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THE EFFECTS OF LOW TEMPERATURE APPLICATIONS ON DORMANCY OF *SALVIA VERTICILLATA* L. AND *RUMEX CRISPUS* L. SEEDS

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Abstract

The effect of low temperature was studied to break the seed dormancy of *Salvia verticillata* L. and *Rumex crispus* L. seeds. *S. verticillata* and *R. crispus* seeds collected in 2017 and 2018 were kept in deep freezer at -80°C for certain periods time. Some of the seeds of both the species were soaked in hot water at 90°C for 5 seconds but some others were left untreated. Germination tests were performed in dark at 20 ±1 ^{0}C according to randomized plots design with four replications. Seed germination rates and germination performance (time) were calculated. The highest germination in *S. verticillata* was obtained from the seeds collected in 2017 (57%) and 2018 (67%) which were kept in deep freezer at -80°C for 4 days and were left untreated. The highest germination rate in *R. crispus* was obtained from the seeds collected in 2017 (83%) which were kept in deep freezer at -80°C for 1 day and were planted without any treatment. Germination rate of fresh *R. crispus* seeds was found to be rather low.

Key words: Seed germination, Dormancy, Low temperature, *Salvia verticillata*, *Rumex crispus*.

Introduction

Seed germination has a key role in the success of plant breeding cycle (Bu *et al*., 2008). Genetic variation of populations can only be protected by seed germination (Fenner & Thompson, 2005; Anon., 2017) and germination after the formation of seed is a critical transition process which has an effect on survival (Larson *et al*., 2015; Gallagher & Wagenius, 2016). Seed germination involves a series of physiological phenomena which are affected by internal (dormancy, physical immaturity and genotype) and external (light, temperature, availability of water and substrate) factors (Kleczewski *et al*., 2010). Seed's ability to delay germination till the availability of suitable time and place is one of the key mechanisms for its survival (Copeland & McDonald, 2008) and it makes sure that the seed is germinated only when the environmental conditions are favorable (Finskelstein *et al*., 2008).

The term 'dormancy' is used to define, "the seed which does not germinate under favorable conditions in a particular time period". Seed germination process is temporarily delayed in some plants due to dormancy (Hilhorst, 1995; Baskin & Baskin, 1993). This is beneficial for plants as the seed remains inactive in this period and endures several environmental stresses and poor climatic conditions helping its survival for generations (Lampter, 1994). While a certain level of dormancy is advantageous during seed development, however, sometimes it is often not demanded for agricultural products in need of fast germination and growth (Bewley, 1997).

Dormancy and seed germination depend on genetic factors and environmental conditions affecting plant growth and development (Sarmandnia, 1996). Testa, embryo and the presence of inhibiting materials in the seed, are among the factors affecting seed dormancy (Latifi, 2001; Elamin *et al*., 2013). *Salvia* spp. and *Rumex* spp. seeds have dormancy because of their impermeable testa. The mucilage-like substance available in testa of *Salvia* seed causes dormancy (Ozcan *et al*., 2014). As seed germination rate is low in *Rumex crispus* and *Salvia verticillata* seeds, it is important to analyze the factors affecting dormancy and germination (Khakpoor *et al*., 2015). Dormancy breaking investigations were carried out to study the impermeable structure of seed testa of *Salvia* and *Rumex* plants (Baskin & Baskin, 1978; Grime *et al*., 1981; Van Assche & Vanlerberghe, 1989; Bozdogan *et al*., 2018). Seed germination studies were conducted by using various methods. These studies revealed interesting results in different environmental conditions. Most of the medicinal plant seeds show variability with regards to their ecologic adaptability to environmental conditions. Therefore, creating optimal conditions and identifying the eco-physiological factors affecting dormancy are important for their cultivation and production (Khakpoor *et al*., 2015). The knowledge of seed quality and creating optimal conditions for seed germination are important for their planting and propagation (Ghasemi Pirbaloti *et al*., 2007).

Even if a hard testa prolongs particularly the life of seed in soil under unfavorable environmental conditions and helps prevention of the extinction of species in nature, it might inhibit utilization of their wild-type relatives or plant species related with improvement or agricultural works (Bewley, 1997; Nair *et al*., 2004). On the other hand, there are various factors that eliminate or reduce seed dormancy by using thermal processes such as immersion in hot water, mechanical or chemical practices such as sand scarification and acid applications (Buyukkartal *et al*., 2013; Karaguzel *et al*., 2004; Hu *et al*., 2009).

R. crispus contains tannins, flavonoids, fixed oil and volatile oils, caratenoids, iron, phosphate and polysaccharide compounds (Ozer *et al*., 2004). *S. verticillata* has antioxidant (Tosun *et al*., 2009; Sarbanha *et al*., 2011; Orhan *et al*., 2013), antimicrobial (Kunduhoglu *et al*., 2011), antidiabetic effect (Eidi *et al*., 2011) and anticholinesterase activities (Matkowski *et al*.,

2008, Kunduhoglu, *et al*., 2011; Orhan *et al*., 2013) and known to contain various polyphenols, essential oils and terpenoids (Matkowski *et al*., 2008). *R. crispus* and *S. verticillata* were selected because of their importance in human health as medical and/or aromatic plants.

It is important to analyze the factors affecting germination of *S. verticillata* and *R. crispus* seeds with seed dormancy, which are among the medicinal and aromatic plants. This research was conducted to analyze the effects of low temperature treatments for breaking the seed dormancy of fresh (1-month-old seeds) and old (13-month-old seeds) of both the species.

Materials and Methods

Seeds of *S. verticillata* and *R. crispus* were collected in 2017 and 2018 from the natural habitat of Battalgazi/ Malatya province-Turkey with an altitude of 725 m (38° 27′ 51″ N 38° 21′ 27″ E). The seed of both the species used in experiments were 1-year-old (13-month-old) and fresh (1-month-old) seeds. The collected seeds were kept at room temperature (24°C) in cloth bags until the experiment.

S. verticillata and *R. crispus* seeds collected in 2017 and 2018 were individually treated twice in those years. In the first treatment, *S. verticillata* and *R. crispus* seeds were put into zip lock bags and kept for 0, 1, 2, 4 and 7 days in deep freezer at -80°C. In the second treatment, the seeds in zip lock plastic bags were kept for 0, 1, 2, 4 and 7 days in deep freezer at -80°C, and immersed in hot water at +90°C and were kept there for 5 seconds before the beginning of the experimental process. The experiments were repeated twice. As there was no difference between the findings, the mean values were taken for evaluation.

Seeds were kept for germination in dark at 20 ± 1.0°C in a temperature-controlled incubator for 7 days. Seeds were placed in a 9 cm-wide petri dish with two layer of Whatman No 1 filter paper and were moistened with 3 ml distilled water. The experimental design was completely randomized plots with four replicates and 50 seeds were placed in petri dishes, in each replication. To calculate the time and rate of germination, tests continued until the 7th day when germination was stable and germinated seeds were taken out of the petri dish depending on the findings of daily counting.

Maximum germination percentage (G_{max}), its angular transformation (arcsine √Gmax-archin) and the time elapsed for the germination of 50% of the seeds (in days) (G_{50}) and the time elapsed for the germination of 10-90% of the seeds (G_{10-90}), were calculated using the total count of germinated seeds (Wiley *et al*., 1993). While the time between 10%-90% of G_{max} (G_{10-90}) is accepted as the estimated germination propagation which is opposite of germination synchronicity, the time up to 50% of Gmax (G_{50}) is a measurement opposite the germination rate (Tiryaki and Topu, 2014).

Statistical analyzes for percentages of total germinated seeds in the experiment were made within the groups and then the results were evaluated. One way (ANOVA) variance analysis was applied in the evaluation of the data. The difference between the applications was determined using the Least Significant Design (LSD) at the $p \leq 0.05$ probability level.

Results

Fresh seeds of *R. crispus* were excluded from evaluation as sufficient rates of germination were not achieved in the two studies conducted in 2018.

Germination rates of *S. verticillata* seeds are given in Figure 1. angular transformation and germination performance and durations are given in Table 1.

Germination rate was found lower in 2017 seeds compared to seeds collected in 2018. In the seeds of 2018, the highest germination rate was in D4 with a percentage of 67%; whereas this percentage was 57% in the seeds of 2017 (Fig. 1). With regards to UT, germination rates of the seeds of 2017 and 2018 were close to each other (24.5% and 23%, respectively). Findings of the other applications showed that; as the time of keeping the seeds in deep freezer was prolonged (D1-7), higher germination rate was observed in the seeds of both years. Germination rates in 'D4+HW', 'D7+HW' and 'HW-only' treatments were lower when compared to UT (Fig. 1).

Table 1. The effect of different applications on germination values of *S. verticillata* seeds (Gmax-archins, G_{50}, G_{10-90}) of 2017-2018.

Treatments	$G_{max\text{-}archins}$ [degree]		G_{50} (days)		G_{10-90} (days)	
	2017	2018	2017	2018	2017	2018
D1 only	[46.73]	[46.70]	2.25	2.00	1.25	0.25
D2 only	[45.00]	[51.80]	2.25	2.00	1.00	0.25
D4 only	[49.29]	[56.58]	2.00	2.00	1.00	0.50
D7 only	[40.55]	[43.03]	2.00	2.00	0.75	0.25
D1 + HW	[25.23]	[35.03]	3.50	4.50	3.00	3.25
D2+ HW	[13.07]	[33.21]	3.75	4.75	4.00	3.75
D4+ HW	[13.42]	[14.60]	4.25	5.25	5.75	4.50
D7+ HW	[27.99]	[22.94]	4.25	4.75	2.75	4.75
HW only	[16.67]	[25.59]	3.75	3.75	3.00	3.50
UT	[29.58]	[28.48]	2.50	5.00	1.00	2.75
LSD 0.05	[7.58]	[13.16]	1.17	1.55	1.58	2.08
Significance	**	**	**	**	**	**

Hw: Hot water, S1-7: Storage of seed in the freezer for 1-7 days, UT: Untreated control seed

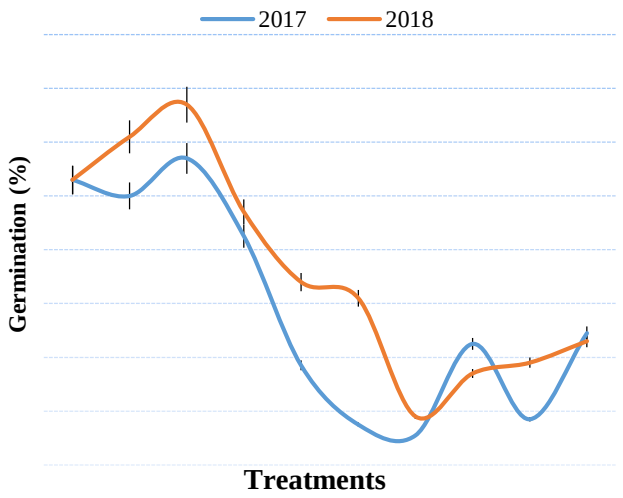

Fig. 1. The effect of various applications on the germination rate of *S. verticillata* seeds collected in 2017 and 2018. Hw: hot water, D1-7: storage of the seed in the freezer for 1-7 days, UT: untreated control seed.

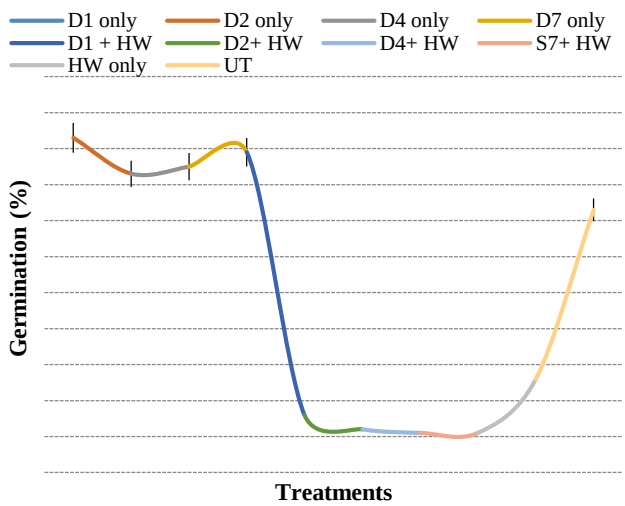

Fig. 2. The effect of various applications on the germination rate (%) of *R. crispus* seeds of 2017. Hw: hot water, D1-7: storage of seed in the freezer for 1-7 days, UT: untreated control seed.

Table 2. The effect of different applications on germination values ($G_{max\text{-}archins}$, G_{50}, $G_{10\text{-}90}$) of *R. crispus* seeds of 2017.

Treatments	G max-archins [degree]	G_{50} (days)	$G_{10\text{-}90}$ (days)
D1 only	[66.20]	3.00	3.25
D2 only	[58.94]	3.00	1.50
D4 only	[60.35]	4.50	3.75
D7 only	[63.24]	3.75	1.75
D1 + HW	[12.06]	4.00	4.75
D2 + HW	[16.83]	4.25	4.25
D4 + HW	[2.89]	1.75	1.75
D7 + HW	[2.89]	1.25	1.25
HW only	[20.53]	3.75	2.25
UT	[52.87]	3.00	1.00
LSD 0.05	[11.02]	--	--
Significance	**	ns	Ns

Hw: Hot water, S1-7: Storage of seed in the freezer for 1-7 days, UT: Untreated control seed

It was clearly shown that germination rates of both years seeds were increased and some differences were detected in hot-treated seeds as the seeds stayed in deep freezer for longer periods (except D7) (Fig. 1 and Table 1). Germination rate was decreased only in hot-treated seeds. Compared to untreated seeds, germination rate of the seeds of 2017 was decreased when they were exposed to hot water after keeping at -80°C for 1 to 7 days. Seed germination rates were the lowest particularly following 'D2+HW' and 'D4+HW' treatments. The lowest rate of germination in the seeds of 2018 was found in 'D4+HW' experiment and the germination rate of the seeds was reduced up to 50% compared to untreated seeds. In addition, 'D1+HW' and 'D2+HW' treatments of the seeds of 2018 revealed higher germination rates compared to untreated seeds and the seeds treated with hot water (Fig. 1 and Table 1).

In the all cold treatments, the duration of germination in *S. verticillata* seeds (G_{50}) were compared with the untreated seeds of 2017. The results revealed shorter germination durations in the seeds kept in deep freezer. The durations were extended in the seeds treated with hot water.

The duration required for 50% germination of the seeds of 2017 was 4.25 days in the experiments 'D4+HW' and 'D7+HW', whereas it was longer in the experiment of 'D4+HW' for the seeds of 2018 (5.25 days).

The lowest germination was obtained from the synchrony ($G_{10\text{-}90}$) of the seeds of 2017 that were kept in deep freezer for 0.75 to 7 days (Table 2). Similar results were obtained from experiments D1, D2 and D7 for the seeds of 2018 (0.25 day). The highest germination was obtained from the synchrony ($G_{10\text{-}90}$) of the seeds of 2017 and 2018 (D4+HW and D7+HW, respectively).

Germination rate of *R. crispus* seeds kept in deep freezer was higher than untreated seeds (Fig. 2). The highest germination rate was obtained in seeds that were kept in deep freezer for 1 day (83%). The germination rate was found as 63% in untreated seeds. Germination rates obtained in hot water treatments following deep freezer treatments were found to be lower compared to untreated seeds and the seeds kept in deep freezer (D-only).

There was no statistically significant difference between the germination rate of *R. crispus* seeds and synchronicity. There were significant differences with respect to Gmax-archins values.

With respect to germination rates (G_{50}), the highest germination was obtained in D4 treatment with 4.5 days and the lowest germination was obtained in 'D7+HW' with 1.25 days. When compared with untreated seeds, the seeds kept only in deep freezer had longer periods for germination. In hot-water treatments, 1.25-4.00 days were required for 50% seed germination (Fig. 1). The lowest germination synchrony ($G_{10\text{-}90}$) was obtained in UT seed with 1 day whereas this rate was higher (but statistically insignificant) in other treatments (Fig. 2).

Discussion

S. verticillata and *R. crispus* are medicinal and aromatic plants and have an important role in human health. There is seed dormancy in these plants due to the impermeability of the testa, which inhibits germination.

The mucilage-like substance available in the testa of *Salvia* seed causes dormancy (Ozcan *et al*., 2014). As seed germination rate is low in these plants, it was important to analyze the factors affecting their dormancy and germination (Khakpoor *et al.*, 2015). Dormancy breaking studies were carried out in *Salvia* and *Rumex* plants (Baskin & Baskin, 1978; Grime *et al*., 1981; Van Assche & Vanlerberghe, 1989; Bozdogan *et al*., 2018). Genetic factors are important in determining the rate of hardness in seed testa (Tinius, 1991, Van Assche & Vandelook, 2010). Also, other factors such as where the seeds are located (Argel & Humphreys, 1983, Dubbern De Souza & Marhos-Filho, 2001), early or late maturation (Ranathunge *et al*., 2010, Hay *et al*., 2010), ecological differences in temperature and relative humidity (Benech-Arnold *et al*., 2000, Gehan Jayasuriya *et al*., 2012) can cause delay in germination.

Previous research findings revealed that *Salvia* seeds required a cold pre-treatment for germination (Young & Young, 1992); the rate of germination was highly increased in *S. cyanescens* seeds, compared to the control group, when they were kept in deep freezer at -5°C for 5 minutes (Yucel & Yılmaz, 2009) and gibberellic acid treatments significantly increased the rate of germination following pre-cooling in certain *Salvia* species (Subası & Gulseven, 2010; Ozcan *et al*., 2014). In this research it was found that, cold pre-treatment of the seeds at -80°C for 4 days accelerated the rate of germination more than 100% in both years compared to the control group. Our results fully agree with the previous reports (Young and Young, 1992; Yucel and Yılmaz, 2009) that cold pretreatment is important for germination.

Only heat treatments caused a decrease in germination rate in both years. This was compatible with the finding of Khakpoor *et al*., (2015) that hot water treatments significantly decreased the germination index of *S. verticillata* seeds.

Hot water treatments significantly decreased the germination index of *S. verticillata* seeds The germination rate of *R. crispus* seeds was similar with *S. verticillata* seeds. Particularly hot water treatments caused a significant reduction in seed germination while cold treatments caused an elevation (Fig. 2). On the other hand; Tavili *et al*., (2009) reported that hot water treatment would have a negative impact on testa and hot water could harm the seed structure.

Comparison of the germination rates of the fresh and 1-year-old seeds of 2017 and 2018 revealed that fresh seeds had a higher germination rate. As reported by Hajyzadeh *et al*., (2017), this was compatible with the finding that fresh anise seeds had a higher germination rate compared to older seeds.

Ates (2017) in his dormancy breaking studies of 1-month-old and 12-month-old seeds reported that the best treatment in 1-month-old seeds was 2000 ppm gibberellic acid with a germination rate of 95.7% and the germination rate was also reached to 100% by the same treatment in 12-month-old seeds. This indicates that germination rates of plants with hard testa can vary.

Bozdogan *et al*., (2018) monitored germination rates of *R. crispus* seeds for 14 days while keeping in light and light/dark environments and found an increase in germination rates compared to control group, and also found that germination rate began to decrease after they were kept at 80°C for 1 minute following cold treatment. On the other hand; Tiryaki & Topu (2014) reported that lupine and broad beans seeds when treated with low and high heat elevated rate of germination. Their findings are compatible with our results.

Seed germination studies were performed with different plants previously with the aim to elevate germination rates mostly by corroding the testa using chemical substances. For *R. crispus* seeds, 120 hours in 3% ethanol (light) and 60 seconds in sulfuric acid (light/dark), were reported to be the most effective ways in breaking dormancy (Bozdogan *et al*., 2018). Similarly, incubating the *Corchorus olitorius* L. seeds in sulfuric acid applications at different time periods was also reported to stimulate germination (Velempini *et al*., 2003; Emongor *et al*., 2004).

In sulfuric acid treatments, there were reductions in germination rates following 15-minute-long exposures. When the duration of exposure to sulfuric acid was increased from 60 seconds to 15 minutes, germination rate increased. When exposure was longer than 15 minutes, germination rate decreased below 20%. Tuncer & Ummuhan (2017) while studying the dormancy problem in Molehiya (*Corchorus olitorius* L.) found that the seeds with sulfuric acid treatment for 5-10 minutes had higher germination rates whereas there were statistically significant reductions in germination rate with the increase of the duration of exposure. In another similar research, Elias & Al-Safadi (2011) obtained the highest germination rate (32%) in 20-minute-long H_2SO_4 pre-treatment in *Capparis spinosa* L. seeds. This rate was decreased in 30-minute-long H_2SO_4 treatment while there was no germination in 45- and 60-minute-long H_2SO_4 treatments.

Corroding testa by mechanical and chemical substances damages the other parts of the seed including the embryo and may reduce seed viability due to the pathogenic and saprophytic organisms, leading to reduction of seed shelf-life. However; this technique gives a slight damage to the testa with regards to water permeability (Loch & Harvey, 1992; Acharya *et al*., 1999). On the other hand; implementation of different types of acids is not preferable and is assumed to be unreliable due to its cost, safety risk and the need for environmental measures; or it does not have the essential qualities for the seeds of other important plant species (Ates, 2011, Loch & Harvey, 1992; Alderete-Chavez *et al*., 2010). Therefore, to low heat treatments in mechanic corrosion could be less harmful. Similar to previous reports (Young & Young, 1992; Yucel & Yılmaz, 2009; Bozdogan *et al*., 2018), cold treatment of *S. verticillata* and *R. crispus* seeds significantly increased germination rates whereas hot treatments following cold treatments had a negative effect on germination rates (Figs. 1 & 2). As for *S. verticillata*, germination rate was 67% in D4 treatment and 83% in D1 treatment, which were higher than 23% and 63% obtained in UT treatment.

In conclusion, the freezing-melting method previously applied for the first time to break the seed dormancy to white-lupine and red clover seeds by Tiryaki & Topu (2014) was also used for the seeds of *S.*

verticillata and *R. crispus*. The present results suggested that the highest germination rate in *S. verticillata* was obtained from the seeds collected in 2017 (57%) and 2018 (67%) which were kept at -80°C for 4 days and were left untreated. However, the highest germination rate in *R. crispus* was obtained from the seeds collected in 2017 (83%) which were kept at -80°C for 1 day and then planted without any further treatment. The present results will be useful for further studies in order to have more, faster and reliable seed germinations.

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