al. (2020), who stated that concentrations of $GA_3 \geq 1.0$ g L^{-1} greatly improved the GP in *M. japonica* seeds. Previous studies also indicated that the seeds of weed species treated with GA_3 ger-

Table 6. The response of germination characteristics of Scotch thistle cypselas following treatment with KNO_3 and GA_3 as a physiological seed dormancy-breaking mechanism.

Treatment	GP (%)	GR	MGT	T_{50}	Radicle length (cm)	Plumule length (cm)
Control	6.80f	0.17d	13.42a	12.39a	0.51f	0.52e
KNO_3, 24 h	20.35e	1.15c	10.64b	9.15b	1.09e	1.40d
KNO_3, 48 h	36.35d	1.73c	10.28b	8.53bc	1.51d	2.23c
KNO_3, 96 h	69.36b	3.30b	9.34b	6.86c	2.07bc	2.46c
GA_3, 12 h	43.09cd	2.60b	10.38b	8.74bc	1.79cd	2.64bc
GA_3, 24 h	52.90c	2.94b	9.59b	8.41bc	2.26b	3.05b
GA_3, 48 h	88.43a	4.96a	9.47b	7.10bc	2.96a	3.57a

Note: Within a column, means followed by the same letter are not significantly different at the 0.05 probability level according to Tukey's multiple comparison test.

minated markedly better than those of the control absent of GA_3 treatment (Rogis et al. 2004). GA_3 signaling, like KNO_3, induces lower ABA concentrations by activation of proteins that regulate the gene or genes encoding the ABA catabolic enzymes to overcome the physiological dormancy in seeds with a dormant embryo. Kucera et al. (2005) revealed that GA_3 promotes seed/fruit germination by activating the synthesis of proteins and other required metabolites for the embryo.

Conclusions

In summary, the germination characteristics significantly improved when sulfuric acid was applied for 10 min. It seems that soaking cypselas in 98% sulfuric acid can help in reducing the concentrations of water-soluble inhibitors in the embryo and cypselas coat, and then cypselas initiate germination. Hence, the chemical scarification of Scotch thistle cypselas could lead to the breaking of physiological dormancy and germination. Despite 72 h moist cold stratification led to cypselas germination up to 34.61%, these results indicated that germination of cypselas would be improved if the duration of soaking cypselas in water-solution was increased. The best germination response was observed when cypselas were soaked in KNO_3 for 96 h and GA_3 for 48 h. These findings could help future studies on Scotch thistle by alleviating cypsela dormancy and thereby aiding in rapid germination.

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